Turnover of Muscle Protein in the Fowl (*Gallus domesticus*)

RATES OF PROTEIN SYNTHESIS IN FAST AND SLOW SKELETAL, CARDIAC AND SMOOTH MUSCLE OF THE ADULT FOWL

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Rates of protein synthesis in skeletal, cardiac and smooth muscle of fully grown fowl (*Gallus domesticus*) were determined *in vivo* by means of the constant infusion method using [^{14}C]proline. In the anterior latissimus dorsi muscle, containing predominantly slow fibres, the average synthesis rate of non-collagen muscle proteins was $17.0 \pm 3.1\%$ per day, a value higher than that obtained for cardiac muscle ($13.8 \pm 1.3\%$ per day) and for smooth muscle of the gizzard ($12.0 \pm 1.9\%$ per day). In the posterior latissimus dorsi muscle, containing predominantly fast fibres, synthesis rates were much lower ($6.9 \pm 1.8\%$ per day). In each case these average rates for the non-collagen protein were similar to the average rate for the sarcoplasmic and myofibrillar protein fractions. The RNA concentration of these four muscles showed that relative rates of protein synthesis were determined mainly by the relative RNA concentrations. The rate of protein synthesis per unit of DNA (the DNA activity) was similar in the two skeletal muscles, but somewhat lower in cardiac muscle and gizzard, possibly reflecting the larger proportion of less active cell types in these two muscles. These quantitative aspects of protein turnover in the two skeletal muscles are discussed in terms of the determination of ultimate size of the DNA unit, and in relation to muscle ultrastructure.

There is little information about the relationship between structure and function in muscle and protein turnover. Although the rate of protein turnover in muscle has long been known to be considerably lower than in other tissues (Waterlow & Stephen, 1968; Millward & Garlick, 1972), it has also been apparent for some time that rates of protein turnover may vary considerably between different muscle types. Protein turnover has been observed to be more rapid in cardiac muscle than in skeletal muscles (Kimata & Morkin, 1971; Millward & Garlick, 1972; Garlick *et al.*, 1973a, 1975), and the rate of incorporation of labelled amino acids into individual skeletal muscles has been shown to be highest in red muscles (Citoliier *et al.*, 1967; Dreyfus, 1967; Goldberg, 1967). However, few of these studies indicate actual rates of protein synthesis and breakdown that have been obtained by reliable methods. In addition, in most mammalian skeletal muscles, mixed populations of several different fibre types are present, so that a clear distinction between muscle types cannot always be made (Franzini-Armstrong, 1973). Further, the difference between some con-

tractile and histochemical properties of the fibre populations of individual muscles becomes more pronounced during postnatal development (Shear & Goldspink, 1971; Gutmann *et al.*, 1974). As a result, the developmental stage at which measurements of rates of protein turnover are made may well affect the extent to which differences in such rates will be observed.

In the present study, we have attempted to determine the rate of muscle protein turnover in relation to fibre type. To do this we have measured rates of protein synthesis *in vivo* with the constant-intravenous-infusion method (Waterlow & Stephen, 1968; Garlick *et al.*, 1973b), infusing [^{14}C]proline into fully grown fowls. The fowl is particularly suited to such a study because of the presence of accessible muscles, which are examples of muscles that are anatomically distinct and can be distinguished by contraction characteristics, e.g. the anterior latissimus dorsi muscle, containing principally slow fibres, and the posterior latissimus dorsi, containing principally fast fibres (Hess, 1961; Asmussen *et al.*, 1969; Page, 1969). Rates of protein turnover in these two types of skeletal muscle fibre are also compared with cardiac and smooth muscle by inclusion of heart and gizzard (a red smooth muscle) in the study.

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Experimental

Fowls

White Leghorn cockerels (35 weeks old), weighing 2.1 ± 0.1 kg, were maintained on an 18% protein commercial chicken-breeder diet [Dixon and Sons (Ware) Ltd., Ware, Herts., U.K.]. Cockerels of this age have virtually ceased to grow as indicated by weight changes of less than 5% during a 3-week preliminary observation period.

