Turnover of Muscle Protein in the Fowl

CHANGES IN RATES OF PROTEIN SYNTHESIS AND BREAKDOWN DURING HYPERTROPHY OF THE ANTERIOR AND POSTERIOR LATISSIMUS DORSI MUSCLES

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Measurements were made of the growth and of the changes in rates of protein turnover in the anterior latissimus dorsi muscle of the adult fowl in response to the attachment of a weight to one wing. Over 58 days there was a 140% increase in the protein content with similar increases in the RNA and DNA contents. The fractional rate of protein synthesis, measured by the continuous-infusion technique using [^{14}C]proline, increased markedly during hypertrophy. This increase was mediated initially (after 1 day) by an increase in the RNA activity but at all other times reflected the higher RNA content. The rate of protein degradation, calculated from the difference between the synthesis and growth rates, appeared to increase and remain elevated for at least 4 weeks. At no time was there any suggestion of a fall in the rate of degradation. The following events are discussed as central to the changes that occur during skeletal-muscle hypertrophy. 1. Nuclear proliferation is necessary to maintain the characteristic synthesis rate because of the inability of existing nuclei to 'manage' increased protein synthesis for more than a limited period. 2. The increased protein breakdown during hypertrophy is consistent with the known overproduction of new muscle fibres and may indicate some 'wastage' during the growth. Such wastage may also be associated with myofibrillar proliferation. 3. Muscle stretch must be recognized as the major activator of growth and as such can be compared with the 'pleiotropic activators' that have been described for cells in culture.

The hypertrophy of skeletal muscle involves a marked increase in the protein content of the tissue (Goldberg, 1967; Hamosh *et al.*, 1967; Laurent & Sparrow, 1977). However, there have been conflicting reports on the relative contributions of changes in rates of protein synthesis and degradation in mediating this growth of the protein mass. There is agreement in all of the published studies that an increase in the rate of synthesis occurs, but the changes in degradation are more controversial. In extensive studies, Goldberg (1968, 1969, 1971*a,b*) has investigated changes in protein turnover during growth of rat plantaris and soleus muscles, after tenotomy of the gastrocnemius. He showed that the incorporation of radioactivity after repeated daily injections of [^{14}C]leucine was elevated in the hypertrophying muscle and concluded that rates of protein synthesis were increased (Goldberg, 1968). When the extent of the increased incorporation was compared with the growth rate, and a value assumed for the rate of protein turnover, Goldberg (1968) suggested that the

rate of protein degradation may have fallen. This possibility was further investigated in a subsequent study (Goldberg, 1969) in which, after an initial pulse dose of [^3H]leucine, the rate of loss of radioactivity from the hypertrophying muscle was compared with that in the control. Because there was a greater retention of radioactivity in the hypertrophied muscle, Goldberg (1969) concluded that the rate of protein degradation was decreased during the hypertrophy. The interpretation of both these studies is open to question, however. In the first study (Goldberg, 1968), the conclusions about the changes in rate of degradation critically depend on the assumed rate for protein turnover in the non-growing muscles and this rate, when compared with more recent measurements of rates of synthesis in young hypophysectomized rats (Millward *et al.*, 1976), was almost certainly too low. In the second study (Goldberg, 1969), increased reutilization of label rather than decreased degradation could have accounted for the results. This seems likely in the light of a study of hypertrophy of fowl muscles (Laurent & Sparrow, 1977), which has shown that increased reutilization occurs despite attempts to

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minimize it by means of an unlabelled amino acid 'chase'.

If the rate of protein synthesis is measured accurately, then the changes in breakdown can be determined by comparing the change in rate of synthesis with the growth rate of the protein pool. Turner & Garlick (1974) applied this approach to the study of protein turnover during hypertrophy of rat diaphragm after unilateral phrenicectomy. The rate of protein synthesis was measured by the continuous-infusion technique, and they showed that the rate of protein synthesis was increased to an extent that was greater than that necessary to account for growth. It was concluded therefore that the rate of protein degradation was also increased. In a further study using the same technique, an increase in protein degradation was also reported during rapid growth of rat hindlimb muscles in the course of nutritional rehabilitation (Millward *et al.*, 1975).

The present study was undertaken to resolve further the mechanism of skeletal-muscle hypertrophy by examining the changes in protein turnover during the hypertrophy of the anterior and posterior latissimus dorsi muscles of the adult fowl that occurs in response to the attachment of a weight to one wing (Sola *et al.*, 1973; Laurent & Sparrow, 1977). In this model the hypertrophy is reproducible and very rapid for the anterior muscle, and, since no surgery is involved, the quantification of the changes in muscle composition is relatively easy (Laurent & Sparrow, 1977). Changes in muscle protein turnover during hypertrophy were investigated by means of a constant infusion of [^{14}C]proline (Laurent *et al.*, 1978a). To understand the mechanism of any changes in protein synthesis better, measurement was also made of muscle RNA and DNA content during the hypertrophy.

