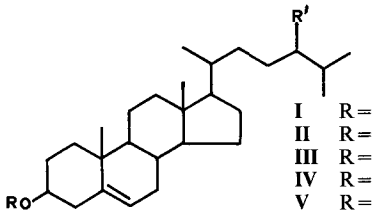
A mixture of [22, 23(n)-$^2H_2$]-β-sitosterol (Amersham, 15 µCi, sp. act. 47 Ci/mmole), 2 mg of fucosterol and 2 mg of isofucosterol, were deposited onto 140 mg of finely grounded oatmeal and fed to 50 (1.4 g) young larvae of *Tenebrio molitor,* which had been starved for 48 h.



I R=–H; R'=–CH–CH_3 (E)
II R=–H; R'=–C_2H_5
III R=–H; R'=–CH–CH_3 (Z)
IV R=–Ac; R'=–CH–CH_3 (E)
V R=–Ac; R'=–CH–CH_3 (Z)

Specific activity of diastereoisomeric ethylidene sterols (and their acetates) formed during the metabolism of β-sitosterol in *Tenebrio molitor*

Compound		Activity (dpm of ^3H/mM × 10^{-6}
Fucosteryl acetate (**IV**)	3rd crystallization	2.07
	4th crystallization	2.12
	5th crystallization	2.08
Fucosterol (**I**)	3rd crystallization	2.11
	4th crystallization	2.08
	5th crystallization	2.09
Isofucosteryl acetate (**V**)	3rd crystallization	1.35
	4th crystallization	1.38
	5th crystallization	1.36
Isofucosterol (**III**)	3rd crystallization	1.33
	4th crystallization	1.36
	5th crystallization	1.34

After the food has been consumed (~4 days), the larvae were freezed, macerated in ethanol and submitted to alkaline hydrolysis. The unsaponifiable material was fractionated on silica gel-celite and from the free sterol fraction fucosteryl acetate (**IV**) (2.3×10^5 dpm of ^3H) and isofucosteryl acetate (**V**) (1.5×10^5 dpm of ^3H) were obtained by acetylation and $SiO_2/AgNO_3$ 3:1 preparative TLC (hexane – benzene 1:1). Each compound was shown to be pure by $SiO_2/AgNO_3$ TLC and GLC (LAC 796 1%, T_c=200 °C), and then diluted with carrier material and crystallized to constant specific activity (table).

Hydrolysis of the acetates (**IV**) and (**V**) produced the free sterols (**I**) and (**III**), the activity of which also is shown in the table. Our experiment indicates that, besides fucosterol, isofucosterol too is a real metabolite of β-sitosterol in *Tenebrio molitor.* This result is in agreement with the formation of the 2 geometrical isomers, fucosterol and isofucosterol, through 2 different and parallel pathways with opposite stereochemistry or, alternatively, with the enzymic isomerization of one of the isomers into the other.

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Disaccharidase rhythm in rat small intestine; no relationship with mitosis rhythm

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Summary. Same circadian difference in the specific activities of sucrase and maltase was observed in the purified brush border fraction as in the crude homogenate of the mucosa of rat small intestine, suggesting that the disaccharidase rhythm is not due to the mitosis rhythm of epithelial cells.

The brush border of the intestinal epithelium contains many hydrolytic enzymes, such as sucrase, lactase, maltase, alkaline phosphatase and leucine aminopeptidase, and some of these enzymes are believed to play important roles in the final stages of digestion. As reported previously[1-4], the activities of these digestive enzymes of the homogenate of the intestinal mucosa show a clear circadian rhythm with the highest activity at midnight in rats kept under normal light-dark cycle and ad libitum feeding condition. When the rats are put on a daytime feeding schedule, the phase of the rhythm shifts about half a day, suggesting that time of feeding is a synchronizer of the enzyme rhythm.

The circadian rhythmicity in the intestinal epithelium has also been described in mitotic index[5], in DNA synthesis[6] and in mitotic rate[7,8], and these rhythms seem to be related to the feeding pattern[8,9]. One explanation about the rhythm in crypt cell proliferation is that more digestive-absorptive cells are required during periods of feeding[9]. According to this view, the enzyme rhythm itself might be secondary to the rhythm in the population of epithelial cells in the intestinal mucosa. To test this, in the present study, we examined the disaccharidase rhythm in the brush border fraction purified from the intestinal mucosa of rats.

Methods. Male Wistar strain rats (200–250 g) were kept at 22±2 °C under 12 h light-dark cycle (light on from 07.00 to 19.00 h) and allowed free access to water and laboratory chow. Individuals were decapitated every 4 h and the upper half of the small intestine was rapidly removed, washed out with cold 0.9% NaCl and everted. The mucosa was scrapped off with a glass slide and homogenized with about 50 vol. of 5 mM ethylenediamine tetraacetate buffer (pH 7.4) in a Warling Blendor for 40 sec. An aliquot of the homogenate was assayed for sucrase and maltase by Dahlqvist's method[10] and for protein by the method of Lowry et al.[11]. The homogenates obtained at 00.00 h and at 12.00 h were centrifuged at 450 × g for 10 min and brush borders were purified according to the method of Forstner et al.[12].

