Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 2242–2245, April 1987
Biochemistry

Adaptive regulation of intestinal nutrient transporters

(induction/repression/vitamins/trace minerals/amino acids)

JARED M. DIAMOND* AND WILLIAM H. KARASOV†

*Department of Physiology, University of California Medical School, Los Angeles, CA 90024; and †Department of Wildlife Ecology, University of Wisconsin, Madison, WI 53706

Contributed by Jared M. Diamond, December 24, 1986

ABSTRACT **Because most eukaryotic somatic cells are bathed in a constant internal milieu, most of their proteins are constitutive, unlike the adaptive enzymes of bacteria. However, intestinal mucosal cells, like bacteria, face a varying milieu. Hence, we tested for adaptive regulation of intestinal nutrient transporters, sought its functional significance, and compared it with regulation of bacterial proteins. All 12 transporters studied proved to be regulated by dietary substrate levels. Regulation in the intestine is slower than in bacteria and shows lower peak-to-basal activity levels. Regulatory patterns vary greatly among transporters: two sugars and two nonessential amino acids monotonically up-regulate their transporters, two vitamins and three minerals monotonically down-regulate their transporters, and two transporters of essential amino acids respond nonmonotonically to levels of their substrates. These varied patterns arise from trade-offs among four factors: transporter costs, calories yielded by metabolizable substrates, fixed daily requirements of essential nutrients, and toxicity of certain nutrients in large amounts. Based on these trade-offs, we predict the form of regulatory pattern for intestinal transporters not yet studied.**

Bacterial cells are directly exposed to a fluctuating environment, so that wide variations in substrate levels for bacterial enzymes and transporters are a normal occurrence. Accordingly, bacteria are found to possess regulatory machinery through which levels of enzymes adapt to medium composition, especially to levels of their substrates or products.

In contrast, most animal cells are bathed in an internal milieu whose composition is held constant within narrow limits by an excretory organ such as the kidney. Thus, wide fluctuations in substrate levels do not occur, and most proteins in animals' somatic cells are constitutive, with regulation of their levels being superfluous. The best-studied exceptions are certain enzymes (1) and transport proteins (2, 3) of liver and adipose tissue, whose levels are regulated by variations in plasma nutrient composition (arising ultimately from variations in dietary solute inputs). *A priori*, one might expect more numerous examples of protein regulation in intestine than in liver or adipose tissue, because intestinal mucosal cells (unlike hepatocytes or adipocytes) are directly exposed to fluctuating inputs of dietary solutes into the intestinal lumen. While dietary regulation of intestinal hydrolases such as sucrase and amino-oligopeptidase is well established (4), there was until recently only limited information (summarized in ref. 5) about regulation of intestinal nutrient transporters.

The present paper summarizes regulatory patterns for intestinal absorption of 18 solutes conveyed by 12 different transporters. Our first goal was to determine the existence of regulation. It turns out, as one might have anticipated from the above-mentioned functional considerations, that every intestinal transporter examined is subject to adaptive regulation. Our second goal was to compare these eukaryotic systems with bacterial systems in regard to time course and peak-to-basal activity ratio for regulation. Our final goal was to understand the functional significance of regulation, whose direction and degree proved to vary greatly among the 12 transporters.

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.

METHODS

Cell membranes of the intestinal mucosal epithelium possess many different transport systems that convey specific classes of sugars, amino acids, vitamins, minerals, and other nutrients from the intestinal lumen to the bloodstream. Other workers have used rat, guinea pig, or chicken intestine to examine regulation of the five separate transporters for calcium (6–9), iron (10–12), phosphate (13), thiamine (14), and vitamin C (15). We used intestines of adult male white Swiss–Webster mice to study 13 solutes and 7 transporters: the sugars glucose, galactose, and 3-*O*-methylglucose, which share a transporter (16–19); the sugar fructose (19) and the dipeptide carnosine (20), each of which uses a separate transporter; and eight amino acids (21, 22) (alanine, aspartate, histidine, leucine, lysine, methylaminoisobutyric acid, methionine, and proline) using four transporters [separate ones (23) for neutral, basic, and acidic amino acids and for imino acids].

