Biochimica et Biophysica Acta, 321 (1973) 262–273

BBA 66995

ALTERATIONS IN THE LEVELS OF PEPTIDE HYDROLASES AND OTHER ENZYMES IN BRUSH-BORDER AND SOLUBLE FRACTIONS OF RAT SMALL INTESTINAL MUCOSA DURING STARVATION AND REFEEDING

YOUNG S. KIM, DENIS M. McCARTHY, WILL LANE AND WILLIAM FONG

Gastrointestinal Research Laboratory, Veterans Administration Hospital, San Francisco, Calif. 94121 (U.S.A.) and Department of Medicine, University of California School of Medicine, San Francisco, Calif. 94121 (U.S.A.)

(Received March 7th, 1973)

SUMMARY

Effects of starvation on the activity of a number of enzymes were studied in rat small intestinal mucosa. Significant reductions in the specific activity of β-fructofuranosidase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26, formerly known as sucrase) and of peptide hydrolases were observed in purified brush-border membranes after 5 days of starvation. By contrast specific activities of a number of peptide hydrolases of the cytosol fraction prepared either from ileal or jejunal segments rose significantly with starvation, associated with a marked fall in tissue protein. Cytosol activities expressed per mg DNA showed similar elevations. Total cytosol enzyme activity per segment remained unchanged. These data suggest that while the cell population decreased during starvation, soluble enzyme activity per cell increased: the brush-border enzymes appeared decreased. When rats were refed a standard chow diet the altered specific activities of peptide hydrolases returned to normal in three days, in both cytosol and brush-border fractions. Refeeding rats with diets consisting of glucose or amino acids or a mixture of a long-chain fatty acid and a monoglyceride, led to marked decreases in the cytosol enzymes with 2 h, irrespective of the type of diet employed.

By the fifth day of fasting specific activity of intestinal fructose-1,6-diphosphatase (D-fructose-1,6-diphosphate 1-phosphohydrolase), a key gluconeogenic enzyme, had risen nearly 2-fold while that of pyruvate kinase, a glycolytic enzyme, had fallen to one half the control value, indicating increased gluconeogenesis and reduced glycolysis. Alanine transaminase (L-alanine:2-oxoglutarate transaminase, EC 2.6.1.2, formerly known as glutamic–pyruvic transaminase) also rose during starvation in keeping with increased gluconeogenesis. Thus it seems possible that during fasting, the cytosol peptide hydrolases of the small intestinal mucosa are involved in the catabolism of endogenous cellular proteins for gluconeogenesis in the intestine, providing metabolic fuel for other vital organs. On the other hand the peptide hydrolases

of the intestinal brush border may be adaptive enzymes similar to β-fructofuranosidase.

INTRODUCTION

Peptide hydrolases catalyzing the hydrolysis of di- and tripeptides occur in small intestinal mucosa as well as in many other tissues[1–7]. In intestine, peptide hydrolases are localized primarily in two subcellular loci, in a soluble form in the cytosol and membrane-bound in the brush border. The enzymes from cytosol and brush-border membranes are both present in multiple forms but their electrophoretic mobilities and heat stabilities are distinct[2,3].

Though much speculation has been made, little is known about the functions of these peptide hydrolases. By analogy with disaccharidases which are similarly located, the brush-border peptide hydrolases have been thought to be involved in the digestion of dietary proteins[2,3]. From the observation that peptide hydrolases in the cytosol fractions of intestinal mucose unlike their brush-border equivalents have zymogram patterns identical with those seen in many other tissues such as liver, kidney, heart and skeletal muscles, it has been suggested that these soluble enzymes might play a more general role in cellular metabolism[2].

Starvation has been shown to cause alterations in enzymes of the intestinal mucosa[8–12]. β-Fructofuranosidase and maltase in the brush border have been shown to be significantly reduced during starvation in rats and in human volunteers, suggesting that they are adaptive enzymes[9,10]. The present study was carried out primarily to measure the respective changes in soluble and membrane-bound peptide hydrolases during starvation in both the jejunum and the ileum of the rat. In an effort to determine the pattern of intermediary metabolism occurring in intestine during these changes in peptide hydrolase activity, the activities of several other enzymes known to be associated with metabolic processes were also studied.

METHODS

Preparation of tissue samples

Male albino Sprague-Dawley rats (230–260 g) were fasted and allowed access only to water, for varying periods of time up to 5 days. Animals were killed by decapitation and all subsequent steps were carried out at 4 °C. Two 15-cm segments of small intestine were obtained, a jejunal segment from just distal to the ligament of Treitz and an ileal segment from the area just proximal to the ileocecal junction. These were rinsed in ice-cold isotonic saline, and everted over a glass rod. The everted gut sacs were gently blotted and the mucosa was scraped with a glass slide. Purified brush borders were prepared from mucosal scrapings according to the method of Miller and Crane[13] as modified by Takesue and Sato[14]. Mucosal scrapings were homogenized in 14% glycerol (10 ml/g). The homogenate was centrifuged at 3000 × *g* for 15 min and the supernatant was passed through two layers of gauze. The filtrate was centrifuged at 105 000 × *g* for 2 h. The resultant post-microsomal supernatant fraction was designated as the soluble or cytosol fraction.

