An investigation into protein digestion with ^{14}C-labelled protein

1. The general pattern of ^{14}C incorporation in body tissues and fluids of the rat up to 3 h after feeding*

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Although a large amount of work on the utilization of food proteins has been done in the last 50 years there is still a considerable degree of uncertainty about several important aspects of the problem. Thus, Fisher (1954) states that the evidence that the products of the digestion of proteins are amino acids is incomplete, that there is little evidence of the essentiality of peptic digestion and little certainty of the occurrence of 'ereptic' digestion, but that tryptic digestion seems to determine the nature of the end-products. He suggests that there is enough evidence to consider seriously the possibility that peptides are the normal currency of protein metabolism and points out that the question of the form in which protein is absorbed from the intestine is fundamental to the whole problem of the intermediary metabolism of protein. The form in which dietary nitrogen is absorbed from the lumen of the intestine is not necessarily that in which it appears in the tissue fluids of the body, for the products of the katabolism of intestinal protein constitute a large part of the substances passing into the body fluids during protein absorption. These katabolic products may be amino acids, but in Fisher's (1954) opinion are more likely to be specific peptides.

Many investigations have been carried out with labelled amino acids, but if amino acids are not the digestion products of dietary proteins the results of these investigations cannot throw any light on the problem of protein digestion. In a few investigations ^{15}N-labelled protein was given to man (White & Parson, 1950; Sharp, Lassen, Shankman, Gebhardt & Hazlet, 1956; Sharp, Lassen, Shankman, Hazlet & Kendis, 1957; Crane & Neuberger, 1960) but the use of human subjects severely restricts the scope of the experiments.

The giving of ^{14}C-labelled protein to rats appeared to offer a convenient and suitable means of obtaining a picture of the assimilation of dietary proteins, and the work to be described was undertaken with this end in view.

* This work formed part of a thesis submitted by one of us (R.D.) to the University of London in partial fulfilment of the requirements for the degree of Ph.D.

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EXPERIMENTAL

^{14}C-labelled algal protein

Uniformly labelled *Chlorella* protein of high specific activity (about 0·1 mc/mg) was obtained from The Radiochemical Centre, Amersham, Bucks. This material was free from fat and carbohydrate, the only likely ^{14}C-labelled contaminant being nucleic acid, much of which would be left behind when the protein was dissolved in phenol (see below) (cf. Kirby, 1956). The labelled protein was diluted with unlabelled *Chlorella* protein, obtained by extracting freeze-dried *Chlorella* cells with 90% (w/v) phenol at 70°. ^{14}C-labelled *Chlorella* protein (1 mg) was dissolved in 10 ml of 90% phenol containing one drop of 30% (w/v) KOH by warming to 70°, and 170 mg of unlabelled *Chlorella* protein were dissolved in 30 ml of the phenol solution. Both solutions were centrifuged to remove insoluble material, combined, and poured into 10 vol. of acetone to reprecipitate the protein. Storage in a refrigerator overnight ensured maximum recovery. The protein was then removed by centrifuging, washed twice with 20 ml acetone, twice with ethanol and twice with diethyl ether. It was then dried in a vacuum desiccator over P_2O_5. The yield was 170 mg and only a small amount ($< 0{\cdot}5\%$) of the ^{14}C activity remained in the supernatant solution.

Experimental animals

Both male and female hooded Norwegian rats of body-weight 250–300 g were used throughout, and until required they were maintained on the Institute stock-colony diet (McKinlay, 1951). After having been starved for 18 h each animal was force-fed with 35 mg of the diluted labelled protein mixed with 0·5 g glucose. Two to three drops of water were added to the mixture and the paste was placed with a spatula in small portions at a time on the back of the animal's tongue. The rats were accustomed to this treatment for 2–3 days before each experiment.

Tissues and fractions examined for ^{14}C incorporation

At 1, 2 and 3 h after administration of the ^{14}C-labelled protein the ^{14}C activity was determined in various tissue fractions: (1) the contents of the stomach and small intestine, (2) the subcellular fractions of the intestinal mucosa, (3) the subcellular fractions of the liver, (4) the protein and amino acid fractions of systemic blood and, in the 3 h experiments, the protein and amino acid fractions of portal blood, (5) stomach, caecum, heart, body musculature, urine and expired CO_2.

