Oecologia (Berlin) (1985) 66:42–49

Oecologia

Relationships between the synthesis and breakdown of protein, dietary absorption and turnovers of nitrogen and carbon in the blue mussel, *Mytilus edulis* L.

A.J.S. Hawkins
Institute for Marine Environmental Research, Prospect Place, The Hoe, Plymouth PL1 3DH, Devon, England

Summary. Seasonal and nutritionally induced changes of whole body protein metabolism have been studied in 45 to 57 mm shell-length *Mytilus edulis* from Whitsand Bay, southwest England. The subtraction of measured net protein balances from coincident rates of protein synthesis, determined in vivo by supplying ^{15}N-labelled alga and monitoring the enrichment of excreted ammonia, enabled computation of protein breakdown rates. Over the range of protein absorption from zero to 0.58% of total soft tissue protein $24\ h^{-1}$, fractional rates of protein breakdown decreased from 0.41 to 0.03%, whereas protein synthesis and net protein balance both increased from 0.25% to 0.39% and from −0.16% to 0.36%, respectively. The progressive reduction in fractional protein degradation with elevated net protein balance represented a "protein sparing" effect, whereby the efficiency of protein synthesis (defined as net synthesis/overall synthesis) confirmed theoretical predictions of as much as 92% during periods of maximal growth. In addition, 38% of breakdown products were recycled directly to synthesis under conditions of zero net balance, with an increasing contribution evident upon further decreases of protein absorption. The overall response was characterized by a consistently conservative elemental turnover of nitrogen relative to carbon, so that as a fraction of each element absorbed, between 1.2 and 1.9 times as much nitrogen was incorporated within structural materials. Such conservation was most pronounced among mussels starved prior to experimentation, indicating nutritionally related efficiencies in the utilization of resources for synthesis. The changing balance between individual processes also effected large alterations in proportional size of the metabolic pool of free amino acids (0.2 to 14.5% of total soft tissue nitrogen). Finally, it is suggested that adjustments of protein synthetic rate may be significant in the regulation of energy expenditure, accounting for at least 16% of basal energy requirements. Results throughout have been compared and contrasted with those for mammals, and whole-body measurements of both protein synthesis and breakdown proposed as a valuable index for environmental effects on instantaneous growth and metabolism.

Protein synthesis is involved in such fundamental processes as repairing damaged tissue and combating infection, quite apart from maintaining normal growth and metabolic homeostasis. Indeed, studies in a variety of mammals indicate that whole body protein synthesis is closely correlated with basal energy metabolism (Garlick et al. 1976; Nicholas et al. 1977; Waterlow 1980; Meier et al. 1981). Despite the obvious importance of such investigations, previous measurements of absolute rates of protein synthesis in adult marine animals have been limited to various tissues of fish (Haschemeyer 1969, 1983; Haschemeyer and Persell 1973; Haschemeyer et al. 1979; Haschemeyer and Smith 1979; Smith et al. 1980; Smith and Haschemeyer 1980; Smith 1981; Pocrnjic et al. 1983; Loughna and Goldspink 1984). Of these studies, none has ascertained rates of whole body protein synthesis, representing the integrated action of all tissues and organs upon body composition. Similarly, coincident rates of protein breakdown, which may be equally important both in adaptive responses (cf. Haschemeyer 1978; Wheatley 1984) and in the determination of net protein balance (cf. Waterlow and Jackson 1981), have rarely been measured to date.

The purpose of this study was to ascertain relationships between dietary absorption and both the synthesis and breakdown of protein in the marine mussel *Mytilus edulis* L. (Bivalvia: Mytilidae). Experiments are based upon a stochastic "end product method" for the analysis of protein turnover (cf. Halliday and Lockhart 1978; Waterlow et al. 1978). Past work has shown that such methods are not only useful comparatively, according to carefully standardized conditions (Waterlow et al. 1978; Reeds and Lobley 1980), but may provide reasonable accuracy when applied under "normal" circumstances of turnover and diet (Golden and Jackson 1983). Results were intended to determine whether rates of synthesis and/or breakdown of protein are sensitive to dietary change, and thus the value of these processes as indexes of instantaneous growth rate. In turn, interrelationships between these processes are considered with respect to the efficiency of protein synthesis and the extent to which breakdown products are recycled. Finally, the overall influence of these interrelations upon metabolic pool size and a marked "protein sparing" effect are quantified, in terms of both carbon and nitrogen, from kinetic analyses of coincident isotopic depurations.

Methods

The collection of 45 to 57 mm shell-length *Mytilus edulis* L. from Whitsand Bay, southwest England, together with conditions of feeding and starvation throughout 5 to 6 wk

periods of laboratory acclimation in March, June and October 1981, have previously been described by Hawkins et al. (1983, 1984). Further, Hawkins and Bayne (1984) have documented the methods of culturing ^{15}N:^{14}C-labelled *Phaeodactylum tricornutum*, feeding this alga to acclimated mussels, and the general procedures for subsequent determination of both ^{15}N and ^{14}C isotope contents within samples.

Rates of protein synthesis

Immediately after mussels had ingested ^{15}N-labelled *Phaeodactylum*, 2 replicate groups of 5 individuals from each seasonal (March, June and October) and nutritional (fed and starved) condition were maintained in membrane-filtered seawater for measurement of the hourly net balance of dissolved nitrogen (Hawkins et al. 1983). Replicate 600 ml aliquots of this seawater, filtered (0.45 µm) upon replacement after 4, 8, 16, 32 and 40 h of incubation, were stored at −20° C for no longer than 10 d prior to processing. The ammonia from individual samples was later collected within boric acid by direct distillation, during which each seawater aliquot was buffered with 4.8 gm of MgO, which raises the pH but does not cause hydrolysis of alkali-labile nitrogen (Bremner 1965). Determination of the isotopic ^{15}N enrichment of this ammonia then enabled estimation of protein synthetic rate by means of a stochastic method described by Waterlow et al. (1978), based upon principles proven by Shipley and Clarke (1972).

