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EFFECT OF CHANGES IN FEEDING SCHEDULE ON THE DIURNAL RHYTHMS AND DAILY ACTIVITY LEVELS OF INTESTINAL BRUSH BORDER ENZYMES AND TRANSPORT SYSTEMS

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SUMMARY

The activities of rat intestinal enzymes, sucrase, lactase, maltase, trehalase, γ-glutamyltransferase, leucylnaphthylamide-hydrolyzing activity, and the transport system for glucose follow diurnal rhythms on ad libitum and restricted feeding regimes. In response to 6 days of restricted feeding, food available between 1400 and 1800 Eastern Standard Time, all rhythms shifted in time and the daily levels of activities were changed. Alkaline phosphatase activity followed a diurnal rhythm only in restricted fed animals.

In restricted fed rats several activity patterns were observed, some with short periods of maximum activity, 3 h or less, and some with plateaus of maximum activity, 5–9 h long. In respect to the time of day of the synchronizer, sucrase peaked before feeding, glucose transport peaked during feeding, alkaline phosphatase peaked after feeding, and the other enzymes had higher levels of activity before, during and after feeding. The effect of restricted feeding on the daily activity levels were: a decrease in leucylnaphthylamide-hydrolyzing activity, no change in alkaline phosphatase, and increases in the others.

These enzyme and transport systems exhibit a large amount of individual regulation or control as reflected by the lack of a uniform activity pattern and response to the synchronizer, and the variation in direction and magnitude of the adaptations to restricted feeding.

INTRODUCTION

Diurnal rhythms of various intestinal brush border enzymes and transport systems of the rat have been described [1–4] The synchronizer for these functions was shown to be the time of day of feeding [1–4]. The activities of these systems were highest around the time of feeding, but comparing the rhythms of these systems to one another and to the time of day of the synchronizer it seems that the response was not

uniform. This was most apparent when feeding time was restricted. In one study [1] the maximum activity of sucrase was observed before that of glucose-galactose transport. In another study [2] the maximum activities of maltase and leucylnaphthylamide -hydrolyzing activity occurred together. In comparing these periods of higher activity to the time of day of the synchronizer, which was different in the various studies, sucrase [1] and L-histidine transport [3] activities peaked before the onset of feeding, glucose-galactose transport [1] peaked during feeding, and maltase and leucylnapthylamide-hydrolyzing activities [2] peaked after feeding.

Restricting and changing the time of day of feeding resulted in an increase in the average daily level of activity of sucrase and glucose-galactose transport [1] as well.

In order to further evaluate this difference in the activity patterns and the general change in activity of intestinal brush border enzymes and transport systems we have studied the rhythms and daily activity levels of a number of systems simultaneously under ad libitum and restricted feeding regimes, with emphasis on the time periods around the artificial feeding period. The transport and enzyme systems studied were glucose-galactose transport, lactase (β-D-galactoside galactohydrolase, EC 3.2.1.23), maltase (α-D-glucoside glucohydrolase, EC 3.2.1.20), sucrase (sucrose α-glucohydrolase, EC 3.2.1.48), trehalase (α, α-trehalose glucohydrolase), γ-glutamyltransferase (EC 2.3.2.2), alkaline phosphatase (orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1), and leucylnaphthylamide-hydrolyzing activity.

These systems were chosen because they represent various aspects of the brush border digestion and absorption of dietary carbohydrates and proteins. The disaccharidases, sucrase, maltase, lactase and trehalase, represent the last digestive reaction for carbohydrates, i.e. the hydrolysis of disaccharides to monosaccharides [5]. In the adult laboratory rat, sucrase and maltase are of major importance for this function while lactase and trehalase are of minor importance since the occurrence of substrate in the adult diet (Purina Lab Chow) and the level of enzyme activity in the small intestine is relatively low [6].

The absorption of free glucose and galactose occurs via the glucose-galactose transport systems. The disaccharidases also have the potential to transport their respective monosaccharide end products [7–9]. Orlowski and Meister [10] have suggested that γ-glutamyltransferase serves a transport function for amino acids.

Alkaline phosphatase, found in high concentrations in the brush border membrane, may have a digestive function for the various phosphorylated compounds found in dietary material, but does not appear to serve any transport function for sugars [8, 11].

Leucylnaphthylamide-hydrolyzing activity, that is the peptidase activity observed with an artificial substrate, may reflect peptidase activity for protein digestion, although this activity can be separated from that of the intestinal mucosal enzymes which act on some of the leucine-containing dipeptides [11, 12].

An abstract of this work has been published [13].

METHODS

In this study we were interested in observing two aspects of these intestinal functions, the existence of diurnal rhythms and the effect of feeding schedule on the

activities of these functions. We, therefore, attempted to provide an environment and daily routine that was as constant and uniform as possible for each of the four groups of animals which comprised the two replicate experiments. The four groups were run consecutively during June to August. Constant lighting was employed to remove a conflicting environmental cue, that is the light-dark cycle.

