ANTAGONISTIC EFFECT OF SOME ANALEPTICS AGAINST DEEP PENTOBARBITAL ANESTHESIA IN DOGS

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ANTAGONISTIC EFFECT OF SOME ANALEPTICS AGAINST DEEP PENTOBARBITAL ANESTHESIA IN DOGS

By

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A THESIS

Submitted to the College of Veterinary Medicine
of Michigan State University in partial
fulfillment of the requirements
for the degree of

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1965

Respectfully Dedicated To:

My Loving Parents

and

My Eldest Brother

Sri. Ramakanta Mishra

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INTRODUCTION

Acute poisoning by the misuse of barbituric acid derivatives has become a problematic phenomenon in the twentieth century; it is growing by degrees, in pace with the rapid advancement of the pharmaceutical industries. In Scandinavian countries, 871 patients, in comparison to 152 in 1932, with acute barbiturate poisoning symptoms were admitted to the hospital in 1947, a sixfold increase of such occurrence within a 15-year period. The ease of obtaining drugs led to serious maladies in the society. In the modern age under the veil of destitution, disease, and desperation, the overwhelming preponderance of the use of barbiturates as the means of self-destruction is far from uncommon. Shulman and co-workers (1955) have stated that over the past decade the amount of barbiturates used on both sides of the Atlantic has more than trebled and the incidence of barbiturate coma has increased fivefold.

On the other hand, reports are not rare of the serious complications arising from the clinical use of the barbiturates--either as sedatives, hypnotics, and narcotics in human practice or as general anesthetic in veterinary use, where the lives of the patients were threatened.

A quest for the prevention of the barbiturate hazard brought forward the existence of another group of drugs, the central analeptics, which are capable of greatly minimizing the mortality from barbiturate intoxication due to their antagonistic action. Even though the detailed mechanism of action of most of the central analeptics is not fully understood, they have been credited with life saving properties against mild and severe types of barbiturate intoxication, because of their CNS stimulating properties. As regards the determination of the most efficacious analeptic for the purpose, controversy still exists though some of the old analeptics, such as nikethamide and amphetamine, have definitely been proved as less effective than the modern ones.

At present, objections have been raised to the use of analeptics against barbiturate poisoning. Some researchers feel that analeptics are effective in mild or moderate barbiturate poisoning but not useful at the time of actual need, i.e., in severe barbiturate intoxication (Mousel, L. H. and co-worker 1941).

There are, however, two schools of thought as to the treatment of acute barbiturate intoxication, though both agree to a thorough and intelligent medical approach.

The conservative school, led by Nilsson, strongly opposes analeptic use, and they claim that these drugs may cause synergistic depression from the production of convulsion in subjects already depressed by barbiturates. Nilsson

explains this phenomenon in the following way:

> An increased activity in the cells of the tissue is created by the cerebral stimulation. An increased activity indicates an increased need for oxygen. An increased need for oxygen in a tissue, which is hypoxic in advance, paves the way for deepening the already existing depression.

Very significant results were obtained by Nilsson (1951). Locket and Angus (1952), Clemmenson (1954), who solely relied on the planned medical management. There are certain disadvantages to this line of treatment: (1) One cannot be sure, in deep barbiturate coma patients, if and when they will regain consciousness, and in more prolonged coma more risk is incurred despite scrupulous care. (2) Serious trouble will arise in hospitals to pay constant attention on prolonged coma cases.

The medical nihilism advocated by Nilsson is condemned by Koppanyi and Fazekas (1952) who strongly claim the definite role of central analeptics in cases of severe barbiturate intoxication. In fact the barbiturate-analeptic antagonism has been evidenced by innumerable clinical and laboratory experimental reports. Because of the lack of adequate information concerning the mode of action of the analeptics, the Council of Pharmacy and Chemistry of the American Medical Association cautions against the routine use of such drugs in barbiturate poisoning cases. Still the following advantages of analeptics can not be denied.

1. Rapid arousal effect due to the cerebral stimulation associated with visible reflex mechanisms.
2. Stimulation of the vital centers, i.e., respiratory and cardiovascular centers in the medulla thereby reinstating adequate respiration and maintaining normal circulation.
3. The prolonged coma stage is converted to a "safe state" and the threat to the patient's life is prevented.
4. Prolonged endotracheal intubation becomes unnecessary.
5. They afford relief from prolonged and strict nursing, favoring minimum hospital management procedures.

In this project, picrotoxin, a very old analeptic, and another fairly recent one, methylphenidate (Ritalin) have been chosen because of the many favorable reports of these two drugs. Their ability to antagonize barbiturates in deeply barbitalised dogs has been studied individually. They are also tried in various fractional dose combinations with the hope of better therapeutic value of the analeptics. Combinations of other drugs were proven to be more effective in previous study in this laboratory (Cairy, C. F., and Sisodia, C. S. 1961; Cairy, C. F., and Giri, Naraian 1965).

CHAPTER I

LITERATURE REVIEW

Picrotoxin

Introduction

Picrotoxin, a unique, controversial drug but unequivocally accepted as a versatile antidote against severe barbiturate poisoning, especially against short-acting ones, had its origin in the early nineteenth century. In the course of time the early premature concepts regarding the chemistry and therapeutic uses of picrotoxin have either been modulated or completely abandoned and a rational, though not concrete, detailed picture of it has been put forth in the modern age.

Boullay, M., discovered picrotoxin in 1812 from cocculus Indicus (Anamirta cocculus) called "Fish berries." Its composition was believed to be $C_{12}H_{14}O_5$. Barth and Kretschy, first to investigate the chemistry of picrotoxin, thought it to be a complex consisting of three constituents: the active component, picrotoxin ($C_{15}H_{16}O_6$); inactive non-poisonous components, picrotin ($C_{15}H_{30}O_6$); and Animirtin.

However in 1911 and 1912 picrotoxin was proved to be a mixture of equal parts of picrotoxinin and picrotin (Cervello 1911; Structural formula, Angelico 1912).

In 1814 Tschudi expressed picrotoxin to be a suitable antagonistic to morphine. J. Chrichton Brown in 1875 suggested it as a possible antagonist against chloral hydrate poisoning. Koppen in 1892 established this effect.

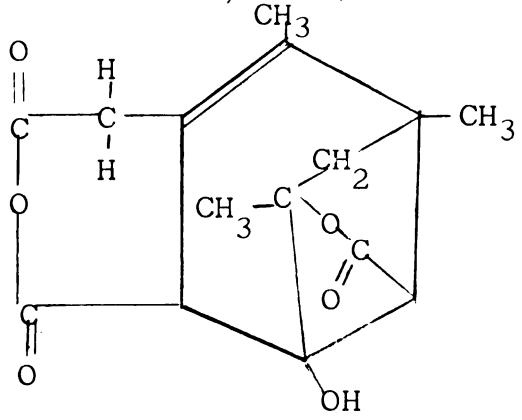
Little attention was paid to this discovery until 1931 when Maloney, Fitch and Tatum, and Maloney and Tatum (1932), on a laboratory animal basis, advocated its ability to combat the central depression resulting from administration of doses higher than LD50 of various barbiturates.

Koppanyi and co-workers (1936) further explored the limits of picrotoxin efficacy by determining the component factors and a rational explanation of their pharmacodynamics, as a striking barbiturate antidote. He was first to treat human barbiturate poisoning. He also pointed out (1936) that picrotoxin, which was once postulated to be helpful in case of morphine poisoning, gives adverse effects due to synergistic convulsive actions.

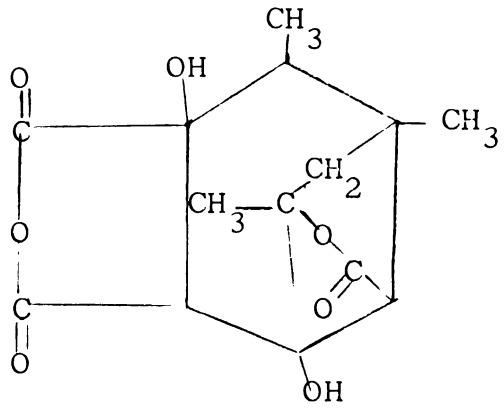
Besides this, many investigators have worked on this drug with diversified results. Because of such inconsistent action, picrotoxin was deleted from United States Pharmacopoeia (U.S.P.), though it was official in the U.S.P. in 1890. It is still officially accepted in British Pharmacopoeia (B.P.).

Chemistry

Picrotoxin is a non-nitrogenous neutral principle of emperical formula $C_{30}H_{34}O_{13}$. It is a loose chemical compound, believed to be an equimolecular mixture of picrotoxinin ($C_{15}O_{16}O_6$) and picrotin ($C_{15}H_{18}O_7$), which break apart on heating and reunite upon cooling. Excepting that picrotin has an additional molecule of water than picrotoxinin, they both are allied chemically, possess identical pharmacological actions but the latter acts more strongly (Angelico 1912). According to another school of thought, picrotin is inert and the entire activity ascribed to picrotoxin resides in the picrotoxinin (Chistoni and others). Some believe picrotoxin has a slight fraction of a third component, animirtin. Above all, picrotoxadiene, a degradation product of picrotin has been synthesized (Conroy 1952). Picrotoxinin indicates no superiority over picrotoxin in barbiturate poisoning trials (Kohn, 1938).



Picrotoxinin



Picrotin

Pharmacodynamics of Picrotoxin

The action of picrotoxin in the system is confined only to the central nervous system and nothing is known of its distribution and fate in the body (Cushny, Textbook of Pharmacology and Therapeutics, 1937). The autonomic nervous system stimulation, especially the parasympathomimetic action of the drug, is purely of central origin (Hatcher, R. A. and Weiss, S. 1923).

Central Nervous System Action

In general stimulation and depression are preceded by severe stimulation. In rats sometimes depression occurs with convulsive doses of picrotoxin.

The C.N.S. stimulation may be divided into the convulsive effect and the stimulation of vital medullary centers.

Picrotoxin as a Convulsant Drug

In normal animals, the C.N.S. stimulation does not appear until convulsion occurs. But in case of patients treated with C.N.S. depressant, it has a critical dose level up to which level beneficial C.N.S. stimulating effects, like arousal symptoms, result, and beyond that a severe convulsion followed with prolonged depression is observed. This post convulsive depression sometimes leads to fatal consequences.

There is a time lag of a few minutes for picrotoxin before any of its C.N.S. action is exhibited. This latency which decreases upon increase doses complicates its analeptic effect as convulsion immediately occurs. This is because picrotoxin has a narrow therapeutic ratio. In rabbits the difference between the convulsant and lethal dose is very small in comparison to pentylenetetrazole.

The typical convulsive effects of picrotoxin is characterized by clonic, asymetrical and coordinated type of response. In the case of frogs with higher doses the spinal stimulation becomes more prominent. Although the convulsions occur spontaneously in cycles alternating with depression, they are prevented by removal of the skin in frogs. Convulsions require the presence of sensory impressions (H. Shriver and Perschmann 1935). Optic and acoustic stimuli do not elicit generalized convulsion in cats treated with picrotoxin. In dogs a simultaneous increase in reflex irritability and a convulsant response in spinal cord is seen (Klein, J. R. 1943).

Site of Convulsion

Although picrotoxin is commonly known as a medullary stimulant, regions of the C.N.S. share in stimulation as the drug dose increases. The actual seat of action moves in an ascending direction as the higher parts of the C.N.S. become more developed. In fish picrotoxin action is medullary as

the convulsion remains unaltered after decerebration, becomes slightly abbrogated with destruction of optic lobes and the typical picrotoxin symptom is completely lost after section from medulla. At this stage, the convulsion due to spinal stimulation looks like a strychnine response. In mammals the site of action is chiefly mid-brain and cerebrum. In man the cerebrum seems to be more particularly involved. The glycogen content of the cortex is increased during picrotoxin induced convulsion (Chance, M. R. A. 1951), and the acid phosphatase in the largest cells of the motor cortex is decreased. However, the brain stem seems to be more involved in picrotoxin convulsion than pentylenetetrazole.

Medullary Stimulation

The life saving property of picrotoxin against barbiturate poisoning is due to the stimulation of the vital centers--respiratory and circulatory--located in the medulla. It increases the rate and depth of respiration, and it increases blood pressure. Besides that, the vomiting, salivary and other centers are also stimulated with the analeptic dose of picrotoxin.

Depressing Effect of Picrotoxin

Post convulsive depression is more pronounced than pentylenetetrazol in rats and rabbits. Loss of placement reaction indicates that cortex is depressed. Confusion followed with unconsciousness proves that the drug first

stimulates and then depresses the cerebral cortex (Dille and Hazleton 1939). Depression duration increases with the increased length of convulsion.

Action on Autonomic System

The autonomic nervous system stimulating effect of picrotoxin is well established. The fact remains debatable whether the parasympathetic or sympathetic action is more pronounced. In any case all possible autonomic effects are of central origin.

The old literature favors the parasympathomimetic action of picrotoxin. In 1909 Grunwald for the first time proved this fact by establishing a variety of parasympathetic nerve action. In his vivid description and rational explanation, he suggests that picrotoxin acts on all autonomic centers; the centers controlling the cranial and sacral autonomic nerves are especially susceptible to this drug. Salivary secretion is increased by the stimulation of the nucleus of the chorda tympani; the heart is slowed by vagus center stimulation. Occulomotor center is stimulated causing constriction of the pupil. In the same way sweating with dilation of skin vessels, contraction of the bladder and uterus, and lacrymation occur. All these effects cease on severing the nerves.

The fall in body temperature is due to direct effect on heat regulating and heat producing center (Harnack). In the late convulsive stage, the mydriasis and tachycardia are due to paralysis of parasympathetic nerve endings. Increased peristalsis of intestine is another feature of late convulsions. Rogers, F. T. (1918) has shown tetanic stomach contraction in turtles with picrotoxin. It induces ovulation in rabbits probably by stimulation of the hypophysis (Brooks, C. M. et al. 1940).

Recent investigators give enough evidence about the sympathetic effect of picrotoxin: pronounced hyperglycemia (Rosenthal, F. E. 1941), raised blood pressure, inhibition of diuresis probably by the constriction of renal arteries and cessation of rectum and bladder contractility in the rabbit. The central origin of these effects is proved on section of spinal cord or appropriate splanchnic nerves.

The peripheral autonomic effect of picrotoxin, if any, is supposed to be negligible. Weakening of the excised frog heart with concentrated picrotoxin solution, stimulation and depression of rabbit intestine (excised) with small and large drug doses respectively, depression of motor nerves and skeletal muscle (Ogawa, M. 1928) and finally hemolysis; these factors contribute to the weak peripheral effect of this drug.

Further advanced study on isolated organs depict many nonspecific effects. There is no indication of neuromuscular junction stimulant action of picrotoxin (Huston, M. J. and Martin, A. W. 1951). It does not inhibit cholinesterase or sensitize the leech muscle to acetylcholine. Its action on the acetylcholine content of the brain depends upon the anesthetic used.

In sum, the predominant effect of parasympathomimetic and sympathomemetic action of picrotoxin establishes its central autonomic nervous action.

Respiratory Action

Picrotoxin is a definite respiratory stimulant in normal and anesthetically depressed patients; full convulsant dose is needed for the former case and subconvulsant dose for the latter. The respiratory response in normal cases is least observed till convulsion sets in, though occasionally respiration is stimulated before convulsions occur.

The pharmacodynamics of picrotoxin is attributed to its direct respiratory center stimulation at the medullary region; not carotid chemoceptor stimulation (Krantz, J. C. and co-workers 1937; Marshall, E. K. and co-workers 1937). Besides, the sensitivity of the inspiratory center to electrical stimulus is increased.

With convulsive doses the respiration becomes irregular during muscular twitching, ceases during clonic spasms and this phenomenon continues alternately until the convulsive stage is overcome or fatality results due to the precipitation of permanent respiratory arrest during spasms.

As a barbiturate antagonist, its success in increasing the respiration, both rate and depth has been suggested by a great number of researchers (Maloney et al. 1931, 1932; Das, S. C. 1939; Chakravarti, M. 1939; Thorp, R. H. 1947). It has been further stated that picrotoxin reinstates adequate respiration and greatly lightens the degree of anesthesia.

Marshall and co-workers (1937) and others have also indicated that it is capable of stimulating respiration in chlorbutanol, trimethylbutanol, paraldehyde and Avertin anesthesia, but ineffective against ethanol. They suggest in these cases picrotoxin behaves as a chemical antidote rather than pharmacological antidote.

Moderate respiratory depression by morphine poisoning is antagonized by picrotoxin (Hahn, F. 1960). Koppanyi and co-workers in 1936, 1941 and others disregard this drug as a morphine antagonist because of its synergestic convulsive response with morphine leading to more adverse consequences.

Cardiovascular Response

Subconvulsant dose of picrotoxin increases blood pressure. An initial rise followed by a fall in blood pressure 8-10 seconds before the onset of convulsion is observed with convulsive doses (Pollock and Tredway 1913 and Pollock and Holmes 1915). The possible cause, they explain, is by one or more of the following mechanisms:

1. Vagus stimulation
2. Stimulation of cardiac inhibiting center; both leading to cardiac slowing
3. Depression of vasomotor center
4. Some cardiac muscle fatigue.

Return of normal blood pressure after the convulsive seizure is apparently due to fatigue of the vagus nerve. The central origin of the vasopressor action of picrotoxin is evidenced by cumulative experimental findings.

Picrotoxin very strongly antagonizes the barbiturate induced circulatory failure by its central action (Maloney, A. H. and Tatum, A. L. 1932) and improvement of the carotid sinus reflex (Chakravarti, M. 1939). The occasional inefficiency of picrotoxin to combat the decreased pressure response in deep barbitualized animals is due to its lack of peripheral activity which is possibly depressed to some extent by barbiturates.

Action on Body Temperature

Reduction in body temperature in normal subject due to picrotoxin is inevitable either by heat loss (Hahn, F. 1960) via sweat and dilatation of skin vessels due to autonomic center stimulation or by decreased hypothalamic function. In the absence of convulsions pronounced fall in body temperature due to skin vessel dilatation and sweating is observed (Harnack). Paradoxically the subnormal body temperature caused by barbiturates and paraldehyde is prevented by picrotoxin (Koppanyi and co-workers 1936; Maloney 1932; and Rosenthal 1941).

As a Barbiturate Antidote

Notwithstanding the few shortcomings of the data on the merits of picrotoxin-barbiturate antagonism, the preponderance of reports leave no room for doubt about its superb life saving effect against severe barbiturate poisoning.

