Variation of Proteins in Myofibrils and Connective Tissue of Chicken Red and White Skeletal Muscles Influenced by Under-nutrition[†]

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The influence of under-nutrition (sub-maintenance feeding) and *ad libitum* feeding on myofibrillar and connective tissue (collagen-like) proteins of red (leg) and white (breast) muscle of growing broilers was studied. The content of 0.3 M KCl-extractable myofibrillar proteins was lower, and that of 6 M guanidine-HCl- and 1% Triton X-100-soluble proteins and connective tissue proteins was higher in red than in white muscle of *ad libitum* fed broilers. The relative amount of some specific proteins in 0.3 M KCl-, 0.6 M KI-, and Triton X-100-extracts differed by more than 20 to 50% between red and white muscle.

Under-nutrition caused some decrease in KCl-extractable proteins of red muscle, and an increase in the connective tissue content of red muscle and in KI-, guanidine-HCl-extractable proteins, and connective tissue of white muscle. SDS-polyacrylamide gel electrophoretograms of 0.3 M KCl extracts showed that the relative concentration of 95 K protein (α -actinin) in both muscles and 59 K protein in red muscle from underfed broilers was lower than that from controls, whereas the relative content of 200 K (myosin heavy chain), 160 K, and 43 K (actin) proteins was rather high in both muscles from underfed broilers. Noticeable changes in the levels of some amino acids of connective tissue were also observed in red and white muscle as a result of under-nutrition.

The classical theory¹⁾ of protein metabolism implies that protein synthesis in adult animals is confined only to the replacement of 'wear and tear' in the tissues. However, it did not account for the metabolism of tissue protein when an animal is kept on inadequate feed. Under these circumstances endogenous protein catabolism provides essential amino acids for specific and indispensable non-protein purposes.^{2~4)} However, Borsook and Keighley⁵⁾ extended the hypothesis that intracellular protein metabolism is not restricted merely to 'wear and tear,' but it is continuously occurring even if the animal is in nitrogen equilib-

rium. This concept has been substantiated by several earlier studies in which ¹⁵N was employed as a tracer.^{6~8)} Apart from these, other investigators^{9,10)} also provided the evidence that endogenous protein continuously undergoes synthesis and breakdown not only during adequate dietary supply but also in the event of subnormal feeding.

With these observations, the concept of labile or reserve or storage protein in tissues also appeared in the literature, suggesting that this protein fraction, which is specifically catabolized when the animal has restricted feed supply, somehow is different from the other

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tissue proteins. This aspect has been discussed at length by Kosterlitz and Campbell¹¹⁾ and Munro.¹²⁾ It seems that the concept of labile protein reserve was developed on the basis of indirect evidence by examining the urinary N:S ratio. Direct tissue fractionation studies, though, indicated some reduction in intracellular proteins of skeletal muscle due to under-nutrition in different species^{13~16)}; it was not ascertained whether or not this reduction represents any discrete form of labile protein in skeletal muscle. In our preceding paper¹⁷⁾ we examined the effect of under-nutrition on different subcellular fractions of sarcoplasm, and on individual proteins in each fraction. The study did show some distinct changes in the relative concentration of several proteins in each subcellular fraction, but there was no evidence of a chemically discrete labile protein or proteins that disappeared from sarcoplasm either of red or white skeletal muscle as a result of under-nutrition. This paper covers the response of myofibrillar and connective tissue proteins of red and white skeletal muscle of broilers to inadequate nutrition.

MATERIALS AND METHODS

The experimental design, growth history of the broilers, and muscle sampling procedure have been described in previous papers.^{17,18)}

Extraction of myofibrillar proteins. A modification of our previous scheme¹⁶⁾ was followed to isolate different myofibrillar fractions (Fig. 1). The myofibrils and connective tissue residue, after the isolation of sarcoplasmic proteins,¹⁷⁾ was extracted twice with 350 ml of 0.3 M KCl in 0.1 M phosphate buffer (pH 6.4). The residue was then extracted once with 500 ml and once with 300 ml of 0.6 M KI in 20 mM phosphate buffer (pH 7.2). The cytoskeletal proteins were isolated from the KI-residue by extracting with 200 ml of 6 M guanidine-HCl solution containing 0.1 M 2-mercaptoethanol.¹⁹⁾ Finally the residue was treated with 1.0% Triton X-100 to remove persistent membrane-bound proteins.

