Motor neurone disease

Further evidence for an abnormality of nerve metabolism

BHAGWAN SHAHANI,¹ G. A. B. DAVIES-JONES, AND W. RITCHIE RUSSELL

From the Department of Neurology, The Churchill Hospital, Oxford

SUMMARY Physiological and biochemical studies were carried out on 16 patients with motor neurone disease (MND). It was confirmed that the peripheral nerves in cases of MND are often unusually resistant to ischaemia especially as regards the large sensory fibres. Biochemical investigations demonstrated a tendency to carbohydrate intolerance and abnormal pyruvate metabolism. When compared with other related studies in MND it seems very likely that there is a metabolic abnormality present in MND which demands further study.

Although motor neurone disease (MND) was documented clinically and pathologically more than a century ago (Hayem, 1869; Duchenne and Joffroy, 1870; Charcot, 1870), no single aetiological factor has been associated with it. Numerous causes have been suggested and the possibility that more than one cause may be responsible has been entertained.

Familial cases, apart from those occurring in Guam, have been noted (Engel, Kurland, and Klatzo, 1959), which suggests some genetic anomaly. Heavy metal and organic poisons have been implicated (Brown, 1954; Kantarjian, 1961; Campbell, 1965). Previous poliomyelitis or other infective agents, associated with encephalitis, have been described in association with MND (Greenfield and Matthews, 1954; Zilkha, 1962), and Zil'ber, Bajdakova, Gardaš'jan, Konovalov, Bunina, and Barabadze (1963) and Kurland (1965) have raised the possibility of a transmissible agent being responsible. Alpers and Farmer (1949) described cases thought to be secondary to trauma, whereas Ask-Upmark and Meurling (1955) described five cases of amyotrophic lateral sclerosis which appeared after gastric resection. They suggested that some deficiency factor may have been responsible for their cases.

Several authors have suggested that some metabolic disturbance might be the cause of MND (Engel *et al.*, 1959; Wohlfart and Gamstorp, 1960; Adams, Denny-Brown, and Pearson, 1962; Partington, 1962). Ask-Upmark (1961) described three patients

with diabetes and MND and another patient who had MND and pancreatitis. Cumings (1962) found abnormal pyruvate metabolism in 15 of 36 cases of MND, liver function tests, serum alkaline phosphatase, SGOT, and SLDH being normal. Oral and intravenous glucose tolerance tests, intravenous tolbutamide test, serum insulin-like activity, and response to exogenous insulin were studied in 11 patients by Steinke and Tyler (1964); nine of these patients showed evidence of chemical glucose intolerance. They attributed this to pancreatic disturbance rather than defective carbohydrate utilization by diseased muscle. Ionășescu and Luca (1964) estimated the arterial venous glucose levels, serum lactate, pyruvate, inorganic phosphate, potassium, and aldolase before and after an oral glucose load in 10 patients. All these parameters were normal, but three patients showed a diabetic type of response to oral glucose. Quick (1969), in 15 patients, found five with a diabetic type response to the intravenous glucose tolerance test. He also found evidence of exocrine pancreatic deficiency in nine patients with MND as measured by the I^{131} triolein and I^{131} oleic acid tests.

Poole (1957) found that post-ischaemic paraesthesiae failed to appear after 10 to 20 minutes of ischaemia in cases of MND. Shahani and Russell (1969) confirmed that ischaemic or post-ischaemic paraesthesiae were either absent or mild in patients with MND. The subjective sensory changes were correlated with the electrophysiological findings, and it was concluded that the absence of paraesthesiae in MND was related to a relatively high resistance of large sensory fibres to ischaemia in this

¹Supported by the association for the Aid of Crippled Children, New York, U.S.A.

disease. It was suggested that this was a consequence of some abnormality in neural metabolism.

This work has been extended to study 16 additional cases of motor neurone disease, with observations also on some aspects of carbohydrate metabolism.

METHODS

We have studied 16 patients with MND, nine males and seven females aged 47 to 68 years. Seven healthy subjects, four males and three females aged 20 to 49 years, and two male patients aged 33 and 53 years with hemiparesis acted as controls for the electrophysiological studies. Fifteen subjects of comparable ages were used as controls for the biochemical studies. In all but one of the patients, clinical diagnosis of MND was supported by electromyographic studies.

The methods used for inducing vascular ischaemia and for electromyographic studies were similar to those described earlier (Shahani and Russell, 1969).

