Conduction velocity in the peripheral nerves of rats with chronic carbon disulphide neuropathy

K. KNOBLOCH¹, J. STETKIEWICZ², AND T. WRÓŃSKA-NOFER³

From the ¹Departments of Toxicology, ²Pathomorphology and ³Biochemistry, Institute of Occupational Medicine, 90-950 Łódź, Teresy 8, Poland

ABSTRACT Effects of carbon disulphide (CS₂) on conduction velocity in sciatic and tibial nerve were investigated in rats exposed to CS₂ vapour at concentrations of 0.9 mg/l or 1.6 mg/l of air for periods of 1.5, 3, 6, 9 and 12 months. The conduction velocity was measured on the day following each exposure period, and again, three and six months after the last day of exposure. The majority of exposed rats showed a reduction in conduction velocity on the day after exposure: the extent of this reduction was dependent on the CS₂ concentration and the duration of exposure. Exposure for 1.5 months to 1.6 mg CS₂/l produced temporary and fully reversible impairment of conduction velocity. Increased exposure to CS₂ (3, 6 or 9 months at 1.6 mg/l; 6 or 12 months at 0.9 mg/l CS₂ in air) caused a reduction in velocity which was only partially reversible.

Peripheral neuropathy caused by carbon disulphide (CS₂) poisoning has been recognised since the second half of the nineteenth century. It has been observed in workers of the viscose rayon industry and induced experimentally in animals exposed to CS₂. Measurement of conduction velocity in peripheral nerves has recently aroused interest as a sensitive electrophysiological method for detecting early functional changes in the peripheral nerves both of men and of animals exposed to CS₂ (Lukas, 1969; Lukas, 1970; Lukas *et al.*, 1974; Seppalainen and Tolonen, 1974; Linnoila *et al.*, 1975; Vasilescu, 1976).

The aim of the study described here was to determine the changes in conduction velocity in the sciatic and tibial nerves of rats exposed to CS₂ at concentrations of 0.9 mg/l air and 1.6 mg/l air, and the dynamics of recovery after exposure had ended.

Methods

One hundred and forty-three white female Wistar rats, initially three months old, were used. They were exposed to CS₂ vapour at concentrations of 0.9 mg/l

(0.8–1.0 mg/l) or 1.6 mg/l (1.5–1.7 mg/l) in an inhalation chamber five hours daily, six days a week in separate groups for 1.5, 3, 6, 9 or 12 months. Both exposed and control rats were given a standard diet and water *ad libitum*.

Conduction velocity was measured the day after the end of any exposure period, and again three and six months later.

Measurements were performed on animals anaesthetised i.p. with 0.3 g chloral hydrate/kg body weight; the animals were kept warm by means of a lamp heater while the measurements were being taken. The anal and hind limb skin temperature during that time remained constant in control and CS₂-exposed rats ($\pm 1^\circ\text{C}$).

The method used was a modification of that described by Fullerton and Barnes (1966). The sciatic and tibial nerves were stimulated at points S₁ and S₂ (Fig. 1) with supramaximal stimuli (250–300 V) for 50 μs . The stimulating cathode was a stainless steel needle inserted through the skin into a para-central region of the thigh, close to the sciatic nerve (S₁) and into the medial side of the ankle close to the posterior tibial nerve (S₂). The action potential from the interosseous and lumbrical muscles on the plantar side of the hind paw was picked up by a concentric needle electrode and recorded on a Hewlett-Packard

Received for publication 13 March 1978

Accepted for publication 24 July 1978

Fig. 1 Arrangement of electrodes. A = anode; S₁ and S₂ = cathodes; R = recording electrode.

electromyograph oscilloscope. The conduction velocity was calculated by dividing the distance between points of stimulation S₁ and S₂ by the difference between the two latencies recorded on the oscilloscope (Figs. 2 and 3).

Results

Obvious general symptoms of CS₂ intoxication, such as loss of body weight, staggering and muscle weakness developed between the fourth and sixth months of exposure to 1.6 mg CS₂/l of air; however, rats exposed to a lower concentration of CS₂ (0.9 mg/l) remained healthy throughout the entire experimental period.

Electrophysiological changes preceded the general symptoms of intoxication. The amount by which conduction velocity was reduced in the sciatic and tibial nerve was dependent on the level and duration of CS₂ exposure and on the length of the recovery period. A reduction attributable to CS₂ at a concentration of 1.6 mg/l was noticeable after 1.5 months' exposure. Longer exposure periods exacerbated the

electrophysiological changes, recorded the day after exposure ended (Table 1).

