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# THE METABOLISM OF AMINO ACIDS BY THE SMALL INTESTINE

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The metabolism of amino acids by the small intestine has not yet been widely investigated. Wiseman (1953) has shown that some L-amino acids, but not the corresponding unnatural enantiomorphs, can be transferred against a concentration gradient from the mucosal to the serosal side of an *in vitro* preparation. Using sacs of everted small intestine Wilson & Wiseman (1954) have shown that these preparations are able to concentrate glucose and L-methionine on to the serosal side. Evidence for the formation of alanine from glutamic acid has been obtained both *in vivo* (Dent & Schilling, 1949) and *in vitro* (Matthews & Wiseman, 1953). Other studies on the metabolism of the small intestine have been primarily concerned with glucose (Dickens & Weil-Malherbe, 1941; Fisher & Parsons, 1949; Darlington & Quastel, 1953; Hestrin-Lerner & Shapiro, 1954).

The results to be presented in this paper show that the isolated rat small intestine and intestinal mucosa from rats and cats are able to metabolize certain *N*-acetyl amino acids. In addition, the production of α-oxo acids from amino acids, as well as the reverse process of amino acid synthesis, has been demonstrated.

## METHODS

*Materials.* Commercial samples (L. Light and Co. Ltd) of racemic amino acids were used without further purification. $^{35}$S-labelled methionine was obtained from the Radio-Chemical Centre, Amersham. The amino acid contained some radioactive impurity but no attempt was made to remove this before preparing the α-oxo acid. Other radioactive amino acids were prepared as indicated below.

*Preparation of radioactive amino acids.* Four amino acids (alanine, valine, leucine and glutamic acid) labelled in the carboxyl group with $^{14}$C were synthesized from K$^{14}$CN essentially as described by Loftfield (1947). Labelled potassium cyanide was prepared on a 1–2 m-mole scale using 100 $\mu$c $Ba^{14}CO_3$. From this, free H$^{14}$CN was collected by acidification of the salt with $H_2SO_4$ in an evacuated transfer unit (Kornberg, Davies & Wood, 1952), the receiver being cooled in liquid air to ensure complete transfer. To this was added 1 ml. of concentrated ammonia and the stoppered tube

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allowed to warm up to room temperature. 0·5 ml. of redistilled acetaldehyde, *iso*butyraldehyde, *iso*valeraldehyde or succinic semi-aldehyde was added, and the mixture treated as described by Loftfield (1947). After removal of ammonia by boiling with NaOH a small amount (approx. 0·1–0·3 g) of unlabelled amino acid was added, the solution filtered and diluted to about 300 ml. It was then passed through an ion-exchange column of Zeo-Carb 215 (Partridge & Westall, 1949) and the amino acid finally displaced with ammonia. The eluate was decolorized by boiling with a small portion of charcoal, filtered and evaporated to dryness. With the exception of leucine, which gave a clean solid when treated in this way, the amino acids were redissolved in hot water, filtered and precipitated with alcohol. The precipitate was washed with alcohol and ether.

The yield of amino acid, based on the radioactivity of the $Ba^{14}CO_3$ and on measurements of the final product was 40–43%, except for glutamic acid which gave only a 12% yield. No attempt was made to improve the yield of any of the amino acids by varying the quantities and conditions used in the syntheses. Trial runs on the KCN synthesis using unlabelled $BaCO_3$ gave yields of 50–75%, which were much lower than those quoted by Loftfield. A relatively low incorporation of $^{14}C$ into the cyanide molecule at this stage is probably responsible for the low yields of radioactivity in the amino acids, since, using unlabelled KCN, yields of 56–82% were obtained in the case of alanine, leucine and valine during test syntheses.