Connective tissue (collagen-like) proteins. The residue left after Triton X-100 extraction was washed thoroughly with distilled water and divided into two equal parts. One part was treated with 0.1 M acetic acid to measure the swelling factor,²⁰⁾ and the other was dried at 110°C to a

constant weight. This represented the connective tissue content (collagen-like protein), and was also used for amino acid analysis.

Amino acid analysis. The dried sample of connective tissue protein was hydrolyzed in 6.0 M HCl at 110°C for 24 hr.²¹⁾ The amino acids in the hydrolyzate were analyzed by a Nihon Denshi model JLC-6AH amino acid analyzer. Norleucine was used as an internal standard. The amino acid composition was normalized to the number of residues/1,000 residues based on the glycine content which constituted about 1/3 of the amino acid residues of connective tissue, and varied only 0.8~3% within groups.

Other methods. The protein content of each extract was measured by the method of Lowry *et al.*²²⁾ with slight modifications.^{17,23)}

Polyacrylamide slab gel electrophoresis with SDS (SDS-PAGE) was done in a 10% acrylamide gel by the method of Laemmli.²⁴⁾

Two-ways analysis of variance, wherever appropriate, was done as mentioned before¹⁷⁾ to find the level of significance of the experimental data.

RESULTS

Myofibrillar proteins of red and white muscles and the influence of under-nutrition

The data in Table I show that the amount of 0.3 M KCl-extractable proteins was significantly lower ($p < 0.05$) in red than in white muscle of *ad libitum* fed broilers. However, KI-extractable protein content was identical in both muscles. A significant difference was also present in the level of guanidine-HCl- and Triton X-100-extractable proteins between red and white muscle. These extracts contained respectively the cytoskeletal and membrane-bound proteins beside some myofibrillar proteins. The white muscle appeared to be much lower in these proteins than red muscle ($p < 0.05$). The swelling factor of connective tissue from white muscle was greater than that from red muscle.

With the exception of a slight decrease in the content of KCl-extractable proteins and an increase in connective tissue of red muscle and in the content of KI- and guanidine-HCl-extractable proteins and connective tissue of white muscle from underfed broilers, the overall content of other extractable proteins was hardly influenced by under-nutrition in either

FIG. 1. Flow-sheet Diagram Showing the Sequential Isolation of Different Myofibrillar, Cytoskeletal, and Connective Tissue Proteins from Chicken Muscles.

type of muscles. The swelling factor of connective tissue from underfed broilers was less than that from controls but the difference was not significant.

Differences in various proteins between red and white muscle, and the influence of under-nutrition

The relative concentration of several proteins differed significantly in KCl-extract from red and white muscle of *ad libitum* fed broilers, although the content of 200 K < (more than 200 K), 200 K (myosin heavy chain), 160 K,

and 43 K (actin) proteins was identical in both muscles (Fig. 2(a)). The relative amount of 95 K (α -actinin), 59 K, and 48 K proteins was greater and that of 39 K (troponin-T) and 35 K (tropomyosin) proteins was less in white than in red muscle (Table II).

The SDS-polyacrylamide gel electrophoretograms of KI-extract (Fig. 2(b)) of red and white muscle were almost identical in all aspects, except that the concentration of a 33 K protein was 40% higher in red than in white muscle (Table II) and that a polypeptide band (~ 40 K) just below 43 K was present in breast

TABLE I. PROTEIN CONTENT OF DIFFERENT EXTRACTS OF MYOFIBRILS FROM LEG (RED) AND BREAST (WHITE) MUSCLES OF *ad libitum* FED AND UNDERFED BROILERS

