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

2.* The transport of ^{14}C-labelled nitrogenous compounds in the rat and cat†‡

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(*Received* 28 *March* 1961—*Revised* 14 *September* 1961)

Dawson & Holdsworth (1962) investigated the general pattern of ^{14}C incorporation into the body tissues and fluids of rats during the 3 h after they had received by mouth ^{14}C-labelled protein. They found the most marked increases in ^{14}C activity in the plasma amino acid fraction and appreciably more ^{14}C activity in portal than in systemic plasma. Although peptides were detected in the contents of the small intestine none were detected either in the cells of the intestinal mucosa or in the portal or systemic blood. From these findings it seemed probable that the digestion products of dietary proteins are amino acids and that dietary protein nitrogen is transported from the intestine in the form of plasma amino acids. In an extension of this work we have now examined the distribution of ^{14}C activity in the various blood fractions at intervals from 0 to 6 h after giving rats ^{14}C-labelled protein. In view of the high protein-synthesizing activity of the intestinal mucosa found by Dawson & Holdsworth (1962) and the consequent possibility of proteins or peptides of mucosal origin being transported from the intestine in the lymph, we also examined the distribution and amounts of ^{14}C activity in the nitrogenous fractions of mesenteric lymph. We were unable to obtain from rats samples of lymph sufficiently large for this purpose, but we were particularly fortunate in securing the help of Dr P. P. Scott of the Royal Free Hospital, London, who made available several cats from which she was able to obtain for us the required quantity.

EXPERIMENTAL

Animals

Rats. Male and female hooded Norwegian rats of body-weight 250–300 g that had been maintained on the stock diet (McKinlay, 1951) in the Institute colony were used for the major part of the investigation. At each of the time intervals studied, samples from three animals were obtained and pooled for examination.

Cats. Male and female cats of 2–4 kg body-weight were supplied and operated on by Dr Scott. They had been maintained on a mixed diet, consisting of raw ox heart

* Paper No. 1: *Brit. J. Nutr.* (1962), **16**, 13.

† 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.

‡ A preliminary report of this work was read at the 5th International Congress on Nutrition held in Washington, September 1960 (Dawson & Porter, 1960).

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supplemented with $CaCO_3$ at the rate of 0·5 g/100 g wet meat, cooked potatoes and Farex (Glaxo Laboratories Ltd), for several weeks before the experiment. This diet was low in vitamin A, but the cats were in good condition and showed no signs of vitamin A deficiency when used.

^{14}C-labelled protein

Uniformly labelled *Chlorella* protein of high specific activity (about 0·1 mc/mg) was obtained from the Radiochemical Centre, Amersham, Bucks.

The protein was diluted and given to the rats as described by Dawson & Holdsworth (1962), each animal receiving 8 μc of ^{14}C activity (7×10^6 counts/min cm^2 as measured at infinite thickness in a windowless flow counter (Model SC 16, Tracerlab Inc., Boston, Mass., U.S.A.)).

The cats were given the labelled protein (less diluted than for rats) mixed with 10 g minced ox heart and 2 g arachis oil; each received 200 μc of ^{14}C activity ($1{\cdot}7 \times 10^8$ counts/min cm^2).

Samples taken from rats

Portal and systemic blood fractions were obtained from each rat as described by Dawson & Holdsworth (1962). The gastro-intestinal tract was washed out with 250 ml isotonic NaCl solution at 37°. The samples from the first group were taken 30 min after feeding and from further groups at 1, 2, 3, 4, 5 and 6 h after feeding.

Samples taken from cats

Preliminary experiments with four cats showed that very little of the experimental meal given to the cats left the stomach during the first 2 h. In a subsequent experiment two cats were given the experimental meal and 3–4 h later were anaesthetized by an intraperitoneal injection of Nembutal (Abbott Laboratories Ltd). The abdomen was opened and lymph was drawn from the mesenteric lymph duct into a heparinized syringe. Up to 5 ml lymph could be obtained by gentle manipulation of the intestine. When these samples had been taken, samples of portal and systemic blood were obtained in the same way as from the rats. The gastro-intestinal tract was then ligated at the oesophagus, pylorus and the lower end of the large intestine. The stomach and intestines were removed separately, freed from connective tissue and stored at 0°.

