Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 7674-7677, December 1983
Physiological Sciences

Regulation of proline and glucose transport in mouse intestine by dietary substrate levels

(active transport/induction/nutrient absorption/carbohydrate/protein)

WILLIAM H. KARASOV, ROBERT S. POND III, DAVID H. SOLBERG, AND JARED M. DIAMOND

Physiology Department, University of California Medical Center, Los Angeles, CA 90024

Contributed by Jared M. Diamond, August, 4, 1983

ABSTRACT Active uptake of D-glucose and L-proline at 50 mM was measured in everted intestinal sleeves of mice whose dietary carbohydrate and protein levels were being varied experimentally. Compared to a nearly carbohydrate-free meat diet, a 50% carbohydrate laboratory chow diet stimulated active glucose uptake in the proximal intestine without affecting proline uptake, passive glucose permeability, or several measures of mucosal mass. Switching from a low-protein high-carbohydrate to a high-protein no-carbohydrate diet reversibly stimulated proline uptake while inhibiting glucose uptake. For each solute and diet switch, the stimulation of transport was complete within 1 day, while the inhibition required several days. The results imply induction and repression of intestinal glucose and proline transport by dietary substrate levels. This mechanism, in conjunction with the normal gradient of nutrient concentrations along the intestine, is probably largely responsible for the gradient in nutrient transport along the intestine.

The mechanisms by which mammalian small intestine absorbs amino acids and sugars have been studied extensively in recent decades. Both of these classes of solutes are taken up against a concentration gradient at the lumen-facing (brush-border) membrane of the intestinal cell, with the energy for uptake coming mainly or exclusively from the Na^+ gradient (1, 2). Our detailed knowledge of the mechanisms themselves contrasts with our limited understanding of their regulation. While uptake of sugars and of at least some amino acids is stimulated by dietary carbohydrate and protein levels, respectively, the molecular mechanisms and signals involved in this regulation remain unknown (refs. 3–9; see ref. 10 for review).

One complicating factor in understanding the regulation of intestinal nutrient transport is that both solute-specific and solute-nonspecific regulatory processes exist (10). On the one hand, pregnancy, lactation, diabetes, and intestinal resection are accompanied by parallel nonspecific increases in both sugar and amino acid uptake. These increases are mediated at least in part by growth of the intestinal mucosa, which tends to yield increased uptake of any solute. A steeper Na^+ gradient would similarly tend to stimulate the uptake of any solute cotransported with Na^+. On the other hand, a nutrient transport mechanism may also be regulated specifically by dietary levels or body stores of its substrate as is well established for intestinal uptake of the minerals calcium and iron.

In the present investigation, we tested for the existence of specific regulatory processes by measuring glucose and proline uptake simultaneously as a function of time while varying dietary carbohydrate and protein levels oppositely. We found that glucose uptake and proline uptake responded in opposite directions and with different time courses, thereby proving that under these conditions they are not coregulated.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Animals. We used adult male white Swiss Webster mice (body weight, 30–40 g) with constant access to water and food.

Solutes. As the actively transported sugar and amino acid to be studied, we chose D-glucose and L-proline, respectively. L-Proline was selected for three reasons: it is a nonessential amino acid; most of its intestinal uptake is by a single carrier; and proline and hydroxyproline are the sole natural amino acids functioning as major substrates for that carrier, which is in effect a "private" one for those amino acids (11, 12). We anticipate that regulation of transport may prove more complex for amino acids that are essential, that are transported by several carriers, or whose major transport mechanism is a "public" one shared with other natural amino acids as major substrate.

Diets. Mice maintained on two pairs of diets were compared. First, "chow mice" on a conventional chow diet (Wayne Lab Blox, Allied Mills, Chicago, IL; 50% carbohydrate, 24.5% protein) were compared with "meat mice" fed commercial ground beef (negligible carbohydrate, protein 65% of dry weight) supplemented by ICN vitamin diet fortification mixture at 1% of dry weight. Second, because chow and meat also differ in other constituents, we then compared "high-carbohydrate mice" and "no-carbohydrate mice" on artificial high-carbohydrate low-protein and carbohydrate-free high protein diets (55% sucrose, 15% casein vs. no sucrose, 70% casein), the two diets being identical in other constituents. Detailed compositions of all four diets are given in ref. 9.

