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ORIGINAL PAPERS

CHANGES IN PHYSIOLOGICAL AND ELECTROPHYSIOLOGICAL PARAMETERS IN RABBITS AFTER SINGLE EXPOSURE TO CHLORPHENVINPHOS

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Abstract. In order to evaluate some of the central effects of an organophosphorus pesticide — chlorphenvinphos (CVP), the time course of changes in the activity of blood cholinesterase (ChE), body temperature and hippocampal EEG were compared in rabbits after acute i.p. exposure. The pesticide was administered twice at an interval of 80—90 days. The Deichmann-LeBlanc scheme of dosing (one animal/one dose) was adopted starting with 22 mg/kg. CVP resulted in a dose-dependent decrease in plasma and erythrocyte ChE activity, a decrease in body temperature ranging from 0.7° to 3.5°C, and in an increase in content of the immobility-related, rhythmic slow activity (I-RSA) in the hippocampal EEG. Changes in body temperature appeared at lower doses than those in the hippocampal EEG. Contrary to the changes in ChE activity, which lasted 4—30 days, those in body temperature and in hippocampal EEG disappeared within 24 hours after the injection. CVP administered at the same dose 80—90 days after the first injection, resulted again in an inhibition of ChE activity, but the effect on the hippocampal EEG was less clear, and that on body temperature was variable; no effect, an increase or a decrease appeared.

The data suggest that: i) body temperature is a more sensitive index of a central action of CVP administered for the first time than the hippocampal EEG; ii) the brain cholinergic mechanisms are relatively resistant to the acute action of the OP and undergo fast adaptive changes; iii) even single exposure to CVP may produce some long-lasting functional changes in the brain of some subjects, which has been proven by the changed response to the second exposure.

INTRODUCTION

The long half-life of chlorinated hydrocarbons (like DDT) and their ability to build up in soil and in fat tissue has resulted in restriction of their use for pest control and their replacement by organophosphorus

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compounds (OP). Owing to this, the domestic and agricultural use of OP-s is steadily increasing and, at the same time, the number of people who may be occupationally or accidentally exposed to these compounds is also on the increase. More detailed studies performed on animals, involving at least the most extensively used OP-s are therefore necessary in order to establish all the possible consequences of exposure for human populations. Here we report our preliminary data concerning the neurotoxicity of chlorphenvinphos (phosphoric acid, 2-chloro-1-/2,4-dichlorophenyl/vinyl Diethyl Ester). This OP is used extensively (in Poland 500 tons/year) as an insecticide, mainly to control the Colorado beetle. To date, studies on the neurotoxicity of chlorphenvinphos (CVP) have not extended far beyond the determination of its ability to inhibit the activity of cholinoesterases (3). The reliability of the level of cholinesterase (ChE) inhibition as the index of OP poisoning has been questioned lately. It has been shown that OP-s may interact not only with ChE but also with cholinergic receptors (8), and that other effects of intoxication do not parallel the decreased level of ChE activity. For example, hypothermia and some changes in behavior may disappear before the return of ChE activity to a normal level (13, 18), but electroencephalographic abnormalities may persist after a complete normalization of enzyme activity (6, 7). What is more, it has been reported that in experimental animals, changes in the EEG may be produced by OP doses insufficient to evoke a detectable inhibition of ChE (5, 17).

The purpose of the present experiments was to compare the time course of changes in the activity of ChE in blood with the time course of changes in body temperature and in hippocampal EEG. There are literature data which allow one to regard both these effects as evidence of a direct action of cholinergic drugs. Hypothermia may be produced in a dose-related manner by direct cholinergic stimulation of the hypothalamus (1). As far as the bioeletrical activity of the hippocampus is concerned, there is an abundance of reports proving that at least one of its forms, the low-frequency (4.0—6.5 Hz) immobility-related rhytmic slow activity (I-RSA), depends entirely on the integrity of the central cholinergic system (see 2 for references). It may be assumed then, that studying the temporal relations between ChE activity, body temperature and the hippocampal I-RSA can help to obtain a better insight into the central action of the OP compound under study on one hand, and to assess the usefulness and reliability to each of the three parameters as possible indexes of OP intoxication, on the other.

