membrane lipids pack in the same manner as lipids in a surface film, this equation shows that the model is satisfied by 87.5 Å^2 as the cross-sectional area for phospholipid and, therefore, 125.5 Å^2 as the unit area for the cholesterol-phospholipid complex. The data suggest that the lipid film most nearly simulates conditions in the bilayer model at a surface pressure of approximately 9 dyne/cm.

Data used by a number of investigators to construct membrane and bilayer models may be evaluated by the area-ratio equation. For example, Maddy and Malcolm (*6*) recently assumed that each phospholipid molecule in a plasma membrane bilayer occupied 70 Å^2, a value obtained from the x-ray diffraction pattern of a human brain phospholipid-water system. This area is considerably less than the area required for a bilayer and indeed yields an R of 1.7. Vandenheuvel (*6*) has described a bilayer model for myelin in which phospholipid-cholesterol complexes occupy 100 Å^2, which was extrapolated from the surface area occupied by an egg lecithin–cholesterol complex at an arbitrary film pressure of 5 dyne/cm. The A_{PL}, 62 Å^2, of the Vandenheuvel model gives an R value of 1.6 in place of the predicted bilayer. Mueller *et al.* (*6*) used Gorter-Grendel and Dervichian-Macheboeuf data to calculate the average area, 25 Å^2, occupied by both cholesterol and fatty-acid chains in a bilayer. They suggested that this area may be required for bilayer stability; however, the A_{PL} is 50 Å^2 and the R is only 1.4 in their model.

ROBERT S. BAR
DAVID W. DEAMER
DAVID G. CORNWELL
Department of Physiological Chemistry, Ohio State University, Columbus

References and Notes

1. J. F. Danielli, in *Surface Phenomena in Chemistry and Biology*, J. F. Danielli, K. G. A. Pankhurst, A. C. Riddiford, Eds. (Pergamon Press, London, 1958), p. 246; H. Davson, *Circulation* **26**, 1022 (1962).
2. E. Gorter and F. Grendel, *J. Exp. Med.* **41**, 439 (1925).
3. D. Dervichian and M. Macheboeuf, *Compt. Rend.* **206**, 1511 (1938).
4. L. L. M. van Deenen, *Progr. Chem. Fats Lipids* **8**, 1 (1965).
5. M. P. Westerman, L. E. Pierce, W. N. Jensen, *J. Lab. Clin. Med.* **57**, 819 (1961).
6. E. N. Willmer, *Biol. Rev. Cambridge Phil. Soc.* **36**, 368 (1961); P. Mueller, D. O. Rudin, H. T. Tien, W. C. Wescott, in *Recent Progress in Surface Science*, J. F. Danielli, K. G. A. Pankhurst, A. C. Riddiford, Eds. (Academic Press, New York, 1964), vol. 1, p. 379; F. A. Vandenheuvel, *Ann. N.Y. Acad. Sci.* **122**, 57 (1965); A. H. Maddy and B. R. Malcolm, *Science* **150**, 1616 (1965).
7. O. H. Lowry, H. R. Roberts, K. Y. Leiner, M. L. Wu, A. L. Farr, *J. Biol. Chem.* **207**, 1 (1954).
8. L. A. Abell, B. B. Levy, B. B. Brodie, F. E. Kendall, *ibid.* **195**, 357 (1952).
9. W. D. Harkins and T. F. Anderson, *J. Amer. Chem. Soc.* **59**, 2189 (1937).
10. L. L. M. van Deenen and J. de Gier, in *The Red Blood Cell*, C. Bishop and D. M. Surgenor, Eds. (Academic Press, New York, 1964), p. 243.
11. D. G. Dervichian, *Progr. Biophys. Mol. Biol.* **14**, 263 (1964).
12. D. W. Deamer and D. G. Cornwell, *Biochim. Biophys. Acta* **116**, 555 (1966).
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Effect of Sugars on Transport of Alanine in Intestine

Abstract. *The effect of glucose and galactose on transport of alanine by rabbit ileum has been investigated. Transmural transport and cellular accumulation of the amino acid were inhibited by the sugars, but alanine influx across the mucosal border of the cells was unaltered.*

Recent evidence (*1–4*) has indicated that D-galactose partially inhibits the transport of neutral amino acids across the wall of the small intestine and decreases the ability of the tissue to concentrate amino acids. Suggestions made regarding the mode of action of this sugar include competition for available energy (*1*), formation of the toxic metabolite galactose-1-PO_4 (*2*), and direct action on specific binding sites of a polyfunctional carrier at the brush border (*3*). However, the precise mechanism involved is unclear, and information regarding the effects of galactose on amino acid fluxes across mucosal and serosal borders of the epithelial cell is necessary in order to understand changes in transmural flux and cellular accumulation. Our experiments were designed to examine the influence of D-galactose on the influx of L-alanine from the solution bathing the mucosa into the cells.

