Diurnal rhythm of active calcium transport in rat intestine[1]

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Summary. The active calcium transport along the small intestine of adult rat was shown to exhibit daily rhythmic variations of considerable amplitude. Both the light-dark cycle and the time of food presentation acted as synchronizers of the rhythm observed.

The rhythmicity of many essential physiological variables has been amply documented in a variety of multicellular organisms[2]. Recent findings concerning diurnal fluctuations in plasma calcium and phosphate concentrations in the rat[3,4] and in human subjects[5] suggest strongly the existence of circadian rhythm in the mechanism controlling calcium homeostasis. We report here that the pattern of active calcium transport in rat intestine exhibits daily changes of very considerable amplitude, and that the periodicity of this rhythm can be shifted both by the light-dark cycle and by the time of food presentation.

Materials and methods. Female Wistar rats 4–5 month old, weighing 230–250 g were fed standard laboratory diet (Bacutil, Poland) containing 0.14% Ca. Everted intestinal sacs were prepared from 3 adjacent contiguous 5 cm long segments taken from the proximal part of small intestine beginning from the pylorus[6]. Segment No. 1 corresponds to duodenum. The sacs were filled with 0.5 ml of medium containing 25 mM Tris-HCl buffer pH 7.4, 20 mM fructose, 70 mM NaCl, 0.8 mM NaH_2PO_4, 100 mM mannitol and 0.4 mM $^{45}CaCl_2$, and incubated in 5 ml of the same medium at 37 °C for 90 min under continuous flow of oxygen. ^{45}Ca radioactivity was determined by liquid scintillation spectrometry[7]. Active transport of calcium was calculated from the difference between the amount of ^{45}Ca in the total final sac content and the initial amount of ^{45}Ca.

Results and discussion. The results of experiments performed on rats maintained under natural lighting with dark

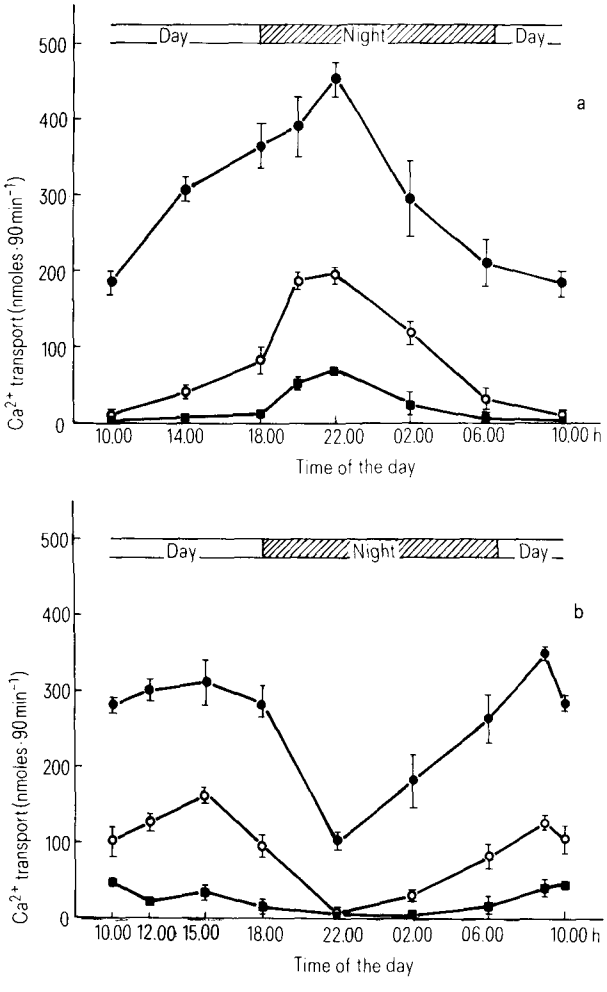

Fig. 1. Diurnal rhythm of active calcium transport in the small intestine of adult rat under natural light conditions with dark night from 18.00 to 6.30 h. *a* rats allowed free access to food; *b* rats adapted for 3 weeks to feeding restricted within 9.00–15.00 h. (●) segment No. 1; (○) segment No. 2; (■) segment No. 3. Each point is the mean of 6 rats. Vertical bars represent SEM.

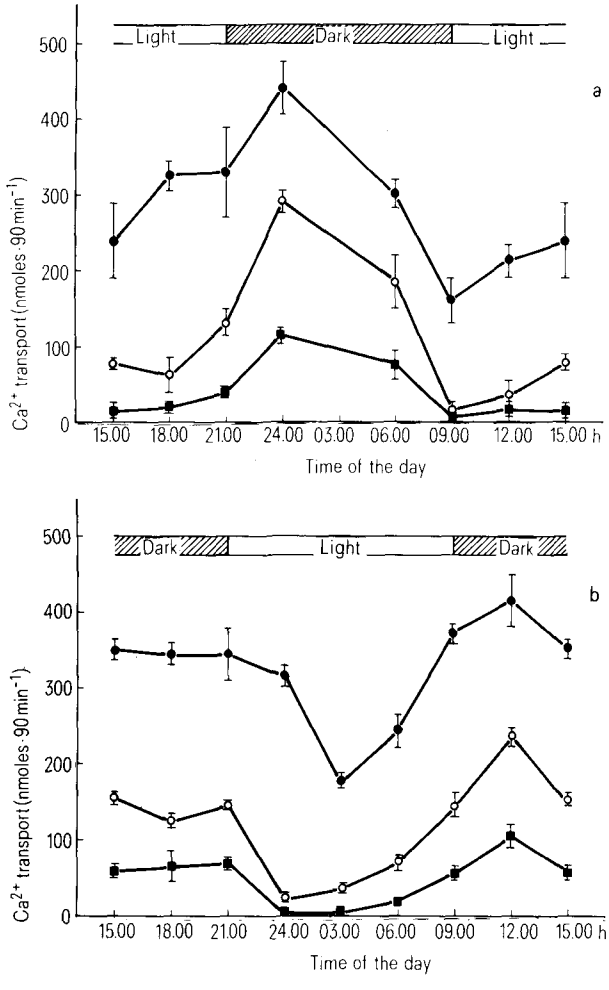

Fig. 2. Diurnal rhythm of active calcium transport in the small intestine of adult rat adapted for 3 weeks to 12 h light-dark cycle and allowed free access to food. *a* light on from 9.00 h to 21.00 h; *b* light on from 21.00 to 9.00 h. (●) segment No. 1; (○) segment No. 2; (■) segment No. 3. Each point is the mean of 6 rats. Vertical bars represent SEM.

