Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 2117–2121, March 1998
Biochemistry

Evolutionary matches of enzyme and transporter capacities to dietary substrate loads in the intestinal brush border

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Contributed by Jared Diamond, December 22, 1997

ABSTRACT **Safety factors of enzymes and transporters are defined as the ratio of V_{max} (maximal reaction rates at high substrate concentrations) to the reaction rate under actual physiological conditions. Although corresponding safety factors have been measured for macroscopic biological structures and for human-engineered structures, safety factors have been little studied at the molecular level. Some evolutionary considerations suggest that safety factors should be modestly in excess of 1.0 ("enough but not too much") and should tend to be similar for the various steps of a pathway consisting of two or more elements arranged in series. Hence we used a preparation of intact mouse small intestine to measure V_{max} values (capacities) of brush-border sucrase (yielding glucose plus fructose) and of the brush-border glucose transporter, for comparison with each other and with dietary sucrose loads. Load was manipulated by varying dietary sucrose level or by studying lactating mice with increased energy requirements. Capacities both of sucrase and the glucose transporter increased with sucrose load (i.e., both proteins are inducible) and remained approximately matched to each other except on a carbohydrate-free diet. Their safety factors decreased from *ca.* 2.7 at low load to 1.0 at high load. Thus, neither sucrase nor the glucose transporter is *the* rate-limiting step for sucrose digestion; both steps are equally limiting. The modest safety factors and matched capacities must be genetically programmed through natural selection, with benefits of excess capacities being balanced against costs of biosynthetic energy and limited membrane space.**

This paper will analyze a series metabolic pathway in terms of evolutionary considerations that are routine in other areas of biology but that have as yet been little explored at the molecular level.

What sets the activities of the enzymes and transporters that constitute metabolic pathways? The proximate factors are already well understood; they comprise the mechanisms of protein synthesis, modification, and degradation and their regulation. However, the ultimate factors are not well understood: how did it come to be that those proximate mechanisms set activities at their actually observed levels, rather than at some higher or lower level?

The qualitative answer to this latter question is clear: proteins, their synthesis, and their activities evolve through natural selection to become adapted to environmental pressures. Specifically, activities of enzymes and transporters are qualitatively matched to prevailing natural loads of substrates. For example, even when different animal species are compared while eating the same ration, the intestinal glucose transporter is genetically programmed to occur at higher activities in herbivores (whose natural diet contains much carbohydrate) than in carnivores (whose natural diet contains little carbohydrate) (1). But how does natural selection ultimately set the numerical values of those activities?

As a framework for addressing corresponding questions about human-built structures, engineers calculate so-called "safety factors": the ratio of a component's designed strength or capacity to the maximum load that it is designed to bear. For instance, the cables of passenger elevators and freight elevators are manufactured with safety factors of about 11 or 7, respectively, meaning that the cable will not break until its payload is 11 or 7 times the advertised legal maximum load (2). This framework has been extended straightforwardly to biological structures such as bones and mollusk shells, whose safety factors are found to lie mostly in the 2–4 range (3, 4). For an enzyme or transporter, the safety factor is the ratio of V_{max} (maximum reaction rate at high substrate concentrations) to the reaction rate with physiological substrate concentrations.

Safety factors of engineered structures are set consciously by engineers, in response to cost/benefit considerations of the marketplace. For example, elevator companies whose cables have too low safety factors go bankrupt because their elevators become shunned as crash-prone, whereas companies whose cables have excessively high safety factors go bankrupt through competition with companies whose elevators are less overdesigned and hence cheaper but still adequate. (Safety factors should exceed 1.0 because both strengths and loads vary somewhat unpredictably and because strengths may deteriorate with age.) Through analogous cost/benefit tradeoffs, natural selection sets biological safety factors unconsciously: animals with too few copies of their enzymes would be limited in their performance, whereas animals with too many copies would squander limited available biosynthetic energy and space. Competition between individual animals, and between elevator companies, selects for economic design (5, 6).

