Selective Absorption of Stereo-isomers of Amino-acids from Loops of the Small Intestine of the Rat

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Amino-acids may be absorbed from the small intestine in two ways, either by diffusion alone, or by a method involving an active process in one or more steps. Previous work has supported both possibilities. Höber & Höber (1937) found that the rate of entry of glycine, alanine and valine was not proportional to the concentration in the gut. They also found that these amino-acids were absorbed faster than polyhydric alcohols of comparable molecular volume and concluded that an active process was involved. On the other hand, Kratzer (1944) reported that the rate of disappearance of amino-acids from the gut was inversely proportional to their molecular volume, while Chase & Lewis (1934), comparing the rates of disappearance of several L- and DL-amino-acids, found no difference between the L-isomers and the racemic mixtures. Simple diffusion would account for these results.

This problem has been reinvestigated using the stereochemically specific methods which are now available for the analysis of amino-acids. It has been found with thirteen amino-acids that the L-isomer disappears more rapidly than the D-isomer from a racemic mixture introduced into the small intestine and this is regarded as evidence for an active process in the absorption of amino-acids.

EXPERIMENTAL

Operative technique

Adult rats were anaesthetized by intraperitoneal injection of nembutal (6 mg./100 g. rat). The ileocaecal junction was located through a midline abdominal incision, and about 15 cm. of small intestine proximal to this was isolated, washed out with distilled water and tied off at one end. A ligature was placed loosely in position around the other end and a blunt hollow needle placed in the lumen of the intestine through the loop of ligature. The ligature was then tightened over the needle and the amino-acid, dissolved in 1 ml., was injected from a syringe. The needle was then withdrawn and the ligature tied off. The loop of gut, with blood supply intact, was then returned to the abdominal cavity and the abdominal wall sutured. The rat was maintained at body temperature during the experimental period, after which it was killed and the loop of gut between the ligatures removed. Its length was measured with the least possible degree of stretching and the interior washed out with distilled water into a 25 ml. volumetric flask. Measured portions of this solution were taken for analysis and compared with standards prepared from the stock solution of amino-acid used for injection. The use of intestinal loops was preferred to the method of Cori (1925), because the uncertainty of gastric emptying time is avoided. Furthermore, it is possible to use the same region of the intestine in every case and to measure the length of loop actually filled with solution. The disadvantages are those of anaesthesia and of the severe operative procedure.

Analytical methods

Analysis of L-*amino-acids.* (*a*) The L-amino-acid oxidase found in the culture medium from *Neurospora crassa* attacks a considerable number of amino-acids (Bender & Krebs, 1950). The mould was grown as described by these authors, and a dry preparation of the enzyme made by the method of Burton (1950). On the 14th day after inoculation the mycelium was filtered off and the medium brought to pH 6. It was concentrated *in vacuo* to about one-tenth of original volume at a temperature below 45°. Salts were partly removed by dialysis for 4 hr. against tap water; the contents of the sac were brought to pH 6·5, cooled to 0° and 1·5 vol. cold acetone added. The precipitate was centrifuged off and dried over P_2O_5. Such preparations retained their activity for several months at room temperature. The determinations were carried out in standard Warburg manometers. Each cup contained a 2 ml. sample, 1 ml. 0·1 M-pyrophosphate buffer pH 8·3 and 1 ml. enzyme solution in the side arm. The insets contained KOH papers. The cups were shaken at 39° in an atmosphere of O_2. The reaction proceeds almost directly to completion without much slowing down as the theoretical value is approached. (*b*) Bacterial decarboxylases for L-histidine, L-lysine, and L-glutamic acid were prepared and used according to Gale (1941) and Gale & Epps (1944). (*c*) L-Aspartic acid was determined by the method of Krebs (1951). In this method aspartic and α-ketoglutaric acids react to give oxaloacetic and glutamic acids. The oxaloacetic acid is decarboxylated with aniline and the glutamic acid with the bacterial decarboxylase.

