CHANGES IN THE ACETHYLCHOLINE SYSTEM OF THE BLOOD AND TISSUES OF RABBITS DURING CHRONIC EXPOSURE TO SMALL CONCENTRATIONS OF CARBON DISULFIDE

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The prolonged action of carbon disulfide produces signs of chronic poisoning accompanied by disturbances in the nervous system [2, 3, 6, 7, 10, 11].

The object of the investigation was to study the action of low concentrations of carbon disulfude on the acetylcholine (AC) system, changes in which are a sensitive index of the functional state of the nervous system [1].

EXPERIMENTAL METHOD

Experiments were carried out on 26 chinchilla rabbits weighing 2.2-3.5 kg. The animals were poisoned for 12 months with a daily exposure of 2 h. The effect of two concentrations of carbon disulfide in the air was studied: 10 mg/m^3, corresponding to the maximal allowable concentration for industrial premises, and 100 mg/m^3. To detect changes in the reactivity of the animals [11], in the course of a year all the animals were immunized three times subcutaneously with typhoid vaccine (1.5 billion bacterial cells/ml). Every two weeks the AC concentration in the blood of the rabbits was determined by a biological method on the isolated frog's lung [12, 17], the acetylcholine esterase (ACE) activity was determined by the method of Fleisher and Pope [15] as modified by M. M. Éidel'man [13], and the butyrylcholinesterase (BCE) activity was estimated by an analogous method using butyrylcholine as substrate (8 μmoles BC in a sample with 0.2 ml blood). At the end of the experiment, some of the animals were sacrificed and the total AC (after homogenization in 0.86% sodium chloride solution and precipitation of the proteins by boiling) and the activity of ACE and BCE (in homogenates prepared in 0.137 M phosphate buffer, pH 7.2) were investigated in the brain, liver, kidneys, and skeletal muscles. The results obtained were analyzed by statistical methods, and differences were considered significant when $P \leq 0.05$ [14].

EXPERIMENTAL RESULTS AND DISCUSSION

The AC content in the blood of the control rabbits changed mainly as the result of immunization. From 3 to 5 weeks after each immunization, the concentration of mediator in the blood fell temporarily (Fig. 1). This decrease was particularly marked after the third immunization, which took place in the control rabbits later than in the experimental (because the titer of agglutinins remained high for a long time). During the first two months of observation and toward the end of the 11th month, the AC concentration in the blood of the control rabbits was indistinguishable from its initial level, while during the period from 4.5 to 8.5 months it slightly exceeded the initial level.

In the case of poisoning with carbon disulfide in a concentration of 100 mg/m^3, an increase in the blood AC concentration was observed after two months of poisoning (Fig. 1). The first immunization, carried out at this time, produced a decrease in the blood AC concentration as in the control series, but the decrease was greater in absolute terms. In the period between 5 and 8.5 months of poisoning AC accumulated in the blood (much more than in the control series), with weakening of the reaction to the second and third immunizations. After 9 months of poisoning, the blood AC concentration fell to its initial level and became significantly below that level at 10 months. Two months after cessation of poisoning

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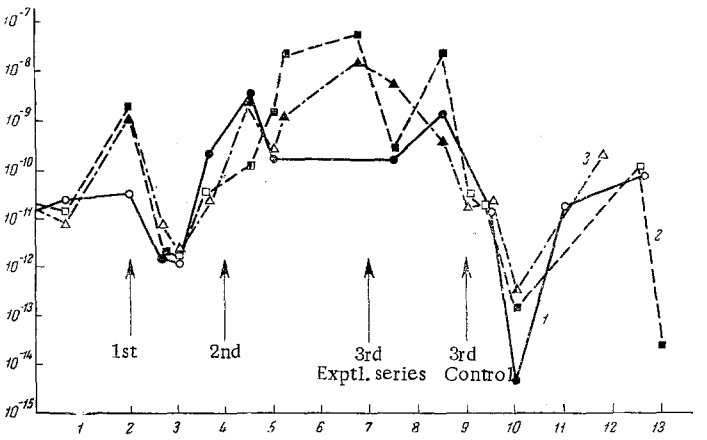

Fig. 1. Dynamics of blood acetylcholine concentration in carbon disulfide poisoning. Abscissa — duration of poisoning (in months), ordinate — acetylcholine concentration in blood (in g/ml, logarithmic scale). 1) Control; 2) carbon disulfide in concentration of 100 mg/m^3; 3) carbon disulfide in concentration of 10 mg/m^3. Symbols — times of investigation; completely shaded — times with significant differences ($P \leq 0.05$) from initial level or preceding time, half shaded — corresponding to $P < 0.1$. Vertical arrows — time of immunization.

