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# Pre- and post-natal growth and protein turnover in smooth muscle, heart and slow- and fast-twitch skeletal muscles of the rat

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1. The growth of one smooth and three individual striated muscles was studied from birth to old age (105 weeks), and where possible during the later stages of foetal life also. Developmental changes in protein turnover (measured *in vivo*) were related to the changing patterns of growth within each muscle, and the body as a whole. 2. Developmental growth (i.e. protein accumulation) in all muscles involved an increasing proportion of protein per unit wet weight, as well as cellular hypertrophy. The contribution of the heart towards whole-body protein and nucleic acid contents progressively decreased from 18 days of gestation to senility. In contrast, post-natal changes in both slow-twitch (soleus) and fast-twitch (tibialis anterior) skeletal muscles remained reasonably constant with respect to whole-body values. Such age-related growth in all four muscle types was accompanied by a progressive decline in both the fractional rates of protein synthesis and breakdown, the changes in synthesis being more pronounced. Age for age, the fractional rates of synthesis were highest in the oesophageal smooth muscle, similar in both cardiac and the slow-twitch muscles, and lowest in the fast-twitch tibialis muscle. Despite these differences, the developmental fall in synthetic rates was remarkably similar in all four muscles, e.g. the rates at 105 weeks were 30–35% of their values at weaning. Such developmental changes in synthesis were largely related to diminishing ribosomal capacities within each muscle. When measured under near-steady-state conditions (i.e. 105 weeks of age), the half-lives of mixed muscle proteins were 5.1, 10.4, 12.1 and 18.3 days for the smooth, cardiac, soleus and tibialis muscles respectively. 3. Old-age atrophy was evident in the senile animals, this being more marked in each of the four muscle types than in the animal as a whole. In each muscle of the senile rats the protein content and composition per unit wet weight, and both the fractional and total rates of synthesis, were significantly lower than in the muscles of younger, mature, animals (i.e. 44 weeks). In the soleus the decreased synthesis rate appeared to be related to a further fall in the ribosomal capacity. In contrast, the changes in synthesis in the three remaining muscles correlated with significant decreases in the synthetic rate per ribosome. Such changes probably influence the mechanical efficiency of the muscles concerned, and may represent an important step leading to heart failure and hence death of these senile animals.

Interest in the developmental and adaptive growth of muscle has increased enormously in recent years. There are probably several reaons for this. Muscle is the largest tissue in mammals, representing some 25–45% of the body mass between birth and the mature adult (Miller, 1969; Young, 1970). Understanding the way that this tissue grows is therefore important in appreciating the growth of the body as a whole. The relatively recent appreciation of muscles' ability to undergo rapid and extensive adaptive growth has also made

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this tissue a focus for identifying the physiological factors that influence tissue growth and for elucidating the cellular mechanisms that effect changes in muscle fibre size and strength, and their metabolic and contractile characteristics (Goldberg, 1971; Pette, 1980; Vrbová, 1980; Goldspink, 1980*a*; Jolesz & Sréter, 1981). In order to perform its contractile function, muscle also synthesizes considerable quantities of unique proteins, thus making this tissue an excellent system for studying aspects of differentiation and growth (Goldspink, 1974; McLachlan & Wolpert, 1980; Goldspink, 1980*a*). However, little reliable information on turnover rates of muscle proteins *in vivo* can as yet be related to the age-related changes in muscle growth (Waterlow *et al.*, 1978). Of the few studies undertaken, most have only investigated a narrow age range, this usually being confined to post-natal life. In addition, instead of carefully defining the rates of protein turnover within individual muscles of well-defined fibre-type compositions, all too often two or more muscles have been combined simply to facilitate analysis. This is unfortunate, since each individual muscle is composed, to varying degrees, of fibres which possess very different biochemical and physiological properties. It is therefore difficult enough to relate even precise turnover rates to the asynchronous development of these different fibre types within any individual muscle. Although it may be convenient to treat the musculature as one body tissue, it is now well established that the different muscle types, which together constitute the musculature, possess very different turnover rates. Further, these muscle types respond very differently to changes in activity (Booth & Kelso, 1973; Goldberg *et al.*, 1974; Goldspink, 1980*b*), nutrition (Young, 1970; Rannels *et al.*, 1977; Waterlow *et al.*, 1978; Millward & Waterlow, 1978) and circulating hormones (Rannels *et al.*, 1977; Ianuzzo *et al.*, 1977; Waterlow *et al.*, 1978; Rannels & Jefferson, 1980; Goldberg, 1980; Flaim *et al.*, 1980; Kelly & Goldspink, 1982).