A related question concerns the safety factors of two or more elements arranged in series. Considerations of economic design suggest that series capacities should tend to be approximately matched, because an excess capacity of a single step would remain unused and constitute wasted expenditure. Nevertheless, numerous other considerations could lead to an imperfect match (5). Indeed, most biochemists believe that one or a few steps in series enzymatic pathways tend to have lower capacities than other steps and hence to be rate-limiting, because of the resulting ease of regulation. But the empirical evidence on this point is conflicting (7): other biochemists report evidence for rate limitation being distributed much more equally over many enzymatic steps in series.

A major difficulty in resolving this biochemical debate is that enzymatic activities measured *in vitro* in tissue homogenates may differ from *in vivo* values because of differences in structural organization, ionic composition, and pH. It seems to

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us that an advantageous system for overcoming this difficulty is the brush-border membrane of the small intestine. That membrane contains numerous hydrolases for dietary nutrients present in solution in the intestinal lumen, operating in series with transporters conveying the reaction products of those hydrolases out of the lumen across the brush border. Those hydrolases and transporters are not intracellular but are part of a membrane facing the luminal extracellular compartment. Hence physiological values of those enzyme and transporter activities can be measured in an intact tissue facing a bulk bathing solution mimicking the normal luminal contents. Thus, *in vitro* V_{max} measurements can be straightforwardly extrapolated to *in vivo* conditions, without the uncertainties introduced by tissue homogenization.

In the present paper we measure capacities (i.e., V_{max} values) of two proteins acting in series in the brush-border of mouse intestine: sucrase/isomaltase, one of whose two active sites splits sucrose to yield glucose plus fructose (8); and the Na^+/glucose cotransporter SGLT1, which transports the resulting liberated glucose (9). We measure the load on these two proteins as the dietary intake of sucrose (hence also of glucose, because digestion of dietary sucrose is virtually complete). We thereby calculate safety factors as the capacity/load ratio for each protein, and we compare the capacities of the two proteins to assess series capacity matching. To simplify the interpretation, we place mice on a diet whose sole carbohydrate is sucrose, so that the dietary loads on sucrase and on the glucose transporter are identical (because 1 mol of sucrose yields 1 mol of glucose upon hydrolysis). When animals are instead consuming their usual natural diets, these often contain other oligosaccharides (besides sucrose) that constitute loads on sucrase/isomaltase, and other sources of glucose (besides sucrose) that constitute loads on the glucose transporter.

Our laboratory previously developed a physiologically realistic preparation of intestinal brush border, termed an everted sleeve, for measuring the V_{max} of the glucose transporter in intact tissue (10). Sucrase activity is instead usually measured in intestinal homogenates (11), so that extrapolation of the measured V_{max} of sucrase to *in vivo* conditions becomes problematic. Hence our paper uses a method, described in the preceding paper (12), for assaying sucrase in intact intestinal tissue by means of everted sleeves. Sucrase and glucose transporter assays are thus directly comparable to each other, because they are measured in the same preparation. They are also comparable to the dietary substrate load consumed by the whole animal, because the assay conditions are relatively physiological. We carry out these comparisons at three different values of dietary sucrose load, manipulated by varying dietary sucrose content and by using both nonlactating and lactating female mice (with low and high dietary intake rates, respectively).

MATERIALS AND METHODS

Animals and Diets. Virgin female Swiss–Webster mice obtained from Charles River Breeding Laboratories were caged individually in a room at 24°C on a constant 13 light/11 dark light schedule. They were supplied *ad libitum* with a pelleted 55%-sucrose ration manufactured by ICN (see ref. 12 for composition). Four mice instead were fed a carbohydrate-free ration, differing only in the replacement of all sucrose with casein. Mice were sacrificed on the 14th day of consuming the ration.

For our studies of lactating mice, four additional female mice were mated, were maintained on the high-sucrose ration, and gave birth to litters of 9–12 pups. Litters were culled to eight pups, and the mothers were sacrificed on the 15th day of lactation, the peak day of lactation for mice, because pups begin to nibble solid food on day 16 (see ref. 13 for details).

