Phospholipid Composition and Metabolism in Mouse Muscular Dystrophy

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1. The composition and metabolism of phospholipids were studied in various tissues from both normal and dystrophic mice of the 129 ReJ strain. Phospholipids extracted from forebrain, spinal cord, sciatic nerve and plasma were fractionated by t.l.c. and measured. 2. Very significant alterations were found in the choline phospholipids from these tissues, except forebrain. Plasma phosphatidylcholine in the dystrophic mouse was increased by 38%. There was a 2-fold increase in lysophosphatidylcholine in the spinal cord of dystrophic mice. The sciatic nerve showed a marked decrease in sphingomyelin content, which is approximately half of that in the controls. 3. Five enzymes involved in phosphatidylcholine metabolism [namely cholinephosphotransferase (EC 2.7.8.2); phospholipases A (EC 3.1.1.4, EC 3.1.1.32); lysophospholipase (EC 3.1.1.5); lysophosphatidylcholine acyltransferase (EC 2.3.1.23); phospholipase C (EC 3.1.4.3)] were studied in tissue preparations from forebrain, spinal cord, sciatic nerves, gastrocnemius muscles and liver. 4. Activities of phospholipases A and C were significantly increased, about 5-fold and 60% respectively, in gastrocnemius muscle of dystrophic mice compared with controls. Phospholipase A also showed 50% higher activity in the sciatic nerves of dystrophic than of normal mice. Lysophosphatidylcholine acyltransferase activities were significantly increased in the sciatic nerves and spinal cord, by 50–100% over that of the controls. The forebrain and spinal cord from dystrophic mice, however, had only 60% of lysophospholipase activities of that of the normal control. Cholinephosphotransferase activity was unchanged in these tissues from both normal and dystrophic mice. 5. It is suggested that a number of features of mouse muscular dystrophy related to altered membrane structure and function can be rationalized in terms of changes in lipid composition and metabolism.

It is commonly observed that there is a pronounced increase in the amount of neutral lipids associated with the diseased muscles in various forms of muscular dystrophy (Shull & Alfin-Slater, 1958; Kunze & Olthoff, 1970). In addition, changes in the contents of membrane lipids have been noted. Amounts of cholesterol and sphingomyelin are increased, whereas the amount of phosphatidylcholine in human and mouse muscle and human erythrocytes is decreased (Kunze & Olthoff, 1970; Hughes, 1972; Kunze *et al.*, 1973). Changes have been noted in the fatty acid composition of phosphatidylcholine from diseased tissues, particularly a marked increase of oleic acid ($C_{18:1}$) and a corresponding decrease of linoleic acid ($C_{18:2}$), and Kunze and co-workers have suggested that the metabolic lesion associated with these changes may be related to a decrease in incorporation of linoleic acid during biosynthesis *de novo* of phosphatidylcholine (Kunze *et al.*, 1973; Kunze, 1975).

There are also changes in turnover of phospholipids in tissues of the dystrophic mouse (Kwok *et al.*, 1976).

The rate of turnover is increased in neural tissues such as brain, spinal cord and sciatic nerve, as well as in skeletal muscle. These changes are particularly interesting, since they may be correlated with the many abnormalities found in membranes from dystrophic tissues (Kleine, 1970; Matheson & Howland, 1974; Bradley & Jenkinson, 1975).

In this paper we report altered amounts of individual phospholipids (particularly the choline phospholipid group) and increased degradation of phosphatidylcholine in tissues of the 129 ReJ *dy/dy* mouse.

Experimental

Animals

Dystrophic mice of the 129 ReJ strain were originally obtained from Jackson Laboratories (Bar Harbor, ME, U.S.A.), and later bred in our animal house. Animals weighing 12–16 g and 8–12 weeks old were used together with carrier (*dy/+*) or normal (*+/+*) litter mates as controls.