Analysis of D-*amino-acids.* These were determined with the D-amino-acid oxidase of pig kidney prepared according to Bender & Krebs (1950). The pig kidney acetone powder (0·5 g.) was ground finely and shaken gently for 5 min. with 10 ml. 0·1 M-pyrophosphate buffer pH 8·3. The mixture was centrifuged and the clear supernatant used in the cups. The manometric determinations were carried out in exactly the same way as for the L-amino-acids except that 1 ml. of the pig kidney extract replaced the *Neurospora* enzyme.

Estimation of total amino-acids. Where only one isomer was determined by a specific method, the value for the other was obtained by difference, the total being determined with

Table 1. *Effect of amount of amino-acid on rate of disappearance*

(The amino-acid was contained in a volume of 1 ml., at pH 7·4. The experimental period was 0·5 hr. in each case. The analyses were made using the specific decarboxylase for the amino-acid concerned.)

Acid	Amount introduced (μmol.)	Amount disappearing (μmol.)	Gut length (cm.)	No. of experiments
L-Glutamic	225	68±5·2	12±1·4	14
	138	50±2·3	17±1·9	8
	68	20±1·9	16±0·6	8
L-Histidine	256	124±2·8	16±0·9	4
	116	84±2·3	13±2·0	15
	64	40±3·3	12±0·8	4

chloramine-T. This reagent appears, in general, to liberate rather more than the theoretical amount of CO_2 (Van Slyke, Dillon, MacFadyen & Hamilton, 1941). The results obtained with the various amino-acids are given in Table 2. Where there was an appreciable departure from theoretical values, the result with chloramine-T was regarded as being proportional to the amount of amino-acid present, and the total adjusted to correspond with the result given by the stereochemically specific method used. The determinations were carried out either at pH 2·4 or at pH 4·6 at 39°. 1 ml. samples with 1 ml. citrate buffer (M at pH 4·6: 0·1 M at pH 2·4) were placed in the main chamber and 1 ml. 10% (w/v) chloramine-T in the side-arm. The gas evolution was complete in all cases after 20 min.

Total histidine. This was determined colorimetrically by the method of Macpherson (1946).

Materials

Amino-acids were commercial samples used without further purification, except for DL-α-aminoadipic and DL-α-aminopimelic acids, for samples of which we are indebted to Prof. H. A. Krebs, F.R.S. The solutions introduced into the gut contained 150–250 μmol./ml. and were brought to pH 7·4. Leucine and norleucine, which were not sufficiently soluble to permit the preparation of solutions of this concentration at pH 7·4, were first dissolved with the aid of a small excess of alkali and brought to pH 7·4 by the addition of the required amount of HCl immediately before introduction into the gut.

RESULTS

Effect of gut loop length on disappearance of amino-acids

As it seemed likely that variation in the length of loop taken would affect the rate of absorption of the amino-acids, attempts were made, so far as possible, to keep the length constant at about 15 cm. There was, however, some variation in length of loop from rat to rat, particularly in the earlier experiments. Examination of the collected results with a view to detecting an effect of loop length yielded equivocal results, as in the case of most amino-acids the scatter of loop length was insufficient to allow of a definite conclusion being drawn. Accordingly, no allowance for length of loop has been made in calculating the results.

Effect of amount of amino-acid introduced on amount disappearing

The amount introduced was kept constant for any one amino-acid, and in all cases equal amounts of D- and L-isomers were placed in the loop. A small number of experiments were, however, carried out with L-glutamic acid and L-histidine, in which the amount of amino-acid introduced was varied. It seemed that, over the range studied, the amount disappearing was proportional to the amount introduced.

Relative rates of disappearance of stereo-isomers

The methods used for each amino-acid are given in Table 2 which also shows the percentage of the theoretical value obtained in each case, except for α-aminoadipic and α-aminopimelic acids which were not available in sufficient quantities for full study. The amounts of D- and L-isomers disappearing were calculated for each rat, and the mean amounts with their standard errors are given for each amino-acid in Table 3. Control experiments with dead rats were carried out with a few amino-acids. The rats were killed by bleeding immediately before the introduction of the amino-acid into the intestinal loop, but were otherwise treated in precisely the same way as the experimental animals. In all cases examined the amounts of both isomers disappearing were small, and the figures correspondingly somewhat uncertain, representing, as they did, small differences between large numbers. The results are shown in Table 4.