Infusions

[^{14}C]Proline [The Radiochemical Centre, Amersham, Bucks., U.K.; $10\mu\text{Ci/mol}$, $3.0\mu\text{Ci/ml}$ in 0.9% (w/v) NaCl] was infused intravenously with a slow infusion apparatus (Scientific and Research Instruments, Croydon, Surrey, U.K.) at a rate of 1.43 ml/h. The infusion was via a cannula (0.5 mm internal diameter) inserted into a wing vein (vena cutanea ulnaris) at the point where it crosses the elbow joint. A second cannula (0.86 mm internal diameter) was inserted about 10 cm into the contralateral vein to enable blood samples to be obtained during the infusion. This cannula was filled with heparinized (0.25 mg/ml) 0.9% NaCl to prevent clotting. Infusions were usually started at 10:00 h and the cockerels were allowed free access to food and water throughout the infusions. After about 6 h the cockerels were injected via the second cannula with sodium pentobarbitone containing 10 mg of heparin. Dorsal feathers were then plucked and the skin above the latissimus dorsi muscles was removed. At this point the infusion was stopped and the cockerels were decapitated. Heart and gizzard were removed, followed by anterior and posterior latissimus dorsi muscles, all of which were immediately cooled by total immersion in ice-cold 0.9% NaCl. The removal of all muscles never took longer than 5 min. Muscles were trimmed of fascia on a cold glass plate, cut into sections for analysis and finally frozen in liquid N_2 .

Treatment of tissues

Plasma was prepared from about 1.5 ml of blood by centrifugation at 1000 g for 10 min. Proteins were precipitated by the addition of an equal volume of 4% (w/v) sulphosalicylic acid, and the supernatant containing the free amino acids was applied directly to the column of the amino acid analyser.

Muscle free amino acids were prepared from a 5% (w/v) trichloroacetic acid homogenate of whole muscle by extracting the supernatant with diethyl ether, evaporating to dryness at 70°C in a rotary evaporator, and finally dissolving the precipitate in the loading buffer (0.3 M-lithium citrate, pH 2.2). The protein precipitate was hydrolysed in 6 M-HCl for

20 h at 110°C in a sealed glass tube. After hydrolysis, the solution was evaporated to dryness and the free amino acids were dissolved in loading buffer.

To extract the sarcoplasmic proteins, a sample of muscle was homogenized in low-ionic-strength buffer (0.05 M-potassium phosphate, pH 7.4), and the combined supernatant from two extractions precipitated with trichloroacetic acid. Myofibrillar proteins were extracted from the insoluble pellet as crude actomyosin with a high-ionic-strength buffer (0.3 M-KCl/0.15 M-potassium phosphate/3 mM-ATP, pH 7.4). The actomyosin was precipitated by dilution to low ionic strength. Sarcoplasmic and myofibrillar proteins were hydrolysed and the amino acids prepared for the amino acid analyser as described above.

Amino acids were separated by means of preparative amino acid analysis with a Locarte amino acid analyser (Locarte Instruments, London W12 9RT, U.K.). A step system of lithium citrate buffers was used (0.3 M, pH 2.8, plus 2.5% ethanol, and 0.3 M, pH 3.5; Kedenburg, 1971). Quantification of the ninhydrin colour was achieved by the inclusion of internal standards with each run: α -amino butyric acid for protein hydrolysates and norleucine for free amino acid solutions. Specific radioactivities of amino acids were determined by split-stream analysis, about 80 and 50% of the column effluent being collected for radioactive scintillation counting after the analysis of the protein hydrolysate and free amino acid solutions respectively. ^{14}C radioactivity was determined in either Packard (2420) or Beckman (LS150) liquid-scintillation counters, equipped with external standardization. A xylene/Triton X-100-based scintillant (Tritosol; Fricke, 1975) was used. When amino acids from about 8 mg of protein, supernatant from 100 mg of wet tissue or 0.2 ml of plasma supernatant were analysed in this way, sufficient radioactivity was collected to enable determination with an error of less than 5% (at the level of 2 standard deviations).

