CLINICAL OBSERVATIONS AND CLINICOPATHOLOGICAL RESPONSES OF SHETLAND PONIES ADMINISTERED OVERDOSAGES OF DICHLOROVOS

by

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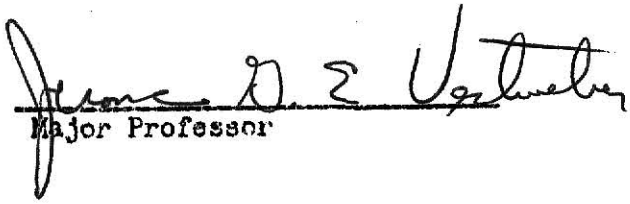

Major Professor

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INTRODUCTION

German scientists began investigating organophosphates as early as 1934. These scientists patented an organophosphate compound in 1937. Work in England was begun in late 1930's on organophosphates which were described as compounds they tested on animals, enzyme systems and themselves. By the end of World War II workers realized some of the insecticidal importance of these compounds and at this same time German research secrets were made available to other countries.

The anticholinesterase property of organophosphates was first discovered by a British group in 1941 while trying to explain the pupil constricting effects of DFP. They found that DFP behaved like eserine, a well known potent anticholinesterase, yet was even more potent than eserine.

The period from 1960 to 1970 was marked by a tremendous increase in work with new antiparasitic organophosphates because of their comparative safety and the resistance developed to chlorinated hydrocarbon insecticides.[19]

The early investigations of parasiticidal properties of organophosphates were primarily in ruminants. These were found to have limited utility as only the trichostrongylid parasites were amendable to organophosphate therapy. Organophosphates are excellent plasticizers for vinyl resins. This plasticizing ability allows the preparation of stable compatible formulations in a plastic vehicle. Dichlorovos, the subject of this paper, belongs to this family of organophosphate plasticizers that has been successfully incorporated with this plastic resin vehicle

allowing release in the gastrointestinal tract at a controlled level which can be adapted to many species. This preparation is now offered in food, encapsulated in gelatin capsules, impregnated in fly strips and flea collars. The plastic resin formulation of organophosphates has given us a very potent, broad spectrum, safe anthelmintic used in swine, dogs, zoo animals, ruminants, horses and even man.[12]

Documented information on the recovery rate of whole blood and plasma acetylcholinesterase (AChE) also called cholinesterase (ChE) in the equine species treated with the anthelmintic paste formula dichlorovos[a] is not complete. Research on other formulations of dichlorovos cannot be used as release time differs with particle size and preparation.[12]

Recovery of ChE in blood of foals was reported to be within normal range of controls in 14 days post treatment with dichlorovos by Albert et. al. Bello et. al. found whole blood and plasma ChE activity to be within normal limits at 21 days post dosage with dichlorovos. There was also conflicting reports in the literature as to the clinical manifestations when administering overdosages of dichlorovos.[1, 3, 4, 6, 12, 21, 29] Knowledge of clinical signs of organophosphate toxicity is important to the veterinary practitioner because of the dangers associated with depressed ChE from recent worming with anti-cholinesterase preparations and the additional use of ChE depressing tranquilizers, chemical restraints or anesthetics.[4, 6, 7, 8, 10, 12, 14, 21, 25, 28, 29] Presently there are safe effective ChE depressing anthelmintic drugs available for use by both veterinarians and horse owners. Their efficacy

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[a]Equigard P. F., SD 1750 7-12-405 P.F.H. Active Ingredient 15% DDVP. Shell Chemical Co., Agricultural Chemical Division, New York, N.Y.

has been well documented in the literature[1, 3, 5, 6, 7, 9, 11] but there is a noteable lack of information on the clinical signs due to accidental overdosage or time interval when whole blood or serum ChE returns to pretreatment levels following this overdosage of adult animals with preparations of dichlorovos. The purpose of this project was to determine the severity of signs of toxicity, to establish if and when a muscarinic blocking agent was necessary and to determine the length of time required for the plasma and whole blood ChE to reach pretreatment levels.

REVIEW OF THE LITERATURE

Cholinesterase

Historically an esterase is defined as any enzyme which hydrolyzes a carboxyester group. There are only two esterases that hydrolyze acetylcholine. Acetylcholine is the chemical mediator at the neuromuscular junction, the parasympathetic neuroeffector junction, all autonomic ganglionic junctions, the neuroeffector junction at the adrenal gland, and the sweat glands in some species. When the "packet" of acetylcholine is released by incoming stimulus it diffuses across the "gap" and stimulates an effector organ, muscle, or post ganglionic nerve fiber. To prevent overstimulation and to control this reaction, cholinesterase is present in excess destroying acetylcholine promptly. If the cholinesterase is inhibited, acetylcholine will accumulate causing at first an excessive stimulation, and finally complete disruption (or block) of the cholinergic system. The final result of this ChE inhibition would be (a) interference at the neuromuscular junction giving rise to fasciculation (rapid twitching) of voluntary muscles and finally paralysis of muscles of respiration; (b) interference with the autonomic nervous system at the cholinergic sites causing symptoms of excessive parasympathetic stimulation: pupil

constriction, excessive lacrimation and salivation, bronchiole constriction, central effects of incoordination and failure of the respiratory center.[2, 10, 13, 19]

Activity of Dichlorovos as a Cholinesterase Inhibitor

The hydrolysis of acetylcholine by cholinesterase is carried out very rapidly (.002 seconds) to form acetic acid and choline. There are several esterases but we are concerned in this study mainly with the two most prominent ones. The first is acetylcholinesterase, (true cholinesterase or cholinesterase I) which is found in nervous tissue, red cells, and human placenta. This type has the greatest activity against acetyl esters and is more pH sensitive than the second type (acetylcholinesterase II, the pseudocholinesterase or non-specific cholinesterase). Like cholinesterase I, cholinesterase II is distributed throughout the body but is found mainly in plasma, and has the greatest action against butyl esters.[2, 10, 19] Dichlorovos is an inhibitor of both the pseudocholinesterase and acetylcholinesterase. The mechanism of hydrolysis of acetylcholine to acetic acid and choline is regulated by the enzyme cholinesterase which includes both types of cholinesterases.[2, 10, 19] There are twenty-two known non-specific ACh hydrolyzing factors in man and horses and others are being investigated.[2]

