TABLE II. Measurements of Oxygen Consumption by an Unsupplemented Guinea Pig Liver Homogenate.

Homogenate concentration, percent (w/v)	Oxygen consumed, μl/hr	
	24.8°	37°
1.0	15.15†± .52‡	28.23 ± 1.88‡
.5	7.54†± 2.23	—
.5 *	5.70 ± 1.50	—
.25*	2.88 ± 1.05	3.34 ± 1.04

* Homogenate frozen and thawed once.

† Mean of 3 determinations, all others of 5 determinations.

‡ Random error ($2\bar{\sigma}$), see Table I.

A homogenate was prepared by blending fresh guinea pig liver in ice cold 8.5% sucrose solution. Reaction flasks contained 0.8 ml pH 7.4 phosphate buffer (0.1 M) and 0.2 ml liver homogenate. The side arms contained 0.15 ml sodium succinate (0.2 M). Control flasks were the same except that distilled water replaced the liver homogenate in the center compartment. Center wells contained filter paper strips soaked in 4N NaOH. After allowing 10 minutes for equilibration, the contents of the side arms were tipped in and measurements started.

20 minutes. The reported rates were determined from 30-minute averages taken over the linear portion of the uptake curve. It can be seen that oxygen uptake is proportional to tissue concentration over the interval studied and that oxygen consumption at rates as low as 3 μliters/hour could be measured with a reproducibility of approximately ± 1 μliter/hour.

Summary. A small differential syringe manometer with a 30 mm horizontal capillary is described. Estimates of accuracy and reproducibility were made by measuring the release of known volumes of nitrogen gas and by measuring the slow respiration rates that occur in unsupplemented liver homogenates. Absorption of oxygen at rates as low as 3 μliters/hour could be measured with a reproducibility of ± 1 μliter. The small size of the apparatus together with the accuracy, reproducibility, and simplicity of measurement recommend its use over conventional vertical column differential manometers.

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Ouabain Effect on Transmural Transport of Potassium by Canine Small Intestine.* (32170)

DANIEL E. WEINER (Introduced by Allan M. Lefer)
Department of Physiology, University of Virginia School of Medicine, Charlottesville

The absorption of Na+ by mucosal epithelium of the small intestine has been shown to be an active process, *i.e.*, Na+ can be transported against an electrochemical gradient, and the kinetics of the relationship between Na+ absorption and NaCl concentration of the luminal solution appear to be those of a saturable carrier system(1,2). In contrast to the situation for sodium, previous work has suggested that potassium absorption can be explained purely on the basis of passive equilibration due to existing electrochemical gradients(3). Recently, however, several reports have suggested that potassium interacts with a mucosal membrane carrier. For example, the apparent affinity of the mobile carrier for the transported sugar, arbutin, is depressed by K+(4), and the Km of the carrier for 6-deoxyglucose is increased from 2 mM to 200-300 mM by K+ loading(5). Another line of evidence that suggests a carrier mechanism is the finding that intestinal K+ absorption can be depressed and Na+ absorption enhanced by aldosterone administration in the rat(6). The present report describes studies that support the hypothesis that K+ interacts

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TABLE I. Effect of Ouabain (10^{-5}M) on Transmural Unidirectional Potassium Fluxes and Membrane Potentials. Values given are the mean and standard error.

	Unidirectional transmural potassium flux (μM/cm^2/30 min)		
	Influx (mucosal → serosal)	Efflux (serosal → mucosal)	Transmural potential (mv) †
N	17	12	12
Control	7.1 ± .3	6.6 ± .7	2.5 ± .4
Ouabain	12.9 ± 1.0*	6.7 ± .5	1.7 ± .3

* Indicates statistically significant difference from control at $p < .001$ level.
† Mucosal surface negative.

with a mobile carrier in the small intestinal mucosal cell.