night from 18.00 to 6.30 h are presented in figure 1. In animals allowed free access to food, intestinal calcium transport exhibited rhythmic changes, being low in the morning and high at night. At 10.00 h active calcium transport was lowest and limited to duodenum only (segment No. 1). The increase in duodenal transport activity occurring at evening and at night was accompanied by a gradual appearance and enhancement of active calcium transport in the contiguous part of the small intestine (segments Nos 2 and 3). The highest transport was achieved at 22.00 h, 4 h after darkness began. Restricted feeding of rats during the daytime from 9.00 to 15.00 h resulted in phase shift of diurnal periodicity of intestinal calcium transport (figure 1, b). The highest transport occurred in the morning during the time of food presentation. At 22.00 h the transport was low and limited to duodenum only. Phase shifting of diurnal rhythm of active calcium transport in the intestine could have been achieved also by the changes in the light-dark cycle. In rats maintained in reversed light-dark schedules (12:12) with free access to food, the transport was always low during light period and high in dark period with a peak 3 h after the start of darkness (figure 2).

The results presented indicate that both light-dark transition and the time of food presentation do play a role as 'Zeitgeber' in determining the phase of daily rhythm of active calcium transport in rat intestine. The time of food intake, however, appears to be a more potent synchronizer than the light-dark cycle. This assumption is supported by the experiments with feeding restricted to light phase (figure 1). On the other hand, even in rats fasted for 48 h, an increase of active calcium transport did occur from 170 ± 18 at 10.00 h to 283 ± 18 nmoles · 90 min^{-1} at 22.00 h in the duodenum; in the intestinal segments Nos 2 and 3 from fasted rats, there was no active calcium transport at 10.00 h whereas at 22.00 h the transport was 83 ± 15 and 40 ± 8, respectively. Thus it appears likely that, although cyclic food intake may be a prerequisite for diurnal changes in intestinal calcium transport, the rise in transport activity is not a simple, direct consequence of food digestion only.

Further studies are needed to elucidate the exact mechanism of the daily rhythm of active calcium transport in rat intestine and its physiological significance. It is of interest to note, however, that the sequential pattern of rhythmic increase of calcium transport along the proximal region of small intestine is inversely correlated with diurnal fluctuations of plasma calcium concentration[3,4] and follows a general time-dependent sequence of adaptive response in active transport ability along intestinal length during pregnancy, lactation and dietary calcium restriction[6,8].

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Lymphocytes, but not cancer cells are able to penetrate into the rat embryo yolk sac wall

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Summary. Normal rat lymphocytes and cells of 2 highly invasive tumors, the L5222 rat leukemia and the V2 rabbit carcinoma, were inoculated in vitro on the mesothelial surface of the visceral wall of the rat embryo yolk sac. After 48 h, lymphocytes, without any damage being inflicted on the mesothelial cells, had penetrated deeply into the yolk sac wall, whereas both kinds of cancer cells had destroyed the mesothelial cells, but not advanced beyond the basal lamina.

It is well established that some classes of normal cells (leukocytes, macrophages) and many cancer cells are able to migrate within the body's extracellular matrix. The mechanism responsible has not been clarified so far. In order to investigate the encounter between cells and extracellular matrix in vitro, we have inoculated normal and neoplastic cells on the visceral wall of the rat embryo yolk sac, which is characterized by a particularly thick layer of collagen-rich material.

Materials and methods. The visceral wall of the rat embryo yolk sac consists mainly of a 7 µm layer of thin, plaited collagen microfibrils embedded into an electronoptically unstructured ground substance. On one side, this layer is covered by mesothelial cells with their basal lamina, on the opposite side by connective tissue and epithelium. Fragments of the whole visceral yolk sac wall were explanted on to a semi-solid mixture of Bacto-Agar and tissue culture medium[1]. The following cells were used for inoculation experiments: Rat lymphocytes obtained by filtration of minced lymph nodes through several layers of gauze; L5222 rat leukemia cells[2] from the peritoneal cavity of BDIX rats; V2 carcinoma cells isolated by trypsinization of 24-h-cultures of cells derived from solid tumors grown in the peritoneal cavity of New Zealand rabbits. The cells were washed in medium, and pellets were deposited on the mesothelial surface of the yolk sac wall explanted 2 h previously. Specimens for transmission electron microscopy were removed 8, 24, 48 and 72 h after inoculation. Up to 48 h neither the yolk sac fragments nor the deposited cells showed any regressive changes. Such alterations, however, were recognizable at 72 h, and exploitation of the model was therefore limited to 48 h.

Results. Ultrathin sections provide evidence for completely different behavior patterns for lymphocytes and cancer cells. In the presence of lymphocytes, the mesothelial cells remain intact throughout the observation period. At 24 h, lymphocytes are either found between mesothelial cells and their basal lamina, or within pouch-like protrusions of the latter extending into the collagen layer (figure 1). Concerning the attainment of the submesothelial position, our data