

THE EFFECT OF CERTAIN ORGANOPHOSPHATES ON
SERUM AND ERYTHROCYTE CHOLINESTERASE IN THE CANINE SPECIES

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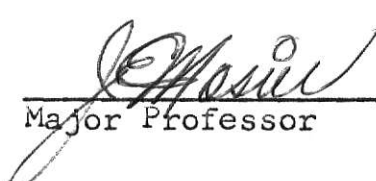
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INTRODUCTION

The use of organophosphates as insecticides and vermifuges has increased following introduction of tetraethyl pyrophosphate by German scientists. Tetraethyl pyrophosphate was a highly effective insecticide, but very toxic to mammals. Subsequently, Parathion was found effective, but less toxic to mammals than tetraethyl pyrophosphate. Further research produced malathion, with reduced insecticidal properties, but still effective and much safer when used on mammals.

The rapid pace of post World War II research produced organophosphate compounds in rapid order. Each product was synthesized to compensate for an inadequacy in its predecessor, either in terms of safety or parasite control.

Organophosphates have an adverse effect on the enzyme cholinesterase. This is the enzyme which catalyzes the hydrolysis of acetylcholine to acetic acid and choline. Erythrocytes, plasma, and other tissues of the body contain the enzyme cholinesterase. Evidence indicates that cholinesterase is not a single enzyme, but can be divided into true cholinesterase and pseudocholinesterase. True cholinesterase, most commonly found in the erythrocytes, nervous tissue, muscles, and glands, has a more specific action on acetylcholine than on choline esters such as butyrylcholine or benzoylcholine. Pseudocholinesterase is found most commonly in plasma, but can be found in nerve and other tissues. Pseudocholinesterase is a non-specific enzyme capable of hydrolyzing a wide range of natural and synthetic

esters, including acetylcholine.

The organophosphate pesticides share certain fundamental characteristic. Structurally, they all contain the phosphorus radical in a combination which permits the compound to competitively inhibit the enzyme cholinesterase. Their success in pest control depends upon the selective toxicity they produce in pests as opposed to that produced in the host.

The pharmacological effects of organophosphate compounds are equivalent to an excessive accumulation of acetylcholine. These compounds are known also to inhibit the activity of certain other enzymes, such as chymotrypsin, trypsin, liver esterase, and milk lipase.

This study was undertaken to investigate normal daily variation in serum and erythrocyte cholinesterase activity in the canine and the effect of some of the more common medicinal organophosphates on the serum and erythrocyte cholinesterase activity. A base line of daily variation of cholinesterase would allow evaluation of the effect of different organophosphates on daily levels of serum and erythrocyte cholinesterase activity. The effect of combining certain organophosphate compounds in therapy were included in the study.

Knowledge of the hazards of the organophosphates should aid the clinician in preventing problems created by lowered cholinesterase activity.

REVIEW OF LITERATURE

Stedman (1932) presented evidence of cholinesterase in blood serum of the horse. Stedman (1935) found that the serum of different species differed with respect to content of cholinesterase and reported that erythrocytes also contained large amounts of enzyme.

Hall (1937) investigated cholinesterase values of normal and pathologic human serum, and concluded that sex, age, diet, activity, heart rate, or blood pressure did not influence the serum cholinesterase. He further noted that while the serum cholinesterase value for an individual was constant, it could vary widely from individual to individual. This observation was confirmed by Vorhaus (1953).

Mendel (1943) reported two different cholinesterases in the body on the basis of substrate concentrations and substrate specificity. They called the cholinesterase of the erythrocyte and brain tissue - true cholinesterase, and the cholinesterase found in serum - pseudocholinesterase.

Sawyer (1947) reported that high concentrations of true cholinesterase were associated with developing erythrocytes in the red bone marrow. Its presence in the perfused lymph node, spleen and thymus would indicate that the lymphocyte may also contain true cholinesterase. Its function in the lymphocyte as in the erythrocyte is obscure.

Witter (1963) proposed that true cholinesterase is of importance in the transmission of the nerve impulse from the

preganglionic fibers to autonomic ganglia, from postganglionic cholinergic nerves to smooth and cardiac muscle and to secretory cells, from motor nerves to striated muscles, and from certain structures within the central nervous system.

The physiologic role of pseudocholinesterase is unknown. Funnell (1965) proposed that it is a regulator of the amount of free choline in the plasma. He postulated the existence of a homeostatic mechanism for the control of free choline in the plasma which was on two opposing systems: in one system, the plasma cholinesterase is responsible for choline ester hydrolysis; and in the other system, it is responsible for choline ester formation.

Neely (1965) concluded that cholinesterase has in its active center two kinds of sites, one is called the esteratic site, and the other the anionic site. O'Brien (1963) reported that the anionic site carries a negative charge, and binds the quaternary nitrogen of acetylcholine, thus increasing the specific affinity of the enzyme for the substrate. The esteratic site is the location at which hydrolysis of the substrate occurs. It is the site that organophosphates attack.

Aldridge (1953) stated that it is generally accepted that inhibition of cholinesterase by organophosphate compounds consist of direct phosphorylation of the active center of the enzyme with formation of a disubstituted phosphoryl cholinesterase. Burgen (1951) reported evidence that phosphorylation of the enzyme is preceded by an initial attachment of the inhibitor to the enzyme. Once phosphorylation has occurred, re-

activation of cholinesterase can be brought about by uncat-
 alysed or enzymatic hydrolysis. The rate of reaction and equili-
 brium points would be determined by the structure of the inhi-
 bitor molecule. Inhibition of cholinesterase by eserine, neo-
 stigmine, and related compounds is due to the formation of a
 reversible complex between the enzyme and the entire inhibitor
 molecule. Duration of action in vivo of anticholinesterase
 inhibitors of this type depend on the rate of dissociation of
 the enzyme inhibitor complex and the rate at which the free
 inhibitor is removed from the body by metabolism and excretion.

Blaber (1960) reported that the erythrocyte cholinesterase
 which is inhibited by organophosphates recovers in one of two
 stages depending on which inhibitor is used. During the first
 stage, the inhibited enzyme undergoes two simultaneous reactions.
 First, there is spontaneous reactivation by hydrolysis and
 aging in which the inhibited enzyme is converted into a form
 which can no longer be reactivated spontaneously. In the second
 stage of recovery, the rate of erythrocyte cholinesterase re-
 covery is the rate which inhibited erythrocytes are replaced by
 new erythrocytes.

Burgen (1951) stated that in comparing the actions of the
 various anticholinesterase agents in vivo, distribution via the
 blood stream, passage from blood to tissues, access to cholin-
 esterase, and kinetics of the cholinesterase inhibitor complex
 must be considered.

Dipterex (Freed^R)

O,O,-dimethyl 2-2,2-trichloro-1-hydroxyethyl phosphonate

Radeleff (1964) found that dipterex absorbed by animals is an inhibitor of cholinesterase, and that the inhibition is readily reversible. Dipterex is absorbed from all surfaces and is rapidly metabolized and eliminated by the body. Dipterex is of a low order of toxicity for most animals, young calves being an exception. The rapid detoxification is at least partially responsible for its low toxicity.

Arthur (1957) studied dipterex in the dog and found that following intravenous injection of 150 mg/kg of labeled compound, the degradation and excretion were rapid. The original blood level of 1780 ppm of dipterex dropped to 85 ppm in 9 minutes and to 8 ppm in 6 hours. The investigator suggested that it was not the metabolic products that were responsible for the anticholinesterase properties.

Metcalf (1959) does not agree with Arthur (1957). He presents evidence that in the housefly, there is in vivo conversion of dipterex to DDVP. DDVP in dipterex-treated flies was identified chromatographically. Metcalf maintains that the anticholinesterase action of dipterex, under physiological conditions, is due to DDVP production and that there is argument for the fact that the same phenomenon is applicable to mammals as well as insects.

DDVP (Task^R)

2,2, dichlorovinyl dimethyl phosphate

Radeleff (1964) reported that DDVP was extremely toxic to parasites, but was only moderately toxic to mammals. DDVP is rapidly metabolized and excreted, thus if there is toxicity,

it should be of short duration. When it is incorporated in certain polymers which restrains the release of the compound for absorption, the apparent toxicity is reduced. If it is mixed with feed, rather than using it as a drench or in capsule form, the absorption is even slower and the toxic dose is much higher.

Shell company, the producer of Task, found the oral LD50 dosage of the unformulated DDVP for dogs to be between 100 and 316 mg/kg. Dogs have also been given 50 ppm of unformulated DDVP for 40 consecutive days before a drop in the plasma and erythrocyte cholinesterase occurred, but there were no visible signs of toxicity. The oral LD50 dosage for formulated DDVP ranges from 483 mg/kg in puppies to 886 mg/kg in adult dogs that were two years of age. A single sublethal dose of DDVP produced a short term generalized paralysis that is usually reversible in 24 to 48 hours.

Radeleff (1957) states that the exact significance of plasma or erythrocyte cholinesterase depression is not known. It is possible for animals to show severe signs of cholinesterase poisoning, even though cholinesterase values may be relatively high. It is also possible to have no detectable cholinesterase activity and no apparent signs of poisoning.

Shell company has observed that dogs may survive long periods of time without detectable cholinesterase values. Experiments in the dog and pig have shown that DDVP does not interfere with liver function. Primary detoxification takes place in the liver. If the liver is affected with a disease such as

hepatitis or cirrhosis, the toxic dose will decrease.

Compound 4072 (Dermaton^R)

2-chloro-1(2,4-dichlorophenyl) vinyl diethylphosphate

Radeleff (1964) stated that this compound is relatively new on the market. Following experimentation, he found that the compound was far more toxic than originally anticipated. His work was in the bovine species.

It is reported by Cooper Co. that dogs survived dosages of 12 gr/kilo with slight toxic signs. The application of a 5% dipping solution caused only minor salivation, while 6 weekly applications of a 1.0% dipping solution was tolerated without toxic signs being manifested.

Ronnel (Ectoral^R)

O-O-dimethyl O-(2,4,5, trichlorophenyl) phosphorothioate

Radeleff (1964) reports that ronnel is absorbed rapidly but eliminated more slowly than some of the other organophosphate compounds. The drug is effective both systemically and as a conventional topical insecticide.

The oral LD50 dosage has been reported by Garner (1967) as 500 mg/kg in the dog. The compound is similar to malathion in that lethal oral doses will cause emesis in the dog.

Radeleff (1957) suggested that ronnel has two modes of action. Signs of the first mode of action are depression, pronounced muscular weakness, incoordination, and prostration. Such signs were usually accompanied by diarrhea. The signs may

appear within 24 hours after treatment and last for several weeks. Second mode of action caused salivation and dyspnea and were not seen till 5-8 days after exposure.

Malathion (Para Bomb-M^R)

O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate

Holland (1952) reported malathion to be relatively nontoxic in warm-blooded animals. The oral LD50 dosage for the 90% technical compound in vegetable oil when administered to mice was 885 mg/kg and for rats was 480 mg/kg. Rats on chronic toxicity studies tolerated 1000 ppm in their ration for 6 months. The toxicity of the compound for dermal absorption in rabbits and guinea pigs receiving single or repeated application does not appear to be a problem.

Bagdon (1955) found mild signs consisting of pupillary changes, depression and diarrhea in dogs receiving 120 mg/kg, 240 mg/kg, and 430 mg/kg of malathion intravenously. Treatment of two dogs with 600 mg/kg resulted in severe signs consisting of rapid shallow respiration, muscular fibrillation, prostration and death about one hour after treatment with the compound. A dose of 240 mg/kg of malathion given to dogs over a 4-6 hour period produced no effects beyond the cardiovascular change of bradycardia and increased pulse pressure. The workers suggested that purity was a factor of importance in considering toxicity of the compound.

Radeleff (1964) reports that malathion is absorbed from all body surfaces and that it is destroyed in the mammalian

liver by the enzyme designated as malathionase. Not all malathion is destroyed in the liver. Some is converted to a compound which is more active as a cholinesterase inhibitor than malathion itself.

Garner (1967) reports that dipping four times at 4 day intervals with a 2% solution was without effect in dogs.

MATERIALS AND METHODS

Nineteen dogs, 7 males and 12 females, ranging in size from 7.0 to 10.4 kilograms were utilized in the study. All dogs were young adults. The dogs were preconditioned for at least 3 months, and were guarded against exposure to organophosphates or carbamates during this period of time. The dogs were randomly divided into four groups: group one served as the control group, group two received specified drugs daily, group three received the specified drugs at a rate and frequency suggested by the manufacturer, and group four constituted a group of animals treated with various combinations of organophosphates.

The commercial organophosphates employed in the study were: Freed¹ which was administered orally, Dermaton² which was administered topically, Ectoral³ which was given both orally and topically, Task⁴ which was given orally, and Para Bomb-M⁵ which was applied topically.

The active principle of Freed was 852 mg of O,O,-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate, which was combined with 4.7 mg atropine, 26.3 mg inert ingredients in each #25 tablet.

Dermaton contained 2-chloro-1 (2,4,-dichlorophenyl) vinyl diethylphosphate (24.5%), and was combined with an aromatic

1. "Freed"; Fort Dodge Laboratories; Fort Dodge, Iowa
2. "Dermaton"; William Cooper and Nephews, Chicago, Illinois
3. "Ectoral"; Pitman-Moore Company, Indianapolis, Indiana
4. "Task"; Shell Chemical Company, New York, New York
5. "Para Bomb-M"; Haver-Lockhart Laboratories; Shawnee, Kansas

petroleum solvent (58.2%), and inert ingredients (17.3%).

