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The Influence of Various Stress Conditions on Cholinesterase Levels of Cattle Treated with an Organophosphorus Insecticide

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THE INFLUENCE OF VARIOUS STRESS CONDITIONS ON CHOLINESTERASE LEVELS OF CATTLE TREATED WITH AN ORGANOPHOSPHORUS INSECTICIDE

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

THE INFLUENCE OF VARIOUS STRESS CONDITIONS ON CHOLINESTERASE LEVELS OF CATTLE TREATED WITH AN ORGANOPHOSPHORUS INSECTICIDE

Abstract

Mitchell J. Wrich

Under the supervision of Dr. Paul H. Kohler

Six-to 8-months-old Hereford heifers categorized as grubby or grub-free calves were used for this research. Forty calves were purchased in 1968 and also in 1969. The grubby calves were obtained from Highmore, South Dakota, an area where calves have a history of heavy grub infestation of both Hypoderma bovis (L.) and Hypoderma lineatum (de Villers). The grub-free calves were purchased in Fargo, North Dakota. Calves raised in this area seldom are infested with cattle grubs. Specified groups of calves were subjected to 30 minutes of continuous exercise or the withholding of feed and water for 24 hours prior to treatment with a pour-on formulation of fenthion. The exercise and ration abstinence simulated stress conditions common to many livestock regions. Pretreatment and posttreatment jugular vein blood samples were evaluated to determine the effects of stress and fenthion on blood cholinesterase (ChE).

According to statistical analysis, exercise and feed and water abstinence had little influence on ChE levels. Insecticide treatment produced the most consistent and significant variation in ChE. Fluctuations in ChE levels suggest that

fenthion absorption occurs within 24 to 48 hours following treatment. Generally, insecticide influence was apparent throughout each of the 4 research phases in 1968 and 1969. Cattle origin and year also contributed to major ChE depression.

Animal toxicosis was minimal throughout this study. One calf did display typical subacute organophosphate side effects. Animal reaction to the insecticide climaxed at 26 hours posttreatment and recovery was uneventful.

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INTRODUCTION

Two species of cattle grubs, Hypoderma lineatum (de Villers) and Hypoderma bovis (L.), exist in the United States. Economic losses attributed to these cattle parasites amount to millions of dollars annually. Attempts to control cattle grubs utilizing insecticides date back to the late 1800's. During the first five decades of 1900 the insecticide rotenone provided some grub control but generally it was unsatisfactory. Cattlemen and scientists recognizing the need for achieving better grub control evaluated hundreds of chemical compounds during this same period.

In 1956, Lindquist (1956) announced that ronnel (O,O-dimethyl O-2,4,5-trichlorophenyl phosphorothioate), a new organophosphate insecticide, was toxic to cattle grubs within their host. Subsequent research with ronnel and other organophosphorus insecticides demonstrated that these chemicals functioned as anticholinesterases and occasionally caused direct and indirect animal toxicosis. Direct toxicosis results following application of higher than recommended chemical concentrations. Research also indicates that animals subjected to various stress factors, including transportation, weaning, castration, and certain feed rations are more susceptible to toxicosis. Dying and decomposing cattle grubs within treated animals, chemical substances, or metabolic end-products produced by toxified

grubs, often promote antiphylatic-type reactions or indirect toxicosis.

Several systemic organophosphorus insecticides are currently registered with the United States Department of Agriculture (U.S.D.A.) for use in controlling cattle grubs. Insecticidal efficacy and toxicological data assembled for each chemical entity prior to registration for commercial use are almost noncomprehendible. The literature is far less extensive relative to the effects of various stress factors affecting cattle treated with a systemic insecticide.

Fenthion, (O,O-dimethyl O-[4-(methylthio)-m-tolyl] phosphorothioate), an organophosphorus insecticide, is a promising new systemic insecticide. This investigation was conducted to determine what effect various pretreatment stress factors have on blood cholinesterase at specific intervals following treatment of cattle with fenthion.

REVIEW OF LITERATURE

Cattle grubs have been considered economically important cattle parasites ever since this insect was introduced into the United States from Europe during the early 1800's. In 1889, it was estimated that the livestock industry lost about 3 1/2 million dollars because of cattle grubs (Riley and Howard, 1889). Pfadt in 1962 placed losses due to cattle grubs at 300 million dollars.

Both the immature or larval stage and adult stage inflict economic livestock losses. Larvae cause damage by destroying connective tissue and viscera as they migrate toward the back. Once larvae reach the back they cut breathing holes in the hide and in doing so destroy choice leather (Smith 1948, Scharff 1950, Metcalf et al. 1951, Laake and Roberts 1952, Pfadt 1962, Khan 1969). When cattle infested with grubs are sent to slaughter, encysted grubs must be trimmed from choice meat, reducing marketable meat (Riley and Howard 1889, Smith 1948, Roberts and Lindquist 1956). Haufe et al. (1966), summarizing Cunkleman's (1966) comments relative to hide damage and meat trim, reported the need for increased efforts to control cattle grubs.

Damage caused by adult warbles, more commonly referred to as heel flies, is indirect as they neither bite, sting nor chew their victims. The mere presence of a heel fly hovering or flying in the immediate vicinity of cattle makes

the livestock extremely nervous, resulting in running, standing in water, hiding in tall grass or brush thus reducing grazing (Roberts and Lindquist, 1956). In addition, cattle are often seen running across the pasture with their tail held high over their back in an attempt to avoid oviposition by the female heel fly (Metcalf et al. 1951); this is referred to as "gadding" (Kohler 1959, Khan 1969).

Even though female heel flies do not bite, sting or chew their victims, it is not completely understood why they cause gadding. Kohler (1959) reported that gadding may be due to the buzzing of the female flies or a tickling sensation caused by the attachment of eggs to hairs during oviposition. Regardless of the cause of gadding, Metcalf et al. (1951) and Pfadt (1962), have reported that during the heel fly season cattle graze less which in turn reduces feed efficiency and consequently lowers meat and milk production.

Cattle grubs in their adult stage are called heel flies primarily because they oviposit on the hair of the lower portions of the hind legs of cattle. Oviposition is not restricted to this area. It occasionally occurs on the upper portions of hind legs and lower hind flanks and stomach, especially on the escutchian (Scharff 1950, Roberts and Lindquist 1956, Pfadt 1962).

Two species of cattle grubs are present in the United States and both occur in South Dakota. They are the common

cattle grub Hypoderma lineatum (de Villers) and the northern cattle grub Hypoderma bovis (L.). Osborn (1896), Bishopp et al. (1949), Scharff (1950), Lofgren et al. (1954), and Pfadt (1962) have discussed the biology of both H. lineatum and H. bovis in detail. The life cycles of both species are similar but there are small differences. Eggs of H. lineatum are attached in rows to individual hairs as though they were stacked one on top another. In contrast, the eggs of H. bovis are laid one at a time and are attached to hairs at random. This species is more responsible for gadding, making deposition of more than one egg at a time difficult.

Heel fly eggs hatch in approximately 5 days. The newly hatched larvae burrow into the animals skin at the base of the hair where the respective eggs hatch. Hypoderma lineatum larvae migrate through connective tissue to the esophagus where they remain for several months after which they journey to subdermal tissue of the back and become encysted. Larvae are in the first instar during this phase of their life cycle. Once encystment occurs, larvae molt and transform into second instar larvae. The last instar, the third, is also spent in subdermal encystment. By contrast, H. bovis larvae reach subdermal encystment areas in the back by migrating first to the spinal canal. Larvae of both species cut breathing holes through the skin after they reach the back area. Contrary to what many people

think, encysted grubs breathe through spiracles located in the caudal rather than the cephalic end of the grub. Larvae remain in the back for 60 to 90 days and then emerge from their cysts, drop to the ground and pupate in the soil or trash among the grass (Scharff 1950, Roberts and Lindquist 1956, Pfadt 1962).

Several products have been employed attempting to control cattle grubs but most were ineffective until the advent of the insecticide rotenone (Roberts and Lindquist 1956). Rotenone is found in the roots of derris and cube plants. When roots are ground up, the finished product contains approximately 5% rotenone. This material is mixed with water and is applied as a spray or wash to the backs of cattle or as a dip treatment approximately 35 days following encystment of the first grubs. Even though rotenone is toxic to encysted cattle grubs, it only provides 60% to 80% control; occasionally 100% control is reported (Wells et al. 1922, Snipes et al. 1948, Laake and Roberts 1952).

During the early 1940's and 1950's when cattle numbers were increasing in the United States (Haeussler 1952), livestockmen and scientists increased their efforts to control cattle grubs with spray, washes and dips containing rotenone but results were variable. Scharff (1950) and McGregor et al. (1952) both reported the ineffectiveness of rotenone in providing 100% grub control; their investigations showed

only about 75% grub control. Lofgren et al. (1954) reported only 43% to 95% grub control in South Dakota field trials. In addition, Lofgren reported that rotenone seemed more toxic to H. lineatum than to H. bovis. Scharff (1950) also reported that rotenone sprays were less toxic to H. bovis.

Concentrated efforts to control cattle grubs with rotenone, paralleled by lack of satisfactory control with this product, demonstrated the need for a better insecticide. During the early 1950's Lindquist coordinated systemic insecticide research at United States Department of Agriculture (U.S.D.A.) Laboratories in Kerrville, Texas, and Corvallis, Oregon. In 1956, Lindquist reported on the results of a new livestock organophosphate insecticide, ronnel, (O,O-dimethyl O-[2,4,5-trichlorophenyl] phosphorothioate). Subsequent research by numerous scientists resulted in Federal registration of ronnel for cattle grub control. Other systemic organophosphates that have been registered for this purpose include coumaphos, (O,O-diethyl O-(3-chloro 4-methyl-7-coumarinyl) phosphorothioate); famphur, (O-[p-(demethyl sulfamoyl) phenyl] O,O-dimethyl phosphorothioate); Imidan®, (O,O-dimethyl S-phthalimidomethyl phosphorodithioate); Ruelene®, (O-4-tert-butyl-2-chlorophenyl O-methyl methylphosphoramidate), and trichlorfon, (demethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate). In addition, considerable research has been conducted on fenthion,

(O,O-dimethyl O-[4-(methylthio)-m-tolyl] phosphorothioate).

Development of a livestock systemic insecticide requires several years cooperation between entomologists, toxicologists, chemists and livestockmen. Entomological investigations are rather simple but the chemical and toxicological investigations are somewhat more complex because of safety concern to humans and livestock.

Lindquist (1956), McGregor and Bushland (1957), and Rogoff and Kohler (1960), in discussing developmental progress of ronnel pointed out the importance of chemical and toxicological research. Additional comments concerning toxicological pecularities of various systemic organophosphorus chemicals have been reported by Wrich (1961), DuBois and Kinoshita (1964), Nelson et al. (1967) and Khan (1969). Extensive toxicological and pharmacological reviews of systemic insecticides are also available (Radeleff and Bushland 1960, Claborn et al. 1960, Radeleff 1964, O'Brien 1967).

Organophosphorus insecticides are known inhibitors of cholinesterase (ChE), an enzyme found in mammals. This enzyme is required for the normal functioning of the autonomic nervous system. It is responsible for hydrolyzing acetylcholine (ACh) into its two inactive components, choline and acetate. Acetylcholine is the chemical mediator for nerve impulses at cholinergic sites in the central

nervous system, the preganglionic synapses of the sympathetic nervous system, neuromuscular junctions, adrenal medulla and the sweat glands (O'Brien 1960, Archer 1963, Radeleff 1964, Gage 1967, Guyton 1967, O'Brien 1967, Khan 1969).

Cholinesterase inhibition results in ACh accumulation at the above sites. These cholinergic responses do not all react the same to organophosphate chemicals, rather they are referred to as having either a muscarinic or nicotinic response. The cholinergic sites of the neuromuscular junctions and the parasympathetic ganglia are stimulated by nicotine. Chemicals which stimulate these sites are referred to as nicotinic drugs. Typical symptoms produced by nicotinic drugs include stimulation of voluntary muscles resulting in paralysis and a disorganized twitching called fasciculation.

Cholinergic sites displaying muscarinic effects are found in neuroeffector junctions of the parasympathetic portion of the autonomic nervous system. Drugs affecting these sites are referred to as muscarinic drugs. Muscarinic stimulation results in salivation, slowing of the heart, urination and constriction of pupils (O'Brien 1960, Radeleff 1964, Guyton 1967, Gonang 1967, O'Brien 1967, Khan 1969). In addition Khan (1969) has reported that bloat and rumen stasis may follow the administration of organophosphate systemics. Khan (1969) also reported that muscarinic

abnormalities became apparent before the nicotinic abnormalities.

Michel (1949), Archer (1963), and Gage (1967) have reported that significant quantities of cholinesterase are found in plasma and red blood cells of humans. Contrarily, research by Stowe (1955) and Radeleff and Woodard (1957a; 1957b) has revealed that plasma of cattle contains little if any cholinesterase and that red blood cells contain considerable quantities of this enzyme. Radeleff and Woodard (1956) also reported that cholinesterase activity of bovine blood is taken as an indication of its activity in the nervous system.

Cholinesterase inhibitions are usually associated with mammalian toxicosis but Radeleff (1964) has cautioned using this characteristic as diagnostic proof of poisoning. Rather, he said, determinations should be used as indicators of exposure to ChE inhibitors. Khan (1969) indicated that even though ChE activity is inhibited with organophosphorus toxicosis, correlation of inhibition and toxicosis is difficult. Khan speculated that the rate of inhibition may be more closely related to the onset of toxicosis. Archer (1963) and Gage (1967) have also elaborated on methods and use of ChE determinations in categorizing animal exposure to ChE inhibiting systemic organophosphate insecticides.

Numerous scientists including Robbins et al. (1958), Drummond (1960), Radeleff (1964), Nelson et al. (1967),

Rogoff et al. (1967), and Rogoff et al. (1968) have used blood cholinesterase determinations when evaluating inhibition potential of organophosphate insecticides. In 1956, Radeleff and Woodard discussed cholinesterase research utilizing uncontaminated cattle and sheep blood.

In South Dakota, as well as other areas of the United States, it is common practice to treat 300-500 pound beef calves during late August through early November with organophosphorus insecticides to control cattle grubs. The insecticides may be applied as a spray, dip, pour-on, or feed additive depending on availability of equipment, economics and practicality (Raun and Herrick 1960, Rogoff and Kohler 1960, Simco and Lancaster 1961, Scharff and Ludwig 1962, Rogoff et al. 1967, Cox et al. 1967, Cox et al. 1967, Kantack and Berndt 1970). Periodically some animals exhibit toxic side reactions as a result of the treatments. The magnitude of the toxicosis can vary from slight salivation to diarrhea, bloat, pneumonia, partial paralysis, or a combination of all, depending on insecticide exposure. Khan (1969) classified these side reactions as being direct and indirect animal toxicosis.