Basically, using the symbols of Waterlow et al. (1978), for any end product X the balance $\dot{Q}$ and protein synthetic rate $\dot{Z}$ are given by:

$$e^*/d^* = \dot{E}_X/\dot{Q} = \dot{E}_X/(\dot{Z}+\dot{E}_T), \text{ so that } \dot{Z} = \dot{Q} - \dot{E}_T,$$

where d^* is the dose of isotope, e^* is the cumulative excretion of isotope in X at time t subsequent to the dose, $\dot{E}_X$ is the end product X excreted during that time t, and $\dot{E}_T$ is the total rate of nitrogen excretion. Such analysis depends upon the assumptions that the precursor pool from which the end product is formed is the same as the precursor pool for protein synthesis, and that the end product is derived only from this pool. Previous stochastic methods have all involved the dosing of a single ^{15}N or ^{14}C-labelled amino acid, and in such studies a cardinal assumption has been that the behaviour of tracer administrated is representative of total amino nitrogen. Significantly, the need for this last assumption has largely been obviated here, since mussels were fed alga grown entirely within a culture medium in which the one potential source of nitrogen (sodium nitrate) was ^{15}N-enriched.

The cumulative excretion of isotope was calculated using exponential decay curves fitted by Model II regression analysis (Sokal and Rohlf 1969) to the data for atom % excess ^{15}N within ammonia collected over each 40 h period following the ingestion of labelled alga. Excretion of ^{15}N during periods of isotopic ingestion, which was included in these cumulative calculations, was computed assuming that atom % excess ^{15}N within ammonia increased linearly to the maximum enrichment when administration of labelled algae was discontinued. Then, to help both minimize and standardize the proportional influence of tracer recycled from labelled protein, cumulative excretion was estimated over the predicted times for atom % excess ^{15}N within ammonia to fall below 25% of the maximum enrichment. Despite the rhythms of ammonia excretion documented by Hawkins et al. (1983), the overall rate of excretion can be assumed effectively constant over the time periods involved (40–88 h). Further, it is known that purines are excreted as ammonia by marine molluscs (Keilin 1959), and that urea contributes less than 5% towards total net nitrogen losses from *Mytilus edulis* (Bayne 1973a, b). Accordingly, expressed as rates, $\dot{E}_X$ was assumed equal to $\dot{E}_T$.

Rates of net protein flux and protein breakdown

Seasonal net balances of protein were computed in terms of mg protein 24 h^{-1} by multiplying measured net balances of nitrogen (mg N 24 h^{-1}) by a conversion factor of 5.8 (Gnaiger and Bitterlich 1984). The quantity of nitrogen consumed was calculated as the product of ingestion (mg dry algal wt h^{-1}; refer Table 2 in Hawkins and Bayne 1984) and the mean organic nitrogen content of *Phaeodactylum* [6.64 ± 0.66% (2 SE, $n=5$)]. Nitrogen consumption, when multiplied by the net absorption efficiency, yields the net absorption. The seasonal net absorption efficencies for organic nitrogen, together with ammonia losses (µmol NH_4·N h^{-1}), have previously been documented by Hawkins and Bayne (1984) and Hawkins et al. (1984), respectively. Nitrogen balance could then be computed as net nitrogen absorbed minus nitrogen excreted, and protein breakdown rate as protein synthesis minus net protein balance (Waterlow and Jackson 1981).

Kinetic analyses of isotopic depuration

Total soft tissues, excluding the shell and bysall threads, were dissected for ^{15}N and ^{14}C analyses at intervals up to 45 d subsequent to the ingestion of labelled alga in March and June 1981. Samples were obtained from both fed and starved mussel groups ($n=5$) that continued to be maintained under experimental conditions identical to those imposed during acclimation. Having been pooled for each sample, the tissues were freeze-dried, weighed, homogeneously powdered and stored at −90° C within airtight glass vials. Tissue samples of 10 to 50 mg dry wt were later rehydrated for radio-assay with 0.5 ml distilled water before digesting over 4 d at room temperature with 1.0 ml of Soluene-350 (Packard) and counting in 10 ml of Dimilume-30 (Packard) liquid scintillation cocktail. Absolute counting efficiencies were determined for each sample using ^{14}C-hexadecane (The Radiochemical Center, Amersham), and counts expressed as disintegrations min^{-1} (DPM) mg^{-1} dry tissue. Alternatively, the organic nitrogen within tissue samples of 30 to 50 mg dry wt were prepared for ^{15}N analysis using the micro-Kjeldahl wet digestion technique, followed by distillation of ammonia into Boric acid indicator solution (Bremner 1965, Hauck and Bremner 1976). Subsequent to measurement within an isotope-ratio mass spectrometer (methods of Hawkins and Bayne 1984), enrichment of ^{15}N was expressed as atom % ^{15}N excess, which represents abundance above natural atmospheric levels of 0.37 atom % ^{15}N (Junk and Svec 1958).

For each isotope, the kinetic approach described by Conover (refer addendum p. 484–488 in Marshall and Orr 1961, also Conover and Francis 1973), which is based upon formulae derived by Robertson (1957), was used to identify two general physiological/chemical components of depuration. Although not symbolising clearly defined anatomical

Table 1. Summary of statistical data derived from regression of atom % excess ^{15}N within excreted NH_4 against time (h) subsequent to the ingestion of labelled alga by 45 to 57 mm shell-length *Mytilus edulis* either fed or starved throughout acclimation in March, June and October 1981

Month and nutritional status during acclimation		log $a \pm 95\%$ CI	$-k \pm 95\%$ CI	Turnover time (h)	n	F	P
March	Fed	1.96 ± 0.26	-0.02 ± 0.01	158	7	22.00	<0.01
	Starved	2.45 ± 0.29	-0.02 ± 0.01	176	10	13.27	<0.01
June	Fed	2.28 ± 0.54	-0.03 ± 0.02	86	6	18.66	<0.05
	Starved	2.38 ± 0.36	-0.02 ± 0.01	120	11	14.39	<0.01
October	Fed	2.10 ± 0.52	-0.02 ± 0.02	143	12	5.04	<0.05
	Starved	2.64 ± 0.37	-0.02 ± 0.02	125	11	10.44	<0.05

Where log a and $-k$ are fitted parameters in the equation $Y = ae^{-kX}$; Y = the atom % excess ^{15}N at any time X (h), a is the initial enrichment (atom % excess) of ^{15}N within ammonia excreted immediately subsequent to isotopic ingestion, k is the turnover rate, F is the F-ratio (Sokal and Rohlf 1969) and P is the probability that any difference in the slope ($-k$) from zero is due to chance alone

