

ABSTRACT: Organophosphate (OP) compounds are present in household and agricultural pesticides as well as in nerve agents. The toxic effects of these chemicals result from their anticholinesterase activity, which disrupts nerve junctions and parasympathetic effector sites, leading to a variety of symptoms and possible death. When the anticholinesterase agents in OP compounds reach the neuromuscular junction, they cause a disruption in the firing of muscle fiber action potentials. This effect has the potential of altering the time course of the electromyographic (EMG) signal detected by surface electrodes. We investigated the association between OP compound dose, surface EMG changes, and overt signs of OP toxicity. Daily doses of 10–15 $\mu\text{g}/\text{kg}$ of diisopropylfluorophosphate (DFP) were injected into the calf muscle of four rhesus monkeys while surface EMG signals were recorded from two thigh muscles bilaterally. With increasing number of doses, the EMG signal presented an increasing number of time gaps. The presence of the gaps was evident prior to any overt symptoms of cholinesterase toxicity. These findings can lead to the development of noninvasive technology for indicating the presence of OP compounds in muscle tissue prior to clinical abnormalities.

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THE ELECTROMYOGRAPHIC SIGNAL AS A PRESYMPTOMATIC INDICATOR OF ORGANOPHOSPHATES IN THE BODY

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Organophosphate (OP) cholinesterase inhibitors form the active ingredient of nerve agents such as sarin and soman, as well as many of the more than 25,000 brands of home and agricultural pesticides.²² The horrific effects of nerve agents have been well publicized, but it is less well known that OP compounds are responsible for over 20,000 reported toxic exposures per year in the USA alone.⁸ Exposure to high concentrations of OP compounds may result in death through respiratory failure and the accumulation of respiratory secretions.²² Exposure to low concentrations of OP compounds produces characteristic but treatable symptoms. In skeletal

muscles, symptoms include muscle twitching, cramping, and weakness; involvement of glandular cells and autonomic ganglia may lead to excessive pulmonary secretions and airway constriction, diarrhea, vomiting, and nausea; whereas, in the central nervous system, OP compounds can produce irritability, depression, apathy, loss of concentration, and memory impairment.^{10,16} OP compounds produce their biological actions through inhibition of the various forms of cholinesterase, the degradative enzyme for the neurotransmitter, acetylcholine. Toxicity is mediated through elevation in the levels in acetylcholine in tissues innervated by cholinergic neurons and via concurrent parasympathetic and ganglionic stimulation.¹⁸

Early recognition of exposure and timely intervention are important for effective treatment; therefore, early detection is beneficial. Various approaches are available for detecting the presence of OP compounds. According to Mason,¹⁵ biological monitoring of the ambient atmosphere is the most

Abbreviations: DFP, diisopropylfluorophosphate; EMG, electromyographic; OP, organophosphate

Key words: cholinesterase; diisopropylfluorophosphate; neurotoxins; organophosphates; surface electromyographic signal

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effective means for early detection. Various systems for this purpose are available, but they can only provide estimates of the likely internal dose absorbed or inhaled by an individual.¹⁵ Force sensors have been used to measure the tremor of a mouse that had been exposed to soman or oxotremomrine.⁶ Alternatively, overt signs and symptoms of OP toxicity may be discerned clinically, but symptoms tend to be nonspecific and absorption of OP compounds such as pesticides may be at levels that produce no overt clinical signs.¹⁷ Measurement of enzyme activity of plasma cholinesterase and acetylcholinesterase in red blood cells has historically been used as the preferred indicators for excessive OP exposure,¹⁴ but large individual differences exist in the levels of enzyme activity in blood. In addition, many diseases, as well as other toxicants and medications, can depress plasma and red blood cell cholinesterase, further limiting the effectiveness of these methods.¹⁵ Generally, assays of urine alkyl phosphate are more sensitive for measuring the amount of absorbed OP than methods based on inhibition of blood cholinesterases. However, urinary alkyl phosphate and phenol analyses are affected by OP compounds in the food chain. There are also practical difficulties to consider, such as the need for multiple urine samples initiated close enough to a potential OP exposure-incident to define adequately the extent of low-level exposure.²⁷