Removal of organs, tissues and body fluids

The animals were anaesthetized with diethyl ether; the abdomen was opened by an incision along the mid-ventral line and the flaps of skin and musculature were folded back to expose the viscera. The portal vein was ligatured and blood was drawn into a heparinized syringe and transferred immediately to a test-tube kept in an ice-bath. Systemic blood was then drawn from the heart into a heparinized syringe and transferred immediately to a test-tube kept in an ice-bath. Cannulas were then inserted into the small intestine at the pylorus and just anterior to the caecum, and the intestinal contents were washed into a beaker by passing 250 ml of isotonic NaCl solution through the intestine.

The intestinal contents were stored at 0–4°. The small intestine was then removed and placed immediately in 0·25 M-sucrose solution at 0°. The liver was removed, quickly washed under the tap, blotted on filter-paper, weighed and placed in 0·25 M-sucrose solution at 0°. The stomach was removed and stored at 0–4°. The heart, a portion of thigh muscle and the caecum were removed and stored at −10°.

When the urine and the expired CO_2 were to be collected, the animal was placed, immediately after feeding, in a metabolism jar and a stream of air was drawn through the jar. The air from the jar was drawn through a set of CO_2 traps containing 2 N-NaOH solution. In the bottom of the jar was a raised wire-mesh screen, beneath which was placed a sheet of filter-paper. Urine voided between feeding and anaesthetizing was collected on the filter-paper.

Fractionation of blood and tissue samples

Blood. The blood samples were separated into plasma and cells by centrifuging at 1500 ***g*** for 15 min at 4° in a refrigerated centrifuge (M.S.E. Ltd, London). The plasma was deproteinized by the addition of one-third of its volume of 40% (w/v) trichloroacetic acid and the plasma proteins were separated from the protein-free supernatant solution by centrifuging.

The blood cells were laked with 2 vol. water, the proteins precipitated by the addition of one-third of the volume of 40% (w/v) trichloroacetic acid and the proteins removed by centrifuging.

Removal of intestinal mucosa cells. The small intestine was cut into about 2 in. lengths. Each section was cut along the line of attachment to the mesentery and laid out flat with the mucosal side uppermost on the lid of a Petri dish packed with crushed ice. The mucosal cells were removed by gentle scraping with a spatula and were transferred immediately to ice-cold 0·25 M-sucrose solution.

Preparation of liver mince. The liver was minced in an ice-cold metal meat mincer and the mince was collected in an ice-cold beaker.

Preparation of homogenates of liver and intestinal mucosa. The liver mince was stirred with 5 vol., and the mucosal-cell suspension with 10–15 vol., of ice-cold 0·25 M-sucrose solution. The suspension was then homogenized in a Potter–Elvehjem-type homogenizer having a loosely fitting plunger made of nylon. The plunger was driven by an electric motor and the suspension was homogenized for exactly 1 min. On each downstroke the barrel dipped into a flask of alcohol containing solid CO_2 in order to chill the homogenate.

Treatment of homogenates. The liver and mucosal-cell homogenates were separated into the various cell components by differential centrifuging in a Spinco Model L ultracentrifuge (Beckman Instruments Inc., Belmont, California) by the method of Hogeboom & Schneider (1955), and the fractions obtained were: (1) cell debris (cell walls, nuclei, intact cells), (2) mitochondria, (3) microsomes, and (4) particle-free cell supernatant solution.

The proteins were obtained from the mitochondrial and microsomal fractions by suspending them in 100 vol. 10% (w/v) trichloroacetic acid and centrifuging. The supernatant solutions were discarded.

The nitrogen compounds of the particle-free cell supernatant solution were fractionated into cell supernatant protein and cell supernatant non-protein nitrogen fractions by addition of one-third of its volume of 40% (w/v) trichloroacetic acid and centrifuging.

Contents of stomach and intestine. The stomach contents were removed by washing out the stomach several times with isotonic NaCl solution. The stomach and intestinal contents were separated into protein and non-protein nitrogen fractions by addition of one-third of their volumes of 40% (w/v) trichloroacetic acid and centrifuging.

Stomach, caecum, heart, thigh muscle and eviscerated carcass. These organs or tissues were homogenized in the smallest amount of water, and protein was precipitated from the homogenate by addition of one-third of its volume of 40% (w/v) trichloroacetic acid.

Expired CO_2. Ammonium chloride (10 g/100 ml) was added to the NaOH solution from the CO_2 traps and the CO_2 was precipitated as $BaCO_3$ by addition of an excess of M-$BaCl_2$ solution.

Urine. The urine was eluted with water from the filter-paper on which it had been collected.

