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BIOCHEMICAL STUDY OF MUSCLE IN PROGRESSIVE MUSCULAR DYSTROPHY [1]

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Current concepts of progressive muscular dystrophy hold that it is a disease in which, through genetic error, there are intrinsic defects in muscle metabolism, although the nature of these hypothetical defects remains unrecognized. It is the purpose of this report to describe one approach to the problem and to show that there are defects in the metabolism of isolated muscle from patients with progressive muscular dystrophy.

METHODS

Assessment of enzymatic activity of skeletal muscle obtained by biopsy presents several difficulties. In the first place, as in any quantitative chemical analysis of mixed tissues, there is the question of an adequate reference base, as discussed by Hoagland (1) and by other investigators (2). A specimen of muscle obtained by biopsy contains varying proportions of fatty tissue, nonmuscular fibrous tissue, vascular tissue, extracellular water, and muscle fibers. Diseased muscle is particularly variable in its heterogeneity, and it has been important to the present study to estimate the degree of fibrous replacement of muscle in biopsy specimens. The reference base selected was non-collagen nitrogen (2), a term applied to nitrogen content of material soluble in dilute alkali, as distinguished from alkali-insoluble collagen and elastin.

A second difficulty associated with assessment of enzymatic activity results from inter-action of enzyme systems present in the muscle sample. Since purification of enzymes is not yet quantitative, it has been necessary to employ impure preparations as test systems. In multi-enzyme systems, such as the glycolytic series, the over-all rate is limited by a single slowest step. This renders quantitative assessment of any other step uncertain, since the rate of the reaction will be proportional to the quantity of enzyme only if that reaction is rate-limiting. The difficulty has been circumvented in the case of phosphorylase by Cori and Cori (3) by inhibiting the next enzyme in the chain, and in the case of aldolase by Sibley and Lehninger (4) by removing from the system the product of the reaction under study. Because suitable methods for their determination were available, these two enzymes, phosphorylase and aldolase were selected for study.

In addition, establishment of non-collagen nitrogen as a reference base provided an opportunity to repeat previous observations on the non-heminic iron content of muscle. Earlier work from this laboratory (5, 6) showed that non-heminic iron (that is, iron not included in chromoproteins, myoglobin, and the cytochromes) occurred in muscle in two varieties; one extractable by reducing agents, the second apparently linked with myosin (7). It had been found that the extractable iron in muscle from dystrophic patients was low in terms either of wet weight of muscle or of fat-free dry weight (8–10).

Biopsies of muscle were obtained under general anesthesia. The samples were frozen at once in liquid nitrogen and analyses were performed without delay. Biopsies from patients without muscular dystrophy, controls, were obtained from the right transversus abdominis during operation for appendicitis. Biopsies from patients with muscular dystrophy were obtained from the left transversus abdominis. After freezing the muscle, an aqueous extract was prepared in the Potter-Elvehjem homogenizer held at the temperature of melting ice. The extracting medium was 20 volumes of 0.1 N sodium fluoride. Aliquots were analyzed for non-collagen nitrogen (11), for total nitrogen, for aldolase concurrently by the method of Dounce, Beyer, and Barnett (12, 13) and by the method of Sibley and Lehninger (4), and for phosphorylase by the Coris' method (3). In addition, in a number of patients the activity of aldolase in serum was also assessed (14). In three patients interpretation of values for non-collagen nitrogen were checked by determination of collagen and elastin (scleroproteins) by the method of Neumann and Logan (15). Extractable iron was determined colorimetrically with orthophenanthroline, using sodium dithionine as a reducing agent (16).

For phosphorylase assay the homogenate was centrifuged, the supernatant extract diluted with two volumes of a mixture of cysteine and NaF, pH 6.0, and left for two minutes at 38°. One-tenth ml. was used for the assay. Final concentration of cysteine was 0.05 M, pH 6.0. For aldolase assay the 1/20 extract was diluted with four volumes of water. Two-tenths ml. were used in the presence of 0.5 ml. 0.01 M fructose diphosphate, hydrazine and collidine buffer pH 7.5.

