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PROTEIN DIGESTION AND ABSORPTION IN THE RAT

BY K. J. CURTIS, Y. S. KIM,* J. M. PERDOMO, D. B. A. SILK AND J. S. WHITEHEAD

From the Gastrointestinal Research Laboratory, Veterans Administration Hospital, San Francisco, California 94121, and the Department of Medicine, University of California School of Medicine, San Francisco, California 94143, U.S.A.

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SUMMARY

1. A randomly labelled ^{14}C protein was synthesized in order to investigate the site and rate of digestion and absorption of dietary protein in the rat.

2. A liquid test meal consisting of protein and a non-absorbable marker, $^{51}CrCl_3$, was administered to rats which were then sacrificed at intervals up to 4 hr after ingestion of the meal. Analysis of intestinal contents showed that as gastric emptying proceeded, the meal moved rapidly to the distal two thirds of the small intestine.

3. Protein digestion and absorption occurred predominatly in this area over a period of 1–2 hr.

4. Amounts of endogenous protein present in the small intestine never exceeded amounts of exogeneous protein during maximum absorption of exogenous protein (0–1 hr). At later time periods (2–4 hr), however, more endogenous than exogenous protein was detected in the intestinal lumen.

5. It is concluded that the digestion and absorption of dietary protein is a rapid process, taking place in the distal two-thirds of the small intestine. Endogenous protein levels do not exceed exogenous protein levels until after the bulk of exogeneous protein is absorbed.

INTRODUCTION

Recent investigation in the field of protein digestion and absorption has led to significant advances in our understanding of various aspects of this complex physiological process. Numerous studies have successfully examined specific entities such as intraluminal proteolysis, and peptide and amino acid absorption (Abibi, 1971; Adibi & Mercer, 1973; Crampton, Lis & Matthews, 1973; Matthews, 1971; Silk, 1974; Silk, Web, Lane, Clark & Dawson, 1974). We have attempted to examine the over-all process *in vivo* hoping to devise a technique which could be used in both the normal and pathological state.

A major difficulty in following the fate of an ingested protein meal has been in distinguishing exogenous dietary protein from endogenous protein secreted into the intestinal tract. To circumvent this problem a number of forms of identifiable protein, both labelled and unlabelled, have been employed by several investigators

* To whom correspondence or reprint requests should be addressed: Gastrointestinal Research Laboratory, Bldg. 12, Room 109, Veterans Administration Hospital, 4150 Clement Street, San Francisco, Ca. 94121, U.S.A.

(Borgström, Dahlqvist, Lundh & Jövall, 1957; Crane & Neuberger, 1960; Dawson & Holdsworth, 1962; Nasset & Ju, 1961; Nasset, Ju & McConnell, 1973; Ochoa-Solano & Gilter, 1968; Parkins, Dimitriadou & Booth, 1960; Salter & Coates, 1971). For various reasons most of these compounds have been technically unsatisfactory. In the present report a simple method for producing a soluble protein randomly labelled with ^{14}C is described. Test meals composed of the labelled protein and a non-absorbable marker, $^{51}CrCl_3$, were utilized to assess intestinal sites and rates of digestion and absorption of dietary protein. In addition, the proportion of intraluminal protein present as endogenous protein was determined.

METHODS

Preparation of randomly labelled protein

^{14}C-labelled protein was prepared using a ^{14}C-labelled amino acid mixture containing L-alanine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-lysine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine and L-valine with specific activities of individual amino acids ranging from 93 to 460 mc/m-mole (New England Nuclear, Boston, Mass.). 50 μc aliquots were injected I.P. into male Wistar rats (340–360 g). Initial studies indicated significant uptake of label into serum and liver protein 3 hr after injection, so that at the end of this time, rats were sacrificed, and both sera and livers of the injected rats were obtained. Livers were homogenized with isotonic saline (10 ml./g) in a Potter-Elvehjem homogenizer (clearance 0·004–0·006 cm; Kontes Glass Co, Vineland, N.J.), and the homogenate contrifuged at 16,000 *g* for 10 min. The pellet was washed with an equal volume of isotonic saline and recentrifuged. Both supernatant fractions and sera were dialysed against isotonic saline for 24 hr, and against two changes of glass distilled water for 24 hr. Retentates were then lyophilized. Subsequent analyses showed that 98 % dry weight of the preparation reacted with Biuret reagent (Dittebrandt, 1948). Aliquots were mixed with 3 vol. 1 % phosphotungstic acid (PTA); 98 % of the radioactivity appeared in the precipitate. The material had a specific activity of 3000 c.p.m./mg protein.

