Turnover of Muscle Protein in the Fowl

COLLAGEN CONTENT AND TURNOVER IN CARDIAC AND SKELETAL MUSCLES OF THE ADULT FOWL AND THE CHANGES DURING STRETCH-INDUCED GROWTH

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The collagen content and the rate of collagen synthesis were measured in the anterior and posterior latissimus dorsi muscles and in heart from fully grown fowl. This was done by measuring the proline/hydroxyproline ratios in the muscle and by a constant infusion of [^{14}C]proline. These measurements were also made during the hypertrophy of the anterior muscle in response to the attachment of a weight to one wing of the fowl. In the non-growing muscles the collagen content was higher in the anterior muscle (22.8% of total protein) than in the posterior muscle (9.5% of total protein) and lowest in the heart (3.8% of total protein). In the two skeletal muscles a little over half of the collagen was accounted for by internal collagen (i.e. perimysium and endomysium). Collagen synthesis in these non-growing muscles occurred at 0.59%/day in each of the two skeletal muscles and at 0.88%/day in the cardiac muscle. During hypertrophy the collagen content of the anterior muscle increased, but not as fast as intracellular protein, so that after 58 days the concentration had fallen from 22.8 to 14.4% of total protein. This may have resulted from an incomplete production of the epimysial sheath, since the concentration of internal collagen did not fall and as a result accounted for over 80% of the total in the enlarged muscle. Collagen synthesis increased 8-fold during the first week of the hypertrophy, but never amounted to more than 4% of the total muscle protein synthesis. When the net accumulation of collagen is compared with the increased rate of synthesis it is concluded that between 30 and 70% of the newly synthesized collagen may have been degraded.

Collagen is the major protein of the connective-tissue matrix in muscle, its presence markedly affecting the tensile strength of the muscle, and its distribution within the muscle regulating the extent to which contraction can occur (see Gould, 1973). The production of collagen during muscle hypertrophy should therefore be an essential part of the process. There are several studies that indicate that collagen synthesis is stimulated during hypertrophy. During cardiac-muscle hypertrophy (Lindy *et al.*, 1972) and skeletal-muscle hypertrophy (Turto *et al.*, 1974) the activity of collagen prolylhydroxylase, the enzyme that hydroxylates protein-bound proline to form hydroxyproline, is increased. Studies by Skosey *et al.* (1972) and Turto & Lindy (1976) have shown that the incorporation of [^{14}C]proline into collagen hydroxyproline is increased 3–4-fold during experimental cardiac hypertrophy in rats. However, Schreiber *et al.* (1970) reported that increased collagen synthesis

was not observed during the first 3 h of a pressure overload in the perfused heart, although the synthesis of myosin and total protein was stimulated.

The study of collagen metabolism is rendered particularly difficult because of the unique features of the structure of the collagen fibrils in connective tissue. Mature collagen fibres in the connective-tissue matrix are either inert or turn over extremely slowly according to most reports of collagen turnover (Neuberger *et al.*, 1951; Neuberger & Slack, 1953; Thompson & Ballou, 1956; Kao *et al.*, 1961). In contrast with other proteins, collagen ages chemically because the cross-linking within collagen fibrils develops relatively slowly, and this would appear to affect not only the solubility of collagen but also its resistance to proteolytic attack and degradation (see Prockop & Kivirikko, 1968; Bornstein, 1974). Pulse-decay experiments of collagen turnover have demonstrated a precursor-product relationship of collagen fractions of decreasing solubility (e.g. Jackson & Bentley, 1960) and have also indicated that a proportion of the young soluble collagen is

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degraded (Nimni *et al.*, 1967; Prockop & Kivirikko, 1968). Thus collagen in connective tissue can include mature molecules, which are nearly inert, as well as young more soluble molecules, which can turn over relatively rapidly, and the relative sizes of these pools depend on whether growth is occurring.

