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IN VITRO AND *IN VIVO* PROTEIN SYNTHESIS RATES IN A CRUSTACEAN MUSCLE DURING THE MOULT CYCLE

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SUMMARY

In vivo protein synthesis rates were measured in the carpopodite extensor muscle of the shore crab, *Carcinus maenas*, following a single, high-dose injection of [^{3}H]phenylalanine, which stabilized specific radioactivities in the free pools. In intermoult animals the percentage of protein mass synthesized per day (the fractional rate of protein synthesis) was 1·15 % day^{-1} for the whole extensor muscle. The small, slow-type tonic fibres in the extensor had fractional rates of protein synthesis some 2·1 times higher than those of the large, fast-type phasic fibres.

Measurement of protein synthesis rates of extensor muscles from intermoult animals using an *in vitro* incubation over 2 h gave fractional synthesis rates three times lower than those found in *in vivo* experiments. Compared with the intermoult animals, six- and three-fold increases in fractional synthesis rates were found in the extensor muscles from stages immediately preceding and following ecdysis, respectively. Microdissection of the muscle fibres revealed that the increased synthesis in postecdysial animals was occurring mainly at the external cuticular end of the muscle fibres. Autoradiographic analysis confirmed the cuticular end of the muscles as the major site of muscle protein synthesis. We conclude that the postecdysial increase in muscle fibre length and the associated increase in the sarcomere number is accompanied by an increase in protein synthesis in the muscles.

INTRODUCTION

Walking leg muscles of some crustaceans lengthen during ecdysis and the early postecdysial period (El Haj, Govind & Houlihan, 1984; Houlihan & El Haj, 1985). Evidence for this growth comes from morphological studies demonstrating that the walking leg extensor muscle fibres from *Carcinus maenas* and *Homarus americanus* increase in length during ecdysis by the addition of sarcomeres. Since addition of new sarcomeres to the muscle fibres will involve an increased rate of protein synthesis, the aim of the present study was to measure the rate of incorporation of amino acids into muscle proteins during the crustacean moult cycle in order to test

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the hypothesis that sarcomere addition over the ecdysial period is accompanied by a stimulation in the rate of protein synthesis.

Earlier studies on protein synthesis in crustacean tissues have used pulse labelling techniques in order to measure amino acid incorporation into proteins (Gorell & Gilbert, 1971; McWhinnie & Mohrerr, 1970; Skinner, 1965, 1966*a*,*b*). The results from these studies are difficult to interpret as the levels of incorporation of radioactivity into protein are expressed simply as counts $min^{-1} g^{-1} min^{-1}$ and no measurements of the specific activities of the intracellular amino acid pools were made. New methods giving stable and measurable free pool specific radioactivities have enabled advances in the measurement of the rates of protein synthesis. Garlick, McNurlan & Preedy (1980) used a single high dose of [^{3}H]phenylalanine to flood the intracellular pools and stabilize the specific activity over the course of the determination in mammals. The rate of protein synthesis is then calculated as the fractional rate of protein synthesis, k_s, the percentage of the protein mass synthesized per day (Waterlow, Garlick & Millward, 1978; Garlick, Fern & McNurlan, 1979).

These techniques have been adapted to measure the fractional rate of protein synthesis both *in vivo* and *in vitro* of an extensor muscle of the walking leg of *Carcinus maenas*. The rates of protein synthesis of the whole extensor have been determined at various stages of the moult cycle and, by microdissection and autoradiography, a correlation has been made between amino acid incorporation into muscle proteins and the region of new sarcomere formation.

MATERIALS AND METHODS

Carcinus maenas of varying size were collected from shores around Aberdeen and maintained in a circulating seawater system at 10–12°C. The animals were fed daily on squid and mussel. Young adult *Carcinus maenas* (>8 mm carapace width) were reared in individual containers adapted from those described by Lang (1975).

In vitro *techniques*

Whole right and left third walking legs were removed from the animal by autotomy. The carpopodite extensor muscle was exposed within the merus by removing the overlying cuticle and closer muscle. The legs were then secured into an aerated incubation chamber at 15°C. The meropodite–carpopodite (MC) joint was held at 90° to ensure the fibres were at rest length. The incubation medium consisted of Pantin's (1969) Ringer with added amino acids based upon the measured intracellular levels (Duchateau, Florkin & Jeaniaux, 1959). The concentration of phenylalanine in the medium was equivalent to the intracellular free pool of the extensor muscle of an intermoult animal. The amount of [^{3}H]phenylalanine in the medium was 1 μCi ml^{-1} (37 KBq ml^{-1}). In one series of experiments incubations were carried out in 150 mmol phenylalanine with [^{3}H]phenylalanine at 1 μCi ml^{-1} This flooding level of phenylalanine would increase the free pool of phenylalanine

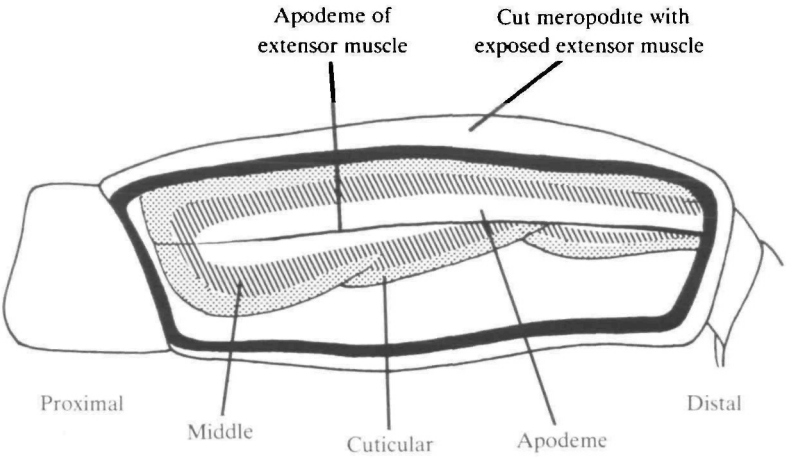


Fig. 1. Diagram of the regions (middle, cuticular and apodeme) of the carpopodite extensor muscle used for the analysis of protein specific activity along the length of the muscle fibres.

several-fold above normal intermoult levels. However, the measured protein synthesis rates of the muscle were found to be similar to those obtained with the lower level of phenylalanine in the incubation medium.

