Cell-Free Protein Synthesis in Heart and Skeletal Muscles from Polymyopathic Hamsters

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1. Cell-free protein synthesis was studied in striated and smooth muscles in an attempt to elucidate the primary genetic defect in polymyopathic hamsters. 2. When washed membrane-free polyribosomes from myopathic and control heart muscle were individually recombined with pH5 enzymes from both types of animals, the pH5 enzymes from myopathic muscle were less active in polypeptide synthesis than those from controls, irrespective of the source of polyribosomes. 3. The same defect was present in skeletal-muscle preparations. 4. Both the initial rate and the maximum extent of incorporation were affected in the defective preparations from myopathic muscle. 5. Concentration differences, with respect to total protein and RNA, were not responsible. 6. Preincubation of the pH5 enzymes resulted in a greater degree of inhibition. 7. The defect in the pH5 enzymes from myopathic muscle was also expressed in poly(U)-directed polyphenylalanine synthesis. 8. Acid proteinase activity in extracts of control and myopathic muscle was the same but general ribonuclease activity in the latter extracts was higher. 9. The defect was also present when both types of pH5 enzymes were prepared in the presence of the ribonuclease-adsorbent bentonite. 10. pH5 enzymes from uterine smooth muscle, brains and livers of myopathic animals were similarly affected in homologous and heterologous combinations. 11. It is concluded that the general tissue defect is both qualitative and quantitative in nature, implying that there is a shortage of some essential soluble component in the pH5 fraction which is accompanied by the presence of an altered substituent. This prevents the attainment of extents of polypeptide synthesis *in vitro* obtained in control extracts from unaffected animals.

An inbred polymyopathic strain of Syrian hamsters (*Mesocricetus auratus*) has been under intensive study since its initial characterization by Homburger *et al.* (1962a). It has been regarded with some justification as an experimentally accessible model for muscular dystrophies and for chronic congestive heart failure in general (Bajusz, 1969). The disease appears to be genetically determined as an autosomal recessive trait (Homburger *et al.*, 1962b). Thus far, only striated muscles have been believed to be primarily affected in these animals, with secondary lesions developing in other organs as a result of defective performance of the heart and skeletal muscles. This does not necessarily imply that the actual genetic lesion is restricted in its expression to differentiated muscle cells, but simply that the most significant effects are experienced there as regards physiological functions. Detailed histopathological or biochemical studies of tissues other than striated muscles, at an early stage of the disease, have not been reported.

The elucidation of the primary genetic defect in these hamsters is important as the first step in a de-

tailed study of the sequence of events, in molecular terms, leading to the severe morphological and functional impairment. Preliminary electron-microscopic studies have suggested that heart-muscle cells are poorly developed at birth in affected animals (Bajusz *et al.*, 1969), but no detailed morphological study is available.

We have studied *in vitro* the synthesis of proteins in heart- and skeletal-muscle preparations from both these hamsters and from control animals, in an attempt to detect the primary genetic abnormality and its biochemical sequelae. Cell-free protein synthesis by smooth muscle, liver and brain fractions has also been investigated for purposes of comparison.

Materials and Methods

Animals

Syrian hamsters (strain BIO 14.6) were imported from the Bio-Research Institute, Boston, Mass., U.S.A. and maintained by random in-breeding in our

hamster colony, in parallel with control hamsters from the London School of Hygiene. They were given EPOL hamster pellets (Vereeniging Consolidated Mills, Cape Town, South Africa) *ad libitum*.

Scintillation chemicals

Fluor alloy obtained from Beckman Instruments, Cape Town, South Africa, was diluted with toluene (chromatography grade) to a 10% (v/v) solution, containing 0.6% 2,5-diphenyloxazole and 0.1% 1,4-bis-(5-phenyloxazol-2-yl)benzene.