Because of these features, measurements of collagen turnover involving pulse-decay experiments are difficult to interpret in terms of the overall rate of collagen synthesis and breakdown, as well as exhibiting the usual problems caused by reutilization of labelled amino acids (e.g. Jackson & Heininger, 1974). In the present study, therefore, the overall rates of collagen synthesis were measured by determining the incorporation of [^{14}C]proline into protein-bound hydroxyproline in total muscle protein during a continuous infusion of [^{14}C]proline (Laurent *et al.*, 1978a). These measurements were made in cardiac and two skeletal muscles (anterior and posterior latissimus dorsi) in fully grown male cockerels (*Gallus domesticus*) and in the anterior latissimus dorsi during the marked hypertrophy that occurs in response to the attachment of a weight to the wing (Sola *et al.*, 1973; Laurent & Sparrow, 1977; Laurent *et al.*, 1978a,b). Since all acid-precipitable collagen is included in the measurements, the problem of the varying solubility of collagen is avoided. Further, the present example of skeletal-muscle hypertrophy is particularly suited to the study of collagen metabolism because no surgery is involved and as a result no collagen-containing scar tissue is present.

Experimental

Hypertrophy and infusion

Hypertrophy was induced in 30 cockerels as described previously (Laurent *et al.*, 1978b) and measurements were made at 1, 3, 7, 28, 34 and 58 days after the attachment of the weight. Details of the continuous intravenous infusion of [^{14}C]proline were as previously described (Laurent *et al.*, 1978a). In all of the birds the anterior latissimus dorsi muscles were examined. In the birds killed at 1, 3 and 7 days during the hypertrophy the posterior latissimus dorsi muscles were examined, and in five birds the heart was examined. The group of birds analysed 34 days after the attachment of the weight were not infused. Only the composition of the two skeletal muscles with respect to total protein, collagen content and its distribution was measured in this group.

Determination of collagen content

The muscles were carefully trimmed free of fascia, leaving the epimysial sheath on the muscles. In the group examined after 34 days of hypertrophy the epimysium was also removed from a sample of the muscle (usually half the muscle), so that only the

internal collagen of the endomysium and perimysium would be included in the analysis.

The determination of the collagen content of the muscles is facilitated by the fact that hydroxyproline can be considered to occur exclusively in collagen, so that protein-bound hydroxyproline will indicate collagen content. Collagen content can be calculated therefore from the hydroxyproline content per unit of tissue, provided considerable care is taken to avoid amino acid degradation in the course of acid hydrolysis. However, in the present experiments there was no need to do this, since the collagen content could be calculated from the ratio of proline/hydroxyproline in the mixed muscle protein (measured in the course of determining the specific radioactivities of protein-bound proline and hydroxyproline), and from a knowledge of the relative amounts of these two amino acids in collagen and non-collagen protein. This was done as follows. Because proline accounts for 15% of collagen (Newman & Logan, 1950) and 3.0% of the non-collagen protein of the anterior muscle (according to our measurements), the relative amounts of collagen and non-collagen protein are related to the distribution of proline according to the expression:

$$\frac{\text{Collagen protein}}{\text{Non-collagen protein}} = \frac{1}{5} \times \frac{\text{collagen proline}}{\text{non-collagen proline}} \quad (1)$$

Since the relative number of proline residues in collagen is $1.27 \times$ the hydroxyproline residues both in pig muscle (McClain *et al.*, 1970) and in a variety of non-muscle tissues (Lowther, 1963) and since hydroxyproline does not occur in non-collagen proteins to an appreciable extent, eqn. (1) can be rewritten solely in terms of proline and hydroxyproline measured in the total muscle protein hydrolysate:

$$\frac{1}{5} \times \frac{1.27 \text{Hyp}}{\text{Pro} - 1.27 \text{Hyp}} = \frac{1}{3.94 \times \left(\frac{\text{Pro}}{\text{Hyp}} - 5 \right)} \quad (2)$$

The ratio of proline/hydroxyproline was calculated from the amino acid analysis of the total tissue protein hydrolysate, and the amount of collagen in the tissue was calculated by multiplying the value obtained in eqn. (2) by the non-collagen protein concentration (i.e. 0.1M-NaOH-soluble protein) measured by the method of Lowry *et al.* (1951).