The mechanism of hydrolysis is represented thusly:

acetylcholine + water $\xrightarrow{\text{HOH}\ /\ \text{ChE}}$ choline + acetic acid[2]

$$\begin{bmatrix} CH_2O - COOH_3 \\ \\ CH_2 - N(CH_3)_3 \end{bmatrix} + HOH \xrightarrow{ChE} \begin{bmatrix} CH_2OH \\ \\ CH_2 - N(CH_3)_3 \end{bmatrix} + CH_3COOH$$

The reaction resembles the classic enzyme substrate reaction except it is non-reversible.

$$\text{Enzyme} + \text{Substrate} \longrightarrow \text{Enzyme substrate complex} \downarrow \text{Enzyme} + \text{end products}$$

$$\text{ChE} + \text{ACh} \longrightarrow \text{Choline} + \text{Acetic Acid}^{2, 27}$$

In this reaction energy release is great and the reaction is irreversible. The effect of ACh on the heart mimmics vagal stimulation.[2, 10, 19]

In man, ox, sheep, goat and rabbit, red blood cells contain more ChE than plasma, whereas the horse, dog, cat and fowl all have the reverse distribution. This difference in ChE amount and distribution can be used to differentiate the equine from these other species. The horse not only has more plasma ChE activity but also has more non-specific substrates that hydrolyze cholinesterases.[2]

The mode of action of organophosphate compounds is inhibition of cholinesterases. Fortunately, the toxic level is reached much more easily in insects and nematodes than in mammals; consequently they are widely used as a control of both internal and external parasites of animals.[12] Phenothiazine and its derivatives potentiate this inhibition activity.[10, 11, 19, 21] In mammals, signs of toxicity with organophosphates are: 1) profuse salivation and lacrimation, 2) dyspnea, 3) urination and defecation, 4) twitching and fibrillation of skeletal muscles, 5) pupillary miosis, 6) convulsions of a clonic nature, and 7) prostration and death. The cause of death from organophosphate toxicity is asphyxiation due to four different mechanisms: 1) bronchoconstriction, 2) lowered blood pressure, 3) neuromuscular

block of the respiratory muscles, and 4) failure of respiratory centers.[13, 15, 19, 20] For the equine species, three major formulations and a technical grade of dichlorovos have been used. In general, the formulated material has a wider range of safety than the technical grade. The minimum toxic dose of the technical grade is 25 mg/kg of body weight orally.[12] At 50 mg/kg the technical grade caused mild toxicity in some horses but no deaths.[8, 13, 27] The gel formulation[b] of dichlorovos administered in oral dosage of 400 mg/kg of body weight in the equine produced no deaths and atropine sulfate was capable of blocking the muscurinic effects.[1, 10] The $L.D._{50}$ range of the resin pellet formulation[c] of dichlorovos is 800 to 1000 mg/kg of body weight orally.[12]

The gel formulation of dichlorovos is applied to the hard palate with a disposable dose syringe having a graduated plunger which corresponds to pounds of body weight of the horse being dosed. The release time of active ingredient (dichlorovos) in this formulation is so rapid it is only effective against first, second, and third instar bots (Gastrophilus intestinalis and G. equorum).[1, 3, 5, 7, 9, 11, 14]

The resin pelleted formulation of dichlorovis is administered in the grain ration. The active ingredient is released over a period of 6 hours. The pelleted formulation is effective against all type bots (Gastrophilus intestinalis, G. nasalis), large strongyles (Strongyles vulgaris, S. equinus, S. edentatus), small strongyles (genera Cyanthostomum, Cylicocercus, Cylicocyclus, Cylicodontaphorus, Tridontophorus, Poteruostomum, Gyalocephalus), pinworms (Oxyuris equi), and adult or mature Ascarids (Parascaris equorum) in horses other than suckling and young weanling foals.[4, 6, 9, 11, 14, 25, 26]

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[b]Equigel[R] Shell Chemical Co., San Ramon, California.

[c]Equigard[R] Shell Chemical Co., San Ramon, California.

Characteristics of Dichlorovos

The chemical names used for dichlorovos are Phosphoric acid 2, 2-dichlorovinyl dimethyl ester, O,O-dimethyl O-(2,2-dichlorovinyl) phosphate, and 2,2-dichlorovinyl dimethyl phosphate. Some of the proprietary names, synonyms and formulations are Atgard C (v-13), Atgard V (v-3), DDVP, Dichlorovos, Equigard V-4, Equigel, Vapona and Task.[16, 20, 23, 24, 25]

Workers in Louisiana found ChE levels of whole blood and plasma to be within the range of pretreatment levels 20 days following treatment with 10 and 20 mg/kg of paste formulations. No effect was noted on the blood.[3] Drudge et. al. treated 40 horses, 1 to 3 years of age, with doses ranging from 25 mg/kg to 100 mg/kg of resin pellet formulation in the feed. Palatability was variable as with any preparation that is offered in the feed. The negative responses were softening of the feces, marked but transient decrease in plasma cholinesterase activity and a mild hemoconcentration. Deleterious effects were not noted in pregnant mares or foals. Drudge treated horses with the resin pelleted formulation at the rates of 12.5 mg/kg/day for 2 days and 10 mg/kg/day for 3 days with the same results. Tracy et. al., working with horses found that erythrocyte ChE was depressed but no change occurred in plasma ChE levels following exposure to dichlorovos vapors for up to 25 days and no clinical manifestations of toxicity were observed.[26] Fowler treated 40 horses, ponies, and donkeys ranging in ages from 20 months to 21 years without toxic clinical signs following treatment at doses up to 45 mg/kg of the resin pelleted formulation.[9]

Albert et. al., treated foals with 20 times the therapeutic dosage of the gel formulation and observed no signs of abdominal pain

even though some foals went into convulsions. ChE blood levels on these foals returned to pretreatment levels in 14 days.[1]

Haas found the feed mixture of resin pelleted formulation the most efficacious for all species of susceptible parasites but like many other workers he found that some horses refused to eat it.[12] The gel formulation is ideal for administration but is only effective against bots (all instar stages) and, 4th instar ascarids.[3, 4, 5, 7, 11] The ideal preparation of dichlorovos is a combination of the gel formulation and the resin pelleted feed preparation designed for application to the hard palate as a paste thus combining the broad spectrum of the resin pelleted formula slow release with the quick acting gel formula in a convenient disposable syringe. This combination is the paste formulation of dichlorovos, the experimental drug in this study.

MATERIALS AND METHODS

Trial I consisted of three female Shetland ponies, five to seven years old, weighing respectively 154 kg, 144 kg, 166 kg for evaluation and recording of clinical signs following administration of 80 mg/kg of body weight of dichlorovos paste formula orally (2.5 times the recommended dosage). The ponies were maintained on a diet of prairie hay and alfalfa hay and were in good physical condition.