Table 2. Calculation of whole body protein synthetic rates (mg 24 h^{-1} $mussel^{-1}$) in 45 to 57 mm shell-length *Mytilus edulis* either fed or starved throughout acclimation in March, June and October 1981

Month and nutritional status during acclimation		d^*	e_A	t	$\dot{E}_A$	$\dot{Q}$	$\dot{E}_T$	$\dot{Z}$
March	Fed	32.73	15.45	86	466.12	274.50	130.08	0.84
	Starved	51.95	21.03	94	379.76	239.52	96.96	0.83
June	Fed	36.17	13.16	46	287.04	411.61	149.76	1.52
	Starved	37.31	15.35	62	295.74	278.26	114.40	0.95
October	Fed	31.77	8.97	70	226.10	274.56	77.52	1.14
	Starved	29.73	7.84	70	119.00	154.72	40.80	0.66

Where d^* = the dose of isotope (μg atom % excess ^{15}N absorbed), e_A = the cumulative excretion of isotope within ammonia (μg atom % excess ^{15}N) after time t (h) subsequent to ingestion, $\dot{E}_A$ = the ammonia excreted during that time t (μg NH_4·N), $\dot{Q}$ = the total flux of nitrogen (μg) 24 h^{-1}, $\dot{E}_T$ = the total rate of nitrogen excretion (μg) 24 h^{-1} (assumed equivalent to ammonia excretion) and $\dot{Z}$ = the rate of protein (mg nitrogen × 5.8; conversion factor of Gnaiger and Bitterlich 1983) synthesis 24 h^{-1}

entities, these components are interpreted as representing biological features that include (i) a major "stable" or "structural" (Lampert 1975) pool, and (ii) a "labile" or "metabolic" (Sprinson and Rittenberg 1949) pool of small size but high turnover rate, being comprised of free amino acids and any unassimilated material in the digestive system. Approximate turnover times for the labile pool were estimated using the exponential decay curves fitted to data for atom % excess ^{15}N within excreted ammonia. Turnover, required as an estimate of the interval before the labile pool was cleared of tracers, was computed as the predicted time taken for atom % excess ^{15}N within ammonia to fall below 5% of the initial enrichment. Straight lines describing isotope depuration from total soft tissues were then fitted to all data obtained subsequent to the coincident turnover time estimated for ^{15}N within the labile pool. These regressions not only enabled the estimation of turnover rates but also, from their projected Y-intercepts, the initial isotopic enrichments within stable compartments. In turn, subtraction of those Y-intercepts from values recorded early in the corresponding depuration profiles enabled the same estimations to be made for the labile pool. Relative pool sizes, expressed as a percentage of both nitrogen and total carbon, were then derived using the expression:

$$a_\infty = X/(1 - e^{-kt})$$

where, for each pool, a_∞ represents the tracer abundance (μg excess ^{15}N and ^{14}C DPM $mussel^{-1}$) at infinite time, k is the rate constant governing uptake, which was assumed equal to the turnover rate, and X is the tracer enrichment after time t, taken to be the maximal abundance immediately subsequent to ingesting labelled alga.

Results

Equations for exponential decay fitted to time courses for the reduction of atom % excess ^{15}N within ammonia excreted by *Mytilus edulis* over 40 h subsequent to isotopic ingestion are satisfactorily representative ($P < 0.05$) of each data set (Table 1). These curves indicate that complete nitrogen turnover within the labile pool occurred at intervals of 4 to 7 d, when substantial enrichments of between 7.11 and 14.04 atom % ^{15}N excess were achieved.

Calculations of whole body protein synthetic rate are summarized in Table 2, and the computed values presented alongside coincident rates of net balance, breakdown and absorption in Table 3. Pearson product moment correlation (Sokal and Rohlf 1969) confirmed that seasonal and nutritionally induced changes of both net protein balance ($r = 0.971$, $n = 6$, $P < 0.01$) and protein synthesis ($r = 0.941$, $n = 6$, $P < 0.01$) varied positively with rates of protein absorption, as illustrated in Fig. 1. A negative relationship, however, was evident between the absorption and breakdown of protein ($r = -0.928$, $n = 6$, $P < 0.01$) (Fig. 1). Elevated net pro-

Table 3. Rates of synthesis, absorption, net balance and breakdown of protein (% total soft tissue protein 24 h^{-1}) within 45 to 57 mm shell-length *Mytilus edulis* either fed or starved throughout acclimation in March, June and October 1981

Month and nutritional status during acclimation		Protein synthesis	Protein absorption	Net protein balance	Protein breakdown
		[(mg protein 24 h^{-1}/mg total soft tissue protein) × 100]			
March	Fed	0.29	0.24	−0.03	0.32
	Starved	0.29	0.00	−0.20	0.49
June	Fed	0.40	0.58	+0.35	0.05
	Starved	0.25	0.00	−0.17	0.42
October	Fed	0.38	0.53	+0.37	0.01
	Starved	0.22	0.00	−0.08	0.30

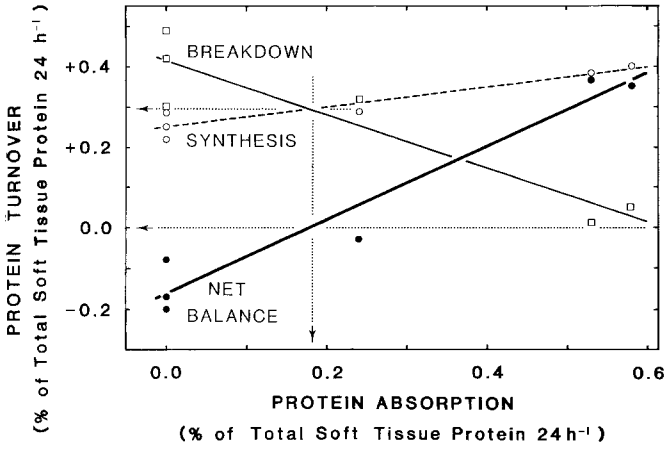

Fig. 1. The synthesis (o--o), net balance (•—•) and breakdown (□—□) of protein in relation to coincident seasonal and nutritionally induced variations of protein absorption (% of the total soft tissue protein 24 h^{-1}) by 45 to 57 mm shell-length *Mytilus edulis*. Straight lines were fitted by the least squares method (Sokal and Rohlf 1969)

tein balance was thus achieved as much, if not more, by decreased protein breakdown as by increased protein synthesis.

