Serum Proteins, Lipoproteins and Glycoproteins. In Muscular Dystrophy of Vit. E-Deficiency.*† (23909)

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Many species of animals develop muscular dystrophy when they are deprived of Vit. E (1) and recover when the vitamin is administered(2,3). The symptoms of this form of nutritional muscular dystrophy resemble in many ways those of patients with muscular dystrophy. Metabolic studies, particularly in rabbits, have revealed a pronounced increase in concentrations of cholesterol and phospholipids in the blood serum and skeletal muscle (4-7). Since the lipids and the bulk of carbohydrates in blood are transported in association with proteins, a closer study of these serum protein complexes seemed to be warranted. It appeared probable that the nature and composition of these lipoproteins and glycoproteins might reflect basic changes in metabolism and thereby give information on the nature of these abnormalities. Previous preliminary investigations(8,9) had shown definite alterations in the moving boundary electrophoretic pattern of blood serum of dystrophic rabbits. In this report the distribution of proteins, lipoproteins and glycoproteins amongst the various electrophoretic fractions of blood sera has been studied in rabbits during development of nutritional muscular dystrophy and during recovery from this disease.

Methods. Muscular dystrophy was produced in young New Zealand rabbits within 3-4 weeks by maintaining animals on Vit. E-deficient diet of Goettsch and Pappenheimer (1), treated with 1% ethereal $FeCl_3$ to eliminate traces of the vitamin. The rabbits weighed 1½ to 2½ lbs when started on this diet. Control rabbits received the same diet and twice a week were given a supplement of 6-7 mg of dl-α-tocopherol (0.25-0.3 ml of 2.5% solution in peanut oil). The same dose of dl-α-tocopherol was administered to effect recovery of dystrophic rabbits. Progress of the disease was followed by determining the ratio of urinary excretion of creatine to that of creatinine(10), which rises to high values in muscular dystrophy. Blood was taken from ear veins after animals had been fasted overnight and the serum separated from the clot by centrifugation. The moving boundary electrophoresis of serum in barbiturate buffer of pH 8.6 and of ionic strength 0.1 was carried out in an Aminco-Stern electrophoresis apparatus at 1°-2°C with a current of 15 mA for 2 hours. Relative concentrations and mobilities were calculated for the ascending pattern. The various electrophoretic fractions were actually separated from each other by means of paper electrophoresis in an LKB apparatus(11). The serum was streaked with Kirk micropipettes across paper strips of 1″ or 1½″ width. Volumes of 10, 30 and 40 λ were applied, depending on whether the electropherograms were to be stained for proteins themselves or for protein-bound lipids or carbohydrates. However, 500 λ were applied on a sheet of 17 cm width when the electrophoretic fractions were to be analyzed for cholesterol and phospholipids. The experiments were done with horizontal strips of Whatman No. 1 filter paper in barbiturate buffer of pH 8.6 and of ionic strength 0.1, at a potential of usually 210 volts and at a current density of 0.1-0.15 mA/cm for about 16 hours at room temperature. The dried strips were stained with Amidoblack 10-B for proteins (11), with Oil Red O for lipoproteins(12), and with periodate-Schiff reagent for glycoproteins(13). The stained bands were scanned with a Photovolt Electronic Densitometer model 525, using narrow band color filters with maximum transmission at 600 mμ, 515 mμ, and 550 mμ respectively. For these measurements the strips stained with Amidoblack 10-B were made translucent by immer-

