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THE UNIVERSITY OF ALBERTA

VASOMOTOR EFFECTS OF ORGANOPHOSPHATE POISONS

by

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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled Vasomotor Effects of Organophosphate Poisons, submitted by Edward Preston Jr., in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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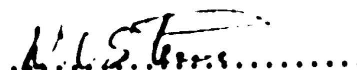

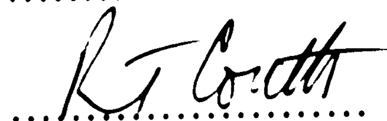

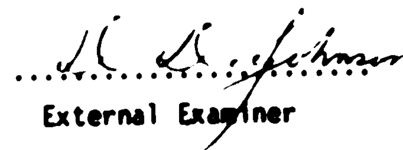

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ABSTRACT

Anesthetized rabbits given systemic organophosphate injection exhibited severe hypotension despite artificial ventilation and atropine sufficient to prevent bradycardia. This non-muscarinic (atropine-insensitive) phenomenon was contrary to and unexplained by previous cardiovascular studies.

The two possible explanations for the hypotension were impairment of the heart or of the vasomotor system. No substantial myocardial impairment was seen in atropinized Langendorff rabbit hearts exposed to high concentrations of soman. The vasomotor effects of soman were therefore investigated in artificially ventilated rabbits.

Systemic soman injection caused vasodilatation in the autoperfused forelimb, and this was concomitant with a fall in arterial pressure. Atropine partly alleviated these effects, indicating that both muscarinic and non-muscarinic vasodilator factors exist.

The denervated autoperfused forelimb vasodilated after systemic soman injection. This depression of basal vascular tone was antagonized by atropine while hypotension still remained, and it suggested that the non-muscarinic effect was located within the neuronal vasomotor pathway.

This postulate was tested by measuring vascular resistance in the forelimb surgically prepared such that it was innervated by but vascularly isolated from a rabbit given intravenous soman. Soman caused hypotension in the atropinized rabbit and a concomitant vasodilatation in its isolated limb, thereby confirming the existence of a neuronal, vasomotor depressant effect. Evidence is presented which strongly

supports the hypothesis that at high soman doses (2 or 3 mg/kg) this action is due to a mechanism other than cholinesterase inhibition.

A final group of experiments demonstrated the effect of soman on the brain-stem cardiovascular centers. Soman was injected into the brain-stem vertebral arterial supply, in amounts having no effect if given intravenously (5 μg/kg or less). Hypotension followed doses as small as 0.2 μg/kg and was caused by bradycardia and vasodilatation (observed in the innervated, isolated forelimb). Bradycardia was due to central vagal excitation, since bilateral vagotomy restored normal heart rate. In vagotomized rabbits the vasomotor inhibition mediated up to sixty-five percent decrease in blood pressure. Vasomotor depression was antagonized by intravenous atropine and, in unatropinized rabbits, by some intrinsic compensatory mechanism that could restore blood pressure within a few minutes despite complete lower brain-stem cholinesterase inhibition. The degree and duration of hypotension appeared unrelated to intravertebral dosage, although the degree of cholinesterase inhibition increased with the latter.

It is concluded that lower brain-stem cholinesterase inhibition is of minor importance in the muscarinic vasomotor depression of systemic organophosphate poisoning, except when its effect is amplified by other vasomotor stresses. It is not directly responsible for non-muscarinic hypotension. However, the depressant effect of soman unrelated to cholinesterase inhibition may be mediated at this central level.

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INTRODUCTION

The primary cause of death from intoxication with the organophosphate cholinesterase inhibitors is anoxic anoxia from respiratory failure. It results from inhibition of respiratory neurone activity in the lower brain-stem, weakened or paralyzed neuromuscular transmission, bronchoconstriction and bronchocongestion (de Candole et al., 1953). It has been suggested that, in addition, the cardiovascular impairment which organophosphates cause can strongly contribute to a rapidly fatal outcome (Holmstedt, 1951).

The objectives in treatment of such poisoning may be broadly defined as follows: (a) to prevent anoxia by artificial ventilation until spontaneous breathing is restored, and (b) to restore and maintain cardiovascular homeostasis.

Work with at least three laboratory species has demonstrated that the blood pressure response to untreated poisoning is variable. This variability results because the response is determined by two opposing factors: (a) a low cardiac output associated with bradycardia and (b) an increased peripheral resistance. Holmstedt (1951)

observed that although cardiac output fell, blood pressure was well maintained until just before death, in untreated rabbits given a slow infusion of tabun.[†] Dirnhuber and Cullumbine (1955) and Fukuyama and Stewart (1961) observed vasoconstriction caused by central vasomotor excitation in the sarin-poisoned rat.[Ψ] Daly and Wright (1956) observed vasoconstriction caused by increased sympathetic discharge and adrenal catecholamine release in sarin-poisoned dogs. So whether blood pressure falls, remains normal or even rises depends on the degree to which vasoconstriction compensates for the severity of bradycardia and low cardiac output present. The latter, especially if severe, causes stagnant anoxia which complements the anoxia of respiratory impairment and, indirectly, leads to vasomotor collapse.

In the above-mentioned studies, atropine restored heart rate and cardiac output and reduced the vasomotor excitement of poisoning. Lubash et al. (1960) observed that the ventilated, atropinized dog maintained normal blood pressure when poisoned by as much as 500 times the LD_{50} of sarin. These facts generally imply that even despite severe intoxication, atropine will ensure cardiovascular homeostasis, provided that lung ventilation is maintained.

More recent study indicates that knowledge of the cardiovascular effects of organophosphates, obtained mainly from dogs, is incomplete and that the present therapeutic measures are inadequate for some species. An example is the work of Wolthuis and Meeter (1968) who found DFP[T] to be directly toxic to the rat heart myocardium in vivo

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† Tabun: ethyl-N-dimethyl phosphoroamido cyanidate
Ψ Sarin: isopropyl methylphosphonofluoridate
T DFP: diisopropyl phosphorofluoridate

and in vitro, an effect unrelated to cholinesterase inhibition. In their investigations anesthetized rats died in three hours from a subcutaneous injection of eight times the LD_{50} for DFP despite artificial ventilation, atropine and oxime therapy. The time at which death occurred varied inversely with dose. Cardiotoxic effects of DFP were also demonstrated in the isolated rat heart. The mechanism of drug action is unknown.

The Problem

During early experience with organophosphates, I observed that sarin, soman* or DFP caused severe hypotension in the rabbit despite prior treatment with atropine and artificial ventilation. This hypotension could not be attributed to bradycardia and was consistently demonstrated only at dosage well above that minimally necessary to kill an untreated rabbit. Such a non-muscarinic effect was in contrast to previous reports that atropinized, ventilated dogs maintained normal blood pressure despite massive doses of sarin (Daly and Wright, 1956; Lubash et al., 1960). It was not described in the literature. Along with the cardiac death phenomenon mentioned above, this was a second example that atropine did not ensure cardiovascular homeostasis in species other than the dog.

The hypotensive properties of organophosphates were investigated in the ventilated anesthetized rabbit. Experiments were directed toward establishing the site(s) and mechanism of organophosphate action. Attention was given to possible effects on (a) myocardial performance (the cardiac toxicity reported by Wolthuis and Meeter, 1968), (b) the

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* Soman: pinacolyl methylphosphonofluoridate

smooth muscle or neuronal elements of the vasomotor pathway, and (c) the brain-stem vasomotor centers, as factors causing hypotension. In this investigation attention was directed principally toward soman.

LITERATURE REVIEW

History

About 1850 M. Moschnine was said to have synthesized the organophosphorus compound tetraethylpyrophosphate (TEPP) in the laboratory of C.A. Wurtz. In 1854 a French nobleman and organic chemist, Phillipe de Clermont, repeated the synthesis in Wurtz's laboratory and gave the first written account a year later (de Clermont, 1855). He described TEPP as "*a thick liquid with a burning taste and peculiar smell*". It is surprising that de Clermont did not succumb to the TEPP he tasted, since this is one of the more potent cholinesterase inhibitors. The synthesis of TEPP was repeated half a dozen times up to 1930, with Nylén first having distilled it to a pure form; but the compound's toxicity remained undiscovered.

The first reported toxic effects of organophosphates came from the laboratory of Willy Lange at the University of Berlin in 1932.

Lange and Gerda von Krueger introduced the P-F linkage for the first time into the organophosphorus compounds and noticed the toxic effects of the vapors upon themselves (Lange and von Krueger, 1932).

In Germany a great development subsequently began in this area. In 1936 Gerhard Schrader, working for the I.G. Farbenindustrie on development of synthetic insecticides, turned his interest to the organophosphorus compounds. By 1946 he had synthesized some 2000 organophosphates, including the highly toxic sarin, tabun, DFP and soman. In 1944 around 200 of the most toxic of these had been classified as secret by the German Ministry of Defence. During the war years numerous chemists studied their convincing military properties; and in January 1940 construction of a large factory began at Duhernfurt, 40 kilometers north of Breslau. By 1945 an estimated 10,000 to 12,000 tons of tabun and 600 tons of sarin had been manufactured.

The first pharmacological experiments with these agents began in Germany in 1937. Eberhard Gross is said to have recognized the enzyme-inhibiting properties of TEPP by 1939 (Schrader, 1952). The parasympathomimetic effects of the nerve gases were clearly recognized by the German pharmacologists and atropine established as an antidote.

In Britain, soon after the start of the war, teams were set up to investigate improved types of chemical warfare agents. Lange and Krueger's published, brief remarks about the toxic effects of organophosphate vapors initiated the first synthetic work with these compounds. In 1940 Kilby prepared samples of dimethyl and diethyl fluorophosphonate and began toxicological studies (Kilby, 1949). The synthesis of DFP was reported by Saunders in 1941 (Saunders, 1957). A team under Lord Adrian

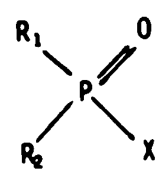
and which included W. Feldberg and B.A. Kilby began investigation of the pharmacological effects of organophosphates. By 1942 it was clear from studies on isolated organs and from biochemical experiments that these agents inhibited cholinesterase in an irreversible manner.

The above was derived from a detailed historical account by Holmstedt (1963). The references given are cited from this author.

General Information

The organophosphate poisons are colorless, watery, relatively odorless liquids which may vary widely in volatility at room temperature. They are lipid soluble, readily penetrate the blood-brain barrier, and are easily absorbed by a variety of routes including the skin.

The general formula for the organophosphate cholinesterase inhibitors may be represented as follows:



R_1 and R_2 can be alcohols, phenols, mercaptans, amides, alkyl or aryl groups attached directly to the phosphorus. The more toxic compounds usually contain alkyl side chains, and branched chains tend to increase toxicity e.g. soman > sarin. A general rule is that when R_1 = alkyl and R_2 = alkoxy, a more toxic compound results than when both are alkoxy e.g. sarin > DFP. X radicals include fluorine (providing greatest toxicity), paranitrophenol, phosphates, cyanide and other groups which make the phosphorus electron-deficient.

It is generally accepted that the organophosphates irreversibly inhibit acetylcholinesterase by phosphorylation of the serine hydroxyl of the esteratic site. This renders the esteratic site inaccessible to its natural substrate moiety, the acetyl portion of acetylcholine. Phosphorylation of the serine by an organophosphate or acetylation by acetylcholine is thought to involve other parts of the esteratic site including an imidazole or carboxyl group in the vicinity, which play a catalytic or intermediary role (Cohen and Oosterbaan, 1963). The important distinction between phosphorylation and acetylation of the esteratic site is that in the former a strong covalent bond (P-O-C) forms which is very resistant to hydrolysis by water.

The enzyme can be dephosphorylated by use of regenerating agents such as pralidoxime (Hobbiger, 1963). This therapeutic oxime attaches to the nearby anionic site and presents a highly nucleophilic group close to the phosphorylated esteratic site. The nucleophile attaches to the phosphorus, the covalent (serine-O-P) phosphoryl linkage is broken, and the enzyme is freed from the phosphorylated oxime and reactivated. It is worth mentioning that the regenerating agents are useless for this function if the phosphorylated enzyme "ages". Aging is due to dealkylation of the phosphoryl attachment e.g. following sarin attachment to acetylcholinesterase (AChE), isopropyl methylphosphonyl-AChE becomes methylphosphonyl-AChE. This process may take minutes or hours, depending upon the type of organophosphate involved. In vitro about 50 percent of soman-inhibited AChE is nonreactivatable in two minutes (Loomis and Johnson, 1966).

Many of the symptoms of organophosphate poisoning result directly from the abnormal accumulation of acetylcholine at nerve endings in

cholinergically innervated tissues. Miosis, lacrimation, salivation, bronchoconstriction and bronchosecretion, abdominal cramps and diarrhea, micturition, sweating, muscle fasciculations followed by muscle weakness or flaccid paralysis, and bradycardia are thus observed. The effects least understood are those involving postulated disturbance of central cholinergic synapses i.e. effects on breathing and vasomotor tone, and convulsions.

Respiratory Effects

The major cause of death is respiratory failure due to three organophosphate effects: (a) bronchoconstriction and associated bronchosecretion, (b) neuromuscular blockade and (c) inhibition of central respiratory neurones (de Candole et al., 1953).

De Candole et al. found that the degree of bronchoconstriction varied with the species and was relatively insignificant in monkeys, slight in rabbits and severe enough in cats to cause complete cessation of lung inflation, and death. This species variation has been related to the relative amount of peribronchial smooth muscle (Johnson et al., 1958). The effects are antagonized by atropine.

The effect of anticholinesterases on the myoneural junction has been reviewed by Holmstedt (1959) and Werner and Kuperman (1963). Acetylcholine accumulation at the motor end-plate accentuates miniature end-plate potential activity and spontaneous induction of action potentials. In addition, a single nerve impulse may cause repeated muscle contractions. These phenomena likely account for the muscle fasciculations following poisoning. Excessive acetylcholine accumulation at the motor end-plate

becomes manifest as a "Wedensky" inhibition i.e. a progressive inability of the muscle to respond with a sustained contraction to repetitive stimulation. Neuromuscular blockade is fully established when the end-plates become persistently depolarized and is sustained into the following period when the end-plate becomes repolarized but refractory to the accumulation of acetylcholine.

