REVISTA ESPAÑOLA DE FISIOLOGIA, 38, 71-78. 1982

Myofibrillar Protein and Collagen Breakdown in Mature Male Rats Fed Protein or Energy Deficient Diets

S. Santidrián *

Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts, 02139 (USA)

(Received on May 26, 1981)

S. SANTIDRIAN. *Myofibrillar Protein and Collagen Breakdown in Mature Male Rats Fed Protein or Energy Deficient Diets.* Rev. esp. Fisiol., 38, 71-78. 1982.

The urinary output of N^{τ}-methylhistidine (3-methylhistidine: 3-Mehis) and total hydroxy proline (OH-pro) were evaluated to assess the effect of a low-protein diet (low-P) or a low-energy diet (low-E), given for 21 days, on the rates of myofibrillar protein and collagen breakdown in mature male rats. As compared to the control rats, the animals fed the deficient diets showed a significant reduction in the rate of growth and plasma insulin. No major changes were found in the weight of several muscles excised from the animals at the end of the experiment. Total OH-pro was significantly reduced in the low-P rats and 3-Mehis was unchanged when results were normalized to either muscle weight or creatinine output. In conclusion, collagen breakdown was found to be very sensitive to protein depletion in the mature rat, but myofibrillar protein breakdown was not notably affected by protein or calorie malnutrition.

It is now generally acknowledged that skeletal muscle is a major tissue in whole body protein metabolism (27) and that both dietary and hormonal factors play an important role in the regulation of the rates of muscle protein development and metabolism (17, 18, 23). WATERLOW and STEPHEN (21) showed a large reduction in protein synthesis in skeletal muscle when rats were fed low-protein or protein-free diets. Refeeding an adequate diet to protein-depleted rats restored greatly the rates of muscle protein synthesis and catabolism (7). Analogous results have been reported by other investigators (27). In a recent study (17) we have shown that the rate of myofibrillar protein breakdown, as measured by the urinary output of N^{τ}-methylhistidine, was transiently increased in young rats fed an energy-depleted diet; this increase was immediately followed by a continuous and progressive reduction in the excretion of the amino acid throughout the experimental period. On the other hand, it has been reported

* Present address: Dep. de Fisiología (División de Nutrición), Universidad de Navarra, Pamplona (Spain).

that diets can act as modulators of aging and longevity (28) and several investigators have studied the effects of aging and nutrient requirements on the rate of muscle protein metabolism (26).

The purpose of this study was to investigate further the effect of both protein and energy malnutrition on the rate of myofibrillar protein breakdown in mature rats. Furthermore, since dietary factors can also affect the rate of collagen metabolism (20), we will report data on the effect of protein and calorie malnutrition on the rate of collagen metabolism by monitoring the urinary excretion of total hydroxyproline (17). Myofibrillar protein breakdown was estimated by measuring the rates of urinary output of N^{τ}-methylhistidine (3-methylhistidine). The vast majority of the N^{τ}-methylhistidine formed in the body is present in skeletal muscle; methylation of histidine occurs after its incorporation into the peptide chains of actin and myosin, and after catabolism of these proteins, the liberated N^{τ}-methylhistidine is not recycled or metabolized, but quantitatively excreted from the body chiefly via urine both in rats and humans. The total amount of N^{τ}-methylhistidine excreted in the urine has, therefore, been proposed as an *in vivo* index of muscle protein catabolism rates (2, 6, 13, 27).

Materials and Methods

Animals and experimental design. Sprague-Dawley intact mature male rats (Charles River Breeding Laboratories, Wilmington, Ma., USA), weighing about 370 g were housed in non-metabolic cages. For three days they were allowed to eat *ad libitum* a purified diet containing 18 % lactalbumin, so that the animals could adapt to the new diet and sorroundings. Then, animals were randomly assigned into three dietary groups of five rats each, and placed into metabolic cages. These groups were arranged as follows: a control group was fed the adequate diet mentioned above; a second group was fed a low-protein diet (1 % lactalbumin), but adequate in energy; and the last group was fed a low-energy diet (half of the carbohydrate content of the adequate diet), but adequate in protein (36 % lactalbumin).