It will also be apparent from the present paper that the rates of protein turnover are linked to, and change with, age-related functions of the different muscle types within the developing animal. All of these considerations call for the most precise information on protein turnover within individual muscles, and when possible in single fibres. In the current absence of sufficiently sensitive techniques to study turnover at the level of individual fibres, we have investigated the changes in growth and protein turnover in well-defined muscle types, i.e. the heart, one predominantly slow-twitch (i.e. soleus) and one predominantly fast-twitch (i.e. tibialis anterior) skeletal muscle, and the smooth muscle of the oesophagus. These parameters have been investigated throughout post-natal life and, where possible, in the latter stages of foetal life also. As in the preceding paper, standardized techniques were rigorously employed throughout, and the age-related changes within these individual muscles compared with the developmental changes in the whole animal (Goldspink & Kelly, 1984).

## Methods

Male albino rats (Charles River, CD strain) were fed and housed in a manner identical with that described in the preceding paper (Goldspink & Kelly, 1984).

Protein synthesis was measured *in vivo* in muscles 10 min after intravenous injection of 150 $\mu$mol of phenylalanine administered in 1 ml of NaCl (0.9%) per 100 g body wt. of the animal concerned, or per 100 g body wt. of the pregnant mothers for foetal muscles. In each case the injection was introduced via a lateral tail vein and included 65 $\mu$Ci of L-[4-$^3$H]phenylalanine/ml (sp. radioactivity 24 Ci/mmol; Amersham International, Amersham, Bucks., U.K.). All animals were decapitated 10 min after commencing the injection. Thoracic and abdominal cavities were immediately opened and the whole animal was submerged in ice-cold NaCl. Hind limbs were simultaneously removed and skinned at decapitation and similarly immersed in ice-cold NaCl. The heart, appropriate leg muscles and oesophagus were dissected out within a few seconds and frozen in liquid $N_2$. The subsequent analysis of these frozen tissues for protein, nucleic acids and rates of protein synthesis and protein breakdown are described in detail in the preceding paper (Goldspink & Kelly, 1984).

## Results and discussion

Before developmental growth could be assessed in terms of the age-related changes in protein turnover, initial experiments were undertaken to determine whether rates of protein synthesis could be accurately measured in the different types of muscle 10 min after the injection of phenylalanine, i.e. at the time originally used by Garlick *et al.* (1980) and in our preceding paper (Goldspink & Kelly, 1984) for two visceral tissues. Changes in the free and protein-bound specific radioactivities of phenylalanine were therefore measured in muscles at intervals up to 30 min after injection of tracer into either 200 g rats or 20-day-pregnant mothers (i.e. for foetal tissues).

The free amino acid in both skeletal and cardiac muscles of the 200 g rats was virtually in-

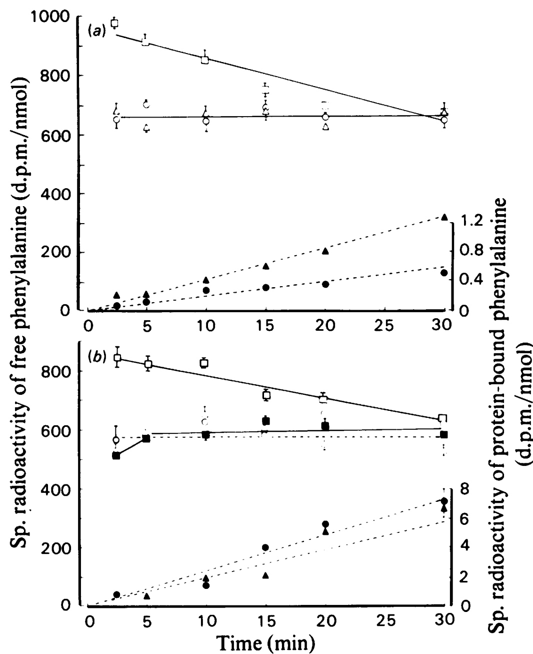


Fig. 1. *Time-related changes in the specific radioactivity of phenylalanine in the plasma, heart and tibialis anterior muscle of foetal and adult rats*

(*a*) Adult rats (200g) were decapitated at various times after receiving an intravenous injection containing 150$\mu$mol of phenylalanine (including 65$\mu$Ci of L-[4-$^3$H]phenylalanine) per 100g body wt. The specific radioactivities of the free (i.e. $S_A$, open symbols) and protein-bound (i.e. $S_B$, solid symbols) phenylalanine in the plasma (squares), cardiac (triangles) and tibialis anterior (circles) muscles were determined at each time as described by Garlick *et al.* (1980). Each value is the mean $\pm$ S.E.M. for at least four individual muscles. (*b*) Here, 20-day-pregnant rats (270 $\pm$ 10g) were injected in a similar manner to the animals above. The specific radioactivity of the free phenylalanine was then determined in both maternal (□) and foetal (■) plasmas, samples being derived from either four injected mothers or 16 foetuses. The free (i.e. $S_A$, open symbols) and protein-bound ($S_B$, solid symbols) specific radioactivities were simultaneously measured in foetal hearts (triangles) and tibialis muscles (circles). These values are presented as means $\pm$ S.E.M. For this nine individual hearts were analysed, i.e. from three foetuses taken at random from each of three injected mothers. A total of 18 tibialis muscles were also studied, in this case as three groups of pooled (six) muscles taken from the same foetuses.