Special controls

Pancreatic duct occlusion was carried out on four rats under light anesthesia according to the procedure of Clowes and MacPherson[15]. Another group of four rats were sham-operated. Animals whose pancreatic ducts had been occluded and sham-operated rats were starved for 3 days, then killed and the mucosal scrapings obtained as described above.

Refeeding

For the refeeding experiments, rats which had been fasted for 5 days were fed with either Purina laboratory chow *ad libitum* or with special elemental diets *via* tubes. The elemental diets used for the refeeding experiments were prepared as follows. Diet I was a 10% (w/v) solution of glucose in distilled water. Diet II was a 5% (w/v) lipid emulsion containing palmitate and monopalmitin (Sigma Chemical Co., St. Louis, Mo.) in the molar ratio 2:1, neutralized with KOH and stabilized by sodium taurocholate (1 mg/ml) (Maybridge Research Chemicals, Lauceston, Cornwall, Great Britain). The emulsion was prepared by treatment with ultrasonic vibration for 10 s. Diet III was a 10% (w/v) solution of hydrolyzed casein (Schwartz-Mann, Orangeburg, New York) in distilled water. Rats starved for 5 days were given 5 ml of a selected diet through a polyethylene tube over a period of 15 min. Control starved rats were given 5 ml of distilled water in a similar manner. Rats were killed 2 h after the tube feeding was completed and the mucosal scrapings obtained as described above.

Enzyme assays

For the assay of peptide hydrolase activity, two previously described methods were used, *i.e.* the method of Matheson and Tattrie[16] using a modified Yemm and Cocking ninhydrin reagent, and also the modification of the method of Josefson and Lindberg[17], previously described from this laboratory and based on recording the rate of change in absorbance at 220 nm, using a Gilford recording spectrophotometer[2]. The following dipeptides and a tripeptide were used as substrates: L-leucyl-L-alanine, glycyl-L-leucine, glycyl-L-valine and L-leucyl-L-leucyl-L-leucine (Sigma Chemical Co., St. Louis, Mo.).

β-Fructofuranosidase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26, formerly known as sucrase) was determined as described by Dahlqvist[18].

Fructose-1,6-diphosphatase (D-fructose-1,6-diphosphate 1-phosphohydrolase) was assayed by the method of Taketa and Pogell[19]. Pyruvate kinase (ATP–pyruvate phosphotransferase) was measured by the method of Bucher and Pfleiderer[20]. Aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1, formerly known as glutamic–oxaloacetic transaminase) and alanine aminotransferase (L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2, formerly known as glutamic–pyruvic transaminase) were assayed by the method of Henry *et al.*[21]. Protein was determined by the method of Lowry *et al.*[22] and DNA content was measured by the method of Ceriotti[23] after a modified Schmidt–Thannhauser alkaline digestion[24].

Zymogram method

Vertical starch gel electrophoresis was performed with the Buchler gel electro-

phoresis apparatus (Buchler Instruments, Fort Lee, N.J.) by the method of Lewis and Harris[6] at pH 7.4 (bridge buffer, 0.1 M Tris–maleate; gel buffer, 0.005 M Tris–maleate). The details of the procedure for the development of zymograms are as described in the previous paper[1].

RESULTS

Effect on body and mucosal weight

As shown in Fig. 1, there were considerable effects of fasting and refeeding on body weights and weights of small intestinal mucosa. The most marked weight loss occurred during the first day of fasting, the rats losing about 35 g in 24 h. Similarly the weight gain was most marked during the first day of refeeding, the weight gain