Maloney and co-workers (1931) were first to proclaim its pronounced barbiturate antagonistic effect and its clinical use. Immediately many investigators pinpointed their attention on this aspect and many research articles came out in support of this; the outstanding, detailed and more rational explanatory reports of Koppanyi and co-workers (1936); Barlow, O. W. (1933); Chakravarti, M. (1939);

Järvinen, P. A. and Vartiainen, A. (1949); Lavenson, G. S. Jr., Plum, F. and Swanson, A. G. (1958) are some of them.

Picrotoxin acts as a physiological antidote and not as a chemical antidote. The blood barbiturate level essentially remains the same in deeply barbitalized control animals and the animals successfully treated with massive doses of picrotoxin (Koppanyi and co-workers 1936). In some rare instances the awakened animals destroy large amounts of hypnotics due to their improved physiological condition and thereby reduce the blood barbiturate concentration. Picrotoxin also does not enhance the elimination rate of the barbiturate concentration. Picrotoxin also does not enhance the elimination rate of the barbiturates (Dille 1938). Further, that picrotoxin and other analeptics cause a complete arousal from barbiturate narcosis before sufficient time is elapsed for the chemical destruction of the barbiturates in the system indicates that the antagonism is purely of physiological nature.

Some general aspects of the review of F. Hahn (1960), Koppanyi and Fazekas (1952), Koppanyi and co-workers (1936), Reifenstein and co-workers (1939), and of the council on pharmacy and of chemistry (1939), on picrotoxin-barbiturate antagonism are discussed below.

Whereas picrotoxin combats the adversities of some barbiturates--sodium barbital, pentobarbital, etc. administered in multiple lethal doses, it is impotent against

certain other barbiturates. Picrotoxin has limited action against barbiturates in the sense that the latter is capable of reversing several LD50's of picrotoxin whereas the reverse is not true. This is perhaps due to the barbiturates' effect on a more extensive area on C.N.S. than the picrotoxin (Koppanyi and co-workers 1936).

When these two drugs are administered together, a direct reversal of the depressed state does not occur but instead a combined form of intoxication of the two drugs intermingled with depression and stimulation is exhibited from which the animal eventually recovers (Council on Pharmacy and Chemistry report 1939).

Picrotoxin is rapidly eliminated from the system (Dille 1938, Dille and Duff 1939). Hence regular repeated doses of the drug are given to counteract the long acting barbiturate intoxication. It gives excellent results against short acting barbiturates with a single dose of 2 mg/kg in dogs. Furthermore, because of the typical distribution nature of the two kinds of barbiturates in the living body, picrotoxin is injected intravenously (i.v.) for short acting barbiturates and either intramuscularly (i.m.) or subcutaneously (s.c.) for long acting barbiturates.

It antagonizes easily higher doses of short acting and sub-lethal doses of long acting barbiturates. Against the lethal dose of the latter barbiturate picrotoxin, within

limits, brings about a cure and in doses beyond that, the efficiency of the analeptic is completely lost. But the life of the patient may be prolonged for a few hours.

Picrotoxin efficiency in poisoning cases depends on the depth of anesthesia, deeper the better is the cure and vice versa, though the upper limit of its perfect usefulness is restricted to two times the lethal dose (Jarvinen, P. A. and co-worker 1949). Thus a conspicuous increase in the speed of recovery against the lethal dose of short acting barbiturate is more prevalent than with the long ones.

A dual action of cortical and medullary stimulating properties of picrotoxin brings about the barbiturate antagonism.

Cortical Action

Koppanyi and co-workers (1936) have established the involvement of higher cortical centers in higher vertebrates by a couple of ways: (1) the barbiturate denarcotising property and (2) the preventive measure against barbiturate narcosis. The participation of cortex is also seen by reactivation of the E.E.G. (Hahn, F. 1960). However this property becomes insignificant with too small doses of the drug (Cass, N. M. 1956).

The cortical arousal effect depends on: (1) the depth of anesthesia and the dose of anesthetics and (2) the type of anesthesia-cortical stimulation is exhibited against

a minimum anesthetic dose, unassociated with convulsion. With higher doses the awakening effect is superimposed on convulsive features. In the laboratory, dogs can be awakened from barbiturate narcosis (long acting and short acting) by picrotoxin injection, but the action against the former type is transient and the animal goes back to deep sleep with the abolition of the analeptic effect, whereas permanent arousal is achieved against short acting barbiturates. This peculiar characteristic behavior of picrotoxin against long acting barbiturates should not be confused with the assumption that it reduces the recovery time in such instances.

Maloney and co-workers (1931 and 1932) have been able to induce sleep in dogs by barbiturates and awakening them with picrotoxin. Cushney (1934) states picrotoxin to be less significant than coriamyrtin in its rapidity of arousal property.

The action which is responsible for the denarcotising effect is not responsible for the convulsion as experimental animals (dog) exhibit convulsion under deep comatose condition (Koppanyi and co-workers 1936). This is further shown by Dille and Duff (1939) with morphine poisoning in dogs.

Sporadic reports indicate prophylactic action of picrotoxin retarding paralysis and accelerating recovery if given prior to administration of narcotics.

Medullary Action

The medullary stimulating property of picrotoxin is by far the most versatile action against barbiturate poisoning as it helps restore the vital body function, i.e., increase in ventilation and blood pressure by direct stimulation of respiratory and vasomotor centers located in the medulla oblongata. Other centers in that region are involved with convulsive doses of the drug. In acute barbiturate poisoning, it dramatically terminates the poisoning effect by reinstating adequate respiration and maintaining normal circulation. These properties have been discussed in detail elsewhere in the chapter.

Maintenance of normal body temperature by picrotoxin in barbiturate poisoning cases are also seen.

Mode of Action as an Analeptic

There is no chemical reaction between picrotoxin and the barbituates in vitro. In vivo also it acts as a physiological antagonist (Koppanyi and co-workers 1936). It has no action on chemical distribution or elimination of the drug from the body (Kahn, J. B. Jr. 1952).

McCulloch, W. S. and Roseman, E. (1943) and Fujita, N. (1956) explains that picrotoxin increases oxygen consumption (in rats) of brain tissue depressed with barbiturates. Krantz, Carr and Beck (1937), however, have shown this is not the direct mechanism to abolish the nerve center

depression. Hence increased oxygen consumption may thus be the consequence, rather than action, of picrotoxin-barbiturate antagonism.

Koppanyi, T. and Fazekas, J. F. (1952) in a generalized view say picrotoxin acts: (1) either by causing arousal or awakening if depression is not too marked; (2) if deep coma exists and arousal is not effected they may be life saving by virtue of their medullary stimulating activity.

Very recently experimental evidence has been presented by VanDerKloot and co-workers (1958, 1959) regarding the counteracting quality of picrotoxin against the peripheral depressing activity of barbiturates. They have shown the antagonistic property of picrotoxin on the inhibitory neuron of the abductor muscle of the crayfish claw. Gamma-aminobutyric acid mimics the effects of the inhibitory transmitter on crayfish muscle. Its action is also blocked by picrotoxin.

Absorption and Elimination

Picrotoxin is effective by oral or parenteral administration, but absorption from the digestive tract is incomplete. Absorption through intact skin is questionable.

Traces of picrotoxin are detected in blood 20 minutes after intravenous injection; by the end of 2 hours it is impossible to demonstrate at all in blood, liver, or muscles (Dille and Duff 1939).

Its distribution in the body is unknown. Perhaps it is readily destroyed inside the system. After massive injection, traces are recovered from urine which indicates that it is disposed of from the body in some unknown way. A convulsant dose of picrotoxin in rabbit was destroyed within one hour and only 10% was excreted in the urine (Dille, J. M. 1938). According to Chistoni, A. (1912) picrotoxin, to some extent, is eliminated unchanged but usually breaks apart to two components, picrotoxinin being completely destroyed and picrotin largely eliminated unchanged in the urine.

Methylphenidate (Ritalin, Ciba)

Introduction

Methylphenidate was for the first time introduced in Europe in clinical practice by Drassado and Schemidt in 1954. In the same year Meier, Gross and Tripod indicated the analeptic effect of the drug against thiopental anesthesia based on laboratory animal experiments.

By now, though not much investigation has been done about its biochemical and detailed pharmacological action on the system, it is being commonly used in human medicine for two purposes--as a mild psychomotor stimulant and as an analeptic, both are the manifestations of central nervous system stimulation. Its use in veterinary practice is still of little significance.

The psychoanaleptic action of methylphenidate in human patients was studied in detail by Ferguson, J. T. (1953 and 1956). Until December 1956, oral administration was the only way of combating regressive psychiatric conditions (Ferguson, J. T. 1955 and 1956; Carter, C. H. 1956) with methylphenidate. Ferguson advocated the superiority of parenterally (i.v.) used methylphenidate to the oral route in 1956 also. Because of its non-toxicity to tissues it may be given by sc., i.m. or i.v. route (Plummer, J. and Yonkman, F. 1958).

The potentiality of methylphenidate against excessive sedation and lassitude exhibited by barbiturates, antihistaminics and tranquilizers has been confirmed by both clinical observation and laboratory experiments. Christensen and Fox have reported the success of methylphenidate injection in hastening the recovery from barbiturate anesthesia used in oral and other surgery.

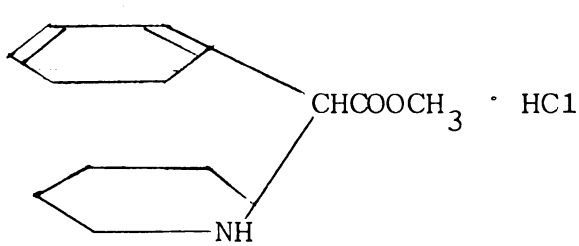
Plummer, J. and Yonkman, F. F. (1958) and Gale, A. S. (1959) have presented a vivid description of various pharmacological actions of methylphenidate, its peculiar dose relationship and especially its antidepressant action. Gale has stated that the effectiveness of methylphenidate is independent of the kind of depressant agent but is more related to the depth of depression and to some extent to the age of the patient; the very old and the very young seem to respond best.

Carter and Maley (1957) reported favorably on the effectiveness of methylphenidate in nine cases of barbiturate overdose. This beneficial action was claimed by Adriani and Smith (1958) to be definite after trying its efficacy against hundreds of barbiturate intoxication patients. According to them it is more effective than older analeptics like picrotoxin and pentylenetetrazole in mild and severe barbiturate intoxication.

However little effort has been made to prove its usefulness in severe barbiturate poisoning cases in veterinary practice. We have tried to solve this aspect to some extent in our laboratory.

Chemically

Phenyl-piperdye-acetic acid methyl ester or methylphenidate



Trade name: Ritalin

Pharmacological Action

Circulatory Effects

Methylphenidate, in effective doses, does not affect the blood pressure level of the patient. As an antidote for psychic depressants (Reserpine etc.), when administered along with reserpine or afterwards, it has little effect in altering the hypotensive effects of the latter drug (Plummer, A. J. and Maxwell, R. A. 1956); Ferguson, J. T. and Funderburk, W. H. 1956). With high doses, capable of causing central motor stimulation (running movements), the blood pressure rise was not so much deviated from the normal level (Maxwell and co-workers 1957). It is useful in optimal doses to counteract the declined blood pressure caused by prolonged use of anesthesia (Gale, A. S. 1958). Much higher doses cause a transient rise in blood pressure of about 20 mm.Hg (Plummer, A. J. and Yonkman, F. F. 1958). Further reports indicated its usefulness in protecting against the postoperative decline in blood pressure greater than 10 mm.Hg, though drop below this limit is not uncommon.

A very rational explanation pertaining to the circulatory response of methylphenidate and interesting information about its synergistic and antagonistic actions with other pressor agents has been presented by Maxwell and co-workers (1957). The drug possesses an antihypertensive

effect of central origin and a potentiating action on the response produced by peripherally acting sympathomimetic drugs. Blockade of elevated pressure elicited by bilateral carotid occlusion, prompt reduction of pressure raised by amphetamine and ephedrine (Gale, A. S. 1958, 1959; Plummer, A. J. and Yonkman, F. F. 1958) and finally initial moderate decline in the blood pressure of unanesthetized renal hypertensive dogs characterizes the centrally mediating mechanism of methylphenidate (Charter, C. H. and Maley, M. C. 1957). On the other hand, it potentiated the pressure response of epinephrine, nor-epinephrine and naphthazoline the peripheral sympathomimetic drugs. This phenomenon was confirmed by Ivey, E. P. (1959). Maxwell, R. A. and Plummer, A. J. have explained the above observations as follows:

> Methylphenidate (Ritalin) antagonizes the pressure response to amphetamine via a central blockade of cardiovascular centers which are influenced by amphetamine and ephedrine. A peripheral blocking effect of it can only be supported if one postulates that amphetamine and ephedrine have different peripheral cardiovascular receptor sites than do epinephrine, nor-epinephrine and naphthazoline, where methylphenidate selectively blocks the former pressure raisers and potentiates the pressure raising effect of the latter groups.

The synergistic pressure response of Ritalin with epinephrine plays a significant role in shock treatment by reducing the epinephrine dose to a great extent (Gale, A. S. 1959).

Central Nervous System Action

The mild C.N.S. stimulating effect of methylphenidate has been reported by many investigators and medical practitioners. But up-to-date information does not indicate the exact site of action of this drug on the C.N.S. However, the accumulating evidences of its psychoanaleptic effect (Ferguson, J. T. 1955 and 1956; Meier at al. 1954) and the stimulation of the vital centers, i.e., respiratory, circulatory centers (Adriane, J. and Smith, B. 1958; Gale, A. S. 1958) against barbiturate and tranquilizer overdoses definitely depicts its cortical and medullary stimulating properties. The cortical arousal activity was further proved by Plummer, A. J. and co-workers (1958) who recorded the first encephalogram activity especially in the leads from mesencephalic reticular formation, following methylphenidate administration. The prompt C.N.S. stimulation property of methylphenidate unaccompanied by depression rebound has been taken advantage of in not infrequent clinical use. In therapeutic doses its convulsant effect is minimal. Even in cases of severe barbiturate intoxication and pronounced tranquilizer depression the drug used in repeated doses exhibits its antidotal potency with least untoward effects.

Methylphenidate is a direct C.N.S. stimulant. It is not a biological competive agent nor does it have blocking agent property (Gale, A. S. 1958). One single optimum dose of methylphenidate maintains C.N.S. stimulation for 3-4 hours.

A peculiar dose relationship has been recorded with regard to the C.N.S. stimulation. Methylphenidate in doses of 0.1-0.2 mg/lb. body weight is most effective. Doses below 0.05 mg/lb. and above 0.4 mg/lb. however, markedly reduce the stimulating property (Gale, A. S. 1958). There is no explanation for this behavior but to observe that other stimulants might show such response in higher doses.

Action Against Cortical Depression

In this country methylphenidate, as a matter of fact, was used clinically as a psychoanaleptic agent against mentally retarded patients (Charter, C. H. and co-workers 1955). Besides the preponderance of evidences in support of this (Ferguson, J. T. 1956; Ferguson and co-workers 1957; Plummer and Maxwell 1956), it has further been established as an antidote against more severe forms of psychophysiologic depressions, depressions which may be drug induced (Charter and Maley 1957). Ferguson acknowledges it as the forerunner of all the drugs tested in controlling, ameliorating or eliminating the abnormal behavior manifestation seen in elderly patients. The dramatic response brought about by a

single parenteral dose is possibly due to a chemicophysiological change within the central nervous system rather than the action at some specific anatomical sites.

Heart rate, blood pressure, respiration and kidney function remains unaltered in cases of prolonged massive methylphenidate injection to mentally ill patients.

Methylphenidate Tranquilizer Antagonism

Many observations have been made about the methylphenidate, reserpine, and chlorpromazine antagonism at various dose levels of the latter groups of drugs and this has been accepted as a very good antidote against the mood normalizing, mild corticomotor depression and oversedative effects of tranquilizers. Plummer and Maxwell (1956) report that methylphenidate produces complete reversal of the so-called side reactions of reserpine (in tranquilizing dose) excepting the hypotensive effects. After the lapse of 3 hours the typical reserpine effects i.e., miosis, nictitating membrane relaxation, bradycardia, etc. are exhibited. Failure of ganglionic blockade principle to check the nictitating membrane relaxation in dogs explains its specific type of antagonism at C.N.S. In fact a clear cut antagonism to the sedative effects and other side reactions of central origin, of tranquilizers has been shown by Plummer and co-workers (1958) in dogs, rabbits and monkeys.

Methylphenidate-Barbiturate Antagonism

Although the sedative and anesthetic dose reversal effect of methylphenidate is agreed upon generally, both in laboratory experiments and clinical use, controversial reports have been presented concerning its efficacy in combating barbiturate intoxication. Gale, A. S. (1959) and Plummer and Maxwell (1958) believe this drug is a moderate antidote against mild and severe barbiturate intoxication, whereas Adriani, S. A. and co-workers (1958) claim it as a superb barbiturate antagonist in comparison to other analeptics like pentylenetetrazole, picrotoxin and nikethamides. Charter and Maley (1957) have also given similar opinions. However in consideration of its apparently specific central stimulation of respiration, "alerting" of consciousness effect and the high therapeutic index, it seems to be a useful analeptic. Further, it can be injected parenterally by s.c., i.m. or i.v. route with similar results. The parenteral injection seems to be well tolerated both locally and parenterally as it does not create any tissue changes at the site of injection, due to extravasation. There was no evidence of phlebitis (Ferguson, J. T. 1956).

Methylphenidate increases the respiratory rate and depth in cases of mild and severe type of barbiturate anesthesia. Prompt recovery is seen against light anesthetic

doses. A reasonable ventilation increase along with rapid arousing effect adds to the efficacy of methylphenidate as a barbiturate antagonist, though the latter effect is not seen promptly in extreme intoxication cases. Unlike other analeptics of phenylisopropylamine type it does not possess cardiovascular stimulating activity. Severe postoperative barbiturate chill and euphoria after intravenous barbiturate anesthetics--these two common complications are greatly eliminated with methylphenidate injection.

It acts better against short acting barbiturates (especially pentothal) rather than long acting ones. Because of transient action of methylphenidate it should be injected at repeated intervals till a significant long acting barbiturate depression reversal effect is observed.

A synergistic analeptic effect has been recorded when injected along with levallorphan tartarate (Lorfan) (Gale, A. S. 1959).