Calculation of the rate of collagen synthesis

The rate of collagen synthesis was calculated by comparing the specific radioactivities of the protein-bound hydroxyproline with that of free proline measured at the end of infusion by the equation of Garlick *et al.* (1973):

$$\frac{S_b}{S_i} = \frac{\lambda_i}{(\lambda_i - k_s)} \left(\frac{1 - e^{-k_s t}}{1 - e^{-\lambda_i t}} - \frac{k_s}{(\lambda_i - k_s)} \right) \quad (3)$$

where S_0 and S_1 are specific radioactivities of bound hydroxyproline and free proline respectively, λ_1 is the exponential rate constant describing the rise to plateau of free proline with time, t , and k_s is the fractional synthesis rate of collagen with units of time⁻¹. In addition to the problems inherent in this approach, which have been discussed (Laurent *et al.*, 1978a, plus Appendix), there are additional problems specific to the measurement of collagen synthesis. There is no reason why the specific radioactivity of proline in the connective-tissue cell, the site of collagen synthesis, should be the same as the measured value, which is principally that of proline in the muscle cells. Further, although these measured values are different in the three muscles (i.e. 85, 64 and 46% of the specific radioactivity of plasma proline for heart, anterior and posterior latissimus dorsi muscle respectively), there is no reason to suppose that the values for connective-tissue proline should also vary between the muscles. We have therefore assumed that the appropriate value of S_1 to be used in the calculation is that of the measured value in the anterior muscle, the median value of the three muscles.

In addition to lack of direct information about the specific radioactivity of proline at plateau value in connective tissue, there is also insufficient information to enable the rate of rise to plateau values to be predicted with any certainty. We have assumed therefore that the value for λ_1 , the rate constant describing the rise to plateau value of the precursor in eqn. (3), is 22.2 days⁻¹, since this gives the closest approximation to the rise to plateau of the specific radioactivity of proline in the anterior muscle (see the Appendix to Laurent *et al.*, 1978a). Any errors resulting from these assumptions are unlikely, in our opinion, to alter the principal conclusions reached in the present paper.

Results

Collagen content

The collagen content of the cardiac muscle and anterior and posterior latissimus dorsi muscles is

shown in Table 1 as wet weight concentration and proportion of total protein. There was less collagen in heart (ventricle) than in either of the two skeletal muscles, and there was also less collagen in the posterior latissimus dorsi than in the anterior muscle. The value for the wet weight concentration in cardiac muscle, 0.56%, is similar to that reported for rat heart (0.57% wet wt.; Skosey *et al.*, 1972), but a little lower than the value reported for human heart (0.9%; Leclercq & Swynghedauw, 1976). This represents less than 4% of the total protein. This difference in collagen content between the anterior latissimus dorsi muscle, which comprises mainly slow red tonic fibres, and the posterior muscle, comprising mainly fast white fibres, is consistent with the many studies that have shown that the collagen content of individual muscles varies (e.g. from 3 to 30% of total protein; see Gould, 1973). The difference in the present experiments was not due to gross differences in the distribution of collagen, since the collagen content of a portion of internal muscle (i.e. with the epimysial sheath removed) was also higher in the anterior muscle (9.4% total protein) than in the posterior muscle (4.9%; Table 3). This indicates that internal perimysial and endomysial collagen accounts for a little over half the total collagen in each case.

During the hypertrophy of the anterior latissimus dorsi muscle collagen accumulated during week 1 at approximately the same rate as intracellular protein, but after that time no further increase was apparent. After 58 days, although total protein had increased by 142%, collagen had only increased by between 30 and 50%. Thus as Table 2 shows, the amount of collagen as a proportion of total protein was maintained at control values during the first week, but fell after this time.

