Effects of Protein Deficiency on the Rate of Radioactivity Loss from Body Constituents in Adult Rats Given $^{14}$C-Amino Acids

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Summary

The effect of protein deficiency on the rate of loss of radioactivity from body constituents was studied in adult rats administered $^{14}$C-Chlorella protein hydrolysate or $^{14}$C-lysine. Rats were kept on a protein-free diet for 3 weeks and then injected with labelled amino acids and fed on a protein-free diet for 3 more days to allow $^{14}$C deposition in tissues. Then they were given experimental diets (protein-free diet, 1% and 10% wheat gluten diets pair-fed with the protein-free diet, and 10% wheat gluten diet ad libitum) for 7 days and sacrificed.

The rates of loss of radioactivity from tissue proteins became low in general with the extent of protein deficiency. This increased capacity of tissues to retain $^{14}$C-amino acids may result from higher efficiency of protein utilization in protein deficiency. The reutilization of free amino acids and the rate of catabolism of tissue proteins are discussed on the basis of the results. The half-life of muscle protein was too long to observe the effects of experimental diets given for 7 days on the rate of loss of radioactivity.

Many investigators have shown that in animals and man increase in the protein content of the diet above the maintenance level decreases the efficiency of dietary protein utilization. On the contrary, at submaintenance levels of protein intake, the efficiency of protein utilization is thought to be constant. However, recently, Inoue et al. (1,2), Said and Hegsted (3) and Young et al. (4) cast some doubts on the latter idea, indicating that the efficiency of protein utilization became higher with decreasing protein intakes, especially with poor quality proteins. In previous studies in this laboratory (5) the efficiency of protein utilization was examined in rats on a protein-deficient diet by measuring the rate of incorporation of $^{14}$C-Chlorella protein hydrolysate into various body constituents and the total

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amount of $^{14}$C retained in these tissues. The results showed that with a low protein intake, both protein synthesis and the ability to retain exogenous protein were enhanced in the liver. In muscle $^{14}$C retention also increased in protein deficiency but protein synthesis decreased. Thus to evaluate protein utilization it seems necessary to investigate the catabolic rate of tissue protein, and this was studied in the present work.

**EXPERIMENTAL**

Male Sprague-Dawley rats were fed a commercial stock diet until they weighed about 300 g. Then they were given a protein-free diet for 3 weeks, to induce better retention of radioactivity in their tissues (5). The rats were used for the following two experiments.

**Experiment 1.** Animals were injected intraperitoneally with 4.0 $\mu$Ci/100 g body weight of $^{14}$C-Chlorella protein hydrolysate2 (40.3 mCi/mg atom C, diluted to 8 $\mu$Ci/ml with saline) and were fed on the protein-free diet for 3 more days to allow $^{14}$C deposition in the tissues. Then they were divided into 5 groups of six animals each. One group was killed (C-Control). The other four groups were fed respectively on protein-free diet (C-0-A), 10% wheat gluten diet *ad libitum* (C-10-A) and 1% and 10% wheat gluten diets pair-fed with the C-0-A group (C-1-P, C-10-P, respectively) for 7 days. The groups pair-fed with C-0-A group were used to observe the effects of only protein intake level on the protein utilization under isocaloric condition because in protein starvation food intake of animals is usually depressed to about half of that of animals fed protein diets.

**Experiment 2.** Experiment 2 was similar to experiment 1, except that rats were given 2.5 $\mu$Ci/100 g body weight of $^{14}$C(U)-L-lysine (280 mCi/mmmole of lysine, diluted to 5 $\mu$Ci/ml with saline) instead of $^{14}$C-Chlorella protein hydrolysate and the group receiving 10% wheat gluten diet *ad libitum* was omitted. Using similar nomenclature to that in experiment 1, the groups were designated as L-Control, L-0-A, L-1-P and L-10-P, respectively.

**Food intake.** Wet diets prepared by adding 60% water by volume were used throughout. Food intake was calculated by drying the remaining wet diet at 105°C and weighing it.