Different extracts	<i>ad libitum</i> Feeding		Under-feeding	
	Leg (red muscle)	Breast (white muscle)	Leg (red muscle)	Breast (white muscle)
0.3 M KCl-extract	1,438 ± 205	1,839 ± 129 ^a	1,362 ± 148	1,807 ± 291 ^a
0.6 M KI-extract	7,223 ± 362 ^a	7,350 ± 323 ^a	7,477 ± 356 ^a	8,350 ± 121
6 M Guanidine-HCl-extract	2,140 ± 308 ^a	1,255 ± 307	2,117 ± 317 ^a	1,522 ± 363
1% Triton X-100-extract	108 ± 42 ^a	42 ± 15 ^b	110 ± 37 ^a	50 ± 23 ^b
Connective tissue (collagen-like protein)	434 ± 53	263 ± 22	1,103 ± 225	358 ± 12
Swelling factor of connective tissue (ml/g of dry weight)	210 ± 45 ^a	231 ± 11 ^b	193 ± 38 ^a	222 ± 21 ^b

The figures carrying the same letter in a horizontal line are not significantly different from each other ($p > 0.05$). Except for the swelling factor, all the data are mg/100 g muscle (wet weight).

TABLE II. RELATIVE DIFFERENCE IN CONCENTRATION OF VARIOUS MOLECULAR WEIGHT PROTEINS IN DIFFERENT EXTRACTS OF MYOFIBRILS FROM RED (LEG) AND WHITE (BREAST) MUSCLES FROM *ad libitum* FED AND UNDERFED BROILERS

Extractable proteins	Molecular weight of proteins	<i>ad libitum</i> Feeding		Under-feeding	
		Leg	Breast	Leg	Breast
0.3 M KCl-extract	200 K (MHC) ≤	N.S.D.	N.S.D.	I.*	I.*
	160 K	N.S.D.	N.S.D.	I.*	I.*
	95 K (α -actinin)	Low**	High**	D.**	D.*
	59 K	Low*	High*	D.*	N.S.C.
	48 K	Low*	High*	N.S.C.	N.S.C.
	43 K (actin)	N.S.D.	N.S.D.	I.*	I.*
	39 K (troponin-T)	High***	Low***	N.S.C.	N.S.C.
	35 K (tropomyosin)	High*	Low*	N.S.C.	N.S.C.
0.6 M KI-extract	~40 K	Low*	High*	N.S.C.	N.S.C.
	33 K	High**	Low**	I.*	N.S.C.
1% Triton X-100-extract	200 K ≤	High*	Low*	N.S.C.	N.S.C.
	90 ~ 95 K	Low*	High*	N.S.C.	N.S.C.
	<43 K	High*	Low*	N.S.C.	N.S.C.

MHC, myosin heavy chain; N.S.D., no significant difference; *, **, and *** indicate that the difference in the level of a protein was respectively more than 20, 40, and 50% between red and white muscles; N.S.C., no significant change due to under-nutrition; I. and D. indicate respectively increase and decrease in level of a protein due to under-nutrition; **, and *** indicate that the change was more than 20, 40, and 50% respectively.

muscle (it was absent in leg muscle irrespective of the nutritional state of the muscles). Similarly the SDS-PAGE profiles of the guanidine-HCl-extracts from both muscles were similar (Fig. 2(c)). In contrast, difference in the concentration of some proteins was quite visible from the SDS-PAGE patterns of the Triton X-100-extracts of red and white muscle (Fig. 2(d)). The relative concentration of 200 K ≤ and some low molecular weight fraction (<43 K) was 20% higher, and that of the 90~95 K protein was lower in red than in white muscle (Table II). Moreover, a 90 K band was present in breast, but it was absent in leg muscle Triton X-100 extract.