The orally administered Ectoral contained 58 mg of O-O-dimethyl O-(2,4,5-trichlorophenyl) phosphorothioate and 192 mg inert ingredients per tablet. The topical preparation of Ectoral was an emulsifiable concentrate containing 33.33% of the active ingredient O-O-dimethyl O-(2,4,5-trichlorophenyl) phosphorothioate, 56.33% of aromatic petroleum derivatives, and 10.34% inert ingredients.

TABLE I

Dogs weight, sex and organophosphate preparation used.

Group No.	Dog. No.	Weight (Kg)	Sex	Compound
1.	1	9.5	M	Control
	2	8.2	F	Control
	3	8.6	F	Control
	4	10.0	M	Control
2.*	5	8.6	M	Freed Orally
	6	7.3	M	Dermaton Topical
	7	9.0	M	Ectoral Orally
	8	10.0	F	Task Orally
	9	10.4	M	Ectoral Topical
	10	10.0	M	Para Bomb-M Topical
3**	11	9.5	F	Ectoral Orally
	12	8.2	F	Dermaton Topical
	13	8.2	F	Task Orally
	14	8.6	F	Freed Orally
	15	9.5	F	Ectoral Topical
	16	7.0	F	Para Bomb-M Topical
4***	17	10.4	F	Para Bomb-M Topical & Ectoral Orally
	18	7.3	F	Dermaton Topical & Task Orally
	19	7.3	F	Ectoral Topical & Task Orally

* Received the organophosphate daily.

** Received the organophosphate on day 0, 3, and 7.

*** Received the organophosphate on day 0, 3, and 7.

Task contained 80 mg of resin coated 2,2-dichlorovinyl dimethyl phosphate in each #5 capsule.

The Para Bomb-M compound contained 0.06% pyrethrins, 0.10% 2,2-thiobis (4-chloro-6-methylphenol), 0.48% piperonyl butoxide, 0.50% malathion (O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate, 0.24% 2,3,4,5,-bis (2-butylene) tetrahydro-2-furaldehyde, 23.62% petroleum distillate, and 75% inert ingredients.

Control dogs 1 through 4 were not subjected to medication. Dogs 5 through 10 were given recommended doses of Freed orally, Dermaton topically, Ectoral orally, Task orally, Ectoral topically, or Para Bomb-M topically. Each were administered daily for 7 days.

Dogs 11 through 16 were each given recommended doses of Freed orally, Dermaton topically, Ectoral orally, Task orally, Ectoral topically, or Para Bomb-M topically on day 0, day 3, and day 7.

Dog 17 was given a recommended dose of Ectoral orally plus Para Bomb-M topically on day 0, day 3, and day 7. Dog 18 was given a recommended dose of Dermaton topically and Task orally on day 0, day 3, and day 7. Dog 19 was given a recommended dose of Ectoral topically and Task orally on day 0, day 3, and day 7.

Freed was given at the rate of 852 mg of active ingredient per 25 pounds orally, Dermaton concentrate was mixed at the rate of 15 ml in one gallon water and the mixture applied to the back of the dog, Ectoral was given orally at the rate of 58 mg

of active ingredient per 5 pounds and topically at the rate of 30 ml of concentrate to one quart of water which was applied to the back of the dog., Task was given orally at the rate of 80 mg per 5 pounds of body weight. Para Bomb-M was sprayed topically over the entire body of the dog.

Blood was collected daily for one week and thereafter at weekly intervals for determination of serum and erythrocyte cholinesterase in the control group. EDTA was utilized as the anticoagulant. Following collection, the blood was centrifuged and the plasma and erythrocytes were separated. The erythrocytes were washed in saline three times. A modified Michel's technique (1949) was used to measure the erythrocyte and plasma cholinesterase activity. The concentration of cholinesterase was determined by electrometrically measuring the drop in pH (Δ pH) following incubation with acetylcholine bromide or acetylcholine chloride at 25 degrees C for 1 hour. Correction was made for the drop in pH resulting from non-enzymatic hydrolysis occurring during the period of incubation. In modifying the technique, the erythrocyte volume was increased by 8 fold, and the plasma volume by 1.7 fold as compared to Michel's original method. Thirty minute readings were made with the total incubation period being one hour. The cholinesterase values were expressed in Δ pH units per hour.

All dogs receiving drugs were kept in individual cages. Control blood samples were taken from each dog 2 and 4 hours before initial treatment. Ten ml of blood from the external jugular vein was utilized for serum and erythrocyte cholinesterase

determinations. Blood samples were taken every twenty-four hours for an 8 day period for cholinesterase determinations and thereafter four weekly samples were taken following the suspension of treatment.

The method for assay of plasma and erythrocyte cholinesterase of the dogs receiving drugs was an automated method utilizing the Technicon Auto-Analyser as described by Ward (1969). Cholinesterase activity was expressed in SH units which is described as the micromoles of sulfhydryl groups liberated in 3 minutes by 1 ml of sample incubated at 37 degrees C at pH 7.4. The incubation period for the particular machine used was found to be 4.75 minutes. To adjust to an incubation time of 3 minutes, the apparent cholinesterase activity was multiplied by 0.632. The plasma and packed erythrocytes were routinely diluted 1:3 resulting in a dilution factor of four. The combined correction factor for the procedure was the incubation correction (0.632) multiplied by the dilution factor of (4) or 2.53 to express the values in SH units of cholinesterase activity.

RESULTS

The daily and weekly variations of normal plasma and erythrocyte cholinesterase activity are summarized in Fig. 1-4 and tables 2 and 3. The variation is expressed in percent of the mean cholinesterase activity. The erythrocyte cholinesterase activity in dogs 1-4 was elevated during the first week in which daily samples were taken. Dog 1 reached 170% on the third day of sampling while dogs 2, 3, and 4 reach 135%, 105%, and 129% respectively on the same day. On day 5, the cholinesterase activity was within the range of 100 - 120% of the mean cholinesterase activity in each dog. Subsequent erythrocyte cholinesterase activity, monitored at weekly intervals remained in the range of 80 - 90% of the mean cholinesterase activity. Tables 2 and 3, express the mean value, minimal and maximal value, standard deviation and coefficient of variation of the erythrocyte and serum cholinesterase activity of each of four dogs.

The daily and weekly variation of the plasma cholinesterase activity of dogs 1-4 is shown on Figs. 3 and 4. Variation is very erratic, and does not follow a particular pattern.

Table 2. Variation of erythrocyte cholinesterase activity in dogs 1-4

Dog #	Mean (delta pH/hr.)	Minimal and maximal Values	Standard Deviation	Coeff. of Variation
1	.41	.34-.70	.13	32
2	.36	.28-.49	.07	19
3	.40	.31-.50	.06	15
4	.24	.20-.33	.05	20

Table 3. Variation of plasma cholinesterase activity in dogs 1-4

Dog #	Mean (delta pH/hr.)	Minimal and maximal Values	Standard Deviation	Coeff. of Variation
1	.47	.25-.63	.12	25
2	.29	.15-.38	.08	28
3	.33	.20-.46	.08	24
4	.27	.17-.41	.07	26

Differences of plasma and erythrocyte cholinesterase activity of dogs 14 and 5 treated with Freed orally are illustrated in figures 5 and 6. Dog 14 was treated with a standard dose of Freed on day 0 and 3, and day 7. The first dose given on day 0, caused rapid depression of the plasma cholinesterase in 24 hours. Activity was 72%, 24 hours following the first treatment. On day 2, the activity increased to 88%, followed by depression to 62% day 3 through 5. On day 6 and 7 an increase in the cholinesterase activity to 88% occurred in dog 14. Following treatment on day 7 activity dropped to 61%. The maximum plasma cholinesterase depression during the treatment period for dog 14 was 61%. Twelve days after the last dose, the plasma cholinesterase activity returned to 115% of the pretreatment control activity. The first dose, given on day 0, caused the erythrocyte cholinesterase activity to be depressed to 80% within 24 hours. Activity on day 3 was 59%. The second dose given day 3, caused depression from 59% to 52% by day 5. Activity was 45% on day 7 and treatment on this day caused depression of activity to 35% on day 8. Maximum depression in the treatment period of dog 14 was 35% which occurred on day 8. 32 days after the last treatment, dog 14 had erythrocyte cholinesterase activity of 89%. The activity did not reach pretreatment control levels in the recovery period. Dog 5 was treated with a standard dose of Freed daily for 7 days. Rapid depression of plasma cholinesterase occurred in the 24 hour period immediately after the first dose on day 0. Activity was depressed to 54% of pretreatment control levels. The maximum depression

during the treatment period occurred on day 7 when activity was 38%. Twelve days after the last dose, plasma cholinesterase activity returned to 110% of pretreatment control activity. Each dose resulted in continued decrease in erythrocyte cholinesterase activity. Maximum depression occurred on day 8 when the activity was 8% of the pretreatment control level. Dog 5 did not reach pretreatment control levels in the recovery period, but had 94% activity 32 days after the last treatment.

Differences of plasma and erythrocyte cholinesterase activity of dogs 6, 12 and 18 treated with Dermaton topically are illustrated in figure 7 and 8. Dog 12 was treated with a standard dose of Dermaton on day 0, day 3, and day 7. The first treatment on day 0, caused the plasma cholinesterase activity to be depressed to 38% within 24 hours. The second dose on day 3 resulted in continued depression from 33% to 28% by day 5. Maximum depression occurred during the treatment period was 28%. Plasma cholinesterase levels returned to 98% by the 26th day following the last treatment. There was an increase in erythrocyte cholinesterase activity the first 48 hours. The activity reached 118% by day 2. Thereafter there was progressive depression of activity to a maximum on day 8 of 67%. Activity returned to a level of 104% by the 12th day following the last treatment.

Dog 6 was given a standard dose of Dermaton topically daily for 7 days. Treatment on day 0 caused the plasma cholinesterase activity to be depressed to 31% within 24 hours. The activity continued to decrease to 18% by day 3. Maximum depression of activity occurred on day 5 when the activity was 16%. Plasma

cholinesterase activity did not return to pretreatment control levels during the recovery sampling period. Erythrocyte cholinesterase activity remained in the range of 102%, for 48 hours following the first treatment, but by day 3 had decreased to 64%. By day 5 it had decreased to 51%. The activity remained in the range of 50% - 60% till day 8. Erythrocyte cholinesterase activity reached 132%, 18 days following the last treatment.

Dog 18 was treated with a standard dose of Dermaton topically and Task orally on day 0, day 3, and day 7. The first dose, given on day 0, caused plasma cholinesterase activity to be depressed to 24% within 24 hours. Activity from day 1 to day 8 decreased from 24% to 14%. During the recovery period the plasma cholinesterase activity reached 81% of the pretreatment levels by the 32nd day following the last treatment. The first dose given on day 0, resulted in a depression of erythrocyte cholinesterase activity to 66% within 24 hours. The activity rebounded to 75% by day 2, then decreased to a maximum depression of 18% on day 8. During the recovery period, the erythrocyte cholinesterase activity reached 76% of pretreatment control levels on the 32nd day following the last treatment.

Differences of plasma and erythrocyte cholinesterase activity of dogs, 11, 7, and 17, treated with Ectoral orally are illustrated in figure 9 and 10. Dog 11 was treated with a standard of Ectoral on day 0, day 3, and day 7. The first dose on day 0, caused the plasma cholinesterase activity to be depressed to 44% within 24 hours. Activity continued to decrease to 36% by day 3. Treatment on day 3 did not result in

further depression of activity. Activity increased to 38% by day 5, and continued to increase to 49% on day 7. Treatment on day 7 resulted in a decrease of activity to 34% of pretreatment control levels. Maximum depression of activity during the treatment period occurred on day 8 when activity was 34%. Control levels of plasma activity were reached in dog 11, 18 days following the last treatment. The first dose given on day 0 caused an increase in the erythrocyte activity to 115% by day 2, however, the activity decreased to 86% by day 3. Treatment on day 3 and day 7 did not affect activity significantly. Maximum depression during the treatment period was 76% which occurred on day 6.

Dog 7 was treated with a standard dose of Ectoral daily for 7 days. The first dose on day 0, caused plasma cholinesterase activity to be depressed to the 56 percentile within 24 hours. By day 3, the activity was 24%. Maximum depression during the treatment period occurred on day 8 when the activity was 23%. Control levels of plasma activity were reached in dog 7 on the 18th day following the last treatment. There was an increase in erythrocyte cholinesterase activity to 112% by day 2 followed by depression to 88% by day 3. Depression was progressive throughout the treatment period with maximum depression of 53% occurring on day 8. During the recovery period the erythrocyte cholinesterase activity reached 78% on the 12th day and 114% on the 18th day following the last treatment.

Dog 17 was treated with a standard dose of Ectoral orally and Para Bomb-M topically on day 0, day 3, and day 7. Maximum

depression of plasma cholinesterase activity occurred on day 8 of the treatment period when activity was 44% of pretreatment control levels. Plasma cholinesterase activity reached 100%, 12 days following the last treatment. There was an increase of erythrocyte cholinesterase activity to 123% by day 2 followed by depression to 81% by day 3. Maximum depression of 71% occurred on day 8. Pretreatment control levels of erythrocyte cholinesterase were reached by 12 days following the last treatment.

