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THE ABSORPTION OF AMINO ACIDS BY TWIN LOOPS OF RAT INTESTINE

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Three methods of studying the removal of amino acids by intestinal tissue have been compared in a previous paper (AGAR, HIRD AND SIDHU[1]). One of these methods —uptake by numbers of small segments (0.5 cm)—has been studied in some detail. Using selected amino acids, L- and D-methionine, as inhibitors, it has been found that L- but not D-methionine inhibits the three processes, the uptake, transfer and absorption of L-histidine. Further, the content of L-histidine in the tissue at the end of the experiment was found to be affected in the same way as the uptake, transfer and absorption. As experiments with uptake gave similar results to the other two methods

References p. 393.

and are also the most convenient and precise to do, it has been concluded that they are a valid way to investigate the problem of absorption.

However, an uncertainty remained. In experiments on absorption using twin ligated loops *in vivo*, it was not practicable to use more than 1 ml of the amino acid solutions. A short experimental period was necessary in these experiments, and nearly all of the amino acid disappearing from the lumen was recovered from the intestinal wall at the end of the experiment. Therefore, little if any was removed from the intestinal wall by the blood stream. This meant that our studies on absorption, while they were *in vivo* studies, differed little in character from the studies on uptake. Accordingly, we have devised a new technique which independently circulates a larger volume of solution of L-histidine through twin loops of intestine. The duration of the experiment has been lengthened so that a much greater proportion of the amino acid, disappearing from the lumen, has been removed by the blood stream. The results show that, under the more physiological conditions used, the absorption of amino acids follows the same pattern as does the transfer and uptake of amino acids in *in vitro* systems.

METHODS

Young female rats, 100–150 g, were prepared essentially as given in a previous paper (AGAR *et al.*[1]) Two adjacent loops (signified upper, adjacent to duodenum, and lower) were cannulised and 20 m of Krebs bicarbonate buffer, at 37° ± 1°, containing the amino acid(s) (10 m*M*) was circulated through each loop, using the principle described by FISHER AND PARSONS[2] to circulate and gas the fluid. The circulation was continued for 40 minutes. At the end of this time both loops of intestine were removed, washed with 20 ml of warm saline and either slit longitudinally, mopped with filter paper, weighed and then dispersed in a glass homogeniser for determination of the histidine content (AGAR *et al.*[3]), or dried in an oven at 110° overnight to determine the dry weight. The hydrostatic head was 13 cm. The intestine and apparatus were drained at the end of the experiment and the volumes measured. It was found experimentally that 0.5 ml of the fluid is left undrained in the apparatus and, accordingly, this correction was applied to the volume of fluid at the end of the experiment.

The net weight of loops varied between 1.1 and 1.7 g. In experiments where pieces of intestine were dispersed for the estimation of histidine a dry weight of 18.2% of the fresh weight as found previously (AGAR *et al.*[1]) has been assumed.

Analytical method. Samples were withdrawn at varying intervals of time and the histidine content was estimated essentially by the method of MACPHERSON[4]; the diazonium salt, however, was prepared at 0°, 30 min before use, and the optical density read in a Beckman spectrophotometer (model DU) at a wavelength of 498 mμ.

RESULTS

The absorption of L-*histidine*

The results given in Tables I, III and IV show that with these rats the amount of L-histidine absorbed under the conditions used is not significantly different between the upper and lower loops. However, the group of rats used to obtain the results in Table II do seem to have a significant difference between the upper and lower loops. Such differences do not account for the inhibition of absorption in the presence of L-methionine since this amino acid inhibits when present in either loop.

The absorption of L-*histidine in the presence of* L- *and* D-*methionine*

Table II shows the effect of an equimolar concentration of L-methionine on the absorption of L-histidine by loops of intestine. The marked inhibition of uptake by L-methionine ($p < 0.01$) occurs when both upper and lower loops of the pairs are used as the

References p. 393.

TABLE I

ABSORPTION OF L-HISTIDINE

Replications	Upper loop Amount absorbed/g dry wt		Lower loop Amount absorbed/g dry wt	
	L-histidine (μmole)	water (ml)	L-histidine (μmole)	water (ml)
1	324	13.9	329	14.9
2	393	16.4	401	14.4
3	307	13.6	304	12.2
4	300	15.9	312	14.0
5	317	14.0	333	13.7
6	300	13.5	332	9.5
Mean	323	14.5	335	13.1

Absorption of L-histidine – upper and lower loop not significantly different. Absorption of water – upper and lower loop not significantly different.

control. The reduction in absorption by L-methionine is also accompanied by a reduction in the amount of L-histidine remaining in the intestinal tissue itself. This suggests that L-methionine inhibits the absorption of L-histidine by inhibiting its entry into the tissue and not on its release into the blood stream for, if this were so, the content of this amino acid in the intestinal tissue would be increased. This is perhaps further evidence to that given by AGAR *et al.*[1] that it is the uptake of amino acids by the tissue and not the release that is active.

