The Accumulation of Amino Acids by Various Preparations of Dog Intestine

In a previous paper[1], the amino acid absorption of tissue preparations from a surgical specimen of human small intestine was determined by in vitro incubation of the preparations in a solution of radioactive amino acid. The results indicated a high uptake of amino acid by the intestinal muscle, even when separated from the mucosa. CRANE and MANDELSTAM[2] have obtained similar results with hamster tissue. In this communication the amino acid uptake of isolated intestinal muscle is characterized to determine the degree of active transport exhibited by the muscle.

Methods. A length of small intestine was obtained from a large dog (30–50 kg) under nembutal anaesthesia (30 mg/kg). The sample was transferred immediately to sterile saline. The intestine was pinned to a board, opened, and the mucosa gently stripped from the muscle. Fragments of muscle and mucosa, of wet weight approximately 25 mg, were incubated for 1 h in oxygenated Krebs buffer at 37°C. The buffer contained low specific activity amino acids. After incubation, the tissue was rapidly washed in buffer, weighed and dissolved in 0.1 ml of 30% KOH. The solution was counted in a liquid scintillation counter as previously described[1], and the activity compared with that of the incubation medium to calculate the accumulation of the amino acid.

Results. L-phenylalanine absorption and its inhibition by 2:4 dinitrophenol (DNP) by the intestinal tissue preparations are shown in Table I. Whereas the mucosa concentrates the amino acid against a concentration gradient, the muscle alone is unable to do so. The absorption of phenylalanine by the mucosa is inhibited by DNP, but the absorption by the muscle is not inhibited by DNP. Absorption of the labelled phenylalanine into the muscle tissue is due solely to diffusion, as this absorption does not produce a concentration gradient nor is it inhibited by DNP.

In the whole tissue, amino acid concentration is less than in the case of the mucosa alone and DNP inhibition is diminished. These findings suggest that there is a high concentration of amino acid in the mucosa and a low concentration in the muscle, rather than a uniform concentration in the whole tissue caused by amino acid diffusing from the mucosa into the muscle. In a second experiment, whole tissue was separated into muscle and mucosa after incubation. The results are shown in Table II. It is seen that the amino acid concentration is much higher in the mucosa than in the muscle.

If two amino acids share the same transport system requiring energy, then each amino acid inhibits the absorption of the other. In Table III the results are shown of the effect of methionine on the absorption of labelled proline. Methionine inhibits mucosal absorption of proline by 36% but the muscle absorption is reduced by only 7%. Active

Table II. Separation of muscle from mucosa after the incubation

Incubation medium	Mucosal concentration	Muscle concentration
5 m*M* L-phenylalanine alone	0.932 ± 0.139	0.441 ± 0.035
5 m*M* L-phenylalanine and 5 m*M* L-methionine	0.492 ± 0.055	0.380 ± 0.057
5 m*M* L-phenylalanine and 1 m*M* DNP	0.531 ± 0.031	0.367 ± 0.033

Incubations of whole tissue in the solutions stated is carried out. After the incubation, the tissue is divided into muscle and mucosa, and the concentration is measured in each separately. Concentrations given in μM/100 mg tissue (wet weight). Each value is the mean of six determinations ± one standard deviation.

Table III. Inhibition of the uptake of L-proline by L-methionine

Preparation used	Absorption Proline alone	Proline with methionine	% inhibition
Mucosa alone	0.592 ± 0.031	0.378 ± 0.019	36.2%
Muscle alone	0.376 ± 0.013	0.348 ± 0.012	7.4%

Fragments of mucosa or muscle incubated in 5 m*M* L-proline with or without the addition of 5 m*M* L-methionine as competitive inhibitor. Each value of absorption (in μM/100 mg wet tissue) is the mean of six determinations ± one standard deviation.

Table I. Inhibition of L-phenylalanine uptake by 2:4-dinitrophenol

Preparation used	Absorption in absence of DNP	Tissue concentration	Absorption in presence of DNP	Tissue concentration	Inhibition by DNP
Mucosa alone	0.792 ± 0.078	9.9 m*M*	0.394 ± 0.041	4.9 m*M*	50.3%
Muscle alone	0.398 ± 0.017	5.0 m*M*	0.403 ± 0.010	5.0 m*M*	—
Whole tissue	0.529 ± 0.051	6.6 m*M*	0.395 ± 0.017	4.9 m*M*	25.4%

Fragments of dog intestine, either intact or divided into component muscle and mucosa, are incubated 1 h at 37°C in a solution of lightly-labelled 5 m*M* L-phenylalanine in oxygenated Krebs bicarbonate buffer (containing 0.2% glucose) in the presence or absence of 1 m*M* 2:4-dinitrophenol. Accumulation of the amino acid in the tissue is expressed in μM/100 mg tissue (wet weight). Each value is the mean of six determinations ± one standard deviation.

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transport is therefore involved in the mucosal uptake of proline but is minimal or absent in the isolated muscle.

