were operating. One was clumping which resulted from centrifugation, the other was inhibitory activities in allantoic fluid. B/GL virus will not combine with cholesterol. The apparent retention was due to mechanical filtration of clumps from saline suspensions by the cholesterol columns. Composition of type B strain is clearly different since it does not combine with cholesterol as do type A viruses. In contrast, adsorption of A/PR 301 strain to cholesterol is affected by inhibitory materials in allantoic fluids. Adsorption increased when this virus was resuspended in PBS as shown by results from either column or slurry experiments. It is also apparent that Formosa allantoic fluids are not free from inhibitory substances because PR 301 strain resuspended in those fluids did not readily combine with cholesterol. An analogous set of reactions occurs with these 2 strains in HI tests with serum which contains non-specific inhibitory substances(5). Here again, the Formosa strain is completely resistant and readily agglutinates erythrocytes, whereas with the A/PR 301 strain, the hemagglutination pattern will not form in presence of serum inhibitors.

With fatty acids tested the inhibitor content of allantoic fluid observed in cholesterol reactions did not appear to play as significant a role. In contrast to its behavior with cholesterol, type B/GL strain readily combined with stearic acid.

Summary. Marked differences were noted among strains of influenza with respect to adsorption to powdered cholesterol, palmitic acid and stearic acid. It has been shown that some strains suspended in allantoic fluids adsorb to lipids less efficiently than when suspended in buffered saline. Behavior of type B/GL strain was significantly different from type A strains, especially with cholesterol or palmitic acid as adsorbent. Mixtures of minimal amounts of influenza virus with cholesterol or stearic acid elicited an enhanced antibody response in animals when tested at 3 weeks.

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Serum Proteins, Lipoproteins and Glycoproteins. II. Muscular Dystrophy and Related Diseases in Patients.* (24698)

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Biochemical changes in various forms of muscular dystrophy have been summarized(1, 2). They include changes in serum proteins but in such investigations in clinical muscular dystrophy, classification of patients on the basis of commonly recognized forms of the disease was not always made uniformly or stated clearly. These uncertainties may account for some discrepancies in results. In this report, various forms of muscle disease were strictly differentiated from each other in tabulation of results. The largest group comprised children with pseudohypertrophic muscular dystrophy (PMD). Other muscle diseases investigated were myositis, myotonia dystrophica and amyotrophic lateral sclerosis. Some patients with scoliosis and with Perthes disease also were studied, since sometimes muscular wasting was present which was of secondary nature. The purpose of this investigation was to find significant changes of serum proteins, lipoproteins and glycoproteins

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in patients with muscle diseases, and to compare the results with our findings in similar, previous studies on muscular dystrophy of vit. E-deficiency in rabbits(3).

Methods. Blood samples were taken by venipuncture from fasting subjects. Serum was separated by centrifugation soon after clotting and usually worked up within a few days, during which period it was kept at about +3°C (in case of very short storage) and otherwise at −15°C. Serum proteins, lipoproteins and glycoproteins in barbiturate buffer of pH 8.6 and of ionic strength 0.1 were fractionated by both moving boundary and paper electrophoretic methods. The procedures for these technics were generally the same as described previously(3). Concentrations of total protein, total and free cholesterol, phospholipids and protein-bound hexoses, hexosamines and sialic acid also were determined by previous methods(3). Furthermore, by differential elution of paper electrophoretic lipoprotein fractions with acetone and petroleum ether, total phospholipids and sphingomyelins were obtained(4). Protein-bound fucose was determined by the cysteine method(5). The data were evaluated statistically by Student's "t" test(6).

Results. The Tables show mean values with standard deviations. Since all patients with PMD, scoliosis and Perthes disease were children, they were compared with healthy children as controls. However, patients suffering from myositis, myotonia dystrophica and amyotrophic lateral sclerois were adults and, therefore, were compared with healthy adults as controls. Those mean values which differed from corresponding ones of their appropriate controls on the statistically significant level of 1% and on borderline level of 2% are underlined in the Tables. Numbers in parentheses are numbers of subjects whenever they differed from those given in second column of Tables.

Table I shows relative concentrations of serum protein fractions in moving boundary electrophoretic patterns. Albumin decreased in myositis and scoliosis and α_2-globulin increased in all muscle diseases. (The increase in case of amyotrophic lateral sclerosis, however, could not be shown to be really significant, presumably because of the small number of cases.) Other globulins did not vary significantly, with the exception of increase of γ-globulin in scoliosis. Total protein concentration did not change significantly and electrophoretic mobilities for corresponding fractions were alike in the various groups.

