Biochem. J. (1992) 286, 915–922 (Printed in Great Britain)

Turnover of glycogen phosphorylase in the pectoralis muscle of broiler and layer chickens

Angela V. FLANNERY, John S. EASTERBY and Robert J. BEYNON*
Proteolysis Research Group, Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

Glycogen phosphorylase is a major sarcoplasmic protein in chicken pectoralis muscle, constituting approx. 4% of the total protein complement. In slow-growing layer chicks phosphorylase accumulated in parallel with muscle accretion, but in fast-growing broiler chicks the concentration of phosphorylase in the muscle increased (from 5 to 8 mg/g wet wt.) with time. In a 5-week period, the total amount of phosphorylase in the pectoralis muscles increased 18-fold in broiler chicks (from approx. 75 to 1400 mg total), but only 3-fold (from approx. 100 to 270 mg total) in layers. Pyridoxal phosphate, the cofactor of the enzyme glycogen phosphorylase, was used as a specific label to measure the rate of degradation of the enzyme in the pectoralis muscle of growing broiler and layer chickens *in vivo*. In young animals, the fractional rate of phosphorylase synthesis was similar in broiler and layer chickens (approx. 15%/day), but the rate of degradation in layers (5%/day) was 5-fold higher than in broilers (1%/day). As the animals aged, the rate of synthesis decreased, but more so in layers than in broilers. The rate of degradation of phosphorylase also decreased in layers, but in broilers it remained at the low level seen in young animals. The dramatically higher rate of phosphorylase accretion in the pectoralis muscles of the broilers is therefore achieved by an initial lower rate of degradation combined with a sustained difference between rates of synthesis and degradation.

INTRODUCTION

In muscles of young animals, the rate of protein turnover is higher than in adults. Since growth reflects discrepancies between the rates of protein synthesis and breakdown, any change in this imbalance will lead to an altered growth rate. Several studies have shown that decreased protein breakdown, together with a variable contribution from protein synthesis, are responsible for the increase in protein turnover under conditions of growth, work-induced hypertrophy or after starvation and refeeding (Millward *et al.*, 1975; Goldberg, 1969; Laurent *et al.*, 1978; Young *et al.*, 1971).

The chicken has been the subject of genetic selection over many generations, leading to the production of inbred strains (broilers) that accrete muscle at a very rapid rate as compared with those selected for egg production (layers); the rate of protein deposition is typically 6 g of protein/day per $kg^{0.75}$ in broilers and 3.5 g protein/day per $kg^{0.75}$ in layers (Fisher, 1980). The molecular basis for this difference in phenotype is not known. Previous studies of protein turnover in which total protein synthesis was determined (Maruyama *et al.*, 1978; Kang *et al.*, 1985) suggested that a lowered degradation rate in broilers during the first few weeks of life was the primary cause of their enhanced breast-muscle accretion. Another study, using layer chicks, suggesting that changes in the rate of synthesis were responsible for the accumulation of breast-muscle protein (MacDonald & Swick, 1981). The method used in these studies entailed labelling of proteins with radioactive amino acids. This measures the average behaviour for total proteins, which, particularly in muscle, is biased by the large contribution made by the contractile apparatus to the protein complement of this tissue. Information is lacking on the turnover of individual proteins, which may provide a more focused view of the relationship between individual cellular proteins and their turnover.

We have developed a method for the measurement of glycogen phosphorylase (EC 2.4.1.1) degradation based on specific labelling of the enzyme by its cofactor, pyridoxal phosphate (Butler *et al.*, 1985; Cookson & Beynon, 1989). After provision of the cofactor in the form of a radiolabelled precursor ([G-^{3}H] pyridoxine), the labelled enzyme–cofactor complex is extremely stable to the extent that the decay of enzyme-bound radiolabel parallels the breakdown of phosphorylase (Beynon *et al.*, 1986; Leyland *et al.*, 1990; Leyland & Beynon, 1991). The pectoralis muscle in the chicken is composed almost exclusively (> 93%) of white type II glycolytic fibres (MacNaughton, 1974). As such, glycogen phosphorylase is abundant, comprising a significant proportion of sarcoplasmic protein. These features have enabled us to develop the methodology for use in broiler and layer chickens and to ascertain the differences in turnover parameters for the enzyme in these two groups of animals.

MATERIALS AND METHODS

Animals

These were 1-day-old male broiler-strain chicks (White Plymouth Rock), obtained from Vale Royal Hatcheries, Frodsham, Cheshire, U.K., and 1-day-old male layer-type chicks (Hi-Sex Brown), obtained from Farm Fresh Hatcheries, Tarleton, Lancashire, U.K. Animals were housed in a heated brooder in a room with continuous lighting. They were fed *ad libitum* on a commercial broiler starter diet containing 19% protein and 10.2 MJ of metabolizable energy/kg (Chick Crumbs; Special Diets Services, Witham, Essex, U.K.). Body weights were recorded daily.

Radiolabelling and preparation of samples

At 14 days post-hatching, chickens were injected subcutaneously with 3.7 MBq of [G-^{3}H]pyridoxine hydrochloride (sp. radioactivity 51.8 GBq/mmol; Amersham International, Amersham, Bucks., U.K.) in 0.2 ml of 0.9% NaCl. In the degradation studies, broilers and layers were labelled with separate batches of [G-^{3}H]pyridoxine. The radioactivity in blood and tissue samples of broilers was only one-third of that in

* To whom reprint requests should be addressed.

layers. Subsequent h.p.l.c. analysis of the two batches of radiolabelled material revealed that the batch used in the broiler experiment had only one-third of the expected [G-^{3}H]pyridoxine content, the remainder of the radiolabel being associated with breakdown products. This had no effect on the kinetics of loss of labelled enzyme or on the derivation of the rate of degradation, which relies on loss of the total labelled pool. When phosphorylase was purified from muscle labelled with this batch of [G-^{3}H]pyridoxine, all of the radioactivity was released into the supernatant fraction after precipitation with 5% (w/v) trichloroacetic acid; thus none of the radioactivity was associated with the protein component of the enzyme. However, the initial extent of labelling differed in the two groups.

