EFFECT OF PROTEIN-CALORIE MALNUTRITION ON INTESTINAL DISACCHARIDASE ACTIVITIES AND DISACCHARIDE ABSORPTION IN THE RAT

by

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We accept this thesis as conforming to the required standard

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ABSTRACT

The purpose of the present investigation was to study the effect of prolonged protein-calorie malnutrition on intestinal disaccharidase activities and on disaccharide absorption, as carbohydrate intolerance is a major problem in children suffering from protein-calorie malnutrition.

Four groups of rats (90 to 120 grams) were fed the following diets for 8 to 9 weeks: control (18% lactalbumin, 66% carbohydrate); low protein low carbohydrate (0.5% lactalbumin, 66% carbohydrate); low protein high carbohydrate (0.5% lactalbumin, 83.5% carbohydrate); and low protein restricted (1% lactalbumin, restricted to 4 grams per day). After 8.5 weeks, part of the group on the 0.5% lactalbumin low carbohydrate diet was fed the control diet (18% lactalbumin, 66% carbohydrate) for 8 weeks.

At the end of the feeding period, the following assays were performed: 1) in vivo absorption of radioactive \(^{14}\text{C}\) lactose, sucrose, and maltose; 2) activities of intestinal lactase, sucrase, and maltase; 3) plasma albumin concentrations; and 4) mucosal protein concentrations.

In the three protein deficient groups (0.5% lactalbumin low carbohydrate, 0.5% lactalbumin high carbohydrate, and 1% lactalbumin), the activity of both the jejunal and ileal disaccharidases and the absorption of lactose, sucrose and maltose were significantly higher when compared with the controls. The jejunal sucrase and maltase activities were significantly higher in the 0.5% lactalbumin high carbo-
hydrate group than in the 0.5% lactalbumin low carbohydrate group, but the absorption of lactose, sucrose and maltose were alike. When the 1% lactalbumin (restricted to 4 grams per day) and the 0.5% lactalbumin low carbohydrate groups were compared, there were no statistically significant differences in the specific activities of the intestinal disaccharidases or the absorption of the disaccharides.

The absorption of lactose, sucrose, or maltose were similar in the controls and the protein repleted group. The disaccharidase activities were also similar in these two groups except for a significant depression of jejunal maltase and ileal sucrase and maltase activities in the protein repleted group.

Therefore, these results indicate that protein deprivation in rats for 8.5 weeks causes an increase in specific activities of the intestinal disaccharidases in both the jejunum and ileum, and that these changes caused by protein depletion may be reversed by feeding a diet high in protein. Also, an increase in the carbohydrate content of the protein deficient diet results in an induction of jejunal sucrase and maltase activities. The high specific activity of the intestinal disaccharidases following protein-calorie malnutrition may be in part due to a preferential loss of structural proteins rather than to an increase in enzymatic protein in the intestinal mucosa.

The increase in the disaccharidase activities in the protein deficient rats is accompanied by an increase in disaccharide absorption which could be due to the higher levels of disaccharidases or to an increase in the transport of the
constituent monosaccharides. The demonstration of statistically significant differences in sucrase and maltase activities between the 0.5% lactalbumin high carbohydrate and the 0.5% lactalbumin low carbohydrate groups without a concomitant increase in sucrose and maltose absorption, supports the view that the higher absorption of sucrose and maltose in the protein deficient rats is a result of increased monosaccharide transport.

The results of this study are not consistent with the suggestion that protein-calorie malnutrition is responsible for disaccharide intolerance in children.
ACKNOWLEDGEMENT

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CHAPTER I

LITERATURE REVIEW

A. Introduction

Protein-calorie malnutrition is still one of the major problems in developing countries. In children, it has been clearly shown to be associated with depressed activities of intestinal disaccharidases (1) which is probably a consequence of alterations of intestinal structure (2). Bowie et al. (3) noted that 60% of children with protein-calorie malnutrition suffered from malabsorption of carbohydrates. However, it is not clear whether the disaccharide intolerance and diminished disaccharidase activities are primarily due to protein deprivation, or to gastrointestinal infection, and deficiencies of nutrients other than protein.

In order to clarify this problem of carbohydrate intolerance in protein-calorie deficiency, a number of investigators have studied the effect of protein deficient diets on intestinal disaccharidase activities in young rats (4,5). The results obtained in those studies were surprising because protein deprivation in rats produced an increase in the specific activities of disaccharidases and particularly of maltase and sucrase. It has been suggested, therefore, that this increase in disaccharidase activities in rats was an adaptation to the high proportion of carbohydrate in the protein deficient diets since most of the investigators
replaced the protein portion of the control diet with carbohydrate. It should also be pointed out that in the experiments designed to show the effect of protein deficient diets on intestinal disaccharidase activities, the experimental animals have not been kept on the protein deficient diets long enough to develop edema which is so characteristic of the full kwashiorkor syndrome in children (4,5,6).

Almost all of the studies on the effect of protein malnutrition on carbohydrate absorption reported in the literature determined changes in intestinal disaccharidase activities without measuring the absorption of disaccharides. Depressed levels of disaccharidases can be implicated as being functionally significant only if variations in disaccharidase levels are reflected in changes in disaccharide absorption as well (7). Also, it is important to study the absorption of the disaccharides by an in vivo technique as procedures which separate the intestine from the animal create a condition in which the blood vessels are no longer present to carry away the absorbed material.

With the preceding ideas in mind, it seemed necessary to further study the effect of long-term protein-calorie deprivation on intestinal disaccharidase activities and on in vivo absorption of disaccharides.

B. Digestion and Absorption of Carbohydrates

1. Review of digestion and absorption of carbohydrates.
Carbohydrates are ingested in complex forms which must be broken down to their simpler components before they can be
absorbed. The process of digestion and absorption of dietary carbohydrates is shown in Table I. The digestion of carbohydrates occurs in the lumen of the small intestine with the action of amylase on the polysaccharides, amylose and amylopectin, and at the surface of the mucosal cells of the small intestine with the action of the disaccharidases (lactase, sucrase, and maltase) on the disaccharides (lactose, sucrose, and maltose). The final products of disaccharide hydrolysis are the monosaccharides: glucose, fructose, and galactose. The monosaccharides are then transported across the mucosal membrane by either active transport, as in the case of glucose and galactose, or facilitated diffusion, as in the case of fructose (8). When the complete process of disaccharide hydrolysis and monosaccharide absorption were studied in man, the action of lactase was found to be the rate-limiting step in the absorption of lactose (9), whereas the mucosal transfer of glucose was the rate-limiting step in sucrose and maltose absorption (10,11).

2. Location of the intestinal disaccharidases. The activities of intestinal disaccharidases, lactase, sucrase, and maltase, are not uniformly distributed along the length of the small intestine with the highest activity being found in the jejunal section and the lowest activity being found in the ileal section in rats (12), pigs (13), and man (14).

The lactase, sucrase and maltase activities are located in the brush border of the mucosa (15) with most of the activity being found along the sides of the villi and not in the crypts (16). The activities of sucrase and maltase
# TABLE I*

**DIGESTION AND ABSORPTION OF CARBOHYDRATES**

<table>
<thead>
<tr>
<th>CARBOHYDRATE</th>
<th>SITES OF DIGESTION</th>
<th>ABSORPTION (MODE OF TRANSPORT)</th>
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<tbody>
<tr>
<td>Intraluminal</td>
<td>Muscosal</td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>amylose</td>
<td>maltose</td>
</tr>
<tr>
<td></td>
<td>amylose → maltose</td>
<td>maltotriose</td>
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<tr>
<td></td>
<td>amylopectin</td>
<td>maltotriose</td>
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<tr>
<td></td>
<td>amylopectin →</td>
<td>maltotriose</td>
</tr>
<tr>
<td></td>
<td>α-amylase</td>
<td>maltase</td>
</tr>
<tr>
<td>Sucrose</td>
<td>sucrase</td>
<td>glucose</td>
</tr>
<tr>
<td>Lactose</td>
<td>lactase</td>
<td>galactose</td>
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have been associated with small knobs on the outer edge of the microvilli (17,18).

3. Changes in intestinal disaccharidase activities with age. At the time of birth in the rat, the levels of lactase activity are high and decrease during the immediate post weaning period to the low levels characteristic of the adult animal. Sucrase and maltase activities are low at birth, and by 4 to 5 weeks of age, increase to levels found in adult rats (19,20).

In man, sucrase and maltase activities (except for maltase I) at birth are the same as in adult life (14). In the case of lactase, the level of activity found at birth may either remain the same throughout the life of the individual or decrease within the first decade of life to a lower level of activity which would then be permanent. The populations in which a decrease in lactase activity after early childhood has been shown to be very prevalent are listed by Herber (14).

4. Effect of dietary carbohydrate on activity levels of intestinal disaccharidases. The activity of the intestinal disaccharidases can be influenced by the carbohydrate content of the diet. It has been demonstrated that after a 3 day fast, a 70% sucrose or maltose diet causes increased jejunal sucrase and maltase activities in the rat when compared to animals fed a carbohydrate-free diet after the fast (7,21). In the case of lactase, some researchers have found an increase in intestinal lactase activity in rats in response to high levels of lactose in the diet after weaning, (20,
(12, 19, 24). Studies done on the effect of dietary carbohydrate on the activities of intestinal disaccharidases in man (25, 26) have shown that an increase in dietary sucrose and fructose results, after 3 to 5 days, in significant increases in sucrase and maltase activities. When subjects were given diets containing 0 to 80% sucrose or glucose, jejunal sucrase and maltase activities increased in the subjects receiving the sucrose or glucose in the diet as compared to the carbohydrate-free group (27). The increases in sucrase, and maltase activities were proportional to the levels of sucrose and glucose in the diet.

C. **Protein-Calorie Malnutrition and Carbohydrate Absorption in Man**

1. **The effect of protein-calorie malnutrition on the intestinal mucosa in man.** In protein-calorie malnutrition, the changes that take place in the mucosal tissue of the gastrointestinal tract include thinning of the intestinal wall and mucosa; broadening and atrophy of the villi; increased cellularity of the lamina propria; and decreased height of the epithelial cells. There is also the presence of lymphocytic infiltration between the epithelial cells on the surface of the villi (28). These changes have been noted in children suffering from protein-calorie malnutrition by Stanfield et al. (2), Burman (29), Barbezat et al. (30),
Brunser et al. (31), and Berkel et al. (32) and in malnourished adults by Tandon et al. (33), and Mayoral et al. (34,35).

