Protein turnover in skeletal muscle of piglets

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1. Rates of protein synthesis and catabolism were measured in longissimus dorsi and hind-limb muscles of suckling piglets.

2. Half-lives for synthesis and catabolism for mixed sarcoplasmic proteins were 4·8 and 9·4 d respectively. The corresponding values for mixed myofibrillar proteins were 5·7 and 16·4 d.

3. The half-lives for synthesis of sarcoplasmic proteins were significantly different from those of myofibrillar proteins and were not confounded by contamination of the sarcoplasmic protein fraction with plasma proteins of higher specific activity.

4. Individual myofibrillar proteins were synthesized and catabolized at rates which were not statistically significantly different. Intramuscular connective tissue also appeared to turnover rapidly, the half-life for synthesis being 8 d and that for catabolism 20 d.

5. Values obtained for the specific radioactivities of aspartate + glutamate in mixed plasma proteins support the view that, in so far as the young of animals larger in mature body size than rats or mice are concerned, muscle assumes a more important role relative to liver in regulating whole body amino acid metabolism.

There have been several recent publications on rates of protein turnover in serum, liver, muscle and brain of rats and mice (Waterlow & Stephen, 1968; Garlick, 1969; Millward, 1970a, b; Gan & Jeffay, 1971; Garlick & Marshall, 1972). To the author's knowledge, however, no published results exist for rate of turnover of muscle proteins in large animals. Furthermore, many of the reports on rates of synthesis and catabolism of individual muscle proteins are complicated by the problem of amino acid re-utilization in muscle (Young, 1970). Some results for the young of one large animal, the pig, are presented in this paper in which rates of synthesis (k_s) and catabolism (k_c) were measured using an experimental procedure similar to that of Millward (1970a, b). In this technique, which largely overcomes the problems associated with amino acid re-utilization, the proteins are labelled by a single injection of $[^{14}C]Na_2CO_3$ solution. After isolation and hydrolysis of the protein to be examined the specific radioactivities (*a*) of incorporated aspartate + glutamate are measured.

Two experiments are reported. In the first, rates of synthesis of myosin, actin, sarcoplasmic and intramuscular connective tissue (IMCT) proteins were measured in 12-d-old piglets. In the second, rates of catabolism were also measured, but in 6-d-old piglets. Various isolation procedures were also compared to determine the effects on the half-lives for synthesis ($_sT_{\frac{1}{2}}$) and catabolism ($_cT_{\frac{1}{2}}$) of contaminating proteins.

METHODS

Animals

Two groups of three male piglets were used for the two experiments. The mean weight of piglets in the first experiment was 3·5 ± 0·2 kg at 12 d. Those in the second

experiment weighed 1.2 ± 0.1 kg at birth and 3.1, 3.6 and 4.2 kg respectively, at slaughter, at 7, 12 and 15 d of age. Each group was allowed to suckle the sow and had access to dry piglet feed until immediately before slaughter.

Isotope administration and slaughter of piglets

Sterile aqueous $[^{14}C]Na_2CO_3$ solution (CFA 2; Radiochemical Centre, Amersham, Bucks.) was injected intraperitoneally from weighed syringes. In the first group each animal received 3 mCi, in the second 1.2 mCi.

Piglets of the first group were killed 1, 4 and 7 d after injection, those of the second group 1, 6 and 9 d after injection. Each piglet was killed by a blow on the skull and severance of the head to allow maximum drainage of blood.

Collection of samples

Expt 1. Longissimus dorsi (LD) and biceps femoris muscles were dissected from the carcass immediately after slaughter, placed in polyethylene bags and stored in ice. IMCT and myofibrillar proteins were prepared from fresh LD muscle. Mitochondria were prepared from fresh biceps femoris muscle by the method of Cheah (1970).

Expt 2. Longissimus dorsi, biceps femoris, semitendinosus, semimembranosus and adductor muscles were dissected from the carcass, trimmed of fat, weighed, chopped finely and mixed well. The latter four hind-limb muscles (HL) (see above) were then pooled for subsequent analysis. Two 10 g samples of LD and HL were frozen at $-20°$. After freeze-drying, these samples were powdered and sampled for composition studies (Helander, 1961). Moisture content was calculated from the equation H_2O (%) $= 100 - 7.08 \times$ protein nitrogen (%) (J. R. Bendall, personal communication). Residual fresh muscle was fractionated by the methods described below.