Extraction of nucleic acids

RNA and DNA were extracted from a 150 mg sample of muscle tissue by methods based on those described by Munro & Fleck (1969). RNA is made acid-soluble by alkaline hydrolysis in 0.3 M-NaOH at 37°C for 1 h, and DNA is solubilized by hydrolysis in 0.8 M-HClO₄ at 70°C for 45 min. RNA was determined by u.v. absorption at 260 nm assuming 1.00 absorbance unit/cm was equivalent to 32 μg of RNA/ml. Correction was made for u.v.-absorbing peptides present in this solution by determining the protein content and by assuming that 1.00 absorbance unit/cm was equivalent to 1 mg of peptide/ml. DNA was determined colorimetrically by the method of Giles & Myers (1965). Protein was determined on the alkaline solution of the tissue and on the RNA

solution by an automated procedure (Lowry *et al.*, 1951), with bovine serum albumin as a standard.

Calculation of rate of protein synthesis

The fractional rate of protein synthesis can be calculated from the ratio of specific radioactivities of protein-bound proline (S_b) to free intracellular proline (S_i) at the end of the infusion, taking into account the way in which the latter has changed during the infusion (Waterlow & Stephen, 1968). In the past an equation relating S_b/S_i to the fractional synthesis rate has been used which approximates the rise to plateau values of S_i , assuming that it can be described by an expression containing a single exponential, although it is known to be more complex than this (Garlick *et al.*, 1973b). The value of the exponential rate constant in the simplified expression is related to the turnover rate of the intracellular pool and can be determined from the fractional synthesis rate, R (the relative pool sizes of amino acid in the protein-bound pool and the free pool in tissue) and the relative specific radioactivity of the intracellular and plasma amino acids at plateau (S_i/S_p). Although this simplified expression is in many cases reasonably accurate and easy to calculate, a more precise value of the fractional synthesis rate can be obtained by means of a full compartmental analysis which recognizes the more complex way in which the specific radioactivity of the intracellular pool rises to its plateau value. In this analysis (described in detail in the Appendix to this paper) the fractional synthesis rate is calculated from the values of S_b/S_i , S_i/S_p and R shown in Table 1 and the exponential rate constant describing the rise to plateau values of the specific radioactivity of the plasma proline S_p . This was determined by fitting an appropriate curve to the experimental data from the serial blood samples obtained during the infusions.

The use of proline in these experiments, although deliberately chosen to enable collagen synthesis to be measured, poses specific problems that must be taken into account in the calculations. First, for a mixture of proteins the calculated fractional synthesis rate will be a mean value weighted according to the relative amounts of the infused amino acid in each of the various proteins. Most amino acids are reasonably uniformly distributed throughout proteins. However, proline is an exception, since it is particularly abundant in collagen, comprising about 15% (w/v) (Neuman & Logan, 1950), which is over five times the abundance in muscle intracellular proteins. Because of this fact the fractional synthesis rate of mixed muscle proteins, calculated from the specific radioactivity of protein-bound proline, will be disproportionately weighted by the collagen proline and, since collagen turnover is very slow, this will underestimate the true mean fractional synthesis

rate. However, the contribution of collagen-bound proline to the overall proline specific radioactivity can be determined by comparing the specific radioactivity of protein-bound proline and hydroxyproline. The latter amino acid occurs almost entirely in collagen and, since it is formed by hydroxylation of proline during or immediately after translation of the collagen message (Bornstein, 1974), it should have the same specific radioactivity as proline in collagen. Further, the ratio of proline/hydroxyproline in collagen is fixed and known to be 1.27 (Lowther, 1963; McClain *et al.*, 1970). This means that the specific radioactivity of the non-collagen proline can be calculated from that of proline and hydroxyproline according to the formula:

$$\text{Specific radioactivity of non-collagen proline} = \frac{\text{d.p.m. of proline} - 1.27 \times \text{d.p.m. of hydroxyproline}}{\text{amount of proline} - 1.27 \times \text{amount of hydroxyproline}}$$

Thus the S_b/S_i used in the calculations (Table 1) was calculated from the non-collagen protein-bound proline. Further, account must be taken of the presence of collagen in the determination of the value of R . The fact that collagen turns over so slowly and probably is not degraded within the contractile cell means that collagen-bound proline will not contribute to the turnover of the intracellular proline within the muscle cells. Thus the R value shown in Table 1 was calculated from the non-collagen protein-bound proline, which according to the above arguments is equal to total protein-bound proline -1.27 times total protein-bound hydroxyproline. The relative amounts of protein-bound hydroxyproline and proline are shown in Table 1. For the sarco-plasmic and myofibrillar protein fractions, the elimination of collagen during preparation meant that it was unnecessary to correct the measured value of protein-bound proline before calculating the fractional synthesis rate.