(the recovery period) the AC concentration in the blood of the rabbits poisoned with carbon disulfide in a concentration 100 mg/m^3 remained low.

During carbon disulfide poisoning in a lower concentration — 10 mg/m^3 — an increase in the blood AC concentration also took place after two months, with accumulation of AC between 4.5 and 8.5 months. The first and second immunizations caused the blood AC level to fall, while the reaction of the rabbits of this series to the third immunization was weakened. After 10 months of poisoning with carbon disulfide in a concentration of 10 mg/m^3 a tendency appeared for the blood AC concentration to fall, but near the end of the period of observation it returned to its initial level (Fig. 1). All the changes in the blood AC concentration in the rabbits of this series were less marked than in the animals of the preceding series.

The ACE activity of the blood of the control animals was essentially indistinguisable from its initial level throughout the period of observation, but showed a tendency to increase by 18-27% between 3 and 8 weeks after each immunization. By the end of the investigation the ACE activity had fallen (Fig. 2).

In carbon disulfide poisoning the blood ACE activity of the experimental animals remained unchanged during the first two months, while from the 3rd to the 8th month at most times it was above the initial level, corresponding to the higher AC concentration in the blood of the experimental animals at this period. In the animals poisoned with carbon disulfide in a concentration of 100 mg/m^3 the ACE activity did not increase in response to the second and third immunizations, but starting with the 9th month it fell below the initial level; by 10.5 months, the ACE activity had fallen on the average by 34% (Fig. 2). After cessation of poisoning the ACE activity in the blood of the rabbits of this series returned temporarily to normal after 1.5 months and again fell by 27% after 2.0-2.5 months. The blood ACE activity in the rabbits poisoned with carbon disulfide in a concentration of 10 mg/m^3 underwent the same changes after the second and third immunizations as in the control series; by the end of poisoning (Fig. 2), a significant decrease in the activity of the enzyme was observed.

The blood BCE activity (Fig. 3) of the control animals at most times was on the average slightly above the initial level, but a significant difference was observed only in the first two weeks. With a carbon disulfide concentration of 10 mg/m^3, the level of this index fluctuated in a sinusoidal manner about the initial level with statistically significant decreased at the 5th and 12th months of poisoning. With a carbon disulfide concentration of 100 mg/m^3 the BCE activity of the blood fell significantly after 4.5 months (by 40-50%), and remained at this level until the end of poisoning and for 2.5 months after its termination.

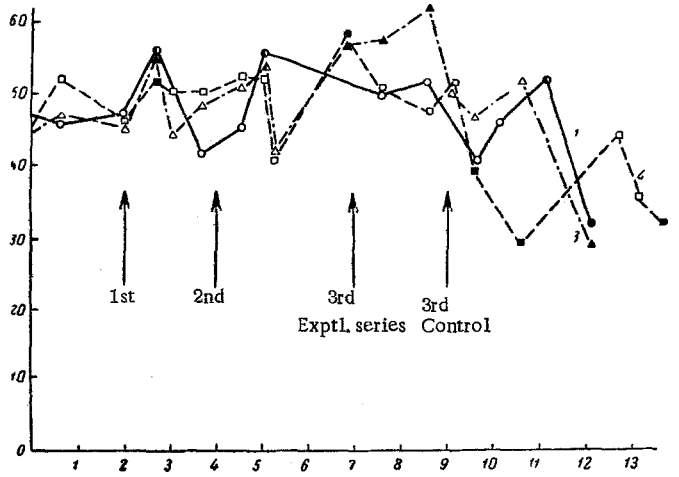

Fig. 2. Dynamics of blood acetylcholine esterase activity during carbon disulfide poisoning. Ordinate — acetylcholine esterase activity (in μmoles AC hydrolyzed by 1 ml blood per hour). Remainder of legend as in Fig. 1.

