Synthesis and degradation of collagens in skin of healthy and protein-malnourished rats *in vivo*, studied by $^{18}\text{O}_2$ labelling

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To explore the effects of growth retardation, caused by restricted protein intake, on collagen turnover in the whole skin, Sprague–Dawley rats ($n = 20$) were labelled with $^{18}\text{O}_2$ and fed on either an adequate (18 %) or a low (3 %) lactalbumin diet. Skin biopsies were obtained at intervals during the following 6 months. Independent groups of animals ($n = 186$) were used to determine the size of the 0.5 M-acetic acid-soluble and -insoluble collagen pools in the entire skin of healthy and malnourished rats. Collagen was estimated by measurement of hydroxyproline. Soluble-collagen synthesis rates were equivalent to $99 \pm 8 \mu\text{mol}$ of hydroxyproline/day in healthy animals and $11 \pm 2 \mu\text{mol}$ /day in malnourished rats. Insoluble-collagen synthesis rates were 32 and $5 \mu\text{mol}$ of hydroxyproline/day in the healthy and protein-depleted rats respectively. The degradation of soluble collagen amounted to 37 ± 8 and $6 \pm 2 \mu\text{mol}$ of hydroxyproline/day in the healthy and malnourished groups respectively. Efflux of collagen from the soluble collagen, defined as the sum of the rate of soluble collagen that is degraded plus that which matures into insoluble collagen, was 70 ± 8 and $11 \pm 2 \mu\text{mol}$ of hydroxyproline/day in the healthy and malnourished groups respectively. Insoluble collagen was not degraded in either group. The fraction of soluble collagen leaving the pool that was converted into insoluble collagen was 0.46 in both diet groups. It is concluded that the turnover of soluble collagen is markedly decreased with malnutrition, but degradation and conversion into insoluble collagen account for the same proportions of efflux from the soluble-collagen pool as in rapidly growing rats.

INTRODUCTION

The consequences of periods of dietary protein and energy deprivation for protein and amino acid metabolism have been explored extensively (Waterlow *et al.*, 1978). A significant focus of attention has been on the effects of protein–energy malnutrition on the synthesis and breakdown of intracellular proteins, especially those in muscle and liver. However, collagen is an extracellular protein, accounting for nearly one-quarter of total body protein, but little is known about the dynamic response of collagen metabolism *in vivo* to dietary protein and energy restriction in the host. Furthermore, skin represents a relatively large protein pool, but again its dynamic responses to various forms of malnutrition has not been investigated in detail. Hence the present studies were designed to evaluate the effect of dietary protein–energy restriction on skin collagen synthesis and degradation in the young rat. For this purpose, we have used a method involving $^{18}\text{O}_2$ labelling of the hydroxyproline of collagen (Molnar *et al.*, 1986) to determine the rates of breakdown of collagen in enriched 0.5 M-acetic acid-soluble and -insoluble fractions of skin. From estimates of the total pool size, the rates of synthesis of collagen in these fractions were determined.

METHODS

Twenty weanling male Sprague–Dawley rats (Charles River Breeding, Kingston, NY, U.S.A.) were given an adequate (18 %-lactalbumin) diet (Young *et al.*, 1971). At 23 days of age they were placed in a specially designed metabolic chamber system (Molnar *et al.*, 1987b) for 36 h and maintained on the adequate diet. During this time they were exposed to an environment of $^{18}\text{O}_2$ (63 atom % excess; Cambridge Isotopes, Woburn, MA, U.S.A.), consisting of approx. 20 % O_2 and 80 % N_2 . The animals were then removed from the chamber and a skin biopsy was obtained, as described below. At this time they were divided randomly into two groups, one of which continued on the 18 %-lactalbumin diet. The other group received a low-protein (3 % lactalbumin) diet (Molnar *et al.*, 1987a). The rats were housed in individual cages, and food intakes *ad libitum* and body weights were determined at regular intervals thereafter.

