Arh. hig. rada toksikol., **30** *(1979) suppl. pp. 503–509*

ON THE MECHANISM OF THE CHRONIC EFFECT OF CARBON DISULPHIDE ON CARBOHYDRATE METABOLISM

V. KUJALOVÁ, I. ŠPERLINGOVÁ and E. FRANTÍK
Institute of Hygiene and Epidemiology, Centre of Industrial Hygiene and Occupational Diseases, Prague, Czechoslovakia

ABSTRACT

Chronic exposure to carbon disulphide (CS_2) demonstrated both in animal experiments as well as in CS_2 exposed workers a change in glucose tolerance. In our study we confirmed the possible binding of xanthurenic acid (XA) to insulin and thus a reduced activity of this hormone. The higher formation of XA caused by chronic CS_2 exposure was proved previously. The XA-insulin complex was isolated from the serum of exposed monkeys *(Macacus rhesus)* and from the serum of workers exposed to CS_2 for a long time. The reduced activity of the isolated XA-insulin complex was demonstrated by the biological effect of glycemia in rabbits.

Some 20 years ago Italian authors[2,3,4] reported the diabetogenic effect of carbon disulphide (CS_2) and the enhanced incidence of diabetes in workers handling CS_2. More recent studies are not unanimous on this point. Goto[5] failed to find initially a significant deviation in the glucose tolerance test (GTT) in a large sample of Japanese workers in contact with CS_2. Since, however, he associated the possible diabetogenic effect of CS_2 with the higher occurrence of retinal microaneurysm in these workers he revised his negative finding by using the prednisolon GTT and observed a deviation in the course of the glycemic curve[6]. Hernberg and co-workers[8] who had found no significant shift in the course of the normal GTT in their sample group, demonstrated a higher level of glycemia in fasting persons who had been working with CS_2 over an extended period. Abramova[1] found a diabetogenic effect of CS_2 in chronic experiments on rats fed on a rich carbohydrate diet. Chronic exposure to CS_2 produced impaired pyridoxine metabolism which manifested itself in a higher formation of xanthurenic acid (XA) which arises with the degradation of tryptophane[14]. A similar increased excretion of XA is concomitant with diabetes[13]. Kotake and co-workers[12] attribute to the increased XA level a diabetogenic effect; in their opinion XA binds to serum insulin, forming a complex the biological activity of which is lower than the activity of insulin alone[11].

In our previous study we found both in experimental animals as well as in workers exposed occupationally to CS_2 for long periods, changes in glucose tolerance[15]. In a combined glucose tolerance test with prior application of corticoid, the glycemic curve showed after load changes that characterise "oxyhyperglycemia" described by Goto and co-workers[7]. The immunoreactive insuline (IRI) curve exhibited a shift to the right and higher peak values than in the case of diabetes. Changes in the levels of lipid metabolism of nonesterified fatty acids and triglycerids were not significant comparing with controls.

MATERIAL AND METHODS

The XA-insulin complex was prepared *in vitro* by XA incubation (40 mg in 5 ml distilled water) with insulin (200 mg in 5 ml distilled water) at pH 8.0 mixed in a 1 : 1 ratio with a phosphate buffer of 0.15 M for a period of 30 minutes at 37 °C[17]. The complex was separated by gel filtration on Sephadex G 50 and eluted with phosphate buffer 0.075 M. The obtained fractions were measured at optical density of 270 and 330 nm. The sample was dialyzed for 48 hours, and after centrifugation the supernatant was freeze dried. The biological effect was assessed by means of the glycemia test in 24-hour fasting silver rabbits (weight 2–2.5 kg) and compared with the insulin effect (Spofa 23.3 mj/mg). Insulin was given i.v. in a dose of 0.5 I.U./kg weight and the complex was given in the same dose for protein concentration[16]. The curve was observed in intervals of 0, 30, 60, 90 and 120 minutes. Glycemia was determined using the o-toluidine method[9].