Experimental conditions

Male Sprague-Dawley rats, 100–125 g, were housed individually in a locked, constant environment suite (animal room with attached preparation room) with free access to water. They were fed commercial rat chow (Purina Lab Chow No. 5001). All handling and feeding were done during the half-hour period from 1330 to 1400 h, except when access to food was restricted, in which case, food was removed from the cages during the 5-min period following the end of feeding at 1800 h. The rats were otherwise left undisturbed.

An extra 15–20 rats were maintained with the experimental animals. This was done to reduce the effect of animal removal on the remaining experimental animals during the 24-h period in which rats were being sacrificed. These animals were fed on the appropriate feeding regime.

Upon arrival the rats were allowed a 3-day acclimatization period during which the light cycle used by the breeder was continued; i.e. light from 0600 to 2000, and feeding was ad libitum. This was followed by a 6-day experimental period during which the rats were either continued on the ad libitum feeding regime (the ad lib group) or restricted in food access to a single 4-h period; namely, 1400–1800 h (the restricted group). Constant lighting was used during the experimental period. Beginning on the morning of the seventh day, groups of three or four rats were sacrificed by cervical dislocation at 13 specific times of day during the ensuing 24 h; namely, 0500, 0700, 0900, 1100, 1200, 1300, 1400, 1500, 1700, 1800, 2100, 0300 and 0500 Eastern Standard Time (EST).

Procedures

In order to observe general activity levels the entire middle three-quarters of the small intestine was removed and used for this work, thus discarding only the most proximal one-eighth and most distal one-eighth of the small intestine. The removed segment was rinsed with buffer and everted. 6-cm portions from the proximal, mid and distal areas were taken for transport studies. The mucosa was scraped from the remainder of the intestine for the enzyme and protein assays.

3-*O*-Methylglucose transport was assessed by a modification of the tissue uptake procedure of Crane and Mandelstam [14]. The uptake of sugar was determined in triplicate in each animal. Two tissue rings, approx. 3–5 mm in width, cut from each of the three areas were distributed to each of three flasks, contained 5 ml of medium. The rings were incubated at 37 °C, for 30 min and shaken at 180 oscillations per min. Krebs-Henseleit bicarbonate buffer, pH 7, equilibrated with O_2/CO_2 (95 : 5, v/v) was used for tissue preparation and as the incubation medium with the additions of: 3-*O*-methylglucose, final concentration, 5 mM; and tracer amounts of 3-*O*-methyl-D-[^{14}C] glucose and D-[1-^{3}H(N)]mannitol. The radioactivity of the tissue supernatant and medium was measured with a liquid scintillation system. Preliminary data on α-aminoisobutyric acid transport was obtained in a similar manner using α-aminoiso-[^{14}C]butyric acid.

The mucosal scrapings were homogenized in 0.9 % NaCl for 3 min in a Waring blender. Aliquots of the homogenate were either used immediately for the assay of sucrase and protein or frozen for the later assay of the other enzymes and the reassay of sucrase. The enzymes were assayed by the following methods: the disaccharidases according to the Lloyd and Whelan [15] modification of the Dahlqvist [16] method; γ-glutamyltransferase according to Naftalin et al. [17]; leucylnaphthylamide-hydrolyzing activity according to Goldbarg and Rutenburg [18]; alkaline phosphatase according to Eichholz [19]; and protein according to Lowry et al. [20] using crystalline bovine serum albumin as standard.

Data calculations and evaluations

The tissue and homogenate of each rat was assayed separately. Transport incubations were done in triplicate and enzyme and protein assays were done in quadruplicate. Mean individual animal values were used in the calculations.

The results of the transport studies are reported as the tissue/medium ratio; i.e. mM 3-*O*-methylglucose$_{tissue}$/mM 3-*O*-methylglucose$_{medium}$ corrected for the mannitol space. This correction factor was calculated for each sample and averaged 36 % of the tissue volume in both groups.

The specific activity of the enzymes was calculated as the μmol of substrate hydrolyzed/min per mg protein.

The results of the replicates were pooled.The rhythmicity of the enzyme activities was similar in each set, but the overall magnitudes differed by about 30 %, necessitating normalization of the data. This was done by setting the daily mean for each replicate equal to 1.00. The variability of the transport activity between the two replicates was not sufficiently great to necessitate normalization.

Condition of animals

Prior to the experimental period the body weight, weight gain and food consumption were similar in both groups. During the experimental period the ad lib group continued to gain weight and increase food consumption at the previous rate; i.e. weight gain of 7 g/day and increase in food consumption of 1 g/day. This pattern was maintained at least through the eighth day in the remaining animals as well.

The restricted group lost weight for the first 2 days of the experimental period. This loss averaged 9 g, about 10 % of the original body weight. From the third day to the end of the experimental period or through the eighth day in the remaining animals body weight increased at the rate of 2 g/day and food consumption increased 1 g/day. One group of rats was maintained on this restricted feeding regime for 23 days. They continued to gain weight and appeared in healthy condition.

By the fourth day, the animals in the restricted group had established a new equilibrium between food intake and weight gain. Once this equilibrium was established, these rats ate similar amounts of food/body weight as compared to the ad libitum animals.

The majority of the eating in the ad libitum group appeared to occur in the early morning hours, although some eating occurred throughout the 24-h period. This eating period is similar to the time of higher enzyme and transport activity presented later.

