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Zectran Fed Orally to Mice . . .

cholinesterase levels in blood determined

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ABSTRACT: Zectran, a carbamate insecticide, is being field-tested against the spruce budworm. Taken in sufficient quantity, it can induce cholinesterase (ChE) inhibition in mammals. In laboratory experiments, Zectran was fed orally to mice. Results indicated that maximum ChE inhibition occurred 15 to 30 minutes after ingestion of Zectran, and that a ChE test is unreliable in the case of Zectran poisoning unless taken while symptoms are apparent.

RETRIEVAL TERMS: Carbamate; poisoning; Zectran; cholinesterase inhibitors; insecticide.

Zectran(R)[1] has many qualities desirable in an insecticide replacement for DDT. It shows high specificity for the spruce budworm (*Choristoneura fumiferana* [Clemens]), a highly destructive forest defoliator. Produced by the Dow Chemical Company, the insecticide (4-dimethylamino-3,5-xylyl methylcarbamate) was field-tested by the U.S. Forest Service during the summers of 1965-66 in aerial sprays. Like the widely used insecticide carbaryl (1-naphthyl-N-methyl carbamate), Zectran is a carbamate that inhibits cholinesterase (ChE) activity. In vitro, this inhibitory action can be readily detected.

Before the 1966 field spray operations began, the normal ChE level of each spray pilot was determined by a blood ChE activity test. While spraying Zectran in the N-butyl ether of ethylene glycol, four pilots experienced dizziness and tightening of the chest. These symptoms receded when the pilots used face masks with respirators. Blood samples taken later that afternoon and stored for an unknown period at 4°C. showed normal ChE levels for the four pilots. This finding, plus the fact that pilots applying Zectran in other solvents did not experience these symptoms, indicated that the solvent was responsible. But it also raised a question as to the reliability of a ChE test in cases of suspected Zectran poisoning.

To determine the degree and rate of Zectran-induced ChE inhibition in mammals, we fed 31 white female Swiss mice each 6 to 10 weeks old, sublethal doses of Zectran, and recorded their blood plasma ChE levels.

Methods and Materials

A colorimetric method of ChE determination[2] was used. It relies on the photometric detection of the yellow anion of 5-thio-2-nitro benzoic acid released by the following steps:

Acetylthiocholine (AcSch) $\xrightarrow{\text{Blood plasma ChE}}$ thiocholine + acetate

thiocholine + dithiobisnitrobenzoate (DTNB) $\longrightarrow$ yellow-colored anion.

The test proved to be fast, accurate, and required little plasma. Using a clinical centrifuge, each blood sample

[1] Trade names and commercial enterprises or products are mentioned solely for necessary information. No endorsement by the U.S. Department of Agriculture is implied.

[2] Ellman, George L., Courtney, K. Diane, Andres, V., Jr., and Featherstone, R. N. *A new and rapid colorimetric determination of acetylcholinesterase activity.* Biochem. Pharmacol. 7:88-95. 1961.