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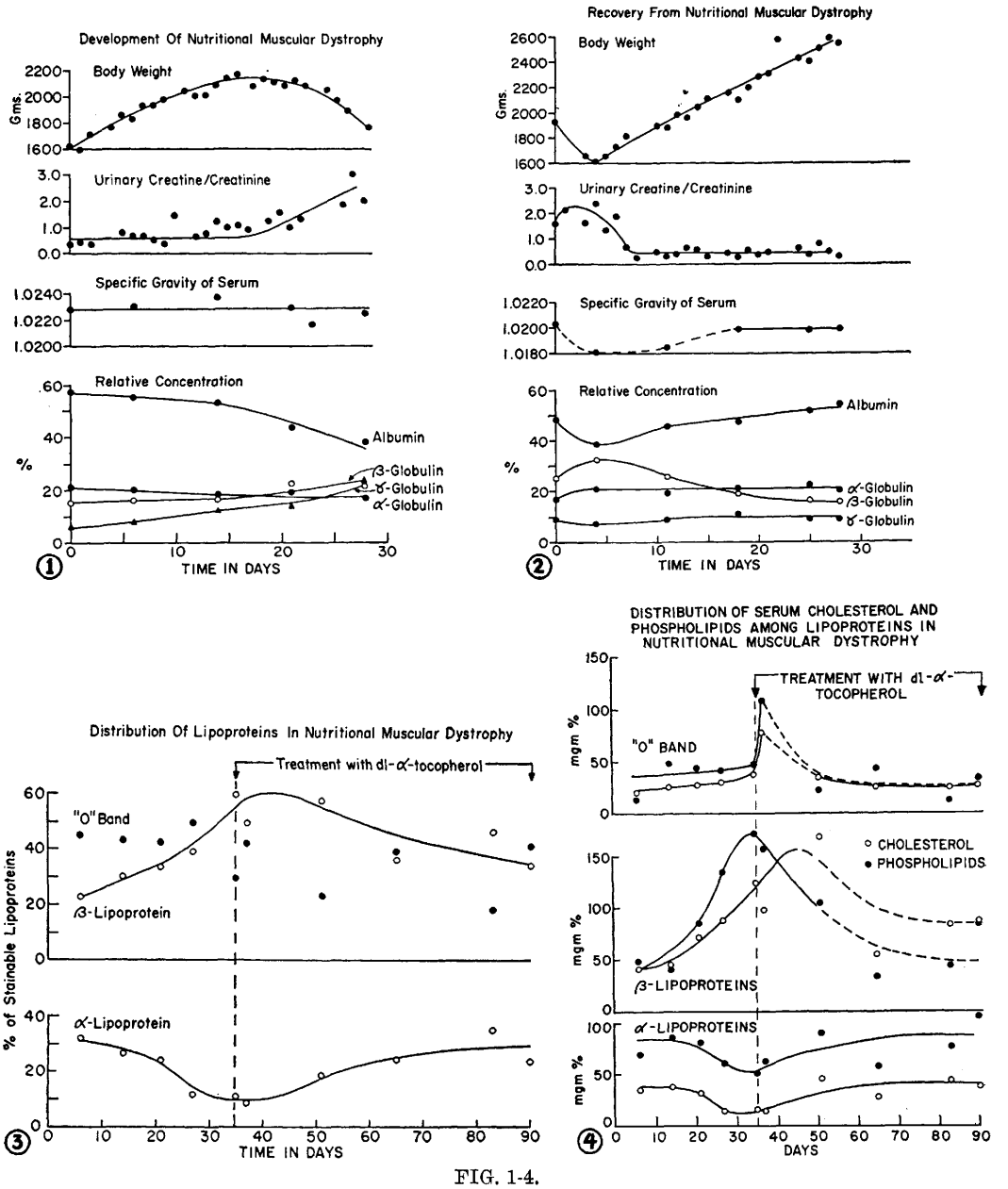

FIG. 1-4.

sion in a mixture of paraffin oil and alpha-monobromonaphthalene(14) or in anisole (15). (Both immersion liquids have a refractive index of 1.51 which is close to that of the paper fibers.) The other stains, however, were measured dry. In the resulting diagrams relative concentrations of electrophoretic fractions were determined with a compensating polar planimeter. Specific gravity of sera was measured in a Lamotte falling drop densitometer(16). The protein nitrogen was determined by the Biuret method(17). The lipoprotein fractions were analyzed for total cholesterol by the Liebermann-Burchard reaction(18) and for phospholipids by the method of Fiske and Subbarow(19). The simultaneous determination of total and of free cholesterol in whole serum was carried out with a

TABLE I. Serum Cholesterol and Glycoproteins in Rabbits during Development of Muscular Dystrophy and during Recovery.