The renewal of the intestinal mucosa as expressed by the mitotic index (which is the percentage of the epithelial cells undergoing mitosis in the crypt of Lieberkühn (36) has been shown to be lower for marasmic patients than in kwashiorkor patients and both are lower than in healthy individuals (31,36).

2. The effect of protein-calorie malnutrition on the intestinal disaccharidases in man. The results of studies on the activity of intestinal disaccharidases in children suffering from protein-calorie malnutrition in various areas of the world including Uganda (2), South Africa (30), and Turkey (32) have demonstrated significantly lower levels of the three disaccharidases, lactase, sucrase, and maltase, when compared to the levels of activity for these enzymes in healthy children of similar age. James (37) studied Jamaican children with kwashiorkor and marasmus and found that 8 of the 19 children had decreased lactase, sucrase, and maltase activities in the jejunal biopsy specimens. The children diagnosed as marasmic had higher levels of lactase, sucrase, and maltase activities than the children diagnosed as marasmic-kwashiorkor or kwashiorkor.

3. The effect of protein-calorie malnutrition on disaccharide absorption in man. Bowie et al. (38) proposed that the diarrhea, which is often a serious complication of
protein-calorie malnutrition, is caused by intolerance to disaccharides. To test this hypothesis, they removed carbohydrates from the diet of 13 malnourished African children suffering from diarrhea. After 3 days on the carbohydrate-free diet, there was a significant decrease in stool weight and a decrease in fecal lactic acid in 9 of the 13 children. Lactose, glucose/galactose, or glucose tolerance tests performed on 3 of the children showed an intolerance to lactose but not to the glucose/galactose mixture or glucose alone.

Further studies by Bowie et al. (3,39) of malnourished children with diarrhea showed that the use of a carbohydrate-free formula corrected the diarrhea in 60 to 65% of the children. The children who improved on the carbohydrate-free diet demonstrated an intolerance to a lactose load but not to an equivalent load of either sucrose, maltose or glucose plus galactose. Lactase activity in the jejunal biopsy samples was below normal in 8 out of 20 malnourished children, sucrase activity was below normal in 3, and maltase activity was below normal in one.

Prinsloo et al. (40) studied the effects of various dietary monosaccharides and disaccharides in the control of diarrhea in children with protein-calorie malnutrition. They divided 120 malnourished Bantu children into 6 groups and gave each group a different source of dietary carbohydrate in their formula. Five of the groups were given the same basic formula except that the carbohydrate source was either
lactose, glucose, sucrose, dextrin-maltose, or carbohydrate-free. The sixth group of children was fed a full-cream powdered milk formula. The children on the formula with lactose or the full-cream powdered milk suffered the most severe diarrhea and had the highest lactic acid excretion in the stools. The severity of the diarrhea was about the same in the groups of children with glucose, sucrose, or dextrin-maltose in their diets and the carbohydrate-free group. The activities of intestinal lactase, sucrase, and maltase for the malnourished children in these studies by Prinsloo et al. (40) were lower than those for normal children with lactase activity being the most affected.

James (41,42,43) studied malnourished Jamaican infants using an intestinal perfusion technique, and demonstrated a generalized defect in glucose, sucrose, and lactose absorption. There was a significant positive correlation between the rate of hydrolysis of the disaccharides and the level of disaccharidase activity in the jejunal mucosa. Treatment for 2 to 4 months with a high protein diet resulted in an increase in the absorption of glucose, sucrose, and lactose.

4. The relationship between lactose intolerance in protein-calorie malnutrition and the incidence of primary lactase deficiency. Lactose intolerance in adults due to primary lactase deficiency exists with varying frequency in difficult ethnic groups as reviewed by Herber (14), and many of the population groups with high prevalence of primary lactase deficiency are populations in which protein-calorie
malnutrition is also prevalent. Wharton et al. (44) and Cook et al. (45) studied the incidence of disaccharide intolerance in the Ugandan Bantu population and suggested that the intolerance to lactose which did not improve with the treatment for protein-calorie malnutrition may be due to the underlying genetic deficiency of lactase activity.

The presence of hereditary lactase deficiency which was verified by family history was reported from India by Chandra et al. (46). Of the 100 infants studied, 50% had low pH (<pH 6.0) stools. When lactose, sucrose, and maltose tolerance tests were performed on those 50 children, 39 were intolerant to lactose, 25 to sucrose, and 16 to maltose. After treatment with a high protein diet for 3 months, 4 of the 50 children were still intolerant to lactose and were considered to have primary lactase deficiency.

Therefore, it has been well documented that disaccharide intolerance and depression of disaccharidase activities is a problem for many children who are suffering from protein-calorie malnutrition. However, it is not clear whether protein-calorie malnutrition is the cause of these problems.

5. The effect of infections and nutritional deficiencies on the activity of the intestinal disaccharidases. Protein deficiency usually does not occur without other accompanying nutritional deficiencies or without the complication of an accompanying infection. In children with protein-calorie malnutrition, admission to the hospital may be the result of an acute episode of diarrhea of unknown cause.
James (43) concluded from his survey on the effects of protein-calorie malnutrition on intestinal absorption that the relevant importance of acute infections or of malnutrition itself on the changes which occur in the intestinal function and structure have not been determined. In the study by Barbezat et al. (30), there was some correlation between the presence of *Giardia lamblia* infestation and the depression of lactase and sucrase activities. In the studies by Prinsloo et al. (40) and Bowie et al. (3,39), however, there was no correlation between the presence of pathogens and the severity of the diarrhea. Mata et al. (47) reported that the studies performed at the clinical unit at INCAP (Institute of Nutrition of Central America and Panama) on 13 children with kwashiorkor or marasmus showed that there was more bacteria in the stomach and jejunum of malnourished children with diarrhea than in malnourished children who were not suffering from diarrhea.

A decrease in intestinal disaccharidase activities may occur due to damage of the intestinal mucosa caused by infestation. Hookworms are an example of this (48,49). Tripathy et al. (50) noted abnormal results of D-xylose absorption tests and mucosal damage in children with heavy *Ascaris* infections.

Therefore, the results of these studies which have just been discussed show that the role of infection in the etiology of disaccharide intolerance in protein-calorie malnutrition is not completely understood and needs to be studied further.
Certain nutritional deficiencies besides protein deficiency have been shown to affect the gastrointestinal tract. When Naiman et al. (51) studied biopsy samples of 14 infants and children who were iron deficient there was a high incidence of abnormalities of gastrointestinal structure and function. The studies of duodenal biopsy samples showed shortening and clubbing of the villi, and increased cellular infiltration of the lamina propria. The absorption of xylose was impaired. When the iron deficiency was corrected, the xylose absorption and structural abnormalities of the villi returned to normal. Although the intestinal disaccharidases were not studied the findings in this specific experiment demonstrate that nutritional deficiencies other than protein may be involved in the damage to the gastrointestinal structure and gastrointestinal function which is found in children suffering from protein-calorie malnutrition.

D. Protein-Calorie Malnutrition and Carbohydrate Absorption in Laboratory Animals.

Various animals have been used to study the effect of protein-calorie malnutrition on carbohydrate digestion and absorption, including monkeys (52,53), baboons (54), pigs (55), and rats (4,5,6,56,57,58,59,60).

1. The effect of protein-calorie malnutrition on the intestinal mucosa in laboratory animals. When the structure of the intestinal mucosa of animals on low protein diets was studied, changes similar to those found in children and adults
suffering from protein-calorie malnutrition have been noted. Deo et al. (52) working with monkeys, were able to demonstrate atrophy of the mucosa after 8 weeks on a protein-free diet, when compared to monkeys on a control diet containing 15 to 20% casein. In the protein deficient monkeys, there was a decreased number of villi per square unit of mucosa and a decrease in the number of cells in the mucosa, however, the shape of the villi did not change. Atrophy of the villi in the monkey after 18 weeks on a diet containing less than 1% protein was also demonstrated by Kumar and Chase (53).

Rats placed on a protein-free diet for periods up to 5 weeks (61) were found to have thinner intestinal walls and the villi were decreased in size. Ultrastructural changes in the epithelial cells included irregular microvilli; a decrease in the endoplasmic reticulum and RNA granules; and changes in the mitochondrial structures. Hill et al. (62) studied the effect of protein deprivation on weanling rats and found that the animals which were protein deficient for up to 45 days did not increase the length of their small intestines and had significantly shorter villi in the ileum as compared to the controls. The villi in the jejunal segment in the protein deficient animals were thinner but not shorter than in the controls indicating that the endogenous protein in the lumen is available to the jejunal mucosa for repair.
2. The effect of protein-calorie malnutrition on intestinal disaccharidases in laboratory animals. The effect of protein calorie malnutrition on intestinal disaccharidases has been studied in laboratory animals in order to clarify the problem of carbohydrate intolerance in children suffering from protein-calorie malnutrition. In the animal model, it is possible to study the individual effects of protein deficiency, infection, and other nutritional deficiencies on the intestinal disaccharidases which cannot be done on human subjects.

Solimano et al. (4) investigated the effect of protein deficiency on the activity of the jejunal disaccharidases in rats and obtained surprising results. Sucrase and maltase activities were significantly elevated after the animals had been fed a protein-free or 5% protein diet for 22 and 44 days respectively. When these rats were then given a protein adequate diet for 7 days, the lactase, sucrase and maltase activities fell from the previously high values to approximately the normal levels found in the controls. The authors suggested that the increase in carbohydrate content in the protein deficient diets may have been responsible for the significant increases in sucrase and maltase activities due to enzyme induction by the high proportion of carbohydrate in the diet.

The experimental diets used in the investigation by Solimano et al. (4) were based on a complete infant food formula to which cornstarch was added to give final protein
concentrations of 10, 5 and 0.4% in the three diets. Extra vitamins and minerals were not added to the 5% and 0.4% protein diets so that the low protein diets were not complete in all the required nutrients. Also the proportion of the carbohydrate in the three diets was not the same. Therefore, the changes in the intestinal disaccharidase activities cannot be attributed only to the protein deprivation as the authors noted. Also, the changes in the intestinal disaccharidase activities were only determined in the jejunum. Therefore, it is not known whether the effect of protein-calorie malnutrition has the same effect on the ileum.

