STUDIES RELATING TO DIGESTION AND ABSORPTION OF PROTEINS.

SARAH ELIZABETH NIXON.

Ph.D.,
University of Edinburgh.

December, 1967.

Abstract

1) Six normal human subjects and two cystinuric subjects were intubated (Blankenhorn, Hirsch and Ahrens, 1955) and fed a test meal of milk protein, gelatine or low protein which included a non absorbable marker (polyethyleneglycol). Samples were then collected from various small intestinal levels.

2) The samples were analysed for :

a) their free amino acid content

b) their total amino acid content after acid hydrolysis and

c) their polyethyleneglycol (PEG) content.

The amino acid concentrations were determined using the semiautomatic procedure of Spackman, Stein and Moore, (1958) and the PEG concentrations by the turbidimetric method of Hyden, (1956)

3) It was shown that at least 95% of the milk protein meal and 99% of the PEG left the stomach within 3 hours. Collections of intestinal contents were made during the 3 hours immediately following the meal.

4) The differences between amino acid concentrations (umoles/mg PEG) in hydrolysates of the intestinal contents and those in hydrolysates of the test meals were attributed to net absorption or secretion. Net absorption after the milk protein meal was most rapid in the upper jejunum and it has been suggested that at least 90% of this meal had been absorbed proximal to a level of 130 cm from the nose. The relative retention of enzyme resistant peptides of gelatine within the lumen was demonstrated. Net secretion was evident in the upper and lower jejunum after the low protein meal.

5) Intestinal samples were fractionated by high speed centrifugation and gel filtration on G-75 and G-25 Sephadex. The proportion of total amino acid in

a) the insoluble material was 8 to 16%

b) the proteins excluded from G-75 Sephadex was 7 to 17%

c)/

c) the proteins or peptides eluted from G-75 Sephadex in a volume equivalent to that in which standard pepsin and trypsin were eluted was 7 to 18%.

d) the peptides and free amino acids which were not excluded from G-25 Sephadex was 36 to 57%

The fractions bore little similarity to the corresponding fractions in the protein test meals.

6) Free amino acid concentrations in the intestinal samples bore little relationship to the amino acid content of the meals or to that of the intestinal samples.

7) Samples collected from two jejunal levels after a milk protein meal were incubated at 37° for periods up to 80 minutes. Theoretical in vivo rates of release of amino acids, calculated from the rates of release observed, were probably fast enough to account for the absorption in the free state of seven amino acids.

8) The addition of 1 gm of ∝ Methyl DOPA to the milk protein test meal had no significant effect on the amino acid concentrations in the small intestine.

9) Retention of lysine, arginine and cystine within the lumen of the small intestine in the cystinuric subjects was demonstrated. The increase in the concentrations observed was due to an increase in the concentrations of the free amino acids.

10) Estimates of the amount of endogenous protein which had diluted the test meal amounted to 2 gm or 8 gm.

References

Blankenhorn, D.H., J. Hirsch and E. H. Ahrens (1955)
Proc. Soc. exp. Biol. Med. 88 356

Spackman, D.H., W.H. Stein and S. Moore (1958)
Analyt. Chem. 30 1190

CONTENTS.

STUDIES ON THE DIGESTION AND ABSORPTION OF DIETARY PROTEIN IN THE HUMAN SUBJECT.

GLOSSARY OF TERMS

α AMINO ACIDS

Asp = Aspartic acid ; Thr = Threonine ; Ser = Serine ; Pro= Proline ; Hypro = Hydroxyproline ; Glu = Glutamic acid ; Gly = Glycine ; Ala = Alanine ; Cys = Cystine ; Val = Valine ; Met = Methionine ; Ileu = Isoleucine ; Leu = Leucine ; Tyr = Tyrosine ; Phe = Phenylalanine ; Lys = Lysine ; His = Histidine ; Arg = Arginine.

α AMINO ACID CONCENTRATIONS

(a) Total Amino Acid Concentrations refers to the concentration of each α amino acid released by acid hydrolysis over a period of 18 hours. The term includes the concentration of the α amino acid in the free state as well as the concentration of peptide linked amino acid. The total α amino acid concentrations are presented in two forms :-

(1) With respect to volume, as μmoles/ml of intestinal contents and

(2) With respect to the intestinal marker compound, as μmoles/mg of polyethyleneglycol (μmoles/mg PEG)

(b) Total Combined Amino Acid Concentrations refers to the sum of the total α amino acid concentrations. The term includes the total concentration of every α amino acid measured.

(c) Free Amino Acid Concentrations refers to the concentration, in the free state, of each α amino acid in intestinal contents.

PEG = Polyethyleneglycol.

α Methyl DOPA = L-alpha-methyl-3.4-dihydroxyphenylalanine

AIMS OF THE STUDIES.

In the work to be described in this Thesis, intubated subjects have received test meals and the processes of protein digestion and absorption have been followed by measuring the amino acid composition of the intestinal contents. Precipitat-:ion and gel filtration techniques have been used to fractionate the intestinal samples and the individual fractions have been analysed for their amino acid content using the automatic amino acid analysis technique of Spackman, Stein and Moore (1958).

These techniques have been used in attempts to answer the following questions:-

(1) From which region of the small intestine are the amino acid constituents of the dietary protein absorbed?

(2) What is the extent of the dilution of dietary protein with endogenous protein?

(3) What is the rate of release of free amino acids from the intestinal contents after a protein meal? Do the amino acids of a protein meal leave the lumen in this form?

(4) To what extent does the intestinal mucosal defect in cystinuria impair the utilisation of the affected amino acids when these are fed as protein? Do the consequences of the defect help in the understanding of absorption in the normal subject?

(5) Does the L amino acid, α Methyl DOPA, known to inhibit amino acid absorption in the rat, interfere with amino acid absorption in man?

SECTION I.

INTRODUCTION.

THE PRESENT STATE OF KNOWLEDGE WITH REGARD TO PROTEIN DIGESTION AND ABSORPTION.

Reviews:

The Role of the Gastrointestinal Tract in Protein Metabolism. Ed. H.N. Munro 1964. Blackwell Scientific Publications, Oxford.

Absorption from the Intestine. G. Wiseman 1964. Academic Press. New York.

Intestinal Absorption. T.H. Wilson 1962. W.B. Saunders. Philadelphia.

Protein Digestion and Absorption in Health and Disease. C.W. Crane. Postgrad. Med. J. 1961 37 745-754.

Some Transport Lessons taught by the Organic Solute. H.N. Christensen. Perspectives in Biology and Medicine 1967. 10 471-494.

The present state of knowledge can be summarised as follows:-

Dietary protein, very often denatured by previous cooking, enters the stomach where it is subjected to attack by the gastric proteolytic enzymes, chiefly pepsin. Gastric proteolysis is limited, partly because the pH of gastric contents is not at the optimum for peptic activity, and partly because the meal begins to leave the stomach as soon as the meal is ingested. The stomach is probably more useful as a smoothing device, allowing the meal to pass into the small intestine at a rate at which it can be dealt with efficiently by the small intestine.

In the small intestine the protein is subjected to the action/

action of the pancreatic and intestinal peptidases. The chief peptidases of the pancreatic secretion are trypsin, chymotrypsin, carboxypeptidases A and B and pancreatopeptidase E. In the absence of pancreatic secretion protein digestion and absorption are seriously impaired. Intestinal secretion contains a peptidase, enteropeptidase, important in the formation of trypsin from its inactive precursor, trypsinogen. The remainder of the intestinal peptidase activity, chiefly Leucine aminopeptidase, appears to be intimately associated with the intestinal mucosa.

The concerted attack of these enzymes on the dietary protein causes rapid hydrolysis to small peptides and free amino acids.

Absorption of amino acids can occur along the length of the small intestine. The form in which the protein amino acids leave the lumen of the intestine is not yet established however. Nevertheless, there is much evidence that it is primarily as free amino acids that the products of protein digestion and absorption enter the blood stream. Some evidence is available for the absorption of small amounts of relatively large proteins and peptides in adult life. That it occurs in newborn animals is well documented.

Amino acid absorption is an active process, requiring energy. At least three different transport mechanisms are present, one for the neutral amino acids, one for the imino acids and possibly glycine and one for the basic amino acids. The requirements necessary for amino acid transport are now fairly well known.

The major route of amino acid transport from the intestine is via the portal blood. A small amount is probably carried in the lymph but the relative flow rates of portal blood and lymph make it likely that the latter is a minor route.

The overall process of protein digestion and absorption is/

is a very efficient one. Not only the major part of the dietary protein, but also the enzymes themselves are digested and absorbed.

This knowledge has been gained over a period of about 200 years by workers in many different specialities including medical practitioners, chemists, biochemists, physicists and agricultural scientists.

METHODS FOR MEASURING PROTEIN DIGESTION AND ABSORPTION IN MAN.

METHODS OF GENERAL APPLICABILITY.

The earliest procedures for measuring protein utilisation depended on their ability to support life and growth. This involved much more than the digestion and absorption of the particular protein.

BALANCE STUDIES.

Nitrogen balance studies have also been used from early times (McCollum, 1956). In this technique the nitrogen excreted is compared with that ingested.

The net absorption of nitrogen =

$$\frac{\text{nitrogen ingested - faecal nitrogen}}{\text{nitrogen ingested}} \times 100 \text{ per cent.}$$

A number of different methods have been used for carrying out balance experiments.

(1) The collection of the faeces over a period corresponding to the period of feeding. Carmine has been widely used as a marker. It is given on the first day of the test and stools are collected as soon as carmine appears. It is given again on the last day and the collection of stools stopped when it appears again.

(2) A constant diet is maintained over a period of time and the daily oral intake and faecal output of nitrogen is compared when equilibrium has been achieved.

(3) The subject is kept on a diet free from the test substance for some time and then given a single dose of the substance. The faeces are collected until it is thought that none of the substance remains in the alimentary tract.

The use of the balance method has been simplified by the/

the use of non-absorbable markers incorporated into the test food. This obviates the necessity for collecting all the faeces. Kreula (1947) found good correlation between the results obtained from a total collection in man and those obtained using chromic oxide (Cr_2O_3) as an indicator. Nakayama, Nakamura, Yamamoto and Tamiya (1960) also used chromic oxide in their study of absorption in patients after total gastrectomy. The percentage intestinal absorption is calculated from the expression

$$\frac{\text{gm protein/gm marker (Test Meal)} - \text{gm protein/gm marker (Faeces)}}{\text{gm protein/gm marker (Test Meal)}} \times 100$$

These techniques can measure only the total net disappearance between the mouth and the anus. That proportion of the faecal nitrogen not affected by the diet can be determined by the previous feeding of a protein free diet although the endogenous faecal nitrogen on a protein free diet is probably not the same as that on a protein containing diet (Nasset, 1952). The methods can give no information on mechanisms or rates of digestion and absorption. Their main importance in man is probably in the detection of gross defects in protein absorption in malabsorptive disease.

PLASMA AMINO ACIDS.

The determination of the increase in free amino acid concentrations in the peripheral venous blood following a protein meal gives much less information than does measurement of the corresponding changes in concentrations in portal blood. Absorption studies based on the appearance in the peripheral venous blood of orally administered substances require a knowledge of the metabolic fates and removal rates of these substances. This is difficult to achieve for the 18 or more amino acids in a protein meal. Variations due to different rates of gastric emptying may be overcome by the introduction of/

of the test substance directly into the duodenum. However, amino acids pass from the intestinal lumen to the peripheral venous blood through two highly metabolically active tissues, the intestinal epithelium and the liver. That amino acids undergo transformation in the intestinal epithelium was shown by Gibson and Wiseman (1951). Frame (1958) showed that notwithstanding the partial conversion of the acidic amino acids to alanine in the intestinal epithelium, the concentration of the latter in the peripheral venous blood after a high protein meal fell below fasting levels at a time when the concentrations of other amino acids were still rising. Glycine is very rapidly removed from the circulation (Junqueira, Hirsch and Rothschild, 1955).

The information on digestion and absorption obtained from these studies is therefore very limited. Richmond and Girdwood (1962) compared the concentrations of 6 amino acids in the peripheral blood of subjects with established malabsorptive disease with those in normal controls. The differences might form the basis of a clinical test for protein malabsorption but they are not likely to elucidate the physiology of protein digestion and absorption.

INTUBATION TECHNIQUES.

If by absorption is meant the disappearance of a test substance from the lumen of the intestine, then a direct measurement of intestinal contents after administration of the particular substance is obviously the method of preferance.

Intubation techniques allow sampling of intestinal contents of normal man. The first description of intestinal intubation was in 1908 but Miller and Abbot (1936) were apparently the first to consider aspiration of intestinal contents by this means. The method was improved by Blankenhorn, Hirsch and Ahrens (1955). A small rubber bag containing 2 to 3 ml. of mercury attached to a length of flexible polyvinyl tubing was swallowed and allowed to pass down the length of the/

the alimentary tract. This technique has been used to measure absorption in man (Borgstrom, Dahlquist, Lundh and Sjovall, 1957).

The technique has been greatly improved by the use of soluble non-absorbable markers to allow for volume changes in the intestines.

INTESTINAL MARKERS.

The necessary properties of a marker substance for use in balance studies, according to Smyth (1961) are:-

(1) That it can be estimated very accurately, preferably by a simple method.

(2) That it can be estimated at low molar concentrations so that its presence will cause mimimum osmotic effects.

(3) That it should not be absorbed from the intestine nor adsorbed to protein.

When used in intubation studies for the calculation of net absorption values over a relatively short period of time, other requirements must be satisfied.

(4) The test substance and the marker must travel along the gastro-intestinal tract at the same rate.

(5) A uniform distribution of the marker and the test substance must occur in the intestinal fluid.

Various compounds have been used as markers. Chromic oxide (Cr_2O_3) was found to be satisfactory in balance studies in animals and was first used in man by Kreula in 1947. In 1953 Sperber, Hyden and Ekman found polyethyleneglycol more suitable and in 1955 Hyden described a rapid turbidimetric method for the estimation of polyethyleneglycol. Borgstrom found almost 100% recovery of this marker from the intestinal tract of two ileostomy patients. Other workers have recovered similar amounts from the intestinal tract in animals and in man (Shaffer and Critchfield, 1947; Smith, 1962; Soergel and Hogan, 1967). Schedl (1966) compared polyethyleneglycol and phenol red/

red and found that the latter was slowly absorbed: Fisher and Parsons (1949) remarked that phenol red stained the intestinal mucosa. Cooper, Levitan, Fordtran and Ingelfinger (1966) compared polyethyleneglycol and bromosulphophthalein and found that the two gave results in very close agreement. Maddrey, Serebro, Marcus and Iber (1967) concluded that polyethylene-:glycol was superior to bromosulphophthalein, indocyanine green and ^{131}I labelled Rose bengal.

Polyethyleneglycol is very soluble in water and in less polar organic solvents (Shaffer and Critchfield, 1947). It has been used therefore for studies involving both water soluble and fat soluble substances.

IN VITRO TECHNIQUES.

An in vitro technique that is feasible in man involves the use of operative or peroral biopsy specimens from the small intestine. Rosenberg, Downing, Durant and Segal (1966) used this method to study the uptake of lysine, arginine and cystine by jejunal mucosa from cystinuric subjects.

METHODS OF RESTRICTED APPLICABILITY.

Many of the techniques which have given valuable information on this topic cannot be applied to man. The most obvious of these is the Cori method (Cori, 1925). This technique, usually used in rats, involves the injection of a test substance into the stomach. After a period of time the animal is killed and the intestine is ligatured at both ends and removed. The contents of the gut are analysed for the particular substance. The difference between that given and that remaining denotes absorption. This method has been used by many workers in the field (Nasset, Schwartz and Weiss, 1955; Reynell and Spray, 1956; Geiger, Human and Middleton, 1958; Twombley and Meyer, 1961; Chen, Rogers and Harper, 1962).

Some of the techniques used in animals have occasionally been made possible in man through some unusual circumstance.

SURGICAL AND PATHOLOGICAL LOOPS WITH FISTULAE.

In 1864 Thiry developed a surgical method of preparing a blind loop of intestine opening onto the abdominal wall. A test solution could be introduced into the loop and samples withdrawn. In 1888 Vella modified the Thiry fistula, allowing both ends of the isolated loop to open onto the abdominal wall. The test solutions could then be introduced into one end and collected from the other. The main problem in this technique was leakage from the fistula. Johnston (1932) modified the method by inserting into the fistula, a catheter firmly anchored by two ballons, one just inside, the other outside, the abdominal wall.

Occasionally Thiry-Vella loops are available in human subjects as a result of surgery.

Orten, over a period of years (Orten, 1963) investigated amino acid absorption in a patient with a Thiry fistula in the ileal/

ileal region of the intestine. Kuroda and Gimbel (1954) studied the absorption of stereoisomers of amino acids in a man with a Thiry-Vella loop, also in the ileum.

SAMPLING FROM THE HEPATIC PORTAL SYSTEM.

London and his school in Leningrad developed a permanent preparation for portal vein sampling. Dent and Schilling (1949) used this rather difficult surgical technique to measure the amino acid concentrations in the portal blood of dogs during protein digestion and absorption.

In patients with portal hypertension the vascular collaterals develop, including superficial abdominal anastomotic veins, permitting sampling of the portal blood in unanaesthet-:ised human subjects. Shiela Sherlock and Walsh (1946) described a man, suffering from hepatic cirrhosis, with a large anterior abdominal wall vein communicating with the portal system. A conspicuous difference existed between the non-protein nitrogen of the abdominal vein and that of the systemic veins following a protein meal.

SAMPLING FROM THE LYMPHATIC SYSTEM.

The thoracic duct is most commonly cannulated because of its size and accessibility. This surgical procedure has occasionally been adopted in man. Bierman, Byron, Kelly, Gilfillan, White, Freeman and Petrakis (1953) introduced polythene tubing into the proximal end of the thoracic duct in 10 patients with advanced neoplastic disease. Linder and Blomstrand (1958) also described a method of cannulation of the thoracic duct in man. They suggested that, whenever, for diagnostic purposes, a supraclavicular lymph node biopsy was necessary, the lymph duct could be cannulated for metabolic studies.

FRACTIONATION OF PROTEINS AND PROTEIN FRAGMENTS.

The methods used are determined by the composition of the mixture to be fractionated and by the proposed subsequent treatment of the fractions produced.

PRECIPITATION TECHNIQUES.

(1) General protein precipitation, is used to remove proteins from a mixture of proteins, peptides and amino acids, usually for further investigation of the latter two fractions. Trichloracetic acid is commonly used as a protein precipitant but when present in filtrates it decomposes with ninhydrin at 100° with the production of CO_2. It cannot therefore be used when subsequent chromatographic analysis of the filtrate on an Automatic Amino Acid Analyser is to be carried out. Other protein precipitants, such as heavy metals, ethanol, tungstic acid and picric acid have been compared (Hamilton and Van Slyke, 1943; de Verdier and Agren, 1948; Stein and Moore, 1954) and picric acid has been found to be most convenient under these conditions.

(2) Fractional precipitation. Precipitants may be used which remove only one of the components of a protein or peptide mixture e.g. fractionation of serum proteins with Na_2SO_4 (Howe, 1921) or separation of the A and B chains of insulin by alteration of pH (Sanger, 1949). These methods have been largely superseded by modern chromatographic techniques.

DIALYSIS TECHNIQUES.

(1) General protein removal. The disadvantages of this method are 1) the length of time necessary for complete equilibration and 2) the increased volume of the final solution/

solution of amino acids or peptides.

(2) Fractionation of a mixture of peptides depending on their rate of diffusion through a dialysis membrane (Craig and King, 1955). The mixture was dialysed for a time well short of that required for equilibration. The dialysate and the contents of the sac were each then concentrated and redialysed against fresh solvent. The process could be repeated a number of times.

CHROMATOGRAPHY.

(1) Paper chromatography has been widely used in the separation of small peptides and amino acids (Sanger, 1952; Oosterbaan and his colleagues, 1958). The spots may be detected by dilute ninhydrin, dilute so that minimum destruction of the amino acids occurs, and then eluted.

(2) Column chromatography allows separation of larger amounts of material than does paper. Ion exchange resins of low cross linkage give satisfactory resolution of mixtures of peptides (Moore and Stein, 1956). Proteins, however, are often denatured by the resin and are difficult to elute from the column. Peterson and Sober (1956) described ion exchange celluloses which were free from these disadvantages and these have found wide application in the fractionation of protein mixtures (Keller, Cohen and Neurath, 1958).

ELECTROPHORESIS.

(1) Paper electrophoresis is used for the separation of small peptides and amino acids (Naughton and his colleagues, 1960; Oosterbaan and his colleagues, 1958). The usefulness of the technique, as in paper chromatography, is limited by the difficulty of obtaining quantitative recoveries and by the small amounts which must be applied to the paper.

(2) Starch gel electrophoresis (Smithies, 1955) gives better resolution/

resolution of larger peptides and proteins than does filter paper, probably because of a filtering action of the gel as well as the differing mobilities of the components in the electric field.

GEL FILTRATION.

Porath and Flodin in 1959 described the use of dextran gels in the separation of substances of different molecular weights. These synthetic dextrans, as opposed to starch, are stable and of well defined composition. Adsorption effects are slight except for aromatic or heterocyclic structures where the adsorption may be of advantage in the separation. Gels of various degrees of cross linkage may be obtained allowing fractionation of substances over a wide range of molecular weights. Since their inception dextran gels have found wide application (Gelotte, 1964; Peeters, 1966).

Various combinations of the described techniques have also been used, such as paper chromatography and electrophoresis (Ingram, 1958) and gel filtration and electrophoresis (Morris, 1966).

AMINO ACID ANALYSIS.

The advent of modern techniques of amino acid analysis has widened the scope of studies on protein digestion and absorption. The early chemical methods were extremely laborious and time consuming. Large quantities of material were necessary. Various methods of separation were attempted. Town (1941) made use of the different solubilities of their copper salts. His techniques required 600gm. of protein.

PAPER CHROMATOGRAPHY.

The attempt by Martin and Synge (1941a) to separate amino acids by counter-current liquid liquid extraction led to column chromatography on silica gel (Martin and Synge, 1941b) and then to paper chromatography (Conaden, Gordon and Martin, 1944). This simple and inexpensive procedure is still considered the method of choice by many workers. Dent and Schilling (1949) were the first to use this technique in the study of protein digestion and absorption.

Quantitative recovery is difficult. The destruction of amino acids which occurs can be minimised by pretreatment of the paper with oxalic acid (Huggins and Moses, 1961). With care the elution of amino acid spots and reaction with ninhydrin can give reproducible results. Identification of spots for elution is difficult on two dimensional chromatograms, however.

The techniques used in paper chromatography are covered in Chromatographic and Electrophoretic Techniques, edited by Ivor Smith, 1960.

ION EXCHANGE CHROMATOGRAPHY.

The first synthetic ion exchange resins were prepared and described by Adams (1935). In 1949 Partridge and Westall attempted to develop a method for amino acid separation on columns packed with Zeocarb 215 and eluted with an ammonium hydroxide/

hydroxide solution. Zeocarb 215, a sulphonated phenol formaldehyde resin was superseded by the more stable uni-:functional polystyrene resins, first Dowex (Moore and Stein, 1954) then Amberlite (Moore, Spackman and Stein, 1958). Moore and Stein then developed an automatic procedure for the separation and quantitative estimation of various ninhydrin positive compounds from a mixture. Resolution of the amino acids was almost complete and recovery from the column was 100$\pm$3% except for Met for which the recovery was 95%.

Estimation of the amino acids in a protein hydrolysate was achieved in 24 hours (Spackman, Stein and Moore, 1958). Ninhydrin is used almost universally for the detection of amino acids. In non-fractionated samples its use is limited by the fact that other substances such as ammonia and urea will react with ninhydrin but in fractionated material this lack of specificity is an advantage. The reaction was first noted by Ruheman in 1911. Van Slyke, Dillon, McFadyen and Hamilton (1941) developed a quantitative gasometric determination of amino acids by measurement of the carbon dioxide produced. Earlier attempts to use the colourimetric method was unsuccess-:ful; the results were not reproducible and the colour yield decreased markedly as the amino acid concentration was reduced. Moore and Stein (1948) made the method quantitative by adding stannous chloride to the reaction mixture to keep the ninhydrin in the reduced form. Cysteine is an exception to the general reaction of amino acids, the colour produced giving a maximum extinction at 470 mu., somewhat similar to that with the imino acids (440 mu). The sensitivity with the imino acids is relatively low.

Ion exchange chromatography, at present, is the method of choice for precise quantitative amino acid analysis on a relatively small number of samples.

MICROBIOLOGICAL/

MICROBIOLOGICAL ASSAYS.

The development of this method stems from the academic studies on the nutritional needs of bacteria and fungi carried out over the last 50 years. Micro-organisms exhibit wide differences in their nutritional needs. Some are capable of synthesizing most nutrients from media of relatively simple composition. Others need a more complex medium containing preformed nutrients.

Of the organisms used for microbiological assays the lactic bacteria are the most useful and the most important. In most cases the lactic acid formed by these organisms in the course of their metabolic activities is directly proportional to the concentration of the test substance in the medium. Direct titration with sodium hydroxide is sufficient to form a standard curve. Agren (1948) pointed out that parallel assays on more than one type of organism were advisable because of a lack of absolute specificity.

The technique has been used by Richmond and Girdwood (1962) and Goldberg and Guggenheim (1962) for the estimation of certain amino acids in plasma during protein digestion and absorption.

ELECTROPHORESIS.

Atfield and Morris (1961) separated amino acids by high voltage electrophoresis. With three buffer systems most of the amino acids in a protein hydrolysate were resolved within 1 - 3 hours. As with paper chromatography quantitative elution is difficult.

THIN LAYER CHROMATOGRAPHY.

Over the last few years thin layer chromatography has become increasingly popular. Its speed and ease of manipulation have/

have made it the technique of choice for a rapid qualitative and semi-quantitative analysis. Quantitation appears to be difficult partly due to adsorption of some of the amino acids onto the support. Substances which have been used as supports include silica and cellulose (Turner and Hedgewell, 1966), rice starch (Petrovic and Petrovic, 1966), cellulose powder (Jones and Heathcote, 1966), Kieselgel G (Fare and Sammons, 1966) and Sephadex gel (Johansson and Ryno, 1962).

This technique will be improved and may supersede paper chromatography.

GAS LIQUID CHROMATOGRAPHY.

Since 1956 the use of gas liquid chromatography has excited much interest. The technique is rapid and highly sensitive. The main problem is, however, the formation in quantitative yield of volatile derivatives. The outstanding ones at this time appear to be the methyl or butyl esters of N-trifluoroacetyl amino acids. Quantitative analysis is still in its infancy.

Review: Methods of Biochemical Analysis (1966).
(Boris Weinstein).

KNOWLEDGE ON TOPICS OF PARTICULAR RELEVANCE TO THIS THESIS.

ENDOGENOUS SECRETIONS INTO THE GASTROINTESTINAL TRACT.

Nasset and Ju (1961) found that when dogs or rats were fed a meal of labelled casein a three to eight fold dilution of the ingested casein with endogenous protein occurred. Nakayama, Nakamura Yamamoto and Tamiya (1960) also found a large secretion of endogenous protein occurred in dogs after a protein meal. They removed parts of the small intestine after a protein meal containing chromic oxide as a marker. Absorption was estimated from the expression -

$$\frac{\text{mg protein (Test Meal)/mg marker} - \text{mg protein (Intestinal contents)/mg marker.}}{\text{mg protein (Test Meal)/mg marker.}} \times 100\%$$

They found that in the duodenum a negative net absorption of about 200% occurred.

Endogenous proteins are derived from gastric, pancreatic and intestinal secretions, bile, mucus and desquamated epithelium from the intestinal mucosa.

A daily volume of 2 to 4 litres of gastric juice, with a mean protein concentration of about 330mg/100 ml. (Richmond, Caputti and Wolf, 1955) is secreted in man. A contribution of 7 to 13gm. of protein per day is therefore derived from the stomach.

Flow of pancreatic juice begins within a very short time of taking food and continues until the stomach is empty (Borgstrom and his co-workers, 1957). The volume of juice secreted daily is generally given as 700 ml. Miller and Wiper, (1944) found that in human subjects with pancreatic fistulae as much as 1770 ml. per day could be collected. The pancreas synthesises/

synthesizes protein very actively. Junqueira, Hirsch and Rothschild (1955) injected ^{14}C Gly and secretin into rats and collected juice from the pancreatic duct. Flow of juice started almost immediately and reached a peak about half an hour later. There was almost complete absence of radioactivity from the pancreatic proteins during the first 50 minutes showing that labelled Gly was not exchanging with preformed protein Gly. Thereafter the pancreatic proteins became radioactive, the radioactivity reaching a peak in 2¾ hours. Injected radioactive plasma proteins did not give rise to radioactive pancreatic juice.

Various figures are given in the literature for the protein content of human pancreatic secretions. Miller and Wiper (1944) give values of 190-340 mg/100 ml. juice collected from pancreatic fistulas. Bartelheimer, Maring and Stimming (1955) collected pancreatic juice by an intubation technique and found the protein content to range from 480-530mg/100 ml. Other authors quote even higher values e.g. 1200mg/100 ml. (Geigy Scientific Tables, 1966). A value of 500mg/100 ml. and a daily volume of 700 ml. give an estimate of endogenous protein from the pancreas of 3.5gm.

An estimated 700-1200 ml. of bile is secreted daily by the liver in man (Popper and Schaffner, 1957). Polonovski and Bourillon (1952) give a figure for the protein content of hepatic bile in man of 180mg/100 ml. A contribution of about 2gm. of protein per day is thus made from the biliary secretions.

Little is known about the amount of protein in intestinal secretions. A daily volume of about 200 ml. with a protein content of 800mg/100 ml. is given in Geigy Scientific Tables, 1966. A rapid turnover of the mucosal cells of the small intestine occurs. This was demonstrated very elegantly by Leblond, Stevens and Bogoroch in 1948. They injected a single dose of radioactive inorganic phosphate into animals and found that the nuclei/

nuclei of the dividing cells of the crypts became radioactive in 2 hours. The subsequent groups of dividing cells in the crypts were nonradioactive. The movement of the labelled cells was followed by autoradiographic methods. After 18 to 24 hours they were on the sides of the villi, after 36 hours on the tips of the villi and later they were in the lumen. In man the mass of mucosal cells shed into the lumen daily may exceed 200gm. (Leblond and Walker, 1956). If 25% of this is protein then 50gm. of protein are contributed in this form. Thus an estimate of 60 to 70gm. per day of protein from endogenous sources can be derived. This is approximately equal to the protein intake per day.

THE DIGESTION OF PROTEINS.

The majority of proteins in their native state exist in a compact well organised form. They are described in terms of their amino acid sequence (the primary structure), the rotation of the peptide chain about its axis (the secondary structure), the folding of the peptide chain (the tertiary structure) and when more than one chain is present, the linking together of the chains (the quaternary structure). The protein can be altered from this well organised structure by various forms of treatment such as acid and heat. This denaturation process results in a general uncoiling of the peptide chain and, by making peptide bonds more readily avail-:able for enzyme action, facilitates digestion.

Pepsin, the chief proteolytic enzyme of gastric secretion, was perhaps the first individual enzyme to be recognised (Spallanzani, 1783) and named (Schwann, 1825, cited by Bovey and Yanari). It is secreted as the inactive precursor pepsin-:ogen which under the action of H^+ and of pepsin itself is converted to pepsin by the loss of small peptides, one of which is inhibitory to pepsin activity. Pepsin has low specificity. It preferentially splits bonds adjacent to an aromatic amino acid residue but also splits other bonds, for instance some involving leucine (Dixon and Webb, 1964). On incubation it gives fragments of much lower molecular weight, enzymatically active but with altered specificity (Perlmann, 1954). Gastric mucosa has been shown to contain other proteolytic enzymes (Ryle, 1964) but their physiological importance is not yet clear. The stomach does not appear to be essential in the digestion and absorption of proteins. The increase in faecal nitrogen after total gastrectomy is probably due to functional impairment of pancreatic secretion. Thus large amounts of food bolus mix with small amounts of pancreatic juices and pass down the jejunum more quickly. Extensive absorption occurs in the distal/

distal third of the small intestine but some of the protein nitrogen escapes this reserve part of the absorbing area (Nakayama and his co-workers, 1960). The impairment of absorption after total gastrectomy is probably due more to the loss of the stomach as a reservoir than as a digestive organ. The acid of the stomach may play an important part in the denaturation of raw protein. In man, however, this is probably rarely necessary; achlorhydria is not a cause of protein malabsorption.

The material which normally enters the duodenum probably consists of denatured proteins, peptides of various sizes and a small amount of free amino acids (Gilligan, Moore and Warren, 1952). In the duodenum it is rapidly attacked by the pancreatic enzymes. The chief proteolytic enzymes of the pancreas are trypsin, chymotrypsin, pancreatopeptidase E and carboxypeptidases A and B, all secreted as inactive precursors. The modes of action of all these enzymes are now well character-:ised, both with synthetic substrates and with proteins. Other peptidases may also be present in pancreatic juice. Leucine aminopeptidase activity has been demonstrated in the acinar cells of the pancreas (Nachlas, Morris, Rosenblatt and Seligman, 1960). Trypsin is secreted as the inactive precursor, trypsinogen. Under the action of enteropeptidase, (formerly known as entero-:kinase) and of trypsin itself trypsinogen is converted to trypsin by the loss of a hexa peptide. It is both a peptidase and as esterase (Schwert, Neurath, Kaufman and Snoke, 1948). Trypsin functions as an endopeptidase splitting mainly protein and polypeptides. It is specific to bonds involving the carboxyl end of an arginine or lysine residue. It is also important in the formation of other pancreatic enzymes from their inactive precursors.

Activation of chymotrypsinogen involves the hydrolysis of/

of 4 peptide bonds. A trypsin catalysed hydrolysis of an Arg-Ileu bond is followed by three other hydrolysees catalysed by chymotrypsin (Desnuelle and Rovery, 1961).

Although less specific than trypsin it shows a marked preference for peptide links involving the carboxyl end of aromatic amino acid residues. It also splits some bonds involving leucine and methionine. Chymotrypsin is also an endopeptidase.

Carboxypeptidase was originally isolated from beef pancreas by Anson (1937) and its activity determined on synthetic substrates by Bergman and Fruton (1941). It has since been discovered that there are two carboxypeptidases, A and B (Neurath, 1960). Both are secreted as inactive precursors, activated by trypsin. Both remove amino acid residues from the carboxyl end of a peptide chain. Carboxypeptidase A is relatively specific to aromatic or branched chain amino acids. It is inactive towards C terminal lysine, arginine or any peptide containing proline or hydroxyproline as the terminal or penultimate amino acid. Carboxypeptidase B is specific to arginine and lysine. The activity is decreased if proline occupies the penultimate position in the peptide chain. Pancreatopeptidase E is most active towards peptide bonds involving neutral amino acids.

Most of the enzyme activity of the small intestine appears to be intracellular. Cajori (1933) and Pierce, Nasset and Murlin (1935) found that centrifuged juice from jejunal loops in dogs had much less activity than uncentrifuged juice. This was particularly so in the case of disaccharidases but was also marked with peptidase activity. The juice, centrifuged or uncentrifuged, did not hydrolyse casein or egg albumin and the activity against even partially hydrolysed protein was not very great. Absorption studies with partially hydrolysed proteins indicated that the hydrolytic activity of the juice was insufficient/

insufficient to account for the peptides that were apparently hydrolysed during absorption (Cajori, 1933). Intestinal mucosa contains a number of dipeptidases (Gailey and Johnson, 1941). Most of this activity is due to leucine aminopeptidase, isolated in a highly purified form by Spackman, Smith and Brown (1955). A wide variety of synthetic amides and peptides are split by this enzyme but it is most active against dipeptides with an N-terminal leucine. Gly-Pro is very slowly hydrolysed. Other dipeptidases have been described, one splitting Ala-Gly, one splitting Gly-Gly and one splitting Pro-Gly (Gailey and Johnson, 1941). Lindberg and his colleagues have recently started a detailed study of intestinal dipeptidases (Lindberg, 1966). A study in man showed that dipeptidase activity is low in the proximal duodenum but mucosa from jejunum and terminal ileum consistently contained large amounts of dipeptidase activity.

An hypothetical picture of events in the small intestine is thus obtained. The endopeptidases split the proteins into peptides of varying length, containing predominantly lysine, arginine and the aromatic amino acids as the C terminal residues. The carboxypeptidases split off the residues from the carboxyl end of these peptides giving a mixture of free amino acids and small peptides. Where the small peptides are eventually split is still a matter of some controversy. Various studies indicate that peptides may enter the mucosal cells from the lumen and can be there hydrolysed before entering the serosal fluid (Newey and Smythe, 1959; Wiggans and Johnston, 1959). Ugolev, Jesuitova Timofeeva and Fediushina (1964) consider that the hydrolysis of the peptides is completed on the mucosal surface of the intestinal wall.

The whole hydrolysis procedure occurs very rapidly in vivo. Dent and Schilling (1949) found the rise in the free amino acids of portal blood in dogs occurred only slightly faster after the ingestion of 100g. of casein hydrolysate as compared/

compared with casein. Crane and Neuberger (1960) found a delay in the urinary excretion of ^{15}N in man of only 10 minutes when feeding yeast protein as compared with the hydrolysate. The protein intake was small (350mg. approximately) in their experiments. Borgstrom and his colleagues, 1957, found that the extent of hydrolysis of the protein of their test meal very rapidly reached 60% in the duodenum.

It is normally assumed that the digestive enzymes are relatively immune from proteolytic attack. It is significant that in the formation of these enzymes from their precursors only a small number of peptide bonds appears to be split. On activation a sharp decrease in optical rotation occurs suggesting that the chains have become more tightly coiled (Desnuelle and Rovery, 1961). This would help to limit further hydrolysis of peptide bonds.

Nasset, Schwartz and Weiss (1955) published results showing that the free amino acids in the intestinal lumen of dogs were present in similar relative concentrations, irrespective of whether the dogs were fed casein, zein or a protein free diet. On the basis of these experiments Nasset has suggested that a homeostatic mechanism exists in the gut. It has been known for a long time through feeding experiments (Geiger, 1947) that the necessary amino acids must be presented simultaneously to the body for maximum utilisation. Nasset's suggestion is that the endogenous secretions mask dietary deficiencies over a short period of time and ensure that absorption of the correct mixture of amino acid occurs. For this hypothesis to hold endogenous protein must be broken down at a rate comparable to that of dietary protein.

ABSORPTION.

ABSORPTION OF INTACT PROTEIN.

The foetal and newborn animal of most mammalian species absorbs intact protein from the small intestine by a process similar to pinocytosis (Clark, 1959). In the human the ability to absorb large molecules and particulate matter in quantity ceases before birth (Wilson, 1962). However, even in adult life the intestine retains some of this ability. This was shown particularly clearly in rats by Sanders and Ashworth (1961) using latex particles. The route to the lymphatics taken by these particles was similar to that described by Palay and Karlin (1959) for fat particles. Alexander, Shirley and Allan (1936) fed egg albumin to adult dogs and detected unaltered ov-albumin by immunological techniques in thoracic duct lymph and systemic blood. Dent and Schilling (1949) concluded from their studies that homologous serum albumin could be absorbed intact by dogs. Insulin by mouth has been shown to lower blood sugar in rabbits and dogs when intestinal proteolytic activity has been inhibited (Eaton and Murlin, 1933; Chisiu, 1966).

