The absorption *in vitro* of alanyl-phenylalanine*

The fate of peptides, liberated from proteins within the intestinal lumen, has been the subject of limited study[1] largely because of the difficulty of direct sampling of the absorbed material. The recently devised *in vitro* technique of WILSON AND WISEMAN[2] provides a convenient method for study of the absorptive process.

The peptides, L-alanyl-L-phenylalanine (L-peptide) and DL-alanyl-DL-phenylalanine (DL-peptide), have been examined in order to ascertain their respective patterns of absorption. Sacs of inverted rat intestine[2] were filled with and incubated in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 200 mg % glucose. Initial peptide concentrations were 0.01 *M*. The sacs were incubated for various time intervals at 37° in an atmosphere of 95 % O_2 and 5 % CO_2. At the completion of the experiment, the sacs were removed from the mucosal solutions, washed, blotted with filter paper, and emptied of their contents. The resulting solutions as well as original samples were analyzed by paper chromatography employing a butanol–acetic acid–water mixture.

In a comparative study of the peptides on the mucosal side, it was observed in a series of experiments that, within the first 8 min of incubation, the L-peptide had been completely hydrolyzed. On the other hand, during the same time interval the DL-peptide was hydrolyzed to only a slight extent. With the L-peptide, the liberated amino acids were found in greater concentration on the mucosal side although a significant amount of the free amino acids had crossed the intestinal wall. Only slight transport of the resulting amino acids across the intestinal wall was observed with the DL-peptide, undoubtedly due to the small amount of peptide hydrolysed as well as to the preferred absorption of the L-species of amino acids[3]. After 2 h of incubation with the L-peptide, more of the amino acid was found on the serosal side than on the mucosal side, confirming an active absorption of these amino acids[3]. However, even after 2 h, the DL-peptide showed residual quantities of peptide on the mucosal side, but as in the L-peptide experiment the majority of the free amino acids were found to be on the serosal side, again indicating absorption against a concentration gradient. In a series of similar experiments over various time intervals, in no case was any penetration of the peptide through the intestinal wall observed.

In order to study possible transport to the mucosal side, the L-peptide was placed on only the serosal side of a series of inverted sacs from a rat intestine, and different experiments terminated at different times up to 120 min. Within the first 30 min, complete hydrolysis of the peptide had occurred as judged by paper chromatographic analysis. At shorter time intervals, residual peptide was found on the serosal side, but no ninhydrin-positive spots were obtained on the mucosal side.

When a gut preparation containing no peptide solution on either side was incubated for 1, 10, or 120 min in a identical fashion to that previously described, aliquots of both the serosal and mucosal solutions, after removal from the intestinal preparation, demonstrated the ability to cleave the L-peptide completely during a subsequent 30 min incubation. However, if the aliquots of the serosal and mucosal solutions were subjected to heat treatment in a boiling water bath prior to contact with the peptide, no proteolytic activity remained. In addition, no hydrolysis of the peptide occurred when incubated in buffer alone. Apparently the peptide-splitting enzyme(s) is released from both sides of the intestinal wall.

Since peptidase activity was found in solutions representing both mucosal and serosal sides, it cannot be concluded from the first two experiments that no transport of intact peptide occurred. It is possible that either of the peptides crossed the membrane at such a slow rate that the resulting concentration of peptide in the presence of hydrolytic enzymes could never accumulate in sufficient quantities for detection with ninhydrin.

In order to determine whether the proteolytic activity of the serosal solution was due to the trauma caused by stripping off the mesentery in the preparation of the sacs, 10 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, were injected into the peritoneal cavity of an intact rat. After 10 min, an aliquot of the peritoneal fluid was recovered. When the L-peptide was incubated with the peritoneal fluid for 30 min at 37°, complete hydrolysis of the peptide occurred. Prior heating of the fluid in a boiling water bath completely destroyed the hydrolytic activity. These observations *in vivo* support the conclusions of the sac experiments that peptidase activity is liberated from the serosal side of the intestinal wall into the peritoneal cavity.

Further investigation of the mechanism involved may elucidate the absorption process for the breakdown products of protein.