Upon sacrifice, smaller fat deposits were seen in the subcutaneous area,

periotoneal cavity and thymic mediasternal region, of the animals in the restricted group. Carcasses of several of these animals were further examined and all organs were within normal size limits. Gross macroscopic examination of the intestinal tracts revealed no difference in the general appearance or length of the small intestine between the groups. However, the size of the stomachs (food holding capacity) was increased in the restricted group. There was no difference in the weight of the individual intestinal mucosal scrapings between groups.

RESULTS

The criterion used to establish the presence or absence of a rhythm in the protein content of the mucosal homogenates and in the activities of the enzymes and transport systems was the reliably periodic aspect of data displayed as a function of time [21]. Activities were determined at two sequential 0500 h times of day to ascertain that the rhythms repeat themselves, additional times were repeated in the two restricted replicates; namely, 1700 h on the sixth and eighth day, and 0500, 1300 and 1800 h on the twenty-third day. There was no significant difference between the values determined at these additional times of day and those obtained on the seventh day of the experimental period. The rhythms appeared to be established prior to the sixth day of restricted feeding and were stable for at least 23 days. We have no explanation for the non-repeating rhythm of alkaline phosphatase (Fig. 8) in the ad lib group. The difference between the 0500-h values were observed on both replicates.

The protein content of the mucosal homogenate was uniform. No diurnal rhythmicity of protein content could be detected and there was no significant difference between the two feeding regimes.

The rhythms of the various enzymes and transport activities in animals from both feeding regimes are combined and displayed in Figs 1–8. In general, in the ad libitum group higher activities occurred at various times between 2400 and 1200 h. Lower activity occurred at various times between 1100 and 2100 h, except for leucylnaphthylamide-hydrolyzing activity (Fig. 7), where lower activity extended to 2400 h, and lactase (Fig. 4) which extended to 0300 h.

Within these high and low periods differences were observed between the various systems. Maximum activity occurred at 2400 h for sucrase (Fig. 2), trehalase (Fig. 3), and 3-*O*-methylglucose transport (Fig. 1); and again at 0500–0700 h, 0700–0900 h, and 0900 h, respectively. For the other enzymes peak activity occurred from 0300–0900 h for γ-glutamyltransferase (Fig. 6), from 0500–0700 h for maltase (Fig. 5), from 0500–0900 h for lactase (Fig. 4), and at 0900 h for leucylnaphthylamide-hydrolyzing activity (Fig. 7).

Minimum activity occurred at 1400 and 1800 h for sucrase (Fig. 2), lactase (Fig. 4), and maltase (Fig. 5) but significantly higher values were observed at 1500 and 1700 h. It is possible that the 1400-h minima were caused by the disturbance of the animals between 1330 and 1400 h for handling, etc. Minimum activity for 3-*O*-methylglucose (Fig. 1) was observed from 1400–1500 h, from 1500–1800 h for trehalase (Fig. 3) and γ-glutamyltransferase (Fig. 6), and from 1800–2100 h for leucylnaphthylamide-hydrolyzing activity (Fig. 7).

In general, there was a 12-h shift in the activity patterns between the ad libitum group and the restricted group. In the restricted group higher activity was observed

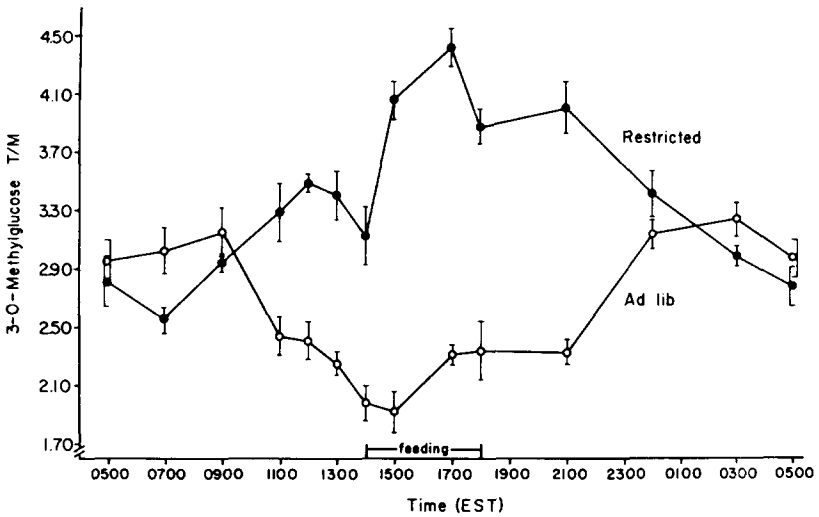

Fig. 1. Diurnal rhythm of 3-*O*-methylglucose transport activity in rats fed ad libitum (○) or restricted to 4 h/day (●). Tissue rings were incubated for 30 min in media containing 5 mM 3-*O*-methylglucose. Mean ±S.E. of tissue/medium ratio values corrected for mannitol space (n = 6–8).

