Comparison of the chemical and biochemical composition of thirteen muscles of the rat after dietary protein restriction

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1. The objective of this study was to determine whether the chemical and biochemical changes induced by muscle wasting caused by dietary protein restriction are different in various skeletal muscles.

2. Rats were fasted for 3 d and then fed on a 10 g protein/kg diet for 21 d. Thirteen muscles from the trunk, forelimb, and hind-limb regions were analysed for muscle weight, and the content of water, fat, cellular and extracellular protein, DNA and RNA. Results were compared to values for an 'initial' control group killed at the start of the experiment.

3. Weight loss was greatest in trunk muscles and least in the distal forelimb muscles. Water content decreased in most muscles, but increased in three forelimb muscles. A significant loss of lipid was found in the gastrocnemius, while the biceps brachii gained lipid. Changes in lipid content of the muscles did not form a distinctive pattern.

4. All muscles except the distal forelimb muscles lost a significant amount of cellular protein, while all muscles except the diaphragm gained extracellular protein.

5. DNA content was unchanged in all muscles. The value for cellular protein:DNA was significantly reduced in the rectus abdominis and the diaphragm. A significant loss of RNA was found in all muscles; the percentage change was greatest in trunk muscles and least in the distal forelimb muscles. The values for RNA:protein and RNA:DNA were significantly lower in all muscles except two distal forelimb muscles.

6. With the exception of the water and lipid content of the muscles, the directions of the changes in the experimental animals were the same for all muscles. The results suggested, however, that the magnitude of changes in certain chemical and biochemical indices of composition may depend to some extent on the anatomical location of the muscle: trunk muscles tended to show the greatest percentage change, while the distal forelimbs changed the least.

The role of striated muscle in the adaptation to severe malnutrition has been the subject of a great deal of study. Typically, the role of the muscle mass in general has been interpreted from analyses of a single muscle, most commonly the gastrocnemius or the quadriceps, although occasionally the specific muscle has not even been identified. Because of growing evidence that not all muscles respond to dietary restriction in the same manner (Joubert, 1956; Dickerson & McCance, 1960; Taskar & Tulpule, 1964; Wechsler, 1966; Rowe, 1968; Goldspink & Waterson, 1971; Turner & Fern, 1974; Dickerson & McAnulty, 1975), there is a need for comparative analysis between different muscles.

It was the purpose of this study to compare several chemical and biochemical indices of composition in different muscles of the rat after 3 weeks of protein restriction, preceded by a 3 d fast. The dietary protocol used was selected to prevent muscle growth and to maximize muscle wasting over a relatively short period (Millward, 1970). The muscles under comparison were heterogeneous in function and in anatomical location. Results from the experimental animals were compared to an 'initial' control group (killed at the start of the experiment) so that the differences observed between the two groups would reflect an absolute change resulting from the dietary restriction, without superimposing the problem of differences due to growth of the controls.

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Table 1. *Composition (g/kg) of the experimental diet for rats*

Maize starch	480
Vitamin mix*	10
Mineral mix†	50
Glucose	150
Wheat flour	150
Lard	150
Casein	10

* Vitamin mix composition (mg): thiamin 6, riboflavin 20, pyridoxine 4, calcium pantothenate 120, nicotinic acid 400, *p*-aminobenzoic acid 1200, biotin 0·4 folic acid 4, cyanocobalamin 0·1; the vitamin mix was diluted with maize starch (44:356, w/w) to give the mixture used in making up the diets.

† Fraser & Alleyne (1974).

MATERIALS AND METHODS

Experimental animals

Male albino rats from the Tropical Metabolism Research Unit colony were used. All rats were approximately 5 weeks old. Eight rats weighing $99 \pm 4{\cdot}5$ g (SD) were used as 'initial' controls. The experimental group consisted of eight animals weighing 100 ± 4 g. The experimental group was starved for 3 d and subsequently maintained on a 10 g protein/kg diet (Table 1) for 21 d. The animals were fed *ad lib.* and had access to water at all times.

Selection of muscles

The animals were killed by diethyl ether anaesthesia, and thirteen pairs of muscles were removed from the trunk (rectus abdominis, psoas, diaphragm), hind-limb (quadriceps femoris, tibialis anterior, gastrocnemius, soleus, gluteus maximus), and forelimb (triceps brachii, biceps brachii, brachialis, flexor carpi radialis and extensor carpi ulnaris). Wherever possible the outer fasciae and any adhering tissue were removed from the muscles. Each muscle was removed with its tendonous attachments by cutting as close as possible to the points of origin and insertion. All muscles from the right side of the body were sequentially removed, followed by sequential removal of the same muscles from the left side. The muscles were blotted, sealed in preweighed polyethylene bags, frozen immediately in a freezing mixture of dry ice and acetone, and stored at $-10°$ for subsequent analyses.

