Chemical changes in the spinal cord in Friedreich's ataxia and motor neurone disease

N. ROBINSON

*From the Department of Chemical Pathology, Institute of Neurology,
The National Hospital, Queen Square, London*

This communication reports a comparison of the chemical constitution of affected and unaffected regions of white matter in the spinal cords of Friedreich's ataxia and motor neurone disease (MND). Friedreich's ataxia is a member of the heredo-familial spino-cerebellar diseases, the mode of inheritance being mainly recessive. Most cases of MND in Europe have no family history (Spillane, 1962) and the duration of the disease is shorter than Friedreich's ataxia. Little is known of the aetiology of the diseases, but in both conditions specific tracts of the spinal cord are involved.

Recent enzyme studies on Friedreich's ataxia suggested that an alteration in carbohydrate metabolism involving the direct oxidation pathway and also a change in the regulation of phosphate were taking place in the spinal cord (Robinson, 1966a, b). In a study of five cases of MND, alterations in oxidative enzyme and phosphatase activities were demonstrated in glia cells of the spinal cord white matter (Robinson, 1966c). In this study the breakdown in metabolism within affected tracts of the spinal cord has been further investigated. Dissection of the affected and unaffected areas of the spinal cords and examination of the chemical composition and integrity of the molecular complexes involved have suggested further changes in the degenerative processes taking place in these regions.

MATERIALS

Spinal cord was obtained at necropsy from seven pathological cases and four controls. Patients with Friedreich's ataxia, durations of the disease, storage before necropsy (in parentheses) and regions of the cord analysed were: Case 1, male age 30 years, duration 13 years, (14 hr) thoracic cord; Case 2, male age 24 years, duration 11 years, (6 hr) thoracic and lumbar cord; Case 3, female age 67 years, duration 32 years, (16 hr) thoracic cord. The patients with MND were: Case 4, male age 21 years, duration 13 months, (9 hr) cervical cord; Case 5, female age 56 years, duration 22 months, (11 hr) lumbar cord; Case 6, female age 54 years, duration

22 months, (9 hr) lumbar cord; Case 7, male age 62 years, duration 24 months, (13 hr) lumbar cord. Control spinal cord was from five cases having no evidence of neuropathological involvement of the brain-stem and cord. Differences between control and pathological material due to post-mortem changes were minimized by using controls of similar ages and subjected to the same conditions at necropsy. Material was rapidly frozen, stored at -25° for the minimum of time to reduce enzymatic action, and analysed.

METHODS

Cylinders of white matter of the spinal cord were removed with a thin-walled capillary tube at 12° from affected posterior and lateral tracts, and also from the unaffected anterior regions of Friedreich's ataxia cases. Cylinders of tissue were removed from the affected lateral tracts and unaffected anterior and posterior areas of the MND cases. The same regions were removed from normal spinal cord. Weighed portions of dissected tissue were dried to constant weight in a vacuum dessicator for dry weights and the remaining fresh tissue treated for extraction of cholesterol (free and esterified), phospholipids, proteolipids, chloroform-methanol-insoluble proteins, and free amino acids.

EXTRACTION PROCEDURES Chloroform, phospholipids, and proteolipids were extracted according to Folch, Lees, and Sloane-Stanley (1957). Cholesterol (free and esterified) was estimated by the method of Sperry and Webb (1950) and the four phospholipids, separated by thin layer chromatography, were estimated for phosphorus content according to Bartlett (1959). Proteolipids in the lower organic phase of the partitioned chloroform-methanol extract were purified as described by Lees, Carr, and Folch (1964), deproteinized with 10% TCA, the protein material washed successively with 95% ethanol and chloroform-methanol (2:1v/v), dried with ether, and the amino acid content estimated as described elsewhere (Robinson and Williams, 1965). Soluble proteins in the upper aqueous phase of the partitioned chloroform-methanol extract were deproteinized with 10% TCA, washed, dried, and analysed for amino acid content as for proteolipid-protein. Free amino acids were extracted and estimated quantitatively as previously described (Robinson and Williams, 1965). Three

separate preparations, analysed in triplicate, were conducted on normal and pathological material.