Prosper et al. (5) investigated the effect of protein-calorie malnutrition on intestinal disaccharidases in 22 day old rats given synthetic diets containing 27 or 0% protein. The increase in disaccharidase activities in rats on the protein-free diet was only statistically significant in the third quarter of the small intestine when compared to the controls. When the animals on the protein-free diet were force fed for 20 days so that the caloric intake would be the same in both the protein-free and the control groups, the only significant differences found were an increase in sucrase activity in the distal half of the small intestine and a decrease in lactase activity in the proximal half of the small intestine in the protein-free group. When total activity of lactase, sucrase, and maltase was determined, the levels of activity were lower in the protein-free group than in the control group. When total enzyme activity was
expressed per 100 grams of body weight, there were no significant differences between the two groups which had similar body weights.

The expression of total enzyme activity per 100 grams body weight may not be valid when comparing healthy control animals to protein deficient animals as there are probably differences in body composition between the two groups (63).

In the study by Prosper et al. (5) the disaccharidase activities were determined along the entire length of the small intestine so that a more complete view of the effect of the protein depletion could be observed. However, as in the study by Solimano et al. (4) the carbohydrate content of the control and protein-free diet was not the same. Therefore, the possibility that the higher proportion of carbohydrate could be influencing the disaccharidase activities in the protein-free group still exists.

In the study by Troglia et al. (56) rats given a restricted intake of rat chow for 5½ to 6½ months had elevated lactase, sucrase and maltase activities when compared to the control groups. There were 3 control groups in this study: one group of controls which was younger than the group with the restricted intake was fed the rat chow ad libitum until the rats reached the final weight of the restricted group, 206 grams; the second control group was the same age as the restricted group and was fed the rat chow ad libitum; and the third control group was meal fed. When total intestinal disaccharidase activities were determined, only the calorie
restricted group had significantly higher total lactase activity. However, the specific activities of lactase, sucrase and maltase were significantly higher in the calorie restricted group than in the control groups.

Kumar and Chase (59) produced a state of undernutrition in infant rats by altering the litter size to 16 animals per mother, 12 hours after birth. After weaning, the rats who had been undernourished received 8 grams of an 8% protein diet per day as compared to the control animals who consumed a mean of 16 grams per day of a 27% protein diet. The disaccharidase activities were studied at various ages starting with 7 days and ending with 115 day old rats. It was found that lactase activity was significantly higher in the undernourished group at all ages except for a temporary depression at 21 days when it was significantly lower in the undernourished group. There were no significant differences in sucrase activity between the two groups at any age. Maltase activity was not determined. Kumar and Chase (59) concluded that the rat was not a good experimental model to use for the study of protein-calorie malnutrition in man.

As in the previous studies of Solimano et al. (4) and Prosper et al. (5) the carbohydrate content of the control and the low protein diets in the study by Kumar and Chase (59) was not kept constant. The protein content of the low protein diet seems to be too high (8%), however, the amount of the diet given each day was restricted so the weight of the undernourished animals was only 20% of the weight of the
controls after 16 weeks of life. The levels of the disaccharidase activities were only determined in the jejunal section.

Kumar and Chase (53) then studied the effect of feeding a low protein diet (<1%) to young adult monkeys for periods of 10 to 18 weeks to see if the results resembled those found in human protein-calorie malnutrition more closely than the results of the studies done with rats. After 6 weeks on the low protein diet, the protein deficient monkeys had significantly reduced lactase activity when compared to the control group which received a 34% protein diet. After 10 weeks, lactase, sucrase, and maltase activities were all significantly reduced in the low protein group. The differences between the results which Kumar and Chase obtained when working with rats (59) and when working with monkeys (53) suggest that the effect of protein-calorie malnutrition on the intestinal disaccharidasises may differ from one species to another. Therefore, one must exercise caution when comparing results obtained from different species.

3. **The effect of protein-calorie malnutrition on absorption of disaccharides in laboratory animals.** The effect of protein-calorie malnutrition on monosaccharide and disaccharide absorption and the activity levels of intestinal disaccharidasises was only studied by Lifshitz et al. (6) They used three types of diets: a control diet with 18% protein, 70% carbohydrate, and 8% fat; a low protein diet with 4% protein, 70% carbohydrate, and 8% fat; and a low protein, low
carbohydrate diet with 4% protein, 45% carbohydrate and 8% fat. Casein was used as the source of protein. The diets were not isocaloric as non-nutritive cellulose was added to make up the casein and glucose deficits in the two protein deficient diets. The rats were fed the respective diets for one to four weeks.

The low protein diets had no effect on the activity of the intestinal disaccharidases and on the absorption of sucrose and maltose. The rate of glucose absorption was significantly greater in the rats on the low protein diets with both 45% and 70% carbohydrate only after 14 days of dietary treatment. There was no change in the absorption of fructose or 3-O-methyl-D-glucose. This was the first study in which the proportion of carbohydrate in the low protein and control diet was kept the same, but the animals were given the respective diets for only 4 weeks. After this short period of time, there were no significant differences in the concentration of mucosal protein between the protein deficient and control animals. This would indicate that 4 weeks of protein deprivation was not long enough to demonstrate all the symptoms of protein-calorie malnutrition. Therefore, the changes in disaccharidase activities or changes in absorption of monosaccharides or disaccharides may not be apparent after only 4 weeks of protein deprivation.

4. The effect of non-protein factors on the activity of the intestinal disaccharidases in laboratory animals. The results of studies dealing with other factors including iron
deficiency or infestation by nematodes support the idea that the associated deficiencies and infections that accompany the state of protein-calorie malnutrition may be important in the disaccharide intolerance found in children suffering from protein-calorie malnutrition. Hoffbrand and Broitman (64) found that lactase, sucrase, and maltase activities were significantly decreased in young dogs who were maintained on an iron deficient diet for 8 weeks when they were compared to the control group.

Sriratanaban and Thayer (57) studied the effect of combined iron and protein deficiency in the rat on the intestinal disaccharidase activities. The animals were divided into 4 groups: the controls (27% protein), low protein (5% protein), low iron, and low iron-low protein. They were maintained on these diets for 75 to 185 days from the time of weaning. As found in previous studies (4,64) the animals on the low protein diet had significantly higher lactase, sucrase, and maltase activities than the controls, and the iron deficient group had lower lactase, sucrase, and maltase activities when compared to the control group. The combination of iron and protein deficiency resulted in an increase in disaccharidase activity in the jejunum and ileum when compared to the controls, but the levels of disaccharidase activities were lower when compared to the protein deficient group.

Bolin et al. (58) studied the effect of worm infestation upon disaccharidase activity in the rat by infesting part of
a group of animals on a control diet containing 30% protein with *Nippostrongylus brasiliensis*. The worm infestation caused a depression of jejunal lactase and maltase activities when compared to the control group.

There are many possibilities for further study on the effects of infections and nutritional deficiencies on the intestinal disaccharidase activities. These must be explored before a decision can be made on the suitability of the rat as a model for studying protein-calorie malnutrition.

E. Objectives of the Present Study

From the preceding review of the literature it appears that well controlled studies are needed in order to elucidate further the effect of protein-calorie malnutrition on the digestion and absorption of carbohydrates.

Therefore, the primary purpose of this investigation is to study the effect of prolonged protein deficiency and protein-calorie malnutrition upon the *in vivo* disaccharide absorption and intestinal disaccharidase activities by feeding rats well controlled diets. The control diet will contain 18% lactalbumin and will be fed *ad libitum*. The protein deficient diet will contain 0.5% lactalbumin and will also be fed *ad libitum*. The protein-calorie malnutrition diet will contain 1% lactalbumin and will be given in restricted amounts (4 grams per day).

The second purpose of this study is to determine whether marked increases of intestinal sucrase and maltase activities previously observed in protein deficient rats (4) could have
been influenced by a high proportion of carbohydrate in the diet. This will be studied by feeding rats protein deficient diets (0.5% lactalbumin) containing different levels of carbohydrate.

The third purpose of this investigation is to determine whether protein repletion leads to a reversal of protein malnutrition symptoms to normal. Rats will be refeed a protein adequate diet after being on a protein deficient diet (0.5% lactalbumin) for 8.5 weeks.

The intestinal disaccharidase activities will be studied at two sites of the small intestine (jejunum and ileum) since it cannot be assumed that a change at one site will occur at all parts of the small intestine.

Results of this investigation should help in clarifying some of the digestion and absorption problems in children suffering from protein deprivation.
CHAPTER II

MATERIALS AND METHODS

A. Animals and Diets

White male rats of the Wistar strain were obtained from the animal unit, Faculty of Medicine, at the University of British Columbia. Their body weights ranged from 90 grams to 120 grams with a mean of 108 ± 9 grams. The rats were divided into four groups and fed the following diets for a period of 8 to 9 weeks, with the time period depending on laboratory convenience. One group of rats received a control diet containing 18% lactalbumin. The second group was given a 0.5% lactalbumin low carbohydrate diet, the third group was given a 0.5% lactalbumin high carbohydrate diet, and the fourth group was given a 1% lactalbumin diet restricted to 4 grams per day. Part of the second group of rats, after being fed the 0.5% lactalbumin low carbohydrate diet for 8.5 weeks, was given the control diet containing 18% lactalbumin for 8 weeks. The composition of these diets is listed in Table II. These diets are based on those developed by Edozien (65) for studying kwashiorkor and marasmus in rats. The only modification of the diets for use in the present experiment was the substitution of cornstarch for dextrose and sucrose. The 0.5% lactalbumin diets with both the high and low levels of carbohydrate were designed to produce symptoms characteristic of kwashiorkor in children which, among others, include edema, loss in body weight, fatty liver, hair dis-
TABLE II

COMPOSITION OF DIETS

<table>
<thead>
<tr>
<th></th>
<th>Control 18% lactalbumin</th>
<th>0.5% lactalbumin Low carbohydrate</th>
<th>0.5% lactalbumin High carbohydrate</th>
<th>1% lactalbumin (Restricted to 4 grams per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactalbumin*</td>
<td>18.0%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>1.0%</td>
</tr>
<tr>
<td>Cornstrach&amp;</td>
<td>66.0%</td>
<td>66.0%</td>
<td>83.5%</td>
<td>66.0%</td>
</tr>
<tr>
<td>Corn oil</td>
<td>10.0%</td>
<td>17.8%</td>
<td>10.0%</td>
<td>17.6%</td>
</tr>
<tr>
<td>Alphacel&amp;</td>
<td>-</td>
<td>9.7%</td>
<td>-</td>
<td>3.4%</td>
</tr>
<tr>
<td>Salt Mix&amp;,®</td>
<td>5.0%</td>
<td>5.0%</td>
<td>5.0%</td>
<td>10.0%</td>
</tr>
<tr>
<td>Vitamin Mix*</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>1.0%</td>
</tr>
<tr>
<td>Choline Chloride*</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

100.0% 100.0% 100.0% 100.0%

*Purchased from Nutritional Biochemical Co., Cleveland, Ohio.