DISCUSSION

The difference in rate of disappearance of the stereo-isomers under identical conditions is regarded as evidence for the existence of a specific mechanism for the active absorption of L-amino-acids, since the rates of diffusion of both isomers would be the same. It seems unlikely that the results can be accounted for by bacterial action in the gut since the control experiments with dead rats did not give striking differences between the D- and L-isomers. Nor is it likely that so wide a range of amino-acids should be

Table 2. *Methods of analysis of amino-acids*

(Kidney and *Neurospora* oxidase: each cup contained 1 ml. 0·1M-pyrophosphate buffer pH 8·3, 2 ml. sample, 1 ml. enzyme, KOH insets, gas phase O_2, temp. 39°.
Chloramine-T: each cup contained 1 ml. 10% (w/v) chloramine-T, 1 ml. sample, 1 ml. M-citrate buffer pH 4·6 or 1 ml. 0·1M-citrate buffer pH 2·4, temp. 39°.
Transaminase: each cup contained in main chamber 0·5 ml. *Clostridium welchii* suspension, 0·2 ml. 2% Cetavlon, 0·5 ml. transaminase preparation, 1 ml. sample, 1 ml. 0·2M-acetate buffer pH 5. In side arm 0·03 ml. aniline, 0·2 ml. 0·2M-acetate buffer pH 5, 0·2 ml. 0·1M-α-ketoglutarate, temp. 39°.)

	L-Form		D-Form		Total		
Amino-acid	Method of analysis	Recovery (%)	Method of analysis	Recovery (%)	Method of analysis	pH	Recovery (%)
DL-Alanine	*Neurospora* oxidase	102	Kidney oxidase	98	—	—	—
DL-Phenylalanine	*Neurospora* oxidase	100	Kidney oxidase	106	—	—	—
DL-Methionine	*Neurospora* oxidase	98	Kidney oxidase	102	—	—	—
DL-Histidine	Decarboxylase	98	—	—	Colorimetry	—	—
DL-Valine	—	—	Kidney oxidase	104	Chloramine-T	2·4	102
DL-Leucine	*Neurospora* oxidase	100	—	—	Chloramine-T	2·4	107
DL-Isoleucine	—	—	Kidney oxidase	96	Chloramine-T	2·4	102
DL-Norleucine	*Neurospora* oxidase	106	Kidney oxidase	96	—	—	—
DL-Aspartic acid	Transaminase	96	—	—	Chloramine-T	4·6	102
DL-Glutamic acid	Decarboxylase	98	—	—	Chloramine-T	4·6	100
DL-α-Aminoadipic acid	*Neurospora* oxidase	—	—	—	Chloramine-T	2·4	—
DL-α-Aminopimelic acid	*Neurospora* oxidase	—	—	—	Chloramine-T	2·4	—
DL-Lysine	Decarboxylase	98	—	—	Chloramine-T	2·4	103

Table 3. *Rates of disappearance of stereo-isomers*

(The amino-acids were contained in 1 ml. at pH 7·4, except for leucine and norleucine where the volume was 1·4 ml. The mean amounts of the isomers disappearing are given in μmol. together with the standard error of the mean in each case.)

Acid	Amount introduced (μmol.)	Experimental period (hr.)	Disappearance (μmol.) L-acid	Disappearance (μmol.) D-acid	L/D ratio	Gut length (cm.)	No. of experiments
DL-Alanine	256	0·5	48±2·3	22±1·8	2·2	14±1·0	12
DL-Phenylalanine	156	0·5	54±3·3	10±1·3	5·4	17±1·6	10
DL-Methionine	272	1·0	104±5·2	64±3·8	1·6	16±1·3	12
DL-Histidine	232	0·5	84±2·3	14±2·1	6·0	13±2·0	14
DL-Valine	222	0·5	69±6·9	22±2·7	3·1	16±1·3	10
DL-Leucine	200	0·5	48±2·8	21±1·7	2·3	18±1·2	14
DL-Isoleucine	184	0·5	54±1·7	25±1·5	2·2	16±1·2	10
DL-Norleucine	282	0·75	55±3·3	32±2·7	1·7	21±1·6	10
DL-Aspartic acid	206	0·5	46±2·9	17±1·8	2·7	19±1·2	10
DL-Glutamic acid	192	0·5	49±3·3	10±2·7	4·9	18±2·0	14
DL-α-Aminoadipic acid	146	0·75	31±4·0	16±2·2	1·9	13±0·9	4
DL-α-Aminopimelic acid	244	0·75	46±9·0	19±4·5	2·4	14±0·9	4
DL-Lysine	168	1·0	38±6·2	10±2·6	3·8	15±2·5	12