Organ-specific OP absorption monitoring methods are absent from the tools currently available for biological monitoring. This deficiency is especially true for the neuromuscular system. The disturbed acetylcholine transmission from OP compounds interferes with signal transmission across the neuromuscular junction and consequently alters the propagation of the action potential along the muscle-fiber membrane.¹² This effect can occur at low doses. Baker and Sedgwick¹ recorded action potentials using invasive single-fiber needle electrodes and noted alterations in the shape and propagation of action potentials that persisted for up to 3 days after exposure to sarin without apparent symptoms of neuromuscular toxicity. Similar changes were noted from a hemidiaphragm preparation that was studied for up to 28 days after exposure to various OP compounds.²³

Given these alterations in action potentials, it is reasonable to expect corresponding alterations in the surface electromyographic (EMG) signal. Consequently, we performed this preliminary study to determine whether the surface EMG signal is altered in a predictable manner following exposure to an OP compound. In addition, we investigated whether

such changes, if present, appear to be dose-sensitive and occur prior to overt signs of cholinesterase toxicity. If so, it should be possible to develop a new technology for monitoring OP compounds absorbed in the neural and muscle tissues.

METHODS

Research Design. Due to the preliminary nature of this study, which was intended to establish association rather than causality between parameters, a self-controlled study design was adopted in which subjects served as their own controls. One of the drawbacks of such a design is the vulnerability to the Hawthorne effect, where changes in behavior following an intervention can occur simply because the subject received special attention by being in the study.⁷ We modified the design to limit this effect by introducing a recovery or “washout” procedure following the intervention to provide further opportunity for establishing an association between dose and effect. Furthermore, other variables, such as serum levels of cholinesterase activity, were included to verify that the effects were likely dose related.

The study was performed using diisopropylfluorophosphate (DFP), a compound commonly employed for studying the effects of OP cholinesterase inhibitors.⁵ Administration of this inhibitor produces classic acute and delayed forms of toxicity, and is relatively safe to use in the laboratory setting because of its low volatility. As is the case with many OP compounds, DFP is rapidly metabolized to an inactive product by blood and tissue esterases. However, the inhibition of cholinesterase is permanent, requiring the synthesis of new enzyme protein for complete recovery. Therefore, even subthreshold doses of DFP may have cumulative effects. DFP is similar to nerve agents in that it does not require *in vivo* modification to an active metabolite, and it is similar to some pesticides in that it responds readily to antidotal measures such as oxime reactivators.²⁵

Experimental Protocol. All animal protocols were approved by the appropriate review boards of the Medical College of Georgia and the Western Institutional Review Board (Olympia, Washington). Four rhesus monkeys, weighing 9.4–12.2 kg, were trained to support their body weight while being restrained in an adjustable primate chair (Fig. 1). They were positioned to stand on their hind limbs and maintain sustained isometric contractions of their lower-limb muscles through the use of a rigid neck collar that was adjusted to a height that required subjects to stand on their hind legs. Subjects had no difficulty

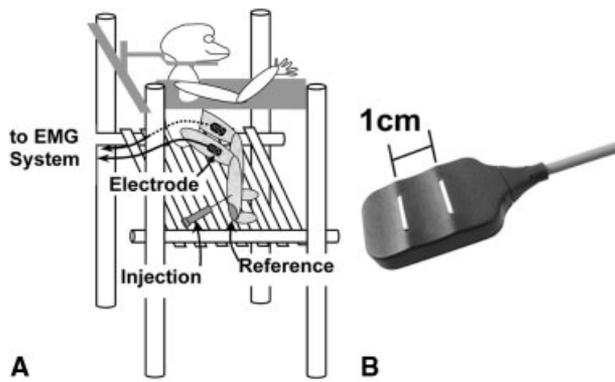


FIGURE 1. Experimental arrangement. Positioning of the subject in the test chair (**A**). The subject is shown supporting its body weight during stance while surface EMG signals are recorded bilaterally from the vastus medialis and vastus lateralis muscle of the thigh (only two of four sensors are shown). The location of the DFP injection site and EMG ground reference electrode is provided. A close-up view of the surface EMG electrode is shown (**B**). The spacing between the parallel bars is 1.0 cm, making this electrode suitable for detecting the surface EMG signal from the relatively small muscles of the animal.