&Purchased from General Biochemicals, Chagrin Falls, Ohio.

coloration, hypoalbuminemia, and gastrointestinal disturbances (63). Kwashiorkor is the deficiency of protein in the diet with an adequate intake of calories. The 1% lactalbumin diet restricted to 4 grams per day was designed to produce the symptoms characteristic of marasmus which is the deficiency of both protein and calories. The symptoms of marasmus in children include muscular wasting, growth retardation, gastrointestinal symptoms, fatty liver, hypoalbuminemia, and irritability. However, the gastrointestinal disturbances, fatty liver, and hypoalbuminemia are not as severe in marasmus as they are in kwashiorkor (63).

Animals on the 1% lactalbumin diet were housed individually in screen bottom cages, and the animals on the other dietary regimens were kept in plastic cages in groups of two to five per cage. Except for the rats on the 1% lactalbumin diet (restricted to 4 grams per day), all other animals were given their diet and water *ad libitum*. The 1% lactalbumin group was fed the 4 grams of diet once a day at 9:30 a.m. and water was given *ad libitum*. All the animals were weighed at weekly intervals.

B. Absorption of Disaccharides

The absorption of lactose, sucrose, and maltose was measured *in vivo* using radioactive disaccharides. The rats were anesthetized with intraperitoneal injections of Nembutal (Sodium pentobarbitol) using 6.0 mg per 100 g body weight for the test animals and 7.1 mg per 100 g of body weight for the controls as the protein depleted animals did not
require as large a dose of the anesthetic as the control animals. The Nembutal dose was injected gradually in three to five portions over a 15 to 30 minute period.

The abdominal wall was opened, and approximately a 10 cm jejunal segment distal to the ligament of Treitz was tied off at both ends. Next, 0.5 ml of the radioactive solution was injected into the lumen of the ligated jejunal segment. The exact amount of radioactive solution injected into the loop was determined by weighing the syringe before and after the injection. The intestinal loop was returned to the peritoneal cavity for a 15 minute period during which the animal was kept warm under a lamp. At the end of this period, the loop was excised and rinsed with 5 ml of 0.9% saline, and the rinsings were collected. The loop was then weighed, slit open and the mucosa was scraped off using a glass slide. The musosal scrapings were weighed and then mixed with 5 ml of 0.9% saline using a glass stirring rod. The luminal rinsings and mucosal scrapings were heated in boiling water in order to inactivate the enzymes and to ensure that all the residual disaccharide was dissolved in the saline solution. The volume of the samples was made up to 10 ml with 0.9% saline and mixed thoroughly. The samples were then centrifuged at 15,000 RPM for 15 minutes. The supernatant fraction was removed and immediately frozen. Radioactivity was determined within 1 to 3 weeks.

The radioactivity of the prepared samples was counted in a Picker Nuclear Liquimat Scintillation Counter using a 0.5 ml aliquot of the supernatant fraction; 2.0 ml of N.C.S.
Tissue Solubolizer (Nuclear Chicago Solubolizer, Amersham/Searle Corp., Arlington Heights, Illinois); and 10.0 ml of scintillation solvent. The scintillation solvent was made up of 7.5 gm PPO and 62.5 mg of POPOP per 1000 ml q.s. toluene which were all obtained from the Fisher Scientific Co. To measure the radioactivity of the injected dose, 0.5 ml of a solution containing approximately 0.01 μCi per ml was used as a standard.

The scintillation samples were counted for 2 minutes with the following channel settings on the Picker Nuclear Liquimat 220:

<table>
<thead>
<tr>
<th>Channel</th>
<th>Upper Limit</th>
<th>Lower Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>780</td>
<td>80</td>
</tr>
<tr>
<td>C</td>
<td>1000</td>
<td>200</td>
</tr>
<tr>
<td>D</td>
<td>1000</td>
<td>320</td>
</tr>
</tbody>
</table>

The amount of disaccharide absorbed was obtained by subtracting the radioactivity which was recovered in the lumen and the mucosa of the ligated segment from the total injected dose. The per cent of dose absorbed was then expressed per 500 mg wet weight of the ligated intestinal segment.

**C. Disaccharidase Activities**

Following the determination of disaccharide absorption, the rest of the small intestine, distal to the ligament of Trietz and up to the ileocecal junction was removed, rinsed
with ice-cold saline to remove the intestinal contents, and placed on an ice-cold stainless steel tray. The small intestine was then divided into four equal parts, and the segment which had been used for the absorption study was accounted for in the first quarter. Lactase, sucrase, and maltase activities were determined in the first and fourth quarters of the intestine. The sections were weighed, the mucosa was scraped off with a glass slide, weighed, and immediately homogenized in ice-cold saline solution using a Teflon Homogenizer at 300 RPM for one minute while being cooled in crushed ice. The mucosal samples were then frozen in sealed polystyrene test tubes and the disaccharidase activities were determined within a week.

The two step TRIS-glucose oxidase method of Dahlqvist (66) with slight modifications was used for the determination of lactase, sucrase and maltase activities. The modifications consisted of the use of 0.1 ml of mucosal homogenate and 0.1 ml of substrate for the incubation mixture instead of 10 μl of each. The absorbance was measured at 420 μm against a reagent blank in a Coleman Hitachi spectrophotometer, Model 101.

Units of disaccharidase activities were defined as micromoles of substrate hydrolysed per minute under the incubation conditions used (66). The specific activity was expressed in units of activity per gram wet weight of mucosa and per gram of mucosal protein.

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1 Teflon Homogenizer, Model K43. TRI-R Instruments, Rockville Center, New York.
D. Other Assays

1. Plasma albumin concentration. For the plasma albumin determination, blood was drawn from the animal by heart puncture and was transferred to test tubes containing heparin. This was done under anesthesia. The blood samples were centrifuged at 15,000 RPM for 15 minutes, and then the plasma was removed and frozen in sealed test tubes. The plasma albumin was determined within three months by the HABA dye method as described by Ness et al. (67) and modified by the Technicon Corporation for use in the auto analyzer (Auto Analyzer Methodology, Method File N-15C). It was further modified in the present experiment by using 0.1 ml of plasma and 5 ml of working HABA dye solution which was made from Stock HABA dye (T21-1079) and Stock Phosphate Buffer (T01-184) from the Technicon Corporation, Ardsley, New York. Bovine Albumin (Sigma Chemical Co., Saint Louis, Missouri) was used as a standard. The absorbance was read at 505 μm in a Coleman Hitachi spectrophotometer, Model 101.

2. Mucosal protein determination. The protein of the mucosa was determined by the method of Lowry et al. (68) using a bovine albumin standard (Sigma Chemical Co., Saint Louis, Missouri). The absorbance was read at 500 μm in a Coleman Hitachi spectrophotometer, Model 101.

E. Materials

The radioactive disaccharides were obtained from the Amersham/Searle Corporation, Arlington Heights, Illinois. The following radio-disaccharides were used for the absorption
studies: lactose (D-glucose-1-C^{14}), freeze-dried solid, specific activity of 10 to 20 μCi per mmole; sucrose uniformly labeled with C^{14}, freeze-dried solid, specific activity of 5 to 15 μCi per mmole; maltose uniformly labeled with C^{14}, specific activity 4 to 10 μCi per mmole.

The 0.5 ml of the incubation mixture used for the absorption studies contained 0.1 μCi of radioactive disaccharide and different amounts of unlabeled disaccharide. In the case of lactose absorption, there was 16 mg of unlabeled lactose per ml; in the case of sucrose absorption, there was 32 mg of sucrose per ml; and in the case of maltose absorption, there was 100 mg of maltose per ml. The reagent grade unlabeled maltose monohydrate and sucrose were obtained from the Fisher Scientific Co., Fair Lawn, New Jersey. The unlabeled beta-lactose, also of reagent grade, was obtained from Eastman Organic Chemical, Rochester, New York.

Glucose oxidase, specific activity of approximately 90 units per ml, A grade, of fungal origin, salt-free and lyophilized and peroxidase, B grade (horseradish) were obtained from Calbiochem, San Diego, California. The glucose standard solution (1 mg glucose per ml) and Triton X-100 were purchased from the Sigma Chemical Co., Saint Louis, Missouri. The TRIS-free base and Phenol reagent 2N solution (Folin-Ciocalteau) were obtained from the Fisher Scientific Co., Fair Lawn, New Jersey. The Nembutal which contained 50 mg sodium pentobarbital per ml was purchased from Abbott Laboratories Ltd., Montreal, P.Q.

The student t test was used for the statistical analysis and the Model 1130 IBM Computer was employed.
CHAPTER III

RESULTS

A. Appearance, Body Weight, and Plasma Albumin Concentration.

The changes in mean body weight of the control, protein depleted, and protein repleted rats are shown in Figure 1. The initial mean body weight of all the rats was 108 ± 9 grams. The control group increased in weight to 359.3 ± 30.6 gm after 8.5 weeks on the 18% lactalbumin diet. Rats on all the three protein deficient diets lost an average of 45 grams from their initial body weight in 8.5 weeks, with most of the loss occurring during the first three weeks. There were no statistically significant differences in the final body weights of the three protein depleted groups. Refeeding the protein depleted rats (rats fed the 0.5% lactalbumin low carbohydrate diet for 8.5 weeks) with the 18% lactalbumin diet produced an abrupt gain in body weight, and after 8 weeks of repletion, the rats had increased their mean body weight to 341 ± 29 grams which was not significantly different from the final mean body weight for the control group (359 ± 31 grams). The initial and final body weights for each dietary group are given in Table III.