Materials

[*Me*-³H]Choline (sp. radioactivity 10.1 μ Ci/mmol), [¹⁻¹⁴C]linolenic acid (sp. radioactivity 60 mCi/mmol), [9,10(n)-³H]palmitic acid (sp. radioactivity 500 mCi/mmol) and CDP-[*Me*-¹⁴C]choline (sp. radioactivity 58 mCi/mmol) were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). 1-[1-¹⁴C]palmitoyl-*sn*-glycero-3-phosphocholine (55 mCi/mmol) was obtained from New England Nuclear (Boston, MA, U.S.A.). ATP was obtained from C. F. Boehringer and Soehne G.m.b.H. (Mannheim, Germany). Silica gel G (type 60) and H (type 60) are products of Merck (Darmstadt, Germany). CoA, CDP-choline, sodium taurocholate, butylated hydroxytoluene (an anti-oxidant), linolenic acid and other lipid standards were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents were of A.R. grade. Organic solvents were obtained from either British Drug Houses (Boronia, Vic., Australia) or Mallinckrodt Chemical Works (St. Louis, MO, U.S.A.) and were used without further redistillation. Silicic acid (100 mesh) was obtained from Mallinckrodt. Bovine serum albumin (Cohn fraction V) was obtained from Commonwealth Serum Laboratories (Melbourne, Australia).

Preparations of isotopically labelled substrates

Radioactive substrates were diluted with unlabelled materials to the specified concentrations. A linolenic acid-albumin complex solution was made by the method of Webster (1967).

[*Me*-³H]Choline-labelled phosphatidylcholine was obtained from rat brain (18 days old) after intracerebral injection of [*Me*-³H]choline (200 μ Ci/20 μ l per brain) (Bickerstaff & Mead, 1967). The mice were killed 6 h later, the brains removed and lipids extracted with chloroform/methanol (2:1, v/v, containing 0.005% butylated hydroxytoluene) by the procedure of Folch *et al.* (1957). The phospholipid fraction was isolated by silicic acid column chromatography by the method described by Sun & Horrocks (1969). Radioactive phosphatidyl[*Me*-³H]choline was purified by preparative t.l.c. on silica gel G (0.5 mm thick) and developed in the solvent chloroform/methanol/25% (w/w) NH₃ (65:25:4, by vol.). Phosphatidyl[*Me*-³H]choline so obtained from different preparations had specific radioactivities ranging from 0.2 to 1.0 μ Ci/ μ mol.

[³H]Palmitoyl-labelled phosphatidylcholine was similarly prepared after intracerebral injection of [9,10(n)-³H]palmitic acid (0.5 mCi/20 μ l per brain). After 24 h incubation *in vivo*, brain lipids were extracted and purified as above. The [³H]palmitoyl-labelled phosphatidylcholine obtained had a specific radioactivity of 0.4 μ Ci/ μ mol.

Homogenate preparation

Tissues such as forebrain, spinal cord, sciatic nerves, gastrocnemius muscles and liver were removed immediately after decapitation. A 10% (w/v) homogenate was prepared in 0.2 M-Tris/HCl buffer, pH 7.4, or in 0.9% NaCl for phospholipases A assay.

Blood was drawn from the abdominal vena cava, into a heparinized syringe, after the animal was killed by ether asphyxia. Plasma was obtained by centrifugation at 1000 g for 10 min.

Assay of enzyme activities

The activities described for all enzymes were linear with respect to time and amount of protein used. Basically the assay procedures in all cases involved the addition of a labelled substrate and a sample of 10% (w/v) tissue homogenate and the radioactive product was purified and counted for radioactivity in a scintillation counter.

Cholinephosphotransferase (EC 2.7.8.2). This was determined essentially as described by Kennedy & Weiss (1956). A 10% homogenate was incubated with shaking at 37°C for 1 h in an incubation mixture containing Tris/HCl buffer (0.1 M, pH 7.4), MnCl₂ (5 mM), ATP (2 mM), CDP-[*Me*-¹⁴C]choline (sp. radioactivity 0.1 μ Ci, 115 nmol) in a final volume of 1 ml. Chloroform/methanol (2:1, v/v, containing 0.005% butylated hydroxytoluene) and carrier phosphatidylcholine (50 μ g) were added and the phosphatidylcholine product was extracted and purified by t.l.c.

Phospholipases A (EC 3.1.1.4 and EC 3.1.1.32). These were jointly assayed at pH 5.0 essentially as described by Webster (1973) with some modifications. The original assay procedure did not selectively measure activities of phospholipases A, as the system contained substantial lysophospholipase (EC 3.1.1.5) activity. Sodium taurocholate in the original method was replaced by equal amounts of sodium taurocholate and sodium deoxycholate, which completely abolished lysophospholipase activity.