To study regulation of sugar transport, we fed mice rations with carbohydrate levels that varied from 0% to 68% by isocaloric replacement with casein. [The resulting changes in sugar transport are due to changes in the dietary carbohydrate rather than protein, since replacement of carbohydrate by fat yields similar results (24).] Carbohydrate was variously supplied in the form of complex carbohydrate (16, 17), the disaccharides sucrose or maltose (16–19), or the monosaccharides glucose, galactose, 3-*O*-methylglucose, or fructose (19).

To study regulation of amino acid transport, we used rations with protein or amino acid levels that varied from 4% to 72% by isocaloric replacement with sucrose, but all rations had adequate levels of essential amino acids. Protein or amino acids were supplied in the form of casein (16–18, 21), an enzymatic partial hydrolysate of casein (20), free amino acid mixtures (20, 21), or casein supplemented with one of seven amino acids [arginine, aspartate, glutamate, lysine, proline, threonine, or valine (22)].

After mice had consumed a ration for 0.5–14 days, we measured transport *in vitro* at 37°C as the uptake of radiolabeled solute by an everted sleeve of small intestine (25). This preparation offers the advantages of maintaining transporter activities at high levels and reducing unstirred layer effects. Solute absorption by the intestine involves steps at two cell membranes in series: uptake from the intestinal lumen across the brush-border membrane into the enterocyte, and exit from the enterocyte across the basolateral membrane into the bloodstream. For a given solute, the

