Effect of Variation of Dietary Intake of Starch and Sucrose on the Activity of Sucrase and Lactase in Jejunum of Adult Rats

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The problem of the regulation of activity of intestinal disaccharidases, especially lactase, has attracted the attention of researchers for many years (6,8); there are still many unresolved questions. This chapter, which deals with the authors’ recent experiments with rats, summarizes data concerning (a) the effect of variation of carbohydrate intake on the activity of sucrase and lactase, (b) the mechanisms involved in changes of the activity of disaccharidases as influenced by the variation of carbohydrate intake, and (c) the locus of the first expression of the effect of the variation of carbohydrate intake along the height of the crypt–villus columns.

METHODS

Rats born in our animal facilities were weaned at 30 days of age and fed a standard laboratory chow (Lab Blox, Allied Mills, Inc., Chicago, Ill.) until 11 to 12 weeks of age. They were then fed isocaloric synthetic diets. (For detailed composition of diets, see refs. 3,14.) The low-carbohydrate diet provided 5% cal. from starch, 21% cal. from protein, and 73% cal. from corn oil. The high-carbohydrate diets contained 72%, 23%, and 5% cal., respectively.

All rats were fed ad libitum and had unrestricted access to water. Fresh food was given every second day. Food intake was measured by weighing the food every morning. Rats were sacrificed by decapitation between 9 and 10 a.m. in a fed state. The entire small intestine was removed, the duodenum was discarded, the jejunoileum was divided into three equal parts along its length, flushed with cold saline solution, and frozen at −20°C. before the assays of activity for disaccharidases and determinations of protein were performed. This chapter summarizes data related only to the proximal third of the jejunum.

Sucrase activity was determined according to Dahlqvist (4), and lactase according to Koldovský et al. (7). The lactase assay mixture contained p-chlo-
romercubenzene [(PCMB) Aldrich Chemical Company, Milwaukee, Wisc.] in order to inhibit any residual lysosomal acid β-galactosidase activity (7). Assays of enzyme activity were performed under the condition of linearity of reaction with time and amount of homogenate used. Protein was determined according to Lowry et al. (10). Enzyme activity was expressed as μmoles of substrate split per mg of tissue protein (specific activity) or per total intestinal segment (total activity). Since specific activity is a ratio between enzyme activity and tissue protein, a change in the value can be caused by a change either of the numerator, or of the denominator (tissue protein or other parameter, e.g., DNA). The denominator can change not only quantitatively, but also qualitatively, i.e., changes in tissue protein other than the structures carrying the hydrolases studied can occur. Expressing the data as total activity removes the possibility of an artifact when data are related to protein, DNA, etc.

To assay the activity of disaccharidases at different levels of the villus, a 5 × 5-mm segment of jejunum was sectioned within a cryostat at −18°C, as previously described (2). Horizontal sections were cut 10 μm thick. A section at a given depth into the villus–crypt unit was mounted on a microscope slide for immediate inspection of histology under a phase-contrast microscope. The tissue blocks were sectioned through the submucosa into the muscular layer. Every 10 consecutive sections were combined and homogenized in 0.5-ml distilled water by vortex shaking.

When cell migration was studied, the technique as described by Ulshen and Grand (13) was used. Simultaneously with the initiation of sucrose feeding, animals received 100 μCi [methyl-3H]thymidine intraperitoneally. From an approximately 10 × 10-mm segment of jejunum, 10 slices of 10 μm thick per tube were prepared using cryostat sectioning. These were solubilized in protosol (New England Nuclear, Boston, Mass.) to which a standard toluene-PPO-POPOP mixture, liquifluor (New England Nuclear) was added. Samples were counted in a Beckman liquid scintillation spectrometer (LS-230). With this technique, similar data to those of Ulshen and Grand (13) were obtained.

RESULTS

Variation of Carbohydrate Intake

Two models were used: (a) starvation, i.e., complete deprivation of carbohydrate and all other sources of energy, and (b) variation of carbohydrate content in isocaloric diets. As already indicated, the protein content of the isocaloric diets remained practically constant; carbohydrate was replaced by fat and vice versa.

Effects on Sucrase

Starvation as well as the decrease of carbohydrate intake leads to a decrease of sucrase specific activity (Fig. 1). Within three days, the specific activity
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decreases more in the animals fed isocaloric diets than in the starved ones. Also, when the data are expressed as total activity per jejunal segment, the decrease in the high-fat low-carbohydrate diet group is more pronounced than in the starved animals (Fig. 2). Feeding the starved animals or the animals fed the low-carbohydrate diet with a high-carbohydrate (starch or sucrose) diet leads to a rapid increase of specific and total sucrase activities (Figs. 1 and 2).