Animals were killed by decapitation after halothane-induced anaesthesia. A blood sample was collected in a heparinized tube, and the pectoralis muscle was rapidly dissected, placed on ice and weighed. A 1 g sample of tissue was taken for analysis and the remainder was frozen immediately at −20 °C. The sample was taken from the same region throughout the growth of the muscle. To ensure that the sample was representative of the whole muscle, an entire pectoralis muscle from an animal in the middle of the experiment (day 12 after labelling) was sectioned into seven portions, cutting both along the fibre length and across. Each portion was assayed for total radioactivity and radioactivity associated with phosphorylase (described below). There was no significant variation between samples, indicating that, after extensive growth of the muscle, the labelled phosphorylase was distributed throughout the muscle bulk.

Tissue samples were homogenized in 9 vol. of 50 mM-potassium phosphate, pH 7.4, and the homogenate was centrifuged at 25000 *g* for 1 h at 4 °C. The supernatant, which contained 87% ± 2% (mean ± S.E.M., $n = 50$) of the glycogen phosphorylase activity, was decanted and filtered through a GF/C filter to remove particulate matter.

SDS/PAGE

This was conducted in 10% acrylamide gels in the Bio-Rad mini P11 system. Gels were subsequently stained for protein with Coomassie Blue or electroblotted on to nitrocellulose.

Assay of phosphorylase activity

Phosphorylase activity was assayed in the direction of glycogen synthesis by a modified procedure (Carney *et al.*, 1978). Butan-1-ol extraction of the phosphomolybdate–vanadate complex was eliminated and the coloured product was measured in the aqueous phase. Background readings corresponding to labile organic phosphate esters were decreased by use of a substrate containing 25 mM-glucose 1-phosphate. The colour yield at 315 nm is linear up to at least 50 nmol of phosphate in a 1 ml volume. Assays performed on crude muscle homogenates gave a linear response ($r = 0.995$) between 1 and 3 nkat (nmol/s) of phosphorylase activity.

Phosphorylase determination

Phosphorylase was quantified by densitometry of Coomassie Blue-stained SDS/PAGE gels of total muscle homogenates with the Molecular Dynamics computing densitometer and Imagequant software package. Purified chicken muscle glycogen phosphorylase (0–5 μg) was used as a calibration standard ($r = 0.996$).

Protein determination

Total muscle protein was assayed by a modification of the biuret method with BSA as a calibration standard.

Sephadex G-25 size-exclusion chromatography

Samples of muscle supernatant (1.0 ml) were chromatographed on a column ($V_t = 12$ ml) of Sephadex G-25 previously equilibrated in 50 mM-potassium phosphate buffer, pH 7.4. The column was eluted at 25 ml/h. Fractions were assayed for protein and radioactivity (Optiphase 'Safe' scintillation cocktail; LKB, Milton Keynes, U.K.). Recoveries of radioactivity and protein from the column were 100 ± 1% (mean ± S.E.M., $n = 15$).

5′-AMP–Sepharose affinity chromatography

Samples of muscle supernatant fraction (0.1 ml) were applied to a column ($V_t = 3.0$ ml) of 5′-AMP–Sepharose (Pharmacia–LKB, Milton Keynes, U.K.) previously equilibrated in 50 mM-potassium phosphate, pH 7.4. The column was eluted at 12 ml/h. After 35 min the buffer was changed to include 10 mM-AMP, and elution was continued at the same flow rate for a further 65 min. Fractions were assayed for phosphorylase activity or for radioactivity. Recoveries of phosphorylase activity and radioactivity from the column were 100 ± 1% (mean ± S.E.M., $n = 50$). The AMP-eluted material contained phosphorylase as the only pyridoxal phosphate-labelled protein (results not shown).

Analysis of data

The data defining the growth rate of animals and the decay of labelled pools were analysed by non-linear curve fitting using the MS-DOS program P.Fit (Biosoft, Cambridge, U.K.), which provides detailed statistical analyses. All fitted parameters are cited ± S.E.M. The program analyses residuals for bias and generates the 95%-confidence bands to indicate the quality of the fitted analysis. Different models were used to describe phosphorylase degradation, based on the time-dependent decrease of phosphorylase cofactor-associated radioactivity in the whole pectoralis. A simple exponential decay model (eqn. 1) assumes stochastic breakdown of phosphorylase throughout the time period of the experiment. This equation best described the degradation of phosphorylase in broilers (t = time, A_t = phosphorylase-associated radioactivity at time t, A_0 = phosphorylase associated radioactivity at time zero):

$$dA/dt = -k_d A \quad (1)$$

$$A_t = A_0 \exp(-k_d t) \quad (2)$$

In layers, the data were consistent with a model in which the rate degradation of phosphorylase fell dramatically throughout the experimental period. Two models were devised to explain this behaviour, both assuming that a key component of the degradative process was being inactivated/removed from the muscle in a second exponential process, defined by k_2. The first model assumed that the rate of degradation of phosphorylase, k_d, fell from an initially high initial value, k_i, to a low final value, k_f, such that:

$$k_d = k_f + (k_i - k_f) \exp(-k_2 t) \quad (3)$$

Substitution for k_d, as defined in eqn. (3), into eqn. (1) yields eqn. (4), which describes the loss of phosphorylase-associated radioactivity in a system in which the rate of degradation is also declining exponentially.

$$A_t = A_0 \{\exp [(-k_f t) + (k_i - k_f)/k_2(\exp(-k_2 t) - 1)]\} \quad (4)$$

For the second model, a simpler form of eqn. (4) is derived when $k_f = 0$, in which instance the equation is:

$$A_t = A_0 \{\exp [k_i/k_2 (\exp(-k_2 t) - 1)]\} \quad (5)$$

Eqns. (2), (4) or (5) were fitted to data describing the loss of cofactor label from the phosphorylase pool over time, by using a Marquardt algorithm. The algorithm requires starting guesses

for A_0 and the rate constants, and the fit was conducted by using several different values for initial guesses. Data were unweighted.