Under conditions of zero net protein balance, rates of breakdown and synthesis were necessarily equal at 0.29% of total soft tissue protein 24 h^{-1} (Fig. 1), which presumably represents the turnover required for maintenance purposes alone. The concurrent rate of protein absorption under conditions of zero net balance was only 0.18% of total soft tissue protein 24 h^{-1} (Fig. 1). This infers that $((0.29-0.18)/0.29) \times 100 = 38\%$ of protein breakdown products were recycled directly to synthesis essential for the normal maintenance of tissues (cf. Wheatley 1984), the remainder being used for energy metabolism. Further, such recycling increases concurrently with decreasing protein absorption (Fig. 1) in a manner compatible with Riley's (1980) hypothesis that essential amino acids are conserved for protein synthesis during prolonged starvation in the oyster, *Crassostrea gigas*.

Simultaneous depurations of ^{15}N and ^{14}C from the total soft tissues are depicted in Fig. 2. Multicompartmental losses of both carbon and nitrogen were readily apparent, for which data describing the initial enrichments and turnovers within each main pool are shown in Tables 4 and 5. Although not statistically significant, differences of turnover rate ($-k$) are consistent with associated findings. Specifically, within the structural pool estimated turnovers of ^{15}N were greatest in mussels starved during March, and of ^{14}C among mussels starved during June (Table 4), at times when both breakdown rates presented here (Table 3) and O:N ratios documented by Hawkins et al. (1984) signified the proportionally greatest catabolism of protein and carbon-rich reserves, respectively.

Table 4. Summary of statistical data derived from regression of time-series for the depuration of ^{15}N and ^{14}C from the structural pool in the total soft tissues of 45–57 mm shell-length *Mytilus edulis* either fed or starved both prior and subsequent to the ingestion of labelled alga in March and June 1981

Month and nutritional status during acclimation		log a ±95% CI	a	$-k$ ±95% CI ($\times 10^{-2}$)	n
^{14}C depuration					
March	Fed	2.31±0.74	10.05	+0.003±0.119	5
	Starved	3.17±0.48	23.83	−0.032±0.089	4
June	Fed	3.73±0.17	41.51	+0.006±0.022	9
	Starved	4.04±0.18	57.00	−0.041±0.036	8
^{15}N depuration					
March	Fed	2.93±0.73	18.69	−0.011±0.124	5
	Starved	3.53±1.45	34.02	−0.123±0.268	4
June	Fed	3.99±0.10	54.22	+0.007±0.006	9
	Starved	4.30±0.10	73.92	−0.011±0.021	8

All symbols and parameters are as in Table 1, but where Y = percentage depuration from the initial atom excess ^{15}N within total soft tissues of *Mytilus edulis* at time X (h) subsequent to isotopic ingestion, and a denotes the percentage of that initial enrichment incorporated within the structural pool alone

Alternatively, there are significant differences ($P < 0.05$) between ordinates (a) of regressions describing depuration from the stable pool (Table 4, Fig. 2). Indeed, changing turnovers associated with the winter/summer alternation from generally catabolic to anabolic states (Table 3) reflected the observation that, relative to March, between 2.1 and 4.1 times as much of both the ^{14}C and ^{15}N absorbed during June were incorporated within structural materials. Further, relative to fed individuals, and despite displaying reduced protein synthetic activity in June (Table 2), mussels starved throughout acclimation regularly incorporated greater fractions of both the ^{15}N and ^{14}C absorbed within body tissues. This infers elevated efficiency in the utilization of resources for synthesis by *Mytilus edulis* when first ingesting food after a period of starvation. Finally, in relation to ^{14}C and regardless of either season or nutritional history, between 1.2 and 1.9 times as much of the ^{15}N absorbed was incorporated within structural materials. Relative to carbon, then, there was a consistently conservative elemental turnover of nitrogen.

Metabolic pool sizes presented in Table 5 primarily concern free amino acids (cf. Cowey and Sargent 1972), for coincident faecal egestions of both ^{15}N and ^{14}C document-

Table 5. The turnover rates and proportions of tracers in metabolic and structural pools, together with estimations of pool sizes at infinite time, within the total soft tissues of 45 to 57 mm shell-length *Mytilus edulis* either fed or starved both prior and subsequent to the ingestion of labelled alga in March and June 1981

Tracer, month an nutritional status during acclimation		Mean initial tracer abundance (μg excess ^{15}N mussel^{-1} or DPM $\times 10^6$ mussel^{-1})	Metabolic pool			Structural pool		
			Turnover rate (day^{-1})[a]	Proportion of tracer	Pool size (% of total N or C)	Turnover rate (day^{-1})[a]	Proportion of tracer	Pool size (% of total N or C)
Nitrogen (^{15}N)								
March	Fed	36.11	0.433	0.813	3.08	0.003	0.187	96.92
	Starved	41.54	0.346	0.660	14.47	0.029	0.340	85.53
June	Fed	60.31	0.433[c] (0.693)	0.458	0.20 (0.13)	0.001[b]	0.542	99.80 (99.87)
	Starved	52.29	0.346[c] (0.693)	0.261	0.21 (0.11)	0.002	0.739	99.79 (99.89)
Carbon (^{14}C)								
March	Fed	4.78	0.462	0.900	2.02	0.001[b]	0.100	97.98
	Starved	6.56	0.396	0.762	6.36	0.008	0.238	93.64
June	Fed	7.31	0.462[c] (0.693)	0.585	0.32 (0.22)	0.001[b]	0.415	99.68 (99.78)
	Starved	7.10	0.396[c] (0.693)	0.430	2.15 (1.28)	0.011	0.570	97.85 (98.72)

[a] Determined as 0.693/half time (days)
[b] Values assumed to be minimally negative, when lines fitted by linear regression did not signify depuration
[c] Values assumed similar to those recorded in March; the effects of a faster turnover (half time 1.0 day), as suggested by the excretion of ^{15}N within ammonia (Table 1), are bracketed