**Effects on Lactase**

In starved animals, the specific activity of lactase increases (Fig. 1b). This increase is only "apparent" and the total activity of lactase (Fig. 2b) does not change; thus, this effect obviously occurs because of "preservation" of lactase activity together with the loss of other unspecific intestinal proteins.

Whereas during starvation sucrase and lactase activities tend to diverge, changes in the carbohydrate content of isocaloric diets are followed by changes of lactase activity that parallel those of sucrase activity (3,14). Refeeding of starved animals leads to an apparent decrease of specific lactase (Fig. 1b) ac-

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**FIG. 1.** Effect of variation of carbohydrate intake on the specific activity of sucrase and lactase in adult rat jejunum. **a:** Symbols not connected, rats fed 2 weeks a high-starch diet. Animals were then fed 14 days a low-starch high-fat diet (Lst), and then were fed a high-starch low-fat diet (Hst) or a high-sucrose low-fat diet (Hsuc). Solid line represents high-starch diet; broken lines, sucrose diet. **b:** Rats fed laboratory chow (Lab Blox) were starved for 3 days and then fed for 24 hr a high-sucrose, low-fat diet. Mean (5–6 animals) and 2 SEM are given. (Constructed from data from refs. 3 and 14.)
tivity with no change of total lactase activity (Fig. 2b). Feeding a high-carbohydrate (starch or sucrose) diet to a low-carbohydrate-fed animal leads to an increase of lactase activity parallel to the changes of sucrase activity (specific and total) (Figs. 1a and 2a).

Studies of animals fed isocaloric diets have thus shown a surprising similarity of the reactivity of sucrase and lactase activity in adult rats to the variation of carbohydrate intake. It is noteworthy that the increase of both lactase and sucrase activity is observed in animals fed high-carbohydrate diets containing either sucrose or starch, that is, the variation in lactase activity is independent of lactose, and changes of activity are affected by α-glucosides.

This finding has led to several other experiments testing the specificity of the sugars involved in the increase of these two disaccharidases activities. Ongoing experiments in our laboratory show that both sucrase and lactase activities increase in jejunal homogenates in animals that have been fed a low-carbohydrate diet for 14 days and then switched for the next 24 hr to high-carbohydrate diets containing either sucrose, fructose, galactose, or lactose.

Another large group of experiments explores the locus where first changes of enzyme activity on the villus-crypt columns are expressed after an introduction of a high-sucrose diet to animals that were previously fed a low-carbohydrate diet. Since the work of Leblond and Stevens (9), it has been recognized that the enterocytes mature both morphologically and biochemically
while migrating from the crypts to the villus. Activity of sucrase and lactase is absent from the crypt cells and increases as the enterocytes migrate toward the tip of the villus (2,11). This leads to a logical question: At which level of the villus, that is, at what time during their lifespan, are the enterocytes capable of responding to a dietary change with an alteration in disaccharidase activity? For sucrase this question has recently been explored by two laboratories (12,13). Both studies have shown that in adult rats, when the sucrase activity is lowered by starvation, feeding with sucrose leads to an increase of sucrase activity in the cells of the crypt. The sucrase activity increases as enterocytes move toward the villus tip.

The results of our experiments with starving rats are in agreement with the previously quoted observations, namely, the increase is first seen in cohorts

![Graph showing lactase and sucrase activity along villus-crypt columns in jejunum of rats that were fed 14 days a low-carbohydrate diet and then for 24 hr a high-sucrose diet. Abscissa depicts total height of the intestinal wall with 100% representing the top part of the villus and 0% representing the bottom of the serosal side. Villus and crypt portions are depicted with rectangles; the overlapping area is the crypt-villus transition (mix) zone. Mean and 2 SEM are given; number of animals per group = 5. Circles, Control group fed low-starch, high-fat diet; triangles, experimental group fed high-sucrose, low-fat diet for 24 hr. Solid symbols depict significantly different values from the low-carbohydrate diet group (p < 0.025) at the same location. The rate of cell migration within 24 hr was the same in both groups and is indicated by the arrow on the abscissa. The arrows from dotted line to solid line indicate that the cohorts of cells present at the start of the high-carbohydrate diet at the level of 30% of the height of the intestinal wall (still in crypt) or at the level of 50% (already in villus) migrate to the levels of 65% and 85% of the height, respectively. Comparison thus shows that the increase of sucrase and lactase during migration occurred in enterocytes that were in crypts and also in the lower villus at the time of the introduction of the high-sucrose diet. (Modified from ref. 15.)