Skin biopsies (about 100 mg wet wt.) were obtained at increasing intervals throughout the study under Halothane anaesthesia and processed as previously reported (Molnar *et al.*, 1986). Briefly, the samples were freeze-dried, defatted and extracted with 0.5 M-acetic acid. By this method, ‘soluble collagen’ was defined as that

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solubilized by this extraction procedure, and 'insoluble collagen' was the insoluble pellet obtained on centrifugal separation. The enriched soluble- and insoluble-collagen samples were hydrolysed for measurement of total hydroxyproline, and a sub-sample was modified for g.l.c./mass-spectrometric analysis of the ^{18}O enrichment of hydroxyproline (Molnar *et al.*, 1986).

Changes in the size of the total soluble and insoluble collagen fractions were assessed with 186 male weanling rats fed and housed similarly to those that had been exposed to $^{18}\text{O}_2$. Whole total skin hydroxyproline in each collagen fraction was estimated by skin removal, processing in a manner similar to the biopsies and measuring hydroxyproline concentration with an automatic amino acid analyser (Beckman Model 119C). These data were used to develop predictive equations (see Molnar *et al.*, 1987a) that could be used to estimate total skin collagen pool size at the time the biopsies were obtained from the rats exposed to $^{18}\text{O}_2$. The predictive equations were as follows: for healthy well-nourished rats, the soluble and insoluble collagen pools (μmol of hydroxyproline) were: $0.414 \times 10^{-3}A^3 - 0.390A^2 + 86.9A - 1800$ and $-0.262 \times 10^{-2}A^3 + 0.793A^2 - 26.5A + 272$ respectively, where A = age of rat (days). Similarly, for the malnourished rats the soluble and insoluble collagen pools were: $0.236A + 155$ and $5.07A - 38.5$ respectively. These results have been described in detail elsewhere (Molnar *et al.*, 1987a).

Analysis of the isotope data

Kinetic modelling in these investigations was based on a two-pool model with unidirectional precursor-product relationship, as previously described (Molnar *et al.*, 1986). Since the collagen pool sizes varied substantially over the experimental period (Molnar *et al.*, 1987a), the conventional assumption of constant pool size in compartment modelling is invalid for these studies. The differential equation for the soluble-collagen pool is given by:

$$\frac{dM_s^*}{dt} = -\frac{F_2 + F_3}{V_s(t)} M_s^* \quad (1)$$

and the differential equation for the insoluble collagen pool is:

$$\frac{dM_i^*}{dt} = F_2 C_s^* - \frac{F_4}{V_i(t)} M_i^* \quad (2)$$

where the subscripts S and I denote soluble and insoluble collagen respectively, the symbol F denotes collagen flux ($\mu\text{mol}/\text{day}$), M represents mass of label, C represents concentration of label and $V(t)$ denotes collagen pool size. In writing these equations we assumed homogeneous and instantaneous mixing of the label, first-order reactions and no additional input of label into the system after $t = 0$. These differential equations are identical with those conventionally applied, but allow for variation in pool size. The solutions to these equations are as follows:

$$M_s^* = M_s^*(0)e^{-(F_2+F_3)\int_0^t V_s^{-1}(u)du} \quad (3)$$

$$M_i^* = F_2 e^{-F_4 \int_0^t V_i^{-1}(w)dw} \int_0^t e^{F_4 \int_0^u V_i^{-1}(z)dz} C_s^*(u)du \quad (4)$$

It should also be noted that, because of the variation in pool sizes, simple rate constants cannot be defined for the experimental period; however, these equations can

be used to determine the fluxes by using the pool-size data. Two observations allowed us to simplify the analysis by taking F_4 to be zero (F_4 = insoluble-collagen degradation rate, as defined in the two-pool model of Molnar *et al.*, 1986). These were: (1) for the insoluble pool, our previous studies demonstrated no apparent degradation in healthy animals, observed for as long as 395 days (Molnar *et al.*, 1986); and (2) data from the present investigation also indicated that there was no degradation of insoluble collagen in malnourished animals.