Composition of test meal

Each meal (final volume 4 ml.) contained approximately 380 mg ^{14}C-labelled protein and $CrCl_3$ labelled with ^{51}Cr (30,000 c.p.m./meal; New England Nuclear, Boston, Mass.) as a non-absorbable marker. $^{51}CrCl_3$ was chosen as the marker substance because previous studies indicate that this compound is absorbed poorly, if at all, from rat stomach or intestine (Donaldson & Barreras, 1966). In the past, however, it has been suggested that small quantities of $^{51}CrCl_3$ became adsorbed to intestinal mucous (Clark & Williams, 1971). To overcome this potential problem whole intestine was always analysed for ^{51}Cr. Less than 20 % of administered ^{51}Cr was found to be adsorbed to intestinal tissue, and recovery of administered ^{51}Cr during the present study was 82 % ± S.E.

Experimental procedure

Male Wistar rats (240–280 g) were fasted for 24 hr. Test meals were delivered intragastrically via oral intubation using a syringe and polyethylene tubing. To ensure complete delivery, 1 ml. H_2O, followed by 0·5 ml., air was drawn up and flushed through the tubing into the stomach. Rats were then killed by ether anaesthesia and decapitation at 0, 0·5, 1, 2, 3, 4 hr after feeding.

All subsequent preparative procedures were carried out in a cold room maintained at 0–4 °C. The abdominal cavity of each animal was opened and ligatures were placed at the pylorus, ileocaecal junction, gastroesophageal junction and rectosigmoid junction. Starting from the ileocaecal junction, the small intestine was removed. Care was taken not to displace intestinal contents. Pairs of ligatures were then tied at the junctions of the proximal third (*A*) and middle third (*B*) and at the junction of the middle third (*B*) and distal third (*C*) of the small intestine. The contents of the stomach, small intestinal segments, and colon were obtained by washing each

segment with 20 ml. cold saline followed by 20 ml. air. Volumes were then made up to 25 ml. and sonicated for 15 sec at 50 W with a probe of terminal diameter 3 mm (Branson Sonic Power Co.; Plainview, N.Y.).

Analyses

^{14}C. 1 ml. aliquots of the gut contents were counted for ^{14}C in duplicate for 1 min using a water miscible toluene PPO–POPOP scintillation mixture containing 33% Triton X-100 (Packard Tri Carb Liquid Scintillation Spectrometer, Model 2230). An external standard was employed to correct for differences in quenching between samples and the results are reported in disintegrations per minute.

^{51}Cr. Two ml. aliquots of the gut contents were pipetted into disposable plastic tubes and were counted for ^{51}Cr (Searle Analytic Automatic Gamma Counting System, Model 1185). The washed whole gut segments were placed in disposable plastic tubes and were counted for ^{51}Cr in a similar manner.

Protein. Protein determinations were performed on the gut contents by the Biuret method as modified by Dittebrandt (Dittebrandt, 1948) using a bovine albumin standard, reading at 555 nm on a spectrophotometer.

PTA precipitation. Cold 1 ml. aliquots of gut contents were mixed with 3 ml. cold 1% PTA. After precipitation on ice for 1 hr the mixture was centrifuged for 10 min and the supernatant and precipitate obtained. The precipitate was resuspended in 4 ml. H_2O. Two ml. aliquots of the supernatant liquid and the resuspended precipitate were counted for 1 min for ^{14}C as described below.

Trypsin. Suitably diluted aliquots of intestinal contents were assayed for trypsin activity by the method of Hummel (Hummel, 1959) using *p*-toluene sulphonyl-L-arginine methyl ester (TAME) as substrate. Results are expressed in units with 1 unit of activity defined as one mole substrate hydrolysed per minute at 25 °C.

Calculation of data

The total intraluminal ^{14}C radioactivity in the intestine of each animal at the time of killing was determined by combining the ^{14}C found in the gut washings with the amount of ^{14}C calculated to be remaining on the intestinal epithelial surface. The latter was determined using the following formula:

$$^{14}C_I = {}^{14}C_W \times \frac{^{51}Cr_I}{^{51}Cr_W}$$

where $^{14}C_I$ = ^{14}C on epithelial surface,

$^{14}C_W$ = ^{14}C in intestinal washing,

$^{51}Cr_I$ = ^{51}Cr on intestinal epithelial surface,

$^{51}Cr_W$ = ^{51}Cr in intestinal washings.

In the above calculation, it is assumed that the ^{51}Cr:^{14}C ratio of material trapped in the intervillous spaces of the intestinal epithelial surface is equal to the ^{51}Cr:^{14}C ratio of the material recovered in the gut washings. While it is recognized that this ratio may vary somewhat from segment to segment, it is also felt that some accounting of material retained on the gut wall must be made. The average amount of ^{51}Cr on the epithelial surface was less than 20% of the total amount of recovered ^{51}Cr.

