CHANGES IN THE CONDITIONED REFLEX AND BLOOD CHOLINESTERASE ACTIVITY OF ANIMALS UNDER THE INFLUENCE OF THE PHOSPHORGANIC INSECTICIDE, PHOSPHAMIDE

T. N. Pan'shina

Toxicological Laboratory (Head, Doctor of Medical Sciences Yu. S. Kagan)
of the Kiev Scientific-Research Institute of Work Hygiene and Occupational Diseases
(Dir., Doctor of Medical Sciences L. I. Medved')
(Presented by Active Member of the AMN SSSR A. I. Cherkes)
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The important role of acetylcholine in realizing the function of the cerebral cortex has been established in the investigations of native and foreign authors [4,8]. In the mechanism of action of the phosphorganic compounds on warm-blooded animals and man, one of the main links is cholinesterase depression. In an objective study of higher nervous activity, the phosphorganic compounds serve as a fine instrument, exluding an important link which is required for the normal course of neural processes [5].

In studying the action of the phosphorganic insecticides (thiophos, carbophos, mercaptophos, methylmercaptophos) on the central nervous system, using the method of conditioned reflexes, disturbances were observed in the higher nervous activity of the animals at early stages in their action on the organism, in the absence of visible signs of intoxication [3].

Phosphamide (rogor, dimethoate)—0,0-dimethyl-S-methylcarbamidomethyldithiophosphate—a new, prospective phosphorganic insecticide, is significantly less toxic for warm-blooded animals than thiophos, metaphos, mercaptophos, or methylmercaptophos. We established that, in experiments on animals in vivo, phosphamide exerts a manifest anticholinesterase action [6].

The purpose of these investigations was to study the influence of phosphamide on the higher nervous activity of animals. Special interest was directed toward the question of how early these changes arise, whether the disturbances in conditioned reflex activity are reversible, and a comparison of the ensuing changes with the degree of cholinesterase depression in the serum and erythrocytes.

EXPERIMENTAL METHOD

The investigations were carried out on cats, in whom conditioned reflexes had been developed according to the motor-alimentary method, with objective recording of the test indices as proposed by E. I. Spynu [7]. A special feature of the method is the development in the animals of a central position between two troughs during the intersignal time period. The animals developed a system of positive and negative conditioned reflexes; the positive conditioned stimulus was a white light and a buzzer, and the differentiating signal was the light from a blue lamp. The stereotype consisted of 10 stimuli. As the indices of the conditioned reflexes, we used the duration of the latent period and the time necessary to cross over.

Cholinesterase activity was determined by Hestrin's colorimetric method [9]. The activity of the enzyme was judged from the amount of acetylcholine broken down (in micrograms).

The phosphamide was introduced into the stomachs of the cats, using a rubber sound, in the form of an aqueous emulsion with OP-7 emulsifier.

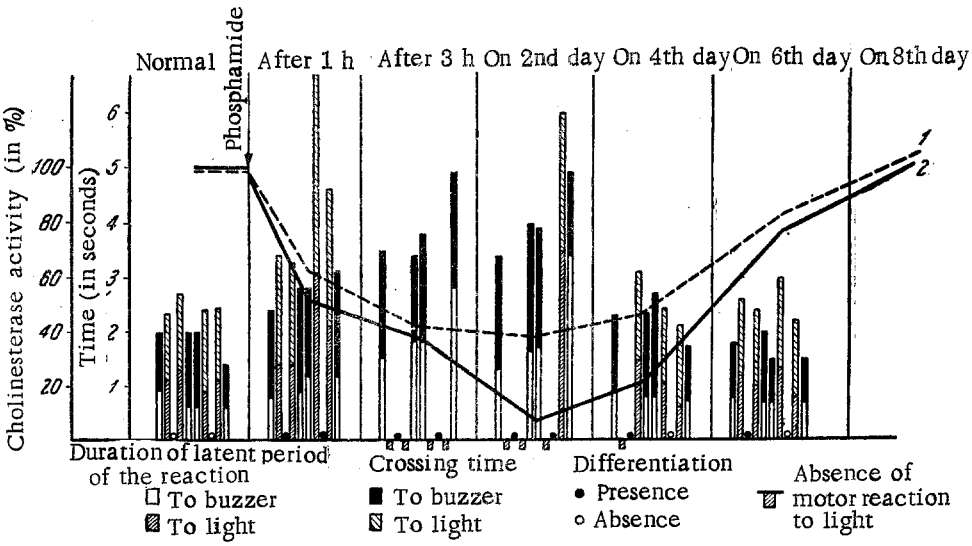

Fig. 1. Changes in the conditioned reflex activity, and cholinesterase activity of the serum (1) and erythrocytes (2), in the cat, Ryzhik, following a single per os administration of phosphamide in a dose of 40 mg/kg.