Evidence for the importance of neuromuscular blockade in respiratory failure has come mainly from phrenic nerve-diaphragm preparations. Douglas and Matthews (1952) showed that following TEPP poisoning in cats, the diaphragm failed to contract in response to phrenic nerve stimulation; yet the nerve still conducted impulses and the diaphragm responded to direct stimulation.

It has been suggested that the degree of neuromuscular blockade in poisoning is importantly influenced by the activity of the respiratory centers. Douglas and Matthews (1952) found that the intensity of blockade increased with frequency of electrical nerve stimulation. They also found that diaphragm paralysis was comparatively severe in those poisoned animals in which the respiratory center was maintained active by atropine. It was therefore suggested that in treatment of poisoning, recovery of neuromuscular transmission might be promoted by suppression of the activity of respiratory neurones by suitable artificial ventilation. Wright (1954) observed that following sarin or TEPP administration to rabbits, partial neuromuscular block which occurred first was followed and enhanced by a spontaneous, temporarily increased duration and frequency of phrenic-nerve discharge.

Recovery of neuromuscular transmission may take place according to three mechanisms: (a) regeneration of some of the original

enzyme, (b) synthesis of new enzyme and (c) adaptation of the synapses to the abnormal situation, (Meeter and Wolthuis, 1968). These authors observed some interesting distinctions between sarin and DFP in this respect. After sarin-induced neuromuscular blockade and subsequent recovery, a second dose of sarin again induced blockade. This suggested that recovery from sarin-induced blockade was due to regeneration of inhibited enzyme. After neuromuscular transmission had recovered following DFP-induced blockade, it could not be blocked again by a second dose. In this case the process of motor end-plate desensitization was believed responsible for recovery.

Atropine is of no direct therapeutic value in restoring respiratory neuromuscular function following organophosphate poisoning (Douglas and Natthews, 1952). The regenerating agents such as pralid-oxime are of great value in this respect except in cases where oxime-resistant agents are involved e.g. soman (Loomis and Salafsky, 1963) or tabun (Wilson and Sondheimer, 1957).

It is generally accepted that organophosphates paralyze respiration by interfering with respiratory centers in the brain-stem. This was concluded from records of phrenic-nerve action potentials which ceased their rhythmical discharge following poisoning (Douglas and Matthews, 1952; de Candole et al., 1953; Wright, 1954). Since then several workers have provided more direct evidence that a central cholinergic mechanism is involved. Metz (1958) correlated brain-stem cholinesterase activity in dogs with potentiation or inhibition of the reflex ventilatory increase that follows electrical stimulation of Hering's nerve. When TEPP was injected intracisternally in low doses (onto the fourth ventricle floor), marked potentiation of the reflex

occurred at cholinesterase levels 84-88 percent of control. The reflex gradually diminished with decreasing cholinesterase activity and disappeared together with spontaneous respiration when enzyme activity was 8-11 percent of normal levels.

Utilizing a stereotaxic microinjection technique, Stewart and Anderson (1968) injected sarin directly into the reticular formation of the rabbit medulla. Bilateral injection into the lateral reticular nucleus at the level of the inferior fovea caused respiratory arrest, which was quickly antagonized by intravenous atropine. These authors interpreted their results as indicating the location of a nervous center concerned with the control of respiratory muscles, and that this is the site of central respiratory paralysis in systemic sarin poisoning.

It has been well demonstrated and is generally accepted that atropine will adequately protect against this central component of respiratory failure (de Candole et al., 1953; Schaumann and Job, 1958; Stewart and Anderson, 1968; Brown, 1960; Douglas and Matthews, 1952). An exception to this has been reported by Meeter and Wolthuis (1968). They compared the length of time required for recovery of spontaneous breathing following soman, DFP or sarin injection in rats. Soman paralysis of respiration long outlasted that of DFP or sarin and was of a central non-muscarinic type, since it remained despite restoration of neuromuscular function and injection of large doses of atropine.

The Hypotension of Poisoning

To achieve a more complete understanding of the fatal effects of organophosphate poisons, their cardiovascular actions have been subjected to much research. These actions are extremely complex. They reflect the sum of excitatory or inhibitory effects of accumulated acetylcholine at several levels of innervation of the heart and blood vessels. The changes observed in blood pressure and cardiac output may be significantly influenced by the species of animal, type of anesthesia, the drug route and dosage and the adequacy of ventilation.

Early reports on the blood pressure effects of DFP, HETP, and TEPP are given by Modell et al. (1946), Heymans and Jacob (1947), Burgen et al. (1949), Verbecke and Votava (1949) and Salerno and Coon (1949). These authors are in general agreement that large doses of anticholinesterase cause a fall in blood pressure concomitant with bradycardia. Small sublethal doses were reported to have either no blood pressure effect e.g. DFP (Salerno and Coon, 1949) or to induce a pressor effect e.g. TEPP and HETP (Burgen et al., 1949). The majority of these early studies dealt in a general way with a variety of systemic effects. It was still not clear at that time to what extent the hypotensive effects were due to reduction in cardiac output or changes in peripheral resistance. It seems to the author that the first positive step in this direction came with Holmstedt's publication on the synthesis and pharmacology of tabun (1951).

Holmstedt measured arterial and venous pressures and cardiac output in unanesthetized, untreated rabbits given a slow intravenous infusion of tabun. Cardiac output began to fall when 25 percent of the

lethal dose had been given and fell to 50 percent of the basal value at 80 percent of the lethal dose. Arterial pressure remained at or above normal until 90 percent of the lethal dose was injected and asphyxia occurred. It was concluded that the circulatory failure of tabun poisoning was due to tabun effects on the heart i.e. bradycardia and possibly impaired contractility. Blood pressure was sustained presumably by vasoconstriction. Peripheral (vasomotor) failure of the circulation was ruled out as a factor contributing to hypotension.

The conclusions drawn from this work were supported by several later investigations (Daly and Wright, 1956; Fukuyama and Stewart, 1961). Daly and Wright measured changes in peripheral vascular resistance in perfused, isolated, innervated organ preparations of the dog. Perfusion was carried out either from the same animal or from a donor dog so that local chemical and neuronal influences could be separated. Sarin and TEPP invariably caused bradycardia and hypotension and produced vasoconstriction in the limbs and splanchnic area. Atropine antagonized all of these effects. These investigators further delineated the vasomotor effects as follows. Vasoconstriction was due both to increased sympathetic discharge and release of adrenal catecholamines. These factors could override a vasodilator effect of the anticholinesterase, which was unmasked in experiments where the perfused bed was denervated and the adrenal influences were removed. The vasodilator effect was attributed to local acetylcholine accumulation, asphyxia, or a local, direct organophosphate effect in the vascular bed.

Fukuyama and Stewart (1961) measured resistance changes in the perfused, innervated, isolated rat's hindlimb subjected only to

neuronally mediated effects of sarin. Sarin caused intense bradycardia paralleled by a vasoconstriction in the isolated limb. Blood pressure usually rose and then fell, the direction and magnitude being governed by the opposing factors of falling cardiac output and vasoconstriction. The return of normal blood pressure following atropine injection was attributed to restoration of cardiac output.

The consensus of opinion has been that the vasomotor system reacts in a compensatory manner to the lowered cardiac output of poisoning (Holmstedt, 1959). Vasodilatation has been occasionally speculated upon as a factor contributing to hypotension (Verbecke, 1949; K.M. Wilson, cited by Daly and Wright, 1956); and Daly and Wright did unmask vascdilator influences in the dog. However, no past study has established vasodilatation-induced hypotension to be a pharmacological effect of the anticholinesterases; and vasoconstriction, rather than vasodilatation, appears to be the predominating influence on blood pressure following poisoning.

Atropine Protection Against Hypotension

It is generally observed that blood pressure rises dramatically when atropine is injected into the hypotensive poisoned animal. This action is attributed to restoration of normal heart rate and cardiac output. This is not observed, however, if a terminal stage of asphyxia has been reached (Daly and Wright, 1956).

Daly and Wright observed that atropine could alleviate the vasomotor excitement of poisoning and attributed this to (a) a possible baroreflex concomitant with the subsequent blood pressure increase,

(b) an increase in blood flow and improved oxygenation of vasomotor centers, or (c) abolition of a possible direct central-excitant action of sarin. The cross-perfusion, isolated-head experiments in dogs by Polet and Schaepdryver (1959) confirmed the existence of a central excitant action of sarin, antagonized by atropine.

It is evident from several studies that atropine offers adequate protection to the cardiovascular system despite severe intoxication. Daly and Wright (1956) and Heymans et al. (1956) observed that the atropinized dog sustained blood pressure and vasomotor tone when very large doses (mg/kg) of sarin were injected intravenously. In the dog the intravenous LD_{50} for sarin is 25 μg/kg (Polet and Schaepdryver, 1959). Lubash et al. (1960) observed that dogs protected by artificial ventilation and atropine sustained normal blood pressure despite intravenous injection of up to 500 times the LD_{50} for sarin.

It is noteworthy, however, that Wolthuis and Meeter (1968) observed death due to cardiac impairment in anesthetized, atropinized, ventilated rats given large doses of DFP. This phenomenon will be discussed later in this review.

Central Vasomotor Effects

In attempts to explain the vasomotor effects of organophosphates, several studies have dealt with their action on the brain-stem vasomotor centers. It was found in dogs that the anticholinesterases have a central excitant action which is antagonized by atropine (Polet and Schaepdryver, 1959; Brown, 1960). Polet and Schaepdryver used the method of cross-perfusion of the vascularly isolated head of the dog connected to its trunk by the spinal cord and vagus-aortic nerves only.

Two or three times the LD_{50} of sarin injected into the vessels perfusing the head produced only excitation of vasomotor output, observed as hypertension in the trunk. Atropine, injected in a similar way, alleviated the central excitation.

Brown (1960) injected average doses of 8.6 or 25.0 µg/kg sarin into the cisterna magna of anesthetized dogs. Central vasomotor excitation commonly occurred, observed as hypertension. Intracisternal or intravenous injection of atropine antagonized the effect. Similar observations were made by Dirnhuber and Cullumbine (1955) in rats. Sarin administered intravenously in near-lethal doses always produced a sustained hypertension and accelerated heart rate. Smaller doses injected intracisternally had the same effect.

These studies in dogs and rats emphasize that any important hypotensive organophosphate action appears to be peripheral rather than central.

One other study, utilizing a different species and experimental approach, should be mentioned at this point. Stewart and Anderson (1964) used a stereotaxic microinjection technique and injected sarin directly into the brain-stem reticular formation of anesthetized rabbits. Bradycardia and hypotension followed bilateral sarin injection. Bradycardia was alleviated by vagotomy indicating that sarin causes central vagal excitation. However, hypotension still remained despite restored heart rate; and normal blood pressure returned following systemic atropine injection. These experiments supported the hypothesis that sarin can depress the brain-stem vasomotor centers by a muscarinic effect.

None of the researchers appear to have attempted to correlate these cardiovascular changes of central origin with measurement of

cholinesterase activity.

Effects on Sympathetic Ganglia

It has been generally found that the organophosphates facilitate transmission in sympathetic ganglia. Such facilitation was demonstrated in experiments which measured the nictitating-membrane response to preganglionic stimulation (Chennels et al., 1947; Burgen et al., 1949; and Holmstedt, 1951). Effects on sympathetic ganglionic transmission are relatively mild compared to those observed on the neuromuscular junction. Paton (1954) in a review discussion contrasted the relative insensitivity of sympathetic ganglia with the great sensitivity of the neuromuscular junction to anticholinesterase agents. This difference may stem from differences in the role of acetylcholinesterase at either of type of synapse. It has been postulated that diffusion plays a major role in transmitter inactivation in sympathetic ganglia and that the esterase function of acetylcholinesterase is mainly presynaptically located (Volle and Koelle, 1961; Koelle, 1962).

Koelle and his colleagues succeeded in correlating the functional changes produced by anticholinesterases in ganglia with varying degrees of cholinesterase inactivation. Kamijo and Koelle (1952) studied in vivo the effect of DFP on the cat nictitating-membrane response to preganglionic stimulation. Partial or complete acetylcholinesterase inhibition facilitated ganglionic transmission. Doses in excess of that necessary to inhibit all cholinesterase depressed the response and the effect was attributed to a direct action of DFP, unrelated to cholinesterase inhibition. Holaday et al. (1954) repeated and confirmed these results by preganglionic stimulation and simultaneous

recording of pre- and postganglionic action potentials over a wide range of DFP doses.

Effects on sympathetic ganglia have been poorly assessed in the phenomena of anticholinesterase poisoning. Nevertheless, the vasomotor excitant effects of organophosphates may be due, at least in part, to their facilitation of sympathetic ganglionic transmission.

Effects on Chemo- and Baroreceptors

Chemo- and baroreflexes merit serious attention in any thorough interpretation of cardiovascular and respiratory phenomena following organophosphate poisoning. Heymans et al. (1956) injected tabun or sarin into the circulation of the carotid body. Sarin had no effect on heart rate, blood pressure or respiration; and the brief hyperpnea that followed tabun injection was attributed to the cyanide moiety that splits off from the tabun molecule. In contrast, Landgren et al. (1952) observed that chemoceptive impulse activity increased in the sinus nerve following close intra-arterial injection of DFP and TEPP. This observation is consistent with early findings that acetylcholine is an effective chemoreceptor stimulant (Heymans et al., 1936; Schweitzer and Wright, 1938). Evidence that acetylcholine has a role as a chemoreceptor transmitter has been more recently provided by Eyzaguirre and Zapata (1968).

It is well known that stimulation of carotid body chemoreceptors by oxygen lack or carbon dioxide induces a reflex tachycardia and rise in blood pressure (Heymans, 1955). Bradycardia has also been shown in the dog following chemoreceptor stimulation (James and Daly, 1969).