Discussion. The experiments reported here show that amino acids are not accumulated by an active transport mechanism by excised dog intestinal muscle. More surprisingly, there is a barrier to diffusion of amino acids from the mucosa into the muscle when the two tissues are incubated together (such as that described by LIFSON and PARSONS[3] for sugars). After an hour's incubation, the amino acid, accumulated to a high degree in mucosal tissue, is found in lower concentration in the muscle cells. This is in agreement with our previous findings that the concentration in the muscle is the same whether it is separated from the mucosa before or after the incubation, but contrary to the results of CRANE and MANDLESTAM[2], who found that for sugars there was free diffusion from mucosa to muscle but amino acids were absorbed by the muscle against a concentration gradient. There may be a species difference (these authors used the hamster) and the hard thick muscle of the dog may present a severe barrier to diffusion.

These results clearly indicate that considerable care should be taken when interpreting the results of in vitro experiments on amino acid uptake because of the barrier which may exist to free diffusion between the component tissues in the samples. Whenever possible, such experiments should be carried out with pure mucosal tissue, especially in the cases where the tissue comes from a large animal, where variations in the proportion of mucosa to muscle can lead to large errors. The major advantages of the accumulation method of AGAR, HIRD, and SIDHU[4] on which this method is based, are the simplicity, the reproducibility and the possibility of using multiple parallels to increase the statistical significance of the results; these advantages are easily lost if allowance is not made for the properties of the muscle tissue in the fragments[5].

Résumé. On a séparé le tissu intestinal en muqueuse et muscle, lesquels ont été incubés in vitro dans une solution d'acide aminé marqué; ainsi on a pu étudier l'absorption intracellulaire de l'acide aminé. On a trouvé que la musculature intestinale ne peut accumuler un acide aminé contre un gradient de concentration. Il semble qu'il existe dans le muscle une barrière contre la diffusion dont la significance est discutée.

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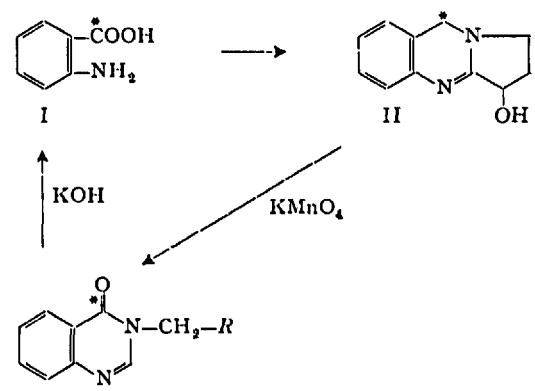
Über den Einbau von Anthranilsäure in Peganin

Zur kleinen Gruppe der Chinazolinalkaloide gehören die Pyrrolidinochinazoline vom Typ des Peganins = Vasicins. Das von HOOPER[1] in der indischen Acanthacee *Adhatoda vasica* Nees gefundene Vasicin (II) ist bisher in fünf Pflanzenfamilien nachgewiesen worden: Acanthaceae, Zygophyllaceae, Scrophulariaceae, Papilionaceae und Palmae. Bei den Zygophyllaceae kommen die Chinazoline vergesellschaftet mit β-Carbolinen vor.

Als Baustein der Chinazoline im Pflanzenreich ist die Anthranilsäure (I) vermutet worden[2]. Wir konnten vor einigen Jahren zeigen, dass Anthranilsäure-(^{14}COOH) von steril aufgezogenen *Peganum harmala*-Pflanzen in Peganin eingebaut wird[3]. Die grösste spezifische Aktivität fanden wir in den Wurzeln. Wir haben damals keine Positionsklärung der Radioaktivität durchgeführt, so dass nicht bewiesen war, ob (I) als Ganzes zur Biosynthese von (II) Verwendung findet. Es bestand noch die Möglichkeit, dass Anthranilsäure decarboxyliert und das CO_2 unspezifisch in Peganin inkorporiert wurde.

Wir haben das Problem erneut aufgegriffen und Anthranilsäure-(^{14}COOH) an bewurzelte Blätter von *Adhatoda vasica* Nees verfüttert. Das gebildete Peganin wurde isoliert, mit inaktivem Alkaloid verdünnt und in Anlehnung an SPÄTH und NIKAWITZ[4] abgebaut (Figur). Die Ergebnisse zeigt die Tabelle. Das eingesetzte Peganin sowie die durch Abbau erhaltene Anthranilsäure haben praktisch die gleiche spezifische Aktivität. Somit ist bewiesen, dass zumindest von *Adhatoda vasica* die Anthranilsäure direkt in Peganin eingebaut wird.

Experimentelles. Anthranilsäure-(^{14}COOH) wurde nach der Vorschrift von MUNSCHE und SCHÜTTE[5] hergestellt: *o*-Nitroanilin wurde diazotiert und mit einer Lösung von



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