RESULTS AND DISCUSSION

Growth characteristics of broilers and layers

Broilers and layers differed in growth characteristics and body weights (Fig. 1*a*). This difference was reflected in the increase in pectoralis weights over time (Fig. 1*b*). The data were subjected to non-linear curve-fitting analysis using both linear and exponential models. The pectoralis in broilers grew exponentially and yielded a fractional growth rate of 0.08/day. For layers, the data were not described by an exponential model; instead, growth over this period was best described as a linear accumulation of mass at 0.7 g/day. The size of the pectoralis as a percentage of total body weight increased over time in broilers (Fig. 1*c*) from 5% at age 20 days to 10% at age 45 days; thereafter it remained constant. In layers the pectoralis constituted 5–6% of total body weight, and this percentage did not change significantly over time.

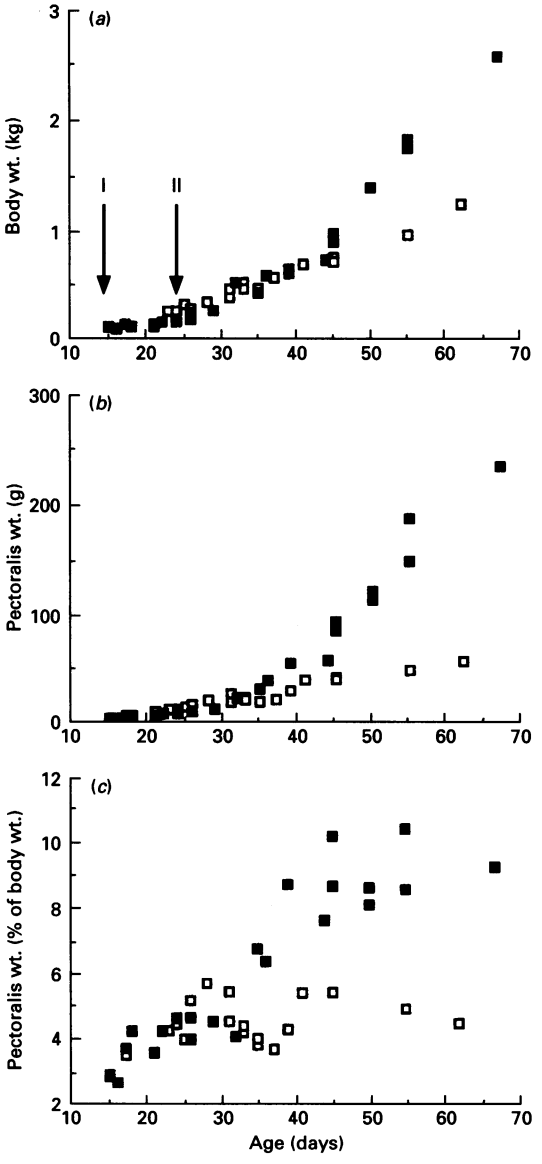

Fig. 1. Growth characteristics of broiler and layer chickens

On each experimental day, the body weight of broiler (■) or layer (□) chickens was recorded (*a*). After killing the entire pectoralis muscles were dissected out and weighed (*b*). The weight of the pectoralis muscle as a percentage of the body weight was calculated (*c*). Markers I and II indicate the days on which the animals were injected and the start of degradation study respectively. Each point represents a single animal.

Protein and phosphorylase accretion in pectoralis muscle

The protein content in the pectoralis of layers remained relatively constant ($P > 0.05$, Student's unpaired t test of 2–3-week-old layers versus 7–8-week-old animals; $n = 5$ for both groups) at 210 ± 12 mg/g (mean ± S.E.M., $n = 24$) over the time period studied (Fig. 2). In broilers, the protein concentration increased with age, 2–3-week-old animals having a protein content of 194 ± 10 mg/g ($n = 5$) and 7–8-week-old animals having a significantly higher ($P < 0.001$, Student's unpaired t test) protein content of 309 ± 12 mg/g ($n = 5$). As the muscle develops in the rapidly growing broilers, the protein content of the muscle increases, a trend that is also reflected in the behaviour of glycogen phosphorylase. In layers, the activity of glycogen phosphorylase in muscle homogenates, assayed in the direction of glycogen synthesis (Carney *et al.*, 1978), was unchanged, at approx. 6.5 ± 0.6 μkat/g throughout an 8-week period, whereas in broilers enzyme activity increased from 5 ± 0.6 to 7.7 ± 0.6 μkat/g over the same period. That this was due to an increase in the mass of phosphorylase in the muscle was confirmed by densitometric analysis after SDS/PAGE of muscle homogenates. In broilers, the amount of phosphorylase in the muscle increased over time, from 5.2 to 8.2 mg/g during the experimental period ($P < 0.01$, Student's unpaired t test for 2–3-week broilers versus 7–8-week broilers; $n = 5$ for both groups). In layers, the amount of phosphorylase protein in the pectoralis muscle remained constant, at approx. 6 mg/g of muscle ($P < 0.05$, Student's unpaired t test of 2–3-week layers versus 7–8-week animals; $n = 5$ for both groups). Quantification of phosphorylase activity and amount enabled an accurate determination of the specific activity of this enzyme. This remained constant during the experimental period, with a value of 1.3 ± 0.12 μkat/mg of phosphorylase ($n = 24$) in layers and 1.1 ± 0.06 μkat/mg of phosphorylase ($n = 27$) in broilers.