An initial time course with walking legs from nine intermoult animals (stages C3–4, see below) ranging from 80 to 100 g body mass was set up over 4 h of incubation. Subsequently, the walking legs from intermoult animals ranging in size from 1 to 100 g body mass ($N = 24$) were incubated for 2 h. Extensor muscles were also used from young adults of 10–20 g body mass at varying stages of the moult cycle, i.e. D1–2, D3–4, A1–2, B1–2 and C1–2. The stage of the moult cycle was determined using the method of Crothers (1967) and by close timing following ecdysis of individual animals in rearing chambers. The duration of the stages was taken from Crothers (1967) i.e. C3–4, 45 %; D1–2, 15 %; D3–4, 4·5 %; E, 0·5 %; A1–2, 2 %; B1–2, 8 %; C1–2, 25 %. In one series of experiments, after incubation individual fibres from extensor muscles of stage A1–2 young adults were fixed in Bouin's *in situ* for 1 h and dissected into three regions, cuticular, middle and apodeme (Fig. 1).

In vivo *techniques*

The *in vivo* free pool technique was adapted from that described by Garlick *et al.* (1980) for rats. A single dose of radiolabelled [^{3}H]phenylalanine was injected into the base of the third walking leg. The injection solution contained 150 mmol phenylalanine with L-(ring 2, 3, 4, 5, 6[^{3}H]phenylalanine) at 50 μCi ml^{-1} (1·85 MBq ml^{-1}) at a dose of 1·0 ml 100 g^{-1} body mass. This amount of phenylalanine should increase the free pool concentration some seven-fold.

A time course using intermoult animals ($N = 16$), ranging in body mass from 50 to 100 g, was set up over 2 h. All animals were maintained in aerated sea water at 15 °C. The time of incubation was lengthened from that of Garlick *et al.* (1980) to account

for the slower rate of synthesis as determined by *in vitro* preparations of crustacean muscle. The carpopodite extensor muscle consists of fast, slow and intermediate muscle fibres and these can be recognized on the basis of fibre diameter and their position in the extensor (Parsons & Mosse, 1982; El Haj *et al.* 1984; Houlihan & El Haj, 1985). From each animal the whole extensor of the right leg was used for protein synthesis measurement and the corresponding left leg extensor was dissected into groups of 10 fast and 10 slow fibres for the determination of protein synthesis of specific fibre types.

Determination of the rate of protein synthesis

Muscle samples from *in vivo* or *in vitro* experiments were immediately homogenized in 0·5 mol l^{-1} perchloric acid at the end of the incubations. After centrifugation, the precipitate containing the protein was washed in 0·2 mol l^{-1} perchloric acid, dissolved in 0·2 mol l^{-1} NaOH and the protein content measured by a modified Lowry method (Miller, 1959) using bovine serum albumin as a standard. The protein was reprecipitated from the NaOH and hydrolysed in 6 mol l^{-1} HCl at 110°C overnight. The HCl was removed by evaporation to dryness and the amino acid dissolved in 0·1 mol l^{-1} citrate buffer at pH 6·3. This solution was used to determine the specific radioactivity of the protein-bound phenylalanine. The supernatant from the initial homogenization, containing the free amino acids, was treated with potassium citrate to precipitate the perchlorate and, after centrifugation, the supernatant was used to determine the free pool specific activity. Free pool (S_a) and protein specific radioactivity (S_b) were determined using the method described by Garlick *et al.* (1980). After the conversion of phenylalanine into β-phenylethylamine (PEA) the concentration of the latter was determined by fluorescence with a standard curve of 0–15 nmol PEA. Standard solutions of phenylalanine were used to quantify the recovery of PEA. Measurements of radioactivity were made on a Beckman LS 7500 scintillation counter using Lumagel scintillant with a counting efficiency of 30–40 %. The experimental results were obtained as S_a, the specific radioactivity of free L-phenylalanine (d.p.m. $nmol^{-1}$), and as S_b, the specific radioactivity of protein-bound phenylalanine (d.p.m. $nmol^{-1}$). The fractional rate of protein synthesis k_s (the percentage of the protein mass synthesized per day) was calculated as:

$$k_s = \frac{S_b}{S_a} \times \frac{100}{t},$$

where t is the duration of the incorporation in days.

Autoradiographic techniques

1·5 μm thick longitudinal sections of Araldite-embedded material prepared from immediate postmoult and intermoult whole extensors were coated with K2 emulsion (Kodak) for light microscope autoradiography. The slides were stored at 4°C for 3, 6 and 9 weeks. After incubation 900 mm^2 transects were taken randomly along at least five fibres from each extensor. The number of grains was counted in each of the three regions, cuticular, middle and apodeme, and expressed in terms of the percentage of

the total number of grains per fibre. The three regions of the muscle are illustrated in Fig. 1.