Endogenous protein was calculated by the following formula:

$$E.P. = T.P. - {}^{14}C.P.,$$

where E.P. = endogenous protein,

T.P. = total protein in intestinal contents as measured by Biuret,

$^{14}C.P.$ = amount of protein present as ^{14}C.

RESULTS

Meal flow

The percentage of the administered non-absorbable marker ^{51}Cr that had left the stomach at different time periods is shown in Fig. 1. Gastric emptying was rapid: 44·9 % of the meal had left the stomach in 30 min, and 84·7 % of the meal had left the stomach in 60 min. Fig. 2 demonstrates the distribution of the non-absorbable marker ^{51}Cr throughout the gut at each time period.

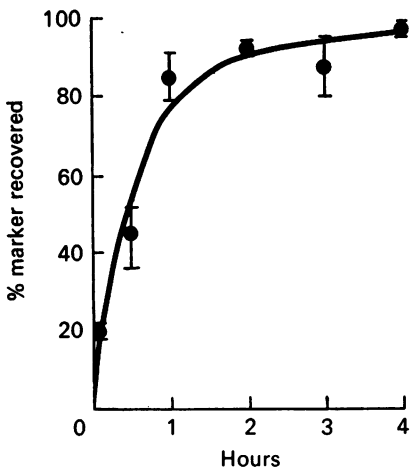

Fig. 1. Rate of gastric emptying. The percentage of the ^{51}Cr marker recovered at the end of each experiment that had emptied from the stomach (vertical axis) at various time intervals after administration of a liquid protein test meal (horizontal axis). Values are the mean ± S.E. of data from at least four individual experiments.

Distribution and sites of absorption of exogenous meal protein

The distribution of ^{14}C-labelled exogenous meal protein throughout the rat gastrointestinal tract at different time intervals after administration is also shown in Fig. 2. As already seen in Fig. 1, exogenous meal protein rapidly left the stomach. At 30 min and thereafter, negligible amounts of ^{14}C were detected in intestinal contents of segment *A* (proximal third of small intestine). At 30 min and 1 hr, ^{14}C accumulated in segments *B* and *C*. At 2, 3 and 4 hr small quantities of ^{14}C were detected in intestinal contents of segment *C* (distal third of small intestine) and the colon. Since $^{51}CrCl_3$ is non-absorbable, a difference in recoveries (% administered dose) of ^{51}Cr and ^{14}C labels at a particular site indicates that absorption of exogenous meal protein has occurred. No such difference was ever observed in segment *A*. In contrast, marked differences in recoveries of the two labels were seen in segments *B* and *C*. Thus, absorption of exogenous meal was not apparent in segment *A*, but occurred in the middle and possibly the distal thirds of rat small intestine.

Total absorption of exogenous meal protein from all intestinal segments is shown in Fig. 3. The results show that absorption occurred rapidly. By 1 hr 65·3 % of administered exogenous meal protein had been absorbed and absorption was virtually complete 2 hr after administration of the meal. Fig. 4 shows that there was