Experimental

Induction of hypertrophy

Skeletal-muscle hypertrophy was induced in 25 fully grown 35-week-old White Leghorn cockerels weighing 2.10 ± 0.10 kg, which were maintained on a commercial chicken-breeder diet [supplied by Dixon and Sons (Ware) Ltd., Ware, Herts., U.K.] containing 18% (w/w) protein. A galvanized iron shackle weighing 180 g was placed around the humerus of one wing of the fowl so that the weight rested on the elbow joint. Each shackle was padded with sponge rubber to prevent bruising to the area of attachment. After 1, 3, 7, 28 and 58 days either four or five fowls were infused intravenously for 6 h with trace amounts of [^{14}C]proline, the anterior and posterior latissimus dorsi muscles were quickly removed, weighed and rates of protein synthesis and protein and nucleic acid contents were measured as previously described (Laurent *et al.*, 1978a). In addition to these fowls, a

group was similarly examined after 14 days, although the rates of protein synthesis were not determined in this case. All analytical methods were as described in the preceding paper (Laurent *et al.*, 1978a).

Measurement and calculation of rates of protein synthesis

This was done as described in the preceding paper (Laurent *et al.*, 1978a).

Calculation of rates of breakdown of protein

The rates of protein breakdown were calculated by subtracting the fractional growth rate of the muscle non-collagen protein pool from the fractional synthesis rate. Measurement of growth rate in this type of experiment can only be made by constructing a growth curve from cross-sectional measurements of groups of fowls at each time point and, although every effort is made to ensure that the whole population is homogeneous, some individual variation is unavoidable. However, in the present experiments, since the growing muscle can be compared with a non-growing control in each fowl, a more accurate growth curve can be constructed, taking into account variability between control muscles. This involves dividing the protein content of the hypertrophying muscle by that of its control and multiplying by the mean value of all the control values. This enables a growth curve to be constructed for a homogeneous population. The fractional growth rate was calculated at each time point from the mean value of the growth rates for the time periods immediately before and after that time point. This method was chosen, rather than calculating the slope of the growth curve from an equation describing the best fit of the experimental data, since it is the simplest and most accurate method. The error associated with the growth rate was calculated from the variances of the mean protein contents at each time point.

Results

Growth of the anterior latissimus dorsi muscle

The response of the anterior muscle to the added weight was very pronounced as has been previously described (Sola *et al.*, 1973; Laurent & Sparrow, 1977). Fig. 1 shows the increases in non-collagen protein, RNA and DNA content. Most of the growth occurred during the first week, but smaller changes occurred after this time so that a new stable state was not reached till after about 6–8 weeks. At this time the protein content had increased 144% above the control value with similar changes in RNA (176%) and DNA (155%). The time course of the changes in protein, RNA and DNA differed, however, since the initial rate of increase in RNA and DNA was faster than that of protein. This was particularly the case with the RNA, where there was considerable 'overshoot',

Fig. 1. Changes in RNA, DNA and protein content during the hypertrophy of the anterior latissimus dorsi muscles. Values for RNA (●), DNA (■) and protein (▲) are shown as percentages of values for the control contralateral muscles. No significant changes occurred in nucleic acid or protein content in the control muscles during the experimental period. Values are shown as means $\pm$ 1 s.d.

with a more than 4-fold increase after 7 days. After this time, RNA was lost from the muscle rapidly up to 14 days and then more slowly. Although not so pronounced, the pattern of response of total muscle DNA was similar since a maximum value was reached after 7 days [$156 \pm 17\%$ above control (mean $\pm$ s.d., $n = 4$)] and subsequent values were all lower. In the case of the protein content there was no apparent overshoot, so that between 7 and 14 days, when RNA and possibly DNA content appeared to be decreasing, protein was still accumulating, albeit at a low rate.

Initially, the total water content of the anterior muscle increased faster than solids. In these experiments during the first 24 h wet weight increased by $36 \pm 6\%$ compared with the $10 \pm 6\%$ increase in protein content. In some experiments we have observed increases in wet weight of over 100% in 24 h, but such increases always, at this time, involve disproportionate increases in muscle water, as have been shown by dry-weight determinations. However, by 7 days the increases in protein and wet weight were proportional, indicating that any excess of water had been lost by this time. During the first few days of the hypertrophy, the amount of free proline increased markedly in the anterior muscle, rising from $0.28 \mu\text{mol/g}$ wet wt. in the control muscles to 1.48 ,

1.32 and $0.63 \mu\text{mol/g}$ wet wt. after 1, 3 and 7 days respectively. Since these increases were out of all proportion to the increases in water concentration, they must have involved increases in proline concentration. It is most likely that this increase involved intracellular proline, since there is no known mechanism by which a concentration gradient of proline between the extracellular space and plasma could be established. The extent to which increases in concentrations of other amino acids occurred during the hypertrophy is not known, although measurements made in muscles after 1 day's hypertrophy indicated increases in only proline and serine and possibly alanine.

The effect of these increases in proline concentration was to alter the R values (see Table 1), the relative pool sizes of protein-bound and free proline. The implication of this change on the measured rate of protein synthesis is discussed below.

