Lysine Fluxes across the Jejunal Epithelium in Lysinuric Protein Intolerance

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ABSTRACT Lysinuric protein intolerance (LPI) is one of a group of genetic diseases in which intestinal absorption of the diamino acids lysine, arginine, and ornithine is impaired. In LPI, the clinical symptoms are more severe than in the kindred disorders. The mechanism of lysine absorption was, therefore, investigated in vitro on peroral jejunal biopsy specimens in seven patients with LPI and 27 controls. The lysine concentration ratio between cell compartment and medium was significantly higher in the LPI group (mean ±SEM, 7.17±0.60) than in the controls (5.44±0.51). This was also true for the intracellular Na concentration (LPI, 73.6±10.8 mM; controls 42.3±3.7 mM). The rate of unidirectional influx of lysine across the luminal membrane was Na dependent and was the same in the two groups. In the absence of an electrochemical gradient, net transepithelial lysine secretion was observed in LPI. This was entirely the result of a 60% reduction of the unidirectional flux from mucosa to serosa. Calculation of unidirectional fluxes revealed the most striking difference at the basolateral membrane, where the flux from cells to serosa was reduced by 62% and the corresponding permeability coefficient reduced by 71%. A progressive reduction in short-circuit current appeared in the epithelia of all four patients with LPI tested after addition of 3 mM lysine. Thus, LPI appears to be the first disease in which a genetically determined transport defect has been demonstrated at the basolateral membrane.

INTRODUCTION

Excessive urinary excretion of the diamino acids lysine, arginine, and ornithine (with or without cystine) characterizes a group of genetic disorders whose clinical manifestations differ considerably (1). In fact, French-Canadian patients with "hyperdibasicaminoaciduria" display no constant symptoms at all (2). In cystinuria, a tendency to develop urinary tract stones is probably the sole clinical problem (1). Protein-tolerant patients with hyperdiaminoaciduria (3) may have gastrointestinal complaints, impaired growth, and mental retardation. In contrast, patients with lysinuric protein intolerance (LPI)[1] have severe symptoms, including hyperammonemia and diarrhea (4).

In most of these diseases there is a defect in the transport of diamino acids, not only in the proximal renal tubules but also in the jejunal epithelium. In both LPI and cystinuria, oral loads have revealed subnormal absorption of diamino acids (5–11). However, puzzling differences in diamino acid transport appear between cystinuria and LPI: (*a*) in jejunal biopsies, lysine accumulation against a concentration gradient is impaired in cystinuria (12–14) but not in LPI (15), (*b*) absorption of the diamino acids from dipeptides is normal in cystinuria (16) but subnormal in LPI (17), and (*c*) transport in the liver is also defective in LPI but presumably normal in cystinuria (18, 19).

This study confirms in vitro that the transport defect in LPI is localized to the basolateral membrane of the epithelial cell. The defect is associated with an increased concentration of intracellular sodium and an unsteady short-circuit current while lysine is present in the incubation medium.

METHODS

Seven patients with LPI (1.1–34 yr) were studied. Two were Moroccan sibs whose parents were not consanguineous. Both had failed to thrive after weaning, suffered from vomiting,

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[1] *Abbreviations used in this paper*: c, cell compartment; C/M, ratio of the intracellular to extracellular L-lysine or Na concentration; I_{sc}, short-circuit current; J, flux; LPI, lysinuric protein intolerance; m, mucosal bathing solution; P, permeability coefficients; s, serosal bathing solution.

and later from protein aversion. Both displayed hepato- and splenomegaly, osteoporosis, short stature (heights −2.5 and −4.0 SD at the age of 10 mo), and hyperammonemia after meals. The concentrations of lysine, arginine, and ornithine were subnormal in plasma but increased in urine. The other patients were Finnish. Three of them were included in an earlier report (4). The two other patients were unrelated children with the symptoms and findings pathognomonic of LPI (4).

All patients were free of gastrointestinal symptoms at the time of the study. Treatment with amino acid supplement (20) was stopped at the latest 2 d before the intestinal biopsy. All pieces of jejunum were histologically normal.