Food allergies are well known. These are probably due to the absorption of small quantities of ingested protein. However, different subjects may differ widely in their response to foreign proteins and the relatively low incidence of food allergies may not necessarily be correlated with a low incidence of absorption of intact protein. Walzer and his colleagues in 1926 to 1928 studied the possibility of protein absorption in man. Passive cutaneous sensitisation was produced in over 100 normal subjects by the intradermal injection of serum from two children, one sensitive particularly to egg protein and the other particularly to fish protein. When the sensitised subjects were fed the corresponding protein a reaction was obtained/

obtained at the site of injection in about 90% of cases. Morris' work on cats (1956) suggested that protein of thoracic duct lymph was diluted by protein from the intestine.

The experiments by Whipple and his co-workers (Robscheit-Robbins and Whipple, 1937; Cruz, Hawkins and Whipple, 1942) demonstrated that protein injected into the blood stream can be utilised by the animal.

Many workers, however, have found no indication of absorption of intact protein. Dawson and Porter (1962) investigated the assimilation of ^{14}C labelled algal protein in rats and concluded from their investigations that the dietary protein was completely hydrolysed to amino acids and transported from the intestine as such. Haemoglobin has been used as a non-absorbable marker by a number of workers (Smyth, 1961). Cajcri (1933) using Thiry loops in dogs recovered 98% of administered egg albumin from the loop after 60 minutes.

It seems likely that under normal conditions the amount of protein absorbed, if it occurs at all, is very small. Under abnormal conditions this may not be true. Crane (1964) found it difficult to reconcile the considerable absorption of protein which occurred in two patients with pancreatic dysfunction with the degree of dysfunction which was found.

ABSORPTION OF PEPTIDE.

In vitro experiments suggest that peptides are very poorly absorbed. From a mixture of di and tri-peptides placed on the mucosal side of an in vitro preparation of rat intestine only Glycyl dipeptides were found to be transferred to the serosal side (Wiggans and Johnston, 1959; Newey and Smyth, 1959). Considerable dipeptidase activity has been found on both the mucosal and serosal sides of the isolated rat intestine (Wiggans and Johnstone, 1959). It is possible that the peptidase activity is such that absorption of peptides, while possible, rarely/

rarely occurs. Of the peptides used by Wiggans and Johnston, the naturally occurring dipeptides were very rapidly hydrolysed with the exception of diGly which was transported. Hydroxy-:proline containing peptides have been found in the blood and urine in man after a gelatine meal (Prockop, Keiser and Sjoerdsma, 1962). Christensen, Cooper, Johnson and Lynch (1947) however, found little evidence of glycyl or alanyl peptides in venous blood of human subjects following the ingestion of 100gm. of gelatine.

London and his colleagues claimed to demonstrate a considerable increase in the polypeptide nitrogen of portal blood in dogs after various protein meals (London and Kotschneff, 1934). Experiments by many workers have failed to confirm this (Dent and Schilling, 1949; Christensen, 1949; Dawson and Porter, 1962; Levenson, Rosen and Upjohn, 1959).

AMINO ACID ABSORPTION.

In 1912 Van Slyke and Meyer using the new Van Slyke method of amino acid estimation demonstrated that the free amino acid content of mesenteric blood doubled when dogs ingested meat. This confirmed the work of Folin and Dennis (1912) who found that the amino acid nitrogen (total non-protein nitrogen - urea and ammonia nitrogen) of portal blood in cats rose following a protein meal.

This rise in the free amino acid concentration of the portal blood after a protein meal has since been confirmed by many workers (Dent and Schilling, 1949; Christensen, 1947, 1949; Levenson and his co-workers, 1959; Goldberg and Guggenheim, 1962; Richmond and Girdwood, 1962).

Dent and Schilling calculated that the rise in the individual amino acids, with the exception of glutamic acid, in the portal blood after casein was similar to that which would have/

have resulted if casein hydrolysate had been added to the fasting blood.

The evidence in support of the theory that protein was hydrolysed to amino acids and that these amino acids were then absorbed was critically examined by Fisher (1954). He suggest-:ed there was still uncertainty about several important points such as the nature of the digestion products absorbed by the mucosa, the time required for enzymic hydrolysis of proteins and the absorption of the digestion products. Although much work has been done since that time these are still questions of interest.

It is now generally accepted that the major part of dietary protein is hydrolysed to free amino acids or very small peptides and leaves the lumen of the intestine as such. In vitro techniques have shown that the small intestine can absorb amino acids at a rapid rate (Wilson and Wiseman, 1954).

MECHANISM OF THE ABSORPTION OF AMINO ACIDS.

Hober and Hober (1937) in in vivo experiments using rat small intestine compared the absorption of amino acids and acid amides and concluded that as had previously been noted for sugars, diffusion could not account for the transport of amino acids. "Their absorption is not like a diffusion but is a process complicated by the presence of an accelerating factor, the effect of which becomes more visible with lower than with higher concentrations".

The structure of the cell membrane on modern theory is of a three layered structure, a bimolecular lipid layer in the centre and protein monolayers on the inner and outer surfaces. The problem therefore becomes one of transport of water soluble molecules through lipid membranes. In the transport of substances from the luminal side to the serosal side of the cell three permeability barriers, two cell membranes and a basement membrane have to be overcome.

A great deal of discussion about the problems involved and possible mechanisms of transport has been published but the mechanism involved is still far from clear (Stein, 1962; Hokin and Hokin, 1963; Lefevre, 1961; Christensen, 1967; Rosenberg, 1948; Albers, 1967).

From the mass of work done certain facts have emerged. The L forms of the amino acids are absorbed at a much more rapid rate than the D forms (Gibson, Clark Smyth and Wiseman, 1951). Absorption of the L forms can occur in vitro against a concentration gradient (Wiseman, 1953) the transport being oxygen dependent. Active transport is the term used to describe the uptake of substance against a concentration or electrochemical gradient with an energy requirement. This has now been shown to occur in vitro for all naturally occurring amino acids with the exceptions of glutamic and aspartic acids. Wiseman/

Wiseman (1953) found that the concentrations of these two amino acids fell in both mucosal and serosal fluids when intestinal segments were perfused. In 1957 Neame and Wiseman did a series of experiments demonstrating that during the absorption of aspartic and glutamic acids from the fluid bathing the mucosal surface of rat small intestine these amino acids underwent transamination reactions with pyruvic acid. Heinz, Pichler and Pfeiffer (1965) have given evidence showing that Glu utilises a different transport system from that of the neutral amino acids (although the evidence is also consistent with the view that Gly is transported by a different mechanism).

Active transport of the basic amino acids was not shown until the work of Milne, Asatoor and Loughridge (1961) showing defective intestinal absorption in cystinuric patients stimulated further work on the basic amino acids (Hagihira, Lin and Wilson, 1961).

Sodium ions play a fundamental role in the transport of the amino acids (Christensen, 1967). During transport the movement of Na^+ into and out of the cells is accelerated. When Na^+ cannot be extruded the efficiency of transport of the amino acids decrease. Pyridoxine is implicated in amino acid transport suggesting involvement of the amino group. The exact role it plays is uncertain however. The stimulatory activity of pyridoxal phosphate is approximately equal to that of pyridoxal. The phosphate appears to be bound only on the surface whereas pyridoxal penetrates readily into the cell, the uptake being completed in about 5 minutes, whereas the amino acid uptake stimulation lasts for about 5 hours (Pal, 1959).

Oestradiol disulphate and diethyl stilboestrol disulphate stimulated glycine uptake by ascites tumour cells to approximate-:ly the same degree as did pyridoxal and pyridoxal phosphate (Christensen, 1960).

In in vitro sacs of small intestine glycine and methionine/

methionine transport were stimulated by glucose but inhibited by galactose (Newey and Smyth, 1964). With both sugars present amino acid uptake was stimulated. It was suggested that the presence of sugars can affect amino acid transport in two ways a) by the provision of energy due to hexose metabolism and b) by the diversion of energy to hexose transport.

Competition experiments indicate that there are at least three different transport systems for amino acids with some overlap between them. One system is involved in the transport of L-lysine, L-arginine and L-cystine. Two systems apparently operate for the neutral amino acids, one for the neutral amino acids and a separate one for proline, hydroxyproline and perhaps glycine.

A further division of the neutral amino acid transport system has been suggested by Christensen. He has given evidence showing that there is a leucine-preferring site (L site) and an alanine-preferring site (A site). The amino acids using the latter site are accumulated to a much greater extent than those using the former site. He suggests that the A site is largely for entry into the cell and that the L site serves for exchange and perhaps for all mediated exit from the cell (Oxender and Christensen, 1963). Following on from this Jéquier, Robinson and Felber (1965) showed that the amino acid accumulation by the A site was energy requiring whereas the reversible efflux by the L site was not energy requiring.

SECTION II.

MATERIALS AND METHODS.

MATERIALS AND EQUIPMENT USED.

Dried Milk Powder: Humanised Trufood. Batch No. 2790, stored at 4° in airtight tins, each containing 450 grammes. (Trufood Ltd., The Creameries, Wrenbury, Nantwich, Cheshire).

Polyethyleneglycol: Molecular weight 4000, Batch No. 4832. (Koch Light Laboratories Ltd., Colnbrook, Bucks).

Gelatine, leaf: (British Pharmacopœia Grade).

Lactose: (British Pharmacopœia Grade).

L Methyl DOPA: (Merck, Sharp and Dohme Ltd., Hoddesdon, Herts)

Trypsin, TRSF 6FA)
Pepsin, PM 718 } (Worthington Biochemical Corporation, Freehold, New Jersey, U.S.A.)

Blue Dextran: (A.B. Pharmacia, Uppsala, Sweden).

Polyvinylchloride Tubing, i.d. 1.6mm, e.d. 2.45mm., hardness value 80, Ref. No. IT/3; Radio-opaque Tubing, i.d. 1.5mm., e.d. 2.5mm. hardness value 85, Ref. No. R/3; Nasogastric Tube G400 F.G.16. (Portland Plastics Ltd., Hythe, Kent).

Dialysis Tubing; Visking tubing 24/32" (H.M.C. 52, Gloucester Place, London, W.1.

Sephadex: G25 and G75 for gel filtration. (A.B. Pharmacia, Uppsala, Sweden.

Dowex 2 (x8) 100-200 mesh. (V.A. Howe and Co. Ltd., 46 Pembroke Road, London, W,11).

pH Meter: EIL Vibron Electrometer, Model 33B with pH measuring unit, Model C33B and radiometer electrode, G297. Temperature 37.5°. Electronic Instruments Ltd., Richmond, Surrey.

THE AUTOMATIC AMINO ACID ANALYSER.

(Spackman, Stein and Moore, 1958).

The unit used was built in 1963 (Evans Electroselenium Ltd., Halstead, Essex). Ninhydrin (Batch No. 14213, Koch Light Laboratories Ltd., Colnbrooke, Bucks) was purchased in a 3 Kgm quantity dispersed in amber bottles each containing 200gm. It was stored in the dark at 4°. The methyl cellosolve was ninhydrin grade (Union Carbide Co., 8 Grafton Street, London, W.1.). Acidic and neutral amino acids were resolved on a 150 x 0.9 cm column of 8% cross linked sulphonated polystyrene resin (EEL graded resin, CG 120, size fraction C1). The column was eluted with 0.2 N sodium citrate buffer at 30ml./hour. A temperature change, from 30° to 50° was used for physiological fluids but protein hydrolysates were all chromatographed at 50°. A buffer change, from pH 3.25 to 4.25 was made at a time which brought Cys off under the influence of the 4.25 buffer, usually between 7 and 9 hours after loading the column. The basic amino acids were resolved on a 15 x 0.9 cm column (EEL graded resin CH120, size fraction B) eluted with 0.35 N sodium citrate buffer at pH 5.28 and at 50. This system did not separate ornithine from lysine but preliminary experiments with a 50 x 0.9 cm column eluted with 0.38 N sodium citrate buffer pH 4.26 at 30/50 showed that ornithine was not present in detectable concentrations except in gelatine hydrolysates where it was present at very low concentrations.

The system was calibrated using a mixture of standard amino acids (EEL standards). Separate chromatographic standards were used for taurine, cystine, ornithine and hydroxyproline (British Drug Houses Ltd., Laboratory Chemicals Division, Poole, England).

The concentration of each amino acid was directly proportional to the area of the extinction peak (the product of/

of the height E570 or 440mu and the width at half the height) produced on the chromatogram.

The variability in the determination of standard amino acids (area/μmole) over the period in which this work was done is shown in Appendix I. The values in the table are the means and the standard deviations.

METHODS.

SUBJECTS.

The subjects used for the experiments, five men and one woman, were aged 21 - 34 years. Three were medical students and three were members of the Department. They had no history of gastro-intestinal disease and standard clinical tests excluded anaemia, glycosuria and proteinuria. Their daily protein and caloric intakes were estimated from dietary histories. One man had a daily intake of 40gm. protein (his total serum protein was 6.8gm/100ml; albumin 3.0gm/100ml), the rest had an intake of 60-70gm/day. Two subjects with cystinuria were studied. Each presented with recurrent attacks of renal colic and each excreted greatly increased amounts of Cys, Lys, Arg and ornithine in the urine. The male subject, (A.D.) was aged 20 years. There was no family history of renal calculus formation. His father, mother and sister each excreted normal amounts of Cys and the basic amino acids. The female subject (H.B.) was aged 23 years. Her parents, her two brothers and her paternal aunt all excreted moderately increased amounts of Cys and Lys. During the period of intubation they led unrestricted lives and took their normal diet. Before the actual experiment they fasted for at least 4 hours and usually overnight.

Test Meals:

The choice of the main test meal was based on the following assumptions:-

(a) The meal should be physiological in quality and quantity. For this reason it is necessary to include fat and carbohydrate as well as protein.

(b) It should mix uniformly with the selected intestinal marker compound/

compound.

(c) It should be reproducible over a period of time in one laboratory and in different laboratories.

(d) It should be palatable without causing undue, and possibly variable, psychic response.

(e) It should be acceptable in a wide variety of conditions. It is intended that the work will be extended to mal-:absorptive states where intolerance to certain dietary constituents may occur. The volume should be small enough to be tolerated by such patients.

The meal satisfying these criteria to the greatest extent was milk. It contains protein (of high biological value), fat and carbohydrate in proportions similar to those found in a normal diet. It could be obtained in quantity as dried milk and stored until required. It was easy to reconstitute. It was palatable and well tolerated by the subjects used in the experiments.

One problem was the large proportion of the very insoluble protein, casein, in milk. However, by the use of a modified milk powder in which the proportion of insoluble casein to soluble protein had been reduced this problem was minimised.

Simple meals consisting of a single protein, a fat and a simple sugar were rejected on the grounds that few normal meals exist in this form and because of the difficulty of obtaining a homogeneous and palatable mixture using such ingredients.

The use of radioactively labelled proteins was rejected, partly on ethical grounds and partly because of the difficulty of obtaining suitable ones. The use of ^{15}N labelled proteins was considered but dismissed on practical grounds because of the lack of mass spectograph facilities.

Two other test meals were used. In one the milk protein was replaced by a protein (gelatine) of low biological value; in the other (the low protein meal) the quantity of milk protein was reduced to trace amounts. These meals were designed to resemble the milk protein meal in all respects other than protein content.

(A/

(A protein free meal using a pure triglyceride and a sugar was difficult to make up in a homogenous and palatable form).

PREPARATION OF MEALS.

A. The Milk Protein Meal:

The meal consisted of 400 ml. of an emulsion prepared from 100gm. of Humanised Trufood and 2.0gm. of polyethyleneglycol in distilled water. The pH of the meal was 6.4.

A tin of Trufood, once opened, was stored at 4° and was not kept for longer than 4 days.

(Humanised Trufood is prepared from ski-med milk, cream and the products of the incubation of rennet with whole milk. These are combined to give a milk of standardised composition which closely resembles human milk. The ratio of soluble protein to casein is increased, the lipid is reduced and the content of lactose is increased. This modified milk is dried at low temperature to give a stable powder suitable for storage).

B. The Gelatine Meal:

15gm. of gelatine, 113 ml. of stock salts solution, 53gm. of lactose and 2.0gm. of polyethyleneglycol were dissolved in hot distilled water. This was allowed to cool slightly and 71gm. of double cream stirred in. The volume was made up to 400 ml. and the meal taken as a lightly set jelly.

The stock salts solution was made up as follows:-

35.73gm. calcium gluconate, 2.71gm. NaCl, 7.52gm/KCl, 11.22gm. $KHCO_3$, 17.90gm$Na_2HPO_4 \cdot 12H_2O$, 7.80gm. $NaH_2PO_4 \cdot 2H_2O$ and 3.92gm. $MgCl_2 \cdot 6H_2O$ were each dissolved individually, then mixed and the total volume made up to 500 ml. Insoluble salts of calcium and magnesium precipitated as a fine suspension. The cream contributed only trace amounts of electrolytes to the meal.

The pH was adjusted to that of the milk protein meal by the addition of concentrated HCl.

C./

Table 1

The composition of the test meals (given per meal)

	gm		
	MP	G	LP
Lactose	53	54	54
Fat	24	26	26
PEG	2.0	2.0	2.0

	mequiv		
Sodium	45	42	47
Potassium	46	46	48
Calcium	38	29	25
Magnesium	9	4	4
Chloride	39	39	45

	mmoles		
	MP	G	LP
Glu	18	10	0.60
Leu	10	3.6	0.62
Asp	9.3	6.3	0.42
Pro	8.5	16	0.50
Ser	7.2	5.1	0.48
Val	7.0	3.3	0.54
Thr	6.8	2.6	0.38
Lys	6.8	3.7	0.40
Ala	6.4	16	0.30
Ileu	5.8	1.9	0.38
Gly	3.9	46	0.34
Phe	3.5	1.8	0.28
Tyr	2.8	0.30	0.16
Arg	2.1	4.9	0.22
His	2.0	0.80	0.26
Met	1.9	0.14	0.12
Cys	0.66	0.30	0.04
Hypro	0.0	12	0.0
Total	103	134	6.0

The values for the gelatine meal include the amino acids derived from the cream.

MP = Milk protein meal

G = Gelatine meal

LP = Low protein meal

C. The Low Protein Meal:

The gelatine was excluded from the above meal.

The three meals were analysed for reducing sugar by a standard automatic ferricyanide method using lactose as standard. The presence of other reducing substances was not ruled out. Triglyceride estimation was carried out by periodate oxidation of the glycerol produced by alkaline hydrolysis (Carlson and Wadstrom, 1959). Insignificant amounts of mono- and di-glycerides were present.

Amino acid content was measured by ion exchange chromatography after acid hydrolysis.

Na^+ and K^+ were estimated by flame photometry, Ca^{++} and Mg^{++} by atomic absorption spectrophotometry and chloride by the back titration of standard silver nitrate.

The composition of the three meals is shown in Table I. With the exception of the protein content the meals differed only slightly. The lower concentrations of Ca^{++} and Mg^{++} in the synthetic meals may have been due to loss of precipitate on glassware during preparation.

POLYETHYLENEGLYCOL (PEG) INTESTINAL MARKER.

Polyethyleneglycol (PEG) was chosen as the non-absorbable marker for reasons given in the Introduction. Justification for its use is also provided in Appendix 2.

The PEG concentrations in intestinal contents were determined by the turbidimetric method of Hyden (1956). Protein was removed by precipitation with $BaCl_2$ and $Ba(OH)_2$ and the PEG then precipitated with CCl_3COOH. A standard curve was obtained using concentrations of 0.25 to 0.75mg. PEG/ml. and the intestinal samples were diluted to give concentrations within this range. Standards were included in each estimation.

The method gave very reproducible results. Estimation of the standards during the period in which this work was done gave the curve shown in Figure 1 (Mean $\pm$ S.D.).

Standard/

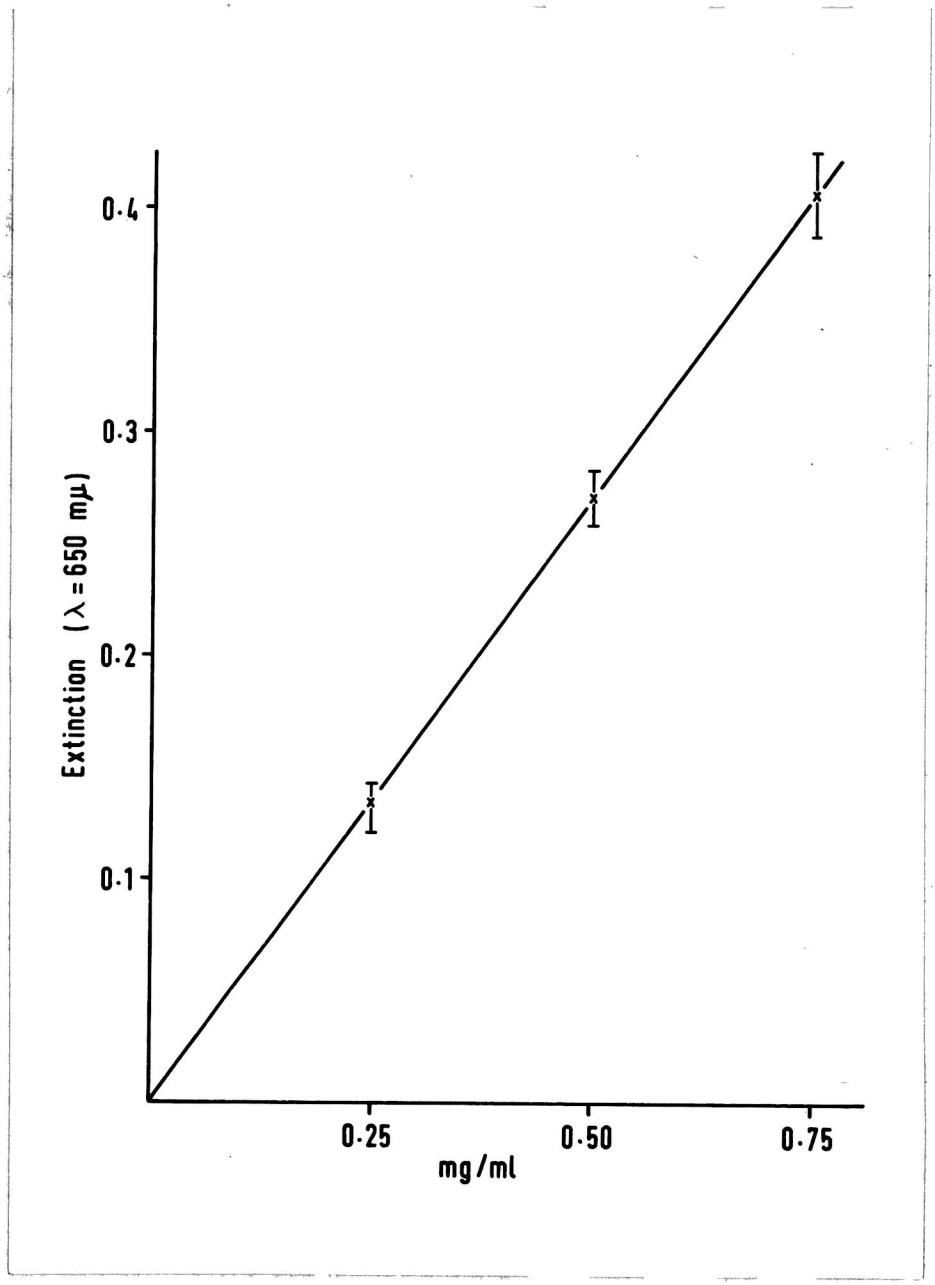

Fig. 1. The polyethyleneglycol standard curve. Extinction (λ = 650) against PEG concentration, mg/ml. Mean $\pm$ s.d. (n = 90)

Standard solutions of PEG in intestinal contents were indistinguishable from the corresponding solutions in distilled water indicating that adsorption to particulate matter was not occurring.

IN VIVO INTUBATION.

Gastric Intubation:

In 6 experiments on two subjects gastric contents were removed by nasogastric tubes, 1, 2 or 3 hours after the ingestion of milk protein test meals. The stomach was washed with four 50ml. volumes of distilled water and the washings and aspirate combined. The total α amino nitrogen and PEG recovered were determined in each case.

Intestinal Intubation:

Polyvinylchloride tubing was passed by the method of Blankenhorn, Hirsch and Ahrens (1955). Six sampling holes, over a distance of about 3cm., were cut in the leading end of the tube, which was connected to the mercury bag by two metres of radio-opaque tube. The tube was passed immediately before a meal and the subjects were instructed to lie on their right sides for an hour after the meal to facilitate the passage of the mercury bag into the duodenum. The tube moved from 50-150cm. per day. No attempt was made to restrict the passage of the tube through the intestinal tract, therefore the exact position of the level of sampling could not be predetermined. An experiment was done when the sampling holes had reached a level of 80-130cm. from the nose. This constituted an "upper level" experiment. A distance of 150-250cm. from the nose constituted a "lower level" experiment. Below a level of 300cm. from the nose it was not possible to collect samples. Occasionally when the sampling holes had travelled too far down the gut a solid coupling was added and a further piece of tubing, with new sampling holes attached. The mercury bag and tube were allowed to/

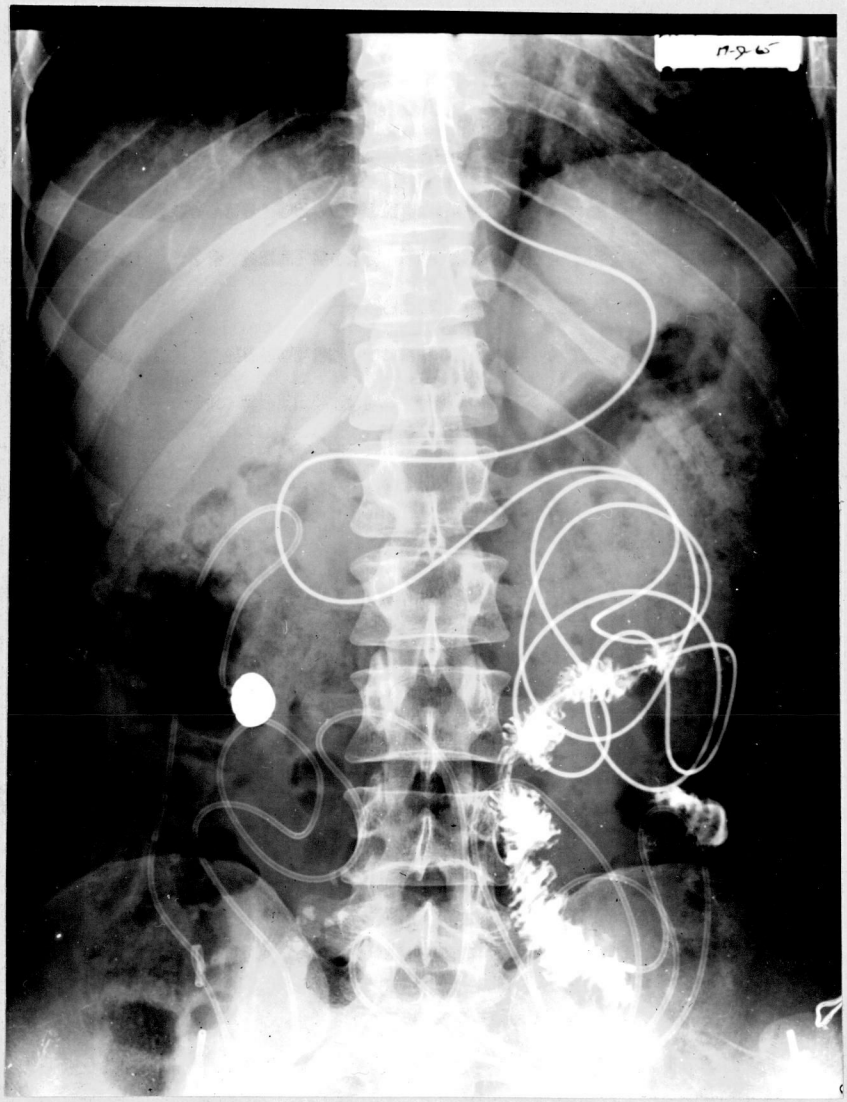

Fig. 2. The tube in position. A suspension of barium was forced down the tube and the outlining of the mucosa at the sampling holes can be seen. The sampling level in this experiment was 188 cm from the nose.

to travel right through the gut. This took five to eight days. The two metres of radio-opaque tube between the mercury bag and the sampling holes prevented pleating of the small intestine proximal to the sampling holes.

The length of the tube between the nose and the sampling holes was determined at the beginning and the end of each experiment and the mean of these values taken as the level for that experiment. At the end of an experiment a suspension of barium was forced down the tube and an X-ray photograph taken. This demonstrated the absence of coils in the stomach and gave confirmatory evidence of the site of sampling by outlining the mucosa at the sampling holes. Figure 2 shows such an X-ray picture. The duodeno-jejunal flexure provided a readily identifiable reference point.

Collection of Samples:

Immediately after the test meal had been ingested, gentle suction by means of a syringe was applied to the nasal end of the tube. When intestinal contents appeared in the tube, suction was stopped and the intestinal contents allowed to siphon off into polythene tubes embedded in crushed ice. Samples were collected in three consecutive hourly fractions. At the beginning of each hour aliquots were removed for pH estimation, free amino acid estimation and for gel filtration, where applicable, as soon as possible. The samples for free amino acid determination were put immediately into picric acid and those for gel filtration frozen immediately and stored at -25°. The rest of each hourly sample was thoroughly mixed and aliquots removed for hydrolysis and polyethyleneglycol estimation. These were also stored at -25° until required.

INCUBATION OF INTESTINAL CONTENTS.

In two experiments intestinal contents, (a) from an upper level, 110cm. from the nose and (b) from a lower level, 170cm. from the/

the nose, were collected into polythene bottles, embedded in crushed ice, for two consecutive hours. The second hourly sample was mixed by swirling gently and 5 ml. aliquots were transferred to 35 ml. stoppered centrifuge tubes. These were fitted into a mechanical shaker and placed in a water bath at 37°. At intervals of 0, 5, 10, 15, 20, 40 and 80 minutes 25 ml. of 1% picric acid was added and the samples subsequently treated as for the free amino acids.

FRACTIONATION OF INTESTINAL CONTENTS AND PROTEIN TEST MEALS BY GEL FILTRATION.

An attempt was made to determine the distribution of amino acid containing components, in terms of molecular size in intestinal contents after the three test meals.

G75 Sephadex was soaked in 0.1% NaCl for 24 hours. After removal of fines it was made up into a column, 85cm. x 1.5cm. and equilibrated at 4° with ammonium acetate buffer, pH 5.6, μ 0.08. The column was developed with the same buffer; a pressure head of 10-20cm. gave a flow rate of 15 ml. per hour. Ammonium acetate was chosen so that the electrolyte could be removed later by freeze drying. The mean pH of the intestinal samples used in these experiments was 5.6.

The column was calibrated with Blue Dextran (molecular weight 2,000.000), Pepsin (molecular weight 36,000; Dixon and Webb, 1964), Trypsin (molecular weight 23,800; Dixon and Webb, 1964) and a mixture of free amino acids.

The positions of the protein and aromatic amino acid peaks were determined by their extinction at 280 mu.

Fractionation of the Dried Milk Powder.

700mg. of Trufood were suspended in ammonium acetate buffer to give a final volume of 10.0 ml. 5.0 ml. was centrifuged for 30 minutes at 23,600g. The supernatant was filtered/

filtered through Whatman No. 5 filter paper which was then washed twice with 1 ml. of buffer. The filtrate was concentrated by rotary evaporation to about 3 ml. which was then loaded onto the column. 3.5 ml. fractions were collected and the extinction at 280 mu determined.

Fractionation of Gelatine.

A solution of gelatine, 10.0mg/ml. was made up in ammonium acetate buffer and 4.0 ml. applied to the column at room temperature. (At 4° the solution set rapidly to a stiff jelly on the surface of the gel). The fractions were collected and the E_{280} determined as before.

Fractionation of Intestinal Samples.

5.0 ml. of intestinal contents collected after a protein containing meal were frozen immediately in polythene tubes and kept at -25° until the time of gel filtration. It was then allowed to thaw at 4° and centrifuged at 23,600g for 30 minutes at 4°. The supernatant was decanted off and the precipitate washed with 1 ml. of the acetate buffer. It was then recentri-:fuged and the washing added to the supernatant. The washing was repeated once.

In the preliminary experiments, using intestinal contents after a milk protein meal, the supernatant and washings were applied directly to the Sephadex column. Fractions of 3.5 ml. were collected and the position of the protein peaks determined by the extinction at 280 mu. It was found that the flow rate was reduced during the elution of the first extinction peak. For all subsequent experiments, therefore, the following procedure was adopted. The supernatant and washings after centrifugation were combined and dialysed against 100 ml. of the ammonium acetate buffer for 24 hours. A cloudiness appeared in the dialysis sac which would not separate on centrifugation and which passed readily through/

through Whatman No.1 filter paper. It was, therefore, filtered at 4° through Whatman No.5 filter paper which was then washed twice with 1 ml. of acetate buffer. The filtrate and dialysate were recombined and freeze dried. The residue was dissolved in the buffer and then applied to the column. After dialysis the flow rate did not vary. Intestinal samples after the low protein meal were treated in a similar fashion but because of the lower protein concentrations the amino acid containing components from 5 ml. (for comparison with the other intestinal samples) and from 20 ml. (for subsequent hydrolysis) of intestinal contents were filtered through the column. The samples were applied in volumes of approximately 4 ml. as a layer between the surface of the gel and the buffer above the column using a fine catheter attached to a 10 ml. syringe.

Gel Filtration on G-25 Sephadex.

In some experiments, using intestinal contents after the milk protein meal, Fraction IV (Figure 15) was subjected to further filtration on Sephadex G-25.

A column, 45 cm. x 1.9 cm. of the Sephadex was made up and equilibrated at 4° with the ammonium acetate buffer. The totally excluded volume was measured with Blue Dextran and human serum albumen. The elution positions of tyrosine, phenylalanine and tryptophan were noted.

Fraction IV was freeze dried and redissolved in ammonium acetate buffer. After application of the sample to the column fractions were eluted and read at 280 mu as before.

A sample of intestinal contents, after protein removal by picric acid, was treated in a similar fashion.

Hydrolysis Procedure.

The dried milk powder was dissolved in 5.8 N HCl containing/

Table 2

Hydrolysis of milk powder and intestinal samples : release of amino acids with time of hydrolysis. (Hydrolysis in 5.8N HCl with $SnCl_2$, 1.5 mg/ml under nitrogen)

	Milk powder µmoles/100 gm				Intestinal contents µmoles/ml		
Time (hours)	12	18	24	72	12	18	24
Asp	8.98	9.26	9.07	8.98	4.77	4.53	4.25
Thr	6.83	6.79	6.55	5.91	3.12	2.99	2.69
Ser	7.22	7.18	6.64	5.08	4.35	4.16	3.79
Pro	8.09	8.46	8.23	8.01	4.50	5.06	4.66
Glu	17.81	17.81	17.83	17.67	8.54	8.55	8.30
Gly	3.74	3.87	3.68	3.71	7.00	7.43	7.40
Ala	6.68	6.39	6.26	6.15	2.80	2.85	2.63
Cys	0.82	0.66	0.63	0.54			
Val	6,23	6.98	6.67	6.97	3.08	3.15	2.95
Met	1.90	1.89	1.56	1.54	0.29	0.23	0.21
Ileu	5.14	5.82	5.82	5.59	2.13	2.23	2.20
Leu	9.78	9.96	9.80	9.68	3.36	3.71	3.65
Tyr	2.68	2.77	2.48	2.35	1.07	0.84	0.83
Phe	3.46	3.51	3.08	2.94	1.33	1.46	1.39
Lys	6.61	6.76	6.75	6.01	2.72	2.94	3.20
His	1.90	2.03	2.00	1.94	1.00	1.22	1.03
Arg	2.11	2.08	2.03	1.99	1.05	1.16	1.11

containing $SnCl_2$ in a concentration of 1.5mg./ml. to give a final protein concentration of 0.5mg./ml. 5.0 ml. aliquots were sealed in nitrogen filled ampoules and heated at 115° for 12, 18, 24 and 72 hours. The hydrolysate was then filtered through glass wool into 500 ml. R.B. flasks. Washings of distilled water increased the volume to about 50 ml. Water and HCl were removed by freeze drying and the dried samples were dissolved in 10.0 ml. of 0.1 HCl. They were then stored at -25° until the chromatographic analysis was done. A similar procedure was used for aliquots of intestinal contents after the milk protein meal for hydrolysis times of 12, 18 and 24 hours. Appropriate concentrations were obtained when the intestinal samples were diluted one in ten.

Amino acid analyses of the hydrolysates was carried out using the semi-automatic method of Spackman, Stein and Moore (1959) with an EEL automatic amino acid analyser. The results of these analyses are shown in Table 2. Destruction of the amino acids was greater in the samples of intestinal contents than in the dried milk. This may have been due to the fact that a greater proportion of the nitrogen was in smaller fragments in the intestinal samples.

Asp, Ser, Thr, Met and Tyr showed decreasing concentrations with increased time of hydrolysis in both cases. Cys was not estimated in these intestinal samples. In the dried milk it showed the greatest degree of destruction over the hydrolysis times.

The exclusion of $SnCl_2$ reduced still further the yield of Cys and increased the destruction of other amino acids, notably Met. Use of $SnCl_2$ and the exclusion of air increased the recovery of added Cys and Met.

Table 3 shows the comparison between the amino acid concentrations yielded by the 18 hour hydrolysis of the milk powder in air and in the presence of nitrogen and $SnCl_2$. The recovery/

Table 3

The yield of amino acids after hydrolysis of milk powder for 18 hours a) in air and b) in N_2 and HCl containing $SnCl_2$: the recovery of added amino acids under these conditions.

	Yield of amino acids µmoles/100 gm		Per cent recovery of added amino acids	
	$SnCl_2$:N_2	Air	$SnCl_2$:N_2	Air
Asp	9.26	9.26	102	100
Thr	6.79	6.77	98	92
Ser	7.18	7.10	87	85
Pro	8.46	8.46	98	93
Glu	17.81	17.82	105	99
Gly	3.87	3.84	105	106
Ala	6.39	6.30	101	105
Cys	0.66	0.46	80	64
Val	6.98	6.94	101	102
Met	1.89	1.21	89	61
Ileu	5.82	5.82	105	106
Leu	9.96	9.94	100	100
Tyr	2.77	2.27	94	76
Phe	3.51	3.31	101	93
Lys	6.76	6.76	96	101
His	2.03	2.05	95	95
Arg	2.08	2.08	96	98

recovery of added amino acids in both these situations is also presented.

No one hydrolysis time gave a maximum yield for all the amino acids. The 18 hour hydrolysis time yielded the greatest values for 7 or more of the 18 amino acids studied. The hydrolysis conditions chosen for all subsequent hydrolyses were, therefore, in 5.8 NHCl containing stannous chloride, 1.5mg/ml., in an atmosphere of nitrogen for a period of 18 hours at 115°.

In order to estimate the extent of destruction of the amino acids during this hydrolysis a series of recovery experiments were done. A standard amino acid mixture was added to the milk powder and intestinal samples to give a total of about 0.5gm. protein + 2.5 umoles of each amino acid in each ampoule. The results are shown in Table 4. Cys, Thr and Ser showed the poorest recovery.

Hydrolysis of the Cream.

50gm. of the cream were weighed into a separating funnel and 20 ml. of 5.8 NHCl containing $SnCl_2$, 1.5mg/ml. added. This was extracted with four 100 ml. volumes of $CHCl_3$. The $CHCl_3$ was then washed with about 50 ml. of distilled water. The aqueous layer and washings were concentrated to just under 20 ml. by rotary evaporation. The concentrate was then transferred to a 50 ml. flask and made up to 50 ml. with 5.8 NHCl containing $SnCl_2$. 5.0 ml. aliquots were hydrolysed as before.