When the initial weights of each group were compared, there were no statistically significant differences between the controls and any of the other groups. However, the 0.5% lactalbumin high carbohydrate group had a significantly lower
FIGURE 1

CHANGES IN BODY WEIGHTS OF CONTROL, PROTEIN DEPLETED, AND PROTEIN REPLETED RATS

- Controls, 18% lactalbumin

Protein depleted groups:

○ 0.5% lactalbumin, low carbohydrate
□ 0.5% lactalbumin, high carbohydrate
△ 1% lactalbumin (restricted to 4 grams per day)

■ Protein repleted (refed the 18% lactalbumin diet after 8 weeks on the 0.5% lactalbumin low carbohydrate diet)
The graph illustrates the body weight in grams over time (weeks) for different groups. The controls show a gradual increase in body weight, while the protein depleted groups exhibit a decrease followed by a recovery. The protein repleted group shows a steady increase in body weight.
<table>
<thead>
<tr>
<th>Dietary Group</th>
<th>Number of Animals</th>
<th>Weight at Start of* Dietary Treatment (grams)</th>
<th>Weight at end of* Dietary Treatment (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, 18% lactalbumin</td>
<td>15</td>
<td>107.3 ± 10.0</td>
<td>359.3 ± 30.6</td>
</tr>
<tr>
<td>0.5% lactalbumin, low carbohydrate</td>
<td>12</td>
<td>113.3 ± 8.8(^a)</td>
<td>62.5 ± 4.8(^b)</td>
</tr>
<tr>
<td>0.5% lactalbumin high carbohydrate</td>
<td>18</td>
<td>101.9 ± 4.2(^a, c)</td>
<td>64.7 ± 6.0(^b, d)</td>
</tr>
<tr>
<td>1% lactalbumin (restricted to 4 grams per day)</td>
<td>18</td>
<td>109.4 ± 7.4(^a, d, e)</td>
<td>62.7 ± 5.0(^b, d)</td>
</tr>
<tr>
<td>Repleted &amp;</td>
<td>12</td>
<td>65.0 ± 6.5(^b)</td>
<td>341.3 ± 28.6(^a)</td>
</tr>
</tbody>
</table>

*Values are Means ± S.D. for the number of rats shown in the second column.

&Refed the 18% lactalbumin diet for 8 weeks after 8.5 weeks on the 0.5% lactalbumin low carbohydrate diet.

\(^a\) Does not differ from the control group (P > 0.05).

\(^b\) Differs from the control group (P < 0.001).

\(^c\) Differs from the 0.5% lactalbumin low carbohydrate group (P < 0.001).

\(^d\) Does not differ from the 0.5% lactalbumin low carbohydrate group (P > 0.05).

\(^e\) Differs from the 0.5% lactalbumin high carbohydrate group (P < 0.001).
initial mean body weight than the 0.5% lactalbumin low carbohydrate and the 1% lactalbumin (restricted) groups \( (P < 0.001) \).

About 40% of the rats fed the 0.5% lactalbumin diets showed a severe loss of hair and became less active as the experiment progressed. The animals on the 1% lactalbumin (restricted) diet did not show as severe hair losses as those on the 0.5% lactalbumin diets. The animals fed the 1% lactalbumin (restricted) diet were hyperactive and aggressive in their behaviour, whereas the animals on the 0.5% lactalbumin diets were very passive. Refeeding the group of protein depleted animals the 18% lactalbumin diet for 8 weeks resulted in a reversal of all the gross symptoms so that the repleted group resembled the controls in both appearance and behaviour. Figures 2 and 3 show the typical appearance of the control and protein depleted rats at the time of sacrifice.

Edema, which is characteristic of the full kwashiorkor syndrome in children, was found in only a few rats on the 0.5% lactalbumin diets, although, a number of animals with no obvious edema showed increased fluid accumulation in the peritoneal cavity at the time of sacrifice. It was noted at the time of sacrifice that the small intestine of the protein deficient rats was very thin, fragile, and small in diameter. The intestinal mucosal layer was also very thin and pale when compared to the control group.

The mortality rate of the rats on the three protein deficient diets was highest during the last two weeks of the experimental period. The percentage of survivors for each dietary group is shown in Table IV.
FIGURE 2

TYPICAL APPEARANCE OF RATS ON THE FOLLOWING THREE DIETS: 1% LACTALBUMIN RESTRICTED TO 4 g PER DAY (LEFT), 18% LACTALBUMIN (CENTER), AND 0.5% LACTALBUMIN HIGH CARBOHYDRATE (RIGHT).
FIGURE 3

A close-up of the rat on the 0.5% LACTALBUMIN HIGH CARBOHYDRATE DIET shown in Figure 2.
**TABLE IV**

**PERCENTAGE OF SURVIVORS ON THE DIFFERENT DIETARY REGIMENS**

<table>
<thead>
<tr>
<th>Dietary Group</th>
<th>Number of Weeks on the Experimental Diet</th>
<th>Percentage of Survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, 18% lactalbumin</td>
<td>8.5</td>
<td>100.0%</td>
</tr>
<tr>
<td>0.5% lactalbumin low carbohydrate</td>
<td>8.5</td>
<td>77.5%</td>
</tr>
<tr>
<td>0.5% lactalbumin high carbohydrate</td>
<td>8.0</td>
<td>86.7%</td>
</tr>
<tr>
<td>1% lactalbumin (restricted to 4 grams per day)</td>
<td>9.0</td>
<td>83.3%</td>
</tr>
<tr>
<td>Repleted*</td>
<td>8.0</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

*Refed the 18% lactalbumin diet for 8 weeks after 8.5 weeks on the 0.5% lactalbumin low carbohydrate diet.
The plasma albumin concentrations in the controls, protein depleted, and protein repleted rats are given in Table V. All the three groups of rats on the protein deficient diets had significantly lower plasma albumin concentrations than the control group ($P < 0.001$). The low plasma albumin confirmed that the animals on the 0.5% lactalbumin and the 1% lactalbumin (restricted) diets were suffering from protein-calorie malnutrition. The levels of plasma albumin in the repleted and the control rats were almost identical. The same was true for the rats fed the 0.5% lactalbumin low carbohydrate and the 0.5% lactalbumin high carbohydrate diets. However, the plasma levels were significantly higher in the 1% lactalbumin (restricted) group than in the 0.5% lactalbumin low carbohydrate group ($P < 0.01$).

B. Intestinal Disaccharidase Activities

The jejunal and ileal lactase, sucrase, and maltase activities of rats fed the various diets are shown in Tables VI, VII and VIII respectively.

The intestinal lactase activity was significantly higher in all the three protein deficient groups (0.5% lactalbumin low carbohydrate, 0.5% lactalbumin high carbohydrate, and 1% lactalbumin restricted to 4 grams per day) in both the jejunum and ileum when compared with the controls fed the 18% lactalbumin diet (Table VI).

When lactase levels of the three protein deficient groups were compared, there were no significant differences among them in either the jejunal or ileal lactase activities. When
TABLE V
PLASMA ALBUMIN CONCENTRATION OF CONTROL, PROTEIN DEPLETED, AND PROTEIN REPLETED RATS

<table>
<thead>
<tr>
<th>Dietary Group</th>
<th>Number of Rats</th>
<th>Plasma Albumin* mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, 18% lactalbumin</td>
<td>15</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>0.5% lactalbumin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>low carbohydrate</td>
<td>12</td>
<td>2.1 ± 0.6\textsuperscript{a}</td>
</tr>
<tr>
<td>0.5% lactalbumin</td>
<td>16</td>
<td>2.2 ± 0.4\textsuperscript{a,b}</td>
</tr>
<tr>
<td>high carbohydrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% lactalbumin (restricted to 4 grams per day)</td>
<td>18</td>
<td>2.7 ± 0.4\textsuperscript{a,c}</td>
</tr>
<tr>
<td>Repleted\textsuperscript{e}</td>
<td>11</td>
<td>4.4 ± 0.5\textsuperscript{d}</td>
</tr>
</tbody>
</table>

\*Values are Means ± S.D. for the number of rats shown in the second column.

\&Refed the 18% lactalbumin diet for 8 weeks after 8.5 weeks on the 0.5% lactalbumin low carbohydrate diet.

\textsuperscript{a}Differs from the control group (P < 0.001).

\textsuperscript{b}Does not differ from the 0.5% lactalbumin low carbohydrate group (P > 0.05).

\textsuperscript{c}Differs from the 0.5% lactalbumin low carbohydrate group (P < 0.01).

\textsuperscript{d}Does not differ from the control group (P > 0.05).
TABLE VI
LACTASE ACTIVITIES IN JEJUNUM AND ILEUM OF CONTROL, PROTEIN DEPLETED, AND PROTEIN REPLETED RATS

<table>
<thead>
<tr>
<th>Intestinal Segment</th>
<th>Dietary Group</th>
<th>Number of Rats</th>
<th>Lactase Activity*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Units per g</td>
<td>Units per g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>wet weight</td>
<td>mucosal protein</td>
</tr>
<tr>
<td>Jejunum</td>
<td>Control, 18% lactalbumin</td>
<td>15</td>
<td>1.3 ± 0.2</td>
<td>9.4 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>0.5% lactalbumin low carbohydrate</td>
<td>12</td>
<td>3.9 ± 1.5b</td>
<td>38.2 ± 14.9b</td>
</tr>
<tr>
<td></td>
<td>0.5% lactalbumin high carbohydrate</td>
<td>18</td>
<td>4.2 ± 1.2b,c</td>
<td>45.6 ± 14.7b,c</td>
</tr>
<tr>
<td></td>
<td>1% lactalbumin (restricted)&amp;</td>
<td>18</td>
<td>3.2 ± 0.8b,c</td>
<td>32.4 ± 7.6b,c</td>
</tr>
<tr>
<td></td>
<td>Repleted®</td>
<td>12</td>
<td>1.0 ± 0.4b</td>
<td>7.7 ± 3.0a</td>
</tr>
<tr>
<td>Ileum</td>
<td>Control, 18% lactalbumin</td>
<td>15</td>
<td>0.2 ± 0.1</td>
<td>2.1 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>0.5% lactalbumin low carbohydrate</td>
<td>12</td>
<td>0.6 ± 0.4b</td>
<td>7.6 ± 5.9b</td>
</tr>
<tr>
<td></td>
<td>0.5% lactalbumin high carbohydrate</td>
<td>18</td>
<td>0.4 ± 0.3b,c</td>
<td>4.5 ± 3.3b,c</td>
</tr>
<tr>
<td></td>
<td>1% lactalbumin (restricted)&amp;</td>
<td>18</td>
<td>0.5 ± 0.2b,c</td>
<td>5.9 ± 1.9b,c</td>
</tr>
<tr>
<td></td>
<td>Repleted®</td>
<td>12</td>
<td>0.2 ± 0.1a</td>
<td>2.1 ± 1.1a</td>
</tr>
</tbody>
</table>

*Values are Means ± S.D. for the number of rats shown in the third column.