Fractionation of blood and lymph samples

The blood samples were separated into plasma and cells by centrifuging at 1500 ***g*** for 15 min. A 0·5 ml portion of each of the pooled rat-plasma samples was taken for examination of the proteins by electrophoresis. The remainder of the plasma and the lymph were deproteinized by the addition of 40% (w/v) trichloroacetic acid; the proteins were removed by centrifuging, washed twice with 10% (w/v) trichloroacetic acid containing 1% hydrolysed casein in order to reduce by dilution any contamination with labelled amino acids, once with 10% (w/v) trichloroacetic acid, twice with ethanol, once with an ethanol–diethyl ether mixture (1:1, v/v) and finally twice with diethyl ether. They were then dried in a vacuum desiccator over P_2O_5, after which the ^{14}C activity was measured by counting at infinite thickness.

The blood cells were laked by the addition of 2 vol. water containing a drop of dilute NH_4OH solution. The proteins were precipitated from the laked cells by the addition of one-third volume of 40% (w/v) trichloroacetic acid and centrifuging. They were washed and dried as already described for the plasma proteins, and the ^{14}C activity was measured by counting the samples at infinite thickness.

The fractions of the blood and lymph soluble in trichloroacetic acid were desalted on $9 \times 0{\cdot}6$ cm columns of ZeoKarb 225 (Permutit Co. Ltd, London) and the total amount of α-amino nitrogen material in each fraction was estimated by the ninhydrin method of Moore & Stein (1954), as described by Dawson & Holdsworth (1962). Preliminary tests showed that recovery of ^{14}C activity was quantitative when deproteinized plasma was desalted in this way. The volume of each fraction calculated to contain 0·1 mg of α-amino nitrogen material was evaporated to dryness on a nickel planchet and the ^{14}C activity was measured by counting at infinite thinness.

Treatment of the samples from the gastro-intestinal tract

Rats. The washings containing the contents of the gastro-intestinal tract were evaporated to dryness on a steam-bath and the residue was ground in a mortar. The residue was weighed and six random samples were taken for measurement of ^{14}C activity at infinite thickness.

Cats. The stomach and its contents, and the intestine and its contents, were homogenized in an Atomix blender (M.S.E. Ltd, London) and the homogenates were freeze-dried. The dried materials were ground and six samples of each were assayed for ^{14}C activity by counting at infinite thickness.

Electrophoresis of plasma proteins

The proteins of the rat-plasma samples were separated by electrophoresis in 0·05 M-veronal buffer at pH 8·6 by the method of Kohn (1957). Duplicate cellulose acetate strips were used for each sample, and after electrophoresis one of the strips was stained with 0·2% Ponceau S in 3% (w/v) trichloroacetic acid. The other was placed in contact with Kodirex X-ray film (Kodak Ltd) and left for 12 weeks in order to determine the distribution of the ^{14}C activity amongst the various components of the plasma proteins.

Measurement of concentrations of amino acids in the plasma of portal and systemic blood

The concentrations of amino acids in the deproteinized plasma of fasting blood of rats, and in the portal and systemic blood during the 6 h after feeding, were determined by the ninhydrin technique of Moore & Stein (1954). The results were expressed as mg of leucine equivalents per ml of blood plasma.

RESULTS

Experiments with rats

Contents of the gastro-intestinal tract. The specific activity of the contents of the gastro-intestinal tract and the total activity remaining in the tract at intervals from 1 to 6 h after the rats had received ^{14}C-labelled protein are shown in Table 1. These values

Table 1. *Specific (counts/min mg cm²) and total (counts/min cm²) activities of the pooled contents of the gastro-intestinal tract of groups of three rats from 1 to 6 h after they had received by mouth 35 mg ¹⁴C-labelled* Chlorella *protein (8 μc of ¹⁴C activity)**