Direct toxicosis results from a chemical overdose. It is caused by the phosphorylation of ChE which subsequently causes an accumulation of ACh at cholinergic sites in the central nervous system (C.N.S.), autonomic ganglia,

postganglionic nerve ending, neuromuscular junctions, adrenal medulla and sweat glands. Stimulation of the C.N.S. is dependent on the ability of the insecticide to pass the blood brain barrier. Depending on the degree of toxicosis, direct effects may be classified as acute, subacute or chronic. Acute and subacute toxicosis may induce central, muscarinic and nicotinic reflexes or signs.

According to Khan (1969), central signs include dullness and depression and these are present in most acutely ill animals. If the toxicosis progresses, excessive salivation, lacrimation, dyspnea and pupil constriction can be observed. These are muscarinic signs and are so named because they resemble the action of muscarine. An animal in advanced toxicosis will exhibit various degrees of shivering, muscular twitching and fasciculation, muscular weakness, ataxia and posterior paralysis. These are nicotinic signs. Direct toxicosis usually can be avoided by following directions printed on the insecticide label.

Indirect toxicosis refers to animal reaction to abnormal metabolic processes initiated by drug administration. Cattle under stress occasionally react more adversely. As early as 1953, Radeleff and Bushland reported that undernourished and emaciated animals may respond differently to insecticides than animals receiving a balanced ration. The stress of shipping, change of environment, feed, and cold weather may

enhance the susceptibility of cattle to some systemic insecticides (Khan et al. 1961). Factors such as these occur routinely in the management of livestock and thus are difficult to avoid. Bushland et al. (1963) stated that stress in any form may alter significantly both toxicological reaction and biological effectiveness. Khan (1967) recommends avoiding applications of certain systemics to distressed animals. Extensive research by Clark et al. (1967) points out the exaggerated toxicological problems associated with coumaphos administration and cattle on high energy feed rations containing 30,000 International Units (I.U.) of vitamin A per ration allowance. Additional research on the complications of vitamin A, high energy feeds, and systemic insecticides has been reported by O'Brien and Wolfe (1959).

Clinical symptoms resulting from indirect toxicosis include severe inflamation, edema, and esophageal occlusion induced by death of the first instar H. lineatum larvae localized in surrounding connective tissue (Scharff et al. 1962). Khan (1964) indicated that lesions caused by disintegrating larvae are due to toxic substances produced by the dying or dead larvae.

Toxicosis resulting from the presence of H. bovis differs from that caused by H. lineatum because they are found primarily in the spinal canal as opposed to the esophagus. Scharff et al. (1962), Radeleff (1964), Rich

(1965), and Nelson et al. (1967), have all observed occasional paralysis of the hind legs, ataxia, and muscular weakness induced by the toxic substances liberated by the affected larvae.

Unlike direct toxicosis which is predictable, indirect toxicosis resulting from exposure to various stress factors is somewhat unpredictable (clinical symptoms are predictable). Even though considerable data are available on stress conditions causing toxicosis, it is not fully understood. For this reason, chemical companies in order to minimize indirect or stress toxicosis, include a broad statement on their insecticide labels to the effect that animals under stress should not be treated. A statement like this is justified.

Scientists and cattlemen are also concerned about stress and toxicosis; the scientist because he is not certain what stress factors or degrees of stress enhance toxicosis and cattlemen because loss of animals reduces their economic return. Even though direct and indirect toxicosis may be inherent with stress and the administration of organophosphorus systemics, these products will continue to be employed because they provide excellent cattle grub control. Nevertheless, a better understanding of the effects of stress is needed.

In many areas of South Dakota cattle are driven various

distances from pastures to corrals prior to treatment with an insecticide. Also, considerable numbers are on high energy feeds prior to treatment. Both of these put stress on the cattle. This study was undertaken to determine if exercise and high energy feed rations, or modifications of each, would enhance toxicosis in cattle treated with the experimental organophosphorus systemic, fenthion. Comparisons between cattle infested with grubs and those free of grubs were of special interest.

METHODS AND MATERIALS

Field Procedures

Two groups of 40 head of weaned Hereford heifers 6 to 8 months of age and weighing between 300-500 pounds were used for this experiment. Older calves were not used because several individuals, including Hadwen and Fulton (1924), Scharff (1950), and Knapp et al. (1959), have reported older animals have fewer grubs. Forty calves were purchased for the 1968 trials and another 40 head for the 1969 trials. Twenty-four calves from each group were obtained from the Highmore, South Dakota area in 1968 and again in 1969. This area is considered to be a locality where cattle normally harbor large numbers of cattle grubs. The balance of the calves of each group were purchased at Fargo, North Dakota, an area where native calves seldom are bothered with heel flies (Noetzel 1965; personal communication). To aid in dissemination of experimental data and, also, for convenience, calves from the Highmore and Fargo areas are referred to in this report as grubby and grub-free calves or animals, respectively.

Following the purchases for the respective years trials, all calves were trucked to South Dakota State University's North Beef Cattle Nutrition Farm located 2 miles north of Brookings, South Dakota. Upon arrival at the Farm, the cattle were weighed, ear-tagged, and observed for 10 days to

determine if any had contracted shipping fever or any other communicable disease. All cattle arrived in good health and no problems occurred during their orientation. The animals were fed a good quality brome hay during this time.

In South Dakota, cattle grubs are present in cattle from early July through late April or early May. Maximum numbers of third instar grubs appear in the back in mid to late March. If cattlemen desire to control cattle grubs, cattle are usually treated with a systemic insecticide sometime between late August and early November as recommended by the South Dakota State University Extension Service (Kantack and Berndt, 1970). Treatments applied at this time exert maximum toxic effect on grubs and cause minimal adverse effects to the animals. For this research, insecticide applications were delayed until early January to enhance the stress of extremely late treatments.

Eleven days after the calves arrived in Brookings, they were allotted according to weight into 4 equal groups for feeding purposes. Also, the original brome hay ration was terminated and the 2 experimental rations were substituted for the remainder of the study. Two groups of calves were given the high energy ration and the other 2 groups the low energy ration. The high energy ration was composed of the following ingredients:

566 pounds of ground shelled corn with vitamin A
1834 pounds of soybean oil meal; 44% crude protein

1000 pounds of dehydrated alfalfa; 17% crude protein
200 pounds of urea; 281% protein equivalent
240 pounds of limestone
160 pounds of trace mineral salt

The ration was fed at the rate of 2 pounds per calf per day, plus 3 pounds of ground shelled corn. Vitamin A was premixed in 52 pounds of the corn to provide 10,000 I.U. per pound of supplement. Animals on the low energy ration were given 15 pounds of an alfalfa-brome hay mixture per day plus dicalcium phosphate and trace mineral salt. All cattle were exposed to their experimental rations for 2 weeks before fenthion was administered.

On the day of treatment with fenthion, the animals were subjected to several routine experimental procedures. First, each calf was palpated to determine the number of encysted cattle grubs. Palpation procedure involved placing one's hands on the calf's shoulders, applying slight pressure to insure positive contact with the hide and then gradually moving the hands toward the rump. An area about 18 inches on either side of the vertebral column was examined. Encysted grubs form small lumps and these vary in size from about 5 to 15 centimeters in diameter at their base. Subsequent palpations were made at monthly intervals until counts revealed that all grubs had emerged from their cysts.

After the calves were palpated, pretreatment blood samples were obtained from each animal via a jugular venous puncture to establish pretreatment control ChE levels.

Blood samples were also withdrawn at 1, 3, 7, 14, and 21 days following collection of the pretreatment samples.

To obtain the blood samples, each animal was placed in a cattle squeeze chute and restrained with its neck extended through the head gate. See figure 1. The individual collecting the blood placed his hip against the side of the animal's head and gently pushed the head to the side exposing the neck. See figure 2. This procedure tightened the otherwise loose, flabby connective tissue of the neck and greatly aided finding the jugular groove. With the animal's neck extended to the side, the operator then placed his left hand on the jugular groove at a point about 14 inches below the jawbone and applied moderate pressure. Pressure on the jugular groove reduced blood flow in the jugular vein causing the vein between the head and the pressure point to swell slightly which facilitated finding the vein.

Once the jugular vein was located a 14 or 16 gauge, 3 inch bleeding needle was inserted into the vein. Care was taken to insert the needle so that the bevelled edge was facing the flow of blood. This insured maximum blood flow through the bleeding needle. Twenty ml test tubes containing 2 drops of 10% ethylenediamine tetraacetic acid (EDTA) anticoagulant were used to collect 10 ml blood samples from each animal. All test tubes were stoppered with rubber

Figure 1. Calf in squeeze chute in preparation for obtaining a blood sample.

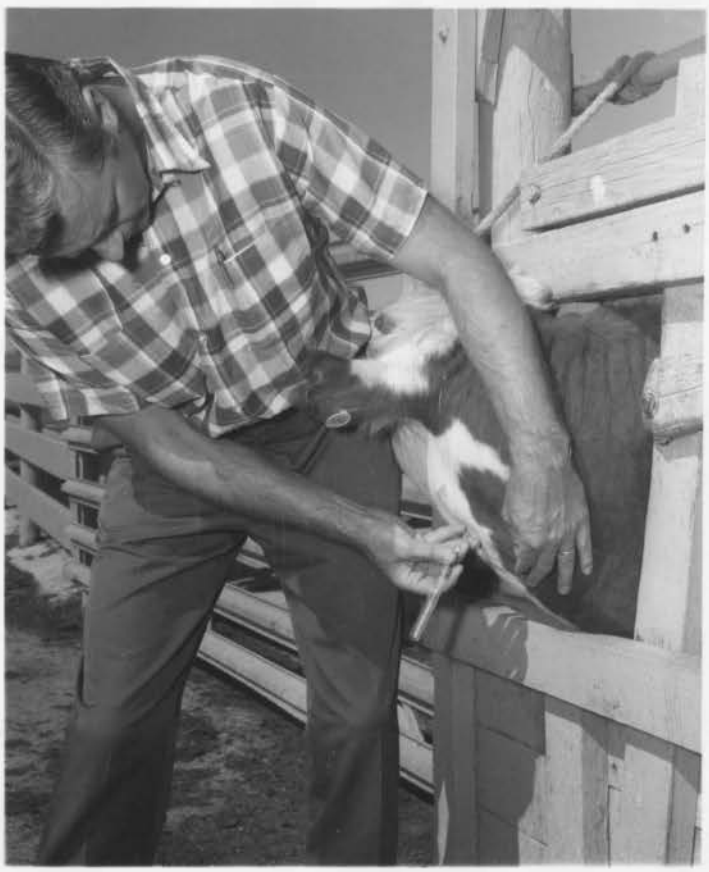

Figure 2. Author obtaining a blood sample from a jugular venous puncture in a calf.

stoppers immediately after the desired volume of blood was obtained. Following collection each sample was placed in an ice water bath to minimize enzymatic activity. All blood samples were returned to the laboratory immediately after the last sample was collected.

Following collection of pretreatment blood samples, cattle in the 2 major feed groups were allotted by selective randomness to their respective phase I experimental groups. Six grubby and 3 grub-free calves on the maintenance feed ration were subjected to the stress factor for this phase, 30 minutes of exercise, while an equal number of similar calves served as no-exercise controls. An additional 2 grub-free calves were classified as untreated, no-exercise controls. The procedures for the 20 calves on the fattening ration were exactly the same. Animal allotment for phase I is shown in Fig. 3.

Exercise consisted of forcing the cattle to trot in feed lot alley-ways for 30 consecutive minutes. This exercise was intended to place the cattle under stress prior to the administration of fenthion and supposedly simulated ranchers rounding up their cattle and driving them from pastures to corrals.

After exercising, cattle were driven into a working chute and the animals allotted for treatment were administered 3% fenthion. A commercially prepared pour-on

EXPERIMENTAL PHASE I

LOW ENERGY FEED RATION

Exercise		No Exercise	
Treat	No Treat	Treat	No Treat
3 Grub-free calves 3 Grubby calves	3 Grubby calves	3 Grub-free calves 3 Grubby calves	3 Grubby calves

Control

2 Grub-free calves

HIGH ENERGY FEED RATION

Exercise		No Exercise	
Treat	No Treat	Treat	No Treat
3 Grub-free calves 3 Grubby calves	3 Grubby calves	3 Grub-free calves 3 Grubby calves	3 Grubby calves

Control

2 Grub-free calves

Figure 3. Animal allotment and designation according to feed ration and stress for experimental phase I; 1968 and 1969.

formulation was used. It was applied in a continuous straight line to the center of the back between the top of the shoulders and rump. The chemical was administered at the rate of one-half ounce of solution per 100 pounds of body weight with the aid of a graduated dipper supplied by the manufacturer. The dipper was held approximately 3 inches above the back as the pour-on was applied (Fig. 4).

Following collection of the last blood sample (21 days after treatment with fenthion), the cattle were permitted to remain in their pens without molestation for 2 weeks. This period was referred to as the rest period. It was also the interval between the succeeding experimental phase. The treatment, bleeding, and rest periods constituted one phase of the experiment. Four phases were observed each year.

Calves utilized in phase I were also the experimental subjects for phase II. Procedures and stress employed in phase II were similar to phase I except for animal allotment. The calves treated with fenthion in phase I were not treated during phase II. These calves were designated as untreated controls during this phase. Contrarily, the untreated calves of phase I were treated with fenthion during phase II. This procedure resulted in twice as many nontreated control animals in phase II as compared to phase I. Animal allotment for phase II is shown in Figure 5.

Stress during phase III was the withdrawal of feed and

Figure 4. Application of fenthion to back of calf, showing area treated and position of dipper at time of application.

EXPERIMENTAL PHASE II

LOW ENERGY FEED RATION

Exercise		No Exercise	
No Treat	Treat	No Treat	Treat
3 Grub-free calves 3 Grubby calves	3 Grubby calves	3 Grub-free calves 3 Grubby calves	3 Grubby calves

Control

2 Grub-free calves

HIGH ENERGY FEED RATION

Exercise		No Exercise	
No Treat	Treat	No Treat	Treat
3 Grub-free calves 3 Grubby calves	3 Grubby calves	3 Grub-free calves 3 Grubby calves	3 Grubby calves

Control

2 Grub-free calves

Figure 5. Animal allotment and designation according to feed ration and stress for experimental phase II; 1968 and 1969.

water from specific animals for 24 hours prior to fenthion treatment. During this phase the insecticide treated and untreated cattle comprised the same animals subjected to these experimental exposures during phase I. Aside from these changes, all other procedures were the same as described previously.

The withdrawal stress was also employed during phase IV and the treated and nontreated groups were reversed as was done in phase I and II. This procedure was used to make maximum use of experimental animals and to determine effects of additive stress. Animal allotment for phases III and IV are shown in Figures 6 and 7. The above procedures were also employed in 1969.