The SDS-polyacrylamide gel electrophoretograms in Fig. 2(a) also indicated pronounced changes in the concentration of some individual myofibrillar proteins in KCl-extracts from both red and white muscles as a result of under-nutrition. For instance, the relative amount of 95 K (α -actinin) protein in both muscles and 59 K protein in red muscle was fairly low and that of 200 K (myosin heavy chain) ≤, 160 K, and 43 K (actin) was high in both red and white muscle from underfed

broilers (Table II). The concentration of 48 K and 39 K (troponin-T) was not affected by submaintenance feeding. The SDS-PAGE profiles of KI-, guanidine-HCl-, and Triton X-100-extracts (Figs. 2(b), 2(c), and 2(d)) did not reveal any striking differences in the relative concentration of individual protein in muscles from underfed and *ad libitum* fed controls (Table II), except that the level of a 33 K protein in KI-extract was higher in red muscle from undernourished broilers than that from controls.

As mentioned in the MATERIALS AND METHODS section, we followed Laemmli's method²⁴) for SDS-PAGE in which the separation gel is 10% acrylamide. Consequently the proteins with molecular weight greater than 200 K were not expected to be separated on this gel. Thus such cytoskeletal proteins as connection (titin) and nebulin, which are extractable in guanidine-HCl buffer, possibly remained in the top concentrating (4% acrylamide) gel. For that matter, it was not possible to examine whether their relative amount varied between red and white muscles. The response of these proteins to under-nutrition was

TABLE III. AMINO ACID COMPOSITION OF CONNECTIVE TISSUE (COLLAGEN-LIKE PROTEIN) ISOLATED FROM RED (LEG) AND WHITE (BREAST) MUSCLE OF *ad libitum* FED AND UNDER-FED BROILERS

Amino acids	<i>ad libitum</i> Feeding		Under-feeding	
	Leg	Breast	Leg	Breast
Hydroxyproline	85 ± 0.0	90 ± 7.5	97 ± 1.0**	98 ± 1.0*
Aspartic acid	52 ± 0.0	50 ± 1.0	53 ± 3.5	48 ± 0.0
Threonine	21 ± 0.5	20 ± 1.5	20 ± 0.5	19 ± 0.5
Serine	29 ± 0.0	31 ± 1.5	29 ± 0.0	28 ± 0.0*
Glutamic acid	79 ± 0.5	76 ± 2.0	81 ± 3.0	75 ± 1.5
Proline	111 ± 1.5	109 ± 1.0	106 ± 1.5*	112 ± 5.0
Glycine	327 ± 7.5	336 ± 3.0	324 ± 5.0	337 ± 10.0
Alanine	99 ± 3.0	97 ± 1.0	104 ± 1.0*	98 ± 1.5
Valine	25 ± 1.0	23 ± 1.5	26 ± 0.5	22 ± 0.5
Half-cystine	N.D.	N.D.	N.D.	N.D.
Methionine	9 ± 0.0	9 ± 0.0	8 ± 0.1	9 ± 0.5
Isoleucine	16 ± 1.0	16 ± 0.5	17 ± 1.0	14 ± 0.0
Leucine	31 ± 1.0	29 ± 1.0	32 ± 1.5	28 ± 0.0
Tyrosine	6 ± 0.0	7 ± 0.0	6 ± 0.5	7 ± 0.0
Phenylalanine	16 ± 1.0	15 ± 1.0	17 ± 1.0	15 ± 0.0
Hydroxylysine	8 ± 0.0	9 ± 0.0	8 ± 0.0	9 ± 0.0
Lysine	27 ± 0.0	28 ± 0.5	21 ± 2.0**	26 ± 0.5**
Histidine	6 ± 0.0	6 ± 0.0	6 ± 0.0	6 ± 0.5
Arginine	53 ± 1.0	49 ± 0.0	48 ± 1.0*	49 ± 1.5

N.D., not determined. Only those mean values of leg or breast muscles containing star (* $p < 0.05$, ** $p < 0.01$) were significantly different from respective controls.

also not clear.

Amino acid composition of collagen-like protein of red and white muscle, and the influence of under-nutrition

Although the total connective tissue (collagen-like protein) content was significantly higher ($p < 0.05$) in red than in white muscle (Table I), there was little difference in amino acid composition of the protein between these muscles, except that the arginine content was higher ($p < 0.05$) in the protein from red than from white muscle (Table III). The total hydrophilic and hydrophobic amino acid balance was the same in the proteins from red and white muscle.