An incubation mixture in a final volume of 0.7 ml, containing 10% homogenate, 0.5 ml of 0.1 M-sodium acetate/0.9% NaCl (pH 5.0), 1.5 mg of sodium taurocholate, 1.5 mg of sodium deoxycholate and phosphatidyl[*Me*-³H₃]choline (0.09 μ Ci, 0.6 mM) was incubated for 30 min. Labelled lysophosphatidylcholine product was co-extracted with carrier lysophosphatidylcholine (200 μ g).

Lysophospholipase activity. An incubation mixture in a final volume of 0.7 ml containing 10% homogenate, 0.5 ml of 0.1 M-acetate/0.9% NaCl (pH 5.0), 3 mg of sodium taurocholate and 1-[1-¹⁴C]palmitoyl-lysophosphatidylcholine (0.1 μ Ci, 0.3 mM) was incubated for 1 h. Carrier palmitic acid (200 μ g) was co-extracted with the radioactive product and separated

on silica gel G-coated t.l.c. in a solvent mixture of hexane/diethyl ether/acetic acid (70:30:1, by vol.).

Lysophosphatidylcholine acyltransferase (EC 2.3.1.23). The method of Webster & Alpern (1964) was used. An incubation mixture containing 10% homogenate, Tris/HCl buffer (60mM, pH 7.4), $MgCl_2$ (5mM), ATP (1mM), CoA (60 μ M), lysophosphatidylcholine (100 μ M), NaF (100mM) and [1- ^{14}C]linolenic acid (0.2 μ Ci, 0.26mM) in a final volume of 1 ml was incubated at 37°C for 1 h with shaking. The reaction was stopped by addition of chloroform/methanol (2:1, v/v, containing 0.005% butylated hydroxytoluene). Phosphatidylcholine was extracted as described for choline phosphotransferase activity.

Phospholipase C (EC 3.1.4.3). An incubation mixture in a final volume of 0.5 ml containing 10% homogenate, Tris/HCl buffer (120mM, pH 7.4), 5mM- Ca^{2+} , 125 μ g of sodium deoxycholate and [3H]palmitoyl-labelled phosphatidylcholine (0.05 μ Ci, 0.3mM) was incubated for 1 h. The radioactive diacylglycerol products were co-extracted with carrier diacylglycerol (200 μ g) and separated on t.l.c. as described for palmitic acid under 'Lysophospholipase activity'.

All reactions were carried out in capped vials (15mm $\times$ 55mm) at 37°C with continual shaking in a metabolic water bath. A preincubation of 2 min was allowed; the reaction was initiated by addition of the radioactive substrate and terminated with 2ml of chloroform/methanol (2:1, v/v, containing 0.005% butylated hydroxytoluene).

Phospholipid extraction and measurement

Carrier phospholipids (100–200 μ g) were added to the vials and the phospholipids extracted with 8 ml of chloroform/methanol (2:1, v/v, containing 0.005% butylated hydroxytoluene). The tubes were vigorously shaken for 1 min and left overnight at room temperature (20°C) to complete the extraction. The extracts were then shaken for 1 min and washed once with 0.2 vol. of Folch upper-phase solution (Folch *et al.*, 1957), consisting of chloroform/methanol/0.88% (w/v) KCl (3:48:47, by vol.) and then with 0.2 vol. of 0.88% (w/v) KCl. The chloroform layer was carefully removed and dried down to a volume of 50 μ l. The radioactive product, phosphatidylcholine or lysophosphatidylcholine, was co-chromatographed with authentic standards on t.l.c. coated with silica gel G (0.25mm thick) in the solvent system described for the preparation of radioactively labelled phosphatidylcholine.

The band corresponding in R_F to the standard compounds was removed with a glass aspirator and eluted into a counting vial with chloroform/methanol (2:1, v/v) (4.5ml), followed by methanol/acetic acid/water (94:1:5, by vol.) (1.5ml), and finally methanol (4.5ml) essentially as described by Skipski *et al.* (1964). The purified radioactive product was measured by

counting radioactivity in toluene scintillator (10ml) containing 0.5% 2,5-diphenyloxazole and 0.03% 1,4-bis-(5-phenyloxazol-2-yl)benzene in a Philips scintillation counter.

Tissue phospholipids were extracted from samples of tissue homogenate by the above procedure. Phospholipids were fractionated by t.l.c. coated with silica gel H (0.25mm thick) and developed in a solvent consisting of chloroform/methanol/acetic acid/water (25:15:4:2, by vol.) (Skipski *et al.*, 1964).

Phospholipid P was determined by the method of Hess & Derr (1975).