Analysis of muscle

Muscles from the left side were oven-dried to a constant weight. Lipid was removed from the dried muscles by repeated immersion in diethyl ether for up to 24 h. Muscles from the right side of the body were homogenized and used for analysis of their protein and nucleic acid content. The recommendations of Munro & Fleck (1966) for the Schmidt–Thannhauser technique (Schmidt & Thannhauser, 1945) were adopted for the determination of RNA content and the extraction of DNA. The indole reagent method (Ceriotti, 1952) was used for the determination of DNA content. Cellular and extracellular protein fractions were prepared by the method of Dickerson & McAnulty (1975) and were analysed by the method of Lowry, Rosebrough, Farr & Randall (1951). The fraction of homogenate which was soluble in 0·2 M-sodium hydroxide was considered to contain cellular protein, while the residue, dissolved in 1 M-NaOH, was considered to contain extracellular protein. Statistical comparisons of differences between the means of the different measurements were made by Students' *t* test. Statistical significance was accepted at $P < 0{\cdot}05$.

Table 2. *Effect of dietary restriction on wet weight, muscle weight: body-weight and dry weight of thirteen different muscles of young rats*

(Mean values and standard deviations for eight rats/group)

		Muscle wet wt			Muscle wt: body-wt			Muscle dry wt		
		mg			mg/kg			mg		
Muscle	Group	Mean	SD	% change	Mean	SD	% change	Mean	SD	% change
Rectus abdominis	C	224	37		210	29		60·7	6·0	
	M	146	18*	−35	223	18	+6	41·2	6·3*	−32
Diaphragm	C	112	15		124	10		28·2	6·0	
	M	81	9*	−28	130	10	+5	24·5	3·7	−13
Psoas	C	241	21		239	23		59·2	4·7	
	M	168	14*	−30	248	14	+4	45·7	3·2*	−23
Quadriceps	C	647	28		664	27		144·6	5·4	
	M	502	63*	−22	739	70*	+11	121·9	17·1*	−16
Tibialis anterior	C	220	15		220	13		61·2	8·1	
	M	167	17*	−24	253	22*	+15	48·0	11·6*	−22
Gastrocnemius	C	537	20		542	19		124·3	9·3	
	M	423	41*	−21	643	57*	+19	107·6	9·3*	−13
Soleus	C	129	8		128	5		34·1	2·1	
	M	97	10*	−25	141	15*	+10	29·2	3·6*	−14
Gluteus maximus	C	478	23		485	23		105·1	10·9	
	M	374	84*	−22	572	138	+18	87·5	19·9	−14
Triceps	C	302	18		298	18		74·8	6·3	
	M	230	27*	−24	311	78	+4	62·0	7·6*	−17
Biceps	C	52	4		51	4		15·9	1·9	
	M	46	3*	−12	68	05*	+33	15·2	1·8	−4
Brachialis	C	60	4		61	7		13·3	1·3	
	M	52	4*	−13	81	19*	+32	10·7	1·6*	−20
Flexor carpi radialis	C	24	2		23	1		5·3	0·4	
	M	24	2	0	35	4*	+52	4·7	0·7	−11
Extensor carpi ulnaris	C	21	2		21	2		4·7	0·5	
	M	21	2	0	31	3*	+48	4·1	0·5	−13

C, 'initial' control (killed at start of experiment); M, malnourished (10 g protein/kg diet for 21 d after a 3 d fast).

* Values for group M were statistically significantly different from those for group C ($P < 0.05$).

RESULTS

At the end of the experimental period the mean (± SD) weight of the experimental group was 67 ± 3 g, a loss of 33 % from the initial weight of the animals. The absolute weights of all muscles except the forelimb flexor and extensor were significantly lower in the experimental group than in the 'initial' control group (Table 2). The percentage change from the initial weight was highest for trunk muscles and lowest for the forelimb muscles. Whereas the relative weights (% body-weight) of the trunk muscles were unchanged, the relative weights of nearly all the limb muscles were increased significantly. The dry weights of most muscles were also significantly lower than those of the 'initial' controls, but no distinctive pattern of change between the muscle groups was evident.