The broiler pectoralis grows very rapidly (Fig. 1), and thus two factors combine to give a very rapid expansion of the phosphorylase pool (defined as the total amount of phosphorylase in the pectoralis muscles): the increase in enzyme content of the muscle, and the rapid growth of the muscle itself. During the experimental period, the pectoralis pool of phosphorylase increased 18-fold, from approx. 75 to 1400 mg. By contrast, the same pool in layers increased only 2.7-fold, from approx. 100 to 270 mg (see Table 1). Although the broiler pool was initially smaller than that of the layer, after 7–8 weeks of growth the former has expanded dramatically, relative to the latter. Such differences in the rate of accretion of this enzyme could be due to differences in either the rate of synthesis or the rate of degradation of the enzyme. Accordingly, the rate of degradation of the enzyme was determined by the cofactor-labelling method.

Kinetics of incorporation of radiolabel into muscle pools

Chickens were injected with [G-^{3}H]pyridoxine and the distribution of the radioactivity (comprising pyridoxine and its metabolites) was monitored. After injection, radiolabel rapidly entered the bloodstream and the intracellular muscle pool. The radioactivity in blood was initially high and then declined rapidly to a value close to zero within 10 days. Intracellular radioactivity was detected at a 10-fold higher level than in blood within the first day after injection and declined slowly thereafter, remaining at a low level throughout the study.

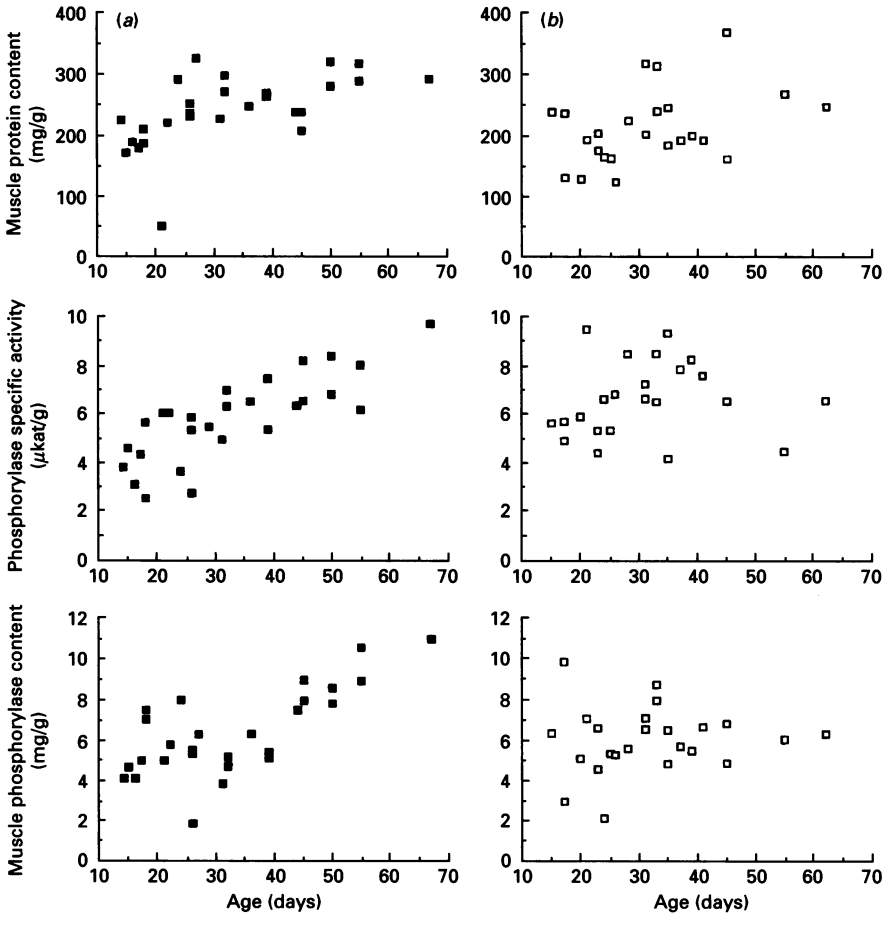

Fig. 2. Age-dependent expression of glycogen phosphorylase in pectoralis muscle of broiler and layer chicks

Pectoralis muscle from broiler (■) and layer (□) chickens between 14 and 64 days old was homogenized in 50 mM-potassium phosphate buffer, pH 7.4. The homogenates were assayed for protein (top panels), phosphorylase activity (middle panels) and phosphorylase quantity by densitometry with pure chicken phosphorylase as a standard (bottom panels). Data are expressed per g wet wt. of muscle. Each point represents a single animal.

Sephadex G-25 size-exclusion chromatography was used to measure the proportion of radioactivity in the low-molecular-mass pool (comprising radiolabelled pyridoxine and its metabolites) and the proportion associated with protein. The data extracted from three time points are shown in Fig. 3*a*. Incorporation of radioactive label into protein occurred over a period of several days, with a concomitant clearance of the low-molecular-mass pool. Affinity chromatography was used to identify the proportion of protein-bound radioactive label associated specifically with phosphorylase. We have previously shown that 5′-AMP–Sepharose affinity chromatography separates phosphorylase from all other pyridoxal phosphate-binding proteins in mouse muscle and have ascertained that this is also the case in chickens. The data extracted from three time points (Fig. 3*b*) reveal the increased incorporation of radiolabel into phosphorylase over time.