The total efflux from the soluble collagen pool, $F_s = (F_2 + F_3)$, and F_2 were determined by least-squares fitting. The fitting was carried out by using MULTICS and the IMSL FORTRAN subroutine for Levenburg-Marquardt non-linear least squares (ZXSSQ). Evaluation of the integrals used the MULTICS subroutine dOlajf. The sample variance for the measurements of isotopic enrichment were used as weights in the least-squares fitting. Goodness of fit was determined by visual inspection, minimization of the gradient norm, the size of the Marquardt parameter and by the estimate of the coefficient of variation for the parameter estimates (NAG, 1983).

The various components of collagen turnover were then determined in a sequential fashion (Molnar *et al.*, 1986). These components were: (a) soluble-pool total-collagen efflux (F_s); (b) the rate of synthesis of soluble collagen, determined as the arithmetic sum of the efflux from the soluble pool and the mass accretion of the soluble collagen (μmol of hydroxyproline) during the period of the study (F_1 = rate of soluble-collagen synthesis, as defined in the two-pool model of Molnar *et al.*, 1986); (c) the rate of synthesis of the insoluble collagen (F_2); and (d) the rate of soluble-collagen degradation (F_3), assessed as the difference between F_s and F_2 .

RESULTS

The growth and food intake of the two groups of rats has been described elsewhere (Molnar *et al.*, 1987a). The well-nourished rats that had been exposed to $^{18}\text{O}_2$ grew

Fig. 1. Change in ^{18}O enrichment (mol% excess) of hydroxyproline in soluble (●) and insoluble (○) collagen in skin of a malnourished (protein-restricted) rat

The continuous and broken lines represent the least-squares fit, as calculated in the Methods section, for changes in ^{18}O enrichment of the soluble and insoluble collagens respectively.

Fig. 2. Change in total ^{18}O (expressed as μmol of labelled hydroxyproline) in soluble (●) and insoluble (○) collagen pool of skin in a malnourished (protein-restricted) rat

at a rate equal to that of non-biopsied rats, and the growth of those receiving the low-protein diet was significantly stunted. Again, the biopsy procedure did not affect the growth response of rats given the low-protein diet. An example of an isotopic (^{18}O) curve is presented in Fig. 1 for a malnourished rat in terms of isotope enrichment of hydroxyproline in the two collagen pools throughout the period of observation. The relationships between the labelling of the soluble- and insoluble-collagen pools reveal a precursor-product relationship (Zilversmit *et al.*, 1943). The change in the total amount of ^{18}O in the two pools is shown in Fig. 2 for a protein-restricted (malnourished) rat. Similar data have previously been presented for well-nourished rats (Molnar *et al.*, 1986).

The decay curves observed in the malnourished animals (Figs. 1 and 2) were qualitatively similar to those for well-nourished animals (see, e.g., Molnar *et al.*, 1986). In each case a precursor-product relationship was evident and, in both dietary groups, there was no apparent decay of collagen in the insoluble pool (see, e.g., Fig. 2).

Initial inspection of the enrichment curve for $^{18}\text{O}_2$ in the soluble-collagen pool for healthy and malnourished rats indicated that there was a precursor pool to the soluble pool being measured (e.g. Fig. 2; Molnar *et al.*, 1986), since the enrichment of ^{18}O in hydroxyproline continued to increase for a brief period after termination of exposure of the rats to $^{18}\text{O}_2$. Thus for purposes of

Fig. 3. Change in total ^{18}O (expressed as μmol of labelled hydroxyproline) in the soluble-collagen pool of skin in a malnourished rat, showing a single (—) and two (---) exponential fit to the data

analysis we used the data that were obtained beginning 7 days after the end of the $^{18}\text{O}_2$ -exposure period.