Preparation of protein fractions for estimation of ^{14}C activity. All the fractions obtained from the fractionations just described were washed once with 10% (w/v) trichloroacetic acid containing 1% hydrolysed casein in order to dilute any adherent radioactive amino acids, twice with 10% (w/v) trichloroacetic acid, twice with ethanol, once with 1:1 (v/v) ethanol–diethyl ether mixture and twice with diethyl ether. They were then dried in a vacuum desiccator over P_2O_5. The protein fractions so obtained included also some nucleic acid, although washing the trichloroacetic acid precipitate with ethanol would remove much of the ribonucleic acids (Venkataraman & Lowe, 1959). It seemed reasonable to assume that the rate of incorporation of ^{14}C-labelled amino acids into nucleic acids would not be greater than that into protein, so that the presence of small amounts of nucleic acids in the fraction designated protein would tend to lower, rather than raise, the measured specific activity of the protein fraction.

Desalting of amino acid fractions. The non-protein nitrogen fractions obtained from the fractionations were desalted on a 15 cm column of cation exchange resin (Zeo-Karb 225, Permutit Co. Ltd) essentially by the method of Boulanger & Biserte (1951). The NH_4OH eluate containing the non-protein nitrogen compounds was freed from NH_3 by distillation under reduced pressure. The total amount of amino nitrogen material in each non-protein nitrogen fraction was determined by the ninhydrin technique of Moore & Stein (1954), and the results were expressed in terms of 'leucine equivalents'.

^{14}C activity of fractions. The ^{14}C activity of the protein fractions was determined by counting replicate samples of each fraction at infinite thickness under an end-window counter, and the ^{14}C activity of the non-protein nitrogen fractions was determined by counting at infinite thinness. For direct comparison of the specific activities of the protein and non-protein nitrogen fractions, a diluted sample of the labelled protein used for feeding was counted at both infinite thickness and infinite thinness, and the

factor relating the two methods of counting was determined. The results have been corrected for method of counting and are expressed as specific activities.

Chromatography of amino acid fractions. The various non-protein nitrogen fractions were examined by one- and two-dimensional paper chromatography. The solvent systems used for the one-dimensional chromatography were: (1) n-propanol/acetic acid/water (150:10:40, v/v), (2) s-butanol/triethylamine/water (150:2:48, v/v), (3) n-butanol/acetic acid/water (100:20:80, v/v). In the two-dimensional chromatography the solvent systems used were either n-butanol/acetic acid/water (100:20:80, v/v) and s-butanol/triethylamine/water (150:2:48, v/v) or n-butanol/acetic acid/water (100:20:80, v/v) and water-saturated phenol.

On completion of chromatography the chromatograms were dried in a hot-air oven at 100° for 30 min, sprayed with 0·2% (w/v) ninhydrin in acetone, and the colour was developed by heating the sprayed sheets at 90–100° for 30 min.

The non-protein nitrogen fraction of the intestinal contents, the cell supernatant non-protein nitrogen fraction of the intestinal mucosa, and the plasma non-protein nitrogen fractions of the portal and systemic blood were examined by two-dimensional chromatography both before and after hydrolysis of the fractions. The hydrolysis was carried out in 6N-HCl in a sealed tube at 110° for 18 h.

Autoradiography of amino acid fractions. The chromatograms of the non-protein nitrogen fractions were run in duplicate. One of the chromatograms was stained with ninhydrin as described above; the other was placed in contact with a sheet of Kodirex X-ray film (Kodak Ltd) and left for 8–12 weeks. The X-ray film was then developed and the chromatogram was stained with ninhydrin. Any darkened zones on the X-ray film were compared with the ninhydrin-positive spots on the chromatogram.

^{14}C activity of portal-plasma amino acids. Owing to the small amounts of material present in the non-protein nitrogen fraction of the plasma, it was not possible to obtain an autoradiograph of this fraction, so the ^{14}C activity of its components was detected in the following way. A two-dimensional chromatogram of the non-protein nitrogen fraction of portal plasma was lightly sprayed with ninhydrin, the ninhydrin-positive spots were cut out and the material was eluted from each spot with 50% ethanol. The eluate was taken to dryness, made to a volume of 1 ml with distilled water, and the amount of material in each eluate was determined by the ninhydrin technique of Moore & Stein (1954). The solution was then transferred to a nickel planchet, evaporated to dryness under an infrared lamp, and the ^{14}C activity was measured under an end-window counter.