Beginning 3 days before sacrifice, we measured food intake and fecal output daily and thereby calculated apparent dry-matter digestive efficiency as (food intake − fecal output)/food intake (see ref. 13 for details). Food intake proved to range from 3.9 ± 0.1 g/day (n = 5) to 4.6 ± 0.2 g/day (n = 6) for different batches of virgin mice consuming the high-sucrose ration. Intake was 19.8 ± 2.7 g/day (n = 6) for lactating mice consuming the same high-sucrose ration and was 3.8 ± 0.1 g/day (n = 4) for virgin mice consuming the carbohydrate-free ration. Despite the 5-fold higher food intake of lactating mice, digestive efficiency proved to be essentially the same for all three experimental groups: 0.75 ± 0.02 (n = 5) to 0.81 ± 0.01 (n = 6) for different batches of virgin mice consuming the high-sucrose ration; 0.81 ± 0.01 (n = 4) for lactating mice consuming the same high-sucrose ration; and 0.76 ± 0.01 (n = 4) for virgin mice consuming the carbohydrate-free ration. On the day of sacrifice all mice were 95–144 days old and weighed 28–44 g.

Everted Sleeve Assays. We measured activity of the brush-border Na^+/glucose cotransporter by the everted sleeve preparation described briefly in the preceding paper (12) and in more detail earlier (10, 14). Brush-border sucrase activity was measured in the same everted sleeve preparation, by measuring colorimetrically the glucose released into the adjacent solution by the action of sucrase on sucrose, after preincubation with the glucose transport inhibitor phlorizin to prevent uptake of the released glucose into the tissue (12). Both the glucose transporter and sucrase assays were carried out at 50 mM concentrations of their respective substrates and were converted to V_{max} values by using the Michaelis–Menten equation (i.e., by multiplying the measured activity at 50 mM by 1.12 and 1.36, respectively to take account of the respective K_m values of 6 mM and 18 mM) (12).

From each of three small intestinal regions (proximal, mid, and distal) of each mouse, we used five sleeves immediately adjacent to each other: the first and fourth sleeves to assay sucrase, the third sleeve as the tissue blank for sucrase assays (see ref. 12), and the second and fifth sleeves to assay the glucose transporter. Thus, we obtained V_{max} values for the glucose transporter and for sucrase from adjacent pieces of intestine from the same mouse, prepared as the same intact intestinal preparation with good control of unstirred layers.

Statistics. Statistical significance was assessed by t tests, one-way and two-way ANOVAs, Tukey tests, and planned comparisons, as discussed elsewhere (13, 15). The $P < 0.05$ level was taken as significant. Values are reported as means ± SEM, with sample size in parentheses.

RESULTS

Calculations of Loads and Capacities. The daily load on intestinal sucrase is the daily intake of sucrose (mmol per day), which we calculated as the measured daily intake of 55%-sucrose ration (grams per day) times the ration's sucrose content (1.61 mmol/g). Because 1 mmol sucrose yields 1 mmol glucose on hydrolysis, the daily dietary glucose load on the glucose transporter is identical. We measured activities (V_{max} values) of the glucose transporter and of sucrase for each region (each third) of the small intestine, in units of nmol per mg of intestinal wet mass per min. By multiplying each regional activity times regional wet mass and summing over the three regions, we obtained the daily capacity of the whole length of the small intestine to hydrolyze sucrose or transport glucose (mmol per day) (14, 16).

Manipulations of Dietary Load. We studied mice at three dietary sucrose loads (Fig. 1): intermediate, high, and zero. Our intermediate dietary load was for virgin mice consuming the high-sucrose diet: 7.5 ± 0.4 mmol sucrose or glucose per day (n = 6).