Water content of muscles from the trunk and hind-limb regions of experimental animals was significantly reduced (Table 3). The largest change was observed in the diaphragm. In contrast, a significant increase in water content occurred in three of the forelimb muscles. Changes in the total lipid content and concentration of most of the muscles were variable.

Table 3. *Effect of dietary restriction on water content, fat content and fat concentration of thirteen different muscles of young rats*

(Mean values and standard deviations for eight rats/group)

		Water content			Fat content			Fat concentration		
		mg/g*		%	mg		%	mg/g†		%
Muscle	Group	Mean	SD	change	Mean	SD	change	Mean	SD	change
Rectus abdominis	C	745	20		11·1	5·3		180	74	
	M	711	22‡	−4·5	8·0	4·0	−28	192	82	+7
Diaphragm	C	724	12		3·2	0·9		112	22	
	M	679	30‡	−6·2	3·7	1·5	+15	149	45	+33
Psoas	C	760	17		3·8	2·3		65	38	
	M	729	14‡	−4·0	4·4	2·5	+16	97	57	+49
Quadriceps	C	773	4		6·6	1·2		79	35	
	M	747	21‡	−2·1	7·4	3·4	+12	57	23	−29
Tibialis anterior	C	747	16		4·0	1·3		66	24	
	M	728	13‡	−2·4	3·8	2·6	+5	59	28	−10
Gastrocnemius	C	768	16		10·1	1·6		82	16	
	M	737	26‡	−4·0	4·6	1·2‡	−54	43	13‡	−48
Soleus	C	738	18		3·5	0·5		103	15	
	M	706	12‡	−4·3	3·6	0·7	+3	125	12‡	+21
Gluteus maximus	C	779	7		4·9	1·3		48	11	
	M	762	16‡	−2·2	3·5	0·9	−29	41	10	−15
Triceps	C	758	25		4·6	1·3		63	23	
	M	740	15	−2·4	4·1	3·6	−11	68	30	+8
Biceps	C	701	30		1·1	0·4		68	22	
	M	673	31	−4·0	1·8	0·4‡	+64	114	17‡	+69
Brachialis	C	778	9		1·2	0·3		91	19	
	M	796	22‡	+2·3	0·9	0·9	−25	87	32	−5
Flexor carpi radialis	C	782	9		0·7	0·2		160	86	
	M	813	20‡	+4·0	0·6	0·3	−14	132	60	−17
Extensor carpi ulnaris	C	782	14		0·7	0·3		161	67	
	M	818	38‡	+4·6	0·6	0·3	−14	149	76	−8

C, 'initial' control (killed at start of experiment); M, malnourished (10 g protein/kg diet for 21 d after a 3 d fast).

* Muscle wet wt.

† Muscle dry wt.

‡ Values for group M were statistically significantly different from those for group C ($P < 0·05$).

However, significant reductions (approximately 50 %) in the total lipid content and lipid concentration were observed in the gastrocnemius muscle, while these values were significantly increased (by approximately 65 %) in the biceps muscle.

A significant reduction in the total amount of cellular protein was found in all muscles except those of the forelimbs of the experimental animals (Table 4). However, the concentration of this protein fraction relative to the dry weight of each muscle was reduced only in the rectus muscle. The total amount of extracellular protein was increased in all muscles except the diaphragm. The increase was significant for the rectus abdominis, gastrocnemius, soleus, brachialis, and extensor carpi radialis. The concentration of this protein fraction relative to dry weight was increased by 35–125 % in all muscles when compared to the initial concentration.

No statistical difference between the total DNA content of 'initial' controls and experi-

Table 4. *Effect of dietary restriction on the content and concentration of cellular and extracellular protein in thirteen different muscles of the young rat*

(Mean values and standard deviations for eight rats/group)