Protein content was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Results

Phospholipid composition of forebrain, spinal cord, sciatic nerve and plasma from normal and dystrophic mice

The results in Table 1 show that the phospholipid content of the sciatic nerve of the dystrophic mice is significantly (46%) less than normal. The most significant decrease was in phosphatidylinositol plus phosphatidylserine (37% less), and sphingomyelin, which is 40% of that in the normal nerve. The total phospholipid content of the forebrain and spinal cord showed no significant difference between the normal and dystrophic mice. However, a significant difference was found in the content of lysophosphatidylcholine, which is increased 2-fold in the cord of dystrophic mice.

However, when the phospholipid composition was expressed as the mol percentage of the individual phospholipid per 100mol of total phospholipid P (Table 2), only spinal-cord lysophosphatidylcholine and sciatic-nerve sphingomyelin from the dystrophic mouse differed significantly from normal. The amount of lysophosphatidylcholine in the spinal cord of dystrophic mice expressed as a mol percentage was twice that in the normal. The sphingomyelin in the nerve of dystrophic mice was only half that of the normal. These results show that there are marked abnormalities in the amounts of specific phospholipids in neural tissues from the dystrophic mice.

The total phospholipid content of dystrophic mouse plasma also differed significantly from that of the controls. The plasma phospholipid content of dystrophic mice is 33% higher than the controls. Here, there are increases in phosphatidylinositol plus phosphatidylserine and phosphatidylcholine (54% and 38% respectively). When these results were expressed as mol percentages only phosphatidylcholine and lysophosphatidylcholine from the dystrophic mouse differed significantly from normal. There was only a 3% increase in phosphatidylcholine in the plasma of the dystrophic mouse when it was expressed

Table 1. *Phospholipids and proteins in some dystrophic mouse tissues*
 Phospholipid contents are expressed as nmol/mg of protein, $\pm$ s.e.m. Plasma protein concentrations are expressed as mg/ml, $\pm$ s.e.m. Numbers in parentheses are the numbers of tissues analysed. All determinations were performed in duplicate. * $t < 0.05$. ** $t < 0.01$. *** $t < 0.001$. r test was used.

Phospholipids	Mice	Brain		Spinal cord		Sciatic nerve		Plasma	
		Normal	Dystrophic	Normal	Dystrophic	Normal	Dystrophic	Normal	Dystrophic
Phosphatidylethanolamine		139.49 $\pm$ 4.38 (11)	143.05 $\pm$ 4.73 (10)	282.8 $\pm$ 17.6 (11)	242.7 $\pm$ 14.8* (11)	257.0 $\pm$ 23.4 (7)	187.2 $\pm$ 21.1** (8)	0.87 $\pm$ 0.05 (6)	1.04 $\pm$ 0.20 (6)
Phosphatidylinositol + phosphatidylserine		52.39 $\pm$ 1.96 (11)	48.23 $\pm$ 1.75 (10)	71.8 $\pm$ 7.6 (11)	65.3 $\pm$ 5.7 (10)	142.1 $\pm$ 9.3 (7)	103.5 $\pm$ 8.8*** (8)	1.00 $\pm$ 0.14 (6)	1.54 $\pm$ 0.11** (6)
Phosphatidylcholine		144.50 $\pm$ 5.15 (11)	150.81 $\pm$ 4.08 (10)	184.3 $\pm$ 10.9 (11)	180.1 $\pm$ 9.5 (11)	185.6 $\pm$ 16.5 (7)	135.6 $\pm$ 16.2* (8)	19.45 $\pm$ 1.05 (6)	26.92 $\pm$ 1.25*** (5)
Sphingomyelin		14.04 $\pm$ 2.08 (11)	12.70 $\pm$ 0.85 (10)	42.0 $\pm$ 4.1 (11)	34.6 $\pm$ 3.5 (11)	104.6 $\pm$ 4.5 (7)	43.2 $\pm$ 8.4*** (8)	0.85 $\pm$ 0.10 (6)	1.04 $\pm$ 0.29 (6)
Lysophosphatidylcholine		0.99 $\pm$ 0.13 (11)	0.98 $\pm$ 0.12 (9)	1.32 $\pm$ 0.19 (7)	2.53 $\pm$ 0.29*** (7)	4.9 $\pm$ 1.5 (7)	5.1 $\pm$ 1.7 (8)	1.75 $\pm$ 0.21 (6)	1.81 $\pm$ 0.32 (6)
Total phospholipids		351.71 $\pm$ 10.20 (11)	355.31 $\pm$ 7.25 (10)	581.6 $\pm$ 31.9 (11)	535.1 $\pm$ 29.1 (10)	694.3 $\pm$ 42.6 (7)	474.2 $\pm$ 47.3*** (8)	23.9 $\pm$ 1.1 (6)	31.9 $\pm$ 1.4*** (5)
Protein								78.4 $\pm$ 2.9 (6)	62.4 $\pm$ 0.9*** (6)