Preparation of protein fractions

Sarcoplasmic proteins. Muscle was homogenized in 2 vol 20 mM-tris, 2 mM-EDTA for 2 min at $0°$ with a Polytron homogenizer. After centrifugation at 12000 g for 10 min at $0°$, two 50 ml samples of the supernatant fraction were removed, brought to pH 5.0 with 0.1 M-acetic acid and incubated at $52°$ for 45 min. The denatured sarcoplasmic proteins (pH 5 sarcoplasmic-protein fraction) were separated from contaminating plasma albumin by repeated centrifugation and washing. Trichloroacetic acid (TCA) was then added to the pH 5.0 sarcoplasmic-protein pellets and to the original 12000 g supernatant fraction (mixed, untreated sarcoplasmic-protein fraction) to a final concentration of 50 g/l. Precipitated proteins were removed, extracted at $70°$ for 30 min with TCA (100 g/l), dehydrated in acetone, extracted twice with diethyl ether and stored under reduced pressure.

Myofibrillar proteins. These proteins were prepared from the tris–EDTA pellets, after sarcoplasmic-protein extraction, by methods devised in this laboratory (J. R. Bendall & I. F. Penny, unpublished observations). Essentially the procedure for actomyosin and myosin involved preliminary extraction with 0.5 M-KCl, purification by dialysis, dissociation in the presence of pyrophosphate and Mg^{2+} and final separation by ultracentrifugation. Actin was obtained by a procedure which dissociated

actomyosin and depolymerized actin in the presence of ATP and ascorbic acid. Both these procedures have been shown, by sodium dodecyl sulphate–acrylamide gel electrophoresis to yield proteins of high purity.

IMCT. Two different procedures were used. In Expt 1 the material collected from the homogenizer shaft and blades and that remaining after actin extraction were pooled and homogenized in 100 vol 0·1 M-KCl. After standing for 4 h, the connective tissue sediment was suspended in 1 M-KCl and extracted overnight at 2° with stirring. After dehydration in ethanol it was then washed three times with acetone, extracted with TCA (100 g/l) at 70° for 30 min, washed in diethyl ether and stored under reduced pressure.

In Expt 2 the IMCT after extraction with 1 M-KCl was incubated for 24 h at 37° in 250 ml 100 mM-sodium phosphate buffer, pH 7·2, containing 0·2 mg trypsin/ml (Type III, Bovine Pancreas; Sigma Chemical Co., London) and 5 mg aureomycin/kg (Lederle Co., London). It was then washed six times in cold phosphate buffer to remove trypsin and prepared with lipid solvents as above. This procedure has been shown to yield IMCT of very high purity as judged by hydroxyproline content (Mohr, 1970).

Other protein fractions. In Expt 2, freeze-dried muscle was extracted with 1·1 M-KI, 0·1 M-sodium phosphate (pH 7·4) according to the method of Helander (1957) to yield KI-soluble protein and stroma-protein fractions. The stroma material was not extracted with TCA but with ethanol–diethyl ether (3:1, v/v) (twice), diethyl ether and acetone. Samples of mixed plasma proteins obtained from heparinized blood collected at slaughter and of mixed mitochondria proteins were extracted with TCA and lipid solvents as described on p. 36 for sarcoplasmic proteins.

Amino acid analysis and isotope assay

Aspartate + glutamate were obtained from protein hydrolysates by displacement chromatography on Deacidite G-I-P resin (Permutit Ltd, London) (Partridge & Brimley, 1949). The method of Yemm & Cocking (1955) was used to estimate concentrations. Radioactivity was measured in a Packard Tricarb Spectrometer (Packard Instruments Ltd, London) using a scintillator based on Triton–toluene. a was calculated as counts/40 min per μmol aspartate + glutamate. Counting efficiencies were checked with internal standard [^{14}C]hexadecane (Radiochemical Centre, Amersham, Bucks.) but, as similar quantities of each protein fraction were subsequently prepared for analysis, this procedure was shown to be routinely unnecessary.