		Protein content						Protein concentration					
		Cellular			Extracellular			Cellular			Extracellular		
		mg		%	mg		%	mg/g*		%	mg/g*		%
Muscle	Group	Mean	SD	change	Mean	SD	change	Mean	SD	change	Mean	SD	change
Rectus abdominis	C	34	7		2·6	0·5		558	59		44	10	
	M	22	5†	−35	3·6	1·0†	+38	446	80†	−20	71	20†	+62
Diaphragm	C	20	2		1·8	0·6		555	71		51	18	
	M	13	2†	−35	1·7	0·4	−5	521	76	−6	68	19	+34
Psoas	C	45	7		1·4	0·3		750	88		25	6	
	M	30	3†	−33	1·7	0·6	+21	757	42	+1	43	16†	+73
Quadriceps	C	112	11		5·2	1·1		719	113		37	15	
	M	88	13†	−21	6·1	1·1	+17	679	101	−6	50	12	+37
Tibialis anterior	C	40	5		1·7	0·4		687	67		36	15	
	M	33	5†	−18	2·0	0·4	+18	688	126	0	45	16	+34
Gastrocnemius	C	96	12		4·9	1·2		671	84		31	12	
	M	77	9†	−20	6·7	0·9†	+37	658	58	−2	59	16†	+90
Soleus	C	22	2		1·3	0·3		638	57		39	9	
	M	16	2†	−27	1·8	0·3†	+38	621	95	−2	70	15†	+80
Gluteus maximus	C	90	7		2·6	0·7		738	122		23	6	
	M	66	9†	−27	3·2	1·2	+23	718	79	−3	33	10†	+47
Triceps	C	54	7		2·3	2·9		746	96		31	6	
	M	41	7†	−24	2·9	0·7	+26	704	66	−6	51	11†	+62
Biceps	C	9	1		0·44	0·20		574	98		28	10	
	M	8	1	−11	0·60	0·22	+36	489	141	−15	38	17	+37
Brachialis	C	11	1		0·35	0·13		850	111		27	10	
	M	10	2	−9	0·53	0·09†	+51	842	148	−1	51	14†	+90
Flexor carpi radialis	C	4	1		0·30	0·14		756	102		57	12	
	M	4	1	0	0·41	0·19	+37	716	141	−5	88	34†	+55
Extensor carpi ulnaris	C	3	1		0·37	0·10		720	154		80	31	
	M	3	1	0	0·70	0·20†	+89	771	180	+7	180	80†	+125

C, 'initial 'control (killed at start of experiment); M, malnourished (10 g protein/kg diet for 21 d after a 3 d fast).

* Muscle dry wt.

† Values for group M were statistically significantly different from those for group C ($P < 0·05$).

Table 5. *Effect of dietary restriction on DNA content and cellular protein: DNA of thirteen different muscles of young rats*

(Mean values and standard deviations for eight rats/group)

		DNA content			Cellular protein: DNA		
		mg/muscle		% change			
Muscle	Group	Mean	SD		Mean	SD	% change
Rectus abdominis	C	0·22	0·06		156	35	
	M	0·27	0·05	+19	84	27*	−46
Diaphragm	C	0·18	0·05		118	37	
	M	0·18	0·03	0	74	16*	−37
Psoas	C	0·22	0·06		186	35	
	M	0·20	0·05	−12	160	40	−14
Quadriceps	C	0·63	0·18		193	76	
	M	0·51	0·15	−18	183	55	−5
Tibialis anterior	C	0·19	0·04		220	71	
	M	0·17	0·04	−12	196	33	−11
Gastrocnemius	C	0·49	0·16		214	73	
	M	0·51	0·08	+5	152	20	−29
Soleus	C	0·14	0·05		177	63	
	M	0·12	0·02	−18	144	14	−19
Gluteus maximus	C	0·42	0·13		224	89	
	M	0·40	0·07	−3	178	40	−21
Triceps	C	0·31	0·06		166	40	
	M	0·29	0·05	−7	151	23	−9
Biceps	C	0·052	0·011		182	56	
	M	0·057	0·014	+10	144	30	−21
Brachialis	C	0·056	0·013		205	40	
	M	0·051	0·016	−9	220	67	+7
Flexor carpi radialis	C	0·036	0·009		142	71	
	M	0·030	0·010	−17	144	65	+1
Extensor carpi ulnaris	C	0·035	0·010		105	37	
	M	0·031	0·017	−11	122	81	+16

C, 'initial' control (killed at start of experiment); M, malnourished (10 g protein/kg diet for 21 d after a 3 d fast).

* Values for group M were statistically significantly different from those for group C ($P < 0·05$).

mental animals was detected in any of the muscles examined (Table 5). A significantly lower value for cellular protein:DNA was found for the rectus abdominis and diaphragm of experimental animals when compared to the 'initial' controls, but none of the other differences were statistically significant. The total RNA content of all muscles of the experimental animals was significantly lower (28–56 %) than that of the 'initial' controls (Table 6). The value for RNA:cellular protein was significantly lower (20–35 %) than that for the 'initial' controls in eleven of the muscles, but the differences were not statistically significant for the flexor carpi radialis and the extensor carpi ulnaris. The value for RNA:DNA was 16–64 % lower than that for the 'initial' controls. The differences were statistically significant in eight muscles.