Uptake Measurements. We measured solute taken up into the intestinal mucosa across the brush-border membrane (*not* solute transported across the entire thickness of the intestine into the serosal bathing solution). The preparation, described in detail elsewhere (13), was an excised, everted sleeve of intestine 1 cm long and secured to a solid glass rod with the intestinal mucosa facing outwards. Briefly, the rod with intestinal sleeve was mounted vertically a few mm over a spin bar rotating at 1,200 rpm to minimize effects of unstirred layers. After preincubation for 5 min in Ringer solution at 37°C, the sleeve was incubated for up to 4 min at 37°C in solution containing radioactive tracers, rinsed for 20 sec in 30 ml of nonradioactive solution at 2°C, removed from the rod, weighed, and prepared for liquid scintillation spectroscopy. Solution composition (in mM) was 128 NaCl, 20 $NaHCO_3$, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, gassed with 5% CO_2/95% O_2 (pH 7.3–7.4). Na^+-Free solution was prepared by replacing NaCl and $NaHCO_3$ with choline chloride and choline bicarbonate.

Uptake values were normalized to a 1-cm length of intestine. Normalization to alternative measures of the quantity of in-

testine, such as weight, protein content, or villous surface area, yielded the same conclusions because the ratio of these measures to length was independent of the diets that we used (see *Results*). With this technique, the coefficient of variation of uptake measurements in adjacent intestinal segments averaged 7% (13). Experimental measurements are expressed as the mean value ± SEM.

Choice of Solute Concentration, Adherent Fluid Marker, and Incubation Time. Uptake of D-glucose or L-proline was studied at 50 mM because this concentration was found to yield uptake rates close to the V_{max} for each of these solutes (9). Some radioactive solute counted with the tissue represents solute in the adherent fluid rather than solute taken up into the tissue. We corrected for this adherent solute by incorporating a second radioactive solute in the incubation fluid as follows (see ref. 13 for details).

In studies of L-[^{14}C(U)]proline uptake, the impermeant probe [1,2-^{3}H]polyethylene glycol (M_r 4,000) was used at trace concentrations to correct for L-[^{14}C]proline in the adherent fluid. We chose a 2-min incubation time because equilibration of [1,2-^{3}H]polyethylene glycol with the adherent fluid is complete at 2 min but not at 1 min and because L-proline uptake at the concentration used is linear with time for at least 2 min. We measured proline uptake both in the presence and absence of Na^+, the former measurement yielding the total uptake (Na^+-dependent and Na^+-independent components) and the latter yielding the Na^+-independent component alone.

In studies of uptake of L-[1-^{3}H]glucose, whose uptake is passive, [1,2-^{14}C]polyethylene glycol at trace concentrations was used as the adherent fluid marker. A 4-min incubation time was chosen because the L-glucose uptake rate is low and 4 min is still within the linear range.

In studies of D-[^{14}C(U)]glucose uptake, L-[1-^{3}H]glucose at trace concentrations was used to correct simultaneously for D-glucose in adherent fluid and for D-glucose taken up passively, yielding the active uptake of D-glucose. Passive glucose uptake was <10% of total glucose uptake (9). We chose a 1-min incubation time because at this time, equilibration of D-glucose and L-glucose with the adherent fluid is essentially complete and D-glucose uptake is still linear with time.

L-[^{14}C(U)]Proline was from ICN Pharmaceuticals, other tracers were from New England Nuclear, and other chemicals were from Sigma.

RESULTS

Uptake Differences Between Chow Mice and Meat Mice. D-Glucose and L-proline uptakes were measured at five positions along the small intestine in mice maintained for at least 14 days on either the chow diet or the meat diet. There was no significant difference in body weight between the two groups of mice.

In both chow mice and meat mice, the active uptake of D-glucose exhibited a gradient in uptake along the small intestine, with uptake minimal in the ileum (Fig. 1 top). Uptake in the proximal jejunum was 700% higher than uptake in the ileum for chow mice ($P < 0.001$ by paired t test) and 179% higher for meat mice ($P < 0.001$). In the proximal half of the intestine, chow mice exceeded meat mice in active D-glucose uptake, the effect being greatest in the proximal jejunum (second point from left in Fig. 1 top; chow mice 92% higher than meat mice) and nonexistent in the ileum. Interpolating uptake rates linearly between consecutive intestinal positions in Fig. 1, one calculates that summed uptake over the whole length of the small intestine is 71% higher in chow mice than in meat mice [22.0 ± 1.7 (n = 5) vs. 12.9 ± 1.1 (n = 7) μmol/min; $P < 0.005$].