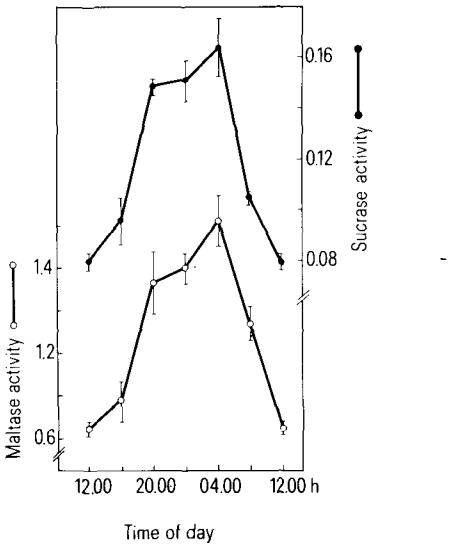
Results and discussion. The figure shows a typical pattern of the circadian rhythm in the activities of sucrase and maltase of the homogenates. The highest activity was observed at 04.00 h and the lowest at 12.00 h, confirming earlier results[1-4]. This pattern of the disaccharidase rhythm seems to be similar to that of the mitosis rhythm reported[7,8].

In the figure, the enzyme activities were expressed as the specific activity based on the protein content of the homogenate of the scrapped mucosa. The changes in the enzyme

Circadian difference in the disaccharidase activities of the crude homogenate and the purified brush border fraction

Killing time of day	00.00 h	12.00 h
Crude homogenate		
Protein content (mg)	314	338
Sucrase		
Total activity	47.7	26.8
Specific activity	0.152	0.0794
Maltase		
Total activity	440	211
Specific activity	1.40	0.624
Purified brush border		
Protein content (mg)	10.4	9.91
Sucrase		
Total activity	22.7	13.3
Specific activity	2.18	1.34
Maltase		
Total activity	203	95.9
Specific activity	19.5	9.68

Crude homogenates of the intestinal mucosa of 4 rats, killed at 00.00 h and at 12.00 h, were pooled and the brush borders were purified from the homogenates. The total activity was expressed as µmole of glucose formed per min, and the specific activity as µmole glucose formed per min per mg of protein.



Circadian rhythms of the disaccharidase activities in rat small intestine. Rats were kept under the condition of normal light-dark cycle and ad libitum feeding. The activities of sucrase (●——●) and maltase (○——○) in the homogenate of the intestinal mucosa were measured and expressed as µmole of glucose formed per min per mg of protein of the homogenate. Values are means of those in 4 rats, with the SE.

activity were similar whether expressed as activity per mg protein or activity per g wet weight of intestine or activity per whole intestine. These results, however, could give no answer as to whether the rhythmic change in the enzyme activity is due to change in the number of enzyme molecules per epithelial cell or change in the population of the cells which bind the enzyme, because the intestinal mucosa contains not only the epithelial cells but also many other cells which do not have the enzymes measured. To discriminate these 2 possibilities, next, the activities of sucrase and maltase were measured in the brush border fractions purified from the homogenates obtained at 00.00 h and 12.00 h, when the enzyme activity was almost highest and lowest, respectively. In the homogenate, the protein content at 00.00 h was nearly equal to that at 12.00 h, but the total activity of sucrase at 00.00 h was about twice as that at 12.00 h (table). Thus, the specific activity of sucrase at 00.00 h was about twice that at 12.00 h. Same relationships were also found in the purified brush border fraction, where the specific activity was about 14 times higher than that in the homogenate. The specific activity of maltase in the brush border fraction at 00.00 h was also about twice that at 12.00 h in the same way as in the homogenate.

If the disaccharidase rhythm itself is secondary to the mitosis rhythm and is due to the change in the population of the epithelial cells and/or brush border, it might be expected that the specific activities at 00.00 h and 12.00 h would approach the same value as the brush border was purified. The results in the table conflict with this expectation. Thus, it could be concluded that the disaccaridase rhythm is not a direct consequence of the mitosis rhythm of the epithelial cell which bind the enzyme, that is, the mechanism controlling the disaccharidase rhythm is different from that controlling the mitosis rhythm. This seems to agree with findings of Stevenson et al.[3], who observed that the pattern of the sucrase rhythm was different from that of the alkaline phosphatase rhythm; if the enzyme rhythm is secondary to the mitosis rhythm, the pattern of the sucrase rhythm would be the same as that of the alkaline phosphatase rhythm. Tutton[8] reported that the mitosis rhythm in the intestinal epithelium disappeared when the rats were sympathectomized by a injection of 6-hydroxydopamine. However, in a preliminary experiment, we observed that the disaccharidase rhythm was not influenced by the treatment of 6-hydroxydopamine at a dose of 100 mg/kg b. wt. These observations also support the conclusion described above.

Recent studies[13,14] on the turnover of enzymes in the intestinal brush border demonstrated that disaccharidases are synthesized and catabolized during migration of the epithelial cells and that individual proteins in the brush border do not all have the same turnover rate. Therefore, the disaccharidase rhythm might be due to changes in the rates of synthesis and of degradation of the enzyme in the matured villus cell. Further studies are needed on the actual molecular mechanism of the enzyme rhythm.

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