Conduction velocity did not differ significantly from control values, within three months of 1.5 months' exposure to 1.6 mg CS₂/l. After rats had been exposed for three months to this level of CS₂ vapour, the conduction velocities were found to be similar, after a three- and a six-month recovery period, to those found in rats exposed for only 1.5 months (Table 1, Fig. 4). The conduction velocity was found also to revert to near-normal values when six months' exposure was followed by three- and six-month recovery periods. During the first three months after a nine-month exposure to 1.6 mg CS₂, impairment of conduction velocity actually increased, and minor improvement was delayed until six months after exposure had ended.

A statistically significant reduction in conduction velocity was also found in rats exposed to 0.9 mg CS₂/litre air for six or for 12 months, but was less marked than that seen in rats exposed to the higher concentration of CS₂. It was most obvious after 12 months' of CS₂ exposure (Table 2). Three months after exposure had ended a further decrease was noted in conduction velocity in this group of animals, and after a total of six months' recovery, only a slight improvement in conduction velocity was found (Table 2, Fig. 5).

Discussion

The results of this study show that decrease in the conduction velocity is related to the concentration of CS₂ inhaled and to the duration of exposure. A similar conclusion was drawn by Lukas (1970) from his study on rats exposed for six and nine months to CS₂ at concentrations of 1.2 and 2.4 mg/l. Linnoila *et al.* (1975) and Lukas (1970) have also found a partial improvement in conduction velocity in rabbits and rats three months after the end of exposure to 2.4 mg/kg CS₂/l. Our observations showed that the improvement in conduction velocity after exposure is also dependent on the level and duration of CS₂ exposure.

It is of particular interest that the conduction velocity actually deteriorated over the first three months after long-term exposure to CS₂ had ended (Figs. 4 and 5). Some improvement could be seen eventually, six months after the end of exposure, but, even then the conduction velocity remained slower than that in control animals of the same age.

Morphological aspects of CS₂ neuropathy were discussed in our previous publications (Szendzikowski *et al.*, 1973, 1974). The basic lesion of the peripheral nervous system, attributable to CS₂, affects the

Fig. 2 Muscle action potentials induced by nerve stimulation at two different points in control rat. Distance between long vertical lines = 2 mV. Latency difference = 0.4 m/s. Distance S_2-S_1 in the hind limb of the rat = 20 mm. Conduction velocity = 50 m/s.

Fig. 3 Muscle action potentials induced by nerve stimulation in a rat exposed to 1.6 mg/l CS_2 for six months. Distance S_2-S_1 in the hind limb of the rat = 22 mm. Latency difference = 1 m/s. Conduction velocity = 22 m/s.

Table 1 Conduction velocity in the sciatic and tibial nerves of rats exposed to 1.6 mg CS₂/l air

Duration of exposure (months)	Conduction velocity† in the sciatic and tibial nerves at various times after end of exposure					
	24 hours		3 months		6 months	
	Control	CS ₂	Control	CS ₂	Control	CS ₂
1.5	54.4 ± 7.2 (9)	42.9 ± 4.1*** (10)	45.6 ± 3.9 (10)	41.3 ± 5.2 (9)	—	—
3	45.6 ± 2.5 (10)	32.2 ± 8.0*** (14)	43.1 ± 4.8 (8)	34.6 ± 3.7** (8)	46.2 ± 5.9 (10)	39.2 ± 4.6* (8)
6	54.6 ± 6.2 (6)	25.1 ± 4.2*** (9)	46.3 ± 5.0 (8)	38.5 ± 1.9** (5)	45.5 ± 6.0 (8)	37.4 ± 7.1* (5)
9	46.9 ± 4.7 (8)	20.3 ± 4.5*** (18)	44.1 ± 6.9 (6)	16.7 ± 1.9*** (7)	49.2 ± 5.0 (13)	34.3 ± 7.5*** (8)

†Results expressed in metres/second as average ± standard deviation. Figures in parentheses represent number of animals used in the experiment. Statistically significant differences from the control group:

*; 0.01 < P < 0.05
 **; 0.001 < P < 0.01
 ***; P < 0.001

Fig. 4 Conduction velocity in the sciatic and tibial nerves of rats exposed to 1.6 mg CS₂/l. The velocities are expressed as percentages of the control values.

