CORRELATION BETWEEN LABILITY AND RELATIVE TURNOVER RATE OF SOLUBLE PROTEIN OF THE RAT LIVER

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(Received February 4, 1977)

Summary A study was made on whether or not the lability of protein generally correlates with its metabolic turnover rate. Two fractions of soluble protein were prepared from rat liver. Using the double isotope method, it was revealed that the fraction precipitated with half-saturated ammonium sulfate had a larger turnover rate than the supernatant one. Both dietary protein depletion and starvation, though they significantly decreased total protein content, did not affect the ratio of the amount of protein in the precipitate to that of the supernatant fraction. These results suggested that both fractions, though they had a different turnover rates, were equally influenced by dietary protein depletion or fasting. In consequence, the results imply that the lability of protein does not necessarily in parallel with the metabolic turnover rate.

It has been recognized that tissue protein contents are affected by dietary protein levels. That protein which can be reversibly depleted and repleted is called "labile protein" because of its lability, or "reserve protein" in view of its function. Though it accounts for only a small percentage of all the body protein (I, 2), its essentiality must not be ignored because of its important role in protein nutrition. On the other hand, characterization of labile protein is very important in order to approach to question of how protein content in tissues or organs is easily altered in response to change in the nutritional status of an animal. The rapid loss of tissue protein observed in protein deficient or starved animals is ascribed to decrease in synthesis and/or increase in degradation rate of the protein.

Munro (3) has stated that the pattern of susceptibility of various tissues to

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protein depletion reflects, in general, the readiness with which the protein of the tissues incorporates labelled amino acids. Though the susceptibility of an individual protein has not yet been elucidated, it is allegedly accepted that, the higher turnover rate of the protein, the more labile it is under protein depletion. In other words, the protein having a large degradation rate in the normal steady state is also rapidly lost from a tissue during protein deprivation or starvation. As loss of protein occurs, when the rate of degradation exceeds that of synthesis, the protein which is lost easily has a high degradation rate in the non-steady state. However, since both synthesis and degradation rates of protein change considerably in the non-steady state (4–6), the magnitude of alteration in the synthesis and degradation rates under such a condition may not always correlate the degradation rate in the normal steady state. CHEE and SWICK (7) doubt the assumption that, while the rate of enzyme synthesis changes instantly, the fractional rate of degradation remains constant even in a transient state of metabolic perturbation. Consequently, it is necessary to estimate degradation rate of protein in the steady and non-steady states so as to clarify the relationship between lability, which means variability of the amount of tissue protein in response to change in the level of dietary protein, and turnover, which is defined as synthesis and degradation of protein in the normal steady state.

The aim of the present work is to elucidate the relationship described above. We measured the effect of protein depletion or fasting on synthesis and degradation rates of two protein fractions of the rat liver, unlike GARLICK et al. (8), who used liver protein as a whole. First, the turnover rate of soluble liver protein fractionated with ammonium sulfate was measured with the double isotope method in the normal steady state. And then the variations of the amount of these two protein fractions were investigated in protein deficient or starved rats instead of direct measurement of the degradation rate of the proteins. We found out no relationship between relative turnover rate and lability of soluble protein of the rat liver.

EXPERIMENTAL

1. Animals

Experiment 1. Male Wistar rats weighing about 240 g were housed individually in wire-bottom cages at 23°C with a 12-hour light-dark cycle. They were fed on the control diet (Table 1) for one week. The animals were injected intraperitoneally with 10 μCi of 14C-(U)-L-leucine (298 mCi/mmole; Daiichi Chemical Reagent Co.) per 100 g of body weight. Four days after the initial injection, 57 μCi of 3,5-3H-L-leucine (25 Ci/mmole; Daiichi Chemical Reagent Co.) per 100 g body weight was given intraperitoneally to the same animals. The animals were killed by heart puncture four hours after the final injection.

Experiment 2. After male Wistar rats weighing 280–350 g were fed on the control diet for 7 days, one group of the animals was fed on the protein-free diet
Table 1. Composition of test diets (%).

| Ingredients       | Control diet | Protein-free diet |
|-------------------|--------------|-------------------|
| a-Starch          | 69.7         | 90                |
| Casein            | 20           | 0                 |
| Soybean oil       | 5            | 5                 |
| Salt mixture b    | 4            | 4                 |
| Vitamin mixture c | 1            | 1                 |

* 0.3% DL-methionine was supplemented.

b Purchased from Tanabe Amino Acid Research Foundation.

c Purchased from Tanabe Amino Acid Research Foundation. 100 g of each diet contained 400 I.U. of vitamin A and 100 I. U. of vitamin D.

for 8 days and the other was starved for 4 days. Drinking water was given *ad libitum*. Then animals were sacrificed as in Experiment 1.