In two normal control subjects and three patients the H reflex was studied under ischaemic conditions before and after an intravenous load of 50 g glucose, which was immediately followed by a maintenance intravenous infusion of 10% dextrose run in at approximately 1.5 g/min during the period of the test.

The response of the serum glucose, lactate, and pyruvate to oral glucose load in the resting, fasting subject was studied in nine patients. The patients had been fasting from 10 p.m. the previous day. After an initial blood sample had been drawn, 50 g glucose dissolved in 284 ml. water was drunk within five minutes, and this dose was repeated immediately after a second blood sample was obtained 30 minutes later. Further blood samples were taken at 60 minutes and 90 minutes after the first glucose drink. All blood samples were taken from the unexercised arm without venous occlusion. The serum lactate and pyruvate were estimated as described by Hohorst, Kreutz, and Bücher (1959) and serum glucose according to Bergmeyer and Bernt (1963). Statistical evaluation of the results was done by use of Student's *t* test.

In three patients a muscle biopsy was performed to determine the routine histology, the ultrastructure, and the histochemical properties of the specimen.

RESULTS

The clinical findings in these patients are described in Table 1. In all but one patient (D.W.) the clinical impression of MND was confirmed by electromyographic studies (Table 2). The maximal motor conduction velocity was normal in all except two patients (M.M. and R.C.). The minimal latency of the median sensory nerve action potential was, however, normal in *all* cases and the maximal conduction velocity in these fibres was also normal. In addition the H reflex could be recorded from the gastrocnemius soleus muscle in all these patients.

The effects of vascular ischaemia in patients with MND are described in Table 3. The time required to block the H reflex was within normal limits (20 to 25 minutes) in five patients and prolonged in eight patients. A definite relationship between the absence of post-ischaemic paraesthesiae in the *upper limb* and the prolongation of time required to block the H reflex in the *lower limb* could not be established in every case. Therefore, in five patients the time required to block by ischaemia the sensory nerve action potentials in the arm was noted by the following procedure. Submaximal shocks (approximately 50% of the maximal) were delivered to the digital branches of the median nerve in the thumb, and the sensory nerve action potentials were recorded from the median nerve at the level of the wrist. In three patients the sensory nerve action potentials were blocked 30 minutes after the vascular ischaemia (Fig. 1). In the remaining two, the sensory potentials could be recorded even after 30 minutes of ischaemia (Fig. 2). In one of these patients, at the end of 30 minutes, gradual reduction in the amplitude of the strength of stimulus (by steps of 5 V from A to E) was made (Fig. 3) in order to see if we were stimulating low threshold fibres. It was found that sensory potentials evoked by very low intensity shocks were not blocked by 30 minutes of ischaemia (Fig. 3B). The prolongation of the time required to block the sensory potentials was correlated with the absence of post-ischaemic paraesthesiae in the same patients (Table 3).

The results of the glucose load test on the serum glucose lactate and pyruvate are given in Table 4, where they are compared with the control subjects. The notable differences are as follows: (1) in the MND patients there is a significantly greater increase in serum glucose after 90 minutes ($P < 0.025$); (2) no significant increase in serum lactate; (3) but a highly significant increase in the serum pyruvate after 60 minutes ($P < 0.005$) which became less significantly elevated after 90 minutes ($P < 0.0125$).

In the three patients who underwent muscle biopsy, Dr. Trevor Hughes kindly studied the distribution of denervation atrophy in relation to type I and type II muscle fibres, and reported that they were equally affected.

DISCUSSION

In two patients (M.M. and R.C.), who had a marked degree of atrophy of the muscles supplied by the ulnar nerve, the maximal motor conduction velocity was slightly slow. The slowing of conduction velocity in motor fibres cannot be attributed to the low temperature of the atrophic extremity because conduction time in the sensory fibres was normal in all cases.

FIG. 1. Patient with MND (J.D.). Sensory nerve action potentials evoked by submaximal shocks to the digital branches of the median nerve. Records taken before vascular occlusion (A) and 5, 10, 15, 20, 25, and 30 minutes of vascular ischaemia respectively (B-G). Calibration time 5 msec. Amplitude 35 μ V.

(Table 2). In a detailed electromyographic study, Lambert (1969) also found slowing of motor conduction velocity in a few patients with motor neurone disease. He explained this finding on the basis of early degeneration of fast-conducting fibres resulting in low values when only slow-conducting fibres survive.

