Copper and zinc levels in peripheral nerve tissues of rats with experimental carbon-disulphide neuropathy

EDGAR LUKÁŠ, PAVEL KOTAS, and IVAN OBRUSNÍK
Institute of Hygiene and Epidemiology, Prague, Centre of Industrial Hygiene and Occupational Diseases, Prague, Tesla—Research Institute for Communication Engineering, Prague, and Institute of Nuclear Research, Řež, near Prague, Czechoslovakia

Lukáš, E., Kotas, P., and Obrusník, I. (1974). British Journal of Industrial Medicine, 31, 288-291. Copper and zinc levels in peripheral nerve tissues of rats with experimental carbon-disulphide neuropathy. By means of neutron activation analysis the levels of zinc and copper in peripheral nerve tissue of two groups of rats with carbon-disulphide neuropathy were estimated. The neuropathy (diagnosed electromyographically) was evoked by inhalation of 3.6 mg CS₂/litre of air (variant 1) or 2.4 mg CS₂/litre of air (variant 2).

Stationary Zn levels were found in control and exposed animals in both of the experimental variants, while the copper levels increased very significantly from 4.2 ± 0.3 ppm to 9.9 ± 1.7 ppm in experimental variant 1 and from 2.4 ± 0.3 ppm to 5.6 ± 0.6 ppm in variant 2.

The experiments suggest that changes in metal metabolism of peripheral nerve tissue deserve attention as one of the possible pathological links in the development of carbon-disulphide intoxication.

The production of thiocarbonates (chelating compounds) by the bonding of carbon disulphide (CS₂) to the amino groups of amino acids and proteins (Mádlo and Souček, 1956; Souček and Mádlo, 1956) aroused the interest of some clinicians and research workers in the elimination of certain metals in the excreta of man and animals, and in the determination of the levels of these metals in different organs as a sign of chronic exposure to carbon disulphide. The results of studies undertaken to investigate these questions were, however, ambiguous.

Djurić, Stojadinović, Bojovic, and Rezman (1968), Asanová (1971), and El-Gazzar, El-Sadik, and Hussein (1973) reported that persons exposed to CS₂ excreted raised levels of zinc or copper in the urine. On the other hand, this finding could not be confirmed by Hernberg and Nordman (1969), who studied zinc and manganese excretion in urine in a relatively small group of employees from a chemical CS₂ plant.

These questions were investigated experimentally by Scheel (1967), who found lowered copper and raised zinc levels in spinal-cord structures of rabbits exposed to carbon disulphide. In addition, this author demonstrated on the growth curves of these animals a protective effect of a diet whose salt content of copper, zinc, cobalt, manganese, iron, and calcium had been raised by 30% (by adding the 4% salt mixture of Phillips and Hart (1935)). Gadaskina and Andreeva (1969) reported raised copper levels in the liver, muscles, and testes of rats exposed to carbon disulphide and at the same time they established enhanced excretion of Cu and Zn in the faeces. The level of copper in the brain tissue of their animals did not change.

Apart from these studies and quite independently of the carbon disulphide problem, there appeared a series of papers explaining the development of swayback, a demyelinating disease in sheep, by copper deficiency in the diet (Campbell et al., 1947;
Campbell, 1963). This work was experimentally verified by Můr, Záruba, and Charamza (1956), who confirmed that primary damage to the myelin sheath, preceding injury of the axon, developed if copper was blocked in experimental animals by intravenous administration of dithio-diethyl-carbamate.

Our own experiments performed in 1968-69 on the development of carbon disulphide neuropathy in rats indicated, though only on a small group of animals and close below the 95% confidence limit, that a diet supplemented with metal salts afforded a certain protection against the development of peripheral neuropathy (Lukáš, 1970). The copper and zinc doses per rat per day had been raised to 1-1 mg Cu and 2-4 mg Zn compared with 0-08 mg Cu and 0-48 mg Zn in the standard diet. This stimulated us to investigate also some other indices of the behaviour of copper in the organism, in particular the level of ceruloplasmin activity in the sera of exposed animals. To our surprise it was found that this activity decreased significantly in exposed rats, the decrease depending on the carbon-disulphide dose and the animals’ constitution (Kocinová and Lukáš, 1969; Kujalová and Lukáš, 1969; 1970). These two partial results of ours, together with the reports referred to above, induced us to undertake the experiments in the present study.

Material and methods

Two groups of male SPF rats (mean weight 200 g) each containing 36 animals, half the animals of either group serving as controls, were inhalation-exposed to 3-6 mg CS₂/litre of air (variant 1) or to 2-4 mg CS₂/litre of air (variant 2) for six hours daily for five weeks a week. Development of neuropathy was followed with stimulation electromyography by measuring conduction velocity in the motor fibres (MCV) of the lumbar plexus to the tibial nerve (including the neuromuscular transmission) of both hind legs, as described previously (Lukáš, 1970). In both experimental variants the animals were sacrificed when they were overtly affected by advanced neuropathy. This stage was reached after 13 weeks of exposure in experimental variant 1 (decrease of MCV to 56-2% of normal values) and after as long as 37 weeks in variant 2 (decrease of MCV to 51-3% of normal values).

The rats were then killed and, using glass instruments for dissection, both the lumbar and brachial plexuses were excised and the material was processed by the chemical method described by Kotas, Obrusník, Lukáš, and Křivánek (1972). Specimens together with mixed Zn and Cu standards were irradiated for 10 hours in a type VVR-S ŠUJ reactor, in a neutron-flux equalling 1 × 10¹³ n cm⁻² s⁻¹. The measurements proper were performed after the chemical separation of the two elements using a semiconductor-(Ge, Li)-detector connected with a multi-channel gamma-ray spectrometer.