RESULTS

As the body mass of *Carcinus* increases, so the muscle wet mass increases according to the relationship $y = 0{\cdot}0027x - 0{\cdot}0088$ ($r^2 = 0{\cdot}93$), where y is muscle wet mass (g) and x is body mass (g). With the increase in muscle masses there is a corresponding increase in total protein content with a linear relationship of $y = 0{\cdot}06x + 0{\cdot}08$ ($r^2 = 0{\cdot}95$), where y is muscle wet mass (g) and x is protein content (mg). Phenylalanine makes up approximately 6% of the total muscle protein. The intracellular or free pool intermoult level of phenylalanine was measured as $200 \pm 24{\cdot}9$ nmol g^{-1} wet mass of extensor muscle. This value is slightly lower than that reported by Duchateau *et al.* (1959).

In vitro *protein synthesis of intermoult muscle*

Fig. 2 demonstrates the viability of the *in vitro* incubation medium as the amino acid uptake into the muscle is monitored with time. The intracellular free pool specific activity remained constant over 4 h with the specific activity of the bound protein increasing over the incubation period. The fractional rate of synthesis, k_s, calculated over separate time intervals remained constant throughout the time of incubation (Fig. 3) at a mean value of $0{\cdot}37 \pm 0{\cdot}06$% per day.

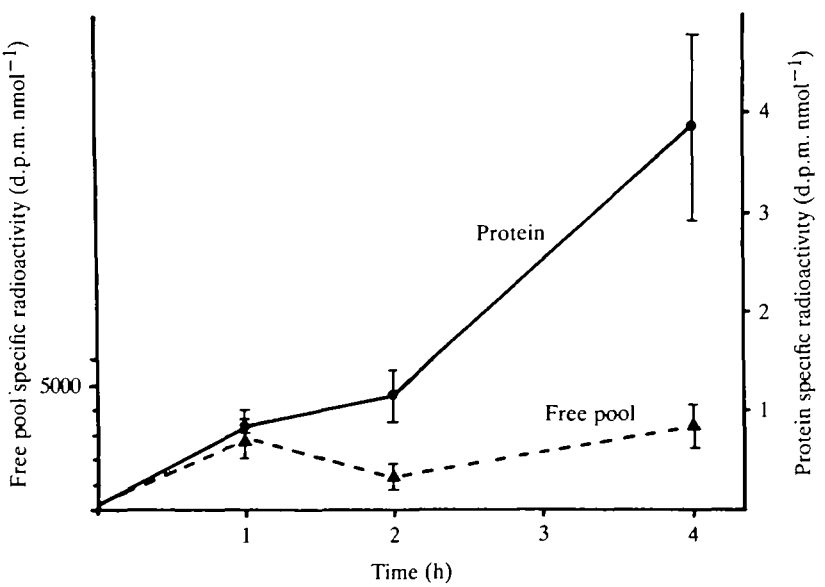


Fig. 2. Time course of the specific activity of phenylalanine in the free pool and in the bound protein during an *in vitro* incubation of the extensor muscle. Each point is a mean ± S.E. of three extensors from three individuals ranging from 80–100 g body mass.

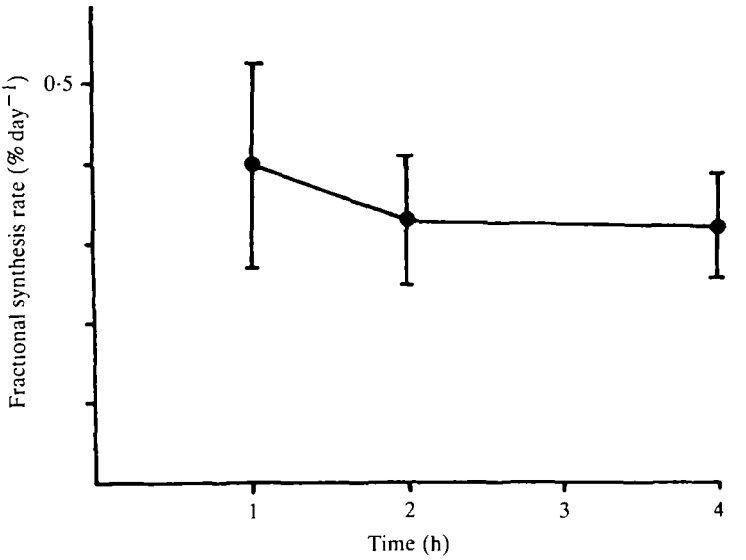


Fig. 3. The fractional rate of synthesis of protein (expressed as % day^{-1}) over the course of an *in vitro* incubation. Each value is a mean ± S.E. of three extensors from three individuals taken from data shown in Fig. 2.

The *in vitro* fractional synthesis rate was measured in extensors from a size range of intermoult animals. Although the mean value of synthesis is slightly lower in small animals, there are no significant differences between the means for the three size groups (Table 1).

