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SEASONAL VARIATION IN THE ACTIVE TRANSPORTING ABILITY AND IN THE MEMBRANE ATPase ACTIVITY OF THE FROG INTESTINAL EPITHELIUM

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Summary

The active sugar and amino acid transport in the small intestine of the American leopard frog (*Rana pipiens*) and a species of European frog (*Rana esculenta*) decreases during the winter months. Parallel with this the (Na^+, K^+)-stimulated ("pump") ATPase activity is markedly depressed. No seasonal changes are observed in the intestine of the tropical bullfrog (*Rana catesbeiana*). It is assumed that the low pump-ATPase activity is caused by the hibernation of the frogs living in moderate or subtropical areas and is connected to a biological clock. The decreased active transport of non-electrolytes appears to be a consequence of the change of the ATPase activity.

The observation that in the isolated small intestine of both homoiothermic and poikilothermic species, the active transport of non-electrolyte nutrient molecules is strongly dependent on the presence of sodium in the medium led to the proposal of a hypothesis that essentially all intestinal active transport processes are dependent upon a functioning sodium pump [1]. On the other hand, there is substantial evidence indicating that a specific membrane-bound ATPase is an integral functioning part of the sodium pump [2]. This ATPase ("pump ATPase") requires in vitro a certain concentration of sodium and potassium for its function and it is specifically inhibited by digitalis steroids [3].

Over the years, we have observed in our laboratory that the small in-

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Abbreviation: S/M ratio, concentration ratio in the serosal versus mucosal solutions.

testine of a frog species native to the North American continent, namely, the leopard frog (*Rana pipiens*), displays a seasonal variation in the active transporting function of the small intestine. It actively transports sugars and amino acids only during the summer months, while in the winter very little, if any, accumulating transport of these non-electrolytes could be observed in vitro and in vivo. These frogs are housed in wet cages at about +3° C continuously without change in the light or temperature.

On the other hand, in the bullfrog (*Rana catesbeiana*) no appreciable seasonal variation was observed in the intestinal active transport. This species is a native of the tropics and over the centuries has not been exposed to climate variations similar to those experienced by the *R. pipiens*.

These sporadic observations prompted a quantitative comparative month-by-month study of the active sugar and amino acid transporting ability in the small intestine of the leopard frog and bullfrog. Concomitantly, the in vitro activity of the sodium/potassium-stimulated, ouabain-inhibited ATPase was also determined in the intestinal mucosal of these animals.

A brief description of the experiments and the preliminary results of a 5-month cycle follows.

The experiments were started in November. All animals were purchased at the same time and housed, without food, in a cold room (average temperature 2–3° C) in wet cages. The cages were thoroughly washed once a week and the animals were given a bath with diluted chloramphenicol to minimize the development of skin infections. Every month three leopard frogs and three bullfrogs were killed by decapitation and pithing of the spinal cord, and their small intestine removed. Two sacs were prepared from each gut: one was injected with ^{14}C-labeled 3-*O*-methyl glucose in a concentration of 2.6 mM in a sulfate containing frog Ringer [4], and the other was injected with ^{14}C-labeled l-phenylalanine dissolved in sulfate Ringer in the concentration of 3.0 mM. The intestines were placed in 2 ml of a solution of identical composition to the one which was injected into the lumen. The containers were oxygenated and gently agitated at 30° C for 3 h. After this, the concentration of the radioactivity was determined by liquid scintillation spectrophotometry in both the serosal and the mucosal medium. The increase of concentration ratio in the serosal versus mucosal solutions (S/M ratio) was calculated as a quantitative measurement of active transport. By definition the initial S/M ratio was 1.0.

It is known that 3-*O*-methyl glucose is not metabolized in animal tissues [5,6]. As for the phenylalanine paper chromatographic separation of the solutions taken at the end of the experiment from both serosal and mucosal compartments, indicated that over 90% of the radioactivity was present in the spot the localization of which was identical with that of the reference amino acid. For this reason it is assumed that the concentration of radioactivity represented the true concentration of the sugar or amino acid, respectively.

