Incorporation of amino acids into soluble and membrane protein fractions of dystrophic hamsters

D. McEwen NICHOLLS,* Robert C. CREASY, Monroe W. CHIN-SEE, James A. CARLISLE,
Angela B. LANGE and Mohammed SALEEM

Department of Biology, York University, Downsview (Toronto), Canada M3J 1P3

(Received 29 January 1980)

The incorporation of labelled leucine was measured in protein fractions of muscle in intact control and dystrophic female hamsters and also in cell-free preparations obtained from these animals. The labelling of the soluble sarcoplasmic protein fraction, the microsomal protein fraction and the sarcolemma protein fraction was increased in the dystrophic hindleg muscle. The specific radioactivities of the sarcolemma protein fraction and other fractions were increased markedly relative to that of free leucine in the dystrophic muscle. In cell-free preparations where ribonuclease effects were avoided, the dystrophic muscle exhibited an increased synthesis of peptide bonds.

Hamsters bearing hereditary myopathy and cardiomyopathy (i.e. dystrophic hamsters) do not exhibit the severe hindleg-muscle wasting nor certain other changes reported for dystrophic mice (Bradley & Jenkison, 1973; Montgomery *et al.*, 1975; Montgomery, 1975).

In the wasted hindlimb of dystrophic mice of the Rej 129 *dy/dy* strain, studies of protein synthesis *in vivo* showed that the incorporation of labelled amino acids into total protein and into soluble protein was not much changed from normal, when the results were corrected for cell size and DNA content (Kitchin & Watts, 1973; Hayashi *et al.*, 1975). In cell-free preparations, however, an increase in ribosomal activity together with a decrease in myosin labelling in the polyribosome fraction has been reported (Nwagwu, 1975; Srivastava, 1972). In our laboratory an inhibitor, acting on the elongation of peptides at a step after aminoacyl-tRNA binding, was detected and purified from the sarcoplasm of dystrophic mouse muscle (Petryshyn & Nicholls, 1976, 1978). It is not known whether such an inhibitor might account for at least a part of the decreased myofibrillar protein content seen in the dystrophic mouse.

Changes in membrane function have been reported in dystrophic hamsters (Sulakhe *et al.*, 1971; Mezon *et al.*, 1974; Dhalla *et al.*, 1975), as well as in dystrophic mice, in dystrophic chickens and in humans with Duchenne dystrophy (see, e.g., Mawatari *et al.*, 1976; Wrogemann & Pena, 1976;

Howland & Iyer, 1977; Duncan, 1978; de Kretser & Livett, 1977; Mrak & Baskin, 1978; Rodan *et al.*, 1974; Sabbadini *et al.*, 1975; Louie & Peck, 1979). However, certain substances such as Ca^{2+} + Mg^{2+} -dependent ATPase, calsequestrin and guanylate cyclase do not appear to be changed (Yap & MacLennan, 1976; Blosser & Appel, 1978).

Whole cell preparations of dystrophic hamster hindleg muscle, incubated *in vitro* with labelled amino acids, exhibited an increased labelling of soluble proteins and of actomyosin at both 60 days and 180–240 days of age; similar changes in the heart and diaphragm occurred in the older age group (Lochner *et al.*, 1971). In addition, the total proteins of the hypertrophied diaphragm of dystrophic hamsters, incubated *in vitro*, exhibited an increase in synthesis and a smaller increase in degradation (Goldspink & Goldspink, 1977). In studying cell-free protein synthesis, however, Bester & Gevers (1973*a,b*) obtained evidence of a defective tRNA, resulting from a 3'-exonuclease activity, which limited cell-free protein synthesis in several muscle types, in brain and in liver of embryonic, 60-day-old and 180-day-old dystrophic hamsters.

Thus it was decided to study intact dystrophic hamsters and to measure the incorporation of labelled amino acids into membrane-bound and soluble protein fractions of muscles and other tissues. The results were compared with those obtained by using cell-free preparations that avoided the influence of the 3'-exonuclease activity. A preliminary report has appeared (Saleem *et al.*, 1978).

* To whom reprint requests should be addressed.

Materials and methods

Chemicals

L-[4,5- ^3H]Leucine (specific radioactivity 40 Ci/mmol), ^3H -labelled L-amino acid mixture and ^{14}C -labelled L-amino acid mixture (specific radioactivity $>50\text{ mCi/mg-atom of carbon}$) were obtained from Amersham Corp., Oakville, Ontario, Canada. Other chemicals were obtained as previously described (Girgis & Nicholls, 1972; Young & Nicholls, 1978).