The decay with time of aspartate + glutamate a was used to calculate k_s, thereby reducing the effects of amino acid re-utilization (Millward, 1970a). Similarly k_c was calculated from the decay with time of the total activity of aspartate + glutamate, using the values for a and muscle composition (Millward, 1970a, b). In the experiments reported here k_c values were determined from the plots of log (mg N of fraction/g fresh muscle × muscle weight (g) × relative counts) against time (d). Relative counts were calculated from the k_s values presented in Table 1, other values being obtained from the growth curves of muscle-protein fractions in Fig. 2, which were in turn derived from values in Tables 2 and 3. Half-lives for synthesis ($_sT_{\frac{1}{2}}$) and catabolism ($_cT_{\frac{1}{2}}$)

Table 1. *Rate constants (k) and half-lives ($T_{\frac{1}{2}}$) of synthesis (s) and catabolism (c) for various protein fractions of longissimus dorsi (LD) and hind-limb (HL) muscles of piglets*

Protein fraction	k_s value (d⁻¹) Expt 1 LD	Expt 2 LD	Expt 2 HL	k_s (d⁻¹)	SE of k_s*	$_sT_{\frac{1}{2}}$ (d)	k_c (d⁻¹) LD	k_c HL	$_cT_{\frac{1}{2}}$ (d) LD	$_cT_{\frac{1}{2}}$ HL
pH 5 sarcoplasmic	—	0·143	0·143	0·145	0·0073 (6)	4·78	0·077	0·071	9·0	9·8
Actomyosin	—	0·121	0·121	0·122	0·0038 (6)	5·68	0·046	0·039	15·0	17·7
Actin	0·112	0·120	0·131	0·115	0·0079 (8)	6·03	—	—	—	—
Myosin	0·116	0·116	0·157	0·133	0·0093 (9)	5·21	—	—	—	—
Mean of actin and myosin	0·114	0·113	0·144	0·124	—	5·59	—	—	—	—
Intramuscular connective tissue	0·086	—	—	0·086	(3)	8·07	0·035	0·031	20·0	22·0
Stroma	—	0·109	0·110	0·109	0·012 (5)	6·33	—	—	—	—

* Figures in parentheses are numbers of observations.

were derived from the expression $T_{\frac{1}{2}} = 0.693/k$, where k is the rate-constant for synthesis or catabolism. The percentages of myofibrillar protein N present as myosin and actin were taken for the purposes of this study as being 54 and 22 respectively (J. R. Bendall, personal communication). It is therefore implicit that $k_s - k_c$ measures the rates of net synthesis (i.e. growth) of each of the protein fractions. Furthermore, the aspartate + glutamate composition of each of the individual protein fractions is not required for the calculation of k_c since each protein fraction is considered to have a constant and characteristic amino acid composition.

RESULTS

The combined results of Expts 1 and 2 for the decay in aspartate + glutamate a with time in the actomyosin, actin, myosin and pH 5 sarcoplasmic-protein fractions are shown in Fig. 1. Although the piglets received large doses of $^{14}CO_3{}^{2-}$ the absolute count rates were low. This necessitated preparation of large quantities of sample material and counting amino acid samples for comparatively long periods (up to 40 min/sample). Furthermore, in Expt 2 the a values were considerably higher than in Expt 1. However, when corrected for differences in isotope dosage, size of animal and counting procedure, good agreement was obtained between the results of the two experiments (Fig. 1 and Table 1).