Methylphenidate Against Other Agents

A high percentage of patients depressed with general anesthetics, barbiturates (pentothal, secobarbital, Neraval, Surital, Na-pentobarbital), alcohols, aspirin, tranquilizers and antihistaminics are reported to improve rapidly and appreciably after methylphenidate injection. But comatose patients recover slowly.

Gale recorded prompt initiation of respiration in some patients anesthetized with general anesthetics with prolonged apnea considered to be due to respiratory center depression rather than muscular relaxation.

Lastly methylphenidate is believed to counteract the respiratory and circulatory effects of anesthetic adjuvants.

CHAPTER II

MATERIALS AND METHODS

Experimental Animals

Dogs, irrespective of age, sex and breed, were chosen for the project. These animals were obtained from the local dog pound and were maintained in individual cages. Commercial dog food* and water were supplied ad libitum. Forty-two dogs, including 6 dogs for control, were used in this experiment. Most of them weighed 10 to 12 kgs, though dogs varying from 7 to 20 kg in weight were used according to availability. Thirty-six dogs were divided into 6 groups and each group was treated either with a single drug or combination of two drugs in different doses. Usually, unused dogs were taken for each experiment. Rarely the same dog was selected a second time. On no occasion were the dogs used which had been anesthetized for any other experimental work in the preceding week. The diseased animals were completely eliminated from the project.

*Purina Dog Chow.

Drugs Used

Sodium pentobarbital 6 per cent solution with 10 per cent ethyl alcohol was employed to anesthetize the dogs.

Picrotoxin was 0.3% soln. (Eli Lilly & Company, Indianapolis and Abbott Laboratories).

Methylphenidate hydrochloride ("Ritalin" - Ciba Pharmaceutical Company, Summit, N.J.). Crystalline powder was used to make 1 per cent solution with sterile saline.

Apparatus Setup

The apparatus consists of a four channel "Grass polygraph"* and a wet test gas meter. All four channels in the polygraph, from top to bottom, were used to record the respiratory rate, ventilation, cuff pressure, and the pulse wave in order and for convenience this principle was observed in all experiments. The third channel used for cuff pressure was calibrated before each experiment in such a way that one cm. deflection represented 100 mm.Hg pressure. The other three channels, except for bringing them to a certain base line, were not calibrated as they were primarily of qualitative importance.

The time was recorded in one-second interval.

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*Grass Instrument Company, Quincy, Mass., U.S.A.

The pulse wave was recorded by means of an electric pulse pickup (Grass Model PTT1) fixed to the interdigital space of one of the hind legs and to the fourth channel of the polygraph.

In order to record the cuff pressure a high pressure transducer was used which was connected to the third channel of the polygraph at one end and at the other end to a 2" wide cuff fixed below the hock region of the hind leg also bearing the pulse pickup. The indirect blood pressure was assessed from the aforementioned two tracings which will be discussed further in the section "Experimental Technique."

A wet test meter was employed to measure expired air as a measure of ventilation volume and respiratory rate. Both of these values were finally recorded on the polygraph. The gas meter was so constructed as to record each 0.25 liters (250 ml) of air by means of an electrically operated signal pointer. This was finally recorded in channel two of the polygraph. As regards to respiratory rate, in each expiration a signal was picked up by a low pressure transducer touching to a thin rubber flutter valve at the gas meter outlet and transmitted to the Grass polygraph.

Experimental Technique

At the outset the dog, clipped at the site of injection, was weighed and sodium pentobarbital was injected intravenously via a radial vein. Forty mg/kg body weight were used to induce deep anesthesia, to decrease the level of minute respiratory volume and eventually to curtail the respiratory rate markedly. A rubber tracheal tube with an inflation cuff around its distal end was then inserted into the trachea through the mouth. The cuff was inflated from outside with an aim to fix the tracheal tube comfortably enough to allow air to pass freely into the trachea and out only through the tracheal tube. The degree of inflation was indicated by a pilot balloon. The tracheal tube was connected to the gas meter by means of a rubber tubing with special valves for inhalation from the room and exhalation through the gas meter.

It was observed in our laboratory that the indirect blood pressure measured by fixing the cuff below the hock region gave reading closer to direct blood pressure than fixing the same above the hock region. At intervals, by squeezing the bulb attached to the cuff, the latter was inflated and pressure was built up around the vessels which caused complete disappearance of pulse wave. This was indicated by the fourth pen drawing a straight line on the graph instead of the usual pulse wave tracings. As pressure was slowly

released from the cuff the third pen gradually moved downward and the fourth one just in the opposite direction till the pulse wave returned. Then the pressure was completely released from the cuff and the cuff pressure curve came down to the base line. A straight line drawn upward from the point of reappearance of the pulse wave to the cuff pressure curve, indicated the indirect systolic blood pressure reading. The indirect blood pressures were recorded from time to time throughout each experiment.

Now after all these arrangements, the pulse wave, indirect blood pressure, ventilation and respiratory rate were recorded for at least 10 minutes. Some dogs responded pretty well to a single pentobarbital dose in which cases minute respiratory volume dropped down to one liter or less. In other cases where the first dose failed to bring about significant reduction in the ventilation, repeated doses of 10 mg/kg or 5 mg/kg i.v. of pentobarbital were injected every ten minutes to lower the ventilation to the desired level, i.e., 1.25 liters or less. Curiously enough some dogs, irrespective of their weights and sizes, failed to respond sufficiently to a total massive dose of 75 mg/kg pentobarbital and the ventilation stayed at 1.75 liters, exhibiting a typical individual drug resistance phenomenon. However, in general, when the minute respiratory volume was lowered to around 1.25 liters, polygraph tracings were made

for 10 minutes to observe the ventilation level. This is the right time for trying the efficacy of the analeptics.

In our laboratory it was observed by different trial that 6 injections of methylphenidate hydrochloride 5 mg/kg i.v. at 20 minute intervals gave the best result for methylphenidate alone; because of this, 6 doses of 5 mg/kg at 20 minute intervals were regarded as the full dose. A 2 mg/kg i.v. single injection of picrotoxin was also considered as the single full dose. Accordingly 6 injections of methylphenidate in 2.5 mg/kg and 1.25 mg/kg i.v. at 20 minute intervals and one injection of picrotoxin 1 mg or 0.5 mg/kg i.v. had been referred in the description as half and one-fourth doses.

A group of 6 dogs ~~were~~ was taken for the study of the deep pentobarbital antagonistic effect of a particular dose of an individual drug or drugs in different combinations. In drug combination injections, both the drugs were taken into different syringes and one was injected immediately after the other; every time picrotoxin was run in first. Immediately, one dose of methylphenidate was given. Then at 20 minute intervals, five more doses of methylphenidate were given.

A tabulation of experimental condition is in Table 1.

Table 1. Experimental Conditions

Group	No. of Dogs	Ave. Wt. (kg)	No. of Deaths	Ave. Dose and Dose Ranges of Pentobarbital Sodium (mg/kg)	Antidotal Drugs	Dose (mg/kg)
1	6	10.6	0 (one dog died next day)	50 (40-65)	none	none
2	6	9.75	0	47.5(40.0-60.0)	Methylphenidate	3 mg/kg i.v. 6 inj. at 20 min. intervals
3	6	9.75	0	47.5(40.0-65.0)	Methylphenidate	5 mg/kg i.v. 6 inj. at 20 min. intervals
4	6	9.666	2	44.14(40.0-50.0)	Methylphenidate	10 mg/kg i.v. 6 inj. at 20 min. intervals
5	6	15.416	0	45.84(40-55)	Picrotoxin	2 mg/kg i.v.
6	6	10.283	0	56.7(40.0-75.0)	Picrotoxin Methylphenidate	1 mg/kg i.v. 2 mg/kg i.v. 6 inj.
7	6	9.833	0	43.4(40.0-45.0)	Picrotoxin Methylphenidate	0.5 mg/kg i.v. 5 mg/kg i.v. 6 inj.

In every case after the end of the last injection, recordings were taken for one and one-half hours to observe the analeptic effect of the drugs.

The radial vein was chosen as the site of injection of analeptics and anesthetics as well. Many times slight increase in ventilation and respiratory rate observed during or immediately before the injection of the analeptics was evidently a reflex mechanism manifestation due to the mechanical stimulus originating at the site of injection.

The reasons for using indirect blood pressure and pulse wave recording devices were three, i.e., to save time, for convenience and lastly to avoid sacrificing the dog.

CHAPTER III

RESULTS

The pentobarbital antagonistic responses of these two analeptics--methylphenidate and picrotoxin, used as four individual drug doses and in two combinations, with respect to ventilation percent increase above the pre-drug level are presented below in the following ascending order of efficiency. These are also shown in the graphs in subsequent pages. Actual ventilations before the injection of analeptics are also shown in graphs at "liters at 0 minute."

1. Six doses of methylphenidate 10 mg/kg i.v. at 20 min. intervals.
2. Deep pentobarbital anesthesia without analeptics (control group).
3. Six doses of methylphenidate 3 mg/kg i.v. at 20 min. intervals.
4. Six doses of methylphenidate 2.5 mg/kg i.v. at 20 min. intervals and picrotoxin 1 mg/kg i.v. one injection.
5. Six doses of methylphenidate 5 mg/kg i.v. at 20 min. intervals.
6. Six doses of methylphenidate 5 mg/kg i.v. at 20 min. intervals and picrotoxin 0.5 mg/kg i.v. one injection.

7. Picrotoxin 2 mg/kg i.v. one dose.

Picrotoxin alone in full dose (2 mg/kg) was found to be most effective in increasing ventilation. Six injections of methylphenidate 10 mg/kg i.v. at 20 minute intervals was least effective. Six injections of methylphenidate 5 mg/kg i.v. at 20 minute intervals used as drug combination produced very satisfactory overall analeptic effect.

Quite frequent return of pedal, corneal, palpebral and occasional cough reflexes coupled with severe paddling movements, raising the head and objecting to the intubation were the arousal signs of analeptics. In addition some dogs coughed for a while soon after the tracheal tube was removed at the end of the experiments.

The arousal effect was further evidenced in some dogs by rapid heart rate, increase in respiratory depth and rate, increased minute respiratory volume, violent running movements, struggling, extreme stage of excitement, twitching of muscles of neck, eyebrow, lower jaw region with occasional general muscular twitching, profuse salivation and so on--all these being more pronounced in case of picrotoxin treatment alone. Details of these effects will be enumerated under the discussion.

Picrotoxin increased blood pressure in all cases whereas methylphenidate, both alone and in combination,

altered blood pressure only slightly.

Abbreviations in the Graphs

M - Methylphenidate

P - Picrotoxin

Pb - Pentobarbital

V - Ventilation

R - Respiratory rate

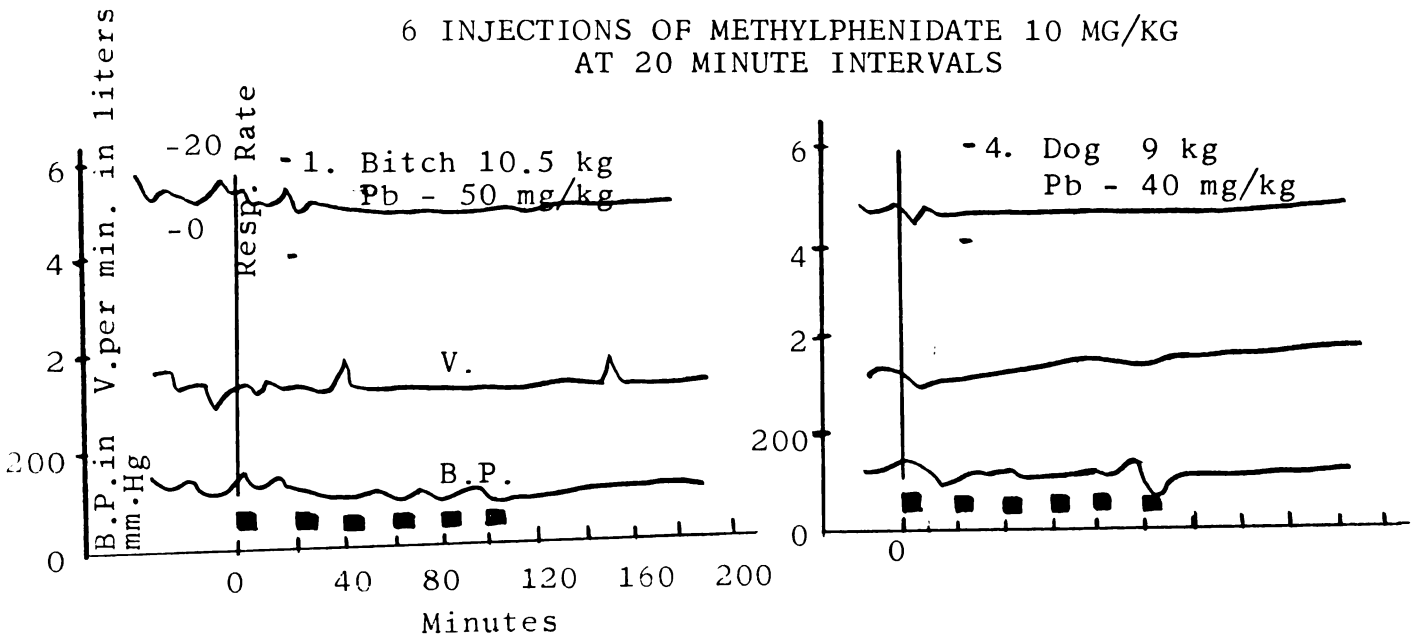
B.P. - Blood pressure

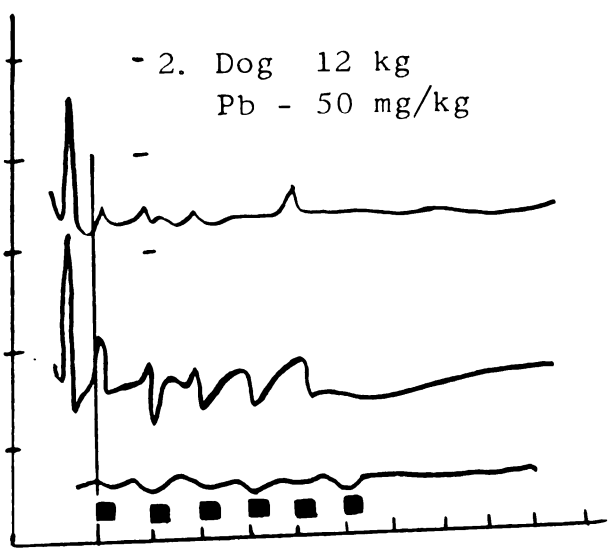
$()^{+}$ - Arousal after analeptics and no more ventilation record due to struggling and excitement

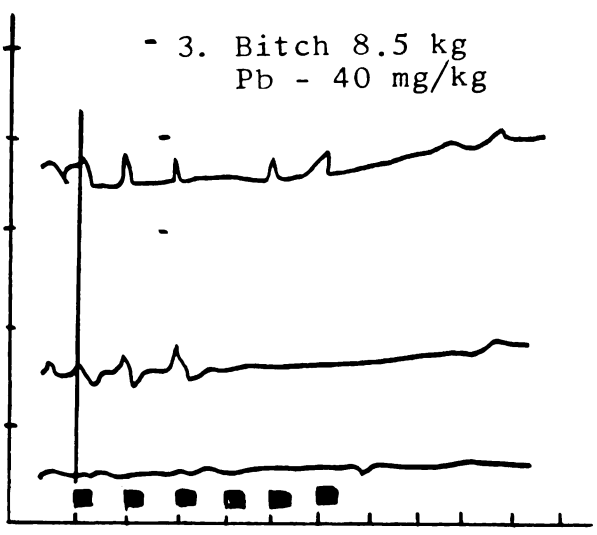
※ - Indirect blood pressure recording stopped as the electronic pulse pickup could not be fixed to the interdigital space due to struggling

■ - Injection of a single drug.