in tolerating this position for trial periods that varied between 5 and 10 minutes. If signs of fatigue or discomfort appeared prior to the allotted trial period, the subject was allowed to return to a seated position in the primate chair and rest before another trial was initiated. Surface EMG electrodes (Delsys, Inc., Boston, Massachusetts; DE 2.1 single-differential, parallel-bar configuration), shown in Figure 1, were attached to the mid-bellies of the right and left vastus lateralis and vastus medialis muscles. EMG signals were detected from each muscle while the subject maintained the isometric sustained contraction during forced standing. EMG signals were filtered during acquisition with a bandwidth of 20–450 Hz (roll-off of 80 dB/dec) and a gain of 1000. The signals were digitized (1024 samples/s) and stored for later processing with data acquisition and analysis software (EMGworks, Delsys, Inc.). Baseline measurements of EMG activity were acquired for varying

durations for each animal, ranging from 2 to 5 days, depending on the availability of the animal and its ability to comply with the experimental protocol.

Following baseline measurements, the animals were injected daily with DFP (10 $\mu\text{g}/\text{kg}$) in the calf muscle for 5 consecutive days. Injection was alternated between the left and right sides to minimize trauma to the muscle from the needle and reduce the likelihood of localized proteolysis from DFP. Over the next 2–4 days, the animals received an additional 15 $\mu\text{g}/\text{kg}$ of DFP daily. As the number of injected DFP doses increased, the animals manifested increased signs of toxicity. Dosing was discontinued at the point of overt symptoms of cholinergic overstimulation. At this point, atropine (0.2 mg/kg) was administered for 4 consecutive days to mitigate the parasympathomimetic effect of the DFP. EMG recordings were made for 14 consecutive days after administration of the first DFP dose. The EMG signal was recorded prior to and immediately following the injections.

Data Processing. Three indices were devised to quantify the progression of the toxicity. The first was a symptom severity index. This was a subjective index that categorized the observed clinical changes in the animals. Severity was categorized according to a method closely modeled after the standardized and validated procedure described by Persson et al.²¹ As summarized in Table 1, clinical categories were identified as “low” if signs were either mild, transient, or spontaneously resolving, and as “moderate” if they were pronounced or prolonged. Specific changes associated with the voluntary and autonomic nervous systems and three different organ systems were evaluated. They included such symptoms as diarrhea and vomiting for the gastrointestinal system; wheezing and dyspnea for the respiratory system; and meiosis, muscle incoordination, and muscle fasciculation for the nervous system. A score of 1 was assigned to each symptom in the moderate category, and a

Table 1. Summary of categories used for symptom severity index.

Organ system	Severity category	
	Moderate (pronounced or prolonged)	Low (mild, transient, or spontaneously resolving)
Gastrointestinal	Vomiting, diarrhea	Vomiting, diarrhea
Respiratory	Wheezing, dyspnea	Wheezing, dyspnea
Nervous	Meiosis, incoordination, muscle fasciculation	Meiosis, incoordination, muscle fasciculation

Signs from three organ systems are indicated. Two severity categories (low and moderate) are indicated for each sign. For each sign, scores of either 1 or 0.5 are assigned for moderate and low categories, respectively. A cumulative index for each trial was computed, as described in the text, by adding all scores for each animal.

score of 0.5 to each symptom in the low category. The cumulative symptom severity index was computed post hoc as the algebraic sum of these scores, and therefore could range between a minimum value of 0 and a maximum of 7. Two observers scored each animal on a daily basis and the average of their scores was used for comparison with other parameters. These observers were not involved in the processing of the other data parameters or their analysis.