The factors, ×0·67 (actin) and ×0·25 (myosin), used in Fig. 1 allow the reader to easily identify results obtained for each protein fraction. They do not influence the results or conclusions subsequently drawn from them. For example, the values for
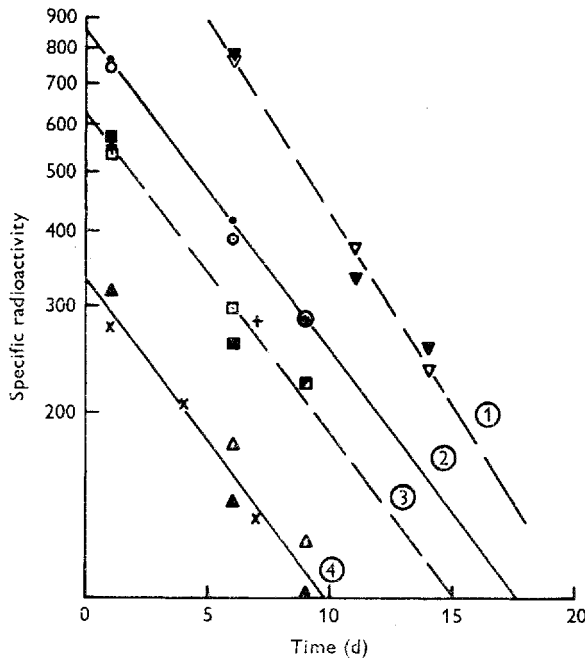
Fig. 1. Combined results of Expts 1 and 2 for the decay of specific radioactivity (a) with time in actomyosin, actin, myosin and pH 5 sarcoplasmic-protein fractions from longissimus dorsi (LD) and hind-limb (HL) muscle of piglets. (1) pH 5 sarcoplasmic-protein fraction: ▽, Expt 2 LD; ▼, Expt 2 HL (results displaced by 5 d from the ordinate). (2) Actomyosin: ⊙, Expt 2 LD; ●, Expt 2 HL. (3) Actin: +, Expt 1 LD; ☐, Expt 2 LD; ■, Expt 2 HL (a values × 0·67). (4) Myosin: ×, Expt 1 LD; △, Expt 2 LD; ▲, Expt. 2 HL (a values × 0·25).

actin (curve 3) when multiplied by 1·0/0·67 become comparable with those obtained for actomyosin (curve 2). The values for myosin (curve 4 − LD) are similarly comparable with those for actin (curve 3 − LD) when both sets of values are multiplied by the appropriate factors, i.e. 1·0/0·25 and 1·0/0·67. In this latter case, however, the myosin a values need also to be corrected for counting efficiency (68 %) as they are expressed as disintegrations/40 min per μmol.

Table 1 summarizes the respective k_s and k_c values obtained for each of the protein fractions of muscle. These results have been calculated by regression correlation from the semi-log plots of a shown in Fig. 1. It is seen that there is good agreement between experiments and also that the results obtained in Expt 2 for LD muscles do not differ significantly from those for HL muscles. So far as individual protein fractions are concerned, the only combined k_s values which were significantly different from actomyosin were those for pH 5·0 sarcoplasmic protein ($P < $ 0·04). IMCT and stroma fractions were excluded from this analysis; they are considered separately later.

It is implicit, therefore, that the significant difference between the correlated values of k_s for sarcoplasmic proteins and actomyosin would be similarly reflected in greater values of a for sarcoplasmic proteins on day 1, and that a would fall more rapidly in this fraction. This was so when mixed sarcoplasmic-protein and actomyosin values

were compared (Expt 2 LD): actomyosin a day 1 724, day 2 403, day 3 289; mixed sarcoplasmic protein a; day 1 991, day 2 479, day 3 301. Unfortunately, further comparisons of sarcoplasmic- protein a values with those obtained for the different myofibrillar-protein fractions are subject to appreciable error as it was noted retrospectively that count values obtained for sarcoplasmic- and plasma-protein extracts were subject to much greater quench. This was possibly due to greater quantities of material having been prepared for analysis. Automatic amino acid analysis failed however to reveal any significant contamination with amino acids other than aspartate or glutamate. Therefore, whereas the values of k_s are comparable with those obtained for the other protein fractions, the results for a are not so easily compared.

It may be concluded therefore that the two major myofibrillar proteins, actin and myosin, are synthesized at the same rate, and since they constitute the major protein fraction of muscle (see Tables 2 and 3), that they account for a major proportion of the total amino acid metabolism.

The results obtained in Expt 2 for mixed untreated sarcoplasmic proteins (see p. 36) for a and k_s were statistically indistinguishable from the values in Fig. 1 and Table 1 for pH 5 sarcoplasmic proteins. Since the untreated fraction contains plasma serum albumin and the pH 5·0 fraction does not, this observation supports the view that the measured differences in k_s and k_c between sarcoplasmic- and myofibrillar-protein fractions do not result from contamination of the sarcoplasmic proteins with plasma proteins of much higher a. Furthermore, independent analyses of mixed plasma proteins for aspartate + glutamate a failed to demonstrate any differences large enough to significantly affect the sarcoplasmic-protein aspartate + glutamate a values and by inference therefore the measured k_s of sarcoplasmic proteins (i.e. plasma-protein aspartate + glutamate a; day 1 1703, day 6 748, day 9 498, whereas mixed sarcoplasmic aspartate + glutamate a (LD); day 1 991, day 6 479, day 9 301). It has been confirmed (D. J. Millward, personal communication) that these differences in plasma- and sarcoplasmic-protein aspartate + glutamate a are much less than those found in the rat. It would appear, therefore, that in large animals muscle assumes a more important role relative to liver in regulating whole body amino acid metabolism.

