Site and rate of intestinal absorption of various amino acids in the chicken in situ

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SPENCER and SAMIY^{15,16)} reported that L-tryptophan and L-phenylalanine was absorbed faster from the middle part of the rat small intestine than from the lower part. MATTHEWS and LASTER⁶⁾ described that the transport of valine, glycine, leucine, alanine and α -aminoisobutyric acid was the slowest in the duodenum and the fastest in the middle to lower-middle part of the hamster small intestine. NATHANS *et al*¹⁰⁾ also observed the maximum transport of moniodotyrosine from the terminal ileum in rats and hamsters. On the other hand, maximum absorption of cystine and cysteine from the rat intestine was observed in the duodenum and minimum absorption in the ileum⁹⁾. In chickens, it was reported that 70~90% of the nitrogen leaving the duodenum was absorbed from the upper half of the jejunum, and very little amount was absorbed from the ileum⁸⁾. Because of these contradictory results, the present experiment was carried out to investigate systematically the site of absorption of various amino acids in the chicken small intestine.

Experimental

Determination of amino acid absorption from the intestine

Single Comb White Leghorn cockerels, weighing 1.3 to 1.8 kg, were used in the present experiment. The birds were fasted for 24 hours prior to the experiment to make the solid matter in the intestine lessen. They were anesthetized by the intravenous injection of sodium pentobarbital at a level of 25 mg per kg of body weight. The abdomen of the birds was opened and an intestinal segment with intact blood circulation was connected to the perfusing unit, then intestinal loop was perfused. Details of the perfusing unit and the perfusion procedure (recirculation method) were described in the report by TASAKI and TAKAHASHI¹⁸⁾.

In order to compare the absorption rate of amino acids from the various parts of the intestine, the segment was taken from the following 5 parts; duodenum, upper jejunum, lower jejunum, upper ileum and lower ileum. The amino acids used in the present perfusion experiment were D- and L-forms of chemically pure grade methionine, alanine, phenylalanine, glutamic acid and lysine. Alfa-aminoisobutyric acid, non-metabolizable in animals⁷⁾, was used as a reference amino acid. Amino acids were dissolved in Krebs-Ringer phosphate buffer (pH 7.4) at the concentration of 10 mM and used as a perfusate. The perfusate was maintained at 41°C during the perfusion. The perfusion was lasted for one hour. The rate of amino acid absorption was determined by the disappearance of amino acid from the perfusate. The micro-Kjeldahl

method was employed to determine the total nitrogen in the perfusate. From the preliminary test, rather high endogenous nitrogen excretion was observed in the duodenum, being $4.2 \mu\text{g/ml}$ of the perfusate, followed by the excretion in the upper jejunum, being $2.8 \mu\text{g/ml}$. The other three segments, the lower jejunum, the upper ileum and the lower ileum, excreted rather little endogenous nitrogen, being less than $1.4 \mu\text{g/ml}$.

Determination of simple diffusion of amino acids

Simple diffusion rate of amino acids was determined by a cellophane membrane diffusion apparatus. Cellophane membrane was attached at the bottom of the inner cylinder into which amino acid solution was drained, and the outer container was filled by Krebs-Ringer phosphate buffer (pH 7.4). Amino acids were dissolved in Krebs-Ringer phosphate buffer (pH 7.4) at the concentration of 10 mM. The inner solution was agitated by bubbling air and the outer fluid was stirred by a magnetic stirrer. The height of both inner and outer solutions was kept at the same level, and the temperature of the solutions was maintained at 41°C . The concentration of the amino acid in the inner solution was determined after one hour diffusion, and an amount of amino acids disappeared was regarded as a rate of simple diffusion.

Results and discussion

Rates of absorption of L- and D-amino acids and α -aminoisobutyric acid from the various parts of the small intestine during one hour perfusion were illustrated in Figure 1.

L- and D-forms of methionine, alanine and phenylalanine were chosen as a representative of neutral amino acids. The maximum absorption of L-alanine and L-phenylalanine, was noted

Fig. 1. Absorption rate of amino acids in various parts of the small intestine during one hour perfusion. Vertical bars show the standard errors of mean. Number of birds used is shown in parentheses.