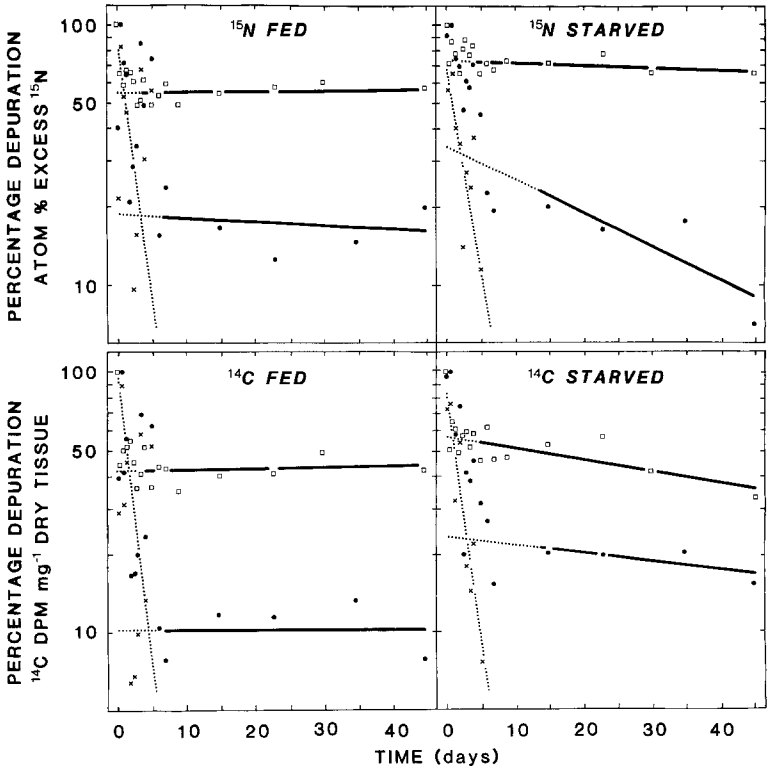

Fig. 2. Percentage depuration from the initial enrichment of ^{15}N and ^{14}C within the total soft tissues following ingestion of labelled alga by 45 to 57 mm shell-length *Mytilus edulis* either fed or starved both during and after acclimation in March (●) and June (□), 1981. Whereas continuous lines (fitted by linear regression, Table 4) represent structural depuration, dotted lines (fitted by eye) signify extrapolations undertaken using procedures described by Conover (refer addendum p. 484–488 in Marshall and Orr 1961). Only labile phases for mussels acclimated in March are represented graphically, for which crosses (x) denote corrected values of more than 5%

ed by Hawkins and Bayne (1984) comprised maxima of between only 5 and 18% of total labile losses. The metabolic pool sizes estimated here for carbon (<2%) and nitrogen (<3%) within fed *Mytilus edulis* are thus comparable with data for both phosphorous in *Calanus finmarchicus* (<6%; Conover, p. 487 in Marshall and Orr 1961) and carbon in *Daphnia pulex* (<3%; Lampert 1975). In addition, despite the errors associated with estimates of turnover rate, it is clear that proportional sizes estimated for labile pools of both carbon and nitrogen were larger during winter than in summer and among starved rather than fed mussels (Table 5). Although effected through the balance between absorption, synthesis and catabolism, such variation appeared most clearly influenced by changing rates of protein breakdown, which were generally maximal when the metabolic pool of free amino acids was largest (Tables 3 and 5).

Discussion

Although fractional whole body rates of protein synthesis in *Mytilus edulis* (0.29 to 0.40% of total soft tissue protein 24 h^{-1}) compare with findings of about 0.4 to 0.7% 24 h^{-1} for muscle protein in various fish (refer Introduction), I am not aware of strictly compatible data for synthetic rate within the whole body of any other marine species. That individual tissues contribute to whole body protein turnover by varying and even opposing degrees is widely recognised (eg. Waterlow 1980; Waterlow and Jackson 1981; Haschemeyer 1983; Pocrnjic et al. 1983). It is thus of interest to compare results for *M. edulis* fed "normally" throughout acclimation (Hawkins et al. 1984) with rates of whole body protein synthesis from mammalian studies. Utilizing a temperature coefficient ($Q_{10}=2.5$) established for protein synthesis among fish (Mathews and Haschemeyer 1978, Haschemeyer et al. 1979; Haschemeyer and Mathews 1983), mussels of an average 0.65 g dry soft tissue weight displayed fractional rates, at 37° C, of between 3.4 and 3.8% 24 h^{-1}. These values are substantially lower than total protein synthetic rates of about 11% 24 h^{-1} predicted for terrestrial homeotherms of comparable size from the relationship whereby protein turnover varies to about the 0.75 power of body weight (Reeds and Lobley 1980; Waterlow 1980;Waterlow and Jackson 1981; Millward et al. 1981). Further, at the time of maximum recorded growth, which coincided with the highest growth efficiencies measured concurrently in terms of calories by Hawkins et al. (1984), as much as 92% of the products of protein synthesis were retained by *M. edulis* (Fig. 1). Although consistent with predicted synthetic efficiencies of between 86 and 97% in ectotherms (Calow 1977, van Es 1980; Schroeder 1981), this contrasts with comparable whole body maxima of about 27% in the growing pig and 33% among children recovering from malnutrition (calculated from Reeds et al. 1980 and Golden et al. 1977, respectively). Mussels are thus markedly more efficient at accumulating protein, but do so at slower fractional rates, relative to mammals for which there are equivalent data.

Smith (1981) has suggested that low rates of muscle protein turnover in fish compared with rats may derive from the reduced supportive and postural role of musculature in an aquatic environment. Indeed, regulated responses of skeletal muscle to the nature and extent of contractile activity, leading to changes in protein accretion, are well documented (Laurent et al. 1978; Smith et al. 1983). This explanation may partially account for the slower fractional rates reported here for *Mytilus edulis* relative to mammals, since the decreased locomotory role of musculature associated with the adult mussels sessile habit will substantially reduce requirements for myofibrilar activity. Further, in contrast to mammals, mussels are known to accumulate protein within non-muscular storage cells (Lubet 1959).