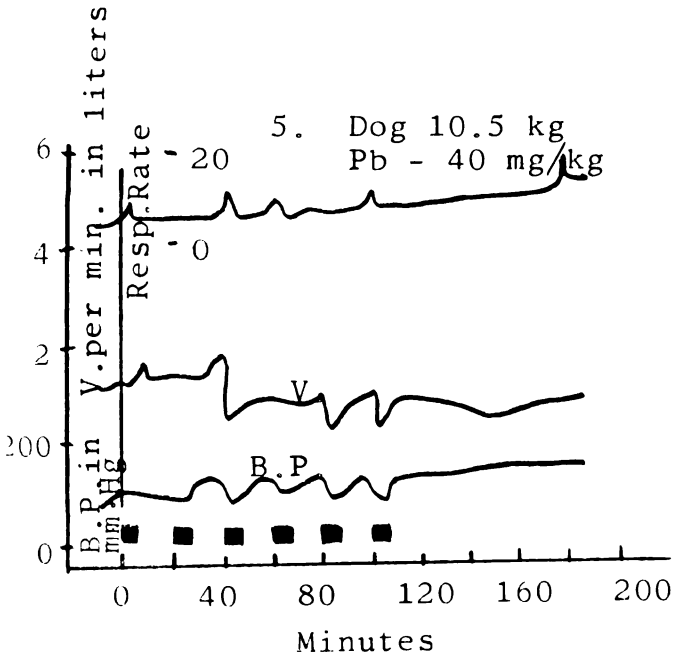
6 INJECTIONS OF METHYLPHENIDATE 10 MG/KG
AT 20 MINUTE INTERVALS

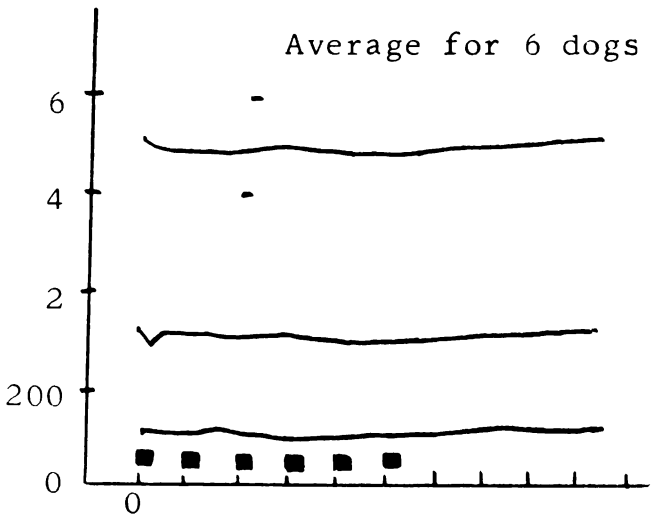


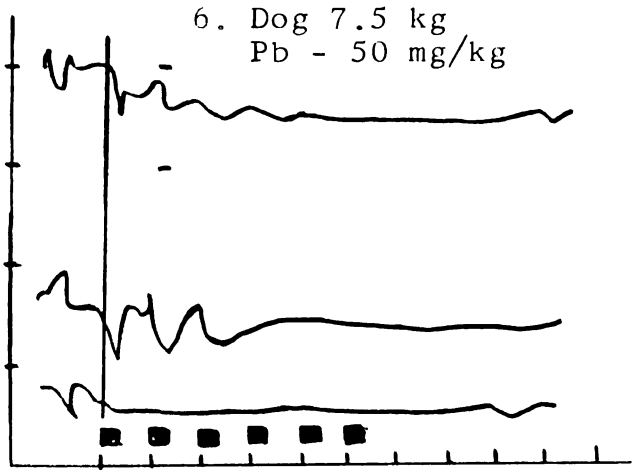


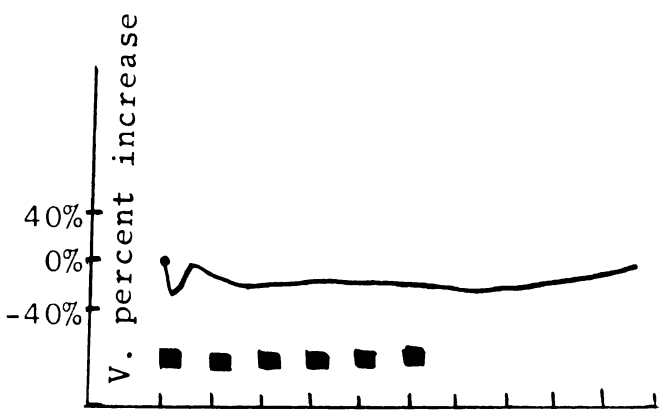


METHYLPHENIDATE 10 MG/KG--Continued

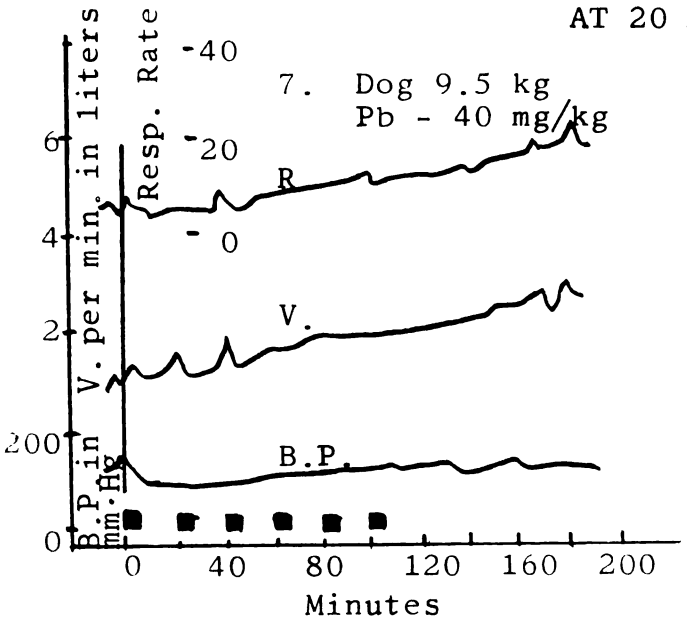


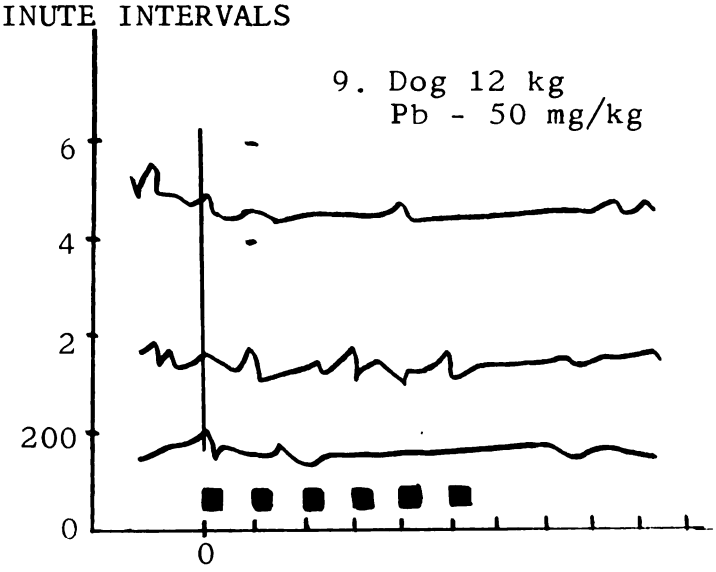


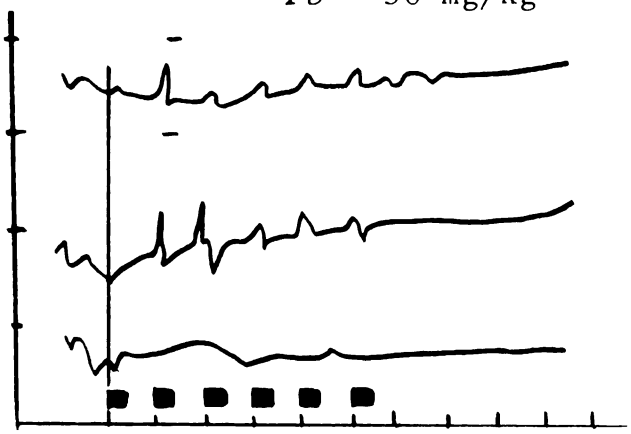


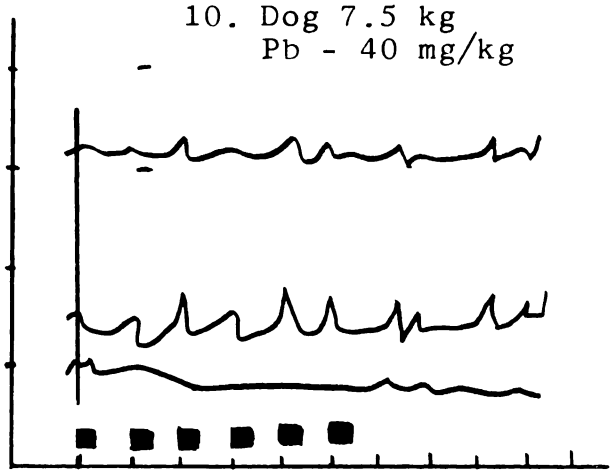


6 INJECTIONS OF METHYLPHENIDATE 3 MG/KG
AT 20 MINUTE INTERVALS









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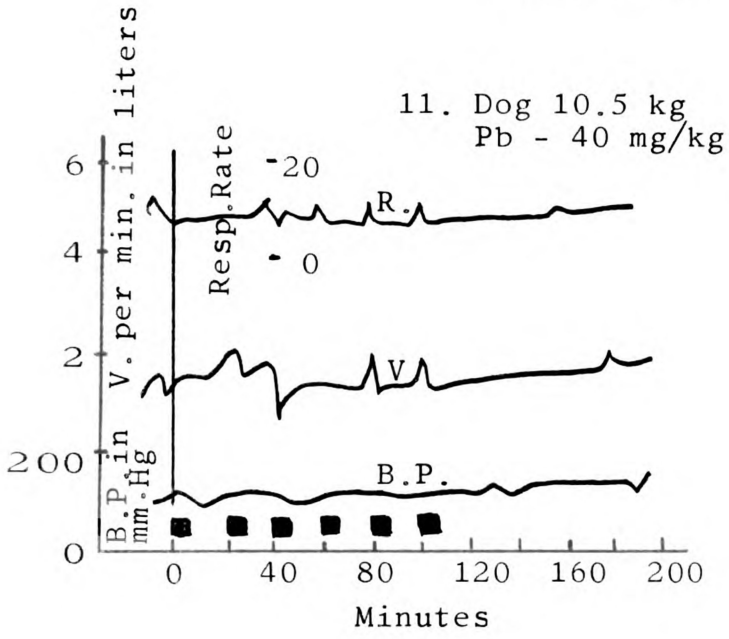
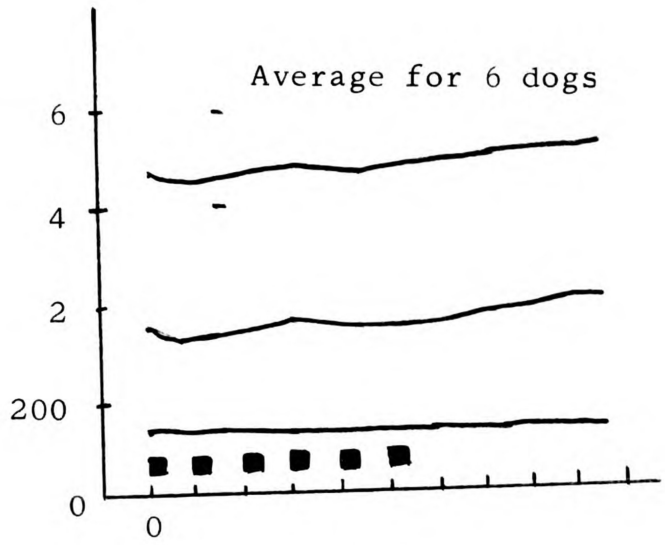
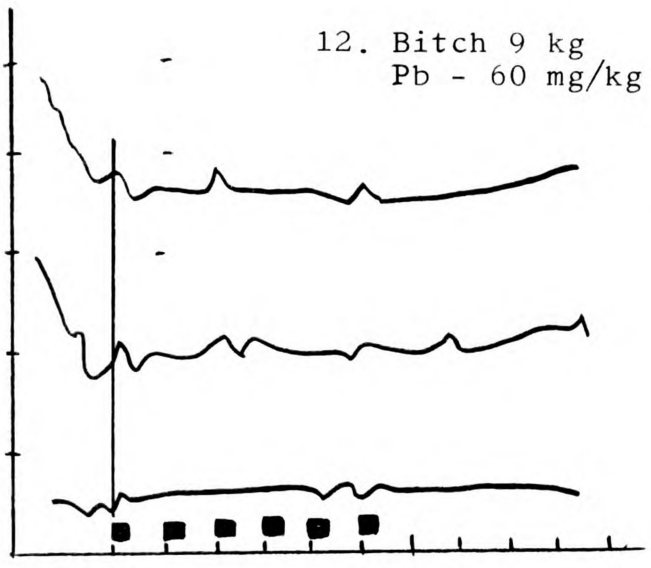
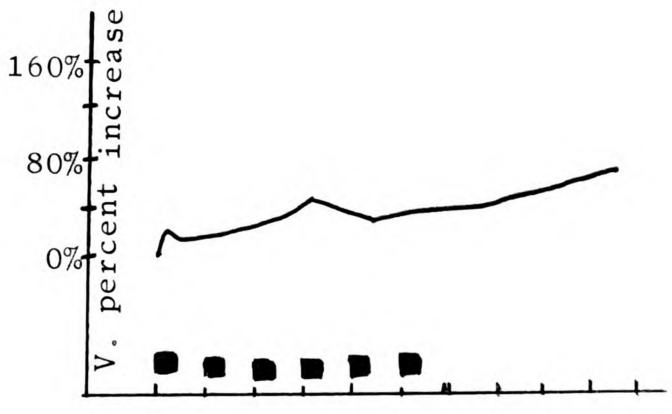
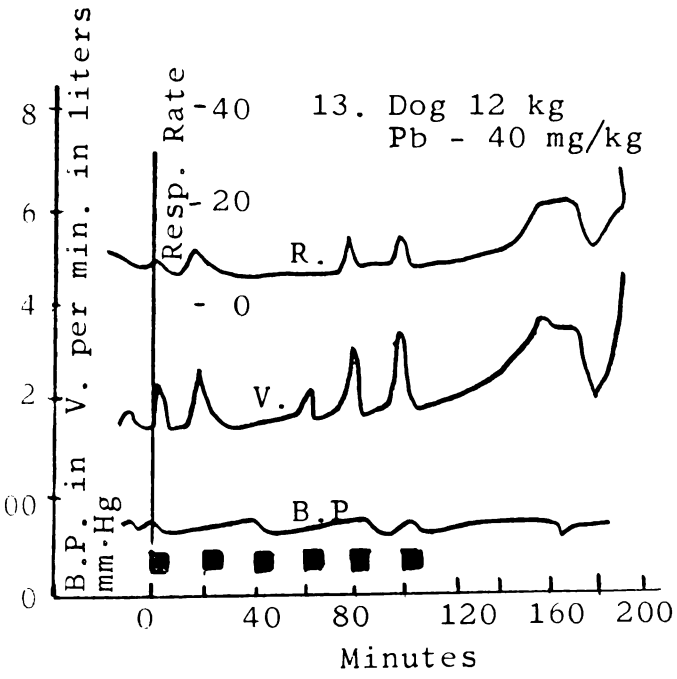