EXPERIMENTAL RESULTS

Changes in conditioned reflex activity, and a lowering of cholinesterase activity in the serum and erythrocytes, arose in the cats under the influence of phosphamide in doses that did not cause apparent signs of intoxication. Changes in conditioned reflex activity were noted with depression of erythrocyte and serum cholinesterase by 40% or more. Thus, with a single per os administration to the cats of phosphamide in a dose of 40 mg/kg, we did not note apparent signs of intoxication in the animals. The blood cholinesterase activity was reduced after only one hour, in the serum by 39-59% of the original level, and in the erythrocytes by 48-82% of the original level; after 3 h, by 57-66% in the serum, and by 58-82% in the erythrocytes; by the 2nd day after introduction of the drug, by 45-62% in the serum, and in the erythrocytes by 67-92% of the original level.

The disturbances in conditioned reflex activity were characterized by elongation of the latent period, and an increase in the amount of time required to cross over, in response to the positive conditioned stimuli. The reflexes to the weaker stimulus (light) were lower than to the strong stimulus (sound), i.e., the changes went through a type of narcotic phase. Lengthening of the latent period and the crossing time, in response to light, was more manifest in the second half of the stereotype. After 3 h and on the 2nd day after administration of phosphamide in a dose of 40 mg/kg, the conditioned reflexes to light dropped out, and in one of the cats, to the buzzer as well (in the second half of the stereotype). Differentiating inhibition was intensified: differentiation appeared in animals in which it had previously not been present.

Figure 1 shows that only one hour after administration of the preparation, changes in conditioned reflex activity were markedly exhibited: we noted elongation of the latent period of the reaction and of the time required for crossing over, in response to light and the buzzer. Cholinesterase activity was reduced in the serum by 39%, and in the erythrocytes by 48% of the original level. After 3 hours, we noted a complete loss of the reflexes to light. On the second day after the experiment, the changes in reflex activity were as marked as before, and the cholinesterase activity continued to fall. Complete restoration of cholinesterase activity was observed in the animals by the 6th-8th day after the experiment. Normalization of the changes in conditioned reflex activity occurred somewhat earlier—by the 4th-6th day.

With introduction of phosphamide into the animals in a dose of 20 mg/kg, the observed changes were of the same character, but were manifested much more mildly. Cholinesterase activity was reduced in the serum by 37-60%, and in the erythrocytes, by 52-73%. Fig. 2 shows that 1 h after administration the drug we did not note significant changes in the conditioned reflex activity of the animal; after 3 h, there was an increase in the latent period and the crossing time in response to light and the buzzer. Cholinesterase activity was reduced: after 1 h, in the serum by 46%, in the erythrocytes by 10%; after 3 h, in the serum by 60%, in the erythrocytes by 73% of the original level. By the 2nd day of the experiment, we noted almost complete normalization of the conditioned reflexes; the cholinesterase

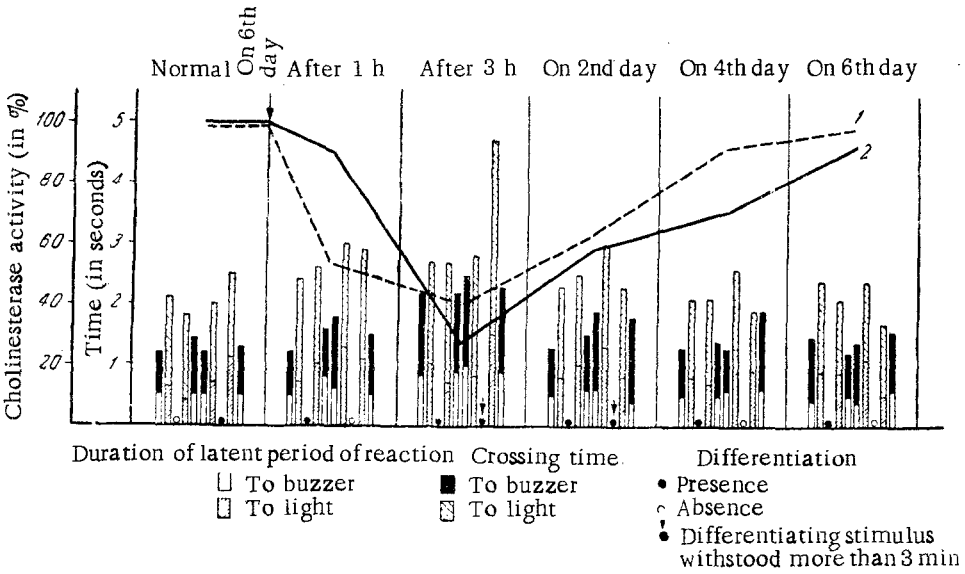

Fig. 2. Changes in conditioned reflex activity, and cholinesterase activity in the serum (1) and erythrocytes (2), in the cat, Pyatnista, following a single per os administration of phosphamide in a dose of 20 mg/kg.