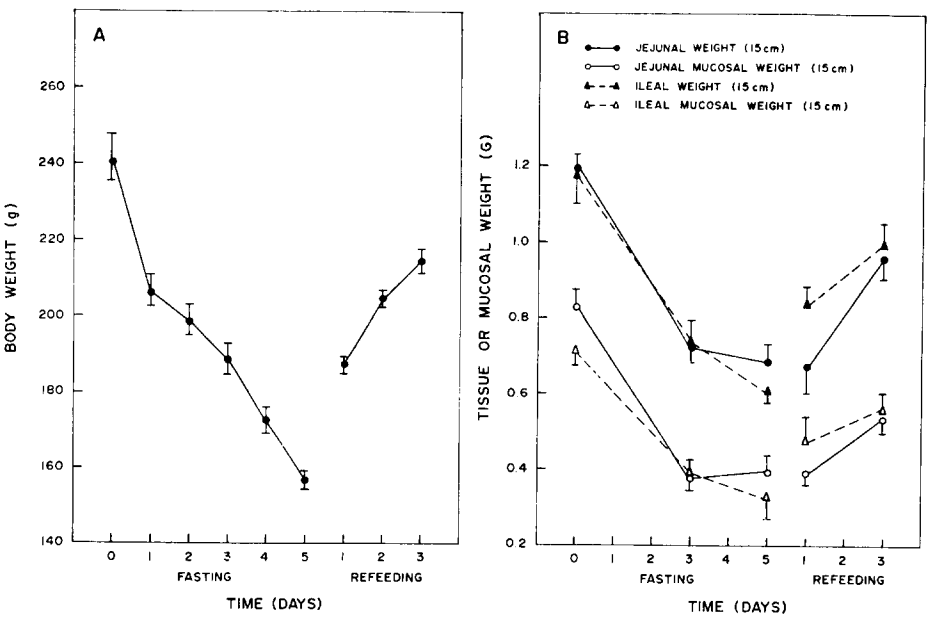

Fig. 1. Comparison of effects of fasting on body weights (A) and weights of standardised intestinal segments (total tissue weights and weights of mucosal scrapings). B(). Each point represents the mean ± S.E. of nine animals.

being about 30 g. Weight of small intestine and of mucosal scrapings per 15 cm segment of jejunum or ileum showed changes similar to those in body weight. When protein concentrations in the soluble fraction and DNA concentrations of the mucosal homogenate are expressed in terms of wet mucosal weight, protein concentrations remained remarkably constant, whereas DNA concentrations unchanged on the third day of fasting, fell considerably by the fifth day in both jejunum and ileum ($P < 0.025$), (Fig. 2). From these data it is apparent that totals of protein and DNA per segment fell considerably during starvation.

Effect of fasting and refeeding on soluble peptide hydrolase activity

The specific activities of soluble peptide hydrolases responsible for the hydro-

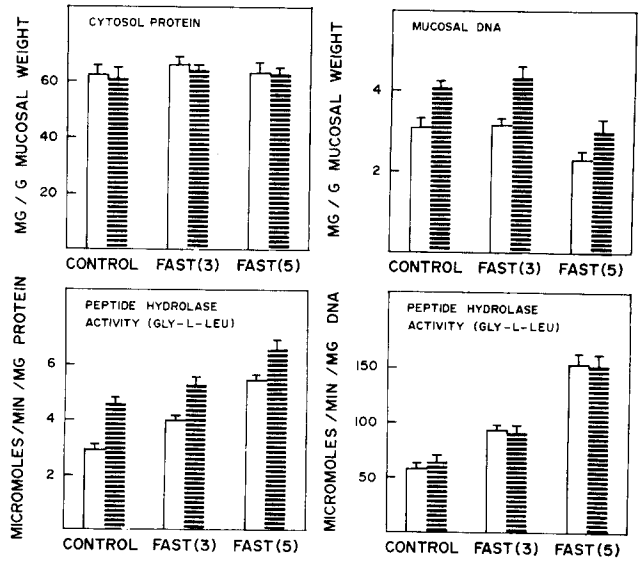

Fig. 2. Effects of 3- or 5-day fasts on jejunal (open histograms) or ileal (hatched histograms) levels of cytosol protein, homogenate DNA, cytosol peptide hydrolase activity per mg of protein and cytosol peptide hydrolase activity per mg DNA, compared with fed controls. Glycyl-L-leucine was used as a substrate. Means ± S.E. of nine animals.

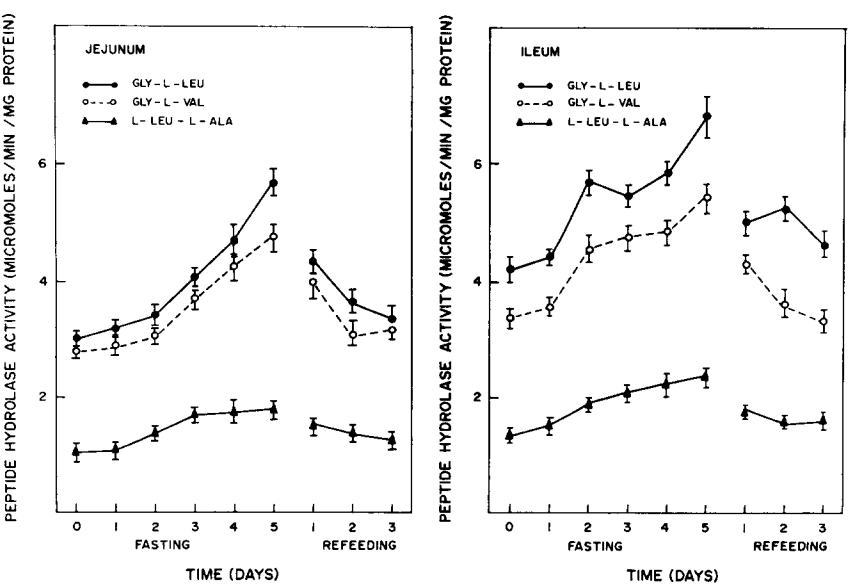

Fig. 3. Relationship of peptide hydrolase activity in the cytosol fractions of intestinal mucosa to duration of periods of fasting or refeeding with chow. Each point represents the mean ± S.E. of nine animals.