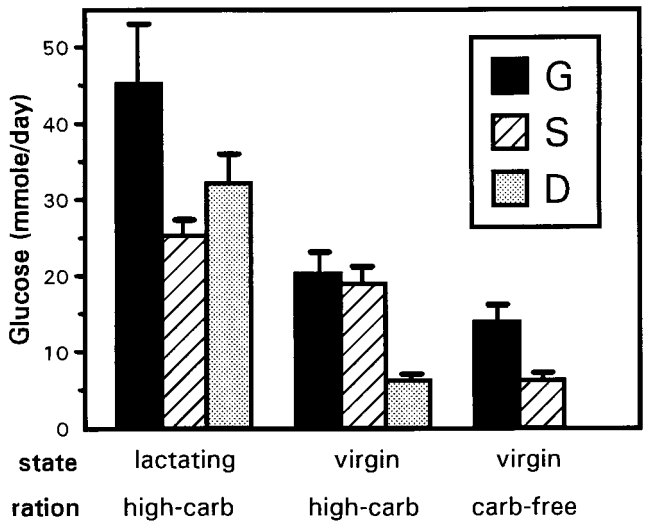

FIG. 1. Comparisons of glucose transporter capacity (G), sucrase capacity (S), and dietary intake of glucose in the form of sucrose (D). The two capacities are for the whole length of the small intestine, and ordinate units are mmol glucose transported, produced, or ingested per day. The comparisons are made at three dietary loads: high (lactating mice consuming 55%-sucrose ration), medium (virgin mice consuming 55%-sucrose ration), and zero (virgin mice consuming no-carbohydrate ration). Each bar gives the mean value ± 1 SEM for 4–6 mice. Note that sucrase capacity equals glucose transporter capacity within experimental error for virgin mice consuming the 55%-sucrose ration; that both capacities significantly exceed the dietary load in virgin mice consuming that ration, but not in lactating mice; that both capacities are down-regulated, but not to zero, on the no-carbohydrate ration; and that glucose transporter capacity might exceed sucrase capacity in lactating mice, but that difference was not statistically significant in our experiments.

For a high dietary load we used lactating mothers at the peak of lactation, consuming the same high-sucrose diet. To provide nutrients for export into milk and to fuel the energetic costs of lactation, the mothers increased their intake 4.3-fold over virgin levels, to 32 ± 4 mmol sucrose or glucose per day (n = 4).

For a zero dietary load of sucrose or glucose, we used virgin mice consuming the carbohydrate-free ration.

Intestinal Adaptation to Lactation. The principal intestinal response of mice to the increased dietary load during lactation was a 78% increase in small intestinal mass, as also found previously (13). Activity per mg of intestinal mass, averaged over the length of the small intestine, was nominally higher during lactation for the glucose transporter (by 24%) and lower for sucrase (by 30%), but these nominal differences were not statistically significant. Because of the increase in intestinal mass, total intestinal capacity increased by 122% (P = 0.005) for the glucose transporter and (not quite reaching statistical significance) by 30% (P = 0.08) for sucrase (Fig. 1).

Intestinal Adaptation to Dietary Carbohydrate Levels. In virgin mice consuming the carbohydrate-free ration, intestinal and body mass, food intake, and apparent dry matter digestive efficiency all remained unchanged compared with virgin mice consuming the high-sucrose ration. However, activities of both the glucose transporter and sucrase declined significantly in both the proximal and mid-intestine. As a result, total intestinal capacity decreased by 31% for glucose transport (P = 0.02) and by 64% for sucrase (P = 0.0002) (Fig. 1). These observations confirm many previous demonstrations that both of these proteins are induced by their substrates (e.g., refs. 17–19). The likely functional explanation of the slighter repression of the glucose transporter than of sucrase on the carbohydrate-free diet is that no sucrose at all enters the intestinal lumen then, but that some glucose continues to diffuse into the lumen down its concentration gradient from the blood and must be reabsorbed.

Regional Gradients of Activity. Under all three loads, activities of both the glucose transporter and sucrase decreased from proximally to distally along the intestine (Fig. 2), as reported by many previous authors (e.g., refs. 14 and 20). These declines are as expected from the declines in sucrose and glucose concentrations along the intestine under physiological conditions, caused by sucrose hydrolysis and glucose absorption.