The XA-insulin complex was isolated from the serum of monkeys *(Macacus rhesus)* and CS_2 exposed humans. Fractions were isolated from the serum of three exposed and two control monkeys. Exposure lasted 6 hours a day, 5 days a week for a period of 20 weeks at a concentration of 1.2 mg CS_2/l of air. After the application of tryptophane the exposed animals had a higher XA excretion. We further isolated the complex from the serum of four persons who had been long exposed to CS_2 in concentrations below 10 ppm. A biochemical examination had revealed in these subjects a positive tryptophane load test, i.e. an increased excretion of XA after application of 2 g L-tryptophane. For the purpose we selected subjects with a not too marked impairment. Low molecular protein fractions, showing the insulin effect, were isolated from the serum on Sephadex G 150 using the procedure described by Murakami and co-workers[18] who had isolated the complex from the serum of diabetics. The column was eluted with a Krebs-Ringer phosphate buffer of 0.05 M, pH 7.4; four fractions of 6 ml were obtained after an hour. Optical density was measured at 270 and 330 nm. The eluate containing the complex was dialyzed for 48 hours and the fraction freeze dried. The biological activity of the complex was assessed in the same manner as was that of the complex prepared *in vitro*.

The i.v. tolerance test was evaluated with the glucose tolerance coefficient according to Korec[10] in which glycemia was monitored after application of 1 g glucose/kg weight in time intervals of 0, 10, 20, 30, 45, 60 minutes.

RESULTS

In vitro xanthurenic acid (XA) forms with insulin, under the conditons described, a complex the biological acitivity of which is lower than the acitivity of insulin alone. Figure 1 shows the result of biological titration. This low--molecular protein complex was isolated from the serum of CS_2 intoxicated monkeys. Figure 2 shows the separation of the serum from a control and intoxicated animal on Sephadex G 150, where the complex had been eluted between the 40th–50th test tubes. The complex from the serum of exposed humans was isolated in the same manner. The impairment of glucose tolerance in the exposed monkeys was demonstrated in the i.v. TT: after 30 minutes the tolerance coefficient showed a reduction from 9.8 in the control to 5.45 in the exposed group, and after 45 minutes a reduction from 14.8 in the control to 8.5 in the exposed group. The biological evaluation of the XA-insulin complex isolated from the serum of exposed monkeys is given in mean values in Figure 3. Figure 4 shows the mean course of the curve after the application of the complex that had been isolated from the serum of exposed humans. The results demonstrate a reduced biological activity of the XA-insulin complex isolated from the serum of both experimental animals and exposed humans. Reproducibility, i.e. the evaluation of the complex in different experiments, was good.

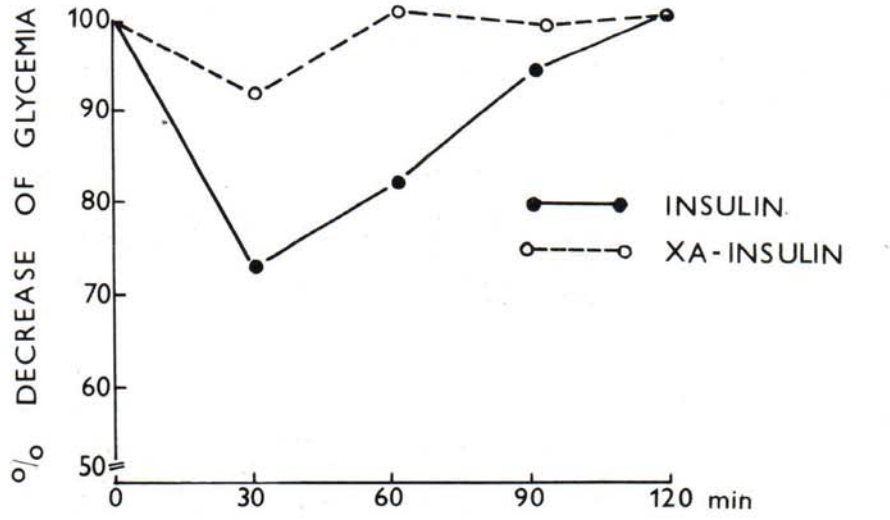

FIG. 1 – Effect of insulin and of the complex XA-insulin prepared *in vitro* on glycemia of rabbits. The points are the mean values for 2 rabbits.