Animals

Normal (strain RB) and dystrophic (strain BIO 14.6) female Syrian hamsters (145–163 days of age) were obtained from the Trenton Experimental Laboratory Animal Co., Bar Harbor, ME, U.S.A., and were allowed free access to water and food (Teklad Inc., Monmouth, IL, U.S.A.). The food was removed 12 h before animals were killed. When labelled amino acid was administered 12–72 h before killing, food was removed 12 h before tracer administration and also 12 h before killing, to avoid differences in the eating habits of the dystrophic animals compared with controls. For studies *in vitro*, ribosomes were prepared from normal male rats of the Wistar strain (180–200 g) that had free access to water and the same food.

At various times before they were killed, the hamsters received intraperitoneal injections of [^3H]leucine ($1\mu\text{Ci/g body wt.}$). In other experiments 5 or $15\mu\text{Ci}$ of a labelled amino acid mixture/animal was used. On the morning of the experiment the animals were decapitated and the tissues (heart, diaphragm, liver, kidney and hindleg muscle) rapidly removed, dissected free of connective tissue, weighed and then placed in ice-cold buffer.

Preparation of microsomal and postmicrosomal-supernatant fractions

All work was performed on ice at 2–4°C. The tissues were homogenized in a glass homogenizer with 12–15 passes of a Teflon pestle in 4 vol. of Buffer 1, containing 0.25 M-sucrose, 50 mM-Tris/HCl (pH 7.6 at 22°C), 80 mM-KCl, 5 mM-MgCl₂ and 10 mM-2-mercaptoethanol. For muscle, the tissue was treated for 30 s in a mixer (model SDT; Tekmar, Cincinnati, OH, U.S.A.) before the homogenization step. The homogenates were filtered through several layers of gauze to remove debris and then centrifuged at 12000 g for 10 min at 2°C in a Spinco type 40 rotor (r_{av} , 5.9 cm). The resulting mitochondrial pellet was discarded and the postmitochondrial supernatant (S15) was centrifuged at 105000 g for 90 min to sediment the microsomal fraction. The microsomal pellet was washed with Buffer 1 and finally resuspended in this buffer. This preparation was stored at –20°C. For rat liver, the microsomal

pellet was used to prepare preincubated salt-washed ribosomes as described previously (Girgis & Nicholls, 1971).

The postmicrosomal-supernatant fraction (S105) was obtained from the 90 min centrifugation step above and was stored at –20°C or was used for the preparation of the pH 5 supernatant as described previously (Petryshyn & Nicholls, 1976).

Preparation of sarcolemma fractions

The procedure used was a modification of that described by Barchi *et al.* (1977). The muscle was homogenized as described above in Buffer II (0.3 M-sucrose/10 mM-Tris/HCl, pH 7.4) and centrifuged at 12000 g for 10 min. The pellet was resuspended in 10 vol. of Buffer III (0.15 M-sucrose, 5 mM-Tris/HCl, pH 7.4, 50 mM-CaCl₂) and centrifuged at 12000 g for 10 min. The resulting pellet was resuspended in 5 vol. of Buffer IV (1 M-sucrose, 5 mM-Tris/HCl, pH 7.4, 1 mM-CaCl₂) and centrifuged at 12000 g for 10 min. The resulting supernatant was kept. The pellet was resuspended a second time in Buffer IV and treated as before. After a further repeat of this step, the pooled supernatant fractions were adjusted to a final sucrose concentration of 15% (w/v) by the addition of Buffer IV with no sucrose. After centrifuging at 105000 g for 30 min, the supernatant was discarded and the pellet was resuspended in 5 mM-Tris/HCl, pH 7.4, and then layered on a continuous linear sucrose gradient (25–45%) in 5 mM-Tris/HCl, pH 7.4. The gradient was centrifuged at 70000 g for 18 h in a Spinco type SW 25.2 rotor. The A_{280} profile of the gradient showed one protein peak at 24–26% sucrose for both the control and dystrophic preparations. The protein (i.e. sarcolemma fraction) was collected carefully from the layer lying between 22 and 29% sucrose and the fraction was stored at –20°C.