Match Between Capacities of Sucrase and the Glucose Transporter. For nearly all groups of both virgin and lactating mice consuming the high-sucrose ration, the capacities of sucrase and the glucose transporter were statistically equal to each other in each intestinal region (Fig. 2). For the whole intestine, the ratio of sucrase to glucose transporter capacity was 1.15 ± 0.21 (n = 5), 0.95 ± 0.08 (n = 6), 0.93 ± 0.12 (n = 6), or 0.83 ± 0.06 (n = 4) for four groups of virgins, 0.61 ± 0.14 (n = 4) during lactation; none of these ratios differs significantly from 1.0 (Fig. 1). (The lower mean value of sucrase capacity compared with glucose transporter capacity in lactating mice does not prove statistically significant, but this conclusion warrants reexamination with a larger sample size.) For virgins consuming the carbohydrate-free ration, the ratio of 0.45 ± 0.07 (n = 4) is significantly (P = 0.004) less than 1.0 (Fig. 1) for the functional reasons discussed above (namely, that some glucose but not sucrose can leak into the intestine from the blood).

Safety Factors. Safety factors, defined as the ratio of total intestinal daily capacity to dietary substrate daily load, are summarized in Table 1 for virgin and lactating mice consuming the high-carbohydrate ration. (Safety factors are nominally infinite for virgin mice consuming the carbohydrate-free ration.) In virgins, both sucrase and the glucose transporter have safety factors significantly greater than 1.0 (2.5 and 2.8, respectively) but not significantly different from each other. In lactating mice, safety factors for both proteins do not differ

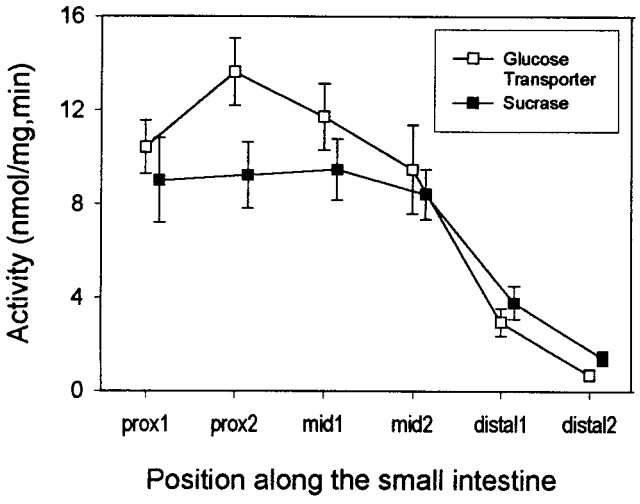

FIG. 2. Gradients of brush-border sucrase activity (■) and glucose transporter activity (□), measured in everted intestinal sleeves of the same mice, at six locations from proximally to distally along the small intestine. At each location, sleeves used for sucrase and glucose-transporter assays were immediately adjacent to each other. Each point represents the mean value, with standard error bars, for six virgin female mice consuming a 55%-sucrose ration. Ordinate units are mmol glucose (produced by sucrase from sucrose, or taken up by the glucose transporter) per mg of wet mass of intestinal sleeve per min. Sucrase data are from fig. 3 of ref. 12. Note that both activities decline in parallel from proximally to distally, and that at each position the two activities are equal or nearly equal to each other within experimental error.

Table 1. Safety factors of the glucose transporter and sucrase

Mice	Glucose transporter	Sucrase
Virgin	2.8 ± 0.3	2.6 ± 0.2
Lactating	1.5 ± 0.3	0.8 ± 0.1

Both virgin and lactating mice were studied while consuming the 55%-sucrose ration. Safety factors were calculated as the V_{max} of the glucose transporter or sucrase summed over the whole length of the small intestine (in units of mmol/day), divided by the dietary intake of sucrose (hence of glucose) in the same individual mouse (also in units of mmol/day). Sample sizes were six virgin mice and four lactating mice. Both safety factors for virgin mice are significantly higher than 1.0 ($P = 0.002$ for the glucose transporter, $P = 0.001$ for sucrase), whereas neither value for lactating mice differs significantly from 1.0 ($P = 0.21$ and $P = 0.06$, respectively).

significantly from 1.0 nor from each other. That is, both proteins possess some reserve capacity in virgin mice, but those reserve capacities become exhausted in lactation: because food intake increases by a much larger factor (4.3 times) than do intestinal mass (1.8 times), glucose transporter capacity (2.2 times), or sucrase capacity (1.3 times).