distinguishable, both tissue pools having rapidly equilibrated with the plasma to attain 70 and 98% of the specific radioactivity in the plasma at 2.5 and 30min respectively (Fig. 1*a*). Unlike the significant time-related decline in the plasma, the free specific radioactivity in both muscles was not significantly changed over the 30min period investigated. Throughout, the protein-bound radioactivities in both muscle types increased linearly. Similar results (not shown) were obtained for the soleus muscle from the same 200g animals. Although the time-related changes in the specific radioactivities were not studied in this smooth muscle of the oesophagus, it has previously been shown by ourselves (Kelly & Goldspink, 1982) and others (Garlick *et al.*, 1980) to be a valid technique for measuring synthesis in other smooth-muscle preparations of the gut. For example, the free tissue specific radioactivity of the smooth muscle of the small intestine (Kelly & Goldspink, 1982; Goldspink *et al.*, 1984) and uterus (A. J. Morton & D. F. Goldspink, unpublished work) fell by less than 9% 10min after injection of the phenylalanine.

A similar sequence of events to that described for the liver and kidney (Goldspink & Kelly, 1984) was also found in the heart and tibialis anterior of the 20-day foetus. Not only did the specific radioactivity of free phenylalanine in the foetal muscles and plasma equilibrate rapidly with that in the maternal circulation, they also remained constant between 2.5 and 30min after administration of the tracer to the pregnant mothers (Fig. 1*b*). Amino acid incorporation into protein was once again found to be linear over 30min in both muscle types. From these combined experiments it was decided that protein synthesis could be accurately determined in these four different muscle types, during both pre- and post-natal life, by using the specific radioactivities measured at 10min after injection of phenylalanine.

*Developmental changes in the heart*

The combined ventricles and atria increased their wet weight (112-fold), protein mass (339-fold) and percentage protein composition (from 8.2 to 24.2%) between 18 days of foetal life and 44 weeks *post partum* (Table 1). This developmental growth, and in particular that of the left ventricle, is in keeping with the increased work load associated with pumping an enlarging blood volume against an increasing peripheral resistance within the cardiovascular system. These changes in the heart were, however, smaller than the changes in the whole animal over the same period (see Goldspink & Kelly, 1984); hence the heart's contribution to the body's protein mass progressively decreased from 1.4 to 0.5%. Both RNA and DNA increased in parallel (their ratio ranging from 1.9 to 2.6) during this time, in accord with the expanding cardiac tissue. However, during post-natal life the increasing tissue mass outstripped the accumulation of RNA and DNA, such that both nucleic acids per g of tissue decreased with increasing age.

Table 1. *Pre- and post-natal changes in the protein and nucleic acid contents of the heart*

Frozen hearts were pulverized between chilled metal plates. The powdered tissues were then allowed to thaw in ice-cold 0.3M-$HClO_4$ (1:50, w/v) and homogenized in a ground-glass homogenizer. Proteins were measured in these homogenates by the method of Lowry *et al.* (1951), and RNA and DNA were extracted and assayed as described by Goldberg & Goldspink (1975). The values at each age are means±S.E.M. derived from a minimum of 12 foetuses or from five animals *post partum*. Values in parentheses give the percentage contribution of the heart towards the whole-body protein and nucleic acid contents; the latter are described by Goldspink & Kelly (1984).

| Age | Protein content (mg) | Protein/ wet wt. (%) | Total RNA P (μg) | RNA P/ wet wt. (μg/g) | Total DNA P (μg) | DNA P/ wet wt. (μg/g) | RNA P / DNA P |
|---|---|---|---|---|---|---|---|
| Foetal (days) | | | | | | | |
| 18 | 1.1±0.1 (1.39) | 8.2±0.3 | 5.1±0.5 (0.86) | 373±31 | 2.7±0.3 (0.57) | 193±17 | 2.07±0.05 |
| 20 | 2.1±0.1 (0.87) | 10.1±0.3 | 9.1±0.6 (0.68) | 466±18 | 5.1±0.2 (0.52) | 247±6 | 1.89±0.08 |
| *Post partum* (weeks) | | | | | | | |
| 3 | 51±1.3 (0.91) | 19.3±4.4 | 86±3.0 (0.74) | 325±7.0 | 61±1.2 (0.95) | 234±8 | 1.45±0.06 |
| 8 | 164±6.0 (0.62) | 21.2±0.9 | 172±5.8 (0.43) | 222±7.0 | 110±2.5 (0.64) | 142±4 | 1.56±0.04 |
| 44 | 383±20 (0.50) | 24.2±2.1 | 304±18 (0.44) | 199±4.0 | 124±8.1 (0.39) | 81±5 | 2.38±0.13 |
| 105 | 247±34 (0.34) | 13.4±1.1 | 316±37 (0.47) | 172±9.4 | 143±17 (0.48) | 78±5 | 2.22±0.11 |

Table 2. *Changes in protein turnover in the heart during pre- and post-natal growth*