*Restricted to 4 grams of diet per day.

@Refed the 18% lactalbumin diet for 8 weeks after 8.5 weeks on the 0.5% lactalbumin low carbohydrate diet.

aDoes not differ from the control group (P > 0.05).

bDiffers from the control group (P < 0.05).

cDoes not differ from the 0.5% lactalbumin low carbohydrate group (P > 0.05).
<table>
<thead>
<tr>
<th>Intestinal Segment</th>
<th>Dietary Group</th>
<th>Number of Rats</th>
<th>Sucrase Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Units per g wet weight</td>
</tr>
<tr>
<td>Jejunum</td>
<td>Control, 18% lactalbumin</td>
<td>15</td>
<td>7.2 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>0.5% lactalbumin low carbohydrate</td>
<td>12</td>
<td>15.6 ± 5.4b</td>
</tr>
<tr>
<td></td>
<td>0.5% lactalbumin high carbohydrate</td>
<td>18</td>
<td>22.5 ± 4.2b,c</td>
</tr>
<tr>
<td></td>
<td>1% lactalbumin (restricted)&amp;</td>
<td>18</td>
<td>18.1 ± 3.2b,d</td>
</tr>
<tr>
<td></td>
<td>Repleted®</td>
<td>12</td>
<td>6.2 ± 1.2b</td>
</tr>
<tr>
<td>Ileum</td>
<td>Control, 18% lactalbumin</td>
<td>15</td>
<td>3.6 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>0.5% lactalbumin low carbohydrate</td>
<td>12</td>
<td>2.9 ± 1.5a</td>
</tr>
<tr>
<td></td>
<td>0.5% lactalbumin high carbohydrate</td>
<td>18</td>
<td>2.7 ± 0.7a,d</td>
</tr>
<tr>
<td></td>
<td>1% lactalbumin (restricted)&amp;</td>
<td>18</td>
<td>1.9 ± 0.7b,d</td>
</tr>
<tr>
<td></td>
<td>Repleted®</td>
<td>12</td>
<td>1.0 ± 0.6b</td>
</tr>
</tbody>
</table>

*Values are Means ± S.D. for the number of rats shown in the third column.

&Restricted to 4 grams of diet per day.

@Refed the 18% lactalbumin diet for 8 weeks after 8.5 weeks on the 0.5% lactalbumin low carbohydrate diet.

aDoes not differ from the control group (P > 0.05).

bDiffers from the control group (P < 0.05).

cDiffers from the 0.5% lactalbumin low carbohydrate group (P < 0.001).

dDoes not differ from the 0.5% lactalbumin low carbohydrate group (P > 0.05).
TABLE VIII
MALTASE ACTIVITIES IN JEJUNUM AND ILEUM OF CONTROL, PROTEIN DEPLETED, AND PROTEIN REPLETED RATS

<table>
<thead>
<tr>
<th>Intestinal Segment</th>
<th>Dietary Group</th>
<th>Number of Rats</th>
<th>Maltase Activities*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Units per g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>wet weight</td>
</tr>
</tbody>
</table>

Jejunum  
Control, 18% lactalbumin  
0.5% lactalbumin low carbohydrate  
0.5% lactalbumin high carbohydrate  
1% lactalbumin (restricted)\&  
Repleted\@  

Ileum  
Control, 18% lactalbumin  
0.5% lactalbumin low carbohydrate  
0.5% lactalbumin high carbohydrate  
1% lactalbumin (restricted)\&  
Repleted\@  

* Values are Means ± S.D. for the number of rats shown in the third column.
\& Restricted to 4 grams of diet per day.
\@ Refed the 18% lactalbumin diet for 8 weeks after 8.5 weeks on the 0.5% lactalbumin low carbohydrate diet.

\a Does not differ from the control group (P > 0.05).
\b Differs from the control group (P < 0.05).
\c Differs from the 0.5% lactalbumin low carbohydrate group (P < 0.01).
\d Does not differ from the 0.5% lactalbumin low carbohydrate group (P > 0.05).
the rats on the 0.5% lactalbumin low carbohydrate diet were repleted with a protein adequate diet containing 18% lactalbumin, the lactase activity levels fell from the previously high values to approximately the levels found in the controls.

The lactase activity in the jejunum is markedly higher than in the ileum for all the dietary groups and the test diets seem to have no effect on the distribution of lactase activity between the jejunum and ileum.

As found with the lactase activity, the jejunal sucrase activities in rats on the 0.5% lactalbumin diets with both high and low levels of carbohydrate and the 1% lactalbumin diet were significantly higher than the levels of jejunal sucrase found in the control group. In the ileum, however, there were no statistically significant differences between the control group and the 0.5% lactalbumin low carbohydrate or the 0.5% lactalbumin high carbohydrate groups. In the 1% lactalbumin group, the level of ileal sucrase activity was significantly lower than in the control group (Table VII).

When the 0.5% lactalbumin low carbohydrate group was compared to the 0.5% lactalbumin high carbohydrate group, the sucrase activity was significantly higher in the animals on the high carbohydrate diet in the jejunum, but there were no statistically significant differences between the level of ileal sucrase activity in these two groups. There were also no statistically significant differences between the 0.5% lactalbumin low carbohydrate and the 1% lactalbumin groups.

The jejunal sucrase activities of the control group and the group which was repleted with 18% lactalbumin for 8 weeks
after 8.5 weeks of protein deprivation were similar. However, the ileal sucrase activities fell in the repleted group to levels which were significantly lower than in the control group.

The sucrase activity was always markedly higher in the jejunum than in the ileum in the dietary groups.

When the intestinal maltase activities were compared in the control and the three protein depleted groups (0.5% lactalbumin low carbohydrate, 0.5% lactalbumin high carbohydrate, and 1% lactalbumin restricted to 4 grams per day), it was found that both the jejunal and ileal levels of maltase were significantly higher in the protein depleted groups than in the controls, and there was a significantly higher level of maltase activity in the jejunum in the 0.5% lactalbumin high carbohydrate group than in the 0.5% lactalbumin low carbohydrate group. There were no statistically significant differences between the last two groups in the ileal maltase activities. There were also no significant differences in the levels of disaccharidases between the groups on the 0.5% lactalbumin low carbohydrate and the 1% lactalbumin diets in either the jejunum or the ileum (Table VIII).

The jejunal and ileal levels of maltase activity in the repleted group had fallen to levels significantly lower than those of the control group.

As found with the lactase and sucrase activities, the maltase activity was also markedly higher in the jejunal than in the ileal segment in all groups of rats.
The mucosal protein concentrations in both the jejunum and ileum of the control, protein depleted, and protein repleted rats are shown in Table IX. The mucosal protein concentration in the jejunum and ileum of the two 0.5% lactalbumin groups and the 1% lactalbumin group were all significantly lower than the mucosal protein concentration in the control group. There was no statistically significant difference between the mucosal protein concentrations of the control and the protein repleted groups.

C. Intestinal Absorption of Disaccharides in Vivo

The intestinal absorption of disaccharides by rats fed the control and the protein deficient diets is shown in Table X. Lactose, sucrose, and maltose absorption by rats fed the 0.5% lactalbumin low carbohydrate diet was significantly higher than in the control group. This greater absorption by the animals on the 0.5% lactalbumin low carbohydrate diet is consistent with the higher levels of disaccharidases found in this group when compared to the controls. The comparison of intestinal lactose, sucrose, and maltose absorption in rats fed the control and the 0.5% lactalbumin high carbohydrate diet demonstrated significantly higher absorption only for sucrose and maltose in the 0.5% lactalbumin group. Lactose absorption was not significantly higher in the 0.5% lactalbumin high carbohydrate group even though the mean value for lactose absorption was 8 times higher than in the control group. This is due to the great variability in the obtained results of the 0.5% lactalbumin high carbo-
### TABLE IX

**MUCOSAL PROTEIN CONCENTRATION IN JEJUNUM AND ILEUM OF CONTROL, PROTEIN DEPLETED, AND PROTEIN REPLETED RATS**

<table>
<thead>
<tr>
<th>Dietary Group</th>
<th>Number of Rats</th>
<th>mg Protein per g wet* weight mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Jejunum</td>
</tr>
<tr>
<td>Control, 18% lactalbumin</td>
<td>15</td>
<td>134.0 ± 8.0</td>
</tr>
<tr>
<td>0.5% lactalbumin low carbohydrate</td>
<td>12</td>
<td>102.7 ± 6.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5% lactalbumin high carbohydrate</td>
<td>18</td>
<td>93.9 ± 10.3&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1% lactalbumin (restricted to 4 grams of diet per day)</td>
<td>18</td>
<td>99.1 ± 5.9&lt;sup&gt;b,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Repleted&lt;sup&gt;&amp;&lt;/sup&gt;</td>
<td>12</td>
<td>133.0 ± 12.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Values are Means ± S.D. for the number of rats shown in the second column.

<sup>&</sup> Refed the 18% lactalbumin diet for 8 weeks after 8.5 weeks on the 0.5% lactalbumin low carbohydrate diet.

<sup>a</sup> Does not differ from the control group (P > 0.05).

<sup>b</sup> Differs from the control group (P < 0.001).

<sup>c</sup> Differs from the 0.5% lactalbumin low carbohydrate group (P < 0.01).

<sup>d</sup> Does not differ from the 0.5% lactalbumin low carbohydrate group (P > 0.05).
TABLE X

LACTOSE, SUCROSE, AND MALTOSE ABSORPTION IN CONTROL, PROTEIN DEPLETED, AND PROTEIN REPLETED RATS

<table>
<thead>
<tr>
<th>Dietary Group</th>
<th>Number of Rats</th>
<th>Lactose* Absorption</th>
<th>Sucrose* Absorption</th>
<th>Maltose* Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(Percentage absorption per 500 mg wet weight intestine)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, 18% lactalbumin</td>
<td>5</td>
<td>7.7 ± 6.4</td>
<td>21.9 ± 4.4</td>
<td>10.4 ± 5.9</td>
</tr>
<tr>
<td>0.5% lactalbumin low carbohydrate</td>
<td>5</td>
<td>46.3 ± 18.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.5 ± 35.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.1 ± 26.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5% lactalbumin high carbohydrate</td>
<td>6</td>
<td>55.5 ± 51.2&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>78.8 ± 25.8&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>75.8 ± 48.6&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1% lactalbumin (restricted to 4 grams diet per day)</td>
<td>6</td>
<td>72.1 ± 38.4&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>91.6 ± 20.9&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>75.2 ± 20.7&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Repleted*</td>
<td>4</td>
<td>13.7 ± 6.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.5 ± 4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.0 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Values are Means ± S.D. for the number of rats shown in the second column.