In vivo *rates of protein synthesis of intermoult muscle*

Preliminary experiments determined that the amount of phenylalanine in the single injected dose was sufficient to both flood the intracellular pool and be incorporated into muscle protein. The specific activity of the free pool equilibrates initially and then falls slowly with time over 2 h following the injection (Fig. 4). The incorporation of radiolabelled amino acids into protein increased with incubation time up to 2 h. The k_s values were calculated from the specific activity of the phenylalanine in the protein (S_b) and the specific activity of the free phenylalanine in

Table 1. *Fractional rates of protein synthesis (k_s) of* Carcinus *carpopodite extensor muscle measured* in vitro

Body mass (g)	Total protein content of the extensor (mg)	k_s (% day^{-1})
1–20	0·4–2·0	0·187 ± 0·025
20–60	2·0–5·0	0·254 ± 0·025
60–100	5·0–10·0	0·249 ± 0·030

Measurements were made from animals in three different size categories.
Each mean (±S.E.) was made from eight animals.

the tissue (S_a). The mean *in vivo* k_s value is $1.15 \pm 0{\cdot}14\,\%\,day^{-1}$ (Fig. 4). The *in vivo* rate is approximately three times higher than the *in vitro* rate for the extensor muscle.

The small, slow-type tonic fibres have a significantly faster rate of synthesis than the large, fast-type phasic fibres; combining the results for 1 h and 2 h, the mean k_s value for large fibres is $0{\cdot}5618 \pm 0{\cdot}218$ and small fibres $1{\cdot}318 \pm 0{\cdot}398\,\%\,day^{-1}$.

In vitro *rates of protein synthesis over the moult cycle*

The concentrations of phenylalanine present in the free pools of the muscles at different stages during the moult cycle are given in Fig. 5. The levels of intracellular phenylalanine are elevated during the immediate pre-ecdysial stage. By stage D3–4, the amount of free phenylalanine in the muscle has risen to a mean value of $316{\cdot}1 \pm 45{\cdot}3\,nmol\,g^{-1}$ wet mass; an increase of 60% over the intermoult and late postmoult levels.

Fig. 6 shows the fractional rate of synthesis ($\%\,day^{-1}$) at each stage of the moult cycle. During stages D3–4, A1–2 and B1–2 there were significant elevations in

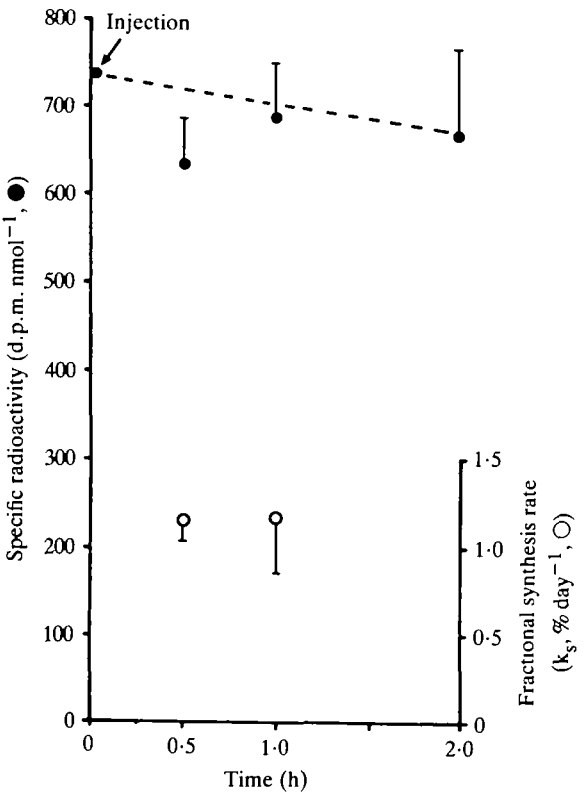


Fig. 4. Specific radioactivity of free pool phenylalanine (●) and calculated fractional rates of protein synthesis (○) for *Carcinus* carpopodite extensor muscle *in vivo* after the injection of [^{3}H]phenylalanine. Each crab was injected with 150 mmol phenylalanine and [^{3}H]phenylalanine at a dose of 1 ml $100\,g^{-1}$ body mass at time zero. The specific activity of the injection solution is indicated on the ordinate. Each mean (±S.E.) was calculated from four animals.

protein synthesis rate. During stage D3–4, the fractional rate of synthesis increased by a factor of 10 above the level at D1–2, to a mean value of 1·68% day^{-1}; at stages A1–2 and B1–2, the fractional rates of synthesis decreased to a mean value of

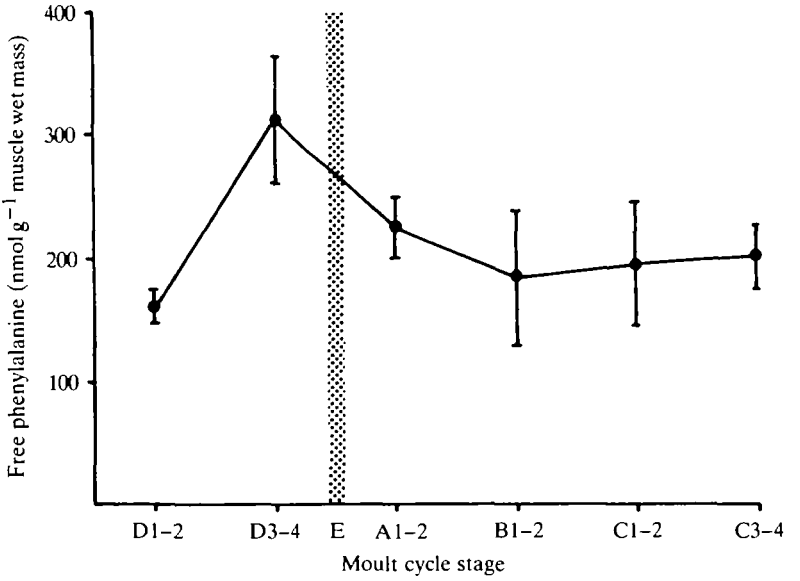


Fig. 5. Levels of free pool phenylalanine during the moult cycle expressed per g of extensor muscle wet mass. Each value is a mean ± S.E. from three individuals. The dotted bar denotes the time of ecdysis.

