THE UPTAKE OF AMINO ACIDS BY THE INTESTINE

by

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It has been shown by WISEMAN[1] and AGAR, HIRD AND SIDHU[2] that isolated loops of rat's intestine transfer L- but not D-amino acids against a concentration gradient. These workers therefore suggested that the process of transfer was an active one. The discovery that cyanide and dinitrophenol interfered with this process (AGAR *et al.*[2]) supports the suggestion. Up to the present no hypothesis based on experimental results has been proposed for the mechanism of active transfer. In the following experiments the uptake of histidine by the intestine was studied in order to test the hypothesis that uptake (presumably by the intestinal epithelium) is part of the mechanism of active transfer of amino acids. Histidine was used because the method of analysis is simple and specific.

In the study of uptake much simpler apparatus may be used than the perfusion apparatus needed for studying transfer across the intestinal wall. Incidentally, the method used makes it possible to provide control preparations. This is difficult in perfusion experiments as the activity of the intestine varies along its length (FISHER AND PARSONS[3]) and there is considerable individual variation between the activities of intestines from different animals. In the experiments here reported a length of intestine was cut into small segments which, after mixing, could be divided at random into two groups, so providing control preparations.

METHODS

The animals used were white rats weighing from 150–200 g. They were starved for 18–24 hours before the experiment in order to reduce the solid matter in the small intestine. The animals were anaesthetised with ether, the abdomen opened and the small intestine, excluding the duodenum, was cut at the upper and lower end, leaving the blood supply intact. The small intestine was washed through with warm saline and then removed. After dipping in warm saline to remove blood, the isolated intestine was placed on a wooden slab and cut into segments of approximately 0.5 cm length with a scalpel. The segments were washed for 5 min in Krebs bicarbonate buffer under the incubation conditions to be described. After washing and thoroughly mixing, the segments were dried with filter paper, divided into two equal groups, and transferred to the incubation vessels which contained 20 ml of Krebs bicarbonate buffer containing 0.5% glucose and either L- or D-histidine HCl. The incubation was carried out in a cup-shaped glass vessel of about 50 ml capacity with a sintered glass floor (No. 3 grade) connected to a glass tube through which 5% CO_2 in O_2 was passed. The sintered glass dispersed the gas into fine bubbles which served to saturate the fluid around the segments of intestine. The vessel was immersed for two-thirds of its height in a constant temperature bath at 38°. A stopper with a small hole was placed in the vessel to prevent loss of spray carried by the escaping gas.

At various intervals of time samples of the fluid were withdrawn for estimation of histidine. At the end of the experiment the segments were removed, dried with filter paper and then, depending on the information required, were either dried at 110° for 5 hours to obtain the dry weight or were homogenized and analysed for histidine after precipitation of the protein with 5% trichloroacetic

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acid. The amounts of histidine taken up by the segments of intestine were expressed as μmole per gram dry weight. The wet weight of tissue at the end of the experiment was about 2 g; the dry weight was between 18 and 23% of the wet weight.

Histidine was estimated by a modification of the method of MAC PHERSON[4]. The optical density was read at 498 mμ in a quartz spectrophotometer, Beckman Model DU, 30 min after colour development.

RESULTS

In studying the uptake of histidine it was more convenient in practice to estimate the loss of histidine from the external fluid than to estimate the amounts actually taken up by the intestine. In many cases, however, the intestinal segments were analysed for histidine. The representative figures quoted in Table I show that the uptake by the segments at the end of an experiment as measured by disappearance from the solution is in good agreement with the figure obtained by direct estimation of the histidine content of the segments.