activity was restored to 60% of the original level, and was completely normalized by the 6th day. A special test on lengthening of differentiation showed that the animal sustains a prolongation of the differentiating stimulus of more than 3 min, while prior to the priming, release occurred on the 20th second. On the 2nd day after the experiment, the animal also withstood prolongation of the differentiating stimulus up to 3 min. By the 4th day, differentiating inhibition was the same as that observed prior to the priming: the cat did not withstand one of the 2 differentiating stimuli.

A dose of phosphasmide of 10 mg/kg caused reduction of the cholinesterase activity in the cats by 10-35%; in the conditioned reflex activity, we noted only a minor tendency toward elongation of the latent period and the crossing time. In addition, in 2 cats in which disruption of differentiation was observed before the priming, stable differentiation appeared.

Phosphamide in a dose of 5 mg/kg depressed cholinesterase activity by 5-17% (such fluctuations in the activity of the enzyme are even observed in healthy animals), and did not give rise to changes in higher nervous activity.

With entrance of phosphamide into the organism via respiratory routes, the changes in conditioned reflex activity were exhibited less markedly, but were of the same character. Phosphamide vapor in a concentration of 0.025 mg/*l* caused a reduction of cholinesterase activity in the cats by 28-80% in the serum, and by 49-69% in the erythrocytes. Changes in conditioned reflex activity were characterized by only a slight tendency toward increase in the crossing time in response to white light. However, application of a special test of extinction, in order to demonstrate fiber changes, showed that extinction of the conditioned reflexes occurred two times faster than before the action of the phosphamide vapor. In addition, differentiating inhibition appeared in cats in which it had not been present before.

With a phosphamide vapor concentration of 0.01 mg/*l*, causing reduction of cholinesterase activity by 25-35% of the original level, only in one of the 3 cats did we note a slight tendency toward elongation of the latent period and the crossing time, in response to white light.

The results of applying tests on frequency increase and prolongation of differentiation show that administration of phosphamide in relatively small doses and concentrations intensifies the processes of differentiating and extinguishing inhibition in the cerebral cortex. Large doses caused prolongation of the latent period.

The disturbances in higher nervous activity observed in the animals with phosphamide intoxication, manifested by intensification of active inhibition (differentiating and extinguishing), and reduction of conditioned reflexes according to the narcotic phase type, are very similar in the character of the changes, to the disturbances in higher nervous activity associated with acetylcholine intoxication. With administration of low doses of acetylcholine, there is an intensification of differentiating and extinguishing inhibition, and a subsequent significant reduction in the conditioned

reflexes, replaced by maximum inhibition [1,2]. The changes in higher nervous activity arising under the influence of phosphamide are probably related to disturbances in acetylcholine metabolism. This is corroborated by the definite dependency, observed in the experiments, between the changes in conditioned reflex activity and the degree of depression of the serum cholinesterase (false) and especially, of the erythrocyte cholinesterase (true).

Essential changes in higher nervous activity arose when the cholinesterase activity was reduced by more than 40% of the original level; this is evidence that depression of cholinesterase activity is an earlier and more sensitive index of intoxication.

With prolonged action of phosphamide on the organism (over a course of 3 months, in the form of a vapor with a mean concentration of 0.005 mg/1, and over a course of 1½ months in the form of a liquid aerosol, with a mean concentration of 0.0015 mg/*l*, the changes in conditioned reflex activity were manifested mildly, but in all the animals we noted a uniform tendency toward prolongation of all the conditioned reflex indices, with their subsequent normalization, despite the continuing action of phosphamide. Changes in the cholinesterase activity were pronounced.

SUMMARY

In studying the action of phosphamide, a phosphorganic insecticide, on the higher nervous activity of animals with the aid of the conditioned reflex method, it was established that in doses and concentrations not provoking any visible signs of poisoning this preparation caused changes in the conditioned reflex activity of cats and depression of cholinesterase activity of the serum and erythrocytes of these animals. Changes in the conditioned reflex activity were observed in depression of cholinesterase of erythrocytes and serum by 40 and more per cent, i.e., depression of cholinesterase activity was an earlier and a more sensitive index of intoxication. Disturbances of the higher nervous activity observed in the animals during the action of phosphamide were manifested in intensified active inhibition (differential and extinction), reduction of conditioned reflexes according to the anesthetic phase type; by the nature of the changes these disturbances were similar to those of the higher nervous activity in acetylcholine poisoning.

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