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of cells that are in the villus–crypt region at the time of beginning of sucrose feeding. A different situation exists in rats where the activity of lactase and sucrase is lowered by feeding a low-carbohydrate diet the preceding two weeks and then switching to a high-sucrose diet. In this case, the sucrase activity is increased along the entire height of the villus [i.e., both in the cells that are in the crypts at the time of the start of the new dietary regimen, and in the cells that are “out” of the villus (Fig. 3)]. Lactase activity exhibits a similar change; its activity increases along the entire height of the villus.

**CONCLUSION**

The data summarized above, as well as other studies from our laboratory (3), clearly show that the activity of sucrase and of lactase depends, in adult rats fed isocaloric diets, on the carbohydrate content of these diets. The effect of carbohydrate intake on sucrase activity is well-established in the literature (1,5,6), but the effect of carbohydrates not containing β-linkage on lactase activity is rather new (3,14,15). Whereas, in the latter case, sucrase and lactase exhibit a close linear correlation (3,14), during short-term starvation they react differently: sucrase activity decreases, but lactase activity does not change.

Feeding starved animals with a high-sucrose diet influences only the sucrase activity of enterocytes present in the crypts. On the other hand, feeding a high-carbohydrate diet to rats previously fed a low-carbohydrate isocaloric diet is followed by an increase of sucrase and lactase activity along the entire height of the villus. Further experiments are needed to analyze the dependency of the reaction of villus cells on the nutrition state of the animal.

The question of functional significance of this dietary manipulation on disaccharidase activity is being explored in studies on absorption capacity of these rats for sucrose and lactose. Furthermore, present studies on adult rats will be followed by studies designed to evaluate the adaptation capability of disaccharidases in suckling rats.

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**REFERENCES**

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DISCUSSION

Dr. Jacquot: Did you measure the actual food intake after changing the diet?
Dr. Koldovský: Yes, we did. The diet is prepared with agar as a caloric diluter.
Dr. Jacquot: So the rats did not change their alimentary habits—they continued eating the foodstuff. The second point I should like to bring up is that I have no idea about the accessibility of lactase as compared to sucrase for pancreatic enzymes. I mean, are they buried in the same way inside the cell membrane or not? I am concerned with the changes in pancreatic exocrine secretion and in changes in the enzymatic digestion of those brush border membranes.
Dr. Kretchmer: I have one statement and one question to make. The statement is related to what Professor Jacquot brought up. I think it is fairly accepted, at least in a hypothetical sense, that pancreatic proteolytic enzymes participate in one or two phases of the biology of the disaccharidases: one phase would probably be the activation of sucrase in the membrane, and the other phase might be the participation of pancreatic enzymes in the degradation of the disaccharidases. The one most studied has been sucrase, lactase being more difficult to study, especially in these age groups that you are dealing with. So that when you manipulate the diet, you have to think of the effects in terms of the two parts of the digestive tract; the effects, whatever they may be, on the degradation or synthesis of a particular disaccharidase or peptidase, or whatever enzyme you want to study, and, two, the effects on the exocrine secretions of the pancreas which might participate in the degradation of the particular enzyme, either slowing it down or speeding it up, which would, of course, affect the activity of the enzyme as you would measure it. It is really, I think, more complex than it is presented.

My question is as follows: one of the conclusions that you gave was that you had evidence to show that the substrate affected the activity—that's what you measured—of the enzyme in the cell after the cell was on the villus, in other words, after the cell had left the crypt. What evidence do you have for that statement?
Dr. Koldovsky: The question first. One of the slides (Fig. 3) shows that the changes of intestinal disaccharidases also occur beyond the 24-hr migration point of enterocytes. As I said, using labeled thymidine, we have measured the rate of migration of the enterocytes; in agreement with data in the literature, the migration time from the crypt to villus is around 48 hr. It is not influenced by the dietary manipulations.

As far as your statement is concerned, I will make another statement in answer. I agree with what you and Professor Jacquot have said, but—as I have stressed—we have measured the activity. Only further experiments will clarify what mechanisms are influencing these changes. The role of pancreatic proteases involved in the regulation of intestinal brush border disaccharidases is quite important [Alpers, D. H., and Tedesco, F. J. (1975): Biochim. Biophys. Acta, 401:28]. We tried to minimize their role in our experiments by using diets in which we did not vary the content of the protein. This, of course, does not exclude the possibility that proteases might have been influenced. Therefore, one of the experiments to be done in the near future is a study under conditions that will exclude pancreatic secretion. The question of changes of activity versus changes of amount of enzymes is one which we can actually begin to answer with the results of experiments that we have done in the last year with Sunshine and Tsuboi from Stanford. Using their technique, we have measured immunochemically the amount of these disaccharidases (namely sucrase, lactase and maltase), their synthesis, and compared those with the synthesis of total mucosal protein. Our experiments indicate that the increase of carbohydrate intake is not only reflected in the increase of disaccharidases activities, but also in their rate of synthesis as well as in the amount of specific enzyme protein.