Differences of plasma and erythrocyte cholinesterase activity of dogs 8, 13, 18, and 19, treated with Task orally are illustrated in figure 11 and 12. Dog 13 was treated with a standard dose of Task on day 0, day 3, and day 7. The first dose given day 0, caused the plasma cholinesterase activity to be depressed to 58% within 24 hours and to 51% by day 3. Treatment on day 3 did not further depress the activity which was maintained at 51% through day 5. Activity then started to increase and reached 71% by day 7. Treatment on day 7 resulted in a depression to 47% on day 8. Plasma cholinesterase activity reached 104% on the 12th day following the last treatment. The first dose, given on day 0, caused the erythrocyte cholinesterase to be depressed to 85% within 24 hours. The activity increased to 93% on day 2, and was again decreased to 65% on day 3. Depression of activity continued with a maximum depression of 33% occurring on day 8. Erythrocyte cholinesterase activity increased to 100%, 26 days after the last treatment.

Dog 19 was treated with a standard dose of Task orally and Ectoral topically on day 0, day 3, and day 7. The first dose given day 0, caused the plasma cholinesterase activity to be

depressed to 52% within 24 hours. By day 2, the activity had risen to 64% and then dropped to 41% on day 3. Treatment on day 3, did not result in further decrease of activity. Activity increased to 48% by day 5. Maximum depression of plasma cholinesterase activity during the treatment period was 41% on day 3. Plasma cholinesterase activity reached 157% by the 12th day following the last treatment. The first dose, given on day 0, caused the erythrocyte cholinesterase activity to be depressed to 79% within 24 hours. Activity increased to 98% on day 2, and decreased to 69% on day 3. Progressive depression of activity then occurred with maximum depression of 38% on day 8. Erythrocyte cholinesterase activity increased to 94% on the 12th day and to 125% on the 18th day after the last treatment.

Dog 8 was treated with a standard dose of Task daily for 7 days. Maximum depression of plasma activity occurred on day 3 of the treatment period. The activity was depressed to 24% of pretreatment control levels. From day 3 to day 6 the activity increased to 48%, but again was depressed to 40% on day 8. Plasma cholinesterase activity reached 120% on the 12th day following the last treatment. Progressive depression of erythrocyte cholinesterase activity occurred during the treatment period with a maximum depression of 10% occurring on day 8. Erythrocyte cholinesterase activity reached 83% by the 32nd day following the last treatment.

Differences of plasma and erythrocyte cholinesterase activity of dogs 9, 15, and 19 treated with Ectoral topically are illustrated in figure 13 and 14. Dog 15 was treated with

a standard dose of Ectoral on day 0, day 3, and day 7. The first dose caused progressive depression of plasma cholinesterase activity to 38% on day 3. The activity increased slightly day 3 through day 7. Maximum depression of 33% occurred on day 8. Plasma cholinesterase activity increased to 104% on the 18th day following the last treatment. There was an increase in erythrocyte cholinesterase activity in the 48 hour period following the first dose. Activity which reached 120% by day 2, was followed by depression to 75% by day 3. Erythrocyte cholinesterase activity remained in the range of 70% - 75% throughout the treatment period and rose to 98% by the 12th day following the last treatment.

Dog 9 was treated with a standard dose of Ectoral topically daily for 7 days. The greatest decrease in plasma activity occurred 24 hours after the first dose. The activity was decreased to 38%. Depression of activity continued to 20% on day 3, and remained at this level through day 8. Pretreatment plasma cholinesterase levels were reached 18 days following the last treatment. Maximum depression of erythrocyte cholinesterase activity occurred on day 8 when the activity reached 37%. Erythrocyte cholinesterase activity reached 125% of pretreatment levels by the 18th day following the last treatment.

Differences of plasma and erythrocyte cholinesterase activity of dogs, 10, 16, and 17, treated with Para Bomb-M topically are illustrated in figure 15, and 16. Dog 16 was treated with a standard dose of Para Bomb-M on day 0, day 3, and day 7. Plasma cholinesterase activity remained near the

pretreatment control levels for 48 hours after the first treatment. On day 3, the cholinesterase activity had decreased to 65%. This was the maximum depression point during the treatment period. Activity increased to 88% on day 6 and stayed at this point till day 8. Following treatment on day 7, the activity decreased to 82% on day 8. Pretreatment plasma cholinesterase activity levels were reached on the 12th day, following the last treatment. On day 1, the erythrocyte cholinesterase activity decreased to 94%, and on day 2 it increased to 108%. By day 3, the activity decreased to 66% of pretreatment cholinesterase levels. The erythrocyte cholinesterase activity stayed in the range of 65% - 75% throughout the rest of the treatment period and returned to 100% by the 12th day following the last treatment.

Dog 10 was treated with a standard dose of Para Bomb-M topically daily for 7 days. Maximum depression of plasma cholinesterase activity occurred on day 5 when the activity was 47%. By the 12th day following the last treatment, the plasma cholinesterase activity had reached 128% of pretreatment levels. There was an increase of erythrocyte cholinesterase activity to 126% on the 48th hour after the first treatment. Activity dropped to 89% on day 3. Maximum depression occurred on day 6, when the activity reached 74%. Erythrocyte cholinesterase activity returned to pretreatment levels of 120% on the 12th day following the last treatment.

DISCUSSION

The organophosphates used in this experiment are in common use in the average practice of veterinary medicine. The compounds have LD50 dosages established, but reports have not appeared in the literature concerning the effect of therapeutic doses on cholinesterase activity. Neither has the effect of combinations of the products on the cholinesterase activity been established. Specific therapeutic schedules have been established by the respective manufacturers. While the dose schedules have been shown to be effective, circumstances are encountered where in fatal toxicity develops.

Consideration of individual susceptibility is suggested by the low cholinesterase activity that an individual may have normally. Considerable individual and normal daily variation does exist. The results of this investigation agree with the results presented by Vorhaus (1953) and Wetstone (1965) for humans to the extent that there is variation between dogs, but it does not agree on the point of minimal daily variation of an individual.

Pritchard (1949) reported that repeated bleeding of up to 20% of blood volume of rats every 24 hours, produced erythrocyte cholinesterase activity 100 percent greater than control levels. He also found that by centrifugation of the blood sample into fractions, the young erythrocytes were in the upper layer and possessed cholinesterase values that were 50% to 70% higher than the lower layers of older erythrocytes. It was reported

by this investigator that rats given a rest period of 14 to 16 days, then bled again would show an erythrocyte cholinesterase activity of only 5 to 15% greater than control levels. Bleeding of the dogs caused stimulation of erythrocytes (Figs. 1 and 2). Following stimulation, young erythrocytes were poured into the cardiovascular system. It should be noted that erythrocytes cholinesterase activity of dog 1 rose to 170% above the mean during the period that blood was being collected daily for cholinesterase determinations. This same response was also shown by dogs 2, 3, and 4. This response did not occur after the 4th bleeding and the erythrocyte cholinesterase values returned close to original levels. Erythrocyte cholinesterase activity remained fairly constant when the dogs were placed on weekly bleeding schedules.

The coefficient of variation (per cent) of erythrocyte cholinesterase activity ranged from 15 to 32%. This is relatively large, and probably would not have occurred to that extent if erythropoiesis was not stimulated during daily bleeding. Dogs 1, 2, and 3, had erythrocyte cholinesterase activity in the range of cholinesterase values reported by Callahan (1967). Callahan reported a range of values for 115 dogs of .29--.67 (delta pH/hr.). Dogs 1, 2, and 3, had a mean value of .41, .36, and .40 (delta pH/hr.) for these respective dogs. Dog 4, had a mean value of .24 (delta pH/hr.). This dog is outside the normal range as reported by Callahan and may be an example of an individual that would be sensitive to the toxic effects of the organophosphates.

The results (Figs. 3 and 4) suggest there is a daily variation in the plasma cholinesterase. The daily variation was quite erratic and did not follow a particular pattern.

The coefficient of variation for plasma cholinesterase, ranged from 24% to 28% in the group of four dogs. Wetstone (1965) found in his study in humans that there was a coefficient of variation of 8.4% in 82 normal adults studied 373 times over periods up to 5 years. Vorhaus (1953) stated that in humans the level of cholinesterase is remarkably constant for each healthy, well fed individual, from day to day and from month to month. Explanation of the variability in the plasma cholinesterase activity cannot be given at the present time.

The results of orally administered Freed on the plasma and erythrocyte cholinesterase suggests that the erythrocyte cholinesterase activity is affected to a greater degree than plasma cholinesterase activity. Daily treatment resulted in depression of erythrocyte activity to 8% of pretreatment activity by day 6.

Dermaton primarily depresses the plasma cholinesterase activity rather than the erythrocyte cholinesterase activity (Figs. 7 and 8). The combination of Dermaton topically and Task orally produced depression of both plasma and erythrocyte cholinesterase activity, 13% and 18% respectively. The activity was very slow in returning to control levels.

Ectoral primarily depressed the plasma cholinesterase (Figs. 9 and 10). The combination of Ectoral orally and Para Bomb-M topically caused less depression of both the plasma and erythrocyte cholinesterase than did oral Ectoral daily, or when

Ectoral was given on day 0, 3, and 7. The effect of Freed orally on erythrocyte cholinesterase and the effect of Ectoral orally on the erythrocyte cholinesterase, indicate the possibility of slower absorption of the Ectoral product from the intestine. Depression of activity occurred on the 3rd day following initial treatment.

Only slight difference was noted between maximum depression of plasma cholinesterase compared to erythrocyte cholinesterase during the treatment period with Task. All dogs showed gradual depression of erythrocyte cholinesterase as compared to acute depression of the plasma cholinesterase. Daily treatment with Task produced depression of activity of erythrocyte cholinesterase to critical levels.

Ectoral topically depressed plasma cholinesterase earlier and to a greater maximum than it did the erythrocyte cholinesterase. Maximum depression of plasma cholinesterase to 20% occurred following 3 days of daily treatment. It took 3 days for the maximum depression of erythrocyte cholinesterase of 47% to occur. Both plasma and erythrocyte cholinesterase rebounded to above control levels after treatment was discontinued.

Ectoral orally in combination with Para Bomb-M topically did not cause significant additional depression of erythrocyte cholinesterase as compared to the use of Para Bomb-M alone. The degree of depression of plasma cholinesterase activity did not differ greatly from the degree of depression of erythrocyte cholinesterase activity.

Craig (1959) reported the activity of erythrocyte

cholinesterase reached an equilibrium well above zero when dogs were exposed to repeated subacute doses of sarin and tabun. Oliver (1961) reported the same result following experimentation with parathion in pigs. Data obtained in this investigation suggested a similar equilibrium resulted with the compounds under study. Oliver (1961) attributes this equilibrium to erythrocyte regeneration and to dissociation of the enzyme organophosphate complex.

Additive toxic effects resulting from combinations of organophosphates administered to animals have been reported. Frawley (1957) undertook to determine whether potentiation of toxicity resulted from the simultaneous administration of combinations of organophosphates. He selected EPN and malathion for his study. Mortality was the end-point and the subacute effects were measured by the use of blood cholinesterase determinations. He found the order of potentiation to be a 50 fold increase when two products were used in combination. Dubois (1958) reported that most pairs of organophosphates produced either additive or less than additive acute toxic effects when mortality was used as the end-point. Combinations of drugs used in this study showed less than additive effect based on the difference of cholinesterase activity of the drug alone in comparison to the combination of two drugs. DuBois (1958) noted that when combinations of organophosphates are more than additive, the compounds usually have the same mechanism of action. The time of occurrence of maximal depression of the cholinesterase activity by each compound should be noted. Certain compounds must be converted to active metabolites with

the onset of signs depending on the rate of conversion of the parent compound to the active metabolites having anticholinesterase activity. Since the rates of absorption and detoxification differ for various organophosphates, additive toxicity may occur if the administration of each compound was spaced so that maximal effect of each occurred simultaneously. Potentiation of toxicity of one organophosphate by another could occur when the enzymatic detoxification of one compound interferes with the enzymatic detoxification of a second compound.

There are at least two factors responsible for recovery of cholinesterase activity following poisoning by organophosphates. If the inhibition of cholinesterase is irreversible, then recovery of cholinesterase activity is due to resynthesis of fresh enzyme. If the inhibited enzyme is unstable, then return of cholinesterase activity may be due to hydrolysis of phosphorylated enzyme. Recovery of cholinesterase activity may occur as a combination of both processes.