Table 2. *Phospholipid composition in some dystrophic mouse tissues*
 Phospholipid contents are expressed as mol % of total lipid P, $\pm$ s.e.m. Numbers in parentheses are the numbers of tissues analysed. All determinations were performed in duplicate. * $t < 0.02$. ** $t < 0.001$. r test was used.

Phospholipids	Mice	Brain		Spinal cord		Sciatic nerve		Plasma	
		Normal	Dystrophic	Normal	Dystrophic	Normal	Dystrophic	Normal	Dystrophic
Phosphatidylethanolamine		39.68 $\pm$ 0.74 (11)	40.06 $\pm$ 0.62 (11)	48.73 $\pm$ 1.38 (7)	46.96 $\pm$ 0.87 (7)	36.60 $\pm$ 1.20 (7)	39.34 $\pm$ 1.44 (8)	3.67 $\pm$ 0.32 (6)	3.45 $\pm$ 0.63 (5)
Phosphatidylinositol + phosphatidylserine		14.93 $\pm$ 0.49 (11)	13.73 $\pm$ 0.56 (11)	13.93 $\pm$ 0.64 (7)	12.17 $\pm$ 0.98 (7)	20.75 $\pm$ 1.46 (7)	22.37 $\pm$ 1.46 (8)	4.26 $\pm$ 0.61 (6)	4.89 $\pm$ 0.55 (5)
Phosphatidylcholine		41.06 $\pm$ 0.76 (11)	42.26 $\pm$ 0.58 (11)	32.33 $\pm$ 1.20 (7)	34.12 $\pm$ 1.41 (7)	26.53 $\pm$ 1.24 (7)	27.97 $\pm$ 1.15 (8)	81.17 $\pm$ 0.69 (6)	84.29 $\pm$ 0.94* (5)
Sphingomyelin		4.01 $\pm$ 0.50 (11)	3.56 $\pm$ 0.21 (11)	6.20 $\pm$ 0.79 (7)	6.24 $\pm$ 0.45 (7)	15.38 $\pm$ 1.00 (7)	8.92 $\pm$ 1.30** (8)	3.66 $\pm$ 0.55 (6)	2.52 $\pm$ 0.63 (5)
Lysophosphatidylcholine		0.28 $\pm$ 0.04 (11)	0.28 $\pm$ 0.03 (9)	0.27 $\pm$ 0.04 (7)	0.52 $\pm$ 0.06** (7)	0.74 $\pm$ 0.22 (7)	1.17 $\pm$ 0.36 (8)	7.23 $\pm$ 0.61 (6)	4.84 $\pm$ 0.59* (5)

as mol percentage of total phospholipid. There was also a corresponding, but significant, decrease in the content of lysophosphatidylcholine.

Phosphatidylcholine biosynthesis de novo

The terminal step in the biosynthesis of phosphatidylcholine *de novo* is catalysed by cholinephospho-

Table 3. *Cholinephosphotransferase activities in various tissues of normal and dystrophic mice*

Activities were determined as described in the Experimental section. Numbers of experiments performed for each tissue are given in parentheses. The results are expressed as nmol of phosphatidylcholine formed/h per mg of protein, $\pm$ S.E.M. In all cases the differences between normal and dystrophic mice were not significant ($t > 0.1$).

Tissue	Mice	Phosphatidylcholine formed	
		Normal	Dystrophic
Brain		1.44 ± 0.40 (4)	1.41 ± 0.19 (4)
Spinal cord		1.01 ± 0.09 (6)	1.05 ± 0.13 (8)
Sciatic nerve		0.70 ± 0.07 (4)	0.56 ± 0.05 (4)
Gastrocnemius muscle		0.23 ± 0.01 (6)	0.22 ± 0.03 (8)
Liver		2.96 ± 0.31 (6)	2.59 ± 0.24 (8)

transferase. No differences were found between normal and dystrophic mice with regard to specific activities of this enzyme in neural tissues, muscle or liver (Table 3).