While the active transport was determined in the intestinal sacs, in another segment of the small intestine from the same animal the (Na^+, K^+)-stimulated ATPase activity was determined as follows [7].

The small intestine was excised and split open and the mucosa was

scraped off with a glass slide and transferred into ice cold 0.25 M sucrose. The tissue was homogenized with a high speed Virtis homogenizer. The homogenate was centrifuged in the cold at approximately 5000 × *g* for 10 min. The resulting pellet was disarded and the supernatant centrifuged for 20 min at approximately 10 000 × *g*. After discarding the pellet, the supernatant was spun at approximately 100 000 × *g* for 60 min. The resulting pellet was then suspended in 0.25 M sucrose and served as an "enzyme". This enzyme was added to a mixture containing (final concentrations) 100 mM sodium + 5 mM potassium + 2 mM magnesium, 5 mM ATP, and 50 mM Tris·HCl buffer (pH 7.5) with or without ouabain (final concentration 10^{-3} mol). The total volume of the mixture was 2.5 ml. It was incubated in a 37°C bath for 1 h. The tubes were then chilled in ice, 1.5 ice-cold 8% perchloric acid was then added to each tube, the content was filtered and the inorganic phosphate in the filtrate was determined by the method of Fiske and Subbarow [8]. The inorganic phosphate produced in the absence of Na^+ and K^+ was subtracted from that produced in the presence of these ions; the difference was considered as the measure of the function of the true "pump ATPase" and expressed as mg P_i/min per g wet tissue.

Fig. 1 shows the results of experiments conducted with the intestine of the *R. pipiens*. It is clear that the active transporting ability of this gut

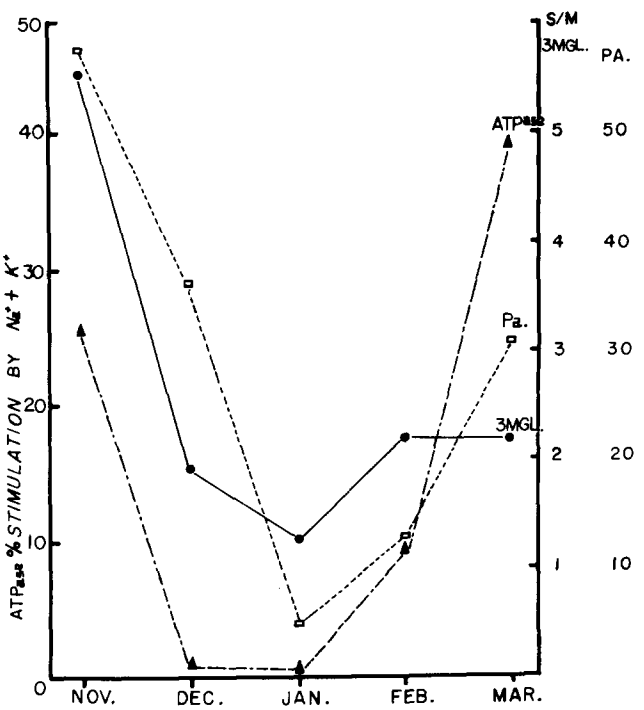

Fig. 1. Active transport of 3-*O*-methylglucose (3MGL) and 1-phenylalanine (Pa) and the (Na^+, K^+, Mg^{2+})-stimulated ATPase activity in the isolated small intestine of the leopard frog (*R. pipiens*) in various months. The transport is expressed as the increased serosal-to-mucosal concentration gradient (S/M) after 3 h incubation (right side ordinate). Left side ordinate: % stimulation by Na^+ and K^+ of the ATPase activity of the microsomal fraction of the small intestinal mucosal homogenate.

steadily decreases from November, reaching an almost zero level around January and February, and increases again to reach optimum in the late spring. The "pump ATPase" activity runs parallel to the active transport ability, almost disappearing during the months of January and February. On the other hand, experiments carried out with the small intestine of the bullfrog are not registered in Fig. 1 as no significant seasonal variation in the active transport ability was found; neither was there any appreciable seasonal variation in the specific ATPase activity in this gut preparation.