MATERIAL AND METHOD

Animals and the surgery. The experiments were performed on seven male New Zealand rabbits about six months old, and of 2.5—3.0 kg body weight at the time of surgery. All the animals were implanted with intrabrain bipolar stainless steel teflon-coated electrodes under barbiturate anaesthesia. The electrodes were placed on the occipital cortex (surface to depth derivations) and into the dorsal hippocampus. The conditions of surgery, the electrodes and the implantation technique have already been described (11). The experiments began about two weeks after surgery.

Test methods. ChE activity in plasma and in erythrocytes was determined in blood samples (2×50 µl) taken from the punctured ear marginal vein using the Voss and Sachsee method (23). Body temperature was measured in the rectum with an electrometer equipped with a thermocouple probe. The hippocampal and cortical EEG-s were recorded with the use of an 8-channel electroencephalograph (M-2, ORMED, Lodz, Poland) with the low and high filters set at 1.0 and 70.0 Hz, respectively. Head movements were also recorded on one of the channels by the „free lead" method, which helped to distinguish I-RSA more precisely from the remaining patterns of the hippocampal EEG. The EEG was recorded while the animal was in an experimental chamber (a wooden box, 60×60×60 cm, with transparent roof and front). In order to facilitate analysis, a sampling procedure was adopted. It consisted in the recording of 5-min samples of EEG separated by 5-min intervals. The I-RSA content, i.e. the percentage duration of activity regarded as I-RSA (expressed as a percentage of the recording time), was calculated in each sample and the mean from three successive samples was regarded as the score. Some qualitative observations concerning the animals' general state were also made during the experiments.

The pesticide and exposure conditions. The pesticide was obtained from a factory (ORGANIKA-AZOT, Jaworzno, Poland), in the form of a concentrate containing 92% of CVP (a mixture of cis and trans isomers at 1 : 9 ratio) and about 8% impurities (enolophosphates). Just before use, it was dissolved in sterile olive oil in order to obtain the required concentration (50 mg/ml). The row of doses was designed following Deichmann and LeBlanc (4). The lowest dose tested was 22 mg/kg. Each successive dose was 50% higher than the preceding one in the row. CVP administration was intraperitoneal. Each animal was given CVP twice, at the same dose, at an interval of 80—90 days providing the animal survived the first injection.

Testing procedures. Before the first injection of CVP, every measure was made at least three times, at a three days interval (the third was the one just preceding the injection), and 30 min, 90 min, 3 h, 6 h, 1 day, 2 days, 4 days, 6 days, 8 days, 17 days, 24 days, 1 month, and 2 months after the injection. The procedure after the second injection was similar but the testing was finished by the 4th day after the injection.

Statistical analysis. Spearman rank correlation analysis was used for statistical evaluation of the obtained data (19).

RESULTS

Effects of CVP on ChE activity. The first injection of CVP resulted in a dose-related inhibition of plasma and erythrocyte ChE (Tables 1 and 2). The peak value of this inhibition ranged from 51.4% (plasma) and 37.0% (erythrocytes) at the 22 mg/kg dose, to 100% (in both cases) at the highest doses (162 and 250 mg/kg). In the case of the plasma ChE the latency of the peak inhibition was negatively correlated with the dose ($r_s=0.93$, $p<0.01$), being the longest in the case of the 22 mg/kg dose. The relationship was less clear in the case of erythrocyte ChE ($r_s=0.47$, N.S.). CVP given in doses of 167 and 250 mg/kg resulted in the death of the animals 240 and 123 min after the injection, respectively. In the survivors, the return of ChE activity to normal, i.e. to at least 85% of the control value, varied from 4 days (dose 33 and 112 mg/kg) to 30 days (dose 50 mg/kg) in the case of both plasma and erythrocyte ChE. There was no correlation between the dose and the rate of normalization of ChE activity.

In all the animals tested, the effect of the second injection of CVP on ChE activity seemed to be more severe (Tables 1 and 2). It was especially pronounced 24 hours after the injection.