Hydrolysis of the Fractions after Gel Filtration on G-75 Sephadex.

The eluate from the column after filtration of the intestinal supernatant could conveniently be divided into three fractions on the basis of the extinction of 280 mu. (Figure 15).

Fraction II corresponded with the elution position of Blue Dextran. Fraction IV included the volume of eluate in which the standard amino acids had been found.

Between/

Table 4

The percentage recovery of added amino acids from hydrolysates of intestinal contents and of milk powder.

	%	S.E.M.	n
Taurine	93	9	5
Asp	96	4	5
Thr	90	2	5
Ser	88	3	5
Pro	98	2	5
Glu	107	4	5
Gly	95	8	5
Ala	98	3	5
Cys	75	4	4
Val	98	3	4
Met	92	2	5
Ileu	101	9	5
Leu	96	2	5
Tyr	99	4	5
Phe	96	2	5
Lys	103	3	5
His	100	4	5
Arg	100	2	5
Hypro	97	4	4

The percentage recovery from an 18 hour hydrolysate of 2.5 μmoles of standard amino acid added to an aliquot of test meal or intestinal contents equivalent to 5 mg protein.

Between these two major peaks was eluted a fraction (Fraction III) with a relatively low E_{280} corresponding with the elution positions of trypsin and pepsin (see Figure 15). The 3.5 ml. volumes of each fraction were combined with washings into a 500 ml. R.B. flask. They were freeze dried and the residue dissolved in 5.8 NHCl containing $SnCl_2$. A suitable concentration was obtained when Fractions II and III were made up to 10.0 ml. and 4.0 ml. aliquots hydrolysed. Fraction IV was made up to 20.0 ml. and 5.0 ml. aliquots hydrolysed.

The residue remaining after centrifugation of the original sample was suspended in 5.8 NHCl containing $SnCl_2$ to give a final volume of 10.0 ml. 4.0 ml. aliquots of the suspension were hydrolysed.

The filter paper, used for filtering the intestinal samples after dialysis, was washed with 5.8 NHCl containing $SnCl_2$ to give a final volume of 10.0 ml. 4.0 ml. aliquots were hydrolysed.

After removal of the aliquots for hydrolysis from Fraction IV, 4.0 ml. was freeze dried directly and dissolved in 10.0 ml. of 0.1 NHCl for the estimation of free amino acid concentrations.

A separate aliquot of intestinal contents, prepared for the estimation of the free amino acid concentrations by picric acid precipitation of protein, was subjected to acid hydrolysis in the same way as Fraction IV.

Determination of Free Amino Acids.

The free amino acids of intestinal contents were estimated by the method of Moore and Stein (1954). Proteins were removed by precipitation with 1% picric acid. Moore and Stein compared picric acid precipitation with ultrafiltration and equilibrium dialysis. The former method was very much quicker (30 minutes) compared with the other two (about 16 hours at/

at 4°). Recovery of added amino acids was within 10% of theory in all cases. Hamilton and Van Slyke (1943) compared various methods of protein precipitation for the gasometric-ninhydrin reaction and found 1% picric acid to be most convenient. They obtained 99-100% recovery of added amino acids. Excess picric acid was removed by the anionic resin, Dowex 2.

Moore and Stein found complete recovery of all amino acids, with the exception of tryptophan, from this resin. Tryptophan was retained and was also labile on the Dowex 2 column, giving only 85% recovery. Tryptophan was not estimated in this study.

5.0 ml. of intestinal contents, taken immediately after collection had started were added to 25.0 ml. of 1% picric acid in a 35 ml. stoppered centrifuge tube. After centrifugation at 1500g. for 15 minutes 25.0 ml. of the supernatant was passed through a 2 x 2cm. column of Dowex 2. The column was washed with four 5 ml. volumes of 0.02 NHCl. The eluate and washings were collected in a 500 ml. R.B. flask and freeze dried. The residue was dissolved in 10.0 ml. 0.1 NHCl and stored at -25° until the chromatographic analysis was done.

An attempt was made to separate free amino acids from small peptides by gel filtration on G25 Sephadex prior to chromato-:graphy. These peptides, in some cases, produced peaks on ion exchange chromatography, overlapping those of the amino acids. The presence of glucose, tested for by Clinistix, in the intestinal samples was utilised to indicate the elution position of the non-aromatic amino acids.

Determination of α Amino Nitrogen.

(a) Removal of NH_3

After hydrolysis and freeze drying of gastric aspirates the residue was dissolved in 10 ml. of 0.1 N NaOH and dried on a rotary evaporator at 50° with 100 ml. 2N H_2SO_4 in the collecting flask/

flask. This was repeated once. The residue was then dissolved in 10.0 ml. of 0.1N HCl. Chromatography of this on the 15cm. column of the Amino Acid Analyser showed that 95-98% of the NH_3 had been removed.

(b) Amino Nitrogen Estimation.

The α amino nitrogen in the samples was measured using a slightly modified version of the Moore and Stein method (1954). Estimations were carried out in citric acid buffer at pH 5.8. The ninhydrin solution was similar to that used for the Amino Acid Analyser but the concentration of ninhydrin was increased by a factor of five. A standard curve was obtained with Asp. Samples were also chromatographed on the Amino Acid Analyser without prior removal of NH_3, to show the relative concentrations of the various amino acids.

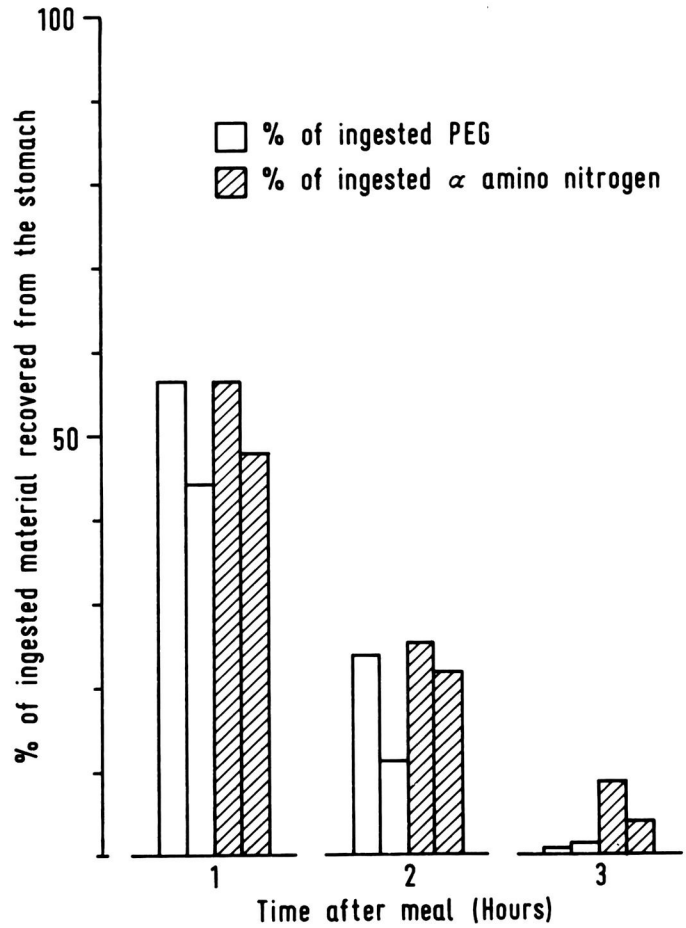

Fig. 3. The percentages of administered PEG and α amino nitrogen recovered from the stomach, 1, 2 and 3 hours after a milk protein meal. The values are given individually for the two subjects.

SECTION III.

EXPERIMENTAL RESULTS.

THE COMPOSITION OF THE GASTRIC RESIDUE.

SUMMARY:

(1) 99% of the PEG marker left the stomach within 3 hours.
(2) PEG, total Leu and total α amino nitrogen left the stomach at similar rates.
(3) The molar concentrations of total Pro, Gly and Asp relative to Leu in the gastric residues rose in each successive hour.
(4) Relatively high concentrations of Pro, Gly, Asp and Glu were found in a sample from the pyloric area after a low protein meal.

RESULTS:

The percentages of administered PEG and α amino nitrogen recovered from the stomach 1, 2 and 3 hours after a milk protein meal in the two subjects are shown in Figure 3.

50% of the protein and PEG left the stomach during the first hour. After 3 hours, in one subject, only, 0.5% of the PEG and 3% of the α amino nitrogen administered was recovered from the stomach. The corresponding values for the other subject were 1.5% and 8%.

Figure 4 shows the comparison between the percentage recoveries of PEG, Pro and Leu. The values used are the means from the two subjects. Three hours after the ingestion of the meal 99% of the PEG marker and not less than 95% of the total Leu had left the stomach.

The molar concentrations of certain amino acids relative to Leu in the gastric residues and in the test meal are shown in Table 5. The values are the means in the two subjects for the residues aspirated 1, 2 and 3 hours after the meal. The ratios of/

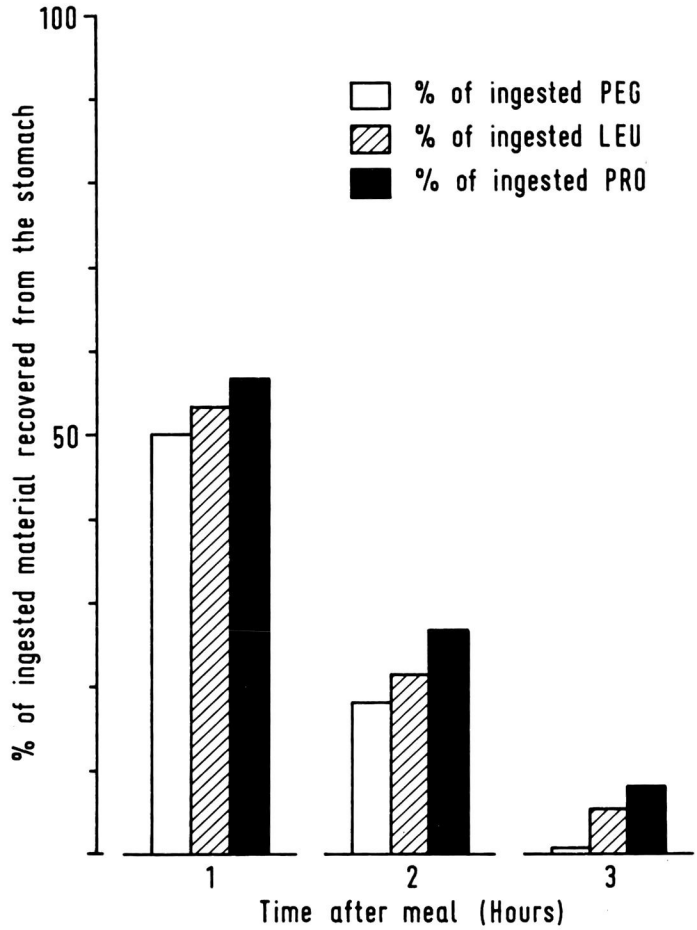

Fig. 4. The percentages of administered PEG, Pro and Leu recovered from the stomach 1, 2 and 3 hours after a milk protein meal. The values are the means from the two subjects.

Table 5

The relative molar concentrations (Leu = 1) of total amino acids in gastric contents compared with those in the test meal.

		Time after meal(hours)		
	Meal	1	2	3
Pro	0.9	0.9	1.0	1.5
Gly	0.4	0.4	0.6	1.1
Asp	0.9	0.9	1.0	1.1
Glu	1.8	1.7	1.7	1.8
Ileu	0.6	0.6	0.6	0.6
Lys	0.7	0.7	0.6	0.7

of Pro, Gly and Asp showed a continued rise over the 3 hours. The values for each of the amino acids are shown individually for the two subjects in Appendix 3.

A small amount of taurine was present in some of these samples.

An intubation experiment was performed with the sampling holes 60 cm. from the nose, the approximate position of the pyloric sphincter. A low protein meal was given and samples of pyloric contents were collected over a period of 3 hours. The samples were not stained with bile pigment and only in the third hour was taurine present in measurable amounts.

The results of this experiment are shown in full in Appendix 4. Table 6 shows the percentage of PEG and total combined amino acids recovered in each hourly period and the total amount recovered over the three hours. 46% of the PEG and total combined amino acid amounting to 50% of that given were recovered during the first hour. Over the three hour period 79% of the PEG and protein amounting to 117% of that given were recovered.

Shown in Table 7 are the molar concentrations of certain amino acids relative to Leu in the combined 3 hour sample and in the test meal. These were very similar to the relative concentrations found in the gastric contents 3 hours after the milk protein meal; Pro and Gly again showed a marked rise in concentration compared with Leu. Appendix 4 shows the comparison between the concentrations of amino acids related to PEG in the low protein meal and in the samples of pyloric contents. The increase in concentration of total combined amino acids in the pyloric samples was due chiefly to Gly, Pro and the acidic amino acids.

DISCUSSION:

The protein and PEG left the stomach at similar rates. The small disparity between the percentages of the two recovered could have been due either to protein lagging slightly behind PEG/

Table 6

The percentage of administered amino acids(total combined) and PEG in samples from an intestinal level of 60 cm from the nose after a low protein meal.

% of administered	0-1 hr	1-2 hr	2-3 hr	0-3 hr
PEG	46	32	0.7	79
amino acids	50	57	10	117

Table 7

The relative molar concentrations (Leu=1) of some amino acids in the low protein test meal and in intestinal samples from a level of 60 cm from the nose.

	Meal	Sample (0-3 hrs)
Pro	0.8	1.7
Gly	0.6	1.1
Asp	0.7	1.1
Glu	1.0	2.1
Ileu	0.6	0.6
Lys	0.6	0.7

PEG in gastric emptying or to the addition of α amino nitrogen from endogenous secretions. It is unlikely that the shift in the relative molar concentrations of the amino acids observed in the gastric residues over the 3 hours was due to selective retention of some amino acids in the stomach. It is probable therefore that the disparity between the percentages of PEG and α amino nitrogen recovered was due to endogenous secretion. Pepsin is reported to have a high content of Asp, Glu, Pro and Ser. Taurine must have been derived from bile salts from the small intestine. Some endogenous amino acids may, therefore, have been derived from protein regurgitated from the small intestine.

If no Leu had been added to the gastric contents during those three hours the relative retention of Leu with respect to PEG would reduce the ratio of Leu to PEG within the intestinal contents by less than 5%.

An estimate of the amount of amino acid added to the low protein meal was obtained by extrapolation to 100% PEG. 2.9 mmoles were added to the 6 mmoles of the low protein test meal over the three hours of the experiment. The amino acid concentration, both as umoles/mg. PEG and as umoles/ml. in the pyloric samples rose during the three hours (Appendix 4). Collection after the third hour i.e. when the PEG had virtually disappeared, was not attempted. If a further sample was obtainable then the above represents a minimum estimate of the total endogenous secretion in response to the low protein meal.

This figure of 3 mmoles of amino acids from endogenous sources can be compared with the percentage of administered amino nitrogen which remained in the stomach in the gastric emptying experiments. A mean value of 6% of the administered protein remained in the stomach after 3 hours as compared with 1% of the PEG. If the disparity between PEG and protein was due to the latter being of endogenous origin then at least 5 mmoles of/

of endogenous α amino acid had been added to the meal. It was probably much greater.

The form of gastric emptying demonstrated in these experiments was similar to that shown to occur in man by Hunt and Spurrel (1951). In their experiments the test meal, consisting mainly of a solution of citrus pectin, contained no fat and emptied from the stomach in approximately 2 hours. The longer period of time, 3 hours, required in these experiments for gastric emptying was probably due to the fat content of the meals.

Since 99% of the marker left the stomach within 3 hours it seemed justifiable to use a 3 hour collection period when sampling from the small intestine after the test meals.

THE COMPOSITION OF THE INTESTINAL RESIDUES.

Six subjects were used for the experiments with the milk protein test meal. A total of 18 experiments was done, each contributing a total amino acid, a free amino acid and a PEG concentration over a 3 hour period. The results from these experiments have, in most cases, been divided into three groups corresponding with levels of 80-90 cm. from the nose (4 experiments); 90-130 cm. from the nose (5 experiments) and 140-260 cm. from the nose (9 experiments).

The experiments with the cystinuric subjects were in the latter two groups.

For the experiments using the other two test meals and the milk protein meal with ∝ Methyl DOPA two subjects of the six controls were used. The experiments with the low protein and the gelatine test meals were all within the level of 90-260 cm. from the nose. Two experiments with ∝ Methyl DOPA were within the range of 80-90 cm. from the nose and two within the range of 130-260 cm. from the nose.

SAMPLE VOLUME AND POLYETHYLENEGLYCOL CONTENT.

SUMMARY:

(1) After the ingestion of the meals there was a variable delay before intestinal contents could be sampled.

(2) The flow through the sampling tube was intermittent.

(3) The proportion of the total intestinal marker recovered in each 3 hour experiment was about 25%.

(4) The largest hourly portion of the marker was collected during the first hour at 80-130 cm. and during the second hour at 140-260 cm.

(5) The test meals at the upper level were diluted by factors of 3 to 5. Even at the lower levels the PEG concentrations/

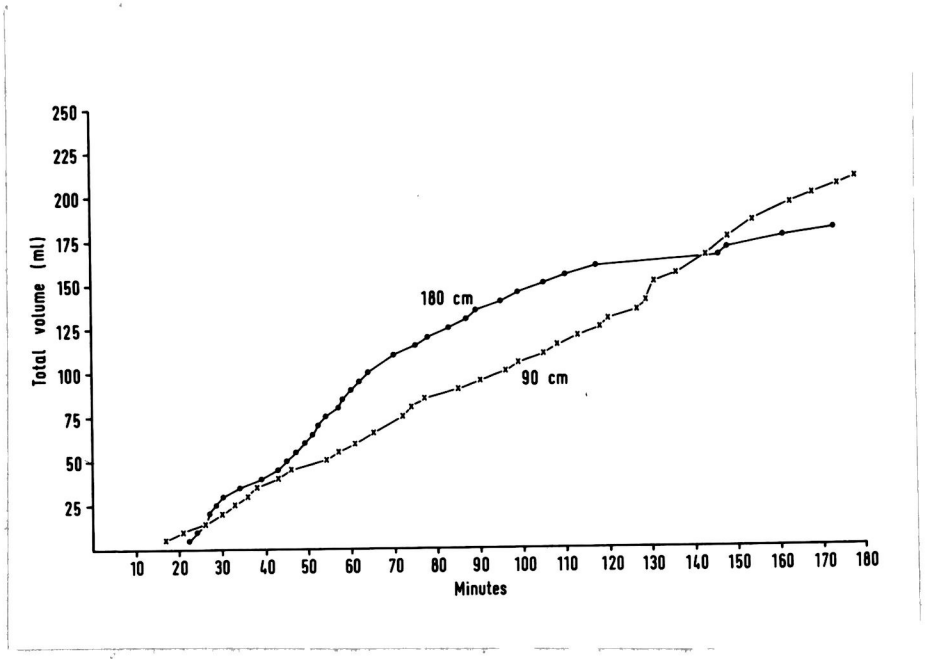

Fig. 5. The total volume of intestinal contents collected in two experiments during the three hour periods after milk protein test meals. The volume produced is plotted against the time of collection. Sampling levels were 90 and 180 cm from the nose.

concentrations were never as high as those in the test meals.

RESULTS:

After the milk protein meal and the low protein meal the flow of intestinal contents started almost immediately from the upper levels and after a delay of 15 to 30 minutes from the lower level. After the gelatine meal, however, a delay of 15-30 minutes occurred before samples could be obtained from the upper level and in both subjects 400 ml. of water were taken by mouth before samples could be obtained from the lower level. In one subject no first hour lower level sample was obtained. This delay with gelatine coincided with a feeling of gastric distension.

Flow through the tube was intermittent, particularly from the lower level. Figure 5 shows the results of one experiment where the volume obtained after a milk protein meal was plotted against time. In this experiment flow started from the upper level (100 cm.), 10 minutes, and from the lower level (180 cm.) 20 minutes, after the ingestion of the meal. 5 ml. volumes were collected. The distances between the points on the graph denote the time intervals during which the samples were collected. In the upper level experiment, during the second hour the time for the collection of 5 ml. varied between 2 and 8 minutes. In the lower level experiment, at the beginning of the third hour, one 5 ml. sample was collected in 30 minutes followed by another in 2 minutes.

Figures 6 and 7 shows the volumes, the PEG concentrations and the PEG recovered as a percentage of that given in the samples obtained after the three test meals. The values for the samples are the means and the ranges.

In two experiments after a milk protein meal the first 5 ml. samples collected from both upper and lower jejunal levels were analysed for PEG and in each case measurable amounts were present/

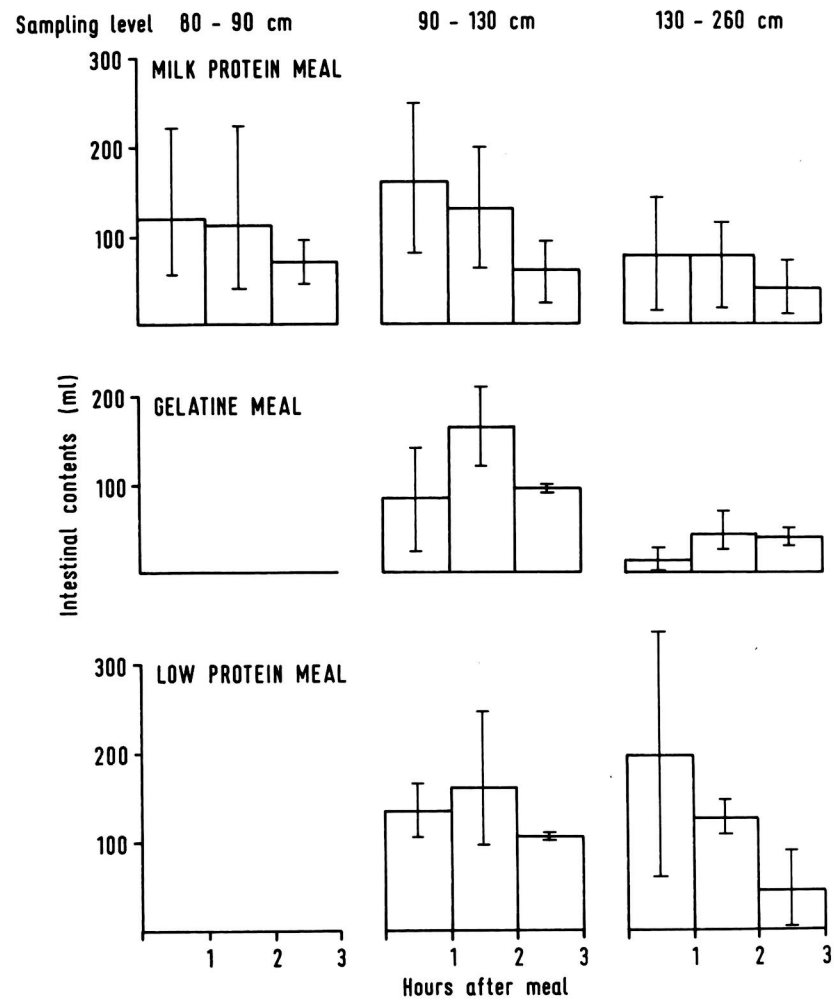

Fig. 6. The sample volumes collected after each of the test meals. Means and ranges.

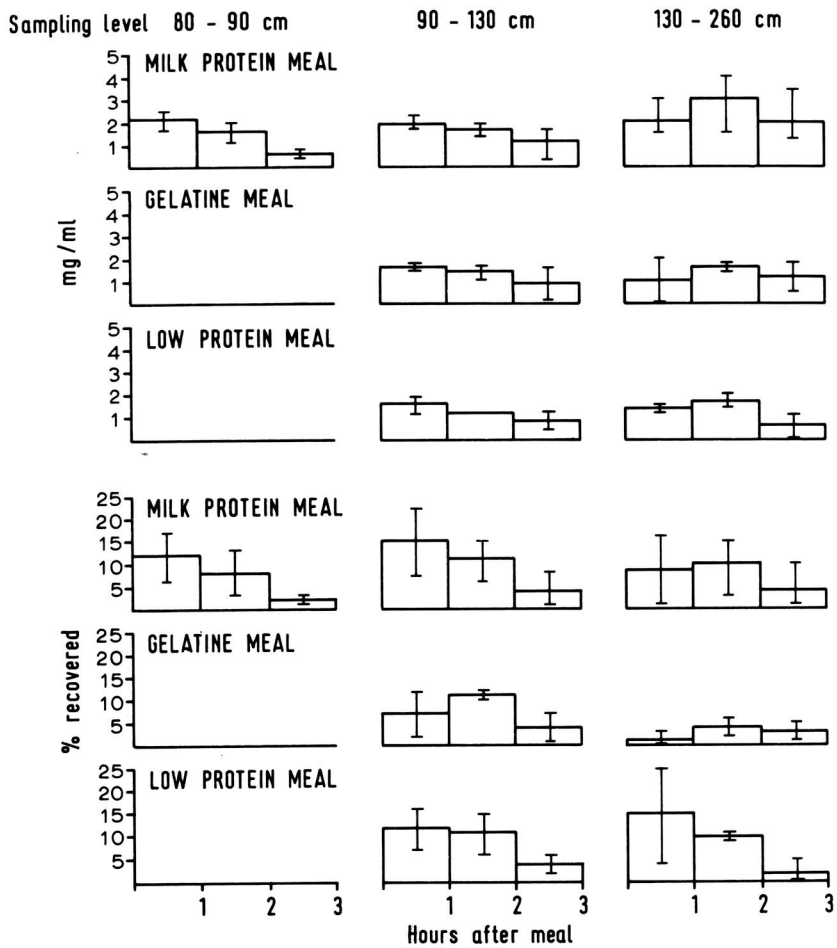

Fig. 7. The PEG concentrations in intestinal samples collected during the three hours after each of the test meals : the percentage of administered PEG recovered in intestinal samples after each of the test meals.

Means and ranges.

present.

DISCUSSION:

In most cases, according to the amount of PEG recovered, the greater part of the test meal passed the duodenal and upper jejunal level sampling holes during the first hour and the lower jejunal level sampling holes during the second hour.

During the periods when the intestinal contents remained static in the tube, proteolysis must have continued.

The ranges in the six control subjects who took the milk protein meal were such that differences in sample volume and PEG after the other two meals could not be attributed to the meals. After each of the three meals, at the upper jejunal level, the subject B.N. tended to reach a steady state of PEG concentration over the three hours. At the lower level this subject varied between the two extremes of donating no sample during the first hour after the gelatine meal and no sample during the third hour after the low protein meal. In the subject G.H. the gelatine meal and the low protein meal appear-:ed to pass the upper jejunal level sampling holes relatively rapidly despite the initial delay in gastric emptying which occurred after the gelatine meal.

The test meals, at the upper levels, were diluted by factors of 3 to 5. Even at the lower level the PEG concentrat-:ions, mg/ml., were never as high as those in the meals given.

Table 8

The mean concentrations (μmoles/mg PEG) of the total combined amino acids in the test meals and intestinal samples over the 3 hour period following the meals.

		Intestinal samples		
cm from nose		80-90	90-130	130-260
	Given			
Milk protein	53	56	42	22
Gelatine	67		57	40
Low protein	3		14	10

THE TOTAL COMBINED AMINO ACID CONCENTRATION.

RESULTS:

Table 8 shows the concentration of total combined amino acid (μmoles/mg. PEG) in each meal, followed by the mean concentrations present in samples of intestinal contents collected during the 3 hours after ingestion. The concentrations were calculated for 3 hour collections in order to minimise those differences between subjects which were due to different rates of transit. It was assumed that the amino acid composition of a sample collected over 3 hours at either level was representative of the composition of the complete PEG containing bolus as it passed the sampling level. The ratios of amino acids to PEG (umoles/mg.) in the 3 hour collection period were calculated from the following expression:-

$$\frac{\sum_{0}^{3} \text{Amino Acid Concentration (μmoles/ml)} \times \text{Volume}}{\sum_{0}^{3} \text{PEG Concentration (mg/ml)} \times \text{Volume}}$$

After each of the protein meals there was net absorption of total combined amino acid between the stomach and the upper jejunum (90-130 cm.) and between this level and the lower level (140-260 cm.).

After the low protein meal, however, net secretion was evident at both levels.

DISCUSSION:

Although absorption can be seen to predominate after the protein meals and secretion after the low protein meal, both processes may be assumed to occur concurrently in each case. If the amino acid content of the low protein meal is neglected, the mean α amino acid concentration measured in the samples from 90-130 cm. level represent a minimum estimate of endogenous amino/

amino acid. This value at least should be added to the protein meals when deriving a minimum estimate of total amino acid absorbed.

Thus, after the milk protein meal at least (53+14) - 42 = 25 μmoles of amino acid per mg. of PEG were absorbed between the stomach and 90-130 cm. The minimum amount of amino acid removed between the stomach and the upper jejunum was therefore of the same order as half the milk protein meal.

This crude calculation gives a striking impression of the rate at which protein digestion and absorption proceeds in the duodenum and upper jejunum.

(The concentrations of total comined α amino acid in hourly samples of intestinal contents are given in Figure 8 in Appendix 5).

Table 9

The effect of the level of sampling on total amino acid concentrations (μmoles/mg PEG) in 3 hour collections of intestinal contents after a milk protein meal.

cm from nose		81	86	100	109	130	169	191	200	233
	Meal									
Asp	4.7	5.1	5.3	4.0	3.8	3.4	2.9	2.5	2.1	1.6
Thr	3.4	3.9	3.5	2.6	2.5	1.8	1.9	1.6	1.3	1.2
Ser	3.6	4.3	3.8	2.8	2.8	2.5	1.9	1.9	1.6	1.3
Pro	4.3	4.8	4.6	3.1	3.5	2.4	2.0	1.6	1.3	1.2
Glu	9.0	8.2	8.3	6.3	5.8	5.2	4.1	3.4	2.9	2.3
Gly	2.0	6.4	7.4	5.8	5.2	5.6	5.5	4.5	3.2	4.0
Ala	3.4	2.8	3.3	2.6	2.2	1.9	1.8	1.3	1.1	1.0
Cys	0.4	0.5	0.5	0.4	0.3	0.4	0.3		0.2	0.3
Val	3.5	3.5	3.7	2.5	2.2	1.9	1.8	1.3	1.2	1.0
Met	1.0	0.8	0.7	0.5	0.5	0.4	0.2	0.2	0.2	0.2
Ileu	2.9	2.7	2.9	2.0	1.7	1.5	1.4	1.0	0.9	0.7
Leu	5.0	4.6	4.6	3.3	3.1	2.5	2.1	1.6	1.3	1.1
Tyr	1.4	1.4	1.5	1.0	1.0	0.9	0.6	0.6	0.5	0.4
Phe	1.8	1.7	1.7	1.1	1.2	1.1	0.7	0.8	0.7	0.4
Lys	3.4	3.4	3.3	2.5	2.6	2.0	1.4	1.4	1.2	0.8
His	1.0	1.1	1.1	0.8	0.9	0.7	0.4	0.6	0.5	0.3
				0.8	0.9	0.7	0.5	0.5	0.4	0.3

THE TOTAL CONCENTRATIONS OF INDIVIDUAL AMINO ACIDS.

SUMMARY:

(1) The total concentration of individual amino acids with respect to the intestinal marker (umoles/mg. PEG) in samples of intestinal contents have been determined after each meal.

(2) After the milk protein meal the concentrations within the duodenum were in most cases greater (net secretion) than those in the meal. The concentrations fell rapidly as the sampling point descended the upper jejunum and then fell more slowly down the lower jejunum and ileum.

(3) A similar pattern followed the gelatine protein meal. However, net secretion of most of the amino acids was still evident in the upper jejunum.

(4) After the low protein meal net secretion was evident down to the upper ileum.

(5) After both the milk protein and the low protein meals the net secretion of Gly was evident at all levels.

RESULTS:

(a) Milk Protein Meal:

Table 9 shows the influence of the level of sampling on the total concentrations with respect to the marker (μmoles/mg PEG) of individual amino acids in each 3 hour collection after a milk protein test meal. The data was derived from nine pairs of adjacent experiments. For comparison the concentrations of amino acids (μmoles/mg PEG) in the test meal are included in the Table.

The/

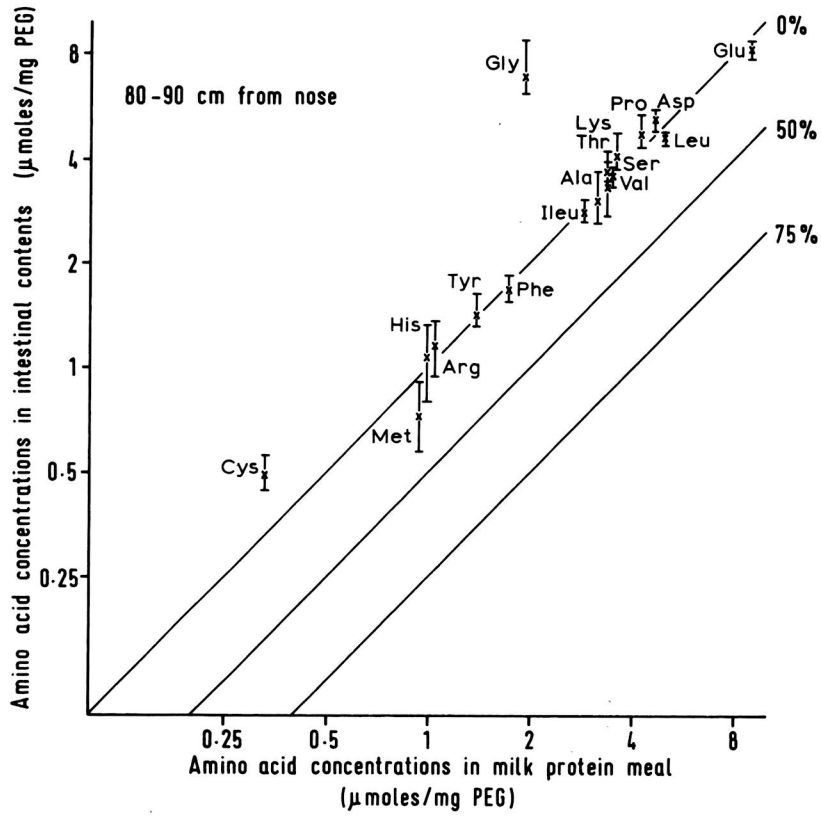

Fig. 9a.

Fig. 9. The relationship between the total amino acid concentrations (μmoles/mg PEG) in the milk protein meal and in the three hour collection of intestinal contents following the meal

a) from a level of 80 to 90 cm from the nose (n = 4)

b) from a level of 90 to 130 cm from the nose (n = 5)

c) from a level of 130 to 260 cm from the nose (n = 9)

Means and ranges. Logarithmic scales have been used.

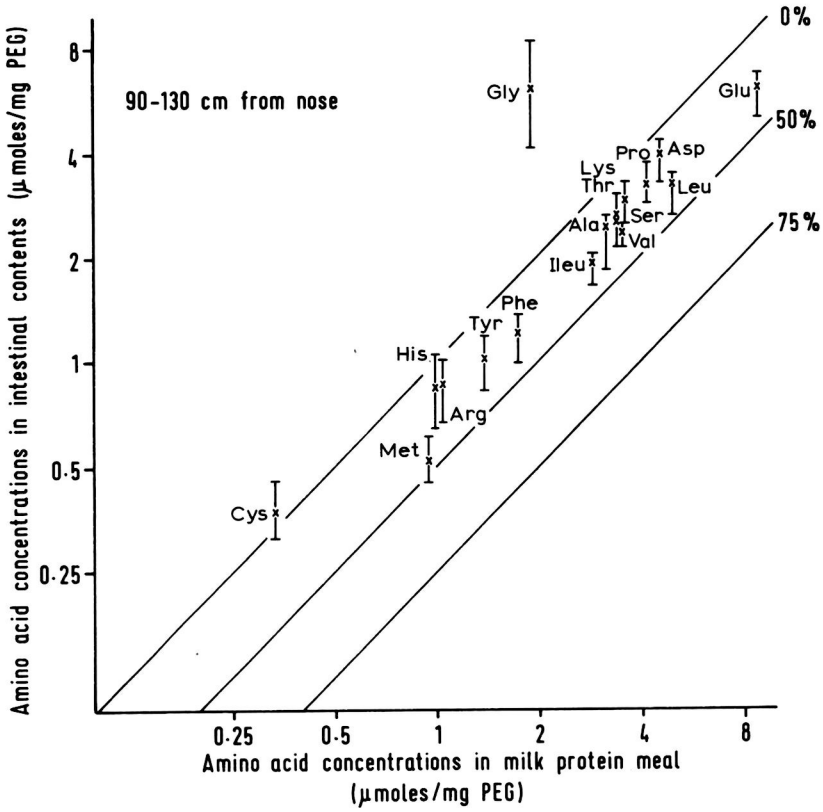
90-130 cm from nose
Amino acid concentrations in intestinal contents (μmoles/mg PEG)
Amino acid concentrations in milk protein meal (μmoles/mg PEG)
0·25
0·5
1
2
4
8
0%
50%
75%
Gly
Glu
Pro
Asp
Lys
Thr
Leu
Ser
Ala
Val
Ileu
Phe
Tyr
His
Arg
Met
Cys

Fig. 9b.

The concentrations in the duodenum (80 and 86 cm. from the nose) were very little changed from those in the meal. Some amino acids e.g. Glu, Ala and Leu, showed a slight drop in concentration; most of the others showed a slight rise. The concentration of Gly had trebled. At levels below the duodenum the amino acid concentrations fell between each pair of experiments with few exceptions. The drop in concentrat-:ion with increasing distance from the nose was steepest between 80 and 130 cm. At a level of 100 cm. from the nose the concentrations of all the amino acids with the exception of Gly were lower than those in the meal. The Gly concentration varied but remained consistently greater than that in the meal.

Figures 9a, b and c show the relationship between the amino acid concentrations (umoles/mg PEC) in the milk protein meal and in the intestinal samples obtained from (a) the duodenum (80-90 cm. from the nose), (b) the upper jejunum (90-130 cm. from the nose) and (c) the lower jejunum or upper ileum (140-260 cm. from the nose) after the ingestion of the meal. Each point on the graph is the mean value for the experiments at these levels; the vertical lines represent the ranges observed. Logarithmic scales have been used.

The vertical distance between the line marked 0% and each mean concentration is a measure of the net absorption or secretion of that amino acid. In the duodenum most of the amino acids were grouped along the line. At the upper jejunal level, with the exception of Gly and Cys, all the amino acids lay between 0% and 50% of the original concentration in the meal. At the lower level the different amino acids maintained similar relative positions but the majority now lay between 50% and 75% of the original concentration.