The 27 control subjects (0.2–39 yr) fulfilled the criteria of absence of diarrhea, normal jejunal histology, and a normal urinary aminogram. The children were biopsied on clinical grounds, but the two adult controls were entirely healthy. Some of the control data have already been published (14). All subjects and/or their guardians had given informed consent to the biopsy.

Biopsy procedure. After a 6-h fast, a jejunal biopsy was taken near the ligament of Treitz with a Carey capsule (21) under fluoroscopic control. Part of the biopsy specimen was examined histologically. The rest was used for transport studies.

In vitro transport studies

Intracellular Na and lysine concentrations. These concentrations were measured as previously described (14) using pieces of jejunal epithelium and the immediately underlying tissue without the muscular layer (22). In brief, the tissue was shaken at 37°C for 60 min in 10 ml of Ringer or Na-free solution containing 3 mM lysine, 1.5 μCi of L-[^{14}C]lysine (New England Nuclear, Boston, Mass.), 1 μCi of ^{22}Na (Comissariat à l'Energie Atomique, France), and 12.5 μCi of [^{3}H]polyethylene glycol (4,000 mol wt) (New England Nuclear). All measurements were made at the 3-mM lysine concentration, which is sufficient for reliable measurement of transmural fluxes and Na dependence. The affinity constant (K_t) values reported for the Na-dependent fluxes are 3 mM in rat jejunum (23) and 10 mM in rabbit ileum (24). The Ringer solution contained, in millimolars, 140 Na, 5.2 K, 1.2 Ca, 1.2 Mg, 120 Cl, 25 HCO_3, 2.4 HPO_4, and 0.4 H_2PO_4 (pH 7.4). The Na-free solution was obtained by replacing NaCl with choline chloride, and NaHCO with $KHCO_3$. Consequently, the potassium concentration of the solution was ~30 mM. After incubation for 60 min, the tissue was washed in cold 0.3 M mannitol, blotted gently, weighed, and extracted in 2 ml of 0.1 N nitric acid. Aliquots of the eluate were measured by liquid scintillation photometry. Results are given as the ratio of the intracellular to extracellular (C/M) L-lysine or Na concentration. According to bidimensional radiochromatography, L-[^{14}C]lysine was not metabolized during the incubation, as has been documented (24). For comparison, the C/M for glucose was measured in five biopsies at 10 mM glucose concentration.

Lysine influx. Lysine influx from the incubation medium across the luminal membrane into the cell (J_{mc}) was determined as previously described (14). After a 30-min preincubation in Ringer's solution, the accurately timed (~60 s) lysine influx was measured in the presence and absence of Na. The extracellular space was estimated with [^{3}H]polyethylene glycol.

Transepithelial fluxes. The biopsy specimen was mounted between two lucite chambers as a flat sheet on filter paper (22). The exposed area was 12.57 mm^2. The tissue was bathed on both sides with identical Ringer solutions (1.5 ml), which were circulated, oxygenated, and maintained at 37°C. The solutions bathing the mucosal and serosal sides were connected via agar bridges to calomel electrodes for measurement of the transepithelial potential difference, and to Ag-AgCl electrodes for passing current through the system. Short-circuit current (I_{sc}) was recorded in the absence of potential difference after appropriate correction for fluid resistance between the potential difference-sensing bridges. It was expressed as μequivalent per hour per square centimeter to make it comparable with the lysine fluxes.[2] After 5 min of steady I_{sc}, 3 mM of L-lysine was added on both sides and 20 μCi of L-[^{3}H]lysine on the mucosal side. When the appearance of ^{3}H into the opposite chamber reached a steady rate (5 min), the flux from mucosa to serosa (J_{ms}) was determined by collecting 200-μl samples from the serosal reservoir at 5-min intervals and replacing them by the same amount of "cold" Ringer-lysine solution. After four flux periods, the bathing solutions were removed, the chambers washed and filled with cold Ringer plus 3 mM L-lysine solution. 2 μCi of L-[^{14}C]lysine was added on the serosal side to allow similar determinations of flux from serosa to mucosa (J_{sm}) during four periods.