Time (h) ...	1	2	3	4	5	6
Specific activity	2280	1880	1096	1416	576	544
Total activity ($\times 10^{-6}$)	13·7	11·3	8·4	7·6	4·5	3·2

* Each animal received a total activity of 7×10^6 counts/min cm², so that each group received 21×10^6 counts/min cm².

for total activity were calculated by multiplying the specific activity, expressed as counts/min mg cm², by the total weight of the fraction in mg. The meal given to each animal had a total activity of 7×10^6 counts/min cm², so that each group of three rats received 21×10^6 counts/min cm². From the figures for total activity given in Table 1 it will be seen that the most marked fall occurred during the 1st hour after feeding, when about 30% of the activity given to the rats had disappeared from the digestive tract. After 2 and 3 h the values were about 50 and 60%, respectively.

Distribution of ¹⁴C activity in blood fractions. The specific activities of the various fractions from pooled portal and systemic blood of rats at intervals from 30 min to 6 h after the rats had received ¹⁴C-labelled protein are shown in Table 2.

Table 2. *Specific activities (counts/min mg cm²) of fractions from pooled portal and pooled systemic blood of groups of three rats from 30 min to 6 h after they had received by mouth 35 mg ¹⁴C-labelled* Chlorella *protein (8 μc of ¹⁴C activity)**

(Equal volumes of blood from the three rats in the group were pooled at each interval)

		Activity at interval after feeding of						
Blood	Fraction	0·5 h	1 h	2 h	3 h	4 h	5 h	6 h
Portal	Blood-cell proteins	12	15	19	13	19	21	22
	Plasma proteins	15	63	139	124	240	260	230
	Blood-cell amino acids	2190	2250	—	—	1150	1120	—
	Plasma amino acids	5000	5140	3390	3020	2250	2430	1560
Systemic	Blood-cell proteins	12	13	16	15	26	21	22
	Plasma proteins	16	60	155	124	230	262	214
	Blood-cell amino acids	1250	1560	—	—	1070	1450	—
	Plasma amino acids	2560	3400	3080	1740	1300	1840	1730

* The specific activity of the protein given to the animals was 2×10^5 counts/min mg cm².

Blood-cell proteins. Only a small amount of ¹⁴C activity was found in the blood-cell protein fraction and there was no significant difference between the portal and systemic blood.

Plasma proteins. The specific activity increased steadily during the first 2 h after feeding; between 2 and 3 h it appeared to decrease slightly, but between 3 and 4 h it doubled, and it attained a maximum value between 4 and 5 h. At 6 h it had fallen slightly. As with the blood-cell protein fraction, no significant difference could be detected between the plasma proteins of the portal and those of the systemic blood.

Plasma amino acids. The most marked changes in specific activity were in this fraction. From Table 2 it will be seen that the plasma amino acids had high specific activity within 30 min of feeding, and that the portal plasma amino acids attained maximum activity between 30 min and 1 h after feeding. Thereafter the activity of this fraction decreased except for a slight increase between 4 and 5 h. Maximum activity in the systemic plasma amino acids was attained between 1 and 2 h after feeding, which was somewhat later than in those of the portal plasma. Thereafter the trend was similar to that observed in the portal blood. Up to 5 h after feeding there was considerably more ^{14}C activity in the amino acids of the portal plasma than in those of the systemic plasma. Thus 30 min after feeding, the portal plasma amino acids had twice the specific activity of those of the systemic plasma, and after 5 h the difference was still about 30%.

Blood-cell amino acids. The differences in ^{14}C activity between the amino acid fractions from the portal and from the systemic blood cells were qualitatively similar to, but quantitatively smaller than, the differences between the plasma amino acid fractions.

Electrophoresis of plasma proteins. Pl. 1 shows the autoradiograph obtained when cellulose acetate strips, on which the plasma proteins had been separated by electrophoresis, were placed in contact with X-ray film. It is apparent that ^{14}C-labelled protein was present mainly in the albumin fraction in both portal and systemic plasma proteins 2 h after feeding (P_2 and S_2, respectively). The activity increased in all the components of the plasma proteins during the 6 h after feeding and at this time (P_6 and S_6) five bands of activity were evident, corresponding in position to the five bands obtained on staining the duplicate strip. No qualitative or quantitative differences in the distribution of ^{14}C activity in the portal and systemic plasma proteins were apparent.