		Cholesterol			Hexoses			
Days	Diet	Free, mg %	Ratio: Free/Total	Esterified, mg %	Total	In seromucoid fraction	Hexosamines	Sialic acid
					mg %			
7	Vit. E-free				75.2	13.0	75.8	65.1
14	*Idem*	28	.22	98	72.4	12.2	73.4	48.7
20	"	55	.29	134	116.5	20.0	91.9	101.0
21	" + tocoph.*							
25	*Idem*	84	.28	213	93.0	10.4	87.6	80.0
28	"	51	.20	202	74.9	10.7	82.4	75.4
32	"	58	.23	196	76.9	11.5	76.9	60.6

* dl-α-tocopherol.

recent method using $FeCl_3$ reagent(20). The glycoproteins were evaluated in terms of the following protein-bound carbohydrates: total hexoses and hexoses in the seromucoid fraction by the orcinol method(21), hexosamines with Ehrlich's reagent(21), and sialic acid with Bial's reagent(22).

Results. Fig. 1 shows the gradual variation of the relative concentrations of serum proteins in moving boundary electrophoretic patterns during development of nutritional muscular dystrophy. It also includes curves for the specific gravity of serum, ratio of urinary creatine:creatinine, and body weight. The latter 2 measurements served to characterize various stages of the disease(2). Decrease of albumin and increase of β-globulin were found in every rabbit investigated and statistically highly significant ($P<.001$). However, changes of the α-globulin fraction which consisted of 3 partly overlapping peaks, and of γ-globulin were not consistent. In contrast to the shift in the distribution of relative concentrations, the mobilities of corresponding serum proteins were alike in dystrophic and normal rabbits. The values for protein nitrogen were also not affected by the progress of the disease. In one rabbit they stayed within the small range of 1.03-1.10 g/100 ml of serum. Fig. 2 shows the effect of treatment of a dystrophic rabbit with dl-α-tocopherol in causing the electrophoretic pattern slowly to return to normal over a period of 4 weeks. Relative concentrations of the various proteins as determined by paper electrophoresis changed with time in a manner generally similar to those in the moving boundary patterns of Fig. 1 and 2.

Distribution of the stainable lipoproteins during development and subsequent cure of muscular dystrophy in a rabbit is presented in Fig. 3. The α-lipoprotein greatly decreased with the progress of the disease and gradually rose again on treatment with dl-α-tocopherol. The β-lipoprotein behaved in a complementary way. Values for the "O" band, which trails the β band and extends towards the origin of the electropherogram(23), scattered considerably, and showed no definite trend.

Fig. 4 shows the amounts of both cholesterol and phospholipids in the α- and β-lipoprotein fractions and in the "O" band. The values have not been corrected for the paper blanks, since the latter fluctuated. The curves varied in a way qualitatively similar to those of the total stainable lipids in Fig. 3. Changes of both lipids in the α-band were more than compensated by their changes in the β-band so that the net result was a considerable rise of their serum levels during the development of the disease and a gradual return to their initial values during period of recovery. The normal values of the ratio of cholesterol to phospholipids for the α- and β-lipoproteins were about 0.5 and 1.0 respectively, which, incidentally, were similar to those reported for human serum(24).

Table I shows that absolute concentrations of both free and esterified cholesterol increased during development of the disease and then decreased slowly during recovery. The ratio of free cholesterol to total cholesterol also increased during progress of the disease, but fell quickly to its original value when the dystrophic rabbit was treated with dl-α-tocopherol.

Variations of protein-bound carbohydrates (hexoses, hexosamines and sialic acid) also are presented in the Table. The concentrations of all of these substances increased, but to a much smaller extent than did those of the lipids when the rabbits became definitely dystrophic, and returned to their normal values within 1-2 weeks after treatment with dl-α-tocopherol had been started.

Staining of protein-bound carbohydrates in the paper electrophoretic fractions did not produce a well-defined pattern. Usually 3 bands were found, at the locations of the α_2-, α_3- and β-globulins. The first 2 bands partly overlapped, which would indicate why occasionally they have been reported as one band in the literature(25). The percentile distribution of these stainable glycoproteins did not seem to vary significantly during development of dystrophy.