Determination of protein and radioactivity

Protein concentration was measured in duplicate tubes by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard. The concentration of free leucine was measured in the cytosol (S105) fraction, after sulphosalicylic acid precipitation of the protein, by using an amino acid analyser (Dionex, Sunnyvale, CA, U.S.A.). The incorporation of labelled amino acids into peptide was measured in duplicate or triplicate tubes after precipitation of the protein by 10% (w/v) trichloroacetic acid, heating in 5% trichloroacetic acid at 90°C for 30 min, and washing as described by Girgis & Nicholls (1971). A portion of the acid-soluble fraction was used to determine the radioactivity of free leucine. The measured radioactivity may not be entirely in leucine if the same situation exists as reported for mouse brain (Banker & Cotman, 1971). The protein was solubilized in 1.0 ml

of Protosol and transferred with scintillation fluid to scintillation vials for counting (Girgis & Nicholls, 1971). Counting efficiency for ^3H -labelled samples was approx. 40% and for ^{14}C -labelled samples was approx. 85%. Radioactivity was expressed as d.p.m./mg of protein. Points plotted on the graphs are means for duplicate samples of two pairs of hamsters. The standard error for radioactivity was less than 10%. The results shown are representative of those obtained in 20 separate experiments carried out over 12 months with dystrophic hamsters and age-matched control hamsters.

Results

Table 1 shows that there was a small but statistically significant decrease (14%) in the body weight of the dystrophic female hamsters. A group of somewhat older male dystrophic hamsters showed a decrease in body weight, but it was not statistically significant either here (Saleem & Nicholls, 1979) or in the study of Goldspink & Goldspink (1977), where the strain of normal controls that was studied was different (i.e. albino AC strain). A small (10%) but statistically significant decrease in hind-leg-muscle weight was also observed in the female dystrophic hamsters. No significant changes in the percentage dry weight, in the free leucine concentration or in the protein concentrations of the S105 supernatant or microsomal fractions were detected. The free leucine content was higher than that reported for rats (Millward *et al.*, 1976). No statistically significant changes in the weight or protein concentration of the other tissues were found (Table 2). The results for the diaphragm of dystrophic females contrast with the results for dystrophic males, where a marked hypertrophy in the dystrophic animals relative to the (AC-strain) controls was reported (Goldspink & Goldspink, 1977).

The weight of the heart, relative to body weight, in these dystrophic female hamsters was increased by

13%. This increase was less than that observed in dystrophic male mice, where the increase was 25% (Petryshyn *et al.*, 1977).

Fig. 1 shows that the incorporation of labelled leucine *in vivo* into the soluble (S105) protein fraction of the hindleg muscle was markedly greater in the dystrophic animals than in the controls. This was seen from $\frac{1}{2}$ h after administration of leucine up to 3 days after administration of leucine. The

Table 2. *Weight and protein concentration of the postmicrosomal-supernatant fraction of several tissues of dystrophic hamsters*

The tissues were removed from 145–163-day-old female hamsters and the postmitochondrial-supernatant fractions were prepared as described in the Materials and methods section. Values given are means $\pm$ S.E.M. for eight pairs of hamsters. *P* values determined by Student's *t* test were not statistically significant at $P < 0.05$.

Tissue	Weight (g)	
	Control	Dystrophic
Diaphragm	0.547 ± 0.070	0.590 ± 0.073
Heart	0.406 ± 0.028	0.394 ± 0.039
Liver	4.01 ± 0.48	2.81 ± 0.29
Kidney	1.30 ± 0.12	1.17 ± 0.09
	S105 protein concn. (mg/100mg)	
	Control	Dystrophic
Diaphragm	7.94 ± 0.55	6.58 ± 0.70
Heart	7.32 ± 0.46	7.94 ± 0.73
Liver	11.9 ± 1.02	10.5 ± 0.82
Kidney	7.03 ± 0.45	7.41 ± 0.61
	Microsomal protein concn. (mg/100mg)	
	Control	Dystrophic
Diaphragm	0.79 ± 0.10	0.78 ± 0.13
Heart	1.95 ± 0.18	1.94 ± 0.28
Liver	2.06 ± 0.11	2.11 ± 0.19
Kidney	2.02 ± 0.10	2.06 ± 0.10

Table 1. *Weight, leucine and protein concentrations of the postmicrosomal-supernatant and microsomal fractions of the hindleg muscle of dystrophic hamsters*

The hindleg muscle of 145–163-day-old female hamsters was removed and the postmicrosomal-supernatant fraction prepared as described in the Materials and methods section. The leucine and protein concentrations of duplicate samples were determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. for eight pairs of control and dystrophic hamsters. *P* values were by Student's *t* test: N.S., not significant ($P > 0.05$).