Table 3. *Concentration (mg/ml) of plasma amino acids in portal and systemic blood of rats from* 30 *min to* 6 *h and in fasting blood* 18 *h after they had received by mouth* 35 *mg* ^{14}C*-labelled* Chlorella *protein*

Time (h) ...	0·5	1	2	3	4	6
Portal blood	0·50	0·50	0·55	0·65	0·30	0·54
Systemic blood	0·36	0·35	0·45	0·43	0·29	0·42
Fasting blood (18 h)	0·25					

Concentration of plasma amino acids. The concentration of plasma amino acids in the blood of fasting rats, and in the portal and systemic blood at intervals during the 6 h after feeding, are shown in Table 3. It will be seen that 30 min after feeding the concentration of plasma amino acids in systemic blood was 50%, and in portal blood 100%, higher than in fasting blood. The highest concentration in both portal and systemic blood was reached 2–3 h after feeding although maximum ^{14}C activity was attained much earlier (see Table 2). It would appear, therefore, that free amino acids are liberated from dietary protein and are absorbed into the blood very rapidly after ingestion.

Experiments with cats

^{14}C activity remaining in gastro-intestinal tract. The specific and total activities of the freeze-dried homogenates of the stomachs and their contents and of the intestinal tracts and their contents, removed 3–4 h after the cats had been given ^{14}C-labelled protein, are shown in Table 4. The total ^{14}C activity given to each animal was $1{\cdot}7 \times 10^8$ counts/min cm^2, so from the values in Table 4 it will be seen that about half of the activity had disappeared from the gastro-intestinal tract.

Table 4. *^{14}C activity remaining in the gastro-intestinal tract of cats 3–4 h after they had received by mouth 500 mg ^{14}C-labelled* Chlorella *protein (200 μc of ^{14}C activity)**

	Specific activity (counts/min mg cm^2)		Total activity (counts/min cm^2)		
Cat no.	Stomach	Intestine	Stomach	Intestine	Gastro-intestinal tract
1	9600	522	$67{\cdot}0 \times 10^6$	$25{\cdot}6 \times 10^6$	$92{\cdot}6 \times 10^6$
2	2900	775	$35{\cdot}6 \times 10^6$	$42{\cdot}7 \times 10^6$	$78{\cdot}3 \times 10^6$

* The total activity given to each animal was 170×10^6 counts/min cm^2.

Table 5. *^{14}C activity in blood and lymph fractions from cats 3–4 h after they had received by mouth 500 mg ^{14}C-labelled* Chlorella *protein (200 μc of ^{14}C activity),* and concentration of amino acids in plasma and lymph*

			Amino acids	
Cat no.	Fraction	Specific activity (counts/min mg cm^2)	Concentration (mg/ml)	Total activity/ml (counts/min cm^2)
1	Portal: plasma protein	139	—	—
	plasma amino acid	2070	0·78	1618
	Systemic: plasma protein	135	—	—
	plasma amino acid	2160	0·47	1012
	Lymph: protein	166	—	—
	amino acid	360	1·90	685
2	Portal: plasma protein	198	—	—
	plasma amino acid	2560	0·61	1570
	Systemic: plasma protein	198	—	—
	plasma amino acid	3240	0·26	840
	Lymph: protein	337	—	—
	amino acid	1710	0·76	1300

* The total activity given to each animal was 170×10^6 counts/min cm^2.

^{14}C activity of blood and lymph fractions. The specific activities of the protein and amino acid fractions of the blood and lymph are shown in Table 5. In each comparison the plasma proteins of the portal blood had the same specific activity as those of the systemic blood, in agreement with the results obtained with the rats. In one cat the specific activity of the lymph proteins was 20–25 % higher than that of the plasma proteins, and in the second cat it was about 60 % higher. The specific activity of the lymph amino acids was considerably lower than that of the plasma amino acids in both cats.