Calculations. The fluxes (J) between the mucosal bathing solution (m), the cell compartment (c), and the serosal bathing solution (s) were calculated as follows:

$$J_{net} = J_{ms} - J_{sm}. \quad (1)$$

$$J_{ms} = \frac{J_{mc} \times J_{cs}}{J_{cm} + J_{cs}}. \quad (2)$$

$$J_{sm} = \frac{J_{sc} \times J_{cm}}{J_{cm} + J_{cs}}. \quad (3)$$

$$J_{cm} = J_{mc} - J_{net}. \quad (4)$$

$$J_{cs} = J_{cm}/[(J_{mc}/J_{ms}) - 1]. \quad (5)$$

$$J_{sc} = J_{cs} - J_{net}. \quad (6)$$

Apparent permeability coefficients (P) across the membrane were calculated as

$$P = J/[\text{lysine}]. \quad (7)$$

The intracellular Na and lysine concentrations, the transepithelial fluxes J_{ms} and J_{sm}, and the influx at the luminal membrane J_{mc} were all obtained experimentally (see Results). Because not all measurements were obtained from each biopsy specimen, calculations were based on the mean values for each group.

Student's *t* test and nonparametric tests (Mann-Whitney U test or Wilcoxon one-sample test) were used to compare means and ranges.

RESULTS

Intracellular lysine and Na concentrations (Fig. 1). When Na was present in the incubation solution, the

[2] The relationship between the current (I) and the ionic fluxes (J) is calculated as follows:

$$J = \frac{I}{F},$$

$$F = 96{,}500\ \mu C/\mu eq.$$

The exposed surface area = 0.1257 cm^2. Thus,

$$J\ (\mu eq/h\ cm^2) = \frac{I_{sc}(\mu A) \times 3{,}600}{96{,}500 \times 0.1257} = \frac{I(\mu A)}{3.37}.$$

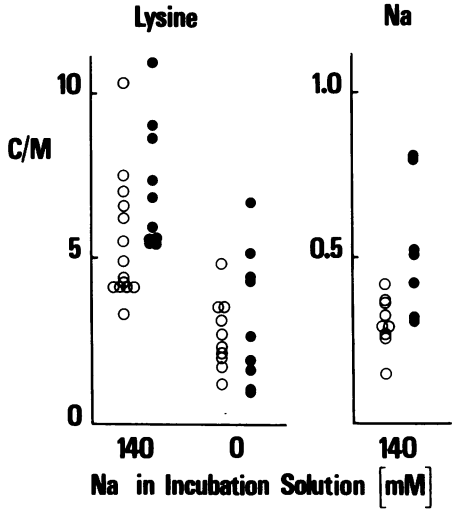

FIGURE 1 C/M in jejunal epithelium cells determined in the presence and absence of extracellular Na. C/M of Na was measured simultaneously on the same pieces of jejunum. ○, controls; ●, patients with LPI.

mean C/M for lysine was significantly higher ($P < 0.05$) in patients (mean±SEM, 7.17±0.60; range 5.37–10.96) than in controls (5.44±0.51; range 4.03–10.29). In the absence of Na, no difference was found (patients: 3.17 ±0.68; range 0.94–6.70; controls: 2.69±0.34; range 1.17 ±4.84) and the ratio was significantly smaller ($P < 0.01$) in both groups.

In the presence of 3 mM lysine, the intracellular Na concentration was higher in patients (73.6±10.8; range 43.4–113.4 mM) than in controls (42.3±3.7; range 21.0–59.4 mM).

With 10 mM glucose in the incubation solution, the C/M for glucose of the five patients (3.85±0.63) did not differ from that of 23 control children (3.97±0.23) (22).

J_{mc} (Fig. 2). When Na was present in the incubation solution, the J_{mc} values of the two groups did not differ significantly (patients: 1.45±0.34, range 0.77–2.68; controls: 1.11±0.21, range 0.54–2.54 μmol/h cm²), nor did they differ in the absence of Na (patients: 0.60±0.09, range 0.36–1.02; controls: 0.57±0.07, range 0.28–0.82 μmol/h cm²). The lysine J_{mc} was equally Na dependent in the two groups.