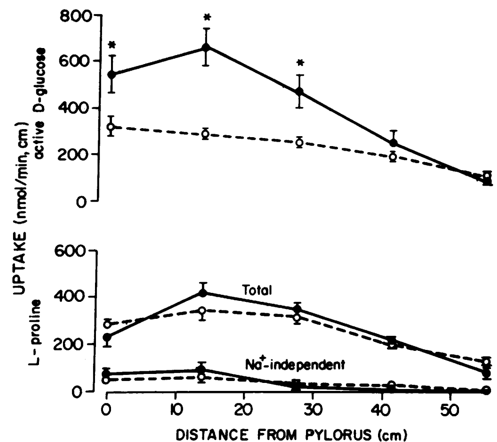

FIG. 1. Active D-glucose uptake at 50 mM (top) and total and Na^+-independent L-proline uptake at 50 mM (bottom) as a function of intestinal position in chow mice (●) and meat mice (○). Each point is based on five to seven mice. Vertical bars give SEM. Asterisks indicate uptake values that differ significantly ($P < 0.05$; t test) between chow and meat mice.

Both total L-proline uptake (i.e., uptake in the presence of Na^+) and Na^+-independent L-proline uptake similarly exhibited gradients along the intestine, with maximal values in the proximal jejunum and minimal values in the ileum (Fig. 1 bottom). In fact, Na^+-independent uptake by the ileum differed significantly from zero in only one of five mice studied on each diet. The proportion that Na^+-independent uptake bore to total uptake was 20% in the proximal jejunum and 8% in the midintestine for mice on both diets. In contrast to the results for D-glucose, chow and meat mice did not differ in total or Na^+-independent proline uptake at any intestinal position or in summed total uptake over the whole length of the intestine [15.3 ± 1.3 (n = 5) vs. 14.6 ± 0.7 (n = 5) μmol/min; $P > 0.5$].

L-Glucose uptake was measured in the proximal jejunum and used to calculate an apparent passive permeability coefficient K_d* for glucose (apparent because uncorrected for effects of unstirred layers). K_d* did not differ between chow and meat mice [0.61 ± 0.08 (n = 11) vs. 0.55 ± 0.12 (n = 10) μl/min per cm; $P > 0.4$].

Four measures of the quantity of intestinal mucosa per centimeter length of jejunum were found not to differ between chow and meat mice. These measures were: dry weight of jejunum [chow 8.4 ± 0.3 (n = 12) vs. meat 8.8 ± 0.5 (n = 10) mg/cm; $P > 0.4$]; dry weight of scrapable mucosa [5.9 ± 0.3 (n = 8) vs. 6.5 ± 0.3 (n = 5) mg/cm; $P > 0.4$]; protein content [100 ± 9 (n = 8) vs. 97 ± 7 (n = 5) mg/g of wet weight; $P > 0.5$]; and surface area at the villus level (9), calculated from measurements of the number and dimensions of villi [5.0 ± 0.5 (n = 10) vs. 5.4 ± 0.4 (n = 10) cm^2/cm; $P > 0.05$].

Thus, an increase in dietary carbohydrate content from negligible to 50% was associated with a specific increase in active D-glucose uptake in the proximal intestine. There were no changes in Na^+-dependent proline uptake, Na^+-independent proline uptake, passive glucose permeability, or mass of intestinal mucosa.

Uptake Differences Between High-Carbohydrate Mice and No-Carbohydrate Mice. Several previous studies (5–7) had demonstrated that intestinal uptake of amino acids increased when dietary protein was increased 5- to 10-fold. Therefore, it was initially surprising that Fig. 1 failed to reveal any difference between proline transport in mice on the high-protein meat diet and mice on the low-protein chow diet. At least four features of our experimental design might have been responsible for this

failure. First, the difference in dietary protein levels in our experiments was only 2.5-fold. Second, because proline uptake is mainly by a private carrier not shared with other amino acids, proline transport might be regulated by dietary levels of proline rather than of protein. Chow protein is relatively richer in proline than is meat protein, with the result that meat is only 2.1-fold richer in proline than is chow (32 vs. 15 mg of proline per g of dry weight). Finally, our chow and meat diets differed not only in protein and carbohydrate content but also in fat, fiber, and mineral content, which might have influenced proline transport and thereby confounded a direct relation between proline content and transport.

