REVERSIBLE AND IRREVERSIBLE MECHANISMS FOR INTESTINAL AMINO-ACID ABSORPTION.

J-Cl.Jéquier, J.W.L.Robinson, and J-P.Felber.

Laboratoire de Biochimie Clinique, Clinique Médicale Universitaire, Lausanne, Switzerland.

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It has been suggested that there are at least two processes involved in amino-acid absorption by the intestinal mucosa, an active transport component, inhibited by 2:4-dinitrophenol (DNP), and a passive diffusion component which occurs simultaneously and which is not influenced by metabolic inhibitors (Milne,1963). In vitro studies have shown that absorption of a lipophilic, neutral L-amino-acid rapidly reaches an equilibrium position (Finch and Hird, 1960), which may be maintained for several hours, and it was suggested that this equilibrium is a dynamic one, consisting of outward diffusion from the cell, followed by reabsorption, to maintain constant the distribution ratio between the intracellular concentration and the concentration in the bathing medium (Agar et al., 1956). Furthermore, these authors showed that when an intestinal segment was saturated with amino-acid and then placed in a solution of buffer without any amino-acid, the amino-acid was released by the tissue to return to the equilibrium distribution ratio between the two compartments. More recently, Oxender and Christensen (1963) have suggested that the exit of amino-acids from cells is also mediated, and at least part of it does not occur by simple diffusion. Other results from the same laboratory (Christensen et al., 1963) have indicated that in vivo the dynamic equilibrium position between amino-acid levels in the plasma and in the intestinal lumen may be attained from either side, by absorption or release of the amino-acid from the intestinal mucosa. This indicates that at least part of the process of amino-acid absorption is reversible, and so, using our modification (Robinson and Felber, 1964) of the method of Agar, Hird, and Sidhu (1954), we set out to study the properties of the release of amino-acids from saturated intestinal cells in vitro.

Methods. The jejunum of a rat is removed under ether anaesthesia, and cut into small segments. The segments are then incubated for one hour at 37°C in a solution of 5 mM L-phenylalanine, lightly labelled with ^{14}C, in oxygenated Krebs bicarbonate buffer, containing 0.2% glucose. After the incubation, the segments are removed from the incubation flask, rapidly washed to remove adhering radioactive solution, and incubated for a second period (of varying length) in one of the following solutions : Krebs buffer containing unlabelled 5 mM L-phenylalanine, Krebs buffer containing unlabelled 5 mM L-phenylalanine and 1 mM DNP, or Krebs buffer alone. Control experiments consisting of a three-hour incubation in the labelled phenylalanine solution are run at the same time. At the end of the incubation period, the segments are removed from the flasks, weighed, and counted, as previously described (Robinson and Felber, 1964). Each experiment consisted of six parallel incubations, and each experiment was carried out at least four times. The results in the tables are expressed as the means of the various experiments ± one SEM. Uptake is expressed in micromoles of amino-acid absorbed per 100 mg. of tissue (wet weight).

Table 1

Inhibition of L-phenylalanine uptake by 2:4-dinitrophenol, and retention of the phenylalanine by the cells throughout a three-hour incubation.

Incubation medium	Incubation time	Uptake	% Inhibition
5 mM L-phenylalanine	1 hr.	1.038 ± 0.0502 (8)	-
5 mM L-phenylalanine + 1 mM DNP	1 hr.	0.467 ± 0.0134 (4)	55.0%
5 mM L-phenylalanine	3 hrs.	0.901 ± 0.0796 (8)	-
5 mM L-phenylalanine + 1 mM DNP	3 hrs.	0.430 ± 0.0112 (4)	52.3%

No. of animals used for the determination of each value given in parentheses after the value. See text for details.

Results. In Table 1, it is shown that during the three-hour incubation, phenylalanine was absorbed to an equilibrium distribution with respect to the concentration in the bathing medium, and that this equilibrium was maintained throughout the experimental period. When the second incubation takes place in an unlabelled

solution of L-phenylalanine, the conditions are essentially the same as for a prolonged incubation, since the tissue is unable to distinguish between labelled and unlabelled amino-acid. Hence isotopic dilution in the tissue compartment is a function of the exchange between L-phenylalanine absorbed already, and L-phenylalanine in the incubating medium. Although the phenylalanine content of the cell remains virtually constant, this isotopic dilution takes place very rapidly (Table 2). Addition of DNP to the medium of the second incubation causes no significant change in the velocity of exchange between tissue and medium. When the second incubation takes place in Krebs buffer without amino-acid, the release is significantly slower than the exchange with unlabelled amino-acid.

