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16. Abstract

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SUBTLE CHANGES IN BRAIN FUNCTIONS PRODUCED BY SINGLE DOSES OF MEVINPHOS (PHOSDRIN)

I. Introduction.

Although the organophosphate pesticides (OPs) have been extensively studied,[10 11 13 18 19 20] many areas of prime significance to aerial applicator personnel remain unexplored. For example, OP poisoning generates a complex mix of symptoms originating in part from effects on peripheral cholinergic synapses and in part from effects on the central nervous system (CNS).[19] Aerial applicator personnel and others who may be exposed to OPs tend to use the peripheral signs—salivation, weakness or tremor, visual blurring, etc.[19 20]—as a danger warning, and will use or seek treatment with atropine and/or pralidoxime to relieve such peripheral symptomatology. Two assumptions underly this course of action: (1) that OPs are not likely to affect CNS functions without concomitant peripheral signs, and (2) that drugs such as atropine and pralidoxime which will eliminate peripheral symptoms will *also* restore CNS functions to normal. If either assumption is wrong, there is a substantial and unrecognized hazard present to aerial applicator personnel in the form of unrecognized CNS dysfunctions. There is little direct evidence concerning either assumption in the literature. The following experiments were undertaken to test assumption No. 1, to see whether an OP, mevinphos, would produce electrophysiological signs of CNS dysfunction at doses too low to produce peripheral symptoms.

Neurophysiological studies on OPs and other acetylcholinesterase inhibitors (AChEIs) have tended to center on their effects on the ascending reticular activating system (ARAS).[7 12 18 12] Thus, OP influences on brain function are usually thought of in terms of minor effects on alertness which may not involve significant performance decrements. However, cholinergic linkages are not unique to the ARAS.[8 12 13 15 17 21] Hippocampus, for example, has a cholinergic input.[4 8 9 15] The fibers originate in and come through the septum,[4 9 15 23] terminate on or near the hippocampal cell body[2 4 5] and are excitatory.[4 23] The powerful excitatory effects of iontophoretically administered ACh on hippocampal cells[4 23] suggest that even small doses of OPs could induce hippocampal dysfunctions without necessarily having much effect on other brain nuclei. Hippocampus is thought to be necessary for certain alerting, orienting and short-term memory storage functions.[1 6 9 12 16 21 22] Thus, a lightly-poisoned pilot required to remember a new procedure or orient to an emergency situation might well be unable to do so, even in the absence of overt signs of poisoning. Therefore, the following experiments were initiated to test whether doses of the OP mevinphos (Phosdrin)„ too small to produce peripheral signs or symptoms, could alter hippocampal electrophysiology. These experiments were undertaken simultaneously with, and in cooperation with the similarly directed behavioral experiments of Dr. M. F. Lewis (AAC–116), reported in OAM Report FAA–AM–72–29.[14]

II. Methods.

Squirrel monkeys with chronically implanted intracerebral electrodes were the experimental subjects. At the beginning of this series, careful consideration was given to the use of human volunteers or poisoned aerial applicator personnel in experiments studying EEG and visual evoked potentials recorded from the scalp. The necessary organophosphate doses were deemed too hazardous for volunteers while the problem of evaluating how much of what chemicals the applicators had absorbed, together with the practical problems of finding them and getting the measurements, made the use of aerial applicator personnel impractical. The major recording electrodes were an insulated stainless steel wire electrode pair (0.25 mm diameter and 1.0 mm tip separation) placed in the right side ventral