However, it is not established whether or not organophosphate poisons facilitate centrally mediated vasomotor excitation (Polet and Schaepdryver, 1959) and bradycardia (Stewart and Anderson, 1964) by a direct pharmacological action on chemoreceptors.

The carotid sinus baroreceptors are not influenced by close intra-arterial injection of DFP or TEPP (Landgren et al., 1952). The baroreceptors are sensitized by low concentrations of acetylcholine (Landgren et al., 1953); but the evidence suggests a pharmacological action, and acetylcholine is not likely involved in the physiological stimulation or inhibition of baroreceptors (Heymans, 1955).

The organophosphates cause oxygen lack. This physiological stimulus to chemoreceptors is likely very important during organophosphate poisoning since the chemoreflexes are the primary mechanism by which oxygen lack stimulates breathing and vasomotor activity. It has been shown that after exclusion of aortic and carotid body chemoreceptors, anoxia no longer induces hyperpnea and hypertension, but inhibits respiration and causes a fall in blood pressure (Bouckaert et al., 1938, 1939, 1941). Chalmers et al. (1967) showed that in rabbits with the buffer nerves intact, blood pressure was maintained during moderate or severe, controlled arterial hypoxia. Similar conditions following section of the sinus and aortic nerves resulted in severe hypotension attributable to vasodilatation.

These observations may explain a phenomenon reported by Daly and Wright (1956). In anesthetized, untreated dogs, sarin caused hypotension, bradycardia and a prolonged vasoconstriction in the limbs and splanchnic area. These events were reversed by atropine. In dogs which had their baro- and chemosensory zones denervated, the

vasoconstrictor response still occurred but was less intense and sometimes quickly interrupted by a permanent non-muscarinic vasodilatation. Daly and Wright's comment was that although the baroreflexes may play some role in the initiation and maintenance of increased vasoconstrictor tone caused by sarin, other mechanisms are also involved. It is likely that chemoreflexes also contributed to the vasoconstrictor response in their normal dogs, since they were not artificially ventilated during this period. The vasodilatation observed in the denervated dogs and its possible relationship to the lack of baro- and chemoreflex capability remains unexplained and uninvestigated.

Cardiac Effects

The effects of anticholinesterases on the heart are reviewed by Holmstedt (1959) and Cullumbine (1963).

The most commonly observed cardiac effect of organophosphate poisoning is a slowing of heart rate. Bradycardia stems, in part, from a postsynaptic, muscarinic action at vagal nerve endings. Heymans et al. (1956) observed that ganglioplegic agents such as hexamethonium will partly alleviate the heart slowing and that the residual bradycardia after such treatment is eliminated by atropine.

These authors concluded that bradycardia was due solely to a peripheral action. Following sarin or TEPP injection into the dog, bradycardia persisted after section of the cervical vagus nerves. Furthermore, injection of sarin into the circulation of the perfused isolated head, connected to its trunk only by the vagus nerves, did not induce bradycardia (also see Polet and Schaepdryver, 1959). It appears, in the dog at least, that these agents do not directly

stimulate the brain-stem, vagal cardio-inhibitory center. In the rabbit, however, this center is stimulated, since bilateral micro-injection of sarin into the reticular formation induces bradycardia, completely reversed by bilateral vagotomy (Stewart and Anderson, 1964, 1968).

The cardiac effects of anticholinesterases are shown in electrocardiogram records as a change in frequency and conduction (Holmstedt, 1951). There is an initial slowing of the normal rhythm followed by progressive prolongation of the P-R interval. A-V conduction defects occur and finally complete heart block with disappearance of the P-wave and atrial contractions. The heart may escape from blockade to resume a very slow rate of beating.

The literature is vague as to whether the anticholinesterases impair myocardial contractility during untreated systemic poisoning. Such a phenomenon has been suggested to contribute to falling cardiac output associated with bradycardia (Holmstedt, 1951; Koelle, 1970). Isolated tissue studies do not generally support this hypothesis, however. Salerno and Coon (1949) found that the Langendorff rabbit heart has little specific sensitivity to HETP or TEPP. HETP, 1 mg, injected into the perfusing aortic cannula caused no change in heart activity; but profound cardiac changes occurred with 0.6 mg/kg HETP injected into the intact rabbit. Murtha and Wills (1964) exposed the isolated cat papillary muscle to bath concentrations of TEPP, DFP, sarin and tabun approximately ten times the expected blood concentration that follows injection of an LD_{50} in the intact animal. These agents had no effect on either spontaneous or electrically stimulated contractions. Preston and Heath (1968) injected repeated small doses of soman,

sarin or DFP into the perfusing cannula of the Langendorff rabbit heart. Although heart rate greatly decreased, contractile performance remained at its control value.

The organophosphates in high doses have a non-specific depressant effect on contractility of the Langendorff rabbit heart, and this is not atropine-reversible. Quilliam and Strong (1949) found that DFP injected into the perfusing aortic cannula caused a partial transient reduction in amplitude (0.25-2.5 mg), temporary stoppage followed by depressed amplitude (2.5-10 mg), permanent severe loss of amplitude (10-25 mg) or permanent stoppage (over 25 mg). Salerno and Coon (1949) reported similar findings with HETP and TEPP.

Wolthuis and Meeter (1968) reported a direct, deleterious action of DFP upon rat heart muscle, independent of its effect on cholinesterase. Anesthetized rats, given atropine and artificial ventilation, died from heart failure in 2, 3 or 8 hrs following subcutaneous injection of 16, 8 or 4 times the LD_{50} of DFP, respectively. The ECG voltage showed progressive diminution followed by gross irregularities in configuration shortly before death. Blood pressure and heart rate remained normal until the above-mentioned irregularities appeared. Oximes restored neuromuscular transmission but did not change the time of death. Exposure of the Langendorff rat heart for five minutes to DFP concentrations of 6 μg/ml or higher decreased both the ECG voltage and contractile force. The mechanism of DFP action or its importance to other species has not been elucidated.

METHODS

Experiments in Anaesthetized Rabbits

New Zealand White rabbits were anesthetized with a 50:50 mixture, by weight, of sodium thiopental and sodium pentobarbital (approx. total dose 40-50 mg/kg i.v.). To maintain surgical depth, small supplemental doses were given during the course of each experiment at times not critical to measurement of cardiovascular parameters. Intermittent positive pressure ventilation with oxygen was provided through a tracheal cannula (19 cm H_2O; 4 sec on, 4 off).

Systemic blood pressure, shown on a Beckman-Offner Dynagraph, was recorded from a cannulated carotid or femoral artery connected to a Statham pressure transducer. Heart rate was counted from oscillations in the pulse pressure trace. Electrocardiogram records were obtained using sternal paste electrodes.

Sarin, soman and DFP were stored as pure liquids in glass-stoppered tubes. These were placed in a desiccator in a fume cupboard. The agents were removed by lambda pipette and dissolved in physiological saline just prior to use. Other drugs were also dissolved in saline and dosage is expressed as weight of the salt per kilogram.

The toxicity of sarin, soman and DFP was initially tested in male white mice*. The stock of soman which was stored and utilized over a prolonged period was subjected to such tests about every three months. This was done to guard against potency loss caused by inadvertent water contamination and subsequent hydrolysis.

Isolated Rabbit Heart Experiments, Langendorff Method

Details of the perfusion apparatus are shown in Figure 1. Hearts were cannulated while immersed in ice-cold oxygenated McEwan's solution (1956)**. During aortic cannulation the semilunar valve was rendered incompetent by pushing the cannula past it and withdrawing it slightly. The heart with cannula was then attached to the perfusion column of McEwan's solution. In systole (an isometric contraction followed by incomplete ejection) the ventricle developed a pulse pressure over the column hydrostatic pressure. The pulse was detected by a calibrated pressure transducer on a side arm of the aortic cannula. The amplitude of the recorded pulse wave was taken as a measure of contractile performance.

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* Toxicity criteria were that the minimal LD_{100} of soman and sarin lay in the range of 65-75 µg/kg and 130-140 µg/kg respectively (i.v. tail vein injection; doses based on personal communications at the Defence Research Establishment Suffield). The LD_{50} of DFP was 2.4 mg/kg, (i.p.).

** Composition of McEwan's solution: NaCl 7.60 g, KCl 0.42 g, $CaCl_2$ 0.24 g, NaH_2PO_4 0.143 g, $NaHCO_3$ 2.10 g, dextrose 2.00 g, sucrose 4.50 g, H_2O 1,000 ml.

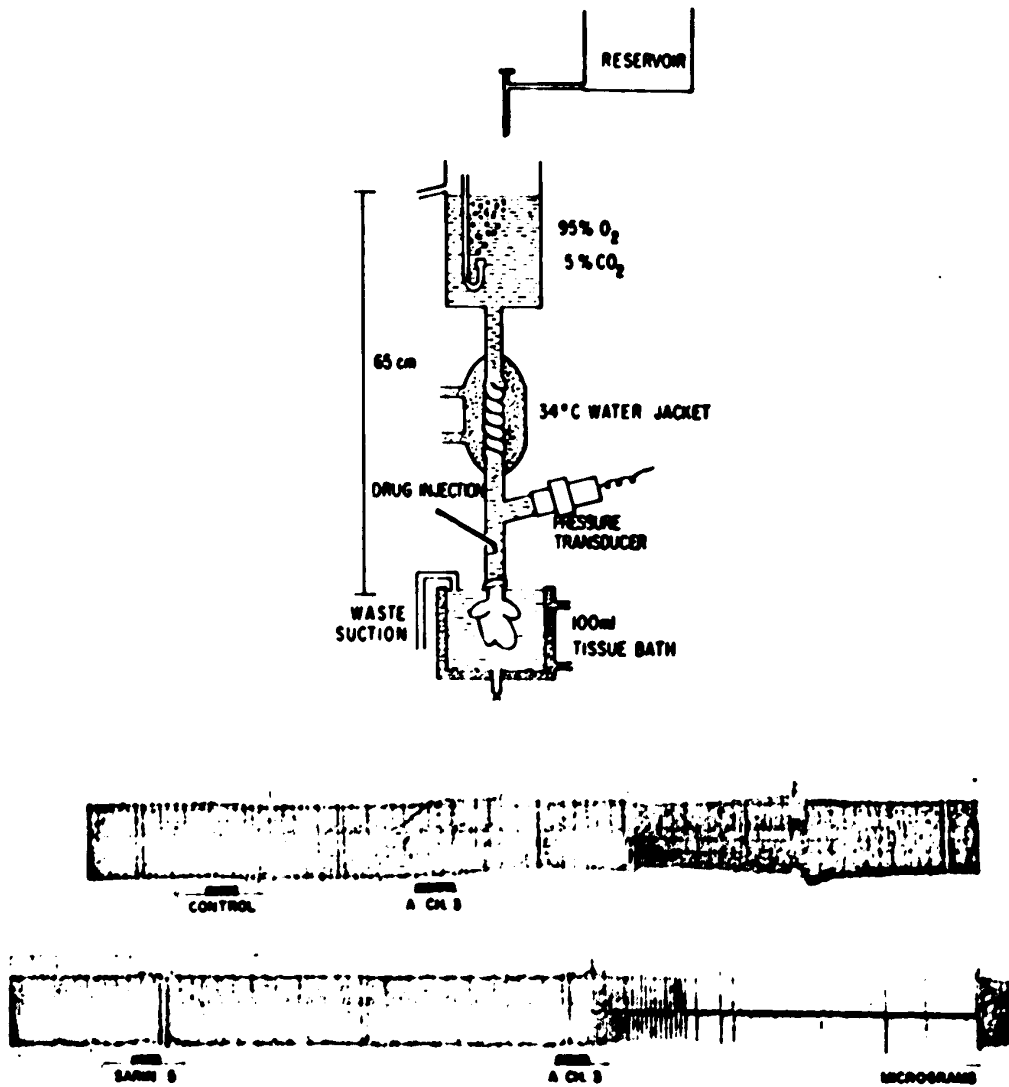

FIGURE 1: LANGENDORFF RABBIT HEART PERFUSION APPARATUS

The pulse-pressure trace shows the response to acetylcholine and its potentiation by sarin.

Vascular Resistance Measurements by Autoperfusion

Details of the autoperfusion circuit are provided in Figure 2. Subclavian arterial resistance changes were monitored by obtaining a pressure die-away curve every few minutes for the duration of an experiment. Typical curves are shown in Figure 7, page 45. Resistance changes were measured by plotting blood flow into the leg, at constant perfusion pressure. Flow at constant pressure was calculated from die-away curves in the following way.

The curves have an exponential decay (Grayson, 1969) and the slope dP/dt at any point is proportional to the outflow of blood from the manometer at that instant. A tangent was drawn by eye to each curve at a specified pressure, usually 65 mm Hg. The slope of the tangent was read directly from the calibrated dynagraph trace in mm Hg/min. It was known from the size of the mercury manometer bore that 100 mm Hg pressure fall represented 4.8 ml blood outflow. Therefore blood flow was calculated from the tangent slope as follows:

Flow = slope x bore characteristic
= X mm Hg/min x 4.8 ml/100 mm Hg
= 0.048X ml/min

This flow across the subclavian bed was driven by the instantaneous pressure head Pt - Pv (Pt = 65 mm Hg and Pv = venous pressure). Venous pressure (less than 1 mm Hg) and its fluctuations were a negligible fraction of the pressure head and ignored. Since this pressure head was a constant in any one experiment, resistance changes in the subclavian bed were inversely proportional to flow changes (an increased blood flow represented vasodilatation).

The major source of error was the subjective one in drawing tangents. It was advantageous therefore to obtain numerous curves, and give them shallow slope by using a fast paper speed, e.g. 2.5 mm/sec, and a wide manometer bore. Investigations which describe the several approaches to flow measurement by this principle are those by Girling, 1951; Nichol et al., 1951; Fukuyama and Stewart, 1961; and Grayson, 1969.