The values presented below are means±S.E.M. of measurements made on the same tissues described in Table 1. Protein synthesis was measured *in vivo* in the hearts 10min after an intravenous injection of 150μmol of phenylalanine per 100g body wt., via pregnant mothers in the case of foetal hearts. The fractional synthetic rates were calculated at each age after measurement of the specific radioactivities of both the free and protein-bound phenethylamine in the hearts (Garlick *et al.*, 1980). The total amount of protein synthesized per day represents the product of the fractional rate and the protein mass (Table 1). The percentage contributions of the heart towards the whole-body synthesis are presented in parentheses. Cardiac growth rates were determined as the daily percentage change in the protein mass, as measured between 1-, 2-, 5- or 7-day intervals in the foetuses, and 3-, 8- or 44- and 105-week-old rats respectively. Breakdown was subsequently calculated by subtracting the measured rates of growth from synthesis.

| Age | Protein/ DNA P (mg/μg) | Fractional rate of synthesis (%/day) | Total protein synthesized (mg/day) | Total protein synthesized (g/day per g of RNA P) | RNA P/ protein (mg/g) | Growth rate (%/day) | Calculated rate of breakdown (%/day) |
|---|---|---|---|---|---|---|---|
| Foetal (days) | | | | | | | |
| 18 | 0.45±0.03 | 74.4±1.3 | 0.76±0.1 (1.8) | 138±16 | 5.1±0.3 | 41.2 | 33.2 |
| 20 | 0.38±0.02 | 47.3±3.8 | 0.96±0.1 (1.1) | 95±8.2 | 4.7±0.2 | 22.6 | 24.7 |
| *Post partum* (weeks) | | | | | | | |
| 3 | 0.83±0.03 | 19.0±0.4 | 9.6±0.3 (0.56) | 113±3.3 | 1.7±0.08 | 4.0 | 15.0 |
| 8 | 1.44±0.03 | 12.0±0.4 | 18.7±0.5 (0.40) | 115±10 | 1.1±0.02 | 1.4 | 10.6 |
| 44 | 3.24±0.08 | 10.3±0.4 | 44.5±1.3 (0.46) | 131±3.9 | 0.8±0.02 | 0.5 | 9.8 |
| 105 | 1.88±0.05 | 6.6±0.3 | 18.2±2.2 (0.23) | 55.8±4.2 | 1.3±0.09 | 0.9 | 5.8 |

The growth of the entire heart during these phases of pre- and post-natal life was associated with both continuously increasing nuclear proliferation (i.e. increasing DNA; Table 1) and protein/DNA values (Table 2; Enesco & Leblond, 1962). Although some polyploidy is believed to exist in a few of the myocardial cells of man and some other animals, this is not thought to be the case for the rat (Kuhn *et al.*, 1974). Even if it were, the magnitude of the changes must still suggest that this cardiac

Table 3. *Post-natal changes in the protein and nucleic acid contents of the slow-twitch soleus muscle*
Soleus muscles were analysed in the same manner as described for the heart (Table 1). Each value represents the mean ± S.E.M. for muscles taken from a minimum of five rats at each age. Values in parentheses indicate the percentage contribution of the soleus muscles of both legs to the whole body's protein and nucleic acid contents (Goldspink & Kelly, 1984).

| Age | Protein content (mg) | Protein/ wet wt. (%) | Total RNA P (μg) | RNA P/ wet wt. (μg/g) | Total DNA P (μg) | DNA P/ wet wt. (μg/g) | RNA P / DNA P |
|---|---|---|---|---|---|---|---|
| *Post partum* (weeks) | | | | | | | |
| 3 | 2.6 ± 0.2 (0.09) | 15.7 ± 0.7 | 4.2 ± 0.2 (0.07) | 262 ± 10 | 2.4 ± 0.1 (0.07) | 149 ± 10 | 1.77 ± 0.04 |
| 8 | 19.8 ± 0.8 (0.15) | 18.5 ± 0.5 | 18.0 ± 0.9 (0.09) | 162 ± 5 | 6.4 ± 0.4 (0.07) | 64 ± 2 | 2.55 ± 0.06 |
| 44 | 53.9 ± 0.4 (0.14) | 24.9 ± 0.7 | 36.5 ± 1.9 (0.11) | 167 ± 9 | 14.7 ± 0.6 (0.09) | 68 ± 3 | 2.33 ± 0.1 |
| 105 | 43.6 ± 4.2 (0.12) | 20.0 ± 1.5 | 20.6 ± 1.6 (0.06) | 104 ± 3 | 15.6 ± 1.3 (0.10) | 72 ± 4 | 1.49 ± 0.1 |

growth arises through a combination of hyperplasia and hypertrophy. Indeed, microscopic studies on hearts between 7 and 95 days *post partum* have demonstrated a progressive increase in the diameter of the contractile cells (Enesco & Leblond, 1962). Nonetheless, the heart does contain a large proportion (50–75%) of fibroblasts, whose proliferation and hypertrophy probably make a significant contribution to the enlargement of the heart during normal growth and its compensatory growth during experimentally induced pressure overloading (Grove *et al.*, 1969; Young, 1970; Kuhn *et al.*, 1974).