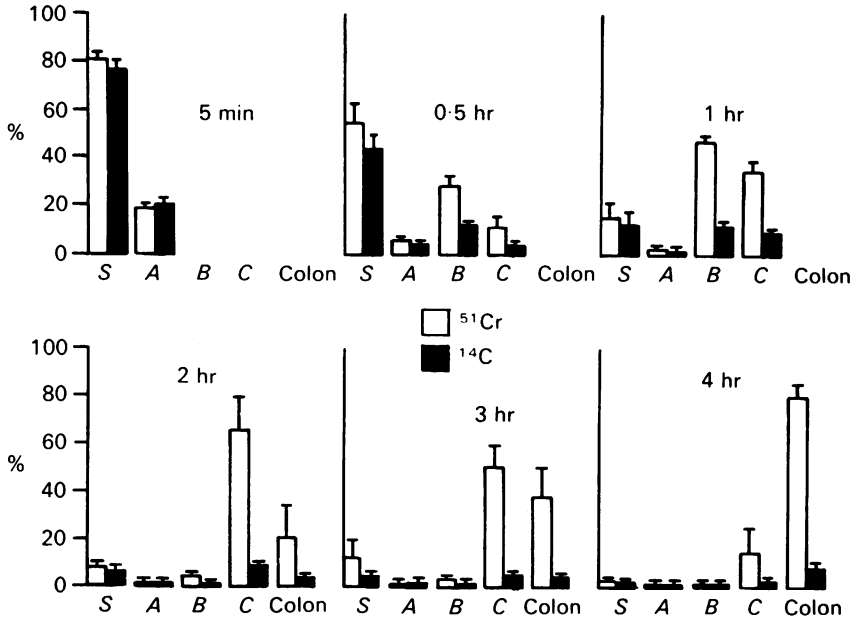

Fig. 2. Distribution of ^{51}Cr and ^{14}C in rat gastrointestinal tract. The percentage (mean ± S.E., $n = 4$) of the ^{51}Cr recovered at the end of the experiments, and the percentage (mean ± S.E., $n = 4$) of administered ^{14}C, corrected for ^{51}Cr recovery (see text), in each intestinal segment at various time intervals after administration of a liquid protein test meal. The open bars represent ^{51}Cr, and the filled bars ^{14}C. *S* refers to stomach; *A*, *B*, *C* refer to proximal, middle and distal thirds of small intestine respectively.

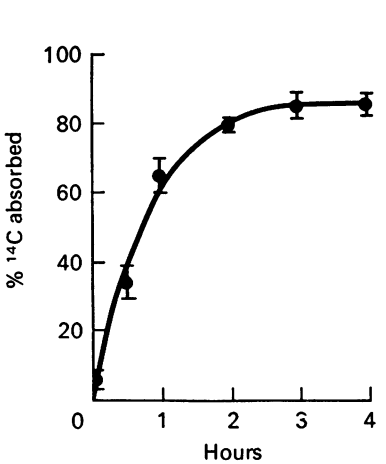

Fig. 3

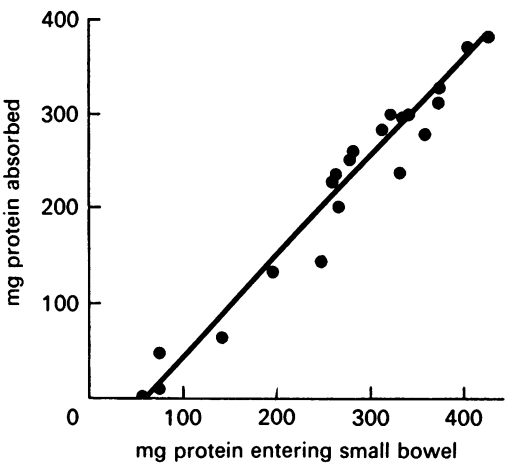

Fig. 4

Fig. 3. Protein absorption. Percentage absorption of administered ^{14}C-labelled protein from rat gastrointestinal tract after administration of a liquid protein meal. Values are mean ± S.E. of data from at least four individual experiments.

Fig. 4. Relationship between gastric emptying and protein absorption. Vertical axis refers to amount of exogenous protein (mg) absorbed from rat gastrointestinal tract, and horizontal axis refers to amount of exogenous proteins (mg) emptied from the stomach (i.e. entering small intestine).

a correlation between the amount of exogenous meal protein leaving the stomach (i.e. entering the small bowel) and amount of meal protein absorbed ($r = 0{\cdot}9$; $P < 0{\cdot}001$).

Protein digestion

The degree of protein digestion is shown in Table 1. Since 98 % of the administered protein meal was precipitable by PTA, the percentage of ^{14}C present in the PTA supernatant reflects the extent of digestion. There is comparatively little ^{14}C present

TABLE 1. % ^{14}C in supernatant after phosphotungstic acid (PTA) precipitation of intestinal contents. Values represent mean ± S.E. of mean of data from four individual experiments

Time* ...	5 min	½ hr	1 hr	2 hr	3 hr	4 hr
Segment						
Stomach	4·1 ± 1·4	1·9 ± 0·8	11·7 ± 2·7	12·5 ± 3·1	13·5 ± 4·4	21·0 ± 2·6
A†	15·6 ± 4·7	55·4 ± 5·1	66·2 ± 0·7	53·3 ± 4·9	45·4 ± 3·6	39·8 ± 6·2
B	—	48·6 ± 5·2	47·8 ± 2·2	51·3 ± 5·1	63·1 ± 6·7	45·2 ± 0·8
C	—	56·5 ± 2·8	39·2 ± 2·8	54·8 ± 6·7	60·2 ± 3·7	62·5 ± 4·3
Colon	—	—	—	44·2 ± 1·9	37·3 ± 8·1	41·6 ± 5·6

* Animals were killed at timed intervals after receiving a liquid protein test meal which contained ^{14}C-labelled protein. Aliquots of intestinal contents were incubated with 1 % PTA and radioactivity measured in resultant pellet and supernatant.

