The hydrolysis of dipeptides by rat intestinal extracts

The capacity of rat intestine to hydrolyse the dipeptides glycylglycine, glycyl-L-leucine, L-leucylglycine and glycyl-L-alanine, is greatest in the ileum and decreases towards the duodenum[1]. This work has been extended to include the colon as well as the small intestine; the dipeptides used were glycyl-L-valine and glycylglycine.

Intestines from adult male rats, Wistar strain, were used. The rats had been starved for 16 h before death to ensure an empty intestine. Mucosal extracts were prepared at 4° by scraping the mucosa from the central portions of duodenum, jejunum, ileum and colon. The scrapings were homogenised in 40–50 vol. of distilled water, with a homogeniser of the Potter–Elvehjem type. After allowing the homogenates to stand at 0° for 2 h, they were centrifuged at 26,000 × *g* for 20 min in a refrigerated centrifuge and the supernatants used as the source of enzyme.

Enzyme activity was assayed by incubating 0.08 ml 0.0625 *M* dipeptide with 0.08 ml of 0.2 *M* Tris buffer at an appropriate pH, and 0.04 ml of enzyme extract, at 37°. Duplicate aliquots of 0.01 ml were removed from the reaction mixture at intervals during a 1-h period and added to 1-ml samples of ninhydrin reagent[2]. These were heated at 100° for 25 min, and the intensity of the colour developed was related to the degree of hydrolysis using a standard graph. The graph was obtained using artificial hydrolysis mixtures and the same method.

The hydrolysis of glycyl-L-valine was measured at the optimal pH value of 8.4. The reaction followed first-order kinetics at the beginning of the incubation period, but the activity of the enzyme slowly declined. When a graph of $\log \frac{s}{s-x}$ (s = initial substrate concentration, x = substrate concentration at time t during the reaction) against t was plotted, a curve was obtained. The first-order rate constant for the reaction was calculated from the tangent to this curve, taken from the origin. Using this method the reaction rate was proportional to enzyme concentration.

Glycylglycine hydrolysis was found to have two pH optima, at pH 7.4 and 8.2 [1]. Determinations were carried out at pH 7.4, in the presence of 0.001 *M* Co^{++}. The reaction had zero-order characteristics and the rate of reaction was proportional to enzyme concentration.

Blanks using boiled enzyme and dipeptide or enzyme alone showed negligible increases in colour.

The nitrogen content of the extracts was determined, using the micro-Kjeldahl technique.

The distribution of the hydrolytic activities in the different intestinal regions is given in Table I. For both dipeptides the specific activity of the ileum is significantly greater than that of the duodenum or jejunum, and that of duodenum is smaller than that of jejunum. The specific activity of the colon is much lower than those of the small intestine. Variation in the specific activities of the small intestine is not explained by excess inactive protein diluting the activity of the extracts from the upper intestine. Measurements of total protein obtained from 2.5-cm segments of intestine from each region showed no significant differences[1].

The low activity found in the colon indicates that the high level of peptidase activity in the small intestine is not common to the whole intestinal tract. Presumably

Abbreviations: Tris, tris(hydroxymethyl)aminomethane.

TABLE I

THE DISTRIBUTION OF PEPTIDE-SPLITTING ACTIVITY IN RAT INTESTINAL TRACT

(1 unit of activity = hydrolysis of 1 μmole substrate in 10 min)

	Expt.	Specific activity (units/mg protein)			
		Duodenum	Jejunum	Ileum	Colon
Gly-Val	1	39.5	42.5	64.5	8.4
	2	39.2	35.2	56.8	2.4
	3	49.5	51.5	62.0	9.9
	4	43.5	43.0	57.5	15.6
	Mean	42.9	43.5	60.2	9.1
	P(D — J) > 0.1	P(J — I) < 0.02		P(D — I) < 0.01	
Gly-Gly	1	1.8	5.3	7.4	1.7
	2	3.6	5.6	6.6	1.4
	3	6.2	6.7	9.8	1.9
	4	7.1	6.8	10.1	—
	5	8.2	6.7	11.3	3.2
	Mean	5.4	6.2	9.0	2.0
	P(D — J) > 0.1	P(J — I) < 0.05		P(D — I) < 0.02	

the high activity of the small intestine is related to its absorptive function. NEWEY AND SMYTH[3] have shown that intact dipeptides enter the mucosal cells *in vitro*, but that the dipeptides are almost completely hydrolysed before they appear on the serosal side of the intestine[4]. This finding is in agreement with the postulate of FLOREY, WRIGHT AND JENNINGS[5] that the function of intestinal peptidases is primarily intracellular.

In view of the high peptidase level in the ileum, it seems likely that absorption of dipeptides and their intracellular hydrolysis may be maximal in this region of the intestinal tract. A high absorptive capacity of the lower small intestine could have two physiological functions. Either maximum absorption occurs in this region, or maximum absorption occurs in the upper intestine, and the high activity in the lower intestine would then make certain of the absorption of remaining traces of food from the lumen, resulting in a highly efficient digestive process.

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[1] G. B. ROBINSON AND B. SHAW, *Biochem. J.*, in the press.
[2] S. MOORE AND W. H. STEIN, *J. Biol. Chem.*, 176 (1948) 367.
[3] H. NEWEY AND D. H. SMYTH, *J. Physiol. (London)*, 152 (1960) 367.
[4] H. NEWEY AND D. H. SMYTH, *J. Physiol. (London)*, 145 (1959) 48.
[5] H. W. FLOREY, R. D. WRIGHT AND M. A. JENNINGS, *Physiol. Revs.*, 21 (1941) 36.

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