Laboratory Procedures

Blood withdrawn from the research animals was returned to the laboratory and placed in a refrigerator. Temperature within the refrigerator was maintained at 2 degrees Centigrade. Normally cholinesterase analyses were conducted immediately after the samples were collected but occasionally they were frozen for short periods prior to analysis. Van Middelem (1963) reported freezing of organic phosphate-containing sample extracts has little effect on enzymatic activity providing samples are not held for extended periods. Rogoff et al. (1967) reported that heparinized bovine blood samples collected in Oregon from cattle treated with Imidan®

EXPERIMENTAL PHASE III

LOW ENERGY FEED RATION

Feed		No Feed	
Treat	No Treat	Treat	No Treat
3 Grub-free calves 3 Grubby calves	3 Grubby calves	3 Grub-free calves 3 Grubby calves	3 Grubby calves

Control

2 Grub-free calves

HIGH ENERGY FEED RATION

Feed		No Feed	
Treat	No Treat	Treat	No Treat
3 Grub-free calves 3 Grubby calves	3 Grubby calves	3 Grub-free calves 3 Grubby calves	3 Grubby calves

Control

2 Grub-free calves

Figure 6. Animal allotment and designation according to feed ration and stress for experimental phase III; 1968 and 1969.

EXPERIMENTAL PHASE IV

LOW ENERGY FEED RATION

Feed		No Feed	
No Treat	Treat	No Treat	Treat
3 Grub-free calves 3 Grubby calves	3 Grubby calves	3 Grub-free calves 3 Grubby calves	3 Grubby calves

Control

2 Grub-free calves

HIGH ENERGY FEED RATION

Feed		No Feed	
No Treat	Treat	No Treat	Treat
3 Grub-free calves 3 Grubby calves	3 Grubby calves	3 Grub-free calves 3 Grubby calves	3 Grubby calves

Control

2 Grub-free calves

Figure 7. Animal allotment and designation according to feed ration and stress for experimental phase IV; 1968 and 1969.

were successfully shipped cold to Richmond, California, for analysis.

Whole blood cholinesterase activity determinations were based on the method of Michel (1949) but modifications of Radeleff and Woodard (1956) and Radeleff (1967; personal communication) were incorporated. Modifications included hemolyzing 0.4 ml of whole blood in 916 ml of 0.01% aqueous saponin. One milliliter of the hemolyzed cells, representing 0.02 ml of cells, was added to 1 ml of red cell buffer in a 5 ml beaker, mixed, and allowed to equilibrate at 25° C. for 10 minutes. At the end of this period, the initial pH (pH_1) was determined with a Instrumentation Laboratory Model 113-S1 pH-Blood Gas Analyzer to the nearest .001 pH unit (Fig. 8). Next 0.2 ml of acetylcholine solution was added with rapid mixing. This preparation was incubated for one hour at 25° C. and then the final pH (pH_2) was taken. A reagent blank was run at the same time to determine non-enzymatic change in pH.

Cholinesterase activity in delta pH units per hour is the difference between pH_1 and pH_2, minus the reagent blank.

Percent ChE inhibition is calculated using the following formula (Archer, 1963):

$$\% \text{ inhibition} = \left[1 - \frac{\text{pH (sample)}}{\text{pH (control)}}\right] \times 100$$

In preparing blood samples for analysis, it is extremely

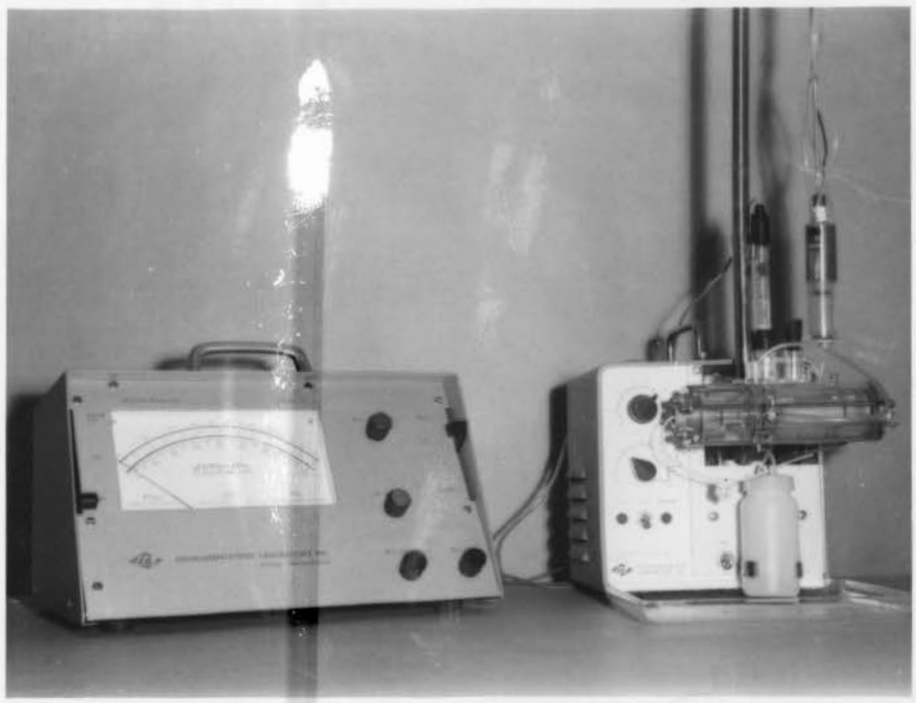

Figure 8. Instrumentation Laboratory pH Blood-Gas Analyzer Model 113-S1.

important to adhere to the time intervals designated for the specific steps. It is essential that a routine be established. The author found it practical to start samples at one minute intervals.

No attempt was made to separate plasma and blood cells when cholinesterase activity was determined because Stowe (1955) and Radeleff and Woodard (1956) reported that there is little or no cholinesterase in plasma of cattle.

The Instrumentation Laboratory pH Blood-Gas Analyzer used to measure pH activity is a very sensitive instrument. It is convenient to use because pH values can be determined with microliter quantities of solution. The solution to be measured is asperated into a glass chamber surrounding an electrode. The electrode unit is then placed in potassium chloride (KCL) and the pH of the solution is read directly from the expanded scale. After the pH has been recorded, the electrode unit is removed from the KCL and the test solution is aspirated out of the electrode chamber. Appropriate buffers and electrode cleaners are then used to clean and prepare the electrode chamber for the subsequent sample. Standard buffers are used routinely to check the pH slope of the recording scale. The data thus obtained were expressed as Δ pH/hour and converted to percentage apparent inhibition for each experimental group by utilizing the mean control value for specific groups. Computations

were also made for individual animals. In all instances the pretreatment value for each animal was regarded as 0 inhibition (Archer, 1963).

Packed cell volumes were determined for each animal each time it was bled. Percent cell volume was obtained by centrifuging microhaematrocrit tubes at about 12,500 rpm's for 4 minutes in an Adams Autocrit™ Centrifuge. Packed cell volumes were determined by averaging 2 aliquots.

As mentioned previously, procedures used in 1969 were essentially the same as those used in 1968 but research in 1969 was begun about a month earlier because of earlier availability of cattle. The individual pre- and post-treatment ChE values obtained from the calves were used to evaluate the influence of stress, year, animal origin, cattle grubs, insecticide treatment and feed ration. All data were subjected to least squares analysis of variance to determine significance.

RESULTS AND DISCUSSION

Phase I: - The importance of exercise as a stress factor in promoting toxicosis in calves treated with fenthion was evaluated by calculating fluctuations in ChE values of whole blood in treated and nontreated animals. Cholinesterase values obtained from pretreatment blood samples of the 40 experimental calves averaged .407 delta pH units activity per hour. Values ranged from a low of .238 delta pH units in one grub-free calf to a high of .557 in a grubby animal. These values are slightly lower than the 253 bovine erythrocyte control samples analyzed by Radeleff and Woodard (1956); these samples averaged between 0.46 and 0.47 delta pH units and ranged from 0.17 to 0.96 delta pH units per hour.

In 1968, palpation of calves on the maintenance ration revealed no grubs in the 8 North Dakota grub-free calves while a total of 76 were present in 5 of 6 South Dakota grubby calves serving as nontreated controls; 1 animal did not have any cattle grubs. Grub counts averaged 12.66 per head and ranged from 9 to 14 per animal. Of the 20 calves on the fattening ration, 5 of 6 grubby, nontreated control animals harbored 55 grubs, for an average of 9.16 per head. Grub counts ranged from 9 to 13 per head in infested calves; one animal had no grubs. Total grubs for the respective feed groups were based on cumulative counts taken January

through April. Based on grubs present in untreated and treated animals, fenthion provided 97.4% grub control. No grubs were present in the grub-free calves.

Palpation counts in 1969 revealed that all untreated grubby controls were infested with cattle grubs. A total of 67 were present in maintenance ration calves and 66 in calves consuming high energy feed. Grub counts ranged from 9 to 18 per head and averaged 11.08 per head. As in 1968, total grubs were based on cumulative counts recorded January through April. No cattle grubs were found in the grub-free, North Dakota cattle.

Phase I data from 1968 and 1969 were evaluated statistically by the least squares analysis of variance. Analytical print-outs were produced for each posttreatment bleeding date. Of all the individual factors and interactions among the factors observed during phase I, the insecticide treatment was apparently most important. Fenthion produced highly significant ChE depression on each bleeding date. However, it is important to note the F value for this factor was the greatest (48.878) at 24 hours after treatment. This value decreased to 13.444 on the final bleeding day (Table 1).

Cattle origin reflecting the presence or absence of grubs, also provided highly significant differences in ChE values in the North Dakota and South Dakota cattle. An F value of 8.123 was recorded for origin effect for these

Table 1. F values of phase I data at specific days posttreatment

Source	F Values Days Posttreatment				
	1.	3.	7.	14.	21.
Year	.029	3.862	1.253	.121	1.017
Origin	8.123**	.330	1.871	.394	.019
Year-Origin	.009	.805	.878	2.352	1.531
Ration	.100	2.440	1.040	1.848	4.840*
Year-Ration	1.047	6.097**	1.040	.919	.158
Origin-Ration	1.047	.023	2.455	3.947	.134
Year-Origin-Ration	3.162	1.297	3.177	1.944	1.079
Stress	.134	.571	.451	.394	.494
Year-Stress	.949	1.797	.327	.003	.046
Origin-Stress	.237	2.440	.043	1.093	.473
Year-Origin-Stress	.171	.074	.000	.588	1.457
Ration-Stress	.534	.023	.165	.292	.239
Year-Ration-Stress	2.507	.841	.116	.121	.210
Origin-Ration-Stress	.433	1.851	.015	.011	.001
Treatment	48.878**	38.294**	25.912**	21.330**	13.444**
Year-Treatment	3.893	.319	5.781*	.953	.785
Origin-Treatment	.000	.001	.000	.001	.000
Ration-Treatment	.000	.001	.000	.001	.000
Year-Ration-Treatment	2.507	3.982	.462	.221	.146
Stress-Treatment	3.338	.104	.046	.700	.183
Year-Stress-Treatment	1.667	.823	.607	.190	.239
Ration-Stress-Treatment	.903	.751	.508	1.619	.926
Year-Ration-Stress-Treatment	.005	.936	.022	.311	.320
Year-Origin-Ration-Stress	1.367	1.906	.105	.700	.631

* = significant at $P < 0.05$
* * = significant at $P < 0.01$

groups at 24 hours posttreatment. This reflects a 9.75% difference in mean enzymatic activity values. The mean ChE value of grub-free calves was 94.29% normal activity at 24 hours posttreatment as compared to 84.54% normal activity in grubby calves. The influence of origin was present in all phase I posttreatment erythrocyte samples but was not significant beyond the 24 hours sample. It is felt that this possibly reflects the rapidity with which the insecticide is absorbed, grubs are destroyed and metabolic products are removed from the animal.

In 1968, ChE values at 24 hours posttreatment ranged from a high of 120% down to 36%. The animal with the highest activity was a grubby calf on the fattening ration in the exercise group; it was treated with fenthion. Maximum depression occurred in a grubby calf in the no-stress group consuming high energy feed (Appendix Table 21). Within 26 hours of fenthion treatment, this animal developed characteristic subacute toxicosis similiar to that described by Khan, 1969.

Radeleff and Woodard (1957), Scharf et al. (1962), and Nelson et al. (1967) have indicated that toxicosis resulting from organophosphorus insecticides may occur within 24 hours of treatment. Utilizing this information as a guideline, phase I animals were treated with fenthion at 10 AM so that the remainder of the day could be used to observe for gross

signs of animal toxicosis. Within 4 hours of insecticide administration, 1 calf was noted as having slightly excessive mucal and saliva discharges. During early stages, salivation was thin and watery and it accumulated at the corners of the mouth where it discharged profusely. Saliva consistency gradually increased and became thick and stringy by 4 PM. This animal also appeared somewhat hypersensitive. There was no obvious deterioration of condition between 4 and 8 PM, consequently no medication was given. Toxicosis intensified slightly through the night. The following morning mild hyperrespiration, body tremors, irregular gait, especially in the posterior quarters, and very slight diarrhea were observed. Regardless of its physical condition, the animal was noted to advance to the feed bunk and eat on several occasions but it failed to consume feed for extended periods. Similar advances to water were also noted. The animal continued to mingle with cattle in the pen as opposed to standing off by itself. The toxicosis reached its peak at about 26 hours posttreatment and then recovery was uneventful. No medication was used to aid recovery.

Examination of mean ChE values for the other 1968 calves revealed that animals on the maintenance ration subjected to exercise prior to fenthion treatment were suppressed slightly more than their counterparts on the fattening ration. These respective groups averaged 23% and 17% ChE

depression or alternately, 77% and 83% normal enzymatic activity. Similarly exposed calves in the no exercise, maintenance ration group did not have lower ChE values than calves on the fattening ration. The respective ChE values for these groups averaged 75% and 60% normal activity. Considering all groups on both rations, maximum group (54%) and individual animal (64%) depressions were recorded from grubby animals on the fattening ration in the fenthion, no exercise group. This information is presented in Table 2. Individual animal ChE values for this group are given in Appendix Table 21.

Data from Table 3 reflects mean ChE values for 1969 phase I research. Individual ChE values are shown in Appendix Table 25. Maximum enzymatic depression at 24 hours occurred in 1 of 3 grubby calves exposed to fenthion, stress and the high energy ration. The most severely affected animal had a ChE value of 64% of normal. This animal continued to display increased depression until 7 days following treatment at which time the ChE value was 44%. At the conclusion of phase I, ChE activity in this calf was 52% of normal. Overall enzyme activity for the 3 calves in this group was only 66% at the end of the test. This contrasts with 85% normal activity in the 3 grub-free calves exposed to the same experimental factors and, to 73% and 84% for grubby and grub-free calves in the no stress, maintenance ration

Table 2. Whole blood cholinesterase activity of 6-8-months-old Hereford heifers subjected to the exercise stress factor and treatment with fenthion; phase I, winter 1968.