Concentrations of amino acids in the plasma and lymph. These are shown in Table 5. Although the systemic plasma amino acids had a higher specific activity than those of the portal plasma, the total activity in 1 ml of portal plasma was considerably higher than in 1 ml of systemic plasma because of the higher concentration of amino acids in the former (see Table 5).

DISCUSSION

The results of Dawson & Holdsworth (1962) showed that dietary proteins were digested very rapidly in vivo with maximum digestive activity during the 1st hour after feeding. After rats had been given ^{14}C-labelled protein, ^{14}C-labelled peptides were present in the contents of the small intestine, but none could be detected in the cells of the intestinal mucosa or in the blood. The work described now is an extension of that work and was done in an attempt further to elucidate the mechanism of the transport of dietary protein nitrogen from the intestine. Values for the ^{14}C activity remaining in the gastro-intestinal tract at various times after feeding were in good agreement with those obtained by Dawson & Holdsworth (1962), and the rapid rise in the ^{14}C activity of the plasma amino acids confirmed that maximum digestion and absorption occurred during the 1st hour after feeding. Similar results were obtained by Crane & Neuberger (1960), who gave normal human subjects ^{15}N-labelled yeast protein and found that maximum ^{15}N activity was attained in the plasma amino acids between 35 and 50 min after ingestion of the labelled protein. There can be little doubt, therefore, that the digestion of protein in vivo occurs much more rapidly than is indicated by the results of the in vitro studies reviewed by Dawson (1960).

The nature of the form in which dietary nitrogen is transported from the intestine has been questioned by Fisher (1954), who is of the opinion that peptides may play a major part in nitrogen transport. Dietary nitrogen must be transported from the intestine as either protein, peptides or amino acids, and in the following discussion the possible contribution made by each of these forms will be considered in the light of the results obtained in our investigation.

Peptides

Several groups of workers have investigated the α-amino nitrogen constituents of plasma. Thus, Stein & Moore (1954), using ion-exchange chromatography, not only identified twenty-eight ninhydrin-positive compounds in deproteinized human plasma but quantitatively determined all except one of them. By this means they were able to account for 95–100% of the α-amino nitrogen of the plasma, but they were unable to detect the presence of any peptides either in fasting blood plasma or in the plasma from blood obtained during the assimilation of protein. Dent & Schilling (1949) and Levenson, Rosen & Upjohn (1959) also were unable to detect the presence of peptides in blood plasma, but they did find amino acids in conjugated form. Levenson *et al.* (1959) isolated from both blood plasma and lymph a conjugate which they showed to be composed mainly of glycine and glutamic acid with some serine and alanine, but its composition was very variable. Neither in their investigation nor in that of Dawson & Holdsworth (1962) was any evidence obtained of the presence of peptides in the

blood plasma. In view of all these findings it seems unlikely that peptides can play any major part in the transport of dietary nitrogen.

Plasma and lymph proteins

Dawson & Holdsworth (1962) showed that the cells of the intestinal mucosa were very active in protein synthesis. It is possible that this mucosal protein could be transferred to the blood or lymph, thereby constituting a transport form of dietary nitrogen. In the investigation described here it was found that maximum ^{14}C activity was attained in the plasma proteins about 5 h after feeding. Further, the activity was not confined to any specific component of the plasma proteins but was distributed amongst them all. At the peak of labelling when the plasma proteins had a specific activity of 260 counts/min mg cm^2 (Table 2), and if a blood volume of 18 ml (Creskoff, Fitz-Hugh & Farris, 1949) and a plasma protein concentration of 6 g/100 ml are assumed, the total activity of the plasma proteins was $260 \times 18 \times \frac{6}{100} \times 10^3 = 2{\cdot}8 \times 10^5$ counts/min cm^2, the activity absorbed during 5 h was $7 \times 10^6 - 1{\cdot}5 \times 10^6 = 5{\cdot}5 \times 10^6$ counts/min cm^2 (Table 1) and the percentage of the dose carried by the plasma proteins was $(2{\cdot}8 \times 10^5) \div (5{\cdot}5 \times 10^6) \times 100 = 5{\cdot}1$. Thus it is evident that even at the peak of labelling only a small proportion of the activity given to the animals could be accounted for in the plasma-protein fractions. This proportion could be significant only if the plasma proteins had a very short half-life. However, the half-life of the plasma proteins has been variously estimated at between 6 and 16 days (Graham, 1937–8; Madden & Gould, 1952; McFarlane, 1956), so that during the 6 h of the experiment only a small proportion of the total plasma proteins would have been resynthesized. It would seem, therefore, that the plasma proteins can make, at most, only a minor contribution to the transport of dietary protein nitrogen.