All experiments were performed on distal ileum from male or female white rabbits maintained on a normal diet and killed by the intravenous injection of pentobarbital. The bathing solution used was a modified Krebs-Ringer-bicarbonate solution containing 140 mM sodium and 12 mM potassium (*5*). Experiments were performed at 37°C, and the solutions were constantly bubbled with a mixture of oxygen (95 percent) and carbon dioxide (5 percent). Radioactive samples were assayed in a liquid scintillation spectrometer. Transmural flux of alanine across the intestine from mucosal to serosal bathing solution was measured by the use of the chamber and methods previously described (*6*). In each experiment, two adjacent pieces of tissue were used. One piece served as a control, and the mucosal bathing solution of the other tissue contained 20 mM galactose or glucose. The tissue was bathed on both sides with Ringer solution containing 5 mM alanine. Alanine labeled with C^{14} was added to the mucosal solution and its rate of appearance in the serosal solution was determined. Influx of alanine across the brush border from mucosal solution into the cells was measured with the technique described by Fuisz *et al.* (*7*). The mucosal surface was exposed for 60 seconds to Ringer solution containing C^{14}-alanine and H^3-inulin, with or without 20 mM galactose or glucose. Influx of alanine was calculated from the uptake of C^{14} by the tissue after correction for the volume of adherent medium as determined by H^3-inulin. The effect of galactose on cellular accumulation of alanine was determined on strips of mucosa removed from the intestine and incubated in Ringer solution containing C^{14}-labeled alanine. Extracellular space was estimated in each tissue by the use of H^3-inulin. The methods for tissue preparation and the calculation of the intracellular concentration of alanine have been described (*5*).

The effects of glucose and galactose on the unidirectional transmural flux of alanine from mucosa to serosa is shown in Table 1. In each experiment, the steady state flux in the presence of sugar was lower than the control flux obtained in contiguous tissue from the same animal. On the average, the unidirectional flux of alanine was inhibited by 53 percent in the presence of 20 mM glucose and by 34 percent in the presence of 20 mM galactose. The influence of galactose on the cellular accumulation of alanine is shown in Table 2 in terms of the ratio of cellular to medium concentrations of alanine observed after a 30-minute incubation. Previous experiments (*5*) have demonstrated that this incubation time is sufficient to obtain maximum intracellular concentrations. Galactose (20 mM) caused a 32-percent reduction in the ratio of cell to medium concentration of alanine compared with the control tissue. Thus, in relative terms, the effects of galactose on the transmural flux and the steady state intracellular accumulation of alanine are quite similar. Phlorizin ($10^{-4}M$), which inhibits

the uptake and transport of sugar by the intestine (*5*), completely inhibited the effect of galactose on the accumulation of alanine. Preincubation of mucosal strips in galactose for 30 minutes prior to an additional 30-minute incubation with alanine in the absence of galactose caused a slight reduction (16 percent) in the accumulation of alanine.

Influx of alanine across the mucosal border in the presence or absence of sugars is given in Table 3. Neither sugar had any detectable effect. In these experiments, the tissue was exposed to the sugar for only the 60-second interval used to measure the flux of the amino acid across the brush border. However, similar results were obtained in other experiments in which the tissue was preincubated for 40 minutes in 20 m*M* galactose prior to determination of alanine influx.

Table 1. Effect of sugars on flux of alanine from mucosa to serosa. Both bathing solutions contained 5 m*M* alanine. Each pair of fluxes is from a single experiment on tissue from the same animal. Mannitol (20 m*M*) was present in the control solutions.

Sugar (20 m*M*)	Alanine flux (μmole per hour per cm^2)	
	Control tissue	Tissue + sugar
Glucose	1.63	0.86
	1.76	.84
	1.17	.47
Galactose	0.85	.69
	.74	.38
	2.02	1.31

Table 2. Effect of galactose on cellular accumulation of alanine. Data based on five sets of duplicate tissues from five animals. Errors are standard errors of the mean. Alanine 3.3 m*M*, galactose 20 m*M*, and phlorizin 10^{-4}*M* were used.

Additions to bathing solutions	Ratio of cell to medium concentration of alanine
Alanine	9.58 ± 0.86
Alanine + galactose	6.49 ± .45
Alanine + galactose + phlorizin	9.21 ± .82
Alanine, preincubation with galactose	8.01 ± .79

Table 3. Effect of sugars on influx of alanine (5 m*M*) across the mucosal border. All control solutions contained 20 m*M* mannitol. Errors are standard errors of the mean.