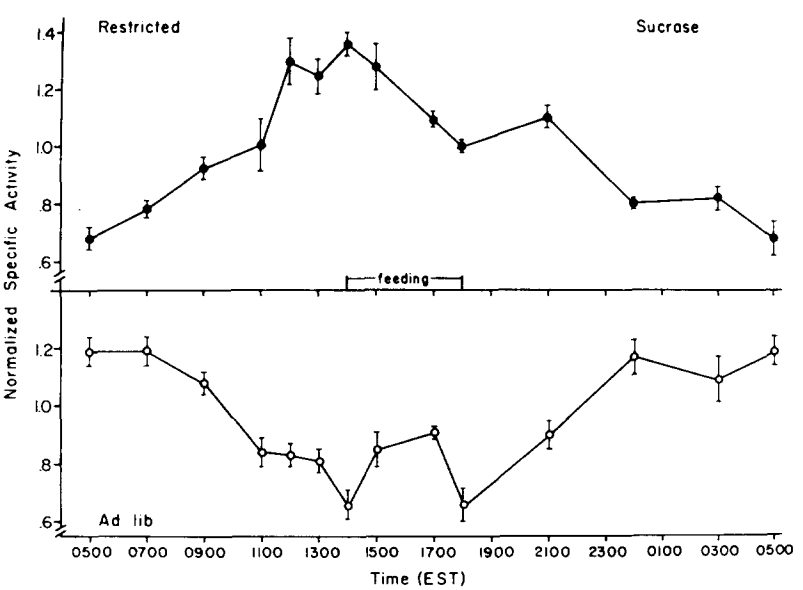

Fig. 2. Diurnal rhythm of normalized sucrase specific activity in rats fed ad libitum (○) or restricted to 4 h/day (●). Mean ±S.E. (n = 6–8).

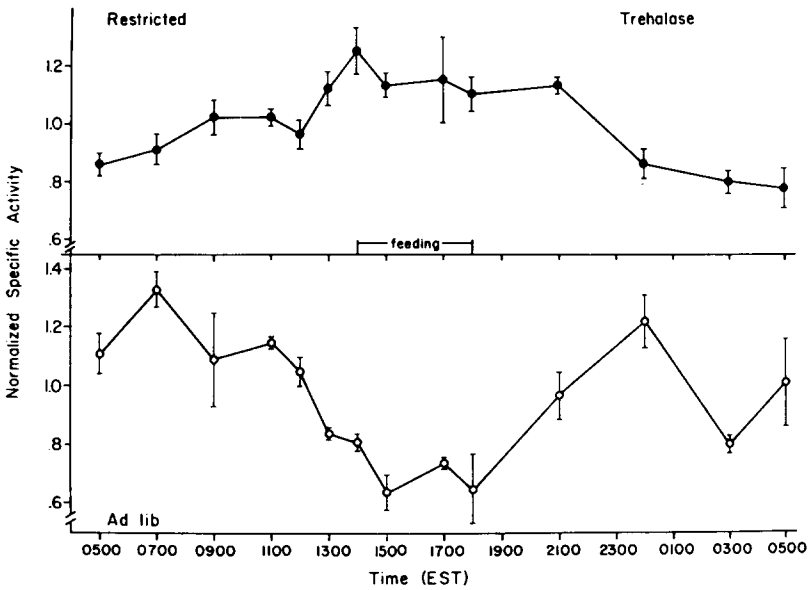

Fig. 3. Diurnal rhythm of normalized trehalase specific activity in rats fed ad libitum (○) or restricted to 4 h/day (●). Mean ±S.E. (n = 6–8).

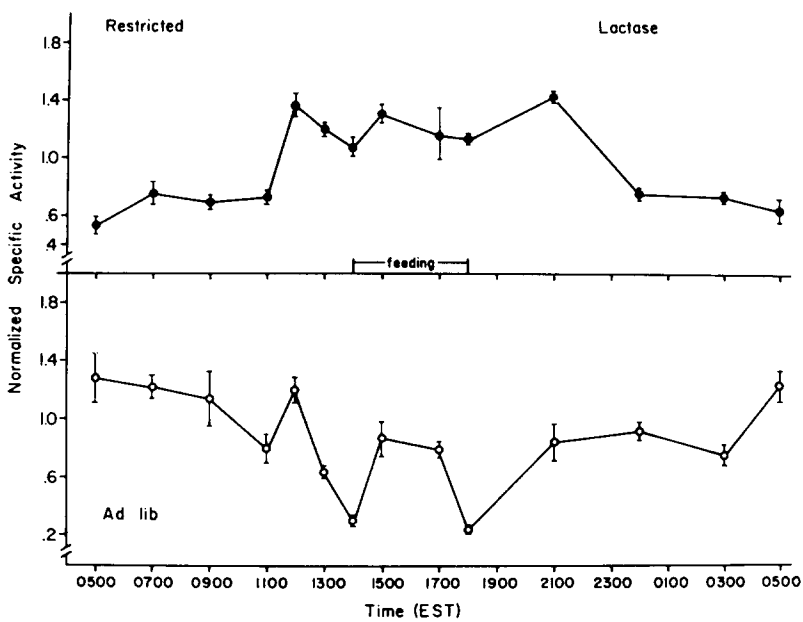

Fig. 4. Diurnal rhythm of normalized lactase specific activity in rats fed ad libitum (○) or restricted to 4 h/day (●). Mean ±S.E. (n = 6–8).