**Metabolic study.** Ten days after isotope injection (3 days for the Control) *i.e.* just after one week’s experimental diet was over, animals were anesthetized with ethylether and blood was withdrawn by cardiac puncture into a heparinized syringe. The liver, gastrocnemius muscles, gastrointestinal tract and various organs (kidneys, spleen, heart and lungs) were removed. The tissues, carcass and

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2 Purchased from Daiichi Pure Chemical Co. The percentage distribution of $^{14}$C was as follows: Lys 4.91; Thr 5.04; Leu 10.60; Ile 4.44; Val 7.06; Met 1.79; Phe 6.28; His 1.77; Arg 6.31; Asp 9.34; Glu 13.65; Gly 6.00; Ala 9.17; Ser 4.65; Tyr 5.54; Pro 3.45; total essential amino acids 48.20; total non-essential amino acids 51.80.
plasma were frozen and stored in a deep freezer until analyzed.

**Analyses.** The preparation of tissue fractions and analytical methods were as described in detail in the previous report (5). Cold 0.5 N perchloric acid (PCA) was added to tissue and carcass homogenates and plasma and the mixtures were centrifuged. The resulting supernatants were designated as the PCA-soluble fractions. The precipitates were washed with 0.5 N PCA and then lipid was extracted with ethanol-ethylether (3: 1, v/v). The residue was referred to as the protein fraction. Glycogen was isolated from fresh liver and muscle by the method of Stetten and Boxer (6) and measured by the anthrone method. The radioactivities in these fractions were measured by scintillation spectrometry. The concentration of free amino acids in the PCA-soluble fractions of plasma and tissues was measured by the method of Matthews et al. (7). Protein was determined by the method of Lowry et al. (8). Crude lipid in the carcass was extracted with ethylether in a Goldfisch apparatus and determined gravimetrically.

**RESULTS**

Animals consumed about 15 g of protein-free diet a day in the 24 day preliminary period (21 days for depletion and 3 days for $^{14}$C deposition). In the succeeding experimental period, their daily intakes were about 12 g of protein-free

| Group b | Body weight | Liver | Gastrocnemius muscle | Gastro-intestinal tract | Viscera a |
|---------|-------------|-------|----------------------|------------------------|---------|
|         | Before experimental diet e | Final d |                     |                        |         |
| C-Control | 241±21    | 7.6±1.6 | 1.17±0.14          | 4.8±0.4                | 3.8±0.3 |
| L-Control | 248±20    | 7.3±1.1 | 1.15±0.45          | 5.2±1.0                | 3.8±0.5 |
| C-0-A    | 242±14    | 7.0±4.8 | 1.19±0.14          | 5.1±0.5                | 3.9±0.4 |
| L-0-A    | 243±16    | 8.1±1.3 | 1.21±0.12          | 5.6±0.5                | 4.4±0.4 |
| C-1-P    | 244±13    | 7.0±1.2 | 1.20±0.10          | 5.3±0.2                | 4.0±0.5 |
| L-1-P    | 245±15    | 7.0±1.2 | 1.20±0.10          | 5.3±0.2                | 4.0±0.5 |

a Means±SD of values in twelve rats except six rats in C-10-A group.
b C-Control indicates rats killed 3 days after administration of $^{14}$C-Chlorella protein hydrolysate. C-0-A, C-1-P, C-10-P and C-10-A indicate groups given $^{14}$C-Chlorella protein hydrolysate and then fed on protein-free diet, pair-fed with the protein-free diet group on 1% or 10% wheat gluten diet and 10% wheat gluten diet ad libitum for 7 days, respectively. Groups administered $^{14}$C-lysine instead of $^{14}$C-Chlorella protein hydrolysate are designated similarly as L-Control, L-0-A, L-1-P and L-10-P, respectively.
c Body weight 3 days after the administration of labelled amino acids.
d Body weight after receiving the respective experimental diets for 7 days.
e Viscera include kidneys, spleen, heart and lungs.
diet and about 15 g of wheat gluten diet, respectively. The animals lost about 50 g body weight in the preliminary period, while during the 7-day experimental period, the rats in the C-10-A group gained 9 g but those in other groups lost 4 to 10 g (Table 1). The weights of liver, gastrocnemius muscle, gastrointestinal tract and viscera were not different significantly in all the animals.