Both the absorption and net balance of protein in *Mytilus edulis* were positively correlated with coincident whole body rates of synthesis (Fig. 1, Table 3). Similar associations among mammals (Millward et al. 1975; Golden et al. 1977; Waterlow 1980; Waterlow and Jackson 1981; Reeds and Fuller 1983) support the hypothesis that muscle mass is regulated primarily by alterations in protein synthetic rate (Rennie et al. 1983). Changes of mammalian protein breakdown, then, are often relatively minor and even paradoxical, exhibiting increases with growth and decreases during wasting (see previous references for synthesis and those quoted by Rennie et al. 1983). Such was not the case for *M. edulis*, however, for which least squares regression indicates that whole body protein breakdown showed a pronounced negative relationship with net protein balance ($a=0.292$, $b=-0.753$, $r=-0.986$, $n=6$, $P<0.001$; Table 3). The high efficiencies discussed earlier, with which mussels accumulated protein were thus largely achieved by a progressive reduction in fractional protein degradation as net protein balance increased. These findings appear generally consistent with those reported for fish muscle, in which levels of protein synthesis correlate with growth rate (Roselund et al. 1983, 1984), and in which both decreased protein synthetic activity (Smith 1981; Lied et al. 1982, 1983; Haschemeyer 1983) and elevated protein breakdown (Smith 1981, Loghna and Goldspink 1984) occur upon starvation.

Reduction of fractional protein degradation with increasingly positive net balance, together with substantial ($\approx 38\%$) recycling of protein degradation products directly to synthesis (refer Results), both contribute towards a marked "protein sparing" effect in *Mytilus edulis*. This effect, evidenced by a consistently lower elemental turnover of nitrogen relative to carbon, is in agreement with previous observations that bivalves utilize carbohydrate as the preferred respiratory substrate (Gabbott and Bayne 1973; Gabbott and Stephenson 1974; Mann 1979a, b; Gallager and Mann 1981; Peirson 1983). At times when carbon-rich reserves are limiting, however, as was increasingly the case with decreasing net growth observed here (Hawkins et al. 1984), *M. edulis* may catabolize protein to satisfy at least 67% of the mass equivalent of maintenance requirments (Hawkins et al. 1984). Millward et al. (1975) have suggested that elevated rates of protein breakdown during mammalian growth are a necessary accompaniment to a mechanistically constrained excess synthesis of myofibrils, with "wastage" of new fibres which fail to reach maturity. The storage of protein within adipogranular cells (Lubet 1959), in addition to muscle, may thus constitute one means by which protein degradation is reduced with growth in *M. edulis*. Whatever the mechanism(s) enabling such conservation of protein, it is clear that this effect may be advantageous to mussels, being sessile organisms which are virtually ubiquitous in coastal ecosystems where pronounced seasonal variations of nitrogen abundance commonly limit organic production (cf. Ryther and Dunstan 1971, Mann 1982, Roman 1983).

Whole body rates of mammalian protein synthesis not only correlate with basal metabolism, but may account for between 15 and 25% of total energy expenditure (refer Introduction). Further, postprandial increases in metabolic rate, termed the specific dynamic action of food (Kleiber 1961), have often been proposed as representing short-term increases in protein synthesis (eg. Jobling 1983). Parry (1983, 1984) has even suggested that changing biosynthetic rate may be the major cause of seasonal variation in oxidative metabolism of ectotherms. The energy cost of protein synthesis, based on what is known of the biochemistry of the process, has been computed as 3.6 $KJ\ g^{-1}$ (Millward et al. 1976). In accordance with estimates for mammals, then, protein synthesis accounted for at least 16% of the estimated basal rate of oxygen uptake in *Mytilus edulis* starved during March (1.78 μmol O_2 h^{-1} or 0.801 $J\ h^{-1}$; Hawkins et al. 1984). The synthesis of ribosomal and messenger RNA, however, over and above the cost of peptide bond synthesis, may considerably enhance this estimate (cf. Waterlow and Jackson 1981), reinforcing the conclusion

that protein synthesis may represent a major proportion of total energy demands. Clearly, protein synthesis is of fundamental significance to normal cell functioning in *M. edulis*. Further, in view of the relatively high costs of synthesis, it may be energetically optimal for this process to be regulated more closely than breakdown. This is consistent with my observations that fractional rates of protein breakdown varied by 0.38% of total soft tissue protein $24\ h^{-1}$ over the range of protein absorption studied, whereas protein synthesis changed by only 0.14%.

Apart from the energetic significance of such adjustments, it has been shown that seasonal and starvation-induced variation of the balance between absorption, synthesis and catabolism of protein effected large changes in the proportional size of the metabolic pool of free amino acids. Ninhydrin-positive-substances act as the main solute for active intracellular isosmotic regulation in bivalve molluscs (Bayne et al. 1976, Gilles 1979). Such changes may thus be of direct ecological significance to *Mytilus edulis*, associated with a suggested seasonal variation in the relative ability to use amino acids for cell volume regulation (refer Table 2 in Livingstone et al. 1979).

In conclusion, this study has shown that the synthesis, breakdown and net balance of protein are sensitive to changing rates of absorption in *Mytilus edulis*. Fractional turnover rates, interrelationships between these variables and ensuing efficiences of protein synthesis differ strikingly from those in mammals, resulting in a distinct and potentially adaptive "protein sparing" effect. Synthetic adjustments are of significance to energy expenditure, and the overall balance may be of relevance to osmoregulatory capability. Finally, it is clear that measurements of both whole body protein synthesis and breakdown represent valuable indexes for short and long term environmental influences on instantaneous growth and metabolism. Although standard elemental balances furnish useful knowledge on net changes, they cannot indicate either metabolic intensity or the manner by which any alteration was effected, for synthesis and breakdown may vary either in the same or in opposite directions.

Acknowledgements. I am especially indebted to Dr. B.L. Bayne for advice and encouragement throughout the course of this study. In addition, I thank Dr. D. Millward for helpful discussion, Dr. N. Owens and Mr. F. Staff for guidance in the use of ^{15}N and ^{14}C isotopes, respectively, Dr. K. Clarke for statistical counselling and Miss M. Day for laboratory assistance. This work was supported by a N.E.R.C. Research Studentship, and represents part of the Adaptive Processes programme of the Institute for Marine Environmental Research, a component of the U.K. Natural Environment Research Council.