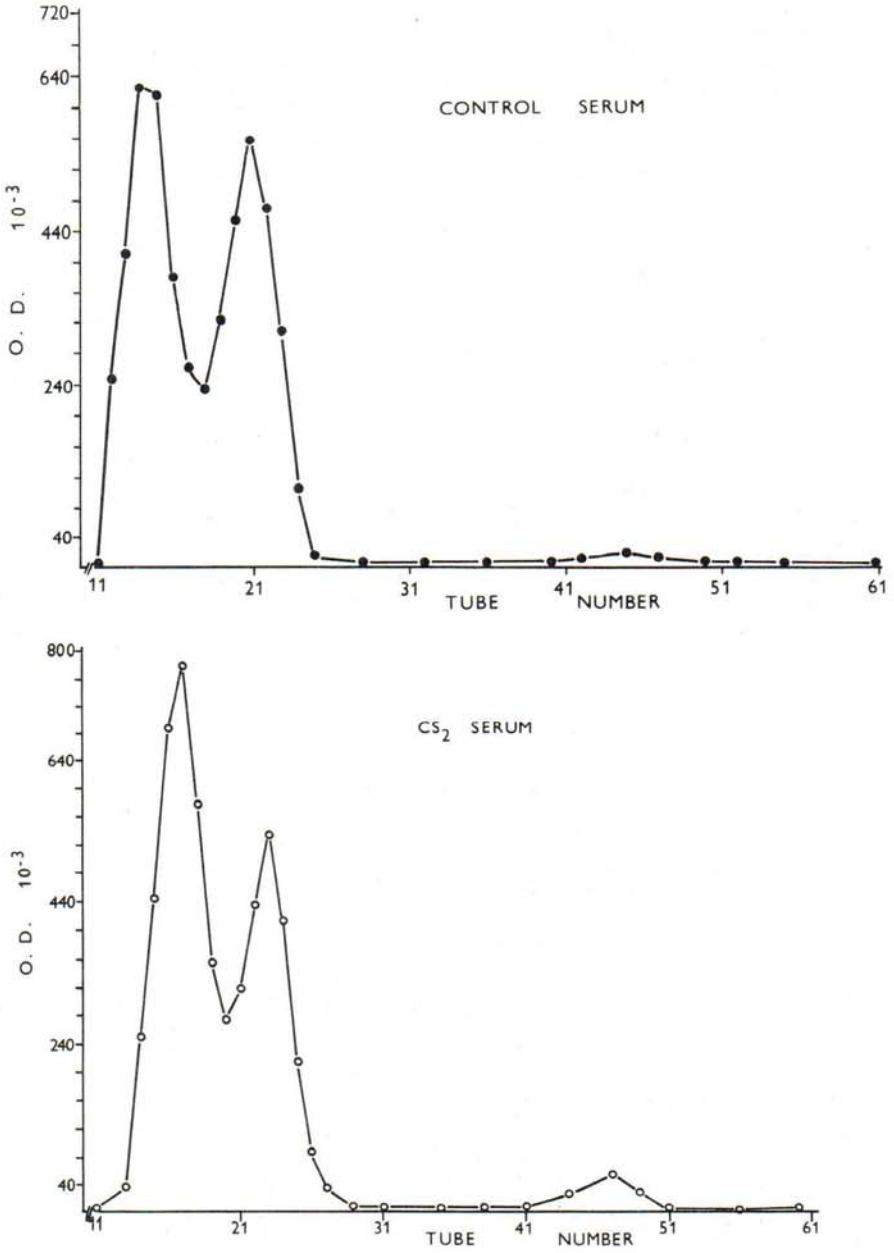

FIG. 2 – Gel filtration of monkey serum on Sephadex G – 150 column. Eluation patterns of monkey sera with Krebs-Ringer-phosphate buffer 0.05 M, pH 7.4 of 6 ml were collected. A 0.2 ml aliquot of fractions was diluted with 4 ml H_2O and measured at an optical density of 280 nm.