Discussion. Changes in the moving boundary electrophoretic pattern during development of nutritional muscular dystrophy were observed earlier than were clinical symptoms and may therefore be used as a more sensitive criterion for onset of the disease. The significant changes in concentrations of albumin and β-globulin represented, to some extent, slow and moderate changes in the proteins themselves as was indicated by paper electrophoretic results. The major changes, however, involved those lipid constituents which are associated with proteins as α- and β-lipoproteins, *i.e.* primarily cholesterol and phospholipids. The large amounts of lipids in the β-fraction probably were mainly responsible for the pronounced increase of β-globulin in the moving boundary electrophoretic pattern during development of the disease. The lipids found in the "O" band are associated only to a small extent with γ-globulin. They consist mainly of substances which have been adsorbed on filter paper, such as chylomicrons (containing neutral fat) and some β-lipoprotein(23).

The observed decrease of α-lipoprotein and corresponding increase of β-lipoprotein are not limited to muscular dystrophy, but have been reported for other diseases in rabbits, particularly atherosclerosis(26,27).

In contrast to the lipids, the protein-bound carbohydrates did not increase nearly as much during progress of the disease and, on treatment, they returned to their normal values more rapidly. The total protein nitrogen did not vary at all. The magnitude of the changes in the lipids and the slow return to normal levels point to the conclusion that disturbances of the lipid metabolism were more severe than were those of the metabolism of proteins and carbohydrates.

Summary. 1. During development of muscular dystrophy of Vit. E-deficiency in rabbits the electrophoretic pattern of serum proteins revealed a decrease of albumin and an increase of β-globulin. These changes were observed earlier than were the usual clinical symptoms and were reversed only gradually during recovery. 2. Concentrations of both cholesterol and phospholipids decreased in α-lipoprotein, but increased greatly in β-lipoprotein so that they rose considerably in whole serum. The magnitude of these changes and the slow return to normal values during recovery indicate severe disturbances of lipid metabolism. 3. In contrast to the lipids, total protein nitrogen did not vary during progress of the disease and the protein-bound carbohydrates increased only moderately and, on treatment, soon returned to their normal levels.

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Effect of Cortisone, Properdin and Reserpine on *Neisseria gonorrhoeae* Endotoxin Activity. (23910)

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In connection with studies on endotoxin derived from *Neisseria gonorrhoeae*(1), we were interested in determining whether certain therapeutic agents would afford a measure of protection against, or enhancement of, toxic properties of endotoxin. We used as therapeutic agents cortisone, reserpine and properdin. Cortisone has been shown by workers to afford some measure of protection against toxic effects of certain bacterial toxins (2). Reserpine and properdin were included since to our knowledge they have not been investigated previously in conjunction with any endotoxin. It was hoped that reserpine and properdin might exert a protective action through interference with possible neurotoxic effect of endotoxin. It has been recently suggested that the properdin system may be a factor involved in natural resistance(3).

Materials and methods. Male CF1 mice weighing 16-18 g, were kept (about 10 animals/cage) in an air-conditioned room and had free access to water and Puritan Mouse Ration. Endotoxin was prepared according to our recent procedure(1). The culture medium, however, has been slightly modified. The nutrient broth has been omitted from the medium and proteose peptone concentration has been increased to 8 g/l. This change resulted in more rapid growth of gonococcus, harvesting being possible in 96 hours. Acetone powder was dissolved in a small volume of 0.05 N sodium hydroxide. The resulting solution was adjusted to pH 7.0-7.4 with 0.25 N acetic acid, diluted to desired concentration with sterile distilled water and administered intraperitoneally. Cortisone (cortone acetate) and reserpine (Serpasil) were diluted with sterile saline to appropriate concentrations and administered intramuscularly. Karber's procedure was used for calculating LD_{50} values and interpreting their statistical meaning(4).

Experiments were conducted as follows: each group of mice was sub-divided into control-challenge and treated-challenge groups. The control-challenge group received only endotoxin, whereas, the treated-challenge group received endotoxin plus one of the 3 agents. Endotoxin controls (LD_{50} 2-3 mg) were run with each experiment since LD_{50} values varied somewhat with each new group of mice. Mice