Table 2 Conduction velocity in the sciatic and tibial nerves of rats exposed to 0.9 mg CS₂/l air

Duration of exposure (months)	Conduction velocity in the sciatic and tibial nerves at various times after end of exposure					
	24 hours		3 months		6 months	
	Control	CS ₂	Control	CS ₂	Control	CS ₂
3	51.2 ± 6.8 (8)	45.4 ± 6.8 (8)	—	—	—	—
6	51.5 ± 6.4 (11)	43.0 ± 2.2** (8)	54.3 ± 6.5 (8)	43.8 ± 4.8* (7)	46.8 ± 5.4 (7)	39.6 ± 3.9* (7)
12	50.7 ± 6.0 (10)	39.0 ± 5.1*** (9)	47.8 ± 4.7 (9)	32.3 ± 4.3*** (6)	37.8 ± 3.3 (7)	32.5 ± 1.9** (5)

Results expressed as in Table 1

axon. The initial changes comprise axonal swellings accompanied by ultrastructural alterations such as accumulation and degeneration of axoplasmic organelles, followed by fibre fragmentation and breakdown; this leads to a considerable reduction in the number of myelin fibres, especially after pro-

longed exposure to CS₂. No signs of primary segmental demyelination have been found.

It seems reasonable to suppose that the electrophysiological changes are caused by injury to axonal fibres in the peripheral nerves. However, the electrophysiological changes appear to precede the develop-

ment of obvious morphological changes, both in histological sections of major peripheral nerves, and in the ultrastructure of myelinated nervous fibres. A similar sequence of electrophysiological and morphological changes following chronic exposure of rabbits to CS₂ has also been described (Linnoila *et al.*, 1975).

These observations suggest that the recovery in conduction velocity observed after withdrawal of CS₂ may be caused by the gradual regeneration of injured fibres in the peripheral nerves.

The importance of conduction velocity measurements in viscose workers has been emphasised (Seppalainen and Tolonen, 1974; Vasilescu, 1976). This technique enables neuropathy to be diagnosed at an early stage before the onset of obvious neurological symptoms. In addition, repeated measurements of conduction velocity are useful for prognosis in the neurological examination of a previously exposed subject. Lack of improvement in conduction velocity indicates irreversible neuropathy (Seppalainen and Tolonen, 1974). Persistent impairment of conduction velocity after protracted exposure to CS₂ has also been confirmed in our present study.

In summary, the following conclusions can be drawn from the results of the experiments described here. First, reduction in conduction velocity is related to the level and duration of exposure to CS₂ vapour. Second, a complete recovery of conduction velocity may follow a short intensive period of exposure to CS₂. Finally, long-term exposure to CS₂ may cause irreparable neuropathy and an irreversible reduction in the conduction velocity of peripheral nerves.

This study has been sponsored by the Polish-American Agreement No. 05-008-2 with Occupational Health Program, Public Health Service, Washington, USA.

References

- Fullerton, P. H., and Barnes, J. M. (1966). Peripheral neuropathy in rats produced by acrylamide. *British Journal of Industrial Medicine*, **23**, 210-221.
- Linnoila, I., Haltia, M., Seppalainen, A. M., and Palo, J. (1975). Experimental carbon disulphide poisoning: morphological and neurophysiological studies. In *Proceedings of VIIth International Congress of Neuropathology, Akadémiai Kiadó, Budapest*, 1975, pp. 383-386. Edited by S. Környei, S. Tariska and O. Gosztanyi.
- Lukas, E. (1969). Leitgeschwindigkeit peripherer Nerven bei Schwefelkohlenstoff ausgesetzten Personen. *Internationale Zeitschrift für Klinische Pharmakologie Therapie und Toxikologie*, **4**, 354-358.
- Lukas, E. (1970). Stimulation electromyography in experimental toxicology. *La Medicina del lavoro*, **61**, 302-308.
- Lukas, E., Kotas, P., and Obrusnik, I. (1974). Copper and zinc levels in peripheral nerve tissues of rats with experimental carbon disulphide neuropathy. *British Journal of Industrial Medicine*, **31**, 288-291.
- Seppalainen, A. M., and Tolonen, M. (1974). Neurotoxicity of long-term exposure to carbon disulphide in the viscose rayon industry. A neurophysiological study. *Work-Environment-Health*, **11**, 145-153.
- Szendzikowski, S., Stetkiewicz, J., Wrońska-Nofer, T., and Zdrajewska, I. (1973). Structural aspects of experimental carbon disulphide neuropathy. I. Development of neurohistological changes in chronically intoxicated rats. *Internationales Archiv für Arbeitsmedizin*, **31**, 135-149.
- Szendzikowski, S., Stetkiewicz, J., Wrońska-Nofer, T., and Karasek, M. (1974). Pathomorphology of the experimental lesion of the peripheral nervous system in white rats chronically exposed to carbon disulphide. In *Proceedings of the Symposium held in Kazimierz upon Vistula, Poland, May 18-20, 1972*, pp. 319-326. Edited by I. Hausmanowa-Petrusewicz and H. Jedrzejowska. Polish Medical Publishers: Warsaw.
- Vasilescu, C. (1976). Sensory and motor conduction in chronic carbon disulphide poisoning. *European Neurology*, **14**, 447-457.