A mono-exponential fit was found to provide an acceptable description to the ^{18}O -decay-curve data obtained for soluble collagen in the healthy rats. In malnourished animals this model was not adequate, and so the data were better approximated by a two-exponential fit (Fig. 3). Hence, in this case, total efflux rate from the soluble pool was taken to be the sum of the two estimated fluxes. The average coefficient of variation of the flux estimates based on the fitted curves was 7.7% for the healthy rats and 19% for the malnourished rats. The fact that a two-exponential fit better described the loss of ^{18}O from the soluble-collagen pool in malnourished rats suggests that the predominant collagen types of skin (I and III) may have been turning over at different rates, whereas their turnover rates appear to be similar in well-nourished animals (Klein & Chandrarajan, 1977).

Table 1 summarizes the results for the various components of collagen turnover measured in this study. The total efflux of soluble collagen from skin was equivalent to 70 ± 8 and 11 ± 2 μmol of hydroxyproline/day for healthy and malnourished rats respectively.

From 32 until 210 days of age, it can be estimated, by using the predictive equations given in the Methods section, that approx. 5750 μmol of insoluble-collagen hydroxyproline accumulated in the healthy animals (5900 μmol maximally at 180 days, minus 150 μmol at 32 days), and 900 μmol of insoluble collagen in the malnourished animals. Therefore, because there was no degradation of insoluble collagen during this same time

Table 1. Synthesis and degradation rates *in vivo* of soluble and insoluble collagens in whole skins of healthy and malnourished rats

Rates (μmol of hydroxyproline/day per rat) are expressed as means $\pm$ S.D. in the first three columns. A mean value is reported in the last column because the accumulation of the insoluble collagen (equivalent to synthesis) was based on a single prediction equation, as discussed in the Methods section (values in parentheses are percentages of total soluble-collagen efflux). Efflux = Rate of maturation into insoluble collagen + true rate of degradation.

Rats	Soluble-collagen synthesis rate	Total soluble-collagen efflux	Soluble-collagen degradation rate	Insoluble-collagen synthesis rate
Healthy	99 $\pm$ 8	70 $\pm$ 8	37 $\pm$ 8	32 (46)
Malnourished	11 $\pm$ 2	11 $\pm$ 2	6 $\pm$ 5	5 (45)

Fig. 4. Fractional efflux (% of pool per day) of soluble collagen in malnourished and healthy rats at various stages throughout the experimental feeding period

■, Malnourished rats; ■, healthy rats.

period (Molnar *et al.*, 1986), 32 μmol of soluble collagen/day matured into insoluble collagen in the healthy animals, and 5 μmol /day in the malnourished animals. The remainder of the soluble-collagen efflux must therefore represent true degradation; this rate (mean $\pm$ S.D.) was 37 $\pm$ 8 μmol /day in the healthy rats and 6 $\pm$ 2 μmol /day in the malnourished rats. On the basis of these various fluxes, 46 % of the soluble collagen leaving the pool matured into insoluble collagen in both the healthy and malnourished rats. From the present data, power-function analysis revealed that our method would have detected a difference ($P < 0.05$) of as small as 7 % for this parameter of collagen turnover.

It should be noted that in arriving at the above estimates we have assumed that the rate of maturation of insoluble collagen was constant at all ages. Our justification for this is supported by the observation that (a) the insoluble-collagen pool increased linearly over the age range of rats used in this study (see also Molnar *et al.*, 1987a) and (b) there was no measurable degradation of the collagen in the insoluble-collagen pool. Hence the maturation rate of soluble collagen can be assumed to be constant for purposes of our analysis. Similarly, the simplifying assumption of a constant rate of total soluble-collagen synthesis is supported by an essentially linear increase in the size of the soluble-collagen pool for the major portion of the study period in the well-nourished rats, and a constant pool size in malnourished rats (e.g.,

Molnar *et al.*, 1987a), together with the constant rate of maturation of soluble into insoluble collagen during this same time period.

Soluble-collagen synthesis rates were approx. 99 μmol /day in the healthy rats (Table 1). Because the size of the soluble pool remained constant in the malnourished rats, the synthesis rate is, in this case, equal to the efflux rate, 11 $\pm$ 2 μmol /day.