To determine whether the changes in collagen content that occurred during the hypertrophy involved any alteration in the distribution of collagen between the surface epimysial sheath and the internal perimysial and endomysial connective tissue (see Gould, 1973), samples of hypertrophied muscle stripped of epimysium were analysed from birds

Table 1. Collagen content of muscles of the adult fowl

Non-collagen protein was determined by the method of Lowry *et al.* (1951). The Pro/Hyp ratio was measured by amino acid analysis of total protein extracted from each muscle. The collagen content is expressed as a percentage wet wt. (calculated from the Pro/Hyp ratio as described in the text) and as a percentage of total protein. Values are means $\pm$ 1 s.d. with number of analyses shown in parentheses.

Muscle	Non-collagen protein (% wet wt.)	Pro/Hyp	Collagen	
			(% wet wt.)	(% of total protein)
Heart, ventricle (5)	15.4 $\pm$ 0.7	7.69 $\pm$ 0.23	0.61 $\pm$ 0.03	3.8 $\pm$ 0.9
Posterior latissimus dorsi (13)	15.6 $\pm$ 0.2	3.70 $\pm$ 0.07	1.63 $\pm$ 0.05	9.5 $\pm$ 0.7
Anterior latissimus dorsi (17)	13.5 $\pm$ 0.3	2.13 $\pm$ 0.01	3.98 $\pm$ 0.07	22.8 $\pm$ 0.3

Table 2. *Changes in collagen content of the anterior latissimus dorsi muscle during hypertrophy*

Non-collagen protein was measured in control and hypertrophy in muscles at each time point, and to correct for variability between birds the measured value was multiplied by the ratio of the mean control value and the actual individual control value (see Laurent *et al.*, 1978b). The Pro/Hyp ratio was measured as described in Table 1 and the amount of collagen per muscle calculated from the Pro/Hyp ratio as described in the text. Values for the Pro/Hyp ratios are shown as means $\pm$ 1 s.d. for four to five fowls in each group.

Time of hypertrophy (days)	Non-collagen protein (mg)	Pro/Hyp	Collagen	
			(mg)	(% of total protein)
0	125	2.13 $\pm$ 0.01	36.9	22.8
1	135	2.02 $\pm$ 0.10	45.6 $\pm$ 5.3	25.2
3	171	2.23 $\pm$ 0.26	45.2 $\pm$ 9.7	20.9
7	229	2.29 $\pm$ 0.18	56.9 $\pm$ 8.6	19.8
28	299	2.42 $\pm$ 0.27	65.9 $\pm$ 12.5	18.0
58	303	2.77 $\pm$ 0.15	51.2 $\pm$ 4.6	14.4

Table 3. *Distribution of collagen in skeletal muscles in the adult fowl and the effect of hypertrophy*

Collagen was measured in a sample of the whole muscle as well as in a sample from which the epimysial sheath had been removed. The Pro/Hyp ratio was determined as described in Table 1 and the collagen content calculated as described in the text. Measurements were made in control anterior and posterior latissimus dorsi muscles and in the hypertrophied anterior latissimus dorsi muscles from a group of birds 34 days after the attachment of the weight to one wing. Values are means $\pm$ 1 s.d. for five fowls.

Muscle	Pro/Hyp		Internal collagen	
	Total muscle	Internal sample	(% of total protein)	(% of total collagen)
Anterior latissimus dorsi				
Control	2.51 $\pm$ 0.27	3.71 $\pm$ 0.40	9.4 $\pm$ 2.1	55 $\pm$ 14
Hypertrophied	3.13 $\pm$ 0.21	3.57 $\pm$ 0.51	9.9 $\pm$ 1.9	83 $\pm$ 9
Posterior latissimus dorsi				
Control	4.35 $\pm$ 0.36	6.25 $\pm$ 0.80	4.9 $\pm$ 0.9	64 $\pm$ 7

Table 4. *Rates of synthesis of collagen in muscles from the adult fowl*

Rates of protein synthesis were measured by means of a constant infusion of [14 C]proline. The rate was calculated from the ratio of the specific radioactivities of protein-bound hydroxyproline (S_b) and free proline (S_f) in the muscles at the end of the infusion. The specific radioactivity of free proline was assumed to be the measured value for the anterior latissimus dorsi muscle in all three cases. The rate was calculated according to an equation that assumed that the specific radioactivity of free proline rose to a plateau value according to a single exponential, the rate constant having the value of 22.2 days $^{-1}$ (see the text). The values are means $\pm$ 1 s.d. for the numbers of measurements shown in parentheses.