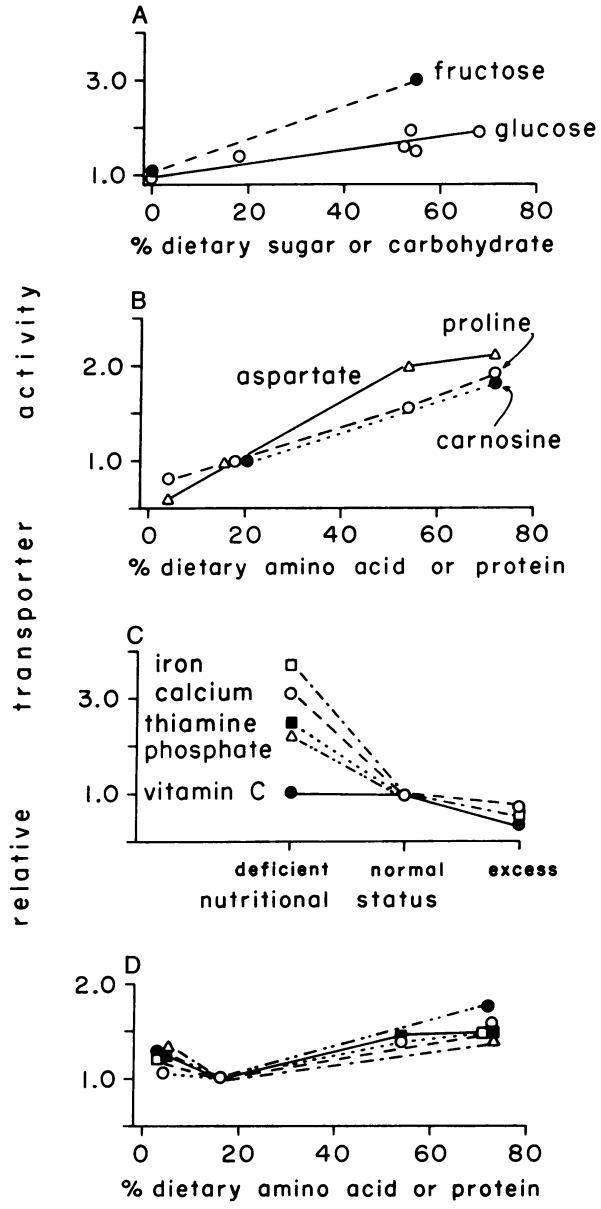

FIG. 1. Summary of how activities of intestinal brush-border nutrient transporters vary with dietary substrate levels or nutritional status. (*A*) Fructose (19) (●) or glucose (16–18) (○) transport as a function of dietary fructose or carbohydrate level. (*B*) Aspartate (21) (△), proline (21) (○), or carnosine (20) (●) transport as a function of dietary level of casein or of an amino acid mixture corresponding to a complete hydrolysate of casein. (*C*) Brush-border receptors for iron in guinea pig proximal intestine (10) (□-----), calcium uptake in chick duodenum (6) (○----), thiamine uptake in rat jejunum (14) (■----), phosphate transport in rat jejunum (13) (△----), and vitamin C uptake in guinea pig ileum (15) (●——), on rations with the given nutrient at inadequate levels, normal levels, or in excess. (*D*) Same as *B*, but for histidine (●-----), lysine (○----), leucine (■——), alanine (□----), and methionine (△-----) (21). *A*, *B*, and *D* are based on maximal transport rates (at substrate concentrations of 25 or 50 mM, far above the K_m), measured *in vitro* in an everted sleeve preparation of mouse intestine and averaged for the proximal and mid-intestine, after mice had been maintained on the indicated ration for 2 weeks [more than sufficient to yield a new steady state of transport activity (16, 17)]. Ordinate values are relative transporter activity: i.e., transport in mice eating a ration with the substrate level shown on the abscissa, divided by transport in mice eating a ration with some standardized substrate level. The standardized level was a fructose- or carbohydrate-free ration in *A*, an 18% casein or 18% amino acid ration in *B* and *D*. *C* shows transport in animals on a ration with the given nutrient in excess or at inadequate levels, divided by transport in animals with the nutrient at adequate levels. The measure of transport used in each case is total uptake, the quantity of physiological interest, except that only the active component of D-glucose uptake is shown [total uptake corrected for the very small passive component by means of the L-glucose uptake (25)]. Most transport is carrier-mediated, as shown by Na^+ dependence or a saturable uptake–concentration relationship. For each point, the sample size was 6–12 animals, and the coefficient of variation averaged 27%. The depicted trends in transporter activity with dietary substrate or nutritional status are all significant at the $P < 0.05$ level or better, except that the differences between vitamin C transport on a deficient and normal diet, and between transport of the five amino acids shown in *D* on a 4% and 18% amino acid diet, are not significant.

brush-border and basolateral transporters are quite different. Our method measures only the brush-border uptake step. [The basolateral exit step is harder to study but has also been shown to be regulated in the cases of glucose (26) and iron transport (11).] By measuring uptake as a function of substrate concentration, we calculated maximal transport velocities (V_{max}) and Michaelis–Menten coefficients (K_m) for transport.

PATTERNS OF REGULATION

Fig. 1 depicts nutrient uptakes as a function of dietary substrate levels, after a time on each ration sufficient for uptake rates to reach a new steady state (see discussion below on time course of regulation). Although dietary substrate levels to which the animals adapted were varied to yield the different abscissa values of Fig. 1, all transport measurements for a given solute were made *in vitro* at the same solute concentration, generally one high enough to yield transport rates approximating the V_{max}. The following conclusions emerge from Fig. 1 and the data used to construct it:

(*i*) Dietary substrate levels reversibly regulate transport in proximal and/or mid small intestine for every solute studied. (Few solutes exhibit regulation in the distal small intestine, the region in which uptake is generally lowest.)

(*ii*) The pattern of regulation varies greatly among solutes. With increasing dietary substrate level, uptake may increase (Fig. 1 *A* and *B*), decrease (Fig. 1*C*), go through a minimum (Fig. 1*D*), or initially remain constant and then increase (Fig. 1*D*).

(*iii*) The form of regulation for a given solute often differs between intestine and liver. For example, amino acid uptake by hepatocytes has been studied intensively and found to decrease with amino acid level (2, 3). In contrast, uptakes of various amino acids by intestine all increase with level at moderate to high amino acid levels (Fig. 1 *B* and *D*).

(*iv*) Different transporters are regulated independently. For instance, replacement of dietary glucose by fructose stimulates fructose transport 2.8-fold without affecting proline transport (19); replacement of dietary lysine by aspartate stimulates aspartate transport but not leucine or glucose transport (22); replacement of dietary carbohydrate by protein stimulates amino acid transport while inhibiting sugar transport (16–18, 21); and lowered levels of nonessential amino acids below the maintenance level inhibit proline transport, stimulate leucine transport, and leave lysine transport unchanged (21).

(*v*) As yet, no intestinal nutrient transporter has been completely purified. Only for the glucose (27) and iron (11) transporters has regulation of transporter site densities been assessed directly by binding studies. In both cases, upregulation of activity is associated with increased site density, and in the former case the factorial increases in V_{max} of transport and in binding site density are identical, proving

that the increase in transport activity is due entirely to increased number of functional copies of the transporter.