Chemicals

ATP, GTP (disodium salts), phosphoenolpyruvate (tricyclohexylammonium salt) and pyruvate kinase were obtained from C.F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. Yeast RNA, bovine serum albumin, sucrose (ribonuclease-free), poly(U) (ammonium salt) and bentonite were obtained from Miles-Seravac, Cape Town, South Africa. Sodium deoxycholate was a product of E. Merck, Darmstadt, Germany. Sephadex G-25 (medium grade) was obtained from Pharmacia, Uppsala, Sweden. L-3-[ring-4-³H]Phenylalanine (20 Ci/mmol) and U-¹⁴C-labelled protein hydrolysate (57 mCi/mg-atom of C) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

Preparation of subcellular fractions

After decapitation of hamsters, the hearts and skeletal muscles (limbs and pectoral area) were removed and dissected free of connective tissue. Homogenates were prepared in medium A, containing (final concns.): 0.25 M-sucrose, 10 mM-MgCl₂, 80 mM-KCl and 50 mM-Tris-HCl buffer, pH 7.8. All procedures were carried out at 0–4°C.

(1) *Polyribosomes*. Polyribosomes from hearts, skeletal muscles and uteri were prepared by the method of Florini & Breuer (1966), with minor modifications. Although the yield of polyribosomes was decreased by the use of a relatively low concentration of KCl, contamination by myosin was also decreased to manageable proportions. Further, Heywood *et al.* (1968) have shown that all classes of polyribosomes co-precipitate randomly with myosin under these conditions, indicating that the polyribosomes prepared were representative of all the species present in the homogenate. Tissues were homogenized in 2 vol. of medium A for two separate periods, each of 15 s, with a chilled Ultra-Turrax homogenizer. The homogenates were filtered through a nylon net (no. 6 mesh) and centrifuged at 10 000g for 15 min in a Sorvall refrigerated centrifuge, to remove all debris, nuclei and mitochondria. Since with skeletal-muscle preparations a heavy fat layer collected at the top, the 10 000g supernatant was filtered again through a

nylon net (no. 9 mesh) and finally centrifuged at 105 000g_{av.} for 120 min in a Spinco Ti 60 rotor to collect the microsomal pellet. The latter was suspended, by using three passes of a loose-fitting Dounce homogenizer, in a medium prepared by adding 3.0 ml of 2.5 M-KCl in 10 mM-MgCl₂ to every 9.75 ml of medium A. The homogenate was treated with a 10% (w/v) solution of sodium deoxycholate to give a final concentration of 1.0% (w/v). After 10 min on ice, the sodium deoxycholate-treated suspension (20.0 ml) was layered on to a 14.0 ml 'cushion' of 1.0 M-sucrose in medium A, and centrifuged for 150 min at 105 000g_{av.} to precipitate the washed polyribosomes. The ribosomal pellet was suspended in medium A and left overnight at 0°C. Denatured and undissolved material was then removed by centrifugation at 1400g for 10 min. The polyribosome solution gave an E_{260}/E_{280} ratio of 1.85.

(2) *pH5 enzyme fractions*. Hamster hearts and skeletal muscles were homogenized in 2 vol. of medium A as described above, and the postmitochondrial supernatant was prepared by centrifugation at 10 000g_{av.} for 15 min in a Sorvall refrigerated centrifuge. This solution was then centrifuged in a Ti 60 rotor (Beckman model L4 ultracentrifuge) at 105 000g_{av.} for 120 min. The upper two-thirds of the resulting supernatant was diluted with 2 vol. of cold water and the pH was adjusted to 5.1 by addition of 1.0 M-acetic acid, with constant stirring. The suspension was left on ice for a further 15 min and the precipitate collected by centrifugation at 10 000g for 15 min. The precipitate was washed once and finally resuspended in medium A, by using a loose-fitting Dounce homogenizer. Denatured insoluble material was removed by centrifugation, and the pH 5 enzyme fractions were stored at –180°C until used. Repeated freezing and thawing over 14 days did not cause loss of activity.

(3) *High-speed-supernatant fractions (S100)*. The postmitochondrial supernatant was centrifuged at 105 000g_{av.} for 120 min as described above. Small quantities of the supernatant (1.0 ml) were passed through columns (1 cm × 30 cm; void volume 5.5 ml) of Sephadex G-25 (fine grade), previously equilibrated with medium A, and eluted with the same buffer before storage at very low temperatures as described above.

(4) *Liver and brain polyribosomes and pH5 fractions*. These were prepared from control and myopathic hamsters by the methods of Hawtrey & Nourse (1966), except that the final precipitates were dissolved in medium A.