lysis of L-leucyl-L-alanine, glycyl-L-valine and glycyl-L-leucine, were higher in the ileum than in the jejunum in the fed state (Fig. 3). With fasting, progressive increase in the soluble enzyme activity ($P < 0.005$) was observed with all three peptide

hydrolases in the cytosol fraction. When these rats were refed with regular chow diet, there was a gradual reduction in the soluble enzyme activity towards normal and only by the third day had the enzyme activity returned to the control values (Fig. 3). When 5-day-fasted rats were refed with any of the special diets (I, II or III) the soluble peptide hydrolase activity had fallen towards normal by 2 h in both jejunum and ileum, as shown in Table I. All three types of diet, that is, glucose, amino acids or fatty acid and monoglyceride mixtures caused similar falls in activity. When cytosol peptide hydrolase activity was expressed per mg of DNA, enzyme activity increased with starvation even more ($P < 0.001$) than when expressed per mg of protein ($P < 0.005$), (Fig. 2). The net result of increasing specific activity and increasing activity per mg of DNA, accompanied by falls in the totals of protein and DNA per segment, was that total peptide hydrolase activity per segment was very similar to that in controls.

Effect of pancreatic duct occlusion and fasting on soluble peptide hydrolase activity

In order to rule out the possibility that the observed elevation of soluble peptide hydrolase activities in intestinal mucosa might be due in part to an increase in binding or absorption of pancreatic peptide hydrolases during fasting, mucosal enzyme activity was examined both in animals whose pancreatic ducts had been occluded and in sham-operated rats after 3 days of fasting. As shown in Table II both groups of rats showed similar increases in the activities of soluble peptide hydrolases during fasting.

Effect of fasting and refeeding on peptide hydrolase and β-fructofuranosidase activity in the purified brush border

In contrast to the peptide hydrolases in cytosol, which increased, Table III shows that the specific activity of both peptide hydrolases and β-fructofuranosidase decreased significantly in the purified brush-border membranes on the third and fifth days of starvation, returning to normal by the third day of refeeding. The observed reduction in activity of β-fructofuranosidase was greater than that of either of the peptide hydrolases.

Effect of fasting on fructose-1,6-diphosphatase, pyruvate kinase, aspartate transaminase and alanine transaminase

Fructose-1,6-diphosphatase activity was over 3 times higher in jejunum than in ileum in fed control animals (Fig. 4). With starvation a significant rise in the activity of these enzymes was observed. Both in jejunum and ileum, fructose-1,6-diphosphatase activity had reached near maximal values by the second day of fasting. Compared with controls the increase was much greater in the ileum (4-fold) than in the jejunum (2-fold), (Fig. 4). Pyruvate kinase activity showed progressive decreases with starvation. By the fifth day of starvation the specific activity of the enzyme was only half of that observed in control fed animals, in jejunum or ileum. The activities of both alanine and aspartate transaminases were greater in jejunum than in ileum in fed control rats (Table IV). By the fifth day of starvation, significant increases in the activities of both enzymes were observed in ileum but in jejunum they differed in their response: the activity of alanine transaminase increased significantly, whereas that of aspartate transaminase was essentially unchanged.

TABLE I

EFFECT OF DIET ON PEPTIDE HYDROLASE ACTIVITIES IN SUPERNATANTS PREPARED FROM MUCOSAL HOMOGENATE OF RAT SMALL INTESTINE

Experimental conditions are described under Methods. Diet I, II, III and water were fed after 5-day fast an rats were killed at 2 h post initiation of refeeding. Enzyme activity is expressed as μmoles of substrate hydr lyzed per min per mg of protein and substrates used were: glycyl-L-leucine, glycyl-L-valine, and L-leucyl-L alanine. Each number represents a mean enzyme activity ± S.E. Probability values apply to differenc between the group that was starved and refed with water and the groups that were starved and refed wit specific diets. N.S., not significant.

Dietary status	*Number of animals*	*Jejunum*			*Ileum*		
		Gly-L-Leu	*Gly-L-Val*	*L-Leu-L-Ala*	*Gly-L-Leu*	*Gly-L-Val*	*L-Leu-L-Ala*
Control fed	9	3.25 ± 0.19	3.01 ± 0.34	1.08 ± 0.12	4.39 ± 0.34	3.49 ± 0.27	1.41 ± 0.09
Starved (5 days)	9	5.62 ± 0.23*	4.75 ± 0.22*	1.78 ± 0.12*	6.88 ± 0.69*	5.47 ± 0.47*	2.38 ± 0.16*
Starved and refed with water	6	5.76 ± 0.41	4.54 ± 0.32	1.89 ± 0.08	6.56 ± 0.47	5.35 ± 0.32	2.45 ± 0.17
Starved and refed with glucose (Diet I)	6	4.87 ± 0.37 N.S.	3.71 ± 0.29 $P < 0.05$	1.27 ± 0.08 $P < 0.01$	5.17 ± 0.38 $P < 0.05$	4.34 ± 0.33 $P < 0.05$	1.76 ± 0.06 $P < 0.01$
Starved and refed with casein hydrolysate (Diet II)	6	4.66 ± 0.21 $P < 0.05$	3.97 ± 0.38 N.S.	1.45 ± 0.15 $P < 0.05$	4.93 ± 0.63 $P < 0.05$	3.88 ± 0.27 $P < 0.01$	1.87 ± 0.18 $P < 0.05$
Starved and refed with lipid emulsion (Diet III)	6	4.25 ± 0.23 $P < 0.01$	3.47 ± 0.21 $P < 0.01$	1.20 ± 0.17 $P < 0.01$	5.05 ± 0.65 $P < 0.05$	3.74 ± 0.20 $P < 0.01$	1.56 ± 0.12 $P < 0.01$