Group	Calves No.	Type	Mean ChE Activity (% of pretreatment value) Days Following Treatment 1 MR**	1 FR**	3 MR	3 FR	7 MR	7 FR	14 MR	14 FR	21 MR	21 FR
No Exercise												
Control	2	G.F.*	103	100	92	98	109	98	105	122	103	122
No Treatment	3	G.*	108	97	104	99	132	99	119	96	119	103
Treatment	3	G.F.	73	74	72	82	69	90	76	87	85	87
Treatment	3	G.	77	46	84	72	75	69	93	86	100	83
Exercise												
No Treatment	3	G.	92	102	99	107	119	109	124	113	128	115
Treatment	3	G.F.	78	88	79	70	70	80	78	85	91	91
Treatment	3	G.	75	79	69	72	85	66	91	67	104	80

* = G.F. = Grub-free cattle (North Dakota); G. = Grubby cattle (South Dakota)
* * = MR = Maintenance ration; FR = Fattening ration

Table 3. Whole blood cholinesterase activity of 6-8-months-old Hereford heifers subjected to the exercise stress factor and treatment with fenthion; phase I, winter 1969.

Group	Calves No.	Type	Mean ChE Activity (% of pretreatment value) Days Following Treatment 1 MR**	1 FR**	3 MR	3 FR	7 MR	7 FR	14 MR	14 FR	21 MR	21 FR
No Exercise												
Control	2	G.F.*	88	87	113	110	94	109	101	95	96	104
No Treatment	3	G.*	95	97	131	95	94	97	107	98	106	99
Treatment	3	G.F.	90	79	91	91	97	72	91	78	94	84
Treatment	3	G.	76	85	85	85	81	82	96	86	89	73
Exercise												
No Treatment	3	G.	97	83	103	87	87	83	100	97	96	97
Treatment	3	G.	74	75	76	72	90	63	92	66	97	66
Treatment	3	G. F.	85	84	94	82	98	75	112	84	114	85

* = G.F. = Grub-free cattle (North Dakota); G. = Grubby cattle (South Dakota)
* * = MR = Maintenance ration; FR = Fattening ration

groups.

Further comparisons of ChE values obtained at 24 hours posttreatment indicates that exercise alone affects enzymatic activity very little irregardless of the feed ration or whether animals are treated with fenthion. In the exercise stress group, fenthion treated grubby calves on the maintenance ration averaged 74% normal enzyme activity as compared to 75% activity in calves on the fattening ration. Grub-free calves in this exercise group and on the above respective rations averaged 85% and 84% ChE activity as compared to 97% and 83% in the no treatment, grubby calves. The higher enzymatic activity in the maintenance ration calves was not anticipated.

Cholinesterase values of grubby and grub-free calves in the no exercise group were comparable to similar animals in the exercise group. The grubby calves averaged 88% normal ChE activity in contrast to 86% for grub-free calves. There was little difference in ChE ratings of the control and no treatment groups of the exercise and no exercise groups. Cholinesterase values ranged from 83% to 97% and averaged 91%. It is also interesting to note that ChE values for all the grubby calves averaged 68.5% normal activity as compared to 78.2% in the grub-free animals. These differences were highly significant.

Blood samples collected in 1968 at 3 days posttreatment

suggested partial correlation of ChE depression in grubby calves subjected to exercise and fenthion administration as compared to grub-free calves exposed to the same stress. The grubby animals averaged 70.5% normal activity compared with 74.5% in the grub-free cattle. These figures are slightly lower than those recorded at 24 hours following treatment; this was anticipated. Cholinesterase values for fenthion treated grubby and grub-free cattle in the no exercise groups were slightly higher than animals in the exercise group. Grubby calves averaged 78% normal ChE activity while grub-free calves recorded a 77% average. There was little difference in ChE readings between the no treatment calves in the exercise and no exercise groups.

The most important observation recorded at this stage of the experiment was the year-feed ration effect. According to blood samples, ChE values of calves on the fattening ration were significantly different from those on maintenance feed. The F value was approaching the highly significant level. Considering year and ration individually, only the feed ration produced a significant effect and this occurred at 21 days after treatment. The year influence reached its highest level at 3 days.

Analysis of blood samples obtained 7 days posttreatment (Table 2.) indicated ChE levels of grub-free calves on the maintenance ration exposed to stress decreased 9% from the

preceding collection whereas animals on the fattening ration experienced a 10% increase in enzyme activity. Contrarily, data obtained from grubby calves on the same respective feed rations revealed distinctively contrasting information. Cholinesterase levels of fattening ration calves averaged 66% normal activity, reflecting continued depression from the onset of this phase. This figure represents maximum depression experienced by these calves during this phase. At 14 days posttreatment this group averaged 67% depression as compared to 80% at the conclusion of this phase. Animals of the maintenance ration group averaged 85% ChE activity at 7 days, up 16% from the previous period. This figure increased to 104% at 21 days.

An examination of data presented in Table 2 for animals in the no exercise, treatment group reveals significant ChE depressions when comparing effects of nontreated calves. Data is particularly apparent for grubby calves on high energy feed. These animals experienced a 54% decrease in enzyme activity versus 26% for grub-free calves 24 hours following treatment. Fattening ration fenthion calves averaged 40% ChE depression compared with an average of 25% depression for calves on the maintenance ration.

At 3 days after treatment all calves in the treatment groups with the exception of grub-free, maintenance ration calves appeared to be recovering from treatment effects as

indicated by ChE values. The ChE curve for these calves did not reflect increased enzyme activity until 14 days after treatment. A significant increase in ChE was recorded at 21 days. Statistical analysis also indicated a significant influence of the year-treatment effect when comparing 1968 and 1969 data. This influence was present at all bleeding periods, but only attained a significant level at 7 days after treatment. It was felt this effect reflects early commencement of the experiment in 1969. Reference is made to Table 1.

Phase II: - The fenthion treatment so apparently influential during phase I had relatively little effect on blood ChE during phase II. According to the analysis of variance of phase II data, the year-treatment combination produced highly significant ChE depressions through the 14 day bleedings; a significant value was recorded for the final bleedings. The influence of year on enzyme activity was also apparent during this phase. Other factors contributing to ChE depressions were various combinations of year, treatment, origin and stress. These data are found in Appendix Tables 6-10 and are summarized in Table 4.

Data collected in 1968 and presented in Table 5 shows that fenthion decreased ChE activity only slightly. At 24 hours posttreatment, maximum depression occurred in the no exercise calves. These animals averaged 84.5% normal ChE

Table 4. F values of phase II data at specific days posttreatment.

Source	F Values Days Posttreatment 1.	3.	7.	14.	21.
Year	6.472*	7.226*	7.203*	1.798	2.635
Origin	.212	2.039	.057	.604	.231
Year-Origin	6.677*	8.157**	2.635	2.539	.716
Ration	.000	1.588	2.040	8.840**	3.430
Year-Ration	.000	.028	1.576	2.601	2.941
Origin-Ration	.010	.002	.866	2.539	1.581
Year-Origin-Ration	.020	.933	.248	1.021	.569
Stress	.293	.933	.047	.046	.640
Year-Stress	7.098*	5.337*	.002	.109	.026
Origin-Stress	1.801	3.733	1.316	.074	.189
Year-Origin-Stress	.177	1.383	.135	.006	.005
Ration-Stress	.004	.298	.607	.064	.716
Year-Ration-Stress	2.257	4.955*	3.628	1.904	.716
Origin-Ration-Stress	.145	1.921	.023	.054	.005
Treatment	.520	.064	.012	.000	2.141
Year-Treatment	9.271**	41.838**	45.892**	14.954**	5.655*
Ration-Treatment	.040	1.695	9.849**	2.239	.301
Year-Ration-Treatment	1.170	6.564*	10.965**	4.822*	.678
Stress-Treatment	7.205*	4.236*	1.631	.907	1.639
Year-Stress-Treatment	.014	.143	.712	.000	.075
Ration-Stress-Treatment	.231	2.547	.712	.463	5.228*
Year-Ration-Stress-Treatment	2.568	2.286	2.496	.019	.678
Year-Origin-Ration-Stress	2.138	6.141*	1.417	.545	.640

* = significant at $P < 0.05$
* * = significant at $P < 0.01$

Table 5. Whole blood cholinesterase activity of 6-8-months-old Hereford heifers subjected to the exercise stress factor and treatment with fenthion, phase II, winter 1968.

Group	Calves No.	Calves Type	Mean ChE Activity (% of pretreatment value) Days Following Treatment 1 MR**	1 FR**	3 MR	3 FR	7 MR	7 FR	14 MR	14 FR	21 MR	21 FR
No Exercise												
Control	2	G.F.*	98	98	99	96	99	89	99	90	99	94
No Treatment	3	G.F.	95	95	94	98	95	98	96	101	96	99
No Treatment	3	G.*	100	95	100	95	99	97	99	100	100	98
Treatment	3	G.	81	88	75	88	80	88	87	92	87	91
Exercise												
No Treatment	3	G.F.	94	91	94	94	95	93	96	96	96	95
No Treatment	3	G.	98	99	98	99	98	98	98	98	98	99
Treatment	3	G.	98	94	92	86	90	82	92	92	97	92

* = G. F. = Grub-free cattle (North Dakota); G. = Grubby cattle (South Dakota)
* * = MR = Maintenance ration; FR = Fattening ration

activity whereas calves in the stress group averaged 96% activity. Maximum enzyme depression (19%) occurred 3 days after treatment in 3 grubby calves in a no exercise, maintenance ration group. Cholinesterase values gradually returned to 87% normal activity at the conclusion of phase II.

The effects of the insecticide treatment were also minimal for animals on the fattening ration. Maximum depression (12%) occurred in cattle in the no exercise group. This was recorded at 24 hours after treatment and it persisted through 7 days. At 14 and 21 days enzyme activity was 92% and 91% of normal. Cholinesterase values of animals on the fattening ration exposed to stress were consistently lower throughout phase II than animals on the maintenance feed. Maximum depression was 18% and it occurred at the 7 day bleeding. Subsequent blood samples had higher enzyme activity values. Recovery to 92% normal activity for this group and 97% for cattle on the maintenance ration was uneventful. Maximum depression for exercise, no treatment cattle was 91% at 24 hours posttreatment. Cholinesterase values for the grubby cattle returned to 99% normal activity while grub-free animals reached 95%.

Also apparent at the initial posttreatment bleeding were significant ChE depression values produced by year effect. Significant values were apparent through 7 days. The F value obtained for the initial bleeding was 6.472, followed by 7.226, 7.203, 1.798, and 2.635 during subsequent

bleeding periods. The F value for significance at the $P < 0.05$ level for this test is 4.080. It was also noted the combination year and cattle origin produced significant differences whereas origin alone was nonsignificant. It was expected this value would have been higher because all animals receiving fenthion were grubby calves. The increased size of the grubs plus their presence in the back as opposed to their presence in connective tissue along the migration route from the esophagus and spinal canal may have contributed to the low F value at this time. In addition, the rapidity of systemic uptake may have also been influential as the F value for cattle origin at 3 days was 2.039. This value decreased to .231 at the termination of phase II.

Nonsignificant data were obtained from the stress effect, but stress in conjunction with year produced significant values as did combinations of stress and treatment.

During the 3 day posttreatment sampling period, the year-origin effect had a highly significant F value of 8.157. Subsequent blood samples indicated nonsignificant F values and each was decreasing with time. Highly significant values were also produced by year-treatment effects as indicated previously. The influence of the year effect was reflected in significant F values where it appeared in combination with ration-treatment and ration-origin-stress. Within the year-ration-treatment source, the 1968 animals

treated with fenthion and fed the maintenance ration had a ChE mean of 96% of normal compared to an 84% mean in 1969. The greatest ChE depression occurred in 1968 in nontreated animals of this group. Enzymatic activity was the greatest in 1969 in nontreated cattle on low energy feed (Table 6). In the year-origin-ration-stress comparison, the 1968 grub-free calves on the maintenance ration in the no exercise group experienced maximum ChE depressions as compared to other cattle but these values were not severe. These animals averaged 18% ChE reductions compared to 111% enzymatic activity in a similar group of calves in 1969. The year effect may be attributable to the fact that research was begun about a month earlier in 1969.

The year effect noted from blood samples collected at 3 days posttreatment was still evident one week following treatment. Significant differences were apparent through 7 days posttreatment. Highly significant differences were reflected in the year-treatment, ration-treatment and year-ration-treatment groups. The appearance of the ration effect in combination with other factors corresponds to a gradual increase in significance for this factor through the previous 2 bleeding periods as well as in the current period. Ration effect achieved a highly significant level at 14 days and then declined to a nonsignificant level at the conclusion of this phase.

Table 6. Whole blood cholinesterase activity of 6-8-months-old Hereford heifers subjected to the exercise stress factor and treatment with fenthion; phase II, winter 1969.

Group	Calves No.	Type	Mean ChE Activity (% of pretreatment value) Days Following Treatment 1 MR**	1 FR**	3 MR	3 FR	7 MR	7 FR	14 MR	14 FR	21 MR	21 FR
No Exercise												
Control	2	G.F.*	115	100	123	104	118	104	110	104	106	97
No Treatment	3	G.F.	111	99	120	101	109	98	100	104	91	103
No Treatment	3	G.*	94	93	95	96	102	91	98	95	92	99
Treatment	3	G.	93	97	79	95	79	96	86	95	95	96
Exercise												
No Treatment	3	G.	99	97	104	96	108	99	102	98	103	96
No Treatment	3	G.F.	97	104	101	104	103	105	99	107	98	101
Treatment	3	G.	88	87	78	86	80	93	82	98	91	101

* = G.F. = Grub-free cattle (North Dakota); G = Grubby cattle (South Dakota)
* * = MR = Maintenance ration; FR = Fattening ration

Treatment-year effect at 14 days posttreatment was highly significant and the F value was 14.954. This value was down from 45.892 recorded the previous week. The treatment-year effect was still significant at the conclusion of this phase.

Year effect decreased to a nonsignificant level at 14 days following treatment-year influence in combination with ration and treatment also decreased drastically and yet remained at a significant level. Blood samples collected at 21 days following treatment revealed that this factor was nonsignificant at that time.

Phase III: - The investigative stress factor for phase III was the withholding of feed and water from specified groups of cattle for 24 hours prior to treatment with fenthion.

Blood samples obtained during this phase in 1968 revealed the continued influence of several experimental factors prevalent during phases I and II. At 24 hours after treatment with fenthion year effect achieved a highly significant level as compared to reaching only a significant level in phase II. Year effect alone and in conjunction with various combinations of origin, ration, and stress remained apparent throughout this period. Cattle origin and feed ration by themselves had virtually no effect but together produced significant values. These 2 factors in

association with year modified effects of the combination to a nonsignificant level. Similar data were obtained from treatment effects. Treatment alone was significantly important but collectively with other factors failed to approach significance. Reference is made to Table 7.