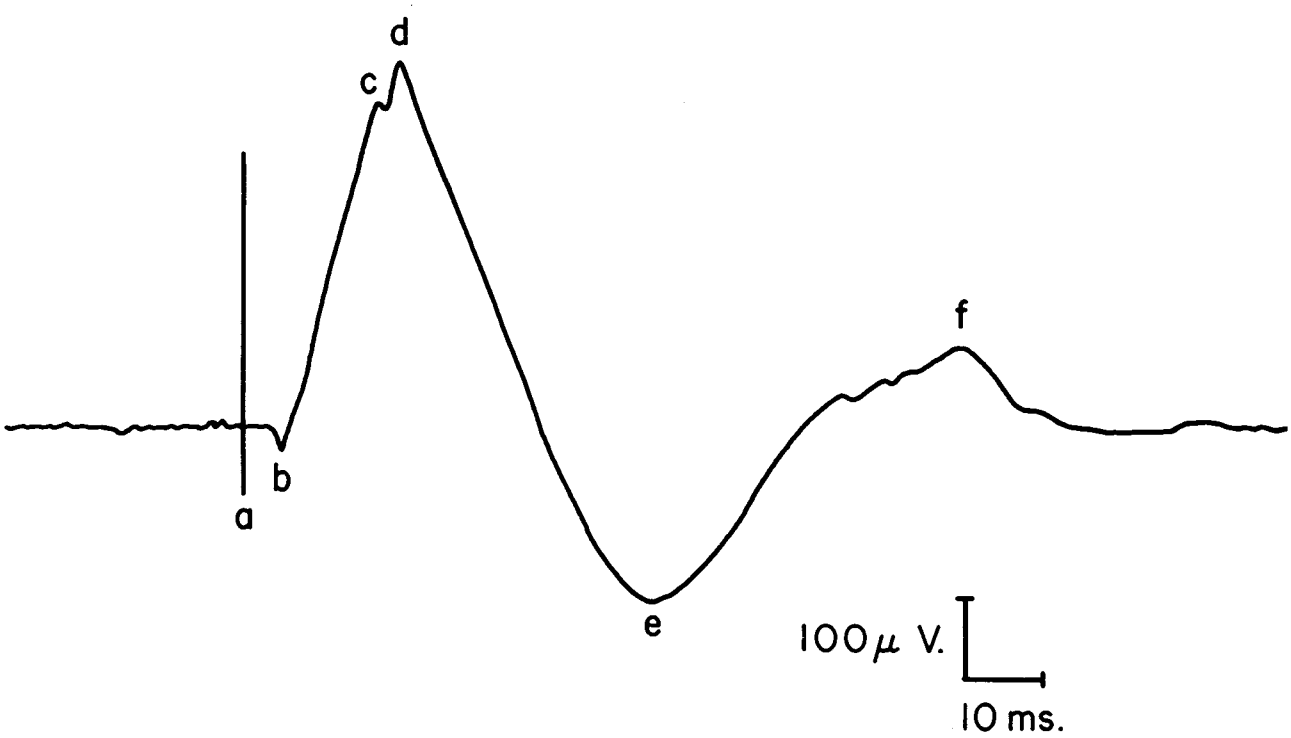

FIGURE 1. This is a recording of a septo-hippocampal averaged evoked potential. This AEP was formed from 10C successive evoked potentials taken at one second intervals. The computer output the AEP onto an X-Y recorder and this figure is a slightly smoothed redrawing of the X-Y recorder tracing. Wave (a) is the stimulus artefact. Waves (c) and (d) represent predominantly excitatory potentials and are conventionally termed negative waves, since that is the sign of the response at the level of the hippocampal pyramidal cell bodies The subsequent waves represent intrahippocampal inhibitory phenomena and other secondary transmission processes, and wave (b) represents activity in the incoming nerve fibers.

hippocampus. Similarly configured stimulating electrodes were placed in right dorsal hippocampus, right lateral septal nucleus, right lateral amygdalar nucleus and the left side dorsal and ventral hippocampus. By use of this electrode array a variety of independent mono- and polysynaptic inputs to hippocampus could be activated[2 5 9] so that any tendency of the OPs to selectively affect hippocampal inputs could be evaluated. After placement, the electrodes were fastened to the calvarium of the monkey with a system of screws and dental repair acrylic, and were connected to the contacts of an ELCO socket also anchored in the dental acrylic mass. After recovery from surgery, the monkey showed perfectly normal behavior, although it could at any time be connected to our apparatus for evoked potential studies.