† *A*, *B*, *C* refers to proximal, middle and distal thirds of small intestine respectively.

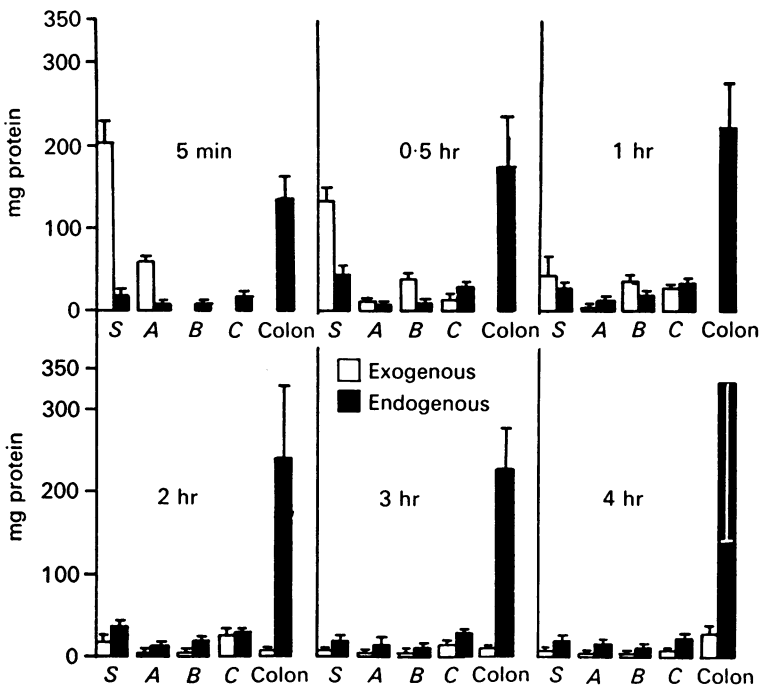

Fig. 5. Intraluminal exogenous and endogenous protein. The amounts (mg) of exogenous protein and endogenous protein detected in contents obtained from segments of gastrointestinal tract at various time intervals after administration of a liquid portion test meal. Values are the mean ± S.E. from four individual experiments. *S* refers to stomach; *A*, *B*, *C* refers to proximal, middle and distal thirds of small intestine respectively.

in the supernatant of the stomach content at the early time periods; however, this increased to 20% by 4 hr. In contrast, there is a significant appearance of ^{14}C in the supernatant of segment *A* immediately following meal administration. Therefore, in the small intestine a substantial portion of the meal in all segments at all time periods examined was present in a PTA-soluble form.

Intraluminal protein content

The amounts of exogenous and endogenous protein in the various segments of the gastrointestinal tract with respect to time are shown in Fig. 5. Immediately after feeding (5 min), exogenous meal protein resides in the stomach and proximal third

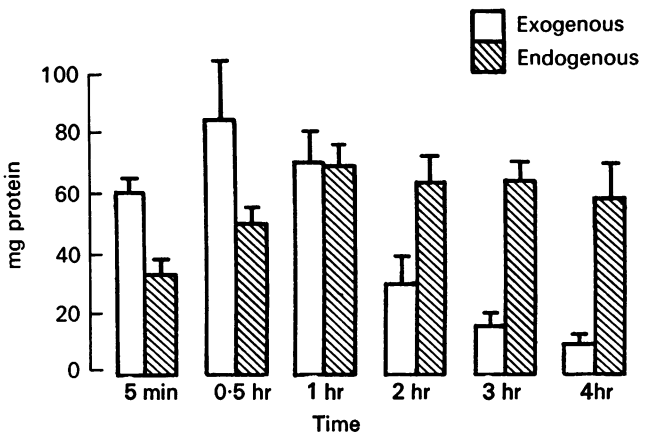

Fig. 6. Exogenous and endogenous protein content of rat small intestine. Total amounts (mg) of exogenous and endogenous protein detected in contents obtained from whole small intestine at various time intervals after administration of a liquid protein test meal. Values are the mean ± S.E. from four individual experiments.

TABLE 2. Trypsin in intestinal contents.* The values represent the mean ± S.E. of data from four individual recipients

Segment† ...	*A*	*B*	*C*	Colon	Total
Time‡ (h)					
½	20·6 ± 2·1	63·0 ± 9·9	82·4 ± 20·9	2·9 ± 1·4	169·1 ± 27·8
1	18·4 ± 3·7	112·7 ± 9·5	105·2 ± 15·5	10·29 ± 7·2	246·6 ± 13·3
2	15·2 ± 1·3	56·4 ± 11·9	106·1 ± 23·9	23·8 ± 11·1	201·6 ± 24·4
3	13·0 ± 2·5	34·7 ± 6·9	106·2 ± 18·5	18·4 ± 9·8	169·1 ± 29·1

* μmole TAME hydrolysed/min at 25 °C by total volume of intestinal contents obtained from each segment of intestine.