Both adequate and low-protein diets were given *ad libitum*. The energy deficient diet was fed half the amount consumed by rats on the adequate diet; since the low-energy diet has 36 % of protein, the total protein intake in this group was the same as on the control group fed the adequate diet. Composition of diets is given in table I. All rats were fed on the respective experimental diet for 21 days. Body weight changes and food intake were recorded daily.

Complete 24 h urine collections (under 0.1 ml of toluene as preservative) were obtained throughout the experiment, but analyses were performed in samples taken each three days and pooled within each dietary group. At the termination of the experiment, rats were killed by decapitation. A sample of blood was then taken from the decapitated body in chilled heparinized tubes. Immediately, livers and gastrocnemius, soleus, tibialis anterior and extensor digitorum longus muscles from hind limbs were carefully excised and weighed.

Urinary analyses. The urines were centrifuged after collection to remove food particles and occasional pellets. Urinary N^{τ}-methylhstidine was determined with the aid of a Beckman 121 amino acid analyzer as previously described by BILMAZES *et al.* (1). Urinary urea-N and creatinine were determined by the methods of FOSTER and HOCHOLZER (4) and OWEN *et al.* (14) respectively. Total hydroxyproline was determined by the method of KIVIRIKKO *et al.* (9) as described in detail by IBBOTT (8).

Plasma insulin determination. Once blood was collected, it was allowed to clot for 1 h, and then centrifuged for 15 min at 2000 rpm. The separated plasma was stored at —20° C until analysis. Plasma insulin concentration was determined by radioimmunoassay method using a kit purchased from Bio-Rad, Louisville, KY., USA.

Statistical procedures. Statistical evaluations were carried out by conventional one- and two-way analysis of variance. Least significant differences were calculated in order to identify differences between means and are given in tables II and III and in figure 1.

Results and Discussion

As pointed out earlier in this study, the urinary output of N^{τ}-methylhistidine is attractive because it reflects the average response of the whole musculature, in contrast with techniques which rely on one or a few muscles (27). However, it has been claimed (12) that non-muscle sources may contribute to a major part of the N^{τ}-methylhistidine excreted in the urine.

Elsewhere, we (4, 20) have concluded from a detailed analysis of the literature on muscle protein turnover and from new experimental data that urinary N^{τ}-methylhistidine is indeed largely derived from skeletal muscle. Furthermore, in a recent paper, HARRIS (6) reported data supporting the validity of N^{τ}-methylhistidine as an *in vivo* index of the rate of myofibrillar protein breakdown in the rat, pointing out serious doubts on the conclusions reached by MILLWARD *et al.* (12) on the turnover rate of muscle protein actin and myosin. These rates have been considerably underestimated by those investigators, and when realistic rates of myofibrillar protein turnover were used, muscle tissue does remain the major contributor of N^{τ}-methylhistidine in urine. Although a considerable amount of evidence (2, 7, 13, 16, 17) confirms the strong relationship between alterations in N^{τ}-methylhistidine output and changes in the rate of myofibrillar protein breakdown, a definitive conclusion about the sources of urinary N^{τ}-methylhistidine may require estimates of release from various individual tissues (specially skin and intestine) by measuring arterio-venous differences. In this paper, the data on N^{τ}-methylhistidine are reported as a total output per animal, and per 100 g body weight (table III, fig. 1). The latter parameter allows for differences in size due to effects of growth, but does not correct for changes in the composition of the body as a whole, or the proportion of muscle to other tissues. Accordingly, the data are also expressed per 100 g weight of the four muscles dissected out at the termination of the experiment and taken to represent the relative size of total skeletal musculature. To provide another index of metabolism

Table I. *Diet constituents: entries are g % dry diet.*

Adequate and low-protein diet were fed *ad libitum*. The low-energy diet was fed at one half the amount of adequate diet consumed by the control animals. The mineral mix was purchased from General Biochemicals, Chagrin Falls, Ohio, USA. Composition of mineral and vitamin mixtures is as described by ROGERS and HARPER (15). Choline was added to the diets as an aqueous solution containing 1 g of choline hydrochloride/5 ml. The corn oil was obtained from Wesson Oil Co., Fullerton, Calif., USA.