RESULTS

In Table I, collagen nitrogen is expressed as per cent of total nitrogen in the biopsy specimen. There was a definite increase in collagen nitrogen

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TABLE I

Ratio of collagenous nitrogen to total nitrogen (in per cent)

Controls	Dystrophics
9.6	36
18.5	50
15	22
10	40
16.6	16
11	52
17	26
10	45
6	22
	33
	36
	50
	40
	21
	38
	35
	20
	33
Mean: 12.6 per cent±1.4	34.2 per cent±2.6

TABLE II

Phosphorylase "a"

	Units per Gm. of wet muscle		Units per Gm. of non-collagenous proteins	
	Controls	Dystrophics	Controls	Dystrophics
	1435	325	9570	4330
	1500	80	8810	1070
	1960	483	10800	3710
	1360	430	8710	3870
	1180	1190	6740	7580
	1140	26	6910	346
		262		1820
		164		1500
		120		1200
		385		4000
		297		2380
Mean:	1429±112	360±96	8590±630	2890±544

TABLE III

Phosphorylase "b" *

	Units per Gm. of wet muscle		Units per Gm. of non-collagenous proteins	
	Controls	Dystrophics	Controls	Dystrophics
	1575	560	10000	7400
	2000	142	11800	1900
	3150	595	17400	4570
	2540	682	16150	6100
	1610	1700	8000	10800
	1995	70	12000	1000
		638		4400
		420		4000
		245		2450
		470		5000
		420		3360
Mean:	2145±226	540±127	12570±1225	4640±840

* By agreement, the total phosphorylase activity in the presence of adenylic acid is called "phosphorylase b activity."

in muscle from patients with muscular dystrophy; the single patient in the high normal range was only slightly affected clinically. In general, the relative collagen nitrogen content was higher in those in whom the disease seemed most advanced, although it never exceeded half the total nitrogen even when enzymatic activity had virtually disappeared, as will be presented later.

Determination of collagen and elastin by the method of Neumann and Logan (15) agreed closely with the value obtained by the simpler method. The content of hydroxyproline in collagen, the amino acid upon which the measurement depends, was presumably the same in normal and dystrophic subjects.

These data confirm the well-known increase in fibrous tissue, seen histologically in dystrophic skeletal muscle.

The activity of phosphorylase "a" and "b" was quite reproducible in normal muscle (Tables II and III), phosphorylase "b" being defined as phosphorylase activity in the presence of adenylic acid. In muscle from all but one of the dystrophic patients, phosphorylase activity was reduced greatly, both in terms of wet weight of muscle or of its non-collagen nitrogen content. In both normal and dystrophic muscle, addition of adenylic acid produced an increase of 30 to 40 per cent in phosphorylase activity. Muscle from two patients with severe dystrophy had almost no phosphorylase activity, although half the nitrogen in the muscle samples from them was non-collagen nitrogen.

Aldolase activity (Table IV) varied in normal muscle to a somewhat greater extent than did phosphorylase activity. Nevertheless, this variation did not obscure a definite decrease in aldolase activity in dystrophic muscle, which was, on the average, only a third that of normal, referred to non-collagen nitrogen. Again, in several patients, aldolase activity was nearly absent.

These decreases in phosphorylase and in aldolase activity conceivably might have been due to the presence of an inhibitor in dystrophic muscle. This possibility was eliminated by the results of experiments in which the extracts of dystrophic muscle were incubated with crystalline aldolase

TABLE IV

Aldolase

Units per Gm. of wet muscle		Units per Gm. of dry non-collagenous proteins	
Controls	Dystrophics	Controls	Dystrophics
1.576	0.17	10	2.26
5.45	0.6	32	4.58
4.85	0.745	26.8	6.7
3.4	1.49	21.8	9.4
2.26	0.10	12.8	1.33
2.385	1.45	14.4	10
2.68	0.64	18.6	6
3.88	0.79	23.9	7.9
	1.085		11.0
Mean: 3.31±0.47	0.78±0.16	20±2.75	6.6±1.18

and phosphorylase. No inhibiting effect was noted.