The overall slowing of the growth rate in the heart up to 44 weeks was particularly dramatic over the 2 days immediately before birth and up to weaning (Table 2). Thereafter, a slower but progressive decline continued through to senility. Broadly similar developmental trends were found for the fractional rates of protein synthesis and breakdown. Both rates decreased by one-third within the last 2 days *in utero*, and this was followed by successive significant ($P < 0.01$) decreases between each post-natal age studied right through to senility (Table 2). The changes in the fractional synthetic rate correlated with the progressive age-related decrease in the cardiac ribosomal capacity (i.e. RNA/protein ratio). Except for two particular ages (i.e. 20 days of gestation and 105 weeks *post partum*), synthesis per ribosome remained reasonably constant (i.e. synthesis/RNA values in Table 2). The total amount of protein being synthesized in the heart (i.e. fractional rate × protein mass) increased with age up to 44 weeks, thereby reflecting the enlargement (i.e. protein mass) of the organ. Nonetheless, the heart's contribution to the synthesis of new proteins within the whole animal gradually diminished from 1.8 to 0.23% (Table 2).

Of particular interest were the developmental changes between 44 and 105 weeks of post-natal life. Although the hearts were very similar in size at each age (i.e. 1.57 and 1.88 g wet wt. respectively), the protein content was significantly lower (36%; $P < 0.01$) and the protein composition approximately halved at 105 weeks. The cellular atrophy (protein/DNA; Table 2) of the cardiac muscle at this age probably impairs the mechanical efficiency of the heart (Harris, 1975) and may be an important step leading to heart failure. This indeed may be one reason why only one-half of the original stock of animals survived to this particular age. Although 18% less protein was synthesized per day in the senile animals as a whole, compared with those at 44 weeks (Goldspink & Kelly, 1984), the changes were even more pronounced in the heart (i.e. a decrease of 59%; $P < 0.001$; Table 2) and elsewhere in the musculature (see below). Interestingly, the fall in total cardiac synthesis at 105 weeks did not appear to arise from a decrease in the tissue's RNA content (Table 1) or ribosomal capacity, but from a marked fall in synthesis per ribosome (Table 2).

*Developmental changes in fast- and slow-twitch skeletal muscles*

This aspect of the study was largely confined to following developmental changes during post-natal life because of the small size of the muscles concerned. Even the larger tibialis anterior had to be pooled, as six muscles from three foetuses, in order to acquire sufficient material for analysis.

The contribution of the soleus and tibialis muscles (i.e. from both hind limbs) to the whole-body protein mass remained fairly constant at 0.09–0.15% and 0.6–0.8%, respectively, between weaning and 44 weeks of age (Tables 3 and 4). Over the same period the accumulation of RNA and DNA within the two muscle types was very

Table 4. *Changes in the protein and nucleic acid contents of the tibialis anterior before and after birth*
All muscles were analysed as described for the heart (Table 1). Each measurement was made on either three groups of pooled (six) muscles taken from 18 foetuses, or single tissues isolated from a minimum of five rats at each age *post partum*. The results for single muscles are presented as means ± S.E.M. Values in parentheses, however, indicate the percentage contribution that both tibialis anterior muscles per rat contribute to that animal's whole-body protein and nucleic acid content at each specified age (Goldspink & Kelly, 1984).

| Age | Protein content (mg) | Protein/ wet wt. (%) | Total RNA P (μg) | RNA P/ wet wt. (μg/g) | Total DNA P (μg) | DNA P/ wet wt. (μg/g) | RNA P / DNA P |
|---|---|---|---|---|---|---|---|
| Foetal (days) | | | | | | | |
| 20 | 0.15±0.01 (0.12) | 6.8±0.1 | 0.5±0.02 (0.07) | 212±14 | 0.5±0.01 (0.09) | 203±13 | 1.04±0.01 |
| *Post partum* (weeks) | | | | | | | |
| 3 | 22.2±0.6 (0.80) | 20.5±0.4 | 19.6±0.7 (0.34) | 180±3.0 | 9.9±0.4 (0.31) | 108±3.0 | 1.98±0.07 |
| 8 | 78.0±3.9 (0.59) | 20.1±1.1 | 69.4±8.7 (0.35) | 180±2.4 | 24.9±1.2 (0.29) | 64±0.3 | 2.84±0.04 |
| 44 | 217±2.0 (0.57) | 23.6±0.4 | 98.6±3.4 (0.29) | 113±3.0 | 30.7±1.1 (0.19) | 35±1.0 | 3.22±0.07 |
| 105 | 163±14 (0.45) | 21.0±0.6 | 65.7±3.8 (0.20) | 88.8±8.9 | 26.4±1.6 (0.18) | 39±4.8 | 2.53±0.1 |