† *A*, *B*, *C* refers to proximal, middle and distal thirds of small intestine respectively

‡ Animals killed at time intervals after receiving a liquid protein test meal.

of the small intestine (*A*) whereas endogenous protein predominates throughout the rest of the gut. At 30 min and 1 hr (the period of maximal absorption of exogenous meal protein, Fig. 3) amounts of endogenous and exogenous protein in small intestinal segments are similar. At 2 hr segments *A* and *B* contained more endogenous than exogenous protein, whereas equal amounts were present in segment *C*. At 3 and 4 hr,

there was more endogenous than exogenous protein in all three small intestinal segments. Throughout the time course of the experiment there was a tendency for increasing amounts of endogenous protein to accumulate in the colon.

Trypsin

Trypsin activity in small intestinal segments and the colon is shown in Table 2. Contents from the distal two thirds of the small intestine (segments *B*, *C*) contained the highest levels of trypsin activity.

DISCUSSION

The experiments described in this paper were designed specifically to investigate the sites and extent of absorption of dietary protein in the rat, and to provide some information about the quantity and fate of endogenous protein. Radioactively labelled dietary protein was used because analysis of luminal contents using standard chemical techniques cannot reliably distinguish between exogenous and endogenous protein. Pancreatic proteolytic enzymes act on protein molecules sequentially by virtue of specific endo- and exopeptidase action of the enzyme (Gray & Cooper, 1971). It is important, therefore, that different amino acid residues of the protein are labelled. Labelling of a single amino acid residue may give rise to false impressions of intraluminal digestion and subsequent absorption if luminal proteolytic enzymes have either a higher or lower affinity for the single labelled amino acid relative to unlabelled amino acid residues. Recent attempts to synthesize two randomly labelled proteins, casein (Nasset *et al.* 1973) and albumin (Salter & Coates, 1971), have resulted in satisfactory preparations, but methodology was cumbersome and time consuming. A third randomly labelled protein (^{14}C-labelled Chlorella protein) is poorly soluble, and we found it difficult to handle in early experiments. The method described in this paper resulted in the production of a randomly labelled protein preparation that was soluble and easy to use. The technique used was simple, reproducible and allowed large amounts of the protein to be synthesized.

The results of the experiments carried out with the synthesized radioactive protein demonstrate that the process of protein digestion and absorption in the rat is rapid and efficient with absorption occurring predominantly in the distal two thirds of the small intestine. In addition, the results provide information about both distribution and quantitation of endogenous protein.

The data highlight the possible importance of the stomach in the regulation of protein absorption. There was a significant correlation between absorption of dietary protein and the total amount of dietary protein entering the small bowel (Fig. 4); if the rates of either luminal digestion or intestinal transport determine the over-all rate of the absorptive processes, then we would not necessarily have expected the linear relationship between stomach emptying and absorption evident in Fig. 4.

Two important facts emerge from the present study regarding the role of the proximal one third small intestine (jejunum) during the handling of a liquid protein test meal. First, there was little evidence of accumulation of $^{51}CrCl_3$ on this region of the small intestine (Fig. 2) despite continual gastric emptying (Figs. 1, 2) with increasing amounts of the marker appearing in the distal two thirds of the intestine

(Fig. 2). These findings are consistent with those of Derblom, Johansson & Nylander (1966) and suggest that in the rat. luminal contents are rapidly propelled through the proximal gut.

Secondly, the rapid transit of the test meal through the proximal intestine was not associated with rapid absorption of the meal in this region, as evidenced by the lack of consistent differences between ^{14}C and ^{51}Cr recoveries at all time periods (Fig. 2). If significant absorption had occurred at this site, ^{51}Cr recoveries would have been greater than ^{14}C recoveries.

In contrast to the lack of consistent differences between ^{51}Cr and ^{14}C recoveries in the proximal intestine, marked differences existed in segments *B* and *C* (Fig. 2). This indicates that the bulk of administered exogenous proteins was absorbed in the distal two thirds of the small intestine. In previous studies significant amounts of either nitrogen (Mettrick, 1970) or ^{131}I label (Parkins *et al.* 1960) were detected in the distal small intestine after administration of casein, albumin and ^{131}I albumin respectively, but it is difficult to be certain where absorption occurred during these experiments because a non-absorbable marker was not used. In general, it has been assumed that the proximal jejunum is the major site of absorption of protein digestion products, both in man (Borgström, Dahlqvist, Gustatson, Lundh & Malmqvist, 1959; Nixon & Mawer, 1970) and the rat (Ochoa-Solano & Gitler, 1968). The present findings suggest therefore that current concepts require re-evaluation as our data point toward the distal two thirds of the small intestine as the major absorptive area. In this context, it is of interest that brush-border peptidases, now thought to play a role in the terminal stages of luminal peptide digestion, are also maximally concentrated in the middle and distal regions of the small intestine (McCarthy & Kim, 1973).