Blood was collected from the left external jugular vein in which an indwelling cannula[d] had been inserted and sutured in place. This blood was used for analysis of plasma and whole blood cholinesterase.[16, 18] The cannula was rinsed with .1 ml. of 10% EDTA after each sample

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[d]Bardic Deseret Angiocath 3½" x 14 ga C.R. Bard, Inc. Murray Hill, New Jersey.

to prevent clotting in the cannula.

Intestinal sounds were monitored with Heart Sounds Amplifier.[e] The diaphragm was pressed under a wide rubber band which was around the pony to hold it on the previously shaven left paralumbar fossa. This was used to record and audit small intestinal movement, possibly some small colon movement, and splenic engorgement. Rectal temperature was measured by a rectal probe and amplified through a Carrier Preamplifier.[f] The rectal probe was inserted approximately three feet into the rectum and colon. Respiration was recorded by an Impedence Pneumograph[g] attached mid-rib at about the eighth rib on each side of the animal. An EKG recording was made using an inverted rhythm lead with the positive bipolar lead attached to the skin on the left shoulder point, the negative bipolar lead was attached to midsternum skin. Four channels of a channel Physiograph[h] was used to record the data with a paper speed of .20 cm per second for two hours.

Blood was taken from each horse prior to oral administration of paste formula dichlorovos for determining the pretreatment ChE level. Following administration additional blood samples were collected at 5 minute intervals for the first hour and every 15 minutes until all clinical signs stablized. Each 5 ml. blood sample was collected in a B-D Vacutainer TM[i] containing 0.1 ml. of 10% EDTA. The samples were

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[e]E & M Instrument Co., Ser. No. 129, Houston, Texas.

[f]E & M Instrument Co., Ser. No. 668, Houston, Texas.

[g]E & M Instrument Co., Ser. No. 2065, Houston, Texas.

[h]E & M Instrument Co., Physiograph MK, Ser. No. 480, Houston, Texas.

[i]Becton Dickinson & Co., Columbus, Nebraska.

refrigerated at 30°C until tested for whole blood and serum ChE activity.[18]

In Trial II six ponies (four treated and two controls) were used to determine the recovery rate of whole blood and plasma ChE levels following oral dosage with 80 mg/kg of dichlorovos. Two treated ponies were exercised and two treated ponies were rested for a comparison of whole blood and plasma ChE activity.

All ponies were penned in individual pens for 24 hours before the trial and were maintained on a diet of alfalfa and prairie hay. All were in good physical condition.

Blood samples were obtained 48, 36, 24 and 12 hours pre-treatment in order to establish pre-treatment blood levels of ChE. Simultaneous with the fourth pre-treatment blood sample an indwelling cannula was placed in the left jugular and sutured in place to facilitate more frequent sampling. Blood samples were taken at 5, 15, 45 minutes post treatment (paste formula of dichlorovos) from all ponies and also at 2, 6, 18, 48, 96, 192 hours and then continued at 96 hour intervals until whole blood and plasma ChE levels approached pre-treatment levels. The blood samples were analyzed for whole blood and plasma ChE by the modified Michel method.[18]

The exercise schedule was as follows: Pony number 131 (untreated control) was rested, Pony 132 (untreated control) was exercised, Ponies 133 and 134 were treated and exercised, Ponies 135 and 136 were treated and rested. The ponies to be exercised were tied to an exercise walker while the rested ponies were loosely confined in the immediate area. Exercise consisted of a fast walk or occasional trot for two hours (See Table 2). The exercised ponies were stopped only long enough to draw

blood samples and the entire group closely observed for changes in clinical signs for 6 hours. All ponies were observed for signs of colic, character and frequency of defecation, urination, pupillary response, muscle fasciculation and perspiration.

The modified Michel method was utilized to determine the whole blood and plasma ChE activity.[18] 0.25 ml. of sample of each whole blood and plasma was diluted with 5 ml. of Michel buffer and pH determined with a pH meter.[j] One ml. of 0.165 molar acetylcholine chloride was added to the solution and incubated for one hour. The change in pH was again determined and ChE activity was determined as a percent of the pre-treatment levels of ChE.

RESULTS

Trial I

Three ponies were observed for clinical signs of organophosphate toxicity after 2½ times the recommended treatment of dichlorovos paste formula. Pony number 311 had an average heart rate of thirty-six beats per minute during the trial. The gastrointestinal sounds were not audible on the preamplifier speaker for fifteen minutes post treatment, although there was a five fold increase of activity on the physiograph recording than the pretreatment period. The horse defecated formed stool in small amounts at five minutes, ten minutes, and again at forty minutes post treatment. The forty minute defecation was followed by a small amount of fecal fluid. Finally at ninety minutes following treatment this horse strained and passed only scant fluid with enough feces

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[j]Orion Research Ionanalyzer Model 601, Cambridge, Mass.

to stain it green. There was some straining and rectal eversion following the final defecation. Gas was passed repeatedly starting at fifteen minutes following treatment with intensified flatus from fifty to seventy-five minutes. Her heart rate remained essentially normal (36/min.) with a barely perceptible slowing over predosage. Signs of apprehension appeared twenty minutes following treatment which was manifested clinically by a raised head, extended nose, and dilated nostrils. Flank fasciculation (involuntary skin and muscle tremors) started at this time and lasted about five minutes. All signs were normal at two hours. See table 1.

Pony number 312, began to show signs of apprehension five minutes post treatment. Signs consisted of extended head, dilation of nostrils, snorting and stomping. Borborygmus was noted between ten and fifteen minutes post treatment and was more frequent but not nearly as loud as pony number 311 and 313. The flank fasciculations began at twenty minutes post treatment and also during this period exhibited hyperpnea. She often turned to look at her flank. These signs were intermittent from twenty to fifty-five minutes post treatment. She defecated formed stool followed by scant fecal fluid at ten minutes post treatment, gas and fluid at forty-five minutes post treatment in very small amounts, and at sixty minutes post treatment had a bowel evacuation that was propelled about four feet to the wall behind her. The evacuation consisted of fluid, liquid feces, and gas. There was straining, rectal eversion, and abdominal pressing for one minute. The rectal probe was pushed out. The heart rate remained at thirty-five beats per minute with no change in the EKG.