TABLE I

AGREEMENT BETWEEN DISAPPEARANCE OF L-HISTIDINE FROM EXTERNAL FLUID AND AMOUNT RECOVERED FROM INTESTINE

Initial concn. in external fluid µmole per ml	*Amount disappearing from external fluid µmole*	*Amount recovered from intestine µmole*
5	22.8	23.6
10	30.1	33.5
15	37.2	43.8
nil	nil	3.9

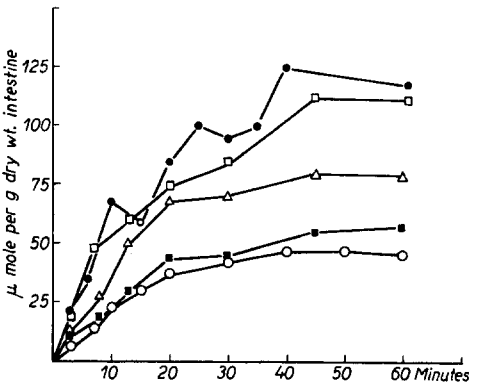

Fig. 1. Time course of uptake of L-histidine by intestinal segments. Curves from below upwards: initial concentration of L-histidine in fluid 5, 10, 15, 20, 30 μ mole per ml. Ordinate: L-histidine disappearing from fluid.

Fig. 1 shows the rate of uptake of L-histidine from solutions of various concentrations using intestines from different animals for each concentration. Each curve is the average of two experiments on segments from the same piece of intestine. The curves show that the amount of L-histidine taken up by the intestinal segments varied with the initial concentration in the external fluid. At the highest concentration the uptake curve became irregular in shape. Such irregular curves at concentrations of 30 μ mole per ml and over were repeatedly obtained.

Fig. 1 also shows that after a certain interval of time an equilibrium was established between the histidine in the tissue and in the surrounding fluid. As the concentration in the external fluid was raised the concentration of histidine inside the tissue cells at the equilibrium point was also raised. At equilibrium it is presumed that molecules of histidine are lost from the cells at the rate at which they are taken up.

Table II, derived from the same experiments illustrated in Fig. 1, shows the equilibrium concentrations in the external fluid and in the total water of the intestinal segments (estimated by loss of weight after 5 hours at 110°). It can be seen that as the concentration of L-histidine in the external fluid is increased the equilibrium ratio of intestinal concentration to outer concentration diminishes. It may be remarked here that if the amino acid is concentrated in only a part of the intestinal wall,

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presumably the epithelial cells, the ratio of concentrations between the active region of the intestinal wall and the external fluid will be higher than the ratios quoted. Moreover, it is possible that not all the intracellular water is available as solvent.

TABLE II

RATIO OF CONCENTRATION OF L- AND D-HISTIDINE BETWEEN EXTERNAL FLUID AND INTESTINE AT EQUILIBRIUM

Isomer used	*Initial external concn. μmole per ml*	*Amount taken up by intestine μmole per g dry weight*	*Conc. in water of intestine μmole per ml*	*Final external concn. μmole per ml*	*Ratio of intestinal to external concn.*
L-	5	45.2	11.3	3.82	2.96
L-	10	57.9	16.5	8.0	2.06
L-	15	80.9	18.4	12.4	1.48
L-	20	113.4	23.6	17.7	1.33
L-	30	126.3	31.6	25.9	1.22
D-	20	29.5	6.3	18.4	0.34

The equilibrium in concentrations of L-histidine between intestine and outer fluid was further illustrated by experiments of which the results presented in Fig. 2 are an example. In this experiment segments of intestine were incubated for 30 min in a medium containing 25 μ mole per ml of L-histidine. The amount of histidine contained in the segments was determined from the amount disappearing from the solution. The segments were then washed and transferred to Krebs solution without added histidine. Samples of the solution were withdrawn at intervals to follow the release of histidine from the intestinal segments. After about 50 min the histidine in the intestine reached equilibrium with the outer fluid (upper curve, Fig. 2). At equilibrium the concentration in the outer fluid was 3.69 μ mole per ml. The lower curve in Fig. 2 shows that the same equilibrium is reached from the other side. In this case segments from another piece of intestine were incubated in Krebs solution containing 5 μ mole per ml of L-histidine. Equilibrium between segments and surrounding fluid was reached at nearly