Changes in general behavior. No clear-cut signs of discomfort of more specific symptoms of intoxication were seen in the animal given 22 mg/kg CVP. In the remaining ones panting, weakness in the limb muscles, salication, excessive secretion of mucus from the nasal cavity, frequent urination and defecation were seen as early as 30 min after the first injection. Spontaneous movements (exploration, grooming) disappeared and the animal remained immobile (usually lying down with paws outstretched). These changes were most pronounced in the two animals which died (doses 167 and 250 mg/kg); obstruction of respiratory tracts and/or paralysis of the respiratory muscles was the assumed cause

TABLE 1. Changes in ChE Activity in Plasma After Two i.p. Injections of Chlorphenvinphos in Rabbits *

Dose (mg/kg) \ Hours after injection	0.5	1.5	3.0	6.0	24.0	48.0	96.0
22.0	81.0	70.5	68.3	66.2	48.6	—	77.8
	40.0	44.5	—	—	38.7	—	79.6
33.0	66.0	50.0	33.0	28.0	38.1	41.3	97.1
	71.5	56.4	8.8	0.0	10.3		89.2
50.0	28.2	17.6	13.7	10.5	15.5	26.1	36.6
	50.9	17.6	26.3	0.0	0.0		52.3
75.0	24.6	4.1	0.0	0.0	10.5	32.1	36.9
	27.4	19.2	0.0	3.3	0.0		14.0
112.0	9.4	0.0	0.0	3.4	17.8	56.5	89.0
	13.9	9.9	0.0	11.4	0.0		21.6
167.0	0.0	0.0	0.0				
250.0	3.3	0.0					
Statistics	$r_s=0.96$	$r_s=1.0$	$r_s=0.9$	$r_s=0.9$	NS	NS	NS
	$p<0.01$	$p<0.01$	$p<0.05$	$p<0.05$			
	NS	NS	NS	NS	NS	—	NS

* Values to the left and up in each cell illustrate the effect of the first exposure. Values to the right and down in each cell illustrate the effect of the second exposure performed between 80 and 90 days after the first one.

of death. In those animals which survived, partial normalization of behavior was seen as soon as 6 hours after the injection. On the next day, signs of gastrointestinal disturbances (diarrhea) were still present but otherwise the rabbits behaved much as they had before the injection. No signs of motor disturbances (suggesting neuropathy (14) appeared in the further periods of testing.

CVP given 80—90 days later in the same dose produced either no clear-cut changes in behavior (doses 22, 33, 50 and 75 mg/kg) or only mild ones (dose 112 mg/kg).

Changes in body temperature. In all the animals, the first injection of CVP resulted in a rapid decrease in body temperature. This effect reached its climax 90—120 min after the injection (it was not related to the time of maximum ChE inhibition) and ranged from 0.7°C (dose 22 mg/kg) up to 3.5°C (dose 250 mg/kg) below the preinjection va-

TABLE 2. Changes in ChE Activity in Erythrocytes After Two i.p. Injections of Chlorphenvinphos in Rabbits *

Dose (mg/kg) \ Hours after injection	0.5	1.5	3.0	6.0	24.0	48.0	96.0
22.0	96.0 85.6	75.6 77.9	69.0 42.1	63.0 44.7	74.3 64.3	— —	74.0 86.0
33.0	68.2 52.3	87.0 —	75.3 15.8	53.2 0.0	38.6 9.1	50.4 —	65.1 31.0
50.0	22.7 49.9	13.2 44.1	7.6 43.1	10.2 0.0	26.4 0.0	37.4 —	46.7 23.7
75.0	15.8 23.0	6.5 20.0	2.0 9.0	8.0 6.5	30.3 8.1	49.3 —	58.3 19.4
112.0	21.2 11.4	18.1 14.0	9.6 0.0	2.2 2.9	21.2 17.0	26.1 —	43.0 53.7
167.0	2.3	1.6	0.0				
250.0	8.1	0.0					
Statistics	$r_s=0.96$ $p < 0.01$ $r_s=0.95$ $p < 0.05$	NS NS	NS NS	$r_s=1.0$ $p < 0.01$ NS	NS NS	NS —	NS NS

* Values to the left and up in each cell illustrate the effect of the first exposure. Values to the right and down in each cell illustrate the effect of the second exposure performed between 80 and 90 days after the first one.

lue. There was no correlation between the peak value of the decrease in body temperature and the dose. (It is possible that the ceiling was reached with the 50 mg/kg dose, which might account for the lack of correlation). The hypothermia appeared to be rather a short-lasting effect. There was a marked normalization of body temperature 6 hours after the injection. After 24 hours its value was within the preinjection range, i.e. $39.3°C \pm 0.5°C$.

After the second injection, a decrease in body temperature, similar to that observed after the first injection, appeared in two animals only (doses 22 and 112 mg/kg), in two they were within the physiological limits (doses 33 and 50 mg/kg) and in one (dose 75 mg/kg) hyperthermia ensued (Fig. 2).