Results and Discussion

Validity of calculated synthesis rates

The constant-infusion method is most useful for measuring rates of protein synthesis if the specific radioactivity of the labelled amino acid infused rapidly reaches a plateau during the infusion. Proline appears to satisfy this criterion. The results in Fig. 1 show that a plateau was reached in the labelling of proline in the plasma, the rise to the plateau value following the equation:

$$S_p = S_{p(\max.)} (1 - e^{-\lambda_p t})$$

where λ_p was about 40 days^{-1} (i.e. 96% of the plateau was reached after 2h). This is similar to the value

observed during an infusion of tyrosine in man ($\lambda_p = 42 \text{ days}^{-1}$; James *et al.*, 1976) and adult rabbits ($\lambda_p = 46 \text{ days}^{-1}$; Nicholas *et al.*, 1977), but somewhat slower than the values observed with tyrosine in mice or rats (60 days^{-1} and 80 days^{-1} respectively; Garlick, 1972), pigs (50 days^{-1} ; Garlick *et al.*, 1976) or dogs (72 days^{-1} ; Everett *et al.*, 1977) or with leucine in newborn lambs (60 days^{-1} ; Soltesz *et al.*, 1973). It is considerably higher than the rate observed in rats during an infusion of glycine ($\lambda_p = 15 \text{ days}^{-1}$; Garlick, 1972) or lysine ($\lambda_p = 20 \text{ days}^{-1}$; Waterlow & Stephen, 1967). Since the plateau is reached fairly quickly during the infusion,

Fig. 1. Specific radioactivity of proline in the plasma of adult fowls during a continuous infusion of [^{14}C]proline. Values are typical results for one bird. Birds were infused via a wing vein and the blood was sampled from the contralateral vein. The rise to the plateau value of specific radioactivity approximated to the equation $S_p = S_{p(\text{max.})} (1 - e^{-\lambda_p t})$ where the exponential rate constant, λ_p , was equal to 40 days^{-1} .

any errors involved in the assumptions made about the time course of the rise to plateau will be minimized.

The relative labelling of the non-collagen protein-bound and free prolines at the end of the infusion and the calculated rates of protein synthesis in the four muscles are shown in Table 1. The extent of the correction of the specific radioactivity of protein-bound proline for the presence of collagen proline was greatest in the anterior latissimus dorsi muscle and least in the heart. This is indicated by the hydroxyproline/proline ratio, which was highest in the anterior latissimus dorsi muscle and lowest in heart. The importance of this correction is indicated by the fact that the mean rate of synthesis in the anterior latissimus dorsi muscle calculated for non-collagen protein was nearly twice the rate calculated for the total muscle protein. As discussed above, the presence of disproportionate amounts of proline in collagen means that the overall rate of synthesis will be unduly weighted by collagen. Since the actual labelling of the hydroxyproline indicated a fractional rate of synthesis of about 0.6%/day (Laurent *et al.*, 1978b) the true weighted mean rate of synthesis for total protein in the anterior latissimus dorsi muscle, which contains 3.6% wet weight of collagen, is 14.5%/day. For the other muscles the true weighted mean rates of synthesis will be closer to the values calculated for the non-collagen protein, since the collagen content is less.

