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11. Samples were extracted ultrasonically in 50 percent methylene chloride in methanol. The extracts were rotary evaporated under reduced pressure and then saponified by reflux in a 20 percent aqueous solution of 1 percent KOH in methanol. The neutral lipids were fractionated into 10 percent diethyl ether in hexane and chromatographed on silica gel by thin-layer chromatography, with 16 percent ethyl acetate in hexane being used as the solvent system. The fatty alcohol band, defined by phytol as the thin-layer chromatography standard, was isolated and analyzed by gas chromatography.
12. Individual fatty alcohols in each mixture were quantified as trimethylsilyl derivatives by capillary/gas chromatography on an apolar fused silica column [OV-1; 25 m by 0.3 mm (inner diameter)] with helium carrier gas (1.2 kg/cm^2), temperature programming (80° to 280°C at 4°C per minute), and 5-α-(H)-cholestane as an internal injection standard. The identity of phytol or dihydrophytol in a sample was confirmed by gas chromatography–mass spectrometry through comparison of sample spectra with those of authentic standards.
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Sodium-Coupled Sugar Transport: Effects on Intracellular Sodium Activities and Sodium-Pump Activity

Abstract. *Intracellular sodium activities, $(Na)_c$, were determined in* Necturus *small intestine before and after addition of galactose to the mucosal bathing solution. In the absence of galactose, $(Na)_c$ averaged 12 millimoles per liter. Within 2 minutes after the addition of galactose to the mucosal solution, $(Na)_c$ increased to a mean value of 20 millimoles per liter and then declined, in parallel with an increase in transcellular sodium transport, to a value that did not differ significantly from that observed in the absence of the sugar. The final steady state in the presence of galactose was characterized by a three- to fourfold increase in the rate of transcellular Na^+ transport in the absence of a significant increase in $(Na)_c$. Thus, the increase in steady-state basolateral pump activity cannot be attributed to an increase in the intracellular sodium transport pool.*

It is generally accepted that the entry of some sugars and all natural amino acids across the apical or luminal membranes of small intestine and renal proximal tubules in various animals throughout the phylogenetic scale is coupled to the entry of Na^+ and is energized by the electrochemical potential difference for Na^+ across those membranes (*1*). These coupled entry processes result in the "secondary active" absorption (*2*) of the sugars and amino acids and also bring about an increase in the rate of active Na^+ absorption by these epithelia. However, the effect of these processes on the intracellular Na^+ activity, $(Na)_c$, and the relation between $(Na)_c$ and the rate of active Na^+ absorption have not been clearly established.

In 1972, Lee and Armstrong (*3*) reported that the addition of 3-*O*-methylglucose to the solution bathing segments of bullfrog small intestine in vitro resulted in a decrease in $(Na)_c$ from an average value of 14.9 m*M* to one of 12.1 m*M*. Although the effect of this sugar on the rate of active Na^+ absorption was not reported, it is reasonable to infer from the results of earlier studies (*4*) that active Na^+ absorption was increased. Thus, one was confronted with two somewhat unexpected and paradoxical findings; namely (i) that $(Na)_c$ decreased despite an increase in the rate of Na^+ entry across the apical membrane and (ii) that the rate of active transcellular Na^+ absorption, which reflects the rate of Na^+ pump activity at the basolateral membrane, increased in the face of a decrease in $(Na)_c$. The single possible shortcoming of these studies was the method used to determine $(Na)_c$. Inasmuch as highly selective liquid ion-exchange resins for Na^+ were not available at that time, the investigators used two closed-tip K^+-selective microelectrodes with different selectivity coefficients for K^+ over Na^+ ($K_{K:Na}$) together with a conventional KCl-filled open-tipped microelectrode. The intracellular Na and K activities were calculated from the results of three separate sets of microelectrode impalements by simultaneously solving two Nicolsky equations (*5*). This approach, unavoidably, introduced a significant propagation of errors and some degree of uncertainty in the final mean values. Nonetheless, to the best of our knowledge, the study by Lee and Armstrong is the only one that directly addressed this issue, and the results have neither been confirmed nor challenged.