2. Preparation of soluble protein of the liver

A rat had scarcely been sacrificed before the liver was perfused through the portal vein with cold physiological saline and excised. Soluble protein of the liver was prepared mainly according to the DICE's method (9). The liver was homogenized in two volumes of ice cold 0.5 m NaCl in 50 mM sodium phosphate buffer, pH 7.6, with a Potter-Elvehjem homogenizer. The homogenate was centrifuged first at 1,000 $\times$ g for 30 min and then 105,000 $\times$ g for 1.5 hr at 4$^\circ$C. The resultant supernatant was treated with an equal volume of saturated ammonium sulfate solution (pH 7.0) for 1.5 hr at 4$^\circ$C and centrifuged at 10,000 $\times$ g for 30 min. The precipitate was washed twice with ice cold half saturated ammonium sulfate solution and dispersed in cold distilled water. Both supernatant and precipitate fractions were reprecipitated by addition of an equal volume of 20% (w/v) trichloroacetic acid (TCA). The precipitates were treated with 5% TCA at 95$^\circ$C for 5 minutes, washed with chloroform–ethanol (3: 1), then with acetone–ethanol (3: 1), and finally with ethyl ether. The samples dissolved in small amount of N-NaOH were measured for radioactivity or protein content.

Protein was determined by the micro-biuret method (10) with bovine serum albumin as a standard.

3. Determination of radioactivity

An aliquot of the sample was mixed with NT-scintillation fluid (11) in a vial and measured for its radioactivity with a Horiba LS-500 scintillation spectrometer. Counting efficiencies for $^3$H and $^{14}$C determined with the internal standard were 32% and 70%, respectively. The percentage of spillover of $^3$H into the $^{14}$C channel was less than 0.1%. Counting error was 5% or less.

RESULTS AND DISCUSSION

The specific radioactivities of $^3$H and $^{14}$C of soluble protein of the liver are
shown in Table 2. The specific radioactivity of $^{14}$C was somewhat low, because a 4-day interval between the first and the second administration of isotopes was used in this experiment, and because the dose of $^{14}$C-leucine was less than that of $^{3}$H-leucine. Although the ratios of $^{3}$H/$^{14}$C in the supernatant and the precipitate fraction are different in each animal, the precipitate fraction has a higher $^{3}$H/$^{14}$C ratio than the supernatant one. This would mean that the precipitate fraction turned over faster than the supernatant one. This result coincides with the observation in the preliminary experiment obtained with the pulse labelling technique (unpublished data).

There are several assumptions inherent to the use of the double isotope technique (12). Among them, the assumption about reutilization of isotope is not valid in this experiment, for the isotopes such as $^{14}$C-leucine and $^{3}$H-leucine were used in our study. But GLASS and DOYLE (13) pointed out that the double isotope method yields an accurate value of degradation rate at least within a range of half-lives of about 1–7 days, though this method can not exclude the reutilization of isotopes. Since turnover rates of the precipitate and supernatant fractions were in this range, the difference in the ratio of $^{3}$H/$^{14}$C seems to reflect that in the turnover rate of the two fractions. In this respect, further experiments with isotopes which can not be reutilized must be carried out.

The result is susceptible to several interpretations because the fractions obtained are a mixture of heterogeneous proteins: the precipitate fraction may be composed of the protein having the rapid turnover rate; the seemingly rapid turnover rate of the precipitate may be attributed to the presence of minor but highly labelled protein; the difference in the relative turnover rates of these two fractions may be reflect the difference in molecular size of proteins composing these two fractions, for there is a general correlation between the relative turnover rates and the molecular size of the soluble protein from the rat liver (9). These questions can not be answered by this experiment alone.

FUNABIKI and KANDATSU (14) demonstrated that the sarcoplasmic protein fraction precipitated with half-saturated ammonium sulfate has a high turnover rate than the supernatant one. The turnover rate of serum globulin is larger than that of serum albumin in rat (15). DICE and GOLDBERG (16) has reported that half-lives of proteins are also related to their isoelectric points. These observations and our present data suggest that the protein prepared with a certain method are degraded similarly.

Next, we intended to investigate the variations of the amount of these two protein fractions under protein deficiency or fasting. If a protein having the higher turnover rate is more labile, the amount of the precipitate fraction will be more decreased compared with that of the supernatant fraction. Even though the total protein of the liver significantly decreased during protein depletion, the decrease in a specific protein fraction has not yet been reported so far.