Table 6. *Effect of dietary restriction on RNA content and concentration of thirteen different muscles of young rats*

(Mean values and standard deviations for eight rats/group)

		RNA content			RNA concentration					
		mg/muscle		%	RNA:cellular protein		%	RNA:DNA		%
Muscle	Group	Mean	SD	change	Mean	SD	change	Mean	SD	change
Rectus abdominis	C	0·74	0·05		22·6	4·3		3·47	0·82	
	M	0·33	0·04*	−56	15·9	4·0*	−30	1·26	0·25*	−64
Diaphragm	C	0·54	0·10		27·6	4·8		3·21	0·89	
	M	0·26	0·06*	−53	19·9	5·9*	−28	1·42	0·25*	−56
Psoas	C	0·95	0·05		21·6	3·1		4·04	0·67	
	M	0·41	0·04*	−56	14·0	1·9*	−35	2·23	0·63*	−44
Quadriceps	C	2·27	0·29		20·3	1·8		3·90	1·49	
	M	1·13	0·13*	−50	13·1	2·0*	−36	2·40	0·90	−39
Tibialis anterior	C	0·77	0·08		19·7	3·4		4·28	1·19	
	M	0·47	0·04*	−39	14·9	1·9*	−24	2·94	0·67*	−31
Gastrocnemius	C.	1·94	0·16		20·4	1·9		4·26	1·20	
	M	1·12	0·12*	−42	14·7	1·9*	−28	2·49	0·47*	−47
Soleus	C	0·48	0·05		21·7	1·8		3·82	1·42	
	M	0·24	0·04*	−50	14·9	2·7*	−31	2·11	0·52*	−45
Gluteus maximus	C	1·70	0·20		20·5	5·0		4·51	1·77	
	M	0·90	0·03*	−47	13·4	1·7*	−35	2·79	1·31	−38
Triceps	C	0·98	0·13		18·3	1·9		3·28	0·73	
	M	0·54	0·05*	−45	13·3	0·9*	−27	1·90	0·36*	−42
Biceps	C	0·20	0·02		23·0	3·0		4·08	0·98	
	M	0·15	0·04	−28	18·3	2·8*	−20	2·86	0·77*	−35
Brachialis	C	0·23	0·04		21·1	4·2		4·40	1·54	
	M	0·14	0·02	−41	13·9	2·9*	−29	3·03	1·13	−31
Flexor carpi radialis	C	0·11	0·01		24·6	3·1		3·39	1·52	
	M	0·06	0·01	−41	18·7	7·8	−24	2·54	1·16	−25
Extensor carpi ulnaris	C	0·09	0·01		27·8	8·3		2·94	1·26	
	M	0·06	0·01	−33	21·8	7·9	−22	2·49	1·42	−16

C, 'initial' control (killed at start of experiment); M, malnourished (10 g protein/kg diet for 21 d after a 3 d fast).

* Values for group M were statistically significantly different from those for group C ($P < 0·05$).

DISCUSSION

Thirteen different muscles representative of trunk, hind-limb and forelimb muscles were analysed. The results suggested that certain chemical and biochemical changes coincident with muscle wasting may differ, depending on the specific muscle examined. However, the specificity involved primarily changes in magnitude rather than in direction, with the exception of changes in water and fat content of the muscles.

Muscle weight

Although other studies in which muscle weight after a period of dietary restriction has been compared to that of an 'initial' control group have yielded conflicting results relative to loss or gain of muscle weight (Cabak, Dickerson & Widdowson, 1963; Montgomery, Dickerson & McCance, 1964; Dickerson, Hughes & McAnulty, 1972; Giovannetti & Stothers, 1975;

Nnanyelugo, 1976), a loss of up to 50 % of the original muscle weight may occur within 4 weeks, provided the dietary restriction is severe enough (Cabak *et al.* 1963; Nnanyelugo, 1976). During the 3-week period of the present study the absolute weights of eleven muscles were reduced by 12–35 % as a result of combined fasting and protein restriction. A definite anatomical pattern of weight loss was evident: the muscles of the trunk lost the most weight, while the loss of weight in hind-limb muscles was less. Forelimb muscles lost the least weight, and in fact two forelimb muscles were unaffected. Since the trunk muscles lost weight in proportion to body-weight, there was no difference in values for muscle weight:body-weight in these muscles of the experimental animals compared to those for the 'initial' controls. In contrast, the values in four of the forelimb muscles were increased by 32–52 %. These results clearly suggest a priority of weight loss by trunk muscles over weight loss by limb muscles, particularly those of the forelimbs.