Pony number 313, the borborygmus was audible and flatus observed

five minutes post treatment. Formed feces was followed by semi-solid feces in small amounts at six minutes post dosage. Because of abdominal straining the rectal thermometer was passed out of the rectum. At ten minutes post dosage a semi-solid stool occurred with sufficient force to reach a wall four feet from the annus. There was projectile defecation at fifteen minutes post dosage. The intestinal sounds were sufficiently strong to dislodge the marking pen from its mounting. There were five defecations in a seventy-five minute period post treatment, with the final bowel movement at seventy-five minutes. This consisted of fecal fluid slightly stained, preceded and followed by gas. Borborygmus was constant in this horse from the initial time of treatment to one hour and forty-five minutes later. Audible gut sounds were noted at five minute intervals. Fairly evenly spaced flatus was noted seven times during the two hour period. Pony number 313 was the only one with a change in heart rate. This change was from an average of thirty-six beats per minute to ninety-eight beats per minute during one period of intense borborygmus. The increased rate lasted for about 30 seconds (Fig. 1). There were several other slight heart rate increases during the recording period, but this was the most pronounced.

Increased urination, salivation, rectal temperature increase or lack of pupillary response were not noted in any of these ponies (table 1).

The whole blood and plasma ChE levels were plotted as median percent activity of all three ponies (Fig. 2). The greatest depression of both whole blood and plasma ChE was within the first 10 minutes post treatment. The greatest depression of the plasma ChE was to a low of 15% of normal activity at 10 minutes post treatment followed by another low

of 20% of normal activity at 40 minutes. The activity of plasma ChE stayed below 70% for 2 hours. This trial was concerned mainly with clinical signs and because these had returned to normal no further blood tests were taken.

Trial II

Six ponies (four treated and two controls) were monitored for ChE activity either following exercise or non-exercise after treatment with 2½ the recommended dosage of dichlorovos paste formula. The clinical signs were not exaggerated except for pony number 136, a non-exercised pony that started salivating at 5 minutes post treatment. These signs lasted 40 minutes. At 5 minutes post treatment perspiration was profuse and this lasted for 25 minutes at which time shivering started; this continued for two hours post treatment. At 7 minutes post treatment a lip twitch was noted; this was followed shortly by a flank twitch which progressed over the entire body and terminated in a clonic spasm which lasted 12 minutes. Signs of abdominal discomfort appeared at 16 minutes post treatment and was manifested by attempting to kick her abdomen, attempting to lie down, and turning of the head toward the flanks. Defecation occurred five times and was a very fluid, forceful, scanty feces. At 30 minutes post treatment this pony was relaxed and comfortable except soaked with perspiration and shivering. Rectal temperature was not taken during this time. All treated ponies had increased quantity or frequency of defecation, some muscle twitching and only two horses, 133 and 136, had a slight increase in salivation (table 2).

Pony number 136 had plasma ChE activity depressed to a low of 2% at 5 minutes post treatment. There was a mean plasma ChE depression

of all horses of 10% at 5 minutes post treatment (Fig. 3). Two hours post treatment the mean plasma ChE activity of all horses had risen to 22%. This was followed by another depression of plasma ChE activity at 48 hours post treatment to 18% with an irregular rise in plasma ChE activity to 63%, 768 hours post treatment. All whole blood and plasma ChE readings were significant through 768 hours post treatment (Table 3&4)

The whole blood ChE followed very closely the plasma ChE readings except the depression of activity was less. Whole blood ChE was depressed to 4 percent activity at 5 minutes post treatment in pony number 134. The mean of whole blood ChE activity in all treated horses at 5 minutes post dosage was 17% (Fig. 4). At 2 hours post treatment this mean had risen to 41%. The next low point in depression of whole blood ChE activity occurred at 48 hours post treatment when the mean was 30% (tables 3 and 4). Post treatment ponies exhibited a significant difference of whole blood ChE from undosed horses during the period from the 5 minute reading to the 864 hour reading (Fig. 4). The 1512 hour whole blood and plasma ChE samples showed no significant difference between the post treatment samplings and the pre-treatment and control samplings.

DISCUSSION

Mild abdominal discomfort to a variable degree is usually seen on administration of dichlorovos paste formula at doses exceeding those recommended by the manufacturer.[1] The discomfort usually consists of a predictable series of events taking place within 10 minutes after treatment. Dichlorovos was administered at two and one-half times the recommended dosage to 7 adult shetland ponies. The signs of discomfort

were flank muscle and skin fasciculations, apprehension, increased intestinal sounds, increased defecation and in one pony clonic spasms, perspiration, colic, and forceful defecation. No significant lacrimation or salivation was observed. Horses monitored for heart and respiration rate, rectal temperature and intestinal motility exhibited only abnormal hypermotility of the gastro-intestinal tract consistently. There were individual variations of the heart rate but there was no temperature change or heart rate decrease. Respiratory rate remained constant in all cases.

Plasma cholinesterase activity was always depressed to a greater degree than whole blood ChE activity. The greatest depression was within 5 minutes post treatment where the mean plasma ChE activity of the experimental group dropped to 10% of normal (Fig. 3, 4, 5). This would correspond to the gel fraction of the paste formula of dichlorovos. Following the initial depression there was a slight irregular rise in plasma ChE values until 48 hours post treatment when the activity was depressed to 18% of normal which would correspond to the release of the pelleted fraction of paste formula of dichlorovos compound. After 48 hours post treatment there was a steady irregular increase of plasma ChE activity to 98% of normal activity at 1412 hours or 63 days.

The whole blood ChE activity was depressed less than the plasma ChE activity with the lowest whole blood ChE activity of 17% occurring at 5 minutes post dosage followed by another low of 30% whole blood ChE at 48 hours. Whole blood ChE activity reached 100% 63 days post treatment.

There was little correlation between the percent activity of cholinesterase in the blood and clinical signs. This was demonstrated

by the fact that pony number 136 displayed signs of organophosphate toxicity but the whole blood and plasma ChE activity were never depressed below 26% and 19% respectively while with pony number 135, the whole blood and plasma ChE activity were depressed to 14% and 2% respectively and no clinical signs were observed. This lack of correlation was found by Bennett et. al.[4]

The irregularity of the ChE levels starting at 384 hours post treatment (Fig. 3 and 4) and continuing through 768 hours post treatment were due to outside influence and not drug related because the control horses were also affected. The net effect of this irregularity was a sudden depression in both whole blood and plasma ChE activity at about 400 hours post treatment. The whole blood ChE depression was more than the plasma ChE depression which is the reverse of the whole blood plasma ChE response to dichlorovos. This pattern resembles the findings of Shri et. al.[22] in their work with the insecticide cholinesterase inhibitor thiophosphate. Other commonly used drugs which affect ChE activity are quaterinary amines and tertiary amines. These were eliminated as causes because there was no evidence of their use but the alfalfa hay was purchased from various dealers and could easily have been contaminated by organophosphates commonly used as insecticides in alfalfa.