Dr. Strong: It does seem very surprising that these enzymes should respond non-specifically. Have you tried sugars that are nonmetabolizable—do they have an effect? Have you tried things that are simply an osmotic load or simply something that produces a mechanical effect, like undigestible cellulose?

Dr. Koldovsky: Some of the experiments that you are suggesting are those we intend to perform in the near future. At the present time, we have some other supporting data that indicate that this is an effect of sugars. (a) Since the intake of isocaloric low- and high-starch diets by the animals and body weight changes are equal, we are not dealing in these experiments with the effect of caloric deprivation, etc. (b) The animals do not exhibit any diarrhea. (c) Furthermore, we have shown the dose-dependent effect of carbohydrate content of the diets on disaccharidase activity [Yamada et al. (1981): Biochim. Biophys. Acta, 676:108]. (d) As far as the sucrase and maltase activity are concerned, the activity changes are in the expected way, that is, the increase of carbohydrate intake is followed by an increase of their activity. (e) The situation which affects the activity of sucrase and lactase differently is starvation. In starved animals the sucrase activity (total and specific) goes down, but lactase activity does not change; (total) specific activity exhibits an increase [Yamada et al. (1983): Am. J. Physiol., 244:G449].

Dr. Greene: I have just been trying to reconcile the data that you presented with the data that Dr. Rosenweig presented in the early 1970s, and I wonder if part of it might not be the method of expression. He expressed his data first in terms of milligrams of protein and decided that this was an inappropriate method of measuring it because of the biopsy technique in which he took various levels of the lamina propria, so that enzyme activity per milligram protein might not have been so much the epithelial cell protein, but many of the supporting structures. For that reason then, when he found no difference in the lactase activity with the various diets, he decided that it might be better to express it in terms of one enzyme activity versus the enzyme that didn’t appear to change (in that instance lactase). Most of his data presented thereafter were the sucrase or maltase activity per unit lactase. I wonder, if he had been able to express the data in the same way—that is, per epithelial cell—do you think he would have had the same findings as you showed?
Dr. Koldovsky: This question has to be answered in several parts. The first is simple. The Rosenweig data you quoted are on man, and our work is on the rat; so there is one possible difference. Furthermore, the number of volunteers was relatively small. Now let us examine the methodology. First I will speak about our methodology which uses the total intestinal mucosa of intestinal segments (jejunum, ileum). Thus, we can express our data not only per protein, but also as total activity per animal. The range of mean lactase activity was (in the mid-jejunum) in different groups between 0.5 and 1.75 μmoles/mg protein/hr. This range was similar for the activity of sucrase which was 1.5–4.5 μmoles/mg protein/hr [Bustamante et al. (1981): J. Nutr., 111:943]. The degree of correlation between activity of sucrase and lactase in these dietary experiments [Bustamante et al. (1981): J. Nutr., 111:943; Yamada et al. (1981): Biochim. Biophys. Acta, 676:108] is very high in all intestinal segments. Second, I stress that when we expressed the data either per protein, per DNA or mucosa wet weight, or per total intestinal mass, we always got the same results. So in our experiments the data are not a result of changes of the denominator, for example, protein. Finally, the data generated together with Sunshine and Tsuboi showing the increase of amount of disaccharidases and their synthesis provide another independent support.

Now, concerning the expression of the data using the ratio of sucrase activity per lactase activity in biopsy specimen: Rosenweig and co-workers assumed that activity of sucrase and lactase are distributed along the height of the villus in a parallel fashion—and this is not true. We have extended in rats [Boyle, Celano, and Koldovsky (1980): Gastroenterology, 79:503] the original observation on a human biopsy specimen [Nordström and Dahlqvist (1973): Scand. J. Gastroenterol., 8:407] that sucrase to lactase ratio along the height of the villus is not identical.Sucrase activity increases along the height of the villus earlier than lactase. Lactase “needs more time” to express its maximal activity. If you have a ratio of sucrase to lactase in the upper villus of about three, then this value is around five in the lower villus [Boyle et al. (1980): Gastroenterology 79:503]. The risk involved in expressing sucrase to lactase data in biopsies depends on the fact that activity of sucrase and lactase do not exhibit on all heights of the villus the same ratio.