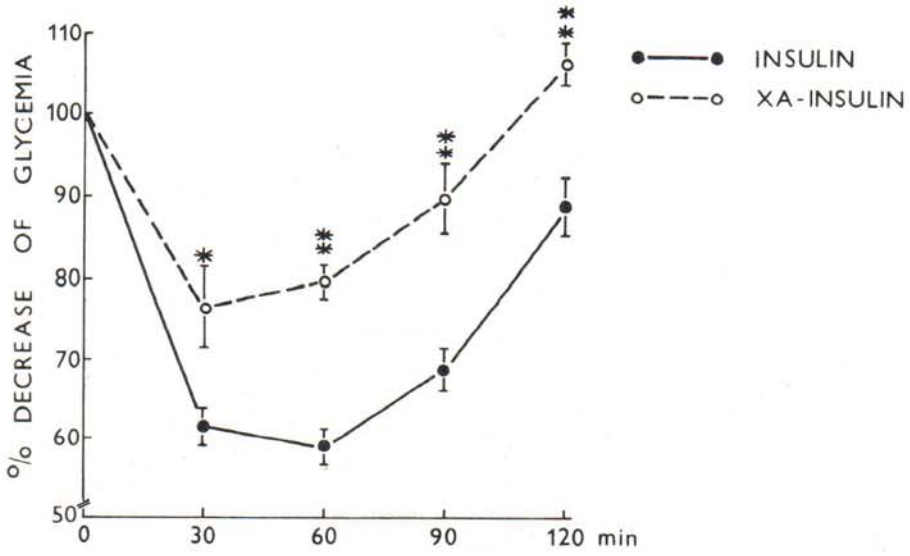

FIG. 3 – Effect of insulin and of the complex XA-insulin isolated from monkey serum on glycemia of rabbits. The values are means ± S.E.

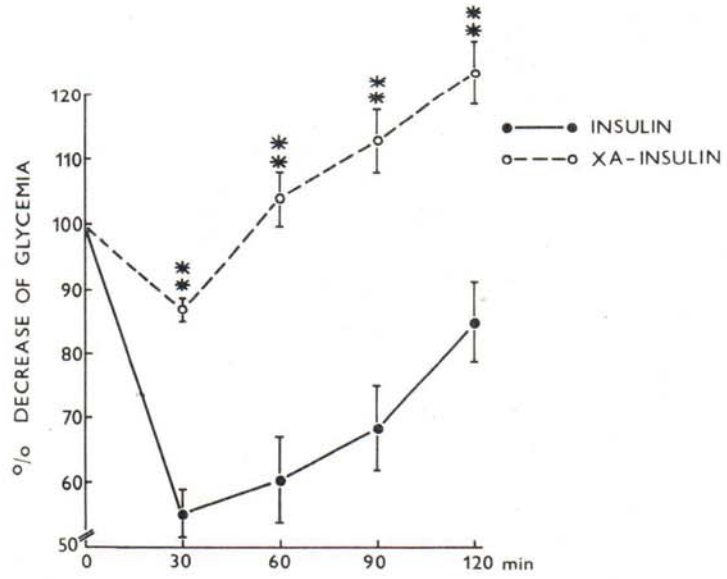

FIG. 4 – Effect of insulin and of the complex XA-insulin isolated from human serum on glycemia of rabbits. The values are means ± S.E.

DISCUSSION

We failed to prove a direct effect of CS_2 on insulin activity. It appears that in case of exposure to CS_2 carbohydrate metabolism is affected indirectly through the increased production of XA which results from changes in pyridoxine metabolism due to chronic exposure to CS_2. Changes in tryptophane degradation give rise to a higher formation of XA. The impaired carbohydrate metabolism has the characteristic of "oxyhyperglycemia". Fasting glycemia values are elevated and GTT, after the application of corticoid, shows higher glycemia values after one or even two hours following the administration of glucose, to return later to normal levels. These changes were found in nearly 60% of the workers[15] exposed to CS_2 for many years. As the effect on insulin activity must of necessity interfere further with metabolism, its study should receive more attention.