RESULTS

The spinal cord white matter mean values of total and free cholesterol, phospholipids, proteolipid-proteins, insoluble and soluble proteins, and free amino acid profiles for the five controls and pathological tissue are shown in Tables I, II and III.

A comparison of control results with other investigations on normal white matter show that cholesterol was lower than reported by Brante (1949) but in general agreement with Davison and Wajda (1962), Soto, De Bohner, and Del Carmen Calviño (1966), and Prensky and Moser (1966). The control spinal cord phospholipids were in general agreement with Soto *et al.* (1966), but higher than in cerebral white matter reported by Prensky and Moser (1966) and Balakrishnan, Goodwin, and Cummings (1961). The mean values for control proteolipid proteins were in agreement with Hess and Lewin (1965) but lower than the values of Prensky and Moser (1966) on infant brain, but for chloroform-methanol-insoluble proteins the mean values were in the lower range reported by Hess and Lewin (1965). No independent figures are available for free amino acids in spinal cord (Table III).

In two of three Friedreich's ataxia cases cholesterol levels in the lateral corticospinal and posterior tracts were low and all three showed minor esterified cholesterol; the levels in MND tissue were normal (Table I).

Total phospholipid-phosphorus content in the affected posterior tracts of two Friedreich's ataxia cases showed differences from the control levels but no one phospholipid was significantly raised. The total phospholipid in all MND cases appeared normal.

Two of three Friedreich's ataxia cases exhibited low proteolipid-protein and high insoluble protein levels but with acceptable total protein levels; in one of these cases 'soluble' protein was above the normal amount partitioned. The Friedreich's ataxia Case 3 exhibited a high 'insoluble' protein level. The MND cases exhibited the same pattern of proteolipid-protein and 'insoluble' protein levels as the two Friedreich's ataxia cases, while the other cases showed loss of proteolipid-protein only. The amino acid profile of the protein hydrolysates indicated that the proteolipid-protein lost from the affected tracts did not involve protein with specifically high acidic or basic amino acid constituents.

The free amino acid content of the spinal cords in Friedreich's ataxia showed low levels of aspartic and glutamic acids in three cases, and leucine and glutamine in two; in MND glutamine, aspartic and

TABLE I

CHOLESTEROL AND PHOSPHOLIPID LEVELS IN SPINAL CORDS OF CONTROLS, FRIEDREICH'S ATAXIA, AND MND
(UNITS: CHOLESTEROL AND TOTAL PHOSPHOLIPID-PHOSPHORUS IN MG/G DRY WT)