Adrenalin at high levels is reported to augment plasma and whole blood ChE.[2, 3] This response was manifested by ponies number 131 and 133 used by basic science students as palpation ponies on day 21 post treatment. These ponies exhibited a rise in the plasma ChE but no change in the whole blood ChE. Control pony, number 131 was very excitable, while the other control pony, number 132, was very calm. Every change in the

handling routine was reflected in the plasma ChE of pony number 131 to a greater degree than in pony number 132.

There was no significant difference in whole blood and plasma ChE activity and clinical signs of the exercised or rested ponies.

The time required for the ponies used in this experiment to reach 100% of their pre-treatment ChE activity was sixty-three days for both whole blood and plasma. At thirty days the mean for all ponies was 63% normal activity for plasma ChE and whole blood ChE activity was 64% of normal activity. All clinical signs in these ponies remained normal after the first two hours post dosage through the entire experiment period. The efficiency of the drug was not tested in this experiment but the safety was checked by whole blood and plasma ChE activity and clinical signs observed. Dichlorovos given at the manufacturer's recommended dosage level should be considered safe, easily administered, and convenient to handle as compared to conventional tube worming.

SUMMARY

Seven Shetland ponies were orally administered paste formula dichlorovos at 80 mg/kg or two and one-half times the recommended dose. Three of the ponies were monitored for clinical signs of toxicity with a physiograph and four were assayed for whole blood and plasma cholinesterase activity for 63 days. The clinical signs of organophosphate toxicity were minimal except for one pony. Mean whole blood and plasma cholinesterase activity was depressed to 10% of normal within the first 10 minutes post treatment. The recovery of whole blood and plasma cholinesterase activity was irregular, reaching pretreatment levels in

63 days. There was no differences in cholesterase activity and clinical signs of ponies exercised versus ponies rested post treatment.

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APPENDIX

TABLE 1

Group I

Clinical signs observed in Shetlands after overdosing with dichlorovos[a] and recorded on a Physiograph

Horse No.	Increased Urination	Increased Defecation	Muscle Fasciculations	Increased Heart Rate	Increased Salivation	Increased Apprehension	Pupillary Response
311 154 kg female	None	5 min. normal 10 min. normal 40 min. semi solid 90 min. fluid	40 min. flank fasciculations through 44 min.	None	None	20 min. through 25 min.	None
312 144 kg female	None	10 min. stool plus fluid 45 min. flatus and fluid 60 min. forceful fluid	20 min. flank through 25 min.	None	None	5 min. through 25 min.	None
313 166 kg female	None	5 min. flatus 6 min. normal plus fluid 10 min. semi solid forceful 15 " 18 " 20 " 40 " 75 "	15 min. flank through 20 min.	36 to 98 for 30 seconds 25 min.	None	5 min. through 15 min.	None

All times are minutes post treatment.

TABLE 2

Group II

Clinical signs observed in Shetland ponies overdosed with dichlorovos[a] and controls

Horse No.	Increased Salivation	Abdominal Distress	Increased Defecation	Increased Urination	Pupillary Response	Muscle Fasciculation	Increased Perspiration
131 Control rest	None	None	5 min. normal 22 min. normal	15 min. normal vol.	None	None	None
132 Control exercise	None	None	6 min. normal	None	None	None	None
133 Treated exercise	15 min. transient slight	None	10 min. fluid 18 min. normal 28 min. normal 60 min. normal	48 min. normal vol.	None	lip 30-40 min. flank 18 min.	None
134 Treated exercise	None	None	30 min. fluid 70 min. solid 90 min. solid	59 min. normal vol. 70 min. slight vol.	None	lip 40-45 min.	None
135 Treated rest	None	None	10 min. solid 15 min. solid	None	None	None	None
136 Treated rest	5 min. transient slight	16 min. through 39 min. attempt to lay down 30-39 min.	10 min. fluid 16 min. fluid 21 min. fluid 31 min. fluid 40 min. fluid-forceful	18 min. normal vol. 33 min. normal vol.	None	lip 7-28 min. flank 18 min. combine through 30 min. spasms 27 through 39 min.	5 min. shivering at 40 min.

Times are minutes post dosage.

TABLE 3

Group II

Percent cholinesterase activity in plasma of control and dichlorovos[a] treated Shetland ponies (80 mg/kg body wt.)

Horse #	Hours Post Treatment .08	.25	.75	2	6	18	48	96	192	288	384	480	576	672	768	864	1512
Control									Percent Activity								
131	119	108	118	112	110	115	114	118	115	104	108	106	102	96	99	101	94
132	97	102	104	103	96	102	97	93	97	96	108	103	91	89	86	99	101
Mean	108	105	111	108	103	109	105	105	106	100	108	104	96	93	93	100	98
STDEV	15	4	10	6	10	9	12	17	13	6	0	2	8	5	9	2	5
S.E.M.	11	3	7	4	7	7	9	12	9	4	0	2	6	4	6	1	3
Treated																	
133	5	35	45	29	37	19	16	29	44	59	70	65	73	65	68	86	99
134	13	18	30	26	28	16	16	17	32	44	59	66	59	54	56	79	99
135	2	3	2	1	24	14	20	24	45	67	80	73	75	74	68	91	97
136	19	15	29	30	35	23	19	21	42	46	64	69	65	62	59	58	96
Mean	10	18	26	21	31	18	18	23	41	54	68	68	68	64	63	79	98
STDEV	8	13	18	13	6	3	2	5	6	11	9	3	7	8	6	14	1
S.E.M.	4	6	9	7	3	2	1	3	3	5	5	2	4	4	3	7	1
T Test	11**	9**	6**	8**	12***	19***	16***	10***	9***	5**	6**	12***	4*	4*	5*	2	0

*P. .05 Significant.
**P. .01 Highly significant.
***P. .001 Very highly significant.

TABLE 4

Group II

Percent cholinesterase activity whole blood of control and treated dichlorovos[a] treated Shetland ponies (80 mg/kg body wt.)

