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Phosphorus in Cell Fractions of Skeletal Muscle from Mice with Hereditary Muscular Dystrophy.* (30036)

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Phosphorus-containing substances play important roles in the metabolism and contraction of muscle. The concentrations of several of them were found in various types of muscular dystrophy to differ considerably from normal levels(1-5). Such studies usually were made on whole muscle. This report deals with the distribution of phosphorus in various forms among the subcellular fractions of skeletal muscle of mice with hereditary muscular dystrophy.

Materials and methods. Dystrophic mice and control littermates of strain 129/Re(6) and of the recently developed hybrid strain 129 RexC57BL/6J(7) were obtained from the Jackson Laboratory, Bar Harbor, Maine. The animals were sacrificed when about 2 months old. The muscles from the hind legs were removed, minced with scissors, and homogenized at 0°-4°C in a Kontes Duall tissue grinder with 10 volumes of 0.1 M KCl, 0.039 M borate buffer, pH 7.1. The homogenate then was fractionated by differential centrifugation into nuclei-myofibrils, mitochondria, microsomes, and supernatant according to the procedure of Winnick and Winnick(8). Each fraction was extracted by a modification of the Schneider method(9) as follows: The acid-soluble substances were extracted at 0°C, once with 5% (w/v) $HClO_4$ and twice with 2% $HClO_4$. Lipids were then removed at room temperature by treatment, twice with 95% ethanol and 3 times with a 3:1 mixture of ethanol and ether. From the air-dried residue nucleic acids were extracted twice for 20 minutes with 5% $HClO_4$ at 70°C; the residue was finally washed with 5% $HClO_4$ at room temperature. Phosphorus, after digestion with 10% $HClO_4$, was determined by the method of Taussky and Shorr(10). The nucleic acids were analyzed by the orcinol and indole tests for ribose and deoxyribose, respectively, according to Ceriotti(11). These tests were calibrated in terms of phosphorus. The data were evaluated statistically by the "t" test (12).

Results and discussion. The results presented in the tables as mean values with standard deviations were obtained with mice of strain 129/Re. Increase or decrease, in the following discussion, means the change in values for the dystrophic mice as compared to those for the controls. Table I shows that total phosphorus was markedly increased in the nuclear-myofibrillar fraction and decreased in the supernatant. The increase in the former was so pronounced that it led to a net increase for the homogenate. (Values for the sum of all fractions checked well with those of the homogenates; usually they were somewhat lower since the various

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TABLE I. Total Phosphorus in Cell Fractions of Skeletal Muscle from Mice.

	mg P/g muscle wet wt		
	Control	Dystrophic	P
Nuclei-myofibrils	1.052 ± .315	2.164 ± .752	<.01
Mitochondria	.162 ± .041	.122 ± .041	
Microsomes	.077 ± .021	.078 ± .019	
Supernatant	1.712 ± .105	1.407 ± .117	<.001
Homogenate	3.245 ± .297	3.761 ± .535	<.05

TABLE II. Acid-Soluble Phosphorus in Cell Fractions of Skeletal Muscle from Mice.

	mg P/g muscle wet wt		
	Control	Dystrophic	P
Nuclei-myofibrils	.617 ± .228	1.325 ± .501	<.01
Mitochondria	.031 ± .014	.023 ± .011	
Microsomes	.009 ± .003	.008 ± .002	
Supernatant	1.552 ± .141	1.307 ± .151	<.01
Homogenate	2.375 ± .454	2.735 ± .540	

manipulations during fractionation led to some loss of material.)

Similarly, acid-soluble phosphorus (Table II) increased in the nuclear-myofibrillar fraction and decreased in the supernatant. It represented the major portion of total phosphorus in the supernatant in both groups of mice. It increased somewhat in the homogenate but not significantly, in contrast to the findings of Sarkar *et al*(5).

Table III indicates a decrease of lipid phosphorus in the mitochondria from dystrophic mice and an increase in the supernatant. The values for the homogenates were alike in the 2 groups, in agreement with the findings of Young *et al*(13). In view of the participation of phospholipids in the structural framework of membranes, their decrease in the mitochondria may be important, since changes in the morphology of mitochondria have been observed with the electron microscope(14). Furthermore, mitochondria from muscle, in contrast to those from other tissues, seem to play a role in protein synthesis(15).