Comparisons of ChE values of animals on stress with those not stressed disclosed some unexpected data. Stressed cattle were affected less by fenthion compared to those remaining on feed. Maintenance ration, grub-free calves had ChE values that averaged 73%, 80%, 73%, 73%, and 76% of normal at the 1,3,7,14, and 21 day bleeding periods compared to values of 71%, 84%, 81%, 92%, and 105% in grubby calves at these same intervals. North Dakota calves (grub-free) exposed to the chemical and feed had ChE values of 59%, 64%, 45%, 51%, and 54% at the 5 respective posttreatment bleeding dates. Comparable values for grubby South Dakota calves at these same intervals were 85%, 70%, 70%, 70%, and 78% of normal (Table 8).

Cattle on maintenance ration feed weighed an average of 548 pounds during this phase compared to 725 pounds for those on the high energy feed. It was expected that the general inferior condition and weight differential of calves on the low energy ration would be exhibited in pronounced differences in ChE values but it was not.

Cholinesterase values for all control and nontreated

Table 7. F values of phase III data at specific days posttreatment.

Source	F Values Days Posttreatment 1.	3.	7.	14.	21.
Year	23.438**	9.735**	38.923**	43.237**	27.068**
Origin	.030	2.815	4.309**	3.028	3.216
Year-Origin	.061	.324	1.063	2.647	5.301*
Ration	.202	.000	2.021	2.522	1.424
Year-Ration	.383	.000	2.956	2.181	1.661
Origin-Ration	5.597*	.152	2.953	2.207	2.637
Year-Origin-Ration	3.061	.808	.318	2.378	3.759
Stress	2.526	.992	.115	.311	.822
Year-Stress	1.880	1.782	1.263	.199	.036
Origin-Stress	2.808	.176	.068	.405	.001
Year-Origin-Stress	.924	.152	.531	.088	.338
Ration-Stress	.107	1.410	11.917**	8.888**	4.411*
Year-Ration-Stress	.092	.823	5.234**	5.876*	2.791
Origin-Ration-Stress	.661	.109	7.788**	2.556	.397
Treatment	5.830*	19.783**	5.050**	4.425*	.957
Year-Treatment	.053	4.929**	4.014*	2.391	.276
Ration-Treatment	.325	.083	1.355	1.273	1.312
Ration-Treatment-Year	.160	1.113	.000	.266	.301
Stress-Treatment	.247	2.027	5.549*	6.499*	4.019*
Year-Stress-Treatment	.043	1.985	2.466	6.449*	5.582*
Ration-Stress-Treatment	2.683	2.468	13.759**	6.933*	2.119
Year-Ration-Stress-Treatment	1.443	.101	1.551	1.826	.658
Year-Origin-Ration-Stress	.411	.704	.159	.370	.640

* = significant at $P < 0.05$
* * = significant at $P < 0.01$

Table 8. Whole blood cholinesterase activity of 6-8-months-old Hereford heifers subjected to the exercise stress factor and treatment with fenthion; phase III, spring 1968.

Group	Calves No.	Type	Mean ChE Activity (% of pretreatment value) Days Following Treatment 1 MR**	1 FR**	3 MR	3 FR	7 MR	7 FR	14 MR	14 FR	21 MR	21 FR
No Feed												
Control	2	G.F.*	100	88	100	92	100	73	100	65	100	58
No Treatment	3	G.*	92	75	102	89	93	74	95	75	97	77
Treatment	3	G.F.	73	92	80	82	73	76	73	79	76	86
Treatment	3	G.	71	73	84	86	81	85	92	86	105	89
Feed												
No Treatment	3	G.	81	79	93	105	77	109	79	109	84	108
Treatment	3	G.F.	59	73	64	64	45	68	51	71	54	74
Treatment	3	G.	85	62	70	75	70	65	70	66	78	74

* = G.F. = Grub-free cattle (North Dakota); G. = Grubby cattle (South Dakota)
* * = MR = Maintenance ration; FR = Fattening ration

animals averaged 86% of normal activity at 24 hours post-treatment. The range was 75% to 100%. Significant changes in ChE readings occurred in control cattle on the high energy ration at the 7, 14, and 21 day periods. Values dropped to a low of 58% activity in the grub-free control calves at the 21 day interval. Rogoff et al. (1960) had indicated that ChE depression in control animals is not unusual and is attributed to handling and other stress factors.

Cholinesterase least square means for year effect at 24 hours after treatment during this phase were 78% for 1968 and 95% for 1969 and the difference was highly significant. A significant difference also occurred during phase II but nonsignificant values were recorded for phases I and IV. Cholinesterase means were consistently lower in 1968 than 1969.

Origin means calculated for grubby and grub-free calves in phase III were both 86% compared to 94% during phase II. However, in phase I the means for grub-free livestock was 94% versus 84% for grubby animals. The higher ChE means observed in phase II possibly reflects the absence of grubs. The original fenthion treatment applied during phase I destroyed the endemic grub population in host South Dakota grubby calves and thus possibly minimized origin effect during subsequent phases.

Considering that research for 1968 and 1969 began during midwinter when temperatures occasionally dropped below zero, it would have been interesting to have reversed the priority of stress factors. It is now felt withdrawal of feed during cold weather would have affected body metabolism more than exercise and that exercise during warmer weather after calves had been exposed to their feed rations for longer periods and had gained weight would have enhanced the stress factor.

Statistical analysis of 3-day ChE values emphasizes treatment effect. A highly significant F value was obtained at this time. A significant or highly significant value at this time was anticipated as several authors have indicated that maximum ChE depression may persist for several days following application of organophosphorus insecticides; this was discussed previously. The ChE mean for treated animals was 83% compared to 96% for untreated cattle. This compares to a mean of 86% for grubby and grub-free cattle. The severest depression recorded in 1968 for grubby calves was 56%. The affected animal was on the low energy ration and was exposed to the stress factor. The calf with least depression was not stressed and was on the fattening ration; its ChE value was 98%. Similar information for 1969 included: lowest ChE value, 83% in a grubby calf exposed to stress and fattening ration; highest ChE value, 105% in a grub-free calf

(Appendix Tables 23 and 27).

It was also noted from the 3-day posttreatment bleeding that year-origin effects produced a highly significant F value of 8.157. Subsequent blood samples indicated nonsignificant F values and each was decreasing with time. Highly significant values were also produced by year-treatment effects as indicated previously. The influence of year effect was reflected in significant F values where it appeared in combination with ration-treatment and ration-origin-stress. Within the year-ration-treatment category, the 1968 animals treated with fenthion and fed the maintenance ration had a ChE means of 96% of normal compared to an 84% means in 1969. The greatest ChE depression occurred in 1968 in nontreated animals of this group. Enzymatic activity was greatest in 1969 in untreated cattle on low energy feed. See Table 9.

In the year-origin-ration-stress comparison, 1968 grub-free calves on the maintenance ration in the no feed group had the lowest ChE values. These animals averaged 18% depression compared to 111% enzymatic activity in a similar group of calves in 1969. It is possible that the year effect may be attributable to the fact that research was begun 30 days earlier in 1969 than 1968.

Using numbers of significant F values occurring at specified bleeding dates as a criteria for overall experimental stress, it appears that maximum stress in phase III occurred at 7 through 14 days following phase initiation.

Table 9. Whole blood cholinesterase activity of 6-8-months-old Hereford heifers subjected to the exercise stress factor and treatment with fenthion; phase III, spring 1969.

Group	Calves No.	Type	Mean ChE Activity (% of pretreatment value) Days Following Treatment 1 MR**	1 FR**	3 MR	3 FR	7 MR	7 FR	14 MR	14 FR	21 MR	21 FR
No Feed												
Control	2	G.F.*	100	100	98	98	98	98	98	98	97	97
No Treatment	3	G.*	98	98	98	98	98	96	99	102	99	103
Treatment	3	G. F.	92	91	89	88	96	89	100	94	106	98
Treatment	3	G.	92	88	92	91	96	102	95	102	96	100
Feed												
No Treatment	3	G.	98	100	99	100	99	100	99	100	99	100
Treatment	3	G.	96	88	99	86	107	85	102	92	114	95
Treatment	3	G.F.	91	88	89	91	93	98	97	99	98	99

* = G.F. = Grub-free cattle (North Dakota); G. = Grubby cattle (South Dakota)
* * = MR = Maintenance ration; FR = Fattening ration

At 7 days posttreatment factors such as year, origin, and treatment had significant values but most important was the influence of stress. Stress had a nonsignificant F value but associated with ration produced a highly significant value. A highly significant value was also recorded for the ration-stress-treatment group. In other groups where stress was a contributing factor, it appeared to enhance effects of associated factors. For example, in combination with year-ration and origin-ration, F values were 5.234 and 7.788, respectively. Individually, these factors were nonsignificant or at best had just achieved significance. The ration-stress-treatment group attained highly significant differences over their counterparts.

Stress and treatment effects were very apparent at 14 days also. In contrast to stress effects which had decreased slightly at this time, treatment effect in conjunction with combinations of stress-year-ration, increased slightly. Decreases in F values were apparent at the termination of this phase.

Phase IV: - As in the previous 3 phases, gross symptoms of animal toxicosis, that is, acute and subacute signs, were not evident. Several calves manifested chronic signs but considering the overall stress of the experiment, and especially the effects of the low energy feed rations, it was impossible to associate anemic appearance with insecticide toxicosis.

Tabulation of data revealed a conspicuous decrease in significant F values at the respective phase IV blood sampling intervals as compared to phase III research. This perhaps reflects animal adjustment to investigative procedures and experimental stress. Contrarily, insecticide influence was still at highly significant levels, with F values higher than those recorded in phase III. It is felt that the higher phase IV insecticide F values represent an additive effect associated with phase I - III treatments.

If the treatment effects recorded at the 5 posttreatment bleeding dates were plotted on a curve, peak or maximum differences would be seen occurring at 7 days after treatment. These data are similar to that presented previously. Highly significant F values at 24 hours and 3 days following fenthion treatment indicate the rapidity with which effects occurred. These values may possibly be related to insecticide absorption by calves. In addition to treatment effects, significant F values were recorded at 7, 14, and 21 days for year-ration-treatment effects. Reference is made to table 10.

Generally, results of phase IV bleedings were unexpected, except for insecticide effects. It was anticipated the generally inferior condition of the calves on the maintenance ration as compared to those on the fattening ration would enhance treatment-stress effects, but it did not.

In 1968, animals withheld from the maintenance ration

Table 10. F values of phase IV data at specific days posttreatment.

Source	F Values Days Posttreatment 1.	3.	7.	14.	21.
Year	.694	.103	.124	.094	.167
Origin	.065	.098	.189	.428	.655
Year-Origin	.139	.088	.162	.326	.483
Ration	.172	.114	.011	.001	.017
Year-Ration	.240	1.823	1.727	1.690	1.932
Origin-Ration	.292	.070	.323	.519	1.078
Year-Origin-Ration	.274	.225	.491	.699	1.329
Stress	.531	.007	.013	.037	.075
Year-Stress	.912	2.307	2.897	3.171	3.282
Origin-Stress	.394	1.875	2.491	2.696	3.227
Year-Origin-Stress	.237	1.177	1.941	2.353	3.282
Ration-Stress	1.005	1.533	1.164	1.070	1.078
Origin-Ration-Stress	.692	.645	.538	.629	.876
Treatment	18.792**	18.629**	22.340**	22.108**	18.469**
Year-Treatment	1.063	.636	1.244	1.900	1.929
Ration-Treatment	1.024	.128	.036	.003	.004
Year-Ration-Treatment	.247	3.325	4.404*	4.393*	4.637*
Stress-Treatment	.321	.324	.179	.015	.005
Year-Stress-Treatment	2.274	1.451	2.440	2.682	2.897

* = significant at P 0.05
* * = significant at P 0.01

and water for 24 hours prior to fenthion treatment averaged a 20% reduction in ChE activity compared to 29% for animals on continuous feed (Table 11.). Calves in the latter groups exhibited decreased enzymatic activity throughout this phase. At the conclusion of the test, they averaged 34% reduction in ChE as compared to a 24% decrease in stressed animals. It was anticipated that ChE values would have been reversed for these groups and considerably lower, reflecting the continuous stress of the maintenance ration.

The no feed stress factor was apparent in cattle withheld from their normal fattening ration prior to insecticide treatment. Cattle in this group averaged a 34% reduction in enzyme activity 24 hours posttreatment. Subsequent ChE values supported rapid animal recovery from the treatment-stress complex. At 3 days following treatment ChE activity was 83% of normal; the value at the conclusion of this phase was 88%.

Cattle on continuous feed, but not subjected to stress, averaged 86% and 101% normal ChE activity at 24 hours and 21 days following treatment.

In 1969, individual animal and group ChE depression values were much higher than 1968 (Table 12.). Twelve per cent depression was recorded for calves exposed to low maintenance ration stress and fenthion versus 96% activity in the no treatment group. The 12% depressions was the maximum

Table 11. Whole blood cholinesterase activity of 6-8-months-old Hereford heifers subjected to the exercise stress factor and treatment with fenthion; phase IV, spring 1968.

Group	Calves No.	Calves Type	Mean ChE Activity (% of pretreatment value) Days Following Treatment 1 MR**	1 FR**	3 MR	3 FR	7 MR	7 FR	14 MR	14 FR	21 MR	21 FR
No Feed												
Control	2	G.F.*	100	97	105	98	104	100	105	100	105	100
Treatment	3	G.F.*	80	63	75	83	74	83	75	84	76	88
No Treatment	3	G.F.	101	98	115	102	119	103	119	103	122	103
No Treatment	3	G.	96	100	105	105	104	106	104	107	103	108
Feed												
Treatment	3	G.	71	86	67	91	66	95	66	97	66	101
No Treatment	3	G. F.	97	96	94	114	97	110	98	120	98	112
No Treatment	3	G.	97	91	103	90	107	92	109	94	110	97

* = G.F. = Grub-free cattle (North Dakota); G. = Grubby cattle (South Dakota)
* * = MR = Maintenance ration; FR = Fattening ration

Table 12. Whole blood cholinesterase activity of 6-8-months-old Hereford heifers subjected to the exercise stress factor and treatment with fenthion; phase IV, spring 1969.

Group	Calves No.	Calves Type	Mean ChE Activity (% of pretreatment value) Days Following Treatment 1 MR**	1 FR**	3 MR	3 FR	7 MR	7 FR	14 MR	14 FR	21 MR	21 FR
No Feed												
Control	2	G.F.*	96	100	94	100	94	100	95	100	96	100
Treatment	3	G.*	88	91	89	86	89	86	92	88	95	90
No Treatment	3	G. F.	96	100	99	99	99	100	98	100	97	100
No Treatment	3	G.	98	99	98	97	98	99	98	99	98	100
Feed												
Treat	3	G.	91	97	89	95	92	93	92	94	93	96
No Treatment	3	G.	97	100	98	100	98	100	98	100	97	100
No Treatment	3	G.F.	97	100	95	100	95	100	96	100	96	100

* = G.F. = Grub-free cattle (North Dakota); G. = Grubby cattle (South Dakota)
* * = MR = Maintenance ration; FR = Fattening ration

recorded for all groups at 24 hours posttreatment. Cholinesterase values returned to 95% normal activity at 21 days. Maximum individual calf depression at 24 hours was 17%; this animal was withheld from its fattening ration for 24 hours prior to treatment with fenthion. Animals within this group averaged 9% depression at 24 hours, 16% at 3 and 7 days and 12% and 10% at 14 and 21 days. Of all groups examined, these animals exhibited the greatest ChE depression (Appendix Table 28.).