Cross-Perfusion of an Isolated Forelimb

The preparation used to measure vasomotor tone in an innervated but vascularly isolated foreleg is shown and explained in Figure 3. The following is a brief description of the surgical procedure to isolate the limb.

The skin of the right thorax was clipped free of hair, incised and peeled back to expose most of the pectoral musculature. The pectoralis major muscle was divided near its origin and spread away to expose the underlying clavicle, the pectoralis minor and subclavius muscles. These structures were divided and spread to expose the brachial plexus, subclavian artery and vein all in close proximity. This bundle of nerves and vessels was left intact. The skin covering the axilla and shoulder was incised and spread to expose the remaining musculature which was divided in the following order: Latissimus dorsi, serratus anterior, rhomboideus major and minor, levator scapulae, and trapezius. All remaining skin connections were divided. At this point the forelimb plus scapula remained together as a unit and except for the intact brachial plexus and subclavian vessels, had no connection with the thorax.

A Bovie electro-surgical unit was used for cutting muscle and coagulating small blood vessels. Larger blood vessels were divided between ligatures. The forelimb with scapula was held in position against the thorax by closing the skin incision with several towel clamps. This helped preserve warmth.

The extracorporeal tygon tubing (inside diameter 3/16 inch) was previously fitted with four polyethylene cannulas (PE 240), filled with heparinized saline and clamped shut. The donor rabbit was anesthetized with urethane (ethyl carbamate, 7.0 ml/kg of a 25% solution), heparinized (8.0 mg/kg; 100,000 units/mg), and the carotid artery and jugular vein cannulated as shown in Figure 3. The recipient rabbit was heparinized and the subclavian artery and vein were tied off and cannulated in that order. The tygon tubing was unclamped to allow perfusion of the leg. The lymphatic duct was cut to allow free drainage from the leg.

Blood flow was measured with a Zepeda extracorporeal electromagnetic probe connected to a Zepeda SWF-1M flowmeter.

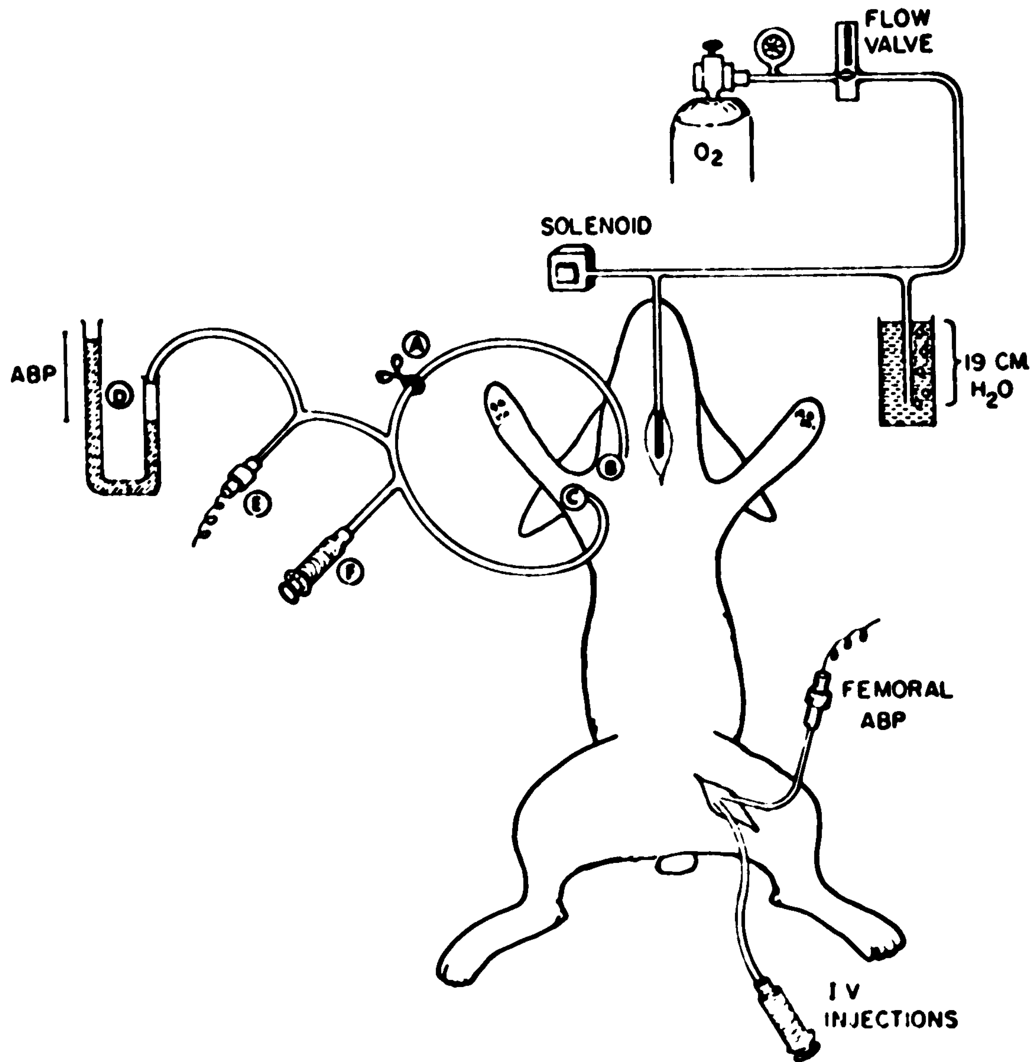

FIGURE 2: AUTOPERFUSION CIRCUIT TO MEASURE RESISTANCE IN THE SUBCLAVIAN ARTERIAL BED

When hemostat A is open, blood flows from the common carotid artery B into the subclavian artery C. Manometer D and transducer E register arterial pressure. When A is clamped shut, mercury column D provides a falling pressure head driving blood into C. Transducer E registers a pressure die-away curve. When systemic pressure (ABP) is low, syringe F is charged with blood and used to pressurize the system. Blood flow at constant pressure head is calculated from the die-away curves.

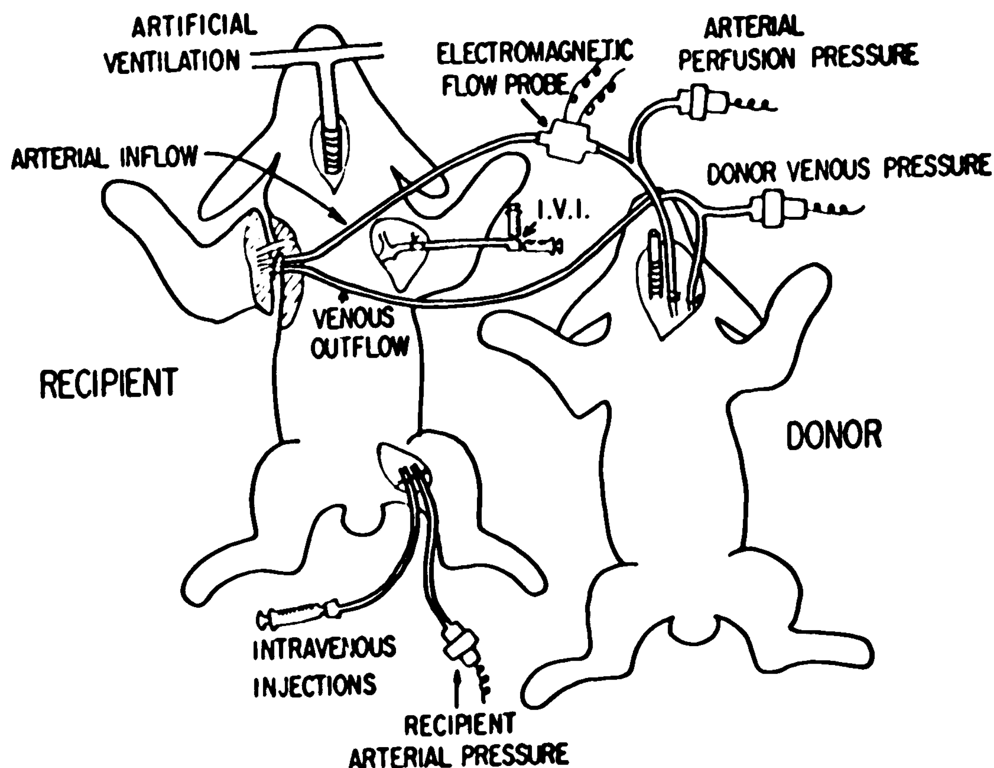

FIGURE 3: METHOD OF CROSS-PERFUSING AN INNERVATED LEG

The recipient's right forelimb is perfused from the carotid artery of a donor rabbit and venous blood is returned to the donor jugular vein. The brachial nerve plexus is the sole connection between recipient and limb. Vascular resistance in the leg (determined by perfusion flow rate and donor systemic pressure) reflects the recipient's vasomotor tone. The leg only demonstrates neuronally mediated effects of drugs injected into the recipient. A drug injected intravertebrally (I.V.I.) in appropriately small amounts affects systemic pressure and vasomotor tone only by action in the brain-stem.

Technique of Intravertebral Injection

It was the objective in this preparation to inject organophosphate cholinesterase inhibitor into the vertebral arterial supply of the brain-stem. This was done by means of a cannula inserted into the left subclavian artery. Its tip, directed toward the heart, was positioned a few millimeters distal to the point at which the left vertebral artery branches off the subclavian. In close proximity to the vertebral artery there are three other subclavian side branches which must be tied off. Prior to cannulation, therefore, the thyrocervical, costocervical, and internal thoracic arteries were exposed and ligated to assure that drug injection passed only into the vertebral artery. This required the following procedure.

The left subclavian artery and vein were exposed in a manner similar to that described in the previous section (isolation of right forelimb). The left sternocleidomastoid, sternohyoid and sternothyroid muscles were severed near their origin on the sternum. The sternum was split along its length from the clavicular notch to the articulation of the fourth rib. This split was biased to the left side of the sternum so that when spreaders were inserted it was the upper left portion of the thoracic cavity that was accessible. The thymus gland was carefully removed, all vascular connections being divided between ligatures. The internal and external jugular veins were severed between ligatures close to their junction with the subclavian vein in forming the left anterior vena cava.

That portion of the subclavian artery that gives off the vertebral artery lies in a position dorsal and rostral to the vena cava. In close apposition with both vessels is a heavy and diffuse layer of

connective and lymphatic tissue which lies in a position ventral to the subclavian artery. This layer was divided between several pairs of ligatures to expose the subclavian artery and its branches. All branches except the vertebral were tied off.

The opening in the thoracic cavity enabled exposure of the right vertebral artery which was then tightly ligated. This was done so that a left intravertebral drug injection would affect both sides of the brain-stem. Normally each vertebral artery provides a strictly unilateral stream in the basilar artery and perfuses only the ipsilateral half of the brain-stem (McDonald and Potter, 1951).

Finally the left subclavian artery was cannulated, ready for drug injection. The volume of the cannula was 0.25 ml. Each drug dose was made up to a volume of 0.2 ml and infused into the cannula at 0.005 ml/sec. It was immediately washed into the vertebral artery at the same rate with 0.35 ml saline.

Measurement of Cholinesterase Activity

The cholinesterase activity of brain tissue was determined by a method described by Aprison et al. (1954) and Metz (1958). The addition of acetylcholine to an active solution of brain homogenate containing phenol red indicator results in acetic acid release, a fall in pH and a color change from red to yellow. The principle of the method was to titrate such a reaction mixture with dilute sodium hydroxide to keep pH at 7.38. During this titration the color of the reaction mixture was kept close to the red color of a standard i.e. a homogenate-phenol red solution buffered to pH 7.38. The rate of sodium hydroxide addition reflected the rate of acetylcholine hydrolysis.

Brain-stems to be analyzed consisted of that tissue lying between a transverse section about 2 mm caudal to the obex and another at the rostral margin of the pons. Cerebellar peduncles and other excess tissue were trimmed away. The brain-stem was washed in saline, blotted, weighed, frozen and stored at -20° C until analysis the following day.

The tissue was homogenized* in ice-cold 0.5% NaCl and further diluted with 0.5% NaCl until the final tissue concentration was 1.8 mg/ml. An aliquot of 3.6 ml of this solution in a test tube was warmed to 36° C in a water bath and 10 drops of 0.02% phenol red added. Dilute NaOH, 3.55×10^{-3} M, was added from an Agla micrometer syringe until the color was equal to that of the color standard** at pH 7.38. At zero time 0.4 ml of 5.0×10^{-3} M acetylcholine bromide was added and the hydrolysis then proceeded at 36° C. For 10 minutes the mixture was titrated with the dilute NaOH and stirred to maintain the light red color shown by the standard. The amount of NaOH added at the end of 10 minutes was recorded in microlitres (μℓ). For each brain-stem four or more aliquots were titrated and the hydrolysis rate obtained as an arithmetic mean.

Before cholinesterase activity could be calculated, the following contributing factors were accounted for.

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* Virtis "23" homogenizer, 10 min at high speed.

** The color standard consisted of 3.6 ml homogenate solution, 10 drops of phenol red, and 0.6 ml of a blood pH reference buffer. It kept a stable light red color at pH 7.38.

Distilled-deionized water was used for all solutions mentioned.

a. Non-Specific Acid Release From The Tissue And The Slow Acid Release Shown By A Solution Of Phenol Red And Water.

The sum contribution of these was measured for every tissue as follows. A homogenate solution, 3.6 ml, containing 10 drops of phenol red was brought to the color of pH 7.38 and during 50 minutes at 36 °C, the amount of NaOH added to maintain this pH was measured.

b. Non-Enzymatic Hydrolysis Of Acetylcholine (ACh) During Ten Minutes And The Acidity Of 0.4 ml ACh Solution Added At Zero Time.

This total quantity of acid was measured by adding 0.4 ml of ACh solution to 3.6 ml water plus 10 drops of phenol red, previously brought to the color of pH 7.38. The amount of NaOH required to raise pH to 7.38 and maintain it through 10 minutes was measured. It was assumed that slow acid release by phenol red plus water contributed to this measurement. It was therefore necessary to measure this factor separately.

c. Slow Acid Release By Phenol Red And Water.