As the number of doses increased, the EMG signal presented an increasing number of gaps that were not characteristic of a normal surface EMG signal (for additional descriptive behavior of surface EMG, see Basmajian and De Luca²). In order to quantify this effect, we constructed two additional indices: a tremor index and a myo-chem index.

The tremor index was designed to measure the 6–12-Hz energy in the modulating signal [measured EMG signal = (modulating signal) × (base EMG signal)] induced on the EMG signal by the presence of tremor.¹² The modulating signal was extracted through a process of rectification and low-pass filtering of the EMG signal. The 6–12-Hz energy within the envelope was estimated via a power density spectrum that was calculated using Welch's method with a half-window overlap.

The myo-chem index was designed to measure the rate of occurrence of gaps that were spaced in an irregular manner, as was observed for the EMG signal at low DFP doses. This index estimates the presence of a gap by comparing local signal power to a long-term average of signal power. We used two moving windows, one with a longer length (W_1), to calculate the long-term average of the signal power:

$$G(z) = \frac{1}{W_1} \sum_{i=z-W_1/2}^{z+W_1/2} X(i)^2$$

and another with a shorter length (W_2) for the local signal power:

$$L(z) = \frac{1}{W_2} \sum_{i=z-W_2/2}^{z+W_2/2} X(i)^2$$

In these equations, $X(i)$ represents the digitized EMG signal and i and z represent data indices.

When local signal power (averaged for statistical stability over a short epoch of the EMG waveform) was significantly smaller than the long-term average of signal power, the local signal value was declared to be within a gap. A single gap was therefore construed

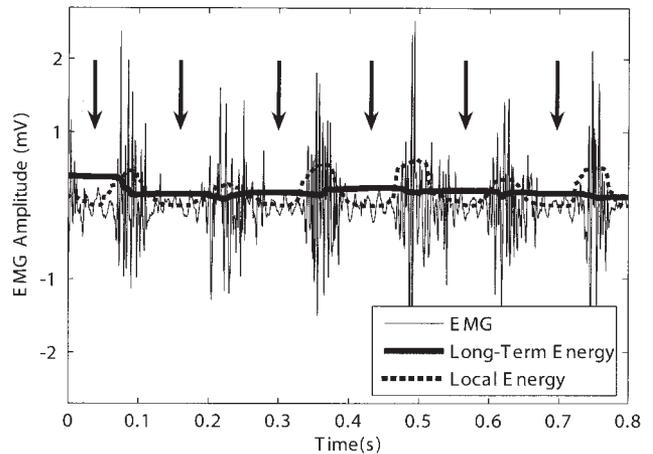


FIGURE 2. A typical record of the surface EMG signal detected from animal 2 after the injection of five doses (10 µg/kg) and three doses (15 µg/kg) of DFP. The near-flat solid line represents the long-term energy and the fluctuating dashed line represents the local energy of the EMG signal. The arrows mark the location of the gaps in the signal.

to consist of consecutive signal values declared to be within that gap. Figure 2 shows a segment of the EMG signal with the estimates of the long-term average power represented by the relatively flat line and estimates of the local signal power represented by the undulating line. The arrows in the figure indicate the presence of gaps. Counts of the number of gaps per second were used as values for the myo-chem index.

A constraint satisfaction approach was used to find values for W_1 and W_2 . The procedure was designed to identify W_1 and W_2 values that satisfied the constraint maximizing the difference between the numbers of gaps in the baseline EMG data compared to the dose-related EMG data. We focused the analysis on EMG data that were derived when the animal had been exposed to the highest cumulative doses of DFP where visible fasciculations were present and there were numerous EMG gaps discernible by eye. Different values of W_1 and W_2 were tried until we were able to equally satisfy the constraint in data from all four animals, and further sampling did not improve the results (i.e., we had reached a plateau). The values computed following this procedure were $W_1 = 128$ samples (125 ms) and $W_2 = 40$ samples (39 ms).