The present study has confirmed the previous findings that the response to vascular ischaemia is altered in most patients with MND (Shahani and Russell, 1969). However, in the present study it was found that the patients who had absent post-ischaemic paraesthesiae in the upper limbs did not

FIG. 2. Patient with MND (N.S.). Experimental conditions as in Fig. 1. Median sensory nerve action potentials before vascular occlusion (A) and 5, 10, 15, 20, 25, and 30 minutes of vascular ischaemia respectively (B-G). Note the preservation of action potentials 30 minutes after ischaemia (G). Calibration time 5 msec. Amplitude 35 μ V.

necessarily require longer time to block the H reflex in the lower limbs. It was therefore decided to demonstrate the response of large sensory fibres to ischaemia in those upper limbs on which the subjective sensory findings had been recorded a few days earlier. In normal control subjects the sensory nerve action potentials are abolished after 30 minutes of vascular ischaemia (Seneviratne and Peiris, 1968). As noted previously in two patients who did not develop ischaemic or post-ischaemic paraesthesiae, the sensory nerve action potentials evoked by submaximal stimulation of digital nerves were preserved even after 30 minutes of ischaemia. Thus the function of large sensory fibres was correlated with subjective sensory changes and objective electrophysiological findings, and it is concluded that the

TABLE 1
MND—CLINICAL FINDINGS

Patient	Sex	Age onset	Duration (mth)	First manifestation	Jaw jerk	Reflexes						Plantar responses	
						Arms	Legs						
								Knee	Ankle				
N.S.	M	54	14	Bulbar weakness	++	++	++	++	±	±	↓	↓	
E.P.	F	54	15	R. foot drop	+	++	++	++	—	—	↑	↑	
W.M.	M	58	12	Bulbar weakness	++	++	+	+	+	+	↑	↑	
D.A.	M	49	21	Fasciculation L. quadriceps	++	++	+	+	+	+	↑	↑	
H.M.	M	52	14	Weakness L. leg	++	++	+	+	+	+	↑	↑	
H.Ma.	F	47	60	Weakness legs	—	+	—	—	—	—	—	—	
P.F.	M	63	78	Wasting L. hand	++	++	+	+	+	+	↓	↓	
R.C.	M	68	8	Generalized fasciculation	+	++	++	++	++	++	↑	↓	
J.D.	F	48	15	Weakness R. hand	++	++	+	+	+	+	↑	↑	
G.M.	F	47	36	Wasting L. hand	++	++	+	+	+	+	↑	↑	
A.B.	M	59	36	Weakness arms	+	—	+	+	+	+	↑	↑	
M.M.	F	53	18	Weakness L. hand	++	++	+	+	+	+	↑	↑	
D.W.	M	50	9	Bulbar weakness	++	++	+	+	+	+	↑	↑	
A.M.	M	64	24	Weakness L. hand	—	—	+	+	+	+	↓	↓	
L.C.	F	57	21	Weakness L. leg with foot drop	++	++	++	++	—	—	↓	↓	
L.Ca.	F	59	7	Weakness L. leg with foot drop	—	+	+	+	+	+	↓	↓	

Protected by copyright.

— absent; + present; ++ markedly present.

TABLE 2
EMG FINDINGS IN MND

Patient	Sex	Age (yr)	Maximal motor cond. veloc. (m/sec)	Med. sensory NAP	
				Latency (msec)	Distance (cm)
W.M.	M	58	59	3.00	14.0
D.A.	M	49	50	2.75	13.0
H.M.	M	52	60	2.75	14.0
M.M.	F	53	36*	2.25	11.5
E.P.	F	54	44	2.00	11.0
L.C.	F	57	58	2.5	13.0
A.B.	M	59	54	2.5	12.0
L.C.	F	59	59	2.0	11.0
P.F.	M	63	55	2.75	12.0
A.M.	M	64	61	2.00	10.0
R.C.	M	68	36*	3.00	13.5
N.S.	M	54	51	3.00	14.0
G.M.	F	47	55	2.50	12.0
J.D.	F	48	61	2.25	11.5
H.Ma.	F	47	60	2.50	11.0