Table 2 shows the concentrations and specific radioactivities of tissue and plasma proteins. The experimental diets did not affect the protein concentrations in any of the tissues, with the minor exceptions of those in the plasma, gastrointestinal tract and viscera. In experiment 1 the specific radioactivities of protein

| Experiment | Group   | Liver     | Muscle    | Plasma    | Gastrointestinal tract | Carcass | Viscera  |
|------------|---------|-----------|-----------|-----------|------------------------|---------|----------|
|            | C-0-A   | 181±10    | 204±8     | 61±6      | 132±3                  | 139±7   | 124±7    |
| Concentration | C-1-P  | 183±11    | 190±10    | 72±4d     | 96±7e                  | 137±19  | 111±13   |
| (mg/g tissue or ml plasma) | C-10-P | 187±5     | 198±2     | 55±4      | 117±20                 | 130±13  | 125±7    |
|            | C-10-A  | 176±11    | 198±17    | 58±7      | 110±7e                 | 141±7   | 107±15e  |
|            | L-0-Control | 176±11 | 216±19    | 60±6      | 120±12                 | 135±14  |          |
|            | L-0-A   | 180±25    | 211±11    | 64±10     | 129±10                 | 130±13  |          |
|            | L-1-P   | 190±13    | 218±9     | 66±5      | 120±19                 | 135±7   |          |
|            | L-10-P  | 187±28    | 215±9     | 65±10     | 135±25                 | 137±9   |          |

Table 2. Concentrations and specific radioactivities of tissue and plasma proteins.a

| Specific radioactivity | experiment | C-Control | C-0-A | C-1-P | C-10-P | C-10-A |
|------------------------|------------|-----------|-------|-------|--------|--------|
|                        | 1          | 100±15    | 100±9 | 100±14| 100±7  | 100±5  | 100±7  |
|                        |            | 62±7      | 130±27| 65±8  | 54±22  | 95±8   | 69±3   |
|                        | 2          | 55±3a     | 113±14| 54±2d | 48±9   | 99±15  | 54±3d  |
|                        |            | 54±3a     | 116±10| 50±5d | 51±20  | 87±16  | 57±8d  |
|                        |            | 45±6a     | 124±16| 42±5d | 34±13  | 79±6a  | 65±10  |

a Means±SD of values in six rats.

b Carcass is whole body excludes liver, kidneys, spleen, heart, lungs and gastrointestinal tracts.

c, d, e Significantly different from values for group C-0-A or L-0-A at levels of 5%, 1% and 0.1%, respectively.

f Expressed as actual values in dpm/mg protein in C-Control and L-Control groups and as percentages of these values in other groups.
in all the tissues other than muscle decreased to different levels during the final 7-day period compared to those in the C-Control group. The rates of loss of labelled amino acids from tissue proteins, particularly those of the liver, plasma and carcass, were significantly higher in the C-10-A group than in the C-0-A group. In groups C-1-P and C-10-P the rates of loss from the tissues and plasma were similar and the specific radioactivities of proteins of the liver, gastrointestinal tract and plasma in these groups were between those of groups C-0-A and C-10-A. The specific radioactivity of muscle protein was maintained or even increased slightly during the period on the experimental diets, irrespective of the diet. In all the groups $^{14}$C loss from carcass protein was less than that from protein of the liver or viscera, because the carcass consists mainly of muscle. In experiment 2, the specific radioactivities of protein decreased in the liver and plasma, increased in the muscle, and increased or slightly decreased in the carcass and gastrointestinal tract. The specific radioactivities of $^{14}$C in the carcass, gastrointestinal tract and plasma were less in group L-10-P than in group L-0-A and the values of group L-1-P were intermediate between those of the former two groups. The L-10-P group retained $^{14}$C in liver protein more efficiently than groups L-0-A and L-1-P. The specific radioactivities of muscle protein in the three groups were similar. The rates of loss of radioactivity from the liver and alimentary tract were very different in experiments 1 and 2. The reason for this is uncertain, but will be discussed later.