Data from Sephadex G-25 and 5′-AMP–Sepharose chromatography defined the distribution of radiolabel between the phosphorylase-bound pool, the non-phosphorylase protein-bound pool and the low-molecular-mass pool in the pectoralis of broilers and layers over a period of 18 days after administration (Fig. 4). Radiolabel is incorporated into the phosphorylase pool over a period of several days, but the distribution attains a steady state 10 days after injection. At this time the low-molecular-mass pool has declined to a low level and the non-phosphorylase protein pool remains low and constant throughout. Broiler and layer profiles reveal no significant differences in accumulation of radiolabel in the various pools. The behaviour of these pools in chickens is very similar to that in the mouse (Cookson & Beynon, 1989; Leyland *et al.*, 1990) and validates the cofactor-labelling methodology in this phosphorylase-rich tissue.

Degradation of glycogen phosphorylase in broilers and layers

In rapidly growing animals, the pectoralis protein pool is expanding. Even in the absence of degradation, pulse-labelled proteins would therefore be diluted by newly synthesized protein. Accordingly, we have based our analyses on the whole pectoralis muscle, on the assumption that the labelled protein would be evenly distributed within the muscle. As the pectoralis grew, it became impractical to analyse the whole muscle, and our data are based on portions of the tissue. Preliminary experiments demonstrated that the labelled phosphorylase was distributed throughout the muscle, and there was no evidence for the retention of labelled material within a core of fibres (see the Materials and methods section). Groups of 20 broiler and layer chickens were injected with [G-^{3}H]pyridoxine as previously described, and the precursor pools were allowed to decline for a period of 10 days. Over the next 50 days, phosphorylase was isolated from muscle supernatants by AMP–Sepharose chromatography, and the associated radioactivity was measured. The rate of breakdown of phosphorylase in broiler pectoralis was

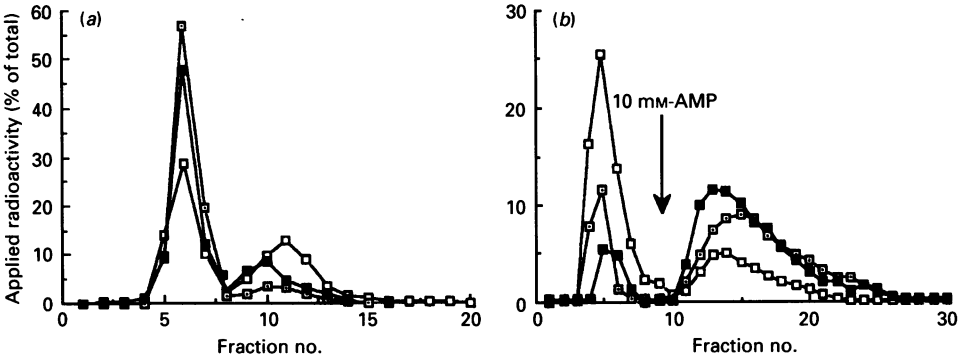

Fig. 3. Partitioning of muscle vitamin B-6 pools after radiolabelling with [^{3}H]pyridoxine

A portion of muscle supernatant was applied to a Sephadex G-25 column and chromatographed as described in the text. The percentage of radiolabel associated with the protein-bound (excluded) and low-molecular-mass (included) fractions was calculated. Data from single broilers, taken at three time points, are shown (*a*). A further portion of muscle supernatant was applied to a 5′-AMP–Sepharose column and chromatographed as previously described. The percentage of radiolabel associated with the phosphorylase pool (bound fractions, eluted after addition of 10 mM-AMP) and other muscle vitamin B-6 pools (unbound fractions) were calculated. Data from three time points are shown (*b*). Time points: □, day 1; ⊡, day 8; ■, day 17.

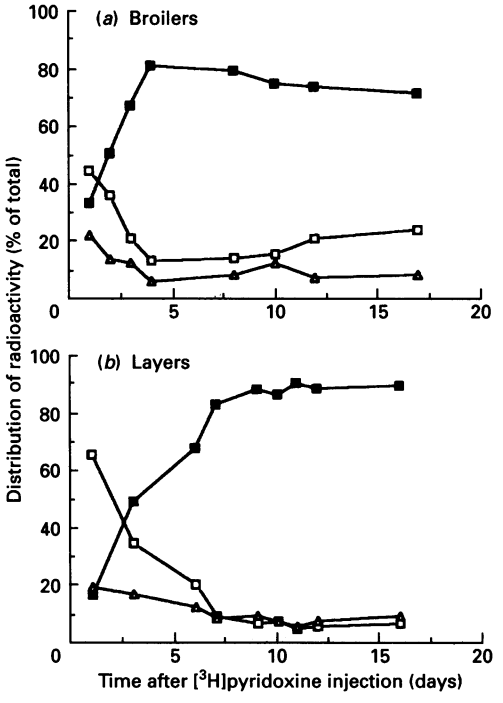

Fig. 4. Distribution of radiolabel in muscle vitamin B-6 pools after injection with [^{3}H]pyridoxine

The distribution of radioactivity between the phosphorylase-bound pool (AMP–Sepharose-bound; ■), the non-phosphorylase protein pool (G-25-excluded minus AMP–Sepharose-bound; △) and the low-molecular-mass pool (G-25-included; □) was calculated at each time point from the data combined from both columns for broilers (*a*) and layers (*b*).