Paper electrophoresis showed relative concentrations of protein fractions alike in patients and controls except for increase of β-globulin in PMD on a 2% level of significance only. In scoliosis, however, albumin was lowered and γ-globulin increased, as in moving boundary electrophoresis; β-globulin increased on a 2% level of significance.

Relative concentrations of total protein-bound lipids in paper electrophoretic fractions in the various diseases did not differ much from those of corresponding controls (Table II), but there was a decrease of α-lipoprotein on the 2% level of significance in PMD and

TABLE I. Moving Boundary Electrophoresis of Serum Proteins in Muscle Diseases.

	No. of subjects	Relative concentrations (%)				
		Albumin	α_1-globulin	α_2-globulin	β-globulin	γ-globulin
Pseudohypertrophic M.D.	17	52.7 ± 2.4	7.0 ± .8	11.7 ± 1.1	14.7 ± 1.8	13.9 ± 2.2
Myositis	9	46.6 ± 2.6	7.9 ± 1.1	12.1 ± 2.1	15.9 ± 1.7	17.5 ± 4.9
Myotonia dystrophica	5	46.3 ±10.3	7.1 ± 1.0	12.5 ± 1.4	17.4 ± 3.4	16.7 ±10.5
Amyotrophic lateral sclerosis	3	54.9 ± 4.7	7.0 ± 1.2	12.0 ± 2.3	15.9 ± 1.2	10.2 ± 7.7
Scoliosis	6	49.9 ± 1.8	7.2 ± 1.2	10.8 ± .9	13.9 ± 1.0	18.2 ± 2.4
Perthes disease	3	52.0 ± 3.5	6.9 ± 1.2	11.3 ± .6	14.1 ± 2.6	15.7 ± 3.2
Normal children	8	55.7 ± 3.0	6.65± .6	10.3 ± .9	14.7 ± 1.3	12.65± 3.4
" adults	10	53.9 ± 2.8	7.4 ± .8	8.9 ± 1.0	13.9 ± 1.9	15.9 ± 1.9

Underlined values, $P < .01$.

TABLE II. Serum Protein-Bound Lipids in Muscle Diseases.

		Paper electrophoresis of lipoproteins			Cholesterol ratio
		Relative conc.			
	No. of subjects	α-l.p.	β-l.p.	"O" l.p.	Free/Total
		%			
Pseudohypertrophic M.D.	16	35.7 ± 7.2	44.1 ± 6.8	20.2 ± 7.5	24.6 ± 3.7 (17)
Myositis	8	28.2 ± 6.8	46.8 ±10.4	25.0 ±11.7	22.2 ± 3.8 (4)
Myotonia dystrophica	3	28.2 ±10.2	56.0 ± 2.5	15.8 ± 8.7	23.8 ± 4.4
Amyotrophic lateral sclerosis	3	35.7 ± 4.7	39.7 ± 1.7	24.5 ± 3.9	
Scoliosis	6	33.1 ± 7.3	44.3 ± 8.4	22.6 ± 8.9	27.8 ± 3.1
Perthes disease	3	30.6 ± 6.1	44.7 ± 8.6	24.7 ±13.6	27.4 ± 2.2
Normal children	9	42.3 ± 5.5	43.8 ± 6.1	13.9 ± 5.5	20.7 ± 3.1 (7)
" adults	10	33.2 ± 7.6	43.2 ± 4.2	23.6 ± 7.5	25.8 ± 2.3 (8)

Underlined values, P <.01. Broken underlined values, P <.02.

definite increase of β-lipoprotein in myotonia dystrophica. Distribution of both total phospholipids and sphingomyelins in these lipoprotein fractions was similar in all groups. The absolute concentrations of total phospholipids and cholesterol did not vary significantly nor did the ratio of the two. The Table shows, however, that the ratio of free to total cholesterol increased in PMD (on the 2% level of significance), scoliosis and Perthes disease.

Table III lists absolute concentrations of protein-bound carbohydrates. Total hexoses were decreased in PMD, scoliosis and Perthes disease and increased, on a 2% level of significance, in myositis. Amounts of hexoses in the seromucoid fraction were similar in all groups and their ratios to total hexoses were fairly constant. Hexosamines varied only in Perthes disease in which they were decreased. Sialic acid was the only protein-bound carbohydrate which increased significantly in PMD. Its apparent elevation in other muscle diseases was not of statistical significance because of large deviations and small numbers of subjects. Fucose, a minor constituent, followed the same trend. The percentile distribution of total protein-bound carbohydrates among paper electrophoretic fractions was alike in all groups.