Therefore, we compared mice maintained for at least 7 days on the artificial high-carbohydrate low-protein and no-carbohydrate high-protein diets, which incorporate the same protein (casein), have identical contents of fat, fiber, and mineral, and differ greatly in protein content (15% vs. 70%) and carbohydrate content (55% vs. 0%). There were no differences between high-carbohydrate and no-carbohydrate mice in body weight, intestinal dry weight, or villous surface area per centimeter of intestine in the jejunum. Active uptake of D-glucose in the proximal jejunum was 68% higher in high-carbohydrate than no-carbohydrate mice [884 ± 16 (n = 7) vs. 527 ± 26 (n = 8) nmol/min per cm; $P < 0.001$], just as in the comparison of chow vs. meat mice. The change in total L-proline uptake was opposite to that for D-glucose: uptake was nearly 2-fold higher on the no-carbohydrate high-protein diet than on the high-carbohydrate low-protein diet [552 ± 24 (n = 8) vs. 282 ± 18 (n = 7) nmol/min per cm; $P < 0.001$]. The Na^+-independent component accounted for the same low percentage of total L-proline uptake in these mice [13.7 ± 2.9% (n = 4) vs. 16.2 ± 2.9 (n = 3); $P > 0.5$] as in the chow and meat mice.

We assume that glucose transport is responding to the change in dietary carbohydrate, while proline transport is responding to the change in dietary protein. Thus, transport of each of these solutes increases with its dietary level. Simultaneous opposite changes in the dietary levels of the two solutes produce specific opposite changes in their transport (see also ref. 7).

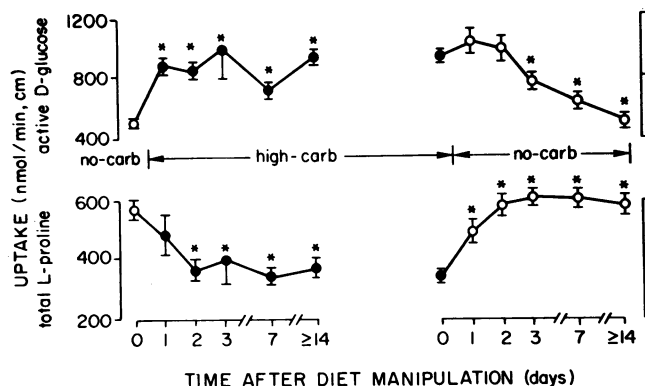

FIG. 2. Time course for effect of diet change on active D-glucose uptake and total L-proline uptake at 50 mM in the proximal jejunum. Left half, mice switched from no-carbohydrate (no-carb) high-protein diet (○) to high-carbohydrate (high-carb) low-protein diet (●) at time $t = 0$; subsequent points give uptake rates for glucose (top) and proline (bottom) after the indicated number of days on the high-carbohydrate low-protein diet. Right half, mice switched from high-carbohydrate low-protein diet to no-carbohydrate high-protein diet at $t = 0$; subsequent points give uptake rates after the indicated number of days on the no-carbohydrate high-protein diet. Vertical bars give SEM (n = 15–24 mice for $t = 0$ points and 5–10 mice for other points). Asterisks indicate uptake values that differ significantly ($P < 0.05$, t test) from the corresponding $t = 0$ value.

Time Course for Changes in Glucose and Proline Uptake. Fig. 2 depicts the results of experiments in which mice were switched from a high-carbohydrate low-protein to a no-carbohydrate high-protein diet or vice versa, and active D-glucose uptake and total L-proline uptake were measured as a function of time. In each mouse two adjacent sleeves of proximal intestine were used for uptake measurements, one for each solute.

When no-carbohydrate mice were switched to a high-carbohydrate diet, glucose uptake was found to have risen by 1 day to a value not significantly different from that for mice maintained on a high-carbohydrate diet for 2, 3, 7, or 14 or more days (Fig. 2 top left). (In a separate study we found no significant increase at 12 hr.) In these same mice there was no significant change in proline transport at 1 day, but there was a significant decrease by 2 days to a value not significantly different from that for mice maintained on a high-carbohydrate low-protein diet for 3 or more days (Fig. 2 bottom left). Conversely, when high-carbohydrate mice were switched to a no-carbohydrate diet, there was no change in glucose uptake at 1 or 2 days, a decrease at 3 days, and further decreases by 7 and 14 days (Fig. 2 top right). In these same mice the uptake of L-proline increased significantly within 1 day (Fig. 2 bottom right).