	Control	Dystrophic	Decrease (%)	<i>P</i>
Body wt. (g)	96.7 ± 3.67	83.0 ± 2.78	14	<0.01
Muscle wt. (g)	7.73 ± 0.17	6.92 ± 0.25	10	<0.02
Dry wt. (as % of wet wt.)	22.4 ± 1.38	21.0 ± 1.91	—	N.S.
S105 protein concn. (mg/100mg)	8.79 ± 0.48	8.34 ± 0.42	—	N.S.
Microsomal protein concn. (mg/100mg)	2.01 ± 0.27	2.21 ± 0.34	—	N.S.
Free leucine concn. ($\mu\text{mol/g}$)	0.262 ± 0.03	0.303 ± 0.04	—	N.S.

difference was greatest at $\frac{1}{2}$ h, but was not much changed for 2h to 3 days. Experiments carried out with mixtures of labelled amino acids also resulted in greater labelling of the dystrophic-muscle cell fractions at various times after administration.

The labelling of the microsomal protein fraction was approx. 5 times that of the postmicrosomal-supernatant fraction (Fig. 2) and was markedly higher in the dystrophic muscle than in the control

muscle. After a peak of labelling at about 3h, the label was lost gradually from the control muscle and more rapidly from the dystrophic muscle. Similar

Fig. 1. Incorporation of [3 H]leucine into protein in the postmicrosomal-supernatant fraction of hindleg muscle of dystrophic hamsters

[3 H]Leucine was administered intraperitoneally at various times before the experiment ($1\mu\text{Ci/g}$ body wt.). The muscle was homogenized and the labelling of the S105 fraction measured as described in the Materials and methods section. ●, Control; ○, dystrophic hamsters.

Fig. 2. Incorporation of [3 H]leucine into protein in the microsomal fraction of hindleg muscle of dystrophic hamsters

[3 H]Leucine was administered intraperitoneally at various times before the experiment ($1\mu\text{Ci/g}$ body wt.). The muscle was homogenized and the labelling of the protein fraction measured as described in the Materials and methods section. ●, Control; ○, dystrophic hamsters.

Table 3. Specific radioactivity of free leucine and relative incorporation of [3 H]leucine into protein fractions

The specific radioactivity of free leucine and of the protein was determined as described in the Materials and methods section. For protein, the specific radioactivity 2h after [3 H]leucine administration was expressed as a percentage of the specific radioactivity of free leucine. Results are means $\pm$ S.E.M., $n=4$. For free leucine, $P>0.05$ at all times. For protein, increases were statistically significant at $P<0.01$.

Time after injection (h)	$10^{-4} \times \text{Sp. radioactivity (d.p.m./}\mu\text{mol)}$		
	Control	Dystrophic	
0.5	18.3 ± 2.8	19.6 ± 3.2	
1	17.9 ± 2.7	18.1 ± 2.5	
2	18.7 ± 2.8	19.8 ± 3.3	
3	17.3 ± 2.6	14.7 ± 2.4	
24	17.2 ± 2.8	13.9 ± 2.4	
48	14.9 ± 3.0	15.8 ± 2.8	
72	12.8 ± 2.0	11.3 ± 1.8	
Relative specific radioactivity (%)			
Fraction			Increase (%)
	Control	Dystrophic	
Microsomal protein	3.48 ± 0.16	5.96 ± 0.26	71
S105 protein	0.66 ± 0.03	0.97 ± 0.03	47
Sarcolemma protein	0.16 ± 0.01	0.36 ± 0.02	125

results were obtained when labelled amino acid mixtures replaced labelled leucine.

The specific radioactivity of the free leucine in the acid-soluble fraction was studied from 0.5 to 72 h after the injection of [^3H]leucine (Table 3). The results for dystrophic muscle were not significantly different from those for control muscle. At the time of greatest labelling (0.5 h), the specific radioactivity of control muscle was $18(\pm 3) \times 10^4$ d.p.m./ μmol and that of dystrophic muscle was $20(\pm 3) \times 10^4$ d.p.m./ μmol . Previously, in dystrophic diaphragm incubated *in vitro* for 2 h, no change in the specific radioactivity of intracellular tyrosine was detected (Goldspink & Goldspink, 1977).

The yield of protein in the sarcolemma fraction isolated by the method of Barchi *et al.* (1977) was (approx.) 0.10 mg/g of muscle and agrees with the yield obtained by Sulakhe *et al.* (1971) using a LiBr-extraction procedure. However, no decrease in the yield was detected in the 145–163-day-old dystrophic animals used in our studies, in contrast with the decrease reported for 240–270-day-old dystrophic animals (Sulakhe *et al.*, 1971). The incorporation of [^3H]leucine into sarcolemma protein fraction was increased significantly in the dystrophic-muscle preparations, based on either mg of protein (Fig. 3), muscle wet weight (results not shown), or on the specific radioactivity relative to that of free leucine (Table 3).