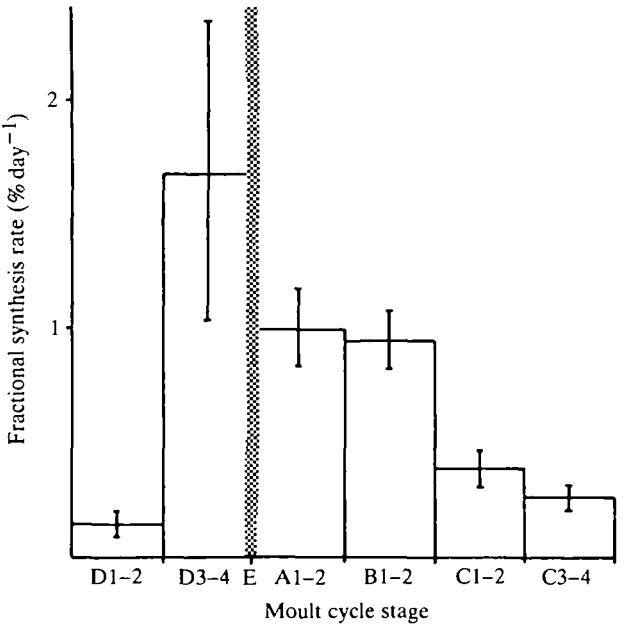


Fig. 6. The mean ± S.E. *in vitro* rate of fractional synthesis of protein in the carpopodite extensor muscle during the moult cycle.

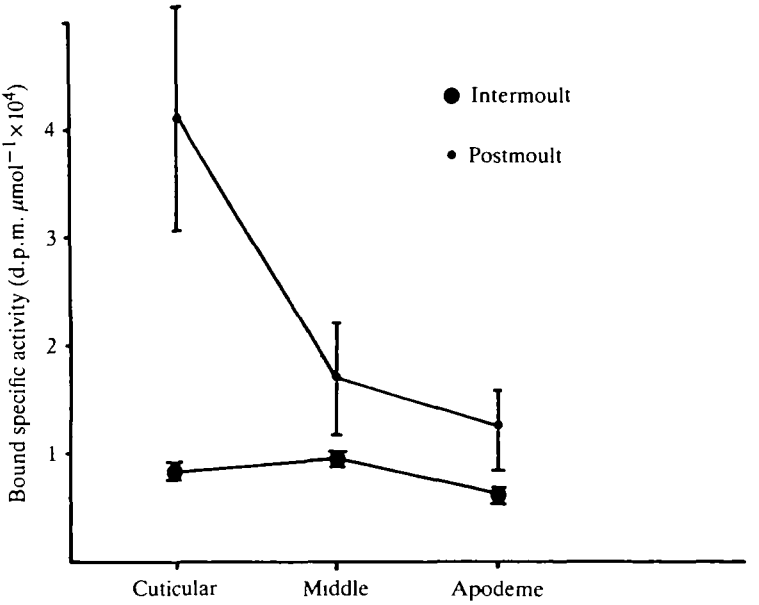


Fig. 7. Specific activity of phenylalanine in the bound protein in different regions of the fibre in intermoult and immediate postecdysial extensor muscles following an *in vitro* incubation and dissection of the fixed fibres. The three regions of the muscle, cuticular, middle and apodeme, are illustrated in Fig. 1. Each value is a mean ± S.E. of fibres from 11 post-ecdysial muscles and three intermoult muscles.

0·98 % day^{-1}. By stage C1–2, the rate of synthesis had fallen to near intermoult levels and there was no significant difference between k_s values at C1–2 and C3–4.

Protein specific activity along the length of the fibre

To investigate further the regions of the muscle responsible for this increased synthesis, the immediate postecdysial fibre, after radiolabelling, was divided into three regions, cuticular, middle and apodeme (Fig. 1). It was found to be necessary to fix the muscles before attempting the dissection and although this resulted in good recoveries of labelled proteins it was not possible to measure the specific activity of the free pools of the fixed tissue. Therefore the results are expressed as the specific activity of the protein-bound phenylalanine rather than the fractional rates of protein synthesis. Fig. 7 illustrates the increase in the protein-bound specific activity in the cuticular (exoskeletal) region of the immediate postmoult muscle fibre. The protein-bound specific activity was higher in all three regions of the muscles when compared with the intermoult fibre treated in the same way, which agrees with the higher fractional rates of protein synthesis described above. In the intermoult muscles the protein-bound specific activity remained constant along the length of the fibre. The increased incorporation of radiolabelled phenylalanine into bound protein supports the theory that new synthesis is taking place in the cuticular region of the fibre.

Autoradiographic analysis of sections of incubated postecdysial fibres demonstrated an increased percentage of the total number of grains in the cuticular or exoskeletal region (Fig. 8) which corresponded with the measured increase in protein specific activity (Fig. 7). A significant difference in the number of grains was found ($P < 0{\cdot}05$) between the middle and apodeme regions and the exoskeletal region of the fibre. In contrast, there was an even distribution of silver grains along the length of the intermoult fibre. Fig. 9 illustrates the distribution of grains in the three regions of a postecdysial fibre. There appears to be no localization around the Z-band, as was found in intermoult muscle (unpublished observation). Contamination of free amino acids during the fixation process (Droz & Warchawsky, 1963) has been cited as a source of error in quantitative analysis of autoradiography; free amino acids, however, should be distributed evenly throughout the fibre and should not affect regional variation in bound specific activity.