We used highly Na^+-selective liquid ion-exchanger microelectrodes to examine the effects on $(Na)_c$ of adding galactose to the solution bathing the mucosal surface of *Necturus* small intestine. Because the villus cells of this epithelium are electrically coupled (*6*), we simultaneously measured $(Na)_c$ and the electrical potential difference across the apical membrane (ψ^{mc}), the latter with conventional microelectrodes, by impaling two nearby cells. In several instances we succeeded in keeping the two single-barreled microelectrodes in place before and after the addition of the sugar to the mucosal solution, so that the results are internally paired.

Necturus maculosa (*7*) were stored in tap water at 4°C until use. The animals were anesthetized in tap water containing 0.1 percent tricainemethanesulfonate (Sigma) until all reflexes were absent. The proximal one-third of the small intestine was excised and stripped of its underlying musculature by blunt dissection, and a segment of this stripped preparation was mounted mucosal surface up in a perfusion chamber with an exposed surface area of 0.19 cm^2. The control perfusion solution contained (in millimoles per liter): Na^+, 110; Cl^-, 117; K^+, 2.5; Ca^{2+}, 1.2; Mg^{2+}, 1.2; MOPS (*7*), 2.5; and mannitol, 15. For the solutions containing galactose, 15 m*M* galactose was substituted for the mannitol. The *p*H of both solutions when equilibrated with air at 23°C was 7.2.

Conventional microelectrodes for the determination of ψ^{mc} were fabricated from glass capillary tubing (outer diameter, 1.5 mm) (*7*) and, when filled with 0.5*M* KCl, had tip resistances (measured in 0.5*M* KCl) of 40 to 60 megohms. The Na^+-selective microelectrodes were fabricated from the same capillary tubing, with the same microelectrode puller used at the same settings. These electrodes were dried by heating in a nitrogen-filled oven at 200°C for 1 hour; then 20 μl of dichlorodimethylsilane was injected into the oven through a gas-tight port and the electrodes were heated for another hour.

Table 1. Effect of galactose on electrical parameters and intracellular Na^+ activity in *Necturus* small intestine (n, number of tissues studied).

State	n	ψ^{mc} (mV)	r^m/r^s	r_t (ohm·cm^2)	I_{sc} (μA/cm^2)	$(Na)_c$ (mM)
Control	12	−27 ± 3	0.82 ± 0.16	113 ± 11	13.4 ± 2.0	12.1 ± 1.1
Galactose						
Peak (1 to 2 minutes)	5	−5 ± 5*	0.18 ± 0.12*	107 ± 7†	17.7 ± 2.3*	20.7 ± 3.4*
Steady state (10 minutes)	7	−19 ± 5*	1.04 ± 0.24†	88 ± 6*	50.7 ± 2.5*	14.1 ± 2.5†

*Difference from control, $P < 0.05$. †Not significantly different from control.

After being cooled, the microelectrodes were back-filled with a small amount of the Na^+-selective liquid ion-exchanger resin described by O'Doherty *et al.* (*8*) and then further back-filled with 0.5*M* KCl. These electrodes were calibrated by determining the slope of the electrode potentials in 1, 3, 10, and 100 m*M* NaCl solutions; the average change in the electrode potential in response to a tenfold change in Na^+ activity was 58.3 ± 0.8 mV ($n = 15$; $r > 0.998$), a value that is essentially identical to that expected for an ideal cation-selective electrode. The selectivity coefficient, $K_{Na:K}$, was determined by recalibrating the same electrodes in the above NaCl solutions, with 100 m*M* KCl added. The value of $K_{Na:K}$ for each electrode was determined graphically as described by O'Doherty *et al.* (*8*), and the same graph was used to determine $(Na)_c$. Thus, the reported values of $(Na)_c$ are automatically corrected for an intracellular K activity of 70 m*M*. The values of $K_{Na:K}$ were between 0.025 and 0.006, with a mean of 0.022. Therefore, since the values for intracellular K activities under the conditions examined in this study are between 50 and 67 m*M* (*6*), any distortion of $(Na)_c$ by interference from cell K^+ is negligible.