(b) Gelatine Protein Meal:

Table 10a shows the relationship between level of sampling/

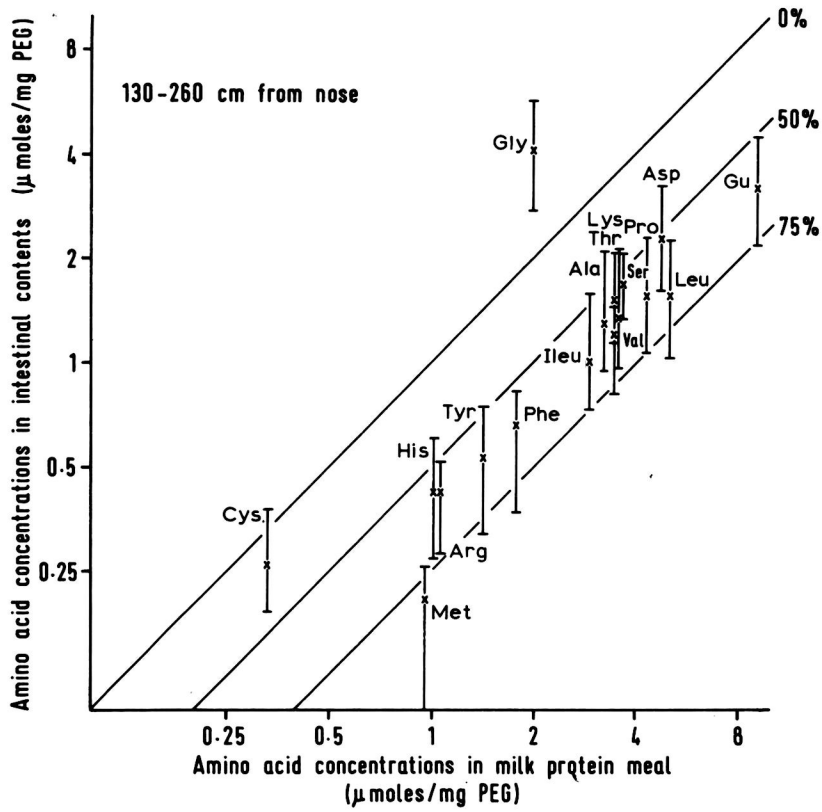
130-260 cm from nose
Amino acid concentrations in intestinal contents (μ moles/mg PEG)
8
4
2
1
0·5
0·25
0%
50%
75%
Gly
Asp
Gu
Lys
Thr
Pro
Ala
Ser
Leu
Val
Ileu
Tyr
Phe
His
Cys
Arg
Met
0·25
0·5
1
2
4
8
Amino acid concentrations in milk protein meal
(μ moles/mg PEG)

Fig. 9a.

Table 10

The effect of the level of sampling on total amino acid concentrations (μmoles/mg PEG) in 3 hour collections of intestinal contents after a) a gelatine meal and b) a low protein meal.

	a) Intestinal contents after the gelatine meal.					b) Intestinal contents after the low protein meal.					
cm from nose		113	126	185	194		111	114	137	195	239
	Meal					Meal					
Asp	3.2	4.0	3.1	2.9	2.6	0.21	1.23	1.21	0.86	0.60	1.08
Thr	1.3	1.8	1.3	1.2	1.1	0.19	0.73	0.73	0.50	0.36	0.66
Ser	2.6	2.8	2.0	1.8	1.7	0.24	0.91	0.81	0.58	0.42	0.76
Pro	8.0	7.3	5.3	4.2	4.8	0.25	0.75	0.73	0.53	0.34	0.63
Glu	5.0	5.3	4.2	3.4	3.3	0.30	1.28	1.21	0.81	0.55	0.88
Gly	22.9	23.2	15.7	11.5	14.9	0.17	5.24	2.82	2.38	1.84	5.38
Ala	8.0	6.7	4.6	3.3	3.9	0.15	0.81	0.81	0.61	0.46	0.72
Val	1.7	2.0	1.3	1.2	1.1	0.27	0.81	0.81	0.57	0.38	0.70
Met	0.07	0.33	0.24	0.21	0.16	0.06	0.12	0.08	0.08	0.04	0.09
Ileu	1.0	1.2	0.80	0.77	0.68	0.19	0.56	0.56	0.38	0.27	0.46
Leu	1.8	2.1	1.4	1.3	1.2	0.31	0.84	0.81	0.58	0.41	0.70
Tyr	0.15	0.56	0.43	0.53	0.34	0.08	0.35	0.32	0.22	0.16	0.32
Phe	0.9	1.1	0.71	0.74	0.56	0.14	0.41	0.48	0.29	0.25	0.34
Lys	1.9	1.9	1.3	1.1	1.2	0.20	0.55	0.56	0.38	0.26	0.39
His	0.4	0.51	0.39	0.41	0.37	0.13	0.25	0.24	0.18	0.11	0.18
Arg	2.5	1.8	1.0	0.67	0.85	0.11	0.36	0.32	0.24	0.15	0.26

sampling and amino acid concentrations after the gelatine meal. The values in the Table were derived from individual experiments in the two subjects.

After the gelatine test meal the drop in concentrations with increasing distance from the nose was less marked compared with the samples obtained after the milk protein meal. At a level of 113 cm. from the nose the concentrations of all the amino acids with the exceptions of Pro, Ala, Hypro and Arg were greater than those in the meal. At no time did the concentrat-:ions of Met or Tyr show a decrease from the very low levels given.

Figures 10a and b. shows the relationship between the amino acid concentrations in the gelatine test meal and in the intestinal samples from upper and lower jejunal levels after the meal. The points in each case are the mean values from the two experiments at each level. At the upper level the concentrat-:ions in most cases were very similar to those in the meal. At the lower level most of the amino acids lay between 0% and 50% of the concentrations in the meal. Ala and Arg, only, lay between 50% and 75% of the original concentrations. Tyr and Met showed net secretion at both levels. The amino acids again maintained similar relative positions at both levels.

(c) Low Protein Meal:

Table 10b shows the relationship between the amino acid concentrations (μmoles/mg PEG) in the low protein meal and those in the intestinal samples obtained at different distances from the nose. Samples obtained from the upper intestinal level after the low protein meal showed a 2 to 6 fold increase in the total amino acid concentrations.

Figures 11a and b. shows the increase in amino acid concentrations in intestinal samples after the low protein meal. The points on the graphs are the means from the two experiments at each level. The experiment at 137 cm. from the nose was not included/

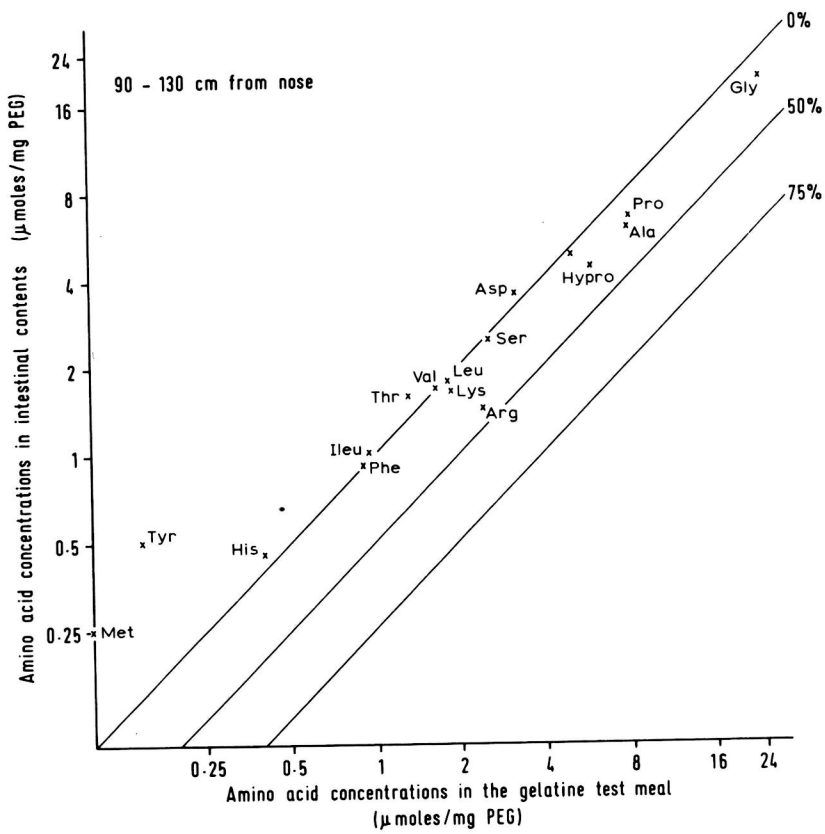

Fig. 10a.

Fig. 10. The relationship between the total amino acid concentrations (μmoles/mg PEG) in the gelatine test meal and in the three hour collection of intestinal contents following the meal

a) from a level of 90 to 130 cm from the nose and

b) from a level of 130 to 160 cm from the nose.

The values are the means from two experiments at each level.

Logarithmic scales have been used.

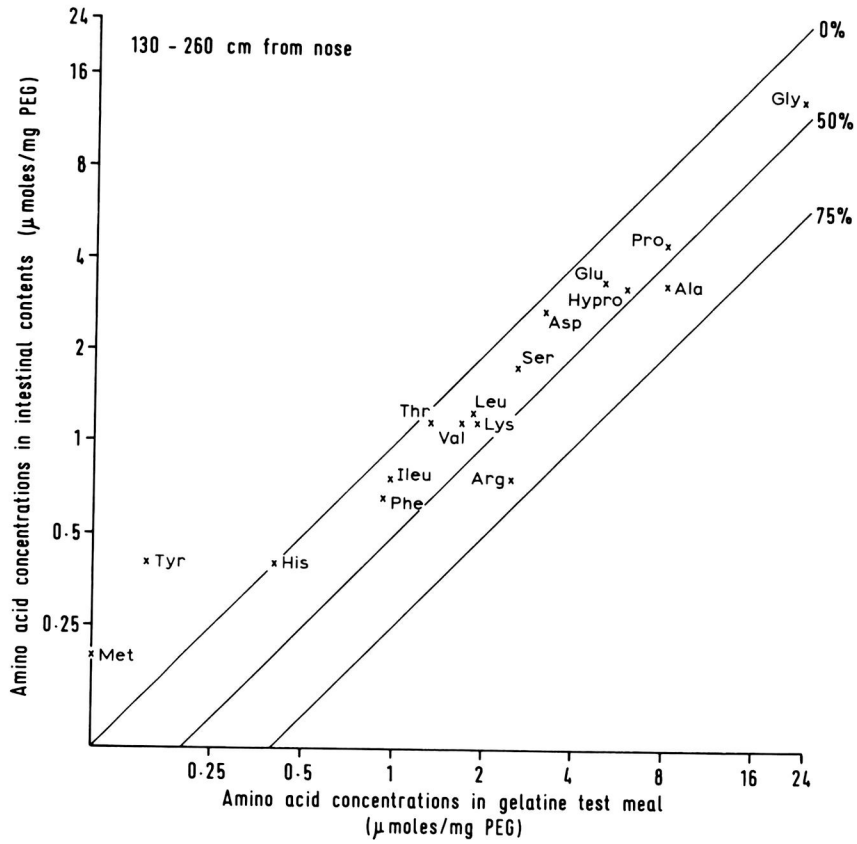
130 - 260 cm from nose
0%
50%
75%
Gly
Pro
Glu
Hypro
Ala
Asp
Ser
Leu
Thr
Val
Lys
Ileu
Arg
Phe
Tyr
His
Met
24
16
8
4
2
1
0·5
0·25
Amino acid concentrations in intestinal contents (μ moles/mg PEG)
0·25
0·5
1
2
4
8
16
24
Amino acid concentrations in gelatine test meal
(μ moles/mg PEG)

Fig. 10b.

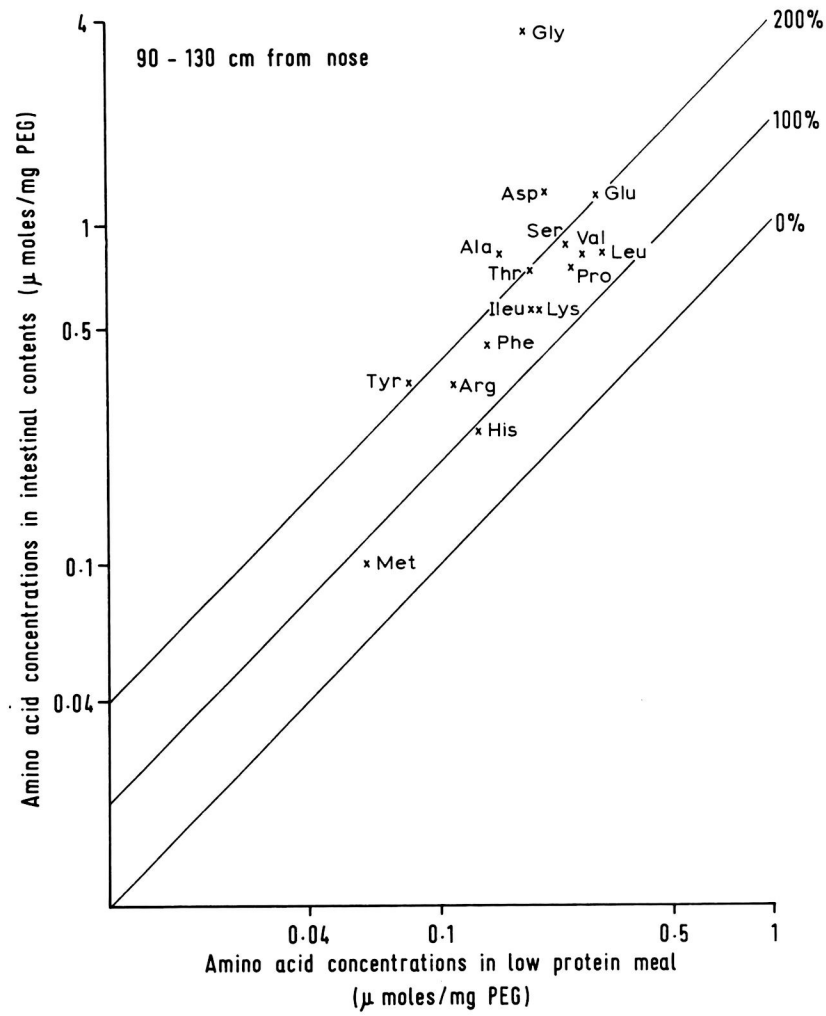

Fig. 11a.

Fig. 11. The relationship between the total amino acid concentrations (μmoles/mg PEG) in the low protein test meal and in intestinal samples following the meal

a) from a level of 90 to 130 cm from the nose and

b) from a level of 130 to 260 cm from the nose.

The values are the means in the three hour collections of intestinal contents from two experiments at each level. Logarithmic scales have been used.

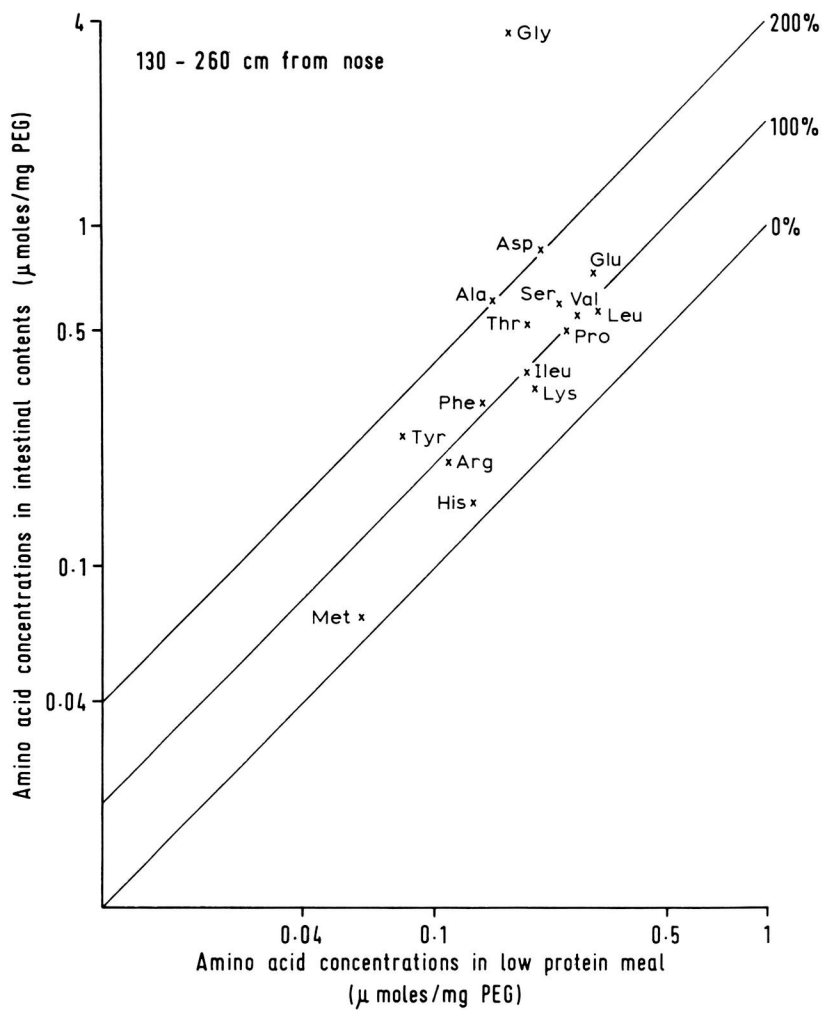
130 - 260 cm from nose
Gly
Asp
Glu
Ala
Ser
Val
Leu
Thr
Pro
Ileu
Lys
Phe
Tyr
Arg
His
Met
200%
100%
0%
4
1
0.5
0.1
0.04
0.04
0.1
0.5
1
Amino acid concentrations in intestinal contents (μ moles/mg PEG)
Amino acid concentrations in low protein meal
(μ moles/mg PEG)

Fig. 11b.

included with either group. The amino acids again showed a similar distribution at both levels.

DISCUSSION:

The amino acid concentrations in the intestinal samples are the net result of secretion and absorption. That some absorption had already occurred in the duodenum after the milk protein meal is evident from the lower concentrations of Gly, Leu and Met (μmoles/mg PEG) in the duodenal samples as compared with the test meal. At a level of 100 cm. from the nose a net disappearance of 30% of the amino acids of the meal (excluding Gly) had occurred. When the milk protein meal had reached a level of 230 cm. from the nose a net disappearance of 75% had occurred. At the lowest level studied, 194 cm. from the nose, after the gelatine test meal, a net disappearance of only 30% of the amino acids present in the meal had occurred. Tyr and Met showed net secretion and the concentration of His was unchanged from that in the meal.

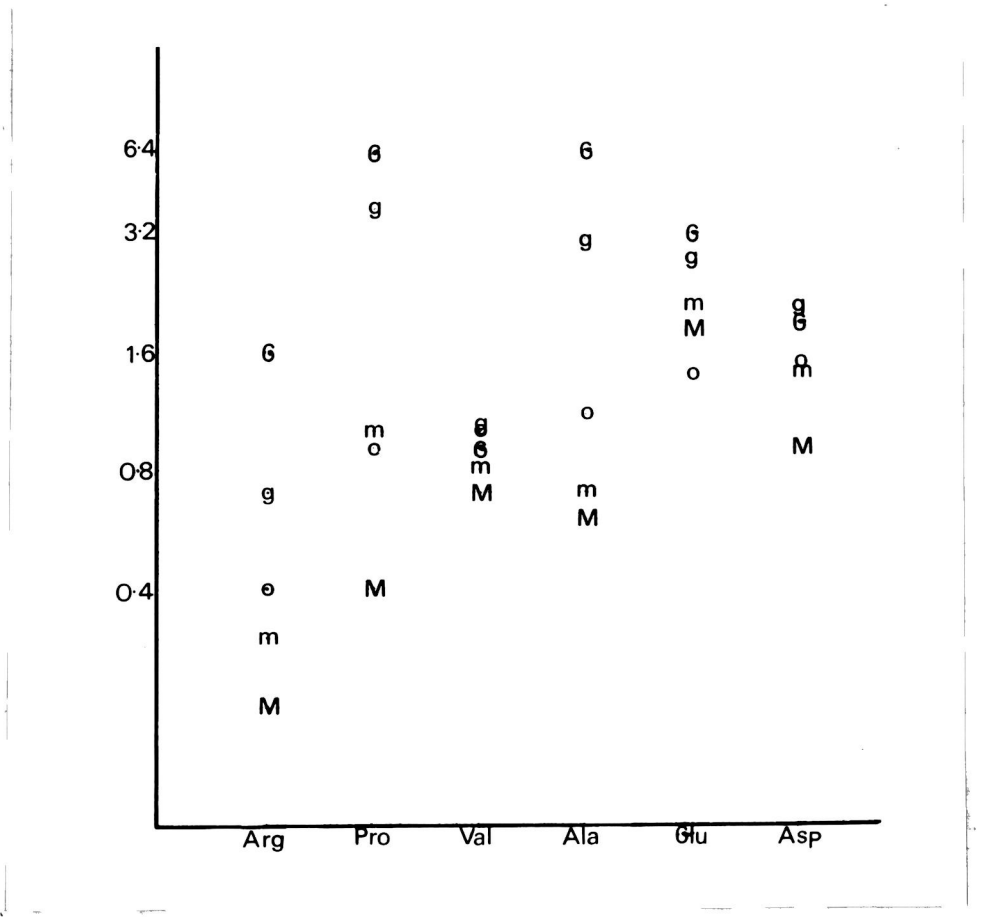

Fig. 12. The molar concentrations relative to Leu (Leu = 1) of 6 amino acids in hydrolysates of the two protein test meals and in the three hourly collections of intestinal contents after the three test meals. The values are the means from all levels.

M = Milk protein meal. G = Gelatine meal.

m = Intestinal samples after the milk protein meal.

g = Intestinal samples after the gelatine meal.

o = Intestinal samples after the low protein meal.

A logarithmic scale has been used.

THE RELATIVE MOLAR CONCENTRATIONS OF THE TOTAL AMINO ACIDS COMPARED WITH LEU IN THE INTESTINAL SAMPLES.

SUMMARY:

The relative molar concentrations (Leu = 1.0) of the individual amino acids in intestinal contents after the milk and gelatine protein meals show progressive changes with time after ingestion and with distance from the nose. In each case the trend is towards the pattern of relative molar concentrations observed in intestinal contents after the low protein meal. Asp and Glu are exceptions to this generalisation.

RESULTS:

The relative molar concentrations of the amino acids in the hydrolysates of intestinal contents differed from the relative concentrations in the corresponding meals.

The molar ratios of the total amino acids compared to Leu in the protein containing meals and in intestinal samples from the various levels after the three meals are shown in full as a Table (Appendix 6).

Leu was chosen as the reference because it was well resolved on all chromatograms and because the relatively high concentrations present made the quantitative estimation more reliable.

Figure 12 shows graphically on a logarithmic scale the ratios of certain selected amino acids.

DISCUSSION:

1. The ratios in general showed a shift from those found in the meal proteins towards those observed in intestinal contents after the low protein meal. The two acidic amino acids were exceptions to this rule. The values used for the calculation of the relative molar concentrations in the intestinal samples were the mean values for the 3 hour collection at all levels. Changes between hours were more marked than changes/

Table 11

The relative molar concentrations (Leu = 1) of total amino acids in hourly samples of intestinal contents after the gelatine meal.

	Samples after gelatine meal. Time after meal(hrs)				Samples after low protein meal.
	Meal	1	2	3	0-3
Asp	1.8	1.9	2.0	1.7	1.5
Pro	4.4	3.7	3.9	1.1	0.89
Glu	2.8	2.7	2.7	1.6	1.4
Gly	12.7	11.5	11.2	8.2	7.0
Ala	4.4	3.6	3.3	1.3	1.1
Met	0.04	0.17	0.16	0.08	0.12
Tyr	0.08	0.20	0.26	0.49	0.41
Arg	1.4	1.0	0.85	0.43	0.41
Hypro	3.2	2.6	2.5	0.13	0.00

The values for the samples after the gelatine meal are the relative molar concentrations in each hourly sample from an upper jejunal level in one subject. The values for the samples after the low protein meal are the mean values from samples collected over the 3 hour period from all levels.

changes between levels and depended on the rate of transit of the meal through the gut. The ratios of some of the amino acids over the 3 hours in a subject after a gelatine test meal are shown in Table 11. Included in the Table were the ratios for the gelatine test meal and for intestinal contents after the low protein meal.

The ratios of Pro, Ala and Arg in samples collected during the first 2 hours after the ingestion of the meal were very similar to those in the meal. The ratios in the sample collected during the third hour were almost identical with those in intestinal contents after the low protein meal. That most of the test meal had passed this point (113 cm. from the nose) by the end of the second hour was shown by the low concentration of PEG (0.1 mg/ml.) in the third hour sample and confirmed by the low ratio of Hypro to Leu in this sample.

2. The shift in ratios in the intestinal samples may have been due in part to selective absorption of amino acids but was also due in large part to dilution by endogenous secretions. In the samples from upper intestinal levels after both protein meals the ratios of the total amino acids in the 3 hour collect-:ion differed from those in the meals (Appendix 6) whereas the concentrations of total combined amino acids (µmoles/mg PEG) in these samples were almost identical with those given in the meals. The endogenous contribution was not great enough to mask the relatively large amounts of Gly, Pro, Hypro, Ala and Arg in the gelatine test meal but was sufficient to alter the ratios of Met and Tyr, present in gelatine in very small amounts, to those found in intestinal contents after the low protein meal.

3. The acidic amino acids held an anomalous position in the general shift observed. The relative molar concentrations in intestinal contents after the two protein meals tended to reach a higher level than those in samples after the low protein meal.

Table 12

The concentrations of taurine (μmoles/mg PEG) in jejunal contents after the three test meals.

Meal	MP		LP		G	
Level of jejunum	Mean ±	s.d.	GH	BN	GH	BN
90-130 cm	1.4	0.5	1.4	1.5	2.1	2.1
130-260 cm	1.3	0.5	1.2	1.5	1.7	0.9

The values for intestinal samples following the low protein and the gelatine test meals are presented individually for the two subjects.

THE CONCENTRATIONS OF GLYCINE AND TAURINE.

SUMMARY:

Gly and Tau together constituted a relatively large proportion of the ninhydrin reacting materials present in hydrolysates of intestinal contents particularly after a low protein meal. All the taurine and a variable proportion of the Gly was derived from conjugated bile salts.

RESULTS:

The hydrolysates of intestinal samples contained high concentrations of glycine and taurine, the actual concentrat-:ions varying with the individual. In samples from upper intestinal levels in the three hour collection after a milk protein meal, the glycine concentration ranged from 7.2 to 14.2 µmoles/ml. The corresponding range for samples from the lower level was from 6.1 to 12 µmoles/ml.

The taurine concentration in samples from the upper level ranged from 1.2 to 3.0 µmoles/ml. and in samples from the lower level from 1.7 to 3.8 µmoles/ml.

The highest concentrations were usually found in the first hourly sample from the upper level and in the second hourly sample from the lower level.

Taurine was present entirely in a conjugated form and 90-98% of the glycine was conjugated even after the gelatine meal. No taurine was detected in hydrolysates of the test meals.

Table 12 shows the concentration, µmoles/mg PEG, of taurine in intestinal samples after the three meals. No significant differences were found between samples from the two levels or after the three meals.

The ratio of the concentration of glycine to taurine in samples taken after the low protein meal and the milk protein meal/

meal was 3.7 ± 0.5 (the mean ± s.e.m.). The relative concentrations in the samples after the gelatine meal were much higher, presumably because of the much larger amounts of glycine given in the meal.

DISCUSSION:

It is probable that this addition of glycine and taurine to intestinal contents was from bile salts. The ratios of glycine-conjugated to taurine-conjugated bile acids in human jejunal contents ranged from 1.3 to 6.3 in the experiments described by Borgstrom and his colleagues (Sjovall, 1959). Some glycine must also have been drived from endogenous proteins.

The combined glycine and taurine concentrations represented a significant percentage of the total combined amino acids + taurine in intestinal contents. After the milk protein meal the percentage of the total combined amino acids + taurine as glycine + taurine ranged from 15 to 20% in samples from the upper level and from 18 to 26% in samples from the lower level. The corresponding values in the two subjects given the low protein meal were 30% and 40% in samples from the upper level and 38% and 45% in samples from the lower level.

In measurements of protein amino acid concentrations in unfractionated hydrolysates of intestinal contents by the ninhydrin method a considerable error is therefore introduced by the presence of bile salts. The error is particularly great in samples after a meal containing fat and carbohydrate but no protein.

FRACTIONATION OF INTESTINAL AMINO ACID CONTAINING CONSTITUENTS OF INTESTINAL CONTENTS BY CENTRIFUGATION AND GEL FILTRATION ON G75 SEPHADEX.

SUMMARY:

1. The upper jejunal contents have been resolved into four crude fractions (I) the insoluble material, (II) the proteins or peptides totally excluded from G-75 Sephadex, (III) the proteins or peptides eluted in an intermediate position and (IV) the peptides and amino acids eluted in a volume similar to the total column water.
2. When the protein meals were fractionated by this method most of the total combined amino acid was contained in Fractions I and II; less than 2% was contained in Fraction IV.
3. Pure samples of pepsin and trypsin were eluted in Fraction III.
4. When samples of intestinal contents were fractionated more than 35% of the total combined amino acid was contained in Fraction IV.
5. The residue of the gelatine test meal was found in Fractions III and IV of the intestinal contents.

RESULTS:

The samples of jejunal contents fractionated were from the region, 100-190 cm. from the nose. Five samples after the milk protein meal were taken over the period 15 minutes to 2 hours after the ingestion of the meal at a level of 110 cm. from the nose. The variations in the concentrations of total combined amino acid, μmoles/ml., suggests there may have been a difference with time (Figure 14).

Two samples after the gelatine meal were obtained from

a/

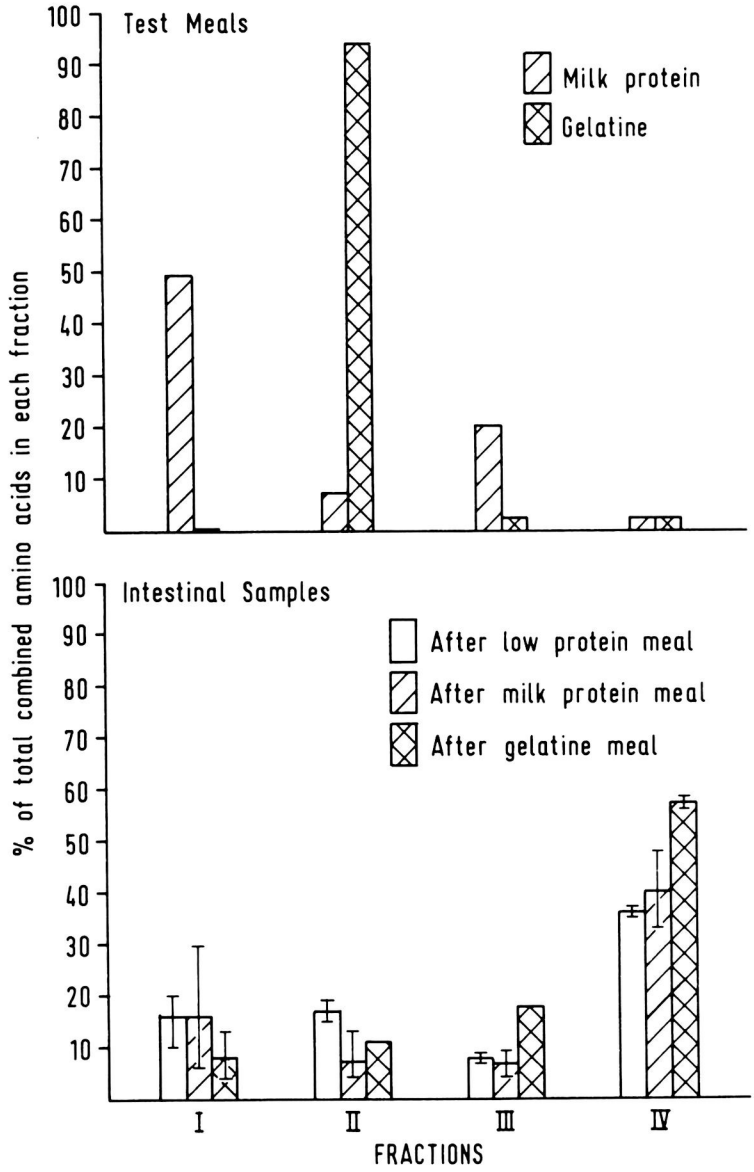

Fig. 13. The percentage of the total combined amino acid of the protein test meals and of intestinal samples in each of four fractions after fractionation by high speed centrifugation and gel filtration on G-75 Sephadex. Means and ranges.

a level of 110 cm. and one from a level of 190 cm. 1 to 1½ hours after the ingestion of the meal. No differences were found in amino acid content between the samples from the two levels.

Fraction I was the insoluble material sedimenting at 23,600g. The microscopic examination of haematoxylin and eosin stained sections showed no evidence of intact mucosal cells in this fraction of intestinal contents.

50% of the total combined amino acid of the milk protein meal was in this fraction. None of the gelatine meal was sedimented (Figure 13). Amino acid containing constituents were found in this fraction of all the intestinal samples (Figure 13). The proportion of the total combined amino acid in this form varied from 8 to 16% after the different meals. (The total combined amino acid content in the samples was derived from acid hydrolysis of the unfractionated material). The concentrations of total combined amino acids varied from 2.5 to 8.5 μmoles/ml. of intestinal contents after the different meals (Figure 14).

The molar concentrations of the individual amino acids relative to Leu in this fraction of jejunal contents showed little variation corresponding with differences between meals (Table 13). The values for Gly, Ala and Pro were, however, raised after the gelatine meal.

The supernatant was separated by gel filtration on Sephadex G-75 into three arbitrary fractions, Fractions II, III and IV on the basis of the u.v. extinction (λ = 280mu.) of the eluate (Figure 15).

Fraction II was that fraction of the supernatant which was totally excluded from G-75 Sephadex and eluted in the volume from 45 ml. to 65 ml. (Figure 15). Although only 7% of the amino acids of the milk protein meal and as much as 94% of the amino acids of the gelatine meal were contained in this fraction, in jejunal contents after the three meals the proportion of the total combined amino acid in this form varied only from/

Table 13

The molar concentrations relative to Leu of the total amino acids in fractions of intestinal contents compared with those in the test meals.

Fraction	1			11			111			1V			Test meals		
	MP	LP	G	MP	LP	G	MP	LP	G	MP	LP	G	MP	LP	G
Asp	1.1	1.7	1.6	1.3	1.4	1.3	1.3	1.5	2.0	1.3	1.5	2.0	0.93	0.67	1.8
Thr	0.75	0.94	0.90	0.88	1.0	1.2	1.3	0.70	1.0	0.78	0.71	0.81	0.68	0.61	0.72
Ser	1.0	1.1	1.3	1.0	1.1	1.0	1.1	1.3	1.7	0.88	0.88	1.5	0.72	0.78	1.4
Pro	0.89	0.89	1.6	0.76	0.93	1.7	1.2	0.90	6.7	0.93	0.71	5.0	0.85	0.81	4.4
Glu	1.5	1.2	1.6	1.2	1.1	1.3	2.3	1.1	3.6	2.3	1.7	3.5	1.8	0.97	2.8
Gly	1.0	2.8	5.0	1.0	1.3	1.9	0.84	4.3	14.1	2.0	7.9	12.8	0.39	0.55	12.8
Ala	0.78	1.0	1.5	1.0	1.0	1.2	0.89	0.90	4.3	0.76	1.1	4.5	0.64	0.48	4.4
Val	0.76	1.1	1.0	0.94	1.1	1.0	1.1	1.0	1.1	0.78	0.88	0.85	0.70	0.84	0.94
Met	0.11	0.12	0.12	0.06	0.05	0.05	0.11	0.10	0.10	0.16	0.18	0.21	0.19	0.19	0.04
Ileu	0.52	0.65	0.64	0.50	0.57	0.57	0.63	0.65	0.55	0.64	0.56	0.50	0.58	0.61	0.56
Leu	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Tyr	0.29	0.47	0.30	0.44	0.36	0.33	0.26	0.45	0.15	0.25	0.35	0.17	0.28	0.26	0.08
Phe	0.39	0.59	0.42	0.44	0.43	0.46	0.29	0.40	0.45	0.35	0.47	0.42	0.35	0.45	0.50
Lys	0.67	0.65	0.68	0.59	0.50	0.61	0.42	0.55	0.84	0.84	0.79	1.3	0.68	0.65	1.1
His	0.22	0.29	0.27	0.26	0.28	0.30	0.24	0.25	0.21	0.23	0.29	0.26	0.20	0.42	0.22
Arg	0.27	0.47	0.47	0.32	0.36	0.39	0.18	0.30	0.34	0.40	0.44	1.1	0.21	0.36	1.4
Hypro									5.0			4.5			3.3

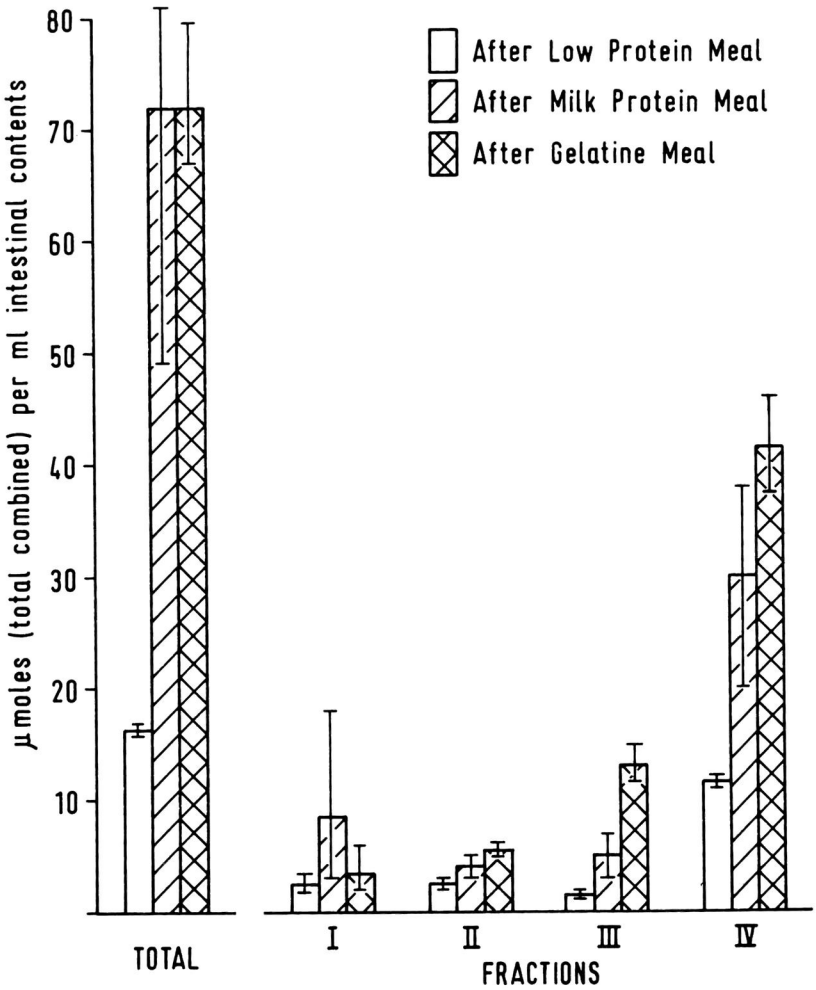

Fig. 14. The concentrations of total combined amino acids (µmoles/ml) in each of the four fractions of intestinal contents after each of the test meals. Means and ranges.