*Preparation of α-oxo acids.* $^{35}S$-labelled α-oxo-γ-methyl-mercapto butyric acid was prepared from radioactive methionine by treatment of 10 mg of racemic material with D-amino acid oxidase prepared from pig kidney (Bender & Krebs, 1950). Concentrated kidney extract (equal volumes of powder and pyrophosphate buffer, pH 8·4, extracted for 5 min and the residue centrifuged off) was pipetted into a Warburg manometer cup containing the methionine and incubated in $O_2$ at 39° C until there was no further gas uptake. Other cups containing a total of 100 mg of unlabelled methionine were similarly treated. The combined solutions were deproteinized by adding approx. 0·2 vol. of 20% (w/v) metaphosphoric acid. The precipitate was centrifuged off and the supernatant liquid extracted continuously with ether until all of the α-oxo acid had been removed. The ether was then evaporated and the residue dissolved in 2 ml. of water. The α-oxo acid was neutralized with alcoholic soda using brom-thymol blue as internal indicator. During this stage an oil tended to form, and subsequent precipitation with alcohol produced a rather dirty solid. Because only a small amount of this labelled material was available, no attempt was made to purify the salt. α-Oxo-*iso*caproic acid was prepared from racemic leucine using D-amino acid oxidase. The Warburg cups each contained 40 mg of the amino acid. After incubation, the contents of twenty-four cups were pooled, deproteinized and extracted with ether. At the suggestion of Mr D. Williamson (personal communication) approx. 10% (w/v) $NaHCO_3$ was used for neutralizing the α-oxo acid, thus preventing the formation of an oil. The neutral solution was evaporated to dryness *in vacuo* and the residue dissolved by warming with a small volume of distilled water. The sodium salt was obtained from this solution by precipitation with alcohol, the crystals filtered and washed with alcohol and ether.

Pyruvic acid and α-oxo-*iso*valeric acids were prepared chemically (Vogel, 1954; Ramage & Simonsen, 1935), and the sodium salts obtained by neutralization of these acids with bicarbonate. These salts, and that of α-oxo-*iso*caproic acid, were 99–100% pure as determined by the permanganate oxidation method of Long (1942). In addition, those prepared from amino acids gave no ninhydrin reaction.

*Acetyl amino acids.* These were prepared by the method described by du Vigneaud & Meyer (1932), and the melting-points agreed with those given by Birnbaum, Levintow, Kingsley & Greenstein (1952). All were free from ninhydrin-reacting material and gave only single spots after chromatographic development and spraying with brom-cresol green indicator (Katz, Liebermann & Barker, 1953).

*Chromatography.* Amino acids were separated by descending chromatography on Whatman No. 1 or No. 4 paper using butanol–acetic acid–water solvent at 21–22° C. After drying, the chromatograms were sprayed with 0·1% ninhydrin in butanol and the colour allowed to develop at room temperature.

2-4-Dinitrophenylhydrazine derivatives were separated in butanol–ethanol–ammonia solvent (El Hawary & Thompson, 1953), using ascending chromatography, and, after drying, the spots were identified in ultraviolet light.

*Ionophoretic separations.* The method described by Neish (1953) was used. Separations were carried out at 0·3–2·0 mA at 300 V or 16–25 mA at 500–600 V on strips of Ford 428 Mill paper wetted with 0·05 M-$Na_2CO_3$. Solutions from the intestine apparatus were deproteinized by the addition of 0·1 vol. 20% (w/v) metaphosphoric acid and filtered. To the filtrate (15–25 ml.) was added 1 ml. of a saturated solution of 2-4-dinitrophenylhydrazine in 2N-HCl. After standing for at least 30 min the solution was extracted with three 10–15 ml. portions of ether. The combined ether solutions were extracted with 5 ml. of 10% (w/v) anhydrous $Na_2CO_3$ and the lower layer extracted once with 15 ml. ether to remove unreacted 2-4-dinitrophenylhydrazine. The sodium carbonate layer was separated, excess concentrated HCl added and the solution extracted with 15 ml. ether. The ethereal layer was removed, washed with 10 ml. water and the ether evaporated. The residue was taken up in a few drops of alcohol and small samples applied to the buffered paper.

*Estimations.* Amino acids were estimated using the methods described by Gibson & Wiseman (1951). Acetyl methionine was separated on silica gel columns as described by Gordon, Martin & Synge (1943), and estimated by titration with baryta. Total α-oxo acids in test solutions were estimated colorimetrically by the direct method of Friedemann & Haugen (1943) using an Ilford Spectrum blue (602) colour filter, and concentrations are expressed as mg sodium pyruvate/100 ml. solution. In the case of solutions containing pyruvate and another α-oxo acid, some error is introduced by the differences in light absorption. Sodium pyruvate estimated in this way has a greater light absorption per unit concentration than the α-oxo acids of glutamic acid, leucine and valine, and the concentrations of α-oxo acids given are therefore minimal values.