RESULTS

Contents of the gastro-intestinal tract

The total ^{14}C activity remaining in the gastro-intestinal tract 1, 2 and 3 h after feeding with ^{14}C-labelled protein is shown in Table 1. The total activity of the fraction was obtained by multiplying the specific activity of the fraction (counts/min mg cm^2) by the total amount of material in the fraction. The total ^{14}C activity administered to each animal, calculated from the activity of a diluted sample of the ^{14}C-labelled protein

measured at infinite thickness under an end-window counter, was 2×10^6 counts/min cm^2. From the results given in Table 1 it is evident that 1 h after feeding about 35% of the labelled protein had been absorbed, and after 2 and 3 h about 50 and 60% respectively had been absorbed. It would appear from these results that the maximum rate of digestive activity occurs within the 1st hour after feeding, and that it slowly diminishes thereafter. About 3% of the ^{14}C activity administered was found in the caecum 2 and 3 h after feeding.

Table 1. *Total ^{14}C activity (counts/min cm^2) remaining in the contents of the gastro-intestinal tract of individual rats 1, 2 and 3 h after they had received by mouth 35 mg ^{14}C-labelled* Chlorella *protein (8 μc of ^{14}C activity)**

	Time (h)			
			3	
Fraction	1	2		
Stomach contents: protein	1185400	598000	494500	490000
non-protein nitrogen	75000	43000	66250	92000
Intestinal contents: protein	10650	200800	60480	18200
non-protein nitrogen	15050	300	30000	15000
Caecal contents: protein	1770	64000	36900	71300
Faeces: protein	50	—	550	—
Total	1287920	906100	688680	686500

* The total activity of the proteins given to each animal was 2×10^6 counts/min cm^2.

Table 2. *Specific activities (counts/min mg cm^2) of fractions from the heart and skeletal muscle of individual rats 1 and 2 h after they had received by mouth 35 mg ^{14}C-labelled* Chlorella *protein (8 μc of ^{14}C activity)**

	Time (h)	
Fraction	1	2
Heart muscle: protein	1·5	5·1
Skeletal muscle: protein	1·2	3·3
non-protein nitrogen	30	54

* The specific activity of the protein given to each animal was 571000 counts/min mg cm^2.

Table 3. *Specific activities (counts/min mg cm^2) and total activities (counts/min cm^2) of the eviscerated carcass, urine and $BaCO_3$ containing the expired CO_2 of individual rats 1 and 3 h after they had received by mouth 35 mg ^{14}C-labelled* Chlorella *protein (8 μc of ^{14}C activity)**

	Time (h)			
	1		3	
Fraction	Specific activity	Total activity	Specific activity	Total activity
Carcass protein	1·07	59000	1·85	170000
Urine	8	80	28	10000
Expired CO_2	2·24	18500	7	120000

* The specific activity of the protein given to each animal was 571000 counts/min mg cm^2 and the total activity 2×10^6 counts/min cm^2.

Heart and body musculature

Very little ^{14}C activity had been incorporated into the proteins of the heart and body musculature up to 2 h after feeding (Table 2). Because of their low activity these tissues were not examined in the 3 h experiments.

Carcass, urine and expired CO_2

The specific activities and total ^{14}C activities of the eviscerated carcass, urine and expired CO_2 (as $BaCO_3$) are shown in Table 3. All the specific activities were very low. However, the total weight of material in the carcass and CO_2 fractions was large, about 25 and 7 g, respectively, so that when the specific activity was multiplied by the weight of the fraction to give the total activity it appeared that a considerable amount of ^{14}C activity had been incorporated into the fraction. (Most of the $BaCO_3$ was derived from atmospheric CO_2 drawn through the apparatus to flush it out.) The accurate measurement of activities only a few counts/min above the background was not possible with our equipment and it must be emphasized that the inherent error in counting such low activity is greatly magnified when the specific activity is multiplied by the weight of the fraction. Thus the figures for the total activities are probably only a rough approximation to the total activity incorporated in the carcass or lost during respiration.