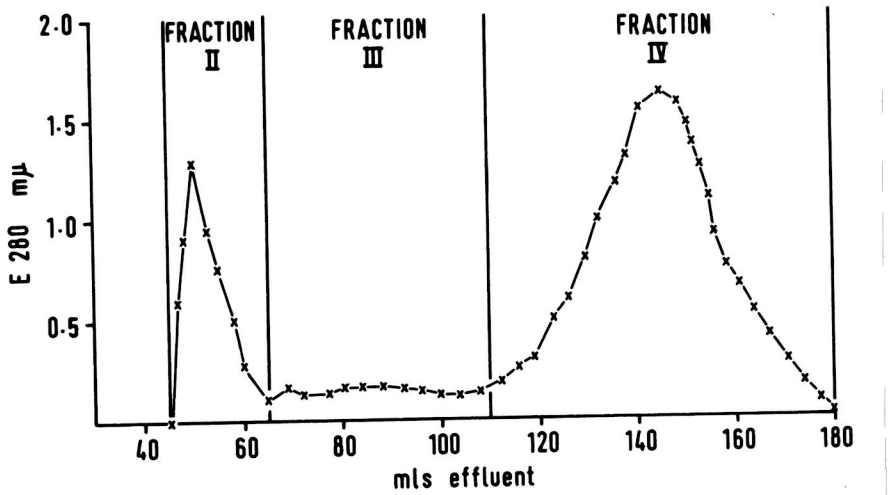

Fig. 15. The relationship between extinction (λ = 280 mμ) and elution volume (ml) from G-75 Sephadex when the supernatant from 5 ml of intestinal contents after a milk protein meal was applied to the column.
Column dimensions ; 85 cm by 1.5 cm, flow rate 15 ml per hour.

from 7 to 17% after the different meals (Figure 13). The concentration of total combined amino acids in this fraction varied from 2.5 to 5.5 µmoles/ml. of intestinal contents (Figure 14). Again the relative molar concentrations (Leu = I) in this fraction of intestinal contents bore little relationship to those in the corresponding meals (Table 13). The samples of intestinal contents resembled each other more closely than they resembled the relevant meals.

Fraction III was the fraction eluting from the column in the volume from 65 ml. to 110 ml. (Figure 15). The reference proteins, pepsin and trypsin, were also eluted in this region of the chromatogram. 20% of the amino acid containing components of the milk protein meal and 2% of the components of gelatine were in this fraction. In this fraction of jejunal contents after the three meals the proportion of the total combined amino acids varied from 7% to 18% (Figure 13).

The concentrations of total combined amino acids varied from 2 to 13 µmoles/ml. of intestinal contents after the three meals. The relative molar concentrations (Leu = I) in this fraction of intestinal contents after the gelatine meal differed from this fraction after the other two meals and bore a resemblance to that in the meal (Table 13). This fraction after the other two meals was similar in amino acid composition and differed from the meals.

Fraction IV was that fraction eluted from the column in the volume from 110 to 180 ml. (Figure 15). It included that region of the chromatogram in which a standard solution of an amino acid mixture was eluted.

Only 2% of the amino acid containing constituents of the protein test meals was eluted in this region whereas 35 to 57% of the total combined amino acids in jejunal contents after all three meals was contained in this fraction (Figure 13). The concentration of total combined amino acids varied from 6.5 to 41.5/

Table 14

Comparison of Fraction IV and intestinal contents after removal of protein with picric acid (PSN) : percentage in each of these fractions of the total amount present in the sample.

	Hydrolysed		Unhydrolysed		The free amino acids as % of the total in each fraction	
	Fr.IV	PSN	Fr.IV	PSN	Fr.IV	PSN
Asp	47	42	5	5	11	8
Thr	43	56	5	4	11	7
Ser	45	48	4	4	8	8
Pro	47	56				
Glu	51	52	6	8	12	16
Gly	54	21	4	4	7	17
Ala	42	44	6	5	13	12
Val	45	44	6	4	14	9
Met	50	46	14	6	27	13
Ileu	47	48	3	2	7	5
Leu	44	38	5	3	11	8
Tyr	37	35	21	15	56	46
Phe	40	46	13	10	32	24
Lys	52	49	25	17	48	35
His	46	36	13	11	28	32
Arg	43	36	27	21	63	59

41.5 µmoles/ml. of intestinal contents after the three meals (Figure 14). The relative molar concentration (Leu = I) in this fraction after the gelatine meal resembled that in the meal and differed from those in this fraction after the other two test meals.

Further Investigation of Fraction IV.

To exclude the possibility that Fraction IV was a result of continued enzyme activity during the gel filtration procedure the amino acid content of this fraction, before and after hydrolysis, was compared with intestinal contents after removal of protein by picric acid immediately on collection (as in the procedure for free amino acid determination). The results of this are shown in Table 14. With the exception of Gly the total amino acid concentrations were very similar in two cases. The lower Gly figure in the picric acid supernatant may have been due to adsorption of bile salts onto Dowex 2 during the removal of excess picric acid. Taurine, a prominent component of Fraction III was absent from the picric acid supernatant.

The amino acid concentrations in the unhydrolysed samples were slightly greater in the Fraction IV than in the picric acid supernatant. This was most noticeable in the case of Met but was also tru of Tyr, Lys and Arg.

Gel filtration of Fraction IV and the picric acid supernatant on Sephadex G-25 gave the elution patterns shown in Figure 16. For comparison the positions of human serum albumin, totally excluded from the gel, Tyr, Phe and Try are shown. The proportions of the total amino acid in Fraction IV and the picric acid supernatant which was present in the free form are shown in the right hand column of Table 14. In the majority of cases the proportion was less than 20%.

The good agreement between the values for the total amino acid content in Fraction IV and the picric acid supernatant suggests/

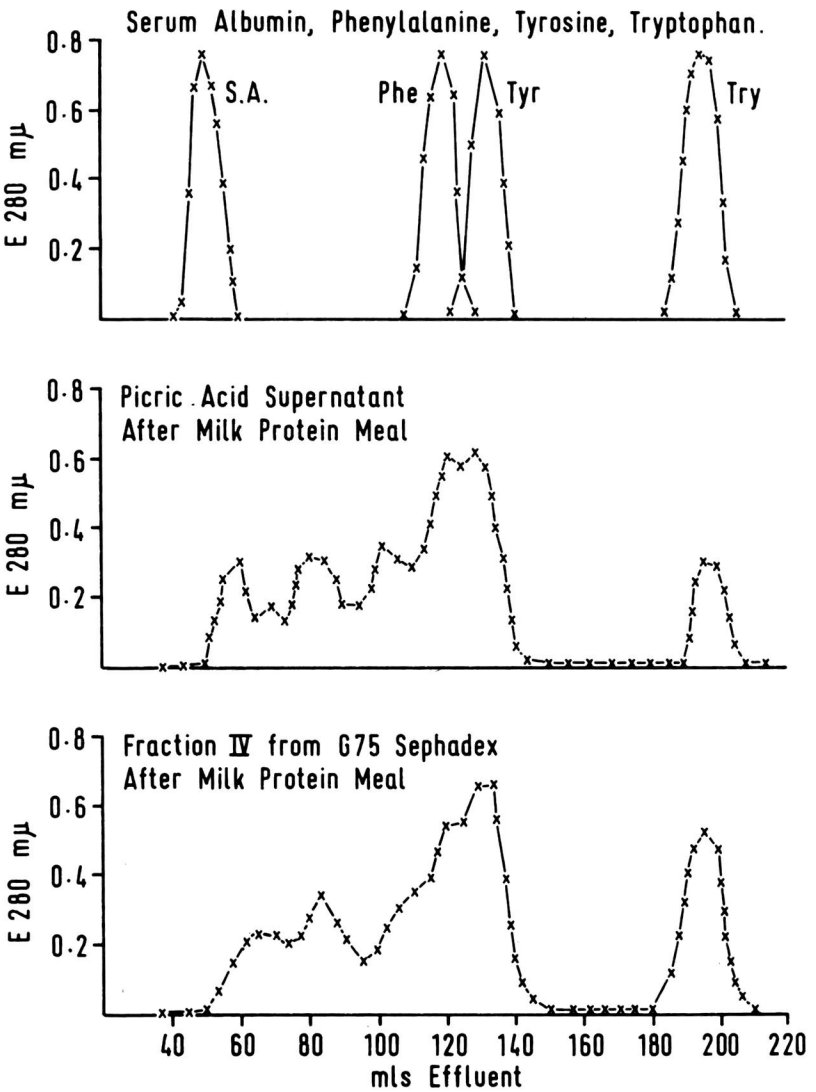

Fig. 16. The elution patterns (E = 280 mμ) from G-25 Sephadex of

a) Intestinal contents after removal of protein by picric acid precipitation (picric acid supernatant)

and b) Fraction 1V from G-75 Sephadex filtration.

For comparison is included the elution positions of serum albumin, phenylalanine, tyrosine and tryptophan.

The column dimensions were 45 cm by 1.9 cm, flow rate 25 ml per hour.

suggests that these are similar fractions derived by two different methods.

Recovery of the Amino Acid Containing Components from the Fractionation Procedure.

The sum of the total combined amino acids in each fraction of test meal or jejunal contents compared with the total combined amino acid content in the original unfractionated material is a measure of the recovery from the procedure. This varied from 68% from jejunal samples after the milk protein test meal to 98% from the gelatine test meal (Table 15). The losses probably occurred prior to gel filtration. The amino acid content of the clear supernatant (of intestinal contents after the milk protein meal) applied to the Sephadex column compared with the amino acid content of the three fractions showed 95 to 100% recovery from the column.

DISCUSSION:

1. In the protein containing test meals the major part of the amino acid containing components was found in Fractions I and II. In the jejunal samples the largest fraction of the amino acid containing components was obtained in Fraction IV. This was not due to continued enzyme action during manipulation of the samples as shown by the comparison with jejunal samples where the protein had been removed with picric acid immediately on collection. In jejunal samples after the gelatine meal a differentiation could be made between dietary and endogenous protein on the basis of the Hypro content (Table 13). On this basis all the residual protein of the gelatine meal was obtained in fractions III and IV of the jejunal samples.

2. Fractions I and II of the samples obtained after each of the three meals were similar in amino acid composition. It is likely that they were all derived from endogenous secretions. Slight/

Table 15

The percentage recovery of total combined amino acid from the fractionation procedure (mean and range)

Meals		Intestinal samples after		
MP	G	MP	LP	G
80	98	62(56-72)	75(74-76)	93(90-98)

Recovery from G-75 Sephadex only

Intestinal samples after MP meal

98 (95-100)

Slight discrepancies which occurred (Table 13) were probably due to contamination with Fraction III and IV. The degree of similarity in relative molar concentrations in Fractions III and IV after the milk protein meal and the low protein meal suggest that the differences in these two fractions after the two meals are quantitative only i.e. they were both derived from endogenous protein in each case but the milk protein meal provided a greater stimulus to secretion than did the low protein meal.

The increased ratios of certain amino acids in these fractions after the gelatine meal may have been due to the resistance of certain peptides to enzymic hydrolysis (Grimm, 1960; Grassman, Hannig and Schleyer, 1960).

3. The reference samples of pepsin (molecular weight 35,000) and trypsin (molecular weight 23,800) were eluted in Fraction III. Chymotrypsin (molecular weight 23,000) and carboxypeptidases A and B (molecular weight 34,000) would probably also be in this fraction. The major proteolytic enzymes of the intestinal contents in their original forms amounted therefore to a maximum mean value of 12% of the total protein amino acids present (Figure 13).

4. G-75 Sephadex excludes molecules of molecular weight over 5000 (Gelotte, 1964). Fraction IV (and intestinal contents after protein precipitation with picric acid) therefore contains mainly peptides of molecular weight under 5000, together with some free amino acids. Snook (1963) found that 70% of the soluble intestinal nitrogen after various types of meal, including a protein free meal was dialysable. He gave a molecular weight of 8000 as the upper limit for the peptides under the conditions employed. The differences between the meals were reflected mostly in this fraction.

5. The percentage recovery of amino acids was greater for gelatine and for intestinal contents following gelatine than it was/

was for the milk protein meal or for intestinal samples following this meal. The reason for this is not clear. It may have been that in intestinal contents following gelatine a greater percentage of the total amino acids was in a soluble form compared with that after the milk protein meal. Certain-:ly the gelatine itself was more soluble than the casein of the milk proteins. This implies that loss has occurred of insoluble proteins, or proteins which may have come out of solution at some time during the procedure. Loss may therefore have occurred (a) of insoluble residue due to non uniform sampling for hydrolysis or (b) of proteins which may have come out of solution during dialysis and adhered to the dialysis sac.

THE CONCENTRATION OF FREE AMINO ACIDS IN INTESTINAL CONTENTS.

SUMMARY:

1. The percentage of the total amino acid in the gut in the free form after the milk protein meal ranged from 2% in the case of Pro to 20% in the case of Arg.
2. The relative molar concentrations of the free amino acids bore little relationship to those in hydrolysates of the test meals or of intestinal samples.
3. The concentrations of some free amino acids e.g. Asp, Thr and Ser were lower in the duodenum than in the jejunum.
4. Free Pro and Hypro were not detected in intestinal samples after the gelatine meal.
5. A bimodal distribution of free Glu and Asp concentrat-:ions in different subjects is postulated.

RESULTS:

The determination of the free amino acids in intestinal contents was complicated by the presence of ninhydrin positive material, probably small peptides, emerging from the ion exchange column immediately after the buffer peak corresponding with the change from pH 3.25 to 4.25 buffer. This was particular-:ly noticeable in samples after the gelatine meal where the peaks corresponding to Cys, Val, Met, Ileu and Leu which were present in small concentrations, were partially obscured by much larger peaks due to unidentified compounds. In many cases it was not possible to determine the actual concentrations of these amino acids. An attempt to separate the free amino acids from the other compounds by gel filtration on Sephadex G-25 prior to chromatography was unsuccessful.

The free amino acid concentrations in the intestinal samples after a milk protein meal were initially measured on each/

each hourly sample but it was apparent that differences between hours were small compared with differences between subjects. In the later experiments with the milk protein meal, therefore, the determinations were made only on pooled aliquots from the three hourly samples. A similar procedure was adopted for samples after the low protein meal and after the gelatine meal. The free amino acid concentrations were similar for most amino acids at upper and lower jejunal levels after each meal.

The concentrations (µmoles/litre) in intestinal samples after the milk protein meal were higher than those after the gelatine meal or the low protein meal.

Figure 17 shows the free amino acid concentrations (mean $\pm$ S.D.) in samples from all levels in the 6 subjects after the milk protein meal. The large subject variation was reflected in the standard deviations which were greatest for Glu and Asp. High concentrations of free Glu and Asp in jejunal contents appeared to be a characteristic feature of two subjects. The Glu concentrations shown in Figure 18 suggest a possible bimodal distribution.

Figure 19 shows the free amino acid concentrations in intestinal contents after the low protein meal. The values are the means of 4 values for two subjects. In most cases the concentrations were approximately 10% of those in the samples after the milk protein meal. Free Gly after the low protein meal was approximately 25% of the corresponding value after the milk protein meal.

Figure 20 shows the free amino acid concentrations in the intestinal samples after the gelatine meal. The values, except for Glu and Asp, are the means of 4 values from two subjects. There was a 10 fold difference between the concentrations of the acidic amino acids in the two subjects (Figure 18). The values are, therefore, given separately. The concentrations in the samples obtained after the gelatine meal were, in general, very similar to those after the low protein meal/

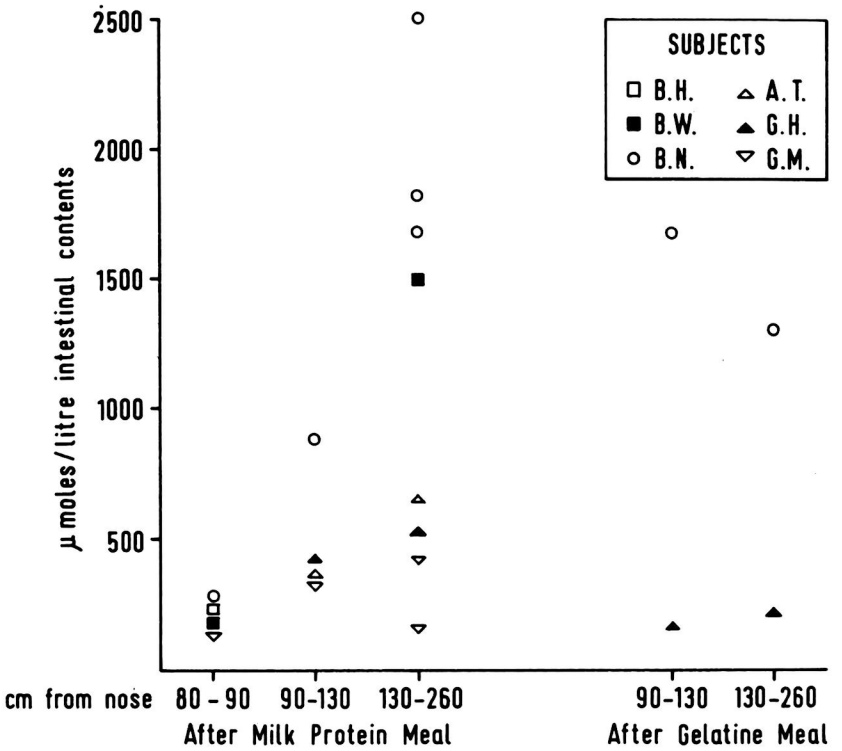

Fig. 18. The concentrations of free Glu (µmoles/litre) in the three hour collection of intestinal contents in the six control subjects. The values are the individual values for each experiment.

meal. Free Gly, Ala, Lys and Arg concentrations were significantly higher, however, in the samples after the gelatine meal. Pro and Hypro were not detected as free amino acids in these samples.

The free amino acid concentrations expressed as percentages of the total amino acids were very similar in intestinal contents after the milk protein meal and after the low protein meal. Figure 21 shows the mean values and the range of the concentrations expressed in this way for the subjects who took the milk protein meal. Figure 22 shows the mean values for the samples obtained after the low protein meal and Figure 23 the mean values for the samples after the gelatine meal (the values for Glu and Asp are again given separately for the two subjects who took the gelatine meal). In the samples after the gelatine meal many of the free amino acid concentrations (% of total) were smaller than after the milk protein meal or the low protein meal. This was particularly notable in the cases of Val, Leu and Ala.

The concentrations expressed as μmoles/litre and as percentages of the totals are presented for each of the three levels in tabular form (Appendix 7).

DISCUSSION:

The concentrations, μmoles/litre of some free amino acids e.g. Asp, Thr, Ser, Ala and His, after the milk protein meal were lower in the duodenal samples than the jejunal samples. Lindberg (1966) found that dipeptidase activity was low in the proximal duodenum but rose sharply in the distal part of the duodenum. The concentrations of free Tyr and Arg were lower in the lower jejunum than in the upper intestinal levels. However, more experiments would be necessary to show significant differences with level of sampling.

The relative molar concentrations of the amino acids in the/

the free state in the intestinal samples bore very little relation to the relative molar concentrations of the total amino acids in hydrolysates of the test meal or of the intestinal contents. After the milk protein meal many of the free amino acids in jejunal samples were present in approxi-:mately equimolar concentrations. The concentrations of Lys and Arg were high and constituted the greatest percentages of the totals. The concentration of Met was relatively low but nevertheless constituted a fairly high percentage of the total Met present.

In intestinal samples from one subject after the gelatine meal, Gly, which represented 30% of the amino acid content of the meal, constituted approximately 50% of all the free amino acid present. Pro and Hypro which, together represented 21% of the amino acid content of the gelatine meal were, however, not detectable in the free state (the limits of detection were approximately 50 umoles/litre in each case). It was shown by Grimm in 1960 using synthetic substrates that a bond between the imino group of a Pro or a Hypro residue and the carboxyl group of an Arg or Lys residue was trypsin resistant. Grassman and his colleagues (1960) concluded that 40% of the total Lys and Arg of procollagen, from which gelatine is derived, (Harding, 1965; Harrington and Von Hippel, 1961) was linked to Pro or Hypro by this trypsin resistant bond. This would account for 15% of the total imino acid. The remainder may also be released very slowly; for example, the carboxypeptidases are less active when an imino acid occupies a terminal (carboxy-peptidase A) or penultimate position (carboxypeptidases A and B).

Lys and Arg are rapidly released from proteins by the combined actions of trypsin and carboxypeptidase B. Tyr and Phe are rapidly released by the action of pepsin, chymotrypsin and carboxypeptidase A. (Dixon and Webb, 1964).

The proportions of the totals present in the free state were/

were greatest for these amino acids after each meal. Met and the branched chain amino acids, relatively rapidly released by chymotrypsin and carboxypeptidase A, were also present in the free state as relatively high percentages of the totals.

Although the total amino acid concentrations were much higher in intestinal contents after the milk protein meal than after the low protein meal the proportions in the free state were similar in both cases. In intestinal samples after the gelatine meal many of the amino acids, in particular Met and Tyr, were mostly of endogenous origin. The free concentrat-:ions of these amino acids were smaller proportions of the totals after the gelatine meal than after the low protein meal. Thus the presence of gelatine or peptides derived from gelatine may inhibit the release of certain amino acids from endogenous protein or peptide.

THE RELEASE OF FREE AMINO ACID DURING IN VITRO INCUBATION OF FRESH JEJUNAL CONTENTS.

SUMMARY:

1. The amino acids fell roughly into three groups with regard to their rate of release on incubation -
   (a) A group comprising Tyr, Met and the basic amino acids.
   (b) A group comprising Ala, Phe, His and the branched chain amino acids.
   (c) A group comprising Thr, Pro, Gly and the acidic amino acids.
2. The rate of release of the rapidly released group, (a), was sufficient to account for their absorption from the gut in the free state.
3. The rate of release of the slowly released group, (c), was not sufficient to account for their absorption in the free state.
4. After 80 minutes incubation at least 90% of the acidic amino acids were still peptide linked.

RESULTS:

The results of the incubation, in μmoles/litre, and expressed as percentages of the totals, are shown in Appendix 8. Samples from two intestinal levels, 110 cm. and 170 cm. from the nose, were incubated. The total amino acid concentrations were very similar in both cases therefore the results from each level will be directly compared.

The amino acids fell roughly into three groups with regard to the rate of increase in free concentrations during incubation. In Figures 24a and b. the concentrations, expressed as percentages of the totals, are plotted against time of incubation for various representatives of the three groups.

(a) Upper Level (Figure 24a).

Arg, in samples from a level of 110 cm. from the nose, increased from 380 μmoles/litre to 625 μmoles/litre during the first/

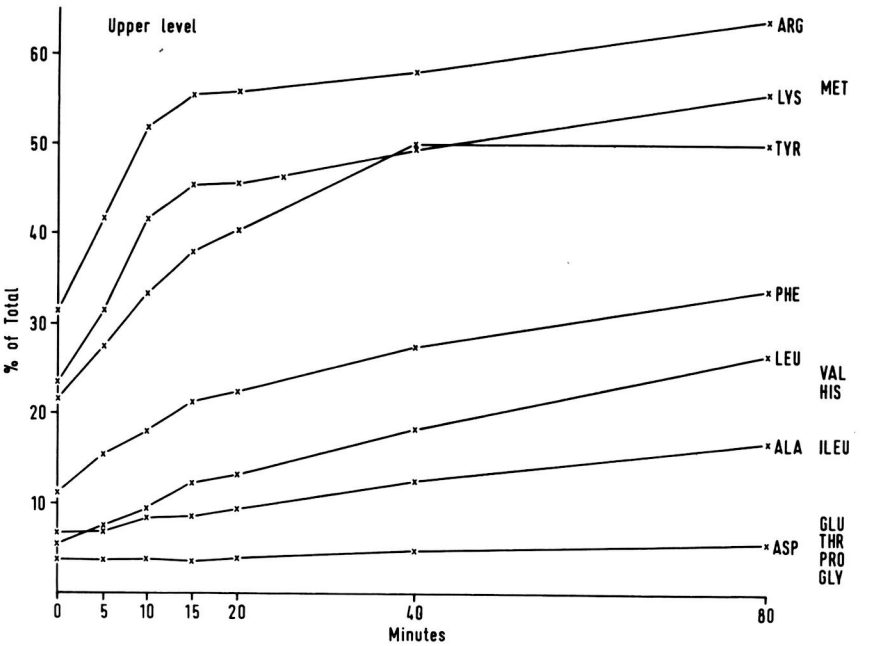

Fig. 24a.

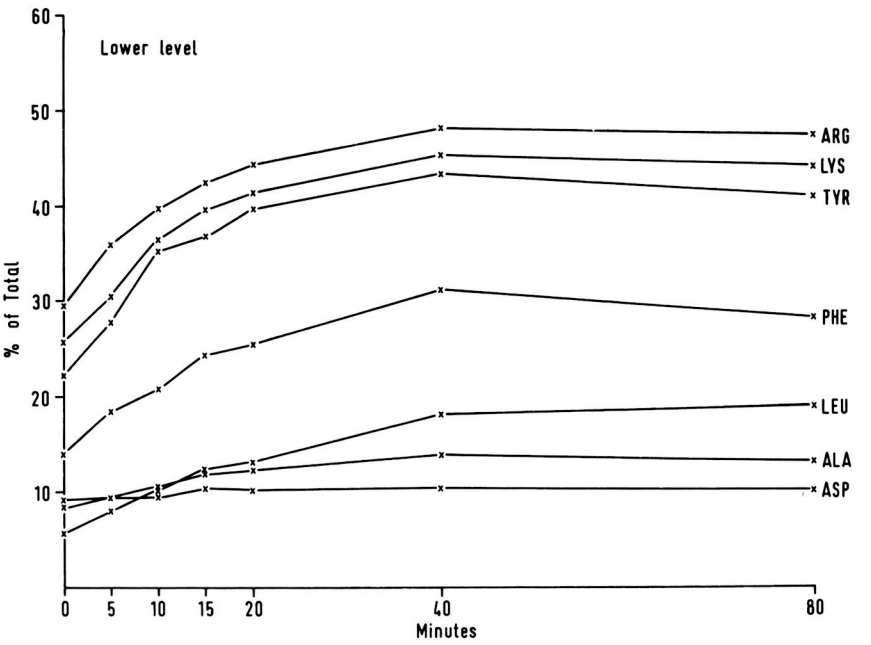

Fig. 24b.

Fig. 24. The rates of release of free amino acids during in vitro incubation of fresh jejunal juice

a) from a level of 110 cm from the nose and

b) from a level of 170 cm from the nose.

The concentrations, expressed as percentages of the totals, are plotted against time of incubation.

first ten minutes of incubation. Thereafter the rate of release declined, the concentration rising to 775 µmoles/litre over the next 70 minutes. This concentration amounted to over 60% of the total Arg present. The proportions of Lys, Met and Tyr in the free state increased in a similar fashion.

At the other end of the scale was a group of amino acids showing little or no increase in concentration over the period of incubation. Pro, Gly and the acidic amino acids were in this group. Where an increase in concentration was observed it appeared to occur at a time when the rate of release of the rapidly released amino acids had declined.

Between these two extremes came a group showing a gradual but continued rise in concentration over the incubation period. His, Ala, Phe and the branched chain amino acids were in this group.

(b) Lower Level (Figure 24b).

The same grouping was observed in samples from the lower level. The initial amino acid concentrations were in some cases slightly greater than those from the upper level sample but in many cases were almost identical. The concentrations of Asp and Glu in the sample from the lower level were approximately double those in the sample from the upper level.

The initial rate of release of the rapidly released amino acids was less in the lower level sample. After 40 minutes of incubation there was no further release of any of the amino acids. The group comprising Phe, Ala, His and the branched chain amino acids showed almost identical results at both levels over the first 40 minutes.

DISCUSSION:

The initial slope of the concentration/time curve gives an estimate of the rate of release of free amino acid from the intestinal contents at the time of removal. Enzyme autolysis, substrate/

The fractions bore little similarity to the corresponding fractions in the

The fractions bore little similarity to the corresponding fractions in the test

The fractions bore little similarity

Fra

Fra Fractionationonabc

Fra Fractionation

Table 16

The estimated rates of release of free amino acids in vivo

	μmoles per minute per ml jejunal contents	
	Upper level	Lower level
Lys	71	40
Val	30	13
Arg	24	12
Leu	21	22
Tyr	20	21
Phe	15	12
Met	14	13
Ala	7.0	8.2
Ser	6.6	7.1
Ileu	5.6	5.8
Glu	5.1	7.1
Thr	3.6	3.7
His	2.1	2.5
Asp	1.7	3.0
Pro	1.5	1.0
Gly	0.5	2.1

substrate exhaustion and product accumulation will each tend to reduce the rate of release of free amino acids in vitro. The slopes during the first 10 minutes appeared to be linear and theoretical rates (μmoles/minute) of amino acid release in vivo were calculated from the rise in free concentration during this interval. In view of the delay between the contents leaving the lumen and the start of the incubation each value will underestimate the actual rate of release in vivo. The estimated rates of amino acid release within the lumen at 110 cm. and 170 cm. are shown in Table 16. The amino acids released most slowly showed only minor increases in free amino acid concentration after 10 minutes. In these cases the slope was calculated from the increase over the initial 40 minute period.

In the case of many of the amino acids the rates of release were almost identical at both levels. However, the release of Lys, Val and Arg was approximately 50% slower at the lower level compared with the upper level. The rates of release of Glu, Asp and Gly were greater at the lower level than at the upper level.

Lys and Arg in Fraction IV of the gel filtration experiments amounted to about 50% of the totals present. It is possible therefore that all the Arg and Lys in Fraction IV was converted to free amino acid on incubation.

Table 17 gives an estimate, based on the theoretical in vivo rates of release (μmoles/minute) at the upper jejunal level, of the time required for the release of all the amino acids of the milk protein meal. The entire content of 7 of the amino acids could probably be released during the 3 hours of the experiments. It is therefore not necessary to postulate anything other than complete digestion within the lumen with subsequent absorption of the free amino acids for Lys, Val, Arg, Tyr, Phe and Met. This might also be true of Leu. However, for the amino acids such as Pro, Gly and the acidic amino acids some other mechanism/

Table 17

The estimated time to release all the amino acids of the milk protein test meal.

Lys	72 minutes
Val	175 "
Arg	66 "
Leu	358 "
Tyr	108 "
Phe	175 "
Met	104 "
Ala	11.6 hours
Ser	13.6 "
Ileu	13.0 "
Glu	45.5 "
Thr	23.6 "
His	11.9 "
Asp	71.0 "
Pro	74.5 "
Gly	98.5 "

mechanism must be postulated.

After 80 minutes incubation much of the total amino acid present was still in peptide form. In samples from both levels 90% or more of the total acidic amino acids were peptide bound at the end of the incubation period.

---

pH OF THE INTESTINAL SAMPLES.

The mean value for the pH of the samples collected from the upper levels after the milk protein meal was 5.75. In every experiment the pH fell with succeeding hourly collect-:ions. The mean values for each hourly collection from the upper levels were 6.28, 5.74 and 5.21.

The pH of the samples collected from the lower level were less affected by the time of collection. The mean value in these samples after the milk protein meal was 6.28.

The pH values of the samples collected after the other two meals were similar to those after the milk protein meal.

AMINO ACID CONCENTRATIONS IN THE LUMEN OF THE SMALL INTESTINE AFTER A MILK PROTEIN MEAL IN CYSTINURIC SUBJECTS.

INTRODUCTION:

In 1908 Garrod described cystinuria as one of the four "inborn errors of metabolism", a term which is still used to describe this condition. It was originally recognised because of the formation of urinary stones, from the relatively insoluble cystine. It was then discovered that the dibasic amino acids were also excreted in large amounts in the urine but that the excretion of other amino acids was normal (Yeh, Frankel, Dunn, Parker, Hugher and Gyorgy, 1947). The abnormality was considered to be essentially renal, the plasma levels of the affected amino acids being either normal or low (Dent and Rose, 1951; Moore and Stein, 1954, Dent, Senior and Walshe, 1954; Robson and Rose, 1957). In normal subjects a rapid intravenous infusion of L-Lys caused a transient increase in the renal clearance of cystine, arginine and ornithine. In cystinuric subjects the infusion of L-Lys caused no further increase in the renal clearance of these amino acids. Glycine infusion caused no increase in urinary cystine, lysine, arginine or ornithine. A common transport pathway in the renal tubule was therefore postulated for these amino acids. It was suggested that cystine, a neutral amino acid, by virtue of its two amino groups was transported in a similar fashion to the dibasic amino acids.

It has been known for many years that the oral ingestion of cystine did not increase the renal clearance of cystine whereas intravenous infusion of cystine did. A transport defect in the intestine, however, was not considered until recently. Milne, Asatoor and their co-workers in 1961 and 1962 demonstrated a similar defect in the mucosa of the small intestine in cystinuric subjects. The absorption defect was barely noticeable at ordinary levels of protein intake but when large doses of lysine or/

or arginine were given to cystinuric subjects a considerable portion was found unchanged in the faeces. Bacterial degradation products of each of these amino acids were demonstrated in faeces and urine. Large doses of lysine or ornithine given orally also increased the amounts of arginine in the faeces. The effect of cystine feeding was not examined but no increase in faecal cystine was observed when large doses of lysine or ornithine were ingested.

The condition has been found to be genetically controll-:ed. Harris and Warren (1953) in a study of the amino acid excretion in 21 cystinuric families found two types of family with regard to the relatives of known cystinuric subjects - (a) those where the heterozygotes were completely normal with respect to the renal clearance of cystine, lysine, arginine and ornithine and (b) where the heterozygotes had moderately increas-:ed urinary lysine and cystine.

Thier, Segal, Fox, Blair and Rosenberg (1965) in a study of jejunal mucosa from cystinuric subjects found that the subjects fell into three types:-

Type 1 : the mucosa could not accumulate cystine, arginine or lysine.

Type 2 : Lysine was not accumulated but some accumulation of cystine occurred.

Type 3 : all the amino acids were accumulated in excess of the concentrations in the medium but not to the same extent as in the normal subject.

The relatives of Type 1 were indistinguishable from normal. The relatives of Type 2 had distinctly increased urinary excretion of cystine and lysine. The relatives of Type 3 had increased urinary lysine and increased or normal urinary cystine.

RESULTS:

6 intubation experiments were performed in the cystinuric subjects/

subjects at intestinal levels ranging from 108 to 250 cm. from the nose.

The samples of intestinal contents collected during 3 hours from these subjects did not differ appreciably in volume, PEG content or total combined amino acid concentration from those obtained from the control subjects. Comparison of individual amino acids, however, showed significant differences between the control subjects and the cystinuric subjects with respect to Lys, Arg and Cys.

TOTAL AMINO ACID CONCENTRATIONS:

Figure 25 shows the comparison between the concentrations, µmoles/mg. PEG of three amino acids at increasing distances from the nose in the control subjects and the cystinuric subjects. The concentrations, µmoles/mg. PEG in the test meal are indicated on the ordinate of the graph. Each column represents the result of a single experiment. Glu, Leu and Met represented the upper and lower ends of the concentration range for the neutral amino acids in the milk protein meal (Table I).

There was no difference between the cystinuric subjects and the controls with respect to the concentrations of Glu, Leu and Met. There was excellent agreement, for instance, between the concentrations of Leu in the experiments on the cystinuric subjects and the values obtained by interpolation between adjacent control experiments (Figure 25).

Figure 26 shows the corresponding values in the two groups of subjects for Arg, Lys and Cys. The differences between the cystinuric subjects and the controls were very marked. Lys, the chief basic amino acid of the meal and of intestinal contents after this meal showed definite evidence of net absorption (the concentration in the meal, µmoles/mg. PEG - the concentration in the intestinal contents, µmoles/mg. PEG) by 130 cm. However, with one exception the values for net absorption were less than half the adjacent control values. Only one experiment showed any net/

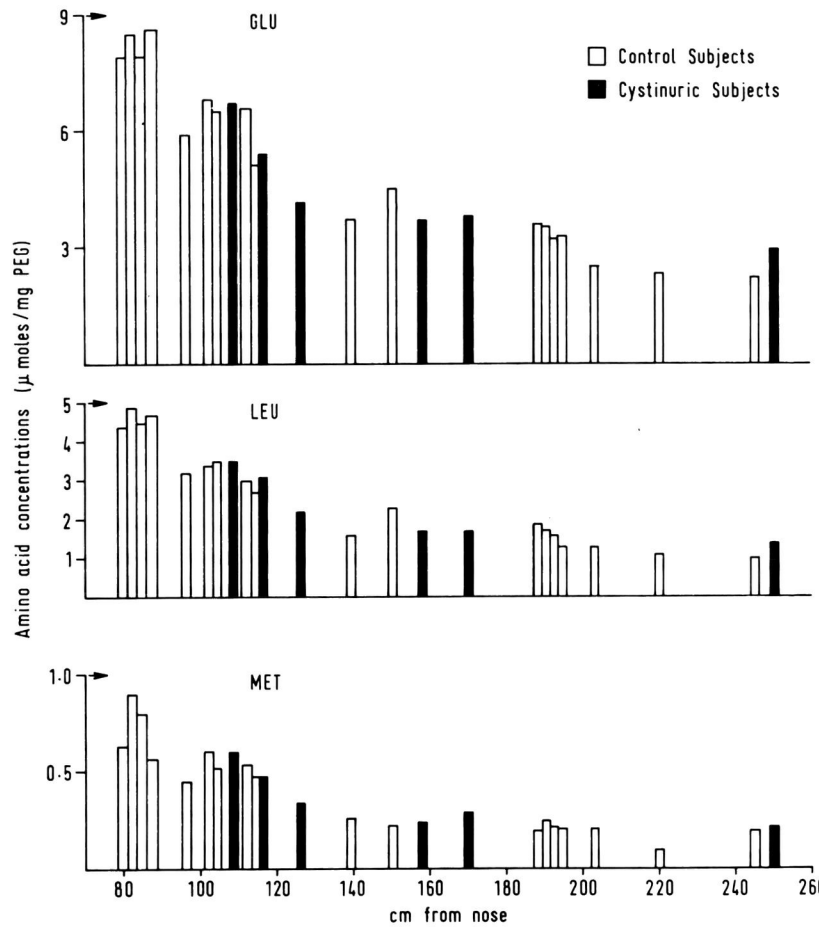

Fig. 25. The concentrations (μmoles/mg PEG) of total Glu, Leu and Met in the three hour collections of intestinal contents from control and cystinuric subjects after a milk protein test meal. The comparison between concentrations and sampling levels. Each value is derived from one experiment. The concentrations in the test meal are indicated on the ordinates

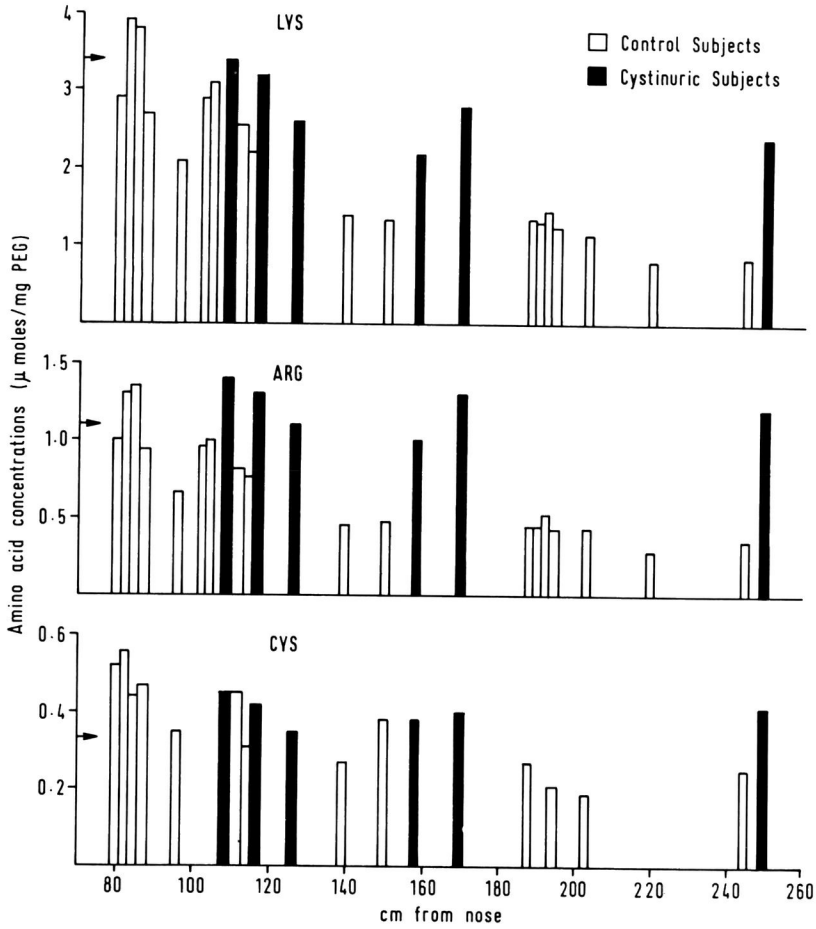

Fig. 26. The concentrations (μmoles/mg PEG) of total Lys, Arg and Cys in the three hour collection of intestinal contents from control and cystinuric subjects after a milk protein meal. Each value is derived from one experiment. The concentrations in the test meal are indicated on the ordinates.