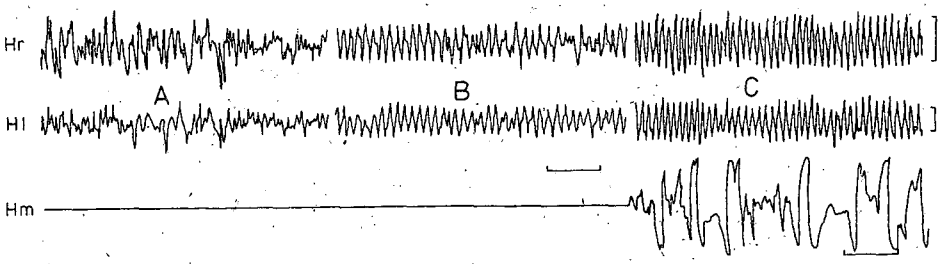

Fig. 1. Fragments of EEG records illustrating the three main patterns of hippocampal electrical activity in the rabbit. Denotation of traces: Hr — right hippocampus, Hl — left hippocampus, Hm — head movements. Calibration: 500 μV, 1.0 s. A — large irregular activity (LIA), B — immobility-related rhythmic slow activity (I-RSA), C — movement-related rhythmic slow activity (M-RSA). Note that the I-RSA frequency is much slower than that of M-RSA.

Effect of CVP on the hippocampal EEG. Three patterns of bioelectrical activity may be distinguished in the hippocampal EEG of awake, undrugged rabbits: a) large irregular activity (LIA) associated with a state of relaxation, b) movement-related rhytmic slow activity (M-RSA) accompanying movements termed as „voluntary" and

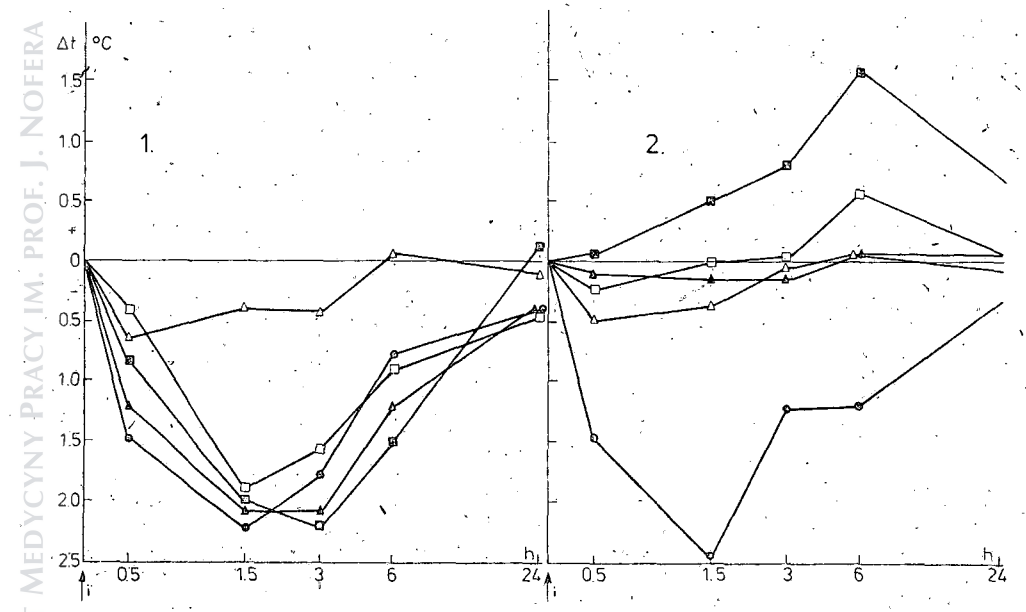

Fig. 2. Changes in rectal temperature in rabbits after i.p. administration of chlorphenvinphos at different doses. Ordinates — temperature difference between the pre- and the postinjection values (centigrade scale), abscissa — time after injection (hours). 1 — effect of the first injection, 2 — effect of the second injection performed between 80 and 90 days after the first one. Denotation of curves: △ — dose 22 mg/kg, □ — dose 33 mg/kg, ▲ — dose 50 mg/kg, ■ — dose 75 mg/kg, ● — dose 112 mg/kg.

c) immobility-related rhytmic slow activity (I-RSA), which is found in the immobile attentive state (ref. 2, 22). Examples are presented in Fig. 1. According to literature, I-RSA is generated in response to activation of cholinoceptive elements within the reticulo-septo-hippocampal axis (22) and as such it may be regarded as an index of the level of cholinergic brain activity. Since in our rabbits the cortical records were strongly contaminated with the hippocampal RSA, only the hippocampal EEG was analysed in detail.