References

Bayne BL (1973a) Physiological changes in *Mytilus edulis* L. induced by temperature and nutritive stress. J Mar Biol Ass UK 53:39–58

Bayne BL (1973b) Aspects of the metabolism of *Mytilus edulis* during starvation. Neth J Sea Res 7:399–410

Bayne BL, Widdows J, Thompson RJ (1976) Physiology II. In: Bayne BL (ed) Marine mussels. International Biological Programme, vol 10. Cambridge University Press, Cambridge, pp 207–260

Bremner JM (1965) Total nitrogen. In: Black CA (ed) Methods of soil analysis, part 2. American Society of Agronomy, Madison, pp 1149–1178

Brown JG, Bates PC, Holliday MA, Millward DJ (1981) Thyroid hormones and muscle protein turnover: the effect of thyroid-hormone deficiency and replacement in thyroidectomized and hypophysectomized rats. Biochem J 194:771–782

Calow P (1977) Conversion efficiencies in heterotrophic organisms. Biol Rev 52:385–409

Conover RJ, Francis V (1973) The use of radioactive isotopes to measure the transfer of materials in aquatic food chains. Mar Biol 18:272–283

Cowey CB, Sargent JR (1972) Fish nutrition. Adv Mar Biol 10:383–492

Gabbott PA, Bayne BL (1973) Biochemical effects of temperature and nutritive stress on *Mytilus edulis* L. J Mar Biol Ass UK 53:269–286

Gabbott PA, Stephenson RR (1974) A note on the relationship between the dry weight condition index and the glycogen content of adult oysters (*Ostrea edulis* L.) kept in the laboratory. J Cons Int Explor Mer 35:359–361

Gallager SM, Mann R (1981) The effect of varying carbon/nitrogen ratio in the phytoplankter *Thalassiosira pseudonana* (3H) on its food value to the bivalve *Tapes japonica*. Aquaculture 26:95–105

Garlick PJ, Burk TL, Swick RW (1976) Protein synthesis and RNA in tissues of the pig. Am J Physiol 230:1108–1112

Gilles R (1979) Intracellular organic osmotic effectors. In: Gilles R (ed) Mechanisms of osmoregulation in animals. Wiley Chichester, New York, pp 111–156

Gnaiger E, Bitterlich G (1984) Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. Oecologia (Berlin) 62:289–298

Golden MHN, Jackson AA (1983) Assumptions and errors in the use of ^{15}N-excretion data to estimate whole body protein turnover. In: Waterlow JC, Stephen JML (eds) Nitrogen metabolism in man. Applied Science Publishers, London, pp 323–344

Golden MHN, Waterlow JC, Picou D (1977) The relationship between dietary intake, weight change, nitrogen balance and protein turnover in man. Am J Clin Nutr 30:1345–1348

Halliday D, Lockhart IM (1978) The use of stable isotopes in medicinal chemistry. Prog med Chem 15:1–86

Haschemeyer AEV (1969) Rates of polypeptide chain assembly in liver "in vitro": relation to the mechanism of temperature acclimation in *Opsanus tau*. Proc natn Acad Sci USA 62:128–135

Haschemeyer AEV (1978) Protein metabolism and its role in temperature acclimation. In: Malins DC, Sargent JR (eds) Biochemical and biophysical perspectives in marine biology, vol 4. Academic Press, New York, pp 29–84

Haschemeyer AEV (1983) A comparative study of protein synthesis in notothenids and icefish at Palmer Station, Antarctica. Comp Biochem Physiol 76:541–543

Haschemeyer AEV, Mathews RW (1983) Temperature dependancy of protein synthesis in isolated hepatocytes of Antarctic fish. Physiol Zool 56:78–87

Haschemeyer AEV, Persell R (1973) Kinetic studies on amino acid uptake and protein synthesis in liver of temperature acclimated toadfish. Biol Bull mar biol Lab Woods Hole 145:472–481

Haschemeyer AEV, Smith MAK (1979) Protein synthesis in liver, muscle and gill of mullet (*Mugil cephalus* L.) *in vivo*. Biol Bull mar biol Lab Woods Hole 156:93–102

Haschemeyer AEV, Persell R, Smith MAK (1979) Effect of temperature on protein synthesis in fish of the Galapagos and Perlas Islands. Comp Biochem Physiol 64:91–95

Hauck RD, Bremner JM (1976) Use of tracers for soil and fertilizer nitrogen research. Adv Agron 28:219–266

Hawkins AJS, Bayne BL (1984) Seasonal variation in the balance between physiological mechanisms of feeding and digestion in *Mytilus edulis* (Bivalvia: Mollusca). Mar Biol 82:233–240

Hawkins AJS, Bayne BL, Clarke KR (1983) Co-ordinated rhythms of digestion, absorption and excretion in *Mytilus edulis* (Bivalvia: Mollusca). Mar Biol 74:41–48

Hawkins AJS, Salkeld PN, Bayne BL, Gnaiger E, Lowe DM (1985) Feeding and resource allocation in *Mytilus edulis*: evidence for time-averaged optimisation. Mar Ecol Progr Ser 20:213–281