An independent check on the validity of the calculation used to correct for the influence of proline from collagen is provided by the rates of synthesis of the sarcoplasmic and myofibrillar proteins (Table 2). These two fractions together comprise most of the intracellular proteins in muscle. For the

Table 1. Relative specific radioactivities of protein-bound (S_b) and free proline (S_f) and calculated rates of protein synthesis for non-collagen mixed proteins from individual muscles of the fowl

Measurements of the relative labelling of free and protein-bound proline were made in each muscle at the end of a 6h infusion of trace amounts of [^{14}C]proline. The labelling of the non-collagen proline was determined by comparing the labelling of protein-bound hydroxyproline and proline as described in the text. The fractional rate of protein synthesis was calculated as indicated in the text by means of a compartmental analysis involving S_b/S_f , the relative pool sizes of non-collagen protein-bound and free proline (R), the relative values of the intracellular and plasma proline specific radioactivities at plateau (S_f/S_p) and the exponential rate constant describing the rise to plateau values of the specific radioactivity of the free proline in the plasma, which was 40 days^{-1} in all cases. Also shown is the ratio of hydroxyproline and proline in the muscle protein, which indicates the magnitude of the correction required in the calculation of R and S_b/S_f . The results show means ± 1 s.d. The numbers of measurements are shown in parentheses.

Muscle	Hyp/Pro	R	S_f/S_p	S_b/S_f	Fractional synthesis rate (%/day)
Anterior latissimus dorsi (21)	0.47 ± 0.2	108	0.641	0.0344 ± 0.0060	17.0 ± 3.1
Posterior latissimus dorsi (21)	0.27 ± 0.07	85	0.461	0.0109 ± 0.0028	6.9 ± 1.8
Heart (5)	0.13 ± 0.06	224	0.850	0.0306 ± 0.0029	13.8 ± 1.3
Gizzard (5)	0.27 ± 0.04	224	0.618	0.0255 ± 0.0040	12.0 ± 1.9

Table 2. *Relative specific radioactivities of protein-bound and free proline and calculated fractional rate of protein synthesis for sarcoplasmic and myofibrillar proteins isolated by differential salt extraction from individual muscles of the fowl* Measurements and calculations were made as described in Table 1. Values are means $\pm$ 1 s.d. The numbers of measurements are shown in parentheses.

Muscle	Sarcoplasmic protein		Myofibrillar protein	
	S_b/S_i	(%/day)	S_b/S_i	(%/day)
Anterior latissimus dorsi (21)	0.0321 ± 0.0074	15.9 ± 3.7	0.0323 ± 0.0050	16.0 ± 2.5
Posterior latissimus dorsi (21)	0.0105 ± 0.0027	6.7 ± 1.8	0.010 ± 0.0029	6.58 ± 1.9
Heart (5)	0.0327 ± 0.0073	14.8 ± 3.3	0.022 ± 0.0043	10.6 ± 2.3
Gizzard (5)	0.0228 ± 0.0055	10.8 ± 2.6	0.0228 ± 0.0049	10.8 ± 2.4

anterior and posterior latissimus dorsi muscles and gizzard there is no significant difference between calculated rates of synthesis of the non-collagen protein and the two protein fractions. In heart, the calculated non-collagen rate of protein synthesis was intermediate between that of the two fractions.

The calculated value of the fractional synthesis rate is correct only if the measured specific radioactivity of the free amino acid is the same as that of the precursor, i.e. the aminoacyl-tRNA. In the present experiments this was not measured directly, so that the calculated fractional synthesis rate, based on the specific radioactivity of free proline in tissue, may be in error to the extent that the true specific radioactivity of the precursor differs from the measured value. The maximum possible value, which would result in a minimum value for the calculated fractional synthesis rate, is that of the plasma. The measured specific radioactivity in free proline in tissue (S_i) was always lower than that of the plasma (S_p), S_i/S_p being equal to 0.85 for heart, 0.64 for the anterior muscle, 0.62 for gizzard and 0.46 for the posterior muscle. Thus synthesis rates calculated, assuming the precursor specific radioactivity to be that of the plasma, would be 11.7%/day for heart, 9.9%/day for the anterior muscle, 7.1%/day for gizzard and 2.2%/day for the posterior muscle.