Discussion. Various changes of relative concentrations of serum protein fractions in electrophoretic patterns in muscle diseases have been reported(7-12). Dryer, *et al.*,(8) found increase of α_2-globulin in moving boundary electrophoresis in PMD, but, unlike our results, decrease of γ-globulin. They described twinning of the α_2-globulin peak as a nearly regular and characteristic feature of the disease, but we observed this only occasionally. The peak was usually asymmetric and broad, indicating electrophoretic heterogeneity. The increase in relative concentration of α_2-globulin resulted from an increase of conjugated non-protein constituents rather than of proteins themselves, since the latter did not change in paper electrophoretic patterns. It probably was related to the increase of sialic acid which has high affinity for α_2-globulin(13,

TABLE III. Serum Protein-Bound Carbohydrates in Muscle Diseases.

	Hexoses	Hexosamines	Sialic acid	Fucose
	mg %			
Pseudohypertrophic M.D.	97.6 ±15.9(24)	99.5 ± 9.4(14)	77.5 ±15.8(17)	10.1 ± 4.4(16)
Myositis	126.5 ±20.2 (5)	110.5 ±17.1 (5)	84.3 ±16.7 (5)	12.3 ± 4.0 (3)
Myotonia dystrophica	105.7 ±22.9 (3)	127.0 ±12.6 (3)	93.1 ±17.6 (3)	12.7 ± 6.2 (3)
Scoliosis	82.5 ± 8.9 (6)	107.9 ± 8.4 (5)	65.5 ± 8.5 (6)	10.8 ± .6 (4)
Perthes disease	75.7 ± 4.6 (3)	92.3 ± 2.3 (3)	69.1 ± 4.0 (3)	8.3 ± 2.6 (3)
Normal children	121.6 ±11.1 (9)	106.6 ± 5.7 (9)	59.3 ± 8.0 (9)	9.5 ± 1.6 (8)
" adults	98.1 ± 9.4 (8)	104.1 ±10.3 (7)	68.1 ±10.7 (8)	8.4 ± 2.0 (8)

Underlined values, P <.01. Broken underlined values, P <.02.

14). It was not specific for muscle diseases, and has been found in other wasting diseases (5).

Diseases with tissue destruction are usually accompanied by high levels of sialic acid, and of the 2 other main protein-bound carbohydrates, hexoses and hexosamines(5). In muscle diseases, however, these 2 constituents did not increase significantly. Since the value for total hexoses in normal adults was lower than usual(5,15), the moderate increase of this substance in myositis may be only apparent. In PMD total hexoses actually decreased, which may be related to their reported low value in cerebrospinal fluid(16).

In contrast to our results Dryer *et al.*,(8) found the level of phospholipids in PMD decreased to less than half normal value. Total cholesterol has been reported to decrease(17), but we found such a decrease only on a 5% level of significance. Similar studies of muscular dystrophy of vit. E-deficiency(3) had revealed decrease of albumin and increase of β-globulin in moving boundary electrophoretic patterns. The rise of β-globulin was related primarily to strikingly elevated levels of cholesterol and phospholipids. Such severe disturbance of lipid metabolism was reflected also by marked shift in distribution of lipoproteins in paper electrophoretic patterns. Another difference between this nutritional myopathy and clinical forms of the disease was the increase, in the former, of all main protein-bound carbohydrates.

Summary. 1. Serum proteins, lipoproteins and glycoproteins were studied primarily in pseudohypertrophic muscular dystrophy (PMD), myositis, myotonia dystrophica and amyotrophic lateral sclerosis, and in scoliosis and Perthes disease with occasional secondary muscular changes. 2. Moving boundary electrophoretic patterns revealed significant, although not specific, increase of a_2-globulin in nearly all muscle diseases. This increase seemed to be related to a high level of sialic acid, particularly in PMD. 3. Distribution of lipoproteins in paper electrophoretic patterns and concentrations of cholesterol and phospholipids did not show the pronounced changes previously found in muscular dystrophy of vit. E-deficiency in rabbits. 4. In contrast to this experimental myopathy and many other wasting diseases, human muscle diseases were not accompanied by significant increase of protein-bound carbohydrates other than that of sialic acid in PMD.

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