A limitation of this experiment was that, during the recording sessions, the animals often moved their limbs and did not always provide us with stationary EMG signals. In order to remove the artifact introduced by these dynamic contractions, we conditioned the signal by high-pass filtering with a sixth-

order Butterworth filter with a cut-off frequency of 100 Hz. A computer algorithm was written to select more stable (stationary) segments of the EMG signals that were free of the relatively high variability in EMG amplitude that is characteristic of movement and force variation.² The algorithm extracted stationary EMG segments of 5 s or longer by computing the ratio of the maximum and minimum of the root-mean-square value of the EMG signal and eliminating those portions of the raw data where this ratio exceeded a value of 2. Root-mean-square value was calculated for 0.25-s windows that were overlapped by 0.1 s using a moving window.

The EMG segments complying with the stationary criteria were collected for all four muscles and were analyzed separately to compute the myo-chem index and tremor index for each day.

RESULTS

We collected baseline data for 2 days on animals 2 and 3, for 3 days on animal 4, and for 5 days on animal 1. The variation in duration was due to scheduling demands. We collected a total of 53 min 45 s of EMG signals for all animals prior to administration of the first dose of DFP (14 min 40 s for animal 1, 16 min 50 s for animal 2, 2 min 50 s for animal 3, and 19 min 25 s for animal 4). When pooled across subjects, this aggregation provided a total of 863 5-s epochs of stationary EMG data for all four muscles. Similarly, pooled data collected from the onset of DFP administration until the completion of the study resulted in a total of 624 5-s epochs of stationary EMG data.

All four animals showed clear signs of toxicity, but to varying degrees. Blood samples taken after five doses of DFP (10 $\mu\text{g}/\text{kg}$) and after the last dose of DFP (15 $\mu\text{g}/\text{kg}$) indicated that the plasma cholinesterase levels were maintained at 89.9% and 87.7%, respectively. Overt signs of toxicity disappeared in all the animals within 6 weeks of the first dose.

Prior to the administration of DFP, there were no visible signs of abnormality in the behavior of the animals and in the appearance of the EMG signal. As the number of DFP doses increased, the toxicity manifested itself as visible changes in the behavior of the animal and in an altered appearance of the EMG signal. Figure 3 presents the sequence of alterations in the EMG signal that were characteristic of the experimental data set. Prior to the administration of the DFP (Fig. 3a), the amplitude of the EMG signal was normal; that is, the amplitude appeared stochastic and stationary in nature.² It was continuous with only occasional gaps in the signal. The first noticeable alterations in the EMG