In Expt 1 results were obtained for mixed mitochondrial proteins from biceps femoris muscle. The results ($_sT_{\frac{1}{2}} = 4\cdot23$ d) were very similar to those for the pH 5 sarcoplasmic-protein fraction in Expt 2 ($T_{\frac{1}{2}} = 4\cdot78$ d).

It will be noted from Table 1 that only results obtained for IMCT treated with KCl (Expt 1) and KI-stroma (Expt 2) have been presented. The reasons for omitting the results for IMCT treated with trypsin (see p. 37) may be considered briefly. It was found that there was an appreciable decrease in the a of aspartate + glutamate in this fraction after treatment with trypsin. This resulted in the calculated $_sT_{\frac{1}{2}}$ and $_cT_{\frac{1}{2}}$ values becoming extremely large ($_sT_{\frac{1}{2}} > 20$ d; $_cT_{\frac{1}{2}} = \infty$), results which are clearly in error. The loss of radioactivity after treatment with trypsin probably resulted from the extraction of soluble telopeptide material of high a, which in these young animals had not been stabilized into the IMCT matrix; this possibility has not been investigated further here.
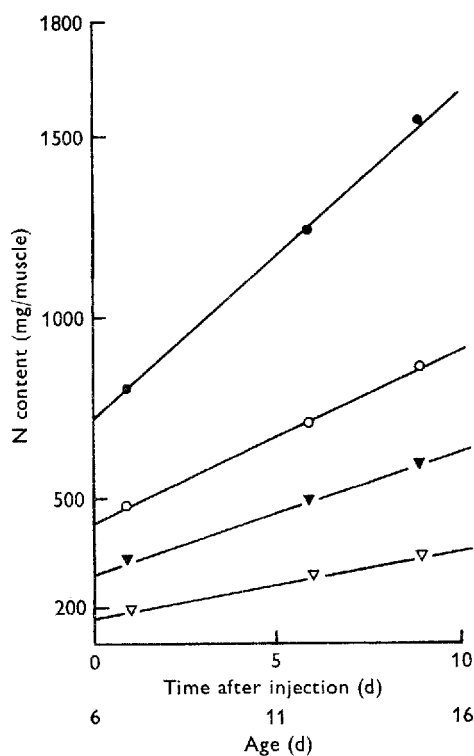
Fig. 2. Growth in total nitrogen contents of subfractions of longissimus dorsi (LD) and hind-limb (HL) muscles of piglets with time. Individual points were derived from results presented in Tables 2 and 3. ●, Myofibrillar N (HL); ○, myofibrillar N (LD); ▼, sarcoplasmic N (HL); ▽, sarcoplasmic N (LD).

Table 2. *Expt 2. Composition of longissimus dorsi muscle of piglets on a fresh-weight basis*

Piglet no.	1		2		3	
Age at slaughter (d)	7		12		15	
Time after injection (d) ...	1		6		9	
	(mg N/g muscle)	(% total N)	(mg N/g muscle)	(% total N)	(mg N/g muscle)	(% total N)
Total N	24·9	—	27·2	—	29·0	—
Total non-protein N	3·1	12·4	3·4	12·5	3·7	12·8
Total protein N	21·8	—	23·8	—	25·3	—
KI protein N	18·2	—	20·1	—	21·7	—
Sarcoplasmic protein N	5·3	21·3	5·5	20·2	5·7	19·7
Myofibrillar protein N	12·9	51·8	14·7	54·0	16·0	55·2
Stroma N	3·3	13·3	3·5	12·9	3·5	12·1
Recovery of N (%)	98·9	—	99·2	—	99·7	—
Muscle wet weight (g)	35·33	—	48·43	—	50·04	—
Water (approx. %)	84·6	—	83·1	—	82·1	—

Table 3. *Expt 2. Composition of hind-limb muscles of piglets on a fresh weight basis*