The subject of secretion of endogenous protein into the gut lumen during absorption of dietary protein is a controversial one. Nasset has suggested that very large amounts of endogenous protein, perhaps two to three times the amount of exogenous protein, are secreted daily into the small intestine (Nasset, 1972; Nasset & Ju, 1961). A role of endogenous amino acids in determining the final composition of luminal free amino acids available in the gut lumen for absorption and subsequent protein synthesis has been proposed.

A major criticism in the past has been that sampling of intestinal contents was performed after absorption of exogenous protein had occurred, with the result that ratios of endogenous and exogenous protein may have been overestimated (Crompton & Nesheim, 1967).

In the present study absorption of exogenous protein was virtually completed 2 hr after administration of the liquid protein meal (Fig. 3). During the first hour, when rapid absorption of exogenous protein occurs, the amount of exogenous protein exceeds or at least equals the amount of endogenous protein (Figs. 5, 6) in the gut lumen. It was only at later sampling periods after maximal absorption of exogeneous protein had occurred (2–4 hr, Figs. 5, 6) that the ratio of endogenous: exogenous protein approached a 2:1 ratio which is more in keeping with the suggestion that large quantities of endogenous protein dilute exogenous protein (Nasset, 1972; Nasset & Ju, 1961). The present data therefore indicate that (1) significant secretion of endogenous protein occurs into the gut lumen after administration of exogenous

protein, but that (2) the amount of endogenous protein in the gut lumen only exceeds the amount of exogeneous protein after maximal absorption of the latter has taken place.

The site or sites of digestion and absorption of endogenous protein remain ill-defined at present. One of the striking findings in the present study was the accumulation of endogenous protein in the rat colon. This is in marked contrast to exogenous protein, which is present in this region of the gut in very small quantities (Fig. 5). While it is recognized that much of the protein in the colon is bacterial in origin, the progressively increasing values following the meal protein ingestion suggest that endogenous protein resistant to hydrolysis in the small intestine is entering the colon. This suggestion is supported by the present observation and that of others (Pelot & Grossman, 1962) that the specific endogenous protein, trypsin, is present in large quantities in the distal small intestine. Depite the accumulation of endogenous protein in the rat colon, balance studies in normal rats have shown that the average amount of nitrogen excreted in the stool is equivalent to only 8% of that ingested (Donaldson, 1967). These data taken in conjunction with present results suggest that the rat colon represents a major site of endogenous protein degradation and absorption. In this context it is noteworthy that tryptic activity in rat colon was one fifth or less than the activity in the distal small intestine (segment *C*, Table 2).

If significant quantities of endogenous protein are degraded in the rat colon, then bacterial enzymes are probably in part or totally involved in the catabolic process. Evidence for this is gained from experiments carried out in germ-free animals, for it has been shown that faecal nitrogen excretion (Loesche, 1968) and faecal pancreatic enzyme activity (Borgström *et al.* 1959) are increased in these animals.

In summary, the bulk of dietary protein ingested by the rat appears to be absorbed in the distal two thirds and not the proximal small intestine. Endogenous protein is secreted into the gut lumen, but not in the quantities previously suggested, and the colon is implicated in the metabolism of this endogenous protein.

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REFERENCES

Adibi, S. A. (1971). Intestinal transport of dipeptides in Man: relative importance of hydrolysis and intact absorption. *J. clin. Invest.* **50**, 2266–2275.

Abibi, S. A. & Mercer, D. W. (1973). Protein digestion in human intestine as reflected in luminal, mucosal, and plasma amino acid concentration after meals. *J. clin. Invest.* **52**, 1586–1594.

Borgström, B., Dahlqvist, A., Lundh, G. & Jövall, J. S. (1957). Studies of intestinal digestion and absorption in the human. *J. clin. Invest.* **36**, 1521–1536.

Borgström, B., Dahlqvist, A., Gustatson, B. E., Lundh, G. & Malmqvist, J. (1959). Trypsin, invertase and amylase content of feces of germ-free rats. *Proc. Soc. exp. Biol. Med.* **102**, 154–155.