<sup>a</sup>Refed the 18% lactalbumin diet for 8 weeks after 8.5 weeks on the 0.5% lactalbumin low carbohydrate diet.

<sup>b</sup>Does not differ from the control group (P > 0.05).

<sup>c</sup>Differs from the control group (P < 0.05).

<sup>d</sup>Does not differ from the 0.5% lactalbumin low carbohydrate group (P > 0.05).
hydrate group as demonstrated by the large standard deviation of the mean.

When the absorption of lactose, sucrose, and maltose in the rats fed the control and the 1% lactalbumin diets were compared, the absorption of all three disaccharides was significantly higher in the 1% lactalbumin group than in the controls. The absorption values in the 1% lactalbumin group were 4 to 10 times higher than those in the control group.

There were no statistically significant differences in the absorption of lactose, sucrose and maltose between the 0.5% lactalbumin low carbohydrate group and the 0.5% lactalbumin high carbohydrate group. Similarly, there were no statistically significant differences between the absorption of the three disaccharides between the 0.5% lactalbumin low carbohydrate and the 1% lactalbumin group. However, the values for lactose, sucrose, and maltose absorption were consistently higher in the 1% lactalbumin group than in the 0.5% lactalbumin low carbohydrate group. The mean value for lactose absorption for the 1% lactalbumin group was almost twice that of the 0.5% lactalbumin low carbohydrate group. The standard deviations of the means in the 1% lactalbumin group were large and a statistically significant difference between these two groups was not demonstrated.

The comparison of the absorption of lactose, sucrose, and maltose in the group of animals fed the control diet (18% lactalbumin) and the repleted group (fed the 18% lactalbumin diet for 8 weeks after 8.5 weeks of protein depletion) showed
that there were no statistically significant differences in the absorption of the three disaccharides.
CHAPTER IV

DISCUSSION

In studying the effect of protein-calorie malnutrition on intestinal disaccharidases in rats it is extremely important to keep the animals on the protein deficient diets for an extended period of time until they develop lesions associated with protein-calorie malnutrition. According to Edozien (65), the changes associated with protein-calorie malnutrition in rats include a marked loss of body weight, severe loss of hair, a marked decrease in plasma albumin concentration, decrease of protein concentration in the intestinal mucosa, fluid accumulation in the peritoneal cavity, and edema.

In the present study the rats on the 0.5% lactalbumin diets developed all of the above symptoms which indicates that the rats had been on the protein deficient diet long enough to be considered protein-calorie deficient. Also the differences in gross appearance, plasma albumin concentration and behaviour between the rats on the 0.5% lactalbumin diets and the 1% lactalbumin diet (restricted to 4 g of diet per day) were similar to those reported by Edozien (65). Therefore it was assumed that the rats on the 0.5% lactalbumin diets were protein deficient and that the rats on the 1% lactalbumin diet (restricted) were protein-calorie deficient.

Solimano et al. (4) reported that protein deprivation in rats produced an increase in the specific activities of intestinal disaccharidases and particularly of sucrase and maltase.
They suggested that this increase in disaccharidase activities was an adaptation to the high proportion of carbohydrates in the protein deficient diets as the protein of the control diet was replaced with carbohydrate in order to produce the low protein diets.

The obtained results in the present study partially support the hypothesis of Solimano et al. (4) that a high proportion of carbohydrate in the protein deficient diet can induce intestinal disaccharidase activities since the disaccharidase levels in the rats fed the 0.5% lactalbumin high carbohydrate diet were statistically significantly increased when compared to the controls. However, the increase in the disaccharidase activities in the 0.5% lactalbumin high carbohydrate group was not entirely due to the high proportion of carbohydrates in the diet because the rats on the 0.5% lactalbumin low carbohydrate diet also showed statistically significant increases in disaccharidase levels when compared to the controls although not to the same extent as the 0.5% lactalbumin high carbohydrate group.

It is not clear why there is an increase in the intestinal disaccharidase activities in the 0.5% lactalbumin low carbohydrate group when compared to the controls. One possible explanation for this increase in disaccharidase levels could be a loss of structural proteins in the intestinal mucosa. If there had been a larger decrease of structural protein in the intestinal mucosa as compared to the decrease of enzymatic proteins, the expression of enzymatic activity per gram of protein or per gram of wet weight mucosa would lead to high results.
McNeill and Hamilton (69) recently studied the effect of fasting on intestinal disaccharidase activities in the rat small intestine and observed a statistically significant increase in lactase activity. They have postulated that fasting might have stimulated lysosomal beta-galactosidase. If a protein deficient diet has a similar effect to fasting, the increase in lactase activity in the protein deficient rats could possibly be a result of the stimulation of the lysosomal beta-galactosidase.

The results of the present study together with those of Solimano et al. (4) differ from the findings by Prosper et al. (5) and Lifshitz et al. (6) in that they find no marked alterations in intestinal disaccharidase activities in protein deficient rats. It should be stressed, however, that their experimental conditions were very different from the present investigation.

In the study by Prosper et al. (5) the test animals were fed a protein-free diet for 45 days and the controls were given a 27% protein diet. In addition their findings should be considered as inconclusive on the basis of the fact that the disaccharidase activities were performed on only 3 protein deficient rats and that there was a large variation in the disaccharidase activities within both the controls and the test animals.

Lifshitz et al. (6) also did not observe a statistically significant increase in disaccharidase activity in protein deficient rats. They fed the protein deficient diet (4% casein) for 1 to 4 weeks, and at the end of 4 weeks the
mucosal protein concentration was not statistically different from the controls. This would imply that the intestinal mucosa had not yet undergone changes associated with protein deficiency because the rats were not kept long enough on the protein deficient diet. It should be pointed out, however, that although there was no statistically significant difference in disaccharidase levels between the protein deficient rats and the controls in the study by Lifshitz et al. (6), the disaccharidase activities were appreciably higher in the protein deficient rats.

There were no significant differences between the intestinal disaccharidase activities in the 1% lactalbumin group and the 0.5% lactalbumin low carbohydrate group. Therefore the restriction of daily intake did not have an effect on the activities of lactase, sucrase, and maltase in addition to the effect caused by protein depletion. This may be due to the fact that towards the end of the 9 week experimental period about one-third of the animals on the 1% lactalbumin diet were not eating the entire 4 grams of diet which they were given each day. Therefore, near the end of the experimental period, these animals were no longer restricted in their caloric intake. The amount consumed by the 0.5% lactalbumin low carbohydrate group or the 1% lactalbumin group was not monitored. However, it would appear that the failure of some of the animals on the 1% lactalbumin diet (restricted to 4 grams per day) to consume the entire portion of food did not affect the development of the caloric deficiency syndrome as these animals had significantly different plasma albumin
concentrations, gross appearance and behavior characteristics from the animals on the 0.5% lactalbumin low carbohydrate diet.

The studies found in the literature dealing with the effect of caloric restriction on the intestinal disaccharidases did not restrict the protein content of the diet to as low a level as in the present experiment (1% lactalbumin). Kumar and Chase (59) found that the restriction of food at birth by increasing the litter size (16 animals per mother) followed by restriction of an 8% casein diet to 8 grams per day until 115 days of age resulted in significantly higher lactase activity in the restricted intake group when compared to the controls who receive a 27% casein diet ad libitum. There were no differences in sucrase activity between the control and the restricted intake group. Maltase activity was not determined.

Troglia et al. (56) used rats weighing between 90 and 110 grams who were then fed a restricted amount of rat chow (an average of 10.5 grams of rat chow per day) for 5½ to 6½ months. At the time of sacrifice, the activity levels of jejunal lactase, sucrase, and maltase were all significantly higher in the restricted group than in the control group.

The results of the present study cannot be compared directly with the studies of Kumar and Chase (59) or Troglia et al. (56) as the protein restriction in the present study (1% lactalbumin) was much more severe. The similarity of the results, however, indicate that the effect on the intestinal disaccharidase activities of restricting the caloric intake of a 1% protein diet does not differ from the effect of
restricting the caloric intake of a diet containing higher protein levels.

In the present study, the protein depleted animals gained weight very abruptly when placed on the 18% lactalbumin diet, and at the end of the 8 week feeding period, they were not grossly distinguishable from the control animals. The plasma albumin concentration was almost identical in the repleted and the control groups. The lactase and jejunal sucrase activities in the repleted group fell to the same levels of activity found in the control group, but the jejunal maltase and ileal sucrase and maltase activities fell to levels which were significantly lower than those found in the control group. The fact that not all the intestinal disaccharidase activities returned to the same level as found in the control group suggests that severe protein depletion may cause prolonged or irreversible changes in intestinal maltase and sucrase activities.

The absorption of lactose, sucrose, and maltose was significantly enhanced in the protein deficient rats when compared to the controls (Table X). This enhancement in the absorption of the disaccharides is consistent with the higher levels of jejunal disaccharidases in the protein deficient rats.

When the 0.5% lactalbumin low carbohydrate and the 0.5% lactalbumin high carbohydrate groups were compared, significant increases in sucrase and maltase activities in the high carbohydrate group were found. However, there were no differences in the absorption of lactose, sucrose, or maltose
between the 0.5% lactalbumin low carbohydrate and the 0.5% lactalbumin high carbohydrate groups. Gray and Ingelfinger (10,11) had demonstrated that in human subjects the rate-limiting step in sucrose and maltose absorption is the transport of the constituent monosaccharides across the mucosal membrane, and not the level of intestinal sucrase and maltase activities. But in the case of lactose absorption, intestinal lactase activity is the rate-limiting step and not the absorption of the constituent monosaccharides (9). It has been shown that either the restriction of dietary protein or semistarvation may initially result in an increased transport of glucose across the mucosal membrane (6,70,71). Therefore, it is possible that the protein deprivation increased the absorption of sucrose and maltose by affecting the rate of glucose transport. Lactose absorption would be increased in response to the increase in lactase activity as that is the rate-limiting step in lactose absorption. The increase in the carbohydrate content of the 0.5% lactalbumin diet from 66% to 83.5% resulted in increases in sucrase and maltase activities but not in sucrase and maltose absorption. This would be expected if the rate-limiting step of sucrose and maltose is the transport of the constituent monosaccharides.