Jobling M (1983) Towards an explanation of specific dynamic action (SDA). J Fish Biol 23:549–555
Junk GA, Svec HJ (1958) Nitrogen isotope abundance measurements. US Atomic energy Commission, Office of Technical Information, ISC-1138, 37 pp
Keilin J (1959) The biological significance of uric acid and guanine excretion. Biol Rev 34:265–296
Kleiber M (1961) The fire of life. An introduction to animal energetics. J Wiley and Sons Inc, New York, p 454
Lampert W (1975) A tracer study on the carbon turnover of *Daphnia pulex*. Verh int Verein theor angew Limnol 19:2913–2921
Laurent GJ, Sparrow MP, Millward DJ (1978) Turnover of muscle protein in the fowl: changes in rates of protein synthesis and breakdown during hypertrophy of the anterior and posterior latissimus dorsi muscles. Biochem J 176:407–417
Lied E, Lund B, von der Decken A (1982) Protein synthesis *in vitro* by epaxial muscle polyribosomes from cod, *Gadus morhua*. Comp Biochem Physiol 72:187–193
Lied E, Rosenlund G, Lund B, Decken A von der (1983) Effect of starvation and refeeding on *in vitro* protein synthesis in white trunk muscle of Atlantic cod (*Gadus morhua*). Comp Biochem Physiol 76:777–781
Livingstone DR, Widdows J, Fieth P (1979) Aspects of nitrogen metabolism of the common mussel *Mytilus edulis*: adaptation to abrupt and fluctuating changes in salinity. Mar Biol 53:41–55
Loughna PT, Goldspink G (1984) The effects of starvation upon protein turnover in red and white myotomal muscle of rainbow trout, *Salmo gairdneri* Richardson. J Fish Biol 25:223–230
Lubet P (1959) Recherches sur le cycle sexuel et l'emission des gametes chez les Mytilidae et les Pectinidae (Moll. Bivalves). Revue Trav Off (scient tech) Pech marit 23:387–548
Mann KH (1982) Ecology of coastal waters, a systems approach. Blackwell, Oxford, p 322
Mann R (1979a) Some biochemical and physiological aspects of growth and gametogenesis in *Crassostrea gigas* (Thunberg) and *Ostrea edulis* L. grown at sustained elevated temperatures. J Mar Biol Ass UK 59:95–110
Mann R (1979b) The effect of temperature on growth, physiology and gametogenesis in the Manila clam *Tapes philippinarum* (Adams and Reeve, 1850). J exp mar Biol Ecol 38:121–133
Marshall SM, Orr AP (1961) Studies on the biology of *Calanus finmarchicus*. *XII*. The phosphorus cycle: excretion, egg production, autolysis. J mar biol Ass UK 41:463–488
Mathews RW, Haschemeyer AEV (1978) Temperature dependency of protein synthesis in toadfish liver *in vivo*. Comp Biochem Physiol 61:479–484
Meier PR, Peterson RG, Bonds DR, Meschia G and Battalgia FC (1981) Rates of protein synthesis and turnover in fetal life. Am J Physiol 240:320–324
Millward DJ, Bates PC, Rosochacki S (1981) The extent and nature of protein degradation in the tissues during development. Reprod Nutr Develop 21:265–277
Millward DJ, Garlick PJ, Reeds PJ (1976) The energy cost of growth. Proc Nutr Soc 35:339–349
Millward DJ, Garlick PJ, Stewart RJC, Nnanyelugo DO, Waterlow JC (1975) Skeletal-muscle growth and protein turnover. Biochem J 150:235–243
Nicholas GA, Lobley GE, Harris CL (1977) Use of the constant infusion technique for measuring rates of protein synthesis in the New Zealand white rabbit. Br J Nutr 38:1–17
Parry GD (1983) The influence of the cost of growth on ectotherm metabolism. J theor Biol 101:453–477
Parry GD (1984) The effect of food deprivation on seasonal changes in the metabolic rate of the limpet, *Cellana tramoserica*. Comp Biochem Physiol 77:663–668
Peirson WM (1983) Utilization of eight algal species by the bay scallop, *Argopecten irradians concentricus* (Say). J exp mar Biol Ecol 68:1–11
Pocrnjic Z, Mathews RW, Rappaport S, Haschemeyer AEV (1983) Quantitative protein synthetic rates in various tissues of a temperate fish *in vivo* by the method of phenylalanine swamping. Comp Biochem Physiol 74:735–738
Reeds PJ, Fuller MF (1983) Nutrient intake and protein turnover. Proc Nutr Soc 42:463–471
Reeds PJ, Lobley GE (1980) Protein synthesis – are there real species differences? Proc Nutr Soc 39:43–53
Reeds PJ, Cadenhead A, Fuller MF, Lobley GE, McDonald JD (1980) Protein turnover in growing pigs. Effects of age and food intake. Br J Nutr 43:445–455
Rennie MJ, Edwards RHT, Emery PW, Halliday D, Lundholm K, Millward DJ (1983) Depressed protein synthesis is the dominant characteristic of muscle wasting and cachexia. Clin Physiol 3:387–398
Riley RT (1980) The effect of prolonged starvation on the relative free amino acid composition of the extracellular body fluids and protein bound amino acids in the oyster, *Crassostrea gigas*. Comp Biochem Physiol 67:279–281
Robertson JS (1957) Theory and use of tracers in determining transfer rates in biological systems. Physiol Rev 37:133–154
Roman MR (1983) Nitrogenous nutrition of marine invertebrates. In: Carpenter EJ, Capone DG (eds) Nitrogen in the marine environment. Academic Press, London, pp 347–384
Rosenlund G, Lund B, Decken A von der (1983) Properties of white trunk muscle from saithe *Pollachius virens*, rainbow trout *Salmo gairdneri* and herring *Clupea harengus*: protein synthesis *in vitro*, electrophoretic study of proteins. Comp Biochem Physiol 74:387–397
Rosenlund G, Lund B, Sandnes K, Braekkan OR, Decken A von der (1984) Muscle protein synthesis *in vitro* of saithe (*Pollachius virens*) correlated to growth and daily energy intake. Comp Biochem Physiol 77:7–13
Ryther JH, Dunstan WM (1971) Nitrogen, phosphorus and eutrophication in coastal marine environments. Science 171:1008–1013
Schroeder LA (1981) Consumer growth efficiencies: their limits and relationships to ecological energetics. J theor Biol 93:805–828
Shipley RA, Clark RE (1972) Tracer methods for *in vitro* kinetics. Theory and applications. Academic Press, New York, p 172
Smith MAK (1981) Estimation of growth potential by measurement of tissue protein synthetic rates in feeding and fasting rainbow trout, *Salmo gairdnerii* Richardson. J Fish Biol 19:213–220
Smith MAK, Haschemeyer AEV (1980) Protein metabolism and cold adaptation in Antarctic fishes. Physiol Zool 53:373–382
Smith MAK, Mathews RW, Hudson AP, Haschemeyer AEV (1980) Protein metabolism of tropical reef and pelagic fish. Comp Biochem Physiol 65:415–418
Smith RH, Palmer RM, Reeds PJ (1983) Protein synthesis in isolated rabbit forelimb muscles. Biochem J 214:153–161
Sokal RR, Rohlf FJ (1969) Biometry. The principle and practise of statistics in biological research. Freeman and Co, San Francisco, p 776
Sprinson DB, Rittenberg D (1949) The rate of interaction of the amino acids of the diet with the tissues proteins. J biol Chem 180:715–1726
Van Es AJH (1980) Energy costs of protein deposition. In: Buttery PJ, Lindsay DB (eds) Protein deposition in animals. Butterworths, London, pp 215–224
Waterlow JC (1980) Protein turnover in the whole animal. Invest Cell Pathol 3:107–119
Waterlow JC, Garlick PJ, Millward DJ (1978) Protein turnover in mammalian tissues and in the whole body. North-Holland Publishing Co, Oxford, p 804
Waterlow JC, Jackson AA (1981) Nutrition and protein turnover in man. Br Med Bull 37:5–10
WheatleyDN (1984) Intracellular protein degradation: basis of a self-regulating mechanism for the proteolysis of endogenous proteins. J theor Biol 107:127–149

Received November 25, 1984