The fractional rate of removal of collagen molecules from the soluble-collagen pool was calculated by dividing efflux by pool size. For malnourished rats, the mean fractional rate was 0.06 day⁻¹ throughout the entire experimental period (Fig. 4). In healthy animals, this rate decreased with the increase in soluble collagen pool size (and age) of the animal (Fig. 4). Thus the fractional efflux rate amounted to 0.12 day⁻¹ at 32 days of age, declining to 0.018 day⁻¹, or about 2 %, at 150 days of age. At this stage the relative fractional rate in the malnourished rats was higher than that for the well-nourished group.

DISCUSSION

In recent years the protein dynamics of muscle and liver during nutritional stress have been the subject of considerable investigation, but studies of the changes in skin have been relatively few. However, this tissue is a quantitatively important organ, accounting for about 10–20 % of the total body weight, depending on the species, and it is second only to muscle as the largest soft tissue of the body (Widdowson & Dickerson, 1960). Furthermore, it is a potentially important protein pool, since approx. 10 % of the protein in adult human (Widdowson & Dickerson, 1960) and 25–30 % of total body protein of the rat is located in the skin (Waterlow, 1959; Waterlow & Stephen, 1966).

Although there have been a number of investigations concerned with the changes in collagen mass and tissue content with malnutrition, there are no reliable studies dealing with quantitative kinetic aspects, examining specifically the rate of skin collagen synthesis and/or degradation. A possible major reason for the limited information is the lack of an adequate method by which to measure precisely and directly collagen degradation *in vivo*.

We have reported previously (Molnar *et al.*, 1986) the reliable application of a kinetic model to study collagen kinetics *in vivo*, based on the approach proposed by Jackson & Heininger (1974, 1975). Using ¹⁸O₂ as a label,

which is incorporated into hydroxyproline as a post-translational modification of peptide-bound proline, it is possible to study collagen degradation with this non-reutilized label and so avoid the large errors that are introduced by the recycling of other labels (Molnar *et al.*, 1986).

Soluble-collagen efflux, or removal of hydroxyproline-labelled soluble collagen, in malnourished animals was found to be (in terms of hydroxyproline) $11 \pm 2 \mu\text{mol/day}$, compared with 70 ± 8 in healthy animals. The synthesis rate of soluble collagen in healthy animals was $99 \mu\text{mol/day}$, whereas in the malnourished animals the synthesis was equal to the efflux rate, $11 \mu\text{mol/day}$. Such kinetic studies have not been previously reported for collagen turnover in skin under conditions of protein-energy malnutrition. Rao & Rao (1980) found a 40% decrease of isotope incorporation into the saline-extractable collagen of skin of rats on a 6%-casein diet. Similarly, McClain *et al.* (1975) found a 33–46% decrease in incorporation of isotope into the skin of rats fed on an 8%-protein diet. Waterlow & Stephen (1966) found as much as a 75% decrease in incorporation of labelled amino acid into the skin of rats fed on a 6%-protein diet. However, these previous studies neither examined absolute rates of collagen synthesis directly nor controlled for possible differences in the size of the precursor pool, which might alter the extent to which label was incorporated into the measured pool.

Urinary hydroxyproline excretion has been used as an index of whole-body collagen turnover. Its excretion is relatively constant throughout growth and development (Waterlow *et al.*, 1978), but is increased during periods of accelerated growth (Jasin *et al.*, 1962; Picou *et al.*, 1965; Anasuya & Narasinga Rao, 1966a,b; Pappas *et al.*, 1971). Although other factors, such as glomerular filtration rate and hydroxyproline oxidation rates, may alter hydroxyproline excretion, it has been suggested that these various findings indicate increased collagen turnover during periods of rapid growth and decreased turnover when growth is stunted for various reasons. Our observations demonstrate a decrease in the absolute rates of synthesis and efflux of collagen from the acetic acid-soluble pool in response to protein-energy deficiency in the young rat.