The body and liver weights of each group are shown in Table 3. The body
Table 2. Relative turnover rate of soluble protein of the rat liver fractionated with ammonium sulfate. Rats were injected intraperitoneally with 10 μCi of 14C-leucine and, 4 days later, with 57 μCi of 3H-leucine. Four hours after the final injection, rats were killed and soluble protein of the liver was prepared. Soluble protein was fractionated with half-saturated ammonium sulfate into two parts, viz. precipitate and supernatant. The radioactivity of the sample mixed with toluene-based scintillation fluid was counted with a Horiba LS-500 scintillation spectrometer.

|        | Precipitate |        | Supernatant |        |
|--------|-------------|--------|-------------|--------|
|        | ^3H (dpm/mg protein) | ^14C | Ratio (^3H/14C) | ^3H (dpm/mg protein) | ^14C | Ratio (^3H/14C) |
| Rat A  | 2,2459      | 631    | 36          | 11,997 | 405 | 30 |
| Rat B  | 1,9696      | 771    | 26          | 12,850 | 695 | 18 |
| Rat C  | 2,4152      | 783    | 31          | 11,559 | 469 | 25 |
| Average| 31          |        |             | 31     |    |    |

Table 3. Body and liver weights of rats fed on the protein-free diet for 8 days or starved 4 days.

| Groups       | Body weight |        | Liver weight |        |
|--------------|-------------|--------|--------------|--------|
|              | Initial (g) | Final (g) | (g)          |
| Protein-free | 275 ± 4     | 264 ± 5 | 8.5 ± 0.6    |
| Control      | 277 ± 4     | 299 ± 3 | 10.4 ± 0.3   |
| Fasting      | 373 ± 7     | 314 ± 7 | 7.1 ± 0.2    |
| Control      | 373 ± 4     | 371 ± 3 | 9.3 ± 0.2    |

Each value is the mean of rats with standard error.

Table 4. Changes in protein content in the liver of rats fed on the protein-free diet for 8 days or starved for 4 days.

| Groups       | Total protein (mg) | Soluble fraction |        |        |        |
|--------------|--------------------|------------------|--------|--------|--------|
|              |                    | Total (mg)       | Precipitate (mg) | Supernatant (mg) |
| Protein-free | 1,064 ± 88*        | 456 ± 44*        | 278 ± 30* | 211 ± 18* |
| Control      | 1,605 ± 75         | 671 ± 11         | 345 ± 32 | 267 ± 21 |
| Fasting      | 1,124 ± 33*        | 393 ± 6*         | 234 ± 11* | 164 ± 10* |
| Control      | 1,396 ± 87         | 542 ± 21         | 291 ± 12 | 253 ± 29 |

Each value is the mean of four to five rats with the standard error.

* Significantly different from control groups (p < 0.05).

weights in protein-free diet and starved groups were 12% and 16% less than that of the control group, respectively. The liver weights were also 18% and 24% less, respectively. The protein content of the liver is shown in Table 4. The content of the total protein in the protein deficient group was 33% lower than that of the con-
trol. These results were very similar to the findings of Konno et al. (17) reported previously. The content of total soluble protein was also significantly low ($p < 0.05$) in both groups. But the ratio of the amount of protein in the precipitate to that of supernatant was 1.32 in the protein deficient group and 1.29 in the control group. The ratios in the starved group and in its control group were 1.43 and 1.15, respectively. The differences in the ratios between the experimental and the control groups were not significant ($p > 0.05$). The result suggests that there is a rapid loss of protein from the liver under protein depletion or fasting, but it does not indicate that the precipitate fraction loses protein more rapidly than the supernatant fraction during the experimental period. These results agreed with many findings that there was no appreciable difference in the percentage of proteins lost from the rat liver. The first experiment showed that the precipitate fraction has a larger turnover rate than the supernatant. Both fractions, however, were affected similarly by protein depletion or fasting. From these two observations, we concluded the idea that the lability of a protein reflects largely its own turnover rate is not always valid. Several findings seem to support our conclusion. For example, the serum albumin, which has a smaller turnover rate, is most labile among several serum proteins (18). The idea that the protein having the large degradation rate in the normal condition is apt to be lost in tissue in protein depletion is based on the assumption that the rate of degradation in the normal steady state remains unchanged even in the non-steady state. However, the loss of protein in the early stage of protein depletion is due to increase in the degradation rate (8). The rate of synthesis (19, 20) and the rate of degradation as well (21) were influenced by protein depletion.

Since the proteins studied here are heterogeneous, many problems must be solved. One of them is the relationship of lability and turnover rate of an individual protein. Many factors are related to the control system of synthesis and degradation of tissue proteins. It is important to know how these factors play the role in degradation and synthesis of protein in the steady state and how they alter the rate of synthesis or degradation in a transition state caused by protein depletion or fasting.

We thank Dr. R. Funabiki for his helpful criticism.

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