Certain differences between the weight changes of anatomically distinct muscles have previously been documented. Severe undernutrition in fowls caused greater muscle wasting in the pectoral muscle than in the sartorius (Dickerson & McCance, 1960) relative to their initial weight. In addition, Dickerson & McAnulty (1975) showed that the weight of quadriceps muscle of undernourished rats increased, while that of the tibialis anterior and the gastrocnemius was unchanged relative to 'weight' controls. No difference between these same hind-limb muscles was noted under the experimental conditions in the present study, in which comparison was made with 'initial' controls instead of 'weight' controls. Differential muscle growth may have occurred in the 'weight' controls in the study by Dickerson & McAnulty (1975).

Water content

When the wet weights of the muscles were compared, it appeared that at least some of the forelimb muscles were spared relative to hind-limb muscles. This difference was obscured, however, when dry weights were compared, since the direction of change in water content of fore- and hind-limbs differed: the muscles of the trunk and hind-limbs lost a small but significant amount of water, whereas three of the forelimbs gained a significant amount of water. Previous reports suggest that the water content of muscle from severely-malnourished animals may be increased relative to that of the 'initial' controls (Dickerson & McCance, 1960; Montgomery *et al.* 1964; Young, Stothers & Vilaire, 1971). The results of the present study therefore differ from other previous reports in showing that significant changes in water content did occur as a result of 3 weeks of dietary restriction, and that water content decreased in most muscles. It has been shown that the increase in muscle water after dietary restriction is mainly extracellular (Dickerson & McCance, 1960), but the relative distribution of water between the intra- and extracellular compartments of muscles which lose water, as was the situation for many of the muscles in this study, is not known.

Lipid content

The lipid content of muscles from malnourished animals has received little attention, although Montgomery *et al.* (1964) reported a marked reduction in lipid concentration of the sartorius muscle of undernourished fowls relative to the 'starting' concentration. The present analyses of lipid content revealed large variations between animals in each group. However, a significant loss of lipid was observed in the gastrocnemius, whereas the biceps brachii gained lipid. The concentration of lipid was reduced by half in the gastrocnemius, but it was significantly increased in the soleus and in the biceps. These results and those of Montgomery *et al.* (1964) suggest that certain muscles, such as the gastrocnemius of the rat and the sartorius of the fowl, appear to contain a lipid store which may be mobilized under certain dietary conditions, while other muscles accumulate lipid under the same circum-

stances. It would be of interest to know the source of the mobilized lipids, since loss of membrane lipids would be likely to have consequences in terms of physiological muscle function (i.e. excitation) and metabolism (i.e. mitochondrial function), while loss of non-structural lipid deposits would be more likely to affect energy reserves. The site of increased muscle lipids is of interest for similar reasons. It should be noted that lipid-filled vacuoles commonly appear in muscle fibres undergoing severe atrophy or degeneration (Adams, 1975).

Cellular protein

Previous reports have shown that total nitrogen, total protein, and total cellular protein are reduced relative to the initial protein content after a period of undernutrition (Dickerson & McCance, 1960; Cabak *et al.* 1963; Montgomery *et al.* 1964; Wannemacher & Cooper, 1970; Nnanyelugo, 1976). The trunk and hind-limb muscles from the experimental animals in the present study lost 20–35 % of their initial cellular protein content. In contrast, no loss of cellular protein was detected in any of the forelimb muscles except the triceps. Loss of cellular protein in the other muscles did not alter the concentration of this fraction relative to the dry weight of the muscle, however, except in the rectus abdominis. This marked response of the abdominal muscle is consistent with the observation that ultrastructural degeneration is more severe in this muscle than in limb muscles (Wechsler, 1966).

The loss of cellular protein affects both myofibrillar and sarcoplasmic proteins (Dickerson & McCance, 1960; Cabak *et al.* 1963; Montgomery *et al.* 1964; Millward, 1970; Wannemacher & Cooper, 1970). The morphological correlate of the loss in cellular protein is apparently a reduction in fibre size without concomitant loss of fibres (Joubert, 1956; Montgomery *et al.* 1964; Goldspink, 1965; Stickland, Widdowson & Goldspink, 1975). A reduction in myofibril size (Goldspink, 1965) or loss of contractile elements (Wechsler, 1966) may also occur within the fibres.

