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Increased plasma levels of lead in patients with amyotrophic lateral sclerosis compared with control subjects as determined by flameless atomic absorption spectrophotometry

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SUMMARY The levels of lead in plasma were determined in 16 cases of amyotrophic lateral sclerosis (ALS) and 18 control subjects, using flameless atomic absorption spectrophotometry. The mean values were 0.52±0.22 μg/100 ml (ALS) and 0.37±0.13 μg/100 ml (controls), the difference is statistically significant (5% level). The values in both groups are lower than reported earlier for normal subjects. The findings are discussed against the background of the possible pathogenetic significance of retrograde axoplasmic flow in ALS.

This report covers one of a series of studies dealing with the turnover and tissue distribution of lead in amyotrophic lateral sclerosis (ALS). The work started as a result of several earlier observations on a relationship between the disease and previous exposure to heavy metals, particularly lead (Wilson, 1907; Currier and Haerer, 1968; Campbell *et al.*, 1970; Felmus *et al.*, 1976). One aim of our studies is to test whether lead is pathologically available to the motor neurones in the disease, and we recently found increased levels of lead in cerebrospinal fluid in patients with ALS as compared with control subjects (Conradi *et al.*, 1976).

The transport of a substance from plasma to the cell bodies of lower motor neurones via the motor endplates and the retrograde axoplasmic flow has recently been demonstrated in the rat in experiments using the enzyme horseradish peroxidase (Broadwell and Brightman, 1976). This route of entry to the central nervous system short circuits the blood–brain barrier, and accordingly the motor neurones might be damaged by substances from plasma transported in this way. Such a mechanism could be operating in ALS, in view of the selective involvement of motor neurones in this disease. The neurotoxicity of lead is well-known from earlier work (see Waldron and Stöfen, 1974).

There are few reports on the concentration of lead in plasma of normal individuals, probably because of earlier methodological problems. The question has to our knowledge not been studied in ALS. Only a small fraction of the lead found in whole blood is considered to be present in plasma, and plasma levels ranging between 1 and 10 μg/dl have been reported in normal subjects (Butt *et al.*, 1964; McRoberts, 1973; Rosen and Trinidad, 1974). After the addition of various amounts of lead to blood in vitro, only about 1 μg/dl was found to be retained in plasma after 15 min (Clarkson and Kench, 1958). Interestingly, the plasma levels of lead have been claimed to be constant over a wide range of lead concentration in whole blood (Butt *et al.*, 1964; Rosen and Trinidad, 1974). This suggests a protective action of the erythrocytes against overloading of the plasma with lead. The binding of lead to plasma constituents is not known in detail, but it has been suggested that the metal is bound both to small molecules, such as polypeptides, and to proteins (Griffin and Matson, 1972).

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In the present study the plasma levels of lead were determined by flameless atomic absorption spectrophotometry in 16 patients with amyotrophic lateral sclerosis and 18 control subjects with non-degenerative neurological diseases.

Patients and methods

Amyotrophic lateral sclerosis was diagnosed on conventional clinical grounds. Patients having symptoms predominantly from either peripheral, central, or bulbar motor neurones were included. The ages of the ALS patients and ages and diagnoses of the patients in the control group are given in the Table. Blood was obtained through antecubital venous puncture using lead-free syringes, and collected in heparinised lead-free test tubes. For the determination of lead in plasma, 10 ml of blood was immediately centrifuged at 5000 rpm for 10 min. The plasma samples were stored for two to 12 weeks before analysis. In the ALS group, the lead content of whole blood was also determined, using the method of Einarsson and Lindstedt (1969). Lead in plasma was determined with an atomic absorption spectrophotometer model AA-6 (Varian Tectron, Melbourne, Australia) with a carbon rod CRA-63 and a temperature control unit, measuring the temperature of the carbon rod with a photodiode. A deuterium lamp was used for background correction. A hollow cathode lead lamp (Varian) was used and operated at 5 mA with use of the lead 217.0 nm resonance line. A slit width of 1.0 nm was used. A standard deviation of $\pm 10\%$ for within day runs and $\pm 15\%$ for runs between days was estimated. The samples of plasma lead concentrations in the two groups were compared using the U-test of Mann and Whitney (see Siegel, 1956).

