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PROTECTIVE EFFECTS OF GLUTATHIONE AND XANTHINOL NICOTINATE AGAINST CARBON DISULPHIDE POISONING IN THE MOUSE

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100/mg/kg of intraperitoneally administered glutathione protected mice against CS_2-poisoning by inhalation at the level of acute mean lethal concentration.

The same dose of xanthinol nicotinate, under similar conditions, protected against subacute mean lethal concentration of CS_2.

The mechanisms of protective action are discussed.

There are indications that cellular metabolism might be disturbed in carbon disulphide poisoning. Back in 1958 *Cohen* (1) put forward the hypothesis that dithiocarbamate esters, known to arise in tissues after CS_2-poisoning (2), bind various divalent metal cations, thus removing essential ions from the metabolic processses and possibly affecting cellular respiration. Two years later a paper was published by *Scheel* et al. (3) reporting an abnormal distribution of copper and zinc ions in the tissues of animals experimentally poisoned with CS_2, which seems to confirm *Cohen*'s theory. On the other hand, several authors reported that some enzymes engaged in cellular respiration, *viz.* glycolase (4), cytochrome oxidase (5, 6), and monoamine oxidase (7), are directly inhibited by CS_2. An important fact however is in favor of *Cohen*'s hypothesis: oral administration of *Philip*'s and *Hart*'s salt mixture largely normalizes the Cu^{++} and Zn^{++} distribution altered by CS_2 poisoning. It also mitigates other symptoms (9), which might be attributed to the restitution of bound ions by ions contained in the mixture.

Considering the damages to cellular respiration potentially caused by CS_2-poisoning we thought that it would be worthwhile to investigate the possibility of protecting animals against CS_2 by means of well established promoters of cellular oxidative processes. We selected two such substances for trial: glutathione and xanthinol nicotinate and we examined their effects in the mouse.

METHODS

The efficiency of both glutathione and xanthinol nicotinate against CS_2-poisoning by inhalation, was examined by means of the equipment shown in Fig. 1, which was operated as follows. Air was drawn through the chamber at a rate of 1.5 litre per minute in order to keep up fresh

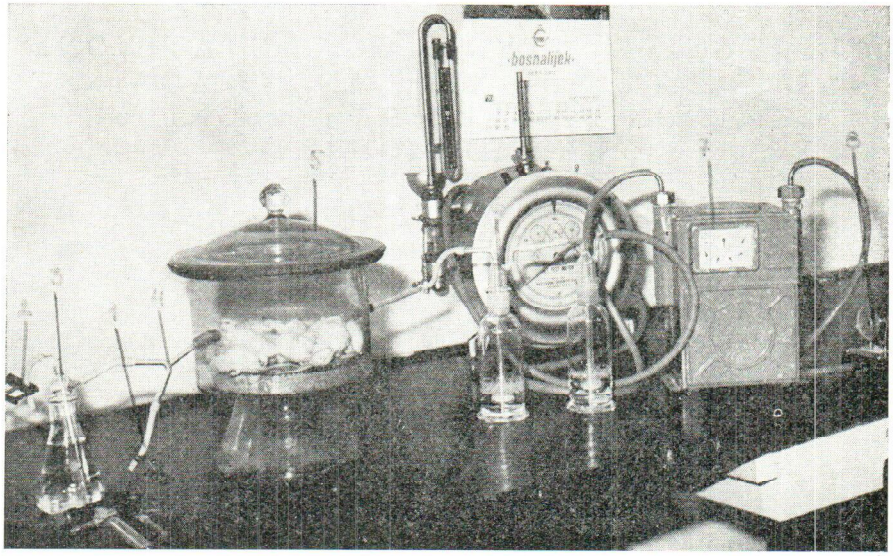

Fig. 1. *Apparatus for inhalation poisoning*
1. Screw clamp regulating the flow of air
2. Screw clamp regulating the dosage of CS_2
3. Wash bottle for CS_2
4. Distributing manifeld
5. Inhalation chamber, adapted from a vacuum desiccator
6. Vessel containing 50 ml of 5% alcoholic KOH
7. Flowmeter
8. Aspirator

breathing atmosphere. Carbon disulphide vapor was then admitted in the quantities necessary to maintain its concentration in the chamber as nearly constant as possible (the constancy of CS_2 vapor concentration was frequently checked by the method of *Jacobs* (8).