REFERENCES

1. *Abramova, J. I.* The questions of pathogenesis, specific prevention and therapy of carbon disulfide intoxication. In: Brieger, H. and Teisinger, J. ed. Toxicology of Carbon Disulfide, Proceedings of an International Symposium, Prague, September 15th–17th, 1966. Excerpta Medica Foundation, 1967, pp. 32–34.
2. *Austoni, M., D'Agnolo, B.* Sulfocarbonismo cronico e diabete mellito. Minerva Med., **48** (1957) 2500–2504.
3. *Ferrero, G. P.* Frequenza del diabete mellito nei lavoratori esposti al rischio di intossicazione da solfuro di carbonio. Med. Lavoro, **60** (1969) 38–42.
4. *Finulli, M., Ghislandi, E.* La frequenza del diabete nella intossicazione cronica da solfuro di carbonio. Med. Lavoro, **50** (1959) 189–192.
5. *Goto, S., Hotta, R.* The medical and hygienic prevention of carbon disulfide poisoning in Japan. In: Brieger, H., Teisinger, J. ed. Toxicology of Carbon Disulfide, Proceedings of an International Symposium, Prague, September 15th–17th, 1966. Excerpta Medica Foundation, 1967, 219–230.
6. *Goto, S., Hotta, R. and Sugimoto, K.* Studies on chronic carbon disulfide poisoning. Pathogenesis of retinal microaneurysm due to carbon disulfide, with special reference to a subclinical defect of carbohydrate metabolism. Int. Arch. Arbeitsmed., **28** (1971) 115–126.
7. *Goto, Y., Sato, S., Ohira, S., Maruhama, Y.* Plasma insulin and free fatty acids in young people with postprandial glycosuria: plasma insulin response in the cases with borderline glucose tolerance test. Diabetologia, **9** (1973) 264–267.
8. *Hernberg, S., Nordman, C., Partanen, T., Christiansen, V., Virkola, P.* Blood lipids, glucose tolerance and plasma creatinine in workers exposed to carbon disulphide. Work-Environ. Health, **8** (1971) 11–16.
9. *Hyvärinen, A., Nikkilä, E. A.* Specific determination of blood glucose with o-toluidine. Clin. Chim. Acta, **7** (1962) 140–143.
10. *Korec, R.* Calculation of the assimilation constant of glucose – K_G. Diabetologia, **9** (1973) 307.
11. *Kotake, Y., Sotokawa, T., Murakami, E., Hisatake, A., Abe, M., Ikeda, Y.* Studies on the xanthurenic acid-insulin complex: II. Physiological activities. J. Biochem. (Tokyo), **63** (1968) 578–581.
12. *Kotake, J., Murakami, E.* A possible diabetogenic role for tryptophan metabolites and effects of xanthurenic acid on insulin. Am. J. Clin. Nutr., **24** (1971) 826–829.
13. *Kotake, Y., Ueda, T., Mori, T., Igari, S., Hattori, M.* Abnormal tryptophan metabolism and experimental diabetes by xanthurenic acid (XA). Acta Vitaminol. Enzymol. (Milano), **29** (1975) 236–239.

14. *Kujalová, V.* Changes in xanthurenic acid and 4-pyridoxic acid excretion due to carbon disulphide exposure in rats and factory workers. In: Abstracts of III. International Symposium on Toxicology of Carbon Disulphide, Cairo, 1974, p. 22.

15. *Kujalová, V.* "Oxyhyperglycemia" as consequence of chronic exposure to carbon disulphide. In: Abstracts of the IV. International Symposium on Occupational Health in the Production of Artificial Fibres, Helsinki, 1977, p. 30.

16. *Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J.* Protein measurement with the folin phenol reagent. J. Biol. Chem., **193** (1951) 265–275.

17. *Murakami, E.* Studies on xanthurenic acid-insulin complex. Preparation and properties. J. Biochem. (Tokyo), **63** (1968) 573–577.

18. *Murakami, E., Kotake, Y.* Studies on the xanthurenic acid-insulin complex III. Distribution on xanthurenic acid and formation of xanthburenic acid-insulin complex in serum. J. Biochem. (Tokyo), **72** (1972) 251–259.