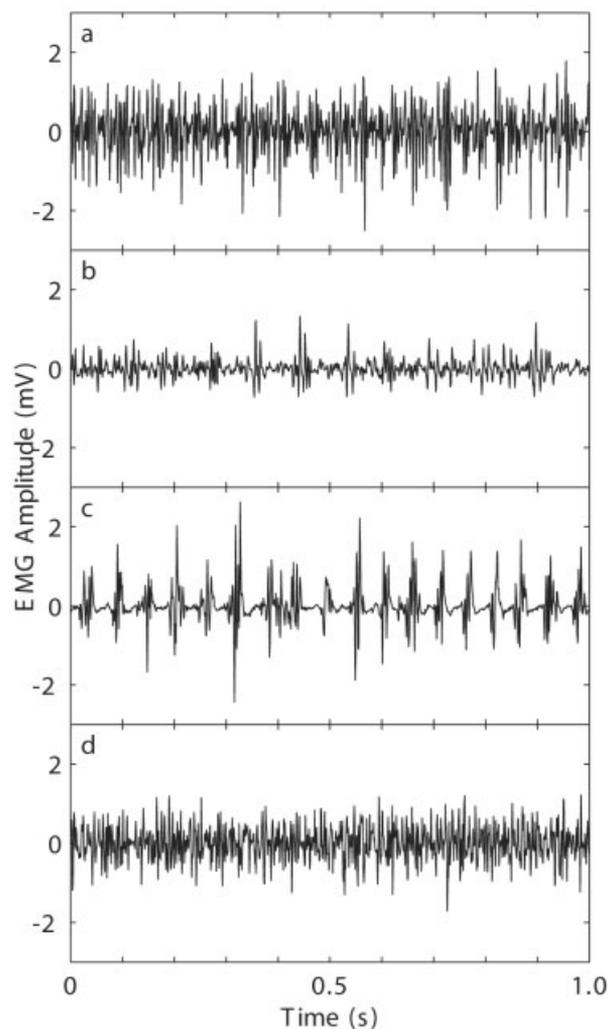


FIGURE 3. Samples of the EMG signal from animal 3 at various stages of the dosing sequence: (a) baseline measurement 1 day prior to the first injection of DFP; (b) first observation of the gaps noted 1 day after the first injection; (c) the most affected record, seen 6 days after first injection; and (d) a return to normal appearance 7 days after the last injection of DFP.

signal were manifested by disorganized clusters and gaps in the signal (Fig. 3b). This clustering became more pronounced up to the day after the last administration, when it appeared as well-synchronized bursts alternating with periods of nearly no activity that appeared as gaps (Fig. 3c). One to 2 weeks after the last injection, the signal began to regain its normal appearance (Fig. 3d). A comparable pattern of signal behavior was reported by Smith²³ in a hemidiaphragm preparation for up to 28 days after exposure to different OP compounds.

No overt clinical changes were observed during the first three consecutive administrations of the 10- $\mu\text{g}/\text{kg}$ dose. Subsequently, we noticed slight