Dr. Guesry: Since we may expect quite a large change of enzyme activity with a change of diet, do you have any data on much younger animals—suckling rats, weaning rats, or even suckling babies?

Dr. Koldovsky: There are several data from the literature. One of them is from Kretchmer’s laboratory [Lebenthal, E., Sunshine, P., and Kretchmer, N. (1972): J. Clin. Invest., 51:1244]. The other group that studied this is F. Raul, P. M. Simon, M. Keding, J. F. Grenier, and K. Haffen. [Biol. Neonate (1978): 33:100]. From their data, the conclusion can be reached that in suckling animals an increased intake of carbohydrates is followed by an increase of the activity of disaccharidases. The question is what are the mechanisms involved; are they the same as in adults or are they different?

Dr. Ransome-Kuti: What I understood is that if you give an experimental rat substance like fructose or sucrose you could increase the activity of lactase. Is this true? Because from all the evidence that we have, we have not been able to increase the activity of lactase in the human being when we have administered lactose itself, so it is a bit puzzling to hear that in adult rats, if you give them a disaccharide which is absolutely unrelated to the enzyme, you could increase the activity of the enzyme. I would like Dr. Koldovsky to confirm that this is what he is finding and comment on whether this has got any relationship whatsoever to what we have observed in the human being. I think he must also know about a Thai experiment in which they fed Thai children with milk and some without milk—they did not find any increase in lactase activity after the absorption of lactose by these children.

Dr. Koldovsky: According to the literature, lactase is probably an adaptive enzyme in the adult mammals, but the question of the specificity of the substrate that will influence lactase activity is not clear. Experiments have been done so far where the
effect on the lactase activity was tested by studying lactose, as a substrate and as an inducer of the activity, and glucose, galactose and sucrose were used as control sugars. In our studies in rats, in adult rats, we have clearly shown that sucrose and starch are equal in their effect on sucrase and lactase activity in animals. The problem that I probably did not state sufficiently is that we are operating, as Dr. Kretchmer also stated, in a range allowed by genetic background or age. For example, in the suckling animal, the specific activity of lactase is between one and two units, and in the adult animal it is between 0.2 and one unit depending on the part of the small intestine and age of the animal that you study. So, within that range, the lactase activity in the adult animal is affected by quantitative changes of carbohydrates—we have clear data on this as far as the effects of sucrose, starch, and glucose are concerned. These are the sugars where we have both quantitative and time-dependent types of responses and these are practically the same. The story is open as far as other monosaccharides which might be affecting this activity. The changes in the sucrase and the lactase activity may be the result of changes of the total glycoprotein synthesis in the small intestine. Maybe the variations of carbohydrate intake affect the total glycoprotein content of the brush border. If we call the changes depending on the intake of some substrate adaptation, we are operating in a given range that is genetically controlled. In other words, this is not an approach that can increase or overcome a genetic deficiency of lactase in mammals, including man.

Dr. Kretchmer: The one question that Dr. Ransome-Kuti asked was what is the functional significance of your findings?

Dr. Koldovsky: The enzymatic activity changes in a ratio of 1 to 5, so there probably will be.

Dr. Kretchmer: Yes, but 1 to 5 when you start at a very low level.

Dr. Koldovsky: If I change it, for example, for an adult animal, it has 100% activity, and I can vary this from 150% to 30% in that range.

Dr. Kretchmer: So, it is your speculation that there will be a functional effect.

Dr. Guthrie: I think Dr. Ransom-Kuti gave us a very important piece of information. I just would like to ask him if he checked to see if by giving lactose he could induce increased lactase activity in these children.

Dr. Ransome-Kuti: What we found was that by the age of 2 to 4 years, the children in Lagos did have a low lactase activity as shown by lactose tolerance tests. Now, the evidence that the lactase activity could not be increased by giving lactose was established by trying it on our medical students; they were fed lactose for about a month, and they were tested before and after with the lactose tolerance test. We found that they did not reactivate their lactase. Similar experiments have been carried out in Thailand where subjects were also fed milk for very long periods, and we have found that there was no reactivation of lactase activity. Even South African Bantus, who regularly drank milk and were able to tolerate large amounts of milk, were found to have very low lactase activity when they were tested. Of course, there was some type of adaptation going on in these individuals in South Africa, but this adaptation was not as a result of lactase activity in the intestine.