DISCUSSION

Approximations. Our methods and calculations simplify natural conditions in at least six obvious respects.

First, we have compared capacities with dietary substrate loads time-averaged over the 24-hr cycle. In reality, the metering action of the pyloric sphincter does not fully smooth out fluctuations in substrate load resulting from the intermittent schedule of food intake (21). Hence loads may be higher, and hydrolase and transporter reserve capacities (defined as excesses of capacity over load) may be lower, at certain hours of peak substrate delivery.

Second, and related to the preceding point, our comparisons of capacities with loads are based on the calculated V_{max} values of sucrase and of the glucose transporter— i.e., on activities measured at luminal sugar concentrations far above the Michaelis constant (K_m value) of sucrase or of the transporter. Measured glucose concentrations in the intestinal lumen of rats consuming our high-sucrose diet fluctuate *in vivo* with time and intestinal position (21). At some times of day, especially in the distal intestine, they may approach or decline below the K_m, yielding actual activities *in vivo* below V_{max} values. However, most absorption still must occur at times and proximal positions of higher glucose concentration and at values near the V_{max}.

Third, we intentionally used a ration containing only a single carbohydrate load (sucrose), so that the load for comparison with capacities would be unambiguous. Normally, however, other dietary carbohydrates (such as starch and glucose) will be present and will constitute additional loads. Under those conditions, one does not necessarily expect the capacities of the glucose transporter and sucrase (actually, sucrase/isomaltase) to be matched to the sucrose load, nor possibly to each other. For instance, dietary glucose would constitute a load on the glucose transporter but not on sucrase.

Fourth, we have not examined whether glucose transporter and sucrase capacities may be modified by direct interaction: e.g., whether the rate of sucrose hydrolysis may be modified by glucose transport or by glucose itself (cf. ref. 22).

Fifth, we measured glucose transporter and sucrase capacities at a pH near neutrality (7.3), but the latter's and possibly the former's activity varies with pH, and intestinal luminal pH varies somewhat with position and time because of arrival of gastric acid and alkaline secretions. However, most hydrolysis and absorption occur at pH values near neutrality.

Finally, sucrose hydrolysis yields two products, glucose and fructose, which are taken up by separate transporters. We have not examined the match between fructose transporter and sucrase capacities.

Conclusions. Our studies yield three main conclusions, besides the values of safety factors. First, when sucrose is the sole dietary carbohydrate, the capacities of sucrase and the glucose transporter are matched to each other within experimental error.

Second, the close match between glucose transporter and sucrase capacities means that, under usual conditions, neither constitutes *the* rate-limiting step in sucrose utilization. (Whether this remains true during lactation requires further study.) Instead, the resistance to sucrose utilization is equally distributed over both steps in this series two-step pathway. This conclusion, based on quantitative measurements of capacities, could have been anticipated from the qualitative observation that dietary sucrose up-regulates the activities of both sucrase (18, 19) and the glucose transporter (17). Up-regulation of both proteins would have been superfluous if only one had been rate-limiting and the other had been present in excess.

Finally, it is important to appreciate that this match cannot be attributed to direct interactions between hydrolysis and transport (e.g., inhibition of sucrase by its glucose product), because we intentionally measured each process in the absence of the other's operation. We measured glucose transporter activity in a sucrose-free solution. We measured sucrase activity with the glucose transporter inhibited, in an initially glucose-free solution, at short times when the released glucose concentration was still low and glucose production was still linear with time (hence when glucose evidently was not inhibiting sucrase).