COMPARISON OF REGULATION IN THE INTESTINE AND IN BACTERIA

Regulation of intestinal transporters differs quantitatively from regulation of bacterial enzymes and transporters in two respects: time course and peak-to-basal activity ratios. These differences emerge from our studies of sugar and amino acid transporters in mouse intestine, as well as from studies of the same transporters in rat intestine (28).

As regards time course, regulation is much slower in the intestine than in bacteria: bacterial enzymes begin to respond to altered substrate levels within a few minutes, but intestinal brush-border transporters show no response to dietary changes for 12 hr and do not reach a new steady state for 1–3 days. While part of the lag for intestinal transporters represents the transit time of food from mouth to intestine, a long lag remains after this transit has been short-circuited [e.g., a lag of several hours for the stimulation of basolateral glucose transport by acute hyperglycemia (26), or for the stimulation of calcium transport by dihydroxy vitamin D_3 (29)]. Similarly, liver enzymes take several hours to respond directly to hormones and longer to respond to dietary alterations (1–3).

For both the glucose and the imino acid transporters of intestine, up-regulation is faster than down-regulation: $\approx$1 and $\approx$3 days, respectively, to reach a new steady-state (16, 17). The reason for this asymmetry is unknown.

As regards peak-to-basal activity ratios, basal levels of adaptive enzymes in bacteria are very low in the absence of their substrates and can be stimulated by up to several thousand-fold by addition of their substrates. In contrast, the ratio of peak-to-basal activity is <4, often considerably less, for all intestinal transporters studied. For liver as well, peak-to-basal activity ratios of adaptive enzymes are much smaller than in bacteria.

We suggest two possible functions of moderate basal activity levels in intestine and liver. One function may be to enable the animal to utilize a brief input of dietary substrate. Given the slowness of the regulatory responses, a single meal would transit the animal before any up-regulation that it triggered had taken effect. Hence, a substrate present in a single meal could be absorbed or metabolized only if the relevant proteins were maintained at significant basal activity levels even in the substrate's absence. Regulation instead serves to match intestinal and liver proteins to a running average of dietary composition over several meals. A second suggested function of moderate basal activities of intestinal transporters stems from the fact that transported substrates circulate in plasma and can diffuse back into the intestinal lumen. Thus, basal transporter levels may serve to prevent nutrient losses by recapturing substrates diffusing down their concentration gradient from blood to lumen in the absence of dietary substrate input.

FUNCTIONAL SIGNIFICANCE OF REGULATION

The diversity of regulatory patterns in Fig. 1 is striking. Substrates may induce, repress, or not affect activities of their transporters, depending on the substrate and its dietary level. What is the functional significance of these diverse patterns?

We identify trade-offs among four factors as setting these patterns:

(*i*) Biosynthetic and other costs (30, 31) of synthesizing and maintaining any protein. Because of these costs, any protein should be repressed if it ceases to yield benefits such as metabolic energy or provision of an essential nutrient.

(*ii*) Caloric pay-off. A metabolizable nutrient yields calories in direct proportion to the amount of the nutrient. Hence, all other things being equal, the activity of a transporter for a metabolizable nutrient should be up-regulated by dietary levels of its substrate.

(*iii*) Fixed daily requirements. Certain nutrients such as vitamins and minerals are required in fixed daily amounts. The transporter for such a nutrient would be most needed when the nutrient was in short supply. The transporter would be less needed at high dietary substrate levels, when absorption by passive diffusion down a concentration gradient could satisfy the fixed daily requirement without transport costs, or when the fixed requirement could be extracted from the higher substrate level by a lower transporter number. Hence, the transporter should tend to be repressed by its substrate.

(*iv*) Toxicity. Toxic nutrients should tend to repress their transporters to protect the animal against risk of intoxication at high dietary substrate levels.

Thus, in combination with factor *i*, factor *ii* should tend to result in a substrate up-regulating its transporter, while factors *iii* and *iv* would tend to result in down-regulation. In 1983, we noted that the combination of these four factors yields straightforward predictions in two sets of cases (5):

First, for nonessential nontoxic nutrients used only as a source of calories (e.g., sugars), transporter activity should increase monotonically with dietary substrate level. Our reasoning was that factors *iii* and *iv* (fixed requirements and toxicity) are irrelevant for a nonessential nontoxic substrate, and that factor *ii* (caloric pay-off) would lead to transporter up-regulation by its substrate. In effect, the costs of adding transporters on a high-substrate ration are more than met by the resulting additional calorie absorption, and this increased absorption is safe because the substrate is nontoxic, while decreased absorption on a low-substrate ration is also safe (because the nutrient is nonessential) and saves transporter costs. Fig. 1*A* confirms the prediction of monotonically increasing activity for two transporters of nonessential nontoxic nutrients used only for calories: the glucose transporter, and probably the fructose transporter (four and two dietary substrate levels tested, respectively).