Incorporation of labelled amino acids into protein

Two procedures were used to measure the incorporation of labelled amino acids into proteins. Reproducibility and counting efficiency were the

same. In the first, the reaction medium used for incorporation studies contained (final concns.) in 1.2ml: 40mM-Tris-HCl buffer, pH 7.8, 150mM-KCl, 6mM-MgCl₂, 0.2M-sucrose, 1.6mM-ATP, 0.6mM-GTP, 5.0mM-phosphoenolpyruvate, 20μg of pyruvate kinase, 10mM-2-mercaptoethanol, ¹⁴C-labelled protein hydrolysate (1.25μCi) plus the components given in the legends to the Tables and Figures. Incubations were carried out under aerobic conditions at 37°C for 30min or for time-periods described in the legends to the Tables and Figures. The reaction was terminated by the addition of 4.0ml of ice-cold 5% (w/v) trichloroacetic acid, the samples were left on ice for 30min, heated for 20min at 90°C, and again cooled on ice for 20min. The protein precipitates were collected on Millipore filters (HA; pore size 0.45μm), and washed with 20ml each of cold 5% (w/v) trichloroacetic acid, ethanol-diethyl ether (1:1, v/v) and ether. The filters were dried before being placed in counting vials with 5 ml of scintillation fluid. Radioactivity was determined in a liquid-scintillation spectrometer (Packard Tri-Carb model 525). In the second procedure, the more convenient method of Mans & Novelli (1961) was used. The final reaction volume was 0.25ml with identical reaction concentrations as described above. Two Whatman 3MM filter papers (diam. 2.5cm) per incubation mixture were used (100μl/filter). All tests were carried out in triplicate and results never differed by more than 5%.

Incorporation of labelled phenylalanine into polypeptide, with poly(U) as template

The reaction medium used contained in 0.20ml: 40mM-Tris-HCl buffer, pH 7.8, 150mM-KCl, 6mM-MgCl₂, 0.2M-sucrose, 1.6mM-ATP, 0.6mM-GTP, 5.0mM-phosphoenolpyruvate, 10μg of pyruvate kinase, 10mM-2-mercaptoethanol, pH5 enzyme (0.5mg of RNA), polyribosomes (1.0mg of RNA) and S100 fraction (0.5mg of protein). The reaction mixture was preincubated for 60min at 37°C to allow polyribosomal 'run-off' from most of the endogenous mRNA and to release free 80S monoribosomes. After the preincubation, [³H]phenylalanine (2.5μCi) and 70μg of poly(U), suspended in 1.0M-MgCl₂, were added to give a total reaction volume of 0.25ml and a final Mg²⁺ concentration of 15mM. Blanks to which poly(U) was not added were incubated in each case. Incubation was carried out for a further 30min at 37°C and terminated by absorption of the mixtures on Whatman 3MM filter papers, which were further processed as described above.

Assay of general ribonuclease activity in pH5 enzyme fractions

The method of Herrington & Hawtrey (1969) was used for the determination of nuclease activity in

pH5 enzyme fractions. Yeast RNA (previously dialysed against four changes of 1000 vol. of water for 24 h to remove free nucleotides) was used as substrate. Tubes containing 1.0ml of pH5 enzyme (60mg of protein), 0.8ml of medium A and 0.2ml of RNA substrate (2mg) were incubated at 37°C. At selected times, 0.5ml samples were removed and placed in tubes kept on ice and containing 5.0ml of 0.5M-HClO₄. After 30min at 0°C, the RNA and protein were collected by centrifugation at 1400g for 10min. The supernatant was assayed for liberated nucleotides by measuring the increase in *E*₂₆₀. Tubes containing no pH5 enzyme were included for analysis at the same selected times during incubation, to correct for RNA breakdown occurring in the absence of added enzyme. Nuclease activity was expressed as increased *E*₂₆₀/mg of pH5 enzyme protein.

Acid proteinase activity in pH5 enzyme preparations

This activity was measured by the method of Barrett (1967). The denatured bovine haemoglobin substrate was in great excess over the endogenous pH5 proteins in solution. Enzyme activity was expressed as increased *E*₂₈₀/mg of pH5 enzyme protein.

Determination of RNA and protein

Protein in all preparations was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. The concentrations of RNA in pH5 enzyme fractions were determined by the orcinol method (Mejbaum, 1939) with hydrolysed yeast RNA as standard.