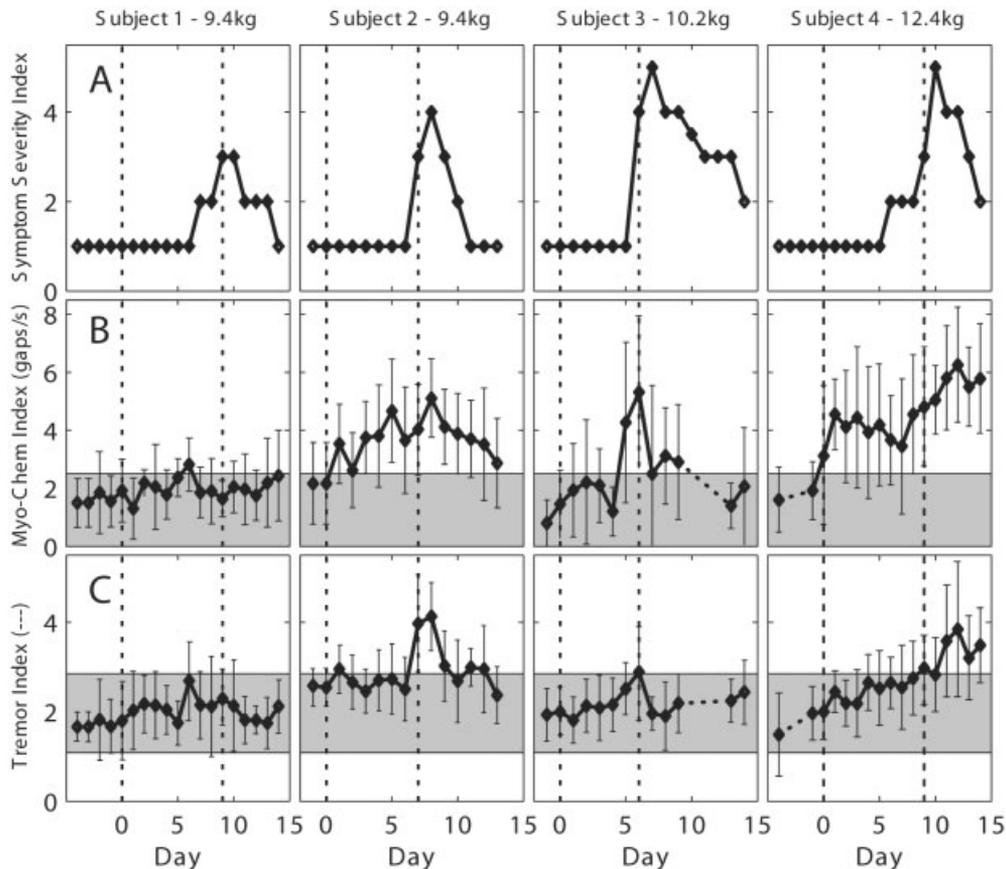


FIGURE 4. The time-course of: **(A)** the symptom severity index; **(B)** the myo-chem index; and **(C)** the tremor index. Note that the EMG signal could not be obtained on each day from some animals. Animal 4 was uncooperative during the baseline measurements and animal 3 became ill and could not be tested for a few days. Those events are indicated by a dashed line. The first vertical dashed line indicated the first day of DFP injection (day 0) and the second vertical dashed line indicates the date of the last injection, which differed among animals. The data were computed from a total of 863 trials across all four animals. The upper and lower bounds of the shaded interval indicate the 95% confidence limits. By common convention, any value of the index above the shaded region is considered significantly different from the baseline value.

fasciculations of muscle fibers in the thigh muscle as well as in other superficial muscles throughout the body. Fasciculations are a common sign of exposure to anticholinesterase drugs and appear as brief, irregular contractions of muscle fibers that are not associated with voluntary movement and involve different muscles randomly.¹² The fasciculations were easily distinguished from voluntary movement, because they occurred at rest and were typically present with myokymia, which appears as slight movement of the skin in the vicinity of the contraction. As the number of injections increased, the clinical changes (see Table 1) became more pronounced. In contrast, the first signs of alterations in the appearance of the EMG signal became apparent as early as 1 day after the first injection in two animals (nos. 2 and 4), and later in the others. Alterations in the EMG signal always appeared before the first sign of overt clinical

change. When the EMG signal was altered, the modification was seen in all four muscles, indicating that the effect was likely systemic.

Figure 4 presents a comparison of the time-course of the three indices as well as their deviations from baseline. The symptom severity index is shown as an averaged value, from scores assigned by two of the experimenters. The myo-chem index and tremor index are shown as average and standard deviation values calculated for all four muscles on each day. The shaded area in the plots of these indices represents the 95% confidence interval for their baseline values. Because the confidence interval was computed from a total of 863 5-s-long baseline trials pooled across all four animals, it has the same dimensions in each animal. Pooled data were used because of our interest in developing a myo-chem index technique that does not require individual baseline measurements for use in the field.

Figure 4 indicates that the myo-chem index averaged for the four muscles and plotted for each experimental day in Figure 4b exceeded the 95% confidence limits for three of the four animals. This deviation from baseline occurred as many as 6 days prior to overt clinical change in two of the animals (nos. 2 and 4) and 1 day prior to observing symptoms in one animal (no. 3). In contrast, the tremor index exceeded its baseline 95% confidence limit in only two of the animals (nos. 2 and 4) and only during peak symptom severity index periods.

DISCUSSION

The overt clinical manifestations of DFP toxicity in this study were similar to those reported previously. That is, it varied among animals; the clinical changes were similar; and the intensity of the changes increased with the number and concentration of administered doses.^{3,18,20}

The novelty of our study concerns the finding that surface EMG signals are modified in association with increased dose of OP compounds, and that this manifestation in the EMG signal occurred prior to overt symptoms of cholinesterase toxicity. Previous reports^{1,23} have described changes in the shape of action potentials detected via needle electrodes, but no associations were made with respect to increases in dose followed by a washout period. Furthermore, none of these studies utilized noninvasive EMG signal detection.