After being mounted, the tissues were perfused with the control solution and short-circuited with an automatic voltage clamp (*9*). At 20-second intervals, bipolar current pulses were passed across the tissue sufficient to displace the transepithelial potential difference (ψ^{ms}) by 10 mV; the duration of each pulse was 3 seconds. Neighboring cells were then impaled with a conventional KCl-filled microelectrode and a Na^+-selective microelectrode under stereomicroscopic vision. After steady-state control data were obtained, the perfusate was switched to one containing 15 m*M* galactose. The methods for recording ψ^{mc}, the short-circuit current (I_{sc}), and the deflection in the potential of the Na^+-selective microelectrode on passing from the mucosal solution into the cell (V_{Na}) have been described (*6, 9*). The transepithelial resistance (r_t) was calculated from the current necessary to displace ψ^{ms} by 10 mV. The ratio of the slope resistances of the apical and basolateral membranes (r^m/r^s) was calculated from the ratio of the deflection in ψ^{mc} ($\Delta\psi^{mc}$) to the deflection in ψ^{ms} ($\Delta\psi^{ms}$) in response to the periodic current pulses. In all reported experiments, there was no discernible difference between the value of (r^m/r^s) determined with the KCl-filled microelectrode and that determined with the Na^+-selective microelectrode.

Results are expressed as the mean ± the standard error. Statistical analyses were carried out with Student's t-test and a value of $P < 0.05$ was considered significant.

The results of an experiment in which both microelectrodes remained in place before the addition of galactose to the mucosal perfusate and after a new steady state was achieved in the presence of this sugar are illustrated in Fig. 1, and the average results for all experiments are given in Table 1. As reported previously (*9*), (i) after the addition of galactose to the perfusate there was a rapid depolarization of ψ^{mc} and a decrease in r^m/r^s; (ii) these initial "peak" responses, which were achieved in 1 to 2 minutes were followed by a slower repolarization of ψ^{mc} and an increase in r^m/r^s to a value that was not significantly different from the control value; and (iii) the I_{sc} increased moderately immediately after the exposure of the tissue to galactose but then slowly increased further, in parallel with the repolarization of ψ^{mc}, finally achieving a mean value approximately four times that observed in the absence of the sugar. The new and important findings are that approximately 1 minute after the addition of galactose to the perfusate ("peak") there is a nearly twofold increase in $(Na)_c$ that is followed by a slow decline to a value that, by 10 minutes, does not differ significantly from that observed in the absence of galactose (*10*).

The initial increase in $(Na)_c$ is consistent with an increase in the rate of Na^+ entry into the cell coupled to the entry of galactose; indeed these findings provide additional direct evidence for this coupled entry mechanism. Two factors may contribute to the subsequent decline in $(Na)_c$: (i) an increase in basolateral Na^+ pump activity and (ii) an increase in cell water content, which accompanies the intracellular accumulation of sugars and amino acids in osmotically active forms (*11*).

The finding that currently defies adequate explanation is that the steady-state rate of active Na absorption—and hence the rate of Na^+ pump activity—in the presence of the sugar is approximately four times that under control conditions, in the absence of a significant increase in $(Na)_c$. This finding, which confirms and strengthens that of Lee and Armstrong (*3*), contradicts the traditional view that an increase in pump activity in response to an increase in the rate of Na^+ entry across the apical membrane is mediated by an increase in the "intracellular Na^+ transport pool." Thomas *et al.* (*12*) reported that the rate of active Na^+ absorption by *Necturus* urinary bladder may increase as much as fourfold in the absence of a statistically discernible increase in $(Na)_c$.

The simplest explanation for this finding, namely that the relation between pump rate and $(Na)_c$ is so steep that the

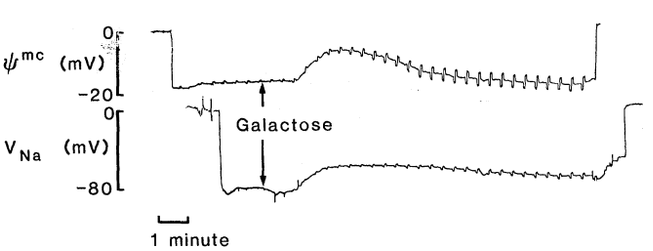

Fig. 1. Simultaneous recordings of the electrical potential difference across the apical membrane (ψ^{mc}) of one villus cell and the electrical deflection of a Na^+-selective microelectrode (V_{Na}) after penetration of a second, nearby cell. As discussed in the text, the periodic deflections provide a measure of r^m/r^s.

small increase in $(Na)_c$ from 12 m*M* to 14 m*M* is sufficient to account for a fourfold increase in pump activity, can be dismissed on the grounds that it is quantitatively inconsistent with any of the known kinetic properties of the basolateral Na^+ pump (*13*). Alternative possibilities are that some signal other than $(Na)_c$ (i) increases the turnover rate or stoichiometry (Na ions pumped per pump cycle) of a fixed number of already operating pumps or (ii) that there is an increase in the number of operating pumps resulting from the activation of pumps already in the membrane that were previously quiescent, or that there is a recruitment of new pumps into the membrane.