Solutions bathing mucosa	Alanine influx (μmole per hour per cm^2)
Control*	2.68 ± 0.17
Galactose (20 m*M*)	2.81 ± .20
Control†	1.55 ± .19
Glucose (20 m*M*)	1.54 ± .17

* Data based on 23 paired samples from seven animals. † Data based on nine paired samples from three animals.

Inhibition by galactose of transmural transport and cellular accumulation of alanine in rabbit small intestine is similar to findings reported for other species (*1–4*). The observation that glucose also inhibits transmural transport of alanine is contrary to the results of Newey and Smyth (*2*) and suggests that this inhibition is unrelated to either the accumulation of galactose-1-PO_4 or the extent to which the sugar is metabolized. Further, the finding that the influx of alanine, which we have shown to be dependent on sodium in the mucosal solution (*7*) and subject to competitive inhibition by other neutral amino acids (*8*), is not inhibited by sugars appears to rule out the hypothesis suggested by Alvarado (*3*).

Since a total of at least four unidirectional fluxes are involved in the overall processes of cellular accumulation and transmural transport, changes in these phenomena cannot be unequivocally ascribed to effects on any single unidirectional flux. Our experiments do, however, suggest that the efflux of alanine from the cell to the mucosal solution is altered by the sugars. If we assume that the transport system can be described by two barriers in series (the mucosal and serosal boundaries of the cell), the transmural unidirectional flux from mucosa to serosa should be given by

$$J_{ms} = J_{mc}\,J_{cs}/(J_{cm} + J_{cs})$$

in which J_{ij} is the unidirectional flux from compartment i to j (*9*). The subscripts m, c, and s denote mucosal solution, cell interior, and serosal solution, respectively. The data in Table 1 show that sugars cause a decrease in J_{ms}. This effect could be due to a decrease in J_{mc} or J_{cs} or to an increase in J_{cm}, but the data in Table 3 show that J_{mc} is unaffected by galactose or glucose. Further, a decrease in J_{cs} should lead to an increase in cellular accumulation of alanine, but accumulation decreases in the presence of galactose (Table 2). Thus, an increase in J_{cm} seems to provide an adequate explanation for the three sets of observations. It is premature to speculate on possible mechanisms of such an effect by sugar since, even if the effect is on this particular flux, there is no information as to the type of transport processes involved. However, the observation that phlorizin prevents galactose inhibition of the accumulation of alanine suggests that the sugar must enter the cell in order to exert its effect. Further studies, including direct measurement of amino acid efflux across the mucosal border, are necessary to clarify the mechanisms involved.

RONALD A. CHEZ
STANLEY G. SCHULTZ
PETER F. CURRAN
Biophysical Laboratory,
Harvard Medical School,
Boston, Massachusetts

References and Notes

1. H. Newey and D. H. Smyth, *Nature* **202**, 400 (1964).
2. S. J. Saunders and K. J. Isselbacher, *ibid.* **205**, 700 (1965).
3. F. Alvarado, *Science* **151**, 1010 (1966).
4. J. H. Annegers, *Amer. J. Physiol.* **210**, 701 (1966).
5. S. G. Schultz, R. E. Fuisz, P. F. Curran, *J. Gen. Physiol.* **48**, 849 (1966).
6. S. G. Schultz and R. Zalusky, *ibid.* **47**, 567 (1964).
7. R. E. Fuisz, S. G. Schultz, P. F. Curran, *Biochim. Biophys. Acta* **112**, 593 (1966).
8. P. F. Curran, S. G. Schultz, R. A. Chez, R. E. Fuisz, unpublished observations.
9. H. H. Ussing and K. Zerahn, *Acta Physiol. Scand.* **23**, 110 (1951).
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Glucose-6-Phosphate Dehydrogenase: Homologous Molecules in Deer Mouse and Man

Abstract. *Two forms of glucose-6-phosphate dehydrogenase, A and B, have been reported in deer mouse tissues. The B enzyme, which showed autosomally controlled polymorphism, is now found to be equally active toward glucose-6-phosphate and galactose-6-phosphate; the A enzyme is specific for the former. Human and horse livers also have two forms of glucose-6-phosphate dehydrogenase which exhibit the same substrate specifities as those in the deer mouse. A wide variety of electrophoretic patterns was seen in the human galactose-active enzyme.*

I have previously reported (*1*) the occurrence of two different forms of glucose-6-phosphate dehydrogenase (G6PD) in tissues of the deer mouse *Peromyscus maniculatus*; called A and B, they were demonstrated by starch