At about 1 week after feeding of the low-protein diet, we observed that both absolute and fractional turnover rates of soluble collagen had decreased several-fold and remained at this rate for the next several months. On the other hand, in the healthy animals soluble-collagen fractional turnover decreased with growth and development. Such age-related decreases in the fractional turnover rate of proteins in the healthy animals also have been reported for muscle (Waterlow *et al.*, 1978).

On the basis of measurement of collagen pool sizes, we previously concluded that the change in the soluble-collagen pool size appears to be related, in general, to growth rate (Molnar *et al.*, 1987a). The present turnover data agree with this hypothesis; in healthy animals, fractional efflux rates were highest during periods of highest growth. On the other hand, in malnourished animals, growth rate was constant, as also was the soluble-collagen fractional efflux rate. In addition, however, we found that, despite a 6-fold decrease in soluble-collagen efflux rates, the rate of formation of insoluble collagen remained a constant percentage (about 46%) of the total efflux from the soluble-collagen

pool in both healthy and malnourished rats. In consequence, the rate of true degradation of soluble collagen remained a constant 53% of the total removal of soluble collagen from the pool under the two nutritional conditions. Previous investigators have reported that one-third to two-thirds of 0.5 M-NaCl-extractable collagen is degraded rather than being transferred to the insoluble pool in the skin of healthy animals (Nimni *et al.*, 1967; Sodek, 1977; Laurent *et al.*, 1978). However, this phenomenon has not been examined previously with respect to the effects of malnutrition. Although Rao & Rao (1980) found an 8% decrease in the rate of maturation of saline-extractable skin collagen in rats given a 6%-casein diet, their study did not control adequately for changes in the collagen pool size.

The reason for the extensive degradation of soluble collagen in relation to the maturation process is not understood. Bienkowski (1984) has suggested that this process occurs intracellularly, and in part serves as a mechanism for removal of defective molecules. It seems possible that this process may continue in an analogous fashion in the extracellular domain (e.g. see also Laurent, 1987). However, Bienkowski (1984) also suggests that about 15% of all collagen molecules that are manufactured correctly undergo degradation intracellularly. Furthermore, it has been observed that the *N*-terminal extension peptides that are cleaved from procollagen during extracellular processing of the molecule exert a feedback inhibition specific for collagen synthesis (Paglia *et al.*, 1979; Wiestner *et al.*, 1979; Horlein *et al.*, 1981; Pinnell, 1984); it could be speculated that collagen degradation at all levels might have such a regulatory effect.

Approx. 80% of the collagen molecule consists of amino acids that are gluconeogenic precursors (Miller & Gay, 1982), but it is unlikely that the degraded soluble collagen plays a significant role in supporting substrate needs for gluconeogenesis. Our estimate of soluble-collagen breakdown in the skin would imply a relatively small contribution to total-body protein turnover. For example, assuming that soluble collagen contains about 10% hydroxyproline, the degradation rate of $37 \mu\text{mol}$ of hydroxyproline/day for soluble collagen in healthy rats is equivalent to about 50 mg of collagen protein. For normal adult rats weighing 400–500 g, whole-body protein degradation is approx. 12 g/day (e.g. Waterlow *et al.*, 1978). Therefore the degradation of soluble collagen in the entire skin appears to represent less than 1% of total-body protein breakdown.

In summary, we have determined the rates of synthesis and degradation of soluble and insoluble collagens in skins of healthy and growth-restricted rats, using the non-reutilizable label $^{18}\text{O}_2$ (see Molnar *et al.*, 1986). Dietary protein restriction caused a prompt and marked decrease in the synthesis of soluble collagen and, in consequence, a decreased rate of formation of insoluble collagen. However, the synthesis of insoluble collagen accounted for about 46% of the molecules leaving the soluble-collagen pool, in both the well-nourished and growth-retarded rats. Hence, under these different nutritional conditions the formation of mature collagen fibres in skin appears to be linked closely to, and possibly regulated by, the rate at which the synthesis of soluble collagen exceeds the rate of net soluble-collagen deposition. Whether collagen pools at other sites, such as in the lung and liver, behave similarly in response to

nutritionally induced growth retardation remains to be investigated.