The content of connective tissue was higher in both muscles from underfed than from well-fed broilers (Table I). Besides quantitative changes, conspicuous changes in some amino acid residues also occurred in connective tissue due to under-nutrition. The data in Table III show that the number of hydroxyproline and

alanine residues was higher, and that of proline, lysine, and arginine was lower in connective tissue of red muscle from underfed broilers than that from *ad libitum* controls. Similarly, the number of hydroxyprolines was greater, and that of serines and lysines was less in connective tissue of white muscle from undernourished chickens than from the controls. Despite this, the hydrophilic and hydrophobic amino acid balance in connective tissue was not affected by under-nutrition. However, the number of essential and charged amino acids decreased, and that of hydroxy and non-essential amino acids increased to some extent. The connective tissue of white muscle from underfed broilers contained more imino acids and less essential amino acid residues than from the *ad libitum* fed broilers.

DISCUSSION

While discussing the effects of under-nutrition, Peret and Jacquot²⁵⁾ concluded that de-

pletion of endogenous nitrogen occurs in three phases when an animal is kept on below-subsistence feed. Initially, the so-called labile protein reserve is catabolized to maintain vital body functions. In the second phase, the degradation of metabolizable protein in the tissue starts. Finally, the terminal phase which correspond to the pre-mortal rise in nitrogen excretion, is a signal for initiating the catabolism of fixed protein from the tissue. However, the results presented in this paper together with those in our preceding communication¹⁷⁾ suggest that no single protein or fraction of muscle proteins can be categorized as reserve protein in the sense of being chemically distinct from the basic cellular proteins, and which disappears from muscle system of underfed animals. The changes that are observed in the relative concentrations of several proteins in skeletal muscles as a result of under-nutrition in this study, appear to be more of the nature of a metabolic adaptation to cope with adverse nutritional constraints. Some other studies also found no evidence for the presence of labile reserved protein in mammalian liver.^{26~28)}

The consensus of most investigators is that the rate of protein synthesis is higher in oxidative red muscle than in glycolytic white muscles.^{29~34)} However, divergent findings have been reported on the issue whether or not the turnover rate of sarcoplasmic and myofibrillar proteins in muscle is identical. While Loble and Lovie³⁵⁾ found the same turnover rate, others^{36,37)} reported faster turnover rates for sarcoplasmic than myofibrillar proteins. Even the different myofibrillar proteins such as myosin subunits, actin, and tropomyosin are shown to turn over at different rates.^{38,39)} Myosin heavy chains are renewed faster than the actin.⁴⁰⁾ These differences may explain the differential response of different proteins in red and white muscle to under-nutrition we observed.

Several studies have indicated that the rate of protein synthesis in fast glycolytic white muscles is greatly decreased by nutritional restriction, but in slow oxidative red muscles the rate is not affected.^{33,41~44)} This general-

ization seems to hold for sarcoplasmic proteins in white muscle,¹⁷⁾ but it may not apply to myofibrillar proteins. There is also evidence that relatively slowly turning over myofibrillar proteins are more sensitive to nutritional deficiency than sarcoplasmic proteins.^{40,44,45)} This seems to hold for myofibrillar protein in red muscle, and may not be applicable to that in white muscle. The guanidine-HCl extractable proteins in white muscle also seem to be sensitive to nutritional conditions whereas those in red muscle were not affected (Table I).

Although a pulse-labeling study by Low and Cerauskis⁴⁶⁾ did not indicate any differences in the synthesis rates of myosin, actin, and actomyosin in rat skeletal muscle in response to nutritional conditions, other investigators^{40,47)} have shown that the synthesis rate of actin was especially influenced by the nutritional state of the animal. In contrast, our study suggests that 95 K (α -actinin) and 59 K protein were affected by nutritional stress, whereas the content of actin and myosin heavy chain was higher in skeletal muscles of underfed broilers than that of *ad libitum* fed controls. Since the absolute weight of leg and breast muscles from underfed broilers were about 30~35% less than those from control broilers, the overall development of these muscles was indeed affected adversely by under-nutrition. However, the available nutrition seems to be used to maintain the critical concentration of different proteins according to the metabolic and physiological needs of the muscles. This is apparent from the differential changes in the concentration of several proteins that occurred in red and white muscle in response to nutritional stress.