One of the investigators (E.G.) conducted experiments on a European frog, *Rana esculenta*, native to the southern part of Italy (Bari) and kept under conditions similar to those used in Lexington. The accumulation of 3-*O*-[*Me*-^{14}C]methylglucose and the microsomal (Na^+, K^+, Mg^{2+})-dependent ATPase activity were measured in the small intestinal epithelium during different months of the year. The sugar accumulation was determined at the end of a 3-h incubation of a loop of isolated small intestine. The microsomal ATPase was assayed as described in Methods, except that the activity was expressed per mg of protein. The latter was measured according to Lowry et al. [10].

Fig. 2 shows the results. Two low peaks in the active transporting capacity were observed in the gut of the *R. esculenta*: one is February—April and another is July—September. The second (summer) low peak is puzzling; perhaps it is related to the fact that those three months are normally very dry in southern Italy. Although the seasonal variations in the APTase activity of the intestinal mucosa of *R. esculenta* are not as dramatic as those of *R. pipiens*, it is significant that even in the European species they strictly parallel the changes observed in the accumulative transport. Thus, the results of the experiments conducted in Bari essentially corroborate those obtained in Lexington.

One of the interesting facts which emerge from these studies is the remarkable quantitative parallelity between the pump ATPase activity of the intestinal epithelium and its ability of actively transporting sugars and amino acids. Thus, in the *R. pipiens* during the winter and early spring months

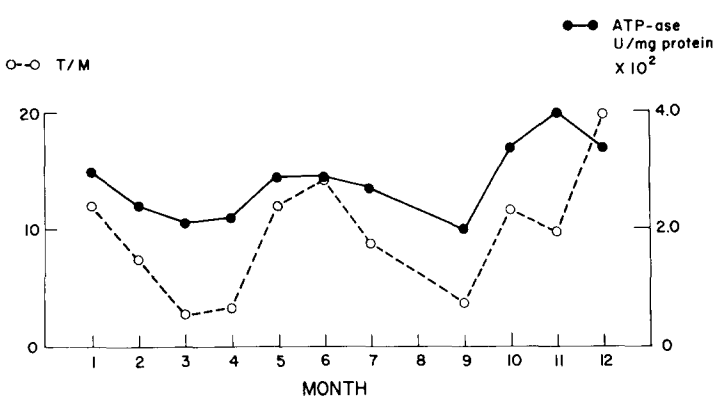

Fig. 2. Active transport of 3-*O*-methylglucose and the (Na^+, K^+)-dependent ATPase activity in the small intestinal mucosa of the European frog (*R. esculenta*). The transport is expressed as the increase of the S/M gradient after 3 h incubation. ATPase expressed as μg P liberated (U)/mg protein. Abscissa: months of the year (January=1, December=12).

when the active transport was very low or even absent, no measurable sodium/potassium-stimulated, ouabain-inhibited ATPase activity could be demonstrated. During the late spring months, the active transport ability was gradually restored and so was the measurable pump ATPase activity. The present study thus offers additional strong evidence to support the concept that the pump ATPase is intimately involved in the active intestinal transport of organic nutrient molecules [9], the most likely involvement being via the sodium pump. It has therefore been proposed that in the intestinal epithelium there is only one "pump", namely, the sodium pump, and all other concentrative transport processes are energized through this single functioning energy transducer [1].

The other interesting finding is the marked seasonal variation in the activity of an enzyme system, in this case the (Na^+, K^+)-stimulated, ouabain-inhibited, ATPase in the intestinal epithelium. Clearly, the activity of this enzyme is regulated by a biological clock. Simple manipulations, such as removing the animal from its normal habitat by increasing the temperature, by force-feeding or thyroxin injection, etc., failed to change the functioning of the biological clock. It appears that the clock developed over thousands of years as the result of the existence of a species in certain climatic conditions. Interestingly, this clock does not seem to be built into the system of the bullfrog, which over thousands of years lived in tropical habitats where the temperature did not fluctuate and where the animal was not forced into hibernation during certain months.

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