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The isolation from wool of a readily extractable protein of low sulphur content

The protein solutions obtained by extracting wool with alkaline solutions of potassium thioglycollate contain several components. One of these, kerateine 2, was obtained in a relatively pure state by fractional extraction from wool[1,2] and was further purified after conversion to the S-carboxymethyl derivative[3,4], SCMK2.

The isolation and purification of a second protein component of reduced wool has now been achieved by the following procedure: solvent-scoured wool top (100 g air–dry weight) was extracted at 50° for 2 h in 3 l 0.1 *M* potassium thioglycollate, pH 10.5, and the proteins precipitated at 20° and pH 5 using acetic acid. The precipitate was redissolved in 1 l 0.1 *M* potassium thioglycollate, pH 8.5, and coupled with iodoacetate at this pH using a two-fold excess. The pH was then adjusted to 6 and the solution dialysed. Electrophoresis in glycine-NaOH buffer at pH 11 and ionic strength 0.1 indicated the presence of at least 3 components (Fig. 1a).

On acidification about two-thirds of the protein precipitated between pH 4.5 and 4.1 and did not redissolve at lower pH values. The remainder of the protein almost completely precipitated at pH 2.9. A sharp separation could thus be effected by precipitation at pH 4.1. The electrophoretic patterns of the precipitate and supernatant obtained at this pH are shown in Figs. 1b and 1c respectively.

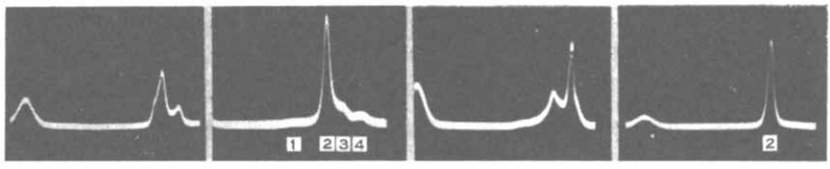

Fig. 1 a–d. Ascending electrophoretic patterns of wool protein fractions run in 0.1 ionic strength glycine–NaOH buffer at pH 11.

The supernatant fraction contained at least 4 components which were not further fractionated. It had a high sulphur content, containing about 6.5% compared with 3.5% in whole wool[5], and if present entirely as S-carboxymethyl cysteine (SCMC) residues, this amino acid would constitute at least 30% of the protein fraction.

The fraction precipitating at pH 4.1 also contained at least 4 components. The component corresponding with the main peak (2), which accounted for about 70% of the total area, was separated by precipitation at —5°, pH 7, and ionic strength 0.01 with acetone at a concentration of 50% (v/v). Two re-precipitations under identical conditions gave a protein which moved with a single boundary on electrophoresis at all pH values from 8.5 to 11 at a protein concentration of 1%. Electrophoresis was difficult below pH 10 because of the high turbidity of the protein solution. Fig. 1d shows a typical pattern obtained at pH 11. Ultracentrifuge runs at pH 11 in 0.1 ionic strength glycine–NaOH buffer containing also 0.2 *M* NaCl gave one peak, the s_{20w} being 2.5 at a protein concentration of 0.9%. Ultracentrifuge runs showed only one component but light-scattering measurements indicated the presence of large aggregates which continually accumulated on standing at room temperature. Of the total protein nitrogen, 3.75% was in the form of SCMC and 0.5% as cystine. In addition there was about 1% of combined sulphur in an unknown form.

The S-carboxymethyl protein derivative obtained by the above process and SCMK2 are similar as regards their electrophoretic mobilities ($7.2–7.8 \cdot 10^{-5}$ and $7.0–7.9 \cdot 10^{-5}$) sedimentation coefficients ($2.5 \cdot 10^{-13}$ and $3–4 \cdot 10^{-13}$) and SCMC content (3.75 and 3.53%) though the latter two parameters differ significantly. The new protein also differs from SCMK2 in its greater ease of salting out at pH 6 with $(NH_4)_2SO_4$ (0.17–0.19 *M*, *cf.* 0.3–0.4 *M*), NaCl (1.0–1.2 *M*, *cf.* 1.0–2.0 *M*), and sodium acetate (0.18–0.20 *M*, *cf.* 0.3–0.4 *M*). The pH for 50% precipitation is also lower (4.6–4.7, *cf.* 4.9–5.1). Preliminary analysis by Dr. D. H. SIMMONDS shows them to have a similar but significantly different amino acid composition.