* Differs from control fed group, $P < 0.005$.

TABLE II

EFFECTS OF FASTING ON SUPERNATANT PEPTIDE HYDROLASE ACTIVITY IN PANCREATIC DUCT-OCCLUDED AND SHAM-OPERATED RATS

Experimental conditions are described under Methods. Enzyme activity is expressed as μmoles of substrate hydrolyzed per min per mg of protein. Substrates used were L-leucyl-L-alanine and glycyl-L-leucine. Each number represents the mean enzyme activity ± S.E. Probability values compare results between either of the operated groups and fed controls.

	Fed controls		*3-day-fasted sham-operated rats*		*3-day-fasted pancreatic duct-occcluded rats*	
	*Jejunum (9)**	*Ileum (9)*	*Jejunum (4)*	*Ileum (4)*	*Jejunum (4)*	*Ileum (4)*
L-Leu-L-Ala	1.08 ± 0.12	1.41 ± 0.09	1.74 ± 0.21 $P < 0.01$	2.34 ± 0.32 $P < 0.01$	1.92 ± 0.24 $P < 0.01$	2.17 ± 0.32 $P < 0.01$
Gly-L-Leu	3.25 ± 0.19	4.39 ± 0.34	4.21 ± 0.35 $P < 0.05$	5.73 ± 0.57 $P < 0.01$	4.08 ± 0.52 $P < 0.05$	5.81 ± 0.48 $P < 0.01$

* Number of animals in parenthesis.

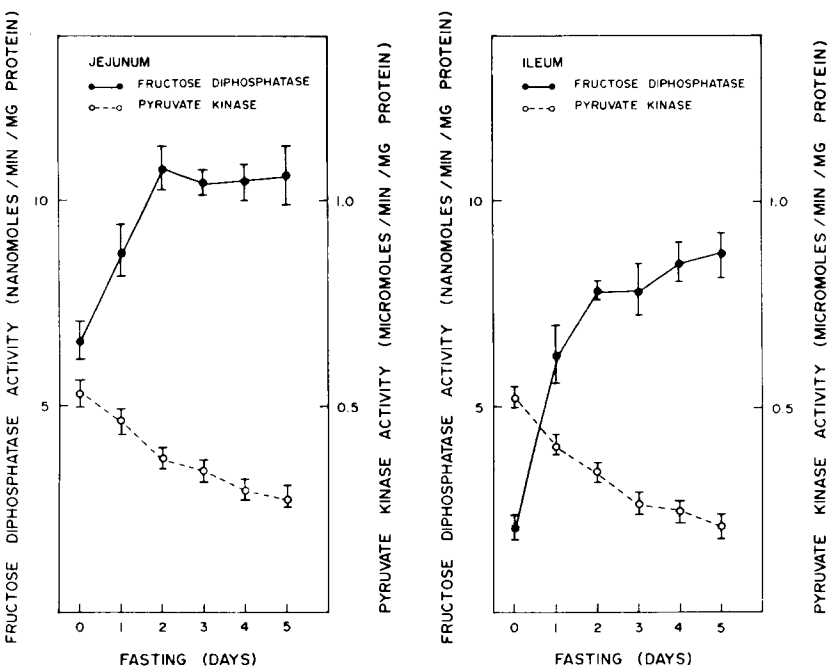

Fig. 4. Relationship of duration of fasting to activity of pyruvate kinase and fructose-1,6-diphosphatase, in cytosol fractions of jejunum and ileum. Means ± S.E. of nine animals.

Mixing experiments

When aliquots of the soluble fractions of both fed and starved animals were mixed to exclude the presence of activators or inhibitors, the assayed enzyme activities of mixtures were the same as the additive values of the aliquots (Table V).

Zymogram studies of fed and fasted animals

The zymogram patterns of soluble and brush-border fractions obtained from fed and fasted rats were compared. Starvation caused no qualitative change in peptide hydrolase profiles in either group.

TABLE III

EFFECT OF FASTING ON β-FRUCTOFURANOSIDASE AND PEPTIDE HYDROLASES IN PURIFIED BRUSH-BORDER FRACTIONS OF RAT SMALL INTESTINE

Experimental conditions are described under Methods. Enzyme activity is expressed as μmoles of substrate hydrolyzed per min per mg of protein. Substrates used were L-leucyl-L-alanine and L-leucyl-L-leucyl-L-leucine. Each number represents the mean enzyme activity ± S.E. Probability values compare results in fasted group and in fed controls.