Intestinal mucosa and liver

The trend in the incorporation of ^{14}C activity into the subcellular fractions was similar in the intestinal mucosa and the liver (Tables 4 and 5). In both, the fraction having the highest specific activity was the non-protein nitrogen fraction of the cell supernatant solution. Of the protein fractions, that of the microsomes had the highest specific activity. In all instances the fractions from the intestinal mucosa had considerably higher specific activities than those from the liver. However, when the total ^{14}C activities of the whole fractions are considered, the mucosal fraction in which the

Table 4. *Specific activities (counts/min mg cm²) of fractions from the intestinal mucosa and the liver of individual rats* 1, 2 *and* 3 *h after they had received by mouth* 35 *mg* ^{14}C*-labelled* Chlorella *protein* (8 *μc of* ^{14}C *activity*)*

Tissue	Fraction	Time (h)			
		1	2	3	
Intestinal mucosa	Mitochondrial protein	12	63	79	54
	Microsomal protein	22	98	98	64
	Cell supernatant protein	14	55	67	50
	Cell supernatant non-protein nitrogen	110	390	200	—
Liver	Mitochondrial protein	0·7	9	12	10
	Microsomal protein	8·5	32	46	20
	Cell supernatant protein	2·1	15	21	11
	Cell supernatant non-protein nitrogen	50	205	60	50

* The specific activity of the protein given to each animal was 571 000 counts/min mg cm².

Table 5. *Total activities (counts/min cm²) of fractions from the intestinal mucosa and the liver of individual rats 1, 2 and 3 h after they had received by mouth 35 mg ^{14}C-labelled* Chlorella *protein (8 μc of ^{14}C activity)**

Tissue	Fraction	Time (h) 1	2	3
Intestinal mucosa	Cell debris (nuclei, cell walls)	2700	7500	12500
	Mitochondrial protein	500	7000	11300
	Microsomal protein	4500	12500	21000
	Cell supernatant protein	9000	30000	28000
	Cell supernatant non-protein nitrogen	1100	8500	3000
Liver	Cell debris (nuclei, cell walls)	1300	5000	19300
	Mitochondrial protein	199	15000	9500
	Microsomal protein	3300	35000	37700
	Cell supernatant protein	1900	25000	25700
	Cell supernatant non-protein nitrogen	600	4500	9300

* The total activity of the protein given to each animal was 2×10^6 counts/min cm².

greatest amount of ^{14}C was incorporated was the cell supernatant protein fraction. In the liver, the fraction showing the greatest total ^{14}C activity was the microsomal protein fraction.

Blood

In the 1 and 2 h experiments systemic blood samples only were taken, since the main object in the earlier part of the investigation was to establish the general pattern of ^{14}C incorporation. From these earlier experiments it appeared that the optimum time at which to study the general pattern of incorporation was 3 h after feeding, and once this had been established a comparison of the distribution of ^{14}C activity in the various fractions of portal and systemic blood was made. In a series of experiments described by Dawson & Porter (1962) differences between the ^{14}C activity of fractions from the portal blood and those from the systemic blood from 0 to 6 h after feeding were investigated.

The specific activities of systemic blood fractions 1 and 2 h after feeding and of portal and systemic blood fractions 3 h after feeding are shown in Tables 6 and 7, from which it will be seen that only the non-protein nitrogen fractions showed appreciable ^{14}C activity up to 3 h after feeding. In several experiments carried out 3 h after feeding, the non-protein nitrogen fraction of portal plasma was found to have a specific activity 50–300% higher than that of the systemic plasma.

The total amounts of amino-nitrogen material present in the non-protein nitrogen fractions of blood, estimated by the ninhydrin technique (Moore & Stein, 1954), were: fasting blood 0·25, systemic blood (3 h after feeding) 0·43 and portal blood (3 h after feeding) 0·65 mg/ml. Thus the concentration of amino-nitrogen material in the portal blood 3 h after feeding was 50% higher than in the systemic blood.

Chromatography of amino acid fractions

After one-dimensional chromatography all the non-protein nitrogen fractions, except that from the intestinal contents, gave similar chromatographic patterns, and

Table 6. *Specific activities (counts/min mg cm²) of fractions from the systemic blood of individual rats* 1, 2 *and* 3 *h after they had received by mouth* 35 *mg* ^{14}C*-labelled* Chlorella *protein* (8 μc *of* ^{14}C *activity*)*

Fraction	Time (h) 1	2	3†	
Plasma proteins	3·2	15	28	20
Plasma non-protein nitrogen	80	523	180	93
Blood-cell proteins	—	—		0·74
Blood-cell non-protein nitrogen	60	190	110	63

* The specific activity of the protein given to each animal was 571 000 counts/min mg cm².
† Values for individual rats.

