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# Blood Lipids in Progressive Muscular Dystrophy*

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Muscular dystrophy is a heredofamilial disease characterized by primary degeneration of certain groups of voluntary muscles. Although several forms of the disease are recognized clinically the basic metabolic defect is probably the same in all cases, regardless of the outward manifestations. In some instances it appears that the genetic defect is sex-linked, while in others it is not. All of the members of a given family may not show the obvious signs of disease, but if a careful study is made the non-affected members often show more or less subtle and asymptomatic defects which may be characterized as abortive forms of the disease. In experimental animals a type of dystrophy may be produced by diets which are deficient in vitamin E, and the resulting dystrophy is accompanied by a markedly increased oxygen consumption of even the resting muscle. At the same time the urine of dystrophic animals, like the urine of dystrophic humans, contains large amounts of creatine and very little creatinine. Since creatine phosphate is intimately involved in the metabolism of skeletal muscle, loss of this vital intermediate is completely in line with the obvious muscular wasting. Attempts at alleviation of human disease by vitamin E therapy have been disappointing. While vitamin E, alone or with inositol (1), corrects the creatinuria, the other degenerative changes are not altered from their progressive course.

For the past ten years we have been engaged in cataloging the electrophoretic patterns of serum and plasma proteins in a variety of diseases, and our interest in muscular dystrophy was recently stimulated by receipt of several samples of serum and plasma as part of this general project. It was observed that the few samples then available were distinctly lactescent or chylous in appearance. Investigation of successive samples from a larger number of individuals indicated that this was a general observation. In some instances post-absorbtive samples were sufficiently opaque to completely obscure large newsprint held directly behind the sample tube (Figure 1). The striking appearance of the samples lead us to investigate the content of some lipoid components. Our findings in this connection constitute the basis of the present report.

## Methods

The total plasma lipids were extracted by Sperry's modification (2) of a procedure due to Folch *et al.* (3). This is essentially a counter-current extraction which has been shown to give more

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complete extraction than the classical methods, and is substantially free of non-lipid contaminants. Free and total cholesterol were measured by the method of Schoenheimer and Sperry (4). Total lipid phosphorus was determined by the method of Youngberg and Youngberg (5). Electrophoresis was performed in the Tiselius apparatus according to techniques which have been in use in this laboratory for more than ten years (6). Samples of plasma were diluted to an appropriate protein concentration with veronal buffer (pH = 8.6, ionic strength = 0.1) before dialysis. Enlarged tracings of the scanned patterns were divided according to the method of Pedersen (7). A few samples were also studied in acetate buffer at pH = 4.5.

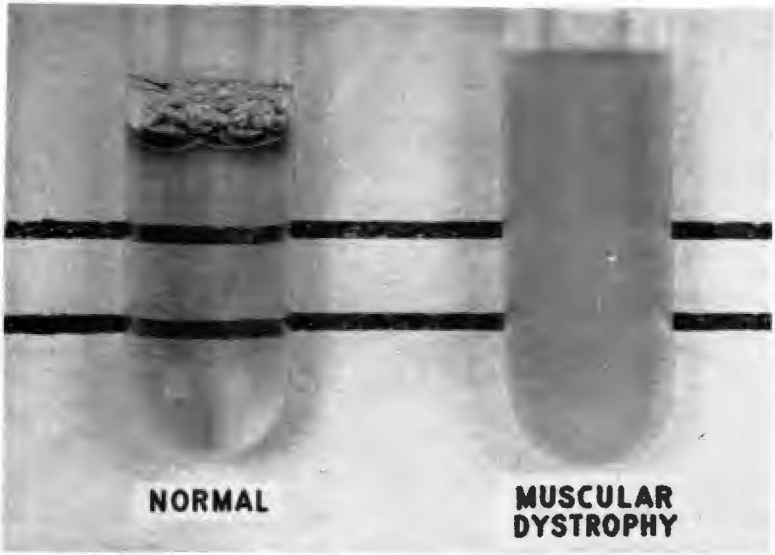
## Results

The observed values for total plasma lipid, plasma free and total cholesterol, and plasma total lipid phosphorus are given in Table I. Since it has been claimed that the relation between cholesterol and lipid phosphorus is perhaps more constant than either measurement taken alone (8), Table I also includes the calculated content of lipid phosphorus predicted from the measured total cholesterol and the regression equation of Peters and Man (9).

In the course of extracting the total lipids from the plasma, the

**Table I**
Lipid Fractionation of Human Dystrophic Plasma

| Total Lipid, Mg%. | Free Cholesterol, Mg%. | Total Cholesterol, Mg%. | Measured Lipid Phosphorus, Mg%. | Predicted Lipid Phosphorus, Mg%. |
|---|---|---|---|---|
| 450 | 23.0 | 142 | 0.4 | 7.9 |
| 600 | 21.5 | 136 | 4.0 | 7.7 |
| 475 | 27.5 | 141 | 0.3 | 7.9 |
| 550 | 19.0 | 125 | 3.5 | 7.4 |
| 450 | 22.0 | 117 | 1.3 | 7.1 |
| 450 | 12.5 | 90 | 3.3 | 6.3 |
| 875 | 34.0 | 191 | 3.4 | 9.4 |
| 500 | 23.0 | 111 | 0.5 | 7.0 |
| 575 | 35.0 | 148 | 4.6 | 8.1 |
| 525 | 25.0 | 127 | 3.4 | 7.4 |
| 700 | 20.5 | 124 | 5.1 | 7.3 |
| 775 | 50.0 | 165 | 4.9 | 8.6 |
| 750 | 52.5 | 194 | 6.9 | 9.4 |
| 600 | 41.0 | 166 | 1.1 | 8.6 |
| 600 | 40.0 | 172 | 0.8 | 8.8 |
| 750 | 41.0 | 199 | 1.7 | 9.6 |
| 825 | 55.5 | 177 | 5.9 | 8.9 |
| 850 | 56.0 | 188 | 4.8 | 9.3 |
| 675 | 44.5 | 152 | 4.0 | 8.2 |
| 775 | 42.0 | 161 | 4.5 | 8.5 |
| | | Dystrophic Mean | | |
| 637 | 34.0 | 151 | 3.2 | 8.2 |
| | | Normal Mean | | |
| 600 | 46.0 | 178 | 7.2 | 8.9 |