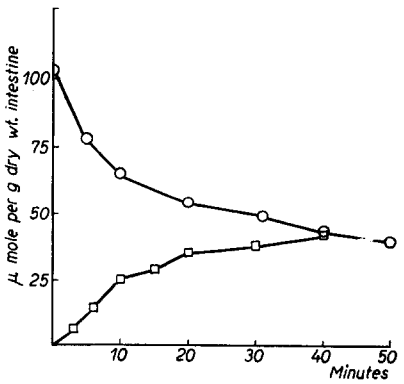

Fig. 2. Attainment of equilibrium between L-histidine in intestinal segments and external fluid. The same equilibrium is approached from either side (see text). Ordinate: L-histidine content of intestine derived from analysis of external fluid.

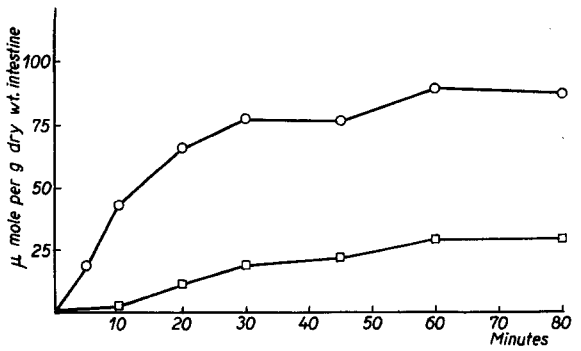

Fig. 3. Comparison of uptake of L- and D-histidine from fluid containing 20 μ mole per ml. Upper curve, L-histidine; lower curve, D-histidine. Ordinate: Histidine disappearing from fluid.

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the same uptake per gram dry weight. The final concentration in the outer fluid was 3.64 μ mole per ml, or almost the same as in the previous case.

The uptake of L- and D-histidine was studied separately in two experiments using segments from the same intestine. Fig. 3 shows that at the equilibrium point the segments take up about three times as much L-histidine as D-histidine when the initial external concentration is 20 μmole per ml in each case. In addition, the slope of the curves shows that the rate of uptake of L-histidine is much greater than that of the D-form. It can be seen from Table II that D-histidine is not concentrated in the total water of the intestine.

In the presence of 10^{-2} M cyanide in stoppered non-gassed vessels the uptake of D-histidine was unchanged. The uptake of L-histidine, on the other hand, was reduced almost to that of D-histidine, the equilibrium ratio of intestinal to outer concentration being 0.51. Fig. 4 shows the effect of $2 \cdot 10^{-4}$ M 2:4-dinitrophenol on the uptake of L-histidine from a solution containing 20 μ mole per ml. It can be seen that this compound reduces the uptake to about one-third of the control value. In this case the equilibrium ratio was 0.31 in the presence of the inhibitor.

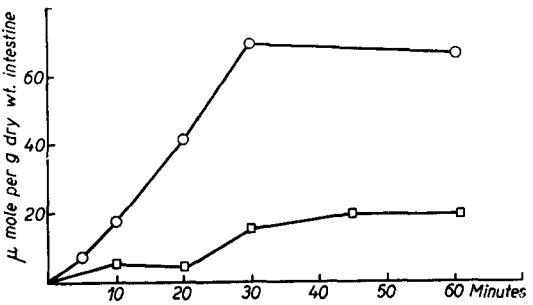

Fig. 4. Effect of $2 \cdot 10^{-4}$ dinitrophenol on uptake of L-histidine from fluid containing 20 μ mole per ml. Upper curve, control; lower curve, DNP. Ordinate: L-histidine disappearing from fluid.