Tissue	Cholesterol		Phospholipids*				
	Total	Free	Total phospholipid-P	PE (% of total phospholipid-P)	PS	PC	S
Controls							
Cervical	122 ± 8.1	122 ± 7.2	7.00 ± 0.27	35.9	17.2	28.0	18.8
Thoracic	123 ± 6.9	123 ± 7.1	7.93 ± 0.34	30.4	17.4	27.7	24.5
Lumbar	138 ± 9.1	137 ± 8.4	7.07 ± 0.34	32.8	17.7	27.1	22.4
Friedreich's ataxia							
<i>Unaffected regions</i>							
Case 1 Thoracic	119 ± 8.2	119 ± 6.7	9.4 ± 0.51	34.1	16.8	27.3	21.8
Case 2 Thoracic	116 ± 7.2	116 ± 6.9	7.71 ± 0.38	30.8	17.7	27.2	24.3
Case 3 Thoracic	103 ± 4.9	101 ± 4.2	8.36 ± 0.48	29.9	15.1	31.8	23.2
<i>Affected regions</i>							
Case 1 Thoracic	132 ± 4.8	126 ± 6.1	11.2 ± 0.50	32.3	17.5	26.5	23.7
Case 2 Thoracic	83 ± 5.4	80 ± 6.1	7.10 ± 0.36	31.4	17.7	31.4	19.5
Case 3 Thoracic	67 ± 3.6	67 ± 4.1	5.67 ± 0.48	30.2	15.6	32.4	21.8
Case 2 Lumbar	89 ± 5.6	80 ± 5.1	6.43 ± 0.32	33.9	15.4	28.8	21.9
MND							
<i>Unaffected regions</i>							
Case 4 Cervical	140 ± 6.9	140 ± 7.4	7.90 ± 0.51	32.6	16.9	27.8	22.7
Case 5 Lumbar	123 ± 6.7	123 ± 8.1	8.10 ± 0.48	31.8	17.4	26.9	23.9
<i>Affected regions</i>							
Case 4 Cervical	120 ± 6.6	120 ± 5.9	7.82 ± 0.29	35.4	18.4	26.5	19.7
Case 5 Lumbar	128 ± 4.6	122 ± 7.2	7.39 ± 0.38	32.1	17.6	28.4	21.9
Case 6 Lumbar	145 ± 6.1	128 ± 7.1	7.94 ± 0.42	33.2	17.5	26.4	22.9
Case 7 Lumbar	160 ± 6.4	159 ± 6.9	7.10 ± 0.44	31.2	18.0	28.1	22.7

*PE = phosphatidylethanolamine, PS = phosphatidylserine, PC = phosphatidylcholine, S = sphingomyelin

glutamic acids were low in two cases. In both conditions some other amino acids were at borderline levels.

DISCUSSION

This investigation was undertaken to examine the chemical composition of normal tracts and tracts showing loss of myelin in the spinal cords of two clinically well-characterized neurological disorders with the possibility that chemical changes may indicate an alternation in a metabolic pathway within the affected tracts.

TABLE II

PROTEIN LEVELS IN SPINAL CORDS OF CONTROLS, FRIEDREICH'S ATAXIA, AND MND (UNITS: MG/G DRY WT)

Tissue	Proteins		
	Proteolipid protein	Insoluble protein	Soluble protein
<i>Controls</i>			
Cervical	46.5 ± 3.6	237.5 ± 18	1.2 ± 0.6
Thoracic	52.4 ± 3.8	245.1 ± 20	3.8 ± 0.6
Lumbar	49.3 ± 3.0	246.5 ± 19	6.6 ± 2.9
<i>Friedreich's ataxia</i>			
<i>Unaffected regions</i>			
Case 1 Thoracic	52.7 ± 4.6	339.0 ± 29	2.8 ± 0.9
Case 2 Thoracic	67.8 ± 2.8	237.0 ± 20	1.9 ± 0.9
Case 3 Thoracic	35.1 ± 4.9	281.9 ± 21	3.2 ± 1.1
<i>Affected regions</i>			
Case 1 Thoracic	63.2 ± 5.8	483.5 ± 51	1.9 ± 1.0
Case 2 Thoracic	41.4 ± 4.9	338.0 ± 35	11.5 ± 3.1
Case 3 Thoracic	9.75 ± 2.9	462.0 ± 43	2.9 ± 0.9
Case 2 Lumbar	31.8 ± 5.0	291.0 ± 31	2.1 ± 1.1
<i>MND</i>			
<i>Unaffected regions</i>			
Case 4 Cervical	51.4 ± 5.1	289.0 ± 18	1.7 ± 0.4
Case 5 Lumbar	55.7 ± 4.9	296.0 ± 22	3.4 ± 1.4
<i>Affected regions</i>			
Case 4 Cervical	34.0 ± 4.1	298.4 ± 21	3.5 ± 1.6
Case 5 Lumbar	26.5 ± 3.4	311.2 ± 36	6.0 ± 3.0
Case 7 Lumbar	28.5 ± 3.4	218.1 ± 34	2.5 ± 0.7

Changes in chemical composition of the affected tracts in Friedreich's ataxia and MND suggested that they were probably secondary processes and not primary biochemical defects since the changes were common to both conditions, which have different genetic aspects and exhibit different clinical and pathological features, although the lateral corticospinal tract investigated in this study was commonly affected.