	Number of animals	*Sucrose*	L-*Leu*–L-*Ala*	L-*Leu*–L-*Leu*–L-*Leu*
Control	7	0.86 ± 0.07	0.83 ± 0.10	0.19 ± 0.02
Fasted (3 days)	6	0.39 ± 0.05	0.56 ± 0.06	0.14 ± 0.01
		$P < 0.005$	$P < 0.01$	$P < 0.01$
Fasted (5 days)	7	0.26 ± 0.03	0.42 ± 0.03	0.11 ± 0.01
		$P < 0.001$	$P < 0.005$	$P < 0.005$
Refed (3 days)	4	0.91 ± 0.11	0.79 ± 0.09	0.20 ± 0.03

TABLE IV

EFFECT OF FASTING ON ALANINE AND ASPARTATE TRANSAMINASES IN JEJUNAL AND ILEAL MUCOSA

Experimental conditions are described under Methods. Enzyme activity is expressed as nmoles of substrate transaminated per min per mg of protein. Each number represents the mean enzyme activity ± S.E. Probability values compare results in fasted group and in fed controls. N.S., not significant.

	Number of animals	*Alanine transaminase*		*Aspartate transaminase*	
		Fed	*Fast (5 days)*	*Fed*	*Fast (5 days)*
Jejunum	9	531.0 ± 34.0	763.3 ± 86.5 $P < 0.01$	230.3 ± 14.9	247.6 ± 23.2 N.S.
Ileum	9	186.7 ± 14.6	298.5 ± 21.8 $P < 0.01$	124.0 ± 6.2	198.4 ± 13.7 $P < 0.01$

DISCUSSION

The specific activities of the peptide hydrolases of purified brush border fell in both jejunum and ileum during starvation but returned to normal upon refeeding. The specific activity of β-fructofuranosidase exhibited similar changes. Reductions in the activities of β-fructofuranosidase, maltase and isomaltase with fasting have previously been reported in man[10] and in rat[9]. It appears from the present study that the brush-border-bound peptide hydrolases may undergo similar adaptive changes and that their activity may be similarly regulated by the availability of substrates.

In striking contrast to the enzyme activity in brush borders, there were significant increases in the specific activities of peptide hydrolases in the soluble fractions during starvation, which fell on refeeding. These were seen in both jejunum and ileum and were established by the second day. Previous studies have shown that following

TABLE V

ACTUAL *versus* PREDICTED PEPTIDE HYDROLASE ACTIVITY IN STANDARD MIXTURES OF ALIQUOTS OF SUPERNATANT FLUID FROM FED AND FASTED RAT INTESTINE

Experimental conditions are described under Methods. Enzyme activity is expressed either as μmoles of glycyl-L-valine hydrolyzed per min per 100 μl of supernatant fraction or as μmoles of glycyl-L-valine hydrolyzed per min per mg of protein.

Mixture	*Volume of supernatant fraction (μl)*		*Enzyme activity (μmoles/min per 100 μl)*				*Enzyme activity (μmoles/min per mg protein)*			
	Fed	*Fasted*	*Theoretical**		*Experimental***		*Theoretical*		*Experimental*	
			1	*2*	*1*	*2*	*1*	*2*	*1*	*2*
1	100	0	0.31	0.27	—	—	3.4	3.1	—	—
2	75	25	0.32	0.30	0.33	0.32	3.4	3.5	3.5	3.7
3	50	50	0.35	0.37	0.35	0.35	4.0	4.4	3.8	4.2
4	25	75	0.37	0.40	0.38	0.40	4.6	4.9	4.6	5.0
5	0	100	0.42	0.43	—	—	5.3	5.6	—	—

* The theoretical values are those predicted from the sum of the individual activity of each aliquot. 1 and 2 represent duplicate experiments.

** The experimental values are those of assays of individual mixtures.

starvation there are significant reductions in the number of cells per unit length of intestine, in the rate of [^{3}H]thymidine incorporation into mucosa, and in mucosal weight[25–28] and the present studies bear this out. However, despite the evidence of a reduction in cell population, there was a marked rise in enzyme activity when expressed per mg of protein or per mg of DNA, reflecting an increased concentration of the enzymes in the surviving cells. This increase in specific activity suggests either increased enzyme synthesis or diminished catabolism, though direct data on this point is lacking.

It is improbable that rises in the peptide hydrolase activities of soluble fractions were due to release of brush-border-bound peptide hydrolases into the cytosol, since zymograms of the soluble fraction of the intestine of fasted rats did not show any evidence of the electrophoretically distinct brush-border peptide hydrolases. The presence of an activator or the absence of an inhibitor in soluble fractions prepared from fasted rats, was ruled out by the data from mixing experiments. Peptide hydrolases are present in the rat pancreatic juice[2,29] and though low in activity there existed the possibility of an increased adsorption of these enzymes in fasted rats: our data on fasted rats whose pancreatic ducts had been ligated exclude this and suggest that the observed changes were due primarily to direct effects of fasting on intestinal cells rather than to any alterations in the pancreatic output of similar enzymes.