Table 7. *Specific activities (counts/min mg cm²) of fractions from portal and systemic blood of individual rats* 3 *h after they had received by mouth* 35 *mg* ^{14}C*-labelled* Chlorella *protein* (8 μc *of* ^{14}C *activity*)*

Blood	Fraction	Animal no.	Specific activity	Mean
Portal	Plasma proteins	1	15·5	21·25
		2	27	
	Plasma non-protein nitrogen	1	147	117
		2	123	
		3	132	
		4	66	
	Blood-cell proteins	2	1·5	1·4
		3	1·5	
		4	1·1	
	Blood-cell non-protein nitrogen	1	32	33
		2	42	
		3	48	
		4	6	
Systemic	Plasma proteins	1	20·4	28·2
		2	36	
	Plasma non-protein nitrogen	1	93	60
		2	87	
		3	47	
		4	18	
	Blood-cell proteins	2	0	1·1
		3	1·1	
		4	2·2	
	Blood-cell non-protein nitrogen	1	63	25
		2	6	
		3	30	
		4	0	

* The specific activity of the protein given to each animal was 571 000 counts/min mg cm².

for most of the ninhydrin-positive spots there were amino acid markers with the same R_F.

After the two-dimensional chromatography no difference could be observed between the non-protein nitrogen fractions of plasma from portal and systemic blood.

The non-protein nitrogen fractions of the intestinal contents, the cell supernatant fluid of the intestinal mucosa, and the portal plasma were examined by two-dimensional chromatography before and after hydrolysis. When the solvent systems s-butanol/triethylamine/water and n-butanol/acetic acid/water were used this fraction of the intestinal contents gave a very distorted picture that was difficult to interpret. The distortion was due to a yellow pigment which after chromatography extended over a considerable area of the chromatogram. The interfering material was not removed by desalting on a column of ZeoKarb 225 (Permutit Co., London). When the fraction was chromatographed in n-butanol/acetic acid/water and water-saturated phenol the distortion, though still present, was reduced sufficiently to permit identification of the amino acid spots. Of the three fractions examined by two-dimensional chromatography before and after hydrolysis, only the intestinal contents showed a marked change in chromatographic pattern after hydrolysis, thus indicating the presence of peptide material. Photographs of the chromatographic patterns of the intestinal contents before and after hydrolysis are shown in Pl. 1 *a*, *b*. Although equal amounts of material were applied to each chromatogram, the colours of the spots after staining with ninhydrin were much less intense on the chromatogram of the unhydrolysed extract than on the chromatogram of the hydrolysed extract, and, as it is well known that peptides give lower colour yields with ninhydrin than do amino acids, this observation clearly indicates the presence of peptides. The cell supernatant amino acid fraction of the intestinal mucosa and the portal plasma amino acid fraction showed only minor changes after hydrolysis, thus indicating that the fractions contained only minor amounts of material other than free amino acids. By the ninhydrin technique no trace of the peptide material shown to be present in the intestinal contents could be detected in either the cell supernatant fluid of the intestinal mucosa or the plasma of the portal blood. It would appear, therefore, that the peptide material present in the intestinal contents was hydrolysed to free amino acids before entering the cell supernatant fluid of the intestinal mucosa.

Autoradiography of amino acid fractions

On autoradiography all the ninhydrin-positive components in the non-protein nitrogen fractions of the intestinal contents, the cell supernatant fluid of the intestinal mucosa and the cell supernatant fluid of the liver were found to be radioactive. The amount of material present in the non-protein nitrogen fractions of the blood was apparently too small to give any positive results on autoradiography.

The various ninhydrin-positive spots on a two-dimensional chromatogram of the non-protein nitrogen fraction of portal plasma were cut out from the chromatogram and the material was eluted from each spot. The eluates were evaporated to dryness, and in every instance the residue was found to be radioactive.

