Serum and Plasma Proteins, Lipoproteins and Glycoproteins. III. Hereditary Muscular Dystrophy in Mice.* (24699)

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Spontaneous occurrence of genetic mutation resulting in hereditary muscular dystrophy in inbred strain of mice was observed recently(1). This report deals with proteins, lipoproteins and glycoproteins in blood serum and plasma of such mice. The results are compared with those found in similar studies on human muscular dystrophy(2) and on myopathy of Vit. E-deficiency in rabbits(3).

Methods. Dystrophic mice and control litter mates of strain 129 were supplied by Roscoe B. Jackson Memorial Lab., Bar Harbor, Me. They were maintained on Purina Laboratory Chow, and sacrificed at ages 6 to 9 weeks. For determination of protein-bound lipids and carbohydrates, the animals were fasted at least 6 hours, and usually overnight, before blood samples were taken. Blood was obtained by decapitation under ether anesthesia or, occasionally, by cardiac puncture. Serum was used for paper electrophoretic runs, and oxalated plasma for determinations of protein nitrogen and of protein-bound lipids and carbohydrates. Procedures for paper electrophoretic fractionation of serum proteins, lipoproteins and glycoproteins were generally the same as described previously(3). Concentrations of protein nitrogen, total and free cholesterol, phospholipids and protein-bound hexoses, hexosamines and sialic acid also were determined as previously reported(3). Some preliminary determinations of amounts of esterified fatty acids were performed(4). In comparison of results on dystrophic mice with those on control litter mates, differences between corresponding mean values were considered to be statistically significant if p-values were .01 or less. In such cases mean values are underlined in Tables showing control values. For most determinations number of mice used was 10 to 15.

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Results. Relative concentrations of serum protein fractions of dystrophic mice did not differ significantly from those for normal litter mates (Table I). The lipoprotein pattern consisted of intense band at location of albumin, (occasionally extending somewhat ahead of the latter), and of a band spread widely among the globulins. The former band showed highly significant decrease to 42.7 ± 8.6% in dystrophic mice ($P<.001$). The other band increased correspondingly, but its diffuse nature did not permit definite differentiation among the various globulins. Total areas of lipid stains were about the same in the 2 groups. The pattern of serum glycoproteins consisted of 3 bands at locations of α_1-, α_2- and β-globulins. The band associated with α_2-globulin was lowered to 40.2 ± 4.8% in dystrophic mice ($P<.01$).

Concentrations, in plasma, of protein nitrogen and of protein-bound lipids and carbohydrates were somewhat lower, but not significantly so in dystrophic mice (Table II). Cholesterol values are similar to those of other workers(5). Ratio of cholesterol to phospholipids was alike in the 2 groups (0.65-0.67).

Discussion. The group of normal litter mates used as controls consisted of both heterozygous and homozygous individuals, depending on type of mating. To exclude the possi-

TABLE I. Paper Electrophoresis of Serum Proteins, Lipoproteins and Glycoproteins of Normal Mice (Strain 129).

	Proteins	Protein-bound Lipids	Protein-bound Carbohydrates
	%		
Albumin	51.6 ± 3.7	59.3 ± 9.3	
α_1-globulin	6.4 ± 3.2		21.1 ± 8.2
α_2- "	14.3 ± 5.1		46.0 ± 4.5
β- "	17.3 ± 3.2		32.9 ± 8.5
γ- "	10.4 ± 2.3		

Single underline, $P<.01$.
Double underline, $P<.001$.

TABLE II. Plasma Proteins and Protein-Bound Lipids and Carbohydrates of Normal Mice (Strain 129).

Protein nitrogen, g %	.838± .096
Total cholesterol, mg %	132 ± 34
Free chol./total chol., %	36.0 ± 13.4
Phospholipids, mg %	204 ± 50
Esterified fatty acids, mg %	398 ±253
Hexoses, "	84.8 ± 17.5
Hexosamines, "	86.4 ± 20.0
Sialic acid, "	128.4 ± 26.7

bility that the heterozygous litter mates were affected by mutation, the same series of determinations was performed with a group of normal homozygous mice. Estimates for the 2 control groups did not differ significantly except for a doubtful decrease in ratio of free to total cholesterol in the homozygous group.

Migration of the main band of stained lipids occasionally ahead of the protein stain for albumin may have been due to prevention by the lipid of staining of the protein.

The significant changes found in this study of dystrophic mice were shifts in patterns of lipoproteins and, to a lesser extent, of glycoproteins. In pseudohypertrophic muscular dystrophy in man changes observed(2) were: increase of α_2-globulin in moving boundary electrophoretic pattern, increase of β-globulin and decrease of α-lipoprotein in paper electrophoretic pattern, increase in ratio of free to total cholesterol and increase of sialic acid. (The first and last changes were at 1% level of significance, the others did not exceed the 2% level.) Myopathy of Vit. E-deficiency in rabbits was characterized by decrease of albumin, increase of β-globulin and lowering of α-lipoprotein together with marked rise of β-lipoprotein(3). This alteration in lipoproteins was assoiated with large increases of both free and esterified cholesterol and of phospholipids. Also the levels of protein-bound hexoses, hexosamines and sialic acid were raised in sera of Vit. E-deficient rabbits, but did not increase in the 2 hereditary forms of dystrophy, with the exception of sialic acid in the human disease. How closely the morbid process in mice resembles the hereditary muscular disease in man or the deficiency state of rabbits is not clear. Lack of large variation in levels of most constituents studied is similar in the 2 hereditary forms of the disease. Distribution pattern of lipoproteins, however, was altered both in hereditary dystrophy of mice and in nutritional dystrophy of rabbits. These 3 forms of muscular dystrophy involve 3 different species so that only cautious comparisons should be made.

Summary. 1. Paper electrophoretic patterns of sera from mice with hereditary form of muscular dystrophy and from normal litter mates as controls showed no change in relative concentrations of the protein fractions themselves, but did indicate a shift in distribution particularly of lipoproteins and, to a smaller extent, of glycoproteins. 2. Concentrations, in plasma, of protein nitrogen, protein-bound lipids (free and esterified cholesterol, phospholipids, esterified fatty acids) and carbohydrates (hexoses, hexosamines and sialic acid) did not vary significantly from those of controls. 3. Results have been compared with those from previous, similar studies on clinical forms of muscular dystrophy and on myopathy of Vit. E-deficiency in rabbits.

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