Table 18

The relative molar concentrations of the amino acids (Leu = 100) in hydrolysates of intestinal contents collected from control and cystinuric subjects during the 3 hours after a milk protein meal.

	Controls (n = 6)		Cystinurics (n = 6)		"t" test
	mean	± S.E.	mean	± S.E.	P
Glu	206.3	9.1	199.0	7.2	>0.5
Asp	136.5	5.7	139.6	7.6	>0.7
Pro	103.1	6.7	94.5	2.3	>0.2
Ser	104.0	7.2	96.5	6.0	>0.4
Val	81.0	4.1	87.8	3.2	>0.2
Thr	92.5	5.2	88.8	5.1	>0.6
Lys	81.2	2.0	129.6	11.6	<0.005
Ala	79.0	3.7	82.7	4.5	>0.5
Ileu	66.3	5.2	68.2	1.6	>0.7
Gly	234.1	41.2	187.3	23.8	>0.3
Phe	40.7	2.6	41.7	1.5	>0.7
Tyr	32.8	1.6	34.0	1.8	>0.6
Arg	27.8	1.3	58.3	7.4	<0.005
His	27.5	0.8	25.8	1.1	>0.2
Met	16.7	0.6	15.8	0.5	>0.2
Cys	14.8	2.0	18.2	2.4	>0.2

net absorption of Arg and Cys.

THE RELATIVE MOLAR CONCENTRATIONS OF TOTAL AMINO ACIDS.

The concentrations, μmoles/mg. PEG, correlated closely with the sampling position particularly in the upper part of the small intestine. This produced a large variance when experiments were grouped. The relative molar concentrations of the amino acids in hydrolysates of intestinal contents were, however, much less dependent on sampling position. This means of expressing total amino acid concentrations was therefore used in Table 18 to evaluate differences between the control and cystinuric groups. Leu was chosen as the common reference; the drop in concentrat-:ion with increasing distance from the nose for both controls and cystinurics appeared to belong to the same continuous sequence (Figure 25). Each experiment on a cystinuric subject was paired with one control experiment performed at a similar level. By this means a balanced design was obtained and inappropriate weighting of the mean control values by the duodenal experiments was avoided.

In the cystinuric subjects the relative molar concentrat-:ions of Lys and Arg were increased to a highly significant extent ($P < 0.005$ on the Student t test). The mean cystine value was increased by 23% but the relatively large standard errors prevented the attainment of statistical significance. There was no evidence for the relative retention within the lumen of the members of any other transport family.

FREE AMINO ACID CONCENTRATIONS:

Table 19 shows the mean concentrations of the free amino acids in intestinal contents from the controls and the cystinuric subjects. The values for the controls were again taken from those experiments matched for level with the experiments in the cystinuric subjects.

The free amino acid concentrations were increased in the cystinuric subjects. Pro, Gly and His were the only exceptions. The/

Table 19

The concentrations (μmoles/L) of the free amino acids in intestinal contents collected from control and cystinuric subjects during the 3 hours after a milk protein test meal.

	Controls (n = 6)		Cystinurics (n = 6)		"t" test
	mean	± S.E.	mean	± S.E.	P
Glu	694	190	837	71	>0.4
Leu	225	39	268	39	>0.4
Asp	244	85	325	48	>0.4
Pro	89	10	85	14	>0.8
Ser	204	31	238	37	>0.4
Val	193	30	219	26	>0.5
Thr	169	25	191	19	>0.5
Lys	688	31	2,870	270	<0.001
Ala	207	27	214	23	>0.8
Ileu	98	20	126	16	>0.2
Gly	238	31	233	22	>0.8
Phe	176	6	208	5	<0.005
Tyr	207	11	266	10	<0.005
Arg	264	34	1,500	150	<0.001
His	133	3	108	8	<0.02
Met	66	6	80	7	>0.1
Cys	113	14	323	43	<0.001

Table 20

The free amino acids of intestinal contents expressed as a percentage of the total amino acid released by hydrolysis.

	Controls (n = 6)		Cystinurics (n = 6)		"t" test
	mean	± S.E.	mean	± S.E.	P
Glu	6.9	1.6	9.2	0.9	>0.2
Leu	5.0	1.2	6.5	1.6	>0.4
Asp	3.6	1.3	5.1	0.8	>0.3
Pro	2.1	0.3	1.9	0.3	>0.6
Ser	4.1	0.5	5.7	1.2	>0.2
Val	5.0	0.8	5.8	1.1	>0.5
Thr	3.8	0.5	4.7	0.7	>0.3
Lys	18.6	1.7	47.8	3.9	<0.001
Ala	5.5	0.9	5.7	0.8	>0.8
Ileu	3.1	0.5	4.3	1.0	>0.3
Gly	2.4	0.5	2.7	0.1	>0.5
Phe	9.0	0.7	11.1	0.7	<0.1
Tyr	13.0	0.6	17.1	0.8	<0.005
Arg	19.8	1.7	56.5	2.8	<0.001
His	9.3	0.9	9.4	0.8	>0.9
Met	8.2	1.0	12.1	2.1	>0.1
Cys	14.0	1.8	39.2	3.8	<0.001

The increase was greatest for Lys, Arg and Cys ($P < 0.001$). The reduction in the concentration of His was also significant ($P < 0.02$).

Table 20 shows the comparison between the free amino acids of intestinal contents expressed as a percentage of the total amino acids released by hydrolysis in the control subjects and the cystinuric subjects. In the cystinuric subjects the values for 13 amino acids showed an increase over those in the control subjects. The increase was highly significant ($P < 0.001$) for Lys, Arg and Cys. The increase in Tyr was significant ($P < 0.005$) but the increase for Phe only reached marginal significance ($P < 0.1$).

DISCUSSION:

The decrease in concentration with increasing distance from the nose of the acidic and neutral amino acids was similar in the control and the cystinuric subjects. The absorption of Lys, Arg and Cys, however, was markedly depressed in the cystinuric subjects. The lowest experiment on a cystinuric subject with a sampling point about 100 cm. from the ileo-caecal valve showed the net absorption of Lys to be 30% of the lysine content of the meal. The absorption of Lys may continue below 250 cm. (Orten, 1957 and Kuroda and Gimbel, 1954 showed that the normal ileum can absorb amino acids) but it seems unlikely that a further increment in net absorption would exceed that between 130 cm. and 250cm. Lys which has passed the ileocaecal valve unabsorbed is probably rapidly decarboxylated by the colonic bacterial flora. Strains of E.coli which were unusually active in the decarboxyl-:ation of Lys have been isolated from the stool in cystinuric subjects (Asatoor, Lacey, London and Milne, 1962). It is possible therefore that a net utilisation of less than half the Lys content of a milk protein meal occurs in the cystinuric subject.

The retention of Lys within the lumen was demonstrated by/

by the highly significant increases in the relative molar concentrations of total Lys (Table 18) and the free Lys concentration (Table 19). The increase in total Lys was due to the increase in free Lys; there was no increase in the Lys chemically combined as protein or peptide (total Lys - free Lys) as shown in Figure 27. Thus there was no impairment of the enzymic release of Lys from protein or peptide.

There was no consistent evidence for any net absorption of Arg by the cystinuric subjects. The total and free Arg were both increased in the same manner as Lys and the increase was again due to the increase in free amino acid (Figure 27). Milne and his co-workers observed that the ingestion of L-Lys in cystinuric patients caused an increase in faecal Arg. They suggested that a defective carrier system was saturated by L-Lys. The relatively high concentrations of free Lys (Table 19) in the intestinal contents after the test meal may therefore have inhibited the uptake of Arg. However, Rosenberg and his colleagues did not observe competition between L-Lys and L-Arg for uptake by jejunal mucosa from Type 1 or 2 cystinuria.

The cystine concentration in the intestinal contents after the test meal was very small and the chemical losses during hydrolysis were relatively large (Table 2). Both these factors contributed to the relatively large standard errors of the mean Cys concentrations (Table 18). Thus the increased relative molar concentrations were not significant. However, Figure 26 shows that there was no consistent evidence for any net absorption of Cys. The other changes resembled those shown by Lys and Arg.

Cystinuria could be considered as a naturally occurring experiment in which it is possible to identify and measure the end products of an in vivo protein digestion because these are allowed to accumulate in the lumen. The concentrations of free Arg and Lys expressed as percentages of the totals were very similar in the intestinal samples from the cystinuric subjects and in the samples from the control subjects after 20 to 40 minutes/

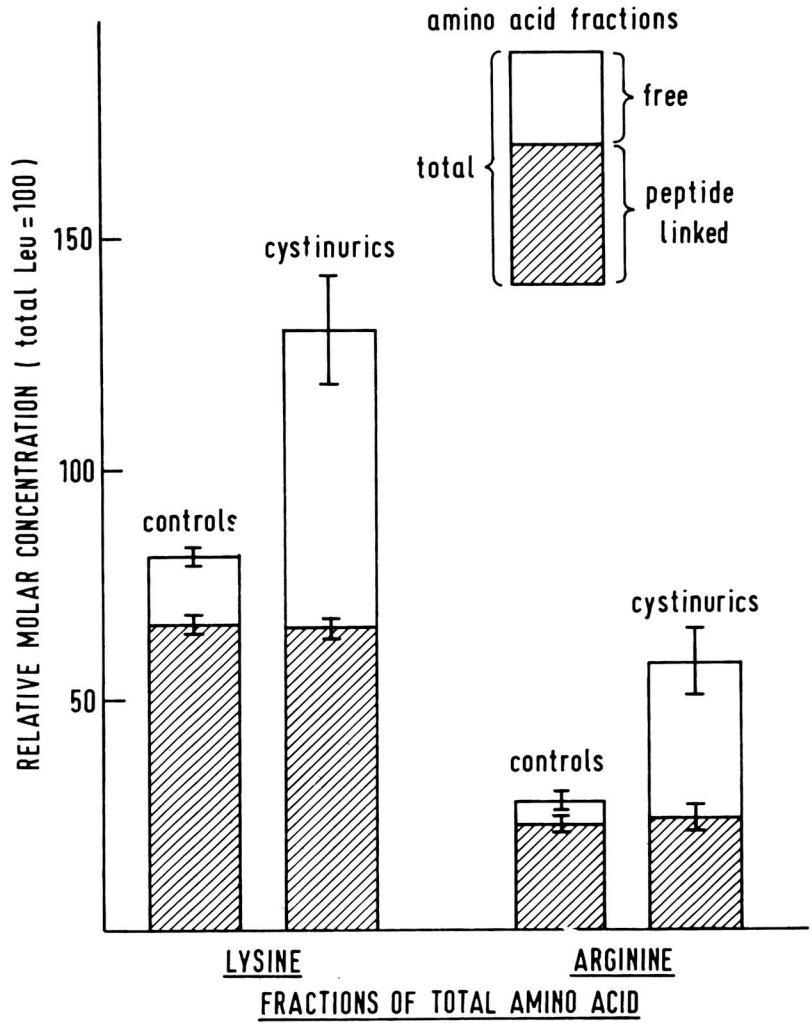

Fig. 27. The relative molar concentrations (total Leu = 100) of Lys and Arg (± S.E.M.) in intestinal contents collected from control and cystinuric subjects after a milk protein meal. The total and free concentrations were determined experimentally ; the peptide linked fraction of each amino acid was calculated by difference.

minutes incubation. The concentration of peptide linked Lys and Arg were the same in samples from the cystinuric subjects and in the controls but the Lys and Arg absorbed by the controls remained within the lumen in the cystinurics in the form of free amino acids.

The two cystinuric subjects belonged to two types (Rosenberg and his co-workers, 1965). The pattern of amino acid excretion in relatives would identify subject A.D. as Type 1 and subject H.B. as Type 2 or 3. The separate results from the two subjects are shown in Figures 28a and b. No clear differences between the subjects were shown but a larger number of more closely matched experiments would be necessary to identify or exclude such differences.

The increased concentrations of free Phe and Tyr (Table 19) had not been expected. Christensen (1967) states that the active transport of L-Phe may be partially inhibited by L-Lys. It is possible therefore that a similar interaction occurs between L-Tyr and L-Lys or L-Arg. The total concentrations of these aromatic amino acids decreased in a similar fashion in the controls and the cystinuric subjects. It is possible that normal absorption of these amino acids could only occur from an increased concentration of the free amino acids.

Apart from the unexplained differences in free His concentration all the differences in the amino acid composition of intestinal contents from the cystinuric subjects may be explained by the recognised defect in the mucosal transport of free Lys, Arg and Cys.

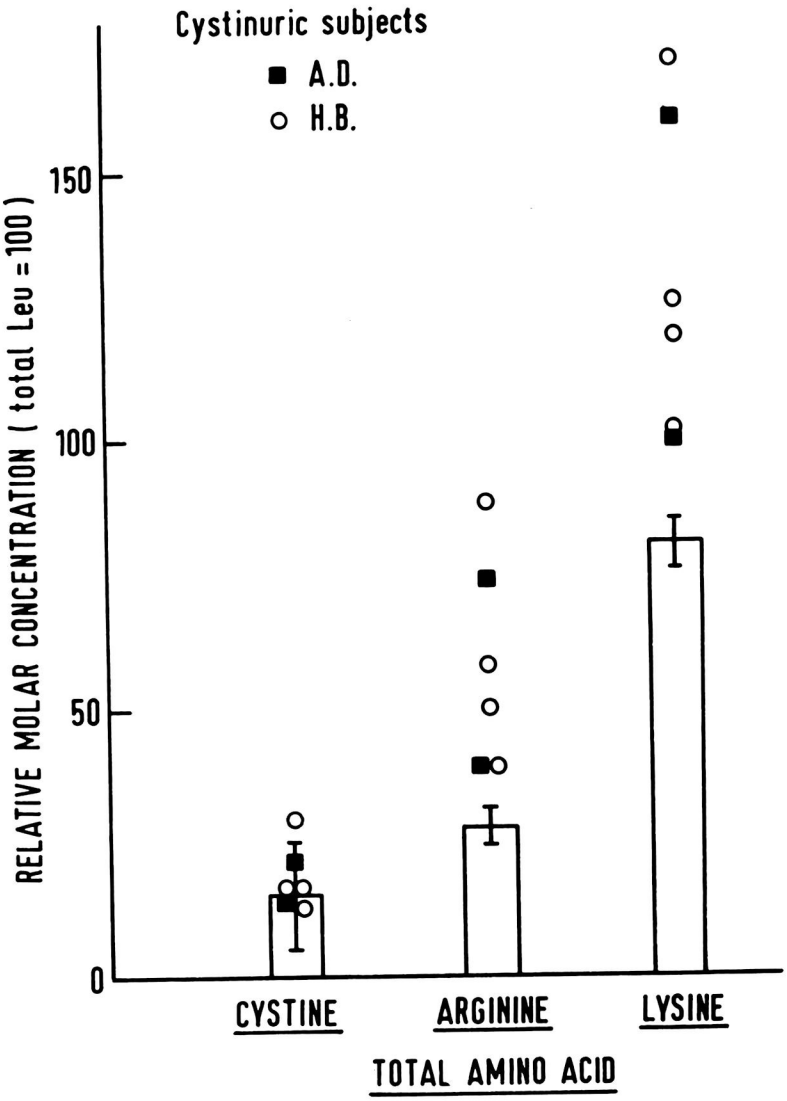

Fig. 28a. The relative molar concentrations (total Leu = 100) of total Cys, Arg and Lys in hydrolysates of intestinal contents collected after a milk protein meal. The control results (mean $\pm$ S.D.) are shown in the histogram. The results of single experiments in the cystinuric subjects A.D. and H.B. are shown as separate points.

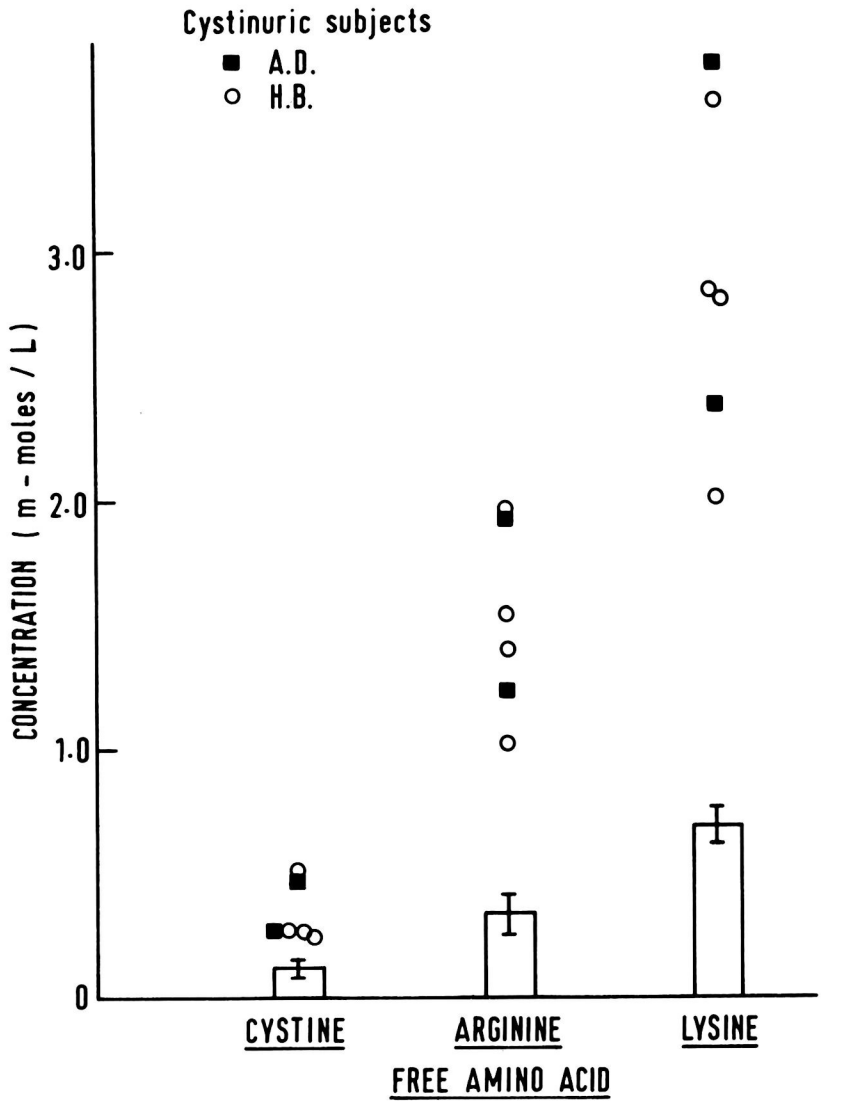

Fig. 28b. The concentrations (mmoles/litre) of free Cys, Arg and Lys in intestinal contents collected after a milk protein meal. The control results (mean ± S.D.) are shown in the histogram. The results of single experiments in the cystinuric subjects A.D. and H.B. are shown as separate points.

THE EFFECT OF L ∝ METHYL DOPA ON THE INTESTINAL HANDLING OF THE MILK PROTEIN TEST MEAL.

INTRODUCTION:

L ∝ Methyl DOPA, a synthetic amino acid, is absorbed by the kidney tubule and by intestinal mucosa by a mechanism other than simple diffusion (Young and Edwards, 1964). Stop flow analysis demonstrated that ∝ Methyl DOPA inhibited the transport mechanism for His, located in the proximal tubule of the kidney. Because of this action in the kidney and because oral treatment in man with L ∝ Methyl DOPA is associated with a mild fall in serum albumin concentrations (Edwards, Young, Jeremy and Neale, 1963) the effects of the drug on the intestinal transport of amino acids were studied by Young and Edwards in 1966 using in vivo loops of rat small intestine. They found that L ∝ Methyl DOPA but not the D isomer had a non-specific inhibitory action on amino acid transport from the lumen of the intestine. The effect was noted particularly with Glu and Lys ($P < 0.001$).

The inhibitory effect was not due solely to the increased osmotic pressure of the test solution as shown by experiments using mannitol in place of ∝ Methyl DOPA. Glucose transport from the test solutions was normal showing that the drug had no deleterious effects on general cell metabolism and that the effect on amino acid transport was confined to a stage in the transport process not shared by the hexose transport process.

Therapeutic doses of L ∝ Methyl DOPA are commonly 2-3gm. daily. Of this only about 50% is absorbed (Dollery and Harrington, 1962); the remainder is excreted as unchanged ∝ Methyl DOPA (Buhs, Beck, Speth, Smith, Trennen, Cannon and Lanagh, 1964). Therefore conditions are optimal for interactions down the full length of the small gut.

RESULTS:

Analysis of intestinal samples after a milk protein meal/

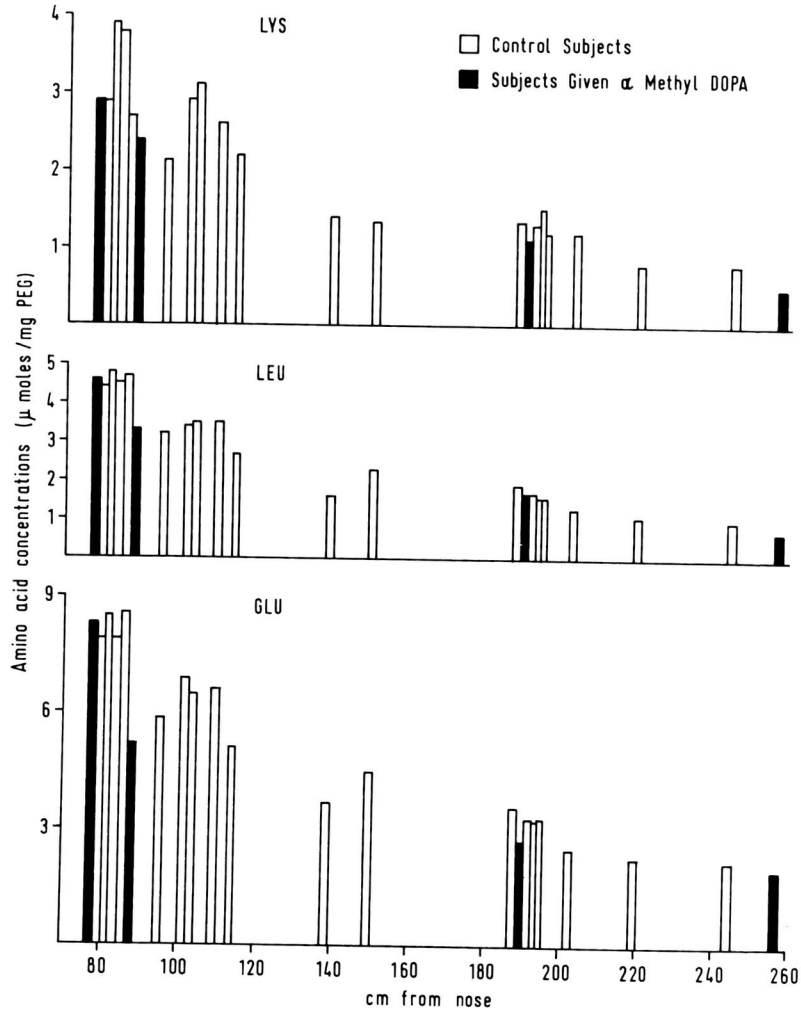

Fig. 29. The concentrations (μmoles/mg PEG) of total Glu, Leu and Lys in the three hour collections of intestinal contents from control subjects and subjects given a test meal incorporating 1 gm of α Methyl DOPA. Each value is derived from a single experiment.

meal containing 1gm. of L ∝ Methyl DOPA gave results showing no appreciable differences from those in the control subjects.

The total amino acid concentrations, μmoles/mg. PEG in the intestinal samples obtained are presented in Appendix 9. The results gave no indication of decreased absorption of any amino acid. Figure 29 shows the sequential concentrations of Glu, Leu and Lys in the control subjects and the subjects given ∝ Methyl DOPA. The amino acid concentrations were similar at the same levels in the two groups of subjects.

The free amino acid concentrations, in μmoles/litre and expressed as percentages of the totals, in the intestinal samples are also shown in Appendix 9. Tyr and Phe, amino acids expected to be affected by the presence of the analogue, ∝ Methyl DOPA, were difficult to estimate because of an unidentified ninhydrin positive component emerging from the column at a position tending to overlap these amino acids. This component was not L ∝ Methyl DOPA. The concentrations of free Tyr and Phe did not appear to be significantly different from those in the control subjects, however.

∝ Methyl DOPA was readily resolved on the Amino Acid Analyser although the intensity of the colour produced by its reaction with ninhydrin was much less than that of the amino acids. A quantitative determination was, nevertheless, possible.

DISCUSSION:

The inhibitory effect of L ∝ Methyl DOPA on amino acid transport observed by Young and Edwards (1966) was, in the case of half the amino acids studied, relatively slight ($P < 0.05$). This occurred with a ratio of ∝ Methyl DOPA to the amino acid under study of 10 to 1. In their experiments 10 mMolar solutions of the amino acids were perfused through the small intestinal loop. A 20% water gain occurred in the loop, therefore the concentration to which the intestinal mucosa was exposed was of the order of 8 umoles/ml. The concentration of Methyl DOPA

was/

was 80 umoles/ml. In the experiments described here 400 ml. of test meal incorporated 1gm (5mmoles) of ∝ Methyl DOPA giving a concentration of 12.5 umoles/ml. In the upper region of the intestine the test meal was diluted approximately 1 in 3 (p. 58), giving a theoretical concentration of drug of approximate-:ly 4 umoles/ml. Direct determination of the drug in upper level intestinal samples from the two subjects given the drug gave concentrations of 3.8 and 3.6 umoles/ml. The sum of the free amino acid concentrations were of the same order. The mean value in the untreated control subjects was 3.3 umoles/ml; in the upper level samples from the two Methyl DOPA treated subjects the concentrations were 2.4 umoles/ml. and 1.7 umoles/ml. The amino acids whose transport Young and Edwards showed to be inhibited to the greatest extent were Glu and Lys. The concentrat-:ion of Methyl DOPA was 5-20 times greater than the concentrat-:ions of either of these amino acids.

At therapeutic dose levels of L ∝ Methyl DOPA no inhibitory effect on the intestinal handling of protein by normal human subjects was demonstrated.

SECTION IV.

GENERAL DISCUSSION.

The experiments were carried out under conditions as close to physiological as possible. Intubation of the subjects was performed at least one day prior to the experiment and by the time of the experiment the subjects were usually almost unconscious of the presence of the tube. Over the three hours during which sampling occurred the subjects read magazines and generally took little interest in the experimental procedure.

The test meals were also designed to provide physiological meals, both in quality and quantity. Thus each of the two protein containing meals provided about one quarter of the daily intake of protein, fat and carbohydrate and gave approximately 500 calories. The low protein meal was initially designed as a protein free meal but for reasons already given double cream was used as the source of fat. This did introduce a small amount of protein. Analysis of the three meals indicated no appreciable differences in fat or carbohydrate content.

The presence of the tube in the small intestine may have caused abnormal peristalsis or secretion. Quantitative changes in the amino acid containing components of intestinal contents may have occurred during the passage up the tube.

However, it is believed that the results approximated closely to the events which occurred within the small intestine after each of these three meals.

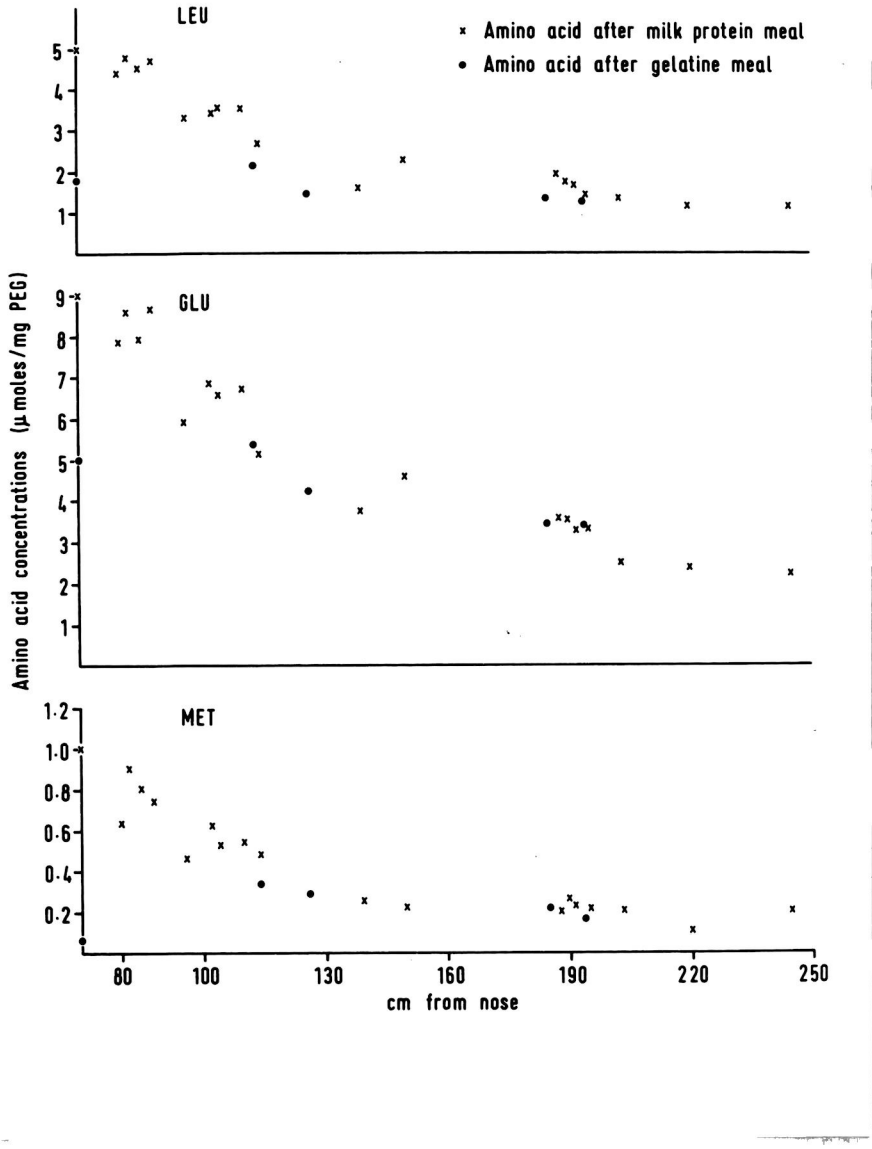

Fig. 30. The sequential concentrations (µmoles/mg PEG) of total Leu, Glu and Met in the three hour collections of intestinal contents after the milk protein and the gelatine test meals. The concentrations in the protein test meals are indicated on the ordinates of the graph.

SITE OF ABSORPTION.

(1) It is suggested that the digestion and absorption of dietary proteins takes place within the duodenum and upper jejunum proximal to 130 cm. from the nose.

(2) As a corollary, the amino acid containing constituents which remain in the lumen below this level are derived from endogenous secretion.

The following observations are consistent with this hypothesis:-

1. After the milk protein meal the total combined amino acid concentration fell steeply as the sampling point passed from the duodenum down the upper jejunum (Table 9). Below 130 cm. the concentration continued to fall but much less steeply. These results would be explained if amino acid or peptide absorption below 130 cm. was retarded by the relative resistance of native endogenous protein to proteolysis.

2. The concentrations (umoles/mg. PEG) of total Leu, Glu and Met in the two protein meals and in the intestinal contents have been compared (Figure 30). After the milk protein meal the concentrations fell steeply as the sampling point approached 130 cm. from the nose. After the gelatine meal,however, the concentrat-:ions rose. Thus after the two meals the concentrations converged and had become similar by 130 cm. These changes would be explained if 15g. of milk protein and 15g. of gelatine had stimulated the secretion of similar amounts of endogenous Leu, Glu and Met (as constituents of secreted protein) and what remained within the lumen at 130 cm. was the residue of this alone. It is suggested that gelatine is an abnormal dietary protein in that it contains a high percentage of peptide bonds involving Pro or Hypro, which are relatively resistant to enzyme hydrolysis (Dixon and Webb, 1964). Residues of this protein may therefore still be present in the lumen of the gut at a level by which the dietary protein has normally been absorbed. The apparent inconsistency/

inconsistency of the results after the low protein meal may have been due to a smaller stimulus to secretion by this meal.

3. It has been postulated that denatured dietary protein is hydrolysed to amino acids within the gut more rapidly than native endogenous protein (Munro, 1964). If the digestion of dietary protein had not been completed by 130 cm., then differences in the rate of release of amino acids during in vitro incubation would have been expected between samples collected from 110 cm. and from 170 cm. During the initial period of incubation no such differences were found (Figure 24) and (Appendix 9). It is concluded that by 110 cm. the amino acids of the milk protein had already been released. However, the concentrations of the amino acids in the free state could account for only 8% of the amount given in the test meal; thus at least 100 - 8% had been absorbed by this level.

4. Borgstrom and his colleagues (1957) found approximately 60% absorption of labelled albumin by 130 cm. However, iodine is a large atom and its presence in a protein may delay enzyme hydrolysis as well as subsequent transport of the iodinated amino acids. The fact that hydrolysis occurred to a degree where 60-70% of the iodinated albumin was in the form of fragments soluble in phosphotungstic acid did not preclude the possibility that some peptides, relatively resistant to enzyme action may have been present. A search of the literature has failed to yield any information on the comparative rates of digestion of iodinated and non-iodinated proteins. Similarly no information has been obtained regarding the effects of iodination on the subsequent transport of amino acids.

In order to test the theory that the digestion and absorption of dietary protein is complete by 130 cm. a null hypothesis may be postulated: the amino acid composition of the intestinal contents below 130 cm. is independent of the nature of the dietary protein. This null hypothesis will be examined in the light of the experimental results.

1./

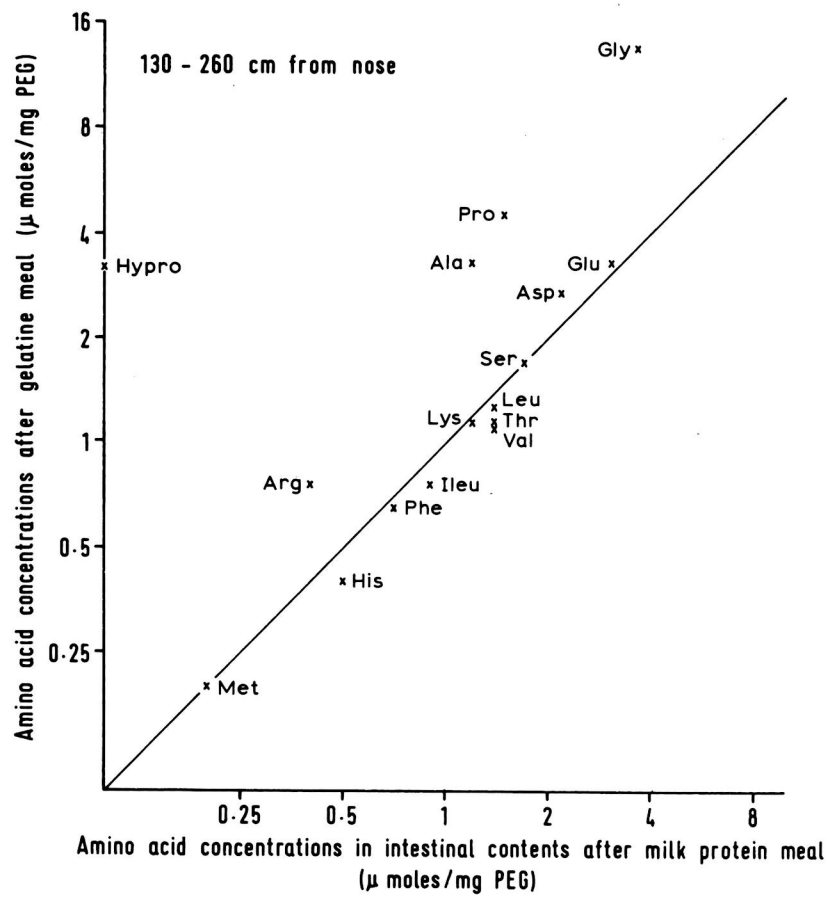

Fig. 31. The total amino acid concentrations (μmoles/mg PEG) in the three hour collections of intestinal contents from a sampling level of 130 to 260 cm after a gelatine meal plotted against the corresponding values in intestinal contents after a milk protein meal. A logarithmic scale has been used. The values are the means after each meal.

1. Total Amino Acid Concentrations:

In Figure 31 the total amino acids, μmoles/mg. PEG, in intestinal contents from a level below 130 cm. after the two protein meals are plotted against each other. A log scale has been used. With the exceptions of Gly, Ala, Arg, Pro and Hypro the concentrations were very similar in both types of intestinal sample. Rogers, Chen, Paraino and Harper (1960) showed that the quantity of nitrogen recovered from rat small intestine after a gelatine meal was greater than that after a casein meal. The increase in nitrogen associated with gelatine was in the TCA soluble, non-amino acid fraction (Rogers and his co-workers, 1962). On the basis of the present work it is suggested that this increase in nitrogen was due to the accumulation of peptides containing Arg, Gly, Ala, Pro and Hypro. These results support the suggestion that by this level the only residues of dietary protein which remain in the lumen are those which are resistant to enzymic hydrolysis.

2. Relative Molar Concentrations (Leu = 1) of Total Amino Acids:

There was a weak correlation between the molar concentrat-:ions of the total amino acids relative to Leu in the two protein containing test meals (r = 0.14) (Biostatistics : Goldstein, 1964). However, there was a strong correlation between the intestinal samples collected from the level below 130 cm. after all three meals (r = 0.85 - 0.96). This is further support for the suggestion that dietary protein digestion and absorption is complete by this level. It is consistent, too, with the suggestion that the smaller total amino acid concentrations after the low protein meal were due to the smaller stimulus to secretion by this meal, but the composition of the secretion was similar to that in response to the protein meals. (The correlation coefficients (r) derived from the data in Appendix 6 are listed in Appendix 10).