TABLE III. Lipid Phosphorus in Cell Fractions of Skeletal Muscle from Mice.

	mg P/g muscle wet wt		
	Control	Dystrophic	P
Nuclei-myofibrils	.205 ± .041	.260 ± .056	
Mitochondria	.101 ± .012	.073 ± .005	<.001
Microsomes	.049 ± .017	.055 ± .011	
Supernatant	.005 ± .001	.009 ± .003	<.01
Homogenate	.400 ± .020	.459 ± .052	

Table IV shows that the pronounced increase of deoxyribonucleic acid-phosphorus (DNA-P) occurred exclusively in the nuclear-myofibrillar fraction. The very small amounts of DNA-P in the other fractions probably resulted from adsorption and occlusion of DNA released from some ruptured nuclei during homogenization. However, some evidence for the occurrence of DNA in mitochondria has been reported recently(16).

Table V reveals that the increase of ribonucleic acid-phosphorus (RNA-P), like that of DNA-P, occurred mainly in the nuclear-myofibrillar fraction. This result may have a bearing on the synthesis of muscle proteins, since Perry and Zydowo(17) found that the bulk of myofibrillar RNA exists as a ribonucleoprotein. RNA-P in the microsomes also increased, on a 2% level of significance. Antoni *et al*(18) observed an inincrease of RNA in both microsomes and mitochondria of muscle from rabbits with muscular dystrophy resulting from vit. E-deficiency. A pronounced increase of both nucleic acids was also found by other investigators in various forms of muscular dystrophy(19-22). However, this finding should be viewed with caution as it may reflect the infiltration of dystrophic muscle tissue by

TABLE IV. DNA-Phosphorus in Cell Fractions of Skeletal Muscle from Mice.

	mg P/g muscle wet wt		
	Control	Dystrophic	P
Nuclei-myofibrils	.074 ± .008	.140 ± .007	<.001
Mitochondria	.001 ± .001	.002 ± .002	
Microsomes	.001 ± .001	.001 ± .001	
Supernatant	.004 ± .002	.006 ± .004	
Homogenate	.085 ± .013	.149 ± .016	<.001

TABLE V. RNA-Phosphorus in Cell Fractions of Skeletal Muscle from Mice.

	mg P/g muscle wet wt		
	Control	Dystrophic	P
Nuclei-myofibrils	.079 ± .012	.115 ± .019	<.01
Mitochondria	.008 ± .002	.007 ± .002	
Microsomes	.004 ± .001	.007 ± .002	<.02
Supernatant	.014 ± .004	.019 ± .006	
Homogenate	.110 ± .014	.158 ± .028	<.01

other types of cells such as macrophages and connective tissue(23), rather than a rise in the level of nucleic acids in the muscle cells themselves.

Corresponding studies with the hybrid strain of the mice gave results similar to those with strain 129.

Summary. Phosphorus in various forms was determined in the subcellular fractions of skeletal muscle from mice with hereditary muscular dystrophy and from their normal littermates as controls. Total phosphorus increased in the nuclear-myofibrillar fraction and decreased in the supernatant, with a net increase for the homogenate. Acid-soluble phosphorus behaved similarly, but did not significantly increase in the homogenate. Lipid phosphorus decreased in mitochondria and increased in supernatant. In the nuclear-myofibrillar fraction, phosphorus of both deoxy- and ribonucleic acids was considerably increased, whereas, in the microsomes, only that of the ribonucleic acid was increased.

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Time Relationship of Injection of Actinomycin D and Antigen to the Immune Response.* (30037)

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We have reported(1) that actinomycin D, given at the same time as antigen (sheep erythrocytes) in the rat, caused a delay in time of appearance of circulating hemagglutinin but had no effect on apparent rate of synthesis or maximal amount of antibody eventually produced at 5 to 6 days. In the mouse, cellular damage, particularly in germinal centers of lymphoid tissue, was most pronounced when the antibiotic was given with antigen(2). Coincident with the cytotoxicity was a delay in appearance of circulating antibody. As restitution and repopulation of lymphoid tissues occurred, hemagglutinin was

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