Amino acid absorption in chicken intestine

in the lower ileum. The absorption rate of L-methionine and L-alanine was almost the same in the duodenum and the jejunum, but the absorption rate of L-alanine in the ileum was larger than that of L-methionine. In all parts of the intestine the absorption rate of phenylalanine was smaller than that of two other amino acids. Compared the absorption rates of L- and D-forms of amino acids, the former was always larger than the latter. This fact means that L-amino acid is more easily absorbed from the intestine than D-amino acid.

SCHEDL and CLIFTON¹³⁾ reported using perfusion method in situ that the absorption rate of L-methionine declined progressively from the proximal to the distal part of the human small intestine. The present experiment showed, on the contrary, that the absorption rate of L-methionine was almost the same in the duodenum and the jejunum, and in the ileum it was larger than the upper three segments though the difference was very small. SPENCER and SAMIY¹⁶⁾ and SPENCER and BRODY¹⁷⁾ reported that the site of the rat and hamster intestine showing the most rapid absorption of L-phenylalanine was the lower jejunum and the upper ileum, and RAMASWAMY and RADHAKRISHNAN¹³⁾ reported that the proximal jejunum and the distal jejunum of rats were the sites showing the most active absorption of L-phenylalanine. In the present experiment the maximum absorption was found at the lower ileum, and the lowest absorption was found at the duodenum. Therefore, the transport pattern of L-phenylalanine in rats seems to be quite different from that of the present experiment in chickens.

As a basic amino acid, lysine was used in the present experiment. There was no difference in absorption of L-lysine from the jejunum and the ileum, but from the duodenum the absorption rate was significantly small. The absorption rate of D-lysine from the jejunum was larger than that from the other segments.

MCLEOD and TYOR⁸⁾ reported that the maximum transport of L-lysine was observed in the middle part of the small intestine in hamsters, but LARSEN *et al*⁵⁾ noted that L-lysine was absorbed along the small intestine at almost the constant rate in rats. FEARON and BIRD³⁾ also reported using everted sacs of the chicken intestine that L-lysine transport was almost the same in segments from the upper jejunum to the lower ileum in one hour incubation. In their report the transport at the duodenum was not indicated. The present experiment showed that in chickens L-lysine was absorbed at the very slow rate from the duodenum, and at the rather rapid rate from the jejunum and the ileum. The absorption of L-lysine and D-lysine from the most parts of the intestine (the jejunum and the lower ileum) was almost the same, whereas the other amino acids studied here showed the different absorption rate between L- and D-forms; L-amino acids were always more rapidly absorbed than D-amino acids. Moreover, D-lysine absorption from the duodenum was more rapid than L-lysine. These phenomena are very specific in the lysine.

As an acidic amino acid glutamic acid was used in the present experiment. L-glutamic acid was absorbed more rapidly in all parts of the intestine than D-glutamic acid. Rapid absorption of L-glutamic acid was observed at the upper jejunum and the lower ileum, but this amino acid was absorbed rather slowly from the duodenum and the upper ileum. In D-glutamic acid the absorption rate from the duodenum and the upper jejunum was very small, and from the lower jejunum and the ileum this amino acid was absorbed at almost the constant rate.

Alfa-aminoisobutyric acid was used as a non-metabolizable amino acid in the present experiment. The absorption pattern of this amino acid was almost the same as that of L-lysine or L-glutamic acid, showing the slowest rate in the duodenum, and almost the constant rate in the other segments.

The site of intestine in D-amino acid absorption has not yet been reported by any worker. In the present experiment, the absorption rate of D-amino acids in various parts of the intestine was investigated in methionine, alanine, phenylalanine, lysine and glutamic acid. As a result, the absorption pattern of D-lysine in the jejunum and the lower ileum was almost the same as that of L-lysine, whereas D-forms of the other 4 amino acids were absorbed more slowly than L-forms of the corresponding amino acids. Moreover, the absorption pattern of L- and D-lysine and L-glutamic acid was almost similar to that of α -aminoisobutyric acid, and the absorption rates of D-glutamic acid at all the sites of the intestine, of D-methionine at the upper jejunum, and of D-phenylalanine at the duodenum and the upper jejunum, were smaller than that of α -aminoisobutyric acid.

JERVIS and SMYTH⁴⁾ reported using everted sacs of the rat small intestine that D-methionine could be moved against a concentration gradient, and this movement was inhibited by dinitrophenol or cyanide treatment, or by the presence of L-methionine. They suggested from this result that the active transfer mechanism was not absolutely specific only for L-methionine, but also for D-methionine, even though D-methionine had a lower affinity for active transfer mechanism than L-methionine. They also described in their report that the active transfer of D-methionine did not necessarily mean that all D-amino acids could be absorbed actively. On the other hand, PAINE *et al.*¹¹⁾ reported using permanent Thiry-Vella fistulae of the chicken intestine that the absorption of D-methionine was not inhibited by a treatment of dinitrophenol. They concluded from this fact that D-methionine was absorbed only by simple diffusion.