Recently various investigators presented evidence that during periods of fasting both in man and in experimental animals, continuous formation of glucose occurs, from amino acids liberated from the endogenous protein of liver, muscle and kidney, to meet the metabolic requirements of vital organs such as brain and heart[30–33]. From the measurements of intracellular amino acid pools in liver and muscle and determinations of the origins of amino acids being metabolized, Gan and Jeffay[33] suggest that during the initial stages of starvation, the liver supplies amino acids to the rest of the body by degrading its endogenous labile protein stores; when the reserve store of labile proteins in liver is depleted, a similar process then occurs in muscle. As shown in these experiments, the intestine also exhibits a marked fall in mucosal weight and in DNA content during starvation, indicating rapid tissue breakdown, similar to that in liver and muscle.

To offset any effects of maldigestion due to pancreatic insufficiency caused by starvation[34,35], some of the refeeding experiments were performed using elemental diets, *i.e.* diets composed of components not requiring digestion prior to absorption. The effect of elemental diets I, II, and III in reducing levels of peptide hydrolase in intestine 2 h after refeeding suggests firstly that absorption is not appreciably impaired and secondly that the turnover of these enzymes is rapid. All three diets were effective in decreasing the peptide hydrolase activity. It therefore appears likely that the elevation in soluble peptide hydrolase activity was not due to lack of a specific dietary component but probably to the general unavailability of any substrate for cellular metabolism.

The increased specific activity of intestinal cytosol peptide hydrolase during fasting is consistent with the observation of Levin *et al.*[36] but differs from that reported by Kumar and Chase[37,38]. Using total small intestinal mucosal homogenate as a source of enzyme, Levin and co-workers measured the peptide hydrolase activity in rats after three days of fasting using two peptide substrates, glycylglycine and L-leucylglycine. They found that these enzymes in the mucosal homogenate were

elevated when expressed as enzyme activity per g of wet mucosal weight. However, the relative contributions by enzymes of the brush border and cytosol were not studied. In contrast, Kumar and Chase[37,38] studied the cytosol peptide hydrolase activity, using L-valylproline and glycylproline as substrates, in small intestinal mucosa of both rats and monkeys on protein deficient diets. They observed that the activity of these enzymes when expressed per mg of cytosol protein decreased progressively with time on the protein deficient diet. This discrepancy may be due either to differences in the peptide substrates used in the assays or to differences between the effects of total starvation and the effects solely of protein deprivation on mucosal metabolism.

Studies showing a decrease in the glycolytic enzyme, pyruvate kinase and an increase in a key gluconeogenic enzyme, fructose-1,6-diphosphatase, in small intestinal mucosa on the third day of fasting have previously been reported[11]. The present study confirms these observations and extends them to the fifth day. These changes suggest that during starvation glycolysis is reduced and gluconeogenesis increased in intestinal mucosa. In the present study a significant increase in alanine transaminase activity occurred both in jejunum and ileum during starvation although aspartate transaminase activity increased significantly only in the ileum. The rises in transaminase activity suggest that amino acids liberated from intracellular proteins by peptide hydrolases may be transaminated and subsequently utilized for gluconeogenesis.

Based on the data obtained in these experiments, together with data on the effect of starvation on various other tissues[30–33], a hypothesis concerning the effect of starvation on small intestinal mucosal metabolism can be formulated. During starvation, labile proteins in the mucosal cells are hydrolyzed by intracellular proteases, yielding di-, tri- and oligopeptides. Accumulation of these peptides within the cell leads to an increase in peptide hydrolases in the soluble fraction with consequent liberation of amino acids. These amino acids may then be transported to other tissues, or may be deaminated or transaminated into keto acids in the intestinal cells, which can then be used for gluconeogenesis. The glucose could be utilized either distally, for energy production by more vital organs such as heart and brain, or locally in the intestine.

However, cytosol peptide hydrolases may also be involved in intestinal function. Recently it has been reported that certain small peptides are preferentially absorbed over amino acids by intestinal cells[39,40]. Administration of peptides, other than glycylglycine or proline containing peptides, leads to the appearance solely of amino acids in the portal circulation[41,42]. From this it has been deduced that hydrolysis of the peptides may occur in intestinal cells. Thus, in addition to their catabolic role in the hydrolysis of intracellular proteins, cytosol peptide hydrolases may act on dietary peptides during absorption. However, the activities of peptide hydrolases in the brush border appear only to be concerned with the hydrolysis of luminal peptides.

ACKNOWLEDGEMENTS

The able secretarial help of Mrs Fay Avrech is acknowledged. This work was supported by a Veterans Administration Research Grant and V.A. RE TR-48. Mr Lane is a Veterans Administration Medical Student Research Trainee.