SUMMARY

The results of this study may be summarized as follows:

1. Normal variation on a daily basis exists for an individual and between individuals with regard to the plasma and erythrocyte cholinesterase activity.
2. Freed caused greater erythrocyte cholinesterase depression than plasma cholinesterase depression. Dermaton caused greater plasma cholinesterase depression than erythrocyte cholinesterase depression. Ectoral topically and orally resulted in greater plasma cholinesterase depression than erythrocyte cholinesterase depression. Task caused slightly more erythrocyte cholinesterase depression than plasma cholinesterase depression. Para Bomb-M caused nearly equal depression of plasma and erythrocyte cholinesterase.
3. The plasma cholinesterase depression resulting from all compounds used in this study occurred sooner than the erythrocyte cholinesterase depression. Pretreatment plasma cholinesterase levels were reestablished in a shorter time than erythrocyte cholinesterase levels after treatment was stopped. Erythrocyte cholinesterase levels returned to normal at the rate at which new erythrocytes were released into the peripheral circulation.
4. The greater the depression of cholinesterase activity, the longer the period of time required for return to pretreatment levels.
5. An equilibrium well above zero was established for the cholinesterase activity in dogs exposed to repeated therapeutic doses of organophosphate compounds used in this experiment.
6. The organophosphate compounds used in the study were found to be less than additive when used in combination and did not potentiate each other.

CONCLUSION

Results of the study suggest that danger from the simultaneous administration of multiple organophosphate compounds are not hazardous to healthy dogs provided the compounds are given at recommended levels. Additionally, the results suggest that there is apparent safety to the utilization of the compounds in a wide variety of doses.

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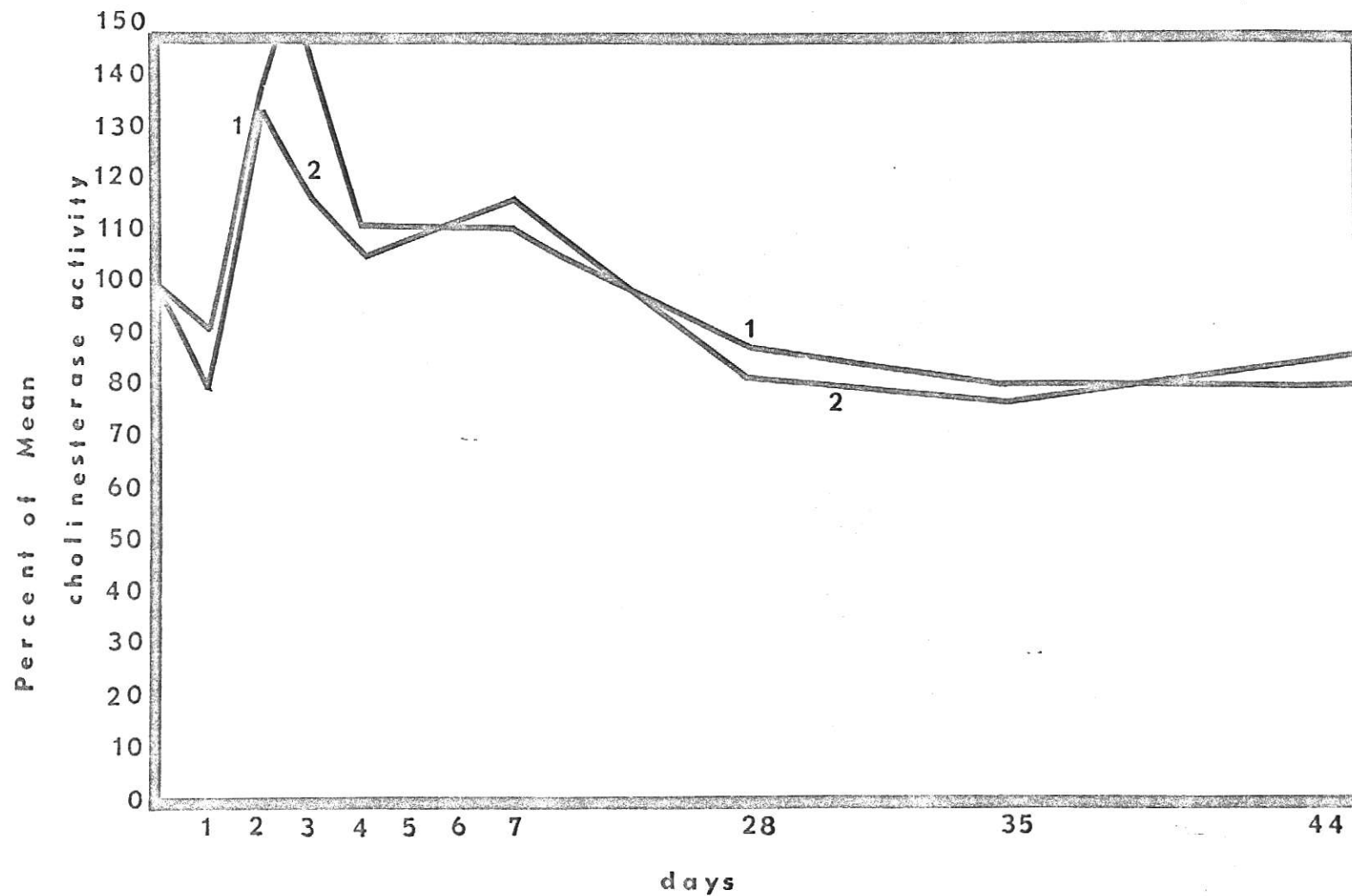


Fig. 1 Normal variation of erythrocyte cholinesterase activity occurring in dogs 1 and 2

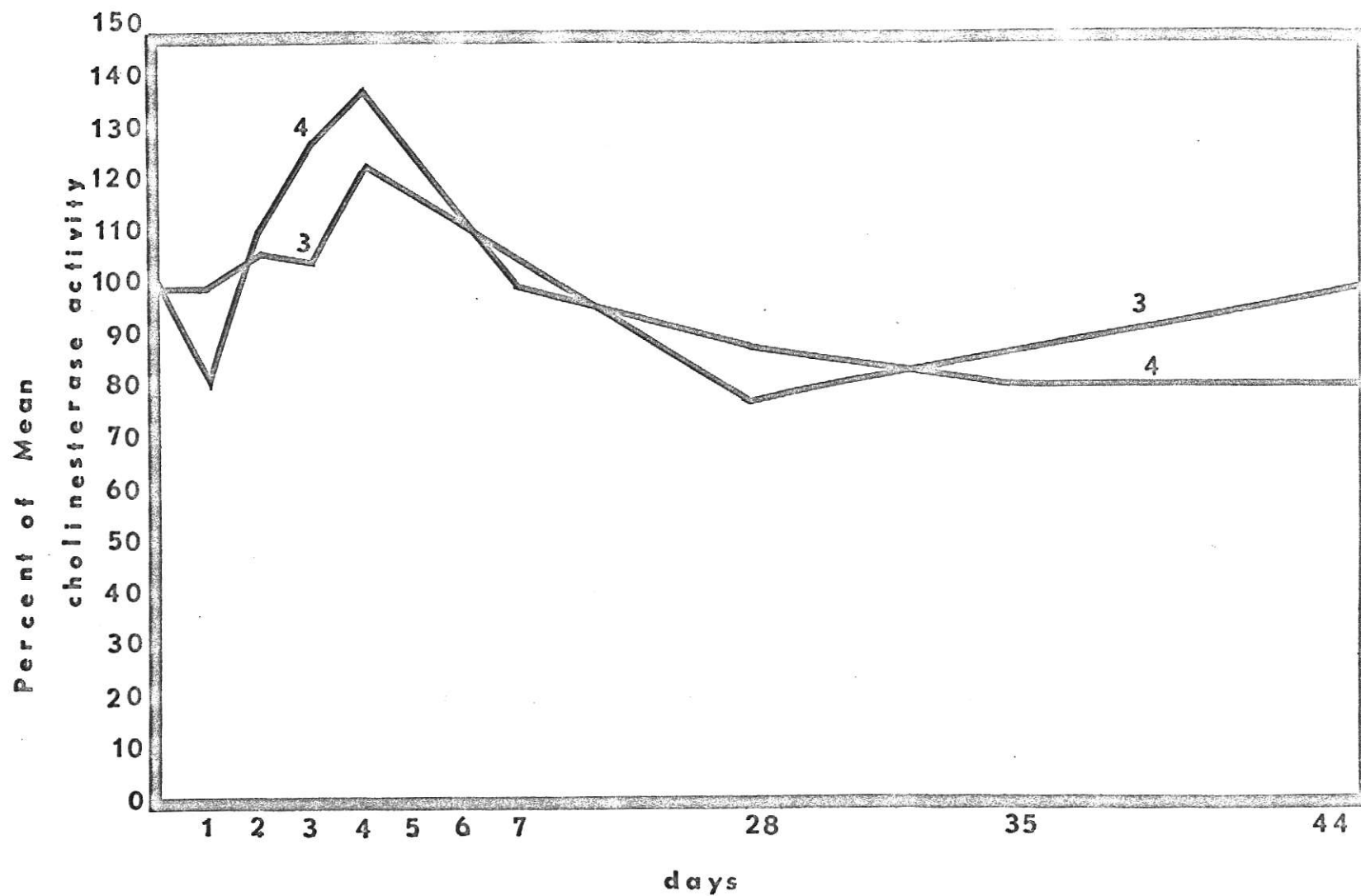


Fig. 2 Normal variation of erythrocyte cholinesterase activity occurring in dogs 3 and 4

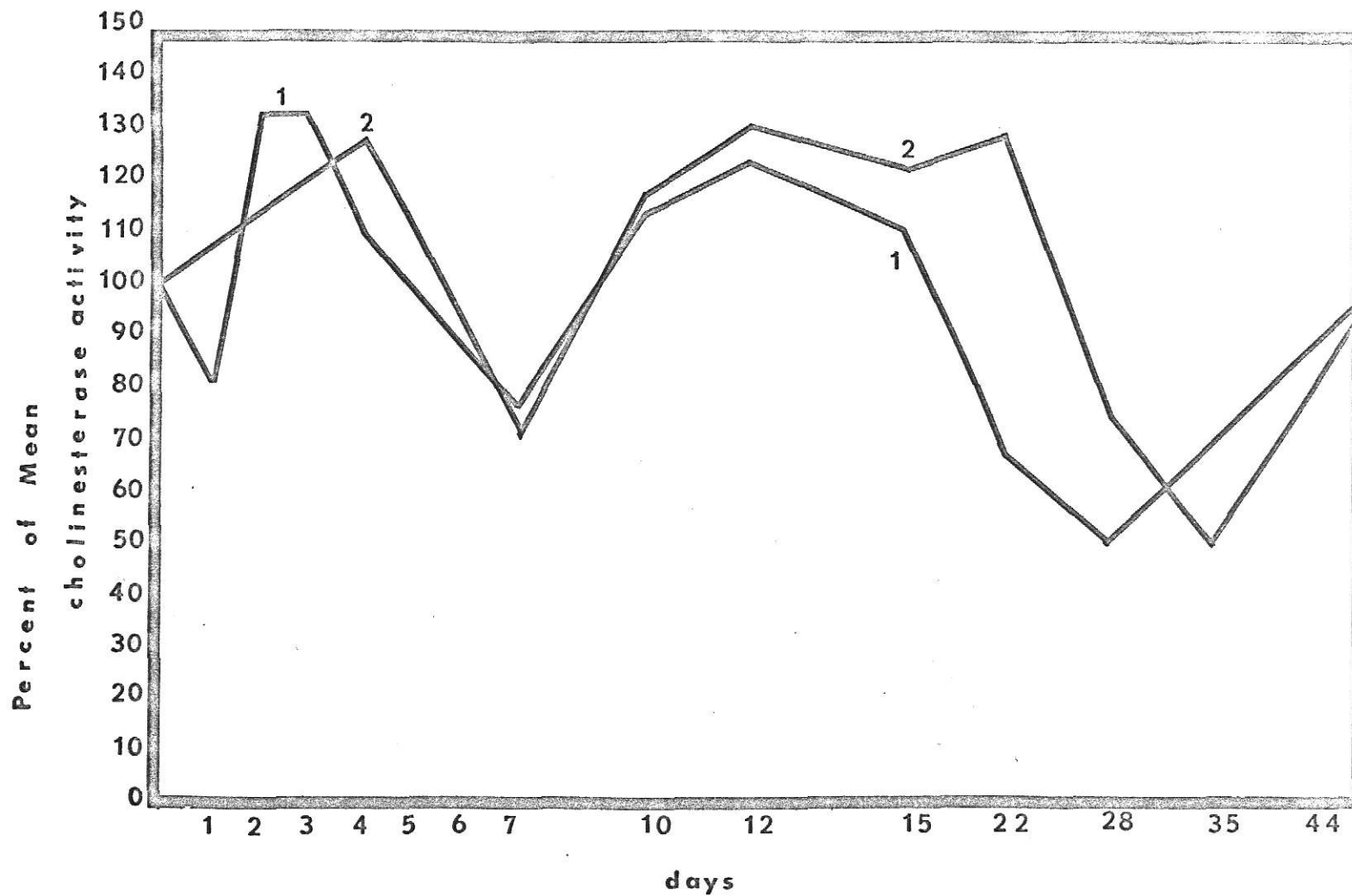


Fig. 3 Normal variation of plasma cholinesterase activity occurring in dogs 1 and 2