Groups of male and female mice, weight 24 $\pm$ 2 g, were placed in the chamber and exposed to the atmosphere containing CS_2. The acute toxicity (by inhalation) of the poisonous agent was measured by the CS_2-concentration sufficient to kill 50 percent of animals in a group within 30 minutes of exposure (mean lethal concentration, LC_m). The assessment of the measure of subacute toxicity required several exposures, and was carried out by exposing groups of animals for periods of 30 minutes for

three consecutive days, to the same CS_2-concentration in each session. The CS_2 concentration sufficient to kill 50 percent of the animals in a group was taken as a subacute LC_m. Both LC_m values were obtained from the experimental data using a standard method (9).

In testing the efficiency, the potentially protective substances, glutathione and xanthinol nicotinate, were dissolved in water and administered intraperitoneally two hours before exposure to LC_m CS_2-vapor. Both

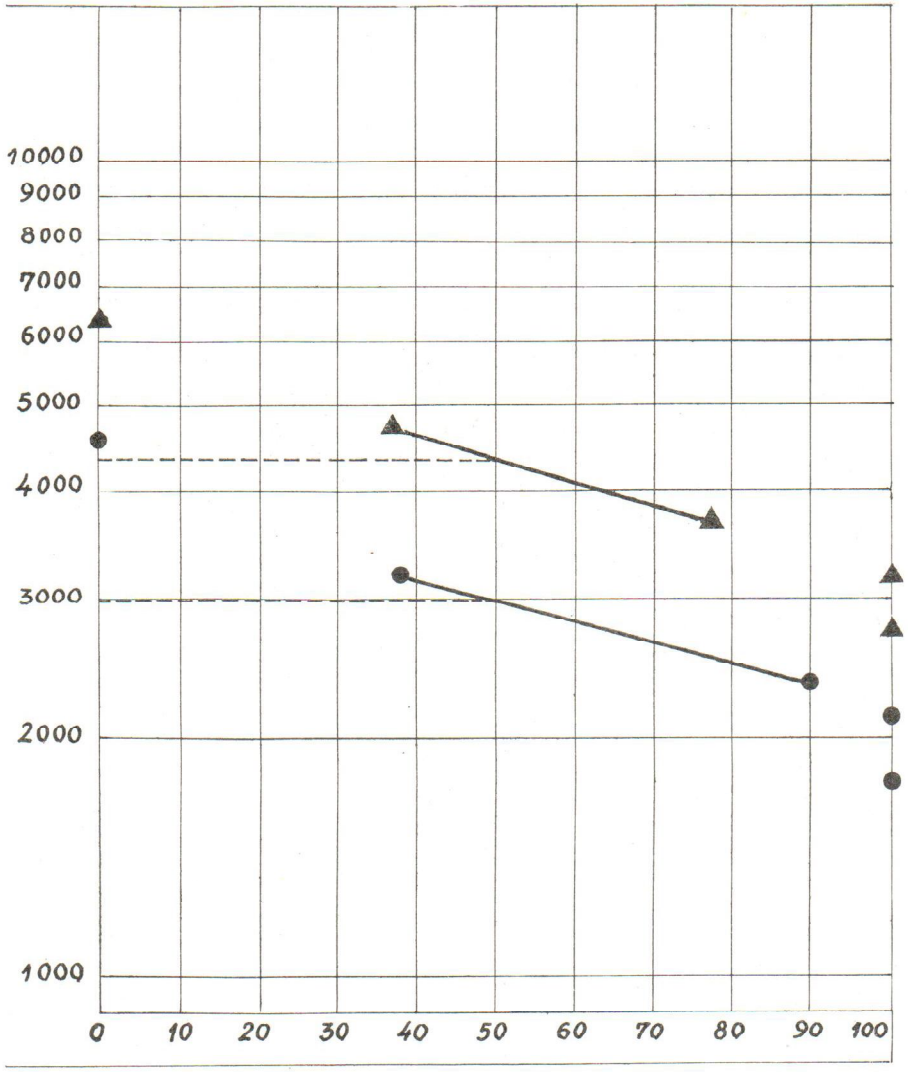

Fig. 2. *Graphical determinations of mean lethal concentrations*

substances were given in equal doses, i. e. 100 mg/kg (controls receiving pure water instead of the drug solution were subjected to the same treatment as experimentals).