Table 3 shows that the relative specific radio-

Fig. 3. Incorporation of [^3H]leucine into protein in the sarcolemma fraction of hindleg muscle of dystrophic hamsters

[^3H]Leucine was administered intraperitoneally at various times before the experiment ($1 \mu\text{Ci/g}$ body wt.). The muscle was homogenized and the labelling of the S105 fraction measured as described in the Materials and methods section. ●, Control; ○, dystrophic hamsters.

activities of the microsomal, S105-supernatant and sarcolemma protein fractions 2 h after injection of labelled leucine were increased by 71, 47 and 125% respectively. Since the specific radioactivity of the free leucine of dystrophic muscle was not significantly different from that of control muscle from 0.5 to 3 h after injection, the relative specific radioactivity of the protein fractions was significantly increased throughout this time.

In the diaphragm of the female dystrophic hamsters, a small increase in the labelling of soluble protein was observed up to 72 h after the tracer injection of [^3H]leucine (Fig. 4). These results for soluble protein resemble those for soluble protein and for actomyosin after a short incubation of whole-cell preparations from diaphragm (Lochner *et al.*, 1971) and for total protein of diaphragm (Goldspink & Goldspink, 1977). The labelling pattern of the microsomal protein of the diaphragm resembled that of the soluble protein, except that the labelling was higher than for the soluble protein.

The labelling of soluble and microsomal proteins was studied in the liver of the female dystrophic hamsters and was found to be greater in the dystrophic animals at $\frac{1}{2}$ and 2 h, but not at later times, after the injection (Fig. 5). Alterations in liver protein synthesis in dystrophic hamsters have previously been reported only for newborn animals (Wrogemann *et al.*, 1977). In the liver of the adult females studied here, the increased short-term incorporation was observed not only after administration of labelled leucine but also after the administration of a mixture of labelled amino acids.

Protein fractions of the kidney and heart of the

Fig. 4. Incorporation of [^3H]leucine into protein in the postmicrosomal-supernatant fraction of diaphragm of dystrophic hamsters

[^3H]Leucine was administered intraperitoneally at various times before the experiment ($1 \mu\text{Ci/g}$ body wt.). The muscle was homogenized and the labelling of the S105 fraction measured as described in the Materials and methods section. ●, Control; ○, dystrophic hamsters.

Fig. 5. Incorporation of [^3H]leucine into protein in the postmicrosomal-supernatant fraction of liver of dystrophic hamsters

[^3H]Leucine was administered intraperitoneally at various times before the experiment ($1\mu\text{Ci/g}$ body wt.). The muscle was homogenized and the labelling of the S105 fraction measured as described in the Materials and methods section. ●, Control; ○, dystrophic hamsters.

Table 4. Incorporation of [^{14}C]phenylalanyl-tRNA into peptide *in vitro* in the soluble fraction of leg muscle of dystrophic hamsters

The pH5-supernatant fraction was prepared as described previously (Petryshyn & Nicholls, 1976). Incubation was for 10 min at 37°C in duplicate tubes with various concentrations of supernatant fraction in 0.40 ml of medium containing 50 mM-Tris/HCl (pH 7.8 at 25°C), 80 mM-KCl, 6 mM-MgCl₂, 10 mM-2-mercaptoethanol, 0.4 mM-GTP, [^{14}C]phenylalanyl-tRNA (13 700 d.p.m., $33\mu\text{g}$ of tRNA), excess salt-washed rat liver ribosomes ($50\mu\text{g}$ of RNA) and $100\mu\text{g}$ of poly(U). The incubation was terminated, and the protein precipitated, washed and counted for radioactivity as described in the Materials and methods section. Values were corrected for unincubated blanks. Similar results were obtained in ten separate experiments, each using either female or male age-matched control and dystrophic hamsters.

Supernatant concn. (μg of protein/tube)	$10^{-3} \times \text{Incorporation}$ (d.p.m./mg of RNA)		Increase (%)
	Control	Dystrophic	
100	10	24	140
200	25	40	60
400	57	80	40
600	83	95	14
1200	86	92	7

dystrophic female hamster, unlike those of the liver, did not exhibit an increased labelling. The result for the soluble protein of heart muscle agrees with that of Lochner *et al.* (1971), since the incorporation that they observed with leucine was not significantly different statistically in the myopathic animals. Their results obtained using lysine, instead of leucine, showed a significant increase in the older dystrophic animals, though not in the younger group (60–90 days of age).