Table 1. Simple diffusion rate of amino acids determined by cellophane membrane

Amino acid	D-enantiomorph	L-enantiomorph
	μ mole/cm ² /hr	μ mole/cm ² /hr
Methionine	3.2 (133)	3.2 (133)
Alanine	4.0 (167)	4.0 (167)
Phenylalanine	2.4 (100)	2.4 (100)
Lysine	2.4 (100)	2.4 (100)
Glutamic acid	2.4 (100)	2.4 (100)
α -Aminoisobutyric acid	2.4 (100)	

Relative diffusion rate (ratio of diffusion rate of amino acid to that of α -aminoisobutyric acid) is shown in parentheses

Table 2. Relative absorption rate* of amino acids in various intestinal segments

Amino acid	Duodenum	Upper jejunum	Lower jejunum	Upper ileum	Lower ileum
L-methionine	204	148	153	162	183
D-methionine	101	55	123	127	157
L-alanine	214	149	155	182	227
D-alanine	151	94	102	104	128
L-phenylalanine	128	110	130	119	176
D-phenylalanine	53	36	73	78	120
L-lysine	85	94	94	100	106
D-lysine	112	92	100	68	92
L-glutamic acid	120	115	107	86	132
D-glutamic acid	47	23	66	61	66
α -aminoisobutyric acid	100	100	100	100	100

* Absorption rate of α -aminoisobutyric acid was taken as 100%

Amino acid absorption in chicken intestine

In order to investigate whether or not D-methionine was absorbed actively, simple diffusion rates of amino acids were determined, and the result is shown in Table 1. The diffusion rates of amino acids are indicated as the quantities passing through the cellophane membrane in one hour (μ moles/cm²/hr), and the relative rates are shown as the ratios of the diffusion rate of amino acids to that of α -aminoisobutyric acid. From the table, the diffusion rates of L- and D-forms of each amino acid studied here are completely the same, being 133% of the relative rate in methionine, 167% in alanine, and 100% in phenylalanine, lysine and glutamic acid.

Ratios of the absorption rate of amino acids to that of α -aminoisobutyric acid are listed in Table 2. From the following facts, AKEDO and CHRISTENSEN¹⁾ concluded that α -aminoisobutyric acid was transported by passive diffusion in rat everted sacs; 1) the transport rate of α -aminoisobutyric acid was almost proportional to the mucosal concentration, and also 2) dinitrophenol did not affect on α -aminoisobutyric acid transfer. The former fact also suggests that the self-inhibitory effect in α -aminoisobutyric acid absorption is not existent. If α -aminoisobutyric acid is not absorbed by active mechanism but only by simple diffusion, the ratios shown in Table 2 indicate whether the amino acids are absorbed by active mechanism or only by simple diffusion.

The relative absorption rate of L-methionine was 148 at the upper jejunum as the lowest to 204 at the duodenum as the highest, whereas the relative diffusion rate was 133. This result indicates that L-methionine is absorbed actively along all parts of the small intestine. The relative absorption rate of L-alanine was 149 to 227, but the relative diffusion rate was 167. Therefore, this amino acid seemed to be absorbed actively from the duodenum and the ileum, but not from the jejunum. L-phenylalanine was also absorbed actively in all parts of the small intestine. The relative absorption rate of D-alanine and D-phenylalanine was smaller than the relative diffusion rate of the corresponding D-amino acid, and this fact indicates that the self-inhibitory effect occurs in the lower ileum except for D-phenylalanine. This phenomenon could be explained by the result of SCHEDL *et al.*¹⁴⁾. They studied the relationship between the absorption rate of α -aminoisobutyric acid in the rat intestine and its accumulation in tissues, and described that the jejunal segment showed high absorption rate but low tissue concentration, and the ileac and the duodenal segment showed high tissue accumulation but low absorption rate. Therefore, the absorption rate of amino acid would partly be regulated by the accumulation of amino acid in the intestinal tissue, and this regulation is considered to be the self-inhibitory effect. However, the reason why such a phenomenon occurs is not solved.