	Piglet no. 1		Piglet no. 2		Piglet no. 3	
Piglet no.	1		2		3	
Age at slaughter (d)	7		12		15	
Time after injection (d)	1		6		9	
	(mg N/g muscle)	(% total N)	(mg N/g muscle)	(% total N)	(mg N/g muscle)	(% total N)
Total N	24·2	—	27·0	—	28·3	—
Total non-protein N	3·0	12·4	3·4	12·6	3·5	12·4
Total protein N	21·2	—	23·6	—	24·8	—
KI protein N	19·3	—	20·9	—	22·2	—
Sarcoplasmic protein N	5·7	23·6	6·3	23·3	6·2	21·9
Myofibrillar protein N	13·6	56·2	14·6	54·1	16·0	56·5
Stroma N	1·8	7·4	2·6	9·6	2·3	8·1
Recovery of N (%)	99·2	—	99·4	—	99·0	—
Muscle wet weight (g)	60·78	—	79·86	—	97·12	—
Water (approx. %)	85·0	—	83·2	—	82·5	—

KI extraction of muscle to yield stroma protein also appeared to produce erroneous values, for Table 1 shows that the $_sT_{\frac{1}{2}}$ value for the stroma fraction (6·3 d) was considerably lower than that for KCl-extracted IMCT (8·1 d). This probably reflects the inability of KI to extract quantitatively any contaminating denatured sarcoplasmic protein of higher a from the IMCT matrix. This would also account for the variability in the contribution of this fraction to the total composition of the muscles (see Tables 2 and 3). The contamination, judging from the a values, amounted to more than 40 % on an N basis. For this reason, catabolic rates shown for IMCT in Table 1 are calculated from the a obtained in Expt 1 for KCl-extracted IMCT, and the values for mean composition in Expt 2. From the muscle composition values (Tables 2 and 3) and growth results (Fig. 2) this would seem to be a reasonable approximation.

The results for skeletal muscle composition presented in Tables 2 and 3 demonstrate that in all but one instance recovery of N was more than 99 %. Freeze-drying and powdering were found to improve sampling and reproducibility, duplicate samples usually agreeing to within 2 % of the mean value. Comparisons with published findings (Dickerson & Widdowson, 1960) also suggest that freeze-drying has little or no effect upon protein extractability. Nevertheless, consistent differences in muscle-protein composition, unattributable to extraction efficiency, were found both between each of the muscle groups and between individual animals within a group. Presumably these reflect differences in the ages and postnatal development of piglets at slaughter.

DISCUSSION

The advantage of using the a of aspartate + glutamate to calculate k_s and k_c of muscle proteins has been discussed in detail elsewhere (Millward, 1970a, b). Garlick & Millward (1972) have also recently reviewed the validity and applicability of this technique, compared with other methods available for measuring protein turnover. To the author's knowledge, however, there are no published findings for large animal

species or for individual proteins isolated from skeletal muscle. The values for the pig, obtained by using an experimental procedure similar to that of Millward (1970*a*, *b*), make possible some comparisons between species.

It is, however, important to consider first some of the problems encountered in using the method to study protein turnover in animals much larger than rats or mice. The technique depends upon labelling aspartate + glutamate, which also provides important information relating to protein catabolism. However, because of the low incorporation efficiency of $^{14}CO_3^{2-}$ into these residues, very careful analytical procedures are required if adequate reproducibility and accuracy are to be obtained. For example, our recent experience has been that the CO_3^{2-} method precluded accurate measurement of protein synthesis and catabolism in individual muscles and muscle proteins of larger pigs (100 kg), even though each animal received 12·5 mCi $^{14}CO_3^{2-}$, because the *a* of aspartate + glutamate residues were even lower than those recorded here. Another problem encountered has been that methods adopted for the isolation of muscle proteins and for measuring muscle-protein composition are both time-consuming and difficult to reproduce exactly. For these reasons, no attempts were made to isolate individual proteins from the sarcoplasmic-protein extract.

Despite these problems, it has been demonstrated statistically that sarcoplasmic proteins are synthesized more rapidly than myofibrillar proteins. The k_c of mixed sarcoplasmic proteins was also greater than that of actomyosin. These results are furthermore not confounded by contamination of the sarcoplasmic-protein fraction with plasma proteins of much higher *a* since care was taken to remove the latter during preparation.