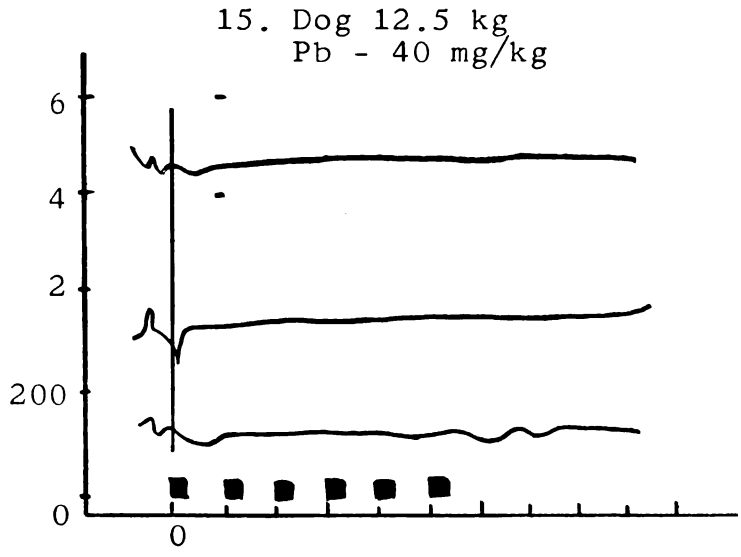


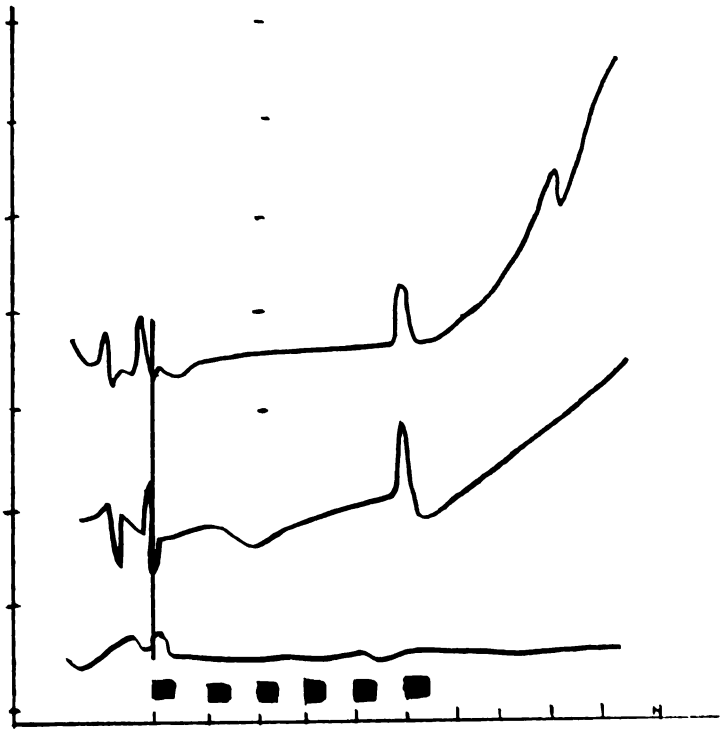


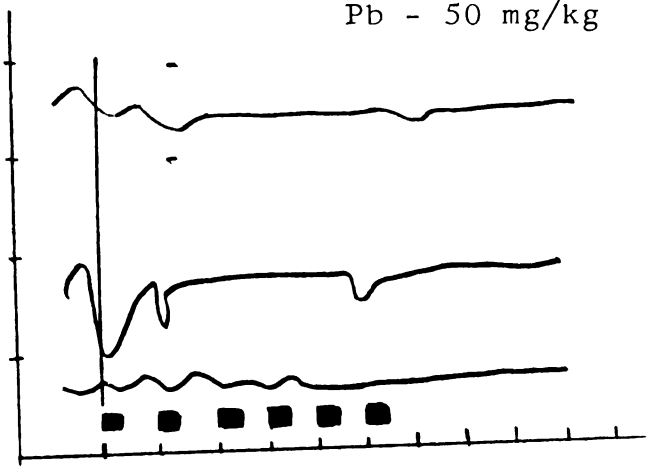


6 INJECTIONS OF METHYLPHENIDATE 5 MG/KG
AT 20 MINUTE INTERVALS









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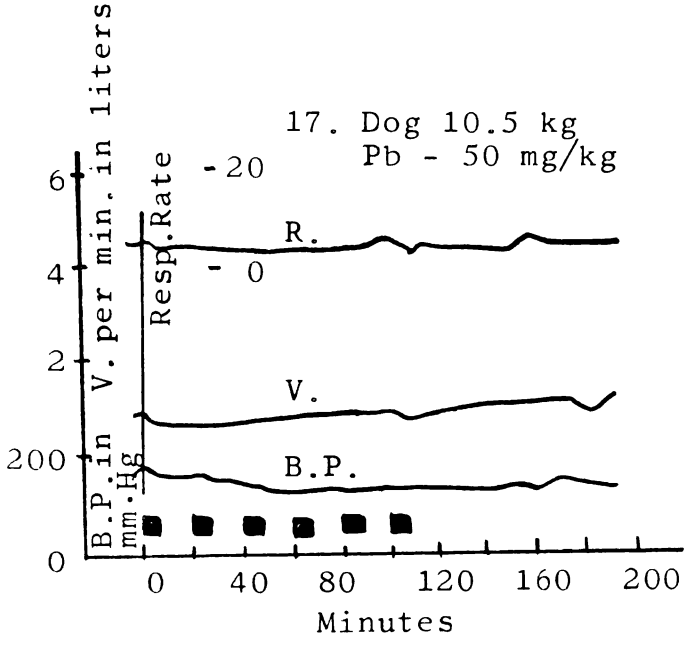
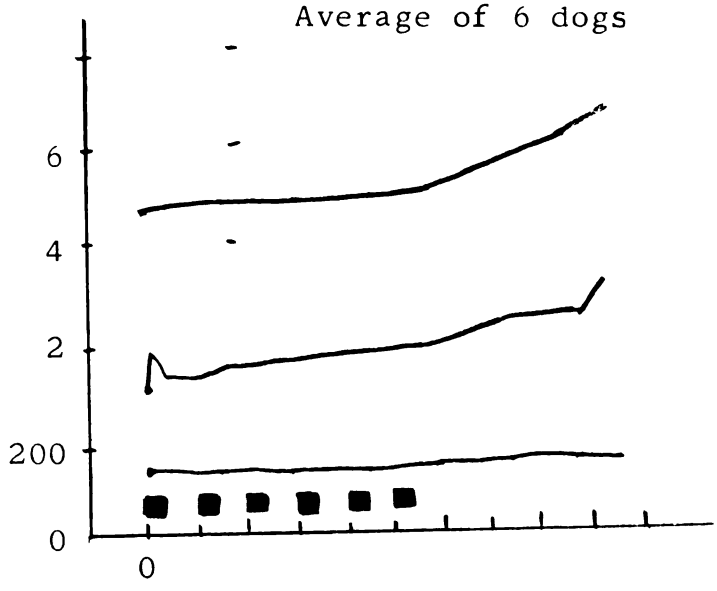
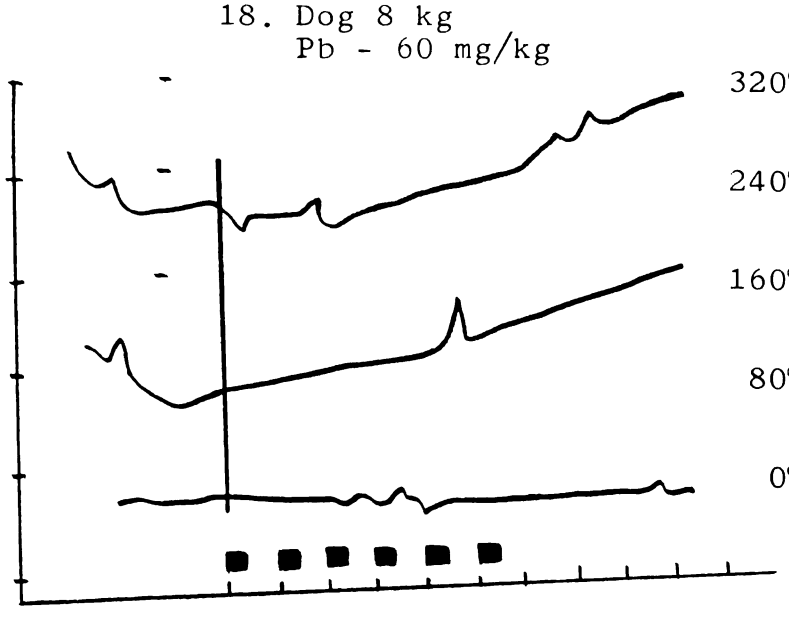
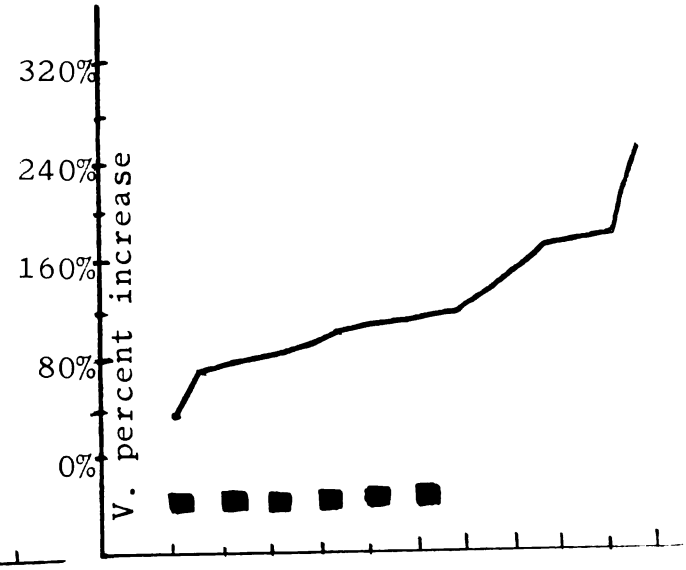