Similarly, the activity of the lymph proteins 3–4 h after feeding can account for only a very small proportion of the activity given to the animals. Thus in the two experiments to determine the distribution of ^{14}C activity in the lymph, the lymph proteins had a mean specific activity of 280 counts/min mg cm^2 and their concentration was 20 mg/ml, so that, if a rate of flow of mesenteric lymph of 1·5 ml/h kg bodyweight is assumed (Morris, 1956), the total activity transported per hour 3–4 h after feeding would be $280 \times 20 \times 1{\cdot}5 \times 3{\cdot}5 = 3{\cdot}6 \times 10^4$ counts/min cm^2. As the total activity absorbed was $1{\cdot}7 \times 10^8 - 0{\cdot}85 \times 10^8 = 0{\cdot}85 \times 10^8$ counts/min cm^2 (Table 4), the percentage of the dose carried by the lymph proteins during this period was $(3{\cdot}6 \times 10^4) \div (0{\cdot}85 \times 10^8) \times 100 = 0{\cdot}04\,\%$.

Plasma and lymph amino acids

The more detailed examination of the blood fractions made in this investigation confirmed the finding of Dawson & Holdsworth (1962) that the most marked changes in ^{14}C activity occurred in the plasma amino acid fraction. The results (Table 2) showed that maximum ^{14}C activity was attained in the plasma amino acids of the portal blood about 30 min after feeding when their specific activity was about 5000 counts/min mg cm^2, about twice that of the amino acids of the systemic blood plasma which reached their peak about 1 h after feeding. The higher specific activity of the portal

plasma amino acids was maintained for several hours, and 5 h after feeding it was still about 30% higher than that of the systemic plasma amino acids.

These differences in specific activity were paralleled by differences in the concentrations of the plasma amino acids (Table 3), the concentration in portal blood being higher than in systemic during the 6 h after feeding. At 30 min after the labelled protein had been given the concentration of plasma amino acids in portal blood was 100% and in systemic blood 50% higher than in fasting blood.

The results in Table 5 show that the specific activities of the amino acid fractions of lymph were appreciably lower than those of portal blood, but that the total activities/ml were not very different. Nevertheless, the contribution to the transport of dietary protein nitrogen made by the lymph amino acids must be extremely small since the rate of flow of lymph (1·5 ml/h kg body-weight) (Morris, 1956) is only about 1/800 of that of portal blood (2000 ml/h kg body-weight) (Dobson & Jones, 1952).

From the foregoing it is apparent that the only fractions transporting appreciable amounts of dietary nitrogen from the intestine were the blood amino acids. It is of interest, therefore, to consider whether in the experiments with rats the difference in ^{14}C activity between the amino acids of the portal and systemic blood could account for the removal of an appreciable proportion of the activity absorbed from the intestine. The total amount of activity removed from the intestine by the portal plasma amino acids would be proportional not only to the difference in activity between the portal and systemic blood but also to the rate of flow in the portal vein. The rate of blood flow in the hepatic vein of the rat was measured by Dobson & Jones (1952) and by Benacerraf, Biozzi, Halpern, Stiffel & Mouton (1957); both groups of workers found values close to 1·3 ml/g liver tissue per min. Dobson & Jones (1952) also found that portal blood flow accounts for some 60–70% of the total hepatic flow. Thus as the mean weight of the livers of the rats used in our experiments was 11·5 g, we may, for the purposes of our calculations, assume that the rate of portal blood flow was $11{\cdot}5 \times 1{\cdot}3 \times 0{\cdot}65 = 11$ ml/min. If the ^{14}C activities (counts/min cm^2) of the plasma amino acids in 1 ml of portal plasma and in 1 ml of systemic plasma are plotted against time, the area between the two curves is proportional to the total ^{14}C activity transported by the portal plasma amino acids during the time of the experiment (Fig. 1). By measuring the area between these curves it was found that during the 5 h after the labelled protein had been given the total activity transported by the portal plasma amino acids was $1{\cdot}97 \times 10^6$ counts/min cm^2. Similarly, it was calculated from Fig. 2 that the total ^{14}C activity transported by the amino acids of the portal blood cells during the same time was $0{\cdot}47 \times 10^6$ counts/min cm^2. Thus the ^{14}C activity added to the plasma amino acids of the portal blood during its passage through the intestinal blood vessels in the 5 h after feeding was $2{\cdot}44 \times 10^6$ counts/min cm^2.