Table 4. *Rates of disappearance of stereo-isomers in dead rats*

(The experimental period was 0·5 hr. in each case, except for DL-lysine for which a period of 1 hr. was allowed. Conditions were as in Table 3 except that the experiments were carried out with dead rats.)

Acid	Amount introduced (μmol.)	Disappearance (μmol.) L-acid	Disappearance (μmol.) D-acid	Gut length (cm.)	No. of experiments
DL-Phenylalanine	156	5±1·7	4±0·9	21±1·8	4
DL-Histidine	232	24±3·3	17±4·9	17±3·7	4
DL-Glutamic acid	192	20±3·6	6±1·7	28±3·5	4
DL-Lysine	168	10±2·0	5±1·7	19±2·1	4

attacked at comparable speeds by the reduced bacterial flora remaining after washing out the gut. It also appears unlikely that the amino-acids are oxidized in the gut, since the intestinal mucosa has not been reported to be a rich source of amino-acid oxidases. It should be stressed, however, that the experiments described here do not establish that the amino-acids are in fact transferred to the blood stream, and it is for this reason that the term amino-acid disappearance has been used in preference to absorption.

It is uncertain how the rate of amino-acid disappearance is influenced by concentration. If the simplest assumption is made, namely that the rate of disappearance is proportional to the concentration, it seems quite unjustifiable to draw quantitative conclusions from the relative rates of disappearance of the different amino-acids, because the percentage of each which disappeared in the experimental period was not the same in every case. The figures for the L/D ratio (Table 3) are open to a similar criticism. They have, however, the merit of being minimum figures. Using, for example, the assumption just mentioned, it is easy to calculate that the L/D ratio for alanine would be 3·2 instead of 2·2 if it were possible to make an instantaneous measurement of the relative rates for the two isomers. On the other hand, to obtain the greatest absolute difference in the amount of the two isomers disappearing, a long experimental period is desirable. If k_1 and k_2 were the velocity constants for the disappearance rates of the two isomers, the optimum length of experiment would be given by

$$t = -\frac{1}{k_1 - k_2} \log k_2/k_1.$$

Thus for DL-histidine the optimum length of experiment would appear to be about 1·25 hr., but the L/D ratio would be only 3·8 as compared with the observed value, for an experimental period of 0·5 hr. of 6·0.

SUMMARY

1. The rate of disappearance of the D- and L-isomers of thirteen amino-acids has been studied in an isolated loop of rat small intestine.

2. In each case the L-isomer disappeared faster than the D-isomer.

3. This is regarded as evidence for an active process in the absorption of amino-acids.

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Methods for Isolating ω-Amino-acids: γ-Aminobutyric Acid from Rye Grass

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Work is in progress here on the chemical reactions which are undergone by dietary constituents, especially those from green leaves, in the course of digestion in the ruminant. The occurrence of ω-amino-acids in plant juices and as products of microbial degradation of common amino-acids in the rumen necessitates systematic procedures for the isolation of these compounds.

Three methods are now described which in suitable combinations permit systematic isolation of the ω-amino-acids. These procedures can be used on the micro scale for metabolic studies with isotopic tracers. They are additional to the ion-exchange methods of Westall (1950) and Lederer & Pavlov (see Lederer, 1949). The present methods have so far been used for isolating γ-aminobutyric acid from among the dialysable constituents of rye grass (*Lolium perenne* L.), as described below, and in studying the production of δ-aminovaleric acid in the rumen of the sheep (cf. Shazly & Synge, 1950).