Instead, the match must be genetically programmed. That is, the activities of both sucrase and the glucose transporter are up-regulated by dietary sucrose, but the quantitative outcome of this genetically specified regulation proves to be similar activities of the two proteins when sucrose is the sole dietary carbohydrate.

There is no structural reason why the activities must be thus matched, and why regulation could not have resulted in a gross mismatch between the two activities. This reasoning is clear from the mismatch actually observed when dietary carbohydrate is removed, so that sucrase experiences no load but the glucose transporter continues to experience the glucose diffusing into the intestine from the bloodstream. Under those conditions, both proteins are down-regulated, but sucrase is down-regulated further, so that its activity becomes half that of the glucose transporter's. Again, we emphasize that this outcome is not an automatic result of direct interactions between hydrolysis and transport, because we assayed everted sleeves from mice fed on high-carbohydrate or carbohydrate-free rations under identical solution conditions; the sleeves differed only in the recent dietary histories of the mice from which they came.

Evolution of Matched Capacities. By what evolutionary mechanism did the genetically programmed match between sucrase and the glucose transporter in sucrose-consuming mice arise, and by what mechanism is it maintained? The mechanism must be the elimination, by natural selection, of animals with mismatched capacities. Such animals would have an unusable excess capacity of either sucrase or the glucose transporter, caused by a deficit of the other protein. That excess would represent in principle a waste of biosynthetic energy, but that cost would be quantitatively minor, because either sucrase or glucose transporter synthesis makes only a trivial contribution to a mouse's total energy budget. Instead, much more important is the "lost-opportunity cost" arising from competition for limited membrane space (23): the brush-border membrane is crowded with membrane-spanning transporters and with external hydrolases, so that a useless excess of

one protein would preempt space that could have been devoted to some other protein. Hence such uneconomical animals would tend to be outcompeted by more economical individuals. Familiar examples of such evolutionary reductions of underused capacities include the reduction of eyes in cave-dwelling animals, the reduction of pectoral muscles and wings in birds that colonize predator-free islands, and the evolution of auxotrophic mutants, i.e., of bacterial strains that evolve to lose specific biosynthetic enzymes as a result of being grown in a medium containing the product of that enzyme (24).

There is already a large literature on the so-called protein burden, i.e., the costs associated with the synthesis of unnecessary proteins (e.g., refs. 25–28). The evolutionary process that we propose for the matching of series capacities through elimination of excessive capacity at one step is no different from the widely recognized process that can lead ultimately to complete loss of unused enzymes.

Safety Factors. The safety factors of both sucrase and the glucose transporter are around 2.7 in virgin female mice. These molecular safety factors are in the same range as those found previously for intestinal brush-border amino acid transporters (13, 29, 30) and for capacities at higher levels of biological organization, such as strengths of bones and spiders' webs (3, 5). Natural selection thus results in biological capacities conforming to the rule "enough but not too much": inadequate capacities would at times limit an animal's performance, but excessive capacities would waste energy and space (5).

The capacities of both sucrase and the glucose transporter are regulated by dietary loads, but only over a 3-fold span (Fig. 1). As a result, at the peak of lactation, when dietary loads increase more steeply than do capacities, safety factors for sucrase and the glucose transporter decline to near 1.0. Conversely, even on a carbohydrate-free diet, some sucrase capacity and glucose transporter capacity are preserved, to reabsorb glucose leaking into the gut from the blood and to be ready for the unpredictable arrival of a sucrose-containing meal. The likely proximate mechanism maintaining some residual capacity on a carbohydrate-free diet is that glucose leaking into the gut induces not only the glucose transporter (31) but also sucrase (19).

Outlook. The considerations that we have developed for intestinal brush-border sucrase in series with the glucose transporter warrant testing on other series pairs of intestinal hydrolases and transporters, such as on lactase or maltase and the glucose/galactose transporter, on sucrase and the fructose transporter, and on peptidases and amino acid transporters. These considerations also warrant testing on other series metabolic pathways elsewhere in the body.

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