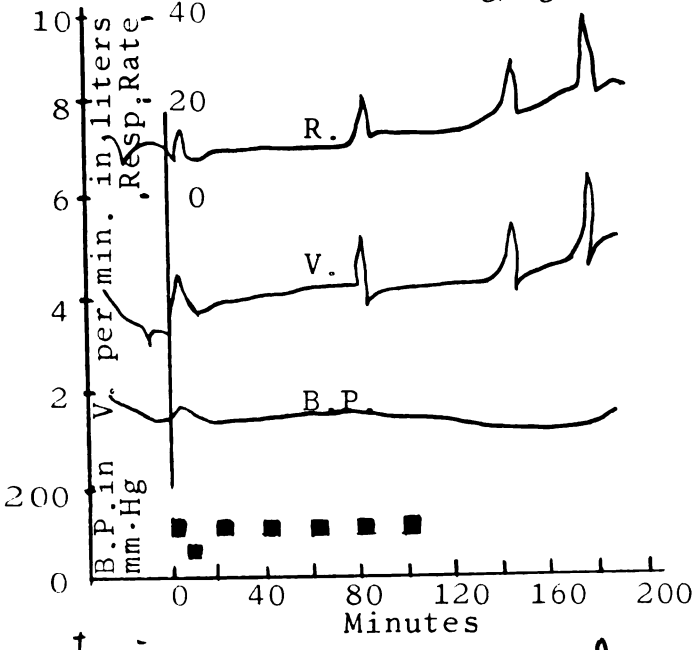




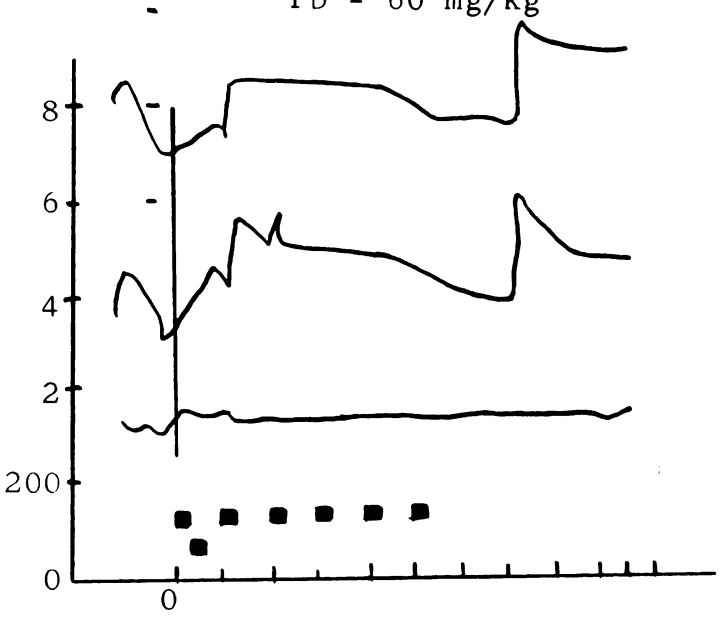


PICROTOXIN 1 MG/KG ONE INJECTION + 6 INJECTIONS OF METHYLPHENIDATE 2.5 MG/KG AT 20 MINUTE INTERVALS

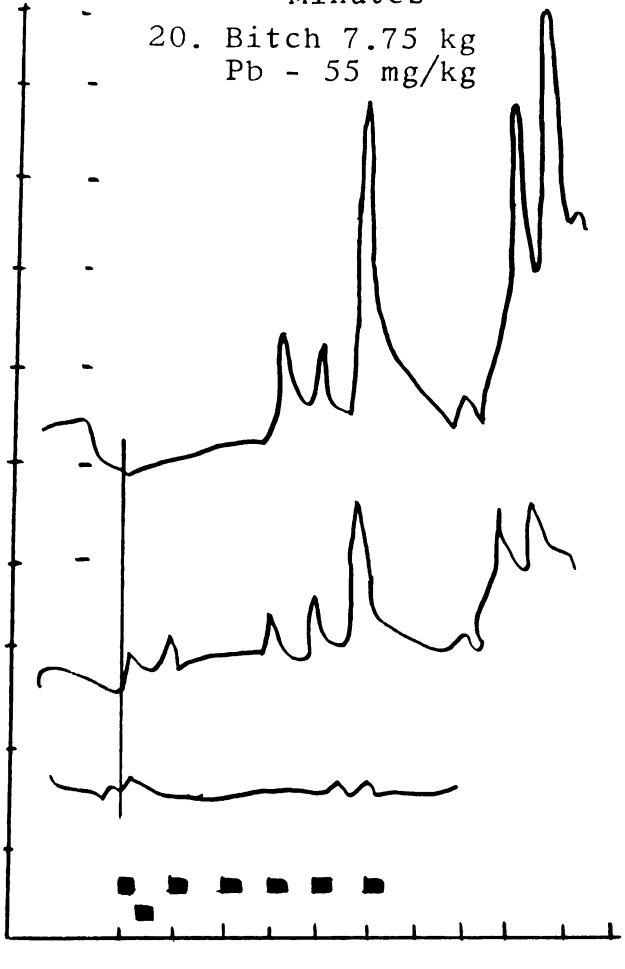
19. Dog 10 kg
Pb - 55 mg/kg



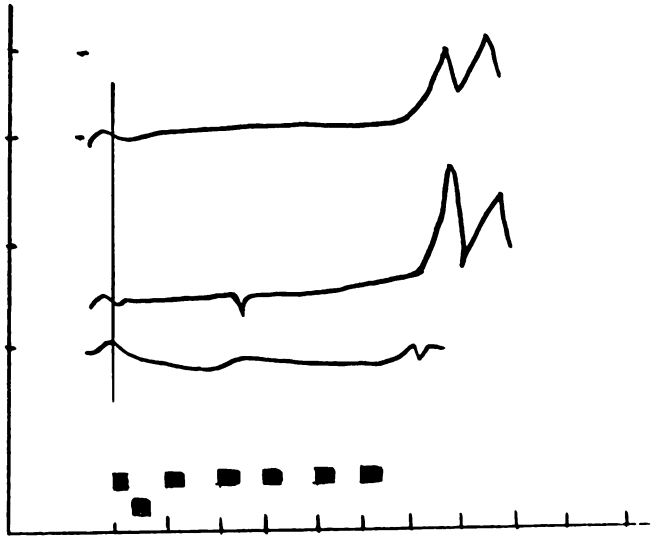
21. Bitch 8 kg
Pb - 60 mg/kg



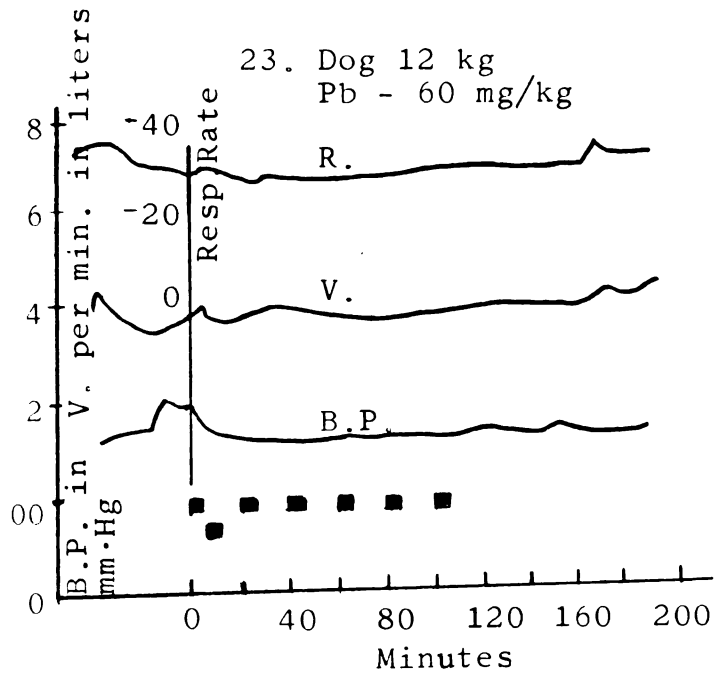
20. Bitch 7.75 kg
Pb - 55 mg/kg

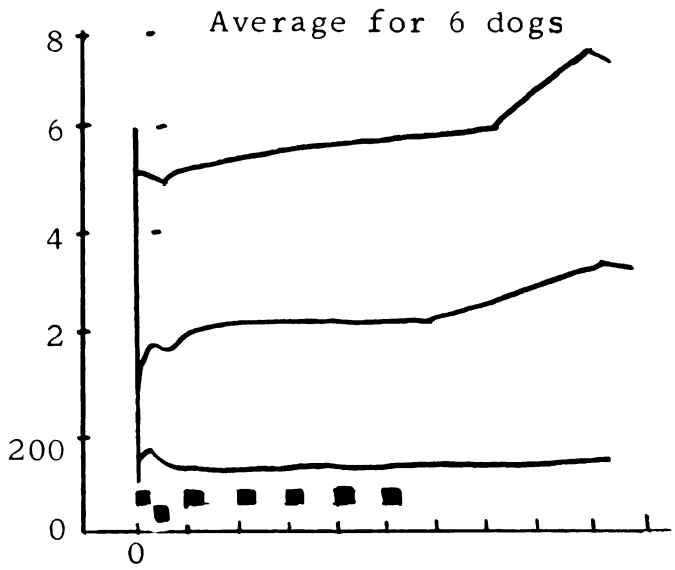


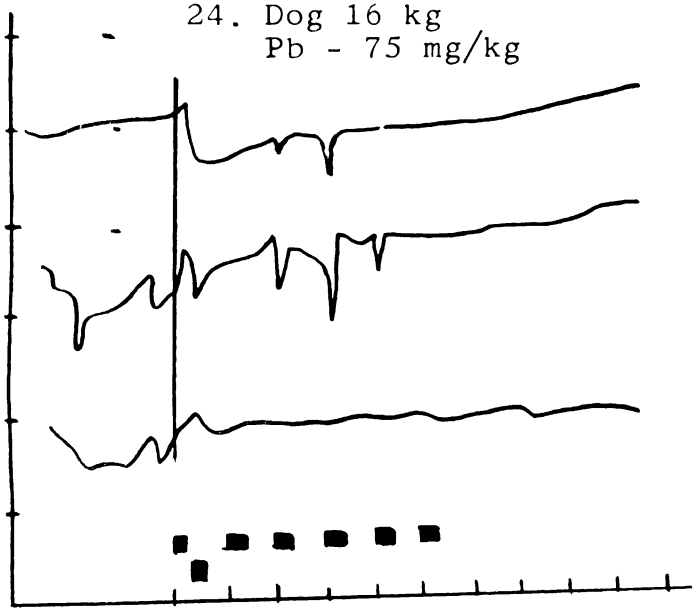
22. Dog 7 kg
Pb - 40 mg/kg

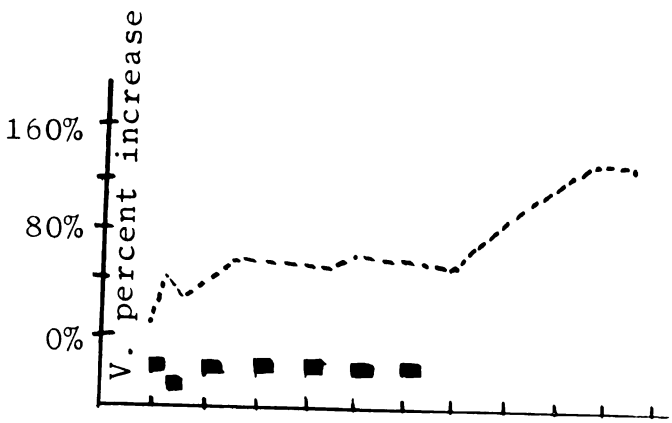


PICROTOXIN 1 MG/KG + METHYLPHENIDATE 2.5 MG/KG--Continued

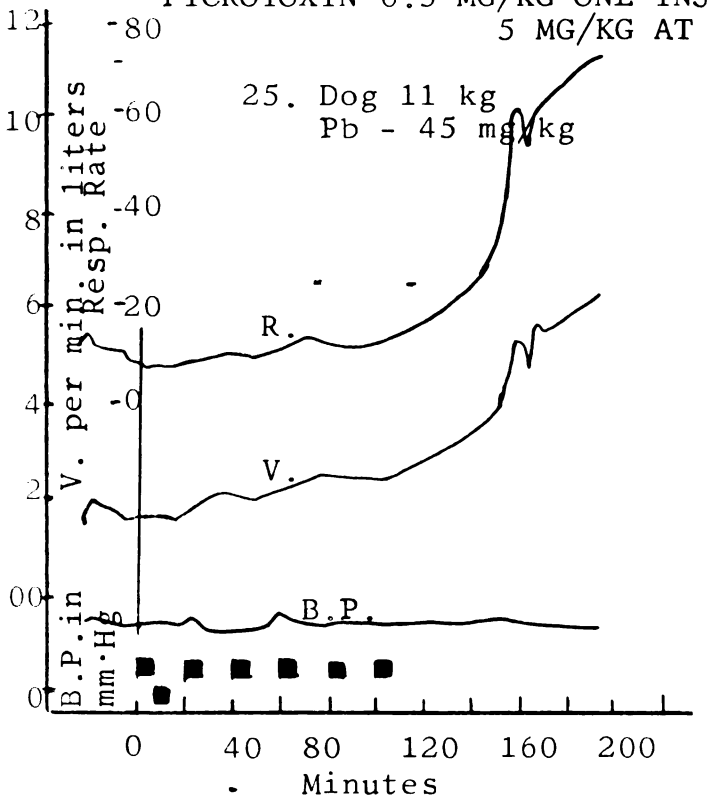


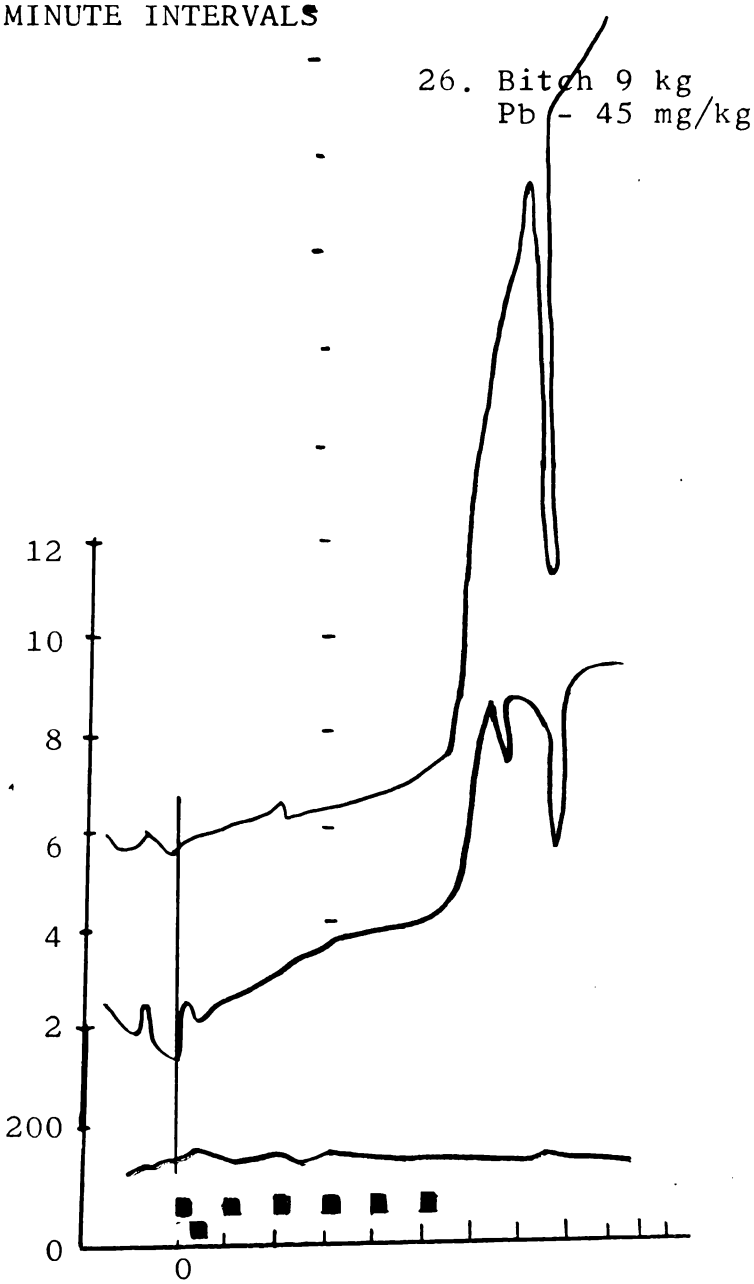


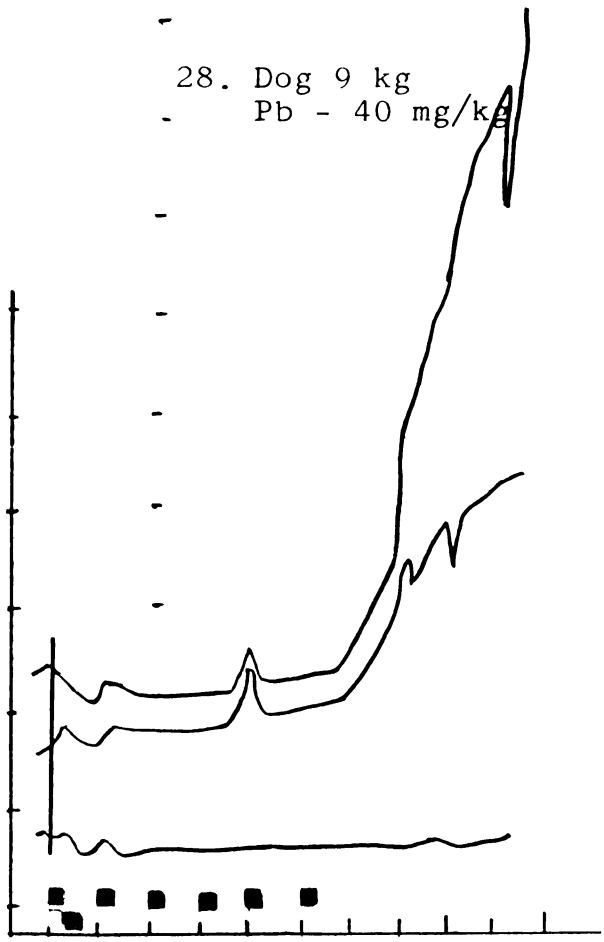


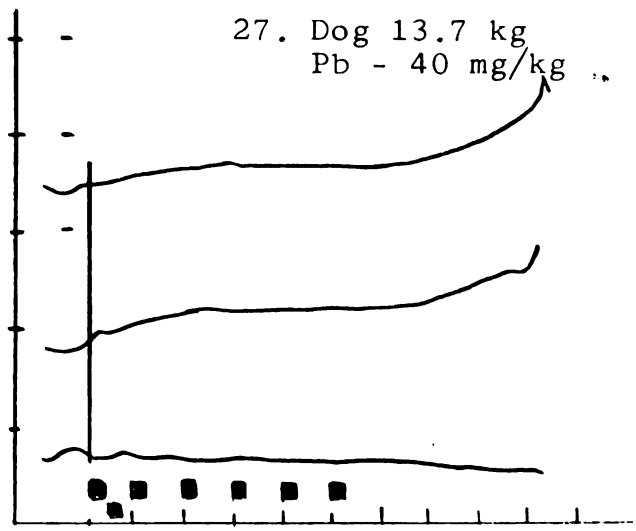


PICROTOXIN 0.5 MG/KG ONE INJECTION + 6 INJECTIONS OF METHYLPHENIDATE 5 MG/KG AT 20 MINUTE INTERVALS

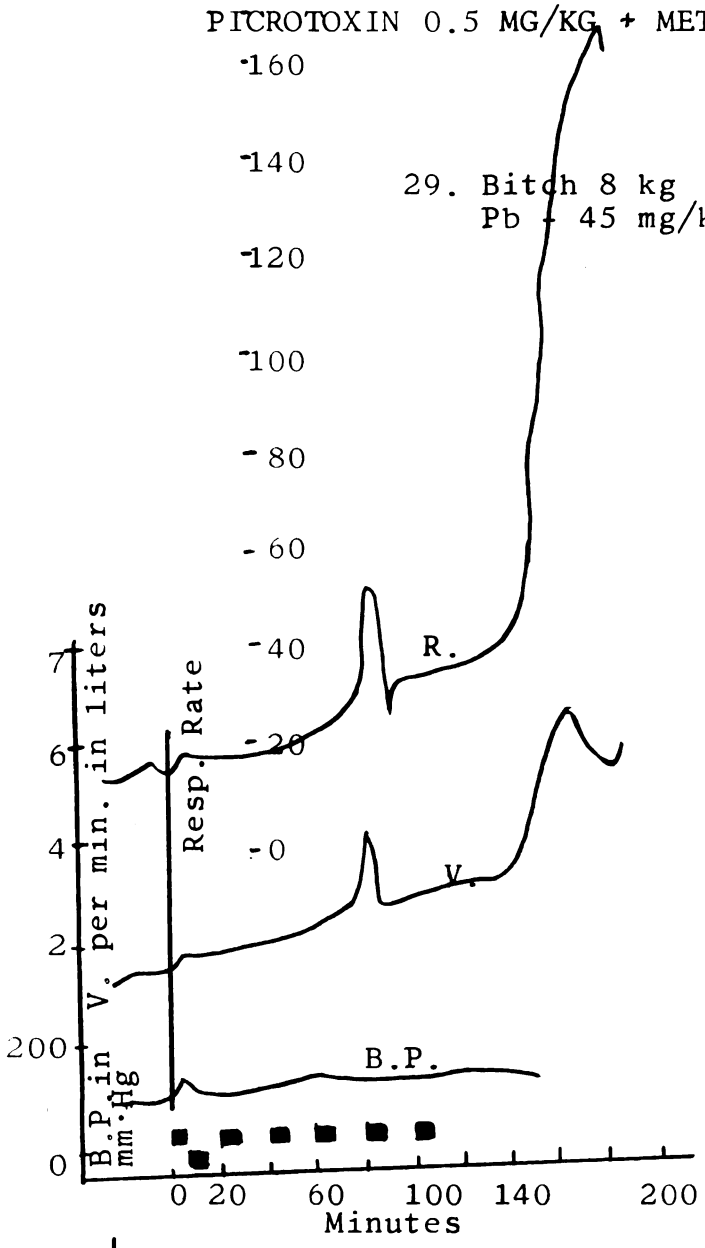


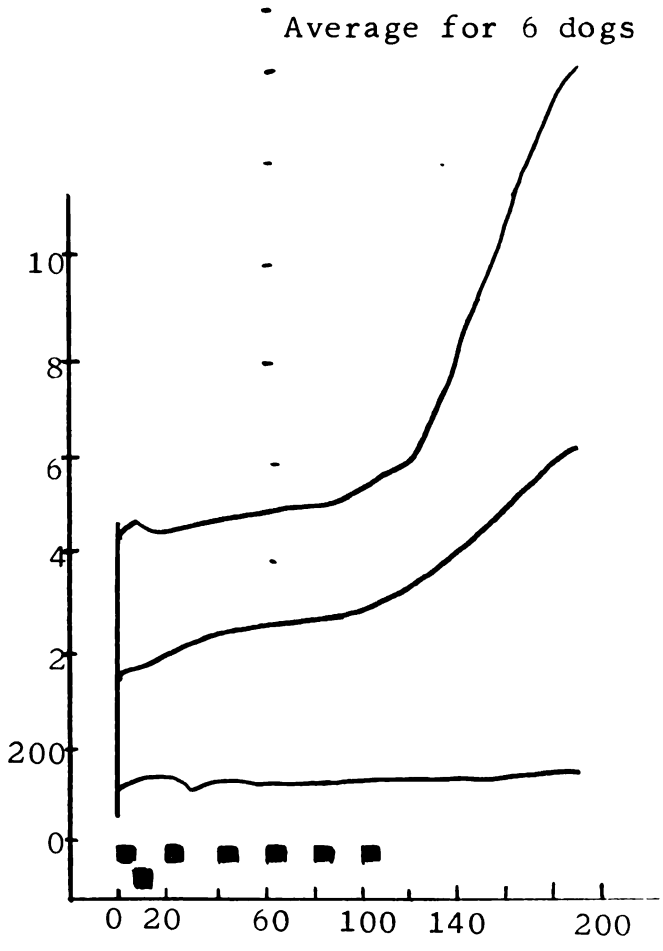


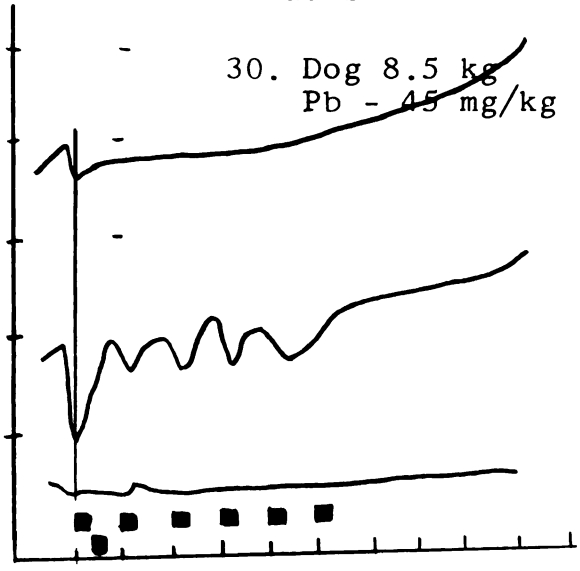


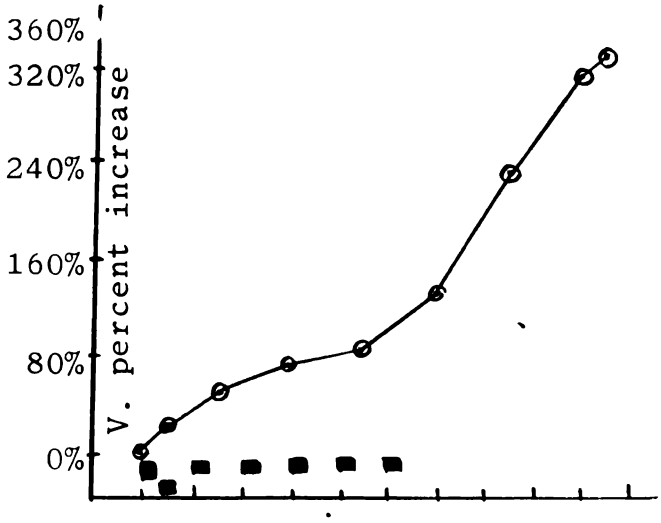


PICROTOXIN 0.5 MG/KG + METHYLPHENIDATE 5 MG/KG--Continued



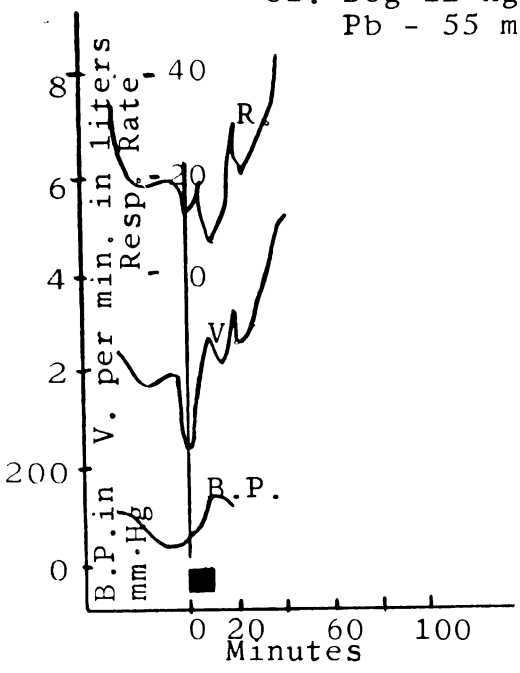




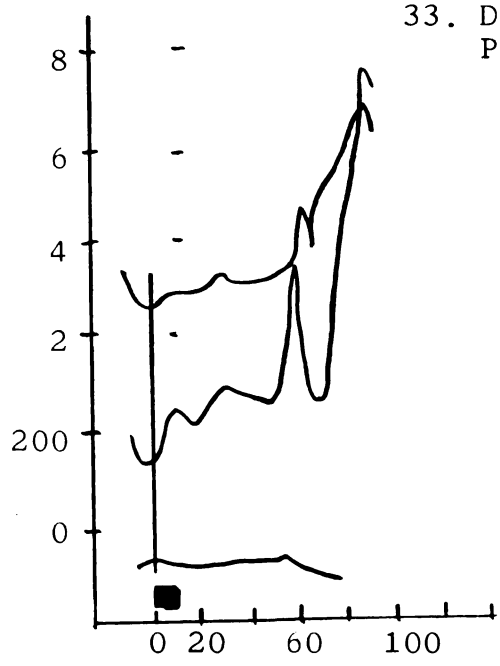


PICROTOXIN 2 MG/KG ONE INJECTION

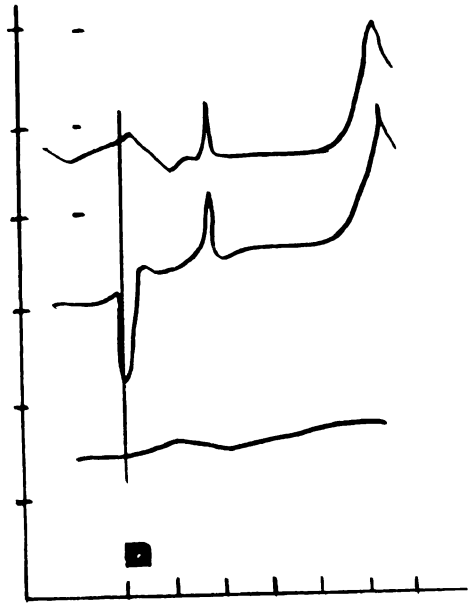
31. Dog 12 kg
Pb - 55 mg/kg



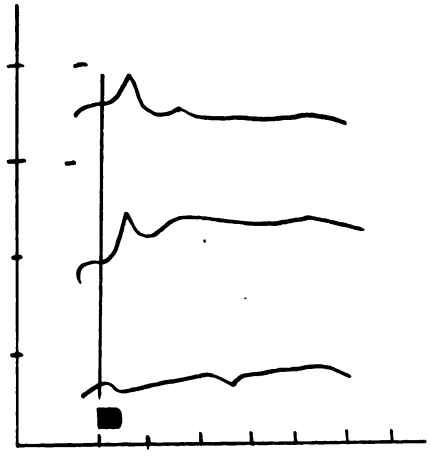
33. Dog 17 kg
Pb - 40 mg/kg



32. Dog 20 kg
Pb - 70 mg/kg

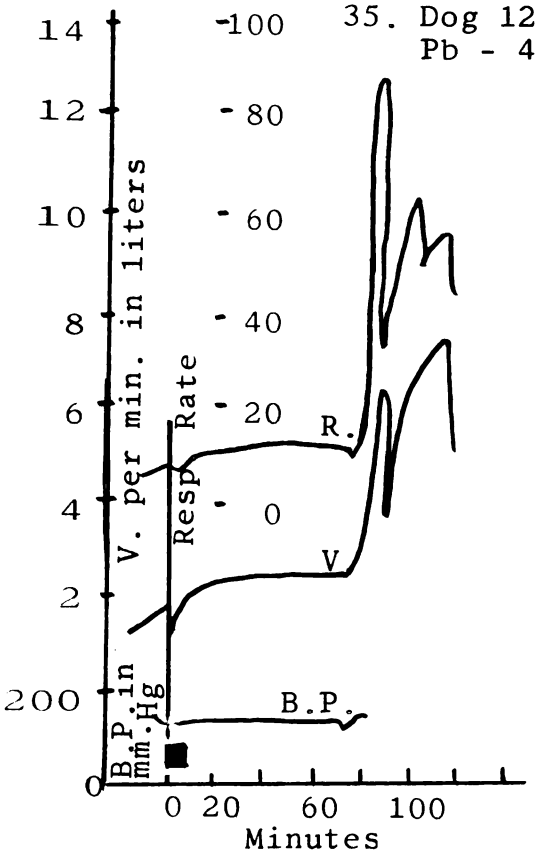


34. Dog 18 kg
Pb - 40 mg/kg

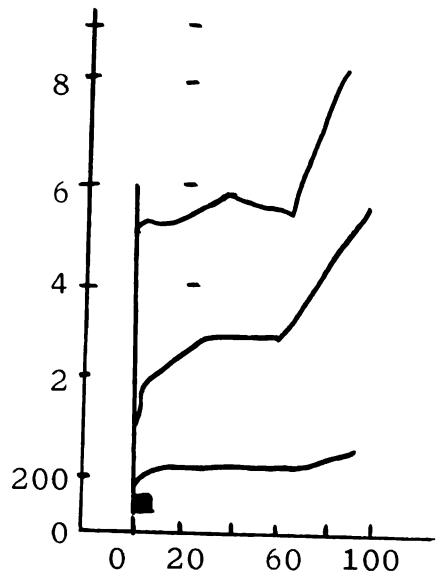


PICROTOXIN 2 MG/KG--Continued

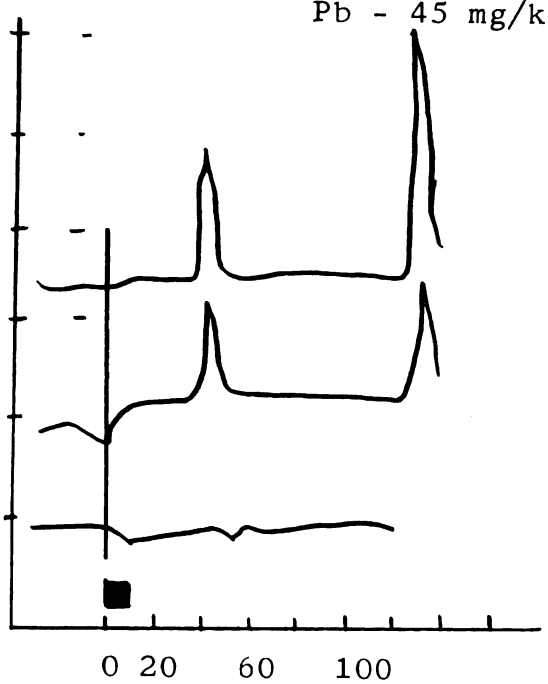
35. Dog 12.5 kg
Pb - 40 mg/kg

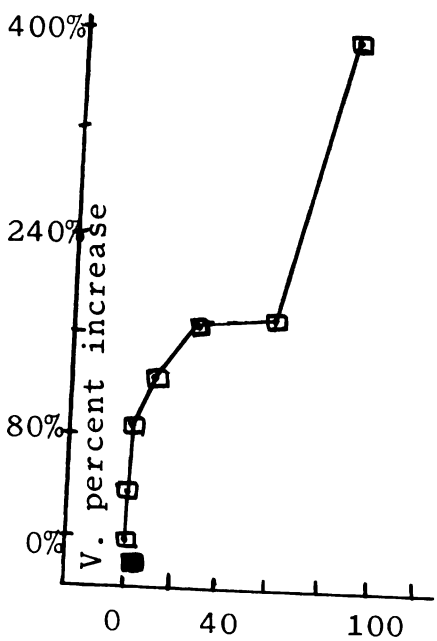