Second, for essential yet potentially toxic nutrients not used for calories (e.g., vitamins and trace minerals), transporter activity should decrease monotonically with dietary substrate level. Our reasoning (5) was that factor *ii* (caloric pay-off) is irrelevant for a nonmetabolized nutrient, and that factors *iii* and *iv* (fixed requirements and toxicity) would both lead to transporter down-regulation by its substrate. Fig. 1*C* confirms the prediction of monotonically decreasing activity for five transporters of essential toxic nutrients not used for calories: the iron and calcium transporters, and probably the phosphate, vitamin C, and thiamin transporters.

For these seven transporters, the regulatory patterns are simple and readily predicted, because all trade-off factors predispose to regulation in the same direction. However, for amino acid and peptide transporters, the predictions from some factors conflict with predictions from other factors. A positive relationship between amino acid or peptide transporter activity and dietary substrate level is expected because amino acids and peptides yield calories; more absorption yields more calories. A negative relationship is expected for three reasons: all amino acids and peptides are collectively essential as a nitrogen source; eight amino acids are specifically essential; and certain amino acids (mainly the essential ones) become toxic at high levels (32). Fig. 1 *B* and *D* shows the resulting regulatory compromises achieved. The simplest is for the nonessential nontoxic amino acids proline and aspartate, whose transporters are positively regulated (Fig. 1*B*), just as for nonessential nontoxic sugars. Among the more toxic essential amino acids, one transporter serves the basic amino acids (e.g., lysine), while one or more other

transporters serve neutral amino acids (23). As shown in Fig. 1*D*, transport of the essential amino acids lysine, leucine, methionine, and histidine increases at dietary levels above the maintenance level (thereby yielding additional calories), but the increase for these toxic amino acids is less steep than for the nontoxic proline and aspartate (thereby reducing risk of intoxication). At dietary levels below the maintenance level, the transport of lysine, leucine, methionine, and histidine does not decline further (as for proline and aspartate) but remains constant or (as for iron, calcium, thiamin, and phosphate) increases, thereby ensuring a supply of these essential amino acids. Thus, the regulatory patterns for essential amino acids combine those for sugars and for trace minerals and vitamins.

It may at first appear discrepant that regulation of the nonessential amino acid alanine at low dietary amino acid levels (□ in Fig. 1*D*) resembles that of essential amino acids rather than that of the nonessential proline and aspartate. However, whereas proline and aspartate have "private" transporters that can be regulated independently (23), alanine shares the "public" intestinal transporter for essential neutral amino acids and is constrained to be co-regulated with them. The dipeptide transporter monitored by carnosine is up-regulated at high dietary protein levels, but its response at low levels has not been measured, so it is unknown whether the dipeptide pattern is closer to Fig. 1 *B* or *D*.

TESTABLE PREDICTIONS

It is evident that intestinal transporters provide numerous systems for studying adaptive regulation in eukaryotic cells. The many transporters whose regulation has yet to be examined will offer tests of our framework for understanding the diverse responses of the transporters studied to date. By the reasoning outlined above, we make four sets of predictions.

(*i*) Specific transporters are known to exist for other water-soluble vitamins besides thiamin and vitamin C, including (33) biotin, choline, folic acid, inositol, nicotinic acid, pantothenic acid, riboflavin, and vitamin B-12. We anticipate that these transporters will be down-regulated as dietary levels of their substrates increase.

(*ii*) Several other essential trace minerals besides iron, calcium, and phosphate may also be absorbed by specific transporters, including cobalt, copper, iodide, magnesium, manganese, and zinc. We predict that these transporters will also be down-regulated by their substrates.

(*iii*) Transporters for short-chain fatty acids, a nonessential source of calories, will be up-regulated by dietary substrate.

(*iv*) The distal ileum has a transporter for reabsorbing bile acids, which are secreted by the liver, are essential for intestinal fat absorption, and may be obtained either from the diet or by synthesis. We predict that this transporter will be down-regulated by its substrates.