Fig. 2 yields two conclusions. (*i*) For each switch in diet, the simultaneous but opposite change in glucose and proline transport occurs at different rates. (*ii*) For each solute, the increase in transport with increased dietary levels occurs more rapidly than the decrease in transport with decreased dietary levels.

DISCUSSION

We have demonstrated nearly 2-fold increases in intestinal transport of glucose and proline with increases in dietary solute level. The separateness of the responses is confirmed by the facts that the two solutes responded in opposite directions and with different time courses in the experiment with high-carbohydrate and no-carbohydrate diets (Fig. 2) and that glucose transport changed without any change in proline transport in the experiment with chow and meat diets (Fig. 1). By a kinetic analysis, we have shown elsewhere (9) that the change in glucose transport involves a change in the V_{max}.

The simplest explanation for the transport changes is that the glucose and proline carriers are specifically induced or repressed by increases or decreases in their substrate levels. Several nonspecific alternative explanations can be eliminated from consideration. The transport changes are not due to changes in the amount of intestinal mucosa because we found no diet-related change in four measures of this amount, and, in any case, glucose and proline transport can respond separately or oppositely. A change in the Na^+ gradient can also be eliminated as the explanation because this would produce parallel changes in glucose and proline transport. The specificity of the responses is confirmed by the lack of change in passive glucose permeability.

The substrate-dependent induction explanation for the diet dependence of transport also provides a straightforward interpretation of the normal gradient in glucose and proline transport along the intestine because the transport gradients parallel the normal gradients in luminal substrate concentrations (14, 15). (The substrate concentrations rise to a maximum in the jejunum because of protein and starch hydrolysis in the duodenum and then decline toward the ileum because of absorption.) When luminal glucose is eliminated by placing mice on a carbohydrate-free meat diet, the glucose transport gradient nearly disappears (Fig. 1 top) and actually does disappear when glucose transport is normalized to villus surface area (figure 6

of ref. 9). Further evidence linking the transport gradient to the substrate gradient is that ileal glucose transport increases when ileal tissue experiences a glucose load because it is transplanted to the duodenum (16), because the jejunum is excised (17, 18), or because a high-glucose diet sufficient to saturate the transport capacity of the duodenum and jejunum is presented (8, 19).

Other tissues in which amino acid or glucose transport increases with dietary substrate levels include liver, in which amino acid uptake increases with dietary casein content (20), and kidney, in which taurine transport increases with dietary taurine (21). Both effects appear to involve the appearance of new transport sites. Similarly, levels of pancreatic amylase and intestinal brush-border disaccharidase increase with dietary carbohydrate, as do the levels of pancreatic proteases and intestinal brush-border amino-oligopeptidase with dietary protein (22–24). However, glucose represses its own transport in cultured porcine kidney cells (25) and in the blood–brain barrier (26).

Among the major unsolved problems posed by dietary regulation of intestinal sugar and amino acid transport, the following four may be mentioned:

(*i*) Is the increased glucose transport with dietary carbohydrate due to increased rates of carrier synthesis (as suggested by the ability of the protein synthesis inhibitor cycloheximide to block the increased glucose absorption that would otherwise follow hyperglycemia, ref. 27), decreased rates of carrier degradation, or increased rates of carrier insertion into the brush-border membrane (as suggested for the regulation of brush-border amino-oligopeptidase activity by dietary protein, ref. 24)?

(*ii*) The replacement half-time for intestinal mucosal cells in mice is 17–18 hr (28, 29). One day was required in our experiments for stimulation of glucose or amino acid transport by dietary substrate. Thus, it is uncertain to what extent the transport changes depend on induction of new carriers in existing cells, as opposed to production of new cells with higher carrier density.

(*iii*) The signal(s) involved in dietary control of intestinal nutrient transport is unknown. Luminal, intracellular, or intravenous levels of the substrate itself are the simplest hypothesis, but some findings suggest the possibility of a hormonal signal (10).

(*iv*) Glucose and proline are not essential nutrients but merely a convenient source of calories. Thus, it is economical of biosynthetic energy for their private transport proteins to be induced by substrate availability and to be repressed by substrate deficit. It would be suicidal for this same pattern to apply to the essential amino acids. Yet, most of the essential amino acids differ from proline in being absorbed not by a private carrier but instead by public carriers that they share with numerous nonessential amino acids. How do these public carriers respond to dietary deficiencies of specific amino acids, to nitrogen-deficient diets, or to diets rich enough in protein to permit metabolism of excess amino acids as calories?