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REFERENCES

- Anasuya, A. & Narasinga Rao, B. S. (1966*a*) *Indian J. Med. Res.* **54**, 849–857
- Anasuya, A. & Narasinga Rao, B. S. (1966*b*) *Lancet* **i**, 94–95
- Bienkowski, R. S. (1984) *Collagen Relat. Res.* **4**, 399–412
- Horlein, D., McPherson, J., Goh, S. H. & Bornstein, P. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6163–6167
- Jackson, S. H. & Heininger, J. A. (1974) *Clin. Chim. Acta* **51**, 163–171
- Jackson, S. H. & Heininger, J. A. (1975) *Biochim. Biophys. Acta* **381**, 359–367
- Jasin, H. E., Fink, C. W., Wise, W. & Ziff, M. (1962) *J. Clin. Invest.* **41**, 1928–1935
- Klein, L. & Chandrarajan, J. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1436–1439
- Laurent, G. J. (1987) *Am. J. Physiol.* **252**, C1–C9
- Laurent, G. J., Sparrow, M. P., Bates, P. C. & Millward, D. J. (1978) *Biochem. J.* **176**, 419–427
- McClain, P. E., Wiley, E. R. & Beecher, G. R. (1975) *Nutr. Rep. Int.* **12**, 317–324
- Miller, E. J. & Gay, S. (1982) *Methods Enzymol.* **82A**, 3–32
- Molnar, J. A., Alpert, N., Burke, J. F. & Young, V. R. (1986) *Biochem. J.* **240**, 431–435
- Molnar, J. A., Alpert, N. M., Burke, J. F. & Young, V. R. (1987*a*) *Growth* **51**, 132–145
- Molnar, J. A., Vizulis, A., Miyatani, S., Cunningham, J. J., Wright, J. D. & Burke, J. F. (1987*b*) *J. Appl. Physiol.* **61**, 1582–1585
- NAG (1983) *Fortran Mini Manual, Mark II*, Numerical Algorithms Group, Downers Grove, IL
- Nimni, M. E., deGuia, E. & Bavetta, L. A. (1967) *Biochem. J.* **102**, 143–147
- Paglia, L., Wilczek, H., Diaz de Leon, L., Martin, G. R., Horlein, D. & Muller, P. (1979) *Biochemistry* **18**, 5030–5034
- Pappas, A. M., Miller, M. E., Anderson, M. & Green, W. T. (1971) *Clin. Orthop. Relat. Res.* **79**, 241–248
- Picou, D., Alleyne, A. O. & Seakins, A. (1965) *Clin. Sci.* **29**, 517–523
- Pinnell, S. R. (1984) *J. Invest. Dermatol.* **79**, 73s–76s
- Rao, J. S. & Rao, V. H. (1980) *Ital. J. Biochem.* **29**, 1–12
- Sodek, J. (1977) *Arch. Oral Biol.* **22**, 655–665
- Waterlow, J. C. (1959) *Nature (London)* **184**, 1875–1876
- Waterlow, J. C. & Stephen, J. M. L. (1966) *Br. J. Nutr.* **20**, 461–484
- Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978) *Protein Turnover in Mammalian Tissues and in the Whole Body*, North-Holland, New York
- Widdowson, E. M. & Dickerson, J. W. T. (1960) *Biochem. J.* **77**, 30–43
- Wiestner, M., Krieg, T., Horlein, D., Glanville, R. W., Fietzek, P. & Muller, P. K. (1979) *J. Biol. Chem.* **254**, 7016–7023
- Young, V. R., Stothers, S. C. & Vilare, G. (1971) *J. Nutr.* **101**, 1379–1390
- Zilversmit, D. B., Entenman, C. E. & Fishler, M. C. (1943) *J. Gen. Physiol.* **26**, 325–332

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