Results

Effectiveness in protein synthesis of pH5 enzyme fractions from myopathic and control hamster striated muscles

When washed membrane-free polyribosomes from myopathic and control heart muscle of identical RNA content, were recombined with pH5 enzyme preparations from both types of animals [RNA content also standardized (Table 1)], the system from myopathic animals was less active than the control in polypeptide synthesis directed by endogenous mRNA (Table 2). Diminished protein synthesis catalysed by polyribosomes from control hamster heart muscle was also obtained by the substitution of pH5 enzyme from myopathic muscle for that prepared from controls. This defect was thus present irrespective of the source of polyribosomes. It could be demonstrated in embryonic hearts, as well as in those of animals aged 2 months and 6 months. The differences were smaller with decreasing age, however, but remained highly significant.

Table 1. *RNA and protein analysis of pH5 enzymes prepared from myopathic and control hamster skeletal muscles*

A pH5 enzyme preparation was diluted to contain identical E_{260} units. For further details see the text.

Source of pH5 enzyme	E_{260} (units/ml)	E_{280} (units/ml)	E_{260}/E_{280}	RNA (mg/ml)	Protein (mg/ml)	RNA/protein ratio
Myopathic	12.40	10.16	1.22	8.72	6.95	1.25
Control	12.40	10.24	1.22	8.70	6.92	1.26

Table 2. *Effectiveness of pH5 enzymes from heart and skeletal muscle in amino acid incorporation by homologous polyribosomes*

The otherwise standard incubation mixture (see the text) contained (in 0.25 ml): pH5 enzyme (0.5 mg of RNA) and polyribosomes (0.8 mg of RNA) in the case of systems derived from hearts, and pH5 enzyme (0.7 mg of RNA) and polyribosomes (1.0 mg of RNA) in those prepared from skeletal muscles. Similar results were obtained in over 20 other experiments.

Age of animals	Incubation system		Radioactivity incorporated (c.p.m.)	
	Polyribosomes	pH5 enzyme	Heart muscle	Skeletal muscle
Embryonic	Control	Control	20050	18850
	Control	Myopathic	16807	15730
	Myopathic	Myopathic	16789	15742
	Myopathic	Control	19917	18817
2 months	Control	Control	19500	17273
	Control	Myopathic	14616	12804
	Myopathic	Myopathic	14579	12921
	Myopathic	Control	18569	17140
6 months	Control	Control	17400	16202
	Control	Myopathic	11379	11343
	Myopathic	Myopathic	11936	11490
	Myopathic	Control	17860	15870

In skeletal-muscle preparations, a similar defect in the pH5 enzyme fractions from myopathic animals was demonstrable (Table 2). In very young animals, more of this tissue was available, and it was therefore preferentially used in certain experiments as a model for protein synthesis in muscle tissues.

To determine whether these differences were influenced by the time-course of events, parallel incubations of both systems were carried out for selected periods (Fig. 1).

After an initial rapid increase of incorporation in both systems, this decreased to zero after 40 min. The rate of incorporation in the system from myopathic animals, however, was definitely slower and reached a lower plateau value, indicating that the observed impairment affected both the initial rate of incorporation and the extent thereof. The negligible incorporation after 40 min may be ascribed to 'run-off' of ribosomes from mRNA molecules, in the absence of further reinitiation.

To ascertain how variations in the concentrations

of polyribosomes and pH5 enzymes might affect the incorporation of labelled amino acids into polypeptides, the polyribosome concentrations in systems from both myopathic and control animals were kept constant and the concentrations of pH5 enzyme varied (Fig. 2a); Fig. 2(b) shows the results when the pH5 enzyme concentration was kept constant and incubated with increasing amounts of polyribosomes.

Increased concentrations of polyribosomes or pH5 enzyme could not eliminate the defect in myopathic animals, indicating that the latter was not simply quantitative but also qualitative in nature. Preincubation at 37°C of the pH5 enzyme fractions from both myopathic and control skeletal muscles resulted in decreased amino acid incorporation into protein by polyribosomes from both control and myopathic muscles (Table 3); the effect was greater in the defective fraction.