DISCUSSION

In vitro studies by Frankel (1916) and Dunn & Lewis (1921) on the enzymic digestion of proteins in which the three digestive secretions, pepsin, trypsin and erepsin, were allowed to act in succession indicated that the time required for complete digestion was about 15 days. In an investigation of the time-course of the excretion of extra glucose and of extra nitrogen after the administration on separate occasions of glucose, protein and an acid hydrolysate of protein to phlorhizinized dogs Janney (1915) found that digestion was completed in about 9 h. A similar conclusion was reached by Kúthy (1930) who determined the rate of assimilation of raw meat by rats and found that the highest concentration of blood α-amino nitrogen occurred 3 h after the ingestion of the protein, at which time 50% of the protein had disappeared from the intestine. Gupta, Dakroury & Harper (1958) found that 2 h after giving rats soluble protein such as casein or beef, or an amino acid mixture, 60% of the protein nitrogen had disappeared from the stomach with little accumulation of nitrogenous material in the intestine. The findings in our investigation are in good agreement with those of the in vivo studies just mentioned. Thus the amount of dietary protein that had disappeared from the intestinal tract 1, 2 and 3 h after feeding, calculated from the amount of ^{14}C activity that had disappeared at these times, was 35, 50 and 60%, respectively. It is evident, therefore, that the absorption of dietary protein occurs very rapidly in the intact animal. The disappearance of ^{14}C activity, however, is no indication that the protein had been digested. Examination of the intestinal contents 3 h after feeding showed that a large part of the ^{14}C activity of the intestinal contents was associated with the trichloroacetic acid-soluble fraction, and chromatography suggested that most of the α-amino material was in the form of peptides. None of these peptides could be detected in the cell supernatant fluid of the intestinal mucosa cells, so there seems little doubt that they were hydrolysed either before entering the mucosal cells or very rapidly inside them. Autoradiographs showed that the peptides present in the intestinal contents and the amino acids present in the mucosal cells were radioactive, so it was reasonable to infer that the amino acids had been derived from the peptides. It seems probable, therefore, that the absorption products of dietary proteins are amino acids. In this connexion it is interesting to note that several workers (Clarke, Gibson, Smyth & Wiseman, 1951; Gibson & Wiseman, 1951; Agar, Hird & Sidhu, 1953; Wiseman, 1953; Matthews & Smyth, 1954; Wilson & Wiseman, 1954) have investigated the absorption of peptides by surviving isolated intestines. Of the small number of dipeptides investigated, most appeared to be rapidly hydrolysed in passing through the intestinal membranes, but small amounts of some were detected on the serosal side of the membrane. The behaviour of one tripeptide, glycylglycylglycine, was also investigated, and it was hydrolysed to the dipeptide glycylglycine and free glycine. No tripeptide, and only a small amount of the dipeptide, could be detected in the serosal fluid. However, it is still possible that other peptides might behave differently.

It is evident from the results given in Tables 4 and 5 that the intestinal mucosa is a very active site of protein synthesis. ^{14}C amino acids were rapidly incorporated into all

the subcellular components, especially the cell supernatant proteins and the microsomal proteins. The microsomes are known to be particularly concerned in protein synthesis, and it is possible that the protein in the cell supernatant fluid is synthesized in the microsomes. The high protein-synthesizing activity of the mucosa is possibly related to the short life of the mucosal cells which are sloughed off into the lumen of the intestine. This investigation has provided no evidence that the protein synthesized in the mucosal cells is transferred to the blood through either the portal vein or the thoracic lymph duct, as the specific activity of both the portal and systemic blood proteins showed only small increases during 3 h after feeding.

The only blood component that showed a sharp rise in ^{14}C activity after ^{14}C-labelled protein had been given was the plasma non-protein nitrogen fraction. Thus, 3 h after feeding, the specific activity of this fraction of blood was 50–300% higher than that of systemic blood and the concentration of non-protein nitrogen in portal plasma was 50% higher than that in the systemic plasma. On chromatography, no peptide could be detected in the plasma, and all the amino acids were shown to be radioactive. It seems likely, therefore, that dietary nitrogen is transported from the intestine in the form of plasma amino acids.

The differences between the specific activities and the concentrations of the portal plasma amino acids and the systemic plasma amino acids indicate that amino acids are removed from the blood by the liver. This finding is confirmed by the large amount of ^{14}C activity incorporated into the proteins of the subcellular components of the liver, showing that active protein synthesis occurs in the liver. It is known that the liver proteins are particularly labile, so that the protein synthesis in the liver may probably be concerned with the formation of a nitrogen store.

SUMMARY

1. ^{14}C-labelled algal protein was given by mouth to rats and, 1, 2 and 3 h after feeding, the distribution of ^{14}C activity was determined in (1) the gastro-intestinal tract, (2) the subcellular components of the intestinal mucosa and the liver, (3) the portal and systemic blood, (4) the urine, faeces, expired CO_2 and body musculature.