Ten drops of phenol red solution were added to 3.6 ml water at 36° C, and the solution brought to the color of pH 7.38. The amount of NaOH added to keep pH at 7.38 was measured for 30 minutes.

Factors b and c were measured in numerous tissue analyses and expected and found to be relatively constant. The means of these values were accepted as correction constants for all cholinesterase activity measurements.

The acid release measured in all titrations was recorded in terms of µℓ NaOH neutralized per 10 minutes. This was converted to common rate units of meq ACh hydrolyzed/mg tissue/min by the following equation.

$$\text{Rate Acid Release} = \frac{10\ \text{min}}{\text{acid release}} \times \frac{1}{\text{mg tissue per aliquot}} \times \frac{1}{\text{titration time}} \times \frac{\text{meq NaOH}}{\mu\ell\ \text{NaOH}}$$

$$= Z\ \mu\ell\ \text{NaOH} \times \frac{1}{6.48\ \text{mg}} \times \frac{1}{10\ \text{min}} \times \frac{3.55\times10^{-6}\ \text{meq}}{\mu\ell}$$

$$= 0.054783 \times 10^{-6}\ Z\ \ \text{meq NaOH/ mg tissue/min}$$

$$= 0.054783 \times 10^{-6}\ Z\ \ \text{meq ACh Hydrolyzed/mg tissue/min}$$

The method for calculation of cholinesterase activity, and its derivation are on page 99 (Appendix).

The micrometer syringe could be read to the nearest 0.2 $\mu\ell$. It is estimated that the minimum amount of 3.55 x 10^{-3} M NaOH that produces a visible color change lies in the range of 1.5-2.5 $\mu\ell$. The subjective error in reaching the titration endpoint is reduced by making repeated titrations. This error becomes less important with tissue aliquots of high activity. A disadvantage with highly active tissues is that the NaOH added during titration e.g. more than 200 $\mu\ell$ for normal medulla, dilutes the color intensity compared with the standard, making endpoint judgement more difficult. Adding more phenol red is not advised since even one added drop turns the color toward yellow. An approach to this problem is to use a second color standard containing less phenol red.

RESULTS

Non-Muscarinic Hypotension in Ventilated Rabbits

The hypotensive effect of intravenous soman in atropinized, ventilated rabbits is shown in Figure 4 in which mean arterial pressure is plotted as percent of control. Actual control values of arterial pressure (before atropine and soman) ranged from 92 to 122 mm Hg. Hypotension increases with soman dosage, indicating that response is probably related to dose level. The minimum effective dose would appear to lie between 25 and 50 µg/kg.

This hypotension was not caused by cardiac slowing since the mean heart rate just prior to the second injection of atropine was 99.6 ± 3.5* percent of its value before soman. Moreover, atropine was unable to antagonize it.

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* Standard error of the mean

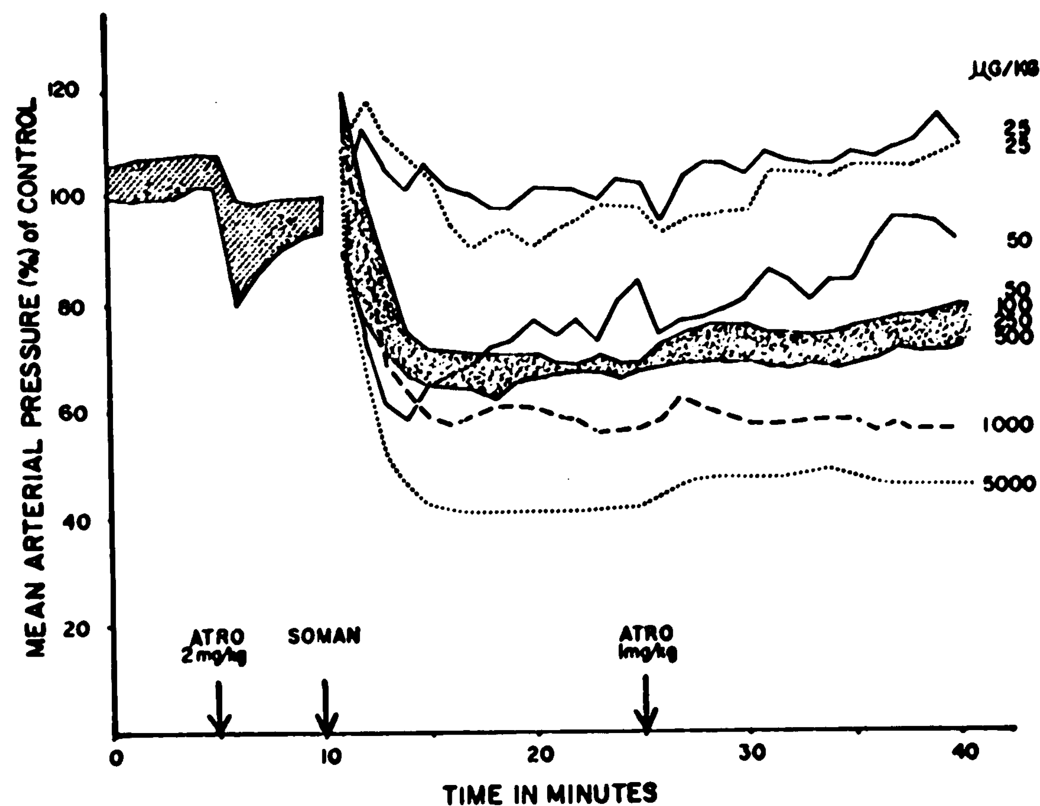

FIGURE 4: HYPOTENSION INDUCED BY VARIED DOSES OF SOMAN

Each broken line is the systemic pressure (% control) of one ventilated rabbit given the dosage indicated on the right. The shaded area before soman injection encloses the upper and lower limits of all experiments.

In eight ventilated atropinized rabbits (Figure 5), sarin or soman was intravenously infused at the rate of 400 μg/min for 200 minutes. DFP was infused at 1500 μg/min in four rabbits. Systemic arterial pressure was recorded over four hours and survivability estimated. Each compound induced hypotension within a few minutes. It was of interest that when the organophosphate infusion ended, sarin or DFP-poisoned rabbits rapidly improved their blood pressures. Such a recovery was absent or minimal in the soman experiments. Control rabbits subjected to similar conditions of anesthesia, atropinization and artificial ventilation* maintained normal blood pressure.

All rabbits but one survived the four-hour duration of the experiment. In one DFP-injected rabbit which died, it is believed that a maintenance dose of sodium thiopental helped precipitate death. Continuous ECG records taken in all other DFP and the sarin experiments showed no measurable change in amplitude over the four-hour period.

In these constant-infusion experiments there was a gradual decrease in heart rate which contributed to the hypotension induced by organophosphate. Mean control heart rate was 258 ± 9 beats per minute in eight experiments.** This value decreased to 215 ± 6 (at the end of 2 hrs), to 203 ± 5 at 3 hrs, and was 210 ± 10 beats per minute at the end of 4 hrs.

In the sarin experiments heart rate increases of 9, 9, 25 and 37 percent contributed to the blood pressure increase at the end of the experiment.

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* Control rabbits were curarized to make them dependent on the ventilation system (tubocurarine chloride pentahydrate, 0.3 mg/kg with maintenance doses of 0.1 mg/kg throughout four hours).

** Mean and standard error from 3 sarin, 3 DFP and 2 soman experiments.

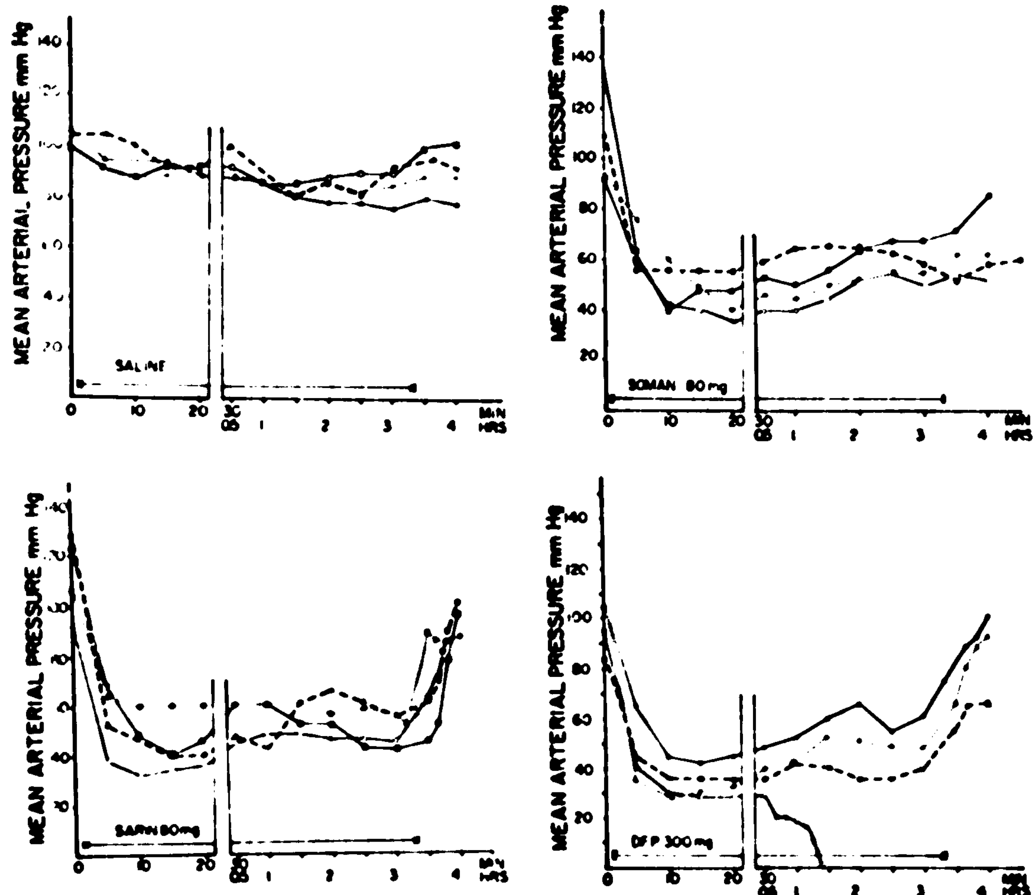

FIGURE 5: HYPOTENSION CAUSED BY CONSTANT RATE INTRAVENOUS ORGANOPHOSPHATE INFUSION

Each broken line represents mean arterial pressure of one ventilated, atropinized rabbit. Atropine sulphate, 1 mg/kg i.v. was injected at the start of infusion and every half-hour to the end of 4 hours. Total infusion volume was 50 ml delivered over 200 minutes.

Langendorff Isolated-Heart Experiments

In each experiment heart rate and contractile performance were measured for four hours in an isolated heart. Soman dissolved in McEwan's solution was injected through a fine catheter into the perfusing aortic cannula at 333 μg/min. Soman concentration in the coronary perfusate ranged about a mean value of 25 μg/ml.* All hearts were atropinized with atropine sulphate added to the perfusion reservoir (0.1 μg/ml).

In Figure 6, mean pulse pressure amplitude and heart rate of six hearts are plotted as a percent of their initial value before soman infusion. Both parameters remained within 15 percent of their initial control value, during the four-hour period.

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* Obtained by dividing drug injection rate by mean coronary flow rate. Perfusate concentration varied inversely with coronary flow rate. Coronary flow rates, measured in each heart, ranged from 10.0 to 16.7 ml/min ($\bar{x}$ = 13.3, S.E. = 1.1, ml/min).

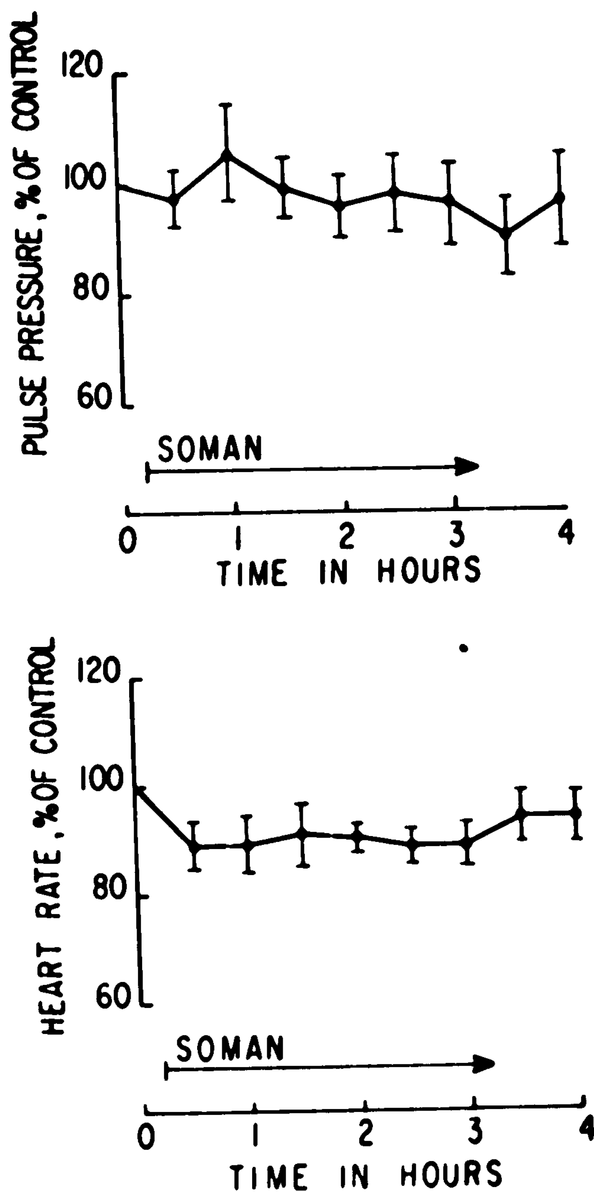


FIGURE 6: **EFFECT OF SOMAN ON THE ATROPINIZED LANGENDORFF RABBIT HEART**

Mean contractility and heart rate are plotted as percent of control values before infusion ($SE_{\bar{x}}$ is shown). Soman concentration in the perfusate ranged about a mean of 25 μg/ml (see text). Original data are in Tables VII and VIII, Appendix.