SUMMARY

Six-to 8-months-old Hereford heifer calves infested with Hypoderma bovis and Hypoderma lineatum and grub-free animals of a similar age were exposed to various stress conditions prior to treatment with fenthion pour-on. Stress factors for this research included 30 minutes of continuous exercise and/or the withholding of food and water for 24 hours prior to insecticide treatment. The effects of stress and fenthion administration were evaluated by determining fluctuations in whole blood cholinesterase levels. This research was divided into 4 phases and was conducted in December through June of 1968 and 1969. Forty calves were used as experimental subjects each year.

Phase I data revealed that exercise stress affected cholinesterase the least and insecticide treatment affected ChE the most. Insecticide influence prevailed through 21 days posttreatment. Cattle origin reflecting the presence or absence of cattle grubs provided a highly significant difference in ChE values for both grubby and grub-free cattle through 24 hours posttreatment. Subsequent values were nonsignificant. One animal exhibited typical organophosphorus acute toxicosis, however, recovery was uneventful and without the aid of medication.

Analysis of phase II data indicated that the most influential factor was year. Year produced significant F

values through 7 days posttreatment. Year in conjunction with cattle origin, feed ration, and stress also contributed significantly in ChE depression. The effects of fenthion treatment were again evident during this phase; highly significant F values were recorded at 3,7, and 14 days following insecticide administration.

Cholinesterase values of animals on the fattening ration exposed to stress were consistently lower throughout phase II than those of animals on maintenance feed. Cholinesterase values of grubby and grub-free calves averaged about 8% depression at 24 hours posttreatment and variations remained minimal through this phase.

During phase III F values for year rose to highly significant levels. Cholinesterase means for 1968 were consistently lower in 1968 than 1969 and this may be the result of initiating 1969 research 30 days earlier than 1968 research. Cattle origin and feed ration decreased to nonsignificant levels.

Data obtained from phase III research revealed a conspicuous decrease of significant F values compared to earlier research. Contrarily, insecticide effects were still prevalent and in some instances more pronounced.

Cholinesterase values of calves on the high energy feed differed little from those on the maintenance feed; this was unexpected. The feed ration abstinence stress had little

effect on the calves. Cattle origin and year also had less effect on ChE levels during phase IV than during phases I - III.

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APPENDIX

Appendix Table 1. Least squares analysis of variance of phase I data; 24 hours posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0000	.029
Origin	1	.0114	8.123[b]
Year-Origin	1	.0000	.009
Ration	1	.0001	.100
Year-Ration	1	.0014	1.047
Origin-Ration	1	.0014	1.047
Year-Origin-Ration	1	.0044	3.162
Stress	1	.0001	.134
Year-Stress	1	.0013	.949
Origin-Stress	1	.0003	.237
Year-Origin-Stress	1	.0002	.171
Ration-Stress	1	.0007	.534
Year-Ration-Stress	1	.0035	2.507
Origin-Ration-Stress	1	.0006	.433
Treatment	1	.0686	48.878[b]
Year-Treatment	1	.0054	3.893
Origin-Treatment	1	.0000	.000
Ration-Treatment	1	.0000	.000
Year-Ration-Treatment	1	.0035	2.507
Stress-Treatment	1	.0046	3.338
Year-Stress-Treatment	1	.0023	1.667
Ration-Stress-Treatment	1	.0012	.903
Year-Ration-Stress-Treatment	1	.0000	.005
Year-Origin-Ration-Stress	1	.0019	1.367
Error	48	.0014	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 2. Least squares analysis of variance of phase I data; 3 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0079	3.862
Origin	1	.0006	.330
Year-Origin	1	.0016	.805
Ration	1	.0050	2.440
Year-Ration	1	.0125	6.097[a]
Origin-Ration	1	.0000	.023
Year-Origin-Ration	1	.0026	1.297
Stress	1	.0011	.571
Year-Stress	1	.0036	1.797
Origin-Stress	1	.0050	2.440
Year-Origin-Stress	1	.0001	.074
Ration-Stress	1	.0000	.023
Year-Ration-Stress	1	.0017	.841
Origin-Ration-Stress	1	.0037	1.851
Treatment	1	.0785	38.294[b]
Year-Treatment	1	.0006	.319
Origin-Treatment	1	.0000	.001
Ration-Treatment	1	.0000	.001
Year-Ration-Treatment	1	.0081	3.982
Stress-Treatment	1	.0002	.104
Year-Stress-Treatment	1	.0016	.823
Ration-Stress-Treatment	1	.0015	.751
Year-Ration-Stress-Treatment	1	.0019	.936
Year-Origin-Ration-Stress	1	.0039	1.906
Error	48	.0020	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 3. Least squares analysis of variance of phase I data; 7 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0037	1.253
Origin	1	.0056	1.871
Year-Origin	1	.0026	.878
Ration	1	.0031	1.040
Year-Ration	1	.0031	1.040
Origin-Ration	1	.0074	2.445
Year-Origin-Ration	1	.0096	3.177
Stress	1	.0013	.451
Year-Stress	1	.0009	.327
Origin-Stress	1	.0001	.043
Year-Origin-Stress	1	.0000	.000
Ration-Stress	1	.0005	.165
Year-Ration-Stress	1	.0003	.116
Origin-Ration-Stress	1	.0000	.015
Treatment	1	.0785	25.912[b]
Year-Treatment	1	.0175	5.781[a]
Origin-Treatment	1	.0000	.000
Ration-Treatment	1	.0000	.000
Year-Ration-Treatment	1	.0014	.462
Stress-Treatment	1	.0001	.046
Year-Stress-Treatment	1	.0018	.607
Ration-Stress-Treatment	1	.0015	.508
Year-Ration-Stress-Treatment	1	.0000	.022
Year-Origin-Ration-Stress	1	.0003	.105
Error	48	.0030	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 4. Least squares analysis of variance of phase I data; 14 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0003	.121
Origin	1	.0010	.394
Year-Origin	1	.0064	2.352
Ration	1	.0050	1.848
Year-Ration	1	.0025	.919
Origin-Ration	1	.0108	3.947
Year-Origin-Ration	1	.0053	1.944
Stress	1	.0010	.394
Year-Stress	1	.0000	.003
Origin-Stress	1	.0029	1.093
Year-Origin-Stress	1	.0016	.588
Ration-Stress	1	.0008	.292
Year-Ration-Stress	1	.0003	.121
Origin-Ration-Stress	1	.0000	.011
Treatment	1	.0585	21.330[b]
Year-Treatment	1	.0026	.953
Origin-Treatment	1	.0000	.001
Ration-Treatment	1	.0000	.001
Year-Ration-Treatment	1	.0006	.221
Stress-Treatment	1	.0019	.700
Year-Stress-Treatment	1	.0005	.190
Ration-Stress-Treatment	1	.0044	1.619
Year-Ration-Stress-Treatment	1	.0008	.311
Year-Origin-Ration-Stress	1	.0019	.700
Error	48	.0027	

* All Original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 5. Least squares analysis of variance of phase I data; 21 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0036	1.017
Origin	1	.0000	.019
Year-Origin	1	.0054	1.531
Ration	1	.0172	4.840[a]
Year-Ration	1	.0005	.158
Origin-Ration	1	.0004	.134
Year-Origin-Ration	1	.0038	1.079
Stress	1	.0017	.494
Year-Stress	1	.0001	.046
Origin-Stress	1	.0016	.473
Year-Origin-Stress	1	.0052	1.457
Ration-Stress	1	.0008	.239
Year-Ration-Stress	1	.0007	.210
Origin-Ration-Stress	1	.0000	.001
Treatment	1	.0479	13.444[b]
Year-Treatment	1	.0028	.785
Origin-Treatment	1	.0000	.000
Ration-Treatment	1	.0000	.000
Year-Ration-Treatment	1	.0005	.146
Stress-Treatment	1	.0006	.183
Year-Stress-Treatment	1	.0008	.239
Ration-Stress-Treatment	1	.0033	.926
Year-Ration-Stress-Treatment	1	.0011	.320
Year-Origin-Ration-Stress	1	.0022	.631
Error	48	.0035	

* All original MS values were divided by .100.
a Significant at $P \leq 0.05$.
b Significant at $P \leq 0.01$.

Appendix Table 6. Least squares analysis of variance of phase II data; 24 hours posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0033	6.472[a]
Origin	1	.0001	.212
Year-Origin	1	.0034	6.677[a]
Ration	1	.0000	.000
Year-Ration	1	.0000	.000
Origin-Ration	1	.0000	.010
Year-Origin-Ration	1	.0000	.020
Stress	1	.0001	.293
Year-Stress	1	.0036	7.098[a]
Origin-Stress	1	.0009	1.801
Year-Origin-Stress	1	.0000	.177
Ration-Stress	1	.0000	.004
Year-Ration-Stress	1	.0011	2.257
Origin-Ration-Stress	1	.0000	.145
Treatment	1	.0002	.520
Year-Treatment	1	.0048	9.271[b]
Ration-Treatment	1	.0000	.040
Year-Ration-Treatment	1	.0006	1.170
Stress-Treatment	1	.0037	7.205[a]
Year-Stress-Treatment	1	.0000	.014
Ration-Stress-Treatment	1	.0001	.231
Year-Ration-Stress-Treatment	1	.0013	2.568
Year-Origin-Ration-Stress	1	.0011	2.138
Error	48	.0005	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 7. Least squares analysis of variance of phase II data; 3 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0034	7.226[a]
Origin	1	.0009	2.039
Year-Origin	1	.0038	8.157[b]
Ration	1	.0007	1.588
Year-Ration	1	.0000	.028
Origin-Ration	1	.0000	.002
Year-Origin-Ration	1	.0004	.933
Stress	1	.0004	.933
Year-Stress	1	.0025	5.337[a]
Origin-Stress	1	.0017	3.733
Year-Origin-Stress	1	.0006	1.383
Ration-Stress	1	.0001	.298
Year-Ration-Stress	1	.0023	4.955[a]
Origin-Ration-Stress	1	.0009	1.921
Treatment	1	.0000	.064
Year-Treatment	1	.0197	41.838[b]
Ration-Treatment	1	.0000	1.695
Year-Ration-Treatment	1	.0031	6.564[a]
Stress-Treatment	1	.0020	4.236[a]
Year-Stress-Treatment	1	.0000	.143
Ration-Stress-Treatment	1	.0012	2.547
Year-Ration-Stress-Treatment	1	.0010	2.286
Year-Origin-Ration-Stress	1	.0029	6.141[a]
Error	48	.0004	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 8. Least squares analysis of variance of phase II data; 7 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0032	7.203[a]
Origin	1	.0000	.057
Year-Origin	1	.0011	2.635
Ration	1	.0009	2.040
Year-Ration	1	.0007	1.576
Origin-Ration	1	.0003	.866
Year-Origin-Ration	1	.0001	.248
Stress	1	.0000	.047
Year-Stress	1	.0000	.002
Origin-Stress	1	.0005	1.316
Year-Origin-Stress	1	.0000	.135
Ration-Stress	1	.0002	.607
Year-Ration-Stress	1	.0016	3.628
Origin-Ration-Stress	1	.0000	.023
Treatment	1	.0000	.012
Year-Treatment	1	.0204	45.892[b]
Ration-Treatment	1	.0043	9.849[b]
Year-Ration-Treatment	1	.0048	10.965[b]
Stress-Treatment	1	.0007	1.631
Year-Stress-Treatment	1	.0003	.712
Ration-Stress-Treatment	1	.0003	.712
Year-Ration-Stress-Treatment	1	.0011	2.496
Year-Origin-Ration-Stress	1	.0006	1.417
Error	48	.0004	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 9. Least squares analysis of variance of phase II data; 14 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0009	1.798
Origin	1	.0003	.604
Year-Origin	1	.0014	2.539
Ration	1	.0048	8.840[b]
Year-Ration	1	.0014	2.601
Origin-Ration	1	.0014	2.539
Year-Origin-Ration	1	.0005	1.021
Stress	1	.0000	.046
Year-Stress	1	.0000	.109
Origin-Stress	1	.0000	.074
Year-Origin-Stress	1	.0000	.006
Ration-Stress	1	.0000	.064
Year-Ration-Stress	1	.0010	1.904
Origin-Ration-Stress	1	.0000	.054
Treatment	1	.0000	.000
Year-Treatment	1	.0082	14.954[b]
Ration-Treatment	1	.0012	2.239
Year-Ration-Treatment	1	.0026	4.822[a]
Stress-Treatment	1	.0005	.907
Year-Stress-Treatment	1	.0000	.000
Ration-Stress-Treatment	1	.0002	.463
Year-Ration-Stress-Treatment	1	.0000	.019
Year-Origin-Ration-Stress	1	.0003	.545
Error	48	.0005	

* All original MS values were divided by .100.

a Significant at $P < 0.05$.

b Significant at $P < 0.01$.

Appendix Table 10. Least squares analysis of variance of phase II data; 21 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0010	2.635
Origin	1	.0000	.231
Year-Origin	1	.0002	.716
Ration	1	.0013	3.430
Year-Ration	1	.0011	2.941
Origin-Ration	1	.0006	1.581
Year-Origin-Ration	1	.0002	.569
Stress	1	.0002	.640
Year-Stress	1	.0000	.026
Origin-Stress	1	.0000	.189
Year-Origin-Stress	1	.0000	.005
Ration-Stress	1	.0002	.716
Year-Ration-Stress	1	.0002	.716
Origin-Ration-Stress	1	.0000	.005
Treatment	1	.0008	2.141
Year-Treatment	1	.0022	5.655[a]
Ration-Treatment	1	.0001	.301
Year-Ration-Treatment	1	.0002	.678
Stress-Treatment	1	.0006	1.639
Year-Stress-Treatment	1	.0000	.075
Ration-Stress-Treatment	1	.0020	5.228[a]
Year-Ration-Stress-Treatment	1	.0002	.678
Year-Origin-Ration-Stress	1	.0002	.640
Error	48	.0003	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 11. Least squares analysis of variance of phase III data; 24 hours posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0321	23.438 [b]
Origin	1	.0000	.030
Year-Origin	1	.0000	.061
Ration	1	.0002	.202
Year-Ration	1	.0005	.383
Origin-Ration	1	.0076	5.597 [a]
Year-Origin-Ration	1	.0042	3.061
Stress	1	.0034	2.256
Year-Stress	1	.0025	1.880
Origin-Stress	1	.0038	2.808
Year-Origin-Stress	1	.0012	.924
Ration-Stress	1	.0001	.107
Year-Ration-Stress	1	.0001	.092
Origin-Ration-Stress	1	.0009	.661
Treatment	1	.0080	5.830 [a]
Year-Treatment	1	.0000	.053
Ration-Treatment	1	.0004	.325
Year-Ration-Treatment	1	.0002	.160
Stress-Treatment	1	.0003	.247
Year-Stress-Treatment	1	.0000	.043
Ration-Stress-Treatment	1	.0036	2.683
Year-Ration-Stress-Treatment	1	.0019	1.443
Year-Origin-Ration-Stress	1	.0005	.411
Error	48	.0013	

* All original MS values were divided by .100.

a Significant at $P < 0.05$.

b Significant at $P < 0.01$.