Both glutathione and xanthinol nicotinate were tested against acute poisoning, while only xanthinol nicotinate was used in the subacute test. The protective efficiency of the compounds was assessed by the increase in survival rate.

RESULTS

Determined LC_m values for acute and subacute tests, were 4.5 and 3.0 g CS_2-vapor per litre respectively (Fig. 2).

Only glutathione proved to have a statistically significant protective efficiency against acute poisoning (Table 1). Xanthinol nicotinate was

Table 1

Survival rate in protected and unprotected animals after acute carbon disulphide poisoning by inhalation

(Protective agent: Glutathione, 100 mg/kg i. p.)

	Number of animals		
	Experimentals	Controls	Total
Died	8	13	25
Survived	22	17	35
Total	30	30	60

$\chi^2 = 5{,}554$ K = 1 P < 0,05

unable to protect against acute poisoning, but in subacute poisoning the same dose of the drug provided a statistically significant degree of protection (Table 2).

Table 2

Survival rate in protected and unprotected animals after subacute carbon disulphide poisoning by inhalation

(Protective agent: Xanthinol nicotinate, 100 mg/kg i. p.)

	Number of animals		
	Experimentals	Controls	Total
Died	12	21	33
Survived	18	9	27
Total	30	30	60

$\chi^2 = 5{,}45$ K = 1 P < 0,05

DISCUSSION

Our assumption concerning a possible protective capacity of cell-respiration promoters in CS_2-poisoning has been supported by the results described above, obtained with glutathione and xanthinol nicotinate. It is remarkable, however, that the latter substance was efficient only against subacute poisoning, and completely inefficient in acute tests. The difference observed between the two substances in acute tests might be due to the ability of glutathione to protect free sulphydryl groupings, which xanthinol nicotinate lacks. Since CS_2 is converted in the organism into dithiocarbamate esters (2), and H_2S is liberated during CS_2-poisoning (11), we believe that a reaction takes place in the poisoned organism, which we previously observed in vitro (10), namely a reaction of dithiocarbamates or CS_2 and free sulphydryl groups due to the liberation of H_2S. This reaction could be prevented by glutathione, what would account, at least partly, for its protective efficiency. Other authors, however, attribute the liberation of H_2S after CS_2-poisoning to the action of specific desulphydrase (11) which is contrary to the above interpretation.

As a typical SH-protecting substance glutathione might also counteract other mechanisms involving SH-groups. It is possible that during CS_2 poisoning certain enzymes are blocked via their SH-groups (2). All these modes of protective action cannot be exerted by xanthinol nicotinate, and therefore this substance is inefficient in acute poisoning; however, as xanthinol nicotinate is known to promote cell-respiration, its action may be directed against secondary – respiratory – effects of CS_2 poisoning so that it can be efficiently used in cases of subacute intoxication.

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Sažetak

ZAŠTITNI EFEKTI GLUTATIONA I KSANTINOL NIKOTINATA KOD TROVANJA MIŠEVA s CS_2

Budući da trovanje s CS_2 dovodi do inhibicije enzima odgovornih za oksidacione procese, ispitivali smo kakve efekte kod letalnih trovanja s CS_2 na nivou LC_m (srednje letalne koncentracije) izazivaju glutation i ksantinol nikotinat, supstance za koje se zna da stimuliraju oksidacione procese na nivou ćelije. Rezultati dobiveni eksperimentalnim putem pokazuju da glutation dat preventivno 100 mg/kg i. p. signifikantno zaštićuje životinje akutno trovane parama CS_2 na nivou srednje letalne koncentracije. Ksantinol nikotinat zaštićuje isto tako miševe od srednje letalne koncentracije kod subakutnog trovanja s CS_2. Diskutira se o mehanizmu zaštitnog efekta ovih dviju supstanca.

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