The relationship between the specific radioactivity of the aminoacyl-tRNA and that of the mean value for free proline in tissue has not been directly determined in muscle *in vivo*. In perfused cardiac muscle, McKee *et al.* (1977) have established that the specific radioactivity of aminoacyl-tRNA has an intermediate value between the specific radioactivity of the amino acid in the perfusate, and in the tissue. This is consistent with the reports that tRNA charging occurs either from a membrane-derived pool in equilibrium with the extracellular pool (Khairallah & Mortimore, 1976) or from two sites, one of which is extracellular (Hod & Herschko, 1976). Other studies (e.g. Mortimore & Ward, 1976) have indicated that compartmentation of the intracellular pool

in liver may partly reflect the presence of lysosomes. Thus free amino acids generated during the course of lysosomal proteolysis comprise a non-expandable amino acid pool which would remain largely unlabelled during incorporation studies. This is one reason why the tissue free amino acid specific radioactivity should always be lower than that of the true precursor. The influence of a 'cold' lysosomal amino acid pool in muscle is probably less than in liver because of the lower turnover rate (Waterlow *et al.*, 1978) and associated smaller lysosomal pool size.

In the present experiments, the measured specific radioactivity of tissue was the weighted mean value of the intracellular and extracellular amino acid pools. Since the specific radioactivity of the extracellular space is the same as that of the plasma, the measured value will be higher than that for the intracellular pool. In these muscles, the size of the extracellular pool, determined with ^{51}Cr -labelled EDTA, was 26 and 15% (anterior and posterior latissimus dorsi muscles), 19% (heart) and 17% (gizzard) (G. J. Laurent, unpublished work). However, because the concentration of proline is higher in the intracellular space than in plasma, the proportion of extracellular proline in the measured tissue proline will be much less than these values for extracellular space. Without further information on the exact site of tRNA charging in muscle, we feel that the most reasonable approach is to assume the specific radioactivity of the precursor to be that of the measured tissue specific radioactivity uncorrected for extracellular space. This assumption is supported by the studies of Fern & Garlick (1973, 1974).

Rates of protein synthesis and RNA and DNA concentrations in muscle

The composition of the muscles in terms of protein, RNA and DNA is shown in Table 3. These values are useful in evaluating the major determinants of protein synthesis rates in the muscles.

The RNA concentration in muscle has long been

Table 3. *Composition of individual muscles from the fowl*

The protein measured is that soluble in 0.3M-NaOH, representing mainly intracellular proteins, since collagen has only limited solubility in 0.3M-NaOH. Nucleic acids were extracted and measured as described in the text. Values are means $\pm$ 1 S.D. The numbers of measurements are shown in parentheses.

	Protein concentration (mg/g wet wt.)	$10^3 \times$ RNA/protein ratio	RNA/DNA	Protein/DNA
Anterior latissimus dorsi (21)	135 $\pm$ 3	7.6 $\pm$ 0.2	2.70 $\pm$ 0.50	384 $\pm$ 14.8
Posterior latissimus dorsi (21)	156 $\pm$ 2	3.4 $\pm$ 0.5	3.05 $\pm$ 0.15	909 $\pm$ 14
Heart (5)	154 $\pm$ 7	9.3 $\pm$ 0.9	1.90 $\pm$ 0.15	188 $\pm$ 14
Gizzard (5)	166 $\pm$ 6	7.0 $\pm$ 0.5	2.00 $\pm$ 0.20	250 $\pm$ 33

Table 4. *Rates of protein synthesis per unit of RNA (RNA activity) and DNA (DNA activity) in individual muscles from fowl*

RNA activities were determined by dividing the fractional synthesis rate for non-collagen protein by the RNA/protein ratio. DNA activities were determined by multiplying the fractional synthesis rate for non-collagen protein by the protein/DNA ratio. Values are shown as means $\pm$ 1 S.D. for the numbers of measurements shown in parentheses.

Muscle	RNA activity (g of protein synthesized/day per g of RNA)	DNA activity (g of protein synthesized/day per g of DNA)
Anterior latissimus dorsi (21)	22.4 $\pm$ 0.6	60.5 $\pm$ 11.3
Posterior latissimus dorsi (21)	20.4 $\pm$ 1.3	62.1 $\pm$ 3.0
Heart (5)	14.9 $\pm$ 2.2	28.3 $\pm$ 2.6
Gizzard (5)	18.6 $\pm$ 0.3	37.4 $\pm$ 3.7