Appendix Table 12. Least squares analysis of variance of phase III data; 3 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0090	9.735 b
Origin	1	.0026	2.815
Year-Origin	1	.0003	.324
Ration	1	.0000	.000
Year-Ration	1	.0000	.000
Origin-Ration	1	.0001	.152
Year-Origin-Ration	1	.0007	.808
Stress	1	.0009	.992
Year-Stress	1	.0016	1.782
Origin-Stress	1	.0001	.176
Year-Origin-Stress	1	.0001	.152
Ration-Stress	1	.0013	1.410
Year-Ration-Stress	1	.0007	.823
Origin-Ration-Stress	1	.0001	.109
Treatment	1	.0183	19.783 b
Year-Treatment	1	.0045	4.929 a
Ration-Treatment	1	.0000	.083
Year-Ration-Treatment	1	.0010	1.113
Stress-Treatment	1	.0018	2.027
Year-Stress-Treatment	1	.0018	1.985
Ration-Stress-Treatment	1	.0022	2.468
Year-Ration-Stress-Treatment	1	.0000	.101
Year-Origin-Ration-Stress	1	.0006	.704
Error	48	.0009	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 13. Least squares analysis of variance of phase III data; 7 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0429	38.948[b]
Origin	1	.0047	4.309[a]
Year-Origin	1	.0011	1.063
Ration	1	.0022	2.021
Year-Ration	1	.0032	2.956
Origin-Ration	1	.0032	2.953
Year-Origin-Ration	1	.0003	.318
Stress	1	.0001	.115
Year-Stress	1	.0013	1.263
Origin-Stress	1	.0000	.068
Year-Origin-Stress	1	.0005	.531
Ration-Stress	1	.0131	11.917[b]
Year-Ration-Stress	1	.0057	5.234[a]
Origin-Ration-Stress	1	.0085	7.788[b]
Treatment	1	.0055	5.050[a]
Year-Treatment	1	.0044	4.014
Ration-Treatment	1	.0014	1.355
Year-Ration-Treatment	1	.0000	.000
Stress-Treatment	1	.0061	5.549[a]
Year-Stress-Treatment	1	.0027	2.466
Ration-Stress-Treatment	1	.0151	13.759[b]
Year-Ration-Stress-Treatment	1	.0017	1.551
Year-Origin-Ration-Stress	1	.0001	.159
Error	48	.0011	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 14. Least squares analysis of variance of phase III data; 14 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0450	43.237[b]
Origin	1	.0031	3.028
Year-Origin	1	.0027	2.647
Ration	1	.0026	2.522
Year-Ration	1	.0022	2.181
Origin-Ration	1	.0022	2.207
Year-Origin-Ration	1	.0024	2.378
Stress	1	.0003	.311
Year-Stress	1	.0002	.199
Origin-Stress	1	.0004	.405
Year-Origin-Stress	1	.0000	.088
Ration-Stress	1	.0092	8.888[b]
Year-Ration-Stress	1	.0061	5.876[a]
Origin-Ration-Stress	1	.0026	2.556
Treatment	1	.0046	4.425[a]
Year-Treatment	1	.0024	2.391
Ration-Treatment	1	.0013	1.273
Year-Ration-Treatment	1	.0000	.266
Stress-Treatment	1	.0067	6.499[a]
Year-Stress-Treatment	1	.0067	6.499[a]
Ration-Stress-Treatment	1	.0072	6.933[a]
Year-Ration-Stress-Treatment	1	.0019	1.826
Year-Origin-Ration-Stress	1	.0003	.370
Error	48	.0010	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 15. Least squares analysis of variance of phase III data; 21 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0384	27.068[b]
Origin	1	.0045	3.216
Year-Origin	1	.0075	5.301[a]
Ration	1	.0020	1.424
Year-Ration	1	.0023	1.661
Origin-Ration	1	.0037	2.637
Year-Origin-Ration	1	.0053	3.759
Stress	1	.0011	.822
Year-Stress	1	.0000	.036
Origin-Stress	1	.0000	.001
Year-Origin-Stress	1	.0004	.338
Ration-Stress	1	.0062	4.411[a]
Year-Ration-Stress	1	.0039	2.791
Origin-Ration-Stress	1	.0005	.397
Treatment	1	.0013	.957
Year-Treatment	1	.0003	.276
Ration-Treatment	1	.0018	1.312
Year-Ration-Treatment	1	.0004	.301
Stress-Treatment	1	.0057	4.019[a]
Year-Stress-Treatment	1	.0079	5.582[a]
Ration-Stress-Treatment	1	.0030	2.119
Year-Ration-Stress-Treatment	1	.0009	.658
Year-Origin-Ration-Stress	1	.0009	.640
Error	48	.0014	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 16. Least squares analysis of variance of phase IV data; 24 hours posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0012	.694
Origin	1	.0001	.065
Year-Origin	1	.0002	.139
Ration	1	.0003	.172
Year-Ration	1	.0004	.240
Origin-Ration	1	.0005	.292
Year-Origin-Ration	1	.0005	.274
Stress	1	.0009	.531
Year-Stress	1	.0016	.912
Origin-Stress	1	.0007	.394
Year-Origin-Stress	1	.0004	.237
Ration-Stress	1	.0018	1.005
Origin-Ration-Stress	1	.0011	.692
Treatment	1	.0343	18.792[b]
Year-Treatment	1	.0019	1.063
Ration-Treatment	1	.0018	1.024
Year-Ration-Treatment	1	.0004	.247
Stress-Treatment	1	.0005	.321
Year-Stress-Treatment	1	.0041	2.274
Error	52	.0018	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 17. Least squares analysis of variance of phase IV data; 3 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0002	.103
Origin	1	.0001	.098
Year-Origin	1	.0001	.088
Ration	1	.0002	.114
Year-Ration	1	.0036	1.823
Origin-Ration	1	.0001	.070
Year-Origin-Ration	1	.0004	.225
Stress	1	.0001	.007
Year-Stress	1	.0045	2.307
Origin-Stress	1	.0037	1.875
Year-Origin-Stress	1	.0023	1.177
Ration-Stress	1	.0030	1.533
Origin-Ration-Stress	1	.0012	.645
Treatment	1	.0369	18.629[b]
Year-Treatment	1	.0012	.636
Ration-Treatment	1	.0002	.128
Year-Ration-Treatment	1	.0066	3.325
Stress-Treatment	1	.0006	.324
Year-Stress-Treatment	1	.0028	1.451
Error	52	.0019	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 18. Least squares analysis of variance of phase IV data; 7 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0002	.124
Origin	1	.0003	.189
Year-Origin	1	.0003	.162
Ration	1	.0000	.011
Year-Ration	1	.0032	1.727
Origin-Ration	1	.0006	.323
Year-Origin-Ration	1	.0009	.491
Stress	1	.0000	.013
Year-Stress	1	.0054	2.897
Origin-Stress	1	.0046	2.491
Year-Origin-Stress	1	.0036	1.941
Ration-Stress	1	.0021	1.164
Origin-Ration-Stress	1	.0010	.538
Treatment	1	.0416	22.340[b]
Year-Treatment	1	.0023	1.244
Ration-Treatment	1	.0000	.036
Year-Ration-Treatment	1	.0082	4.404[a]
Stress-Treatment	1	.0003	.179
Year-Stress-Treatment	1	.0045	2.440
Error	52	.0018	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 19. Least squares analysis of variance of phase IV data; 14 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0001	.094
Origin	1	.0008	.428
Year-Origin	1	.0006	.326
Ration	1	.0000	.001
Year-Ration	1	.0031	1.690
Origin-Ration	1	.0009	.519
Year-Origin-Ration	1	.0013	.699
Stress	1	.0000	.037
Year-Stress	1	.0059	3.171
Origin-Stress	1	.0050	2.696
Year-Origin-Stress	1	.0044	2.353
Ration-Stress	1	.0019	1.070
Origin-Ration-Stress	1	.0011	.629
Treatment	1	.0413	22.108[b]
Year-Treatment	1	.0035	1.900
Ration-Treatment	1	.0000	.003
Year-Ration-Treatment	1	.0082	4.393[a]
Stress-Treatment	1	.0000	.015
Year-Stress-Treatment	1	.0050	2.682
Error	52	.0018	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix Table 20. Least squares analysis of variance of phase IV data; 21 days posttreatment.

Source	DF	MS*	F Value
Total	72		
Year	1	.0003	.167
Origin	1	.0012	.655
Year-Origin	1	.0009	.483
Ration	1	.0000	.017
Year-Ration	1	.0037	1.932
Origin-Ration	1	.0020	1.078
Year-Origin-Ration	1	.0025	1.329
Stress	1	.0001	.075
Year-Stress	1	.0062	3.282
Origin-Stress	1	.0061	3.227
Year-Origin-Stress	1	.0062	3.282
Ration-Stress	1	.0020	1.078
Origin-Ration-Stress	1	.0016	.876
Treatment	1	.0354	18.469[b]
Year-Treatment	1	.0037	1.929
Ration-Treatment	1	.0000	.004
Year-Ration-Treatment	1	.0088	4.637[a]
Stress-Treatment	1	.0000	.005
Year-Stress-Treatment	1	.0055	2.897
Error	52	.0019	

* All original MS values were divided by .100.
a Significant at $P < 0.05$.
b Significant at $P < 0.01$.

Appendix table 21. Animal ChE values calculated from pretreatment bleedings. Percent values represent fluctuations in ChE activity at days posttreatment as compared to pretreatment control values. Phase I, 1968.

Animal Group & Number	Animal Designation*	Pretreatment Δ pH values	ChE activity expressed as % of pretreatment Δ pH control values				
			Days Posttreatment				
			1.	3.	7.	14.	21.
No Exercise			Maintenance Ration				
870	GF-C	.394	99	103	105	108	102
873	GF-C	.261	107	102	113	102	103
547	G-T	.452	103	104	108	107	105
808	G-T	.439	103	92	99	99	99
676	G-T	.252	119	115	156	151	153
863	GF-NT	.333	69	55	79	88	92
865	GF-NT	.433	70	56	61	72	86
872	GF-NT	.443	81	72	67	68	77
678	G-NT	.402	76	88	101	109	118
837	G-NT	.336	55	68	56	67	71
839	G-NT	.435	99	97	94	104	111
Exercise							
802	G-T	.344	88	80	129	138	154
825	G-T	.391	84	126	127	131	133
583	G-T	.577	103	92	102	102	98
861	GF-NT	.282	97	85	82	95	98
864	GF-NT	.574	61	62	50	59	74
867	GF-NT	.238	77	90	79	85	101
807	G-NT	.312	69	53	99	113	124
850	G-NT	.426	78	78	69	89	94
853	G-NT	.452	79	76	87	108	93
No Exercise			Fattening Ration				
681	GF-C	.450	99	98	95	131	114
871	GF-C	.421	101	99	92	131	129
811	G-T	.414	92	98	115	113	112
823	G-T	.539	99	99	92	97	102
827	G-T	.459	99	99	96	94	96
862	GF-NT	.426	48	75	89	89	88
866	GF-NT	.405	96	93	93	100	100
869	GF-NT	.535	78	79	87	86	86
687	G-NT	.450	36	60	51	66	79
829	G-NT	.375	42	84	93	94	94
845	G-NT	.517	54	73	62	97	75
Exercise							
805	G-T	.269	92	81	90	94	95
806	G-T	.357	120	148	150	147	148
826	G-T	.488	95	93	88	97	102
831	G-NT	.454	97	86	61	74	83
832	G-NT	.399	76	66	58	52	73
575	G-NT	.547	63	63	69	76	84
868	GF-NT	.288	95	65	78	86	96
874	GF-NT	.417	76	75	71	74	76
875	GF-NT	.293	94	69	90	95	102

* G; GF; C; T; NT = Grubby calf; grub-free calf; control; treatment and no treatment

Appendix table 22. Animal ChE values calculated from pretreatment bleedings. Percent values represent fluctuations in ChE activity at days posttreatment as compared to pretreatment control values. Phase II, 1968.

Animal Group & Number	Animal Designation*	Pretreatment ΔpH values	ChE activity expressed as % of pretreatment Δ pH control values — Days Posttreatment 1.	3.	7.	14.	21.
No Exercise			Maintenance Ration				
870	GF-C	.567	96	97	97	97	98
873	GF-C	.497	100	100	100	100	101
547	G-T	.597	87	83	83	84	91
808	G-T	.511	80	76	75	78	89
676	G-T	.451	75	67	83	98	81
863	GF-NT	.366	93	94	96	98	96
865	GF-NT	.602	100	97	96	95	95
872	GF-NT	.489	93	92	93	96	97
678	G-NT	.603	102	102	101	102	101
837	G-NT	.429	100	99	99	99	100
839	G-NT	.554	98	98	97	98	99
Exercise							
802	G-T	.629	96	95	92	92	94
825	G-T	.524	99	94	92	95	98
583	G-T	.569	98	88	86	88	98
861	GF-NT	.405	98	98	98	99	99
864	GF-NT	.546	102	99	99	100	100
867	GF-NT	.289	81	84	87	88	89
807	G-NT	.482	95	96	96	97	97
850	G-NT	.583	97	97	98	98	99
853	G-NT	.426	101	100	99	100	100
No Exercise			Fattening Ration				
681	GF-C	.551	101	100	100	100	100
871	GF-C	.566	95	92	78	81	87
811	G-T	.461	91	93	94	96	95
823	G-T	.580	86	87	87	89	89
827	G-T	.433	87	85	83	92	89
862	GF-NT	.453	99	97	98	99	99
866	GF-NT	.398	99	103	103	104	102
869	GF-NT	.581	86	94	94	101	95
687	G-NT	.376	86	86	90	100	93
829	G-NT	.485	99	99	100	100	100
845	G-NT	.440	100	101	100	100	100
Exercise							
805	G-T	.251	99	89	75	100	98
806	G-T	.512	100	94	98	100	98
826	G-T	.662	84	76	73	77	81
831	G-NT	.435	103	100	100	99	100
832	G-NT	.529	92	96	95	98	96
575	G-NT	.603	103	101	100	98	100
868	GF-NT	.332	96	97	94	97	97
874	GF-NT	.366	97	98	98	99	98
875	GF-NT	.392	79	88	87	92	89

* G; GF; C; T; NT = Grubby calf; grub-free calf; control; treatment and no treatment

Appendix table 23. Animal ChE values calculated from pretreatment bleedings. Percent values represent fluctuations in ChE activity at days posttreatment as compared to pretreatment control values. Phase III, 1968.