To determine whether this general pH5 enzyme defect could also be expressed in a much more limited

and well-defined system, poly(U)-directed polymerization of [¹⁴C]phenylalanine was studied. Here, initiation of peptide-bond formation could be

assumed to be non-enzymic at the high Mg²⁺ concentration used (15mM) (Stanley *et al.*, 1966) and only one tRNA (for phenylalanine) would be required. To study the activity of polyribosomes and pH5 enzyme fractions from control and myopathic animals, with identical unrelated combination partners, polyribosomes and pH5 enzymes were prepared from rat liver and combined with the separate fractions from both types of hamsters in all possible combinations.

The pH5 enzymes from both heart and skeletal

Fig. 1. Time-course of amino acid incorporation into protein by polyribosomes incubated with pH5 enzyme, both from control skeletal muscles (○) and by polyribosomes with pH5 enzymes, both of myopathic origin (●)

The reaction conditions are described in the Materials and Methods section.

Table 3. Effect of preincubation of pH5 enzyme on protein synthesis by polyribosomes

pH5 enzyme preparations (0.2mg of RNA) from both myopathic and control skeletal muscles were preincubated for 30min at 37°C before being added to a complete polypeptide-synthesizing system. Reaction conditions are described in the Materials and Methods section. Incubations were carried out at 37°C for 30min.

Incubation system		Radioactivity incorporated (c.p.m.)
Polyribosomes	pH5 enzyme	
Control	Control	1280
Control	*Control	1025
Myopathic	Myopathic	821
Myopathic	*Myopathic	572

* pH5 enzymes preincubated.

Fig. 2. Limitation of amino acid incorporation by polyribosome or pH5 enzyme concentration

(a) Increasing concentrations of pH5 enzyme from myopathic (●) and control (○) skeletal muscles were incubated with fixed amounts of homologous polyribosomes from both sources (0.6 mg of RNA). (b) ¹⁴C-labelled amino acid incorporation by pH5 enzymes (0.6mg of RNA) from control and myopathic animals, with increasing concentrations of homologous polyribosomes from control (○) and myopathic (●) animals. Conditions for the incubation mixture are described in the Materials and Methods section.

Table 4. *Poly(U)-stimulated polyphenylalanine synthesis in a cell-free system, with components from both heart and skeletal muscles*

Reaction conditions were as described in the Materials and Methods section. Incubations were carried out at 37°C. Radioactivity incorporated in the absence of added poly(U) never exceeded 1700 c.p.m.

Incubation system		Radioactivity incorporated (c.p.m.)
Polyribosomes	pH 5 enzyme	
Rat liver	Control skeletal	45175
Rat liver	Myopathic skeletal	31817
Rat liver	Control heart	42849
Rat liver	Myopathic heart	26877
pH 5 enzyme	Polyribosomes	
Rat liver	Control skeletal	97832
Rat liver	Myopathic skeletal	112303
Rat liver	Control heart	28763
Rat liver	Myopathic heart	37725

muscles of myopathic animals were less active than their control partners in poly(U)-directed polyphenylalanine synthesis, when both were combined with the same liver polyribosomes (Table 4). However, when the two types of muscle polyribosomes were incubated with the same pH 5 enzyme from rat livers, the polyribosomes from myopathic animals were more active than those from controls. Thus, the pH 5 enzyme defect observed also affects poly(U)-directed phenylalanine incorporation at high Mg^{2+} concentrations, where initiation of polypeptide synthesis by the initiation-codon-specific fMet-tRNA mechanism is not required. The initiation factors for protein synthesis are believed to be largely bound to polyribosomes (Heywood, 1970), which particles appear to be unaffected by the existence of myopathy. At high Mg^{2+} concentrations with poly(U) as messenger, no release of chains takes place (Gilbert, 1963).