Component	Adequate	Low-protein	Low-energy
Dextrine	44.2	55.5	28.4
Sucrose	22.1	27.8	14.2
Lactalbumin	18.0	1.0	36.0
Mineral mix	5.0	5.0	5.0
Vitamin mix	0.5	0.5	0.5
Choline	0.2	0.2	0.2
Corn oil	10.0	10.0	10.0
Agar	4.0	4.0	4.0
Water	100.0	100.0	100.0

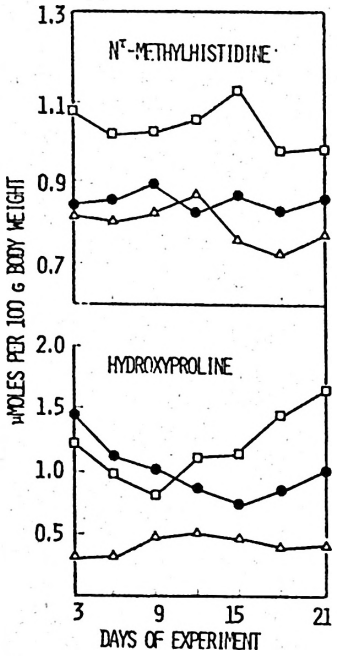

Fig. 1. *Urinary output of N$^{\tau}$-methylhistidine (3-methylhistidine) and total hydroxyproline by mature male rats fed either an adequate diet (●), or a low-protein diet (△) or a low-energy diet (□) for 21 days.*
Each point represents results of analyses of pooled samples of urine from 5 rats. Least significant differences for N$^{\tau}$-methylhistidine and total hydroxyproline are 0.12 and 0.35 respectively (two-way ANOVA) for $p < 0.05$.

related specifically with muscle and to minimize variability from daily urine excretion, creatinine output was measured.

As judged by changes in body weight (table II), results showed that both protein and calorie malnutrition caused a significant impairment ($p < 0.05$) in the slow rate of growth displayed by the mature rat. However, per unit of body weight, the weight of the four muscles excised at the end of the experiment, representing predominantly red (soleus), white (extensor digitorum longus) and mixed (gastrocnemius and tibialis) fibers, were unchanged among the three dietary groups. This fact indicated that the net reduction of weight shown by those muscles paralleled the reduction of the whole animal, and, as a consequence, their relative weight remained unchanged. The urinary output of N$^{\tau}$-methylhistidine was significantly increased ($p < 0.05$) in the energy-depleted animals, and slightly reduced in the rats fed the low-protein diet. These facts could indicate that in the mature rat protein and energy malnutrition have opposite effects on the rate of myofibrillar protein breakdown, which would be in agreement with previously reported data on muscle protein turnover in young rats (7, 20). However, when N$^{\tau}$-methylhistidine output was expressed per unit of either the sum of the four excised muscles weight or per unit of creatinine, no significant differences were found among the experimental groups. This effect indicated that the rate of myofibrillar protein breakdown was similar in the three dietary groups, and, therefore, mature rats have a different response to protein and calorie malnutrition to that seen in the young rat. This finding points out to the fact that one should be careful in interpreting data from N$^{\tau}$-methylhistidine output, in the sense that this parameter must be expressed in relation with a proper muscle reference such as creatinine output or the weight of specific muscles, and not only to total body weight.