Transepithelial lysine fluxes (Fig. 3). In the control group, the two unidirectional lysine fluxes across the whole epithelium were not significantly different. Consequently, the net flux was minimal. In contrast, in the epithelia of the patients, the net result was lysine secretion. This was entirely the result of a 60% lower rate in J_{ms} ($P < 0.01$), with no difference in J_{sm}. Because the fluxes and the apparent permeability at the basolateral membrane could not be determined experimentally, but were calculated from Eqs. 4–6, we fur-

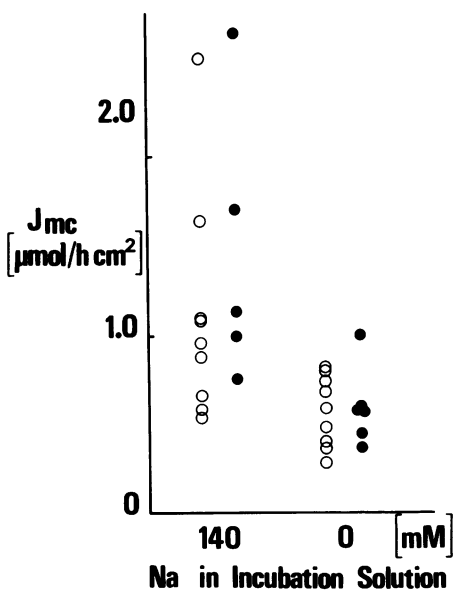

FIGURE 2 The unidirectional lysine flux (J_{mc}) across luminal membrane of jejunal epithelium cells determined in the presence and absence of extracellular Na. ○, controls; ●, patients with LPI.

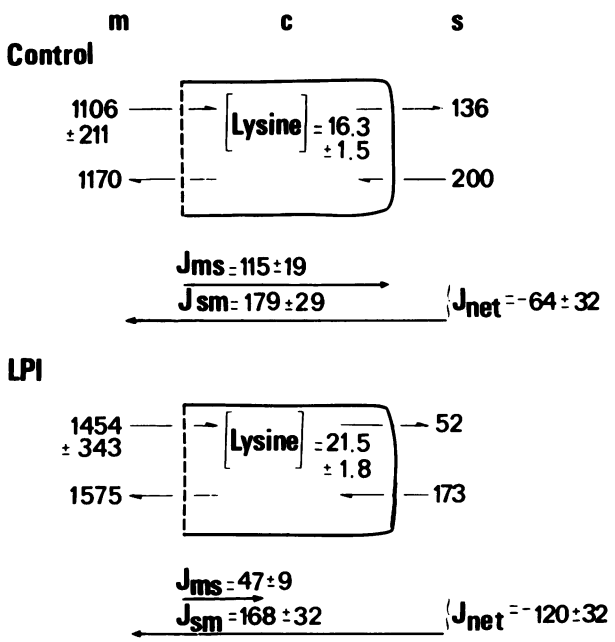

FIGURE 3 Lysine fluxes (mean±SEM) across the control epithelia (upper panel) and LPI epithelia (lower panel). The transmural fluxes J_{ms} and J_{sm}, the unidirectional flux J_{mc}, and the intracellular lysine concentration were obtained experimentally. The other fluxes across the two membranes (J_{cm}, J_{cs}, and J_{sc}) were calculated from the measured mean values, assuming that the tissue constitutes a single compartment between mucosal and serosal bathing solutions. Fluxes are expressed in nanomole per hour per square centimeter. Note the clearly subnormal J_{ms} and J_{cs} in LPI.

TABLE I

Calculated Apparent Permeability Coefficients across the Luminal and Basolateral Membranes in the Jejunal Biopsies

	P_{mc}	P_{cm}	P_{cs}	P_{sc}	P_{mc}/P_{cm}	P_{cs}/P_{sc}
			cm/h			
Controls	0.369	0.073	0.0085	0.067	5.05	0.13
LPI*	0.484	0.075	0.0025	0.057	6.45	0.04

* Note the low exit permeability from the cell across the basolateral membrane.

ther reexamined the relationship between the transmural unidirectional fluxes and the unidirectional fluxes across the luminal and basolateral membranes (Eqs. 2 and 3). The reduction of J_{ms} with no change in J_{sm} could obviously be related to a decrease in J_{mc} or in J_{cs}. Because the J_{mc} values were found, experimentally, not to differ significantly in the two groups, the alteration in net transepithelial fluxes in patients is clearly the result of the 62% reduction in J_{cs}. Comparison of the apparent permeability coefficients at each membrane (Table I) indicated a 71% reduction in cell-to-serosa permeability (P_{cs}) in the patients, but no difference in the other three permeability coefficients.