3/

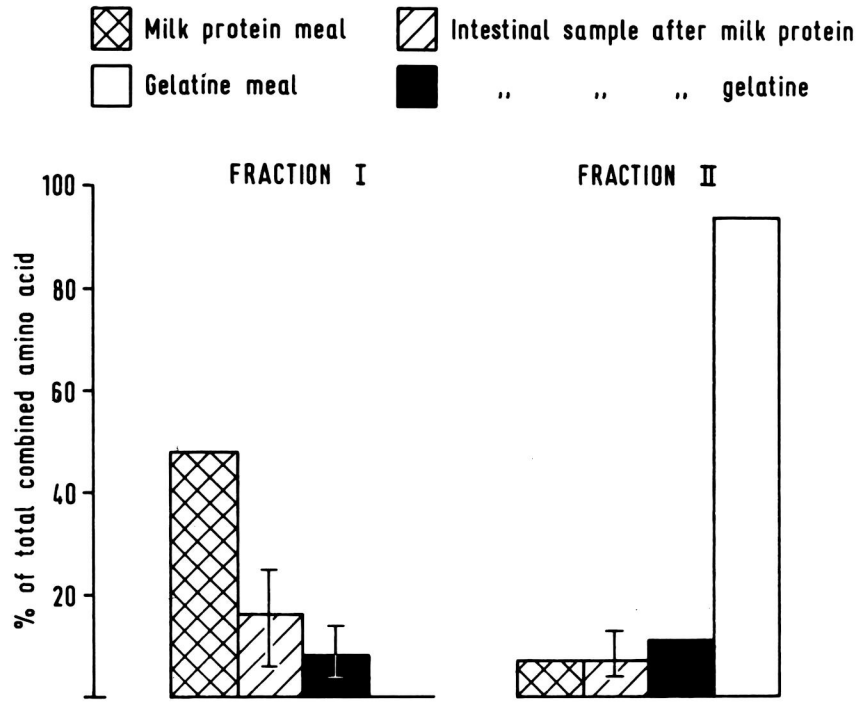

Fig. 32. The percentages of the total combined amino acids in Fractions 1 and 11 of the protein test meals and in intestinal samples following the three test meals. Means and ranges.

3. Fractions after Gel Filtration:

(a) In the case of Fractions I and II the samples of jejunal contents resembled each other closely and differed from the respective meals.

(i) Figure 32 shows the percentage of the total combined amino acid in Fractions I and II of the protein meals and of the intestinal samples.

(ii) There was a strong correlation between the relative molar concentrations (Leu = 1) of the total amino acids in these fractions after the three meals:
Fraction I. $r = 0.90 - 0.94$: Fraction II. $r = 0.90 - 0.98$.

(b) In the case of Fractions III and IV the similarity did not extent to the samples after the gelatine meal.

(i) The amount of total combined amino acid in these two fractions of samples after the gelatine meal was greater than after the milk protein meal (Figure 14). In Fraction III the increased concentrations after the gelatihe meal were due largely to the increased concentrations of Gly, Pro, Ala, and Hypro (Figure 33). In Fraction IV, however, subtraction of these amino acids from the total combined amino acids showed that there was an increased concentration of the remaining 12 amino acids in samples after the milk protein meal (Figure 33). Although the free amino acid component of Fraction IV was greater after the milk protein meal, this increase in the free amino acid concentrations did not account for the increase in the total concentrations of these 12 amino acids in this fraction after the milk protein meal (Figure 33).

(ii) The relative molar concentrations of the total amino acids in Fractions III and IV showed weak correlations between the three types of intestinal samples:
Fraction III. $r = 0.50 - 0.79$; Fraction IV. $r = 0.57 - 0.94$.
The weakest correlations were observed in comparisons involving/

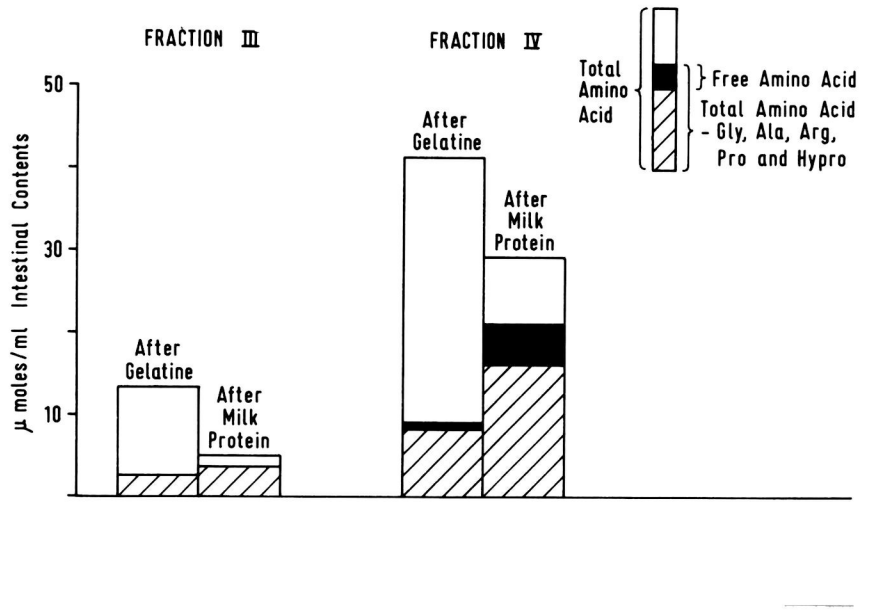

Fig. 33. The amino acid concentrations (µmoles/ml), mean values,

a) total combined amino acid

b) total combined amino acid less total Gly, Ala, Arg, Pro and Hypro

c) total combined amino acid less the free amino acids and total Gly, Ala, Arg, Pro and Hypro

in Fractions 111 and 1V of intestinal samples after the milk protein and the gelatine test meals.

involving the intestinal samples after the gelatine meal. When the amino acids, Gly, Pro, Ala and Arg were excluded from the calculations the remaining 12 amino acids showed strong correlations between all three types of intestinal samples in both Fraction III ($r = 0.79 - 0.94$) and Fraction IV ($r = 0.97$).

(The correlation coefficiencts (r) derived from the data in Table 13 are listed in Appendix 10).

The hypothesis thus breaks down to the extent that the total concentration of each amino acid with the exceptions only of Gly, Ala, Pro and Arg was greater in Fraction IV after the milk protein meal than after the gelatine meal. In these fractionation experiments, however, dilution factors have not been taken into account. The retention of the enzyme resistant peptides of gelatine may have caused retention of water too.

4. The Free Amino Acid Concentrations:

(a) The free amino acid concentrations (µmoles/litre) with the exception only of Gly, were higher in samples after the milk protein meal than in samples after the other two meals (Figures 17, 19, 20 and Appendix 7).

(b) With the exceptions of Gly, Leu, Ser, Thr and the dicarboxy amino acids the concentrations expressed as percentages of the totals were higher in samples after the milk protein meal than after the other two meals (Figures 21, 22, 23 and Appendix 7).

(c) With the exception only of Gly the concentrations expressed as percentages of the totals were similar in samples after the gelatine meal and after the low protein meal (Figures 22, 23 and Appendix 7).

This fraction of intestinal contents was therefore not dependent of the nature of the meal. Hydrolysis had occurred to a greater degree in samples after the milk protein meal. This may have been due to relative protection of the endogenous proteins/

proteins by the enzyme resistant peptides of gelatine (Snook and Meyer, 1964). It is possible, however, that this fraction represents the residue of the milk protein. This would amount to 8% of the milk protein meal.

The results are thus consistent with a slightly modified hypothesis; that at least 90% of the milk protein meal had been digested and absorbed by a level of 130 cm. from the nose. Any dietary protein which remained by this level was contained in the free amino acid or small peptide fraction.

Definite evidence was obtained for the presence of residual gelatine peptides in both Fractions III and IV.

THE AMOUNT OF ENDOGENOUS PROTEIN.

An attempt has been made, using the results obtained in this work, to give an estimate of the amount of endogenous protein which had diluted the protein of the test meal. This has involved various assumptions which may not always have been valid. There was no way of differentiating between amino acids of dietary origin and those of endogenous origin.

The estimated amounts of endogenous protein have been derived from the following:-

(a) From a comparison of the amino acid concentrations at 110 cm. after the low protein meal with those in the meal. At 110 cm. from the nose after the low protein meal the amino acid concentration (μmoles/mg., total combined), excluding Gly, in the 3 hour collection period was 10 μmoles/mg. PEG. 3 μmoles/mg. PEG was the corresponding concentration in the low protein test meal. Therefore at least 7 μmoles of amino acids per mg. PEG had been derived from endogenous protein and 14 mmoles had been added to the PEG containing bolus (2gm. PEG) during its passage to the sampling holes. 14 mmoles of amino acids in the appropriate relative concentrations amounted to 1.8gm. protein. This must be an underestimate of the actual endogenous protein secretion for the following reasons:-

1. No account has been taken of absorption proximal to this level and
2. Gly from endogenous protein was not included. (A large part of the Gly estimated in these experiments was probably derived from bile salts).

(b) From the increase in total Arg observed in intestinal contents in the cystinuric subjects. In samples from the upper jejunum (110 - 120 cm.) the mean concentration of Arg was 1.3 μmoles/mg. PEG. The concentration in the test meal was 1.05 μmoles/mg. PEG. Therefore at least 1.3 - 1.05 = 0.25 μmoles/mg. PEG/

Table 21

The theoretical concentrations of total amino acids in intestinal samples after the gelatine test meal when 8 gm of endogenous protein is added to the meal and 40% absorption has been assumed compared with the concentrations actually found.

	Theoretical conc. (μmoles/mgPEG)	Actual conc. (μmoles/mg PEG)
Asp	4.1	4.0
Thr	2.1	1.8
Ser	3.2	2.8
Pro	6.1	7.3
Glu	5.3	5.3
Ala	6.3	6.7
Val	2.5	2.0
Met	0.26	0.33
Ileu	1.6	1.2
Leu	1.4	2.1
Tyr	0.72	0.56
Phe	1.9	1.1
Lys	1.5	1.9
His	0.69	0.51
Arg	2.2	1.8

PEG were derived from endogenous protein i.e. 0.5 mmoles of Arg were secreted as constituents of the endogenous secretion which diluted the protein of the test meal. If the amino acids were present in the same relative molar concentrations as in the intestinal contents after the low protein meal a combined total concentration (excluding Gly) of at least 14 mmoles had been added to the milk protein test meal. Thus a second estimate of approximately 2 gm. of endogenous protein is obtained.

(c) From a comparison of the Met concentrations in samples after the gelatine meal with those after the low protein neal.

In upper intestinal samples after the gelatine meal the concentration of Met, umoles/mg. PEG, was approximately three times the concentration of Met in the samples after the low protein meal. The amounts given in the meals were almost identical, the Met in the gelatine meal being almost entirely derived from the cream. Thus the endogenous contribution of Met to the gelatine meal must have been greater than that to the low protein meal by a factor of approximately four*

* (Concentration of Met in intestinal contents after the low protein meal = 0.12 umoles/mg. PEG. Given = 0.06 umoles/mg. PEG. Concentration of Met in intestinal contents after the gelatine meal = 0.33 umoles/mg. PEG. Given = 0.07 umoles/mg. PEG. Endogenous Met after the low protein meal = 0.06 umoles/mg. PEG. Endogenous Met after the gelatine meal = 0.26 umoles/mg. PEG.)

Various studies have shown that enzyme secretion, qualitatively and quantitatively, is directly related to the composition of the meal (Sumie, 1958; Gadzieva, 1956; Snook and Meyer, 1964). If the composition of the endogenous secretion after the gelatine meal was similar to that after the low protein meal then at least 8gm. of endogenous protein amino acids, excluding Gly, had been added to the gelatine meal. The addition of 8gm. of protein, with the amino acids in the same relative concentrations as were found in the intestinal samples after the low protein meal, to the protein of the gelatine meal, followed by/

by say 40% absorption of each component would give concentrations, umoles/mg. PEG, in the samples after the gelatine meal very similar to those actually found as shown in Table 21.

Differences in rates of hydrolysis of the proteins from the two sources and differences in rates of transport of individual amino acids could account for the differences between the theoretical and the actual concentrations. For instance the observed concentration of Pro, µmoles/mg. PEG in intestinal samples after the gelatine meal is higher than the theoretical concentrations. This may be due to the impaired digestion of proline containing peptides of gelatine.

(d) From the total combined amino acid concentrations at 130 cm. after the milk protein meal. It has been suggested that by this level the milk protein has been absorbed and what remains is derived largely from endogenous protein. At 130 cm. from the nose the concentration was 29 umoles/mg. PEG (Table 9). Thus an estimate of 58 mmoles or approximately 8gm. of endogenous protein added to the milk protein meal is obtained. Table 22 shows the theoretical concentrations of total amino acids, umoles/mg. PEG, in the intestinal contents collected from the duodenum if 8gm. of protein in the same relative concentrations as in the intestinal contents after the low protein meal are added to the milk protein meal and 40% absorption of the mixture had occurred. For comparison is shown the concentrations actually found.

The minimum estimates of endogenous protein derived by these methods thus range from 2gm. to 8gm. The results did not indicate the large secretion (a six fold dilution of the meal) of endogenous protein shown by Nasset and Ju (1961) to occur in dogs and rats and by Nakayama and his co-workers (1960) to occur in dogs. Differences in the rates of gastric emptying could account for part of the discrepancy. The gastric emptying time of 3 hours in these experiments, with 50% leaving the stomach during the first hour is consistent with other work in man (Hunt and Spurrel/

Table 22

The theoretical concentrations of total amino acids in intestinal samples after the milk protein test meal when 8 gm of endogenous protein is added to the meal and 40% absorption has been assumed compared with the concentrations actually found.

	Theoretical conc. (μmoles/mg PEG)	Actual conc. (μmoles/mg PEG)
Asp	5.5	5.1
Thr	3.8	3.9
Ser	4.3	4.3
Pro	4.4	4.8
Glu	8.5	8.2
Ala	4.0	2.8
Val	4.0	3.5
Met	0.89	0.77
Ileu	3.1	2.7
Leu	5.0	4.6
Tyr	1.7	1.4
Phe	2.0	1.7
Lys	3.4	3.4
His	1.2	1.1
Arg	1.5	1.2

Spurrel, 1951; Borgstrom and his colleagues, 1957). However, many workers have found that a period of 6 hours or more is usually necessary for the whole of a test meal to leave the rat stomach (Geiger and his colleagues, 1958; Rosenthal and Nasset, 1958; Chen, Rogers and Harper, 1962). Pancreatic secretions enter the gut 10 to 15 minutes after ingestion of the meal and continue until the stomach is empty (Borgstrom and his colleagues, 1957). Slower gastric emptying in the dog and rat may increase the amount of endogenous protein by prolohging the secretion of pancreatic enzymes.

The amount of total combined amino acids collected during the third hour from the upper level after the milk protein meal was still considerable (Appendix 5). This occurred at a time when the amount of PEG recovered was very small suggesting that the protein in these third hour samples was mostly of endogenous origin. After the low protein meal, in samples obtained from the upper level, a continued rise in total combined amino acid concentrations with no diminution in the volume of sample, occurred over the three hours (see Appendix 11). In one subject a sample was obtained at the beginning of the fourth hour, from the upper level, after the low protein meal. The intestinal contents were still flowing freely although no PEG was detectable. The total combined amino acid concentration, excluding Gly, was 24 µmoles/ml. This was double the concentration observed in the sample collected during the first hour. A greater endogenous response to the meal occurring over a longer period of time than that required for the meal to pass the sampling holes may, therefore, have been demonstrated if sampling from the gut had been continued as long as flow continued.

DIGESTION.

The evidence obtained from the work of this Thesis cannot demonstrate the extent to which hydrolysis proceeds prior to absorption. However,

(1) In vitro incubation experiments showed that the seven amino acids, Lys, Val, Arg, Leu, Tyr, Phe and Met were released rapidly from the jejunal contents (Table 16). Extrapolation to the milk protein meal suggests that these seven components may be absorbed in the free state (Table 17).

(2) The experiments in the cystinuric subjects demonstrated that the in vivo rate of release of at least two of these amino acids, Lys and Arg was similar to the in vitro rate of release. Therefore Lys and Arg, at least are probably absorbed mainly in the free form.

(3) The rate of release of the remaining amino acids from the jejunal contents was slow. If these amino acids are absorbed in the free form then two possibilities exist -

a) that the enzyme(s) normally releasing them are particularly labile or

b) that the enzyme(s) are not free in the lumen of the gut.

The available evidence suggests that the second of these possibilities is the correct one. It has been known for many years that the peptidases of the intestinal mucosa are intimately associated with the mucosa (Gailey and Johnson, 1941; Wiggans and Johnston, 1959) and that centrifugation of intestinal juice results in decreased enzyme activity (Cajori, 1933; Pierce and his colleagues, 1935). Microscopic examination of the insoluble residue of intestinal contents did not indicate the presence of mucosal cells. Pierce and his co-workers (1935) found very few mucosal cells in the solid matter and suggested that this insoluble residue either adsorbed the intestinal enzymes or were themselves/

themselves the enzymes. The solid matter was present in the jejunal samples incubated in these experiments; it cannot therefore be concerned with the release of Gly, Pro or the acidic amino acids. Both Cajori and Pierce and his colleagues concluded that the enzymic activity of the intestinal secretion was insufficient to account for the amount of peptone that was absorbed.

(4) Borgstrom and his colleagues (1957) found that the degree of hydrolysis of iodinated albumin rapidly reached 50 to 60% in the duodenum. The extent of hydrolysis was determined by the decrease of ^{131}I which was precipitable in phosphotungstic acid with a concomitant increase in the ^{131}I associated with the phosphotungstic acid soluble material. The work being described has shown, however, that protein fragments associated with the acid soluble fraction may well have included fairly stable peptides such as the Gly, Pro, Hypro, Ala, Arg peptides of the gelatine or those peptides containing Asp and Glu after the milk protein meal which did not release the free amino acids on incubation.

(5) Peptides resistant to hydrolysis in the lumen may therefore -

a) be hydrolysed at the mucosal surface of the cell with subsequent transport of the free amino acids or

b) be absorbed as such with probably subsequent hydrolysis intracellularly (Wiggans and Johnston, 1959).

Evidence exists that Hypro containing peptides of gelatine may be absorbed as such (Prockop, Keiser and Sjoerdsma, 1962). In the work being described almost 50% of the Hypro of the gelatine meal had disappeared by a level of approximately 190 cm. yet free Hypro was never demonstrated in intestinal contents (the approximate limits of detection were 50 µmoles/ml.). Gly peptides which have also been demonstrated to be relatively resistant to enzyme hydrolysis can be transported across the gut wall as such (Wiggans and Johnston, 1959; Newey and Smythe, 1959). One factor/

factor, therefore which may determine the form in which protein amino nitrogen is absorbed may be susceptibility to hydrolysis by the intestinal enzymes. Chisiu (1966) found that rabbits fed a mixture of ovalbumin and insulin showed marked hypoglycaemia. Similar results were noted in alloxan diabetic rabbits. This effect was attributed to an antityptic factor in the investigated proteins. Eaton and Murlin (1933) had obtained a similar response to oral insulin in pancreatectomised dogs when peptic activity was inhibited by increase in pH.

(6) Despite the endogenous contribution the overall conservation is efficient, therefore not only enzyme resistant peptides but also the enzymes themselves must be absorbed. Autoproteolysis of the enzymes must therefore occur. Snook and Meyer (1964) using various criteria, found that the structural properties of trypsin and chymotrypsin were altered in the small intestine and because the enzymes were protected by dietary protein attributed these structural changes to proteolysis of the enzyme molecules concurrent with retention of activity during the early stages of digestion. After dialysing intestinal contents weak proteolytic activity in the dialysate was noted. No information, however, was given on the maximum size of the molecules able to pass through the dialysing membrane. In these experiments although at least 35% of the total amino acids in intestinal contents was in the form of molecules under 5,000 molecular weight the incubation experiments showed that enzymic activity could continue. Borgstrom and his co-workers (1957) demonstrated high concentrations of trypsin and chymotrypsin far down in the ileum; the enzymes have also been found in freshly voided faeces (Crane, 1964; Pelot and Grossman, 1962). However, the enzymes may not have been in the same form as when secreted. It is known that in certain enzymes a proportion of the amino acids may be removed without affecting activity although specificity may be altered (Koshland, 1960). Perhaps the best known example of this is papain where approximately two thirds of the 180 amino acid residues/

residues can be removed by aminopeptidase without loss of enzyme activity (Fruton and Simmonds, 1960). Perlmann in 1954 found enzymatically active fragments in the dialysate after autodigestion of pepsin. At least two active components were isolated after separation on 4% crosslinked Dowex 50. The active fragments were inactivated by heating to 100° for 5 minutes or on prolonged incubation at 37°. Although the specific activity of the dialysate against haemoglobin was only 3% of that of a freshly prepared enzyme solution, the activity against acetyl-lPhe-Ala-diiodotyrosine was 64% of that of the freshly prepared enzyme solution. Bresler, Champagne and Ya Frenkel (1961) showed that tryptic autolysates, non-sedimenting in the ultracentrifuge and which passed through a cellophane dialysing membrane, retained an appreciable part of the proteolytic activity. In the investigation of enzyme concentration by the activity against small synthetic peptides (Borgstrom and his colleagues, 1957) active fragments of the enzymes may therefore be contributing to the total.

The final breakdown of the endogenous protein fragments may occur as a result of the dipeptidase activity associated with the mucosa.

The evidence is therefore consistent with the view that absorption mainly in the free state occurs for Lys, Arg, Met, Tyr, Phe and Leu. It is not consistent with the view that Gly, Thr, Ser, the imino acids or the dicarboxylic acids are released in the lumen prior to absorption.

RATES OF ABSORPTION.

After a milk protein meal the net absorption of the amino acids appeared to occur at rates roughly dependent on the concentrations in the meal. Thus by the time the meal had reached an intestinal level of about 200 cm. from the nose a net absorption of approximately 70% of Glu and of Arg had occurred. This amounted to 12 mmoles of Glu and 1.5 mmoles of Arg. If the concentrations of the amino acids at the upper (80-90 cm.) and lower (250 cm.) levels are compared absolute absorption of amino acids along this segment of gut can be compared. This amounts to 3.9 umoles of Glu/mg. PEG and 0.6 umoles of Arg/mg. PEG.

When an equimolar solution of amino acids is perfused through loops of small intestine, in vitro and in vivo, Arg is absorbed rapidly and Glu slowly (Gitler and Martinez-Rojas, 1964; Orten, 1963). However, when a solution of amino acids in the same relative concentrations as is found in egg albumin with the concentration of Glu three times that of Arg, the rate at which absorption of Glu occurred was markedly increased (Gitler and Martinez-Rojas, 1964. These workers suggested that although competition for carriers occurred the concentrations were such that the slowly absorbed amino acids could compete effectively. The implication is that in a normal diet the concentration of Glu is such that it can compete effectively for transport mechanisms. However, when normal intestinal processes are considered the rate of breakdown to transportable fragments is an important factor. In these experiments the free amino acid concentrations in the lumen of the intestine bore little relationship to the concentrations in the meals given or to the concentrations of total amino acids in the intestine. Thus many of the free amino acids in the samples after the milk protein meal were present in approximately equimolar concentrations. Met was present in smaller concentrations; the mean values for the concentrations/

concentrations of the free acidic and basic amino acids were greater. However, the concentrations of the acidic amino acids showed great subject variation and were in many cases similar to, or even smaller, than the concentrations of the neutral amino acids.

If absorption of Glu occurs in the form of peptides then glutamyl peptides may compete with amino acids for transport mechanisms. If hydrolysis of the peptides occurs at the mucosal surface of the cell then possibly by binding of the released amino acids at the surface further transport of Glu is accelerated.

COMMENTS AND CRITICISMS.

1. The test meals were not ideal. They contained a high concentration of potassium ion and the lactose content would limit their use in many cases of malabsorption where a deficiency of disaccharidases is a complicating factor (Littman and Hammond, 1965). The fact that the meals were fluid or semi-fluid would cause gastric emptying to be more rapid than after a normal type of meal containing a higher percentage of solid matter. Thus the results obtained in these experiments cannot be applied to other more normal meals. The high concentration of gelatine in the gelatine test meal caused some degree of gastric distension and discomfort. Chen and his colleagues (1962) found that although rats would eat 5gm. of a test meal, which was protein free or contained 22% of casein or zein in 30 minutes, they would not eat 5gm. of diets containing 22% of amino acids or gelatine in this time. They attributed this to osmotic effects causing the uptake of water into the gastric contents.

2. PEG was found to be a satisfactory marker. It was relatively easy to estimate by the turbidimetric method (Hyden, 1956). A complete recovery from the gut was not attempted but in vitro experiments showed that it did not adsorb onto the solid matter of the intestinal samples.

3. Guminski and Naismith (1959) using young rats demonstrated the separation of the soluble components of milk from casein in the stomach after suckling with subsequent passage of "whey proteins" into the intestine. There was no evidence of this in the experiments described here; the soluble components as exemplified by the PEG and the total protein apparently left the stomach together.

4. The sampling period of 3 hours may have been too short. Although in most cases the test meal had passed the upper level sampling holes during this time, in one or two experiments the third/

third hour sample still contained considerable concentrations of PEG. Because of the rapid transit of the meal down that part of the small intestine under study it is probable that the major part of the meal also passed the lower level sampling holes during the three hour period. Collection for four or more hours would have verified this as well as giving more information regarding the amount of endogenous secretion in response to the meal.

5. The presence of the tube in the gut may have caused abnormal peristalsis or increased endogenous secretion, possibly of mucus. Its presence may also have had unrecognised psychological effects, affecting particularly, gastric emptying.

Various qualitative and quantitative changes may have occurred in the amino acid compartments of the intestinal samples while passing through the tube. This would be most liable to occur in samples from the lower level a) because of the greater length of tube to be traversed and b) because the flow from the lower level was less uniform.

6. Without full recoveries from the gel filtration procedure, definitive statements regarding the partitioning of the amino acid containing components can not be made.

7. The method of estimating hydroxyproline was not sufficiently sensitive to detect very small concentrations which may have been present in the free state in the intestinal samples after the gelatine meal.

8. Cystine estimation was unsatisfactory, partly because of the small amounts present and partly because of destruction during the hydrolysis procedure.

9. The free amino acid estimations on the Amino Acid Analyser would have been more reliable if a method of separating the free amino acids from small peptides had been found.

It is hoped to remedy some of these criticisms in future work.

CONCLUSIONS.

1. At least 90% of a milk protein meal was digested and absorbed by an intestinal level of 130 cm. from the nose.

2. Peptides, relatively resistant to enzymic hydrolysis, were demonstrated to occur in gelatine.

3. In vitro incubation experiments do not support the view that protein hydrolysis goes to completion in the lumen of the gut prior to absorption.

4. Estimates of the amount of endogenous protein suggest that it is of the same order as that in the meal.

5. Definite evidence for impaired absorption of Lys, Arg and Cys from a normal protein meal in cystinuria was obtained.

6. No evidence for the suggestion that α Methyl DOPA in therapeutic doses inhibits amino acid transport has been obtained.

ACKNOWLEDGEMENTS.

I am very grateful for the help and encouragement I have received from my two Supervisors, Professor R.H. Girdwood and Dr. G.E. Mawer.

Professor Fisher has provided some stimulating discussions.

I am grateful for access to the cystinuric subject, A.D. who was a patient of Professor S.W. Stanbury. Professor H. Harris provided the information about the relatives of the cystinuric subject, H.B.

I am grateful to my colleagues for allowing themselves to be intubated.

Trufood Ltd. gave a large amount of dried milk powder.

The work was supported by a grant from the Distillers Co. Ltd.

Appendix 1

The area of the extinction peaks (λ = 570 or 440) produced by standard amino acids during the period in which this work was done.

	Area/μmole	± S.E.M.	n
Taurine	17.0	0.6	8
Asp	18.0	0.4	20
Thr	18.4	0.4	22
Ser	19.2	0.4	22
Pro	5.74	0.01	22
Glu	18.7	0.4	22
Gly	18.4	0.3	22
Ala	18.0	0.5	21
Cys	19.9	0.3	5
Val	20.4	0.4	21
Met	19.3	0.2	22
Ileu	20.5	0.3	22
Leu	20.4	0.2	22
Tyr	20.2	0.3	22
Phe	20.7	0.3	20
Lys	28.4	0.8	16
His	23.1	1.7	16
Arg	24.6	0.6	17

Appendix 2

The justification for the use of polyethyleneglycol as an intestinal marker in these studies.

Evidence that polyethyleneglycol (PEG) can be completely recovered from the intestinal tract comes from various sources (Borgstrom and his colleagues, 1957 ; Smith, 1962 ; Soergel and Hogan, 1967 ; Maddrey and his colleagues, 1967). It has been shown in this work that the dietary protein and the PEG left the stomach at similar rates. It was shown by Goulston, Olsen and Harris (1966) that the PEG concentration of a sample of perfusate in gut segments was representative of the perfusate at the sampling point. Confirmatory evidence that a uniform distribution of the test meal protein and PEG occurred in the intestinal tract came from this work. The concentrations of two amino acids originating solely in the test meal and both poorly absorbed, namely α Methyl DOPA and Hypro, closely followed the concentrations of PEG at the duodenal level.

Appendix 3

The relative molar concentrations (Leu = 1) of total amino acids in gastric contents compared with those in the milk protein test meal. (Subjects GM and BN)

		Time after meal (hours)					
	Meal	1		2		3	
		GM	BN	GM	BN	GM	BN
Asp	0.93	0.90	0.93	0.92	1.07	1.01	1.22
Thr	0.68	0.60	0.65	0.58	0.67	0.65	0.67
Ser	0.72	0.65	0.66	0.65	0.77	0.73	0.83
Pro	0.85	0.92	0.91	0.90	1.06	0.95	2.04
Glu	1.80	1.64	1.73	1.49	1.92	1.50	2.06
Gly	0.39	0.30	0.42	0.63	0.54	0.83	1.42
Ala	0.64	0.62	0.67	0.54	0.70	0.56	0.61
Cys	0.07	0.09	0.08	0.10	0.08	0.11	0.12
Val	0.70	0.70	0.72	0.77	0.77	0.75	0.79
Met	0.19	0.13	0.11		0.16	0.15	
Ileu	0.58	0.56	0.59	0.55	0.61	0.56	0.54
Leu	1.00	1.00	1.00	1 00	1.00	1.00	1.00
Tyr	0.28	0.28	0.26	0.30	0.28	0.33	0.37
Phe	0.35	0.35	0.32	0.38	0.33	0.42	0.52
Lys	0.68	0.68	0.67	0.63		0.64	0.74
His	0.20	0.22	0.20	0.24		0.24	0.31
Arg	0.21	0.25	0.24	0.28		0.30	0.49

Appendix 4

The results from an intubation experiment with the sampling holes 60 cm from the nose. Samples were collected over a 3 hour period after a low protein meal.

	Meal	Intestinal samples			
		0-1hr	1-2hr	2-3hr	0-3hr
Volume (ml)	400	248	202	18	468
PEG (mg/ml)	5.0	3.7	3.2	0.8	
PEG (% of that given)		46	32	0.7	79
Amino acids					
µmoles/ml	15	12	17	31	
µmoles/mg PEG	3	3.3	5.5	37.4	
% of that given		50	57	10	117

Amino acids µmoles/mg PEG

	Test meal	Sample (0-3hr)
Asp	0.21	0.41
Thr	0.19	0.21
Ser	0.24	0.28
Pro	0.25	0.61
Glu	0.30	0.78
Gly	0.17	0 39
Ala	0.15	0 22
Val	0.27	0.29
Met	0.06	0.07
Ileu	0.19	0.21
Leu	0.31	0.37
Tyr	0.08	0.07
Phe	0.14	0.17
Lys	0.20	0.24
His	0.13	0.10
Arg	0.11	0.11

Appendix 5

The total combined amino acid concentrations in hourly samples.

a) Differences between samples.

The total combined amino acid concentrations, with respect to volume (μmoles/ml) in the intestinal samples are shown in Fig. 8a. The values are the means and the ranges observed. (No sample was collected during the first hour from the lower level in one subject after a gelatine meal.)

The concentrations in samples after the two protein meals were similar and greater than those after the low protein meal. After the protein meals the concentrations in general fell from hour to hour at each level and between levels in each hour. After the low protein meal the concentrations in the sample obtained from the upper level during the third hour were higher than those in the preceding two hourly samples. There was little, or no, decrease between levels.

The total combined amino acid concentrations with respect to the marker, (μmoles/mg PEG) are shown in Fig. 8b. These were greater in samples after the gelatine meal than in samples after the milk protein meal. This was most marked in the second and third hour samples from the lower level. The variation in concentrations, μmoles/mg PEG, in samples obtained during the third hour from the upper level was due to variation in PEG concentrations during this period. The values for the concentrations expressed in this way in samples after the low protein meal were lower than the corresponding values after the protein meals. The differences were especially marked in samples obtained during the first two hours from the upper level. Poor agreement was noted between the results from the lower level experiments in the two subjects given the low protein meal. In one of these subjects very rapid transit of the meal apparently occurred ; a first hour sample from the lower level contained 25 % of the PEG given.

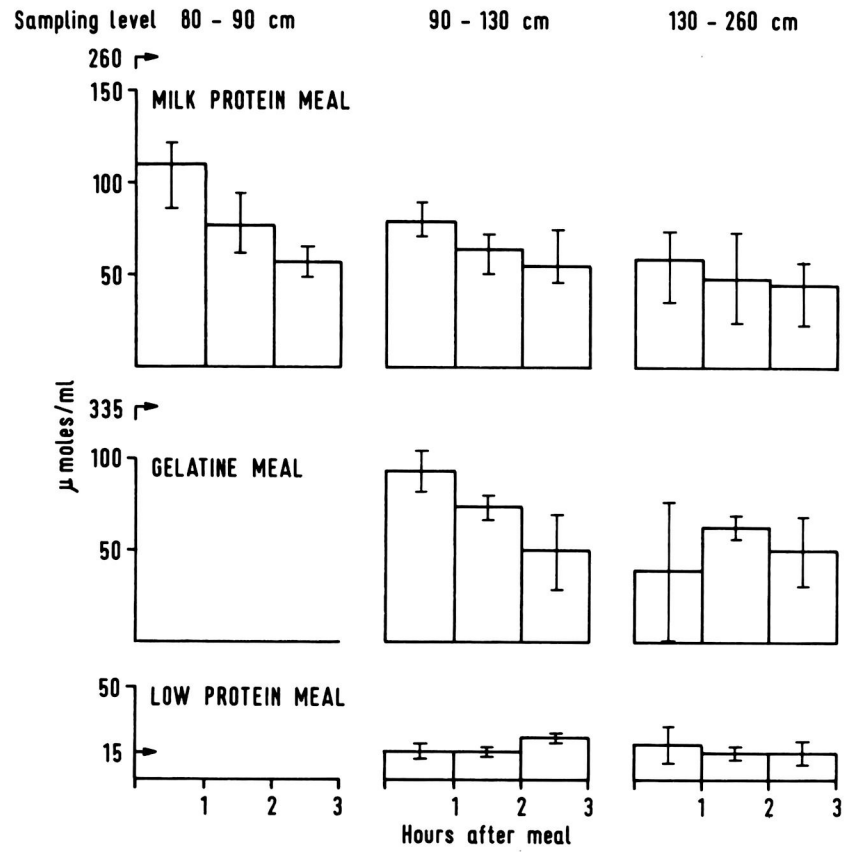

Fig. 8a. The total combined amino acid concentrations (µmoles/ml) in intestinal contents during the three hours after each of the test meals. Means and ranges. The arrows on the ordinates indicate the concentrations in the test meals.

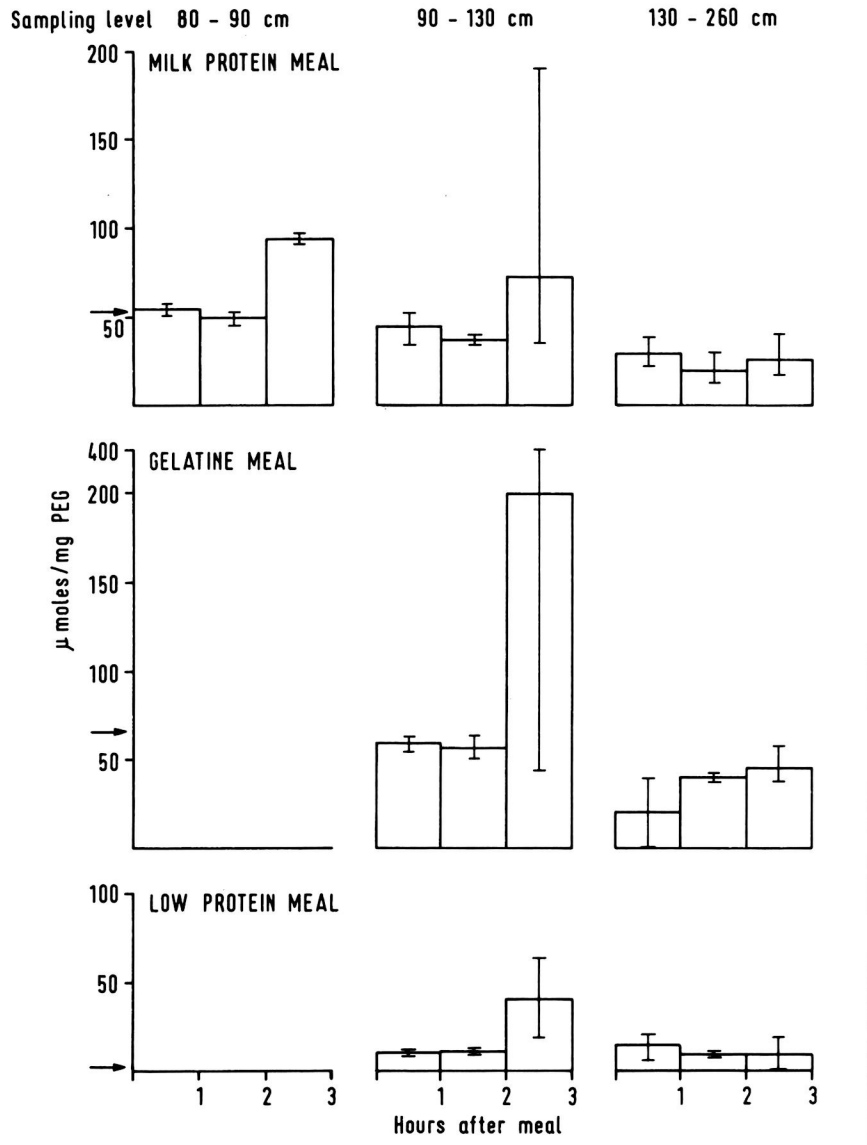

Fig. 8b. The total combined amino acid concentrations (µmoles/mg PEG) in intestinal contents during the three hours after each of the test meals. Means and ranges. The arrows on the ordinates indicate the concentrations in the test meals.

b) Differences between samples and the corresponding meals

The arrows on Figs. 8a and b denote the amino acid concentrations in the test meals. After the two protein meals the total combined amino acid concentrations, μmoles/ml, in the intestinal samples were at all stages of the experiments lower than those in the corresponding meals. In contrast the concentrations after the low protein meal were of the same order as that in the meal.

There was also, in most cases, a drop in the concentrations with respect to the marker (μmoles/mg PEG) between the protein meals and intestinal samples following these meals. During the third hour at the upper levels the concentrations (μmoles/mg PEG) were very often higher than those in the meals. The concentrations of PEG in the third hour upper level samples were normally very low (see Fig. 7) giving a greatly increased ratio of amino acids to PEG in these samples. After the low protein meal the ratio of amino acids to PEG in all the intestinal samples was greater than that in the meal.

The relative molar concentrations (Leu = 1) of total amino acids in the protein test meals and in intestinal samples following the three test meals.