The ^{14}C-labelled *Chlorella* protein used had a specific activity after dilution of 2×10^5 counts/min mg cm^2 when counted at infinite thickness in a gas-flow counter, and the 35 mg given to each rat had, therefore, a total ^{14}C activity of 7×10^6 counts/min cm^2. However, it is evident from Table 1 that $1{\cdot}5 \times 10^6$ counts/min cm^2 of this activity was still present in the gastro-intestinal tract 5 h after feeding. Thus protein equivalent to a total of $7 \times 10^6 - 1{\cdot}5 \times 10^6 = 5{\cdot}5 \times 10^6$ counts/min cm^2 was assimilated

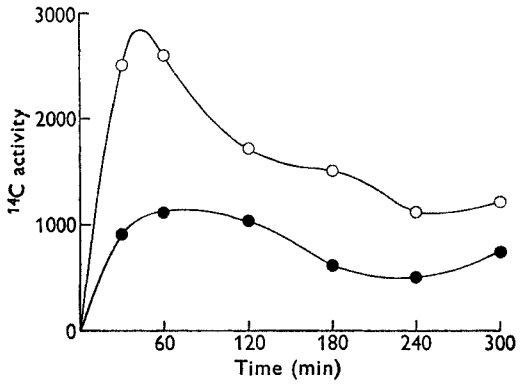

Fig. 1

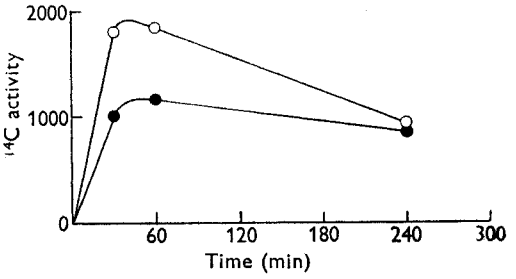

Fig. 2

Fig. 1. ^{14}C activity (counts/min ml) of plasma amino acid fractions taken from rats during 300 min after they had received by mouth 35 mg ^{14}C-labelled *Chlorella* protein (containing 8 μc of ^{14}C activity). ○, portal plasma; ●, systemic plasma.

Fig. 2. ^{14}C activity of the amino acids of the blood cells taken from rats during 300 min after they had received by mouth 35 mg ^{14}C-labelled *Chlorella* protein (containing 8 μc of ^{14}C activity). ○, portal blood; ●, systemic blood.

during the 5 h of the experiment. The calculations set out above showed that the differences between the specific activities of the amino acids of portal and systemic blood could account for the transport of activity of $2{\cdot}44 \times 10^6$ counts/min cm^2, that is equivalent to $(2{\cdot}44 \times 10^6) \div (5{\cdot}5 \times 10^6) \times 100 = 41\,\%$ of the ^{14}C-labelled protein assimilated. These calculations are made on the basis that all the activity assimilated is passed into the blood stream, but it is evident that some of the labelled nitrogenous compounds may be used in the intestinal mucosa. It is not possible for us to estimate the extent of this occurrence; it is clear, however, from the work of Dawson & Holdsworth (1962) that the mucosal cells were particularly active in protein synthesis, so it is possible that an appreciable amount of ^{14}C-labelled amino acids was used directly by these cells.