The total amounts of $^{14}$C in tissue and plasma proteins are expressed as percentages of the amount administered in Table 3. Three days after $^{14}$C admin-

| Experiment | Group     | Liver (dpm) | Plasma (dpm) | Carcass (dpm) | Viscera (dpm) | Gastrointestinal tract (dpm) | Sum (dpm) |
|------------|-----------|-------------|--------------|---------------|---------------|-----------------------------|-----------|
| 1          | C-Control | 3.4±0.5     | 2.4±0.5      | 18.3±1.0      | 1.3±0.3       | 1.2±0.1                    | 26.5±0.5  |
|            | C-0-A     | 1.9±0.2     | 1.4±0.2      | 18.2±1.3      | 0.8±0.0       | 0.9±0.2                    | 23.2±1.1  |
|            | C-1-P     | 1.5±0.1c    | 1.2±0.1      | 19.1±2.1      | 0.7±0.1c      | 0.5±0.1d                   | 22.9±2.3  |
|            | C-10-P    | 1.6±0.3e    | 1.0±0.2d     | 15.8±2.0d     | 0.7±0.1c      | 0.7±0.2                    | 19.8±1.8  |
|            | C-10-A    | 1.5±0.1d    | 1.0±0.2d     | 15.6±0.9d     | 0.8±0.2       | 0.5±0.2e                   | 19.4±1.2  |
| 2          | L-Control | 6.2±0.4     | 2.5±0.3      | 26.5±5.6      | 0.8±0.1       |                            | 35.9±5.8  |
|            | L-0-A     | 3.2±0.3     | 1.8±0.3      | 28.7±6.5      | 1.0±0.1       |                            | 34.7±6.1  |
|            | L-1-P     | 3.5±0.4     | 1.7±0.2      | 25.0±2.1      | 1.1±0.3       |                            | 31.3±1.9  |
|            | L-10-P    | 4.6±0.6e    | 1.6±0.2      | 22.6±1.9d     | 0.7±0.2e      |                            | 29.5±2.3  |

a Means±SD of values in six rats.
b The total plasma volume was calculated as 3.5% of the body weight.
c, d Significantly different from values for group C-0-A or L-0-A at levels of 5% and 1%, respectively.

istration, about 27% and 36% of the dose administered were recovered in body proteins in experiments 1 and 2, respectively, indicating that exogenously ad-
ministered amino acids were metabolized rapidly in the first few days after their administration. Up to 7% of the administered radioactivity was lost during the 7-day experimental periods in experiments 1 and 2. The differences of the total \(^{14}\)C in respective tissues and plasma proteins in the dietary groups were similar to the differences of the specific radioactivities of their proteins in both experiments. About 70 to 85% of the \(^{14}\)C recovered from all body proteins were in carcass proteins in all the animals, so the amount of \(^{14}\)C in the carcass is very important in considering protein utilization. In experiment 1, the recoveries of total \(^{14}\)C in whole body proteins were similar in groups C-0-A and C-1-P and in groups C-10-P and C-10-A and the values of former groups were higher than those of latter groups and the values in experiment 2 were more in the order of groups L-0-A, L-1-P and L-10-P.

Table 4 shows the concentrations and the specific radioactivities of free amino acids. The amino acid concentrations in the tissues and plasma were similar in the different groups, so only data on control rats are shown. The specific radio-

| Experiment | Liver | Muscle | Plasma | Carcass | Viscera |
|------------|-------|--------|--------|---------|---------|
| Concentration\(^b\) |      |        |        |         |         |
| 1 C-Control | 0.68±0.16 | 0.64±0.12 | 0.06±0.01 | 0.45±0.04 | 0.66±0.10 |
| 2 L-Control | 0.67±0.06 | 0.65±0.05 | 0.06±0.01 | 0.62±0.11 |         |

Table 4. Concentrations and specific radioactivities of tissues and plasma free amino acids.\(^a\)

| Specific radioactivity\(^c\) | dpm/mg NH\(_2\)-N |    |        |        |         |
|----------------------------|------------------|----|--------|--------|---------|
| of C-Control | 10.9±4.3 | 0.8±2.0 | 278±153 | 8.6±0.9 | 60.7±2.5 |
| (×10\(^3\)) |         |        |        |        |         |
| C-Control | 100±39 | 100±29 | 100±55 | 100±16 | 100±4  |
| 1 C-0-A | 54±16 | 107±72 | 37±14 | 90±8 | 57±6 |
| C-1-P | 46±8 | 118±21 | 43±17 | 74±6 | 44±3\(^d\) |
| C-10-P | 70±26 | 90±25 | 37±18 | 87±19 | 46±2\(^e\) |
| C-10-A | 48±16 | 94±47 | 32±5 | 83±15 | 46±7\(^e\) |

| of L-Control | 16.2±4.6 | 3.6±1.7 | 79.7±39.3 | 10.4±2.8 |
| (×10\(^3\)) |         |        |        |         |
| L-Control | 100±28 | 100±47 | 100±49 | 100±27 |
| 2 L-0-A | 72±9 | 103±11 | 57±24 | 107±50 |
| L-1-P | 67±7 | 89±17 | 51±16 | 91±6 |
| L-10-P | 73±10 | 81±11 | 87±45 | 107±18 |