*Radioactivity measurements.* $Ba^{14}CO_3$ obtained from respiratory $CO_2$ or from ninhydrin decarboxylation of amino acid, was washed by centrifugation, three times with $CO_2$-free distilled water and once with acetone. Finally the precipitate was suspended in acetone and poured on to a tarred aluminium counting plate of standard diameter and allowed to dry. It was then reweighed to determine the quantity of $Ba^{14}CO_3$. The plate was counted under standard conditions (background radiation 10–13 counts per min) to an accuracy of ±2%. All counts were corrected for background radiation and the dead time of the counter, and determined as counts per min at infinite thickness. Comparisons and calculations were made from such counts.

*Tissue preparations.* Rats and cats were used as sources of intestinal mucosa. Each animal was anaesthetized with pentobarbitone sodium (Nembutal, Abbott Laboratories Ltd), the abdomen opened and the small intestine cannulated at the jejunal end. It was then washed out with cold Krebs phosphate saline gassed with oxygen (Umbreit, Burris & Stauffer, 1949). The intestine was stripped from the mesentery, quickly rinsed free of blood and put into a dish of cold saline. 10–15 cm lengths were cut open, the mucosa scraped off and suspended in cold phosphate saline containing 0·1% glucose to give 15–40 mg/ml. dry weight of tissue. Acetyl amino acids were made up in the Krebs phosphate saline with 0·1% glucose and the pH readjusted to 7·4. 2 ml. of the mucosal suspension and a similar volume of saline with or without acetyl amino acid substrate were pipetted into Warburg manometer cups of approx. 25 ml. capacity. The centre well contained NaOH for absorption of $CO_2$, and after attachment to the manometers the system was gassed with oxygen and incubated at 39° C with continual shaking.

In other experiments rat small intestine was used without removal of the mucosa. The method was similar to that described by Wiseman (1953) except that a modified apparatus (Smyth & Whaler, 1953) was used, and during the setting up of the preparation the intestine was rinsed and continually bathed in ice-cold phosphate saline. In this type of *in vitro* preparation three 15–17 cm lengths of rat small intestine are attached to part of the apparatus in such a way that a continual circulation of saline through the lumen of each loop is maintained, using a small reservoir and an oxygen lift. This part of the apparatus fits into a vessel of small volume containing more oxygenated saline which bathes the serosal surfaces of the intestine. Thus both the mucosal and serosal surfaces are bathed in oxygenated Krebs saline. The whole apparatus is placed in a water-bath at 37–39° C.

Substrates were neutralized before being added to the phosphate saline used in the apparatus. Glucose (0·4%) was present in all experiments in both mucosal and serosal fluids. Respiratory $CO_2$ was collected in approx. 0·3 N-$Ba(OH)_2$, and the amount of $CO_2$ produced estimated by back-titration of the excess alkali using standard HCl with thymol blue as internal indicator.

## RESULTS

*Deamination of amino acids by the* in vitro *small intestine preparation.* The presence of α-oxo acids in the fluids bathing both surfaces of the intestine was regularly noted at the end of experiments. The mucosal and serosal fluids were treated as described earlier, and the 2-4-dinitrophenylhydrazones separated by ionophoresis. Further samples were run in butanol-ammonia solvent on Whatman No. 1 filter-paper. Before deproteinization filtered samples (3 ml.) of the two solutions were examined for total α-oxo acid by the direct method of Friedemann & Haugen (1943).