The apparent anomaly shown in Fig. 1, which indicates the *a* values for sarcoplasmic proteins to be little greater at day 1 after injection of isotope than those for actomyosin or actin, although the k_s for sarcoplasmic proteins was statistically significantly greater, probably reflects small errors in amino acid analyses and differences in counting efficiencies. However, as for each individual protein fraction similar quantities of material were prepared, analysed in batches and shown to give consistent results (see p. 37), the values for k_s and k_c of the different proteins should be compared, rather than those for *a*. The similar results obtained from the two separate experiments, for each of the different protein fractions (see Table 1), suggest this conclusion to be more correct. Nevertheless, allowing for greater variability in *a*, the failure to demonstrate differences between sarcoplasmic- and plasma-protein *a* values as great as those found in the rat (D. J. Millward, personal communication) supports in part the hypothesis (Munro, 1969; Millward & Garlick, 1972) that, in animals of greater mature body size than rats or mice, muscle assumes a more important role relative to liver in regulating whole body amino acid metabolism.

The rates of synthesis and catabolism obtained for mixed myofibrillar (actomyosin)- and pH 5 sarcoplasmic-protein fractions are very similar to those found for 100 g rats by Millward (1970*a*, *b*) and Gan & Jeffay (1971). These similarities may, however, be purely fortuitous, since 100 g rats are far more mature than the young piglets investigated here, and have presumably nearly reached a plateau level of synthesis and catabolism, whereas the piglets certainly have not.

The good agreement between Expts 1 and 2 and failure to demonstrate significant differences in k_s between LD and HL muscle in Expt 2 are interesting, since anatomical dissections of pigs from birth to 40 kg (unpublished observations) indicate that HL muscles collectively possess marginally greater growth coefficients than LD. To what extent, therefore, the differences in k_c for LD and HL are significant (see Table 1) or reflect summed errors in the respective analytical procedures would seem to be an important question, because limits to the size and rates of growth of muscles may not only be due to differences in k_s, and presumably due to differences in coding, but may also be controlled by differing specific activities of skeletal-muscle cathepsins and proteases, presumably also coded differently. Clearly some independent assessment of protein catabolism (e.g. 3-methyl-histidine entry rate) would be needed to assess this possibility further.

The fact that differences between k_s and k_c measure net synthesis (i.e. growth), and changes in both k_s and k_c will therefore influence growth is interesting, for results we have obtained from two 100 kg pigs (see p. 43) killed 1 and 6 d after injection of [^{14}C]Na$_2$CO$_3$, gave, with mixed myofibrillar–sarcoplasmic proteins (10 mM-NaOH, pH 8 extracted), $_sT_{\frac{1}{2}}$ of 4·2, 9·0 and 6·2 d in psoas major (PS), biceps femoris (BF) and semimembranosus (SM) muscles respectively, i.e. values which were very similar to those obtained with piglets. In view of the few observations made (n), the low aspartate + glutamate a values and relatively large standard errors (PS day 1; 151·6 ± 0·3 $n = 2$; day 6; 57·0 ± 4·3 $n = 3$; BF day 1; 117·7 ± 8·3 $n = 7$; day 6; 82·8 ± 7·7 $n = 8$; SM day 1; 115·9 ± 4·8 $n = 4$; day 6; 65·3 ± 0·8 $n = 2$) these results must however be considered with caution. They do nevertheless indicate a field of study warranting further investigation.

The finding that, in skeletal muscle, myosin, actin and crude actomyosin all appear to be synthesized and catabolized at similar rates is in excellent agreement with the findings of Chain & Sender (1972). They were unable to show any differences in the a of cardiac myofibrillar proteins after separation on sodium dodecyl sulphate–acrylamide gel columns when the proteins were labelled by constant infusion rather than by the single-shot labelling technique (cf. Schimke, 1970; Young, 1970).

The wide discrepancies in results obtained for k_s and k_c of IMCT when different extraction procedures were followed have been noted already. They confirm the difficulty of identifying the composition of mixed protein fractions from muscle. The extractability of IMCT seems to be particularly affected by the stage of development and age of the animal. This arises no doubt as a consequence of changes in the physical and chemical constitution of collagen which are physiologically and chronologically ordered (Shimokomaki & Bailey, 1971).

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