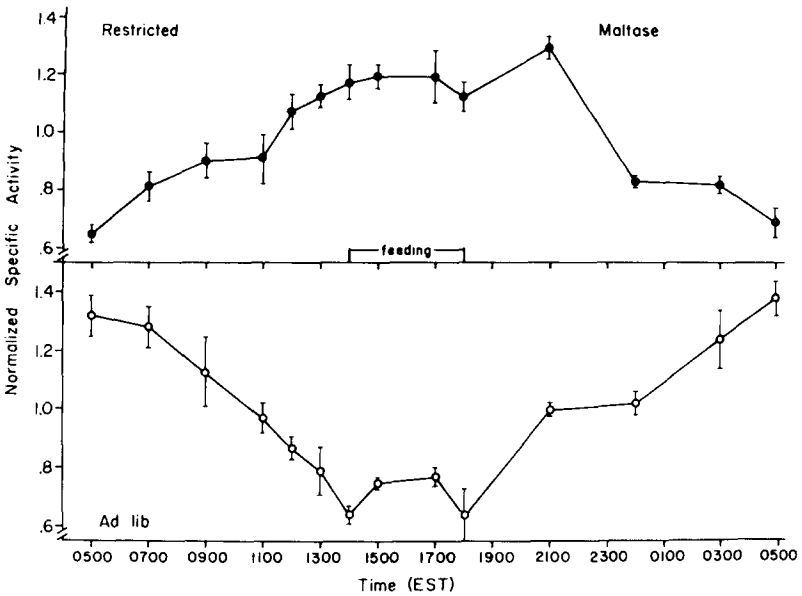

Fig. 5. Diurnal rhythm of normalized maltase specific activity in rats fed ad libitum (○) or restricted to 4 h/day (●). Mean ±S.E. (n = 6–8).

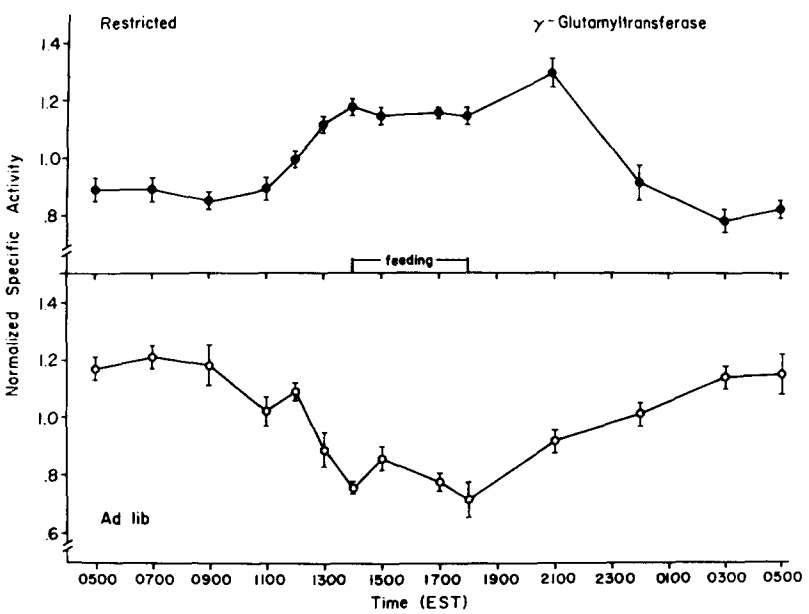

Fig. 6. Diurnal rhythm of normalized γ-glutamyltransferase specific activity in rats fed ad libitum (○) or restricted to 4 h/day (●). Mean ±S.E. (n = 6–8).

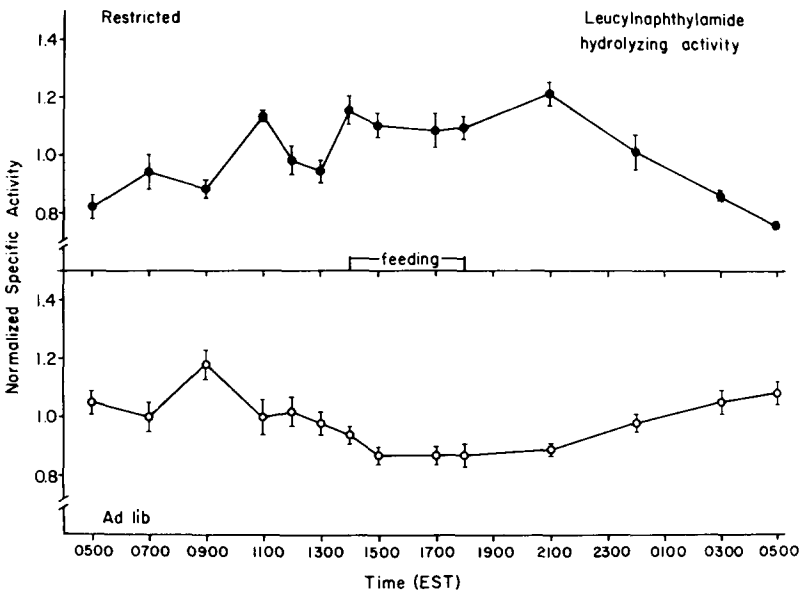

Fig. 7. Diurnal rhythm of normalized leucylnaphthylamide-hydrolyzing activity specific activity in rats fed ad libitum (○) or restricted to 4 h/day (●). Mean ±S.E. (n = 6–8).