recognized as an index of the intensity of protein synthesis (Munro, 1969; Goldberg, 1967). The relationship is most conveniently described in terms of the capacity for protein synthesis, indicated by the RNA concentration, and the activity of the RNA, which is equal to the rate of protein synthesis per unit of RNA (Millward *et al.*, 1973, 1976). The most appropriate expression of RNA concentration or capacity that can be directly compared with a fractional rate of protein synthesis is the RNA/protein ratio. This ratio was considerably higher (Table 3) in the anterior latissimus dorsi muscle, heart and gizzard than in the posterior latissimus dorsi muscle. In contrast, calculation of the RNA activities (Table 4) indicated that there was little difference between the values for the two skeletal muscles and gizzard, although the RNA activity in heart was somewhat lower. This means that the relative synthesis rates in these muscles reflected mainly variation in the capacity for protein synthesis. Wool *et al.* (1968) reported that the rate of incorporation of amino acids *in vitro* into ribosomes isolated from red and white rat skeletal muscles was the same. Measurements *in vivo* with the constant-infusion method in adult rats have also shown that the higher synthesis rates in red oxidative muscles (soleus and diaphragm) compared with predominantly white glycolytic muscles (plantaris, gastrocnemius, quadriceps, psoas and quadratus lumborum;

Millward *et al.*, 1978) reflect different RNA capacities rather than activities (D. J. Millward, P. C. Bates & C. C. Lo, unpublished work).

The magnitude of the RNA activity in these muscles, as in any tissue, depends on the proportion of total RNA that is ribosomal, the proportion of RNA in translating ribosomes, and the elongation rate. We have no direct information on any of these factors in the present experiments. It is noteworthy, however, that the RNA activities, at least for the two skeletal muscles, are higher than those generally observed in rat skeletal muscle (Millward *et al.*, 1973, 1975, 1976), particularly since even higher values of the RNA activity in the anterior latissimus dorsi muscle are observed during its hypertrophy (Laurent *et al.*, 1978a). This is possibly related to the higher body temperature of the fowl (41.7°C compared with 37.3°C in the rat; Brody, 1945), which would increase the elongation rate. Assuming that the Arrhenius activation energy is such that a 10°C rise in temperature doubles the elongation rate (about which there is some doubt; Haschemeyer, 1976) the elongation rate in the fowl would be 35% higher than in the rat.

When the ribosome or RNA concentration is compared with the DNA concentration in the tissue, it is obvious that the RNA/DNA ratio is higher in the two skeletal muscles than in the heart and gizzard (Table 3). We might conclude then that there is a

higher concentration of ribosomes per nucleus in the two skeletal muscles. However, the RNA/DNA ratio is the mean value of all the cell types present in these muscles and may not be a good indicator of the number of ribosomes per nucleus in the contractile cells specifically. In skeletal muscle at least 70% of all nuclei are associated with the contractile cells (Enesco & Puddy, 1964; Cheek *et al.*, 1971), the rest comprising fibroblasts, vascular cells, adipocytes etc. However, in cardiac muscle, nuclei of contractile cells appear to account for a much smaller proportion, since as many as 75% of the total nuclear population have been reported to occur in non-muscle cells (Grove *et al.*, 1969; Morkin & Kimata, 1974). If these non-muscle cells in heart are less active in protein synthesis, this would account for the lower RNA/DNA ratio in heart. Little is known about the distribution of cell types in gizzard.

Since both the RNA activity and the RNA/DNA ratio is the same in the two skeletal muscles, the rate of synthesis per unit of DNA (the DNA activity) will also be the same, as shown in Table 4. Because the DNA activity in the two skeletal muscles is the same, it follows automatically that the fractional rates of protein synthesis (Table 1) vary inversely with the protein/DNA ratio (Table 3). This fact is of interest, since it may be important in the regulation of ultimate muscle size. Muscle growth involves increases in both numbers of nuclei (i.e. total DNA) as well as in the amount of associated protein (i.e. protein/DNA; Enesco & Leblond, 1962) in the muscle fibres, which are in effect multi-nucleate contractile cells (Goldspink, 1972). To rationalize such growth, Cheek *et al.* (1971) defined the muscle DNA unit as an imaginary volume of cytoplasm within the contractile cell managed by a single nucleus. Thus total DNA and protein/DNA ratios define muscle size in terms of numbers and sizes of DNA units, and since the sizes of DNA units vary between muscles, as shown by these results (Table 3), both total DNA and protein/DNA ratios can be considered to be variables in the determination of ultimate muscle size. The pertinent question then becomes what determines size of the DNA unit? DNA-unit size can be determined by the rate of protein synthesis or breakdown if either of these two processes is kinetically first-order. Accepting the conventional viewpoint that protein synthesis is zero-order (e.g. a fixed rate per unit of DNA, the DNA activity) and that breakdown is first-order (a fractional rate, k_d), DNA-unit size is determined by the expression:

$$\text{Protein/DNA} = \text{DNA activity}/k_d$$

This is the expression that is often used to define the steady-state concentration of an enzyme (see Schimke, 1970) and it means that since DNA activity in the anterior and posterior muscles is the same, the

different DNA-unit size reflects the different fractional breakdown rates. However, recognition that the characteristic breakdown rate of a muscle determines DNA-unit size crucially depends on the assumption that protein breakdown is a first-order process and although it is usually assumed to be so, there is little convincing evidence that it is, at the level of the whole tissue (Millward, 1978). If, for example, protein breakdown is zero-order (e.g. a rate per unit of DNA), then there would be no causal relationship between size of DNA unit and rates of protein breakdown. The determination of the true reaction order of synthesis and breakdown of protein is obviously important.

Turnover rate in relation to muscle structure and function

The difference in turnover rate between the posterior latissimus dorsi and the other three muscles (Table 1) is marked. It is unlikely that this difference in turnover reflects compositional differences, since in the anterior and posterior muscles and gizzard the turnover rates of the two principal intracellular protein fractions, the sarcoplasmic and myofibrillar proteins, are the same (Table 2) and similar to that for the mixed non-collagen protein fraction (Table 1). This result was surprising, since in the rat the contractile proteins turn over more slowly than the sarcoplasmic fraction (Millward, 1970), as is observed in heart in the present experiments (Table 2).

Why turnover rate in the anterior latissimus dorsi muscle, heart and gizzard should be higher than in the posterior muscle is not known, although, as indicated above, several studies have shown differences between red and white muscles. The high turnover rate in the gizzard, a smooth muscle that is red in appearance, is of interest. Its structure appears to be similar to other visceral smooth muscles (Toner & Carr, 1968). This is, at least to our knowledge, the first report of a turnover rate for a smooth muscle. The relative turnover rate of the posterior latissimus dorsi muscle and heart in this study is similar to the relative turnover rate of mainly glycolytic skeletal muscles such as plantaris, psoas, gastrocnemius (4%/day) and cardiac muscle (11%/day) in adult rats (Millward *et al.*, 1978). However, in the rat no individual skeletal muscle has been described with a turnover rate as high as or higher than cardiac muscle. In soleus and diaphragm muscles, generally recognized as red oxidative muscles, turnover rates are intermediate between heart and the glycolytic muscles (i.e. 8%/day; Millward *et al.*, 1978). Thus the turnover rate of the anterior latissimus dorsi muscle stands out as unusually high. Although there can be no doubt that protein turnover in this muscle is as fast as in heart, because of the uncertainty about the site of tRNA charging and the assumptions made

in the compartmental analysis, we cannot be sure that its turnover rate is significantly higher than in heart without more information.

As far as the structure and function of the anterior latissimus dorsi muscle is concerned, it is a well-studied example of a true slow contracting skeletal muscle (Hess, 1961) with a twitch time 6–8 times slower than that of the posterior muscle (Page, 1969). The ultrastructure of the anterior muscle can be described as somewhat looser and more irregular than that of the posterior muscle (Page, 1969; Franzini-Armstrong, 1973). Whether this looser structure is causally related to its faster turnover is not established by the present experiments.

The activities of cathepsin D and other lysosomal enzymes are higher in the anterior than posterior muscle (Syrový *et al.*, 1965; Stauber *et al.*, 1977) and this raises the possibility that the relative turnover rates in the two muscles simply reflects the relative concentration of the proteolytic system which degrades muscle proteins. However, if this is so then it still has to be determined whether concentrations of proteolytic enzymes have arisen in response to a need for protein turnover at a particular rate, determined by structural and functional aspects of the muscle, or for some other reason.

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