Methods. Isolated preparations of jejunal mucosa were obtained from fasted dogs anesthetized with sodium pentobarbital (30 mg/kg). Intact membranes, consisting of mucosal epithelial cells attached to supporting connective tissue, were obtained by blunt dissection from the submucosa (with the blood supply intact) according to the method of Hakim *et al*(7). The luminal surface of this membrane will be referred to as the mucosal surface, and the surface dissected from the submucosa will be identified as the serosal surface. The mucosal membranes were mounted between lucite half-chambers exposing 2.5 cm^2 of the membrane surface to the incubation solutions. The incubation medium placed in both half-chambers was a modified Krebs-Henseleit bicarbonate buffer containing 10 mM glucose, 140 mM Na+ and 12 mM K+. The chambers were maintained at 37°C, stirred, and gassed with a mixture of 95% O_2 and 5% CO_2. The pH after equilibration with the gas mixture was 7.4. The volume of one of the half-chambers was large (250 ml) so that isotope activity remained constant, whereas the volume of the non-isotope containing chamber was small (35 ml).

At the beginning of each experiment, K^{42} was added to one incubation solution to serve as a tracer for potassium movement. All jejunal membranes were mounted in pairs, and at the end of an equilibration period of 30 minutes, ouabain (10^{-5} M) was added to one of the pairs. Tissue samples were digested in nitric acid and all samples were made up to a constant volume for counting in a well-type scintillation detector. Flux rates were calculated as micromoles of K+ transported per 30 minutes per cm^2 surface area.

In separate experiments, the flux of K+ into the cell interior was measured by labeling the incubation solution facing one cell surface with K^{42} and labeling the other solution with Rb^{86}. No systematic difference between the influx of potassium across either cell surface was noted when the isotopes were alternated as to site of placement. When solutions of high isotopic activity were placed in the large chambers, cross contamination of the solutions due to transmural fluxes was less than 1%. Potassium influx into the cell interior was calculated for 30-minute incubation periods.

Transmural potential measurements were made with calomel-KCl bridge electrodes, using a Beckman high impedance (10^9 ohms) electrometer and Offner type RB Dynograph. The chambers were not stirred or gassed during the measurement of transmural potentials.

Results. The effect of ouabain (10^{-5} M) on the unidirectional transmural flux across the mucosal membrane and the membrane potential is shown in Table I. Ouabain exerted an effect on potassium transport only when added to the serosal incubation solution; addition to the luminal incubation solution was without effect. Ouabain at a concentration of 10^{-6} M altered potassium transport, but the onset of peak activity occurred late, at 60-90 minutes after the addition of the drug. Therefore a higher concentration, (10^{-5} M), which gave a peak effect at 30 minutes was chosen for these experiments.

Ouabain markedly accelerated the potassium transmural influx (mucosal to serosal

TABLE II. Effect of Ouabain (10^{-5}M) on Unidirectional Influx of Potassium Across Mucosal and Serosal Surfaces. Values given are the mean and standard error.

	Potassium influx (μM/cm^2/30 min)	
	Mucosal cell surface	Serosal cell surface
N	8	8
Control	5.1 ± .2	6.4 ± .2
Ouabain	4.9 ± .2	4.4 ± .1*

* Indicates statistically significant difference from control at $p < .001$ level.

solutions) across the mucosal membrane, the difference between the control and ouabain being statistically significant ($p < .001$) (Table I). The small decrease in transmural potential in the presence of ouabain was not statistically significant ($p > .05$).

The potassium permeability of the mucosal and serosal cell surfaces in the presence and absence of ouabain is shown in Table II. The permeability of the two cell surfaces was determined simultaneously by labeling the solution bathing one surface with K^{42} and labeling the other solution with Rb^{86}. Addition of ouabain did not affect the influx across the luminal cell surface, but did significantly decrease the influx across the serosal cell surface.

Discussion. One possible explanation for the increased transmural influx induced by addition of ouabain to the serosal incubation is an alteration of the passive driving forces for potassium. Passive factors that could have operated are concentration gradients, electrical gradients, and solvent drag. In these experiments, concentration gradients were maintained at essentially constant levels in both ouabain and non-ouabain treated systems by using large incubation volumes. The transmural potential changes were not statistically significant and were of the wrong magnitude to have possibly produced the observed alteration in potassium influx. Intracellular potentials were not measured, but might be expected to decrease in the presence of ouabain(8). A decreased intracellular potential, however, would be expected to affect both the mucosal to serosal and serosal to mucosal transmural fluxes, and thus cannot provide an explanation for the alteration in only one unidirectional flux. Water flux rates have not been measured in these experiments, but there are several reasons for believing that the ouabain-induced potassium influx changes cannot be accounted for by solvent drag due to increased water flux. Ouabain, (10^{-6} M), has been shown to inhibit the active transport of a variety of sugars(9,10) and of sodium(11) by the small intestine. A decreased water flux due to a reduced transepithelial transport of osmotically active substances would therefore reduce, rather than increase, the apparent transmural potassium influx.