Growth of the posterior latissimus dorsi muscle

The response of the posterior muscle to the added weight was much less obvious than observed for the anterior muscle (Fig. 2). There was a small initial increase in protein after 3 and 7 days (less than 10%); however, after 14 days the protein content was not different from the control, indicating a loss of any protein accumulated in the initial response. The total muscle RNA had increased by $77 \pm 40\%$ after 3 days, but this extra RNA was subsequently lost. Similarly,

Fig. 2. Changes in RNA, DNA and protein content during the hypertrophy of the posterior latissimus dorsi muscles. Values for RNA (●), DNA (■) and protein (▲) are shown as percentages of values for the control contralateral muscles. No significant changes occurred in nucleic acid or protein content in the control muscles during the experimental period. Values are shown as means $\pm$ 1 s.d.

there was a transient increase in DNA (25–33%), although this too was subsequently lost.

As with the anterior muscle, the free proline concentration increased initially, rising from the control value of $0.56 \pm 0.19 \mu\text{mol/g}$ to $1.3 \pm 0.5 \mu\text{mol/g}$ after 3 days. After 7 days, however, the proline concentration was similar ($0.59 \pm 0.19 \mu\text{mol/g}$).

Rates of protein synthesis in the anterior latissimus dorsi muscle related to changes in RNA and DNA

The rates of protein synthesis during hypertrophy, calculated by compartmental analysis, are shown in Table 1. Within 24 h of attachment of the weight, the rate of synthesis of mixed non-collagen proteins had doubled. This high value was maintained for most of the first month, after which time it returned to the control value.

The more precise measurement of the rate of protein synthesis in this study enables the comparison between increases in protein synthesis and RNA and DNA content, which have previously been reported (Laurent & Sparrow, 1977), to be evaluated in a quantitative way. We have calculated the RNA and DNA activities (Table 2), as has been done for non-growing muscles of the fowl (Laurent *et al.*, 1978a). At day 1 there was a significant increase ($P < 0.01$) in the RNA activity, which can account for most of the increased rate of synthesis at this time, although the capacity for protein synthesis (reflected in the RNA concentration measured as RNA/protein) was also increased (by 27%). However, after the first day the RNA activities in the hypertrophying muscle were not significantly different from control values, so that the increase in the fractional synthesis rate at those

Table 1. *Rates of non-collagen protein synthesis during hypertrophy of the anterior latissimus dorsi muscle*

The fractional rate of protein synthesis was calculated from the relative specific radioactivity of the non-collagen protein-bound proline (S_b) and free intracellular proline (S_i) after 6 h infusion of [^{14}C]proline by compartmental analysis as described in the Appendix to Laurent *et al.* (1978a). This takes into account the values of R , the relative pool sizes of non-collagen bound and free proline, S_i/S_p , the relative specific radioactivities of intracellular and plasma free proline after 6 h, and λ_p , the exponential rate constant describing the rise to plateau values of plasma proline. S_i/S_p and λ_p were the same at all time points and equal to 0.64 and 40 days $^{-1}$ respectively. Values shown as means $\pm$ 1 s.d.

Days	R	S_b/S_i	Fractional synthesis rate (%/day)
1 Control	108 ± 8	0.0318 ± 0.0035	15.9 ± 1.8
Hypertrophy	22 ± 5	0.0558 ± 0.0061	32.4 ± 4.3
3 Control	108 ± 8	0.0291 ± 0.0150	14.5 ± 7.0
Hypertrophy	28 ± 6	0.0579 ± 0.0051	32.2 ± 2.9
7 Control	108 ± 8	0.0371 ± 0.0030	18.4 ± 1.3
Hypertrophy	72 ± 12	0.0638 ± 0.0064	31.5 ± 4.3
28 Control	108 ± 8	0.0354 ± 0.0041	17.6 ± 2.2
Hypertrophy	128 ± 15	0.0536 ± 0.0054	25.8 ± 2.7
58 Control	108 ± 8	0.0385 ± 0.0042	18.9 ± 2.6
Hypertrophy	146 ± 20	0.0370 ± 0.0049	17.9 ± 3.4

Table 2. *Rates of protein synthesis per unit of RNA (RNA activity) and per unit of DNA (DNA activity) during hypertrophy of the anterior latissimus dorsi muscle*

RNA activity was calculated by dividing the fractional synthesis rate by the RNA/protein ratio, whereas DNA activity was calculated by multiplying the fractional synthesis rate by the protein/DNA ratio. Values are means $\pm$ 1 s.d.