Ribonuclease and proteinase activity in pH 5 enzyme fractions

An examination of general nuclease and acid proteinase activities in the pH 5 enzyme preparations was carried out to determine whether the observed defect in pH 5 enzyme from myopathic muscle might be due to hydrolysis of endogenous tRNA or mRNA by ribonuclease, or to degradation of the newly synthesized polypeptides by proteinases. Although the endogenous nuclease activities were a little greater in the pH 5 enzymes from myopathic animals than in those from controls (Fig. 3), these differences were unlikely to account for the inhibition (by nearly 30%) of amino acid incorporation into proteins seen

Fig. 3. *Nuclease activity on yeast RNA in pH 5 enzymes from hamster skeletal muscles*

pH 5 enzymes from control (●) and myopathic (○) animals were tested by using procedures described in the Materials and Methods section.

in the reconstituted cell-free system. There was no difference between the acid proteinase activity in the two types of pH 5 enzymes; this was very low in either case (Fig. 4).

Since bentonite is an effective absorbent of ribonuclease (Littauer & Sela, 1962), pH 5 enzymes from myopathic and control animals were prepared in the presence of a very high concentration (5.0 mg/ml) of bentonite (purified by the method of Nisman, 1968), to eliminate the ribonuclease effect.

From the results (Table 5) it is evident that bentonite absorption of ribonuclease permitted a higher rate of amino acid incorporation by pH 5 enzymes from control and myopathic skeletal muscles, but a 20% difference between the two extracts was still found. It is thus improbable that the impairment in protein biosynthesis as catalysed by the pH 5 enzyme from myopathic animals, was due to contamination by endogenous endonucleases, but more likely that a general pre-existing defect in the enzyme fractions was responsible.

Performance of pH 5 enzymes from hamster brains, livers and uteri in cell-free protein biosynthesis

It was decided to ascertain whether the pH 5 enzyme defect was restricted to striated-muscle preparations, taking into account the prominence in these affected hamsters of heart failure and muscular dystrophy. Polyribosomes and pH 5 enzymes were isolated from control and myopathic hamster brains, livers and uteri and were incubated in different combinations to measure cell-free protein biosynthesis.

When polyribosomes and pH 5 enzymes from myo-

Fig. 4. Acid proteinase activity on denatured bovine haemoglobin in pH5 enzymes from myopathic (○) and control (●) hamster skeletal muscles

Procedures used are described in the Materials and Methods section.

Table 5. Effectiveness of pH5 enzymes from myopathic and control skeletal muscles, prepared in the presence of bentonite (5.0mg/ml of homogenizing medium), in amino acid incorporation by polyribosomes from normal animals

The otherwise standard incubation mixture (see the text) contained in 0.25ml: pH5 enzyme (0.4mg of RNA) and control polyribosomes (0.6mg of RNA). Incubations were carried out at 37°C for 30min.

Source of pH5 enzyme	Radioactivity incorporated (c.p.m.)
Control	14 348
Myopathic	9242
*Control	22017
*Myopathic	17707

* pH5 enzyme prepared in the presence of bentonite.

pathic brain tissue were used, a defect in pH5 enzyme was observed (Table 6). The brain pH5 enzyme derived from affected animals was less active by 20% than that from controls in polypeptide synthesis catalysed by control polyribosomes. This defect was also expressed when pH5 enzymes and polyribosomes, both from the brains of the abnormal animals, were recombined.

A similar defect in cell-free protein biosynthesis was demonstrated when subcellular components from myopathic livers of animals were used (Table 6). The liver pH5 enzyme from myopathic animals was about 20% less active than that prepared from controls. This result is especially noteworthy in the light of the known presence of ribonuclease inhibitor in liver-derived cell sap. This would again emphasize

Table 6. Incorporation of ¹⁴C-labelled amino acid into proteins by liver, brain and uterus polyribosomes and pH5 enzymes from control and myopathic hamsters

The standard incubation mixtures (see the text) also contained (in 0.25ml): pH5 enzyme (0.4mg of RNA) and polyribosomes (0.6mg of RNA). Incubations were carried out at 37°C for 30min.

Incubation system		Radioactivity incorporated (c.p.m.)
Polyribosomes (brain)	pH5 enzyme (brain)	
Control	Control	9261
Control	Myopathic	7429
Myopathic	Myopathic	7104
Myopathic	Control	8634
Polyribosomes (liver)	pH5 enzyme (liver)	
Control	Control	
Control	Myopathic	15803
Myopathic	Myopathic	12624
Myopathic	Control	12816
Myopathic	Control	15620
Polyribosomes (uterus)	pH5 enzyme (uterus)	
Control	Control	
Control	Myopathic	8379
Myopathic	Myopathic	6300
Myopathic	Control	6278
Myopathic	Control	8290

that the impaired performance of soluble fractions of myopathic origin in polypeptide formation was not an artifact produced during our preparative procedures, but probably existed in the intact tissue before homogenization.