I_{sc} (Fig. 4). On the same pieces of tissues, we also recorded the I_{sc}. In the control specimens, 3 mM lysine stimulated I_{sc} by 0.18 μeq/h cm². This remained stable throughout the 60-min observation period. In all four specimens from the patients, we found a progressive decrease in I_{sc} after addition of lysine. A negative reversed current appeared after 30 min. The overall decrease during the 60-min recording was 0.71 μeq/h cm². However, the isotopic lysine fluxes remained constant throughout the experiment in epithelia from both controls and patients.

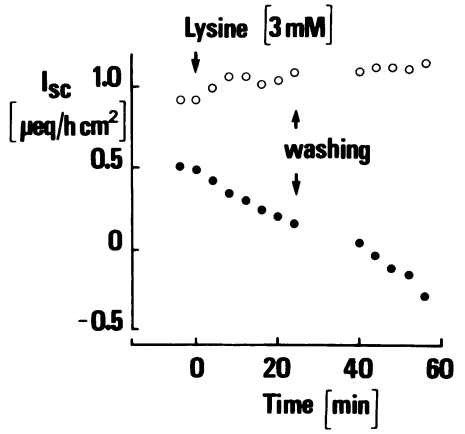

FIGURE 4 I_{sc} in controls (○) and patients with LPI (●). I_{sc} was continuously recorded before and after addition of lysine to the Ringer solution on both sides of the epithelium (n = 4). On the same piece of tissue, lysine transepithelial fluxes were measured; after four J_{ms} flux periods, the bathing solutions were removed and the chambers washed to allow J_{sm} determination.

DISCUSSION

Our results indicate that, in LPI, the lysine transport defect is located at the basolateral membrane in cells of the jejunal epithelium. As compared with controls, there was a 62% reduction in the J_{cs} and a 71% decrease in the corresponding permeability coefficient. Such a defect is entirely consistent with the smaller J_{ms} and the greater lysine accumulation ratio found in LPI in the presence of Na. In an earlier study, Kekomäki (15) measured C/M at 0.1 mM in five patients with LPI, one control, and one cystinuric subject. The ratios were 6.0 (mean) (range 4.0–6.9), 3.9, and 1.8, respectively. In an intestinal two-lumen perfusion study, he did not find any difference between two patients and one cystinuric subject in the rate of disappearance of ^{3}H-labeled lysine from the jejunal lumen. His results in LPI can now be accurately explained by the basolateral transport defect. The present in vitro findings are also compatible with the lack in the patients of an increase in plasma lysine concentration after oral loads of lysine dipeptides (17). Thus, LPI appears to be the first disease in which a genetically determined transport abnormality has been demonstrated at the basolateral membrane.

Lysine malabsorption in LPI and in cystinuria is the result of very different mechanisms. In cystinuria, the defect is located at the luminal membrane, as shown by impaired Na-dependent lysine accumulation (12–14), reduced lysine permeability at the luminal membrane (14), and normal in vivo lysine absorption from dipeptides (16). In contrast, in LPI the Na-dependent lysine transport at the luminal membrane is intact, as indicated by the accumulation of lysine observed in the presence of Na and the unaltered lysine influx across this membrane (Figs. 1 and 2). Nothing is yet known about the precise location of the transport defect in other types of hyperdiaminoaciduria and related diseases.

The present results justify speculation as to the nature of the basic defect in LPI. Very little is known about the mechanism of transport across the basolateral membrane. However, recent findings indicate that

sugars and amino acids are translocated by a facilitated transport system that is independent of Na (25–27). This would make the maximum J_{net} across the basolateral membrane a function of the lysine concentrations in the cell, $[Lys]_c$, and the solution bathing the serosa, $[Lys]_s$, and also the Michaelis-Menten transport constants of the two sides of the membrane, K_c and K_s. Thus, assuming that J_{max} across the basolateral membrane are equal for the two directions,

$$J_{net} = J_{max}\left[\frac{[Lys]_c}{K_c + [Lys]_c} - \frac{[Lys]_s}{K_s + [Lys]_s}\right]. \quad (8)$$

In normal human jejunal epithelium, as in the ileal epithelium of the rabbit (24), lysine transport displays an asymmetry, expressed by the fact that $P_{cs} < P_{sc}$ (Table I) or that $K_c < K_s$. This asymmetry may be connected with the positive charge on lysine. In LPI, the present results suggest a decrease in K_c.