Degradation of phosphatidylcholine by phospholipases

The metabolism of phosphatidylcholine is diagrammatically presented in Scheme 1. Phosphatidylcholine can be degraded by various phospholipases. One important pathway involves two phospholipases A (EC 3.1.1.4 and EC 3.1.1.32) which catalyse the deacylation of phosphatidylcholine to give lysophosphatidylcholine. The present results (Table 4) showed combined activities of phospholipases A₁ and A₂. The enzyme activities in nervous tissues were linear up to 1 h and 1.5 mg of protein, whereas its activity in muscle showed linearity up to 40 min. Ca²⁺ ions up to 10 mM and bovine serum albumin up to 3 mg had no effect on the overall enzyme activities. In the presence of equal amounts of sodium taurocholate (1.5 mg) and sodium deoxycholate (1.5 mg) lysophospholipase activity was completely abolished.

Table 4 shows that the specific activity of these enzymes was elevated 5-fold in the gastrocnemius muscle and 50% in sciatic nerves from the dystrophic

Scheme 1. Some metabolic interactions of phosphatidylcholine

mouse. No difference was observed in the forebrain and spinal cord between normal and dystrophic mice.

Lysophospholipase and lysophosphatidylcholine acyltransferase activities

The lysophosphatidylcholine formed by the action of phospholipases A can be further degraded to *sn*-glycero-3-phosphocholine, the release of free fatty acid being catalysed by lysophospholipase, or can be reacylated to form phosphatidylcholine via the mediation of lysophosphatidylcholine acyltransferase.

Table 4. *Phospholipases A activities in various tissues of normal and dystrophic mice*

For details see the Experimental section. Numbers of experiments performed are given in parentheses. The results are expressed as nmol of lysophosphatidylcholine released/h per mg of protein $\pm$ S.E.M. Abbreviation: NS, not significant ($t > 0.1$).

Tissue	Mice	Lysophosphatidylcholine released		<i>t</i>
		Normal	Dystrophic	
Brain	...	1.52 $\pm$ 0.24 (6)	1.38 $\pm$ 0.10 (8)	NS
Spinal cord	...	1.86 $\pm$ 0.30 (9)	1.56 $\pm$ 0.24 (10)	NS
Sciatic nerve	...	2.16 $\pm$ 0.20 (6)	3.35 $\pm$ 0.46 (6)	<0.02
Muscle	...	0.36 $\pm$ 0.00 (6)	1.74 $\pm$ 0.30 (6)	<0.001

Table 5. *Lysophospholipase activities in various tissues of normal and dystrophic mice*

Results are expressed as nmol of palmitic acid produced/h per mg of protein, $\pm$ S.E.M. for six experiments.

Tissue	Mice	Palmitic acid produced		<i>t</i>
		Normal	Dystrophic	
Brain	...	0.53 $\pm$ 0.05	0.32 $\pm$ 0.00	<0.001
Spinal cord	...	0.29 $\pm$ 0.05	0.19 $\pm$ 0.01	<0.01
Sciatic nerve	...	1.10 $\pm$ 0.30	0.95 $\pm$ 0.14	NS
Muscle	...	0.24 $\pm$ 0.03	0.23 $\pm$ 0.03	NS

Table 6. *Lysophosphatidylcholine acyltransferase activities in various tissues of normal and dystrophic mice*

The results are expressed as nmol of phosphatidylcholine formed/h per mg of protein $\pm$ S.E.M., for the numbers of experiments given in parentheses.

Tissue	Mice	Phosphatidylcholine formed		<i>t</i>
		Normal	Dystrophic	
Brain	...	9.19 $\pm$ 0.44 (7)	10.98 $\pm$ 1.27 (5)	NS
Spinal cord	...	16.47 $\pm$ 1.56 (6)	30.39 $\pm$ 3.88 (6)	<0.001
Sciatic nerve	...	2.75 $\pm$ 0.35 (7)	3.90 $\pm$ 0.43 (8)	<0.05
Gastrocnemius muscle	...	7.09 $\pm$ 0.75 (6)	8.41 $\pm$ 1.73 (6)	NS
Liver	...	10.30 $\pm$ 0.64 (6)	13.71 $\pm$ 2.45 (6)	NS

Neither bovine serum albumin up to 3 mg, fluoride ions (70 mM) nor Ca^{2+} ions (7 mM) had any influence on lysophospholipase activity in the tissues tested. There was no significant difference in lysophospholipase activity in sciatic nerve and gastrocnemius muscle between normal and dystrophic mice. Forebrain and spinal cord from dystrophic mice had significantly lower lysophospholipase activity, being 60–65% of that of the normal. The difference is particularly significant in the forebrain (Table 5).