TABLE 1. Production of α-oxo acids *in vitro* by rat small intestine

| | Amino acid used | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Alanine | | Valine | | Methionine | | Leucine | | Glutamic acid | |
| Oxo acids present | M | S | M | S | M | S | M | S | M | S |
| Pyruvate | + | + | + | + | + | + | + | + | + | + |
| α-Oxo-*iso*valeric acid | − | − | + | + | − | − | − | − | − | − |
| α-Oxo-*iso*caproic acid | − | − | − | − | − | − | + | + | − | − |
| α-Oxo-glutarate | + | + | − | + | − | − | + | + | + | + |
| α-Oxo-γ-methyl-mercapto butyric acid | − | − | − | − | + | + | − | − | − | − |
| Oxaloacetate | ? | ? | − | ? | − | − | ? | ? | ? | ? |
| Total final α-oxo acid (mg) | 0·05 | 0·33 | 0·15 | 0·32 | 0·25 | 0·84 | 0·10 | 0·34 | 0·10 | 0·47 |
| Final concn. α-oxo acid (mg/100 ml) | 0·3 | 1·3 | 0·6 | 1·3 | 1·2 | 4·0 | 0·5 | 1·4 | 0·4 | 1·9 |
| Duration of expt. (min) | 82 | | 77 | | 120 | | 76 | | 90 | |

α-Oxo acid after 75 min when no amino acid present { M = 0·3 mg/100 ml. Total 0·07 mg. S = 1·0 mg/100 ml. Total 0·24 mg.

α-Oxo acid was determined using standard curve (see Methods section) for sodium pyruvate. M = mucosal fluid. S = serosal fluid. + = substance shown to be present after electrophoretic separation. − = substance not seen after electrophoretic separation. ? = trace of material present but not certainly identified.

The results for five experiments, each one with a different amino acid, are given in Table 1. Other experiments gave similar results. With the exception of methionine, amino acid was initially present in the mucosal fluid only, at concentrations of 10–25 mM. The results for methionine are taken from an experiment in which the serosal concentration of L-isomer was 14 mM and the mucosal 2 mM; these concentrations altered little during the course of the experiment. When alanine, leucine or valine were used, the final concentration of L-amino acid in the serosal fluid was equal to, or greater than, in the mucosal fluid, which fell progressively during the experimental period.

Glutamic acid disappeared from the mucosal fluid, but the disappearance was not paralleled by a similar increase in the serosal fluid.

Although α-oxo acids could not be detected within 2–3 min after setting up the preparation, pyruvate was always present in the final mucosal and serosal solutions. Frequently this was accompanied by α-oxo glutarate and occasionally by traces of a compound running in the oxaloacetate position. In addition, the α-oxo acid derived from the amino acid under investigation was present.

TABLE 2. Diffusion and metabolism of two α-oxo acids

| Expt. | α-Oxo acid used | Temperature | Sample time (min) | Concentration of α-oxo acid (mg/100 ml.) Mucosal fluid | Serosal fluid |
|---|---|---|---|---|---|
| 1 | Pyruvate | 37° | 3 | 71 | 0·5 |
| | | | 40 | 59 | 1·2 |
| | | | 75 | 50 | 1·5 |
| | | | 120 | 40 | 2·0 |
| | Total pyruvate disappearance in 120 min = 7·7 mg = 36% original. | | | | |
| 2 | Pyruvate | 7–9·5° | 2 | 70 | 0·2 |
| | | | 53 | 68 | 0·2 |
| | | | 93 | 67 | 1·4 |
| | | | 143 | 64 | 2·1 |
| | Total pyruvate disappearance in 188 min = 1·1 mg = 5% original. | | | | |
| 3 | α-Oxo-γ-methyl-mercapto butyric acid | 37° | 0 | 170 | 0 |
| | | | 140 | 130 | 25 |

Total α-oxo-γ-methyl-mercapto butyrate disappearance in 140 min = 2·8 = 8% original. α-oxo acid determined using a standard sodium pyruvate curve.

In all these experiments pyruvate was produced, but the preparation can also metabolize this substance (Table 2). Even in the presence of glucose added sodium pyruvate disappears from the mucosal fluid without any similar increase in the serosal fluid. Reduction of the temperature reduces the amount of pyruvate which disappears. By contrast, α-oxo-γ-methyl-mercapto butyrate does not disappear so completely, much of the substance leaving the mucosal fluid being recovered on the serosal side of the intestine. In all these experiments the tissue itself accounts for approx. 5% of the total volume, and it is probable that the losses in Expts. 2 and 3 (Table 2) mainly represent material in the tissue itself.