In addition, we reported data on the calculation of fractional rates of myofibrillar protein breakdown from urinary output of N$^{\tau}$-methylhistidine in the three dietary groups. These calculations are approximations and based on data previously reported (7) for both young and mature rats (table IV). On the other hand, it should be emphasized that in the present experiment body composition of the rats did not change dramatically, an important assumption in order to apply the calculations described in table IV, with the exception of liver in the energy-depleted

Table II. *Body weight gain, food intake, organ weight and plasma insulin levels of mature male rats fed either an adequate control diet, or a low-protein (Low-P) diet or a low-energy (Low-E) diet for 21 days.*

Entries are mean values ± S.E.M. for five rats in each group. EDL, extensor digitorum longus muscle. Both organs and blood were collected at the termination of the 21-day experimental period. LSD, least significant difference (one-way ANOVA) for $p < 0.05$.

Group	Initial b. wt. g	Body weight gain g/day	Food intake g/100 g b. wt.	Organ weight/100 g body weight					Plasma insulin level μl.U/ml
				Liver g	Gastrocnemius g	Soleus mg	Tibialis mg	EDL mg	
Control	373±5	2.4±0.4	7.2±0.2	3.3±0.3	1.26±0.10	97±3	376±26	106±7	160±2
Low-P	370±4	—2.9±0.3[a]	7.0±0.7	3.4±0.4	1.24±0.09	92±6	398±30	106±3	42±3[a]
Low-E	370±5	—3.2±0.11[a]	4.3±0.3[a]	2.2±0.1[a]	1.40±0.08	111±11	417±23	116±5	39±1[a]
LSD		0.9	1.3	0.4	0.15	13	42	14	6

[a] $p < 0.05$ as compared to the animals fed the adequate diet (control) (one-way ANOVA).

Table III. *Urinary output of urea-N, creatinine, total hydroxyproline (OH-proline) and N^{τ}-methylhistidine (3-Mehis) of mature male rats fed either an adequate control diet, or a low-protein (Low-P) diet or a low-energy (Low-E) diet for 21 days.*

Entries are mean values ± S.E.M. of the pooled samples taken every three days within each group during the 21-day experimental period. LSD, least significant difference (two-way ANOVA) for $p < 0.05$.

Group	Output (mg/100 g body wt.)		OH-proline (μmoles/24 hours)		3-Mehis (μmoles/24 hours)			
	Urea-N	Creatinine	Total	Per 100 g b. wt.	Total	Per 100 g b. wt.	Per 100 g leg muscle	Per mg creatinine
Control	28±3	2.30±0.23	4.4±0.4	1.10±0.10	3.40±0.05	0.86±0.01	47±1	0.39±0.05
Low-P	29±5	2.53±0.14	1.4±0.2[a]	0.40±0.03[a]	2.64±0.10[a]	0.81±0.02	44±1	0.32±0.02
Low-E	122±9[a]	2.62±0.18	4.8±0.5	1.30±0.14	3.49±0.11	1.02±0.03[a]	50±1	0.39±0.03
LSD	21	0.45	0.9	0.35	0.29	0.12	5	0.12

[a] $p < 0.05$ as compared to the animals fed the adequate diet (control (two-way ANOVA).

rats. An analogous approach has been recently published (20). Results (table IV) correlated with those of other investigators (7, 20) and showed that as compared to the well-nourished animals, no major differences were appreciated in the mature rats fed either depleted diet. However, a slight increase in the fractional rate of myofibrillar protein breakdown (and therefore, a slight reduction in half-life) was found in the energy-depleted rats as compared to the control ones, in agreement with data reported by other investigators (7, 17, 20) that measured N^{τ}-methylhistidine as an *in vivo* index of myofibrillar protein breakdown. This observation also agreed with *in vitro* studies on muscle protein turnover (10).