REFERENCES

1 Peters, T. J. (1970) *Biochem. J.* 120, 195–203
2 Kim, Y. S., Birtwhistle, W. and Kim, Y. W. (1972) *J. Clin. Invest.* 51, 1419–1430
3 Heizer, W. D., Kerley, R. L. and Isselbacher, K. J. (1972) *Biochim. Biophys. Acta* 294, 450–461
4 Rhodes, J. B., Eichholz, A. and Crane, R. K. (1967) *Biochim. Biophys. Acta* 135, 959–965
5 Behal, F. J., Anderson, B., Dawson, F. and Hardman, J. (1965) *Arch. Biochem. Biophys.* 111, 335–344
6 Lewis, W. H. P. and Harris, H. (1967) *Nature* 215, 351–355
7 Schwabe, C. (1969) *Biochemistry* 8, 783–794
8 Kim, Y. S., Lane, W. and Sleisenger, M. H. (1971) *Clin. Res.* 19, 395
9 Blair, D. G. R., Yakimets, W. and Tuba, J. (1963) *Can. J. Biochem.* 41, 917–929
10 Knudsen, K. B., Bradley, E. M., Lecocq, F. R., Bellamy, H. M. and Welsh, J. D. (1968) *Gastroenterology* 55, 46–51
11 Anderson, J. W. and Zakim, D. (1970) *Biochim. Biophys. Acta* 201, 236–241
12 Desai, I. D. (1971) *Can. J. Biochem.* 49, 170–176
13 Miller, D. and Crane, R. K. (1961) *Biochim. Biophys. Acta* 52, 293–298
14 Takesue, Y. and Sato, R. (1968) *J. Biochem. Tokyo* 64, 885–893
15 Clowes, G. H. A. and MacPherson, L. B. (1951) *Am. J. Physiol.* 165, 628–638
16 Matheson, A. T. and Tattrie, B. L. (1964) *Can. J. Biochem.* 45, 95–103
17 Josefsson, L. and Lindberg, T. (1965) *Biochim. Biophys. Acta* 105, 149–161
18 Dahlqvist, A. (1964) *Anal. Biochem.* 7, 18–25
19 Taketa, K. and Pogell, B. M. (1965) *J. Biol. Chem.* 240, 651–662
20 Bucher, T. and Pfleiderer, G. (1955) in *Methods in Enzymology* (Colowick, S. P. and Kaplan, B. O., eds), Vol. 1, pp. 435–440, Academic Press, New York
21 Henry, R. J., Chiamori, N., Golub, O. J. and Berkman, S. (1960) *Am. J. Clin. Pathol.* 34, 381–318
22 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275
23 Cerriotti, G. (1952) *J. Biol. Chem.* 198, 297–303
24 Munro, H. N. and Fleck, A. (1966) *Analyst* 91, 78–88
25 Ju, J. S. and Nassett, E. A. (1959) *J. Nutr.* 68, 633–649
26 Steiner, M., Bourges, H. R., Freedman, L. S. and Gray, S. J. (1968) *Am. J. Physiol.* 215, 75–77
27 McManus, J. P. and Isselbacher, K. J. (1970) *Gastroenterology* 59, 214–221
28 Hall, S. C. and Kim, Y. S. (1970) *Gastroenterology* 58, 957
29 Keller, P. J. (1968) in *Handbook of Physiology, Sect. 6, Alimentary Canal* (Code, C. F., ed.), Vol. V, pp. 2605–2628, American Physiological Society, Washington, D.C.
30 Pozefsky, T., Felig, P., Tobin, J. D., Soeldner, J. S. and Cahill, Jr, G. F. (1969) *J. Clin. Invest.* 48, 2273–2282
31 Felig, P., Owen, O. E., Wahren, J. and Cahill, Jr, G. F. (1969) *J. Clin. Invest.* 48, 584–594
32 Felig, P., Marliss, E., Pozefsky, T. and Cahill, Jr, G. F. (1970) *Am. J. Clin. Nutr.* 23, 986–992
33 Gan, J. C. and Jeffay, H. (1967) *Biochim. Biophys. Acta* 148, 448–459
34 Ju, J. S. and Nasset, E. A. (1959) *Proc. Soc. Exp. Biol. Med.* 100, 834–837
35 Webster, P. D., Singh, M., Tucker, P. C. and Black, O. (1972) *Gastroenterology* 62, 600–605
36 Levin, R. J., Newey, H. and Smyth, D. H. (1965) *J. Physiol. London* 177, 58–73
37 Kumar, V. and Chase, H. P. (1971) *J. Nutr.* 101, 1509–1514
38 Kumar, V. and Chase, H. P. (1972) *Am. J. Clin. Nutr.* 25, 485–489
39 Craft, I. L., Geddes, D., Hyde, C. W., Wise, I. J. and Mathews, D. M. (1968) *Gut* 9, 425–437
40 Sadikali, F. (1971) *Gut* 12, 276–283
41 Peters, T. J. and MacMahon, M. T. (1970) *Clin. Sci.* 39, 811–821
42 Prockop, D. J., Keiser, H. R. and Sjoerdsma, A. (1962) *Lancet* 2, 527–528