Muscle	$10^3 \times \frac{S_b}{S_f}$	Rate of synthesis (%/day)
Heart (5)	1.73 $\pm$ 0.3	0.88 $\pm$ 0.12
Posterior latissimus dorsi (13)	1.14 $\pm$ 0.48	0.59 $\pm$ 0.29
Anterior latissimus dorsi (17)	1.14 $\pm$ 0.20	0.59 $\pm$ 0.08

after 34 days of hypertrophy. The results in Table 3 show that the internal muscle sample from the hypertrophied muscle contained the same ratio of collagen to total protein as in the control muscle, but

because the collagen concentration in the whole muscle was decreased, internal collagen (perimysial and endomysial) accounted for a much higher proportion (83%) than in the control muscles (55%). This means that the proportion of collagen in the surface epimysial sheath was markedly decreased in the hypertrophied muscle.

Collagen synthesis

Rates of collagen synthesis in cardiac and the two skeletal muscles are shown in Table 4 (expressed in terms of % per day). Although the rate of synthesis for collagen in heart was a little faster than in the two skeletal muscles, the rates were very low in all cases. It is reasonable to assume that the muscle collagen pool is in a steady state so that the rates of synthesis and degradation are equal; these rates indicate average half-lives for collagen of 79 days for cardiac muscle and 117 days for the two skeletal muscles.

During the hypertrophy of the anterior muscle the synthesis rate of collagen was markedly increased (Table 5). After 24 h, the rate of collagen synthesis doubled, and by day 3 there was a 7-fold increase, which was maintained for the rest of the first week. After this time the rate fell and, although increased collagen synthesis was still apparent in some in-

Table 5. Rates of collagen synthesis in comparison with rates of total protein synthesis in the anterior latissimus dorsi muscle during hypertrophy

Rates were measured and calculated as described in Table 4 from the relative specific radioactivities of protein-bound hydroxyproline and free proline. Rates of synthesis of non-collagen protein are from Laurent *et al.* (1978b) and absolute rates of synthesis have been calculated by comparing the fractional rates with the amounts of collagen and non-collagen protein shown in Table 2.

Time of hypertrophy (days)	Non-collagen protein synthesis		Collagen synthesis		Protein synthesis (% of total)
	(%/day)	(mg/day per muscle)	(%/day)	(mg/day per muscle)	
Initial	17.0	21.25	0.59 ± 0.07	0.22	1.00
1	32.4	43.7	1.25 ± 0.13	0.56	1.28
3	32.2	55.1	4.5 ± 0.7	2.03	3.69
7	31.5	72.1	4.2 ± 0.2	2.38	3.30
28	25.8	77.1	2.2 ± 0.6	1.45	1.88
58	17.9	54.2	1.05 ± 0.4	0.54	0.99

dividual birds after 28 and 58 days, in others it appeared to have returned to normal.

Discussion

Collagen content of the different muscles

There were considerable differences between the collagen contents of the cardiac and two skeletal muscles examined in this study. However, a considerable part of the total collagen in skeletal muscle occurs in the epimysial sheath, which supports the individual muscle (in contrast with perimysial and endomysial collagen, which supports fibre bundles and individual fibres; see Gould, 1973). In the two skeletal muscles epimysial collagen accounted for 45 and 36% of the total collagen in the anterior and posterior latissimus dorsi muscles respectively. However, cardiac muscle does not have a prominent epimysial sheath, which is one reason for its lower collagen content. When compared with an internal sample of the posterior muscle (i.e. the perimysial and endomysial collagen, which was 4.9% of total protein), the collagen content of heart (3.8% of total protein) is not very different. However, it is still much lower than in the internal sample of the anterior muscle (9.4% of total protein). The higher collagen content of the anterior muscle no doubt reflects its function as a tonic postural muscle, involved in the continuous support of the wing. It was suggested some years ago that muscles that are capable of finely graded movements, as postural muscles generally are, tend to have more connective tissue (Fernand, 1949). However, the studies by Beatty *et al.* (1966) led them to suggest that the collagen content of red muscle was only half that of white muscle. In contrast, the results of Garcia-Bunuel & Garcia-Bunuel (1967) indicated that the collagen content of the rat soleus muscle containing mainly red oxidative fibres was greater than that of gastrocnemius muscle (principally white glycolytic fibres). More information will be required

to establish whether there is a general relationship between the structure and function of muscle and collagen content.