The pH5 enzyme defect was also present in smooth muscles. When pH5 enzyme from uteri of myopathic hamsters was combined with polyribosomes from uteri of control or myopathic animals, a similar defect was found (Table 6).

Discussion

It is clear that the number of causes of genetically determined muscular dysfunction may be very large because of the complicated interrelationship between energy production and the contractile mechanism in the general physiology of muscle cells. For example, increased myocellular 'acid' cathepsin activity has been described in chicken muscular dystrophy, where it is believed to be responsible for the loss of muscle protein (Iodice *et al.*, 1966). Pennington (1963) has made similar observations in dystrophic skeletal muscle of mice, including an increased activity of 'alkaline' proteinase. Studies of creatine kinase in

mouse muscular dystrophy have revealed the presence of an abnormal enzyme molecule lacking one of the two essential thiol groups of the normal dimeric enzyme (Hooton & Watts, 1966). Defects in sarcosomal oxidative phosphorylation have been detected at late stages of polymyopathy in the same Syrian hamster strain we have used for our studies (Lochner & Brink, 1967; Lochner *et al.*, 1968; Schwartz *et al.*, 1968). An abnormal foetal-type met-myoglobin has been detected in the muscles of patients with progressive muscular dystrophy (Whorton *et al.*, 1961).

In all these instances the primary defects are likely not to be the rather specific biochemical abnormalities reported, but some unknown lesions which lead to the different histopathological and functional syndromes and, *inter alia*, to detectable secondary changes. Only in 'acid' maltase deficiency (glycogenosis type II of Hers, 1963) has the genetic defect causing a polymyopathy been localized to an inactive lysosomal enzyme, and even here the full picture of the disease process cannot be rationally explained on the basis of the single abnormality postulated (Engel, 1970). The tremendous difficulty of distinguishing between primary and secondary phenomena needs hardly to be emphasized. On the other hand, elucidation of the true primary lesion, and a detailed description of the evolving secondary changes, is likely to be extremely valuable for our understanding of muscle physiology and pathology.

We have detected an abnormality in the 'soluble' cytoplasmic machinery involved in reconstituted protein-synthesizing systems from the polymyopathic Syrian hamster, not only in the heart and skeletal muscles, but also in uterine smooth muscle and in the liver and brain. The defect in the muscle system is both qualitative and quantitative in nature, implying that there is a shortage of some essential soluble component in the pH5 enzyme fractions, which is accompanied by the presence of an altered substituent which prevents the attainment of extents of polypeptide synthesis *in vitro* seen in control extracts from unaffected animals.

The use of fractions standardized with respect to RNA and protein concentrations obviates the need to consider these factors as causes of the impaired performance of soluble components from myopathic animals. We have in fact found that the yield, per heart, of polyribosomes and pH5 enzymes is increased in parallel in older myopathic animals compared with controls. Watts & Reid (1969) have concluded that the cell-free protein-synthesizing mechanism in myodystrophic Bar Harbor mice is similar to that of normal mice when the activity is expressed in such a way as to allow for the decrease in muscle volume occurring in the abnormal muscle mass.

It is difficult to define the cellular origin of isolated enzyme and polyribosome fractions when there may

be significant variations in the distribution of myocytes, phagocytes and connective-tissue cells in heart muscle at different phases of development, both in the normal hamsters and in the polymyopathic animals where there is clear evidence for such changes as the disease progresses (Caulfield, 1966). This consideration would be more significant had our results indicated an increased activity in protein synthesis rather than the observed decrease, since the non-myocytic cells would be expected to become metabolically more active in diseased muscle. Grove *et al.* (1969) have demonstrated that increases in DNA synthesis observed in hypertrophying heart muscle are caused exclusively by fibroblastic proliferation and not by myocytic events, whether nuclear or mitochondrial.

The existence of a generalized tissue defect, demonstrable even at the embryonic developmental stage, may thus be closely related to the primary genetic defect causing the syndrome of polymyopathy in the BIO 14.6 strain of hamsters.

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