Most of the studies done with laboratory animals on the effect of protein-calorie malnutrition on disaccharidase activities which have been reported in the literature have only measured the specific activities of the intestinal disaccharidases without measuring the absorption of the disaccharides. Only Lifshitz et al. (6) investigated both
disaccharidase activities and absorption of disaccharides and monosaccharides in rats suffering from protein-calorie malnutrition. They found no statistically significant differences in the absorption of glucose, fructose, 3-0-methyl glucose, sucrose, or maltose between the controls and the rats on the low protein diets after 28 days of dietary treatment. There were also no significant changes in disaccharidase activities in the protein depleted animals after 28 days. There was a transitory increase in the rate of glucose, sodium, and water transport in the animals on the low protein diet after 14 days of dietary treatment, but there was no change in the transport of fructose or 3-0-methyl glucose during that period indicating that the change was probably due to increased glucose utilization in the rats on the low protein diets. Similar findings of an initial compensatory response to semi-starvation in the rat which resulted in an increase in glucose transport had been reported by Kershaw et al. (70) and Hindmarsh et al. (71).

It is difficult to compare the results obtained in the present study with the results reported by Lifshitz et al. (6) because of the differences in the length of time that the animals were subjected to the low protein diets (28 days in the study of Lifshitz et al. (6), and 60 days in the present study). However, the results of both studies indicate that disaccharide absorption is not impaired in experimental protein-calorie malnutrition in the rat.

The results obtained in the present investigation together with those reported by other authors (4,5,6,56,59) do not
support the view that disaccharide intolerance in protein deprived children is the result of decreased intestinal disaccharidases (2,30,32,37) if we may apply the results for rats directly to children. There are several possible explanations for the lack of agreement between the human and animal response to protein-calorie malnutrition.

When the animal models are being used to study the effect of protein deprivation on intestinal disaccharidase activity, the experimental conditions can be closely controlled. Therefore, the diets can be made adequate in all other nutrients besides protein, whereas the diets of the children in underdeveloped countries are frequently deficient in protein as well as other essential nutrients. In addition, the malnourished children suffer from gastrointestinal infections. The presence or absence of infections can, however, be controlled in the animal experiment.

Sriratanaban and Thayer (57) have studied the effect of iron and combined iron-protein deficiency on the intestinal disaccharidases in the rat to help clarify this point. They used four groups of animals in their study: a control (27% casein), low protein (5% casein), low iron (27% casein), and low iron-low protein (5% casein). The low iron group had significantly lower levels of lactase, sucrase, and maltase activity when compared to the controls. The protein deficient group had significantly higher disaccharidase activities when compared to the controls. The low iron-low protein group did not demonstrate statistically significant differences when compared to the controls, however, the disaccharidase
activities were somewhat higher than in the controls in both the jejunum and ileum and lower than in the controls in the duodenum. These results indicate that other nutritional deficiencies may affect intestinal disaccharidase activities, and therefore, may be closely involved with the observed decreases in intestinal disaccharidases in children. This area needs further work.

Bolin et al. (58) has also studied the effect of other factors besides protein deficiency on the intestinal disaccharidases and found that an infestation of a group of adequately nourished rats with *Nippostrongylus brasiliensis* resulted in a decrease in the activities of lactase and maltase showing that infestation may be an important factor in the etiology of disaccharidase depression and disaccharide intolerance in protein-calorie malnutrition.

The study of the interrelationship between infection and malnutrition by Scrimshaw et al. (72) has shown that malnutrition generally alters the resistance of the host to infection and that infectious disease exaggerated existing malnutrition. The mechanisms involved in the decreased resistance to infection caused by malnutrition include a reduced capacity of the host to form specific antibodies, a decrease in phagocytic activity of microphages and macrophages, alterations in wound healing and collagen formation, alterations in tissue integrity, diminished inflammatory response, and effects originating in alterations of intestinal flora (72). Since infection and malnutrition are so closely linked it will be necessary to fully explore the role of infection in the development of
disaccharide intolerance in protein-calorie malnutrition before the difference between the results of the present study and the symptoms of children suffering from protein-calorie malnutrition are understood.

Another possible explanation for the difference between the results obtained with rats and with children was suggested by Kumar and Chase (53). They felt that the failure of experiments on protein-calorie malnutrition using rats to show a decrease in disaccharidase activities may mean that the rat is not an adequate model for studying human protein-calorie malnutrition. When these authors studied protein-calorie malnutrition in monkeys, many of the same symptoms found in children suffering from protein-calorie malnutrition were present in the monkeys including marked villous atrophy and decreased intestinal disaccharidase activities after 18 weeks of protein deprivation (<1% protein). The undernourished monkeys also suffered from intermittent diarrhea of an unknown cause. From these results, Kumar and Chase (53) concluded that the monkey was a better model for studying protein-calorie malnutrition than the rat.

The rat cannot be excluded as a suitable model for protein-calorie malnutrition until further work has been done on the effect of the non-protein factors including infection and nutritional deficiencies on the intestinal disaccharidase activities.
CHAPTER V

SUMMARY AND RECOMMENDATIONS

A. Summary

The purpose of the present investigation was to study the effect of prolonged experimental protein-calorie malnutrition on intestinal disaccharidase activities and on disaccharide absorption, as carbohydrate intolerance is a major problem in children suffering from protein-calorie malnutrition.

Four groups of rats (90 to 120 grams) were fed the following diets for 8 to 9 weeks: control (18% lactalbumin, 66% carbohydrate); low protein low carbohydrate (0.5% lactalbumin, 66% carbohydrate); low protein high carbohydrate (0.5% lactalbumin, 83.5% carbohydrate); and low protein restricted (1% lactalbumin, restricted to 4 grams per day). After 8.5 weeks, part of the group on the 0.5% lactalbumin low carbohydrate diet was fed the control diet (18% lactalbumin, 66% carbohydrate) for 8 weeks.

At the end of the feeding period, the following assays were performed: 1) in vivo absorption of radioactive (¹⁴C) lactose, sucrose, and maltose; 2) activities of intestinal lactase, sucrase, and maltase; 3) plasma albumin concentrations; and 4) mucosal protein concentrations. The disaccharide absorptions were expressed as the percentage of the administered radioactivity absorbed per unit weight of intestine. The disaccharidase activities were expressed as the units of enzyme activity per gram of wet weight mucosa or per gram of mucosal
protein; the latter was considered to be the more satisfactory.

In the three protein deficient groups (0.5% lactalbumin low carbohydrate, 0.5% lactalbumin high carbohydrate, and 1% lactalbumin), the activity of both the jejunal and ileal disaccharidases and the absorption of lactose, sucrose, and maltose were significantly higher when compared with the controls. The jejunal sucrase and maltase activities were significantly higher in the 0.5% lactalbumin high carbohydrate group than in the 0.5% lactalbumin low carbohydrate group, but the absorption of lactose, sucrose, and maltose were alike. When the 1% lactalbumin (restricted to 4 grams per day) and the 0.5% lactalbumin low carbohydrate groups were compared, there were no statistically significant differences in the specific activities of the intestinal disaccharidases or the absorption of the disaccharides.

The absorption of lactose, sucrose, and maltose were similar in the controls and the protein repleted group. The disaccharidase activities were also similar in these two groups except for a significant depression of jejunal maltase and ileal sucrase and maltase activities in the protein repleted group.

Therefore, these results indicate that protein deprivation in rats for 8.5 weeks causes an increase in specific activities of the intestinal disaccharidases in both the jejunum and ileum, and that an increase in the carbohydrate content of the protein deficient diet results in an induction of jejunal sucrase and maltase activities. The high specific activity of the intestinal disaccharidases following protein-
calorie malnutrition may be in part due to a preferential loss of structural protein rather than to an increase in enzymatic protein in the intestinal mucosa.

The results obtained on the protein repleted rats have shown that the changes caused by protein deficiency are of a reversible nature.

The increase in the disaccharidase activities in the protein deficient rats is accompanied by an increase in disaccharide absorption. The increases in disaccharide absorption could be due to the higher levels of disaccharidases or to an increase in the transport of the constituent monosaccharides in the protein deficient rats. The demonstration of statistically significant differences in sucrase and maltase activities between the 0.5% lactalbumin high carbohydrate and 0.5% lactalbumin low carbohydrate groups, without a concomitant increase in sucrose or maltose absorption, supports the view that the higher absorption of maltose and sucrose in the protein deficient rats is a result of increased monosaccharide transport.

The results obtained in this study are not consistent with the suggestion that protein-calorie malnutrition is responsible for disaccharide intolerance in children. Since children suffering from protein-calorie deficiency are usually also deficient in other nutrients and suffering from infections, it is not easy to relate disaccharide intolerance and depressed levels of disaccharidases to protein-calorie deficiency only. It is possible, however, that there may be a species difference in the response to protein-calorie deficiency.
B. Recommendations

The following recommendations should be considered if further work is to be done on the effect of protein-calorie malnutrition on the intestinal disaccharidases and the \textit{in vivo} absorption of disaccharides.

Absorption studies using glucose should be carried out together with the studies of lactose, sucrose, and maltose absorption, in order to confirm whether the increases in absorption of the three disaccharides is due to increased disaccharidase activities or due to increased glucose transport.

The rate of absorption over a 30 minute period should be determined rather than the total absorption after 15 minutes, as the rate of absorption is a more satisfactory measure for studying the absorption capacity of the small intestine.

In the present study, the disaccharide absorption was only expressed per unit weight of intestine. Since there is a difference in the diameter of the intestine and the thickness of the intestinal mucosa between the larger control animals and the smaller protein depleted animals, it would be more satisfactory to express the absorption results per unit of surface area of the intestine.

The expression of disaccharidase activity per unit of DNA would clarify whether there was a change in the ratio of the structural to enzymatic proteins in the intestinal mucosa of the protein deficient rats.

It would be informative to study the effect of protein-calorie malnutrition on the intestinal disaccharidases and
disaccharide absorption as a function of time. This would allow one to determine the earliest time at which the intestinal disaccharidase activities and disaccharide absorption increase, and the temporal relationships between the increases. It would also demonstrate whether there were further changes in the intestinal disaccharidase activities or disaccharide absorption as the animals become moribund.
CHAPTER VI

BIBLIOGRAPHY