Table IV. *Estimation of myofibrillar protein turnover from urinary N^{τ}-methylhistidine excretion for well-nourished (control), protein-depleted (Low-P) and energy-depleted (Low-E) mature rats.*

Muscle mass was taken to be 45 % of body weight (11), and muscle protein was taken as 17 % of muscle mass (27). Myofibrillar protein was considered to be 60 % of total muscle protein, and actin and myosin were 30 and 60 % respectively of myofibrillar protein (24). Values for N^{τ}-methylhistidine content of actin (19 μmol/g) and myosin (2 μmol/g) are taken from a previous study (27).

	Control	Low-P	Low-E
Body weight (average value for the entire experiment) (g)	396.5	341.0	338.5
Muscle mass (g/rat)	178.4	153.4	152.3
Muscle protein (g/rat)	30.3	26.1	25.9
Myofibrillar protein (g/rat)	18.1	15.7	15.5
Myosin (g/rat)	10.9	9.4	9.4
Actin (g/rat)	5.4	4.7	4.7
Total N^{τ}-methylhistidine (μmol/rat) in:			
Actin	102.6	89.3	89.3
Myosin	21.8	18.8	18.8
Skeletal muscle	124.4	108.1	108.1
Daily excretion of N^{τ}-methylhistidine (μmol/rat)	3.40	2.64	3.49
Daily proportion of N^{τ}-methylhistidine pool excreted (fractional rate of myofibrillar protein breakdown k_d) (% day)	2.7	2.4	3.2
Half-life for myofibrillar protein ($t_{1/2} = ln\ 2/k_d$) (days)[a]	25.7	28.9	21.7

[a] Time required for a given amount of protein to turn over half of this amount.

In this study, plasma insulin was measured at the termination of the experiment in all rats. As compared to the control animals, both protein and energy-depleted rats exhibited a marked reduction ($p < 0.05$) in the concentration of this hormone in plasma (table II). Insulin is a regulator of muscle protein synthesis and breakdown (23), and it has been reported that an inadequate dietary supply of protein causes changes in the protein synthesis apparatus of skeletal muscle in rats similar to those changes reported to take place in diabetic rats (22). The reduced plasma insulin levels found in the two malnourished groups in this study agrees with published evidence both in rats (5, 25)

This mild increase in the fractional rate of myofibrillar protein breakdown in the energy-depleted rats could be interpreted in the sense that after protein degradation the resulting amino acids can be utilized for resynthesis of protein, converted into other metabolites or oxidized as a source of energy. It seems possible that amino acids derived from protein breakdown in muscle could be used as an energy source to compensate the reduced energy intake in the rats fed the low carbohydrate diet. However, as judged by the significant reduction in liver weight ($p < 0.05$), as well as by the dramatic rise ($p < 0.05$) in urea-N excretion (table III), it appears clear that the liver was affected by the shortage of energy to a greater extent than skeletal muscle. Similar observations have been reported in a recent paper from this laboratory (2).

and humans (3). Nevertheless, with the present results we cannot determine whether this low plasma insulin is due to low pancreatic insulin reserve, impaired insulin release, or to alterations in the enteric receptor mechanism (25).

Finally, the rate of collagen breakdown was measured by evaluating the urinary output of total hydroxyproline. As compared to the control animals, protein-depleted rats had a marked reduction ($p < 0.05$) in the output of hydroxyproline, and, therefore, a reduced rate of collagen protein breakdown (table III). However, energy restriction in the mature rat did not have that outstanding effect on the rate of collagen breakdown. This fact was supported by the calculated fractional rates of collagen protein turnover (table V). Collagen protein was taken to be 25 % of total body protein, which is 15 % of body weight (19). Each μmol of hydroxyproline in urine was taken to represent the breakdown of 0.1 g of collagen protein (19). It should be pointed out that for the calculation of the fractional rates of breakdown in both myofibrillar and collagen protein we have taken as body weight within each experimental group the average weight of the rats for the entire experiment, and not daily values. On the other hand, fractional rates of collagen degradation are relative and based on a number of assumptions made in human studies (19, 20). Nevertheless, from these data it seems that collagen breakdown is markedly reduced by protein malnutrition in agreement with results published by other investigators (20).