The activities of lysophosphatidylcholine acyltransferase were increased in the spinal cord (Table 6), but there was also a smaller increase in the sciatic nerve. No significant difference was observed in enzyme activity from brain, muscle or liver.

Degradation of phosphatidylcholine by phospholipase C

In addition to the 'deacylation-acylation' cycle, phosphatidylcholine can also be degraded by phospholipase C, which catalyses the formation of diacylglycerols and phosphocholine. This pathway has a considerable metabolic influence on pools of phosphocholine and diacylglycerols. Diacylglycerols play a very important role as intermediates in the synthesis of glycerophospholipids and triacylglycerols.

There was no significant difference in the activities of this enzyme in the nervous tissues and liver from

Table 7. *Phospholipase C activities in various tissues of normal and dystrophic mice*

The results are expressed as nmol of diacylglycerols produced/h per mg of protein, $\pm$ S.E.M., for the numbers of experiments given in parentheses.

Tissue	Mice	Diacylglycerols produced		<i>t</i>
		Normal	Dystrophic	
Brain	...	1.14 $\pm$ 0.05 (12)	1.03 $\pm$ 0.07 (11)	NS
Spinal cord	...	1.30 $\pm$ 0.11 (12)	1.58 $\pm$ 0.12 (12)	NS
Sciatic nerve	...	1.22 $\pm$ 0.11 (11)	1.42 $\pm$ 0.17 (12)	NS
Muscle	...	0.37 $\pm$ 0.01 (9)	0.59 $\pm$ 0.06 (9)	<0.001
Liver	...	0.77 $\pm$ 0.04 (6)	0.82 $\pm$ 0.02 (6)	NS

normal and dystrophic mice. Muscle of the dystrophic mouse, however, showed a significantly (60%) higher specific activity than that of the normal mouse (Table 7). Such an increased activity would have a considerable bearing on the lipid composition in dystrophic muscles.

Discussion

The lipid composition of the muscle of the dystrophic mouse has been described by Hughes (1965), but very little work has since been carried out on the nervous tissues from this animal. We have previously shown that the total phospholipid content of sciatic nerve of the dystrophic mouse differs from the normal (Kwok *et al.*, 1976). There were 46% less phospholipids than in the corresponding control. There is now good evidence that the amount of the choline phospholipid group (phosphatidylcholine, sphingomyelin and lysophosphatidylcholine) are markedly affected in tissues from various forms of muscular dystrophy (Hughes, 1965, 1972; Kunze & Olthoff, 1970; Chio *et al.*, 1972; Kunze *et al.*, 1973; Kunze, 1975; Kalofoutis *et al.*, 1977).

Sphingomyelin is a very important component of the myelin in the nervous tissues. Its marked decrease in the sciatic nerve is consistent with the deficiency of myelination in the cranial nerves and spinal nerves in the dorsal and ventral roots at the exit of the cord (Biscoe *et al.*, 1974; Bradley & Jenkison, 1975; Bray & Aguayo, 1975). This could result from either a lesion in its metabolism or a deficiency in its transport from the nerve cells.

The alterations in the phospholipid composition in spinal cord, sciatic nerve, muscle and erythrocytes from muscular dystrophy of various forms would affect the integrity of the biomembranes. As a result of changes in the lipid micro-environment, membrane-associated enzymes are thereby affected in one way or another, depending on the tissues and the type of membranes. There is ample evidence supporting structural and functional alterations in membranes from muscular dystrophy. However, the problem is so complex that no single factor has yet been delineated as the primary lesion in muscular dystrophy.

Previous work (Kwok *et al.*, 1976) has shown that the turnover of phospholipids in neural tissues and muscle of the dystrophic mouse is faster than in the corresponding tissues of controls, but it was not possible to determine whether this was the result of an increased rate of synthesis and breakdown or an alteration in rate of exchange of precursors between blood and tissue. That the increased rate of metabolism of phospholipids in the gastrocnemius muscle and sciatic nerves is due to an increase in the acyl-group turnover rather than synthesis is supported by the finding that there are elevated activities of phospholipases A and no change in the activity of choline

phosphotransferase in these tissues (Tables 3 and 4). The result of these elevated activities of phospholipases A would be an increase in lysophosphatidylcholine, a potent cytolytic agent. Increased activity of phospholipase A has also been reported in human erythrocytes from patients with Duchenne and myotonic muscular dystrophy (Iyer *et al.*, 1976).