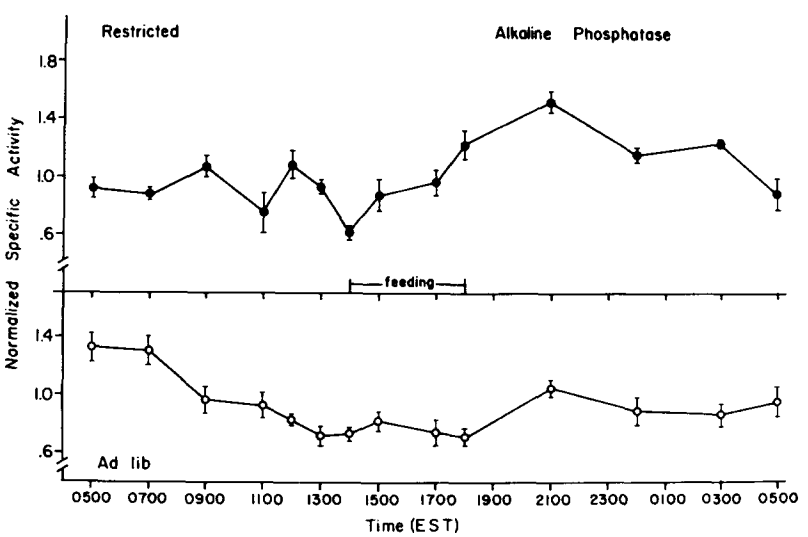

Fig. 8. Diurnal rhythm of normalized alkaline phosphatase specific activity in rats fed ad libitum (○) or restricted to 4 h/day (●). Mean ±S.E. (n = 6–8).

between 1200 and 2100 h for all systems except alkaline phosphatase (Fig. 8), which was higher from 1800 to 0300 h. Lower activity occurred between 2400 and 1400 h for all systems except leucylnaphthylamide-hydrolyzing activity (Fig. 7) which was lower from 0300 to 0900 h and alkaline phosphatase (Fig. 8) which was lower from 0500 to 1700 h.

The activities of these functions followed several different patterns. Sucrase (Fig. 2) and 3-*O*-methylglucose transport (Fig. 1) activities had short, discrete peaks of maximum activity of about 3 h duration. Perhaps alkaline phosphatase (Fig. 8) follows this pattern but its activity peak was at a time when sampling was only every 3 h. The maximum activity of sucrase (Fig. 2) occurred from 1200 to 1500 h. 3-*O*-methylglucose transport (Fig. 1) peaked at 1700 h, and alkaline phosphatase (Fig. 8) peaked at 2100 h.

Trehalase (Fig. 3) may also have a short period of maximum activity peaking at 1400 h, although considering the variation in activities observed at 1700 h, this enzyme may have a plateau of maximum activity from 1400 to 1700 h. Lactase (Fig. 4) has two peaks of activity, one at 1200 h and the other at 2100 h with a slightly lower plateau between. Preliminary data on α-aminoisobutyric acid transport indicates diurnal rhythmicity in activity with a pattern resembling that of lactase.

Maltase (Fig. 5), leucylnaphthylamide-hydrolyzing activity (Fig. 7) and γ-glutamyltransferase (Fig. 6) activities have similar patterns, rising at 1200, 1300 and 1400 h, respectively, leveling off or continuing to rise slightly and peaking at 2100 h.

Looking at these activity patterns with respect to the time of day of the synchronizer, sucrase (Fig. 2) rises before feeding begins, peaks and starts to fall within 1 h of the onset of feeding. 3-*O*-methylglucose transport (Fig. 1) rises, peaks and falls during feeding. Alkaline phosphatase (Fig. 8) rises from its minimum level at the start of feeding and continues to rise to a peak after feeding. The other enzymes, lactase (Fig. 4), maltase (Fig. 5), leucylnaphthylamide-hydrolyzing activity (Fig. 7), trehalase (Fig. 3), γ-glutamyltransferase (Fig. 6), and perhaps α-aminoisobutyric acid transport rise to high levels within the 2 h preceding feeding and during feeding and fall after feeding. Peak activities of these enzymes occur after feeding, except for trehalase (Fig. 3) which peaks at the start of feeding. Lactase (Fig. 4) and α-amino isobutyric acid have an additional peak period before feeding.