It is also evident from the finding of Dawson & Holdsworth (1962) that the expired air contained $^{14}CO_2$, that a proportion of the amino acids given was katabolized, and it is possible that part of this katabolism took place in the intestinal wall. In this connexion it is noteworthy that only small amounts of glutamic and aspartic acids, or their amides, appear in the blood after even a quite large meal of casein, a protein rich in these amino acids (Stein & Moore, 1954; Stein, Bearn & Moore, 1954). Aspartic and glutamic acids constitute almost 18·5 % of the component amino acids of *Chlorella* protein and it seems likely that this proportion of the ^{14}C activity given to the rats does not pass into the blood stream.

Having enumerated some of the factors that could explain why we can only account directly for the absorption in the form of amino acid of about 40 % of the ^{14}C-labelled protein assimilated, it is only fair to conclude by mentioning the possibility that factors may also be operating in the opposite direction. Perhaps the most important of these is the recycling of ^{14}C activity which undoubtedly must occur through the secretion of ^{14}C-labelled digestive enzymes into the intestinal tract during the course of the experi-

ment. Further work is necessary to establish the extent to which it occurs, but it is evident that when it does happen we are not only overestimating the radioactivity removed by the portal blood, but we are also overestimating the radioactivity found in the intestinal tract at the end of the experiment.

Although we think that we can reasonably conclude from our findings that plasma amino acids constitute the main form of transport of dietary nitrogen during assimilation of protein from a fat-free diet, we recognize that our findings on nitrogen transport in lymph are of a preliminary nature and consider that it would be of interest in further studies to determine the levels of protein and amino acids in lymph taken at different times after ingestion of protein both in fat-free and in fat-containing diets.

SUMMARY

1. ^{14}C-labelled algal protein was given by mouth to groups of three rats. At intervals from 0 to 6 h after feeding samples of portal and systemic blood were taken and the distribution of ^{14}C activity in the various blood fractions was determined. ^{14}C-labelled protein was also given to cats, and 3–4 h after feeding samples of mesenteric lymph and of portal and systemic blood were taken and the distribution of ^{14}C activity in these samples was determined.

2. In the rats the labelled protein was rapidly digested and assimilated, and 3 h after feeding about 60% of the labelled protein had been assimilated.

3. The blood fraction showing the most marked changes in ^{14}C activity was the plasma amino acid fraction. Maximum activity was attained in the plasma amino acids of the portal blood between 30 min and 1 h after feeding and in those of the systemic blood between 1 and 2 h after feeding.

4. The plasma amino acids of the portal blood had a considerably higher specific activity than those of the systemic blood for several hours after feeding and, during the same period, the concentration of plasma amino acids was higher in the portal than in the systemic blood.

5. Calculations showed that at least 41% of the ^{14}C that had been absorbed had been transported from the intestine in the form of plasma amino acids.

6. At the peak of ^{14}C labelling in the plasma-protein fraction only about 5% of the ^{14}C absorbed could be accounted for in this fraction.

7. The protein and amino acid fractions of lymph taken from the mesenteric lymph duct of cats 3–4 h after feeding could account for only a very small proportion of the ^{14}C transported from the intestine.

8. The significance of these findings is discussed.

We are grateful to Dr S. K. Kon for his advice and encouragement during this investigation and to Professor R. B. Fisher for discussion, criticism and helpful suggestions. We thank Dr M. E. Coates for her guidance and help in the operations on the rats and Miss M. V. Chapman for looking after them. We are indebted to Dr P. P. Scott for supplying and operating on the cats, and to Drs R. F. Glascock and H. Gutfreund for providing us with radioactivity counting facilities.

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EXPLANATION OF PLATE

Distribution of ^{14}C in the components of the plasma proteins, separated by electrophoresis, from the portal (P) and systemic (S) blood of rats taken at hourly intervals from 1 to 6 h after they had received by mouth ^{14}C-labelled *Chlorella* protein. The figures show time of sampling (h).

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