In order to avoid the results of any liberated nuclease activity in cell-free preparations, we examined the capacity of the pH 5-supernatant fraction for protein synthesis by using previously charged [^{14}C]phenylalanyl-tRNA as labelled precursor. The incorporation was markedly higher in the dystrophic-muscle preparations when limiting amounts of the pH 5-supernatant fraction, containing elongation factors 1 and 2, were present. When excess amounts of the pH 5-supernatant fraction were added to the incubation mixture, the incorporation was the same in the dystrophic-muscle preparation as it was in the control preparation (Table 4). When a postmitochondrial-supernatant (S15) preparation was used to provide uncharged tRNA species and elongation factors, the incorporation of labelled [^{14}C]phenylalanine was no longer elevated in the dystrophic-muscle preparations (Table 5), confirming the observation of Bester & Gevers (1973a,b).

Table 5. Incorporation of [^{14}C]phenylalanine into peptide *in vitro* in the postmitochondrial-supernatant fraction of leg muscle of dystrophic hamsters

The postmitochondrial-supernatant fraction (S15) was prepared as described in the Materials and methods section. Incubation was for 10 min at 37°C in duplicate tubes with various concentrations of S15 fraction in 0.40 ml of medium containing 50 mM-Tris/HCl (pH 7.8 at 25°C), 80 mM-KCl, 6 mM-MgCl₂, 10 mM-2-mercaptoethanol, 0.4 mM-GTP and $0.25\mu\text{Ci}$ of [^{14}C]phenylalanine. Endogenous mRNA and ribosomes were present in the S15 fraction. The incubation was terminated, and the protein precipitated, washed and counted for radioactivity as described in the Materials and methods section. Values were corrected for unincubated blanks.

S15 concentration (μg of protein/tube)	$10^{-3} \times \text{Incorporation}$ (d.p.m./mg of RNA)		Increase (%)
	Control	Dystrophic	
50	2.0	2.2	10
100	2.6	2.7	4
200	2.8	3.0	7
400	3.1	3.2	3
600	3.2	3.2	Nil

Discussion

The changes observed here in the incorporation of amino acids into proteins in dystrophic hamsters *in vivo* and *in vitro* further underline the difference between the hamster and mouse muscular dystrophies. In the dystrophic mouse, the incorporation of labelled amino acids into total protein and into soluble protein of the wasted hindleg muscle studied in the intact animal is not changed on a cell-size or DNA basis (Kitchin & Watts, 1973; Hayashi *et al.*, 1975). However, the capacity of the supernatant fraction for the elongation of peptides is markedly decreased in older animals of both sexes, owing to a proteinaceous inhibitory activity (Petryshyn & Nicholls, 1976, 1978). The present studies on intact dystrophic hamsters show that there is an increased incorporation of labelled amino acids into the soluble protein fraction and also into the microsomal and sarcolemma protein fractions of the hindleg muscle. Moreover, the activity of the soluble supernatant fraction, which contains most of the elongation factors 1 and 2 for peptide synthesis, is markedly increased *in vitro*, provided that the 3'-exonuclease activity described by Bester & Gevers (1973*a,b*) is avoided and that intact exogenous charged tRNA is supplied to the cell-free system (Tables 4 and 5). Additional studies of this increased elongating activity of the soluble supernatant fraction have revealed that it is present in both male and female dystrophic hamsters from 30 to 190 days of age and can be attributed to an increased aminoacyl-tRNA-binding activity (Saleem & Nicholls, 1979). No such change was detected in liver or brain of the dystrophic animals.

The present results with intact animals are in agreement with those obtained when muscle slices or diaphragm were prepared from dystrophic hamsters and incubated *in vitro* in the presence of labelled precursors (Lochner *et al.*, 1971; Goldspink & Goldspink, 1977). Our results extend these studies to the microsomal protein fraction and the sarcolemma protein fraction as well as to cell-free systems from muscle.

A microsomal fraction of leg muscle has been used by others to prepare sarcoplasmic reticulum and free and bound polyribosomes. The results show that Ca^{2+} -sensitive ATPase and calsequestrin are synthesized on the membrane-bound polyribosomes non-co-ordinately and are inserted stepwise into the sarcoplasmic reticulum (Zubrzycka & MacLennan, 1976; Greenway & MacLennan, 1978; Boland *et al.*, 1974; Chyn *et al.*, 1979). The activities of these substances do not appear to be changed in dystrophic chicks (Yap & MacLennan, 1976). The marked increase in the labelling of the microsomal fraction *in vivo* in the diseased animals studied here suggests that the synthesis and activity of Ca^{2+} -

stimulated ATPase and calsequestrin of this fraction should be studied in dystrophic hamsters.