DISCUSSION

The intermoult mean fractional rate of protein synthesis of *Carcinus maenas* extensor muscle is 0·37 % day^{-1} *in vitro* and 1·15 % day^{-1} *in vivo*. Having defined a method for the measurement of the fractional rate of synthesis (k_s), it is possible to compare the rates of synthesis for invertebrate muscle with those for vertebrate muscle. The measured crustacean k_s values for mixed fibre extensor muscle are 10–20 times less than values for mammalian muscle tissue, which range from 3 to

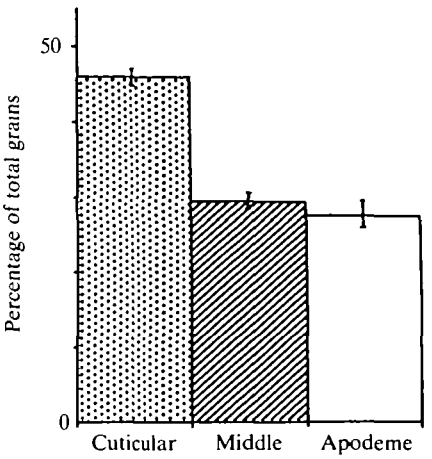
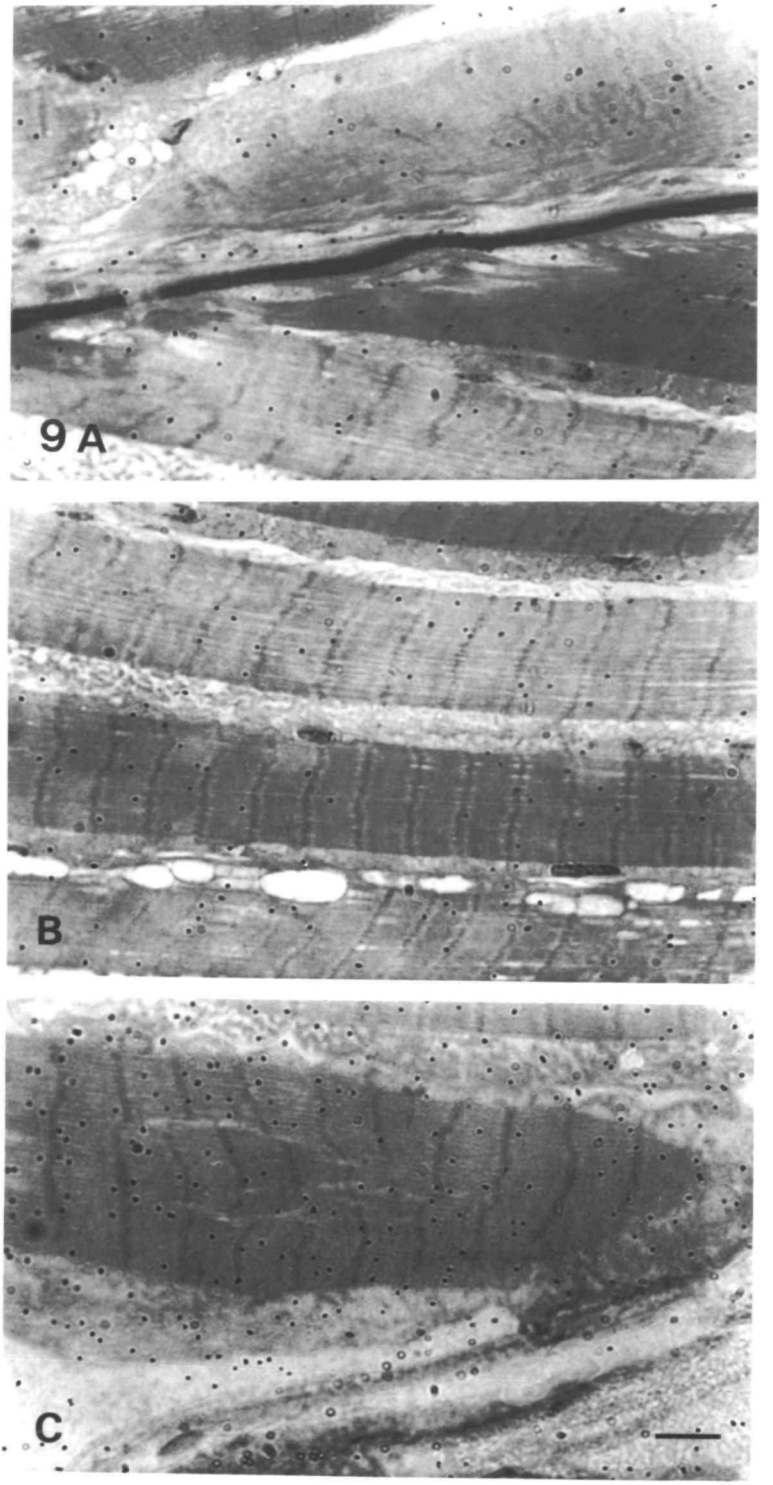


Fig. 8. The percentage of total silver grains counted from autoradiographs from three regions of the immediate postecdysial extensor muscle. Autoradiographs were made from muscles following *in vitro* incubation. The shading corresponds to the diagram of the muscle given in Fig. 1. Each value is a mean ± S.E. of 25 fibres.