Average for 6 dogs

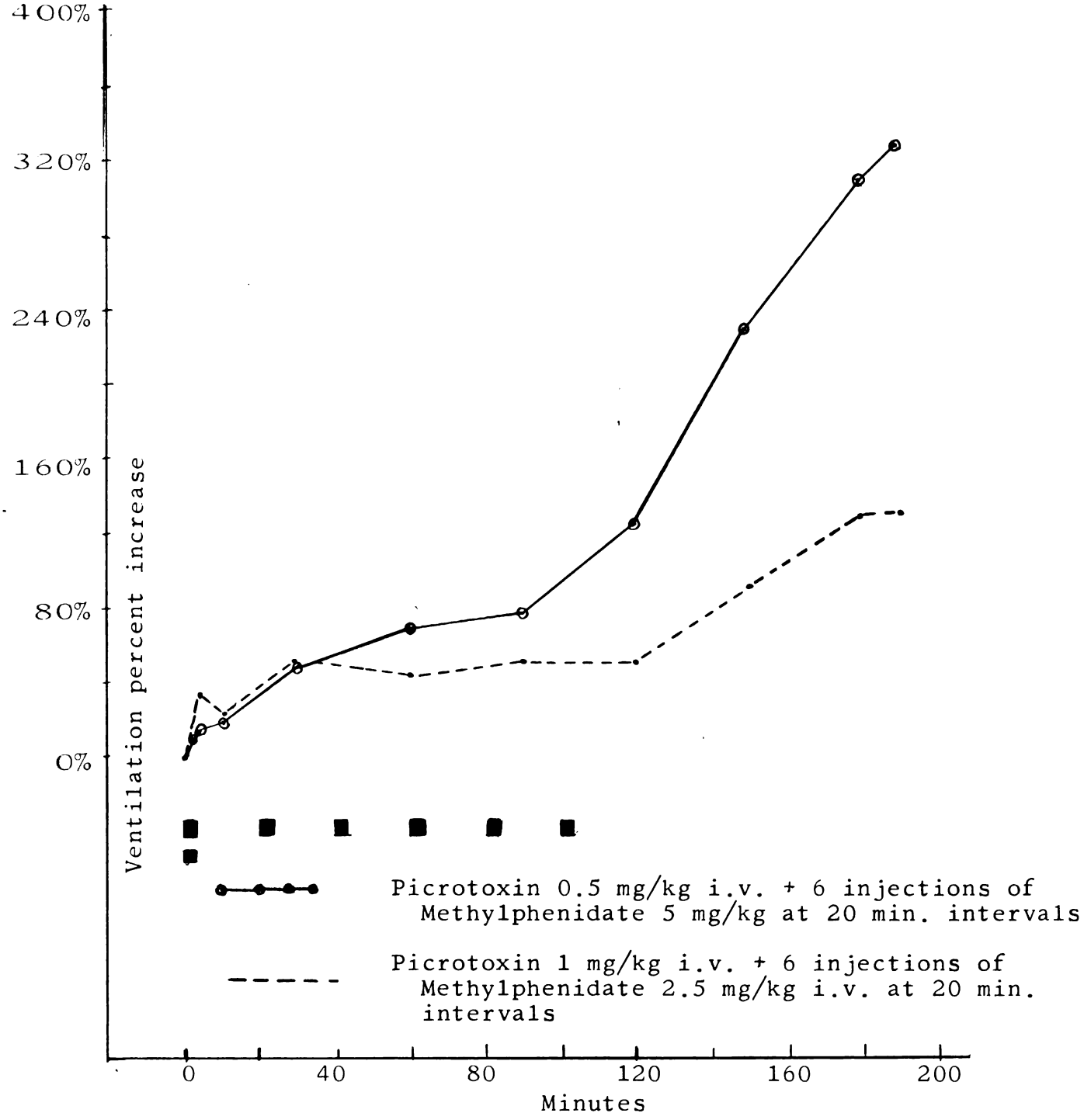


36. Dog 13 kg
Pb - 45 mg/kg

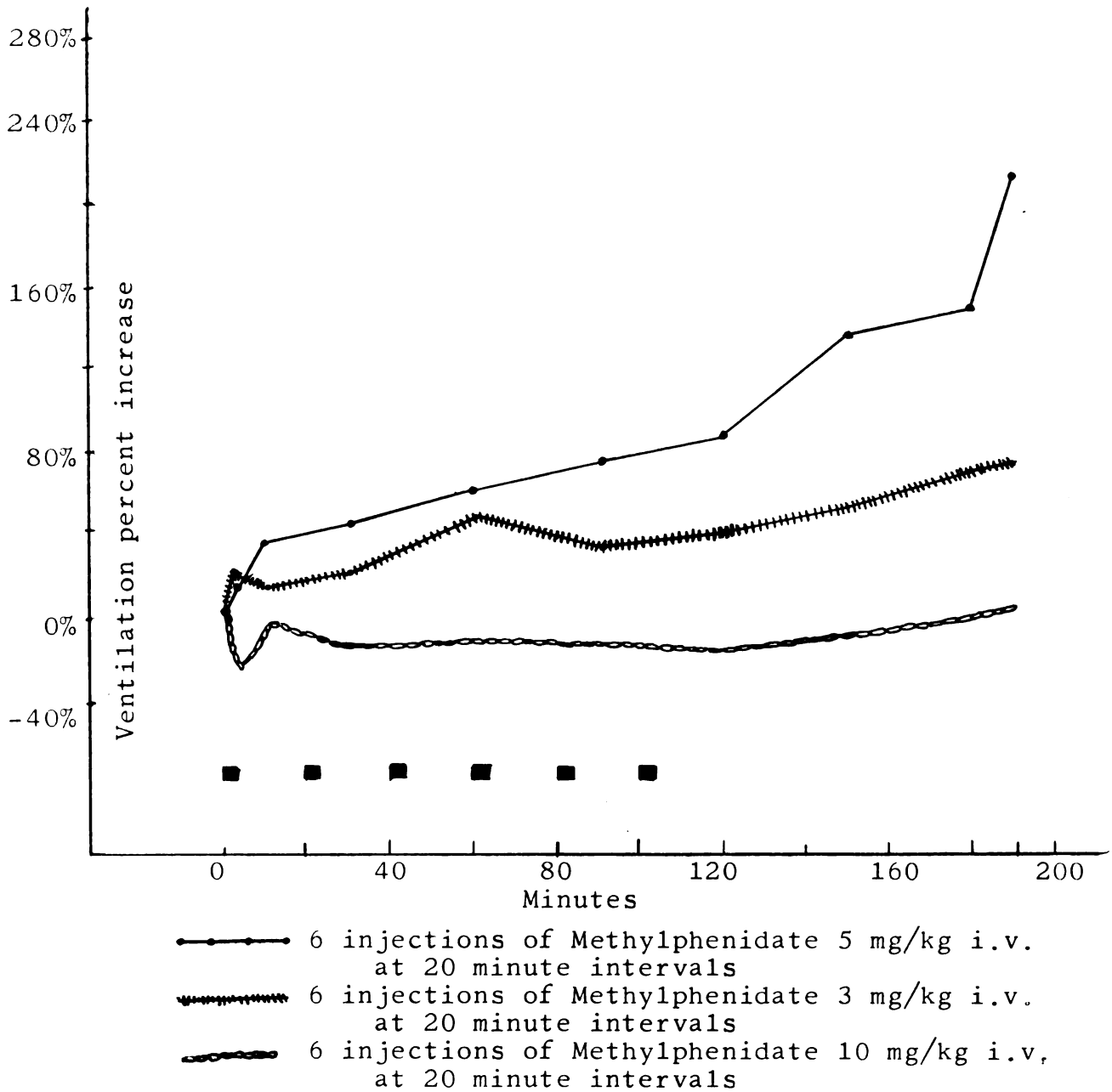




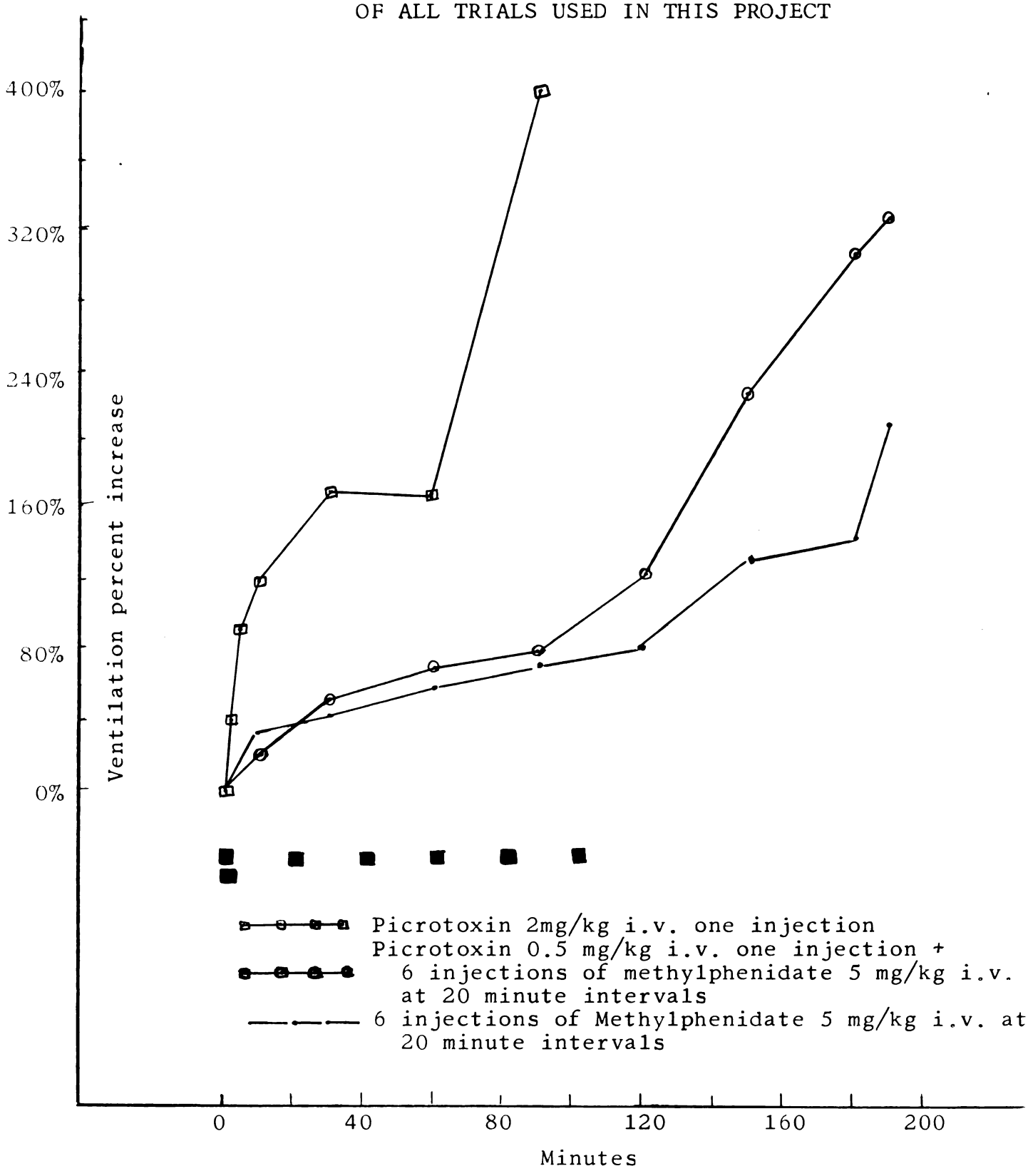
AVERAGE PERCENT IN VENTILATION AT TWO DIFFERENT DOSE LEVELS OF PICROTOXIN AND METHYLPHENIDATE



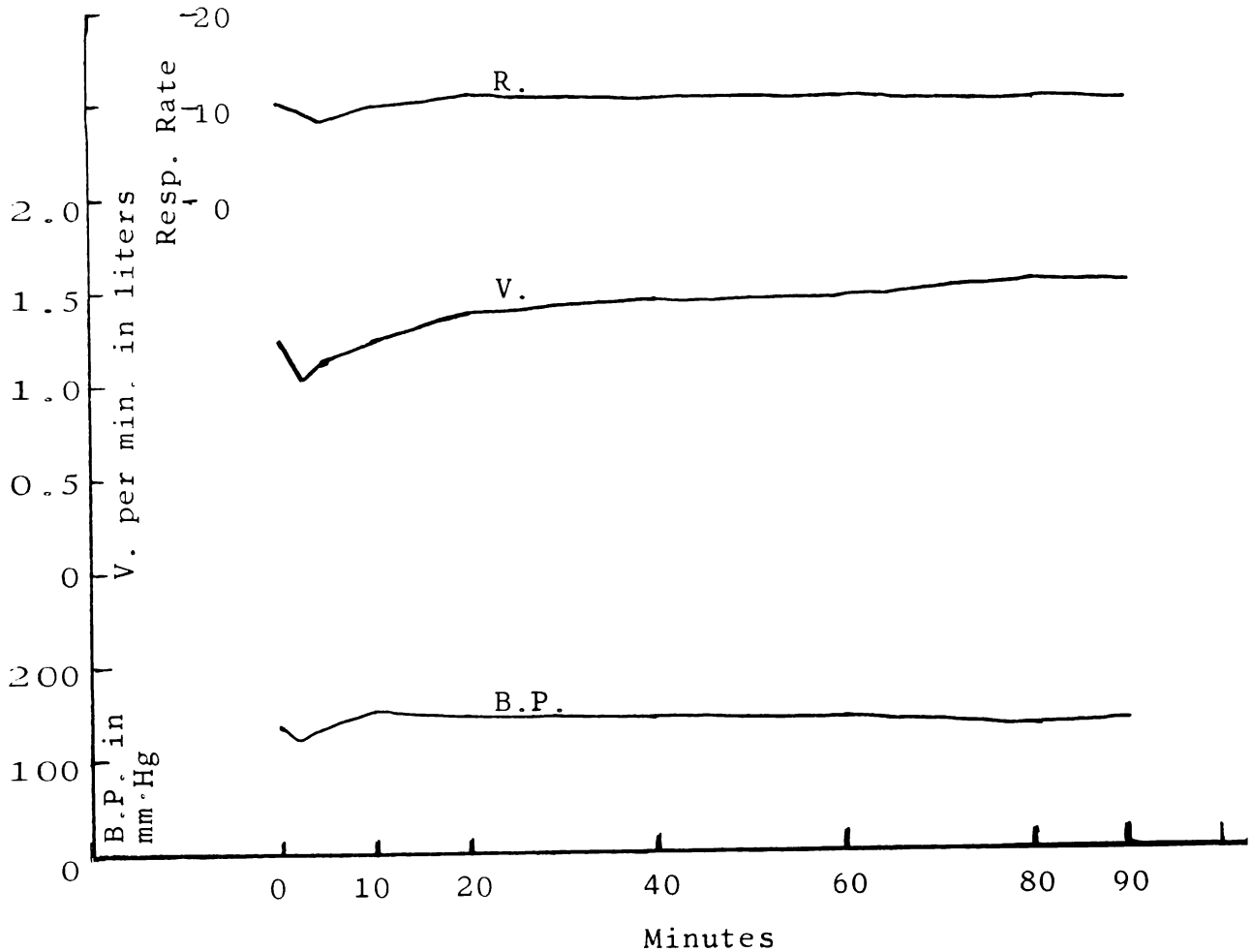
AVERAGE PERCENT INCREASE IN VENTILATION AT THREE DIFFERENT DOSE LEVELS OF METHYLPHENIDATE ALONE



THREE BEST "AVERAGE VENTILATION PERCENT INCREASE"
OF ALL TRIALS USED IN THIS PROJECT



AVERAGE RESPIRATORY RATE, VENTILATION IN LITERS PER MINUTE AND BLOOD PRESSURE OF SIX DEEPLY BARBITALIZED DOGS WITH NO ANALEPTIC TREATMENT



CHAPTER IV

DISCUSSION

Picrotoxin

Picrotoxin alone in doses of 2 mg/kg i.v. in deeply barbitalized dogs, did an excellent job to increase the ventilation level. Immediately after injection, there was a 40% increase in minute respiratory volume; within 30 minutes ventilation sharply increased by as much as 170%, stayed there for another half hour, and by the end of one and one-half hours, it was escalated to a highly commendable level, approximately a 400% increase. This pehnomenon indicates that picrotoxin is a pharmacological barbiturate antagonist. The effect is brought about by the direct central nervous system stimulation, especially the cortex and the vital centers in the medulla oblongata, i.e., respiratory and circulatory centers; this agrees thoroughly with the vivid pharmacological action of picrotoxin described earlier. However, in contrast to many investigators' reports, none of the 6 dogs treated with picrotoxin exhibited the 15-20 minute time lag which is usually cited as the greatest drawback in its therapeutic use.