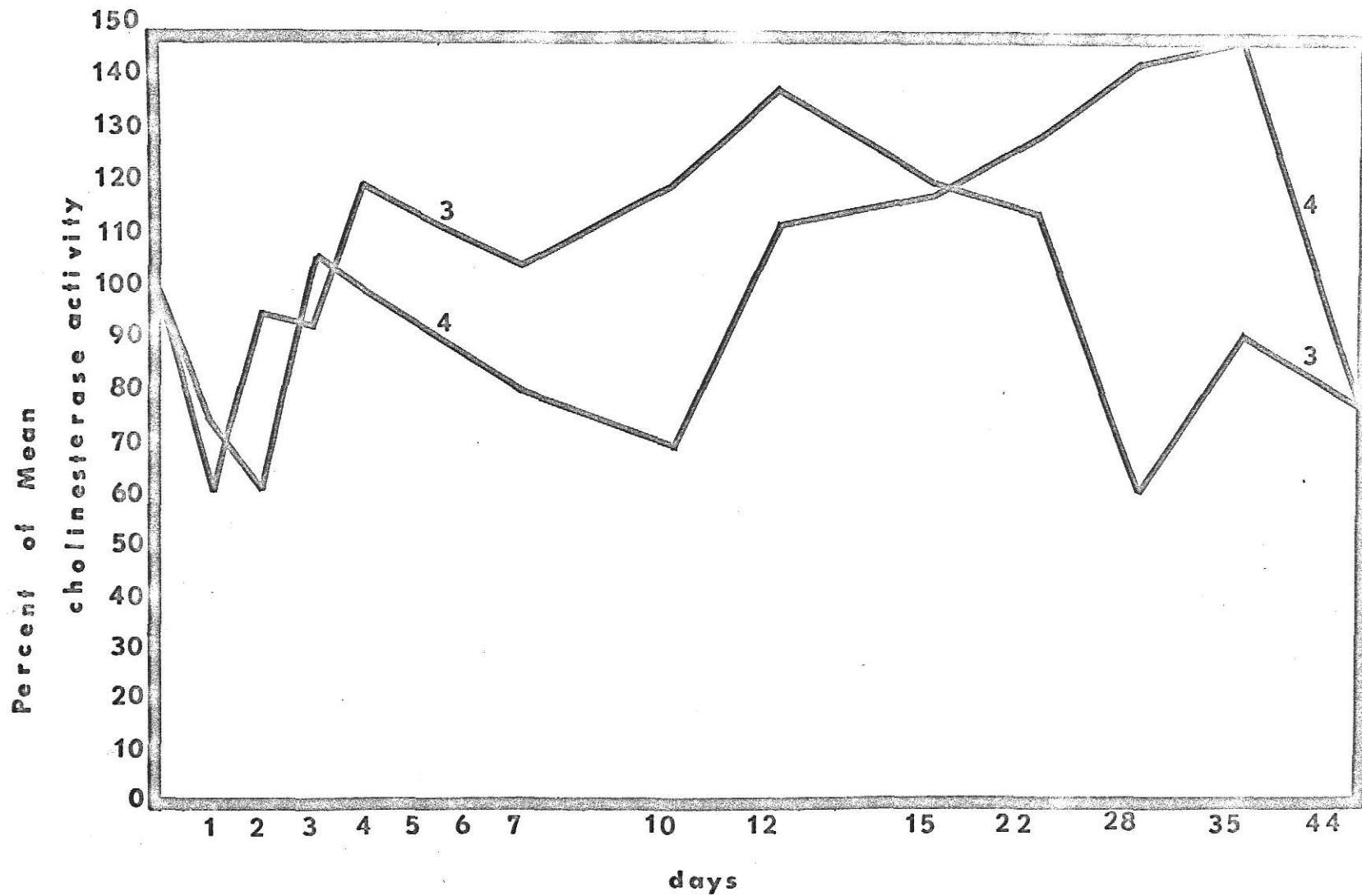


Fig. 4 Normal variation of plasma cholinesterase activity occurring in dogs 3 and 4

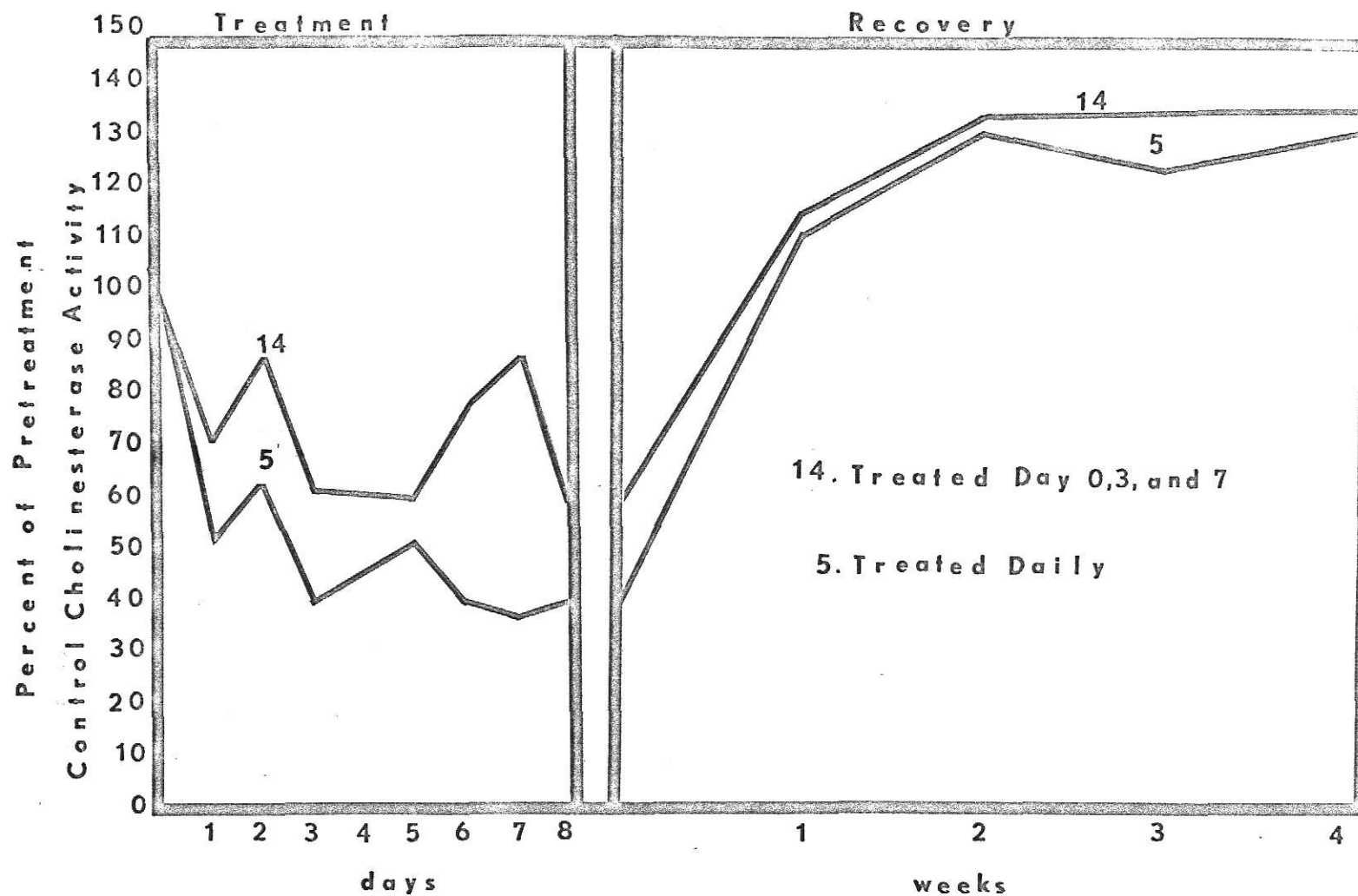


Fig. 5 Plasma Cholinesterase Activity of Two Dogs Treated With Freed^R Orally

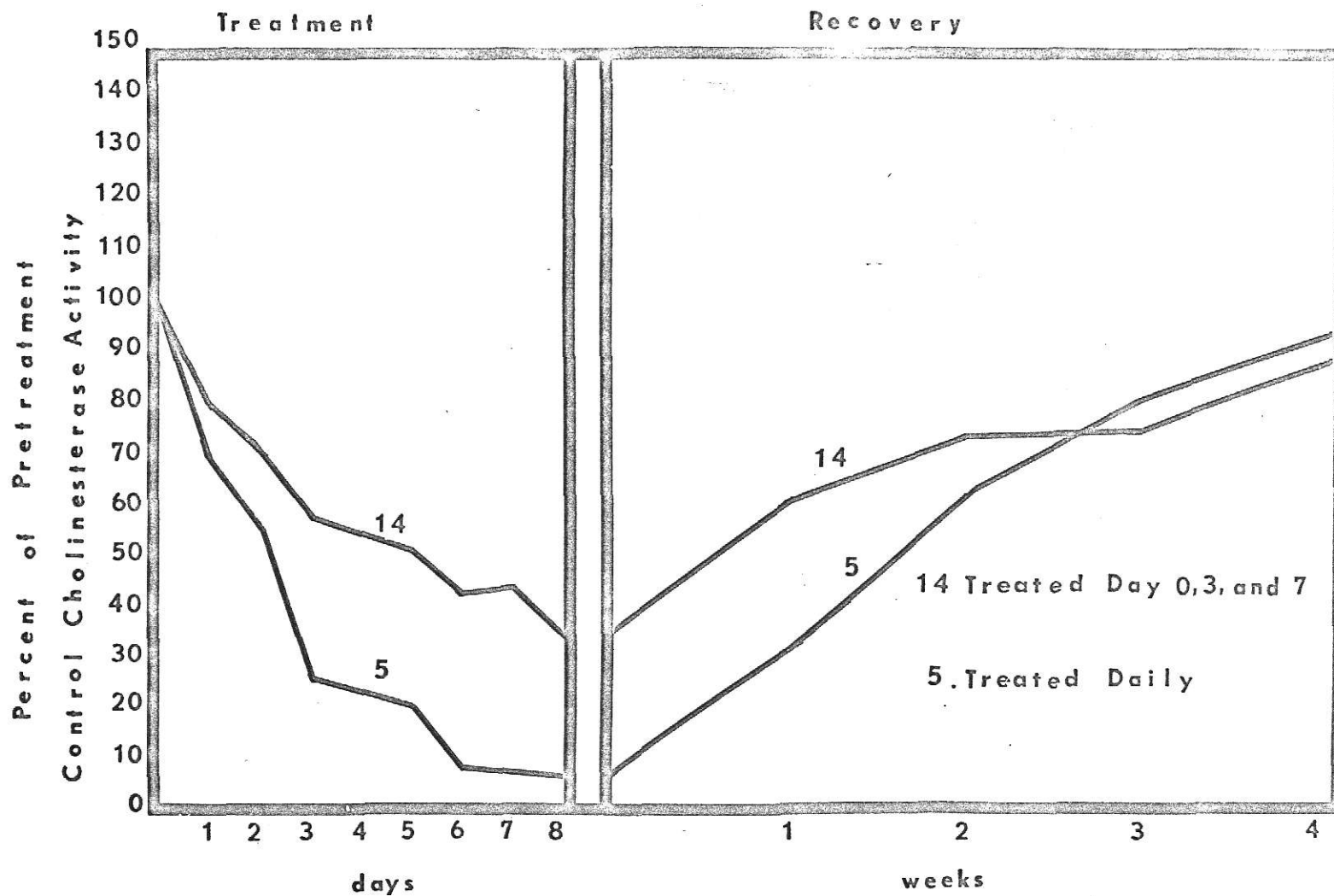


Fig. 6 Erythrocyte Cholinesterase Activity of Two Dogs
Treated With Freed^R Orally

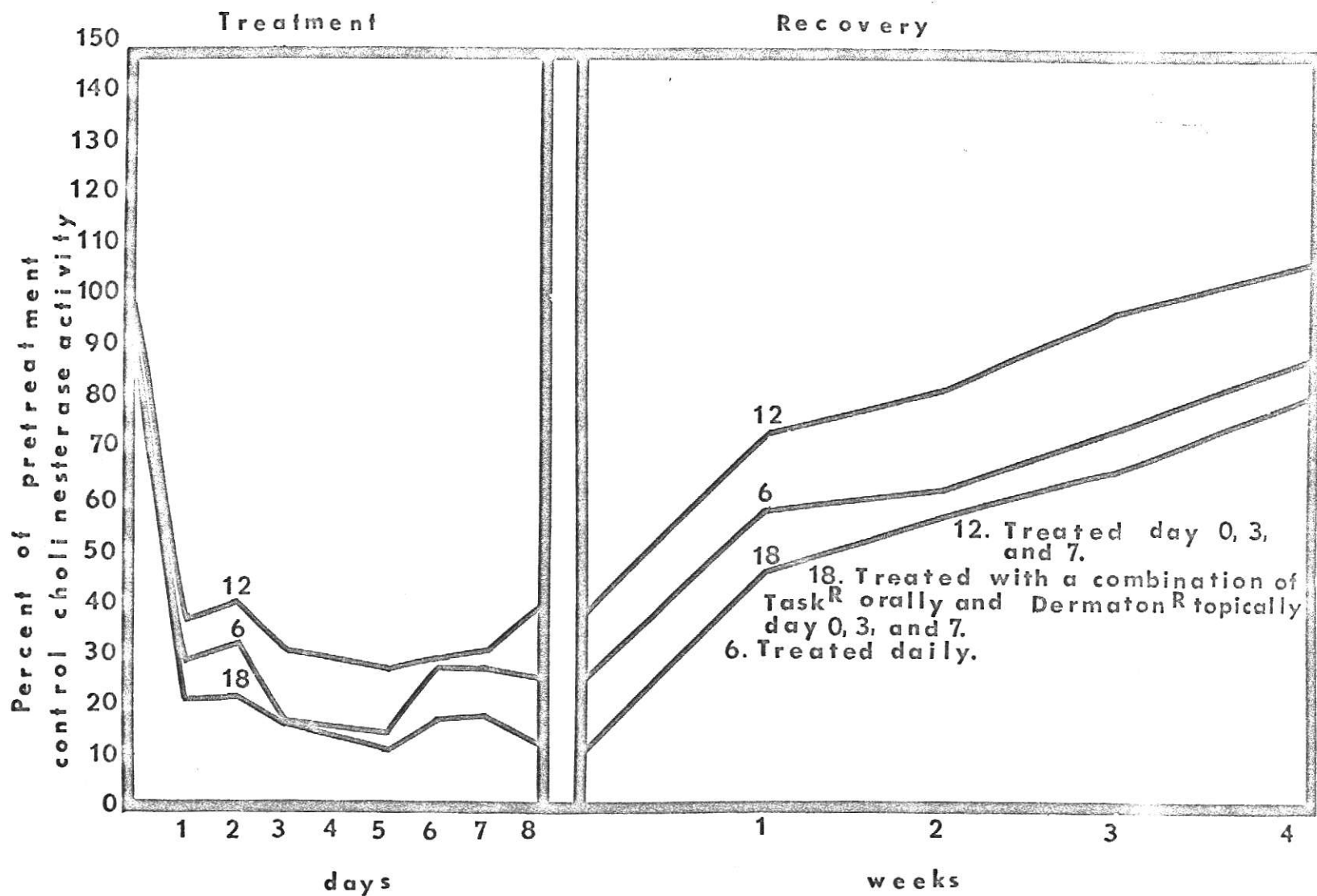


Fig. 7 Plasma cholinesterase activity of three dogs treated with Dermaton^R topically