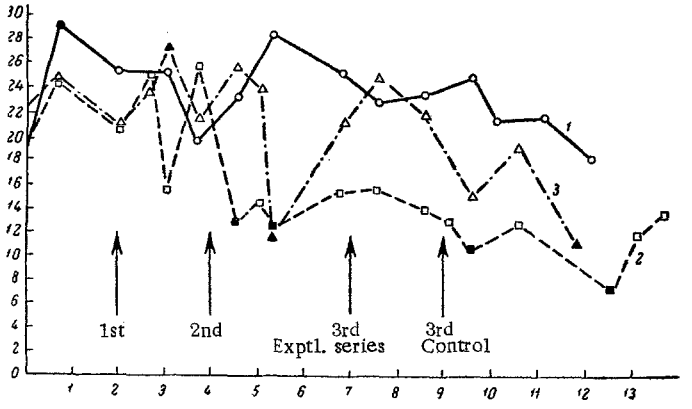

Fig. 3. Dynamics of butyrylcholinesterase activity of the blood during carbon disulfide poisoning. Ordinate — butyrylcholesterase activity (in μmoles BC hydrolyzed by 1 ml blood per hour).

Some of the animals were sacrificed 11-12 months after the beginning of the experiment to investigate the AC system in the tissues (three rabbits from each series of experiments using a concentration of 10 mg/m^3 and the control series and four normal intact animals). The only statistically significant difference between the control animals and the normal was an increase in the ACE activity of the brain. In the rabbits poisoned with carbon disulfide (see Table 1), on the other hand, this index was lowered. The BCE activity of the liver also was lowered. The greatest changes were observed in the kidney, where the AC concentration was increased and the activity of both types of cholinesterase was decreased.

On the basis of the results obtained in these experiments reflecting the dynamics of the AC system in the blood of the rabbits during chronic carbon disulfide poisoning and the reaction of this system to immunization, three principal periods can be distinguished: the first 2-4 months of poisoning — an increase in AC concentration with normal ACE and BCE activity and a usual or intensified reaction to immunization (i.e., a fall in AC concentration and increase in ACE activity); 4.5-8.5 months from the beginning of poisoning — an increase in the AC and ACE content, but with the higher dosage of carbon disulfide a decrease in BCE activity and a tendency for the reaction of AC and ACE to immunization to diminish; 9-11 months — with the higher concentration of carbon disulfide a lowering of all the AC indices, but with the lower concentration restoration of the normal level of the indices followed by a decrease of ACE and BCE. A

TABLE 1. Concentration of AC and Activity of ACE and BCE in Organs of Animals Poisoned with Carbon Disulfide in a Concentration of 10 mg/m^3

	AC (in g/g tissue)	ACE (in μmoles AC/g/h)	BCE (in μmoles BC/g/h)
	Brain		
Normal	$-7{,}992 \pm 0{,}9094$ $(1 \cdot 10^{-8})$	$359{,}4 \pm 32{,}19$	$90{,}0 \pm 23{,}42$
Control	$-7{,}780 \pm 0{,}5292$ $(1{,}7 \cdot 10^{-8})$	$553{,}3 \pm 41{,}13$	$144{,}7 \pm 44{,}92$
Experiment	$-7{,}030 \pm 0{,}4123$ $(9{,}3 \cdot 10^{-8})$	$274{,}2 \pm 5{,}003$	$67{,}8 \pm 13{,}96$
	Kidneys		
Normal	$-9{,}817 \pm 0{,}3435$ $(1{,}5 \cdot 10^{-10})$	$9{,}90 \pm 1{,}67$	$23{,}3 \pm 6{,}34$
Control	$-9{,}817 \pm 0{,}4099$ $(1{,}5 \cdot 10^{-10})$	$8{,}70 \pm 1{,}46$	$9{,}8 \pm 3{,}24$
Experiment	$-8{,}950 \pm 0{,}4159$ $(1{,}1 \cdot 10^{-9})$	$4{,}16 \pm 1{,}84$	$4{,}8 \pm 1{,}42$
	Liver		
Normal	$-9{,}813 \pm 0{,}5070$ $(1{,}5 \cdot 10^{-10})$	$32{,}4 \pm 3{,}49$	$40{,}9 \pm 7{,}75$
Control	$-9{,}920 \pm 0{,}2449$ $(1{,}2 \cdot 10^{-10})$	$33{,}1 \pm 3{,}77$	$52{,}6 \pm 3{,}76$
Experiment	$-9{,}800 \pm 0{,}9633$ $(1{,}6 \cdot 10^{-10})$	$32{,}8 \pm 0{,}64$	$27{,}0 \pm 6{,}64$
	Muscle		
Normal	$-9{,}240 \pm 1{,}261$ $(5{,}8 \cdot 10^{-10})$	$8{,}55 \pm 2{,}68$	$2{,}1 \pm 0{,}47$
Control	$-9{,}407 \pm 0{,}1517$ $(3{,}9 \cdot 10^{-10})$	$8{,}61 \pm 1{,}94$	$2{,}3 \pm 0{,}664$
Experiment	$-8{,}000 \pm 0{,}9381$ $(1 \cdot 10^{-8})$	$6{,}90 \pm 2{,}81$	$2{,}6 \pm 0{,}808$