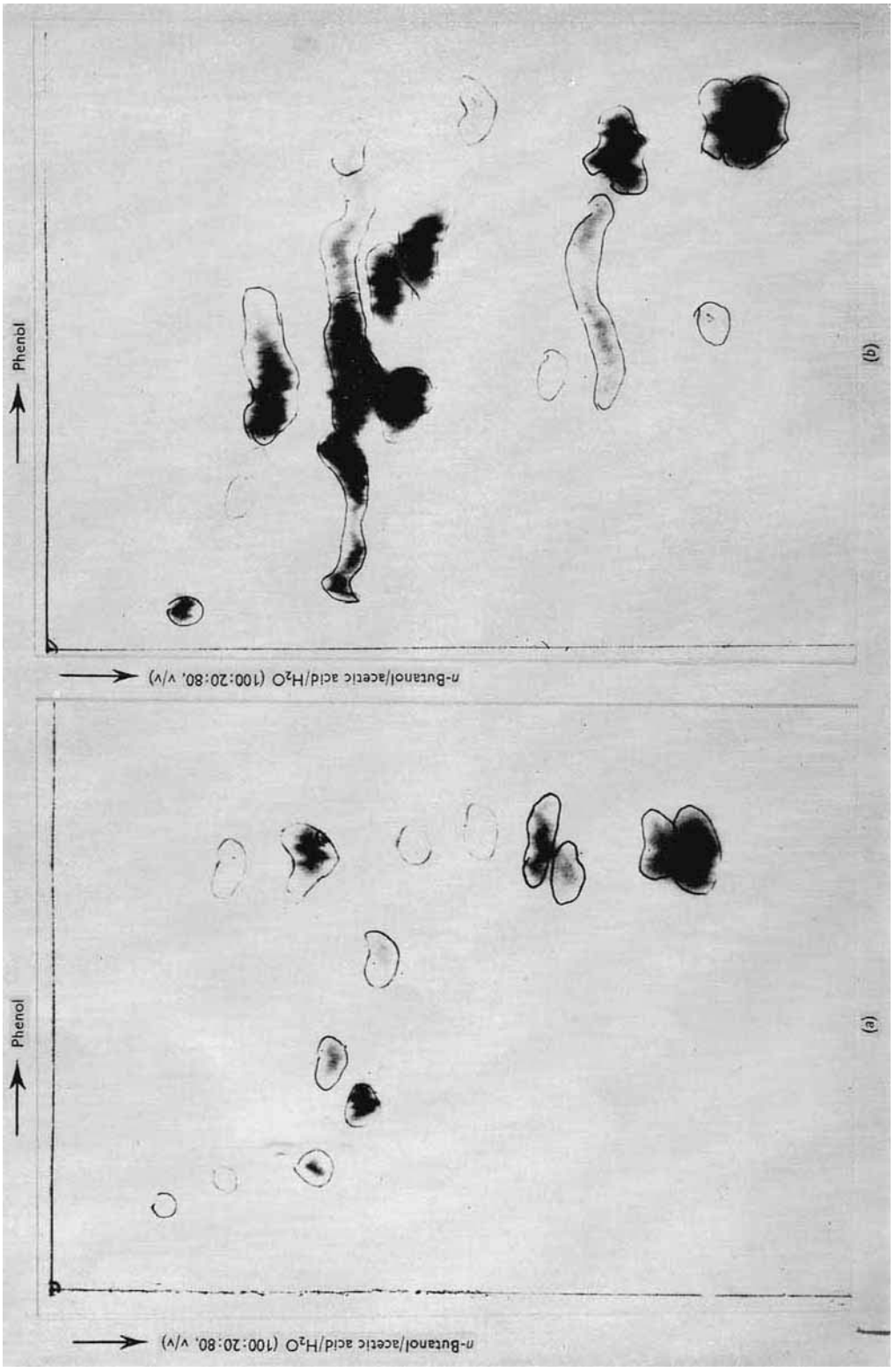
2. Chromatography and autoradiography showed the presence of radioactive peptides in the intestinal contents, but no trace of peptides was found in the intestinal mucosa or the blood.

3. The plasma amino acid fraction had the highest specific activity and the activity of portal plasma amino acids 3 h after feeding was 50–300% higher than that of systemic blood.

4. The concentration of plasma amino acids in the portal blood was 50% higher than in the systemic blood and 100% higher than in fasting blood.

5. Two of the tissues examined, the intestinal mucosa and the liver, were found to be very active in protein synthesis.

6. The significance of these findings is discussed.



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EXPLANATION OF PLATE

Two-dimensional chromatograms of (*a*) the unhydrolysed and (*b*) the hydrolysed trichloroacetic acid-soluble fraction of the intestinal contents of rats 1 h after a meal of ^{14}C-labelled *Chlorella* protein.

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