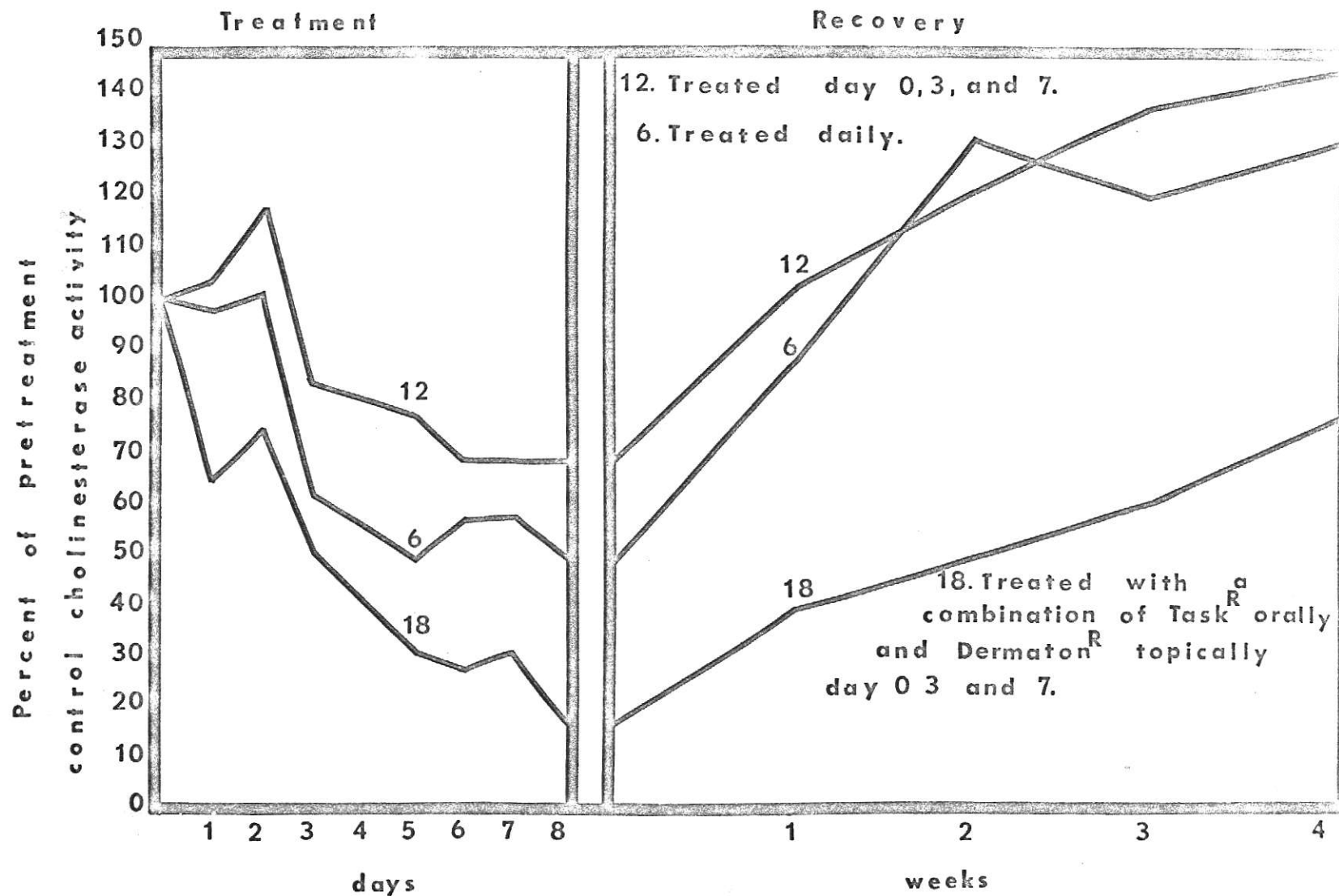


Fig. 8. Erythrocyte cholinesterase activity of three dogs treated with Dermaton^R topically

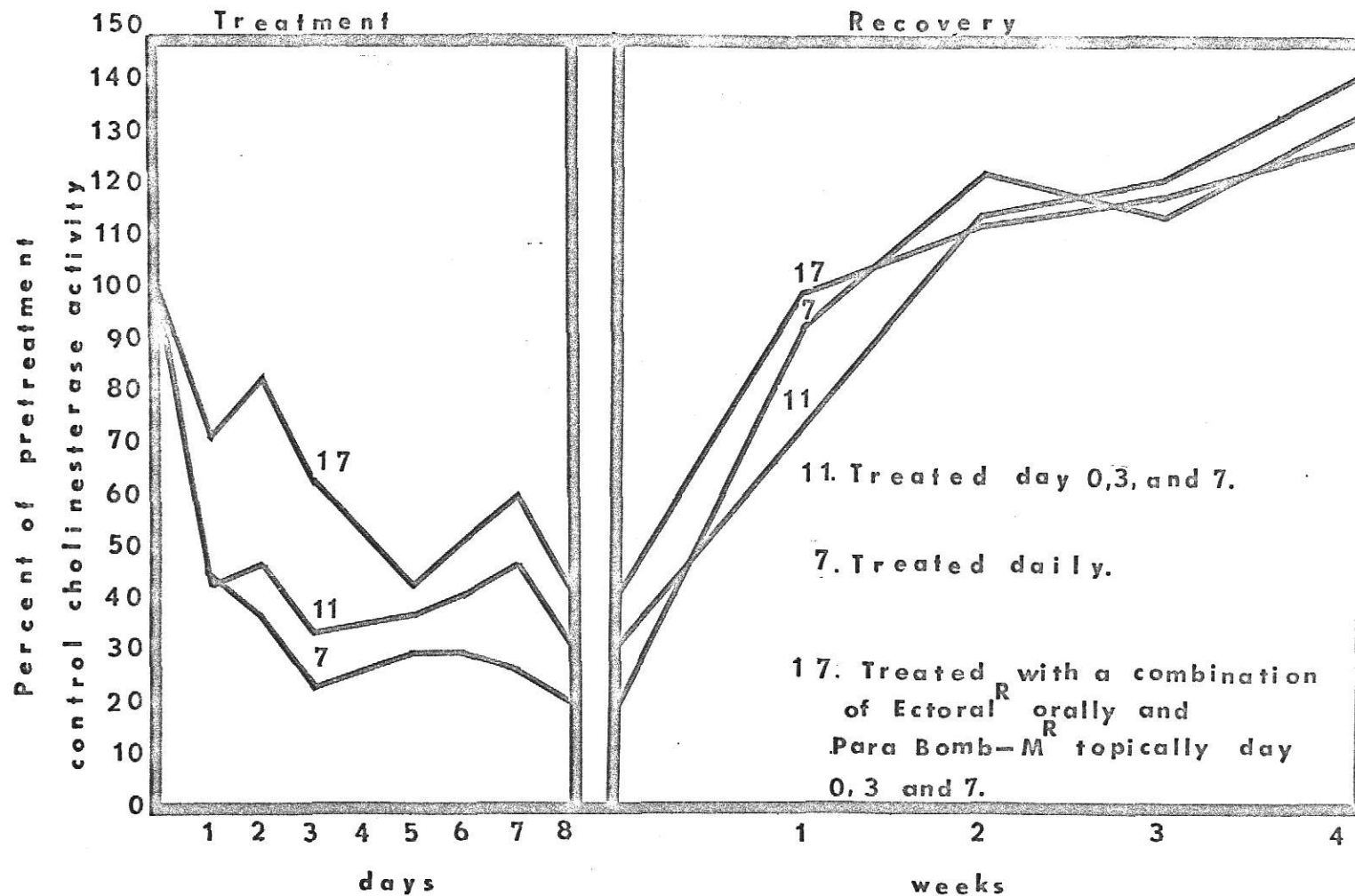


Fig. 9 Plasma cholinesterase activity of three dogs treated with Ectoral^R orally

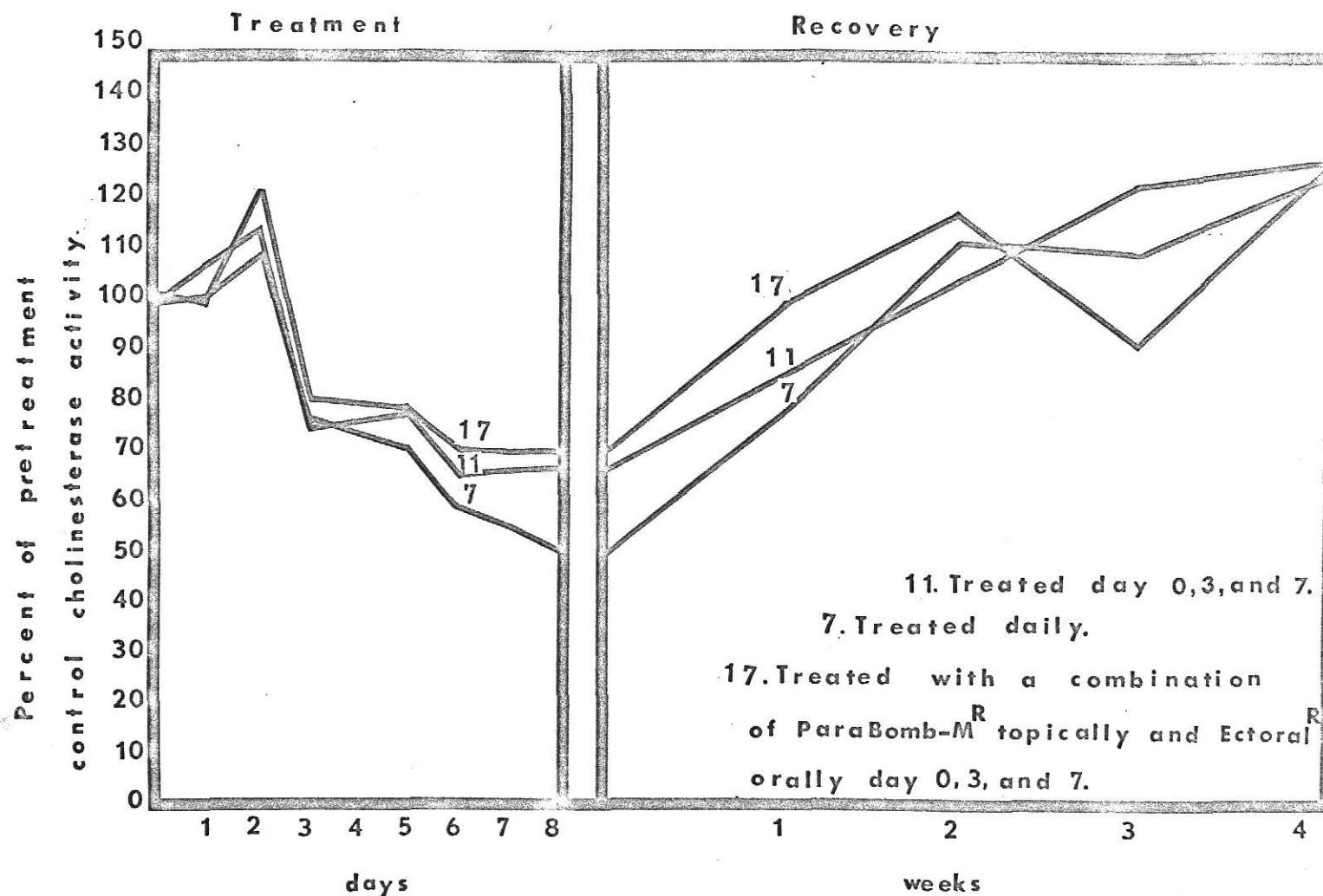


Fig. 10 Erythrocyte cholinesterase activity of three dogs treated with Ectoral^R orally