\(^a\) Means±SD of values in six rats.
\(^b\) mg NH\(_2\)-N of free amino acids/g tissue or ml plasma.
\(^c\) Expressed as actual values in dpm/mg NH\(_2\)-N of free amino acids in the C-Control and L-Control groups and as percentages of these values in other groups.
\(^d, e\) Significantly different from the values for the C-0-A group at levels of 5% and 1%, respectively.
activities of the liver, plasma and viscera decreased while those of the muscle and carcass did not change during the 7-day period in experiment 1 and these changes were comparable with those of tissue protein. However, the values were similar in all the groups except that the value for viscera in group C-0-A was higher than those in the other groups. In experiment 2, the specific radioactivities of free amino acids in the liver and plasma decreased in the 7-day experimental period, while those in the muscle and carcass did not change or reduced slightly. The values for all the tissues and plasma except muscle were similar in the different groups, as in experiment 1. Amino acid metabolism in muscle may have a specific nature since the specific radioactivities of muscle free amino acids changed only a little and the specific radioactivity of muscle protein increased during the experimental period in both experiments.

Data on glycogen in the liver and muscle and on carcass lipids are shown in Table 5. The concentrations and specific radioactivities of glycogen in the liver and muscle and those of lipids in the carcass were similar in all the groups with a few exceptions.

| Liver glycogen | Muscle glycogen | Carcass lipid |
|----------------|----------------|--------------|
| Concentration (mg/g liver) | S.A. (dpm/mg glycogen) | Concentration (mg/g muscle) | S.A. (dpm/mg glycogen) | Concentration (% of weight) | S.A. (dpm/mg lipid) |
| C-Control | 62±17 | 20±10 | 7±2 | 21±8 | 23±7 | 20±8 |
| C-0-A | 64±20 | 11±4 | 8±2 | 34±9 | 25±9 | 19±5 |
| C-10-P | 98±52 | 15±15 | 8±2 | 24±7 | 25±2 | 17±6 |
| C-10-A | 56±19 | 15±3 | 6±1 | 21±2b | 28±9 | 16±4 |
| L-Control | 92±14 | 8±3 | 8±1 | 25±7 | 25±4 | 17±3 |
| L-0-A | 90±15 | 8±2 | 7±2 | 17±4 | 28±3 | 16±4 |
| L-1-P | 111±20 | 11±4 | 9±1 | 19±5 | 25±3 | 17±7 |
| L-10-P | 68±6b | 12±4 | 8±2 | 19±5 | 28±8 | 17±3 |

a Means±SD of values in six rats.
b Significantly different from values for group C-0-A or L-0-A at a level of 1%.

Table 6. ¹⁴C Distribution among various fractions in liver and muscle of C-Control and L-Control groups (%).a

| Tissue | Protein | PCA-soluble | Lipids | Glycogen |
|--------|---------|-------------|--------|----------|
| C-Control | Liver | 67.5 | 11.5 | 19.1 | 2.0 |
|         | Muscle | 70.2 | 21.7 | 7.5 | 0.6 |
| L-Control | Liver | 85.8 | 8.1 | 5.2 | 0.8 |
|         | Muscle | 84.4 | 8.4 | 6.7 | 0.5 |

a Means of values in six rats.
Table 6 shows the $^{14}$C distribution in four fractions of liver and muscle in control groups. Distribution in other groups was very similar and so is not shown. The recoveries of radioactivity in the lipids of liver and muscle were about 19% and 8%, respectively in experiment 1, and about 5% and 7% in experiment 2, respectively.

It was surprising that the recoveries of radioactivity in glycogen and lipid shown in Tables 5 and 6 were so high in animals on the protein-free diet.