The increased labelling in the muscle fractions of dystrophic hamsters contrasts with the absence of a change in kidney and with the transient increase in the soluble and microsomal fractions of the liver of dystrophic hamsters. In newborn hamsters, Wrogemann *et al.* (1977) studied the incorporation *in vivo* of labelled leucine into the soluble proteins of liver and found an increased synthesis of haemoglobin in dystrophic hamsters younger than 3 days and a decreased synthesis in 6-day-old dystrophic hamsters. The significance of the increased labelling in the liver fractions of the adult dystrophic hamsters here is not clear, especially since no parallel increase was observed in the activity of the postmicrosomal-supernatant preparations tested *in vitro* (Saleem & Nicholls, 1979).

Estimating the rate of synthesis of proteins *in vitro* requires measurement of the specific radioactivity of the product at a time when it is not being degraded or removed and also of the specific radioactivity of the precursor amino acid, or, better, aminoacyl-tRNA at the site of synthesis, i.e. the ribosomes (see, e.g., Peters & Peters, 1972; Waterlow *et al.*, 1978). In muscle, as well as other tissues, there is conflicting evidence about the location of the amino acid precursor pool, but Li *et al.* (1973) have provided evidence that the specific radioactivity of intracellular amino acid can be used in many circumstances without appreciable error. In the present experiments we have used the specific radioactivity of free leucine as an estimate of intracellular leucine. Extracellular leucine represents only about 6–10% of certain rat muscles incubated *in vitro* (Goldspink, 1976). Since the specific radioactivity of the microsomal protein, soluble supernatant protein and sarcolemma protein fractions, relative to the specific radioactivity of free leucine, were increased from 0.5 to 3 h after tracer administration, it appears that protein-synthesis rates of these fractions are elevated in dystrophic muscle, especially the membrane fractions. The results obtained with cell-free preparations of dystrophic hamster muscle (Table 4; Saleem & Nicholls, 1979) provide further evidence that there is an increase in protein synthesis.

Millward *et al.* (1976) have provided evidence that muscle protein mass in normal animals is regulated primarily through alterations in protein synthesis rather than protein degradation, although increased rates of protein breakdown are a necessary accompaniment to muscle growth, resulting from the way in which myofibrils proliferate (Millward *et al.*, 1975). In the dystrophic hamster, hypertrophy and regeneration of some muscle fibres and marked atrophy of other fibres is an important histological feature (Homburger *et al.*, 1966;

Johnson & Pearse, 1968) and suggests that both synthesis and degradation may change markedly. In the dystrophic hamsters studied by Goldspink & Goldspink (1977), the hypertrophied diaphragm exhibited large increases in protein synthesis and smaller increases in degradation. They proposed that the increased synthesis rates might be due to elevated activities of enzymes of translation and of ribosomes. Evidence for the former has been reported in dystrophic chicks (Weinstock & Markiewicz, 1974), in the heart of dystrophic mice (Petryshyn *et al.*, 1977), and in dystrophic-hamster leg muscle in our studies here and elsewhere (Saleem & Nicholls, 1979).

This investigation was supported by the Muscular Dystrophy Association of Canada and the National Research Council of Canada. M. S. held a Postdoctoral Fellowship and R. C. C. and M. W. C.-S. Predoctoral Fellowships of the Muscular Dystrophy Association of Canada.