Table III shows a similar experiment with D-methionine. The presence of D-methionine does not inhibit the uptake of L-histidine. Further, there has been no reduction in the histidine content of the intestinal tissue at the end of the experiment.

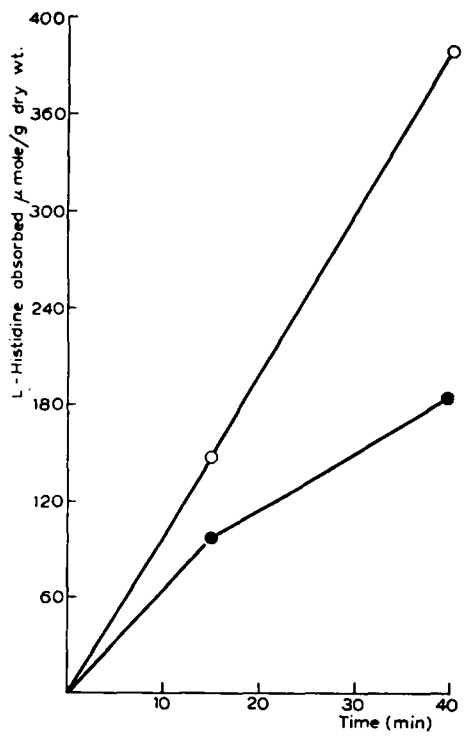

Fig. 1. Absorption of L-histidine in the presence and absence of L-methionine by twin loops of intestine; O—O upper loop L-histidine; ●—● lower loop L-histidine plus L-methionine.

Tables I and II show that the percentage recovery of histidine in the intestinal tissue at the end of the experiment over the histidine disappearing from the lumen is usually much less than 25%. It is assumed that the remainder has been removed by the blood stream.

Fig. 1 shows a typical time progress curve for the absorption of L-histidine in the presence and absence of L-methionine. It can be seen that the rate of absorption of L-histidine is linear during the whole of the experimental period and that L-methionine inhibits the absorption of L-histidine over the whole experimental period also. A similar result is obtained when the L-methionine is added to the upper loop.

References p. 393.

TABLE II

ABSORPTION OF L-HISTIDINE IN THE PRESENCE OF L-METHIONINE

Replications	L-Histidine: Position of loop	L-Histidine: Amount absorbed/g dry wt: L-histidine (μmole)	L-Histidine: Amount absorbed/g dry wt: water (ml)	L-Histidine: L-Histidine recovered from intestine (μmole/g dry wt)	L-Histidine: % Histidine recovered / Histidine absorbed	L-Histidine/L-Methionine: Position of loop	L-Histidine/L-Methionine: Amount absorbed/g dry wt: L-histidine (μmole)	L-Histidine/L-Methionine: Amount absorbed/g dry wt: water (ml)	L-Histidine/L-Methionine: L-Histidine recovered from intestine (μmole/g dry wt)	L-Histidine/L-Methionine: % Histidine recovered / Histidine absorbed
1	Upper	346	11.2	31	9	Lower	159	8.3	11	7
2	Upper	341	15.3	33	10	Lower	100	5.9	23	23
3	Upper	400	10.0	37	9	Lower	181	6.6	17	9
4	Lower	304	9.5	39	13	Upper	206	12.7	21	10
5	Lower	201	5.4	24	12	Upper	137	8.9	21	15
6	Lower	251	6.0	39	16	Upper	143	9.7	27	19
	Mean	307	9.6	34	11.5		154	8.7	20	13.8

Absorption of L-histidine – upper and lower loop significantly different, $p < 0.01$. Absorption of water – upper and lower loop significantly different, $p < 0.05$. Absorption of L-histidine in the presence of L-methionine significantly different, $p < 0.01$.

TABLE III

ABSORPTION OF L-HISTIDINE IN THE PRESENCE OF D-METHIONINE

Replications	L-Histidine: Position of loop	L-Histidine: Amount absorbed/g dry wt: L-histidine (μmole)	L-Histidine: Amount absorbed/g dry wt: water (ml)	L-Histidine: L-Histidine recovered from intestine (μmole/g dry wt)	L-Histidine: % Histidine recovered / Histidine absorbed	L-Histidine/D-Methionine: Position of loop	L-Histidine/D-Methionine: Amount absorbed/g dry wt: L-histidine (μmole)	L-Histidine/D-Methionine: Amount absorbed/g dry wt: water (ml)	L-Histidine/D-Methionine: L-Histidine recovered from intestine (μmole/g dry wt)	L-Histidine/D-Methionine: % Histidine recovered / Histidine absorbed
1	Upper	226	12.9	49	22	Lower	204	8.5	43	21
2	Upper	339	10.9	48	14	Lower	264	6.5	45	17
3	Upper	268	10.8			Lower	247	10.9		
4	Lower	254	7.2	42	16	Upper	257	15.4	41	16
5	Lower	295	9.1	40	14	Upper	298	14.8	36	12
6	Lower	288	10.5			Upper	292	12.3		
	Mean	278	10.2	45	16.5		260	11.4	41	16.5