The theoretical interest of these questions is matched by their practical importance, in view of the role of protein-deficient diets as one of the world's leading public health problems.

It is a pleasure to acknowledge our debt to Susan Gass for help in the experiments and to Ernest Wright for valuable suggestions. This research was supported by National Institutes of Health Grants GM 14772 and AM 17328 (Center for Ulcer Research and Education) and by a grant from the American Diabetes Association.

1. Kimmick, G. A. (1981) in *Physiology of the Gastrointestinal Tract*, ed. Johnson, L. R. (Raven, New York), pp. 1035–1061.
2. Schultz, S. G. & Curran, P. F. (1970) *Physiol. Rev.* **50**, 637–718.
3. Hahn, P. & Koldovský, O. (1966) *Utilization of Nutrients During Postnatal Development* (Pergamon, Oxford).
4. Ginsburg, J. M. & Heggeness, F. W. (1968) *J. Nutr.* **96**, 494–498.
5. Lis, M. T., Crampton, R. F. & Matthews, D. M. (1972) *Br. J. Nutr.* **27**, 159–167.
6. Scharrer, E. (1972) *Experientia* **28**, 267 (abstr.).
7. Scharrer, E., Wolfram, S., Raab, W., Amann, B. & Agne, N. (1981) in *Mechanisms of Intestinal Adaptation*, eds. Robinson, J. W. L., Dowling, R. H. & Riecken, E.-O. (MTP, Lancaster, England), pp. 123–137.
8. Bode, Ch., Eisenhardt, J. M., Haberich, F. J. & Bode, J. Ch. (1981) *Res. Exp. Med. (Berlin)* **179**, 163–168.
9. Diamond, J. M. & Karasov, W. H. (1984) *J. Physiol. (London)*, in press.
10. Karasov, W. H. & Diamond, J. M. (1983) *Am. J. Physiol.* **245**, G443–G462.
11. Munck, B. G. (1981) in *Physiology of the Gastrointestinal Tract*, ed. Johnson, L. R. (Raven, New York), pp. 1097–1122.
12. Stevens, B. R., Ross, H. J. & Wright, E. M. (1982) *J. Membr. Biol.* **66**, 213–225.
13. Karasov, W. H. & Diamond, J. M. (1983) *J. Comp. Physiol.* **152**, 105–116.
14. Reynell, P. C. & Spray, G. H. (1956) *J. Physiol. (London)* **131**, 454–462.
15. Mettrick, D. F. (1970) *Comp. Biochem. Physiol.* **37**, 517–541.
16. Dowling, R. H. (1973) in *Intestinal Adaptation*, eds. Dowling, R. H. & Riecken, E.-O. (Schattauer, Stuttgart, FRG), pp. 35–45.
17. Dowling, R. H. & Booth, C. C. (1967) *Clin. Sci.* **32**, 139–149.
18. Urban, E. & Haley, D. P. (1978) *Am. J. Dig. Dis.* **23**, 531–540.
19. Nunn, A. S. & Ellert, M. S. (1967) *Am. J. Physiol.* **212**, 711–716.
20. Fafournoux, P., Rémésy, C. & Demigné, C. (1982) *Biochem. J.* **206**, 13–18.
21. Friedman, A., Albright, P. W. & Chesney, R. W. (1981) *Life Sci.* **29**, 2415–2419.
22. Koldovský, O., Bustamante, S. & Yamada, K. (1981) in *Mechanisms of Intestinal Adaptation*, eds. Robinson, J. W. L., Dowling, R. H. & Riecken, E.-O. (MTP, Lancaster, England), pp. 153–166.
23. Solomon, T. E. (1981) in *Physiology of the Gastrointestinal Tract*, ed. Johnson, L. R. (Raven, New York), pp. 873–892.
24. Reisenauer, A. M. & Gray, G. M. (1983) *Gastroenterology* **84**, 1283 (abstr.).
25. Moran, A., Turner, A. J. & Handler, J. S. (1983) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42**, 1285 (abstr.).
26. Gjedde, J. & Crone, C. (1981) *Science* **214**, 456–457.
27. Csáky, T. Z. & Fischer, E. (1981) *Diabetes* **30**, 568–574.
28. LeBlond, C. P. & Messier, P. (1958) *Anat. Rec.* **132**, 247–260.
29. Questler, H. & Sherman, F. G. (1959) *Exp. Cell Res.* **17**, 420–438.