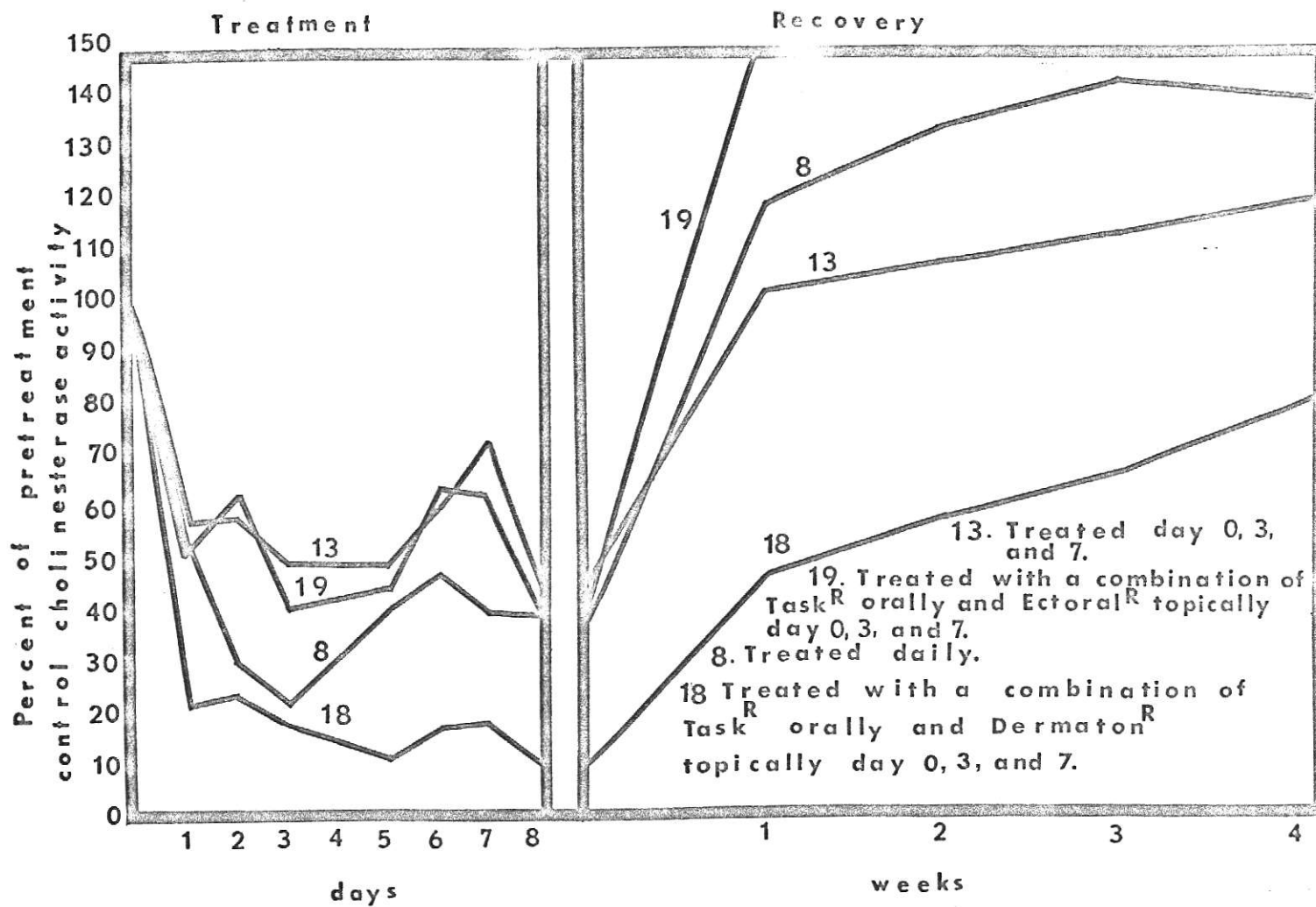


Fig. 11 Plasma cholinesterase activity of four dogs treated with Task^R orally

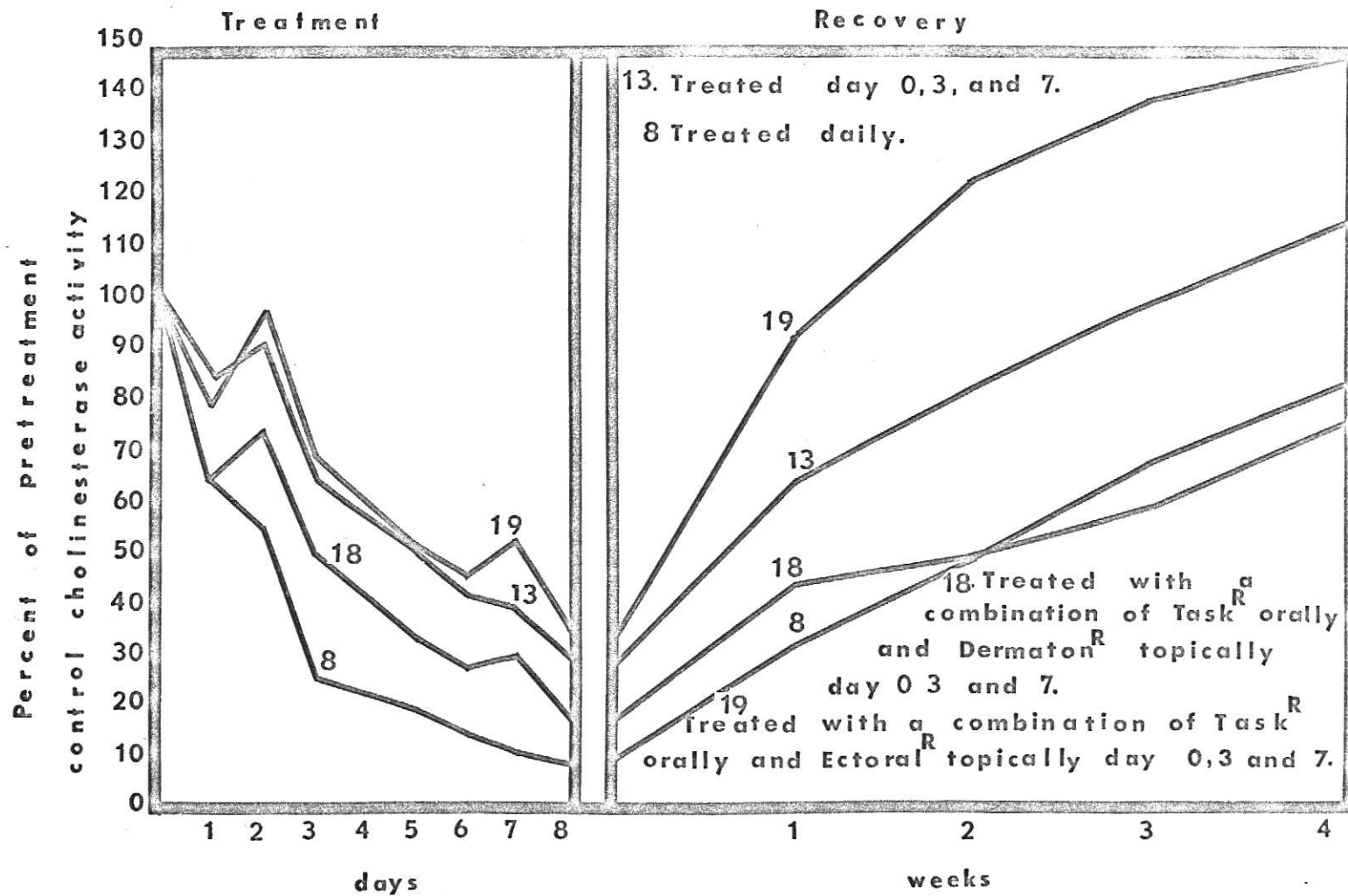


Fig. 12 Erythrocyte cholinesterase activity of four dogs treated with Task^R orally

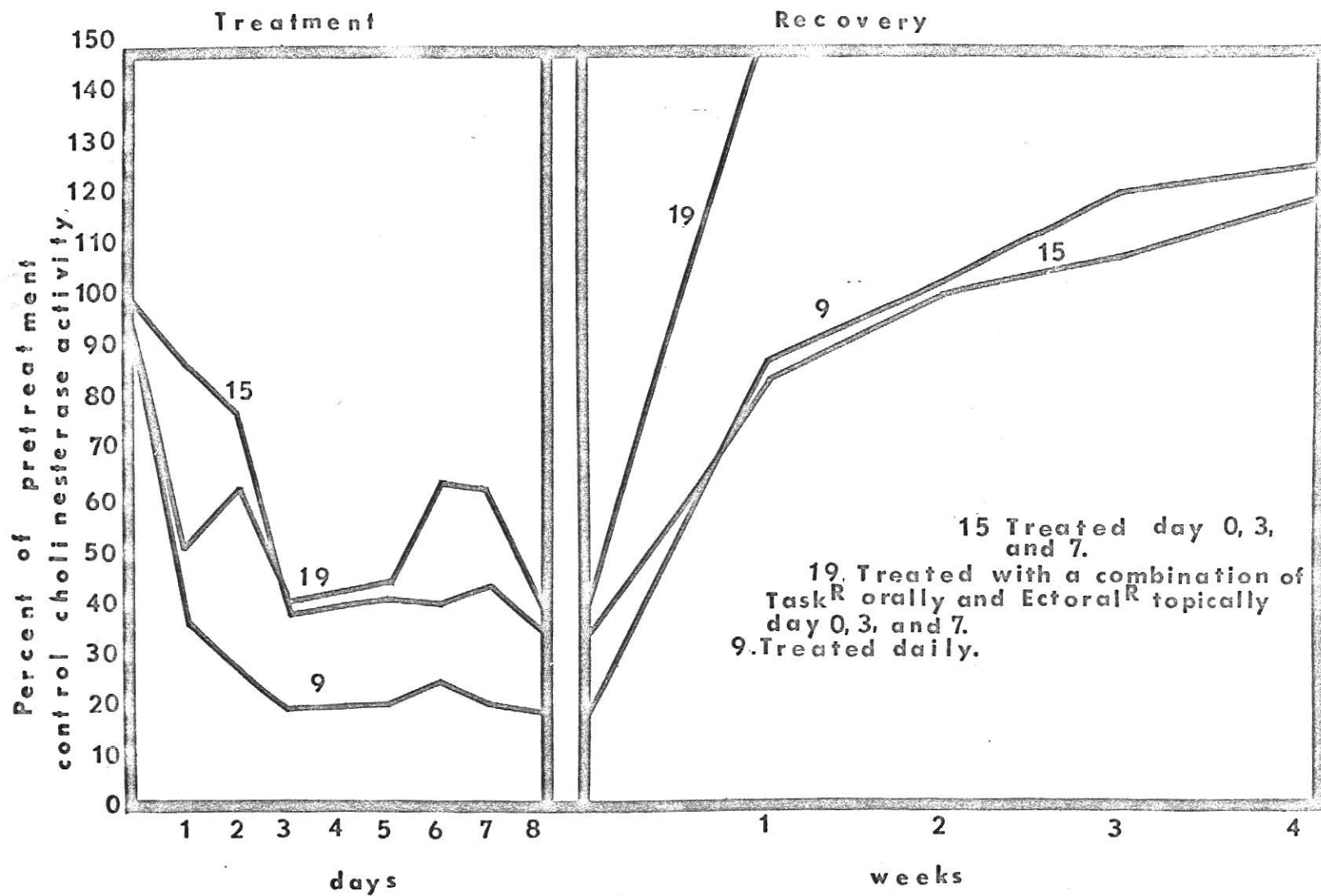


Fig. 13 Plasma cholinesterase activity of three dogs treated with Ectoral^R topically.