**DISCUSSION**

Previously we reported that when the protein intake was restricted, even poor quality protein like wheat gluten was efficiently utilized by human subjects and that the efficiency of protein utilization decreased curvilinearly as the protein intake approached the maintenance level (2). Similar results were obtained by SAID and HEGSTED (3) and KISHI et al.3 on rats. To confirm these findings obtained by measuring the nitrogen balance and by analysis of the carcass, further study was made on the rates of incorporation of $^{14}$C-Chlorella protein hydrolysate into various tissue components of protein-deficient adult rats (5). The latter study showed more rapid turnover of liver protein in protein-deficient rats than in controls, suggesting that their protein synthesis and reutilization of pool amino acids were enhanced. Furthermore, protein-deprived animals retained more radioactivity in body proteins than well-nourished animals, although their rate of incorporation of $^{14}$C-amino acids into muscle proteins, which constitute a large part of the bodily proteins, was decreased. These findings suggested that protein-deficient animals used the small amount of ingested protein available very efficiently. To investigate this possibility, the effect of the diet on tissue protein degradation must be examined, because the net utilization of protein is determined by the balance between protein synthesis and degradation.

In the present work, tracers were injected into animals maintained on protein-free diet for 3 weeks. Then after a 3-day labelling period animals were given various diets and the effects of these diets on the rate of loss of label from tissue proteins were investigated. Prolonged feeding of the protein-free diet resulted in more labelling with tracers than that seen in animals on normal diet, as shown in an earlier report (5), and also reduced the amounts of labile tissue proteins. SOLOMON and TARVER (9) found that the rate of loss of labelled amino acids from tissue proteins may be related to the content of labile tissue proteins. However, our results on the rate of loss probably represent the rate of loss of “fixed protein” in the tissues, because the animals were depleted of labile protein by being fed on protein-free diet for 3 weeks before the experiments.

The total recoveries of radioactivity in body proteins three days after administration of $^{14}$C-Chlorella protein hydrolysate and $^{14}$C-lysine were 26.5% and

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3 Unpublished data.
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35.9\% of the injected doses, respectively. Aguilar et al. (10) also reported that \(^{14}\)C retention was higher in rats which received \(^{14}\)C-lysine than in those which received \(^{14}\)C-Chlorella protein hydrolysate. They found that when growing rats consuming a normal diet were given \(^{14}\)C-Chlorella and \(^{14}\)C-lysine, about 61\% and 70\%, respectively of the labelled amino acid were recovered in the body after 24 hr. The difference in the retentions of tracers in the body in experiments 1 and 2 may be mainly due to the presence of labelled nonessential amino acids in algal protein, amounting to about 50\% of the total labelled amino acids.\(^3\)