The lipid profile of the affected regions of white matter compared with unaffected regions and the same tracts in the controls showed no significant differences in the relative amounts of each component of the myelin, except the loss of lipid which is in conjugation with protein as proteolipid, the protein component appearing in the chloroform-methanol-insoluble protein fraction. It is possible that the proteolipid content may have been reduced—a substantial reduction of white matter proteolipids has been reported in a maple syrup urine case (Prensky and Moser, 1966)—and the protein moiety estimated in the extracts was being reinforced by protein derived from proliferating glia cells or swollen astrocytes and macrophages which are known to have high protein contents (Dixon, 1954; Rubinstein and Smith, 1962). However, the insoluble protein levels were normal and it is fortuitous if reduced lipoprotein-protein was being so closely compensated by protein from gliosis. It is feasible that a hydrolytic reaction due to factors yet unknown but which may include poor arterial supply of the abnormally high astrocytic enzyme activity (Robinson, 1966a, b, c) may have been producing dissociation of the lipid-protein complex in affected tracts.

The spinal cord free amino acids in Friedreich's ataxia and MND showed abnormally low levels of

TABLE III

FREE AMINO ACIDS (μ-MOLE/G DRY WT TISSUE) IN SPINAL CORDS OF CONTROLS AND AFFECTED REGIONS OF FRIEDREICH'S ATAXIA AND MND CASES

Amino acid	Controls		Friedreich's ataxia			Motor neurone disease			
	Mean	Range	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Alanine	14.4	8.91—19.9	13.2	16.3	15.6	14.9	8.80	17.7	9.06
Arginine	2.26	1.97—2.55	2.94	2.01	1.89	2.14	1.69	2.42	1.88
Aspartic acid	6.03	4.60—7.46	2.27	2.22	3.10	2.78	1.52	4.80	4.40
Cystathionine	5.14	3.40—6.88	10.0	6.16	5.82	4.13	4.13	3.94	7.11
Cysteic acid	1.20	—	—	1.0	—	0.98	—	—	—
Glutamic acid	18.9	17.1—20.7	10.5	9.70	10.4	9.31	9.11	18.3	15.3
Glutamine	14.3	11.9—16.7	15.4	8.8	7.61	16.62	4.61	10.5	8.42
Glycine	5.98	4.63—9.33	8.86	4.90	8.90	7.30	6.72	8.33	4.52
Histidine + lysine	5.09	4.06—6.12	4.24	3.99	4.62	5.14	4.01	3.89	3.98
Leucine + isoleucine	7.38	5.56—9.20	0.94	2.90	5.10	6.21	5.30	6.91	5.38
Serine	7.67	5.35—9.99	8.61	7.28	7.74	8.98	6.25	7.18	5.10
Taurine	3.40	2.40—4.40	2.65	3.05	2.98	2.10	2.54	3.01	1.96
Threonine	4.20	3.15—5.25	4.88	3.00	5.14	4.62	5.24	3.52	2.98
Tyrosine	2.50	1.56—3.44	2.79	1.48	2.62	3.52	3.41	2.20	1.45
Valine + methionine	4.27	2.80—5.74	5.62	4.81	3.80	5.54	4.17	3.94	2.72

two amino acids (aspartic and glutamic) that play a central role in brain metabolism; another amino acid, leucine, was low in Friedreich's ataxia. Factors governing a raised cystathionine cannot be explained, however; a pyridoxine deficiency can cause its accumulation in the brain (Tallon, 1962) but no evidence of such a deficiency has been reported in the two pathological conditions. Alterations in the profile of free amino acids in these disorders may be due to two factors: (1) artificially raised amino acids are known to restrict the passage of some other amino acids into the brain; (2) the amino acid abnormalities may have resulted from changes in composition of the blood at the terminal stages, which in turn produced alterations in amino acid flux and the exchange rates between protein uptake and the amino acid pool (Lajtha, 1964). The differences were, however, dissimilar in character and magnitude to those in necropsy brains of infants with maple syrup disease.