Results

The results of the determinations are shown in the Table and diagrammatically in the Figure. The lead concentration in plasma of all the samples

Table *Age, sex, and plasma levels of lead in patients with amyotrophic lateral sclerosis and control subjects*

Patient	*Sex*	*Year of birth*	*Diagnosis*	*Plasma lead concentration (μg/dl)*	*Lead/whole blood (μg/dl)*
GB	M	1928	ALS	0.9	20
SR	M	1932	ALS	0.7	11
MB	M	1946	ALS	0.5	< 10
ES	F	1943	ALS	0.3	< 10
SJ	F	1922	ALS	0.6	< 10
SE	F	1900	ALS	1.0	< 10
HB	F	1915	ALS	0.3	12
EL	M	1925	ALS	0.5	10
HT	F	1903	ALS	0.7	< 10
NL	M	1917	ALS	0.4	17
BS	M	1919	ALS	0.5	17
EO	F	1901	ALS	0.3	< 10
NW	M	1914	ALS	0.3	13
EH	F	1913	ALS	0.3	13
KP	F	1910	ALS	0.4	13
MG	F	1915	ALS	0.5	11
	Mean age	57 years		Mean 0.52 ± 0.22	
MA	F	1922	Herpes zoster	0.6	
DB	F	1919	Cerebral haemorrhage	0.5	
RE	M	1918	Myasthenia gravis	0.6	
BL	F	1947	Charcot-Marie-Tooth disease	0.5	
EH	M	1925	Multiple sclerosis	0.4	
RN	M	1934	Polyneuritis	0.4	
EN	F	1949	Disseminated lupus erythematosus	0.4	
PZ	M	1911	Polyneuritis Pyelonephritis	0.3	
VS	F	1927	Cervical spondylosis	0.4	
BD	M	1923	Paraparesis	0.4	
EH	M	1900	Epilepsy	0.3	
BG	M	1911	Cerebral infarction	0.2	
IS	F	1910	Transient ischaemic attacks	0.3	
AK	F	1915	Subarachnoid haemorrhage	0.2	
UK	F	1945	Lumbago	0.2	
DT	M	1906	Cerebral infarction	0.4	
TH	M	1944	Glioma	0.2	
AW	M	1913	Cerebral infarction	0.3	
	Mean age	53 years		Mean 0.37 ± 0.13	

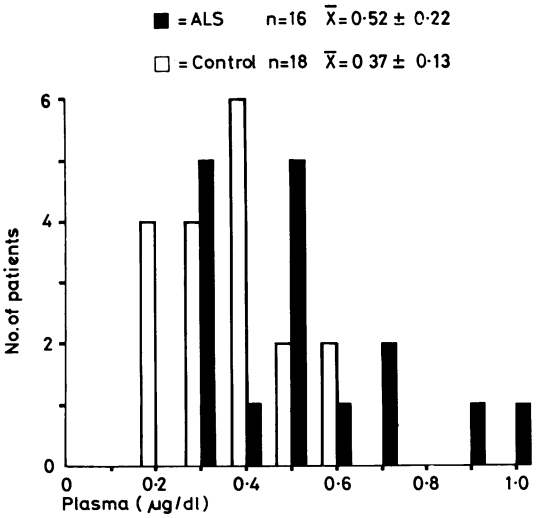

Figure *Diagram of the results in the Table.*

varied between 0.3 and 1.0 μg/dl. The values for normal subjects were concentrated around 0.4–0.5 μg/dl, as were the lowest values of the ALS group, but the range was larger in the ALS group. There was a statistically significant difference ($P<0.05$) between the groups. The values for lead in whole blood were normal.

Discussion

The plasma levels of lead reported here for both normal subjects and patients with ALS were low, and this puts high demands on the sensitivity of the method of analysis. Despite the errors of detection present at these very low levels, the findings were highly reproducible, and the differences between the individual estimates on each sample were small. Our values from the control material were considerably lower than those reported earlier in normal subjects (Butt *et al.*, 1964; McRoberts, 1973; Rosen and Trinidad, 1974). The difference might have to do with methodological factors since none of the earlier estimations was performed using a flameless method. Further, the accuracy of the procedure used at centrifugation of the blood is important, since it is well-known that the lead can easily be removed from the erythrocytes (Clarkson and Kench, 1958; Witschii, 1965).