The difference in swelling factors of collagen-like protein between red and white muscle (Table I) and the variation of amino acid composition of the protein due to the nutritional state of the broilers may be ascribed to the types of collagen, which differ in amino acid composition and in other aspects. There are at least four isoforms of collagen, collagen types I, III, IV, and V in skeletal muscle^{48,49)} and they predominate respectively in epi-

peri-, and endo-mysium,⁵⁰⁾ and the basement membrane of muscle fibers.^{51,52)} Since the diameter of white fibers is larger than that of red fibers,⁵³⁾ this difference is likely to affect the proportion of different collagen types in muscle. The metabolism of one type of collagen may be more sensitive to nutritional stress than the other types. However, this aspect requires further investigation.

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REFERENCES

- 1) O. Folin and W. Denis, *J. Biol. Chem.*, **11**, 87 (1912).
- 2) T. B. Osborne and L. B. Mendal, *J. Biol. Chem.*, **17**, 325 (1914).
- 3) E. V. McCollum, N. Simmonds and W. Pitz, *J. Biol. Chem.*, **29**, 341 (1917).
- 4) H. H. Mitchell, W. B. Nevens and F. E. Kendall, *J. Biol. Chem.*, **52**, 417 (1922).
- 5) H. Borsook and G. L. Keighley, *Proc. Roy. Soc. London*, **B118**, 488 (1935).
- 6) R. Schoenheimer and S. Ratner, *Ann. Rev. Biochem.*, **10**, 197 (1941).
- 7) M. Heidelberger, H. P. Treffers, R. Schoenheimer, S. Ratner and D. Rittenberg, *J. Biol. Chem.*, **144**, 555 (1942).
- 8) H. Tarver and C. L. A. Schmidt, *J. Biol. Chem.*, **146**, 69 (1942).
- 9) W. J. Kerr, S. H. Lurwitz and G. H. Whipple, *Am. J. Physiol.*, **47**, 356 (1918).
- 10) A. T. Cameron and F. A. Sedirak, *Am. J. Physiol.*, **58**, 7 (1921).
- 11) H. W. Kosterlitz and R. M. Campbell, *Nutr. Abstr. Rev.*, **15**, 1 (1945).
- 12) H. N. Munro, "Mammalian Protein Metabolism," Vol. 1, ed. by H. N. Munro and J. B. Allison, Academic Press, New York, 1964, p. 381.
- 13) J. C. Waterlow and C. B. Mendes, *Nature (London)*, **180**, 1361 (1957).
- 14) E. M. Widdowson, J. W. T. Dickerson and R. A. McCance, *J. Nutr.*, **14**, 457 (1960).
- 15) A. Asghar and N. T. M. Yeates, *Agric. Biol. Chem.*, **43**, 437 (1979).
- 16) A. Asghar, A. M. Pearson, W. J. Magee, M. A. Tahir, *J. Nutr.*, **111**, 1343 (1981).
- 17) A. Asghar, J.-I. Morita, K. Samejima and T. Yasui, *Agric. Biol. Chem.*, **50**, 1931 (1986).
- 18) A. Asghar, J.-I. Morita, K. Samejima and T. Yasui, *Agric. Biol. Chem.*, **48**, 2217 (1984).
- 19) K. Maruyama, S. Kimura, K. Ohashi and Y. Kuwano, *J. Biochem.*, **89**, 701 (1981).
- 20) A. Asghar and N. T. M. Yeates, *Agric. Biol. Chem.*, **38**, 1851 (1974).
- 21) D. H. Spackman, W. H. Stein and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).
- 22) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