Extracellular protein

Despite the loss of cellular protein, total extracellular protein actually increased by 20–90 % in every muscle except the diaphragm. Furthermore, the concentration of this protein fraction relative to muscle dry weight was increased by 30–90 % in every muscle. These results are in agreement with most previous reports which suggest that the extracellular fraction of muscle from malnourished rats and fowl is increased relative to the initial content and concentration (Mendes & Waterlow, 1958; Dickerson & McCance, 1960; Cabak *et al.* 1963; Montgomery *et al.* 1964; Dickerson & McAnulty, 1975). A reduction in extracellular protein concentration relative to the initial concentration has been reported, however, in the gastrocnemius muscle of young rats after either 10 d of starvation or 21 d on a protein-free diet (Wannemacher & Cooper, 1970). It should be emphasized that the entire tendonous attachment of each muscle was included in the analyses in the present study. Whether this was done in the reports cited is not entirely clear.

It is not certain whether the increase in extracellular protein represents increased collagen within the interstitial spaces of the muscle (a change which could impair the contractile function of the muscle fibres), or whether it represents an increase in the collagen content of the tendon. Elongation of the femur continued during the experimental period (C. Spence, unpublished results). Several other reports have also suggested that long-bone growth continues during at least the initial period of dietary restriction (Montgomery *et al.* 1964; Dickerson *et al.* 1972; Lee, 1976). Either muscle or tendon elongation or both must accompany long-bone growth. Since cellular protein was lost, it seems most likely that tendon elongation occurred in the experimental animals. This suggestion is supported by our observation of increased extracellular protein in all the muscles analysed which would have

been affected by long-bone growth or increased body length, whereas there was no change in the collagen content of the diaphragm, a muscle which would not have been significantly affected by longitudinal growth (Dickerson *et al.* 1972). An increase in interstitial collagen cannot be completely discounted, however, since morphological observations of muscle biopsies from malnourished children suggest that interstitial collagen may be increased (Montgomery, 1962; F. Hansen-Smith, unpublished results).

DNA

The measurements of total DNA in each muscle indicated that neither loss nor gain of nuclei occurred during the experimental period. Thus the increase in muscle DNA content due to increases in length and girth during normal growth (Enesco & Puddy, 1964; Gordon, Kowalski, & Fritts, 1966; Cheek, Holt, Hill & Talbert, 1971; Burleigh, 1977) were prevented. These observations are in line with other similar studies of hind-limb muscles which show little or no change in total DNA content relative to 'initial' controls (Howarth & Baldwin, 1971; Dickerson & McAnulty, 1975). It should be noted that in one report, however, a significant reduction in DNA content of rat quadriceps muscle was found after 28 d on a 50 g protein/kg diet (Dickerson *et al.* 1972). Goldberg & Goldspink (1975) reported no change in muscle DNA content after 48 h of starvation. It is assumed that approximately 65 % of the DNA measured reflects myonuclei in the muscles from controls as well as from the experimental animals. This assumption has been tested for controls (Enesco & Puddy, 1964) but not for malnourished animals. This percentage of myonuclei has, however, also been found in muscle from malnourished infants (Hansen-Smith, Picou & Golden, 1978) and well-nourished children (F. Hansen-Smith, unpublished results). Direct counts of the total numbers of myonuclei in the flexor digiti brevis of pigs have shown neither loss nor gain in total myonuclei after 1 year of malnutrition (Stickland *et al.* 1975). Also, no loss of myonuclei was detected by direct counts of myonuclei in pectoral muscle of chickens starved or undernourished for a few days (Moss, 1968). While a small proportion of the nuclei actually belong to the muscle satellite cell (Allbrook, Han & Helmuth, 1971), this proportion is apparently not altered by chronic dietary restriction in rats (F. Hansen-Smith, unpublished results).

The ratio, cellular protein:DNA, an index of a hypothetical muscle 'cell' size, was significantly reduced only in the rectus abdominis and diaphragm, despite the significant reduction of cellular protein content in most of the muscles. The lack of significant change in cellular protein:DNA in the other muscles was unexpected, but apparently results from the large variability between individual rats. The literature is not clear regarding the influence of dietary restriction on muscle 'cell' size. A modest, but not statistically significant, reduction in cellular protein:DNA has been reported for rat gastrocnemius muscle after 4 d of fasting (Millward, Nnanyelugo, James & Garlick, 1974), and a significant reduction in protein:DNA has been found in hind-limb muscles of rats after 3 weeks of protein deprivation (Nnanyelugo, 1976). In contrast a slight increase in cellular protein:DNA has been reported for the quadriceps and gastrocnemius muscle of rats after 4 weeks of food restriction (Dickerson & McAnulty, 1975).