For these experiments, the animal was placed in a conventional 2-plate restraint chair in an isolation chamber. Amygdalo-hippocampal septo-hippocampal and/or trans-hippocampal evoked potentials were generated with Tektronix pulse generators. The amplified responses were sent to the LINC-8 computer which formed averaged evoked responses (AERs) and stored them on tape for later analysis. Animals were examined daily. AERs were taken at 15 minute intervals. After the third AER, the animal was given an intramuscular injection of 10μl of fluid per kilogram body weight. The injected fluid was normal saline either by itself or containing mevinphos. AERs were then taken for a further 300 minutes, and the animal was returned to his cage. For these acute studies mevinphos was administered no more than once weekly at dose

ranging from 0.025–0.400 mg/kg. The animal was closely observed at all times through a "one-way" window in the isolation chamber.

Six experiments were also done in pentobarbital anesthetized monkeys held in a stereotaxic apparatus. Qualitatively, the results were identical to those seen in the chronic preparations.

III. Results.

Figure 1 shows a "typical" septo-hippocampal AER. Amplitudes of response peaks were measured. The primary negative wave (d) of the AER was most sensitive to mevinphos action and all future references to AER amplitudes will refer to wave (d).

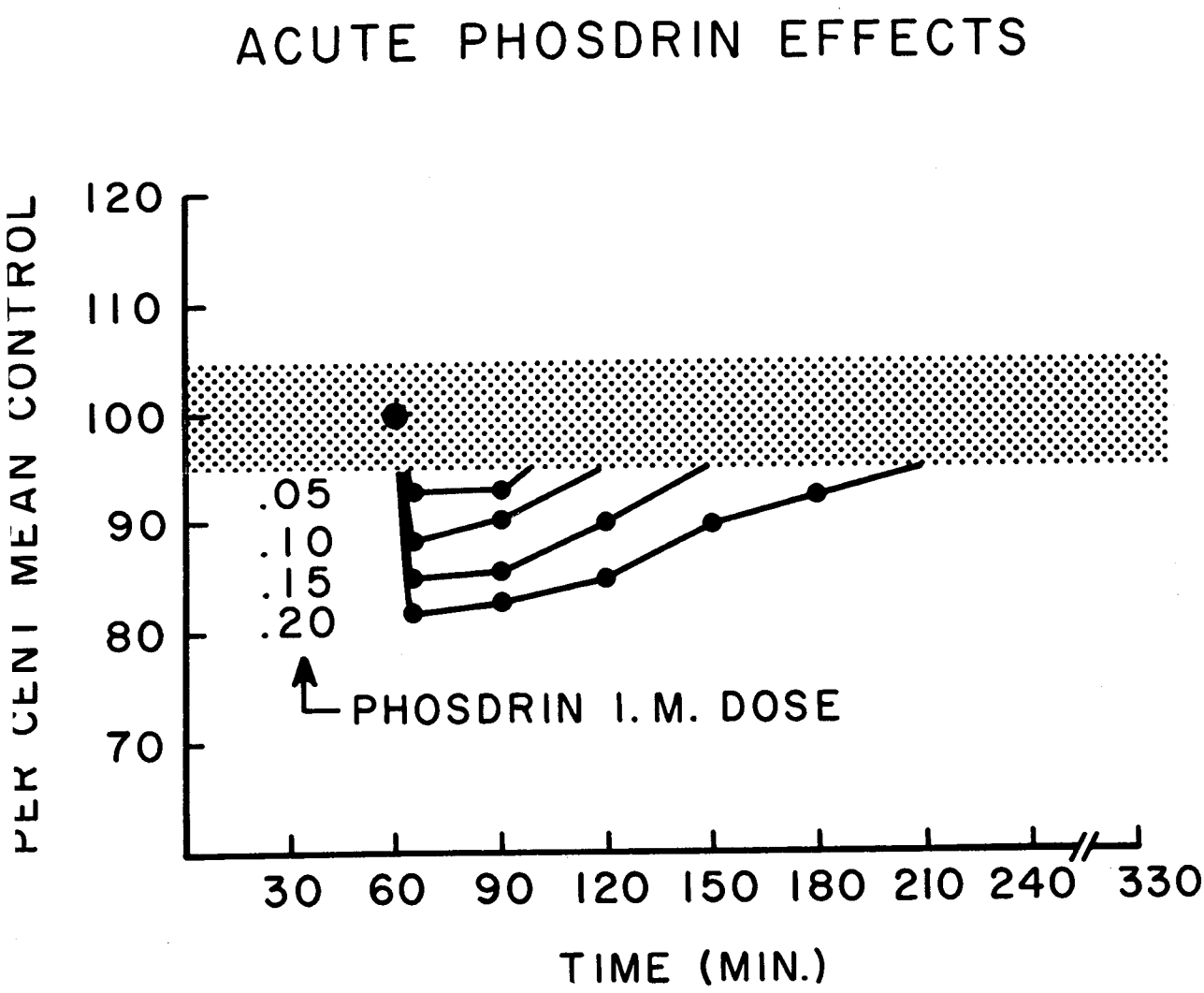

FIGURE 2. A plot of the effects of mevinphos on hippocampal evoked potential amplitudes. For the calculation of these points, the pre-drug control values in each run were averaged. This reference average value was taken as 100%. All potential amplitudes were then plotted as a percentage of this reference average, which is represented by the single dot in the dotted band. The extreme variation of the pre-drug control amplitudes was within 5% of the reference average. This zone of variation is represented by the dotted zone in the figure. AEP peak values occurring within 5% of the reference average were not considered significant and were not plotted. Pre- and post-drug AEP amplitudes were also tested with the Mann-Whitney U-test. The plotted points were all significant at the 0.05 level. In non-drug control studies the AEP amplitudes always remained within the 5% deviation band. Each plotted point above represents the means of 3 to 5 tests. As can be seen the administration of mevinphos induces an inhibition of AEP amplitudes. The extent and duration of this inhibition are proportional to dose within this tested dose range.

Doses of mevinphos below 0.050 mg/kg produced no AER changes. Doses of 0.40 mg/kg produced hippocampal seizures (although generalized CNS seizures were not usually seen), together with marked peripheral signs of OP poisoning (i.e., salivation, pupillary constriction, tremor, weakness, etc.). Post-ictal AERs were not taken since hippocampal seizures alter hippocampal bioelectrogenesis for up to 24 hours (Revzin & Costa, unpublished observations; Gergen & McLean, personal communication).