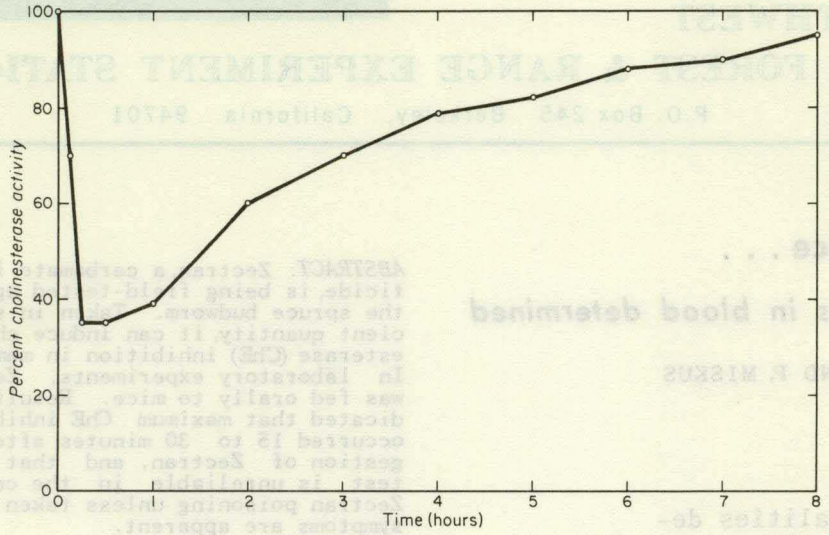

Figure 1.--*Composite cholinesterase level of 31 mice fed 0.5 mg. Zectran.*

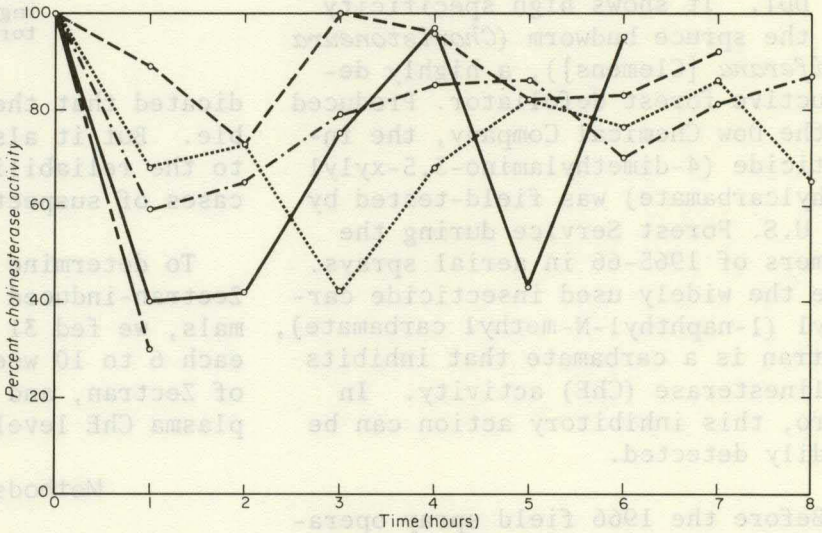

Figure 2.--*Individual cholinesterase levels of five mice fed 0.5 mg. Zectran showed wide fluctuation.*

was centrifuged to separate cells and plasma. Dilutions of 1.8 µl. of plasma were made in 6 ml. of 0.2M sodium phosphate buffer (pH 7.3). After 100 µl. of AcSch solution (54 mg. AcSch in 5 ml. H_2O) were added, the entire solution was incubated 20 minutes at 37°C., and 100 µl. of DTNB solution (40 mg. DTNB + 15 ng. $NaHCO_3$ in 10 ml. 0.1 M sodium phosphate buffer, pH 8) were added. The color that developed immediately was measured as absorbance on a Bausch and Lomb Spectronic 20 at 412 mµ.

A 25-µl. sample of blood was drawn from the tail of each mouse before Zectran feeding. These samples provided each mouse with her own control ChE level. All mice were fed orally 0.5-mg. pellets of Zectran--half the minimum LD_{50} dosage of 15 mg./kg. body weight established by the Dow Chemical Company. We extracted blood samples from the tails 5, 15, and 30 minutes after mice were fed Zectran; and then at intervals of 60 minutes for 8 hours or less.

Results

A curve averaged from the ChE activity of the 31 poisoned mice(fig.1) shows that maximum inhibition occurred 15 to 30 minutes after ingestion of Zectran. Average ChE inhibition was 65 percent

below normal. Maximum inhibition varied from 41 to 89 percent for mice given the same 0.5-mg. dosage of Zectran. The eight mice that died had ChE levels 70 to 89 percent below normal, although several mice with equally low levels survived. The fatally poisoned mice died an average of 40 minutes after ingestion of Zectran

Signs of severe poisoning included involuntary muscle twitching and cramps, constriction of the pupils, excessive bronchial secretion and uncontrollable salivation, accompanied by whitening of the eyelids. They appeared when ChE was inhibited at least 67 percent.

ChE levels were 30 percent below normal 3 hours after poisoning and 5 percent below normal by the eighth hour. Within 2 hours after ingestion of Zectran, external symptoms of poisoning were gone though the mice were less active than usual for another hour or two.

ChE activity curves for five individual mice (fig. 2) show considerable fluctuation during the recovery period. But the trend was toward an immediate increase in ChE level after maximum inhibition.

Discussion and Conclusion

Zectran shows characteristic carbamate anticholinesterase activity in mammals. It is also a rapidly reversible ChE inhibitor, and slight variation in assay time may produce a significant difference in ChE level.[3] ChE recovery from Zectran poisoning is practically complete after 8 hours.

Besides a depressed ChE level, moderate to severe cases of anticholinesterase poisoning are accompanied by distinctive symptoms. In mild cases of poisoning in which the symptoms are less distinctive and can easily be confused with symptoms of other conditions, a ChE test is important. The ChE level fluctuates widely during the recovery period; therefore, a false impression of the subject's condition may be obtained during this period.

The ChE test appears to be unreliable unless taken while the characteristic toxic symptoms are apparent. Furthermore, the ChE test should be performed immediately without intervening storage time, because the carbamate-esterase combination is readily reversed. Even storage in the cold could possibly reverse the Zectran-cholinesterase complex. If a suspected case of mild poisoning is confirmed by ChE test, proper measures should be taken to prevent further exposure.

If nerve poisoning is diagnosed, but the anticholinesterase agent is unknown, ChE recovery time will distinguish between a carbamate and an organophosphate. Unlike carbamates which form easily broken bonds with ChE, organophosphates attach themselves to the enzyme with strong irreversible phosphate bonds. ChE recovery from moderate organophosphate poisoning may take a week or longer.[4]

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[3] Baron, R. L., Casterline, J. L., Jr., and Oryel, R. *In vivo effects of carbamate insecticides on mammalian esterase enzymes.* Toxicol. Appl. Pharmacol. 9:6-16. 1966.

[4] Grob, D. *The manifestations and treatment of poisoning due to nerve gas and other organic phosphate anticholinesterase compounds.* Amer. Med. Ass. Arch. Intern. Med. 98:221-239. 1956.

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[5] Koelle, G. B. The manifestations and treatment of poisoning due to nerve gas and other organic phosphate anticholinesterase compounds. Amer. Med Ass. Arch. Intern. Med. 98: 221-239. 1956.