A second possible explanation for the increased ouabain transmural influx is an increased membrane permeability to K+. This could result from an increased size of water filled pores or extracellular channels, or from the activation or alteration of a membrane carrier system. A change in size of water filled pores would be expected to alter both the mucosal to serosal flux and the serosal to mucosal flux proportionately. Thus, the finding of an increased mucosal to serosal potassium flux in the absence of any change in the serosal to mucosal flux argues against this explanation. Solvent drag through enlarged pores might produce a disparity between the unidirectional fluxes, but as has been discussed above, increased water flow in the absence of significant sodium and glucose transport seems unlikely.

Finally, alteration of a mobile membrane carrier or fixed absorption sites by ouabain must be considered. Certainly, one characteristic action of ouabain is on the sodium pump(8). Acceleration of the unidirectional potassium flux is consistent with the existence of a carrier transport system. If this carrier system does exist, it seems likely that it is an example of facilitated diffusion, *i.e.*, a carrier system that is not dependent upon metabolic energy. Several lines of evidence suggest this possibility. Several workers have failed to show the transport of potassium against a concentration gradient by the small intestine(12,13). In addition, the Q_{10} for potassium exchange across both the mucosal and serosal cell surfaces is 1.28 ($N = 6$). This latter evidence is consistent with the hypothesis that potassium entry into the

mucosal cell is a diffusion-limited process.

Summary. Evidence of the alteration of the unidirectional, mucosa to serosa, transmural potassium flux by ouabain has been presented. This evidence suggests several conclusions concerning the transport of potassium by the small intestinal mucosal cell. It seems likely that potassium actually passes through the cell, rather than passing through extracellular channels between cells as is commonly suggested. This alteration in cellular potassium transport would appear to be carrier linked, and the carrier involved in the ouabain-enhanced transmural flux is on the serosal surface of the mucosal cell. Because potassium cannot be shown to be transported against an electrochemical gradient, and potassium transport has a low Q_{10} suggestive of a diffusion process, the carrier process involved in potassium transport may be an example of an equilibrating carrier system. Whether potassium has a carrier mechanism regularly available, or whether it is able to use the sodium carrier when the latter is altered by the addition of ouabain to the system is an important question which cannot be answered by these experiments.

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Inhibition of Enzymes by the Anthocyanin Malvidin-3-Monoglucoside.*† (32171)

O. R. WHEELER, J. A. CARPENTER, J. J. POWERS, AND M. K. HAMDY
Department of Food Science, University of Georgia, Athens, Ga.

Partially demethylated malvidin was demonstrated to be bactericidal toward *Escherichia coli*(1,2). Pratt *et al*(3) and others (4,5,6) established that anthocyanin compounds influence growth and glucose oxidation by many organisms. Somaatmadja *et al* (7) showed that cyanidin and leucocyanidin inhibited reproduction of *Staphylococcus aureus, Lactobacillus casei,* and *E. coli.* Keith and Powers(8) found that phenolic acids and esters derived from anthocyanin compounds inhibited respiration of *E. coli, Proteus vulgaris, Aerobacter aerogenes,* and *Pseudomonas aeruginosa* and that in absence of other carbon sources these compounds were utilized by the test organisms. Hulme and Jones(9) established that anthocyanins and anthocyanidins inhibited succinic oxidase, whereas leucoanthocyanins inhibited both succinic oxidase and malic acid dehydrogenase. Powers(10) demonstrated that malvidin-3-monoglucoside inacivated yeast hexokinase. The present studies demonstrate the mechanism by which malvidin-3-monoglucoside affects enzyme system of *Salmonella enteritidis* and enzymes from other sources.

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