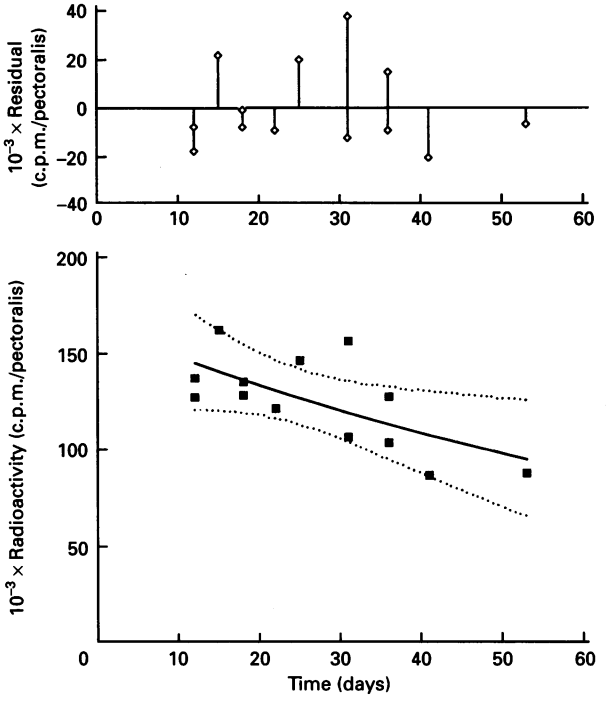

Fig. 5. Degradation of the glycogen phosphorylase pool in the pectoralis muscle of broiler chicks

Broiler chicks (14 days old) were injected with 3.7 MBq of pyridoxine. After 10 days, the rate of loss of label from affinity-purified pectoralis glycogen phosphorylase was measured (lower panel). The data were analysed by non-linear curve fitting as a single exponentional function ($A_t = A_0 \exp(-k_d t)$ where A_t and A_0 are the protein-bound radioactivity at time t and time 0 respectively, and k_d is the degradation rate constant; the continuous line represents the fitted function, and the dotted lines delineate the 95% confidence band for the fitted function. The goodness of fit is also indicated by the plot of residuals (differences between theoretical and actual values) at each time point (top panel). Each point represents a single animal.

very low (Fig. 5). Analysis of the data by non-linear curve-fitting yielded a fractional rate of degradation (k_d) of 0.0106 ± 0.0037/day (a half-life of 66 days). All but two of the data points lay within the 95% confidence bands (dotted lines) for the fitted curve.

The breakdown of phosphorylase in layer pectoralis was initially more rapid than that seen in broilers (Fig. 6), but inspection of the residuals after fitting a simple exponential decay (eqn. 2) indicated that this model was inappropriate (chi-squared value, a measure of goodness of fit, was 2.7×10^7), and inspection of the residuals for the monoexponential fit indicated a marked bias in their distribution (results not shown). The data were consistent with a model in which the rate of degradation fell throughout the experimental period. Curve fitting of a more

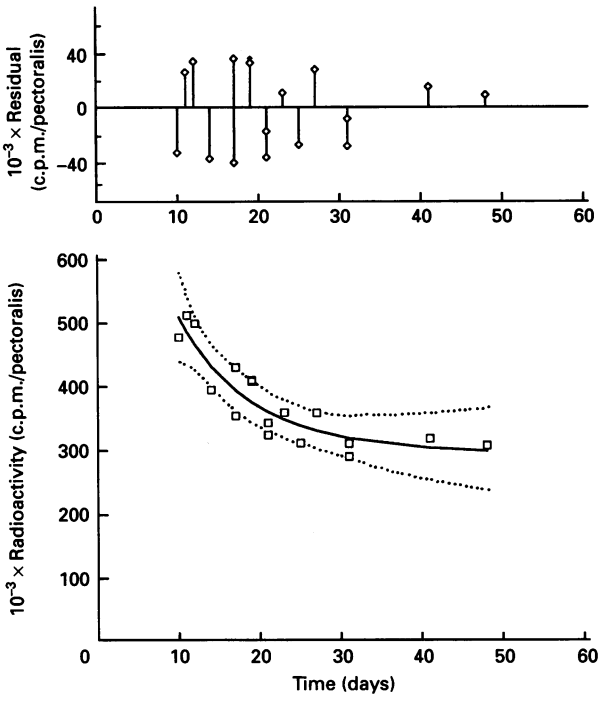

Fig. 6. Degradation of the glycogen phosphorylase pool in the pectoralis muscle of layer chicks

Layer chicks (14 days old) were injected with 100 μCi of pyridoxine. After 10 days, the rate of loss of label from affinity-purified pectoralis glycogen phosphorylase was measured (lower panel). The data were analysed by non-linear curve fitting as a complex exponentional function, $A_t = A_0 \{\exp k_i/k_2 (\exp(-k_2 t) - 1)]\}$, where A_t and A_0 are the protein-bound radioactivity at time t and time 0 respectively, k_i is the degradation rate constant at time 0 and k_2 is the rate of decay of k_i; the continuous line represents the fitted function, and the dotted lines delineate the 95% confidence band for the fitted function. The goodness of fit is also indicated by the plot of residuals (differences between theoretical and actual values) at each time point (top panel). Each point represents a single animal.

complex equation, in which the rate of degradation (k_d) fell from k_i to k_f during the experimental period, failed to converge on any solution that gave a non-zero value for k_f. This is consistent with the data in which the rate of loss of label in older layers has declined virtually to zero. Accordingly, the data were analysed by using the simpler model in which $k_f = 0$ (eqn. 5). This model fitted the data well (chi-squared value $= 1.4 \times 10^7$), and there was no bias in residual distribution (Fig. 6). Moreover, all of the data lay within the 95% confidence band for the fitted curve (dotted lines, Fig. 6). The fitted curve is defined by a degradation rate constant (k_d) of 0.05/day at day 10, which falls to 0.002/day by day 50. The rate of degradation (k_d) declines in this model with a rate constant of 0.09 ± 0.03/day.