*Utilization of amino acids.* The foregoing section has shown that deamination of amino acids can take place in the *in vitro* intestine preparation. That further degradation could take place was shown by experiments in which $^{14}C$-carboxyl labelled amino acids were added to the mucosal or serosal fluids and the respiratory radioactive $CO_2$ collected. The results of these experiments are given in Table 3. The figures for decarboxylation of an amino acid, or more probably a metabolite derived from it, are calculated from the specific activity

and amount of the respiratory $CO_2$ collected, and the specific activity of $CO_2$ obtained by ninhydrin decarboxylation of a sample of the amino acid.

The essential amino acids leucine and valine were only metabolized to a small degree, very much less than was the case with alanine and glutamic acid. When presented to the serosal surfaces, amino acids were much less readily utilized than when present in the fluid bathing the mucosal surfaces. This may be because the mucosa is the site at which these metabolic changes occur. There is some support for this conclusion in the fact that when radioactive amino acid is present initially in the serosal fluid some diffusion of labelled amino acid into the lumen takes place and the low utilization under these conditions may be due to the fact that the mucosa has only small amounts of labelled material available for metabolism.

TABLE 3. Decarboxylation of amino acids

All experiments were carried out at 37–39° in phosphate saline with 0·4% glucose. In those marked 'M' the amino acid was present initially only in the fluid bathing the mucosa, although at the end of the experiment the concentration on the serosal side was equal to, or greater than, that of the mucosal fluid (glutamic acid excepted). In those marked 'S', amino acid was added to both sides of the preparation with approx. six times more on the serosal side than on the mucosal side, and all the radioactivity on the serosal side.

| Amino acid | Initial total* (μmole L-) | Decarboxylated (μmole) | % of initial L-amino acid decarboxylated | Duration of expt. (min) |
|---|---|---|---|---|
| Alanine (M) | 264 | 40 | 15·2 | 82 |
| Alanine (S) | 1620 | 16 | 1·0 | 97 |
| Alanine (S) | 1855 | 27 | 1·4 | 112 |
| Glutamic acid (M) | 265 | 64 | 24·1 | 87 |
| Leucine (M) | 135 | 5 | 3·7 | 71 |
| Leucine (S) | 990 | 11 | 1·1 | 120 |
| Valine (M) | 348 | 1·7 | 0·5 | 73 |

* Assuming the amino acid was a 50/50 mixture of the two isomers.

*Synthesis of amino acid by intestinal tissue.* Paper chromatography of the mucosal and serosal fluids at the end of experiments using glutamic acid, leucine, valine and methionine consistently showed a pronounced amino acid spot with an $R_F$ equivalent to alanine. In the case of glutamic acid, which has been studied in some detail by Matthews & Wiseman (1953), these authors have concluded that the amino acid is in fact alanine. Using alanine, leucine, valine and methionine, traces of an amino acid spot in the glutamic acid position have been observed. In addition, there were usually small amounts of ninhydrin-reacting material which ran in butanol–acetic acid solvent more slowly than did glutamic acid.

The appearance of alanine suggested that some synthesis of this amino acid had occurred. It seemed unlikely that alanine and the more slowly moving amino acids were derived from tissue protein since no other amino acids (leucine, phenylalanine, etc.) running more rapidly than alanine have been observed, unless one of these was added at the start of an experiment.

The ability of the isolated intestine preparation to synthesize amino acid has been tested using phosphate saline containing added glucose, α-oxo acid and ammonia.

Using the sodium salts of α-oxo-*iso*valeric and α-oxo-*iso*caproic acids at approx. 0·1 M concentration with 0·02–0·04 M-$(NH_4)_2CO_3$ in the intestinal preparation, the corresponding amino acid was synthesized as shown by the appearance of a valine or a leucine spot on the chromatogram. In all experiments the amino acid was concentrated on the serosal side of the preparation. With sodium pyruvate there was an apparent increase in the alanine spot but, since alanine normally appears, it was not possible to say with certainty that the amount of amino acid was greatly increased. Control experiments showed that synthesis of amino acid did not take place when the α-oxo acid, ammonia and phosphate saline were incubated in the absence of tissue.