Table V. *Estimation of collagen protein turnover from urinary total hydroxyproline (OH-proline) excretion for well-nourished (Control), protein-depleted (Low-P) and energy-depleted (Low-E) mature rats.*
Collagen protein was assumed to be 25 % of total body protein, which is 15 % of body weight (19). Each μmol of OH-proline in urine was taken to represent the degradation of 0.1 g of collagen protein (19).

	Control	Low-P	Low-E
Body weight (average value for the entire experiment) (g)	396.5	341.0	338.5
Total body protein (g)	59.5	51.2	50.8
Collagen protein (g/rat)	14.9	12.8	12.7
Daily excretion of OH-proline (μmol/rat)	4.4	1.4	4.8
Collagen degraded (g/day)	0.44	0.14	0.48
Daily proportion of OH-proline-pool excreted (fractional rate of collagen breakdown k_d) (% day)	3.0	1.09	3.8
Half-life collagen protein ($t_{1/2} = ln\ 2/k_d$) (days)	23.1	63.6	18.2

In conclusion, it appears that in the mature male rat collagen breakdown was very sensitive to protein malnutrition. However, the rate of myofibrillar protein breakdown was basically unchanged in either protein or energy-depleted rats, as compared to the wel-nourished rats, since no significant differences were found in the output of N^{τ}-methylhistidine when expressed per unit of muscle mass, or per unit of creatinine output.

Acknowledgement

The author wishes to express his gratitude to Miss Christine Bilmazes for the help in urinary N^{τ}-methylhistidine determinations, as well as to Drs. V. R. Young and H. N. Munro for their continuous encouragement and support. This study was carried out during Dr. S. Santidrián's leave of absence for Post-Doctoral training at MIT, from the Department of Physiology (Division of Nutrition), University of Navarra, Pamplona (Spain).

Resumen

Se determinan la excreción urinaria de N^{τ}-metilhistidina (3-metilhistidina: 3-Mehis) e hidroxiprolina total (OH-pro) con el fin de valorar el efecto causado al alimentar ratas macho

adultas con dietas bajas en proteína (b-P) o en energía (b-E) durante 21 días sobre las velocidades de degradación de las proteínas miofibrilares y del colágeno. Comparado con los animales control, las ratas alimentadas con las dietas experimentales muestran una reducción significativa en el crecimiento y en la concentración de insulina en plasma. No se encuentran cambios significativos en el peso de diferentes músculos extraídos de las ratas al final del experimento. La excreción de OH-pro total es significativamente menor en los animales alimentados con la dieta b-P, mientras que no se detectan variaciones en la excreción de 3-Mehis cuando los resultados se normalizan con los pesos de músculos o con la excreción de creatinina. Se concluye que en la rata adulta, la degradación del colágeno es muy sensible a dietas b-P, mientras que la degradación de las proteínas miofibrilares no se afecta por la malnutrición proteica o calórica.