similar to the increases in the whole animal, as evident from the relative constancy of their percentage contributions to whole-body values. Such parallel changes in these values in skeletal muscle (i.e. tibialis anterior) and the whole body were not, however, shared *in utero*, with the tibialis of the 20-day foetus making much smaller contributions to the total protein and nucleic acid contents (Table 4). Clearly the much greater post-natal use of the limbs in weight bearing and locomotion is a major stimulus for muscle growth. Undoubtedly some of the developmental increase in muscle DNA (Tables 3 and 4; and Enesco & Leblond, 1962) represents an increase in the number of myonuclei, these being derived from the mitotic division and subsequent migration of satellite cells (Moss & Leblond, 1971). The proliferation of interstitial cells will also contribute to the enlargement of the whole muscles. Their contribution to the increasing DNA content (approx. 25–30%) will, however, be proportionately greater than to the enlarging protein mass, since these non-contractile cells possess relatively little cytoplasm. The existence of more satellite and interstitial cells within slow-twitch muscles probably explains the greater values for DNA per g of muscle in the soleus, compared with the faster tibialis muscle (Tables 3 and 4).

Age for age the fractional rates were always higher in the slower soleus muscle, being throughout almost double that in the tibialis anterior. This clearly means that slow-contracting fibres, as well as whole muscles, must possess higher turnover rates. Similar differences in turnover rates between fast and slow muscles at specific ages have previously been described (Goldberg, 1967; Rannels *et al.*, 1977; Millward & Waterlow, 1978; Waterlow *et al.*, 1978; Watt *et al.*, 1982; Kelly & Goldspink, 1982). These differences in the synthesis rates can be attributed to both the higher ribosomal capacity and rate of synthesis per ribosome within the slow-twitch soleus (Tables 5 and 6). However, because of its much larger size (i.e. protein mass) the total rate of synthesis per day was invariably higher in the tibialis anterior.

Correlating with their post-natal development and declining growth rates were the age-related decreases in the fractional rates of synthesis in both muscles (Tables 5 and 6). Between weaning and senescence the rates decreased almost identically (3-fold) in both muscle types, these correlating with a similar overall fall in the ribosomal capacity. Synthesis per ribosome *post partum* was largely unchanged, however (Tables 5 and 6). Similar developmental trends in synthesis have been measured *in vitro* (Srivastava & Chaudhary, 1969; Goldspink & Goldspink, 1977; Saleem & Nicholls, 1979) and *in vivo* (Waterlow *et al.*, 1978; Millward & Waterlow, 1978). The post-natal contributions of the soleus and tibialis muscles towards whole-body synthesis, however, remained around 0.1 and 0.3% respectively (Tables 5 and 6). Although fluctuating, the corresponding rates of protein breakdown over the same period were largely unchanged. Nonetheless, consistent with synthesis, these degradative rates were higher in the slower soleus muscle (Tables 5 and 6).

As in the heart (Tables 1 and 2), the previously increasing protein mass, protein/DNA values and total rates of synthesis in both muscle types were reversed at 105 weeks *post partum*. Measurements

Table 5. *Post-natal changes in protein turnover in the soleus muscle*

Rates of protein synthesis were measured in the same muscles described in Table 3. The percentage contributions made by the soleus muscles of both limbs to whole-body synthesis (Goldspink & Kelly, 1984) are presented in parentheses. Growth rates in the soleus muscle were measured as percentages of the protein mass accumulated over the time intervals described in Table 2. Breakdown was calculated, by subtraction, from the appropriate fractional rates of synthesis and growth.

| Age | Protein/ DNA P (mg/μg) | Fractional rate of synthesis (%/day) | Total protein synthesized (mg/day) | Total protein synthesized (g/day per g of RNA P) | RNA P/ protein (mg/g) | Growth rate (%/day) | Calculated rate of breakdown (%/day) |
|---|---|---|---|---|---|---|---|
| *Post partum* (weeks) | | | | | | | |
| 3 | 1.2±0.1 | 18.8±1.1 | 0.52±0.08 (0.06) | 116±5.6 | 1.7±0.08 | 10.6 | 8.2 |
| 8 | 3.0±0.1 | 14.8±0.7 | 2.9±0.1 (0.13) | 158±6.0 | 1.0±0.02 | 3.2 | 11.6 |
| 44 | 3.7±0.1 | 9.6±0.6 | 5.2±0.3 (0.11) | 144±11 | 0.7±0.08 | 1.3 | 8.3 |
| 105 | 2.6±0.2 | 5.7±0.2 | 2.7±0.3 (0.07) | 135±8.8 | 0.5±0.05 | 0.7 | 5.1 |

Table 6. *Pre- and post-natal changes in protein turnover in the fast-twitch tibialis anterior muscle*

Both the fractional and total rates of protein synthesis were measured in the same muscles as described in Table 4. The percentage contributions of both muscles (i.e. per animal) to whole-body synthesis (Goldspink & Kelly, 1984) are presented as values in parentheses. Growth rates and protein breakdown were determined in a manner identical with that described for the heart (Table 2).