DISCUSSION

The absorption of amino acids is one of the functions of the intestine and it has been shown that isolated perfused lengths of rat intestine will transport some amino acids from the lumen to the peritoneal side against a concentration gradient (WISEMAN[1]; AGAR *et al.*[2]). This finding makes it likely that one of the first steps in the overall transport of amino acids across the intestinal wall is their uptake by the epithelial cells. The results presented in this paper show that the uptake of L-histidine by intestinal segments takes place against a concentration gradient, that this process is specific for the L-form of this amino acid, and further that the process is inhibited by cyanide and 2:4-dinitrophenol. There are therefore close similarities between the processes of uptake and of transport by the intestine. Further, it has previously been shown (AGAR *et al.*[2]) that, at the end of an experiment on amino acid transport, an appreciable amount of amino acid is to be found in the intestinal wall.

If it is assumed, on the basis of these similarities, that the uptake process is a part of the transport process, then a hypothesis to explain the overall mechanism of transport can be made. The hypothesis suggested involves the further assumption that the mechanism for the transfer from the extracellular to the intracellular environment is asymmetrically distributed at the brush border surface of the epithelial cells. If this were so, then amino acid molecules would enter the cells against a concentration gradient from the lumen side, be distributed inside the epithelial cells and then diffuse out of the cells down a concentration gradient. The net result in such a system would be the transport of the amino acid across the intestinal wall against a concentration gradient.

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ACKNOWLEDGEMENT

One of us (G.S.S.) is grateful to the Commonwealth Technical Cooperation Scheme (Colombo Plan) for a Junior Fellowship and for a grant towards the expenses of the work.

SUMMARY

The uptake of L- and D-histidine by isolated segments of rat's intestine was studied. At about 50 minutes an equilibrium was reached between L-histidine in the intestine and in the surrounding fluid. At equilibrium the L-histidine reached a higher concentration in the total water of the intestine than in the surrounding fluid.

D-histidine was taken up in less amount than the L-form and did not become more concentrated in the intestine than in the surrounding fluid. Cyanide and dinitrophenol reduced the uptake of L-histidine approximately to that of the D-form.

It is suggested that the uptake of amino acids against a concentration gradient by intestinal cells is an important step in their active transfer across the intestinal epithelium.

RÉSUMÉ

Les auteurs ont étudié la résorption de la L- et de la D-histidine par des segments intestinaux de rat isolés. Après 50 minutes environ s'établit un équilibre entre la teneur en L-histidine dans l'intestin et sa teneur dans le liquide environnant. A l'équilibre, la concentration en L-histidine est plus élevée dans l'eau totale de l'intestin que dans le liquide qui l'entoure.

La D-histidine est moins absorbée que la forme L- et sa concentration n'est jamais plus élevée dans l'intestin que dans le liquide environnant.

Les auteurs suggèrent que la résorption des aminoacides en présence d'un gradient de concentrations par les cellules intestinales constitue une étape importante dans leur transfert actif à travers l'épithélium intestinal.

ZUSAMMENFASSUNG

Die Aufnahme von L- und D-Histidin aus isolierten Segmenten von Eingeweiden von Ratten wurde untersucht. Nach ungefähr 50 Minuten wurde ein Gleichgewicht zwischen L-Histidin in den Eingeweiden und in der umgebenden Flüssigkeit erreicht. Im Gleichgewichtszustand besitzt L-Histidin im gesamten Wasser der Eingeweide eine höhere Konzentration als in der umgebenden Flüssigkeit.

D-Histidin wurde in geringerer Menge als die L-Form aufgenommen und erreichte in den Eingeweiden keine höhere Konzentration als in der umgebenden Flüssigkeit. Cyanid und Dinitrophenol setzten die Aufnahme von L-Histidin ungefähr gleich stark wie die der L-Form herab. Es wird vermutet, dass die Aufnahme von Aminosäuren von den Eingeweidezellen gegen einen Konzentrationsgradienten einen wichtigen Schritt bei ihrer aktiven Übertragung im Eingeweideepithelium darstellt.

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Received November 21st, 1953