Differing from results in experiment 1, in experiment 2 higher retention of \(^{14}\)C-lysine was observed in liver protein in the group on higher protein diet (L-10-P) than in those on lower protein diets (L-0-A and L-1-P). This observation was also in contrast to results on the rates of loss of label from tissues other than liver in experiments 1 and 2 (Tables 2 and 3). A possible explanation of these findings is based on the results of Elwyn (11), who showed that in dogs the amino acid pattern of the diet was more similar to that of the portal vein than to that of the hepatic vein. This was interpreted as due to the role of the liver in buffering the impact of the meal on peripheral tissues by regulating the synthesis and breakdown of protein and amino acids, urea production and reutilization of amino acids in the liver in rapid response to changes in amino acid intake. Thus when wheat gluten is ingested, the unbalanced amino acid pattern might be corrected in the liver by sparing of lysine, the first limiting amino acid of wheat gluten. The degree of lysine deficiency in the group on the higher protein diet was relatively greater than that of groups on low-protein diets, so in the former group more of the \(^{14}\)C-lysine released from tissue proteins was retained in the liver. Since other tissues were supplied amino acids well-balanced by the liver, the retention of \(^{14}\)C-lysine was similar to that of animals receiving \(^{14}\)C-Chlorella protein hydrolysate. In these tissues the rates of loss of labelled substances were influenced more by the level of protein intake than by lysine deficiency. The above considerations indicate that results using \(^{14}\)C-lysine do not necessarily reflect the general pattern of protein metabolism. However, changes in the specific and total radioactivities of proteins in the tissues and plasma generally showed similar tendencies in experiments 1 and 2 and \(^{14}\)C retention in general increased with the severity of protein deficiency except that \(^{14}\)C retention in muscle was affected little by diets in both experiments (Tables 2 and 3). In other words, loss of labels from these tissues was smaller in severe protein deficiency, and this is in accord with the observations of Solomon and Tarver (9) and Waterlow and Stephen (12). However, it is uncertain whether the reduced rate of \(^{14}\)C release from tissue proteins in severe protein deficiency implies an actual decrease in tissue protein catabolism or increased reutilization of pool amino acids for tissue protein synthesis, because there are reports that labelled amino acids released by breakdown of tissue proteins, especially in the liver, may be reincorporated into newly synthesized proteins without leaving the tissues (13–19) and that the rate of amino acid reutilization
depends on the nutritional status (20). In this connection it is noteworthy that Dallman and Manies (19) studied the effect of dietary protein on the rate of loss of radioactivity from liver proteins in adult rats after injection of reutilizable \(^3\)H-arginine and non-reutilizable \(^{14}\)C-guanido-arginine and found that using \(^{14}\)C-guanido-arginine the rates of loss were comparable in rats on low and normal protein diets, whereas using \(^3\)H-arginine the rate was lower in the former than in controls. Furthermore, Stephen and Waterlow (21) studied the effect of dietary protein on the apparent and true half-lives of liver proteins in rats injected with U-\(^{14}\)C-arginine and \(^{14}\)C-guanido-arginine and found that the true half-life was identical regardless of the diet, while the apparent half-life was about three times longer in protein-deficient rats than in controls. Thus, both groups of investigators interpreted the increased retention or incorporation of the labels into tissues of protein-deprived animals as enhanced reutilization only, not as decreased catabolism of tissue proteins. However, Lane (14) reported that the greater retention of labels in protein deficiency was due to decrease in the catabolic rates of tissue proteins, as well as to increased recycling of amino acids. From these reports it seems probable that the increased \(^{14}\)C retention in protein-free diet group of experiment 1 was caused partly by increased reutilization of amino acids in the pool. However, it is uncertain from the results whether reduction in protein catabolism actually occurred and this requires further study.

The specific radioactivities of muscle proteins remained constant or increased during the 7-day experimental period and no significant differences were observed between values in groups on different diets. Previously we\(^4\) and others (9,13,22) also observed that the specific radioactivities in muscle were maintained in growing rats receiving \(^{14}\)C-Chlorella protein hydrolysate or \(^{35}\)S-methionine. This may be because the half-life of muscle proteins is too long (23–25) to allow detection of any decrease in specific radioactivity or differences between the specific radioactivities in animals fed on slightly and severely protein-deficient diets. Moreover, it is possible that the half-life of muscle protein is prolonged by administration of protein-deficient diet for three weeks (26,27). However, as there is evidence to the contrary (28), this problem requires further study. The reutilization of protein is much less in muscle than in liver (18,21,29–31), but the total mass of muscle is so large that reutilization in this tissue significantly affects the efficiency of protein utilization. However, there have been few detailed experiments on the effect of dietary conditions on the reutilization of muscle protein and this should also be studied.

Unlike in muscle protein, in carcass protein the rate of loss of label was lower in severely protein-deficient animals (C-0-A, L-0-A, C-1-P and L-1-P) than in animals receiving 10\% gluten diets, although at least 50\% of the carcass protein is muscle protein. This indicates that the metabolism of body proteins other than muscle proteins was appreciably affected by dietary protein. Thus, the increased

\(^4\),\(^5\) Unpublished data.
capability of most of tissues and plasma proteins to retain $^{14}$C-amino acids may lead to the higher efficiency of protein utilization observed in severely deficient animals.

As stated above, in the intestine retention of label from $^{14}$C-lysine increased or remained constant during the 7-day experimental period, whereas label from $^{14}$C-Chlorella protein hydrolysate decreased considerably (Table 2). The reason for this discrepancy is not clear. It is interesting in this connection that Fujita et al. showed that the free lysine concentration of the small intestine in adult rats on wheat gluten diet was significantly higher than that in control rats on egg protein diet. Comparing with the results obtained in experiment 2, the retention of $^{14}$C in gastrointestinal tract and also in liver after administering $^{14}$C-lysine must be further studied on rats fed a diet containing good quality protein.

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