Mevinphos, in doses of 0.050 to 0.200 mg/kg, caused an inhibition of the AERs (Figure 2). The peak inhibition was dose-related and ranged from 7% at 0.050 mg/kg to 28% at 0.200 mg/kg. The duration of the effect was also dose-dependent and ranged from 45 to 180 minutes. As seen in Figure 2 the variance was quite small, even in this pooled data from all animals. Inhibition was seen in all components of the AER and in all AERs studied—although drug effects tended to be more marked on the septo-hippocampal response.

No peripheral signs were seen at dose levels of 0.05 to 0.20 mg/kg, although two animals developed brief (2–3 Sec) hippocampal seizure episodes after 0.20 mg/kg. That is, there was no observable tremor, weakness, pupillary constriction, or salivation. As mentioned, such signs were seen at doses above 0.20 mg/kg.

IV. Discussion.

Microiontophoretic and other neuropharmacological studies have demonstrated that there are powerful excitatory cholinergic receptors on or near the pyramidal cell somata in hippocampus.[4 23] Therefore, acetylcholine will cause depolarization of hippocampal pyramidal neurones, and nerve endings in their vicinity.[4 13] The negative wave of the evoked response (ER) measured in this study is associated with excitation of nerve cells.[2] That is, peak (d) of the ER is predominantly the vector sum of excitatory post-synaptic potentials (epsp's). Now, the amplitude of the epsp and the amount of transmitter released from the nerve ending by each impulse are both directly proportional to the absolute magnitude of the membrane potential of the neuron soma or ending.[3 24] Since mevinphos is an AChE inhibitor it will increase the concentration of free ACh in hippocampus.[13] This free ACh will depolarize endings and cell bodies. Thus mevinphos should, as we have shown, decrease hippocampal evoked response amplitudes both by reducing epsp magnitudes and by reducing the total amounts of transmitter released by stimulation. ACh also increases membrane conductance[4 24] in hippocampal neurones, and this, in effect, partly short-circuits all post-synaptic potentials in hippocampus.[3 24] This effect also contributes to the reduction of ER amplitudes produced by mevinphos. Since these effects are generalized effects on nerve cell membrane, all hippocampal ERs may be expected to be affected—as was found. Furthermore, the cholinergic synapses in hippocampus originate in septal neurones and through-fibers from midbrain running through the septum.[15 23] Therefore, one would expect that the septo-hippocampal ER would be most affected by mevinphos since the cholinergic nerve endings and associated post-synaptic membrane would, presumably, be exposed to the highest concentrations of free ACh. Though there was a tendency in this direction, the differences between the septo-hippocampal ER inhibition and the others were not significant. There is no conclusive explanation for this disparity. However, the cholinergic fibers are only a small percentage of the nerve elements which can be actuated by septal stimulation.[5 9] Thus, even dramatic changes in evoked activity at cholinergic sites might be partly masked by other events going on at the same time.