In 36 of 38 patients with muscular dystrophy, aldolase activity of serum was higher than in normal subjects. The mean value was 10 times greater than that of controls. Patients with other neuromuscular disorders and marked wasting of muscle did not show abnormal serum aldolase activity. Details of this study are reported elsewhere (17).

Results of determination of non-heminic iron appear in Table V, with data from earlier studies. Although the iron content was reduced when referred to wet weight of dystrophic muscle, when referred to non-collagen nitrogen, the iron content of dystrophic muscle was exactly the same as that of control muscle. On the contrary, in atrophy of nervous origin, the iron content was increased.

DISCUSSION

Data reported herein demonstrate a real decrease in the activity of two glycolytic enzymes in skeletal muscle of patients with muscular dystrophy and, confirming the observation on two cases by Sibley and Lehninger (14), an increase in the concentration of one of them, aldolase, in serum. It is too soon to say whether these enzymatic defects are the cause of the dystrophy or whether they are simply

TABLE V

Muscle iron

	mg. per 1000 Gm. wet muscle			mg. per 1000 Gm. dry defatted muscle			mg. per 1000 Gm. dry non-collagenous protein		
Muscle	Control	Progressive muscular dystrophy	Atrophy	Control	Progressive muscular dystrophy	Atrophy	Control	Progressive muscular dystrophy	Atrophy
Transversus	25.5	10		125	72		170	133	
	18	14.5		75	72		107	200	
	12	18.4		60	90		66	140	
	30	8		140	39		214	70	
	17.6	15.5		85	77		100	100	
		12.6						87	
		16.4						150	
		8.3						88	
Gluteus maximum	18.5	8.5	20.5	100	50	195			
	17	4	30	75	25	185			
	13.2	15.3	44	68	53	280			
	12.9	9.8	42	65	110	235			
		10.9	32.5		67	210			
		11.5	32		122	170			
		17.5			66				
		11.8			78				
		15.6			90				
		15.6							
Triceps		17	32.5		90	162			280
		8	25		38	135			
		7.5	35		37.5	178			
		17.5	28		112				
		9.3			67				
		12.4			70				
		12			50				
		8.8			63				
		11.7							
Pectoral major		9.6		70					
		24.5		120					
Mean	18.1	12.2	32.15	90	70	195	131	121	

sequelae of a more primary biochemical flaw. Mirski and Wertheimer (18) Humoller, Hatch and McIntyre (19), other investigators (20) and we (unpublished observations) have demonstrated decreased muscle phosphorylase activity following transsection of the sciatic nerve, and we have observed it also in muscle made atrophic by tenotomy. Fischer (21) similarly described a fall in muscle aldolase activity following sciatic section. In all these cases the decrease in enzyme activity was secondary to the atrophy and was detected by reference to fresh weight of tissue. It may also be noted that decrease in enzyme activity appeared late in these situations when atrophy was well-established, although it cannot be implied that the decreases in dystrophic muscle represent an early biochemical lesion.

Nor can it be stated whether or not the decrease in aldolase and phosphorylase activity is unique. It is entirely possible that more widespread disturbance of glycolytic activity, and even of other enzymes may occur in muscular dystrophy.

SUMMARY

1. Collagen and elastin content of dystrophic muscle was increased.

2. In muscle obtained by biopsy from patients with progressive muscular dystrophy there was a decrease in the activity of phosphorylase and of aldolase. This decrease was demonstrable either when the values were expressed in terms of the wet weight of the sample or in terms of its remaining muscle fibrillar protein, non-collagen nitrogen.

3. Aldolase activity of serum in dystrophic patients was increased many times above the normal value.

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