The Transport of L-Histidine across Rat Small Intestine *in vitro*: an Effect of Semi-starvation and Energy Substrate Requirements

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Gilman & Koelle (1960) have reported on the ability of various intermediates of glycolysis and the tricarboxylic acid cycle to support transfer of ions in the small intestine of the rat. In view of their results and the numerous reports in the literature (e.g. Curran, 1968) of the linked fluxes of ions and non-electrolytes in transport studies, it was decided to investigate the ability of various energy substrates to support the transport of the amino acid L-histidine. The present communication gives the results of this exploratory investigation and also the effect of semi-starvation on the transport system.

The velocity of transport of L-histidine was studied across the small intestine of male Wistar rats (approx. 200g.) by using the everted-sac method, based on that described by Wilson & Wiseman (1954). The animals were allowed food and water *ad libitum* except when being semi-starved, when no food was given for 5 consecutive days (free access to water was allowed during this time). Five sacs were prepared from the whole of the small intestine and were chosen at random based on a latin-square design. Four of these sacs were used to follow the transfer of histidine; the fifth sac was filled with the solvent system and acted as a reagent blank in the subsequent assay procedure. The mucosal and serosal solutions were initially at the same concentration (5mM) and were prepared with bicarbonate-buffered saline, pH 7.2 (Krebs & Henseleit, 1932). The sacs were incubated for 45 min. at 37° in O₂+CO₂ (95:5). Except in the control series and in experiments with semi-starved animals, when no added energy substrate was included, all incubation mixtures contained 0.5% D-glucose or the appropriate iso-osmotic concentration of the alternative energy substrate under study. The concentration of L-histidine in the samples was measured by a fluorimetric method similar to that described by Shore, Burkhalter & Cohn (1959) for the assay of histamine. The amount transported was calculated after correction for water movement as described by Parsons, Smyth & Taylor (1958). The results of these exploratory studies are given in Table 1.

The velocities recorded in Table 1 are not necessarily initial velocities (no test being made of

this point), and so one cannot draw any conclusions on the effect of the various substrates or semi-starvation on the basic parameters of the histidine-transport system. However, a report on the ability of the various substrates or the nutritional state of the animal to support the transport of L-histidine is possible.

All the substrates tested seem able to support the active transport of L-histidine, pyruvate being the least effective. It is noteworthy that both glucose and the ionized moieties can presumably approach the mitochondrial sites and so allow the cell to produce energy. This allows the transport to proceed for a longer period of time than in the control series, where presumably the endogenous energy supply is rate-limiting. The lower ability of pyruvate to support the transport activity might be due to the inability of this acid to approach the appropriate site in the cell or to the lack of catalytic amounts of one of the carboxylic acids, e.g. malate, required to give the continuous supply of oxaloacetate necessary to condense with the acetyl-CoA formed from pyruvate. It is known that transport activity varies along the length of the intestine (Barry, Matthews & Smyth, 1961), and information on the detailed mechanism of action is lost when various substrates are compared

Table 1. *Velocity of transport of L-histidine across the intestinal mucosa of the rat*

Results are given as means $\pm$ S.E.M., with the numbers of determinations in parentheses.

Substrate	Velocity (μ moles/hr./g. initial wet wt.)
D-Glucose (0.5%) (well-fed)	19.4 $\pm$ 3.52 (8)
Sodium pyruvate (0.25%) (well-fed)	3.74 $\pm$ 1.39 (8)
Sodium citrate (0.125%) (well-fed)	9.57 $\pm$ 4.26 (8)
Sodium succinate (0.167%) (well-fed)	11.17 $\pm$ 3.06 (8)
No substrate (well-fed, i.e. control series)	1.83 $\pm$ 1.59 (8)
No substrate (semi-starved)	11.86 $\pm$ 2.79 (16)

on a 'total-intestinal' sac basis. However, it was not thought justified to analyse the results in separate segments at this stage of the investigation because of the limited data.

It is considered that the results obtained with semi-starved animals merit the most emphasis. Neale & Wiseman (1968) have shown that the amount of endogenous D-glucose in the intestine is greatly decreased in the semi-starved condition. It was therefore considered that intestines from semi-starved rats with no exogenous energy substrate would be the most appropriate control in a comparison of the various test substrates for their ability to support the transport of L-histidine. However, the amount of histidine transported by these intestinal sacs was found to be as great as that with succinate as substrate and far greater than that of the well-fed animals with no exogenous D-glucose added.

Enhanced ability to transport L-histidine by the intestines of semi-starved rats has been shown by Kershaw, Neame & Wiseman (1960), but always with D-glucose added to the incubation medium. These workers argued that the concentration of L-histidine in the subepithelial space is greater than in controls and this, together with the decreased thickness of the intestinal wall, gives an increased diffusion from the subepithelial space. It seems probable that a similar explanation is valid here. However, as no glucose was added to the incubation in this instance, and in view of the results from the control series, it seems unlikely that glucose could

provide energy for the transport process. This statement is all the more probable in the light of the work of Neale & Wiseman (1968).

It is known that during starvation animals derive energy first from the combustion of their carbohydrate stores. Next the fat reserves mainly are drawn upon to provide energy for a longer period of time (Best & Taylor, 1966). It is possible that fat, through β -oxidation, is being broken down to supply the energy for the transport of L-histidine in the intestines of the semi-starved animals, and this allows the continued transport of histidine over and above that of the control sacs from well-fed animals. The failure of these sacs to continue the transport is probably due to the exhaustion of their endogenous energy supply, as mentioned above.

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