TABLE 3
EFFECTS OF ISCHAEMIA IN MND

Patient	Sex	Age (yr)	Post-ischaemic paraesthesia	Time to block H reflex (min)	Time to block sensory NAP (min)
W.M.	M	58	M	30	
L.C.	F	57	M	25	
L.C.	F	59	N	30	
A.M.	M	64	N	30	
A.B.	M	59	A	30	
R.C.	M	68	A	30	
H.M.	M	52	A	30	> 30
D.W.	M	50	M	30	
D.A.	M	49	M	35	< 30
N.S.	M	54	A	25	> 30
G.M.	F	47	M	25	< 30
J.D.	F	48	N	25	> 30
P.F.	M	63	N	20	< 30

A—absent
M—mild
N—normal

TABLE 1—continued

Cramp pains	Sensory symptoms/signs	Weakness				Fasciculation			Wasting			
		Arms	Hands	Legs	Bulbar muscles	Arms	Legs	Tongue	Arms	Hands	Legs	Tongue
Calf and proximal arm muscles	—	+	—	—	+	+	+	+	+	+	+	+
—	—	—	+	++	—	+	—	—	—	+	+	—
—	—	—	—	+	+	+	+	+	—	—	+	+
—	—	+	+	+	+	+	+	+	+	++	+	+
—	—	+	++	+	+	—	+	—	+	++	+	+
—	—	—	—	++	—	+	—	—	—	—	++	—
—	—	++	++	—	+	+	—	—	++	++	—	—
—	—	+	+	+	—	+	+	+	+	+	—	—
—	—	+	+	+	+	+	+	+	+	++	—	—
—	—	+	+	+	—	+	+	—	+	++	—	—
—	—	+	+	+	—	+	+	—	+	+	+	—
—	—	+	+	+	+	+	+	+	+	++	+	+
—	—	+	+	+	+	+	+	+	+	++	+	+
—	—	++	+	—	—	+	+	—	++	++	+	—
—	—	++	+	++	+	+	+	+	++	++	++	+
—	—	—	—	L. leg only	—	—	—	—	—	—	—	—

TABLE 4

MEAN SERUM PYRUVATE, LACTATE, AND GLUCOSE IN PATIENTS WITH MND AND IN CONTROL SUBJECTS

Pyruvate				
	0	30	60	90
Control (15)	0.062 ± 0.018	0.063 ± 0.020	0.070 ± 0.028	0.077 ± 0.023
MND	0.065 ± 0.019 (9)	0.080 ± 0.027 (9)	0.103 ± 0.027 (9)	0.101 ± 0.021 (8)
Lactate				
	0	30	60	90
Control (10)	0.861 ± 0.429	0.846 ± 0.338	0.942 ± 0.214	1.049 ± 0.164
MND	0.676 ± 0.155 (9)	0.760 ± 0.318 (9)	0.996 ± 0.247 (8)	0.880 ± 0.145 (7)
Glucose				
	75 ± 14	114 ± 34	102 ± 39	77 ± 23
Control (15)				
MND (8)	66 ± 9	107 ± 23	114 ± 21	103 ± 27

Pyruvate and lactate concentrations in μ -mole/ml. whole blood.
Glucose concentration in mg/100 ml.

FIG. 3. Extension of the experiment demonstrated in Fig. 2. Strength of stimulus reduced from A to E. Note that a very low intensity shock in E can evoke the sensory potential (E). Calibration time 5 msec. Amplitude $35 \mu V$.

large sensory fibres are resistant to ischaemia in some patients with motor neurone disease.

The biochemical studies carried out on our patients support the view that there is an abnormal response to glucose loading tests in MND. It may be noted that carbohydrate intolerance producing a diabetic type of glucose tolerance test has been reported previously in MND (Matthews, 1958; Steinke and Tyler, 1964; Ionășescu and Luca, 1964; Quick, 1969). It seems unlikely that the intolerance to glucose was secondary to muscle wasting (Engel *et al.*, 1969), because we found no direct correlation between the degree of wasting and abnormality of glucose tolerance curve in our patients. Steinke and Tyler (1964) attributed the glucose intolerance in their patients to pancreatic disturbance as indicated by the inadequate response to tolbutamide.

Cummings (1962) found abnormal pyruvate metabolism in patients with MND who were neither spastic nor immobile. The present study has confirmed his findings. As seen from Table 4, there was a significant increase in the mean pyruvate concentration 60 minutes after the oral glucose load, which became less significantly increased after 90 minutes.