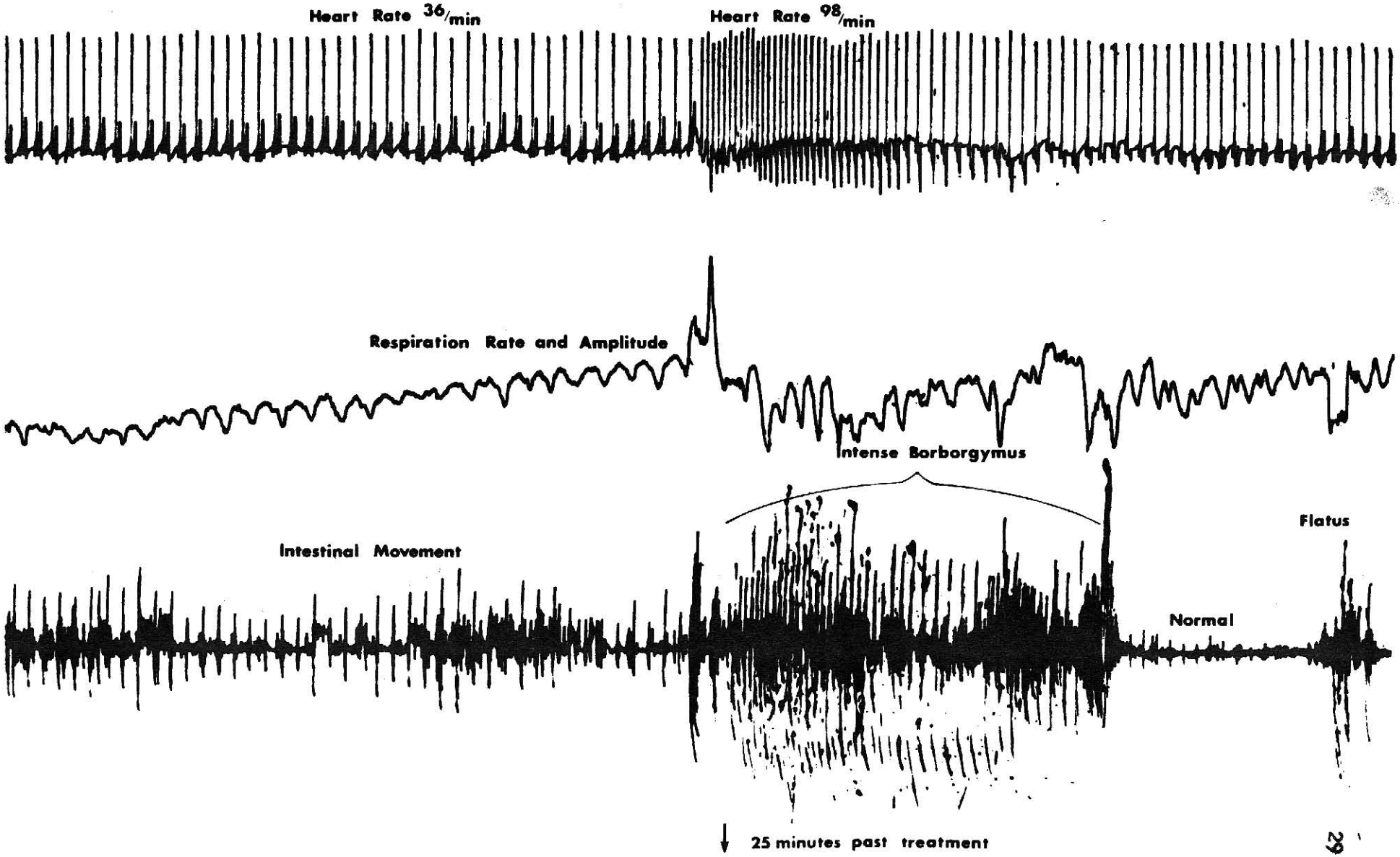
Horse #	Hours Post Treatment .08	.25	.75	2	6	18	48	96	192	288	384	480	576	672	768	864	1512
Control								**Percent Activity**									
131	108	106	110	106	120	115	110	99	90	98	108	101	96	97	92	100	107
132	98	104	103	100	96	96	95	95	96	95	107	88	88	92	91	100	102
Mean	103	105	107	103	108	105	103	97	93	97	107	95	92	94	91	100	104
STDEV	7	1	5	4	17	14	11	3	4	2	1	9	6	4	0	0	3
S.E.M.	5	1	4	3	12	10	8	2	3	2	0	6	4	3	0	0	2
Treated																	
133	24	59	65	51	59	39	34	41	50	58	68	44	62	68	76	89	108
134	4	24	46	28	74	70	37	31	48	50	50	64	63	55	59	90	106
135	14	33	41	43	32	22	27	27	41	55	64	59	60	63	61	81	94
136	26	30	46	42	50	27	23	24	36	47	50	58	57	55	59	82	94
Mean	17	37	49	41	54	39	30	31	44	52	58	55	60	60	64	86	100
STDEV	10	15	11	10	18	21	6	7	6	5	10	9	3	6	8	5	7
S.E.M.	5	8	5	5	9	11	3	4	3	3	5	4	1	3	4	2	4
T Test	11***	5**	7**	8**	4*	4*	11***	12***	10***	11***	7**	5**	10***	7**	4*	4*	1

*P. .05 Significant.
**P. .01 Highly significant.
***P. .001 Very highly significant.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