The accumulation of lysophosphatidylcholine can be prevented by the action of lysophospholipase and/or lysophosphatidylcholine acyltransferase. Apparently this is not effective in muscles and erythrocytes (Kunze & Olthoff, 1970; Kalofoutis *et al.*, 1977) from muscular-dystrophy sufferers or in sarcolemma from dystrophic-mouse muscles (De Kretser, 1977). Though there is no apparent increase in activities of phospholipases A in the spinal cord (Table 4), there is a marked increase in lysophosphatidylcholine in the dystrophic mouse (Tables 1 and 2). This may be due to the decreased activity of lysophospholipase (Table 5) in the spinal cord, although this enzyme activity is also decreased in the forebrain from dystrophic mouse without a corresponding change in lysophosphatidylcholine. However, there is also an increase in the activity of lysophosphatidylcholine acyltransferase activity, particularly in the spinal cord (Table 6). This may reflect an attempt by the tissue to compensate for the accumulation of lysophosphatidylcholine, but, if so, it is not completely successful in the spinal cord. It should be noted that in some human muscle diseases (Kunze *et al.*, 1971, 1973), alterations in the activity of lysophosphatidylcholine acyltransferase have been found. The rate of incorporation of linoleic acid was decreased in muscle but increased in erythrocytes.

The increased amounts of lysophosphatidylcholine could have a serious impact on the structural and functional properties of membrane such as those reported in muscular dystrophy. For example: (1) the increased number of echinocytes, crenellated forms of erythrocytes (Morse & Howland, 1973; Matheson & Howland, 1974) that can be induced by lysophosphatidylcholine (Marikovsky *et al.*, 1976); (2) dysmyelination of the sciatic nerves (Okada *et al.*, 1976), which can also be induced by injection of lysophosphatidylcholine (5–6 µg) or of phospholipase A (Hall & Gregson, 1971); (3) altered membrane properties, both physical and enzymic (Bray, 1973; Butterfield *et al.*, 1974; Roses & Appel, 1974); (4) altered rates of axoplasmic transport (Komiya & Austin, 1974; Tang *et al.*, 1974); (5) decrease in sphingomyelin content in the sciatic nerve; lysophosphatidylcholine inhibits sphingomyelin biosynthesis (Ullman & Radin, 1974). Furthermore, lysophosphatidylcholine at relatively high concentrations (0.7 mM) completely blocks phospholipid exchange between subcellular membrane fractions from brain (Miller & Dawson, 1972).

It is further noteworthy that phospholipases C activity is also increased in the muscle from dystrophic

mouse (Table 7). This may have a bearing on the increased amounts of triacylglycerols and sphingomyelin in the muscles of dystrophic patients and mice (Shull & Alfin-Slater, 1958; Hughes, 1965; Kunze & Olthoff, 1970). The products resulting from phospholipase C, diacylglycerols and phosphocholine, are precursors in the biosynthesis of triacylglycerols, glycerophospholipids and sphingomyelin. Phosphatidylcholine as phosphocholine donor for sphingomyelin biosynthesis has been well documented in viral-transformed mouse fibroblasts, rat subcellular fractions and rat brain (Marggraf & Anderer, 1974; Ullman & Radin, 1974; Kwok, 1975). Phospholipase C has been shown to be capable of catalysing the formation of sphingomyelin from phosphatidylcholine and ceramide (Kanfer & Spielvogel, 1975). The increased amounts of triacylglycerols and sphingomyelin in the dystrophic muscle may be a response to increased phospholipase C activity. Accumulation of triacylglycerols is not due to a lack of lipase activity in the dystrophic muscle (Jato-Rodriguez *et al.*, 1974).

The present results provide further support for the involvement of nervous tissues in the aetiology of mouse muscular dystrophy, though this may reflect a generalized membrane defect. The latter is supported by the observations that different membranes have differential enzyme responses to the expression of the disease. Thus the alterations in membrane permeability and axoplasmic transport, the 'amyelination' in spinal roots and the alterations in erythrocytes may be due to the altered lipid composition and the elevated activities of phospholipases. However, the identity of the primary event in the disease process has not yet been fully elucidated.

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