More certainly to identify the valine and leucine which were synthesized, further procedures were carried out. The fluid bathing the serosal side of the preparation was evaporated to dryness *in vacuo* at 30–35° C and the residue dissolved in the minimum volume of hot water. Ethanol was added to 90% final concentration and the precipitated salts removed by centrifugation. The supernatant solution was concentrated on one or two sheets of Whatman No. 4 filter-paper (57 × 47 cm), being applied in a thin streak about 5 cm from the top of the paper. The sheets were developed in butanol–acetic acid solvent and then dried in air. After heating the sheets at 70–80° C (Woiwod, 1949) the areas containing the desired amino acid were identified in ultraviolet light. These were cut out, the amino acid eluted with water and finally concentrated to small volume. This procedure separated the required amino acid from others and from the corresponding α-oxo acid. After treatment with L-amino acid oxidase of *Neurospora crassa* (Bender & Krebs, 1950) the 2-4-dinitrophenylhydrazone was prepared and samples separated by ionophoresis. The derivatives had the same mobility as α-oxo-*iso*caproic acid and α-oxo-*iso*butyric acid controls and a mixture of test and control did not separate.

In a single experiment using $^{35}S$-labelled α-oxo-γ-methyl-mercapto butyrate (specific activity 600 counts/min counted as the 2-4-dinitrophenylhydrazone) in the absence of ammonia but with added unlabelled DL-methionine, some further evidence for amino acid synthesis was obtained. After incubation, samples of the initial and final solutions were treated with 2-4-dinitrophenylhydrazine, extracted thoroughly with ether, and the aqueous layer, which contained the amino acid, was neutralized and treated with *Neurospora crassa*. Unlabelled carrier α-oxo acid was then added and the 2-4-dinitrophenylhydrazone prepared and counted. The radioactivity of the initial solution was not greater than background (12 counts/min), indicating that the radioactive α-oxo acid initially present had all been removed, while the final serosal solution had a count of 33/min above background, suggesting that some

amination of labelled material had taken place. Only a small count was expected because unlabelled α-oxo acid would dilute considerably the activity of any methionine synthesized from the added labelled α-oxo acid.

*Deacetylation of acetylated amino acids.* The production of free amino acid from the racemic *N*-acetylated compound has been shown to take place using the *in vitro* intestine preparation and also using mucosal scrapings.

With the former, racemic acetyl derivatives of leucine, methionine and alanine were tested separately. Incubations were at 37–39° C in phosphate saline containing 0·4% glucose. Samples of initial and final solutions, as well as controls incubated in the absence of tissue, were put on to filter-paper and developed in butanol–acetic acid solvent. Free leucine and methionine were produced as shown by the appearance of ninhydrin-reacting spots with the same $R_F$ as control amino acids. Some increase in the alanine spot was observed when acetyl alanine was used, but the difficulty of studies involving this amino acid have been mentioned already.

TABLE 4. Deacetylation of acetyl methionine

| Sample | Acetyl methionine (mg/ml.) | Total acetyl methionine (μmole) | Total amino acid* (μmole) |
|---|---|---|---|
| Initial mucosal | 0·90 | 118 | 0 |
| Initial serosal | 0 | 0 | 0 |
| Final mucosal | 0·67 | 87 | 30 |
| Final serosal | *c.* 0·005 | *c.* 0·5 | 32 |

Alanine and methionine present in the final solutions as demonstrated chromatographically. Duration of experiment = 120 min.

* Estimated by L-amino acid oxidase.

By overspraying the ninhydrin-developed chromatograms with brom-cresol green indicator, the acetyl compounds could be identified. Only traces of these substances ever appeared on the serosal side of the preparations. Table 4 gives the results of an experiment using acetyl methionine where this substance was separated on silica gel columns and estimated by titration. In this experiment there was no gas uptake when samples of the final solutions were incubated with D-amino acid oxidase, suggesting that the acetyl D-methionine had not been deacetylated.