Fig. 9. Autoradiographs of three regions of postmoult fibres after *in vitro* incubation for 2h at 15°C in radiolabelled phenylalanine. Slides were coated with K2 emulsion. (A) Apodeme region, longitudinal section; (B) middle region, longitudinal section; (C) cuticular or exoskeletal region, longitudinal section. Scale bar, 5 μm.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8 % day^{-1} *in vitro* and 12–20 % day^{-1} *in vivo*, with a four-fold difference in rate between the two conditions (Waterlow *et al.* 1978). Using the flooding technique, the k_s values for fish epaxial white muscle are in similar range to that of Crustacea, e.g. fed summer toadfish (*Opsanus* sp.) *in vivo* rates of 0·09–0·71 % day^{-1} at experimental temperatures ranging from 11 to 22°C (Pocrnjic, Mathews, Rappaport & Haschemeyer, 1983) and rainbow trout (*Salmo gairdneri*) with rates of 0·5 ± 0·04 % day^{-1} for white muscle and 1·3 ± 0·09 % day^{-1} for red muscle (Houlihan, McMillan & Laurent, 1986). The differences can be related to environmental temperatures (Pocrnjic *et al.* 1983) as well as any possible body size, seasonal and nutritional fluctuations (Houlihan *et al.* 1986; Smith & Haschemeyer, 1980; Pocrnjic *et al.* 1983; Waterlow *et al.* 1978).

The rate of synthesis for a given muscle will also vary according to the predominant fibre type (Goldberg, 1967; Smith & Haschemeyer, 1980). The crustacean extensor muscle used in this study is composed of at least three fibre types, fast phasic, intermediate and slow tonic (Parsons & Mosse, 1982; Houlihan & El Haj, 1985; Houlihan & Mathers, 1985). The slow tonic muscles had a higher rate of protein synthesis, or k_s value, than the fast phasic muscles. This agrees with *in vivo* studies on mammalian and fish muscle (Goldberg, 1967; Houlihan *et al.* 1986).

Comparisons of the relative rates of synthesis during the moult cycle can be made between this study and previous studies. Our *in vitro* k_s values in the walking leg extensor muscle varied according to the stage of the moult cycle with the highest levels recorded during the pre-ecdysial and the immediate postecdysial phase. Skinner (1965, 1966*a*,*b*) measured the rate of protein synthesis in the chelae muscle at three broad stages, premoult, postmoult and intermoult, and found elevated rates, of twice the intermoult level, at premoult and at a late stage of the postmoult. The chelae muscle undergoes atrophy prior to the moult and the major growth phase is during the late postmoult period (Skinner, 1966*b*). The increased synthesis rate during the premoult has been attributed to an increase in the synthesis of digestive enzymes during this stage, whereas the increased synthesis rate during the late postmoult corresponds to the period of muscle growth.

In contrast, the extensor muscle of *Carcinus* does not atrophy prior to the moult (Houlihan & El Haj, 1985; Mykles & Skinner, 1982) and indeed, the extensor muscle lengthens during ecdysis and the immediate postecdysial period by the addition of sarcomeres (El Haj *et al.* 1984; Houlihan & El Haj, 1985). Thus it is unlikely that the similar elevation of protein synthesis rate prior to ecdysis is related to an increase in digestive enzymes as suggested for chelae muscle. The rate of muscle growth (g day^{-1}) is a function of the rate of protein degradation (g day^{-1}) as well as protein synthesis (g day^{-1}) (Garlick *et al.* 1979). Due to the variation in animal size, no direct measurement was made of the growth rate or the degradation rate of the muscles in this study. Therefore, it is not possible to determine whether there is a corresponding increase in the rate of degradation with the increased rate of synthesis during the premoult stage, which would explain the previous evidence that growth is not occurring during this phase (as described by El Haj *et al.* 1984; Houlihan & El Haj, 1985). One possible explanation for the fluctuation in protein turnover during

The premoult period may be related to higher levels of ecdysone during this stage (Chaix, Mavaldi & Secchi, 1981). The hormonal influence on the protein synthesis and degradation rates of various mammalian tissues has been well documented (Waterlow *et al.* 1978) but the possible effects of ecdysone on the rates of protein turnover in Crustacea has yet to be investigated.

The immediate postecdysial increase in synthesis could account for the sarcomere addition and subsequent elongation found in the extensor muscle of *Carcinus* (Houlihan & El Haj, 1985). These results are in contrast to results for the chelae muscle and the previous suggestion that tissue growth is occurring during the late postecdysial stage, stage C1–2 (Warner, 1977). In this study, there was no evidence of elevated levels in the fractional rate of synthesis during the stage C1–2.

Further evidence of muscle growth and sarcomere addition comes from quantitative autoradiographic analysis and the measurement of protein specific activity along the immediate postmoult fibres. An increase in bound specific activity correlated with an increased number of grains indicates that there is uptake of labelled phenylalanine into the external cuticular region of the fibres during the postmoult stage, providing further evidence for an increase in muscle length brought about by sarcomere addition during the ecdysial period. The longitudinal addition of sarcomeres onto the ends of vertebrate muscle fibres has been described by Williams & Goldspink (1971) using a label, adenosine, specific for myofibrillar synthesis. Quantitative radiographic analysis in that study demonstrated an increased amount of radiolabel at both ends of the fibres, in contrast to the present findings in the *Carcinus* extensor, where sarcomere addition appears to be occurring at the exoskeletal junction alone.