We have demonstrated that the inhibitory effect of DFP on cholinesterase activity in the synapses is associated with changes in EMG signal characteristics detected from the surface of the skin. Our study provides preliminary evidence that a noninvasive method based on recording EMG signals may be of practical value as a biological monitor for OP compounds. Furthermore, because the alterations in the surface EMG signal occurred before any overt physiological manifestations were observed in most subjects, the EMG signal may be capable of providing presymptomatic detection of OP toxicity. In three of the four animals (only two animals responded above baseline after one dose) the myo-chem index responded to the presence of DFP after only one dose of 10 $\mu\text{g}/\text{kg}$ had been administered. Not only did a change in this index precede one in the symptom severity index (i.e., it was more sensitive), but it also increased with the number of doses and returned to baseline after injection of DFP was discontinued and atropine was administered. In animal 4, the myo-chem index continued to increase as the severity index decreased after reaching a peak. This is possi-

bly due to the fact that the severity index contains contributions from the parasympathetic nervous system, whereas the myo-chem index represents only the effect on the voluntary nervous system.

The fact that these animals responded to the administration of DFP with intersubject variability in severity of clinical effects and EMG response is not unexpected. Sensitivity to cholinesterase inhibitors is variable among animals and humans.⁴ Animals within a study group will have varying sensitivities to all but the highest doses, so that a dose that affects one animal might have no effect at all on another. These varying sensitivities to cholinesterase inhibitors may be due to differences in the expression and sensitivity of cholinergic receptors and to the fact that narrow dose windows are a characteristic of this class of drugs.¹⁹ Predisposition to toxicity from cholinesterase inhibitors as a class is partly under genetic control. For example, peripheral pseudocholinesterases or aliterases that compete for circulating cholinesterase inhibitors can decrease the amount of DFP available to phosphorylate acetylcholinesterase.¹¹ Thus, although plasma cholinesterase inhibition is a useful indicator of exposure, it is rarely predictive of symptom expression or lethality.^{4,26} We monitored cholinesterase levels in this study to show that there was rapid inhibition soon after initial dosing, which was relatively constant throughout the study, and for comparative purposes with other published studies and for future studies.

The clustering-and-gaps behavior of the EMG signal that was associated with DFP toxicity was not caused by a simple tremor phenomenon. Although tremor in the frequency range of physiological tremor²⁴ was evident in two of the primates, it was only noticeable when the myo-chem index was at its highest level, corresponding to the greatest cumulative dose of DFP. At lower doses, the myo-chem index was more sensitive than the tremor index. The modification in the EMG signal at lower doses may be associated with fasciculations that were noticed in this and previous studies.¹² As the dose was increased, spontaneous fasciculations became more pronounced and more organized into a tremor, which is a common sign of acute OP poisoning.¹² The myo-chem index is able to track the evolution of the fasciculations into the more organized tremor, thus providing a parameter that continuously represents the degree of disruption in the EMG signal associated with OP cholinesterase inhibitor activity.

The clustering effect seen in the EMG signal may thus be better understood as a phenomenon that blocks the activation of the postsynaptic membranes in a near-regular manner rather than as one that

likely originates in the diencephalon, as does physiological tremor. The increasingly well-defined gaps that indicate an almost silent activation of all the motor units in the detection volume of the surface electrode point to a grouped effect at the level of the motoneuron pool. A possible explanation is that, at the administered doses, DFP is able only temporarily to block acetylcholine transmission across most of the neural synapses. During these gaps, the cholinesterase lowers the acetylcholine and postsynaptic excitation occurs for a short period that is manifested as clusters in the EMG signal. This process repeats continuously as the presynaptic excitation continues.

It is unlikely that the reported effects were due to mechanisms other than the systemic cholinesterase effects of DFP. Although administration of DFP may be proteolytic and lead to myofibular damage,¹³ or may have biological effects by mechanisms other than phosphorylation,⁹ these possibilities are unlikely because intramuscular injections of DFP were administered in muscle groups other than those used for recording the EMG signal, and the effect on the myo-chem index was reversed by the injection of atropine.

The results of this study are preliminary and based on the data from experiments with four animals. We mitigated these limitations by emphasizing rigorous analysis techniques and conservative interpretations. Nonetheless, our results provide evidence that a disruption in the surface EMG signal is present prior to symptomatic manifestations of the physiological effects of injected OP compounds and that the disruption is related to the time-course of exposure to OP compound. Further investigations should establish the causality and mechanisms.

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