Autoperfusion Experiments: Resistance Changes in the Intact Subclavian Vascular Bed

In Figure 7 sample portions of the record from an autoperfusion experiment show the die-away curves before and after soman injection and the systemic pressure changes which accompanied them. The increase in slope of the curves after soman shows increased blood flow rate into the forelimb i.e. a decreased resistance to flow. Blood flow driven by a constant pressure head was calculated from each die-away curve and plotted along with systemic arterial pressure and heart rate as experiment A, Figure 8, to be discussed below.

Autoperfusion experiments demonstrated that soman has both muscarinic and non-muscarinic vasodilator effects. Four of these experiments are shown in Figures 8 and 9 (experiments A to D).

In experiment A the increase in blood flow following soman injection was about 160 percent. This reflected a proportional decrease in vascular resistance and largely accounted for the low systemic arterial pressure of 40 mm Hg. The vasodilatation was partly reversed by further atropine injection which also increased heart rate and blood pressure. Blood flow increased after section of the brachial nerve plexus, indicating that some degree of sympathetic vasomotor drive was still left after soman. Further dilatation following intra-arterial injection of a nembutal-pentothal mixture toward the leg demonstrated the locally mediated (basal) vasomotor tone in the limb.

In experiment B the non-muscarinic vasodilatation caused by 2.5 mg/kg soman was augmented by a second dose. It is unlikely that the subsequent atropine injection caused the sudden increase in resistance, since blood pressure did not reflect the change. A thrombus lodged in

some arterial branch in the leg could have been responsible.

In experiment C, Figure 9, a large component of the vasodilatation and hypotension following soman injection was muscarinic since atropine substantially alleviated these effects.

It was found that smaller doses of soman induce non-muscarinic vasodilatation. In experiment D the depressant effects were well established by the time a total dose of 100 μg/kg was infused. However, in two experiments not shown, 100 μg/kg injected as a single intravenous dose had no depressant effects on blood pressure or resistance in atropinized rabbits. Non-muscarinic vasodilatation and hypotension comparable to that already shown were observed in two out of three experiments at 200 μg/kg soman and, at higher doses of 300-1000 μg/kg, in all of six further experiments.

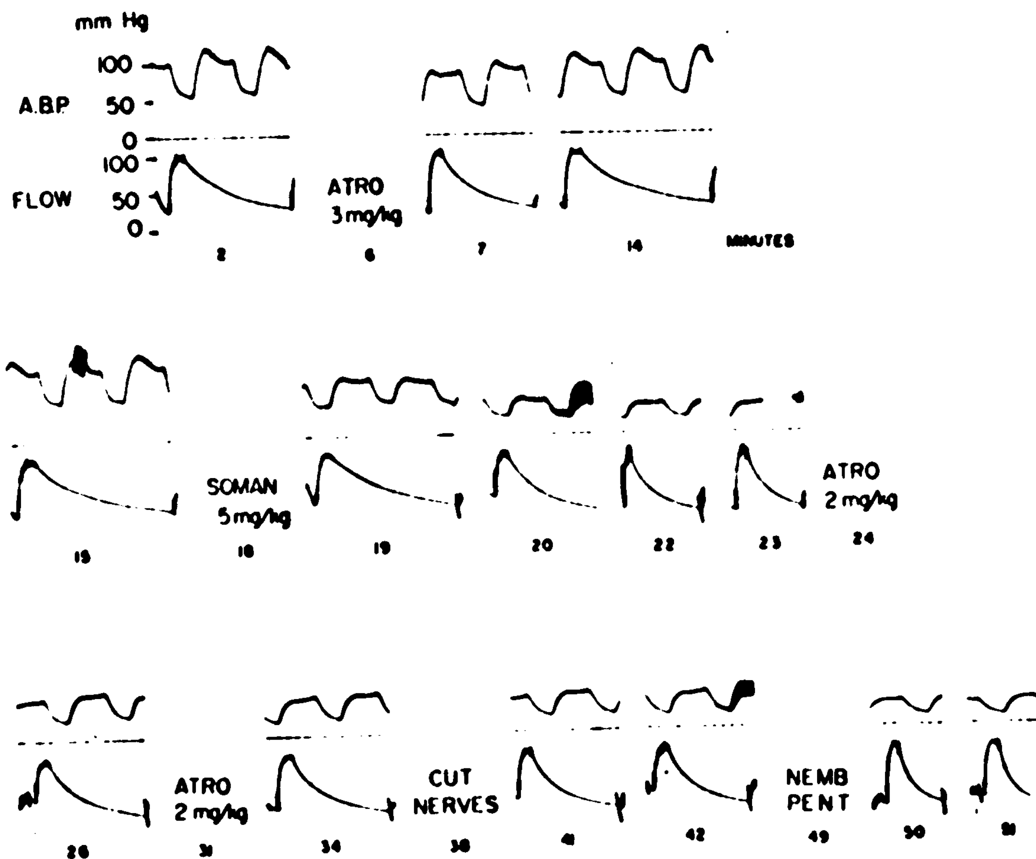


FIGURE 7: AUTOPERFUSION OF THE SUBCLAVIAN VASCULAR BED

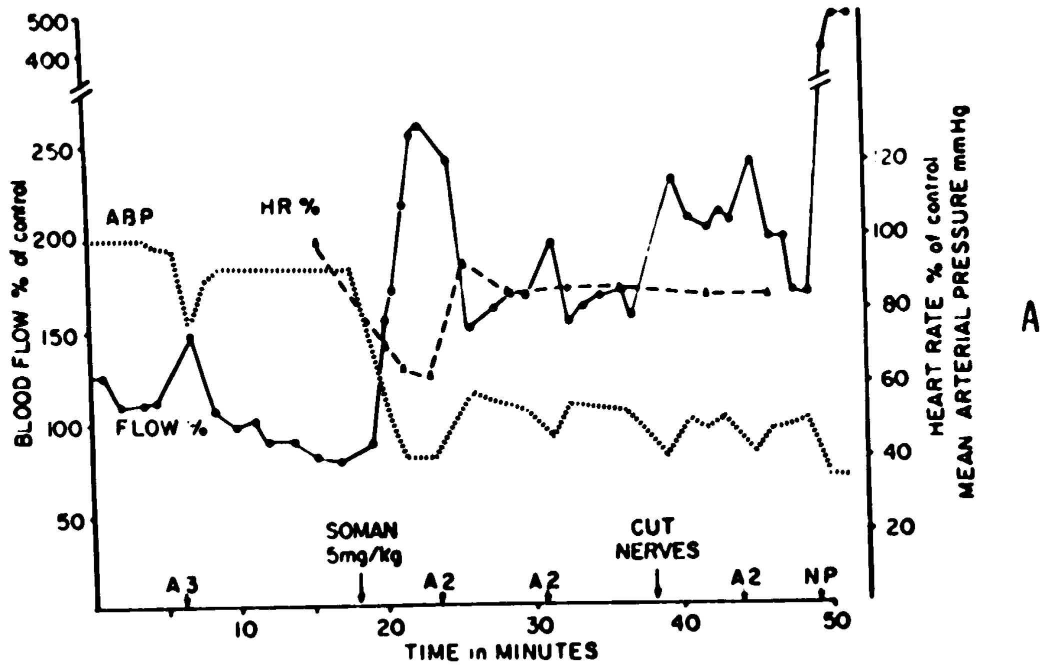
The increase in slope (dP/dt) of the die-away curves after soman reflects a decreased resistance to blood flow (arteriolar dilatation) and accounts for the fall in systemic blood pressure. Cyclic blood pressure excursions are due to artificial ventilation.

FIGURES 8 and 9: AUTOPERFUSION EXPERIMENTS:
RESISTANCE CHANGES IN THE INTACT SUBCLAVIAN VASCULAR BED

Each graph shows arterial pressure, heart rate and vascular resistance changes in one artificially ventilated rabbit. Forelimb blood flow, percent of control, is driven by a constant pressure head (control flow is the mean of all pre-soman flow values). Increased flow indicates vasodilatation. Soman was injected intravenously.

A1, A2 etc. - intravenous injection of atropine sulphate, 1 mg/kg or 2 mg/kg

NP - intra-arterial injection of a 50:50 nembutal-pentothal mixture, 30 mg toward the leg.



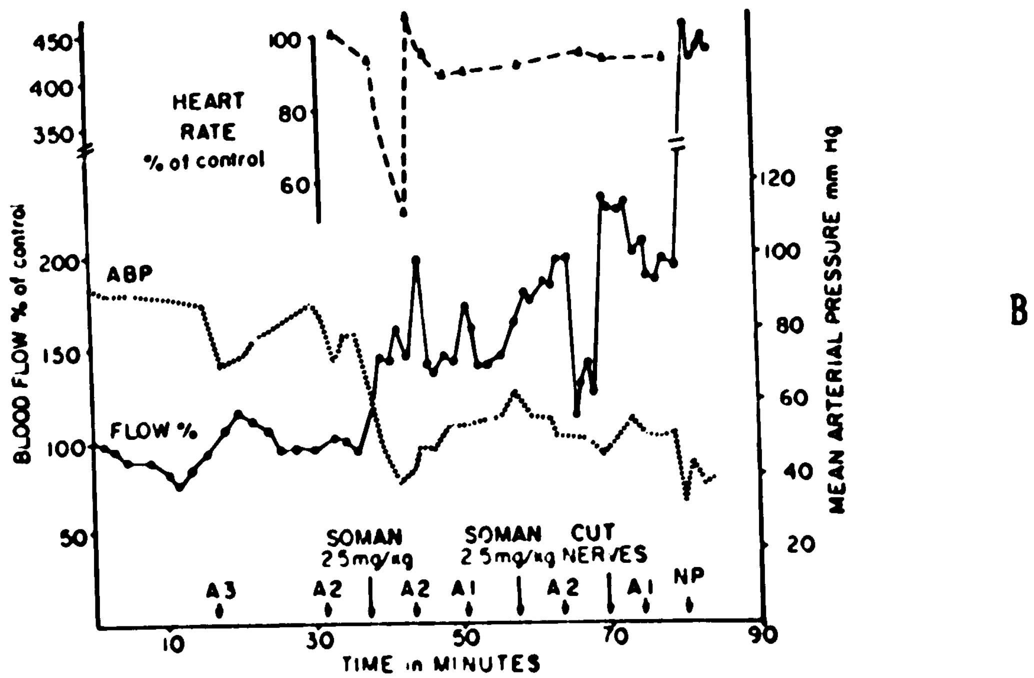
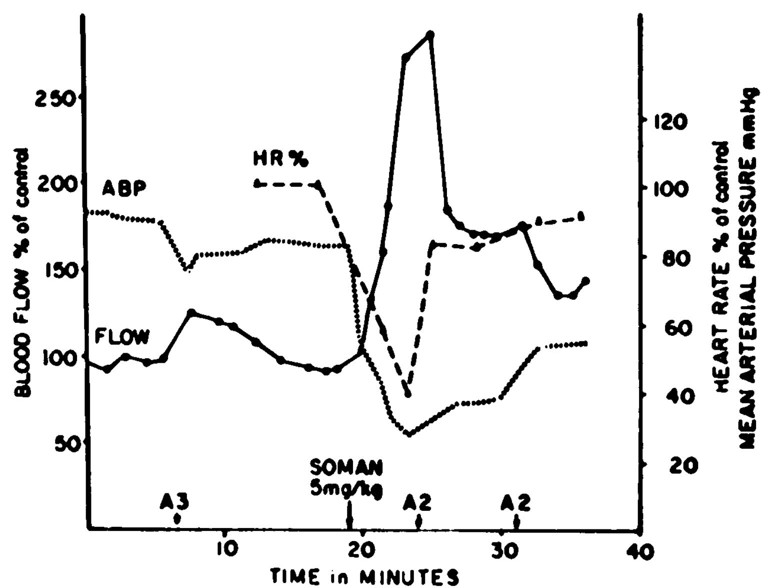


FIGURE 8: EXPERIMENTS A AND B



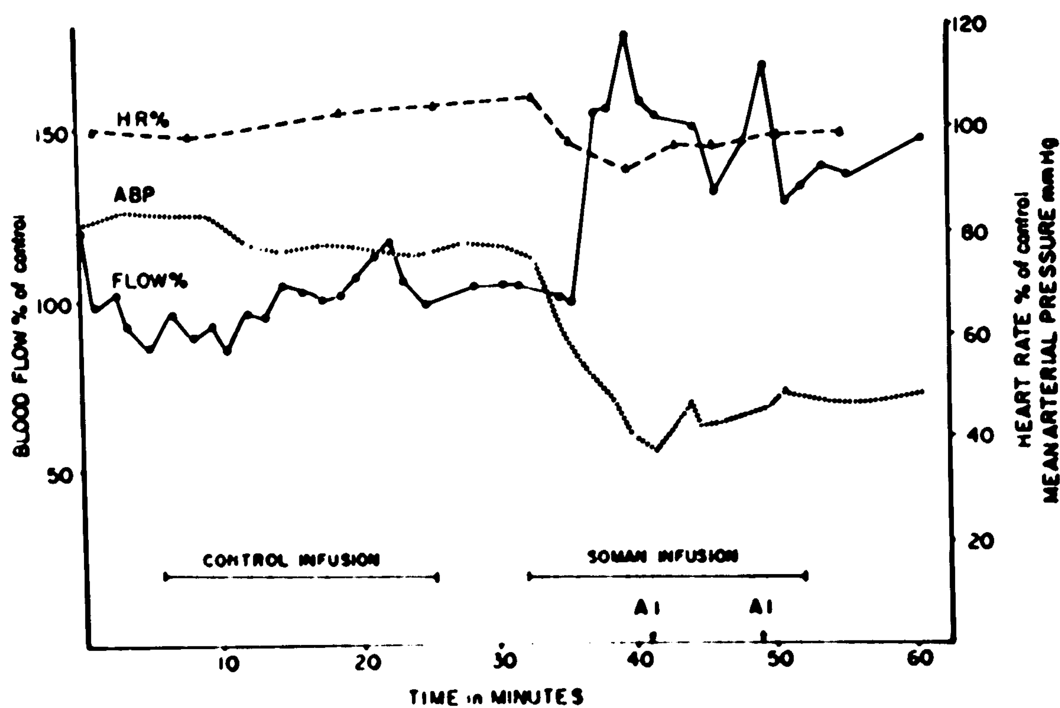


FIGURE 9: EXPERIMENTS C AND D

In experiment D soman was infused at 25 μg/kg/min by motor driven syringe:
Control Infusion = 4 mg/kg atropine in 10 ml saline
Soman Infusion = 4 mg/kg atropine + soman in 10 ml saline

Resistance Changes in the Autoperfused Denervated Forelimb

Four of the autoperfusion experiments (Figures 10 and 11) show the effect of soman on basal vasomotor tone. This is defined as the locally mediated, myogenic vascular tone which remains following denervation (in this case, denervation of the perfused forelimb by section of the brachial nerve plexus).