Absorption of L-histidine – upper and lower loop not significantly different. Absorption of water – upper and lower loop significantly different, $p < 0.05$. Absorption of L-histidine in the presence of D-methionine not significantly different.

The absorption of L-*histidine in the presence of* L-*glutamate*

Analysis of the results given in Table IV do not show any significant effect of L-glutamic acid on the rate of absorption of L-histidine.

TABLE IV

ABSORPTION OF L-HISTIDINE IN THE PRESENCE OF L-GLUTAMIC ACID

Replication	*L-Histidine*			*L-Histidine/L-Glutamic acid*		
	Position of loop	*Amount absorbed/g dry wt*		*Position of loop*	*Amount absorbed/g dry wt*	
		L-histidine (μmole)	*water (ml)*		*L-histidine (μmole)*	*water (ml)*
1	Lower	310	10.0	Upper	260	10.7
2	Lower	363	17.0	Upper	416	17.3
3	Lower	401	16.1	Upper	377	14.3
4	Lower	332	13.9	Upper	348	12.0
5	Lower	238	11.1	Upper	270	10.6
6	Upper	243	10.7	Lower	241	3.6
7	Upper	299	11.7	Lower	241	8.4
8	Upper	458	20.8	Lower	417	19.6
9	Upper	257	11.4	Lower	260	9.7
10	Upper	214	11.9	Lower	165	8.5
	Mean	311	13.5		299	11.5

Absorption of L-histidine – upper and lower loop not significantly different. Absorption of water – upper and lower loop just significantly different, $p \approx 0.05$. Absorption of L-histidine in the presence of L-glutamic acid not significantly different.

The absorption of water

The results given in Tables II, III and IV, but not Table I, show a significantly lower absorption of water in the lower loop. This may account in part for the lower rate of absorption of L-histidine in the animals represented (replications 4–6) in Table II. It does not, however, account for all the inhibition given by L-methionine, because this is still present when this amino acid is added with L-histidine to the upper loop.

DISCUSSION

A comparison of the relationship between rate and duration of absorption given in the present paper (Fig. 1) and a similar relationship in experiments on uptake by segments (AGAR *et al.*[3]) shows that the former is linear but the latter is asymptotic at a much lower final figure of removal of L-histidine from the medium. As L-histidine is not measurably metabolised by segments of gut over the experimental period used (AGAR *et al.*[3]) the additional process removing histidine in the intact animal must be the blood stream. It is clear from Tables II and III that of the amount of amino acid disappearing from the lumen more than 75% has been removed by the blood stream. Such experiments are, therefore, more similar to *in vivo* conditions than are experiments involving uptake *in vitro*.

The inhibition of the absorption of L-histidine by L- but not by either D-methionine or L-glutamic acid, over a period of time when the blood stream is carrying an amino acid away from the intestine, establishes similarities between absorption *in*

References p. 393.

vivo and uptake *in vitro*. It seems from the present and a previous paper (AGAR *et al.*[1]) that not only does L-methionine inhibit the rate of removal of an amino acid from the medium but also the accumulation of that amino acid in the tissue both *in vivo* and *in vitro*.

These results suggest then that the rate of absorption is governed initially by the rate of uptake from the lumen presumably by the epithelial cells and that the rate of removal by the blood stream is determined by the concentration of amino acid accumulated in these cells; the amount of amino acid actually present in the tissue being a steady state resultant of these two processes. The inhibition of the uptake (*in vitro*) of L-histidine by L-methionine (AGAR *et al.*[1]) is much greater than the inhibition of absorption (*in vivo*). This is probably due to the continuous contribution of diffusion during the latter process in which the blood stream would accelerate the contribution by diffusion by preventing the attainment of an equilibrium.

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SUMMARY

A technique is described for studying the absorption, *in vivo*, of amino acids by twin loops of intestine in the rat. It has been found that L-methionine but neither D-methionine nor L-glutamic acid inhibits the absorption of L-histidine. The presence of L-methionine reduces the amount of histidine present in the intestinal wall during absorption. The implications of these results are discussed with reference to previous work using *in vitro* systems.

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