There was no significant change in the serum lactate after the oral glucose load. Several factors may have been responsible, to a greater or lesser degree, for the abnormal pyruvate concentration in these patients. Dysphagia secondary to bulbar involvement, malabsorption and vitamin B1 deficiency due to pancreatic dysfunction (Quick, 1969), and spasticity (McArdle, MacKenzie, and Webster, 1960) can all give rise to abnormal serum pyruvate levels. These factors, however, were probably not relevant because there was neither evidence of malnutrition, nor any relationship between the abnormal pyruvate metabolism and the degree of spasticity.

It could be that the resistance of large sensory fibres to ischaemia in MND and diabetes is a direct consequence of hyperglycaemia. However, this is probably not so as, in our preliminary studies, hyperglycaemia produced by an acute intravenous glucose load and maintained by continuous intravenous glucose infusion did not cause a prolongation in the time taken for the H reflex to disappear. It seems more likely, therefore, that in MND the tendency to carbohydrate intolerance, abnormal pyruvate metabolism, and resistance of sensory fibres to ischaemia are manifestations of a common metabolic anomaly. A further link between MND and carbohydrate metabolism is provided by histopathological studies. Fullmer, Siedler, Krooth, and Kurland (1960) reported that dermal connective tissue of most patients with MND possesses excessive amounts of carbohydrate as seen in periodic acid Schiff preparations. Also the epidermal cells seem to contain more glycogen than normal. On the basis of these findings it was suggested that there was perhaps an abnormality of the carbohydrate metabolism of connective tissue and epidermal cells. Robinson (1968) has demonstrated similarities in the chemical changes, suggesting an alteration in the metabolic pathways, of the spinal cord of patients with MND and Friedreich's ataxia, who are known to have a high incidence of diabetes mellitus (Hewer, 1968).

The observations reported here and elsewhere therefore indicate that there is physiological, biochemical, and histopathological evidence suggesting some abnormality of carbohydrate metabolism in MND which merits further intensive study.

We are grateful to Professor Sir Hans Krebs and Dr. Derek Williamson of the M.R.C. Unit of Metabolism at the Radcliffe Infirmary for the biochemical studies.

REFERENCES

- Adams, R. D., Denny-Brown, D., and Pearson, C. M. (1962). *Diseases of Muscle* (Second edition), p. 541. Harper: New York.