- 23) G. L. Peterson, "Methods in Enzymology," Vol. 91, ed. by C. H. W. Hirs and S. N. Timasheff, Academic Press, New York, 1983, p. 75.
- 24) U. K. Laemmli, *Nature (London)*, **227**, 680 (1970).
- 25) J. Peret and R. Jacquot, "Protein and Amino Acid Functions," ed. by E. J. Bigwood, Pergamon Press, Oxford, 1972, p. 73.
- 26) J. M. Luck, *J. Biol. Chem.*, **115**, 491 (1936).
- 27) C. Dumazert and S. Grac, *Arch. Sci. Physiol.*, **1**, 339 (1947).
- 28) S. Sorof, B. Claus and P. P. Cohen, *Cancer Res.*, **11**, 873 (1951).
- 29) A. L. Goldberg, *Nature (London)*, **216**, 1219 (1967).
- 30) F. A. Short, *Am. J. Physiol.*, **217**, 307 (1969).
- 31) H. G. Leberherz, M. J. Sardo, J. K. Petell and J. E. Shackelford, "Protein Turnover and Lysosome Function," ed. by H. L. Segal and D. J. Doyle, Academic Press, New York, 1978, p. 655.
- 32) G. L. Laurent, M. P. Sparrow, P. C. Bates and D. J. Millward, *Biochem. J.*, **176**, 393 (1978).
- 33) K. N. Frayn and P. F. Maycock, *Biochem. J.*, **184**, 323 (1979).
- 34) D. J. Millward, "Comparative Biochemistry," **19B**, 153 (1980).
- 35) G. E. Lobley and J. M. Lovie, *Biochem. J.*, **182**, 867 (1979).
- 36) B. N. Perry, *Brit. J. Nutr.*, **31**, 35 (1974).
- 37) P. C. Bates and D. J. Millward, *Biochem. J.*, **214**, 587 (1983).
- 38) R. Zak, A. F. Martin, G. Prior and M. Rabinowitz, *J. Biol. Chem.*, **252**, 3430 (1977).
- 39) A. F. Martin, *J. Biol. Chem.*, **256**, 964 (1981).
- 40) P. C. Bates, G. K. Grimble, M. P. Sparrow and D. J. Millward, *Biochem. J.*, **214**, 593 (1983).
- 41) J. B. Li and A. L. Goldberg, *Am. J. Physiol.*, **231**, 441 (1976).
- 42) M. N. Goodman, M. A. McElaney and N. B. Ruderman, *Am. J. Physiol.*, **241**, E321 (1981).
- 43) M. J. Rennie, R. H. Edward, D. Halliday, D. E. Matthews, S. L. Wolman and D. J. Millward, *Clin. Sci.*, **63**, 519 (1982).
- 44) V. R. Preedy and P. J. Garlick, *Biochem. J.*, **214**, 433 (1983).
- 45) P. C. Bates and D. J. Millward, *Biochem. Soc. Trans.*, **6**, 612 (1978).

- 46) R. B. Low and P. W. Cerauskis, *J. Nutr.*, **107**, 1244 (1977).
- 47) D. J. Millward, P. C. Bates, P. Broadbent and M. J. Rennie, "Clinical Nutrition," ed. by R. I.-C. Wesdorp and P. B. Soeters, Churchill-Livingstone, Edinburgh, 1982, p. 190.
- 48) A. J. Bailey and T. J. Sims, *J. Sci. Food Agric.*, **28**, 565 (1977).
- 49) J. J. Wu, T. R. Dutson and Z. L. Carpenter, *Meat Sci.*, **7**, 161 (1982).
- 50) V. C. Duance, D. J. Restall, H. Beard, F. J. Bourne and A. J. Bailey, *FEBS Lett.*, **79**, 24 (1977).
- 51) A. J. Bailey, D. J. Restall, T. J. Sims, and V. C. Duance, *J. Sci. Food Agri.*, **30**, 203 (1979).
- 52) J. R. Sanes and J. M. J. Cheney, *J. Cell Biol.*, **93**, 442 (1982).
- 53) G. F. Gauthier, *Z. Zellforsch.*, **95**, 462 (1969).
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