SUMMARY

The lipid, proteolipid, protein, and amino acid composition of affected regions of white matter in the spinal cords of cases of Friedreich's ataxia and motor neurone disease are compared with adjacent unaffected regions and normal spinal cord.

Two changes seen were (1) the affected regions in both diseases showed lipid lost from proteolipids, the protein residue retained as insoluble protein; (2) the free amino acid content in affected tracts of the spinal cords in both conditions showed changes in glutamine, aspartic and glutamic acids. Possible factors in the metabolism of the spinal cord effecting these changes are discussed.

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REFERENCES

- Balakrishnan, S., Goodwin, H., and Cumings, J. N. (1961). The distribution of phosphorus containing lipid compounds in the human brain. *J. Neurochem.*, **8**, 276-284.
- Bartlett, G. R. (1959). Phosphorus assay in column chromatography. *J. biol. Chem.*, **234**, 466-468.
- Brante, G. (1949). Studies on lipids in nervous system with special reference to quantitative chemical determination and topical distribution. *Acta physiol. Scand.*, Suppl. 63, **18**, 1-189.
- Davison, A. N., and Wajda, M. (1962). Analysis of lipids from fresh and preserved adult human brains. *Biochem. J.*, **82**, 113-117.
- Dixon, K. C. (1954). Cytochemistry of a cerebral scar. *J. Path. Bact.*, **68**, 85-88.
- Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissue. *J. biol. Chem.*, **226**, 497-509.
- Hess, H. H., and Lewin, E. (1965). Microassay of biochemical structural components in nervous tissue. II. Methods of cerebroside, proteolipid proteins, and residue proteins. *J. Neurochem.*, **12**, 205-211.
- Lajtha, A. (1964). Protein metabolism of the nervous system. *Int. Rev. Neurobiol.*, **8**, 1.
- Lees, M. B., Carr, S., and Folch, J. (1964). Purification of bovine brain white matter proteolipids by dialysis in organic solvents. *Biochim. biophys. Acta. (Amst.)*, **84**, 464-466.
- Prensky, A. L., and Moser, H. G. (1966). Brain lipids, proteolipids and free amino acids in maple syrup urine disease. *J. Neurochem.*, **13**, 863-874.
- Robinson, N. (1966a). Friedreich's ataxia: A histochemical study. Part 1. Enzymes of carbohydrate metabolism. *Acta Neuropath. (Berl.)*, **6**, 25-34.
- (1966b). Friedreich's ataxia: A histochemical study. Part 2. Hydrolytic enzymes. *Acta Neuropath. (Berl.)*, **6**, 35-45.
- (1966c). A histochemical study of motor neurone disease. *Acta Neuropath. (Berl.)*, **7**, 101-110.
- , and Williams, C. B. (1965). Amino acids in human brain. *Clin. chim. Acta*, **12**, 311-317.
- Rubinstein, L. J., and Smith, B. (1962). Triphosphopyridine nucleotide (TPN) diaphorase and TPN-dependent dehydrogenase activity of reactive macrophages in tissue necrosis. *Nature (Lond.)*, **193**, 895.
- Soto, E. F., De Bohner, L. S., and Del Carmen Calviño, M. (1966). Chemical composition of myelin and other subcellular fractions isolated from bovine white matter. *J. Neurochem.*, **13**, 989-998.
- Sperry, W. M., and Webb, M. (1950). A revision of the Schoenheimer-Sperry method for cholesterol determination. *J. biol. Chem.*, **187**, 97-106.
- Spillane, J. D. (1962). Discussion on motor neurone disease: the clinical aspects. *Proc. roy. Soc. Med.*, **55**, 1019-1020.
- Tallon, H. (1962). *Amino Acid Pools*, p. 471. Elsevier, Amsterdam.