- Alpers, B. J., and Farmer, R. A. (1949). Role of repeated trauma by pneumatic drill in production of amyotrophic lateral sclerosis. *Arch. Neurol. (Chic.)*, **62**, 178-182.
- Ask-Upmark, E. (1961). Precipitating factors in the pathogenesis of amyotrophic lateral sclerosis. *Acta med. scand.*, **170**, 717-723.
- Ask-Upmark, E., and Meurling, S. (1955). On the presence of a deficiency factor in the pathogenesis of amyotrophic lateral sclerosis. *Acta med. scand.*, **152**, 217-222.
- Bergmeyer, H. U., and Bernt, E. (1963). Determination with glucose oxidase and peroxidase. In *Methods of Enzymatic Analysis* (edited by H. U. Bergmeyer), pp. 123-130. Academic Press: New York.
- Brown, I. A. (1954). Chronic mercurialism. A cause of the clinical syndrome of amyotrophic lateral sclerosis. *Arch. Neurol. (Chic.)*, **72**, 674-681.
- Campbell, A. M. G. (1965). A survey of 190 cases of motor neurone disease. *Riv. Pat. nerve. ment.*, **86**, 211-217.
- Charcot, J. M. (1870). Note sur un cas de paralysie glosso-laryngée suivie d'autopsie. *Arch. Physiol.*, **3**, 247-260.
- Cummings, J. N. (1962). Biochemical aspects. *Proc. roy. Soc. Med.*, **55**, 1023-4.
- Duchenne, G., and Joffroy, A. (1870). De l'atrophie aiguë et chronique des cellules nerveuses de la moelle et du bulbe rachidien, à propos d'une observation de paralysie glosso-laryngée. *Arch. Physiol.*, **3**, 499-515.
- Engel, W. K., Kurland, L. T., and Klatzo, I. (1959). An inherited disease similar to amyotrophic lateral sclerosis with a pattern of posterior column involvement. An intermediate form? *Brain*, **82**, 203-220.
- Fullmer, H. M., Siedler, H. D., Krooth, R. S., and Kurland, L. T. (1960). A cutaneous disorder of connective tissue in amyotrophic lateral sclerosis. A histochemical study. *Neurology (Minneapolis)*, **10**, 717-724.
- Greenfield, J. G., and Matthews, W. B. (1954). Post-encephalitic parkinsonism with amyotrophy. *J. Neurol. Neurosurg. Psychiat.*, **17**, 50-56.
- Hayem, G. (1869). Note sur un cas d'atrophie musculaire progressive avec lésions de la moelle. *Arch. Physiol.*, **2**, 391-406.
- Hewer, R. L. (1968). Study of fatal cases of Friedreich's ataxia. *Brit. med. J.*, **3**, 649-652.
- Hohorst, H. J., Kreutz, F. F., and Bücher, T. (1959). Über Metabolitegehalte und Metabolit-Konzentrationen in der Leber der Ratte. *Biochem. Z.*, **332**, 18-46.
- Ionășescu, V., and Luca, N. (1964). Studies on carbohydrate metabolism in amyotrophic lateral sclerosis and hereditary proximal spinal muscular atrophy. *Acta neurol. scand.*, **40**, 45-57.
- Kantarjian, A. D. (1961). A syndrome clinically resembling amyotrophic lateral sclerosis following chronic mercurialism. *Neurology (Minneapolis)*, **11**, 639-644.
- Kurland, L. T. (1965). Amyotrophic lateral sclerosis: A reappraisal. In *Slow, Latent and Temperate Virus Infections*, p. 13. Edited by D. C. Gajdusek, J. C. Gibbs, Jr., and M. Alpers. National Institute of Neurological Diseases and Blindness: Washington.
- Lambert, E. H. (1969). Electromyography in amyotrophic lateral sclerosis. In *Motor Neurone Disease*, edited by F. H. Norris, Jr., and L. T. Kurland, p. 146. Grune and Stratton: New York.
- Matthews, W. B. (1958). Discussion on some clinical, genetic and biochemical aspects of metabolic disorders of the nervous system. *Proc. roy. Soc. Med.*, **51**, 859-863.
- McArdle, B., MacKenzie, I. C. K., and Webster, G. R. (1960). Studies on intermediate carbohydrate metabolism in multiple sclerosis. *J. Neurol. Neurosurg. Psychiat.*, **23**, 127-132.
- Partington, T. (1962). Motor neurone disease. *Postgrad. med. J.*, **38**, 383-395.
- Poole, E. W. (1957). Ischaemic and post-ischaemic paraesthesiae in motor neurone disease. *J. Neurol. Neurosurg. Psychiat.*, **20**, 225-227.
- Quick, D. T. (1969). Pancreatic dysfunction in amyotrophic lateral sclerosis. In *Motor Neurone Disease*, edited by F. H. Norris, Jr., and L. T. Kurland, pp. 189-198. Grune and Stratton: New York.
- Robinson, N. (1968). Chemical changes in the spinal cord in Friedreich's ataxia and motor neurone disease. *J. Neurol. Neurosurg. Psychiat.*, **31**, 330-333.
- Seneviratne, K. N., and Peiris, O. A. (1968). The effect of ischaemia on the excitability of human sensory nerve. *J. Neurol. Neurosurg. Psychiat.*, **31**, 338-347.
- Shahani, B., and Russell, W. R. (1969). Motor neurone disease. An abnormality of nerve metabolism. *J. Neurol. Neurosurg. Psychiat.*, **32**, 1-5.
- Steinke, J., and Tyler, R. H. (1964). The association of amyotrophic lateral sclerosis (motor neuron disease) and carbohydrate intolerance, a clinical study. *Metabolism*, **13**, 1376-1381.
- Wohlfart, G., and Gamstorp, I. (1960). Chronic progressive disorders of the lower motor neuron. In *Neuromuscular Disorders*, edited by R. D. Adams, L. M. Eaton, and G. M. Shy, p. 664. Williams and Wilkins: Baltimore.
- Zil'ber, L. A., Bajdakova, Z. L., Gardaš'jan, A. N., Konovalov, N. V., Bunina, T. L., and Barabadze, E. M. (1963). Study of the etiology of amyotrophic lateral sclerosis. *Bull. Wld Hlth Org.*, **29**, 449-456.
- Zilkha, K. J. (1962). Discussion on motor neurone disease. *Proc. roy. Soc. Med.*, **55**, 1028-1029.