Day	$10^3 \times \text{RNA/protein}$	Protein/DNA	RNA activity (g of protein synthesized/day per g of RNA)	DNA activity (g of protein synthesized/day per g of DNA)
1 Control	7.6 ± 0.8	375 ± 54	20.9 ± 3.2	69 ± 12
Hypertrophy	9.7 ± 1.6	308 ± 50	33.4 ± 2.3	99 ± 16
3 Control	7.1 ± 1.1	401 ± 54	20.4 ± 2.5	59 ± 8
Hypertrophy	18.0 ± 5.9	294 ± 30	17.9 ± 5.6	93 ± 9
7 Control	7.5 ± 0.4	358 ± 34	24.6 ± 2.4	65 ± 7
Hypertrophy	16.9 ± 1.8	264 ± 30	18.6 ± 5.3	82 ± 10
28 Control	8.1 ± 0.8	346 ± 67	21.7 ± 4.6	61 ± 12
Hypertrophy	10.3 ± 2.4	413 ± 60	24.5 ± 2.0	102 ± 16
58 Control	9.4 ± 3.8	405 ± 30	18.2 ± 6.0	77 ± 7
Hypertrophy	10.4 ± 2.5	447 ± 91	17.3 ± 6.0	77 ± 18

later times appeared to reflect the higher RNA concentrations. After 28 days, although the fractional synthesis rate was still markedly elevated, the variability in the measured RNA concentrations and the RNA activities were such that, although both values increased, the increase was not significant in either case. After 58 days there was no difference between hypertrophied and control muscles with respect to fractional synthesis rate, RNA/protein ratio or RNA activity.

The rate of protein synthesis per unit of DNA (the DNA activity) increased markedly during the first 28 days of hypertrophy (see Table 2). These changes in DNA activity can be related to changes in either the

RNA/DNA ratio or the RNA activity. The reason for the increase initially (at day 1) was an increased RNA activity, since the RNA/DNA ratio at this time did not differ from the control (i.e. 3.0 ± 0.3 as opposed to 2.8 ± 0.5 in the control). However, after this time the ratio increased (to 5.8 ± 1.2 , 4.4 ± 0.3 and 4.17 ± 0.6 after 3, 7 and 28 days respectively), so that DNA activity was elevated even though the RNA activity was not changed.

The changes in the fractional synthesis rate during the hypertrophy of the anterior muscle involved equal increases in the rates of synthesis of both the sarcoplasmic and contractile protein fractions (Table 3). At no time during the hypertrophy was the rate of

Table 3. *Rates of synthesis of sarcoplasmic and contractile proteins during hypertrophy of the anterior latissimus dorsi muscle*

The two protein fractions were extracted in buffers of different ionic strength and the fractional rates of protein synthesis calculated by full compartmental analysis from the values of the specific radioactivities of the protein-bound (S_b) and free (S_f) proline and the other parameters shown in Table 1. Values for the control muscles have been pooled and are shown at day 0. All values are means ± 1 s.d.

Day	Sarcoplasmic		Myofibrillar	
	S_b/S_f	Fractional synthesis rate (%/day)	S_b/S_f	Fractional synthesis rate (%/day)
0	0.0331 ± 0.0097	16.4 ± 4.8	0.0309 ± 0.0089	15.3 ± 4.4
1	0.0496 ± 0.0113	28.7 ± 6.5	0.0503 ± 0.013	29.1 ± 4.9
3	0.0592 ± 0.0115	33.0 ± 5.4	0.0784 ± 0.013	44.1 ± 7.5
28	0.0510 ± 0.0120	24.4 ± 6.07	0.0383 ± 0.009	18.2 ± 4.5

Table 4. *Growth rates of the anterior latissimus dorsi muscle non-collagen protein during hypertrophy and rate of protein breakdown*

Actual values for control and protein contents of hypertrophied anterior latissimus dorsi muscle are shown. To correct for variability between the individual fowl the protein content of the hypertrophied muscle was multiplied by the mean protein content in all the control muscles (shown as the protein content at time = 0) and divided by the actual protein content in the individual control muscle. These corrected mean values are shown. The fractional growth rate was calculated from the mean daily rate of increase before and after each time point. The fractional breakdown rate was calculated by subtracting the growth rate from the fractional synthesis rate shown in Table 1. Values are shown as means ± 1 s.d. The s.d. of the growth rate was calculated from the variances of the mean daily growth rates.

Day	Non-collagen protein (mg)	Corrected values (mg)	Growth rate (%/day)	Breakdown rate (%/day)
0		125	0	17.0 ± 1.2
1 Control	128 ± 9			
Hypertrophy	138 ± 19	135	10.4 ± 1.0	22.0 ± 3.9
3 Control	120 ± 10			
Hypertrophy	164 ± 10	171	9.5 ± 0.4	22.2 ± 2.4
7 Control	128 ± 17			
Hypertrophy	234 ± 38	229	4.13 ± 0.3	25.0 ± 3.7
14 Control	119 ± 12			
Hypertrophy	247 ± 40	260	1.39 ± 0.18	—
28 Control	116 ± 11			
Hypertrophy	277 ± 35	299	0.57 ± 0.09	24.6 ± 3.1
58 Control	139 ± 20			
Hypertrophy	336 ± 90	303	—	17.9 ± 3.5

synthesis of these two fractions significantly different, and further, for the most part the fractional synthesis rate of each of the protein fractions was similar to the fractional synthesis rate of the total non-collagen protein fraction.