Table 2

Retention of L-phenylalanine by saturated cells during the course of a second incubation in different media.

Time of second incubation	1. In 5 mM unlabelled L-phenylalanine	2. In 5 mM unlabelled phe. + 1 mM DNP	3. In Krebs buffer alone
0 time	1.038 ± 0.0502	1.038 ± 0.0502	1.038 ± 0.0502
5 minutes	0.758 ± 0.0665	0.697 ± 0.0803	0.953 ± 0.0835
15 minutes	0.497 ± 0.0647	0.557 ± 0.0836	0.711 ± 0.1032
30 minutes	0.307 ± 0.0489	0.352 ± 0.0884	0.487 ± 0.0538
1 hour	0.172 ± 0.0274	0.170 ± 0.1007	0.299 ± 0.0781
2 hours	0.058 ± 0.0161	0.072 ± 0.0154	0.082 ± 0.0192

By application of Student's paired t-test, to test the significance of the difference between Columns 1 and 3, the value of P is found to be less than 0.05 in the case of the 30 minutes' incubation, between 0.05 and 0.10 in the case of the 5 minutes' and the 15 minutes' incubations, and greater than 0.10 in the case of the 1 hour's and 2 hours' incubations. No difference between Columns 1 and 2 gives a value of P that is less than 0.10. Each value is the mean of 4 experiments with different animals.

Discussion. These results indicate that, although DNP strongly inhibits the initial absorption of L-phenylalanine before equilibrium is attained (Table 1), it has no effect on the reabsorption or release during the maintenance of the dynamic equilibrium. They suggest that amino-acids are accumulated against a concentration

gradient, energy being expended during the process, but after equilibrium has been reached, mediated release of amino-acids occurs, which is coupled to mediated uptake without further need of exogenous cellular energy. This could not be accounted for by outward diffusion followed by reabsorption by the usual pathway (as suggested by Agar et al., 1956), since the second process would be inhibited by DNP. Even if the release of amino-acid into the buffer alone (Table 2, column 3) occurs by simple diffusion - which is doubtful (Oxender and Christensen, 1963) - the more rapid exchange in the presence of unlabelled amino-acid must be mediated by a non-energy-requiring pathway.

It thus appears from our results that two processes exist in the intestinal mucosa for the transportation of amino-acids. The cells are able to concentrate amino-acids against a concentration gradient from either lumen or plasma (Christensen et al., 1963), this being a rapid process in the case of a lipophilic uncharged amino-acid. But there is also a second site which mediates an exchange cycle, which provides its own energy, and plays an important role in mucosal transport.

Our results show many similarities to those of Oxender and Christensen (1963), who gave evidence of dual mediation of amino-acid uptake by the Ehrlich ascites tumour cells. According to their results, the amino-acid is concentrated by the "alanine-preferring site" (which is equivalent to our DNP-sensitive site), whereas reversible efflux occurs via the "leucine-preferring site" (which is equivalent to our DNP-resistant site). We suggest that the intestinal mucosa absorbs all amino-acids via the DNP-sensitive site, the accumulation being dependent on exogenous energy supply, whereas the DNP-resistant site only comes into operation once a concentration gradient has been set up. The amount of amino-acid accumulated before the DNP-resistant site takes over, and the different affinities for the DNP-resistant site might explain the different countertransport effects and differential inhibitive properties of the various amino-acids, as enumerated by Oxender and Christensen (1963).

In vivo, the mucosa maintains a concentration of amino-acid that is higher than both the concentration in the serum and the concentration in the lumen' (unpublished results from this laboratory).

By mediated exchange via the DNP-resistant site with each of these compartments, without further expenditure of energy, it is able to maintain the constant distribution ratio between serum and lumen. But before this can take place, it has to accumulate amino-acid in high concentrations via the DNP-sensitive site, and thus may be able to act as a barrier and controlling mechanism between the blood and lumen.

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