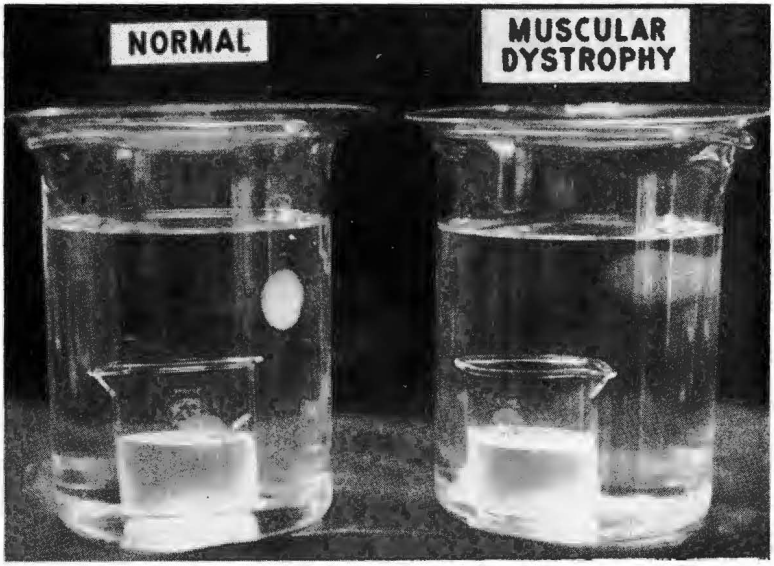




Figure 1. Lactescent Appearance of Dystrophic Plasma Sample.
Figure 2. Formation of Interfacial Precipitate in Extraction of Plasma Lipids.

free lipids are contained in a subnatant chloroform phase, the water-soluble tissue components are held in a supernatant aqueous phase, and a mixture of lipoproteins and proteolipids are found as a fluffy precipitate which forms at the interface between the two layers. Figure 2 is a photograph of actual separations as carried out on normal and dystrophic plasmas. The increased amount and apparent density of the "fluff" produced in the dystrophic sample pictured was observed in nearly all the dystrophic samples examined.

The electrophoretic patterns of the dystrophic plasma samples showed that in most instances two striking peculiarities existed. In a group of 22 samples studied by this method 19 showed a twinning of the alpha-2 globulin peak. The area distribution of the total electrophoretic pattern was considerably altered by this abnormality. If the sum of the twin peaks was nominally designated as alpha-2 globulin the area under this portion of the pattern was, on the average, 13.7% for plasma. By contrast the normal figure for the same part of the area is, on the average, 9.0%. The second peculiarity was found in the gamma globulin area, which averaged 9.6% compared with a normal average of 12.5% for this component. None of the patterns contained a beta globulin spike. Complete details of the electrophoretic studies will be published elsewhere in the near future.

## Discussion

The determined values of the lipid components are rather surprising in view of the appearance of the samples. The level of total plasma lipids is, in all cases examined, within the normal limits. Obviously the appearance of the samples is not based on simple hyperlipemia, as was at first suspected. The free cholesterol levels are surprisingly low, and the total cholesterol levels are at best in the low normal range. By far the most outstanding abnormality is the level of total plasma lipid phosphorus. This usually has a value from 6 mg% to 9 mg%, but in the series of dystrophic plasma samples only one value can be found even in the low normal range, and the mean for the dystrophic series is about one half of the lower limit of normal. The chemical findings are made even more impressive by the observations (10) that experimental dystrophy in rabbits is accompanied by elevated levels of lipid phosphorus and cholesterol. The difference between the observed level of lipid phosphorus and that predicted by the regression equation of Peters and Man is good evidence that the decrease observed in these plasma components is not in any simple proportion.

A few diseases other than progressive muscular dystrophy may give rise to lactescent plasma in the absence of demonstrable hyperlipemia. The most common of these is xanthomatous biliary cirrhosis, but when the lipids are extracted from such cirrhotic samples by

the same procedure employed here there is no evidence of the increased interfacial precipitate, nor do such samples show the peculiar distortion of the electrophoretic pattern. The implication is therefore strong that in human dystrophy some abnormal relation between the lipids and the proteins exists which may be of consequence in establishing the basic pathological mechanisms of this disease. Whether an abnormal complex exists or whether a normal complex is absent is the subject of continuing studies.

## Conclusions

Postabsorbtive samples of plasma from 22 cases of human progressive muscular dystrophy were examined by electrophoresis. In 19 cases, the patterns demonstrated an abnormal twinning of the alpha-2 globulin peak and a decrease in the gamma globulin. The majority of the samples were markedly lactescent, but chemical analysis demonstrated a normal level of total lipid. The cholesterol and lipid phosphorus levels were depressed, the phosphorus level was on the average one half the normal value. Extraction of the lipids was accompanied by a significant increase of interfacial precipitate not usually found when the method of Sperry is employed.

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