Note: Mean content of AC and its error given in logarithmic form, for the sake of clarity all the mean values were converted to antilogarithms (the geometric mean values obtained are given in parentheses).

noteworthy feature was the more marked disturbances in the AC system with the higher concentration of carbon disulfide, when the indices had not returned to normal 2.5 months after poisoning was discontinued.

The first and, possibly, the second phases of the changes described indicate activation of cholinergic mediation at particular stages of carbon disulfide poisoning (what D. E. Al'pern [1] describes as the phase of humoral compensation), in agreement with results obtained by other workers [2, 3, 6]. The third phase may be explained by the gradual development of decompensation. On the whole, the results obtained concur with the periodization of the changes in the central and autonomic nervous system suggested by É. A. Drogychina [5] in certain forms of chronic poisoning.

The decrease in BCE activity in the blood may be explained by depression of the production of this enzyme, which enters the blood stream from the liver [16]. This explanation is supported by reports in the literature describing the severity of the lever damage in carbon disulfide poisoning [4, 10] and also by the decrease in BCE activity observed in the present experiments in the tissue of this organ.

The severity of the disturbances in the AC system in the kidneys corresponds to data indicating the accumulation of carbon disulfide in these organs [7-9] and the development of functional changes during chronic poisoning [7].

The early appearance of the changes described and their distinct pattern of change during development of poisoning confirm that the state of the AC system is a sensitive index of the reaction of the organism to chronic external factors.

LITERATURE CITED

1. D. E. Al'pern, Cholinergic Processes in Pathology [in Russian], Moscow (1963).
2. E. N. Artem'ev and T. S. Konovalova, Ter. Arkh., No. 11, 88 (1962).
3. B. K Baikov, Gigiena Sanit., No. 3, 3 (1963).
4. N. K. Gorbadei, Ter. Arkh., No. 9, 95 (1962).
5. É. A. Drogychina, in the book: Problems in Industrial and Agricultural Toxicology [in Russian], Kiev (1964), p. 57.
6. M. A. Kazakevich, Gigiena Truda, No. 7, 20 (1963).
7. V. A. Kisilenko and G. G. Lysina, Vrach. Delo, No. 9, 973 (1956).
8. N. V. Lazarev, (Editor), Harmful Substances in Industry [in Russian], Leningrad (1963).
9. G. G. Lysina, Vrach. Delo, No. 12, 1283 (1956).
10. G. G. Lysina, Gigiena Truda, No. 6, 54 (1958).
11. V. K. Navrotskii, Vestn. Akad. Med. Nauk SSSR, No. 3, 57 (1960).
12. Kh. S. Khamitov, Kazansk. Med. Zh., No. 4, 48 (1960).
13. M. M. Éidel'man, Lab. Delo, No. 10, 29 (1963).
14. N. Bailey, Statistical Methods in Biology [Russian translation], Moscow (1962).
15. Y. H. Fleisher and E. Y. Pope, Arch. Industr. Hyg., 94 (1954), p. 323.
16. Th. Gürtner, G. Kreutzberg, and A. Doenike, Acta Anaesth. Scand., 7 (1963), p. 69.
17. M. Korsten, Pflüg. Arch. Ges. Physiol., Bd 244, S. 281 (1940).
18. J. Löwy, Occupational Diseases [Russian translation], Moscow-Leningrad (1925).