Animal Group & Number	Animal Designation*	Pretreatment ΔpH values	ChE activity expressed as % of pretreatment ΔpH control values — Days Posttreatment 1.	3.	7.	14.	21.
No Feed			Maintenance Ration				
870	GF-C	.561	100	100	100	100	100
873	GF-C	.495	100	101	101	101	101
547	G-NT	.549	89	92	99	99	99
808	G-NT	.530	100	100	100	98	98
676	G-NT	.430	87	115	81	87	93
863	GF-T	.564	69	63	54	52	48
865	GF-T	.478	86	101	78	80	91
872	GF--T	.487	63	76	87	88	88
678	G-T	.488	67	94	86	99	116
837	G-T	.416	76	71	84	96	103
839	G-T	.468	71	87	72	81	95
Feed							
802	G-NT	.625	51	74	72	76	83
825	G-NT	.624	94	92	49	58	67
583	G-NT	.699	99	114	109	103	103
861	GF-T	.490	52	66	46	68	79
864	GF-T	.624	68	66	46	46	48
867	GF-T	.540	56	60	44	38	35
807	G-T	.395	87	56	70	71	93
850	G-T	.544	73	77	71	71	73
853	G-T	.521	95	76	70	67	67
NoFeed			Fattening Ration				
681	GF-C	.657	96	103	81	76	70
871	GF-C	.554	81	80	64	51	45
811	G-NT	.406	44	73	50	52	53
823	G-NT	.566	75	86	96	96	96
827	G-NT	.601	107	108	76	76	83
862	GF-T	.460	79	86	69	68	78
866	GF-T	.405	98	72	72	81	81
869	GF-T	.439	100	87	88	89	99
687	G-T	.476	47	71	71	72	79
829	G-T	.429	86	98	98	100	102
845	G-T	.474	86	88	85	86	87
Feed							
805	G-NT	.272	68	92	110	114	114
806	G-NT	.385	75	112	102	100	98
826	G-NT	.391	93	112	114	114	113
831	G-T	.451	75	77	65	68	83
832	G-T	.547	59	76	65	68	70
575	G-T	.543	51	73	64	63	69
868	GF-T	.411	75	62	66	67	71
874	GF-T	.365	71	76	80	87	90
875	GF-T	.502	73	54	58	59	60

* G; GF; C; T; NT = Grubby calf; grub-free calf; control; treatment and no treatment

Appendix table 24. Animal ChE values calculated from pretreatment bleedings. Percent values represent fluctuations in ChE activity at days posttreatment as compared to pretreatment control values. Phase IV, 1968.

Animal Group & Number	Animal Designation*	Pretreatment ΔpH values	ChE activity expressed as % of pretreatment Δ pH control values				
			Days Posttreatment				
			1.	3.	7.	14.	21.
No Feed			Maintenance Ration				
870	GF-C	.491	100	100	98	99	98
873	GF-C	.316	100	110	110	111	111
547	G-T	.519	78	65	64	65	65
808	G-T	.518	100	81	78	78	79
676	G-T	.512	61	78	80	83	83
863	GF-NT	.261	100	133	144	146	153
865	GF-NT	.480	101	106	104	103	103
872	GF-NT	.427	101	106	109	109	109
678	G-NT	.607	97	97	97	97	95
837	G-NT	.431	99	101	101	101	100
839	G-NT	.429	93	116	115	115	114
Feed							
802	G-T	.632	66	60	59	57	58
825	G-T	.526	71	77	76	77	77
583	G-T	.693	77	64	63	63	64
861	GF-NT	.475	96	91	96	96	97
864	GF-NT	.399	96	95	96	97	97
867	GF-NT	.378	99	97	99	100	100
807	G-NT	.399	92	95	101	106	107
850	G-NT	.546	100	114	113	113	113
853	G-NT	.349	99	101	108	109	109
No Feed			Fattening Ration				
681	GF-C	.493	91	89	94	96	96
871	GF-C	.450	102	107	105	104	105
811	G-T	.381	59	92	92	95	100
823	G-T	.545	72	84	84	83	87
827	G-T	.603	57	72	72	75	78
862	GF-NT	.430	98	106	106	107	106
866	GF-NT	.325	97	108	108	108	107
869	GF-NT	.446	99	92	95	95	97
687	G-NT	.374	100	94	95	96	97
829	G-NT	.430	98	89	92	95	96
845	G-NT	.429	102	131	130	130	130
Feed							
805	G-T	.319	81	72	77	81	93
806	G-T	.386	84	92	100	102	104
826	G-T	.439	94	110	108	107	107
831	G-NT	.445	70	84	88	91	93
832	G-NT	.450	102	109	103	103	103
575	G-NT	.451	102	76	86	88	95
868	GF-NT	.415	80	116	115	114	114
874	GF-NT	.353	93	110	109	108	108
875	GF-NT	.379	115	115	115	113	115

* G; GF; C; T; NT; = Grubby calf; grub-free calf; control; treatment and no treatment

Appendix table 25. Animal ChE values calculated from pretreatment bleedings. Percent values represent fluctuations in ChE activity at days posttreatment as compared to pretreatment control values. Phase I, 1969.

Animal Group & Number	Animal Designation*	pretreatment Δ pH values	ChE activity expressed as % of pretreatment Δ pH control values				
			Days Posttreatment				
			1.	3.	7.	14.	21.
No Exercise			Maintenance Ration				
705	GF-C	.358	107	131	91	117	91
707	GF-C	.615	68	95	97	85	100
479	G-NT	.522	99	114	93	95	93
452	G-NT	.537	96	132	84	114	121
455	G-NT	.520	91	150	105	111	103
708	GF-T	.602	90	85	86	86	93
709	GF-T	.483	97	96	113	95	103
710	GF-T	.607	84	83	93	93	87
408	G-T	.574	74	92	92	85	92
414	G-T	.567	74	95	59	81	85
475	G-T	.442	79	69	93	122	90
Exercise							
415	G-NT	.487	87	103	72	99	84
500	G-NT	.472	106	106	90	102	106
425	G-NT	.577	99	99	99	99	99
440	G-T	.597	83	81	89	91	90
453	G-T	.465	72	66	82	88	97
483	G-T	.480	68	81	99	98	104
712	GF-T	.397	86	98	96	101	96
714	GF-T	.428	71	99	100	107	105
716	GF-T	.328	98	84	102	128	141
No Exercise			Fattening Ration				
704	GF-C	.575	90	98	74	89	77
706	GF-C	.436	84	122	143	102	131
431	G-NT	.414	96	94	94	95	96
447	G-NT	.437	97	100	100	100	100
463	G-NT	.539	99	92	96	100	100
402	G-T	.526	80	83	47	80	74
413	G-T	.429	80	101	86	93	100
498	G-T	.537	77	88	84	61	79
701	GF-T	.555	91	96	66	79	70
703	GF-T	.434	95	98	103	110	87
715	GF-T	.671	69	61	76	69	63
Exercise							
404	G-NT	.478	77	67	90	126	125
424	G-NT	.380	97	105	58	87	92
460	G-NT	.548	75	90	102	78	73
474	G-T	.784	64	63	44	53	52
436	G-T	.734	75	73	59	62	56
480	G-T	.505	87	81	87	83	91
702	GF-T	.545	90	84	64	72	70
711	GF-T	.431	82	90	79	99	102
713	GF-T	.466	81	72	82	82	83

* G; GF; C; T; NT = Grubby calf; grub-free calf; control; treatment and no treatment

Appendix table 26. Animal ChE values calculated from pretreatment bleedings. Percent values represent fluctuations in ChE activity at days posttreatment as compared to pretreatment control values. Phase II, 1969.

Animal Group & Number	Animal Designation*	Pretreatment Δ pH values	ChE activity expressed as % of pretreatment Δ pH control values				
			Days Posttreatment				
			1.	3.	7.	14.	21.
No Exercise			Maintenance Ration				
705	GF-C	.595	97	100	99	99	99
707	GF-C	.549	132	146	137	121	112
479	G-T	.515	97	90	89	92	98
452	G-T	.615	86	70	70	77	87
455	G-T	.542	97	78	79	89	101
708	GF-NT	.639	98	119	110	86	83
709	GF-NT	.530	126	129	107	104	88
710	GF-NT	.511	108	111	109	111	102
408	G-NT	.568	107	98	100	98	80
414	G-NT	.460	98	101	106	98	102
475	G-NT	.488	87	87	101	99	94
Exercise							
415	G-T	.574	83	78	73	71	82
500	G-T	.474	99	92	93	99	106
425	G-T	.630	81	65	73	77	85
440	G-NT	.502	96	100	110	113	111
453	G-NT	.498	101	106	111	87	96
483	G-NT	.504	99	107	103	107	101
712	GF-NT	.477	95	92	89	92	97
714	GF-NT	.469	99	110	118	107	100
716	GF-NT	.480	97	101	103	99	96
No Exercise			Fattening Ration				
704	GF-C	.489	100	100	100	98	99
706	GF-C	.450	99	107	107	109	94
431	G-T	.547	101	101	98	93	91
447	G-T	.474	95	91	87	89	92
463	G-T	.442	100	94	103	103	106
402	G-NT	.500	91	85	80	93	96
413	G-NT	.500	94	95	99	96	98
498	G-NT	.395	93	109	95	96	104
701	GF-NT	.420	102	102	96	116	109
703	GF-NT	.576	96	102	98	96	100
715	GF-NT	.692	100	100	100	100	100
Exercise							
404	G-T	.725	74	83	94	94	97
424	G-T	.534	94	85	93	108	104
460	G-T	.454	92	90	91	91	101
474	G-NT	.529	93	94	95	99	96
436	G-NT	.450	97	98	98	98	100
480	G-NT	.545	101	97	104	98	92
702	GF-NT	.511	101	98	104	113	104
711	GF-NT	.431	115	110	104	111	107
713	GF-NT	.569	96	105	107	108	92

* G; GF; C; T; NT = Grubby calf; grub-free calf; control; treatment and no treatment

Appendix table 27. Animal ChE values calculated from pretreatment bleedings. Per cent values represent fluctuations in ChE activity at days posttreatment as compared to pretreatment control values. Phase III, 1969.

Animal Group & Number	Animal Desig-nation*	Pretreatment Δ pH values	ChE activity expressed as % of pretreatment Δ pH control values Days Posttreatment 1.	3.	7.	14.	21.
No Feed			Maintenance Ration				
705	GF-C	.485	100	100	100	100	99
707	GF-C	.620	100	96	95	95	95
479	G-NT	.615	99	99	99	98	99
452	G-NT	.558	99	98	97	99	101
455	G-NT	.604	97	98	99	99	97
708	GF-T	.479	91	90	98	106	123
709	GF-T	.493	88	90	99	101	101
710	GF-T	.564	98	87	91	94	93
408	G-T	.480	89	88	93	93	93
414	G-T	.510	98	99	98	95	93
475	G-T	.516	88	90	97	97	102
Feed							
415	G-NT	.551	95	97	97	97	97
500	G-NT	.516	100	99	99	99	99
425	G-NT	.425	99	99	100	99	99
440	G-T	.597	93	95	108	102	92
453	G-T	.439	98	98	101	102	100
483	G-T	.465	98	105	111	103	115
712	GF-T	.452	92	95	99	100	100
714	GF-T	.435	92	89	91	95	96
716	GF-T	.421	89	84	90	95	99
No Feed			Fattening Ration				
704	GF-C	.512	101	96	96	95	94
706	GF-C	.662	100	99	100	100	100
431	G-NT	.455	98	97	89	100	101
447	G-NT	Dead	--	--	--	--	--
463	G-NT	.519	98	98	103	104	105
402	G-T	.535	81	86	103	101	102
413	G-T	.485	95	97	105	100	97
498	G-T	.465	87	90	97	106	102
701	GF-T	.475	89	89	91	95	99
703	GF-T	.475	93	88	88	95	97
715	GF-T	.435	92	88	89	93	98
Feed							
404	G-NT	.480	100	100	100	101	102
424	G-NT	.598	99	100	99	99	99
460	G-NT	.620	100	99	99	99	99
474	G-T	.549	91	91	92	98	103
436	G-T	.425	85	84	87	93	94
480	G-T	.545	89	83	76	86	89
702	GF-T	.415	82	89	102	103	102
711	GF-T	.617	92	89	95	96	96
713	GF-T	.541	91	91	97	99	99

* G; GF; C; T; NT = Grubby calf; grub-free calf; control; treatment and no treatment

Appendix table 28. Animal ChE values calculated from pretreatment bleedings. Percent values represent fluctuations in ChE activity at days posttreatment as compared to pretreatment control values. Phase IV, 1969.

Animal		Pretreatment	ChE activity expressed as % of pretreatment Δ pH control values				
Group & Number	Designation*	Δ pH values	Days Posttreatment 1.	3.	7.	14.	21.
No Feed			Maintenance Ration				
705	GF-C	.390	94	91	93	93	95
707	GF-C	.551	97	97	95	96	96
479	G-T	.370	88	84	86	91	95
452	G-T	.455	88	87	87	90	95
455	G-T	.475	88	95	95	95	95
708	GF-NT	.475	101	101	101	100	100
709	GF-NT	.342	101	99	100	97	95
710	GF-NT	.410	96	97	96	96	96
408	G-NT	.403	98	97	98	97	98
414	G-NT	.421	97	97	96	95	96
475	G-NT	.400	98	101	101	101	101
Feed							
415	G-T	.350	86	86	92	95	96
500	G-T	.430	97	93	94	93	92
425	G-T	.452	90	89	89	89	92
440	G-NT	.430	98	98	97	97	96
453	G-NT	.450	100	100	99	100	100
483	G-NT	.475	93	97	97	97	96
712	GF-NT	.537	93	91	91	92	92
714	GF-NT	.513	98	95	95	96	97
716	GF-NT	.492	99	100	100	100	100
No Feed			Fattening Ration				
704	GF-C	.491	100	100	99	100	100
706	GF-C	.651	100	100	100	100	101
431	G-T	.450	98	96	95	96	98
447	G-T	Dead	--	--	--	--	--
463	G-T	.591	83	75	77	80	82
402	G-NT	.510	98	98	98	99	99
413	G-NT	.421	100	94	100	99	100
498	G-NT	.455	100	99	100	100	101
701	GF-NT	.489	100	99	101	100	100
703	GF-NT	.495	100	99	100	99	100
715	GF-NT	.481	101	100	100	100	100
Feed							
404	G-T	.473	100	99	95	94	95
424	G-T	.491	94	94	93	93	95
460	G-T	.489	96	92	92	94	97
474	G-NT	.495	100	101	100	100	101
436	G-NT	.555	100	99	99	100	99
480	G-NT	.486	99	101	101	101	101
702	GF-NT	.474	100	101	101	101	100
711	GF-NT	.511	100	99	100	99	100
713	GF-NT	.575	99	100	99	101	101

* G; GF; C; T; NT = Grubby calf; grub-free calf; control; treatment and no treatment