It has been argued that the concentration of lead in plasma, rather than that in whole blood, is the most relevant to the action of metal on various tissues—for example, in lead intoxication (McRoberts, 1973)—despite the plasma pool of lead being minute compared with that of whole blood. The present findings must be regarded against this background. The raised plasma lead levels in the ALS group do indicate a pathological turnover of the metal, increasing the amounts of lead in the biologically active plasma pool. Our earlier findings of raised lead levels in CSF of ALS patients (Conradi *et al.*, 1976) also support this view, since the choroid plexus is thought to behave like a "sink," concentrating lead (O'Tuama *et al.*, 1976). There are great interindividual variations regarding susceptibility to develop lead intoxication (McRoberts, 1973). This probably reflects variability of the factors determining the turnover of lead—for example, the capacity and kinetics of the binding of the metal to the erythrocytes and to various plasma constituents. Furthermore, these binding capacities can change under special conditions, such as low pH (Witschii, 1965). Obviously, several other parameters than the whole amount of lead present in plasma at a given moment are relevant to the availability of the metal to various tissues. Further discussions on this point must, therefore, await a more thorough knowledge on the mechanisms of binding of lead in blood. It should also be pointed out that since nearly all the lead in whole blood is bound to the erythrocytes, damage to these cells during the preparation is critical. Accordingly, reported differences in plasma lead levels between samples could be the result of a different vulnerability of the erythrocytes.

The retrograde axoplasmic flow of lower motor neurones has been shown to transport various substances, mainly macromolecules, from the motor endplates to the cell bodies of these neurones (Kristensson, 1975). The fact that macromolecules can enter this route from plasma has been shown by intravenous horseradish peroxidase injections (Broadwell and Brightman, 1976), where the spinal cord showed a nearly selective labelling of the motor neurones. This similarity between enzyme distribution and the involvement of neurones in ALS is striking and merits further consideration, especially from the standpoint of noxious substances gaining access to the retrograde flow.

As shown earlier for horseradish peroxidase (Heuser and Reese, 1973), the substance to be transported by the retrograde flow enters the endplates through a process of pinocytosis, the capacity of which parallels the turnover of synaptic vesicles. Obviously, the concentration of the substance to be taken up in interstitial fluid of skeletal muscle is pertinent to the uptake, regardless of whether the substance originates from

plasma or from skeletal muscle. Further, the uptake of substance in the endplates of individual motor neurones will be influenced by impulse activity and total endplate surface formed by the neurone. Motor neurones supplying large motor units will then show a large surface of uptake of foreign substance. If we assume a constant load of a foreign noxious substance provoking degeneration of motor neurones and being taken up by the motor and endplates, this would explain a progressive involvement of motor neurones. Because of collateral reinnervation of denervated muscle fibres (Edds, 1953) the endplate surface of regenerating motor neurones will increase, which would tend to increase their uptake.

It must also be considered whether damage to descending pathways going to motor neurones taking up a noxious substance can be caused by trans-synaptic spread and further transport through retrograde axoplasmic flow. Actually, trans-synaptic spread between adjacent motor neurones must also be considered, according to recent observations in our laboratory (Cullheim *et al.*, 1977). Spread of foreign substances between central neurones has earlier been observed by intraneuronal injection of dyes (Zieglgänsberger and Reiter, 1974). The involvement of neurones making synaptic contact with damaged motor neurones might then be decided by affinity to the substance and the size, number, localisation, and activity of their synapses on the motor neurone surface. Besides transport of noxious substances by retrograde axoplasmic flow, damage to the motor neurones through interference with the retrograde flow can, of course, be accomplished in a variety of ways.

The present results have thus shown that, in all probability, motor endplates are overexposed to lead in ALS. It is not known whether this can damage the motor neurones; the question is currently being studied experimentally in our laboratory. It must, however, be pointed out that such overexposure of motor neurones to lead in ALS might have been going on for a long time.

The results have been presented in part at the XI World Congress of Neurology in Amsterdam in 1977. The studies were supported by Stiftelsen MS-fonden and the funds of Karolinska Institutet. We would like to thank Mrs Gun Nise for qualified help.

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Notes