Rates of protein breakdown

In Table 4 the growth rate of the protein mass is shown together with the breakdown rate calculated as the difference between the rate of synthesis and the growth rate (Table 2). The initial rate of growth was very rapid at 10.4%/day, falling off to 4.1%/day after 7 days. After this time, growth was much slower at 1.4 and 0.6%/day after 14 and 28 days respectively. After 28 days there was little further change in the protein content. At no time during the hypertrophy were the growth rates of the muscle protein mass as great as the increases in the rate of protein synthesis. In other words, at no time was there any suggestion that the rate of protein breakdown was decreased. On the contrary, the breakdown rate appeared to be increased and this was particularly marked at 7 and 28 days, when the growth rates were falling off but the high rates of protein synthesis were still maintained. The breakdown rate in the muscle after 58 days, when the hypertrophy was complete, was the same as the control value.

Table 5. *Rates of non-collagen protein synthesis during the transient hypertrophy of the posterior latissimus dorsi muscle*

Rates were calculated as described in Table 1. The control values for all time points have been pooled (i.e. as time = 0). The mean control values for the three groups were not significantly different. Values are shown as means $\pm$ 1 s.d.

Day	R	S_0/S_1	Fractional synthesis rate (%/day)
0	85	0.0109 ± 0.0009	6.92 ± 0.70
1	58	0.0103 ± 0.0010	7.05 ± 0.69
3	35	0.0184 ± 0.0020	12.47 ± 1.70
7	95	0.0122 ± 0.0010	7.41 ± 0.60

Rates of protein synthesis and breakdown in the posterior latissimus dorsi

In the posterior muscle there were transient changes in protein turnover during the early period after the attachment of the weight. The fractional synthesis rate was increased by 80% at 3 days (Table 5), although by 7 days it appeared to have returned to the control value. The increase in the fractional synthesis rate at 3 days reflected the increased capacity for protein synthesis indicated by change in the RNA/protein ratio (Table 6), since the RNA activity was not significantly different from the control value. As a result mainly of this higher RNA concentration the rate of synthesis per unit of DNA after 3 days was increased by 60% to 104 ± 22 g of protein synthesized/day per g of DNA, a value as high as that observed in the hypertrophying anterior muscle.

Because the growth of the posterior muscle was not very pronounced, it was not possible to determine with any degree of certainty whether changes in protein breakdown occurred. However, the magnitude of the increase in the fractional synthesis rate at day 3 was certainly sufficient to account for the growth that occurred at that time.

Discussion

Different responses of the anterior and posterior latissimus dorsi muscles

Whereas the anterior muscle underwent an immediate and extensive hypertrophy in these experiments the hypertrophy of the posterior muscle was only transient. Since the anatomy of these two muscles is different, the posterior muscle not normally being responsible for the sustained support of the wing, its transient response most likely results from the fact that it is only stretched during the first few days, before the anterior muscle has been able to grow sufficiently to raise the wing back to a near-normal position. Although the rest of this discussion will only be concerned with the response of the anterior muscle, this should not be taken to indicate that the posterior

Table 6. *Rates of protein synthesis per unit of RNA (RNA activity) and per unit of DNA (DNA activity) during the transient hypertrophy of the posterior latissimus dorsi muscle*

RNA and DNA activity were calculated as described in Table 2. Values are means $\pm$ 1 s.d.

Days	RNA/protein	Protein/DNA	RNA activity (g of protein synthesized/day per g of RNA)	DNA activity (g of protein synthesized/day per g DNA)
0	3.4 ± 0.1	901 ± 132	20.35 ± 1.9	62.1 ± 7.4
1	3.2 ± 0.1	799 ± 119	22.0 ± 2.3	57.2 ± 7.1
3	5.3 ± 0.7	877 ± 200	23.5 ± 2.2	104 ± 22
7	4.5 ± 0.5	744 ± 82	16.5 ± 1.4	54 ± 5

muscle, containing white fibres, cannot hypertrophy (see Sola *et al.*, 1973).

Validity of rates of protein turnover

The continuous-infusion technique is dependent on a reasonably accurate determination of the specific radioactivity of the precursor pool for protein synthesis. The general problems relating to the assessment of precursor labelling and the specific problems related to the use of a labelled proline have been discussed (Laurent *et al.*, 1978a). However, during the hypertrophy there were marked changes in the amount and distribution of tissue water and in the free proline concentration, which raises additional problems about the precursor specific radioactivity. Because the concentration of proline increased so markedly (i.e. 5-fold) during the initial stages of hypertrophy, and assuming that concentration gradients can only be maintained between the intracellular and extracellular space, extracellular proline can be considered to have had a negligible effect on the overall measured specific radioactivity of proline. Because of the concentration gradient that normally exists for proline between intra- and extra-cellular space, it is unlikely that even in the control muscles the extracellular proline labelling contributed more than 10% of total radioactivity. This is supported by the fact that there was no difference between the labelling of free proline in control or stretched muscles at any time. What is not known, however, is whether these changes in the distribution of free proline will affect the relationship between the labelling of the tRNA-bound proline and the measured value. A lysosomal pool of (unlabelled) proline derived from breakdown of protein would lower the apparent specific radioactivity of tissue proline below that of the true precursor, as probably occurs in liver (Mortimore & Ward, 1976). The large increase in proline concentration in extralysosomal pools, as in the present case, will probably mean that the measured labelling of proline will be closer to that of the precursor.