The pattern of accumulation of collagen in the anterior muscle during hypertrophy is difficult to explain. Although during the initial period collagen appeared to accumulate at the same rate as intracellular proteins, after the first week the accumulation of collagen appeared to stop. Further, there was an apparent redistribution of collagen. After 34 days, collagen concentration within the muscle (i.e. perimysial and endomysial collagen) was maintained at control values with much less in the epimysial sheath. We cannot be certain that this finding reflects a dissection problem. The removal of the muscle involves a degree of subjective assessment about the distinction between fascia and epimysium, and, although this can be standardized in controls, it is more difficult during the hypertrophy. This is because the growth of the muscle alters its relationship to surrounding muscles and fascia. If part of the epimysium were removed during dissection, this would result in an underestimation of total collagen content. However, as discussed below, when the rate of accumulation is calculated from the measured rate of synthesis it would appear that total collagen production does indeed lag behind that of the intracellular proteins. Thus the fact that after 34 days of hypertrophy internal collagen appeared to occur at normal concentrations suggests that the production of perimysial and endomysial collagen had matched that of the intracellular proteins, but that epimysial collagen production was incomplete.

Previous studies on changes in collagen content during muscle hypertrophy have produced a variety of findings. Most is known about cardiac hypertrophy. In this case there is generally an increase in total collagen content, and, during hypertrophy induced by a pressure overload, there is generally an increase in the collagen concentration (Grove *et al.*,

1969b; Skosey *et al.*, 1972; Spann *et al.*, 1971). On the other hand, Bartosova *et al.* (1969) have induced hypertrophy by a variety of means and have shown that there may be an increase or decrease in collagen concentration, depending on the stimulus used to induce hypertrophy. These changes can be rationalized if cardiac hypertrophy is divided roughly into two types. In hypertrophy due to volume overload (e.g. in marathon runners or endurance-trained rats or in anaemia) the total myosin adenosine triphosphatase increases, but its concentration remains constant as the fibres of the ventricle get longer and thicker. We would expect the collagen content to similarly increase but not the concentration. Indeed, since the surface area/volume ratio for a fibre would fall, the collagen concentration might even fall. In pressure overload the heart muscle work-load is not really increased in the same way and much of the 'hypertrophy' is simply a thickening of the wall due to increased connective tissue. (This is seen in weight lifters.) Under these circumstances one would expect to see an increase in collagen concentration, which is what Bartosova *et al.* (1969) found in the pure pressure-overload model. It appears that the hypertrophy of the anterior latissimus dorsi muscle is more akin to the volume-overload situation and we might therefore not find the changes surprising. Certainly previous reports of hypertrophy in skeletal muscle have suggested that there is an increase in total collagen but no increase in the collagen concentration (Turto *et al.*, 1974). This is in line with the present results.

Rates of collagen synthesis

The measurements of rates of collagen synthesis described here involve some uncertainty because of the lack of information about the labelling of the actual precursor proline for collagen synthesis in the connective-tissue cells. The assumed value for all three muscles was the measured specific radioactivity of proline in the anterior muscle and if the real value was less than this, the calculated rates would be underestimates. For example, a lower specific radioactivity for proline in the connective-tissue cell could occur if there was a substantial synthesis of proline *de novo* from glutamate, but there is no reason why this should occur in these birds with a high-protein (and therefore proline) dietary intake. In the case of the comparative measurements, in heart and the two skeletal muscles, this uncertainty about the precursor is unlikely to influence the main conclusion that the synthesis of collagen is very slow in all cases and occurs at the same rate at least in the two skeletal muscles, even though the turnover rate of the intracellular proteins in the heart and the red muscle is 2–3 times faster than in the white muscle. This is in accord with the argument that the relative turnover rate of intracellular proteins reflects the different

structure and function of individual types of muscle fibre (Laurent *et al.*, 1978a), whereas there is no reason to expect the metabolism of fibroblasts to vary qualitatively between muscle types.