Turnover parameters of glycogen phosphorylase

The degradation rates, in combination with data on the rate of accretion of phosphorylase, allow us to estimate the turnover of the phosphorylase pool in the pectoralis. Because of the time-dependent decline in phosphorylase turnover, and to facilitate comparisons with previously published data on total protein turnover (Maruyama *et al.*, 1978; Kang *et al.*, 1985), we have compared data collated from early (2–3 weeks old, $n = 5$) and older (7–8 weeks old, $n = 5$) animals. Although there will be an overall trend in the parameters over these time windows, they are short relative to the whole experiment, and errors in the estimates are low. The turnover data are expressed as the mass of phosphorylase from the densitometry data, but similar results would be obtained from activity assays; the specific activity of the enzyme in broiler or layer muscle is unchanged throughout the experimental period. The expansion of the pectoralis pool of phosphorylase is calculated from the simple expression

$$k_g = k_s - k_d$$

where k_g, k_s and k_d are the fractional rates of phosphorylase accretion, synthesis and degradation respectively. The k_d is determined directly in this study, and thus knowledge of k_g allows calculation of k_s. Calculation of k_g is based on the measured rate of increase in the pectoralis phosphorylase pool divided by the total pool size. For layers, the level of phosphorylase in the pectoralis does not change with time, and during the course of this experiment the muscle grows linearly. In broilers, where the rate of degradation is constant but where the total phosphorylase pool is expanding, and the concentration of phosphorylase within the muscle is also increasing, it has proved difficult to derive a simple expression for accretion of phosphorylase within the pectoralis. Accordingly, we fitted linear functions to the data at the early (2–3 week) and late (7–8 week) regions of the curve to give local rates. From these data, we were able to calculate the third parameter, k_s. These data are summarized in Table 1. When the turnover parameters are expressed per g of muscle, the relatively large effect of degradation on phosphorylase accumulation in layers, as compared with broilers, is notable. This is most marked in younger animals, but is nevertheless still obvious in the later stages of the study. The net effect of these parameters, combined with muscle growth, on the pectoralis is also shown in the turnover of phosphorylase in the whole muscle. A dramatic increase in the total accumulation of phosphorylase occurs with time in broilers, whereas in layers accumulation remains constant over the same period.

Comparison of broilers and layers

In 2–3-week layers, the rate of synthesis of phosphorylase (about 13%/day) is approximately the same as in broilers (about 15%/day). The rate of degradation (5%/day) is about 5-fold higher than in broilers (1%/day). As the animals grow, the rate of synthesis in layers falls to 4%/day and that in broilers remains at a higher value, of 7.5%/day. The rate of degradation in layers falls to 0.6%/day, whereas in broilers the rate is unchanged, at about 1%/day. The dramatic accretion of phosphorylase in broilers is therefore attained by maintenance of the high rate of synthesis and a very low rate of degradation. By contrast, the accumulation of the enzyme in layers is a function of the more dramatic decline of synthesis rate as the animals mature, and a commensurate decrease in the rate of degradation. The accretion of muscle phosphorylase in broilers results primarily from a sustained high level of synthesis relative to a low rate of degradation. This is supported by analyses of turnover rates of total protein *in vitro* (Klasing *et al.*, 1987). The rate of degradation in broilers is 4-fold lower in young broilers than in layers, and it seems that these animals have been selected in part for a lower activity of the degradative systems by which phosphorylase turnover normally occurs.

Turnover of phosphorylase relative to total muscle protein

There have been relatively few studies on protein turnover in broiler and layer chicks, but it is of interest to consider phosphorylase turnover in the context of the turnover parameters derived from these studies (Maruyama *et al.*, 1978; Kang *et al.*, 1985). In a study of young (2-week) broiler and layer chickens, the turnover parameters for total pectoralis protein were: synthesis 16%/day, degradation 13%/day and accumulation 3%/day for layers, and synthesis 18%/day, degradation

Table 1. Growth and turnover parameters for glycogen phosphorylase in broiler and layer chicks

Data from 3-week- and 8-week-old broiler and layer chicks ($n = 5$ in all groups) were collated. The data for phosphorylase amount are based on densitometric analysis, and are expressed in terms of unit muscle weight and the total muscle pool. Data are presented as means ± S.E.M. The turnover parameters for broilers and layers of the same age group were compared by Student's t test [$^*P < 0.05$, $^{**}P < 0.001$; n.s., not significant ($P > 0.05$)].

	Broilers		Layers	
Age	3 weeks	8 weeks	3 weeks	8 weeks
Pectoralis weight (g)	12.5±3.3	158.2±22.6	14.7±0.9	39.9±4.4
Phosphorylase activity (μkat/g)	5.0±0.6	7.7±0.6	6.8±0.5	6.5±0.6
Total phosphorylase (μkat)	68.1±20	1260±271	101.6±11.2	267±23.5
Phosphorylase content (mg/g)	5.2±0.4	8.2±0.5	5.1±0.2	6.6±0.8
Total phosphorylase (mg)	74.5±22	1410±292	99.5±10.4	269±24.2
Phosphorylase specific activity (μkat/mg)	1.01±0.17	0.95±0.08	1.26±0.2	1.06±0.2
Phosphorylase turnover (per g of muscle)				
Synthesis (mg/day per g)	0.89±0.031	0.60±0.064	0.76±0.050$^{n.s.}$	0.23±0.020**
Degradation (mg/day per g)	0.06±0.004	0.09±0.006	0.20±0.017**	0.04±0.010**
Accumulation (mg/day per g)	0.84±0.310	0.51±0.068	0.56±0.040$^{n.s.}$	0.19±0.010*
Phosphorylase turnover (whole muscle)				
Synthesis (mg/day per pectoralis)	7.56±0.18	89.5±2.78	10.96±0.10**	9.62±0.34**
Degradation (mg/day per pectoralis)	0.66±0.18	14.1±2.77	2.86±0.09**	1.57±0.34**
Accumulation (mg/day per pectoralis)	6.9	76	8.1	8.1