The compartmental analysis used to determine the rate of synthesis in these experiments gives the best possible estimate of the rate of synthesis from the assumptions inherent in a three-compartment model [see the Appendix to Laurent *et al.* (1978a)]. Only by determining the time course of labelling of tRNA-bound proline could more accuracy be obtained, and this would be a logistically very difficult and expensive task. A problem exists because the enlargement of the intracellular proline pool means that the turnover rate of the intracellular pool is markedly decreased, delaying the rise to plateau of the intracellular compartment [see the Appendix to Laurent *et al.* (1978a)]. Calculation of the rate of synthesis by assuming a precursor-labelling time course similar to that of plasma proline would result in lower synthesis rates

than those in Table 1 (79, 82 and 93% of the values at 1, 3 and 7 days). However, such differences would not alter the principal conclusions of the present study about changes in rates of protein synthesis and breakdown.

Error associated with the rates of breakdown can only reflect errors involved in the measurement of protein synthesis and growth. There is a possibility that the measured rate of synthesis differs from the average rate for each 24 h, the time over which growth rate was measured. However, we feel that this is unlikely. In the first place, in meal-fed rats the difference between the rate of synthesis of muscle protein measured over any individual 6 h period and the average value for the 24 h was less than 10% (Garlick *et al.*, 1973). In the second place, Goldberg's (1971a) studies on work-induced muscle growth have clearly shown that increased work alone will induce growth without any need for nutritional or hormonal support. Since the stretch was continuous in the present experiments the increased protein synthesis associated with the stretch might also be expected to be continuous. For these reasons we feel that the conclusion that the rate of breakdown is not decreased at any time, but is, on the contrary, increased from the end of the week 1 and for at least the following 3 weeks, is reliable.

Changes in rates of synthesis of the sarcoplasmic and contractile proteins

The pattern of change in synthesis and breakdown of protein observed for the mixed non-collagen proteins in the stretched anterior muscle was the same as that observed for the sarcoplasmic and contractile fraction. This is of interest, since Goldberg (1969) reported apparent differential changes in turnover of the two fractions during work-induced growth of the rat soleus muscle. However, in the rat the turnover rate of these two fractions is different (Millward, 1970), so that when there are marked changes in the overall rate of synthesis, the relative rate of synthesis of these fractions must change to avoid marked alterations in composition (Bates & Millward, 1978) and the compositional changes in the hypertrophying soleus are small and transient (Goldberg, 1969; Hubbard *et al.*, 1975). In the anterior muscle, because the rates of synthesis of the two fractions are the same, and because there are no marked compositional changes (G. J. Laurent, unpublished work), differential changes in the turnover of the two fractions during the hypertrophy would not be expected.

Changes in protein synthesis related to changes in RNA and DNA

The changes in the rate of protein synthesis in the stretched anterior muscle followed for the main part the changes in RNA concentration, except at day 1

when a disproportionate increase in synthesis occurred as a result of increased RNA activity. Increased RNA activity was also observed in rat skeletal muscle during nutritional rehabilitation (Millward *et al.*, 1975). As discussed previously (Laurent *et al.*, 1978a), RNA activity can be influenced by the proportion of RNA in translating ribosomes as well as by the elongation rate. We do not know which of these two factors caused the 60% increase in RNA activity. Although there are many studies showing the marked changes in ribosomal aggregation that occur in response to nutritional (Young, 1974) or hormonal factors (Jefferson *et al.*, 1974, 1977), little is known about whether elongation rates change in muscle. A reasonable estimate of the magnitude of the elongation rate in the anterior latissimus dorsi muscle can be made from the maximum observed value of the RNA activity, by assuming that 80% of tissue RNA is ribosomal (Young, 1970), of which a maximum of 80% could be translating [judging by the size of the ribosomal subunit and monomer pool in fed Ehrlich ascites cells (Hirsch *et al.*, 1973)], and knowing that the molecular weights of RNA and peptides are 2.45×10^6 and 110 respectively. On this basis an RNA activity of 33.4 g of protein synthesized/day per g of RNA is equivalent to 13.3 amino acids/s per ribosome and, given the assumptions made, this is probably a minimum value. The rectal temperature of the fowl is 41.7°C compared with 37.3°C in the rat (Brody, 1945), so that, assuming a Q_{10} of 2 (i.e. a doubling of the rate of synthesis for each 10°C rise in temperature; Haschemeyer, 1976), this rate is equivalent to 9.8 amino acids/s per ribosome at 37.3°C. This is similar to the value for rat skeletal muscle, i.e. 8.2 amino acids/s per ribosome (estimated from the value of the RNA activity, 20.3 g of protein synthesized/day per ribosome, observed during catch-up growth; Millward *et al.*, 1975).