Clark, R. J. & Williams, J. A. (1971). The value of phenol red and chromic chloride as non-absorbable gastric indicators. *Gut* **12**, 389–392.

Crampton, R. F., Lis, M. T. & Matthews, D. M. (1973). Sites on maximal absorption and hydrolysis of two dipeptides by rat small intestine *in vivo*. *Clin. Sci.* **44**, 583–594.

CRANE, C. W. & NEUBERGER, A. (1960). The digestion and absorption of protein by normal man. *Biochem. J.* **74**, 313–323.

CROMPTON, D. W. T. & NESHEIM, M. C. (1967). Amino acid patterns during digestion in the small intestine of ducks. *J. Nutr.* **99**, 43–50.

DAWSON, R. & HOLDSWORTH, E. S. (1962). An investigation into protein digestion with ^{14}C-labelled protein. *Brit. J. Nutr.* **16**, 13–24.

DERBLOM, H., JOHANSSON, H. & NYLANDER, G. (1966). A simple method of recording quantitatively certain gastrointestinal motility functions in the rat. *Acta. chir. scand.* **132**, 154–165.

DITTEBRANDT, M. (1948). Application of Weichselbaum biuret reagent to determination of spinal fluid protein. *Am. J. clin. Path.* **18**, 439–441.

DONALDSON, R. M. (1967). Role of enteric microorganisms in malabsorption. *Fedn Proc.* **26**, 1426–1431.

DONALDSON, R. M. & BARRERAS, R. F. (1966). Intestinal absorption of trace quantities of chromium. *J. Lab. clin. Med.* **68**, 484–493.

GRAY, G. M. & COOPER, H. L. (1971). Protein digestion and absorption. *Gastroenterology* **61**, 535–544.

HUMMEL, B. C. W. (1959). A modified spectrophotometric determination of chymotrypsin, trypsin, and thrombin. *Can. J. Biochem. Physiol.* **37**, 1393–1399.

LOESCHE, W. J. (1968). Accumulation of endogenous protein in the cecum of the germ-free rat. *Proc. Soc. exp. Biol. Med.* **129**, 380–387.

MCCARTHY, D. & KIM, Y. S. (1973). Changes in sucrose, enterokinase, and peptide hydrolase after intestinal resection. The association of cellular hyperplasia and adaptation. *J. clin. Invest.* **50**, 942–951.

MATTHEWS, D. M. (1971). Protein absorption. *J. clin. Path.* **24**, suppl. 5, 29–40.

METTRICK, D. F. (1970). Protein nitrogen, amino acid and carbohydrate gradients in the rat intestine. *Comp. Biochem. Physiol.* **37**, 517–541.

NASSET, E. S. (1972). Amino acid homeostasis in the gut lumen and its nutritional significance. *World Ref. of Nutr. and Dietetics*, chap. 14, pp. 134–153.

NASSET, E. S. & JU, J. S. (1961) Mixture of endogenous and exogenous protein in the alimentary tract. *J. Nutr.* **74**, 461–465.

NASSET, E. S., JU, J. S. & MCCONNELL, K. P. (1973). Comparative digestibility of casein and pancreatic juice proteins in the rat. *Nutr. Rep. Int.* **7**, 643–646.

NIXON, S. E. & MAWER, G. E. (1970). The digestion and absorption of protein in man. 2. The form in which digested protein is absorbed. *Br. J. Nutr.* **24**, 241–258.

OCHOA-SOLANO, A. & GITLER, C. (1968). Digestion and absorption of ingested and secreted proteins labelled with ^{75}Se-seleno-methionine and ^{35}S-methionine in the gastrointestinal tract of the rat. *J. Nutr.* **94**, 249–255.

PARKINS, R. A., DIMITRIADOU, A. & BOOTH, C. C. (1960). The rates and sites of absorption of ^{131}I-labelled albumin and sodium ^{131}I in the rat. *Clin. Sci.* **19**, 595–604.

PELOT, D. & GROSSMAN, M. J. (1962). Distribution and fate of pancreatic enzymes in small intestine of the rat. *Am. J. Physiol.* **202**, 285–288.

SALTER, D. N. & COATES, M. E. (1971). The influence of the microflora of the alimentary tract on protein digestion in the chick. *Br. J. Nutr.* **26**, 55–69.

SILK, D. B. A. (1974). Peptide absorption in man. *Gut* **15**, 494–501.

SILK, D. B. A., WEB, J. P. W., LANE, A. E., CLARK, M. L. & DAWSON, A. M. (1974). Functional differentiation of human jejunum and ileum: A comparison of the handling of glucose, peptides and amino acids. *Gut* **15**, 444–449.