Obviously, if the depolarization caused by ACh is sufficiently marked, the neurones will begin spontaneous firing and seizure activity can result. Biscoe and Straughn reported this in their microiontophoretic experiments[4] and it is probably the explanation for the hippocampal seizures seen after mevinphos doses of 0.20 mg/kg or more. As usual in hippocampal seizures, the ictal activity did not propagate beyond hippocampus.[1 2 9] Thus, our results are in full accord with what is known of cholinergic functions in hippocampus.

Dr. Lewis and co-workers have reported quantitatively similar data on the effects of mevinphos on behavior in the squirrel monkey. That is, they reported behavioral inhibition over a dose range of 0.050–0.250 mg/kg, the duration and severity of the inhibition being dose-dependent. The parallelism between their behavioral data and the present electrophysiological findings is striking and, perhaps, suggests the possibility

of a causal relationship between the changes in hippocampal potentials seen here and their behavioral inhibition. As in the present study, no peripheral symptomatology was seen at doses of less than 0.250 mg/kg.

Perhaps the most noteworthy finding was that mevinphos could induce hippocampal biopotential and behavior[14] changes—including seizure activity—without any of the usual peripheral manifestations of OP poisoning. This is a bit surprising, and disquieting. Although mevinphos is said to penetrate the blood-brain barrier more readily than most other OP pesticides, it does not penetrate freely.[11,20] Thus, one would expect that peripheral effects would tend to develop before the CNS effects of the OP. That this order is reversed suggests that the effects of mevinphos on AChE in the CNS differ from those in the periphery and/or that mevinphos has actions in the CNS not directly related to its effects on AChE.

More importantly, the data suggest that aerial applicator personnel exposed to mevinphos—and, perhaps, the other OPs—can suffer significant CNS dysfunctions, even local hippocampal seizures, in the absence of the usual "peripheral" pathognomonic signs. Since hippocampus "plays a crucial role in the programming of acquired sensory-response patterns,"[6] the hazards to the aerial applicator may be substantial in absolute terms, the more so since patients seem generally unaware of hippocampal dysfunctions, even though substantial deficits in performance, consciousness or memory may be present.[19] As a practical matter these data reinforce previous emphasis on the need for extreme caution in handling the organophosphate pesticides.

V. Summary.

Mevinphos (Phosdrin) was found to inhibit the amplitude of hippocampal evoked potentials in unanesthetized squirrel monkeys with chronically indwelling electrodes. The threshold dose was 0.050 mg/kg and the maximal dose studied was 0.200 mg/kg. Doses above 0.200 mg/kg induced hippocampal seizures. Within the dose range of 0.050 mg/kg to 0.200 mg/kg the amplitude and duration of the inhibition were directly proportional to dose. No peripheral signs of poisoning, such as tremor or salivation, were seen at doses of 0.200 mg/kg or under. The discussion emphasizes that mevinphos produces changes in brain function in the absence of the peripheral symptomatology usually taken as indicators of poisoning by aerial applicator personnel. Therefore, it is concluded that exposure to mevinphos may be unexpectedly hazardous since the aerial applicators may be unaware that they have been poisoned.

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