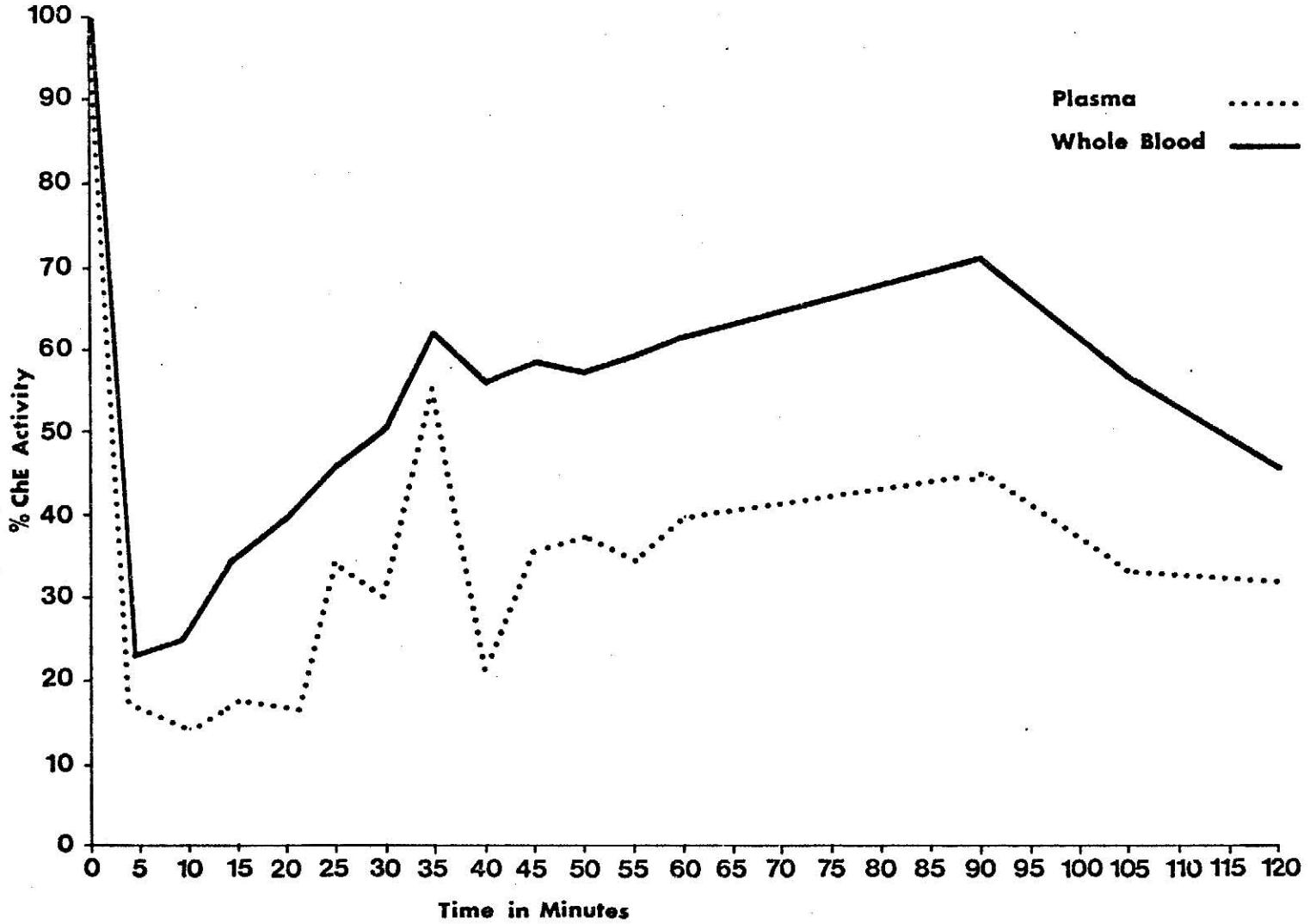
THIS IS AS RECEIVED FROM CUSTOMER.

figure 1



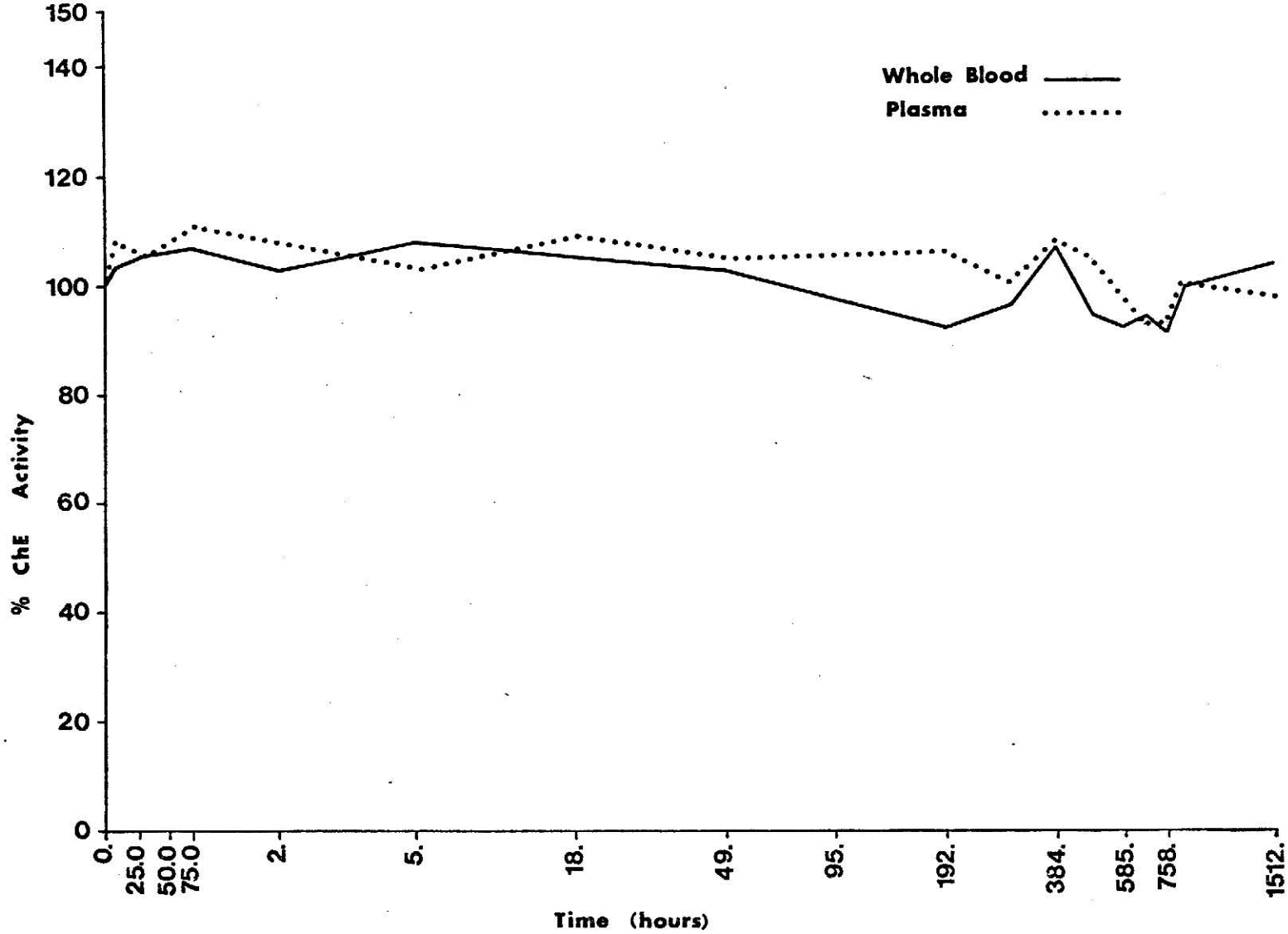
Physiograph Recording of Shetland Pony No. 313 (treated 80mg/kg body wt. dichlorovos[2])
paper speed .20 cm. per second