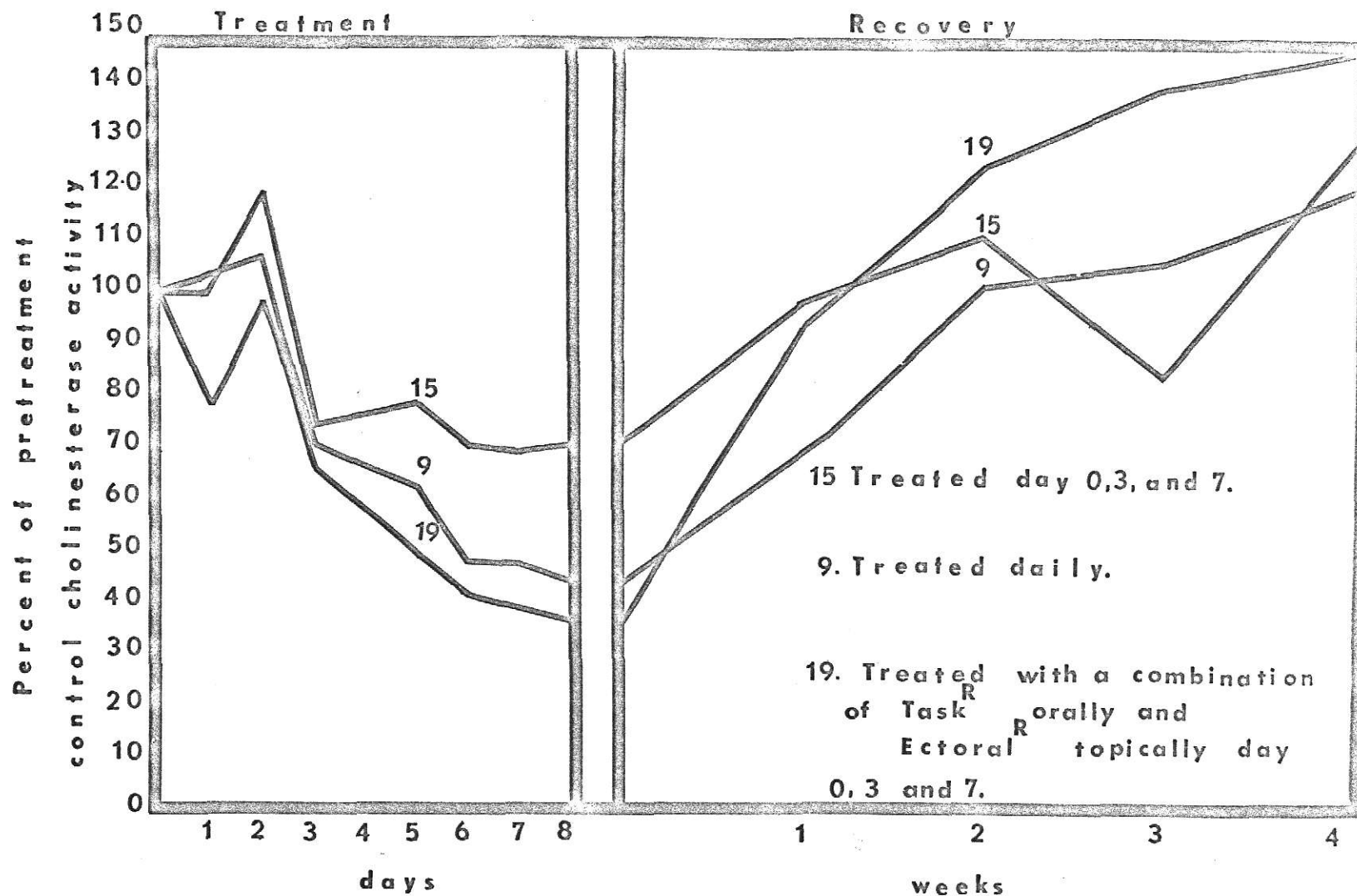


Fig. 14 Erythrocyte cholinesterase activity of three dogs treated with Ectoral^R Topically.

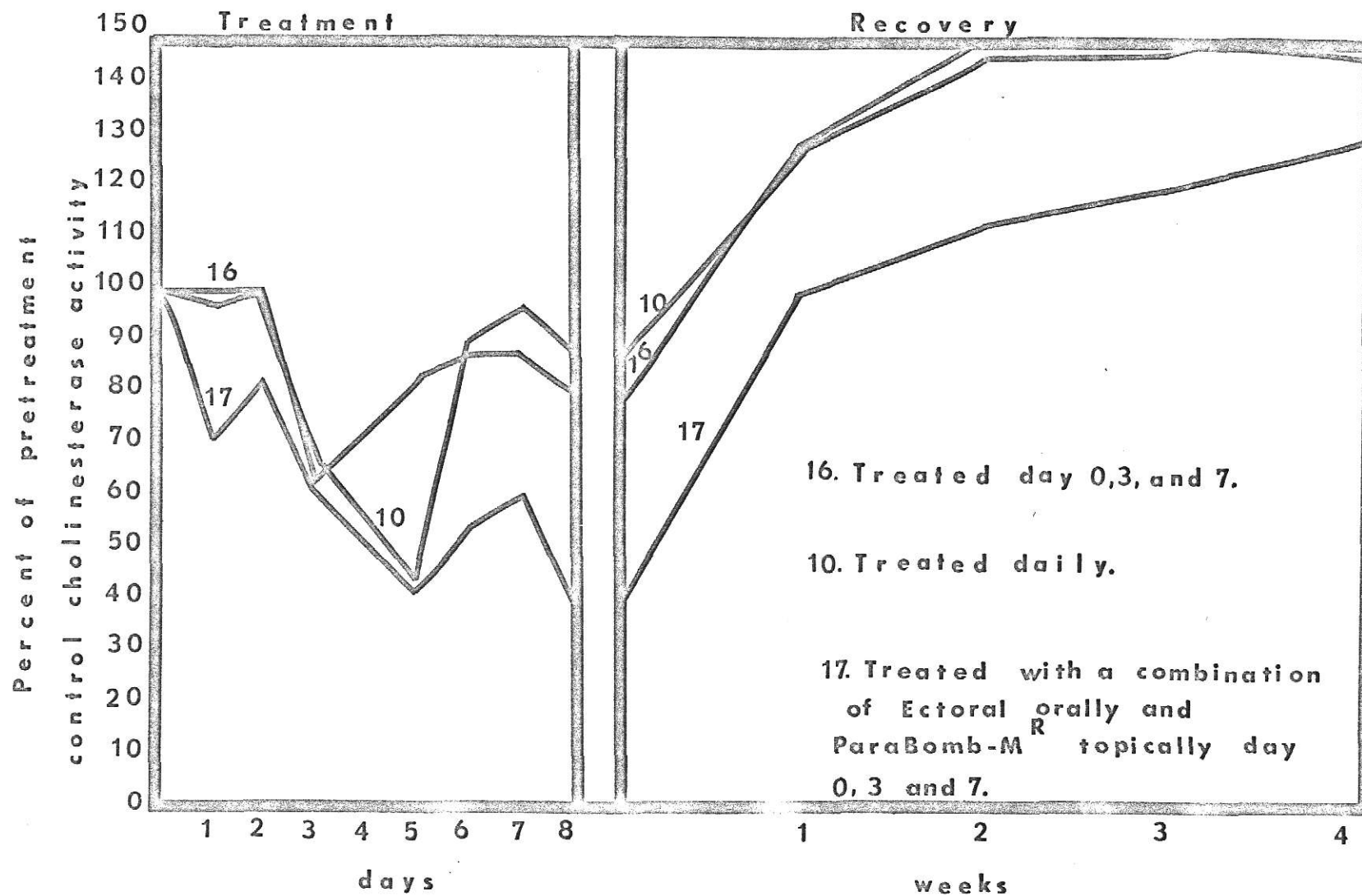


Fig. 15 Plasma cholinesterase activity^R of three dogs treated with ParaBomb-M topically.

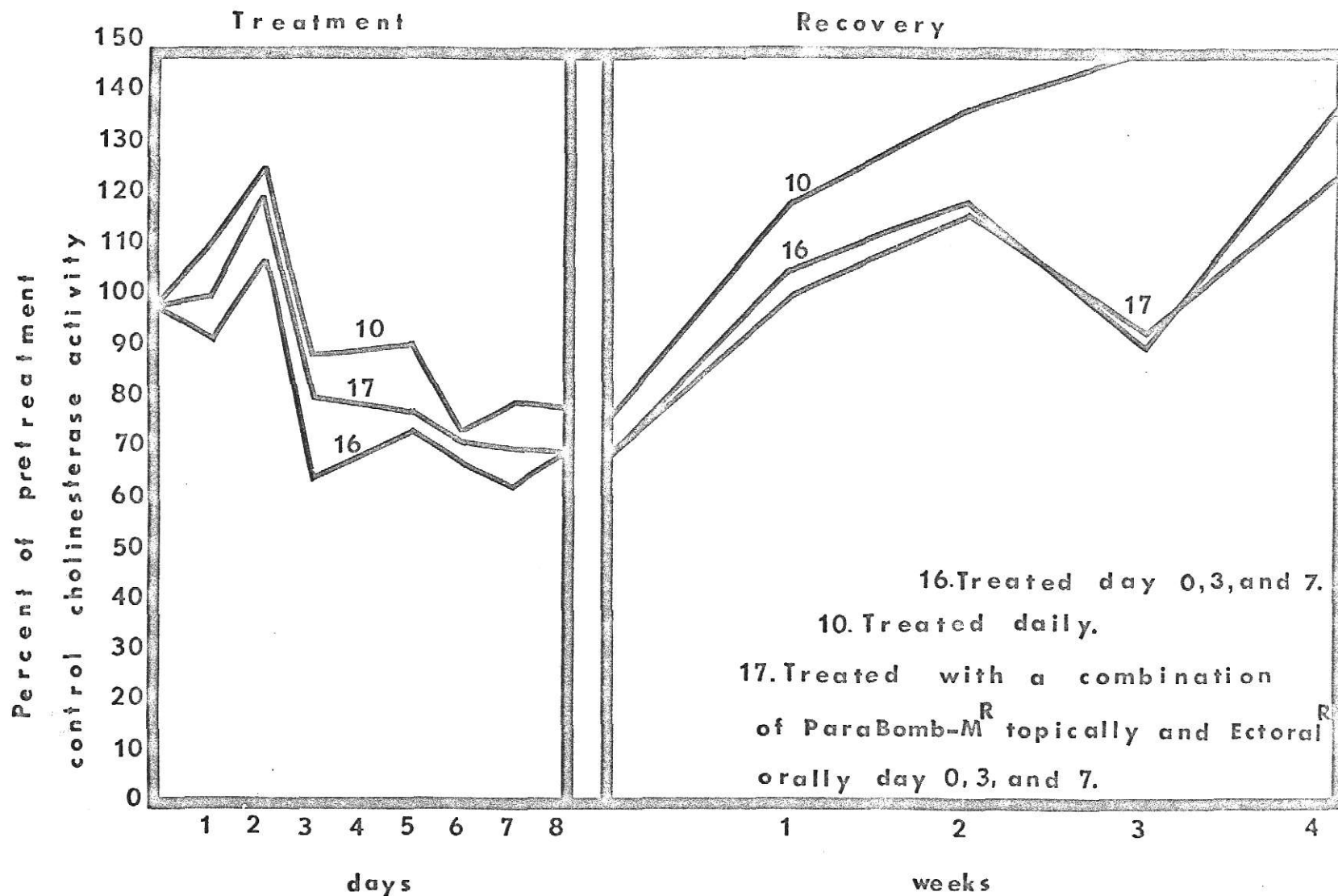


Fig. 16 Erythrocyte cholinesterase activity of three dogs treated with ParaBomb-M^R topically.

THE EFFECT OF CERTAIN ORGANOPHOSPHATES ON
SERUM AND ERYTHROCYTE CHOLINESTERASE IN THE CANINE SPECIES

by

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AN ABSTRACT OF A MASTER'S THESIS

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Certain organophosphate compounds used in the every day practice of veterinary medicine may cause subclinical toxicity. These organophosphates have an adverse effect on the enzyme cholinesterase which catalyzes the breakdown of acetylcholine to acetic acid and choline. Where the therapeutic dosage schedule of the organophosphates is not followed or if the recommended dose is exceeded, toxic signs due to excessive depression of cholinesterase levels may result. The signs are those of excessive parasympathetic stimulation caused by increased levels of acetylcholine.

The project was designed to determine if a daily variation in the serum and erythrocyte cholinesterase activity occurred in the canine species, the effect of the more common organophosphates on serum and erythrocyte cholinesterase levels, and the effect of combinations of therapeutic organophosphates on serum and erythrocyte cholinesterase in the canine species.

Nineteen dogs were divided into four groups. Blood samples were taken daily for 5 days and then weekly for three weeks from Group I which constituted the control group. Michel's modified technique was used for determination of the erythrocyte and serum cholinesterase activity of Group I.

Five compounds were administered to a second and third group of dogs. Freed^R orally, Dermaton^R topically, Ectoral^R orally and topically, Task^R orally, and Para Bomb-M^R topically. Determinations of the plasma and erythrocyte cholinesterase activity were made utilizing the Technicon Autoanalyzer.

The dogs in Group IV were given combinations of at least two of the compounds used in the second and third dog groups.

The plasma cholinesterase coefficient of variation ranged from 24 to 28% in the four untreated dogs, while the erythrocyte cholinesterase activity coefficient of variation ranged from 15 to 32% for the four dogs.

Freed and Task caused greater erythrocyte cholinesterase than plasma cholinesterase depression. Dermaton and Ectoral caused greater plasma cholinesterase depression than erythrocyte cholinesterase depression. Para Bomb-M resulted in nearly equal depression of plasma and erythrocyte cholinesterase activity.

In no instance did the serum or erythrocyte cholinesterase levels approach zero per cent levels. Topically, Dermaton caused the greatest depression of the cholinesterase activity. The plasma cholinesterase depression caused by all compounds in the study occurred earlier than the erythrocyte cholinesterase depression. The return to pretreatment control levels occurred sooner for the plasma cholinesterase following cessation of the drug administration.

Combination of organophosphates did not cause potentiation of cholinesterase activity depression.

Results of this study suggest that the danger from the simultaneous administration of multiple organophosphate compounds are not hazardous to healthy dogs provided the compounds are given at recommended levels. Additionally, there is apparent safety to the utilization of the compounds in a wide variety of doses.