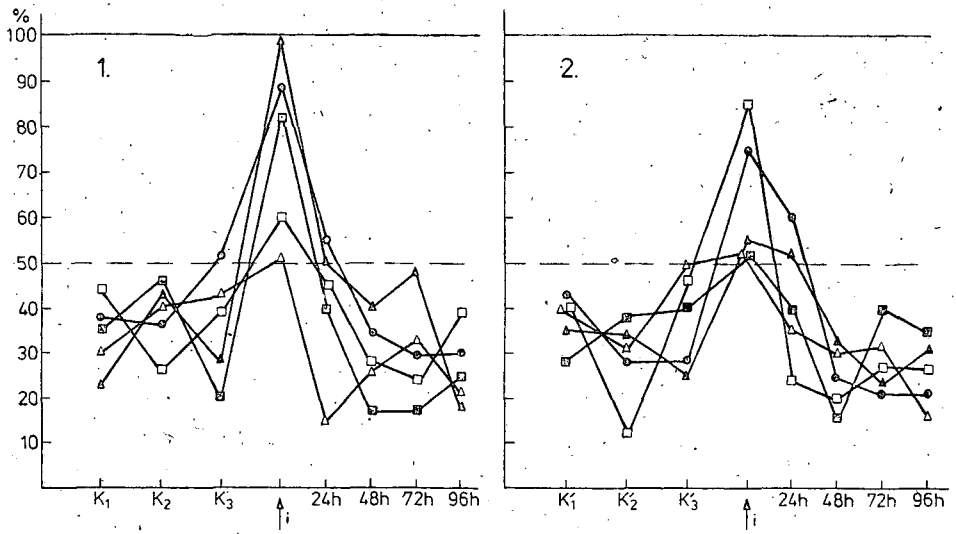

Fig. 3. I-RSA content in the hippocampal EEG in rabbits in the control sessions (K_1, K_2 and K_3) and after i.p. injections of chlorphenvinphos at different doses. Ordinates — percentage of I-RSA in the analysed EEG samples, abscissa — time after incjection. Remaining denotations as in Fig. 2 (The value pointed by "I" is the maximum RSA content in a 5 min EEG sample, which was recorded on the day of injection).

The changes appearing after the CVP injections consisted mainly in an increase of the I-RSA content (at the expense of M-RSA and LIA). Before the injection, the I-RSA content varied from 20 to 50%. After injection it might increase even up to 100% depending on the dose ($r_s = 0.9$, $p < 0.5$). This relation, however, was clearly seen only after 6 hours from the time of the injection, whereas the maximum effect occurred 90—180 min after the time of the injection. In the two rabbits given the highest doses (167 and 250 mg/kg), long before death, the frequency and amplitude of I-RSA decreased and a fast activity superimposed on I-RSA appeared. It evolved gradually into a tonic-clonic seizure activity lasting tenths of seconds. 24 hours after the first injection, the

I-RSA content in the hippocampal records was within the control range in all the animals.

After the second injection of CVP, the changes in the I-RSA content were less pronounced and less consistent (Fig. 3). No relationship between the dose and the effect was found.

DISCUSSION

The anticholinesterase properties of CVP and the acute symptoms of OP poisoning are both well known and there is no need to discuss them here in detail. The new data concerning the CVP are: the relationship between the changes in the bioelectrical activity of the hippocampus on one hand and the level of ChE inhibition on the other, b) the surprisingly rapid disappearance of changes in general behavior, body temperature and hippocampal EEG, much before the normalization of ChE activity, and c) the variable effects of the second injection of CVP on body temperature and on hippocampal EEG.