References

1. BILMAZES, C., UAUY, R., HAVERBERG, L. N., MUNRO, H. N. and YOUNG, V. R.: *Metabolism*, 27, 525-530, 1978.
2. BURINI, R., SANTIDRIÁN, S., MOREYRA, M., BROWN, P., MUNRO, H. N. and YOUNG, V. R.: *Metabolism*, 30, 679-687, 1981.
3. CAHILL, G. F., HERRERA, M. G., MORGAN, A. P., SOELDNER, J. S., SEINKE, J., LEVIG, P. L., REICHARD, G. H. and KIPNIS, D. M.: *J. Clin. Invest.*, 45, 1751-1769, 1966.
4. FOSTER, L. B. and HOCHOLZER, J. M.: *Clin. Chem.*, 17, 921-925, 1971.
5. GREY, N. J. S., GOLDRING, S. and KIPNIS, D. M.: *J. Clin. Invest.*, 49, 881-889, 1970.
6. HARRIS, C. I.: *Biochem. J.*, 194, 1011-1014, 1981.
7. HAVERBERG, L. N. OMSTED, P. I., MUNRO, H. N. and YOUNG, V. R.: *Biochem. Biophys. Acta*, 405, 67-71, 1975.
8. IBBOT, F. A.: In «Clinical Chemistry, Principles and Techniques» (Henry, R. J., Cannon, D. C. and Winkelman, J. W., eds.). Harper and Row, publishers, Hagerstown, Maryland, 1974, pp. 565-638.
9. KIVIRIKKO, K., LAITINEN, O. and PROCKOP, D. J.: *Anal. Biochem.*, 19, 249-255, 1967.
10. LI, J. B. and GOLDBERG, A. L.: *Am. J. Physiol.*, 231, 441-448, 1976.
11. MILLER, S. A.: In «Mammalian Protein Metabolism» (Munro, H. N., ed.), vol. 3. Academic Press, New York, 1969, pp. 183-233.
12. MILLWARD, D. J., BATES, P. C., GRIMBLE, G. K. and BROWN, G.: *Biochem. J.*, 190, 225-228, 1980.
13. MUNRO, H. N. and YOUNG, V. R.: In «Proceedings Conference on Human Nutrition» (Waterlow, J. C. and Stephen, J., eds.), 1981, in press.
14. OWEN, J. A., IGGO, B., SCANDRETT, F. J. and STEWART, C. P.: *Biochem. J.*, 58, 426-437, 1965.
15. ROGERS, Q. R. and HARPER, A. E.: *J. Nutr.*, 87, 267-273, 1965.
16. SANTIDRIÁN, S., YOUNG, V. R. and MUNRO, H. N.: *Rev. esp. Fisiol.*, 36, 255-264, 1980.
17. SANTIDRIÁN, S.: *Rev. esp. Fisiol.*, 37, 23-30, 1981.
18. SANTIDRIÁN, S., MOREYRA, M., MUNRO, H. N. and YOUNG, V. R.: *Metabolism*, 30, 798-804, 1981.
19. WANNEMACHER, R. W.: In «Total Parenteral Nutrition: Premises and Promises» (Ghadimi, H., ed). John Wiley and Sons, New York, 1975, pp. 85-153.
20. WANNEMACHER, R. W. and DINTERMAN, R. E.: *Biochem. J.*, 190, 663-671, 1980.
21. WATERLOW, J. C. and STEPHEN, J. M. L.: *Clin. Sci.*, 35, 287-305, 1968.
22. WOOL, I. G., CASTLER, J. J., LEADER, D. P. and FOX, A.: In «Handbook of Physiology» (Steiner, D. F. and Freinkel, N., eds.). American Physiological Society, Washington D.C., 1972, Sect. 7, vol. 1, pp. 385-413.
23. YOUNG, V. R.: In «Mammalian Protein Metabolism» (Munro, H. N., ed.). Academic Press, New York, 1970, vol. 4, pp. 585-674.
24. YOUNG, V. R., BALIGA, B. S., ALEXIS, S. D. and MUNRO, H. N.: *Biochem. Biophys. Acta*, 199, 297-300, 1970.
25. YOUNG, V. R., VILAIRE, G., NEWBERNE, P. M. and WILSON, R. B.: *J. Nutr.*, 103, 720-729, 1973.
26. YOUNG, V. R.: In «Pharmacological Intervention in the Aging Process» (Roberts, J., Adelman, R. C. and Christofel, V. J., eds.). Plenum Press, New York, 1977, pp. 85-110.
27. YOUNG, V. R. and MUNRO, H. N.: *Fed. Proc.*, 37, 2291-2300, 1978.
28. YOUNG, V. R.: *Fed. Proc.*, 38, 1994-2000, 1979.