In summary, during the immediate postecdysial period, elevation of the rates of protein synthesis in the extensor muscle can be related to the production of new sarcomeres at the external cuticular attachment region of the fibres. Pre-ecdysial increases in the synthesis rate have yet to be fully explained and require further work on fluctuations in the growth rate and the rate of degradation of muscle, as well as hormonal effects on crustacean muscle protein synthesis.

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REFERENCES

CHAIX, J. C., MAVALDI, J. & SECCHI, J. (1981). Variation of ecdysone titre and haemolymph major proteins during the moult cycle of the spider crab, *Acanthoryx lumulatus*. *Comp. Biochem. Physiol.* **69**B, 709–714.

CROTHERS, J. H. (1967). The biology of the shore crab, *Carcinus maenas* (L.). *Field Study* **2**, 407–434.

ROZ, B. & WARCHAWSKY, H. (1963). Reliability of autoradiographic technique for the detection of newly synthesized protein. *J. Histochem. Cytochem.* **11**, 426–435.

DUCHATEAU, G. H., FLORKIN, M. & JEANIAUX, C. L. (1959). Composante amino acide des muscles de *Carcinus maenas* (L.). Lois du passage de l'eau de mer a l'eau saumatre et au coins de la mue. *Archs int. Physiol. Biochim.* **67**, 489–500.

EL HAJ, A. J., GOVIND, C. K. & HOULIHAN, D. F. (1984). Growth of lobster leg muscle fibres over intermolt and molt. *J. crust. Biol.* **4**, 536–545.

GARLICK, P. J., FERN, E. B. & MCNURLAN, M. A. (1979). Methods for determining protein turnover. In *Processing and Turnover of Proteins and Organelles in the Cell* (ed. S. Rapoport & T. Schewe), Proc. of 12th FEBS Meeting, pp. 85–94. London: Pergamon Press.

GARLICK, P. J., MCNURLAN, M. A. & PREEDY, V. R. (1980). A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of ^{3}H phenylalanine. *Biochem. J.* **192**, 719–723.

GOLDBERG, A. L. (1967). Protein synthesis in tonic and phasic skeletal muscle. *Nature, Lond.* **216**, 1219–1220.

GORELL, T. A. & GILBERT, L. I. (1971). Protein and RNA synthesis in the premoult crayfish, *Orconectes virilis*. *Z. vergl. Physiol.* **73**, 345–356.

HOULIHAN, D. F. & EL HAJ, A. J. (1985). Muscle growth. In *Crustacean Growth* (ed. A. Wenner), pp. 15–30. Rotterdam: AA Balkeman Press.

HOULIHAN, D. F., MCMILLAN, D. N. & LAURENT, P. (1986). Growth rates, protein synthesis and protein degradation rates in rainbow trout; effects of body size. *Physiol. Zool.* **59**, 482–493.

HOULIHAN, D. F. & MATHERS, E. (1985). Effects of captivity and exercise on the energetics of locomotion and muscle of *Carcinus maenas* (L.). *J. exp. mar. Biol. Ecol.* **92**, 125–142.

LANG, F. (1975). A simple culture system for juvenile lobsters. *Aquaculture* **6**, 389–393.

MCWHINNIE, M. A. & MOHRERR, C. J. (1970). Influence of eyestalk factors, intermoult cycle and season upon ^{14}C-leucine incorporation into protein in the crayfish (*Orconectes virilis*). *Comp. Biochem. Physiol.* **34**, 415–437.

MILLER, G. L. (1959). Protein determination for large numbers of samples. *Analyt. Chem.* **31**, 964.

MYKLES, D. L. & SKINNER, D. M. (1982). Crustacean muscle: atrophy and regeneration during moulting. In *Basic Biology of Muscles: A Comparative Approach* (ed. B. M. Twarog, R. J. C. Levine & M. M. Dewey), pp. 337–357. New York: Raven Press.

PANTIN, C. F. A. (1969). *Notes on Microscopical Techniques for Zoologists*. London: Cambridge University Press.

PARSONS, D. W. & MOSSE, P. R. L. (1982). The leg flexor muscle of *Carcinus maenas*. II. Distribution of muscle fibre types. *J. exp. Zool.* **224**, 169–175.

POCRNJIC, Z., MATHEWS, R. W., RAPPAPORT, S. & HASCHEMEYER, A. E. V. (1983). Quantitative protein synthesis rates in various tissues of a temperate fish *in vivo* by the method of phenylalanine swamping. *Comp. Biochem. Physiol.* **74B**, 735–738.

SKINNER, D. M. (1965). Amino acid incorporation into protein during the moult cycle of the land crab, *Geocarcinus lateralis*. *J. exp. Zool.* **160**, 226–233.

SKINNER, D. M. (1966*a*). Breakdown and reformation of somatic muscles during the moult cycle of the land crab, *Geocarcinus lateralis*. *J. exp. Zool.* **163**, 115–123.

SKINNER, D. M. (1966*b*). Macromolecular changes associated with the growth of crustacean tissues. *Am. Zool.* **6**, 235–242.

SMITH, M. A. K. & HASCHEMEYER, A. E. V. (1980). Protein metabolism and cold adaptation in Antarctic fishes. *Physiol. Zool.* **53**, 373–382.

WARNER, G. F. (1977). *The Biology of Crabs*. London: Elek Science.

WATERLOW, J. C., GARLICK, P. J. & MILLWARD, D. J. (1978). (ed.) *Protein Turnover in Mammalian Tissues and in the Whole Body*. Amsterdam: Elsevier.

WILLIAMS, D. E. & GOLDSPINK, G. (1971). Longitudinal growth of striated muscle fibres. *J. Cell Sci.* **9**, 751–767.