The average daily activity levels of these systems changed in the restricted group as compared to the ad libitum group (Table 1). The exception to this was alkaline phosphatase. Its activity level was the same in both groups. Leucylnaphthylamide-hydrolyzing activity decreased 17 % while the other activities increased. These increases range from 26 % for 3-*O*-methylglucose transport up to 72 % for lactase. The direction of these changes could be anticipated from observations of the general increase or decrease in the magnitude of the non-normalized activity patterns of the restricted group as compared to the ad libitum group. An example of this is the increase in the tissue/medium ratios for 3-*O*-methylglucose transport in the restricted group over those in the ad libitum group (Fig. 1). In the case of alkaline phosphatase (Fig. 8), the maximum and minimum activity levels observed in the restricted group were of equal magnitude above and below, respectively, the rather uniform ad libitum level.

One result of both this generalized change in the magnitude of the activities and the shift in rhythms with respect to time is that there are large differences in the

TABLE I

AFFECT OF RESTRICTION OF FEEDING ON THE AVERAGE DAILY ACTIVITIES OF VARIOUS DIGESTIVE-ABSORPTIVE FUNCTIONS

Activities for 3-*O*-methylglucose transport are tissue/medium ratio values, corrected for mannitol space. Tissue rings were incubated for 30 min in media containing 5 mM 3-0-methylglucose. Activities for the brush border enzymes are non-normalized specific activity (μmol substrate hydrolyzed/min per mg protein). The 0500-h values were averaged. The mean $\pm$S.E. ($n = 13$) are reported. Significance of the difference between the values for the two feeding regimes was calculated with a two tailed *t*-test. n.s., not significant.

Function	Average daily activities		Restricted/ Ad libitum	*P*
	Ad libitum	Restriction		
3-*O*-methylglucose transport	2.69 $\pm$0.12	3.40 $\pm$0.16	1.26	< 0.0005
Maltase	0.234 $\pm$0.011	0.313 $\pm$0.015	1.35	< 0.0005
Sucrase	0.0582 $\pm$0.0048	0.0759 $\pm$0.0033	1.36	< 0.0005
Trehalase	0.0193 $\pm$0.0010	0.0307 $\pm$0.0013	1.59	< 0.0005
Lactase	0.00398 $\pm$0.00024	0.00684 $\pm$0.00041	1.72	< 0.0005
γ-Glutamyltransferase	0.0233 $\pm$0.0017	0.0386 $\pm$0.0022	1.66	< 0.0005
Leucylnaphthylamide-hydrolyzing activity	0.0344 $\pm$0.0010	0.0285 $\pm$0.0017	0.83	< 0.005
Alkaline phosphatase	0.533 $\pm$0.033	0.529 $\pm$0.032	1.00	n.s.

TABLE II

MAXIMUM DIFFERENCE IN THE ACTIVITY LEVEL OF VARIOUS DIGESTIVE-ABSORPTIVE FUNCTIONS BETWEEN AD LIBITUM AND RESTRICTED FED RATS AND THE TIME OF DAY OF THESE DIFFERENCES

Activities for 3-*O*-methylglucose transport are tissue/medium ratio values, corrected for mannitol space. Tissue rings were incubated for 30 min in media containing 5 mM 3-*O*-methylglucose. Activities for the enzymes are non-normalized specific activities, μmol of substrate hydrolyzed/min per mg protein. Percent difference is the ratio of the mean activity ($n = 6$–8) in the restricted group to that in the ad libitum group $\times 100$, except where noted.

Function	Time of day	Difference (%)
3-*O*-methylglucose transport	1700	190
Maltase	1400	214
Sucrase	1400	290
Trehalase	1800	240
Lactase	1800	341
γ-Glutamyltransferase	1800	240
Leucylnaphthylamide-hydrolyzing activity	0900	156*
Alkaline phosphatase	1400	131*
	2100	136

* Ratio of the mean activity in the ad libitum group to that in the restricted group.

activity levels between the ad libitum and the restricted groups at certain times of the day. The maximum differences and the time they occur are shown for each system in Table II. In all systems the maximum difference is greater than 100 % and go as high as 341 % in the case of lactase. Also, there are times when there are no differences in the activity levels between these groups.

DISCUSSION

Diurnal rhythmicity appears to be a common characteristic of brush border transport and enzyme systems and the time of day of feeding appears to be a common synchronizer for these systems. It might be expected that those enzyme and transport systems which function in absorption and digestion would have higher levels of activity at times that coincide with the time of feeding. However, we have found a considerable variability in the temporal relationship of these rhythms to the synchronizer. Some systems have peak activity before, some during and some after the synchronizer. Others have a broad maximum plateau throughout the duration of the synchronizer. This stresses that these functions respond to the prior pattern of feeding, not to the stimulus of feeding on the day the experiment is performed.

There is also considerable variation between shapes of the activity patterns of different functions. Some systems have patterns with short periods of maximum or minimum activity, while others have patterns with long plateaus of maximum or minimum activity. The rate at which activity levels change can be either rapid or slow, and the magnitude of the difference in activity between the highest and lowest levels is different.

The activity pattern of sucrase and its relationship to the synchronizer is distinctly different from the other disaccharidases. Lactase and trehalase show rhythmicities synchronized by time of day of feeding, even though lactose is less than 2 % of the diet and trehalose may be present in trace amounts (personal communication Purina technical consultant). γ-Glutamyltransferase and leucylnaphthylamide-hydrolyzing activity also show similar rhythmicities although dietary substrates have not been identified for these systems.