A second problem, inherent in the present approach to the study of collagen metabolism, arises from the fact that the kinetics of turnover of collagen are complex (see Bornstein, 1974). There is evidence that the younger more soluble collagen fractions appear to be the source of most of the hydroxyproline-containing peptides that are excreted in the urine, suggesting that there is some wastage of early synthesized collagen due to its degradation before it is fully cross-linked and relatively inert (see Prockop & Kivirikko, 1968). The present measurements would underestimate the true rate of synthesis if a significant proportion of this newly synthesized collagen was degraded during the 6h of the infusion. Steinberg (1973) has suggested that some degradation of procollagen may occur even before it is secreted from the connective-tissue cells. However, there is very little reliable information about the extent of collagen wastage. Nimni and co-workers (Nimni & Bavetta, 1964, 1965; Nimni *et al.*, 1967) investigated the precursor-product relationship between fractions of collagen of varying solubility in rabbit skin and concluded that over two-thirds of the soluble collagen reached the insoluble stage, one-third being degraded en route. If all of this wastage occurred during the first few hours, then the present measurements would underestimate the actual synthesis rates by one-third. Studies of the half-lives of the newly synthesized soluble collagen fractions do not in general indicate the proportion of the turnover of each fraction that is actually degraded rather than converted into a less-soluble form. Since labelled hydroxyproline is excreted for many days after a pulse label (see Prockop & Kivirikko, 1968), it would appear that collagen fibrils that are much older than 6h are degraded so that the underestimate of the rate of synthesis would be much less than one-third and could probably be ignored.

The wastage of a proportion of the newly synthesized collagen means that the calculation of a half-life of the most stable collagen is difficult. Assuming that one-third of all collagen synthesized is wasted before forming mature collagen, as reported by Nimni *et al.* (1967), it follows that the fractional synthesis rate of mature collagen in these muscles was about 0.6%/day for heart and 0.4%/day for the two skeletal muscles. If there was no net deposition of collagen, these rates would indicate half-lives of 115 and 173 days. Any growth of collagen would mean lower replacement rates and longer half-lives, and the possibility that mature collagen is totally inert cannot be over-ruled.

These very low fractional rates of collagen synthesis mean that only a very small proportion of

protein synthesis in muscle is accounted for by collagen synthesis, 0.2% of total protein synthesis in heart, and just under 1.0% in the two skeletal muscles. In view of the fact that fibroblasts are usually assumed to be a high proportion of the non-muscle-cell population, which in turn represents about 70% of the total cell population in heart (Grove *et al.*, 1969a) and 20–30% in skeletal muscle (Enesco & Puddy, 1964), these rates of collagen synthesis indicate that such cells are metabolically quiescent, at least as far as this aspect of their metabolism is concerned.

Synthesis of collagen during hypertrophy

One of the purposes of this study was to determine the extent to which synthesis of proteins in non-muscle cells was an important component of the overall increase in protein synthesis that accompanies skeletal-muscle hypertrophy, and collagen is an easily identifiable non-muscle protein. Since the deposition of new collagen always appears to be associated with increased hydroxyproline excretion (Kivirikko, 1970) and because of the susceptibility of newly synthesized collagen to proteolytic attack (Prockop & Kivirikko, 1968; Steinberg, 1973), it was possible that during the hypertrophy of the anterior muscle new collagen formation could have required a considerable amount of collagen synthesis in the connective-tissue cell.