figure 2



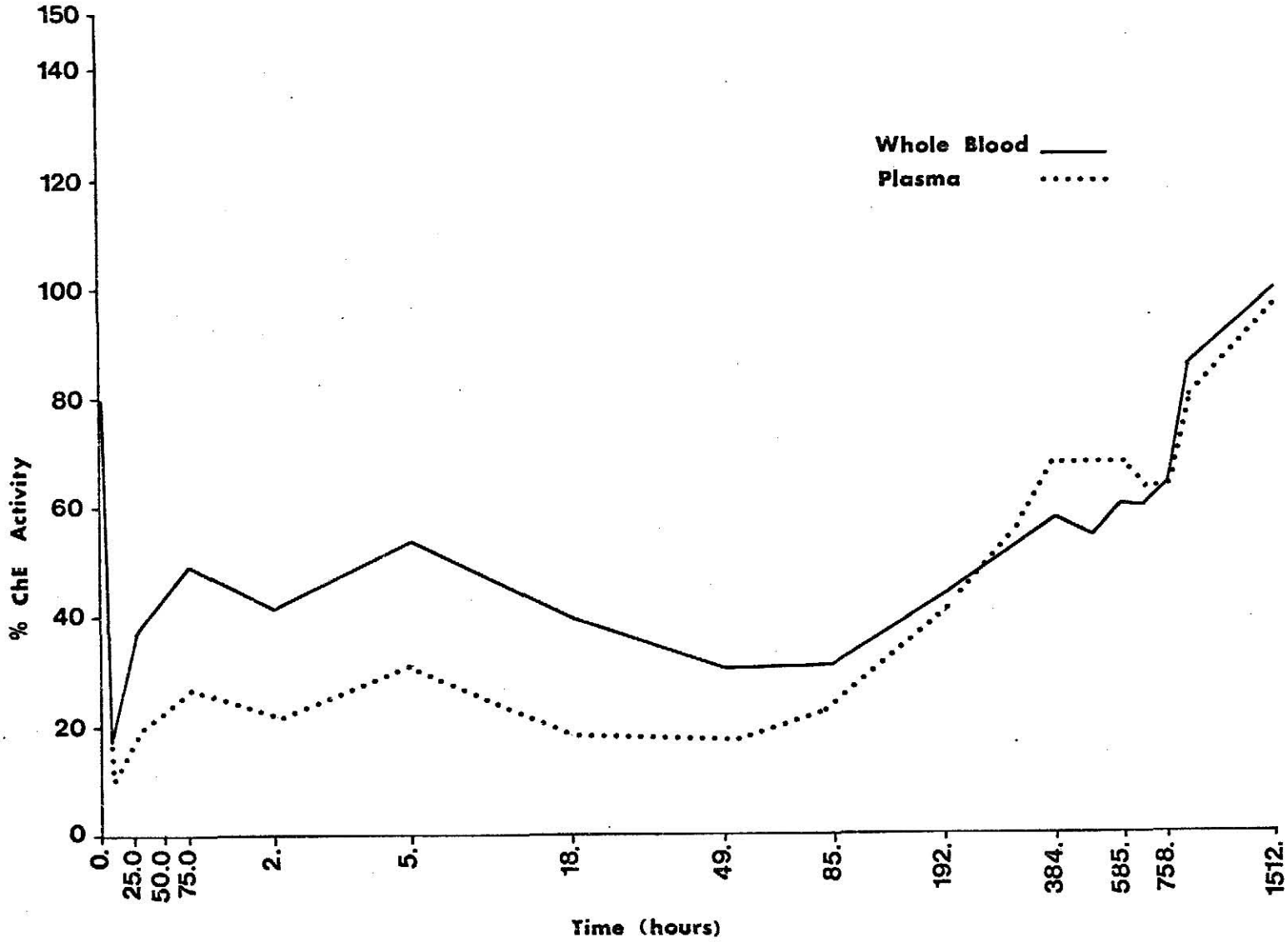
Means of Treated Shetland Ponies Group I ($^{80\ mg}/_{kg}$ body wt. dichlorovos[2])

figure 3



Means of Control Shetland Ponies Group II

figure 4



Means of Treated Shetland Ponies Group II ($^{80mg}/_{kg}$ body wt. dichlorovos[2])

CLINICAL OBSERVATIONS AND CLINICOPATHOLOGICAL RESPONSES OF SHETLAND PONIES ADMINISTERED OVERDOSAGES OF DICHLOROVOS

by

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Kansas State University, 1970

AN ABSTRACT OF A MASTER'S THESIS

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1975

ABSTRACT

Present preparations of dichlorovos have limitations which render them undesirable in a complete parasite control program for horses. The gel formulation[a] of dichlorovos has limited antihelmintic activity because of rapid release. The resin pelleted formulation[b] of dichlorovos offers broad activity; however, when offered in the feed, horses often refuse to eat it. A new combination[c] of the pelleted formulation[b] of dichlorovos and the gel formulation[a] of dichlorovos was evaluated for clinical signs of abdominal discomfort and cholinesterase (ChE) inhibition of both whole blood and plasma. The combination[c] was administered at two and one half times the recommended dosage to nine shetland ponies. Whole blood and plasma cholinesterase values were measured for percent activity. Clinical signs were monitored with physiograph recording of intestinal sounds, heart rate, temperature and respiration.

The only consistently significant change in clinical signs was hypermotility of the gastrointestinal tract. Maximum depression of plasma ChE activity was 2% of normal at five minutes post-treatment and whole blood ChE was 4% of normal at five minutes post-treatment. The time required to reach control and pretreatment ChE levels was sixty-three days.

Toxic signs were minimal except for one pony which exhibited classical signs of clonic spasms, perspiration and abdominal distress even though the blood level of ChE was not inhibited in this pony to the extent of others which showed no signs of toxicity. The blood level of

cholinesterase is of little diagnostic value in correlation with clinical signs of toxicity. There was no difference in clinical signs or ChE activity of ponies exercised or rested post-treatment in this trial.

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a. Equigel[R], Shell Chemical Co., San Ramon, Cal.

b. Equigard[R], Shell Chemical Co., San Ramon, Cal.

c. Equigard P. F., S. D., 1750, 7-12-40-5, PFH, 15% DDVP, Shell Chemical Co., New York, New York.