In standard testing of the OP, neurotoxicity electrophysiological methods are not frequently used in spite of the fact that, according to some authors (5, 17), EEG may appear to be the most sensitive and reliable index of OP poisoning. The hippocampal I-RSA, a pattern of bioelectrical activity controlled entirely by the cholinergic system of the brain (2, 22) seemed to be particularly useful for the assessment of the functional state of this system. In our experiments, however, changes in I-RSA content were clearly seen only when higher doses of CVP, resulting in a deep inhibition of blood ChE, were used. It appears then, that at least in the rabbit, the hippocampal EEG ist not as sensitive an index of OP poisoning as has been expected. It is possible, however, that inhibition of ChE activity in the brain after i.p. administration of CVP may be less pronounced than in the periphery. At present, we have no relevant data supporting or denying such a supposition. Nevertheless, the changes in body temperature — the second index of the central action of CVP — make the above supposition less likely; the 22 mg/kg dose, resulting only in 37% ChE inhibition in erythrocytes (the maximum value) and in no discernible changes in the hippocampal EEG, was sufficient to produce hypothermia. It may be concluded then, that — at least in the case of CVP — body temperature is a more sensitive index of the central action of the pesticide than the hippocampal EEG. However, the rapid disappearance of electrophysiological and temperature changes (in spite of the persisting low level of ChE), as well as the fact that the effects of the second exposure may differ from those of the first, render both these indexes useless in the routine testing of OP neurotoxicity. Nonetheless, when studied in

a conjunction with the changes in ChE activity, they may provide valuable information concerning the dynamics and duration of adaptive changes in the central nervous system following OP exposure.

The relatively rapid disappearance of changes in body temperature and in the hippocampal EEG (as well as those in general behavior) could be ascribed to the process of desensitization of cholinergic receptors (8) and/or to the reduced acetylcholine outflow from synaptic endings due to the activation of presynaptic autoreceptors (12, 20). Both these phenomena may be produced by ChE inhibitors. The lack of effect of CVP on body temperature and on hippocampal EEG noted in some rabbits after the second injection, in spite of the normal effect on ChE activity, may suggest that tolerance developed. This phenomenon is reported quite frequently in OP literature. However, repetitive dosing is usually necessary for tolerance to develop, and the effect disappears when the level of ChE activity returns to normal (16, 18, 20). Our data allow one to assume that one exposure to CVP may be sufficient to produce tolerance and that it may persist long after normalization of ChE activity. It is worth emphasizing that this effect appeared in some animals only. This suggests that some individual predispositions or the height of the dose may have had some part to play.

According to literature, the mechanism of tolerance to anticholinesterases consists in a reduction (degradation) in the number of cholinergic receptors on postsynaptic membranes (9, 10, 16, 20). To what degree such changes may be regarded as a disfunction remains a matter of further studies. One may assume, however, that if it happens to exposed workers it may increase the feeling of safety (owing to the lack of subjective symptoms of exposure), which may in a long run, appear to be extremely harmful.

SUMMARY AND CONCLUSIONS

1. In rabbits, first intraperitoneal exposure to an organophosphorus pesticide, chlorphenvinphos (CVP) — in doses ranging from 22 to 250 mg/kg (Deichmann and LeBlanc'c design) resulted in: i) changes in general behavior typical for an anticholinesterase poisoning, ii) dose-related inhibition of plasma and erythrocyte cholinesterase (ChE), iii) a decrease in body temperature, and iv) dose-related increase in content of a specific, immobility-related rhythmic slow activity in the hippocampal EEG. Doses of 167 and 250 mg/kg appeared to be lethal.

2. The changes in behavior, body temperature and hippocampal EEG disappeared within 24 hours after the injection, irrespective of the dose. The return of ChE activity to a normal level took from 4 to 30 days.

3. Body temperature appeared to be a more sensitive index of CVP poisoning than the hippocampal EEG. Changes in the former were seen after exposure to lower doses of CVP and at lower levels of ChE inhibition than those resulting in discernible changes in the later.

4. Second exposure to CVP, 80—90 days after the first, resulted in a more severe depression of ChE but the effects on general behavior and hippocampal EEG were weaker, and those on body temperature became unpredictable; no effect, neither an increase nor a decrease were observable.

5. It may be concluded that: i) body temperature and hippocampal EEG are not reliable indexes of organophosphorus neurotoxicity, but when observed together with the changes in ChE activity they may be of help in the assessment of the adaptive processes in the brain, ii) brain cholinergic mechanisms are relatively resistant to disturbances in ChE activity produced by CVP. A significant decrease in ChE activity is necessary for the changes to be seen, and the process of adaptation proceeds at a fast rate (hours), iii) in some animals changes in functional state of some brain cholinergic mechanisms produced by a single exposure to CVP may persist much longer than the changes in ChE activity.

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