Our findings on the role of Na in lysine transport in the intestinal epithelium are in accord with those reported earlier (24). The absence of Na from the incubation medium affected the accumulation ratio of lysine as much in the patients as in the controls. However, the higher intracellular Na concentration and the decreasing I_{sc} observed in LPI in the presence of Na are puzzling. According to the Na gradient hypothesis, a less steep gradient should lead to an increased efflux of lysine across the luminal membrane (28). The lack of a significant difference between the luminal influxes in specimens from patients and controls, together with the existence of net transepithelial secretion in the patients, suggests that the efflux is increased in LPI, although we were unable to demonstrate this. We may also legitimately infer that the rise in intracellular Na concentration is associated with a decreased transserosal potential difference (29), which would lower the K_c of the basolateral membrane. However, further experiments are required to elucidate the origin of the Na disturbances and their effects on other Na-dependent solutes.

In LPI, the reduced cell-to-serosa efflux of lysine and also, presumably, of the other diamino acids, arginine and ornithine, might be responsible for impaired in vivo absorption of these amino acids in the free form and as short oligopeptides. It is tempting to speculate that the transport defect in the proximal tubules of the kidney is similar (30). Furthermore, in LPI, the liver also shows the defect in diamino acid transport, suggesting a relation between the transport mechanism at the liver plasma membrane and the basolateral membranes of the epithelium.

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REFERENCES

1. Scriver, C. R., and L. E. Rosenberg. 1973. Nature and disorders of cystine and dibasic amino acid transport. *In* Amino Acid Metabolism and its Disorders. C. R. Scriver, and L. E. Rosenberg, editors. W. B. Saunders Company, Philadelphia. 1st edition. 155–186.
2. Whelan, D. T., and C. R. Scriver. 1968. Hyperdibasicaminoaciduria: an inherited disorder of amino acid transport. *Pediatr. Res.* **2**: 525–534.
3. Kihara, H., M. Valente, M. Porter, and A. L. Fluharty. 1973. Hyperdibasicaminoaciduria in a mentally retarded homozygote with a peculiar response to phenothiazines. *Pediatrics.* **51**: 223–229.
4. Simell, O., J. Perheentupa, J. Rapola, J. K. Visakorpi, and L-E. Eskelin. 1975. Lysinuric protein intolerance. *Am. J. Med.* **59**: 229–240.
5. Brown, J. H., L. F. Fabre, G. L. Farrel, and E. D. Adams. 1972. Hyperlysinuria with hyperammonemia. A new metabolic disorder. *Am J. Dis. Child.* **124**: 127–132.
6. Awrich, A. E., W. J. Stackhouse, J. E. Cantrell, J. H. Patterson, and D. Rudman. 1975. Hyperdibasicaminoaciduria, hyperammonemia and growth retardation: treatment with arginine, lysine, and citrulline. *J. Pediatr.* **87**: 731–738.
7. Kato, T., E. Tanaka, and S. Horisawa. 1976. Hyperdibasicaminoaciduria and hyperammonemia in familial protein intolerance. *Am. J. Dis. Child.* **130**: 1340–1344.
8. Oyanagi, K., T. Sogawa, R. Minami, T. Nakao, and T. Chiba. 1979. The mechanism of hyperammonemia in congenital lysinuria. *J. Pediatr.* **94**: 255–257.
9. Rajantie, J., O. Simell, and J. Perheentupa. 1979. Intestinal absorption in lysinuric protein intolerance: impaired for diamino acids, normal for citrulline. *Gut.* In press.
10. Milne, M. D., A. M. Asatoor, K. D. G. Edwards, and L. W. Loughridge. 1961. The intestinal absorption defect in cystinuria. *Gut.* **2**: 323–337.
11. Asatoor, A. M., B. W. Lacey, D. R. London, and M. D. Milne. 1962. Amino acid metabolism in cystinuria. *Clin. Sci. (Oxf.).* **23**: 285–304.
12. McCarthy, C. F., J. L. Borland, Jr., H. J. Lynch, Jr., E. E. Owen, and M. P. Tyor. 1964. Defective uptake of basic amino acids and L-cystine by intestinal mucosa of patients with cystinuria. *J. Clin. Invest.* **43**: 1518–1524.
13. Thier, S. O., S. Segal, M. Fox, A. Blair, and L. E. Rosenberg. 1965. Cystinuria: defective intestinal transport of dibasic amino acids and cystine. *J. Clin. Invest.* **44**: 442–448.
14. Coicadan, L., M. Heyman, E. Grasset, and J-F. Desjeux. 1980. Cystinuria: reduced lysine permeability at the brush border of intestinal membrane cells. *Pediatr. Res.* **14**: 109–112.
15. Kekomäki, M. 1968. Intestinal absorption of L-arginine and L-lysine in familial protein intolerance. *Ann. Paediat. Fenn.* **14**: 18–22.
16. Hellier, M. D., C. D. Holdsworth, D. Perret, and C. Thirumalai. 1972. Intestinal dipeptide transport in normal and cystinuric subjects. *Clin. Sci. (Oxf.).* **43**: 659–668.
17. Rajantie, J., O. Simell, and J. Perheentupa. 1980. Baso-