	Meal MP	Intestinal samples after the MP meal			Intestinal samples after the LP meal	Intestinal samples after the G meal		Meal G
		80-90 cm	90-130 cm	130-260 cm	all levels	130-260 cm	90-130 cm	
Leu =		4	5	9	5	2	2	
Asp	0.93	1.1 (1.1-1.2)	1.2 (1.1-1.2)	1.5 (1.3-1.8)	1.5 (-)	2.2 (-)	2.1 (1.9-2.2)	1.8
Thr	0.68	0.79(0.72-0.96)	0.79(0.74-0.85)	0.99(0.85-1.1)	0.88(0.86-0.94)	0.92(-)	0.89(0.85-0.92)	0.72
Ser	0.72	0.87(0.80-1.1)	0.88(0.81-0.94)	1.1 (0.90-1.3)	1.0 (1.0-1.1)	1.4 (-)	1.4 (1.3-1.4)	1.4
Pro	0.85	1.0 (0.96-1.1)	0.99(0.82-0.94)	1.0 (0.91-1.2)	0.88(0.83-0.91)	3.6 (3.2-4.0)	3.6 (3.5-3.8)	4.4
Glu	1.8	1.8 (1.7-1.8)	1.9 (1.8-2.0)	2.1 (1.9-2.5)	1.4 (1.3-1.5)	2.7 (2.6-2.8)	2.8 (2.5-3.0)	2.8
Gly	0.39	1.5 (1.3-1.9)	1.9 (1.2-2.4)	2.8 (1.7-4.1)	5.2 (3.5-7.7)	10.6 (8.8-12.4)	11.2 (11.0-11.3)	12.7
Ala	0.64	0.65(0.54-0.78)	0.74(0.68-0.77)	0.84(0.74-0.98)	1.0 (0.96-1.1)	2.9(2.5-3.3)	3.2 (-)	4.4
Cys	0.07	0.10(0.09-0.10)	0.12(0.10-0.16)	0.16(0.14-0.24)				0.08
Val	0.70	0.77(0.73-0.80)	0.72(0.61-0.83)	0.87(0.76-0.98)	1.0 (0.92-1.0)	0.92(-)	0.94(0.92-0.95)	0.94
Met	0.19	0.15(0.12-0.18)	0.15(0.13-0.18)	0.13(0.09-0.19)	0.11(0.09-0.14)	0.15(0.13-0.16)	0.16(0.15-0.17)	0.04
Ileu	0.58	0.60(0.59-0.64)	0.58(0.49-0.62)	0.67(0.56-0.92)	0.66(0.65-0.69)	0.58(0.56-0.59)	0.57(-)	0.56
Tyr	0.28	0.30(0.29-0.34)	0.30(0.30-0.33)	0.34(0.26-0.40)	0.40(0.37-0.45)	0.34(0.28-0.40)	0.28(0.26-0.30)	0.08
Phe	0.35	0.35(0.33-0.38)	0.36(0.33-0.38)	0.42(0.33-0.51)	0.53(0.48-0.60)	0.51(0.46-0.56)	0.51(0.50-0.52)	0.50
Lys	0.68	0.71(0.57-0.83)	0.78(0.65-0.87)	0.80(0.59-0.93)	0.63(0.55-0.69)	0.92(0.84-1.0)	0.91(0.90-0.92)	1.1
His	0.20	0.22(0.16-0.28)	0.25(0.19-0.29)	0.28(0.18-0.38)	0.28(0.25-0.31)	0.31(-)	0.26(0.24-0.27)	0.22
Arg	0.21	0.24(0.19-0.29)	0.25(0.20-0.29)	0.27(0.21-0.33)	0.39(0.36-0.42)	0.61(0.51-0.70)	0.78(0.71-0.85)	1.4
Pro						2.6 (2.2-3.0)	2.5 (2.3-2.6)	3.2

The values for the intestinal samples after the two protein meals are the means and ranges at each level ; the values for the samples after the low protein meal are the means and ranges at all levels.

Appendix 7

The free amino acid concentrations (μmoles/litre) in intestinal contents after

	LP	MP	LP	LP	G	G
	Duodenum	Jejunum	Jejunum		Jejunum	
Asp	44(41-49)	156(70-340)	7	12	205	20
		231(82-823)	6	10	244	19
Thr	60(53-65)	149(105-211)	28	23	57	37
		164(91-264)	22	18	49	40
Ser	89(71-99)	185(110-287)	30	30	95	62
		198(137-291)	28	21	96	68
Pro	123(120-126)	160(78-195)	21	17		
		93(65-120)	17			
Glu	239(201-271)	500(262-944)	21	37	420	42
		920(324-1680)	14	28	329	61
Gly	143(73-198)	231(162-299)	74	50	1745	714
		234(129-348)	61	41	1244	925
Ala	77(69-88)	196(131-259)	45	30	198	107
		197(140-323)	32		99	87
Cys	90(81-100)	92				
		138(120-152)				
Val	89(69-120)	168(113-223)	21	20		26
		189(130-266)	28			42
Met	46(31-65)	74(58-91)	11	6		6
		53(40-58)	6	5		7
Ileu	55(45-71)	94(64-159)	13	10		4
		98(71-100)	18	9		32
Leu	116(81-174)	215(162-314)	33	26		25
		229(182-227)	32	20		39
Tyr	229(194-228)	286(224-408)	40	26	19	38
		173(127-200)	19	19	25	26
Phe	179(149-209)	233(180-323)	33	20	26	42
		144(128-175)	17	18	22	27
Lys	642(504-742)	947(580-1400)	75	62	216	265
		681(570-960)	32	28	145	161
His	132(97-181)	229(252-597)	15	12	31	23
		177(115-262)	8	7	19	15
Arg	364(280-455)	394(252-597)	39	40	250	690
		226(145-335)	15	14	78	108

The free amino acid concentrations in intestinal contents expressed as percentages of the totals after

	MP	MP	LP	LP	G	G
	Duodenum	Jejunum	Jejunum		Jejunum	
Asp	0.5(0.5-0.6)	2.2(0.9-4.6)	0.5	0.3	5.0	0.5
		5.8(1.4-14.0)	0.4	1.0	4.9	0.6
Thr	1.0(0.7-1.2)	2.9(2.7-3.7)	3.0	3.0	3.2	2.3
		4.6(2.2-6.9)	2.0	2.8	2.3	3.1
Ser	1.4(0.9-1.7)	3.0(2.0-3.8)	3.8	3.2	2.9	3.2
		5.3(2.9-7.6)	2.9	3.9	3.3	3.3
Pro	1.6(-)	1.9(1.6-2.1)	1.9			
		1.9(1.7-2.2)	1.8			
Glu	1.8(1.6-1.9)	4.6(2.3-7.9)	1.0	2.9	6.2	0.8
		10.7(4.1-20.8)	1.2	3.1	6.2	2.0
Gly	1.4(0.9-1.9)	2.0(1.1-2.4)	1.3	1.8	7.3	3.3
		2.8(1.1-4.4)	0.7	2.2	6.2	5.4
Ala	1.7(1.3-2.0)	4.2(2.9-5.4)	3.8	3.4	2.8	2.2
		7.0(4.9-10.5)	3.3		2.4	2.3
Cys	12.1(9.8-14.2)	9.7				
		21.1(15.4-27.3)				
Val	1.6(1.2-2.1)	3.6(2.7-4.5)	2.2	2.9		1.1
		7.1(3.5-13.8)	2.8			3.2
Met	3.9(2.3-6.0)	7.2(6.5-8.0)	6.9	5.3		2.2
		9.9(7.6-12.5)	3.9	9.2		4.1
Ileu	1.2(1.1-1.6)	2.6(1.8-3.9)	2.2	2.3		1.3
		4.3(3.0-5.6)	3.2	2.4		3.2
Leu	1.6(1.0-2.5)	3.3(2.9-3.9)	3.3	3.3		1.2
		7.1(4.6-10.6)	3.4	3.0		2.0
Tyr	10.0(9.1-11.5)	13.6(11.9-15.1)	9.2	7.4	3.2	5.9
		13.8(10.5-16.5)	4.3	8.2	3.1	4.4
Phe	6.6(5.6-7.7)	9.3(8.4-10.4)	8.2	3.4	2.1	3.9
		9.5(6.5-12.0)	3.4	4.9	2.1	3.2
Lys	11.6(8.3-14.5)	17.4(13.3-19.5)	11.4	9.7	10.4	12.8
		22.6(17.2-27.0)	6.2	6.9	6.9	10.8
His	7.9(4.6-10.0)	11.6(8,4-17.5)	4.3	4.3	4.8	4.9
		15.4(8.1-20.9)	3.5	4.2	3.2	3.2
Arg	19.0(13.0-25.2)	21.6(18.0-25.4)	9.2	9.8	15.8	36.2
		20.7(13.1-33.0)	4.3	6.2	7.0	10.8

cont. over page.

The values in the tables are a) the concentrations in the three hour collections of intestinal contents. The means and ranges are given for the samples from subjects given the milk protein meal. The concentrations are given separately for the two subjects given the gelatine and the low protein test meals. The upper figure in the column marked jejunum indicates the concentration of that amino acid in samples from intestinal levels of 90 to 130 cm from the nose ; the lower figure indicates the concentration in samples from levels of 130 to 260 cm.

b) the concentrations in the three hour collections expressed as a percentage of the totals in the three hour collections.

Appendix 8

The increase in free amino acids in intestinal contents on incubation at 37

mins	0		5		10		15		20		40		80	
	conc	%	conc	%	conc	%	conc	%	conc	%	conc	%	conc	%
Asp	250	3.8	232	3.5	243	3.7	224	3.4	262	3.9	317	4.8	368	5.5
	686	9.2	703	9.4	708	9.5	770	10	756	10	806	11	765	10
Thr	222	4.9	216	4.8	266	5.9	252	5.5	284	6.2	366	8.0	488	11
	273	6.1	310	6.9	397	8.9	368	8.2	400	8 9	420	9.4	382	8.6
Ser	334	6.7	352	7.1	426	8.6	434	8.7	467	9.3				
	440	9.1	475	9.9	564	12	606	13	725	15	614	13	682	14
Pro	122	2.5	112	2.3	113	2.3	148	3.0	146	3.0	180	3.7	360	7.3
	134	2.9	106	2.3	120	2.6	160	3.5	162	3.5	174	3.8	209	4.6
Glu	838	7.9	794	7.5	840	8.0	825	7.8	908	8.6	1040	10	1180	11
	1380	12	1450	13	1483	13	1630	14	1650	15	1665	15	1570	14
Gly	290	3.3	269	3.0	278	3.1	278	3.1	270	3.0	310	3.5	388	4.4
	400	5.3	431	5.8	450	6.0	498	6.7	508	6.8	485	6.5	413	5.5
Ala	298	6.7	304	6.9	368	8.3	377	8.5	410	9.2	548	12	750	17
	376	8.7	417	9.6	458	11	518	12	532	12	609	14	580	13
Val	105	2.5	270	6.5	406	9.7	425	10	516	12	703	17	983	24
	272	6.5	346	8.3	403	9.6	433	10	502	12	660	16	654	16
Met	132	15	190	21	273	31	321	37	351	40	396	45	507	58
	128	17	173	23	256	34	270	36	266	35	290	38	287	38
Ileu	132	4.0	171	5.2	188	5.7	262	7.9	293	8.9	412	13	602	18
	125	4.0	170	5.4	183	5.8	237	7.5	247	7.8	366	12	409	13
Leu	297	5.4	400	7.2	507	9.2	658	12	722	13	1010	18	1485	27
	295	5.8	408	8.0	515	10	630	12	674	13	935	18	958	19
Tyr	373	22	466	27	571	33	646	38	691	40	856	50	855	50
	350	22	438	28	556	35	583	37	630	40	690	44	650	41
Phe	246	11	335	15	397	18	468	21	495	22	605	27	727	34
	248	14	328	18	372	21	435	25	455	26	556	31	505	28
Lys	914	24	1100	31	1625	42	1765	45	1772	46	1930	50	2170	56
	974	26	1150	30	1376	36	1500	40	1578	42	1725	46	1680	44
His	127	10	141	12	160	13	153	12	168	14	212	17	274	22
	140	11	157	13	165	13	177	14	186	15	211	17	212	17
Arg	381	31	505	42	625	52	672	56	678	56	705	58	775	64
	362	30	440	36	486	40	518	42	542	44	590	48	584	48

The concentrations are μmoles/litre of intestinal contents ; the percentages are of the totals present. The upper value for each amino acid is derived from the incubation of jejunal juice from a level of 110 cm. the lower one from a level of 170 cm.

Appendix 9

The total amino acid concentrations (μmoles/mg PEG) in intestinal samples from two subjects given α Methyl DOPA compared with those in control subjects.

	Controls	α Me DOPA		Controls	α Me DOPA	
	80 - 90 cm	80cm	90cm	130 - 260 cm	192cm	257cm
Asp	5.2 (4.8-5.5)	4.7	3.3	2.3 (1.6-3.3)	2.0	1.4
Thr	3.7 (3.5-4.3)	3.0	2.1	1.5 (1.1-2.1)	1.3	0.7
Ser	4.1 (3.8-4.8)	2.8	2.2	1.7 (1.3-2.1)	1.4	0.8
Pro	4.7 (4.2-5.4)	4.2	3.1	1.6 (1.1-2.3)	1.3	0.7
Glu	8.2 (7.9-8.6)	8.3	5.2	3.2 (2.2-4.5)	2.7	2.0
Gly	6.9 (6.1-8.7)	6.2	7.6	4.1 (2.7-5.7)	4.5	2.3
Ala	3.0 (2.6-3.7)	2.3	2.2	1.3 (0.94-2.1)	1.5	0.7
Val	3.6 (3.3-3.8)	3.7	2.7	1.3 (0.96-2.1)	1.3	0.8
Met	0.72(0.57-0.90)	0.73	0.34	0.21(0.10-0.26)	0.21	0.13
Ileu	2.8 (2.6-3.0)	2.9	2.2	1.0 (0.73-1.6)	1.1	0.63
Leu	4.6 (4.4-4.8)	4.6	3.3	1.5 (1.0-2.3)	1.7	0.7
Tyr	1.4 (1.3-1.6)	1.3	0.9	0.53 (0.32-0.8)	0.6	0.24
Phe	1.7 (1.5-1.8)	1.6	1.2	0.66(0.37-0.8)	0.6	0.32
Lys	3.3 (2.7-3.9)	2.9	2.4	1.2 (0.8-1.5)	1.1	0.52
His	1.1 (0.8-1.3)	0.9	0.8	0.43(0.27-0.6)	0.44	0.24
Arg	1.2 (0.9-1.4)	1.1	0.8	0.42(0.28-0.5)	0.38	0.19

The free amino acid concentrations (as per cent of total) in intestinal samples from two subjects given α Methyl DOPA compared with those in control subjects.

	Controls	α Methyl DOPA		Controls	α Methyl DOPA	
	80 - 90 cm	80 cm	90 cm	130 - 260 cm	192cm	257cm
Asp	0.5 (0.5-0.6)	1.3	0.5	5.8 (1.4-14)	0.9	23
Thr	1.0 (0.7-1.2)	2.0	2.0	4.6 (2.2-6.9)	3.0	5.8
Ser	1.4 (0.9-1.7)	2.9	2.7	5.3 (2.9-7.6)	3.9	6.6
Pro	1.6 (-)			1.9 (1.7-2.2)	2.0	5.8
Glu	1.8 (1.6-1.9)	2.8	1.6	10.7 (4.1-21)	3.0	33
Gly	1.4 (0.9-1.9)	1.0	1.0	2.8 (1.1-4.4)	1.0	1.9
Ala	1.7 (1.3-2.0)	2.6	2.8	7.0 (4.9-11)	3.3	5.4
Val	1.6 (1.2-2.1)	1.2	2.8	7.1 (3.5-14)	5.9	
Met	3.9 (2.3-6.0)	4.1	8.8	9.9 (7.6-13)	3.4	10
Ileu	1.2 (1.1-1.6)	1.6	1.8	4.3 (3.0-5.6)	4.4	4.9
Leu	1.6 (1.0-2.5)	1.8	2.7	7.1 (4.6-11)	7.1	8.3
Tyr	10.0 (9.1-12)	12	13	14 (11-17)	17	17
Phe	6.6 (5.6-7.7)	8.9	10	9.5 (6.5-12)		
Lys	12 (8.3-15)	11	12	23 (17-27)	15	18
His	7.9 (4.6-10)	7.4	8.8	15 (8.1-21)	8.3	19
Arg	19 (13-25)	12	17	21 (13-33)	14	15

The values for both total and free amino acid concentrations are the means and ranges for the control subjects and the individual values for the subjects given α Methyl DOPA.

Appendix 10

The correlations between the relative molar concentrations (Leu = 1) in the protein test meals and in intestinal samples after the three meals.

Correlation coefficient between the protein test meals — r = 0.14

Correlation coefficient between intestinal samples

a) after milk protein	v after low protein	r = 0.89
b) after milk protein	v after gelatine	r = 0.85
c) after low protein	v after gelatine	r = 0.96

Correlation coefficients between fractions of intestinal samples.

Fraction 1

a) after milk protein	v after low protein	r = 0.94
b) after milk protein	v after gelatine	r = 0.92
c) after low protein	v after gelatine	r = 0.90

Fraction 11

a) after milk protein	v after low protein	r = 0.98
b) after milk protein	v after gelatine	r = 0.86
c) after low protein	v after gelatine	r = 0.90

Fraction 111

a) after milk protein	v after low protein	r = 0.79
b) after milk protein	v after gelatine	r = 0.60
c) after low protein	v after gelatine	r = 0.50

Fraction 1V

a) after milk protein	v after low protein	r = 0.94
b) after milk protein	v after gelatine	r = 0.57
c) after low protein	v after gelatine	r = 0.62

With the exclusion of Gly, Pro, Ala, and Arg from the calculations

Fraction 111

a) after milk protein	v after low protein	r = 0.79
b) after milk protein	v after gelatine	r = 0.94
c) after low protein	v after gelatine	r = 0.79

Fraction 1V

a) after milk protein	v after low protein	r = 0.97
b) after milk protein	v after gelatine	r = 0.97
c) after low protein	v after gelatine	r = 0.97

Appendix 11

The rise in the protein concentrations of intestinal contents over the three hour period from the upper level after the low protein meal was unexpected. It may have been due to the greater dilution of the endogenous secretion by the meal during the first two hours although in one subject the sample volume and the PEG concentrations were almost identical in the first and third hour collections. It may have been due to temporal differences in the secretion of pancreatic stimulatory hormones. Secretin, formed in the mucosa of the duodenum stimulates the secretion of copious amounts of pancreatic juice which is relatively deficient in enzyme activity. Pancreozymin, formed by intestinal mucosa, stimulates the secretion of enzymes by the pancreas. The enzyme secretion stimulated by the latter hormone may follow the watery secretion stimulated by secretin. Twombley and Meyer (1961) in an investigation of endogenous protein secretion in rats found a peak in the small intestinal contents of nitrogen (mg), 8 hours after a protein free meal and two peaks, one at $\frac{1}{2}$ hour and the other at 8 hours after a protein containing meal. The peak at 8 hours was attributed to the gradual accumulation of slowly digested enzymes or mucosal cells. It may, however, have been due to a continued rise in enzyme production. The increase in concentrations observed over the three hours at the upper level in these experiments did not occur in samples from the lower level indicating that accumulation was not occurring. The differences in time scale between the experiments described by Twombley and Meyer and the present experiments may have been due to differences in the rates of gastric emptying.

REFERENCES

Adams,B.A. and E.L. Holmes. (1935)

J. Soc. Chem. Ind. 54 1T

Adibi,S.A. and S.J. Gray. (1967)

Gastroenterology 52 837-845

Adibi,S.A., S.J. Gray and E. Mendon. (1967)

Am. J. clin. Nutr. 20 24-33

Agar,W.T., F.J.R. Hird and G.S. Sidhu. (1954)

Biochim. biophys. acta 14 80-84

Agren,G. (1948)

Acta. chem. scand. 2 797-802

Albers,R.W. (1967)

Ann. Rev. Biochem. 36 727-756

Alexander,H.L., K. Shirley and D. Allen. (1936)

J. clin. Invest 15 163-167

Anson,M.L. (1937)

J. Gen. Physiol. 20 663

Asatoor,A.M., B.W. Lacey, D.R. London and M.L. Milne (1962)

Clin. Sci. 23 285

Atfield,G.N. and C.J.O.R. Morris (1961)

Biochem. J. 81 606-614

Balls,A.K. (1962)

J. Gen. Physiol. 45 47-56

Bartelheimer,H., H. Maring and H.J. Steining (1955)

Klin. Wschr. 33 160-174

Ben Abdiljlil,A., A.M. Visani and P. Desnuelle (1963)

Biochem. Biophys. Res. Commun. 10 112-116

Bergman, ... and J.S. Fruton (1941)

Adv. Enzymol. 1 63-98

Bierman,H.R., R.L. Byron,Jr, ..H. Kelly, R.S. Gilfillan, L.P. White, ...D. Freeman and N.L. Petrakis. (1953)

J. clin. Invest. 32 637-649

Blankenhorn,D.H., J. Hirsch and E.H. Ahrens,Jr. (1955)

Proc. Soc. exp. Biol. Med. 88 356

Bresler,S.E., M. Champagne and S. Ya Frenkel. (1961)

Biokhimiya 26 909

Börgstrom,B., A. Dahlquist, G. Lundh and J. Sjövall. (1957)

J. clin. Invest. 36 1521

Bovey,F.A. and S.S. Yanari (1960)

The Enzymes. Vol. 4. Ed. P.D. Boyer, H. Lardy and K. Myrbback. Academic Press. New York.

Buhs,R.P., J.L. Beck, ...C. Speth, J.L. Smith, N.R. Trennen, P.J. Cannon and J.H. Lanagh. (1964)

J. pharmacol. and exp. therap. 143 205-214

Cajori,F.A. (1933)

Am. J. Physiol. 104 659-668

Carlson,L.A. and L.B. Wadstrom (1959)

Clinica chim. Acta. 4 197

Chen,M.L., Q.R. Rogers and A.E. Harper (1962)

J. Nutr. 76 235-241

Chinn,A.B., P.S. Lavik, R.M. Stitt and G.W. Buckaloo (1952)

New Engl. J. Med. 247 877-880

Chisiu,N. (1966)

Studii Cerc. Biochim. 9 267-276

Christensen,H.N., P.F. Cooper,Jr, R.D. Johnson and E.L. Lynch. (1947)

J. biol. Chem. 168 191-196

Christensen,H.N. (1949)

Biochem. J. 44 333-335

Christensen,H.N. (1960)

Adv. Protein Chem. 15 239-305

Christensen,H.N. (1967)

Perspect. Biol. Med. 10 471-494

Clark,E.M., G.H. Gibson, I.H. Smyth and G. Wiseman (1951)

J. Physiol. 112 46P

Clark, .L.,Jr. (1959)

J. biophys. biochem. Cytol. 5 41-50

Consden,R., A.H. Gordon and A.J.P. Martin (1944)

Biochem.J. 38 224

Cooper,H., R. Levitan, J.S. Fordtran and F.J. Ingelfinger. (1966)

Gastroenterology 50 1-8

Corbett,J.L., P.B. Miller, E.M. Clarke and C. Florence. (1956)

Proc. nutr. Soc. 15 v

Cori,C.F. (1925)

J. biol. Chem. 66 691-715

Craig,L.C. and F.P. King (1955)

J. Am. chem. Soc. 77 6620-6624

Crane,C.W. and A. Neuberger (1960)

Biochem. J. 74 313-323

Crane,C.W. (1961)

Postgradm med. J. 37 745-754

Crane,C.W. (1964)

The Role of the Gastrointestinal Tract in Protein Metabolism.
Ed. H.N. Munro.

Cruz, .C., .B. Hawkins and G.M. Whipple (1942)

Am. J. med. Sci. 203 848-854

Dawson,R. and J.M.G. Porter (1962)

Br. J. Nutr. 16 27

Dent,C.E. and J.A. Schilling (1949)

Biochem. J. 44 318-333

Dent,C.E. and G.A. Rose (1951)

Q. Jl. Med. 20 205

Dent,C.E., B. Senior and J.M. Walshe (1954)

J. clin. Invest. 33 1216

Desnuelle,P. and M. Rovery (1961)

Adv. Protein Chem. 16 139-195

Dixon,M. and E.C. Webb (1964)

Enzymes. Longmans, Green and Co. Ltd. London.

Dollery,C.T. and M. Harrington (1962)

Lancet 1 759-763

Eaton,A.G. and J.R. Murlin (1933)

Am. J. Physiol. 104 636-649

Edin,H., G. Kihlen and S. Nordfeldt (1944)

Annals Agr. Coll. Sweden 12 166

Edwards,K.D.G., J.A. Young, D. Jeremy and F.C. Neale (1963)

Mem. Congr. Mundial. Cardiol. 4a 124

Fare,G. and D.C.H. Sammons (1966)

Experientia 22 668

Fisher,R.B. (1954)

Protein Metabolism Methuen and Co. Ltd. London.

Fisher,R.B. and D.S. Parsons (1949)

J. Physiol. 110 36-46

Folin,O. and W. Dennis (1912)

J. biol. Chem. 11 87-95

Frame,E.G. (1958)

J. clin. Invest. 37 1710-1723

Fruton,J.S. and S. Simmonds (1958)

General Biochemistry. John Wiley. New York.

Gailey,F.B. and A.J. Johnson (1941)

J. biol. Chem. 141 921-929

Geiger,E. (1947)

J. Nutr. 34 98-111

Geiger,E., L.E. Human and M.J. Middleton (1958)

Proc. Soc. exp. Biol. Med. 97 232-234

Gelotte,B. (1964)

New Biochemical Separations. Ed. A.T. James and L.J. Morris
D. Van Nostrand. Toronto

Gibson,Q.H. and G. Wiseman (1951)

Biochem. J. 48 426

Gilligan,D.R., J.R. Moor and S. Warren (1952)

J. natn. Cancer Inst. 12 657-676

Gitler,C. and D. Martinez-Rojas (1964)

The Role of the Gastrointestinal Tract in Protein Metabolism.
Ed. H.N. Munro

Goldberg,A. and K. Guggenheim (1962)

Biochem. J. 83 129-135

Goldstein,A. (1964)

Biostatistics : An Introductory Text. The Macmillan Co. New York

Grassman,W., K. Hannig and M. Schleyer (1960)

Hoppe-Seylers Z. physiol Chemi. 322 71-95

Grimm,L. (1960)

Dissertation. University Munchen.

Guminski,T. and L.J. Naismith (1959)

Proc. nutr. Soc. 18 xxxv-xxxvi

Gupta,J.D., ... Lakroury and A.E. Harper (1958)

J. Nutr. 64 447-456

Hagihira,H., L.C.C. Lin and T.H. Wilson (1961)

Biochem. Biophys. Res. Commun. 4 478-480

Hamilton,P.B. and D.D. Van Slyke (1943)

J. biol. Chem. 150 231-250

Harding,J.J. (1965)

Adv. Protein Chem. 20 109-190

Harrington,W.F. and P.H. von Hippel (1961)

Adv. Protein Chem. 16 1-138

Harris,H. and F.L. Warren (1953)

Ann. Eugen. 18 125

Heinz,E., A.G. Pichler and B. Pfeiffer (1965)

Biochem. Z. 342 542-552

Hill,R.L. (1965)

Adv. Protein Chem. 20 37-107

Hober,R. and Hober,J. (1937)

J. cell. comp. Physiol. 10 401-422

Hokin,L.E. and Hokin,M.R. (1963)

Ann. Rev. Biochem. 32 553-578

Howe,P.E. (1921)

J. biol. Chem. 49 93

Hsia,D. Yi-Yung. (1964)

Diseases of Metabolism. Ed. G.G. Duncan. W.B. Saunders Co.
Philadelphia and London.

Huggins,A.K. and V. Moses (1961)

Nature. Lond. 191 668

Hunt,J.N. (1951)

J. Physiol. 113 169-184

Hunt,J.N. and W.R. Spurrel (1951)

J. Physiol. 113 157-168

Hyden,S. (1956)

K. LantbrHogsk. Annlr. 22 139

Ingram,V.M. (1958)

Biochim. biophys. acta. 28 539

Jequier,J-Cl., J.W.L. Robinson and J.P. Felber (1965)

Biochem. Biophys. Res. Commun. 18 507-511

Johansson,B. and L. Ryno (1962)

Acta chem scand. 16 2067-2068

Johnston,C.G. (1932)

Proc. Soc. exp. Biol. Med. 30 193-196

Jones,K. and J.G. Heathcote (1966)

J. Chromatog. 24 106-112

Josefsson,L and T. Lindberg (1966)

Acta physiol. scand. 66 410-418

Junqueira,L.C.U., G.C. Hirsch and H.A. Rothschild (1955)

Biochem. J. 61 275-278

Keller,P.J., E. Cohen and H. Neurath (1958)

J. biol. Chem. 233 344-349

King,T.P. and P.S. Norman (1962)

Biochemistry 1 709-720

Kinter, W.B. (1961)

in Wilson,T.H. Intestinal Absorption. (1962)

Knox,W.E. (1966)

Metabolic Basis of Inherited Disease. Ed. J.B. Stanbury, J.B. Wyngaarden and D.S. Fredrickson. McGraw-Hill New York.

Koshland,D.E.,Jr. (1960)

Adv. Enzymol. 22 45-97

Kreula,M.S. (1947)

Biochem. J. 41 269

Kuroda,Y. and N.S. Gimbel (1954)

J. appl. Physiol. 7 148-150

Leblond,C.P., C.E. Stevens and R. Bogoroch (1948)

Science 108 531-533

Leblond,C.P. and B.E. Walker (1956)

Physiol. Rev. 36 255

Lefevre,P.G. (1961)

Pharmacol. Rev. 13 39-70

Levenson,S.M., H. Rosen and H.L. Upjohn (1959)

Proc. Soc. exp. Biol. Med. 101 178-180

Levine,P.A. and D.D. Van Slyke (1912)

J. biol. Chem. 12 301

Lindberg,T. (1966)

Acta physiol. scand. 66 437-443

Linder,E. and R. Blomstrand (1958)

Proc. Soc. exp. Biol. 97 653-657

Littman,A. and J.B. Hammond (1965)

Gastroenterology 48 237-249

London,E.S. and N. Kotschneff (1934)

Hoppe-Seylers Z. physiol. Chem. 228 235-248

Maddrey,W.C., H.A. Serebro, H. Marcus and F.L. Iber (1967)

Gut 8 169

Marcus,C.S. and F.W. Lengemann (1962)

J. Nutr. 76 179-182

Martin,A.J.P. and R.L.M. Synge (1941a)

Biochem. J. 35 91-121

Martin,A.J.P. and R.L.M. Synge (1941b)

Biochem. J. 35 1358-1368

McCollum,E.V. (1956)

A History of Nutrition. Houghton Miffin Co. Boston.

Miller,T.G. and W.O. Abbot (1936)

Am. J. med. Sci. 187 595-599

Miller,J.M. and T.B. Wiper (1944)

Ann. Surg. 120 852

Milne,M.D., A. Asatoor and L.W. Loughridge (1961)

Lancet 1 51-52

Milne,M.D., A.M. Asatoor, K.D.G. Edwards and L.W. Loughridge (1961)

Gut 2 323

Mitchell,H.H. and R.J. Block (1946)

J. biol. Chem. 163 599-620

Moore,S. and W.H. Stein (1948)

J. biol. Chem. 176 367-387

Moore,S. and W.H. Stein (1954)

J. biol. Chem. 211 907-913; 915-926

Moore,S. and W.H. Stein (1956)

Adv. Protein Chem. 11 191

Moore,S. D.H. Spackman and W.H. Stein (1958)

Analyt. Chem. 30 1185

Morris,C.J.O.R. (1966)

Protides of the Biological Fluids. Ed. H. Peeters (1966)

Morris,E. (1956)

Q. J. exp. Physiol. 41 326

Nachlas,M.M., B. Monis, D. Rosenblatt and A.M. Seligman (1960)

J. Biophys. Biochem. Cyt. 7 261-264

Nakayama,K., T. Nakamura, K. Yamamoto and T. Tamiya (1960)

Gastroenterology 38 946-953

Nasset,E.S., P. Schwartz and H.V. Weiss (1955)

J. Nutr. 56 83-94

Nasset,E.S. and J.S. Du (1961)

J. Nutr. 74 461-465

Nasset,E.S. (1962)

J. Nutr. 76 131-134

Naughton,M.A., F. Sanger, B.S. Hartley and D.C. Shaw (1960)

Biochem. J. 77 149-163

Neame,K.D. and G. Wiseman (1957)

J. Physiol. 135 442-450

Neurath,H. (1960)

The Enzymes. Vol. 4 Ed. P.D. Boyer, H. Lardy and K. Myrback.
Academic Press. New York.

Newey,H. and D.H. Smyth (1959)

J. Physiol. 146 11P

Newey,H. and D.H. Smyth (1964)

Nature Lond. 202 400-401

Oosterbaan,R.A., P. Kunst, J. Van Rotterdam and J.A. Cohen (1958)

Biochim. biophys. acta 27 549-555

Orten,A. (1963)

Fed. Proc. 22 1103

Oxender,D.L. and H.N. Christensen (1963)

Nature Lond. 197 765-767

Pal, P.R. (1959)

Fed. Proc. 18 298

Palay,S.L. and L.J. Karlin (1959)

J. biophys. biochem. Cytol. 5 373-384

Partridge,S.M. and R.G. Westall (1949)

Biochem. J. 44 418-428

Pelot,D. and M.L. Grossman (1962)

Am. J. Physiol. 202 285-288

Peeters,H. (1966)

Ed. Protides of the Biological Fluids. Elsevier. Amsterdam

Perlman,G.E. (1954)

Nature Lond. 173 406

Peterson,E.A. and H.A. Sober (1956)

J. Am. chem. Soc. 78 751-755

Petrovic,S.M. and S.E. Petrovic (1966)

J. Chromatog. 21 313-317

Pierce,H.B., E.S. Nasset and J.R. Murlin (1935)

J. biol. Chem. 108 239-250

Pinsky,J. and E. Geiger (1952)

Proc. Soc. exp. Biol. Med. 81 55-56

Polonovski,M. and R. Bourillon (1952)

Bull. Soc. chim. Biol. 34 703-711

Popper,H. and F. Schaffner (1957)

Liver : Structure and Function. McGraw-Hill Book Co. Inc. New York.

Porath,J. and P. Flodin (1959)

Nature Lond. 183 1657-1659

Prockop,L.J., H.R. Keiser and A. Sjoerdsma (1962)

Lancet 2 527-528

Reynell,P.C. and G.H. Spray (1956)

J. Physiol. 131 452-462

Richmond,V., R. Caputto and S. Wolf (1955)

Gastroenterology 29 1017

Richmond,J. and R.H. Girdwood (1962)

Clin. Sci. 22 303-314

Robscheit-Robbins,F.S. and G.H. Whipple (1937)

J. exp. Med. 66 565-578

Robson,E.B. and G.A. Rose (1957)

Clin. Sci. 16 75-93

Rogers,Q.R., M.L. Chen, C. Peraino and A.E. Harper (1960)

J. Nutr. 72 331-339

Rønnov-Jessen,V. and J. Hansen (1965)

Blood 25 224-230

Rose,W.C., M.J. Oesterling and M. Womack (1948)

J. biol. Chem. 176 753-762

Rosenberg,L.E., S. Downing, J.L. Durant and S. Segal (1966)

J. clin. Invest. 45 365-371

Rosenberg,T. (1948)

Acta chem. scand. 2 14033

Rosenthal,S. and E.S. Nasset (1958)

J. Nutr. 66 91-103

Ruhemann,S. (1911)

J. chem. Soc. 99 792 ; 1306 ; 1486

Ryle,A.P. (1964)

The Role of the Gastrointestinal Tract in Protein Metabolism.
Ed. H.N. Munro

Sanders,E. and C.T. Ashworth (1961)

Exp. cell Res. 22 137-145

Sanger,F. (1949)

Biochem. J. 44 126-128

Sanger,F. (1952)

Adv. Protein Chem. 7 1-67

Schedl,H.P. and J.A. Clifton (1963)

Nature Lond. 199 1264-1267

Schedl,H.P. (1966)

Gut 7 159-163

Schultz,S.G., P.F. Curran, R.A. CHez and R.E. Fuisz (1967)

J. Gen. Physiol. 50 1241-1260

Schwert,G.W., H. Neurath,S. Kaufman and J.E. Snoke (1948)

J. biol. Chem. 172 221

Shaffer,C.B. and F.H. Critchfield (1947)

J. Am. Pharm. Ass. 36 152-157

Sherlock,S. and V. Walshe (1946)

Clin. Sci. 6 113-121

Sjovall,J. (1959)

Acta physiol. scand. 46 339-345

Smith,I. (1960)

Chromatographic and Electrophoretic Techniques.
William Heinemann. Medical Books. Ltd. London

Smith,R.H. (1962)

Biochem. J. 83 151-163

Smithies,O. (1955)

Biochem. J. 61 629-641

Smyth,D.H. (1961)

Meth. med. Res. 9 260-272

Snook,J.T. and J.H. Meyer (1964)

J. Nutr. 82 409-414

Snook,J.T. and J.H. Meyer (1964)

J. Nutr. 83 94-102

Soergel,K.H. and W.J. Hogan (1967)

Gastroenterology 52 1056

Spackman,D.H., W.H. Stein and S. Moore (1958)

Analyt. Chem. 30 1190

Sperber,I., S. Hyden and N.J. Ökman (1953)

Ann. Agr. Coll. Sweden 20 337

Spallanzani (1770)

Text Book of Physiology and Biochemistry. Bell,G.H., J.N. Davidson and H. Scarborough. E. and S. Livingston. Edinburgh

Stein,W.H. (1953)

J. biol. Chem. 201 45

Stein,W.H. and S. Moore (1954)

J. biol. Chem. 211 915

Stein,W.D. (1958)

Nature Lond. 181 1662-1663

Thier,S.C., S. Segal, M. Fox, A. Blair and L.E. Rosenberg (1965)

J. clin. Invest. 44 442

Thiry,L. (1864)

Natur. KI. 1 50 77-96

Town,B.W. (1941)

Biochem. J. 35 417

Turner,N.A. and R.J. Redgewell (1966)

J. Chromatog. 21 129-132

Twombley,J. and J.H. Meyer (1961)

J. Nutr. 74 453-460

Ugolev,A.M., N.N. Jesuitova, N.M. Timofeeva and I.N. Fediushina (1964)
Nature Lond. 202 807-809

Van Slyke,D.D. and G.M. Meyer (1912)
J. biol. Chem. 12 399-410

Van Slyke,D.D., R.T. Dillon, D.A. McFadyen and P. Hamilton (1941)
J. biol. Chem. 141 627-669

Vella,L. (1888)
Untersuch. Naturl. Mensch u Thiere 13 40-74

de Verdier,C-H. and G. Agren (1948)
Acta chem. scand. 2 783-796

Walzer,M. (1926)
J. Immunol. 11 249-252

Walzer,M. and M. Brunner (1928)
Arch.intern. Med. 42 172-179

Weinstein,B. (1966)
Meth. biochem. Anal. 14 203-293

Wiggans,D.S. and J.M. Johnston (1959)
Biochim. biophys. acta 32 69-73

Wilson,T.H. and G. Wiseman (1954)
J. Physiol. 123 116-125

Wilson,T.H. (1962)
Intestinal Absorption. W.B. Saunders and Co. Philadelphia and London.

Wiseman,G. (1953)
J. Physiol. 120 63-72

Wiseman,G. (1955)
J. Physiol. 127 414-422

Yeh,H.L., W. Frankl, M.S. Dunn, P. Parker, B. Hughes and P. György (1947)

Am. J. med. Sci. 214 507-512

Young,J.A. and K.D.G. Edwards (1964)

J. Pharmacol. exptl. Therap. 145 102

Young,J.A. and K.D.G. Edwards (1966)

Am. J. Physiol. 210 1130-1136

Zimmerman-Nielson,C. and F. Schønhyder (1962)

Biochim. biophys. acta 63 201-203