| Age | Protein/ DNA P (mg/μg) | Fractional rate of synthesis (%/day) | Total protein synthesized (mg/day) | Total protein synthesized (g/day per g of RNA P) | RNA P/ protein (mg/g) | Growth rate (%/day) | Calculated rate of breakdown (%/day) |
|---|---|---|---|---|---|---|---|
| Foetal (days) | | | | | | | |
| 20 | 0.34±0.01 | 20.9±0.3 | 0.03±0.002 (0.067) | 65.4±5.3 | 3.1±0.2 | | |
| *Post partum* (weeks) | | | | | | | |
| 3 | 2.5±0.2 | 11.6±0.3 | 2.6±0.3 (0.30) | 123±5.7 | 0.89±0.02 | 5.9 | 5.9 |
| 8 | 3.2±0.2 | 9.1±0.4 | 7.2±0.7 (0.31) | 101±4.5 | 0.87±0.1 | 1.7 | 7.3 |
| 44 | 6.5±0.7 | 4.5±0.3 | 10.6±0.5 (0.22) | 103±5.5 | 0.44±0.1 | 0.2 | 4.3 |
| 105 | 6.8±0.6 | 3.8±0.4 | 5.0±0.2 (0.13) | 59.1±6.7 | 0.45±0.04 | 2.0 | 1.8 |

of protein/DNA are of limited value in skeletal muscle, because its fibres are multinucleated. Nonetheless the changes in protein/DNA, together with the 20 and 25% decreases in the protein masses of the soleus and tibialis muscles respectively, are consistent with the known fibre atrophy and decreased force output of aged muscles (Campbell *et al.*, 1973). The significantly ($P < 0.01$) decreased total rates of synthesis at 105 weeks, relative to earlier stages, must feature prominently in this muscle atrophy. The loss of DNA from the tibialis anterior (Table 4) at 105 weeks could be associated with the known loss of motor units. This degeneration of fibres in some muscles may be partially compensated for by the hypertrophy of others (Rowe, 1969), such limited adaptive growth possibly accounting for the small increase in the growth rate in this aged muscle (Table 6). Although evident in both muscle types, the decrease in synthesis appears to arise in different ways. In the soleus muscle the decreased rate appeared to correlate with a fall in the RNA content and ribosomal capacity (Table 5). In contrast, the change in the tibialis appeared to be an expression of a decreased rate of synthesis per ribosome, the ribosomal capacity being unchanged (Table 6).

Table 7. *Post-natal changes in the proteins, nucleic acids and protein synthesis of the oesophageal smooth muscle*
Portions (approximately lower half) of the oesophagus were taken from between the lower lobes of the lungs and its entry into the stomach. Proteins, nucleic acids and rates of protein synthesis were measured in a manner identical with that described in the previous Tables. Values are presented as the means ± S.E.M. for at least five preparations studied at each age.

| Age | Protein/ wet wt. (%) | Nucleic acids/wet wt. (μg/g) RNA P | DNA P | RNA P / DNA P | Protein/ DNA P (mg/μg) | Rate of protein synthesis (%/day) | (g/g per day of RNA P) | RNA P/ protein (mg/g) |
|---|---|---|---|---|---|---|---|---|
| *Post partum* (weeks) | | | | | | | | |
| 3 | 7.2±0.3 | 178±5.9 | 143±7.4 | 1.26±0.08 | 0.51±0.03 | 41.6±2.3 | 168±13 | 2.50±0.1 |
| 8 | 11.2±0.7 | 172±9.3 | 101±2.2 | 1.70±0.08 | 1.10±0.06 | 25.1±1.3 | 161±5.1 | 1.56±0.07 |
| 105 | 8.5±0.6 | 133±10 | 86.2±7.6 | 1.56±0.1 | 1.10±0.07 | 13.7±0.8 | 88.8±4.7 | 1.57±0.1 |

*Post-natal changes in the smooth muscle of the oesophagus*

The lower two-thirds of the oesophagus contains only smooth muscle, whereas the non-striated muscle of the remaining upper one third is mixed with skeletal muscle. Consequently, only the lower portion of the oesophagus was analysed. Inevitably, this gave rise to less overall information, compared with the other three complete muscle preparations (see above). The developmental changes in the oesophageal smooth muscle (Table 7) can, however, be compared with the fuller data derived from the non-striated muscle of the small and large intestine (Goldspink *et al.*, 1984).