A number of experiments using mucosa have been carried out. In such cases, neutralized acetyl amino acids were incubated in Warburg cups with mucosa from rats or cats. At the end of the period of incubation, the cup contents were transferred to centrifuge tubes and heated in a boiling water-bath for 5 min. The precipitated protein was centrifuged off and samples of the supernatant fluid treated with L-amino acid oxidase. The amino acid content of the control cup (no acetyl derivative) was subtracted from that of the test cup and the difference taken as a measure of the deacetylation which had taken place (Table 5). Paper chromatography of the final solutions showed

that the amino acid corresponding to the acetyl compound used was present as a strong spot, while in the control cup only a trace of ninhydrin-reacting material was present. In both test and control cups some protein breakdown took place, as shown by the presence on chromatograms of a number of amino acids, all present in small amount. No amino acid was produced when acetyl amino acids were incubated in the absence of mucosa. When acetyl tryptophane was used as substrate no free tryptophane could be demonstrated in either control or test solutions.

TABLE 5. Deacetylation of acetylated DL-amino acids by intestinal mucosa

| Expt. | Acetyl amino acid | Incubation time (min) | Total amino acid per cup (μmole) | | Increase of test over control (μmole/cup) | Added substrate (μmole) | Remarks |
|---|---|---|---|---|---|---|---|
| | | | No added acetyl derivative | Acetyl acid present | | | |
| 1 | Methionine | 50 | 16·8 | 34·8 | 18 | 37 | Rat mucosa: approx. |
| | | 100 | 21·6 | 43·2 | 21·6 | 37 | 37 mg dry wt./cup |
| 2 | Leucine | 2 | 5·1 | 4·3 | — | 98 | — |
| | | 30 | 13·0 | 17·3 | 4·3 | 98 | Rat mucosa |
| | | 80 | 19·1 | 30·2 | 11·1 | 98 | — |
| | | 120 | 25·5 | 38·8 | 13·3 | 98 | — |
| 3 | Alanine | 0 | 0 | 0 | — | 120 | — |
| | | 30 | 7·2 | 12·6 | 5·4 | 120 | Rat mucosa |
| 4 | Valine | 60 | 12·3 | 14·3 | 2·0 | 174 | — |
| | | 125 | 11·6 | 18·0 | 6·4 | 174 | Cat mucosa: approx. 80 mg dry wt./cup |
| | Leucine | 60 | 12·3 | 19·3 | 7·0 | 174 | — |
| | | 125 | 11·6 | 21·2 | 9·6 | 174 | — |
| | Methionine | 60 | 12·3 | 25·2 | 12·9 | 140 | — |
| | | 125 | 11·6 | 30·6 | 19·0 | 140 | — |

All incubations in phosphate saline at 39°.

## DISCUSSION

Krebs (1935) showed that rat ileum contains an L-amino acid deaminase, and the presence of α-oxo acids in the solutions taken from the *in vitro* intestine apparatus may be the result of this enzyme acting upon the natural forms of added amino acids. Effective deamination might also be expected if transamination between oxaloacetic, α-oxoglutaric or pyruvic acids and an amino acid took place. Such a reaction would lead to the production of aspartic and glutamic acids and alanine, and it is found that frequently amino acid spots corresponding to these, especially to alanine, do appear. Both *in vivo* and *in vitro* (Dent & Schilling, 1949; Matthews & Wiseman, 1953) glutamic acid disappears from the intestine with corresponding production of some alanine. Mucosa of small intestine has a high $Q_{O_2}$ (Dickens & Weil-Malherbe, 1941), and no difficulty in the supply of transaminating α-oxo acids would be expected, since all are known to take part in the general metabolism of respiring tissues.

Synthesis of alanine *in vitro* has been shown to take place in liver by Canzanelli, Rapport & Guild (1950), and by Kritzmann (1947) who has, on the basis of his results, suggested a possible mechanism for the synthesis. The results reported here do not indicate any particular mechanism, although they show that some amino acids, including certain of the essential ones, can be synthesized by the small intestine when the corresponding α-oxo acid is supplied.

The α-oxo acids corresponding to leucine, isoleucine and methionine have been shown to support growth in rats deficient in these amino acids (Rose, 1937; Cahill & Rudolph, 1942), and it would seem likely that, in view of the valine synthesis which can occur *in vitro* α-oxo-*iso*butyric acid would have a similar effect if fed to valine-deficient animals. This does not appear to have been tested. The relative importance of intestinal synthesis, compared, for example, with that of the liver, is not known, but it may well be that under the conditions existing *in vivo* the intestine may hold an important position in this type of reaction.