We reported that after the addition of galactose to the solution bathing the mucosal surface of *Necturus* small intestine there is a slow increase in the K^+ conductance of the basolateral membrane that parallels the increase in pump activity. This increase is blocked by Ba^{2+} and by prior treatment of the tissue with metabolic inhibitors (*14*). We further reported that all of these effects can be mimicked by exposure of the tissue to a 12 percent hypotonic solution, which presumably results in cell swelling. The increase in K^+ conductance could be caused either by an increase in the conductance of a fixed number of already operating channels or an increase in the number of conductive channels; the latter could result from the activation of quiescent channels already present in the membrane or from the recruitment of new channels into that barrier.

Regardless of underlying mechanism, these two sets of findings clearly indicate that in response to an increase in Na^+-coupled solute entry into the cell across the apical membrane there is an increase in the ability of the cell to actively extrude Na^+ across the basolateral membrane, with no increase in $(Na)_c$, and simultaneously to recycle K^+ across that barrier. Both processes act in concert to prevent potentially large increases in cell Na^+ and K^+ activities in response to large increases in the rate of Na^+ entry and in Na^+ and K^+ pump activity (*15*). It is possible that these responses are "triggered" by cell swelling and serve to prevent inordinate increases in cell volume that would otherwise ensue. The immediate signal (or signals) for these responses and their underlying mechanisms remain to be elucidated.

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$$\frac{I'_{sc}}{I_{sc}} = \frac{1 + [K_{Na}/(Na)_c]^n}{1 + [K_{Na}/(Na)'_c]^n}$$

where I_{sc} and $(Na)_c$ are the control values, and the primes designate the values in the presence of the sugar. Assuming $n = 3$, and substituting the data given in Table 1 into the above equation, we obtain a negative value for K_{Na}, an excluded solution; the situation is even worse if we chose a noncooperative interaction between Na and the pump! Further, it can be readily shown that it is impossible to reconcile the above equation with the experimental data with reasonable values of K_{Na} or n unless one assumes that K_{Na}, $(I_{sc})_m$, or n, or a combination of these, are affected by the Na-sugar cotransport process.
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Allylamine Derivatives: New Class of Synthetic Antifungal Agents Inhibiting Fungal Squalene Epoxidase

Abstract. *A new class of synthetic antifungal agents, the allylamines, has been developed by modification of naftifine, a topical antimycotic. SF 86-327, the most effective of these compounds so far, is highly active in vitro against a wide range of fungi and exceeds clinical standards in the oral and topical treatment of guinea pig dermatophytoses. SF 86-327 is a powerful specific inhibitor of fungal squalene epoxidase, a key enzyme in sterol biosynthesis.*

The search for new antimycotics is currently focused on structures related to the azole-based antimycotics (clotrimazole, miconazole, ketoconazole, and others) (*1*). There have been comparatively few reports in the scientific and patent literature of new structural classes.

Naftifine (*2–4*), a new topical antimycotic, is structurally distinct from all other antifungals (Fig. 1). It was first obtained after acid hydrolysis of heterocyclic spiro-naphthalenones (*5*), and its antifungal activity was discovered during routine screening. Naftifine provided the starting point for synthetic modifications aimed at the development of more potent, orally active compounds. Many related compounds synthesized were found to have considerable antifungal

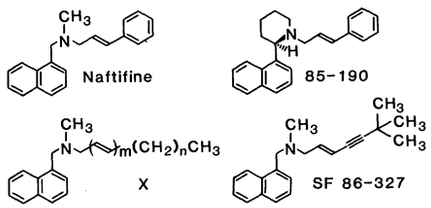

Fig. 1. Chemical structure of naftifine, piperidine derivative 85-190, compound X, and SF 86-327.