lateral membrane transport defect for lysine in lysinuric protein intolerance. *Lancet*. In press.
18. Simell, O., and J. Perheentupa. 1974. Defective metabolic clearance of plasma arginine and ornithine in lysinuric protein intolerance. *Metab. Clin. Exp.* **23:** 691–701.
19. Simell, O. 1975. Diamino acid transport into granulocytes and liver slices of patients with lysinuric protein intolerance. *Pediatr. Res.* **9:** 504–508.
20. Rajantie, J., O. Simell, and J. Perheentupa. 1979. Lysinuric protein intolerance: treatment with citrulline and lysine. *Pediatr. Res.* **13:** 78. (Abstr.)
21. Carey, J. B. 1964. A simplified gastrointestinal biopsy capsule. *Gastroenterology.* **46:** 550–557.
22. Grasset, E., M. Heyman, A-M. Dumontier, H. Lestradet, and J-F. Desjeux. 1979. Possible sodium and D-glucose co-transport in isolated jejunal epithelium of children. *Pediatr. Res.* **13:** 1240–1246.
23. Munck, B. G., and S. N. Rasmussen. 1979. Lysine transport across rat jejunum: distribution between the transcellular and the paracellular routes. *J. Physiol. (Lond.).* **291:** 291–303.
24. Munck, B. G., and S. G. Schultz. 1969. Lysine transport across isolated rabbit ileum. *J. Gen. Physiol.* **53:** 157–182.
25. Hajjar, J. J., R. N. Kuri, and P. F. Curran. 1972. Alanine efflux across the serosal border of turtle intestine. *J. Gen. Physiol.* **60:** 720–734.
26. Naftalin, R., and P. F. Curran. 1974. Galactose transport in rabbit ileum. *J. Membr. Biol.* **16:** 257–278.
27. Danisi, G., Y. H. Tai, and P. F. Curran. 1976. Mucosal and serosal fluxes of alanine in rabbit ileum. *Biochim. Biophys. Acta.* **455:** 200–213.
28. Kimmich, G. A., C. Carter-Su, and J. Randles. 1977. Energetics of Na^+-dependent sugar transport by isolated intestinal cells: evidence for a major role for membrane potentials. *Am. J. Physiol.* **233:** E357–E362.
29. Rose, R. C., D. L. Nahrwold, and M. J. Koch. 1977. Electrical potential profile in rabbit ileum: role of rheogenic Na transport. *Am. J. Physiol.* **1:** 5–12.
30. Simell, O., and J. Perheentupa. 1974. Renal handling of diamino acids in lysinuric protein intolerance. *J. Clin. Invest.* **54:** 9–17.