In experiments E, F and G, following injection of 5 mg/kg soman, heart rate and arterial pressure of the rabbit fell. This was accompanied by vasodilatation in the denervated leg (blood flow increased though driven by a constant pressure head). It was therefore demonstrated that soman can depress basal vasomotor tone.

In each of these experiments further injection of atropine was followed by recovery of vascular resistance to a level equal or close to that before soman. In each case there was some recovery of heart rate and blood pressure.

In experiment H, following infusion of atropine soman was infused at 270 μg/kg/min for 22 minutes. Neither blood flow nor heart rate changed significantly, yet arterial pressure fell from 75 to 50 mm Hg.

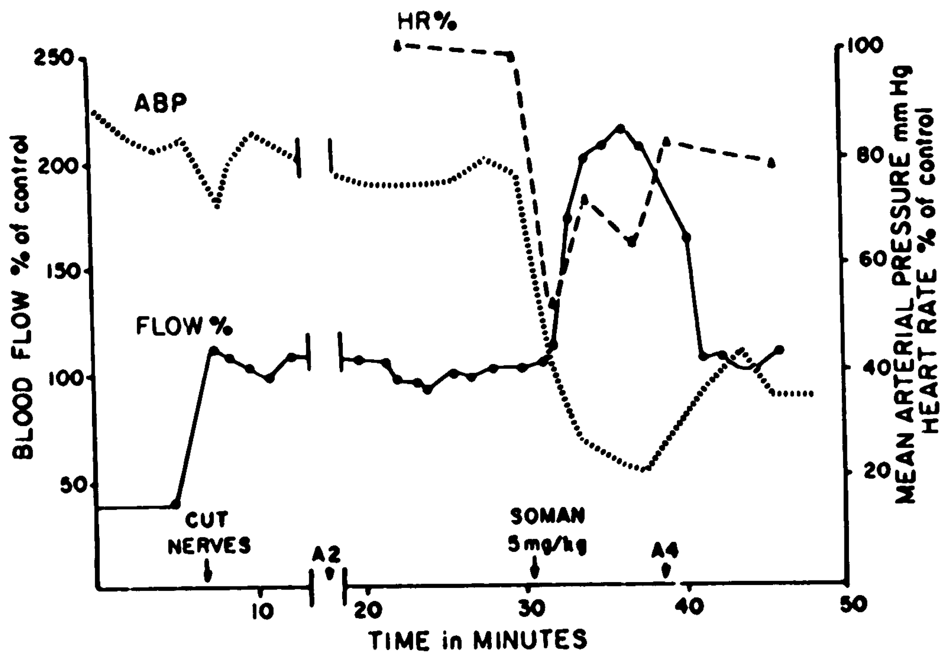
In the above experiments it was most significant that hypotension remained though depression of basal vasomotor tone was well antagonized by atropine.

FIGURES 10 and 11: AUTOPERFUSION EXPERIMENTS: RESISTANCE CHANGES IN THE DENERVATED SUBCLAVIAN VASCULAR BED

Each graph shows arterial pressure, heart rate and forelimb basal vascular resistance in one ventilated rabbit. Increased blood flow indicates a proportional vasodilatation. Soman was injected intravenously.

A1, A2 etc. - intravenous injection of atropine sulphate, 1 mg/kg or 2 mg/kg.

NP - intra-arterial injection of a 50:50 nembutal-pentothal mixture, 30 mg toward the leg.



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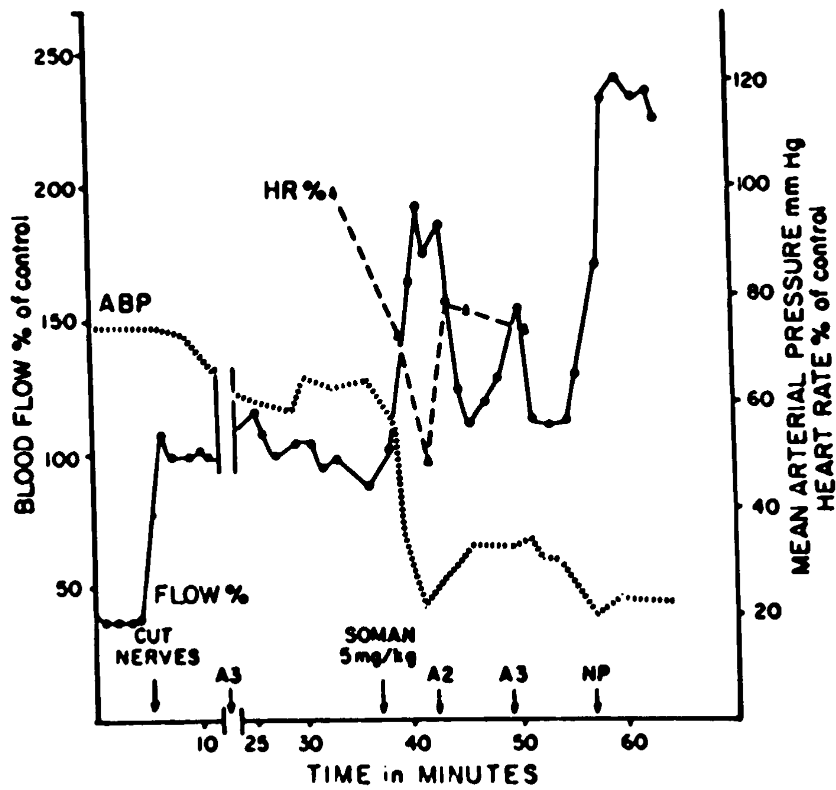
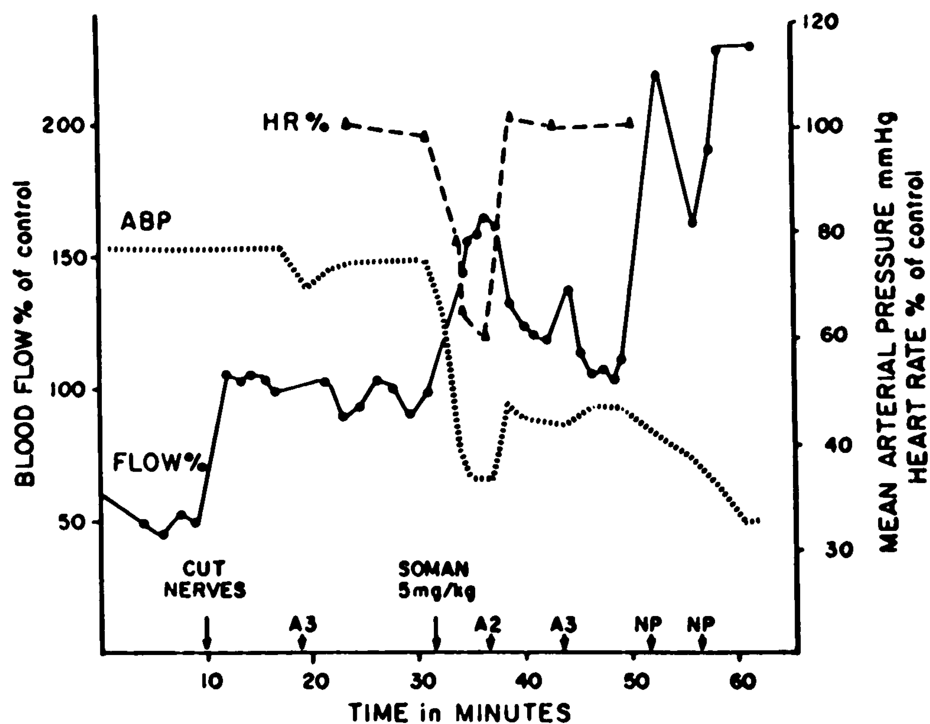
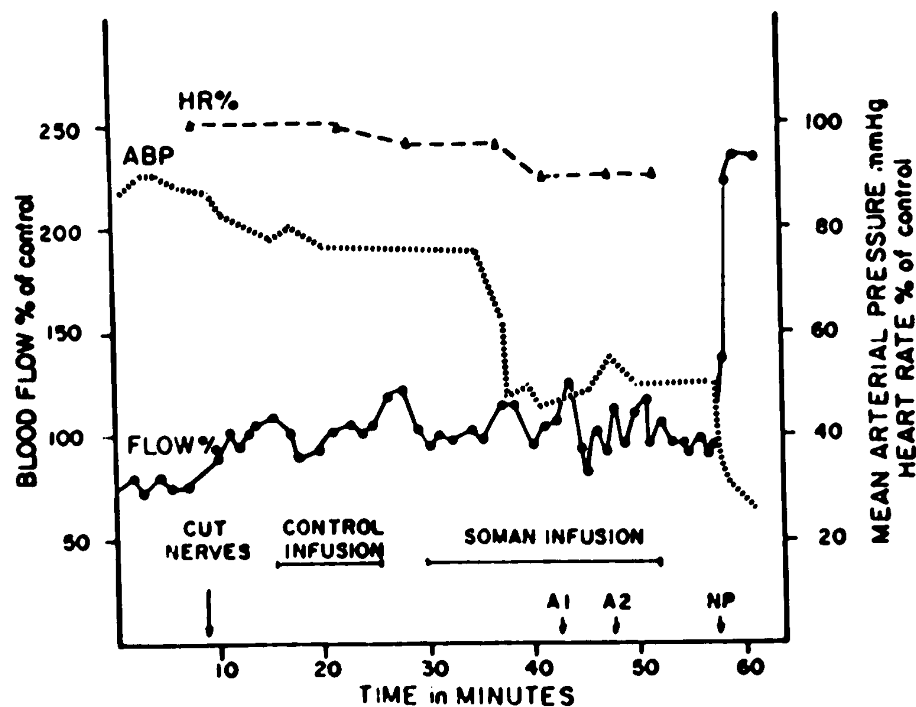


F

FIGURE 10: EXPERIMENTS E AND F



G



H

FIGURE 11: EXPERIMENTS G AND H

In experiment H soman was infused at 270 µg/kg/min with a motor-driven syringe.
Control Infusion = 2 mg/kg atropine in 10 ml saline
Soman Infusion = 2 mg/kg atropine + soman in 10 ml saline

Cross-Perfusion Experiments

The effects of soman on the neuronal elements of the vasomotor pathway were investigated using the cross-perfusion preparation shown in Figure 3. Since the isolated, innervated forelimb was free from direct drug effects, resistance changes in the limb reflected changes in vasomotor output of the poisoned recipient rabbit. Nine of these experiments, experiments A to J, have been grouped in Figures 12 to 15 and deal only with the effects of soman injected intravenously into the recipient rabbit.

In experiments A, B, and C, soman injected into the atropinized recipient rabbit caused increased forelimb blood flow at constant perfusion pressure (PPL). This vasodilatation indicates depressed vasomotor output and explains the concomitant fall in arterial blood pressure (ABP). In each case further treatment with atropine failed to reverse these events. It was concluded that soman had a non-muscarinic depressant effect in the neuronal vasomotor pathway.

This non-muscarinic phenomenon did not occur predictably at lower dose levels of 50 μg/kg or 100 μg/kg. In experiments D, E, F and G, any non-muscarinic vasodilatation was negligible and in the latter two cases the non-muscarinic hypotension was of a minor degree.

Soman also had a muscarinic depressant effect on neuronal elements of the vasomotor pathway. In experiment D, the dose of atropine following 50 μg/kg soman caused a rapid decrease in blood flow, indicating a recovery of vasomotor tone. In experiment F the vasodilatation following 3 mg/kg soman was largely reversed by atropine. In experiment H,

15 μg/kg soman in an unatropinized rabbit caused vasodilatation which was largely reversed by atropine.

A third observation was that a large dose of soman augmented hypotension and vasodilatation in rabbits already poisoned well beyond total cholinesterase inhibition. Near the end of experiments E, G and H, soman doses of 2 or 3 mg/kg caused a large non-muscarinic vasodilatation and concomitant blood pressure fall despite the fact that the rabbits had previously received 250, 300 and 215 μg/kg soman, respectively.

It was often observed that soman injected into an atropinized ventilated rabbit caused a brief, mild blood pressure increase before hypotension set in. This was evident in the blood pressure data of Figure 4 and occurred in cross-perfusion experiment B. The cross-perfusion experiments showed that a prolonged pressor effect could be unmasked if the rabbit was in a state of hypotension from previous soman injection. This is seen in the blood pressure rise following 200 μg/kg soman injection in experiments E and G. In the experiment of Figure 16, 200 μg/kg soman caused blood pressure to remain elevated for about 15 minutes. It is clear that vasomotor excitation mediated the increase in pressure since the isolated leg vasoconstricted during this phase. Part of the Dynagraph trace of this same experiment (experiment J, Figure 15) shows the pressor response and another example of the overriding depressant effect of 3 mg/kg soman, previously discussed. In two cross-perfusion experiments not shown, 200 μg/kg soman elevated blood pressure and caused vasoconstriction for 8 and 10 minutes in hypotensive, atropinized rabbits which had previously received 55 μg/kg soman.