At all ages the percentage of protein within the smooth muscle (Table 7) was lower than in the heart and skeletal muscles (Tables 1, 3 and 4). Initially this value increased between 3 and 8 weeks *post partum*, but subsequently decreased with senescence. Although the nucleic acid concentrations (i.e. values per unit wet wt.) decreased with increasing age, these changes (between weaning and senescence) were less pronounced than in the other three muscle types. Growth of the smooth muscle between 3 and 8 weeks probably involves some hyperplasia (insufficient results available) as well as a doubling of cell size (i.e. protein/DNA; Table 7). This post-natal growth was accompanied by a 67% overall fall in the fractional rate of synthesis between 3 and 105 weeks, this being very similar to that found in the three striated muscles between the same ages. The declining synthesis rate between 3 and 8 weeks was accompanied by a near-identical fall in the ribosomal capacity, with the rate of synthesis per ribosome being unchanged (Table 7). The subsequent fall in synthesis per ribosome, i.e. at 105 weeks, is possibly linked to a fall in the total rate of synthesis in association with senescence, i.e. in a similar manner to the cardiac and tibialis anterior muscles (Tables 2 and 6).

*Further comparisons of the four muscle types*

Age for age, the fractional rates of synthesis showed a definite trend within the four muscle types, i.e. smooth muscle > heart and slow-twitch soleus > fast-twitch tibialis anterior. The close similarity of the fractional synthetic rates in cardiac and slow-twitch muscle (soleus) is presumably related to their similarities of function. Both muscle types exhibit either continuous, or high degrees of, contractility, are fatigue-resistant muscles and rely heavily on an oxidative type of metabolism. In addition, they tend to be relatively insensitive to hormones that influence protein turnover. Their activity patterns contrast with the lower recruitment frequency of the fast oxidative glycolytic and fast glycolytic fibres present in the tibialis anterior (Ariano *et al.*, 1973; Watt *et al.*, 1982). These fibres are recruited when movements need to be speeded up and in association with higher power output. In accord with these functions, the fast fibres (in particular, the fast glycolytic) possess higher myosin ATPase activities and rely primarily on glycolysis for generating their ATP substrate. The greater protein/DNA values of the tibialis are consistent with the greater diameters and force generation of fast-twitch fibres (Tables 4 and 6). Muscular activity in the oesophagus is spasmodic, taking the form of peristaltic waves of contraction initiated by mechanical and neural reflexes associated with swallowing. These propulsive contractions are, however, less frequent and prolonged than the combined propulsive and non-propulsive contractions within the non-striated muscle of the small and large intestine. These differences in activity may therefore correlate with the lower fractional rates of synthesis in the oesophageal smooth muscle, compared with that in the intestine (Goldspink *et al.*, 1984) and uterus (e.g. 40%; A. J. Morton & D. F. Goldspink, unpublished work). It is now becoming increasingly apparent that, as with skeletal muscle, the different types of smooth muscle (e.g. single and multi-unit) possess distinctive biochemical as well as physiological characteristics. Differences in workload and in sensitivities to circulating hormones, either separately or in an inter-related manner, are likely to be major factors influencing

the rates of protein synthesis and breakdown in the different muscle types (Goldberg, 1971; Goldspink, 1980*b*; Goldspink *et al.*, 1983).

The developmental changes in the fractional synthetic rates were very similar in all four muscle types. The values measured at 105 weeks *post partum* were 30–35% of the rates found at weaning (Tables 2, 4, 6 and 7). Further, when the rates for the heart and tibialis anterior at 105 weeks were compared with those of their respective foetal tissues (i.e. 20 days gestation) they remained similar, but now at 14.0 and 18.1%, respectively. The similarity of these developmental changes in protein synthesis differ from the earlier work of Waterlow *et al.* (1978), who observed greater changes in skeletal muscle than in the heart. Nonetheless, despite this slight discrepancy these age-related changes in synthesis in cardiac, skeletal and smooth muscle are more pronounced than in other tissues (see Goldspink *et al.*, 1984).

Initially at birth, most skeletal muscles possess relatively mixed fibre-type populations and uniformly slow contractile properties (Close, 1964). Subsequent differentiation involves the transformation of contractile proteins [e.g. myosin heavy chains (Whalen *et al.*, 1979)] from foetal to either adult fast or slow forms, progressive modifications in the relative proportions of fast- and slow-twitch fibres and the consequent changes in their overall metabolic and contractile characteristics (Bass *et al.*, 1975). Such changes must be reflected in the differing rates of turnover between the various muscle types. For example, at 105 weeks *post partum* the half-lives of the smooth, cardiac and slow and fast skeletal muscles were 5.1, 10.4, 12.1 and 18.3 days respectively. Since the contractile proteins are known to be turned over more slowly (Waterlow *et al.*, 1978), the age-related decrease in protein turnover *post partum* is probably related, in part, to the increasing ratio of myofibrillar to sarcoplasmic proteins. This occurs soon after birth as the fibres acquire more myofilaments to increase their force output in response to increased functional demands and weight bearing. The changing proportion of contractile to cytosolic proteins cannot, however, completely explain the age-related fall in the average turnover rates, since the turnover of the contractile proteins themselves is known to decline with increasing age (Waterlow *et al.*, 1978). Nonetheless, the smaller proportion of contractile proteins probably explains the higher rates of turnover in most smooth muscles (Table 7, and Goldspink *et al.*, 1984).

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