REFERENCES

1. E. De Robertis, G. Rodriguez de Lores Arnaiz, L. Salganicoff, A. Pellegrino de Iraldi and L. M. Zieher, *J. Neurochem.* **10**, 225 (1963).
2. V. P. Whittaker, I. A. Michaelson and R. J. A. Kirkland, *Biochem. J.* **90**, 293 (1964).
3. K. Kadota and T. Kanaseki, *J. Biochem.* **65**, 839 (1969).
4. T. Kanaseki and K. Kadota, *J. cell. Biol.* **42**, 202 (1969).
5. V. P. Whittaker, *Biochem. J.* **72**, 694 (1959).
6. M. H. Aprison and P. Nathan, *Archs Biochem. Biophys.* **66**, 388 (1957).
7. L. A. Barker, J. Amaro and P. S. Guth, *Biochem. Pharmac.* **16**, 2181 (1967).
8. K. Takeno, A. Nishio and I. Yanagiya, *J. Neurochem.* **16**, 47 (1969).
9. K. Kuriyama, E. Roberts and J. Vos, *Brain Res.* **9**, 231 (1968).

Biochemical Pharmacology, Vol. 20, pp. 2114–2115. Pergamon Press, 1971. Printed in Great Britain

The level of pyridoxal phosphate (PLP) in the blood plasma of rats exposed to carbon disulphide

The mechanism by which carbon disulphide brings about metabolic disturbances is not clearly understood. Some authors[1] suggested that interaction between carbon disulphide and vitamin B_6 might represent the basic primary site of action, and some indirect observations seemed to support this hypothesis.[1-3]

In this experiment an attempt was made to obtain direct proof for the interaction *in vivo* between carbon disulphide and the most essential component of vitamin B_6, pyridoxal phosphate.

Experimental

The experiments were performed on white female rats of the Wistar strain, body weight about 200 g, fed standard LSM diet.* The animals were exposed to carbon disulphide in two groups differing in the kind of exposure:

(a) *Long-term exposure.* A dynamic experimental chamber was used into which the CS_2 vapours were introduced automatically at a constant rate.[4] The exposure lasted for 6 months, 6 days a week, 5 hr a day. The concentration of CS_2 in air was measured colorimetrically[5] and ranged from 1·5 to 1·8 mg/l. The rats were fed *ad lib.* throughout the experiment. The exposure started when rats were 3 months old.

(b) "*Continuous*" *exposure.* Rats were kept in a small dynamic chamber[6] continuously for 2 days, the concentration in air being 0·8–1·2 mg/l. All rats (experimental and control) were fasted during time of exposure. The animals used were 5 months of age.

For both above groups controls were also studied. These were subjected to the same conditions, but were not exposed to carbon disulphide.

In both groups animals were killed by decapitation immediately after exposure. The blood plasma was obtained as described by Wachstein *et al.*[7] The determination of pyridoxal phosphate (PLP) was based on its coenzyme function in a tyrosine decarboxylase system, as described by Boxer *et al.*,[8] and modified later by Wachstein *et al.*,[7] using the apoenzyme of tyrosine decarboxylase (Sigma Chem. Co.). An internal standard was used for the calculation of results.

Results and discussion

The results presented in Table 1 show a distinct decrease of PLP in the blood plasma of rats exposed to CS_2. The drop in the level of PLP in exposed animals was roughly 60 and 75 per cent of the control values, for chronic long-term exposure, and 2 days continuous exposure of rats, respectively.

* Standard diet for rats and mice (containing 0·03 mg vitamin B_6/100 g diet) manufacturer: Wytwórnia Pasz, Łowicz.

TABLE 1. THE LEVEL OF PYRIDOXAL PHOSPHATE IN BLOOD PLASMA OF RATS EXPOSED TO CS_2

	Exposed rats		Control rats	
Kind of exposure	No. of animals	PLP (mμg/ml)	No. of animals	PLP (mμg/ml)
6 months, daily	7	85 ± 30* (46–127)	5	221 ± 65 (156–331)
2 days, continuous	8	44 ± 18† (24–84)	7	169 ± 32 (146-240)

Values are mean ± S.D.; range in brackets.
Statistical significance with respect to control: * $0.001 < P < 0.01$.
† $P < 0.001$.

These results represent the first direct evidence of an interaction occurring between carbon disulphide and the active form of vitamin B_6 *in vivo*. Indirect evidence had been obtained earlier by Abramova[2] and also Kujalova and Tintera[3] basing on the tryptophan-load-test and the decrease of urinary 4-pyridoxic acid.

From the presented results it is evident that the drop of PLP in blood is not dependent upon the long duration of exposure, which is characteristic for chronic intoxications. Even deeper changes were obtained in a single exposure kept continuously for a sufficiently long time. Direct binding by carbon disulphide metabolites seems possible first of all with respect to pyridoxamine.[1] However, most probably an equilibrium exists between different forms of vitamin B_6, because they can be enzymatically converted into one another.[1,9] Thus, the drop in the PLP in blood may reflect in an indirect way the binding of other forms of vitamin B_6, especially of pyridoxamine resp. its phosphate.

Whether or not significant drop of PLP occurs also *in vivo* in other tissues remains to be answered: using another indirect method we failed formerly to find any essential drop of the activity the PLP-dependent enzyme, kynureninase, in the liver of rats under prolonged exposure, while a decrease of the activity of this enzyme found in 2-days continuous exposure seemed independent of the deficiency of coenzyme.[6]

Acknowledgement—Thanks are due to Dr. Habil. J. K. Piotrowski, Chief of the Department, for his interest and kind assistance in preparing the manuscript.

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REFERENCES

1. V. VAŠÁK and J. KOPECKÝ, in *Toxicology of Carbon Disulphide* (Eds. H. BRIEGER and J. TEISINGER), p. 35, Excerpta Medica Foundation, Amsterdam (1967).
2. J. I. ABRAMOVA, in *Toxicology of Carbon Disulphide* (Eds. H. BRIEGER and J. J. TEISINGER), p. 32, Excerpta Medica Foundation, Amsterdam (1967).
3. V. KUJALOVA and J. TINTERA, *Med. Lav.* **61**, 227 (1970).
4. K. SZYMCZYKIEWICZ, *Med. Pracy* **4**, 235 (1957).
5. T. DUTKIEWICZ, I. KĘSY-DĄBROWSKA and J. PIOTROWSKI, in *Oznaczanie związków toksycznychw powietrzu*, p. 133, PZWL, Warszawa (1965).
6. R. GÓRNY and J. K. PIOTROWSKI, *Med. Pracy* **21**, 241 (1970).
7. M. WACHSTEIN, J. D. KELLNER and J. M. ORTIZ, *Proc. soc. exp. Biol. Med.* **103**, 350 (1960).
8. G. E. BOXER, M. P. PRUSS and R. S. GOODHART, *J. Nutr.* **63**, 623 (1957).
9. E. E. SNELL, *Vitam. Hormon.* **22**, 485 (1964).