RNA

The results of the RNA analyses are in agreement with other reports which show a reduction in RNA content and concentration in hind-limb muscles of malnourished rats relative to 'initial' controls (Wannemacher & Cooper, 1970; Howarth & Baldwin, 1971; Nnanyelugo, 1976). Muscle RNA has been shown to be very closely and rapidly regulated by dietary intake (Howarth, 1972; Millward, Garlick, James, Nnanyelugo & Ryatt, 1973; Millward *et al.* 1974; Goldberg & Goldspink, 1975). The use of constant-infusion techniques for

measurement of protein metabolism has shown that initial effects of dietary restriction are mediated by a reduction in the efficiency of ribosomal protein synthesis. In later stages the predominant, and quantitatively the most important effects are due to a reduction in tissue RNA content and concentration (Young & Alexis, 1968; Millward *et al.* 1973). Since the total RNA content and the RNA concentration of all muscles were reduced by the dietary restriction it can be concluded that all muscles had a lower capacity for protein synthesis. Increased protein catabolism, particularly during the initial period of fasting (Millward, 1970), combined with a reduced capacity for replacement of protein thus resulted in a net loss of cellular protein from the muscles.

Millward & Garlick (1972) have shown a close relationship between RNA:protein and the intensity of protein synthesis in muscle. In the present experiment, RNA:protein was reduced, but no clear pattern emerged to indicate differences in the intensity of protein synthesis between different muscles, despite the apparent differences between the magnitude of changes in total cellular protein of trunk, hind-limb, and forelimb muscles. In the present experimental model the added factor of increased muscle catabolism would be expected to obscure such a relationship. It is of interest to note, however, that RNA:DNA, in contrast to RNA:protein, correlated well with the relative changes in total cellular protein by individual muscles; the correlation coefficient was calculated as 0·888 ($P < 0·05$).

Whereas a loss of cellular protein was observed in the muscles studied, extracellular protein, presumably mainly collagen, accumulated, thus indicating that synthesis of certain proteins continued despite an over-all reduction in the capacity for protein synthesis. This means that a portion of the RNA remaining after the experimental period was present in the interstitial cells, particularly in the fibroblasts which produce collagen. The presence of interstitial cells has largely been ignored for the purpose of most biochemical analyses of dietary effects on muscle. However, the RNA content of the fibroblast may have quantitative significance when collagen accumulation occurs in muscle, as is evident in the report by Jablecki, Heuser & Kaufman (1973), which dealt with muscle hypertrophy. Expression of results, including values for RNA:protein and RNA:DNA, must be interpreted with this possibility in mind.

Differences between muscles

In summarizing the results of this study, a distinction should be made between the magnitude and the direction of responses by the individual muscles. The direction of the changes by the various muscles were the same for nearly all the measurements. For many measurements, however, the magnitude of change tended to be related to anatomical location, i.e. trunk, hind-limb or forelimb. It might be argued that the large differences in size of the individual muscles in different anatomical regions biased the measurements by regions. Comparison of measurements from two muscles which were identical in weight but were located in different regions, i.e. the rectus abdominis and the tibialis anterior, argues against this objection for muscles in the size-range found in the trunk and hind-limb. However, four of the forelimb muscles were considerably smaller than the other muscles, and errors which would be insignificant in the larger muscles would be of importance in the smaller muscles. The largest forelimb muscle, the triceps, did not always follow the trends established by the four smaller distal muscles. Whether this may be related to its size or its proximity to trunk muscles is not clear.

Factors which differed between muscles within an anatomical region, such as tonic *v.* phasic activity, and differences in metabolic rate, were of no importance for the measurements reported in this study. For example, the soleus and the tibialis anterior differ in function and metabolism, but these muscles were essentially identical in their response. It should be noted, however, that these same factors do influence measurements of enzyme

activity (Taskar & Tulpule, 1964; Goldspink & Waterson, 1971; Turner & Fern, 1974; Hansen-Smith, Van Horn & Maksud, 1977). It is of interest that two muscles most known for their tonic activity and high rate of metabolism, i.e. the diaphragm and the soleus, differed in the magnitude of their response to the experimental conditions. Contrary to the proposal by Wechsler (1966), tonic activity by these muscles failed to prevent muscle wasting or even to spare them relative to less-tonically-active muscles.

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