That many acetylated amino acids lose their acetyl groups *in vivo* has been made clear by the beneficial results on feeding such derivatives to animals deficient in the corresponding amino acids (e.g. Neuberger & Webster, 1946). Acetyl tryptophane supports growth in tryptophane-deficient rats (Berg, Rose & Marvel, 1929) and it seems that, in contrast to acetyl methionine, acetyl valine and acetyl leucine, this compound can only be deacetylated in sites other than the small intestine.

Birnbaum *et al.* (1952) have reported the isolation of a highly active acylase from hog kidney. This readily reacts with a number of *N*-acetylated amino acids, including the acetyl derivatives of methionine, valine and leucine, but not of tryptophane. It is suggested that this enzyme may be responsible for the present results, especially in view of the apparent inactivity towards acetyl tryptophane. Further tentative support for this view is the rate of deacetylation shown in Expt. 4, Table 5, with acetyl methionine most readily attacked, then acetyl alanine and finally acetyl valine. A similar order of activity is shown by the acylase of Birnbaum *et al.* (1952). On the basis of the figures provided by these authors for the activity of their acylase preparation before purification, it can be calculated from the figures for acetyl methionine in Expt. 1, Table 5, that on a wet-weight basis the intestine contains about one-sixth of the acylase present in the kidney. The comparison cannot be carried too far because the kidney preparations were homogenates, whereas the intestinal mucosa was mainly whole cells and permeability factors may have influenced the rate of reaction. In addition, in the experiments from which these calculations were made, the amount of acetyl methionine present was almost certainly a limiting factor, and the figure of one-sixth probably much too low.

In view of the results of Bloch & Rittenberg (1944, 1946) it would seem that there is in the intestine a transacetylating enzyme. These authors showed that when deuterium-labelled acetyl amino acids and phenylaminobutyric acid were fed together, the isolated acetyl phenylaminobutyric acid had an isotope concentration similar to that of the acetyl group originally given. The acylase and the transacetylase must be closely associated since, if the latter enzyme were not present in the intestine, the labelled acetyl amino acid fed would have been deacetylated to some degree during absorption and the isotope concentration of the acetyl phenylaminobutyric acid synthesized would have been low, i.e. of a similar order to that found when the compounds were injected.

The significance of these findings is not clear. It is possible that intestinal acylase may be concerned in some way with the 'active' absorption from the small intestine of certain L-amino acids. The kidney tubule and small intestine have in common the property of absorbing amino acids, and this hypothesis accounts for the presence of a kidney acylase (assuming that this enzyme is common to the kidneys of animals other than the hog) rather more satisfactorily than does the one outlined below. A second important function might be in the synthesis or turnover of protein. The intestinal mucosa has a very rapid turnover of protein (Friedberg, Tarver & Greenberg, 1948; Tarver & Morse, 1948) and, because of enzyme production and the continued sloughing of the mucosa, must have a large net protein synthesis. Investigation of liver might be rewarding since this tissue synthesizes a considerable amount of protein, and an acylase should be present there if it is concerned with protein synthesis. It has already been shown that liver can synthesize acetyl amino acids (Bloch & Borek, 1946), but only in relatively small amounts. The authors have pointed out, however, that the acetyl compounds may have been immediately utilized, without a chance to accumulate, since acetyl phenylaminobutyric acid, a compound thought to be metabolically inert, accumulated to a much greater extent than the acetyl derivatives of leucine and phenylalanine.

## SUMMARY

1. The synthesis of four $^{14}C$-carboxyl-labelled amino acids is described.

2. Some aspects of the *in vitro* amino acid metabolism of rat small intestine and of rat and cat intestinal mucosa have been investigated.

3. Deamination of amino acids takes place in the *in vitro* preparation and other reactions, leading to the loss of the carboxyl-carbon of the amino acid or of a derived metabolite, can occur.

4. Synthesis of leucine and valine takes place if the corresponding α-oxo acid is present.

5. An acylase is present in intestinal mucosa and this would appear to be similar to that present in hog kidney. Some consideration has been given to the possible role of this enzyme in the metabolism of the small intestine.

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