The increased RNA activity observed at day 1 was not maintained, since after this the increased rates of synthesis reflected the higher RNA concentrations. Indeed the increase in RNA concentration was such that, had the elevated RNA activity been maintained, at 3 days the rate of synthesis would have been over 60%/day, which would have induced growth at 40%/day. It would appear then that at these later times RNA was not rate-limiting.

The increases in RNA content could have involved increases in rates of RNA synthesis or decreases in breakdown, although the turnover rate of rRNA would have to be much higher than in rat skeletal muscle (6.0%/day; Grimble & Millward, 1977) for degradation changes to be important. There is indirect evidence for increased RNA synthesis, since Sobel & Kaufman (1970) have reported enhanced RNA polymerase activity in skeletal muscle during hypertrophy, and Goldberg (1971b) has reported

increased incorporation of [^3H]orotic acid into such muscles. Further, in heart muscle, for which a technique for the separation of cell types has been developed (Cutilletta *et al.*, 1977), increased RNA polymerase type I (the ribosomal RNA polymerase) has been observed specifically in the muscle cells (A. F. Cutilletta, M. Rudnik & R. Zak, personal communication). A rather surprising finding is that of Jablecki *et al.* (1973), who report radioautographic studies in which increased [^{14}C]orotate uptake is confined to connective-tissue cells. This has been interpreted as indicating that most of the new RNA is involved in the synthesis of non-muscle cell proteins (Goldberg *et al.*, 1975). This could only be the case if the activity of existing muscle cell ribosomes was able to increase to values that would require elongation rates several times faster than that discussed above, and it is unlikely that such an increase is kinetically possible. In any case the synthesis of the most abundant non-muscle cell protein, collagen, never accounts for more than 3–5% of the total protein synthesis during the hypertrophy of the anterior latissimus dorsi muscle (Laurent *et al.*, 1978b) and there is no evidence that non-muscle cells produce substantial amounts of non-collagen proteins.

The relationship between the growth of the stretched muscle in terms of DNA-unit number (total DNA) and size (protein/DNA; see Cheek *et al.*, 1971) and the rates of protein turnover is of interest because of the apparent inverse relationship between the DNA-unit size and the fractional breakdown rate (Laurent *et al.*, 1978a). This follows from the fact that, in the anterior and posterior latissimus dorsi muscles as well as in various rat skeletal muscles (Millward *et al.*, 1978), the rate of protein synthesis per unit of DNA (the DNA activity) is constant, which suggests that the amount of cytoplasmic protein synthesis that can be 'managed' by a single myonucleus is fixed. The present results enable this empirical relationship to be tested. When the hypertrophy was complete the DNA activity was the same as in the control muscles, and, since the breakdown rate was unchanged, the size of the DNA unit was also the same as the original value. Thus the extra nuclei (and hence the DNA units) accumulated during the hypertrophy can be seen as essential to the growth of the muscle if the characteristic rate of protein turnover is to be maintained, because of the inability of the existing nuclei to sustain indefinitely the increase in the cytoplasmic protein synthesis that they can 'manage'.

This model is not entirely adequate even as an empirical one, since during the hypertrophy the DNA activity did increase considerably (by about 50%). Similar transient increases in DNA activity involving increases in RNA/DNA ratios and in RNA activity have also been observed in rat muscle during their nutritional rehabilitation (Millward *et al.*, 1975).

Why this increased DNA activity is not maintained is not known.

Another possible inadequacy of this approach results from the fact that it assumes that all extra nuclei accumulate in the contractile cells and we do not know with any certainty the relative contributions of the different cell types to the increase in DNA content. The most likely source of new nuclei is satellite cells, which are known to be mitotically active during skeletal-muscle hypertrophy (Schiaffino *et al.*, 1972) and to be the source of new nuclei for the muscle fibre during growth of the immature muscle (Moss & Leblond, 1970, 1971). It is, in our opinion, unlikely that the increased DNA results from a disproportionate increase in non-muscle cells such as endothelial fibroblasts. Certainly the morphological studies of Sola *et al.* (1973) did not indicate this. Further, although there is a marked increase in collagen synthesis during the hypertrophy (Laurent *et al.*, 1978b), this is only transient and could be achieved by an activation of existing quiescent connective-tissue cells.

Nature of the increased protein breakdown

The pattern of change in the breakdown rate observed here, a somewhat delayed increase to a value that is maintained after the major growth spurt has stopped, seems to be a common feature of accelerated muscle growth, since it is also observed during the transient hypertrophy of the rat hemidiaphragm (Turner & Garlick, 1974), as well as in rat hind-limb muscles during nutritional rehabilitation (Millward *et al.*, 1975; see Millward *et al.*, 1978).