References

- Banker, G. & Cotman, C. W. (1971) *Arch. Biochem. Biophys.* **142**, 565–573
- Barchi, R. L., Bonilla, E. & Wong, M. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 34–38
- Bester, A. J. & Gevers, W. (1973a) *Biochem. J.* **132**, 193–201
- Bester, A. J. & Gevers, W. (1973b) *Biochem. J.* **132**, 203–214
- Blosser, J. C. & Appel, S. H. (1978) *J. Biol. Chem.* **253**, 3088–3093
- Boland, R., Martonosi, A. & Tillack, T. W. (1974) *J. Biol. Chem.* **249**, 612–623
- Bradley, W. G. & Jenkinson, M. (1973) *J. Neurol. Sci.* **18**, 227–247
- Chyn, T. L., Martonosi, A. N., Morimoto, T. & Sabatini, D. D. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1241–1245
- de Kretser, T. A. & Livett, B. G. (1977) *Biochem. J.* **168**, 229–237
- Dhalla, N. S., Singh, A., Lee, S. L., Anand, M. B., Bernatsky, A. M. & Jasmin, G. (1975) *Clin. Sci. Mol. Med.* **49**, 359–368
- Duncan, C. J. (1978) *Experientia* **34**, 1531–1672
- Girgis, G. R. & Nicholls, D. M. (1971) *Biochim. Biophys. Acta* **247**, 335–347
- Girgis, G. R. & Nicholls, D. M. (1972) *Biochim. Biophys. Acta* **269**, 465–476
- Goldspink, D. F. (1976) *Biochem. J.* **156**, 71–80
- Goldspink, D. F. & Goldspink, G. (1977) *Biochem. J.* **162**, 191–194
- Greenway, D. C. & MacLennan, D. H. (1978) *Can. J. Biochem.* **56**, 452–456
- Hayashi, Y., Suzuki, H. O. & Totsuka, T. (1975) *J. Biochem. (Tokyo)* **77**, 761–768
- Homburger, F., Nixon, C. W., Eppenberger, M. & Baker, J. R. (1966) *Ann. N.Y. Acad. Sci.* **138**, 14–27
- Howland, J. L. & Iyer, S. L. (1977) *Science* **198**, 309–310
- Johnson, M. & Pearse, A. G. E. (1968) *Proc. Symp. Res. Muscular Dystrophy 4th*, pp. 171–183
- Kitchin, S. E. & Watts, D. C. (1973) *Biochem. J.* **136**, 1017–1028
- Li, J. B., Fulks, R. M. & Goldberg, A. L. (1973) *J. Biol. Chem.* **248**, 7272–7275
- Lochner, A., Brink, A. & Brink, A. J. (1971) *Clin. Sci.* **40**, 89–94
- Louie, D. D. & Peck, E. J. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 358
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mawatari, S., Schonberg, M. & Olarte, M. (1976) *Arch. Neurol. (Chicago)* **33**, 489–493
- Mezon, B. J., Wrogemann, K. & Blanchaer, M. C. (1974) *Can. J. Biochem.* **52**, 1024–1032
- Millward, D. J., Garlick, P. J., Stewart, R. J. C., Nnanyelugo, D. O. & Waterlow, J. C. (1975) *Biochem. J.* **150**, 235–243
- Millward, D. J., Garlick, P. J., Nnanyelugo, D. O. & Waterlow, J. C. (1976) *Biochem. J.* **156**, 185–188
- Montgomery, A. (1975) *Exp. Neurol.* **46**, 87–102
- Montgomery, A., Park, D. C. & Pennington, R. J. (1975) *Comp. Biochem. Physiol. B* **49**, 357–392
- Mrak, R. E. & Baskin, R. J. (1978) *Biochem. Med.* **19**, 47–70
- Nwagwu, M. (1975) *Eur. J. Biochem.* **56**, 123–127
- Peters, T., Jr. & Peters, J. C. (1972) *J. Biol. Chem.* **247**, 3858–3863
- Petryshyn, R. A. & Nicholls, D. M. (1976) *Biochim. Biophys. Acta* **435**, 391–404
- Petryshyn, R. A. & Nicholls, D. M. (1978) *Biochem. J.* **176**, 907–917
- Petryshyn, R. A., Creasy, R. C. & Nicholls, D. M. (1977) *Biochem. Med.* **18**, 139–151
- Rodan, S. B., Hintz, R. L., Sha'afi, R. I. & Rodan, G. A. (1974) *Nature (London)* **252**, 589–591
- Sabbadini, R., Scales, D. & Inesi, G. (1975) *FEBS Lett.* **54**, 8–12
- Saleem, M. & Nicholls, D. M. (1979) *Biochem. J.* **180**, 51–58
- Saleem, M., Creasy, R. C. & Nicholls, D. M. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 1334
- Srivastava, U. (1972) *Can. J. Biochem.* **50**, 409–415
- Sulakhe, P. V., Fedelsove, M., McNamara, D. B. & Dhalla, N. S. (1971) *Biochem. Biophys. Res. Commun.* **42**, 793–800
- Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978) *Protein Turnover in Mammalian Tissues and in the Whole Body*, pp. 1–164, North-Holland, Amsterdam, Oxford and New York
- Weinstock, I. M. & Markiewicz, L. (1974) *Biochim. Biophys. Acta* **374**, 197–206
- Wrogemann, K. & Pena, S. D. J. (1976) *Lancet* **i**, 672–673
- Wrogemann, K., Quillam, N. M., Nylen, E. G., John, M. D. & Clancy, J. F., Jr. (1977) *Can. J. Biochem.* **55**, 894–900
- Yap, J. L. & MacLennan, D. H. (1976) *Can. J. Biochem.* **54**, 670–673
- Young, E. T. & Nicholls, D. M. (1978) *Biochem. J.* **172**, 479–486
- Zubrzycka, E. & MacLennan, D. H. (1976) *J. Biol. Chem.* **251**, 7733–7738