We thank Ronaldo Ferraris, David Solberg, and Eric Stein for information; Richard Weiss and Ernest Wright for criticism of the manuscript; and the National Institutes of Health for support through Grants GM 14772 and AM 17328 (University of California, Los Angeles Center for Ulcer Research and Education).

1. Walker, R. (1983) *The Molecular Biology of Enzyme Synthesis* (Wiley, New York).
2. Kilberg, M. S., Han, H., Barber, E. F. & Chiles, T. C. (1985) *J. Cell. Physiol.* **122,** 290–298.
3. Shotwell, M. A., Kilberg, M. S. & Oxender, D. L. (1983) *Biochim. Biophys. Acta* **737,** 267–284.
4. Reisenauer, A. N. & Gray, G. N. (1985) *Science* **227,** 70–72.
5. Karasov, W. H. & Diamond, J. M. (1983) *Am. J. Physiol.* **245,** G443–G462.
6. Morrissey, R. L. & Wasserman, R. H. (1971) *Am. J. Physiol.* **220,** 1509–1515.
7. Swaminathan, R., Sommerville, B. A. & Cure, A. D. (1977) *Br. J. Nutr.* **38,** 47–54.
8. Allen, L. H. (1982) *Am. J. Clin. Nutr.* **35,** 783–808.
9. Favus, M. J. (1985) *Am. J. Physiol.* **248,** G147–G157.
10. Kimber, C. L., Mukherjee, T. & Deller, D. J. (1973) *Am. J. Dig. Dis.* **18,** 781–791.
11. Linder, M. C. & Munro, H. N. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36,** 2017–2023.
12. Charlton, R. W. & Bothwell, T. H. (1983) *Annu. Rev. Med.* **34,** 55–68.
13. Lee, D. B. N., Walling, M. W. & Brautbar, W. (1986) *Am. J. Physiol.* **250,** G369–G373.
14. Patrini, C., Cusaro, G., Ferrari, G. & Rindi, G. (1981) *Acta Vitaminol. Enzymol.* **3,** 17–26.
15. Rose, R. C. & Nahrwold, D. L. (1978) *Int. J. Vit. Nutr. Res.* **48,** 382–386.
16. Karasov, W. H., Pond, R. S., Solberg, D. H. & Diamond, J. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7674–7677.
17. Diamond, J. M. & Karasov, W. M. (1984) *J. Physiol. (London)* **349,** 419–440.
18. Karasov, W. H., Solberg, D. H., Chang, S. D., Hughes, M., Stein, E. D. & Diamond, J. M. (1985) *Am. J. Physiol.* **249,** G770–G785.
19. Solberg, D. H. & Diamond, J. M. (1987) *Am. J. Physiol.*, in press.
20. Ferraris, R. P., Diamond, J. M. & Kwan, W. (1987) *Am. J. Physiol.*, in press.
21. Karasov, W. H., Solberg, D. H. & Diamond, J. M. (1987) *Am. J. Physiol.*, in press.
22. Stein, E. D., Chang, S. D. & Diamond, J. M. (1987) *Am. J. Physiol.*, in press.
23. Karasov, W. H., Solberg, D. H., Carter, S., Hughes, M., Phan, D., Zollman, F. & Diamond, J. M. (1986) *Am. J. Physiol.* **251,** G501–G508.
24. Hahn, P. & Koldovsky, O. (1986) *Utilization of Nutrients During Postnatal Development* (Pergamon, Oxford).
25. Karasov, W. H. & Diamond, J. M. (1983) *J. Comp. Physiol.* **152,** 105–116.
26. Maenz, D. & Cheeseman, C. I. (1986) *Biochim. Biophys. Acta* **860,** 277–285.
27. Ferraris, R. P. & Diamond, J. M. (1987) *J. Membr. Biol.* **94,** 77–82.
28. Wolfram, S. & Scharrer, E. (1984) *Pfluegers Arch.* **400,** 34–39.
29. Wasserman, R. H., Brindak, M. E., Meyer, S. A. & Fullmer, C. S. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7939–7943.
30. Dykhuizen, D. (1978) *Evolution* **32,** 125–150.
31. Diamond, J. M. (1986) *Nature (London)* **321,** 565–566.
32. Harper, A. E., Benevenga, N. J. & Wohlheuter, R. N. (1970) *Physiol. Rev.* **50,** 428–558.
33. Rose, R. C. (1980) *Annu. Rev. Physiol.* **42,** 157–171.