On the other hand, alkaline phosphatase, an enzyme expected to have a digestive function for a variety of dietary substances, has an activity pattern which is distinct from the others. Its activity is at its minimum at the start of feeding, rises slowly during feeding, reaches a higher level and peaks after feeding. The activity pattern of this enzyme is similar to what one would expect in a stimulus-response condition leading to speculation that the delivery of dietary nutrients to the intestinal tissue is the stimulus for the increase in its activity. Also, it is questionable if alkaline phosphatase activity has a marked diurnal rhythm under ad libitum feeding conditions.

The changes in the average daily level of activities between the ad libitum fed animals and those on the restricted feeding regime is of interest. These changes are not due to dietary induction as observed with some enzyme and transport systems in rats [4, 22, 23] or in man [24–26] since the diet was not altered during the experiments. Again alkaline phosphatase is different from the other systems in that there is no change in activity level. Leucylnaphthylamide-hydrolyzing activity decreases. Those systems with transport function (including the disaccharidases because of hydrolase-related transport) all increase.

Considering the minor role of lactase and trehalase in carbohydrate digestion and absorption and the small amount of substrate present in the diet it is unexpected that these two enzymes, along with γ-glutamyltransferase would have the largest increases in activity. The lactase increase was of special interest in view of the conflicting reports of dietary induction of this enzyme [27, 28]. Perhaps those reports indicating dietary induction of lactase were in fact reporting increases due to changes in eating pattern. There are striking similarities between the increases in sucrase, maltase and lactase reported here and those reported by Bolin et al. [29], where the diet contained either no lactose or 10 % lactose. In fact a greater increase in lactase activity is observed when the time and duration of feeding are changed than when the diet is changed.

While not determining average daily activity levels, others have reported changes in transport activity under more restricted conditions than used in this work [30–32]. In these studies, transport through everted sacs of intestine was assessed. Restricted feeding enhanced the serosal accumulation of some of the sugars, including 3-*O*-methylglucose, amino acids and dipeptides tested, but had no effect on others. This effect may be temporary, inasmuch as the uptake of L-methionine returned to ad libitum levels when rats were maintained on the restricted regime for 51 days. The cause of these changes in transport was not clear and could not be completely accounted for by the changes in the general condition of the epithelial cell surface.

In the present report changes in the general condition of the epithelial cell surface also do not appear to be the cause of the change in average daily activity since the intestinal length, mucosal protein content and weight of the mucosal scrapings were similar in the two groups of rats.

The possibility that the changes in activity are due to the presence of an activator(s) or inhibitor(s) in the intestinal homogenate from the restricted rats cannot be ruled out. Deren et al. [23] looked for such agents in their work, where rats were fasted for 3 days and then refed for 24 h, but were unable to demonstrate their presence. In the results presented here it would seem unlikely that the changes in average activity are caused by a general activation or inhibition. Such agent(s) would be required to have no effect on the activity of one enzyme, decrease that of another enzyme and increase, to various extents, the activities of five other enzymes and one transport system.

Although the cause of these changes in activities are not immediately clear, it may be that some of these changes result from restriction in the amount of time food is available. The restricted group are stressed animals in the sense that availability of food for only 4 h out of 24 and the concomitant "gorging eating pattern" are not normal conditions for laboratory rats. Several laboratories [33, 34] have observed that although the major eating period is normally 12 h or less, rats eat through the 24-h period. We also observed eating activity through the 24-h period in our ad libitum animals.

One adaptation to this probable stress might be an elevation in the activity levels of digestive and/or absorptive functions. The increases in the activity of the disaccharidases and 3-*O*-methylglucose transport would seem to indicate this adaptation had occurred. However, it is not clear why trehalase, lactase and γ-glutamyltransferase show greater increases than maltase, sucrase or 3-*O*-methylglucose transport. Further, if alkaline phosphatase and leucylnaphthylamide-hydrolyzing activity

do have a digestive function, these systems should increase, and they do not.

In fact, the distrinctive behavior of alkaline phosphatase, and the decrease in daily activity level of leucylnaphthylamide-hydrolyzing activity leads one to speculate that these two enzyme systems are not involved in digestion and absorption functions in the same sense as the other systems.

Whatever the explanations of these results may be, several observations from this work should be taken into consideration in the experimental design and interpretations of results of studies on adaptation of intestinal enzymes and transport systems: (1) the activity of many, if not most, of these systems exhibits diurnal rhythmicity; (2) the time of day of feeding is the synchronizer for these rhythms; (3) all of the rhythms do not respond to the synchronizer in the same pattern or in the same temporal framework; (4) changes in the general activity level of these systems, though not always in the same direction or of the same magnitude, can be induced with a constant diet solely by manipulation of the feeding schedule. Thus, manipulation of the feeding schedule, either directly, or indirectly by feeding diets of different acceptability so that animals or man may alter their own eating pattern, can change the activity pattern of these systems both qualitatively and quantitatively.

It seems unavoidable, therefore, that correlations between diet and intestinal enzyme or transport activity level can be made with confidence only after both the entire diurnal rhythmicity pattern, and the mean daily activity levels have been determined.

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