The present results show that this was not the case, since, although there was a marked increase in collagen synthesis within the first few days of the hypertrophy which was disproportionate to the increase in muscle cell protein synthesis, the rate of collagen synthesis never accounted for more than a small percentage of the total muscle protein synthesis. These results are difficult to rationalize with the report of Jablecki *et al.* (1973) that, 2 days after the induction of compensatory hypertrophy in rat soleus muscle, radioautographic studies showed that the incorporation of [^3H]uridine was almost entirely limited to fibroblast cells. Furthermore during the hypertrophy of skeletal muscle (Schiaffino *et al.*, 1972) and cardiac muscle (Morkin & Ashford, 1968; Grove *et al.*, 1968b) there is increased uptake of labelled thymidine into connective-tissue cells.

These reports are often interpreted as indicating that the proliferation and increased activity of fibroblasts in the course of increased collagen production during hypertrophy account for a major part of the new RNA that accumulates in muscle at this time. This is unlikely. According to Vuust & Piez (1972) the elongation rate of collagen is 3.5 amino acid residues/s at 37°C, which is somewhat lower than estimates of elongation rates of rat skeletal-muscle proteins (about 6 amino acid residues/s; Millward *et al.*, 1976). If this is the case, then more ribosomes will be involved in a given rate of synthesis of collagen than in the synthesis of non-collagen

proteins. Thus in the anterior latissimus muscle at 3 and 7 days during the hypertrophy the synthesis of 3–4% of total protein accounted for by collagen synthesis would require no more than 5–7% of all translating ribosomes.

Another purpose of the present study was to observe the time course of any change in collagen synthesis compared with the changes in muscle cell protein synthesis. As far as the changes after 24 h are concerned, it would appear that roughly comparable increases in the rates of synthesis of collagen (112%) and muscle cell proteins (90%) occurred. This means that measurements would have to be made at earlier times to determine whether there was any difference between the rate of response to the weight attachment by the two cell types.

Although we would include the activation of collagen synthesis in the overall 'pleiotypic' response of the muscle to stretching (Laurent *et al.*, 1978b), the nature of the signal and the events within the connective-tissue cell are largely unknown. The RNA content of these cells would most likely increase, and, since changes in RNA synthesis appear to be associ-

Fig. 1. Changes in rate of collagen synthesis and the predicted rate of collagen accumulation compared with the accumulation of non-collagen protein during the hypertrophy of the anterior latissimus dorsi muscle in the adult fowl. The rate of collagen synthesis (●) was measured by means of a constant infusion of [^{14}C]proline. The predicted increase in collagen content as % of the initial value (○) was calculated from the area under the synthesis-rate curve, less the turnover component. This assumes that all of the collagen synthesis above the basal rate (0.59%/day) was deposited. The rate of increase in non-collagen protein (□) is also shown.

ated with changes in RNA polymerase activity, at least during cardiac hypertrophy (Nair *et al.*, 1968), an increase in RNA polymerase activity might be an expected event. However, according to the measurements of Cutilletta and colleagues (A. F. Cutilletta, M. Rulnik & R. Zak, personal communication) assays of the various RNA polymerases in separated muscle and non-muscle cells (Cutilletta *et al.*, 1977) showed that, although the total RNA polymerase activity was increased in both cell types, the RNA polymerase I activity (responsible for rRNA synthesis) was only stimulated in the muscle cells. This could indicate that the mechanism for increased RNA synthesis in muscle and fibroblast cells during hypertrophy might be different.

The extent to which the newly synthesized collagen is 'wasted' owing to proteolytic attack during the hypertrophy can be estimated by comparing the observed amount of collagen accumulated with the calculated amount, assuming that all synthesized above the basal turnover rate was deposited. As shown in Fig. 1, the increased synthesis of collagen could account for a maximum increase in collagen content of 103% over the 58 days, compared with the 142% increase in non-collagen protein. As discussed above, the measured increase in collagen content was between 30 and 50%, which would mean that between 50 and 70% of the newly synthesized collagen was wasted. Since, as discussed above, the collagen accumulated might be underestimated owing to excessive dissection of the perimysium, the actual wastage may be less than this and perhaps closer to the value of 33% estimated by Nimni *et al.* (1967).

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