We further noticed that when picrotoxin was injected slowly and continuously within a period of 4-5 minutes, its side effects, like severe convulsion and profuse salivation, were greatly minimized, though vomition, micturition, defecation and occasional sporadic muscular twitches were observed in one or the other animal used in this experiment. The reduced convulsant effect is possibly due to the combination of the following two properties of picrotoxin:

1. It is rapidly destroyed in the system.
2. The concentration of the drug in the cortical region is not sufficient for the precipitation of convulsions. Instead this drug level is capable of producing desired cortical stimulation so as to awaken the deeply depressed dogs.

Two out of the six dogs did not show typical rise in ventilation or arousal when treated with picrotoxin. This may be attributed to individual variation to drug response. Another two dogs which showed muscular twitching, excessive salivation, vomition and defecation, took a full 20 hours to stand up, which is believed to be due to picrotoxin post-treatment exhaustion.

In general picrotoxin seemed to be an excellent barbiturate antagonist in 100% of the cases by either awakening the dogs from severe C.N.S. depression or by keeping the patient in a "safety zone" from where it slowly recovers to

normal health.

Besides, picrotoxin raises the blood pressure of the deeply anesthetized animals by virtue of its direct vasomotor center stimulation. In this experiment picrotoxin raised the blood pressure by 35 mm·Hg above the original level.

Picrotoxin alone in 2 mg/kg i.v. dose was found to be most effective barbiturate antagonist of all tried in this project.

Methylphenidate Hydrochloride ("Ritalin") Hydrochloride

In search for the best methylphenidate dose against severe barbiturate depression in dogs, various single and repeated doses were tried in this laboratory. The author has taken into consideration only three groups of dogs for comparative study of methylphenidate efficiency when injected intravenously in 3, 5 and 10 mg/kg doses; such six injections being given at 20 minute intervals.

Since the experiment was based primarily on the study of ventilation increase, various methylphenidate antidotal dose trials which did not show any substantial elevation in ventilation were rejected.

A deeply barbitalized dog treated with a single dose of the analeptic - 30 mg/kg i.v. gave a decrease in ventilation. Another dog injected first with 15 mg/kg i.v. produced

slight increase in ventilation which died down to a minus level soon after the same dose was repeated after twenty minutes. Identical results were obtained with 10 mg/kg i.v. doses. But then 5 mg/kg i.v. injection in regular repetitive doses did not decrease the ventilation; instead a clear cut gradual ascending trend of ventilation was demonstrated. As a result, the previous doses were abandoned as the possible barbiturate antidotal doses.

A group of 6 dogs was treated each with 3 doses of methylphenidate - 5 mg/kg i.v. given at 20 minute intervals and then watched for one and one-half hours after the last injection for any change in ventilation level. On an average, a 100% increase in ventilation was recorded with a minimum of arousal effect.

With the expectation of further beneficial results, another series of 6 dogs was treated with 6 doses of 5 mg/kg i.v. at 20 minute intervals, and as usual the effects were recorded for one and one-half hours. In general a very remarkable rise in ventilation, rate and depth of respiratory rate and finally typical arousal symptoms were exhibited. Thus, this was considered to be the standard barbiturate poisoning antidote in this experiment. Two other groups of dogs were also studied with 3 and 10 mg/kg i.v. 6 doses at 20 minute intervals and their results were obtained which will be discussed separately later in this chapter. Six

doses of 5 mg/kg methylphenidate was found to be the best of three individual doses of the drug used in this project.

In the case of a 5 mg/kg analeptic dose, ventilation increased by 35% at the tenth minute, it then gradually increased to 75% by the end of the 6th injection. There followed a somewhat abrupt elevation of ventilation which increased by as much as 230% at the end of the experiment. This was accompanied by a very significant change in respiratory rate and depth also. But the blood pressure was little affected in the course of treatment. In certain cases cortical stimulation aroused the animals also.

There was some initial fluctuation in ventilation level with the 3 mg/kg dose of methylphenidate. After a transient 20% increase in ventilation it dropped down to 12% then rose to 45% after one hour, followed with a drop to 30% by the nineteenth minute, and finally a slow and steady rise up to 70% by the end of the experiment. There was also some rise in the respiratory rate.

The 10 mg/kg dose produced an entirely different picture. Ventilation remained reduced from the beginning of the analeptic therapy till to the end of the experiment. Soon after the first injection, ventilation decreased by 24%. Then it went up to -4% by the end of the tenth minute followed with a gradual descent to -20% for one hour and forty minutes. Then a very slow rise in ventilation developed,

terminating at last on a par with the original pre-treatment ventilation level. In fact, the 10 mg/kg dose proved to be the poorest of all trials in this project. Two animals died 24 hours after the therapy as they failed to recover from the barbiturate depression.

From the above discussion it is strongly believed that methylphenidate bears a definite dose relationship with its therapeutic value. When the standard dose (6 injections of 5 mg/kg at 20 minute intervals) is doubled or reduced to 3 mg/kg, a great deviation from the usual analeptic effect is seen. The variation is more amplified as the standard dose is doubled. This clearly indicates that any other dose of methylphenidate but the standard one will act less effectively to combat the barbiturate depression. A very similar type of observation has been made by Gale, A. S. (1959) in his laboratory. Unfortunately this phenomenon is hardly explained. Perhaps a certain optimum methylphenidate concentration is needed for desired C.N.S. tissue cell excitation.

Methylphenidate used alone or in combination with other drugs against barbiturate poisoning seems to be devoid of any cardiovascular action. Even though it does not raise the depressed blood pressure in this experiment, it is believed to protect the animals from the drastic fall in blood pressure expected from barbiturates.

Over and above all, any significant rise in ventilation with methylphenidate is noticed after the end of the sixth injection only. Although considerable time is lost in the meanwhile methylphenidate seems to maintain the animals in a very safe stage by increasing the ventilation to some respectable height.

Methylphenidate and Picrotoxin Combination

Picrotoxin 1 mg/kg i.v. + 6 injections of methylphenidate 2.5 mg/kg i.v. at 20 minute intervals (A) and picrotoxin 0.5 mg/kg i.v. + 6 injections of methylpehnidate 5 mg/kg i.v. at 20 minute intervals (B) were the two combinations used in this project out of which the latter combination was found to be more effective than the former. As a matter of fact, so far as improvement in ventilation is concerned, this combination's efficiency ranks second from the top, with a maximum 326% increase over the original level.

Picrotoxin at 0.5 mg/kg with 6 doses of methylphenidate - 5 mg/kg raised the ventilation by 20% initially. Then there was a gradual increase in ventilation up to 80% by the end of one and one-half hours. Following that, it immediately shot up, reaching a 326% increase by the end of the experiment. This big peak of ventilation is considerably more than the increase observed with 6 doses of methylphenidate alone, though it was in less than the single dose of

picrotoxin 2 mg/kg i.v. It is therefore believed that a full dose of methylphenidate and one-fourth dose of picrotoxin synergistically increase the ventilation. Blood pressure remained uniform throughout the experiment in all six dogs; methylphenidate is not expected to alter blood pressure and one-fourth a full dose of picrotoxin may be too small to affect blood pressure. In addition, methylphenidate may antagonize the pressor effect of picrotoxin. Besides, side effects are minimum. Recovery time is greatly reduced. All six dogs were able to walk within three to six hours. The 2-drug-cortical stimulation synergism and the protecting property of methylphenidate from rebound depression--these two behaviors might be attributed as a possible explanation of the aforesaid phenomenon.

Half and half dose combinations of the two drugs, however, increased ventilation in deeply barbitalized dogs very poorly in comparison with the preceding one; even the response in this respect is less than the full dose effect of methylphenidate alone. In accordance with the efficiency of this combination on ventilation percent increase, it occupied the fourth position in the series. Within the first four minutes, ventilation went up by 36% (twice as much as the rise by the other combination at the same time) reduced to 24% by the tenth minute, then rose to 50% by the end of thirty minutes (same as the other combination at this point);

but unlike the previous combination, it remained flat at this level for one and one-half hours followed with a somewhat appreciable rise in ventilation which was increased by as much as 130% at the end.

So, it was observed that there was less ventilation percent increase (130%) with 1 mg/kg i.v. one injection of picrotoxin and 6 doses of methylphenidate 2.5 mg/kg i.v. than with 0.5 mg/kg i.v. one injection of picrotoxin and 6 injections of methylphenidate 5 mg/kg i.v. In the latter combination ventilation percent increase was 326%. Deviation from the optimum methylphenidate dose (discussed earlier in this chapter) might be a possible reason for this response. Doubling the picrotoxin from 0.5 mg/kg to 1 mg/kg could not compensate the ventilation percent drop as this is still a very ineffective analeptic dose of picrotoxin. When two dogs were treated with 1 mg/kg i.v. picrotoxin, ventilation percent increase averaged to be a mere 50% which is a very insignificant result in comparison to 400% increase by the 2 mg/kg injection of picrotoxin. At any rate the 130% increase by half and half drug combination is definitely a consequence of the additive, rather slight synergestic, effect of methylphenidate and picrotoxin since 3 mg/kg dose of the former drug and 1 mg/kg dose of the latter drug separately increased the ventilation from the original level 70% and 50% respectively.

The arousal effect was less pronounced and recovery time slightly increased in comparison with the other combination.

Blood pressure as usual remained almost constant from the beginning to the end probably due to the failure of the 1 mg/kg picrotoxin and 2.5 mg/kg methylphenidate doses to stimulate the vasomotor center centrally.

SUMMARY

Methylphenidate and picrotoxin alone and in two combinations were tried in this project to study their ability to improve ventilation in dogs overanesthetized by barbiturates and were found to be in the following descending order of efficiency:

1. Picrotoxin 2 mg/kg i.v. one dose.
2. Six doses of methylphenidate 5 mg/kg i.v. at 20 minute intervals and one dose of picrotoxin 0.5 mg/kg i.v.
3. Six injections of methylphenidate 5 mg/kg i.v. at 20 minute intervals.
4. Six injections of methylphenidate 2.5 mg/kg i.v. at 20 minute intervals and picrotoxin 1 mg/kg i.v. one injection.
5. Six injections of methylphenidate 3 mg/kg i.v. at 20 minute intervals.
6. Deep pentobarbital anesthesia without analeptics (control group).
7. Six injections of methylphenidate 10 mg/kg i.v. at 20 minute intervals.

Picrotoxin alone was found to be excellent to increase the ventilation. Six doses of methylphenidate

10 mg/kg were poorest of all. In fact this dose was completely ineffective. It rather produced regressive effects. Occasionally picrotoxin prolonged recovery time due to post treatment exhaustion.

Methylphenidate, in therapeutic doses, also worked as a very good barbiturate depression antagonist. It was found to possess less side effects and to be free from rebound depression. Further, methylphenidate used alone or in combination did not change the blood pressure.

Picrotoxin and methylphenidate were also observed to be potent enough to bring about arousal effects in barbitalized dogs. The superior awakening property was invariably noticed with picrotoxin alone, not infrequently with drug combinations and occasionally with 5 mg dose of methylphenidate alone. As evidenced from the data, picrotoxin had better arousal effect than methylphenidate in deep pentobarbital anesthesia. No chemical or pharmacological antagonism was observed between picrotoxin and methylphenidate when used in combinations.

So far as early recovery time is concerned (when the animal is fully able to stand up that time is considered in this project as recovery time) picrotoxin 0.5 mg/kg i.v. one injection and 6 doses of methylphenidate 5 mg/kg i.v. at 20 minute intervals combination appeared to be the best of all

trials against deep pentobarbital anesthesia. Under such conditions the side effects were least and ventilation percent increase was quite significant.

The author strongly believes methylphenidate could be of value in veterinary practice either alone or with other analeptics in the treatment of severe barbiturate poisoning.

BIBLIOGRAPHY

1. Adriani, John and Bobby Smith. Anesthesiology, 19:115, 1958.

2. Barlow, O. W. J. Pharmacol., 55:1-23, 1935.

3. Browne, J. Chrichton. Brit. Med. J., 1:409-411, 422-444, 1875.

4. Cairy, C. F., Leash, A. and Sisodia, C. S. Journal A.V.M.A., 3:129-135, 1961.

5. Cairy, C. F., Giri, S. N. and Sisodia, C. S. (in publication).

6. Carter, C. H. Psychiatric Research, 4:44-48, 1956.

7. Carter, C. H. and Maley, M. C. Diseases Ner. System, 18:146, 1957.

8. Cass, N. M. J. Anaesth., 28:325-336, 1956.

9. Chakravarthi, M. J. Pharmacol., 67:153-174, 1939.

10. Chance, M. R. A. Brit. J. Pharmacol., 6:1-7, 1951

11. Chistoni, A. Zentr. Bioch. Bioph., 13:556, 1912.

12. Clemmensen, C. Acta, Med. Scand., 148:83, 1954.

13. Conroy, H. J. Amer. Chem. Soc., 74:3046-3051, 1952.

14. Cushny, A. Text Book of Pharm. and Therapeutics, 1939.

15. Das, S. C. Quart. J. Exp. Physiol., 29:355-365, 1939.

16. Dille, J. M. J. Pharmacol., 64:319-329, 1938.

17. Dille, J. M. and Hazleton, L. W. J. Pharmacol., 67:276-289, 1939.

18. Duff, D. M. and Dille, J. M. J. Pharmacol., 67:353-357, 1939.

19. Ferguson, J. T. Ann. N. Y. Acad. Sci., 61:101, 1955.

20. _____. J. Pharmacol and Exp. Therap., 116:20, 1956.

21. Ferguson, J. T., Linn, F. V. Z. et al. J. A. M. A., 162:1303, 1956.

22. Ferguson, J. T. and Funderburk, W. H. J. A. M. A., 160:259-263, 1956.

23. Fujita, N. Jap. J. Pharmacol., 5:151-173, 1956.

24. Gale, A. S. Anesthesiology, 19:101, 1958.

25. _____. Anesthesia & Analgesia, 38:406, 1959.

26. Grunwald. Arch. F. Exp. Path. U. Pharm., 60:249, 1909.

27. Hahn, F. Pharmacological Reviews, 12:477-481, 1960.

28. Huston, M. J. and Martin, A. W. Arch. int. Pharmacodyn., 87:493-501, 1951.

29. Ivey, E. P. J. A. M. A., 1959.

30. Jarvinen, P. A. and Vartiainen, A. Ann. Med. Exp. Fenn., 27:78-86, 1949.

31. Kahn, J. B. Jr. Curr. Res. Anesth., 31:130-135, 1952.

32. Klein, J. R. J. Biol. Chem., 151:651-657, 1943.

33. Kohn, R. J. Amer. Pharm. Ass. Sci. Ed., 27:286-290, 1938.

34. Koppanyi, T., Linegar, C. R. and Dille, J. M. J. Pharmacol., 58:199-228, 1936.

35. Koppanyi, T. and Fazekas, J. F. Amer. J. Med. Sci., 224:577-585, 1952.

36. Koppen. Arch. F. Exp. Path. U. Phar. 29:327, 1892.

37. Krantz, J. C. Jr. et al. J. Pharmacol., 61:153-161, 1937.

38. Lavenson, G. S. Jr. et al. J. Pharmacol., 122:271-280, 1958.

39. Locket, S. and Angus, J. Lancet., 1:580, 1952.

40. Maloney, A. H. et al. J. Pharmacol., 41:465-482, 1931.

41. Maloney, A. H. and Tatum, A. L. J. Pharmacol., 44:337-352, 1932.

42. Marshall, E. K. Jr. et al. J. Pharmacol., 60:472-486, 1937.

43. Maxwell, R. A. et al. Arch. Internat. Pharmacodyn., 112:26, 1957.

44. McCulloch, W. S. and Roseman, E. Fed. Proc., 2:34, 1943.

45. Meier, R., Gross, F. and Tripod, J. Klin. Wehnschr., 32:450, 1954.

46. Mousel, L. H. and Essex, H. E. Anesthesiology, 2:272, 1941.

47. Nilsson, E. Acta. Med. Scand., 139 Suppl:253, 1951.

48. Ogawa, M. Folia. Pharm. Jap., 7:326-334, 1928.

49. Pollock, L. J. and Holmes. Arch. Int. Med., 16:213, 1915.

50. Pollock, L. J. and Tredway. Arch. Int. Med., 12:445, 1913.

51. Plummer, A. J. and Yonkman, F. F. Anesth. and Analgesia, 37:371, 1958.

52. Plummer, A. J., Maxwell, R. A. et al. Fed. Proc. 15: 468, 1956.

53. Reifenstein, E. C. Ann. Int. Med., 13:1013-1027, 1939.

54. Rogers, F. T. Amer. J. Physiol., 45:553-554, 1918.

55. Rosenthal, F. E. J. Pharmacol., 71:305-314, 1941.

56. Shriver, H. and Perschmann, G. Comp. Rend. Soc. De. Biol., 120:410, 1935.

57. Shulman, A. et al. British Med. Journal, 1:1238-1244, 1955.

58. Thorp, R. H. J. Pharmacol., 2:93-99, 1947.

59. VanDerKloot, W. G. et al. Science, 127:521-522, 1958.

60. VanDerKloot, W. G. and Robbins, J. Experientia, 15:35-36, 1959.