In most parts of the intestine D-methionine (except for the lower ileum) also showed the self-inhibitory effect, but this amino acid seemed to be absorbed actively from the lower ileum. The relative absorption rate of L- and D-lysine (except for the upper ileum) was almost the same as the relative diffusion rate, being about 100. It could be considered that these amino acids were absorbed by simple diffusion. Only in the lower ileum the active absorption was significantly observed in L-glutamic acid. In contrast with L-glutamic acid, the relative absorption rate of D-glutamic acid was much smaller than the relative diffusion rate, and this fact showed the very high self-inhibitory effect occurred in D-glutamic acid.

Summary

Studies have been made on the sites and the rates of absorption of representative neutral, basic and acidic L- and D-amino acids in the chicken intestine in situ. L-methionine and L-alanine were absorbed more slowly from the duodenum and the jejunum than from the ileum.

D-methionine and D-alanine were absorbed maximumly in the lower ileum. The absorption rates of L- and D-phenylalanine had a trend to increase progressively from the duodenum to the lower ileum. The absorption rate of L-form was always larger than that of D-form in these 3 amino acids. L-lysine, a basic amino acid, was absorbed more rapidly from the jejunum and the ileum than from the duodenum, but D-lysine from the jejunum than from the other segments. The most outstanding characteristic of this amino acid is that the absorption rate of L- and D-form was almost the same except for the duodenum and the upper ileum. L-glutamic acid, an acidic amino acid, was rapidly absorbed from the upper jejunum and the lower ileum than from the other segments. D-glutamic acid was absorbed minimumly in the duodenum and the upper jejunum. The absorption pattern of α -aminoisobutyric acid, a non-metabolizable amino acid, was very similar to that of L-lysine. If α -aminoisobutyric acid was absorbed only by simple diffusion, the ratio of absorption rate of amino acids to that of α -aminoisobutyric acid indicates the extent of the active amino acid absorption. As a result, L-methionine, L-alanine (except for the jejunum) and L-phenylalanine from all the sites of the intestine was absorbed by active mechanism, but L-lysine and D-lysine from the most part of the intestine was not absorbed actively but by simple diffusion. Moreover, most of D-amino acids showed the self-inhibitory effect, which resulted in the lowering of the absorption rate.

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鶏腸管におけるアミノ酸吸収の部位による変動

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鶏腸管からのアミノ酸吸収が、腸部位によって、どのように変動するかを観察するために本実験を実施した。

実験鶏として、約4カ月令、体重1.3~1.8kgの白色レグホン種雄を使用した。小腸を十二指腸、空腸前部および後部、回腸前部および後部の5部位に分け、田先・高橋の *in situ* 還流装置を用い、Krebs-Ringer phosphate buffer に10mmの濃度に溶解したアミノ酸について、1時間の還流実験を行なった。

中性アミノ酸である L-メチオニンおよび L-アラニンは、回腸よりも十二指腸および空腸で多く吸収された。D-メチオニンと D-アラニンは、回腸下部で最大吸収を示した。フェニールアラニンは、L型もD型も部位が下るほど吸収量は多くなった。塩基性アミノ酸のリジンは、空腸および回腸に比較して、十二指腸での吸収量は多く、D-リジンの吸収量は空腸で多かった。リジンの特徴は、十二指腸および回腸上部を除いて、L型とD型の吸収量がよく似ていることである。酸性アミノ酸である L-グルタミン酸は、空腸下部および回腸下部で吸

収量は多い。D-グルタミン酸は、十二指腸および空腸上部で吸収量が少ない。また、L型がD型より全部位を通じて吸収量が多かった。動物体内では代謝されないとされている α -アミノイソ酪酸は、L-リジンとほぼ同程度に吸収された。

α -アミノイソ酪酸が単純拡散で吸収されると仮定すると、おのおのアミノ酸の吸収率と α -アミノイソ酪酸のそれとの比は、各アミノ酸の活性吸収の程度を示す指標になると考えられる。この比より判断すると、L-メチオニン、L-アラニン(空腸は徐く)および L-フェニールアラニンはすべての部位で活性吸収が行なわれているが、L-リジンおよび D-リジンはむしろ拡散により吸収されるものと考えられる。L-グルタミン酸は、部位により活性吸収が存在する。D型アミノ酸では、多くの場合、特に D-フェニールアラニンや D-グルタミン酸などで、自己吸収抑制作用があらわれて、吸収が阻害される。