10%/day and accumulation 8% day for broilers (Jones *et al.*, 1986). By contrast, the corresponding values for phosphorylase are synthesis 13%/day, degradation 5%/day and accumulation 8%/day for layers, and synthesis 15%/day, degradation 1%/day and accumulation 14%/day for broilers. Phosphorylase is turned over at a lower rate than 'average' protein, a value that is biased heavily by the major contractile proteins. This is perhaps unsurprising; phosphorylase is subject to stringent allosteric and covalent regulation, and would not be expected to undergo a high turnover. Moreover, the massive accumulation of phosphorylase in broiler muscle is attained by a high rate of synthesis, with virtually no degradation, which contrasts with the behaviour of total protein. It has been proposed that there are two distinct phases in muscle growth; structural maturation (in which the contractile apparatus is laid down) and metabolic maturation (in which the enzymic machinery is established) (Nakamura *et al.*, 1990). The rate of turnover, and developmentally related changes in this rate, need not therefore be linked to the behaviour of the contractile proteins.

In overall terms, however, the degradation of phosphorylase matches the behaviour of total protein, inasmuch as young broilers exhibit a rate of phosphorylase degradation that is about one-third of that of layers. As the animals age, the rate of degradation of phosphorylase in broilers changes only slightly, whereas that for layers approaches the broiler value. This again reflects the pattern seen with total protein (Kang *et al.*, 1985). Fractional protein synthesis rates are much more closely matched in the two groups of animals, and this is seen in the present study. Thus our data, which focus on the behaviour of a single sarcoplasmic enzyme, extend previous observations but emphasize the role of protein degradation in rapid accumulation of the enzyme in broiler muscle. Moreover, our analysis is derived from a degradation study, in which we have calculated k_s from k_d and k_g, whereas previous studies have measured k_s and k_g, and calculated k_d.

At present, the nature of the degradative machinery that contributes to phosphorylase turnover is unknown, and in this respect it is noteworthy that there is no difference in the activities of calpains or calpastatins in broiler and layer muscle (Ballard *et al.*, 1988). The role of the lysosome in muscle protein metabolism is contentious (Wing *et al.*, 1991), and there is evidence for the ubiquitin-mediated degradative pathway in this tissue (Arnold & Gevers, 1990; Fagan *et al.*, 1987). Examination of the activities of these proteolytic mechanisms in muscle from the two strains may add to our understanding of the role of protein degradation in muscle growth.

This work was supported by A.F.R.C. Grant AG26/194 and by N.A.T.O. (CRG 910134). We are grateful to Dr. M. M. Crerar for critical reading of the manuscript, and for useful discussions.

REFERENCES

Arnold, J. E. & Gevers, W. (1990) Biochem. J. **267**, 751–757
Ballard, R., Bardsley, R. G. & Buttery, P. J. (1988) Br. J. Nutr. **59**, 141–147
Beynon, R. J., Fairhurst, D. & Cookson, E. J. (1986) Biomed. Biochem. Acta **45**, 1619–1625
Butler, P. E., Cookson, E. J. & Beynon, R. J. (1985) Biochim. Biophys. Acta **847**, 316–323
Carney, I. T., Beynon, R. J., Kay, J. & Birket, N. (1978) Anal. Biochem. **85**, 321–324
Cookson, E. J. & Beynon, R. J. (1989) Int. J. Biochem. **21**, 975–982
Fagan, J. M., Waxman, L. & Goldberg, A. L. (1987) Biochem. J. **243**, 335–343
Fisher, C. (1980) in Protein Deposition in Animals (Buttery, P. J. & Lindsay, D. B., eds.), pp. 251–270, Butterworths, London
Goldberg, A. L. (1969) J. Biol. Chem. **244**, 3217–3222
Jones, S. J., Aberle, E. D. & Judge, M. D. (1986) J. Amin. Sci. **62**, 1576–1583
Kang, C. W., Sunde, M. L. & Swick, R. W. (1985) Poult. Sci. **64**, 370–379
Klasing, K. C., Calvert, C. C. & Jarrell, V. L. (1987) Poult. Sci. **66**, 1189–1196
Laurent, G. J., Sparrow, M. P. & Millward, D. J. (1978) Biochem. J. **176**, 407–417
Leyland, D. M. & Beynon, R. J. (1991) Biochem. J. **278**, 113–117
Leyland, D. M., Turner, P. C. & Beynon, R. J. (1990) Biochem. J. **272**, 231–237
MacDonald, M. L. & Swick, R. W. (1981) Biochem. J. **194**, 811–819

MacNaughton, A. F. (1974) J. Anat. **118**, 171–186
Maruyama, K., Sunde, M. L. & Swick, R. W. (1978) Biochem. J. **176**, 573–582
Millward, D. J., Garlick, P. J., Steward, R. J. C., Nnanyelugo, D. O. & Waterlow, J. C. (1975) Biochem. J. **150**, 235–243
Nakamura, M., Yao, Y. & Hirabayashi, T. (1990) Cell. Differ. Dev. **32**, 61–69
Wing, S. S., Chiang, H.-L., Goldberg, A. L. & Dice, J. F. (1991) Biochem. J. **275**, 165–169
Young, V. R., Stothers, S. C. & Vilaire, G. (1971) J. Nutr. **101**, 1379–1390

Received 16 December 1991/4 March 1992; accepted 7 April 1992