There is morphological evidence that suggests that there are structural changes occurring in the anterior latissimus dorsi muscle during the hypertrophy consistent with the increased protein breakdown. Firstly new fibres are formed during the hypertrophy, some of which arise *de novo* (Sola *et al.*, 1973) as also observed in embryonic tissue (Kelly & Schotland, 1970; Guth & Samaha, 1972). According to Sola *et al.* (1973) not all of these new fibres reached maturity. Thus some of them are likely to have been 'wasted' with the degradation of their contents (including the nuclei) and this would contribute to the increased protein breakdown.

Fibre proliferation also occurs as a result of fibre splitting (Sola *et al.*, 1973), which appears to be a common feature of hypertrophy (Reitsma, 1970; Edgerton, 1970; Hall-Craggs, 1972). Since, according to Hall-Craggs (1972), there is widespread mitochondrial degeneration in such hypertrophying and split fibres, associated with newly formed lysosomes (Morton & Rowe, 1974), this is also consistent with the increased protein breakdown.

In addition to these increases in fibre number that are unique to muscle hypertrophy, the more than doubling of the contractile protein mass would almost

certainly have involved increases in the diameter and number of myofibrils. Since proliferation of myofibrils involves longitudinal splitting of pre-existing myofibrils (Goldspink, 1972), this may also involve 'wastage' as a result of the degradation of myofibrils isolated during the splitting.

The increased breakdown can, at least according to these arguments, be rationalized into a scheme in which it is an accompaniment to the remodelling of the muscle to accommodate the extra contractile units during growth.

Stretch as a pleiotypic activator

The attachment of the weight to the wing of the fowl in these experiments stretches the latissimus dorsi muscles and will induce tension development via the stretch reflex, since the muscles are innervated. There are therefore passive and active components of the stretch and both are included in the general term as used in this discussion. (Since these muscles also hypertrophy when similarly stretched after denervation, the stretch reflex would appear not to be a necessary component of the stimulus for growth.) Stretch invariably induces muscle growth even in the absence of any nutritional or hormonal support (Goldberg, 1971a), so that it must be accepted as the most important factor regulating muscle growth. Hershko *et al.* (1971), noting that cellular growth appeared to involve the co-ordinated response of metabolically unrelated reactions to single factors, suggested the term 'pleiotypic' to describe the response, factors such as insulin being 'pleiotypic activators'. In this respect, stretch can be defined as the main 'pleiotypic activator' for muscle growth.

One possible biochemical link between stretch and the anabolic response may be the increased membrane transport that has been observed (Goldberg & Goodman, 1969; Narahara & Holloszy, 1974; Goldberg *et al.*, 1974). Since in the present experiments the intracellular concentrations of serine, proline and alanine were increased after 24h (G. J. Laurent, P. C. Bates & D. J. Millward, unpublished work), this suggests that increased transport was occurring. However, there is no necessary link between increased transport and the activation of the anabolic processes. It may be that the increased amino acid transport is a result of a stretch-induced alteration in the cell membrane which itself, through some other mechanism, induces the anabolic responses. Narahara & Holloszy (1974) report that the electrical stimulation of frog sartorius muscle has the same effect as insulin or even trypsin treatment in stimulating glucose and possibly amino acid uptake. They suggest that this involves an alteration of the organization of the cell membrane. Since insulin is a potent stimulator of protein synthesis in muscle (Jefferson *et al.*, 1974, 1977), presumably as a result of events that follow its reaction with membrane receptors, it

Scheme 1. Postulated key events during stretch-induced skeletal-muscle hypertrophy

As discussed in the text, stretch can be likened to 'pleiotypic activation', which induces anabolic responses in muscle myofibre cells, satellite cells and connective-tissue fibroblast cells. However, during the increase in number and size of the myofibres some protein (and possibly DNA) is wasted as a result of the increased degradation. The enlarged muscle contains increased numbers of DNA units, but of the same size, thus maintaining its characteristic turnover rate.

may be that the same series of events can be initiated by a stretch-induced alteration of membrane structure.

An additional factor in muscle is that events in the contractile cell must be linked to the stimulation of mitosis of the satellite cells, the apparent source of the new nuclei during muscle growth. However, since the satellite cells are in close contact with the plasma membrane of the contractile cell, and since they are partially committed myogenic cells, it would not be surprising if their stimulation was part of the pleiotypic response initiated by muscle stretch. Perhaps more difficult to understand is the anabolic response of the non-muscle cells such as the fibroblasts, which also exhibit an immediate increase in the rate of protein synthesis in the stretched muscle (Laurent *et al.*, 1978b). However, we might suppose that an anabolic response to an alteration in membrane structure induced by stretching is a common feature of all cell types, so that any cell within the stretched muscle will behave in the same way.

In summarizing the response of the anterior latissimus dorsi muscle to stretch (Scheme 1) it should be noted that one difference between the observed response and that after the pleiotypic activation of cultured cells as described by Hershko *et al.* (1971) is the fact that increased rather than decreased protein breakdown is a feature of the muscle response. However, as we have argued, this aspect of muscle growth most likely reflects the way in which the functional units of muscle contraction (i.e. both myofibrils and myofibres) increase, and as a result is probably unique to muscle and not a characteristic feature of the growth of other tissues.

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