![Gamma-ray spectrum](image)

**FIG. 1.** Gamma-ray spectrum after chemical separation of zinc and copper.

### TABLE

**Motor Fibre Conduction Velocity in Rats: Lumbar Plexus—Tibial Nerve**

<table>
<thead>
<tr>
<th>Variant 1</th>
<th>CS₂</th>
<th>MCV (mean weight 200 g)</th>
<th>Control</th>
<th>Zn (ppm)</th>
<th>Cu (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-6 mg/l.</td>
<td></td>
<td>31·1 m/sec (27·6-33·2)</td>
<td>17·5 m/sec (16·03-18·24)</td>
<td>n = 17</td>
<td>n = 16</td>
</tr>
<tr>
<td>13 weeks’ exposure</td>
<td>100%</td>
<td>56·2%</td>
<td></td>
<td>28·8 ± 4·5</td>
<td>25·6 ± 2·0</td>
</tr>
<tr>
<td>2·4 mg/l.</td>
<td></td>
<td>30·8 m/sec (28·1-32·9)</td>
<td>16·3 m/sec (14·8-17·7)</td>
<td>n = 11</td>
<td>n = 14</td>
</tr>
<tr>
<td>37 weeks’ exposure</td>
<td>100%</td>
<td>51·3%</td>
<td></td>
<td>22·3 ± 4·2</td>
<td>23·2 ± 3·3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variant 2</th>
<th>CS₂</th>
<th>MCV (mean weight 200 g)</th>
<th>Control</th>
<th>Zn (ppm)</th>
<th>Cu (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2·4 mg/l.</td>
<td></td>
<td>30·8 m/sec (28·1-32·9)</td>
<td>16·3 m/sec (14·8-17·7)</td>
<td>n = 11</td>
<td>n = 14</td>
</tr>
<tr>
<td>37 weeks’ exposure</td>
<td>100%</td>
<td>51·3%</td>
<td></td>
<td>22·3 ± 4·2</td>
<td>23·2 ± 3·3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Zn (ppm)</th>
<th>Cu (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CS₂</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>n = 17</td>
<td>n = 16</td>
<td>n = 17</td>
</tr>
<tr>
<td></td>
<td>28·8 ± 4·5</td>
<td>25·6 ± 2·0</td>
<td>4·2 ± 0·3</td>
</tr>
<tr>
<td></td>
<td>0·6564</td>
<td>t test</td>
<td>3·095**</td>
</tr>
<tr>
<td></td>
<td>0·353</td>
<td>t test</td>
<td>9·864**</td>
</tr>
</tbody>
</table>

1Mean values in our first paper (Lukáš, 1970) are 29·9 ± 0·95 m/sec

2"
The contents of the metals were calculated by comparison with the activities of known amounts of Zn and Cu in the standards. Figure 1 demonstrates a protocol record of a gamma-ray spectrum obtained after chemical separation of the two metals.

Results

Seventeen control and 16 experimental animals were taken for the final analysis in experimental variant 1 and 11 and 14 animals respectively in experiment 2. The Table and Fig. 2 present the absolute figures obtained, in relation to tissue dry weight (ppm), and give a comparison of the relative values, the control values being taken as equal to 100%.

As shown a practically stationary Zn level was found in control and exposed animals in both of the experimental variants, while the levels of copper increased by 135% in experiment 1 and by 127% in experiment 2. These differences are statistically significant (p < 0.01).

![Graph showing conduction velocity in nerve tissue in relation to zinc and copper levels in exposed and control (K) animals.]

FIG. 2. Conduction velocity in nerve tissue in relation to zinc and copper levels in exposed and control (K) animals.

Discussion

Comparison of the relative values shows that the shifts are constant and reproducible and that they correlate well with the electromyographic findings in the affected peripheral nerve tissues. A certain advantage in studying the progress of toxic neuropathy is that the presumably primary damage to the myelin sheath will first of all affect the nerve in its ability to transmit impulses, in our case electric stimuli. Thus, if the rate of nerve transmission is measured by electromyography, it is not only possible to detect the damage but, by taking repeated measurements, to follow its progress and to quantify it.

We consider our results to be reliable also for the additional reason that the deviations in Zn and Cu levels were obtained under exposure to different concentrations of carbon disulphide fumes but in the presence of approximately equal clinical states, i.e., at a time when in both experiments the rate of conduction velocity in the nerves studied decreased to approximately 51-56% of the normal values.

An interesting fact is that only the level of copper rose, whereas that of zinc practically did not change as compared with the control groups. We consider that a rôle may have been played here by the higher constant stability of the chelate bond of dithiocarbamates in relation to copper than to zinc, as had already been pointed out by Scheel (1967).

The rise in the copper level after chronic carbon-disulphide intoxication suggests in our opinion that the organism, in the present case the peripheral nerve tissue, has as it were a tendency to supplement the bound copper by an additional supply of this metal from reserve sources. However, this hypothesis would require support in the form of knowledge of the amount of chelated (and consequently probably biologically inactivated) copper; but so far we do not know the answer to this question.

The experience accumulated so far, the lowered ceruloplasmin activity in the sera of exposed rats, the apparently favourable effects of Zn and Cu salt-enriched diet, as well as the results of the present work, suggest that changes in metal metabolism deserve attention as one of the possible pathogenic links in the development of carbon-disulphide intoxication.

References


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Edgar Lukás, Pavel Kotas and Ivan Obsrunik

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