The pressor effect of soman was demonstrated in five rabbits that previously received ≥50 μg/kg soman (experiments E, G and J). The overriding non-muscarinic depressor effect of soman occurred in four rabbits that previously received ≥215 μg/kg soman (experiments E, G, H and J). Table I shows the lower brain-stem cholinesterase activity of five rabbits given soman doses ranging from 20 to 50 μg/kg.
It is evident that in the cross-perfusion experiments, 50 μg/kg soman would inhibit more than 98 percent of lower brain-stem cholinesterase activity in the recipient.

FIGURE 12 - 15: CROSS-PERFUSION EXPERIMENTS

The dynagraph records on the following pages are obtained from the experimental preparation shown in Figure 3.

ABP - recipient rabbit's arterial pressure

FLOW - blood flow into the recipient's isolated forelimb

PPL - perfusion pressure driving blood into the isolated forelimb, i.e. the donor's arterial pressure

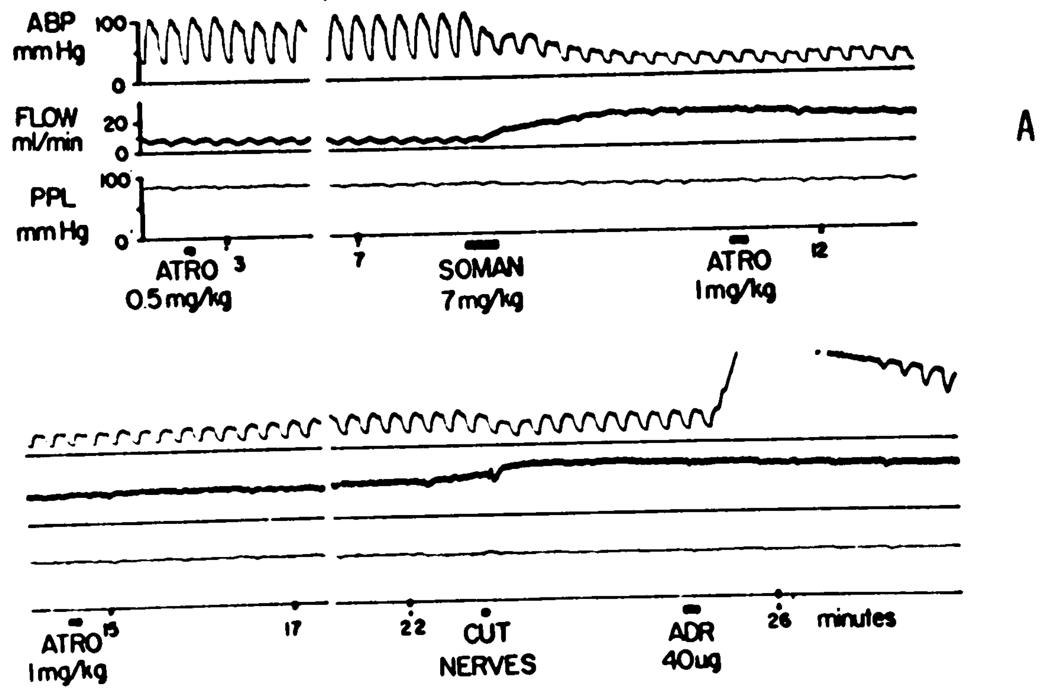
HR - recipient's heart rate, beats per minute

ATRO - atropine sulphate

ADR - adrenaline hydrochloride

The donor rabbit is significant only as a source of perfusion pressure, PPL. All drugs were injected intravenously into the artificially ventilated recipient rabbit. Increased FLOW at constant PPL represents vasodilatation in the forelimb. The foreleg is subject to neuronally mediated, but not direct drug effects.

A



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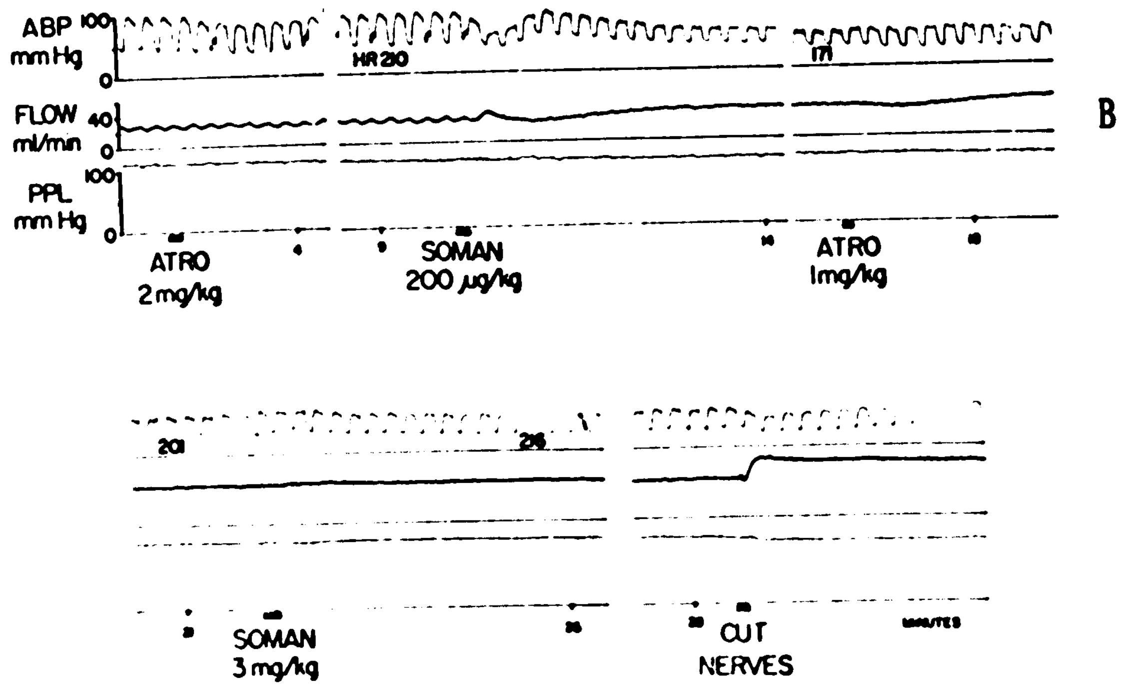


FIGURE 12: EXPERIMENTS A AND B

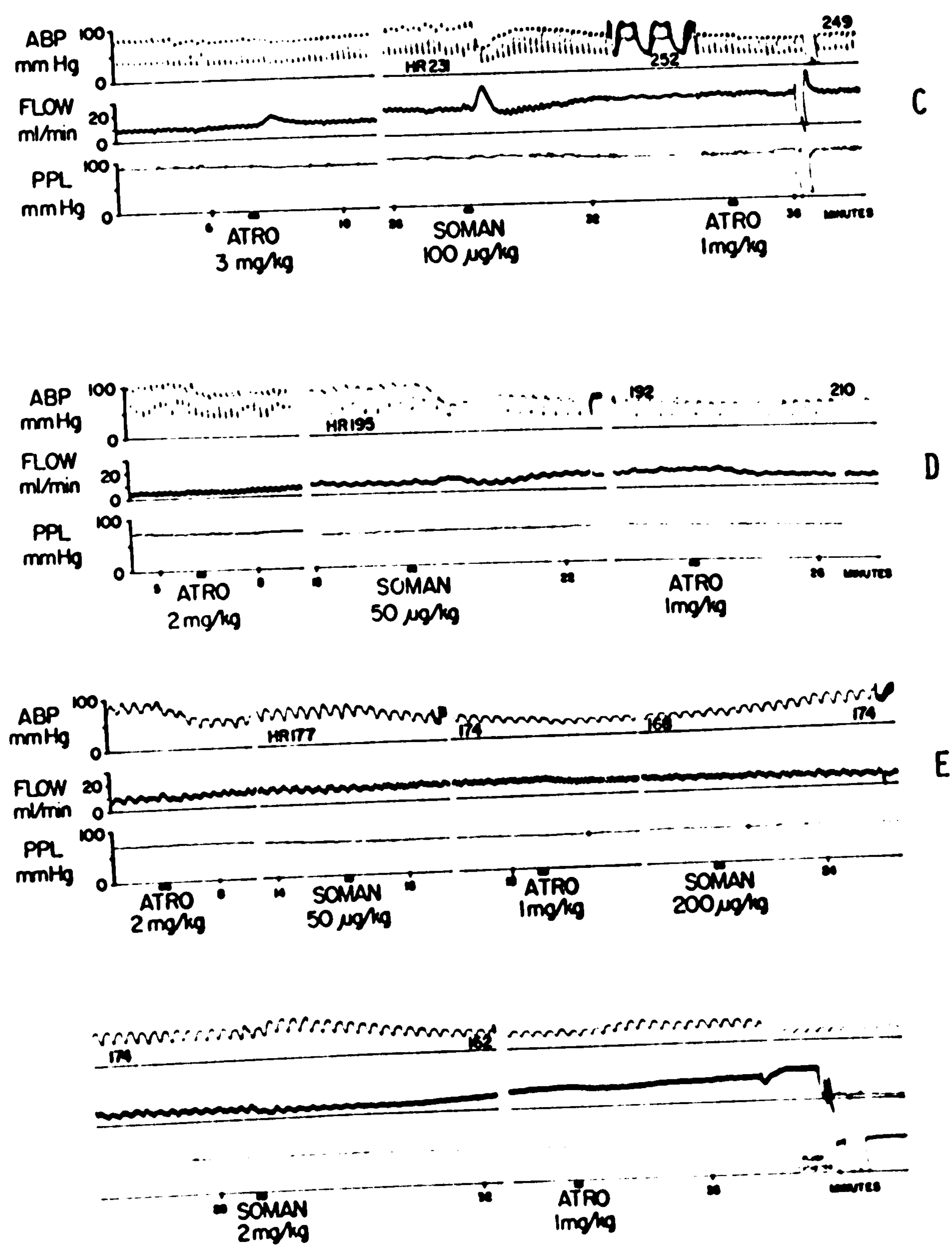
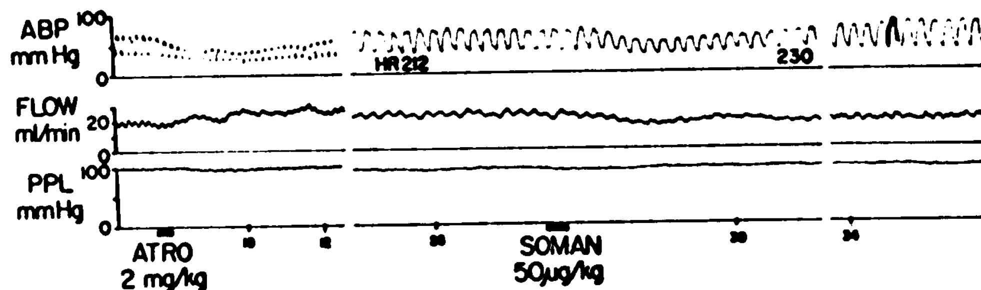
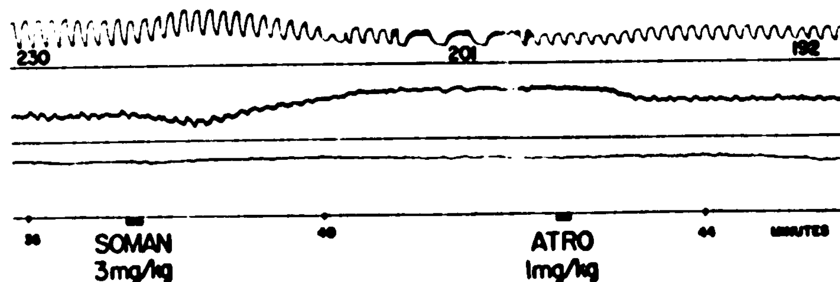


FIGURE 13: EXPERIMENTS C, D AND E





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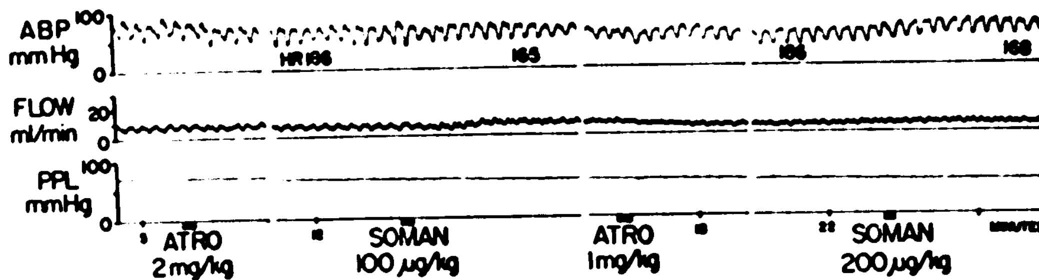

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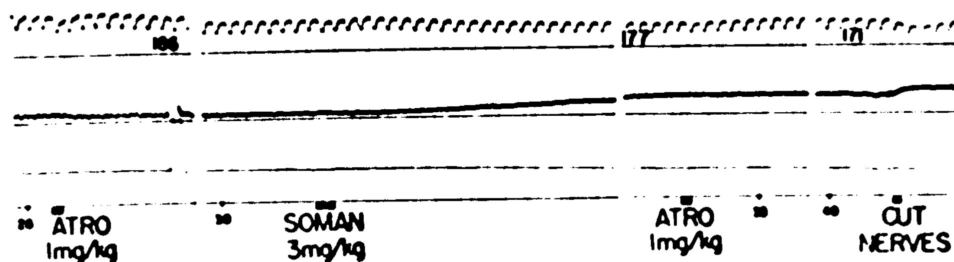


FIGURE 14: EXPERIMENTS F AND G

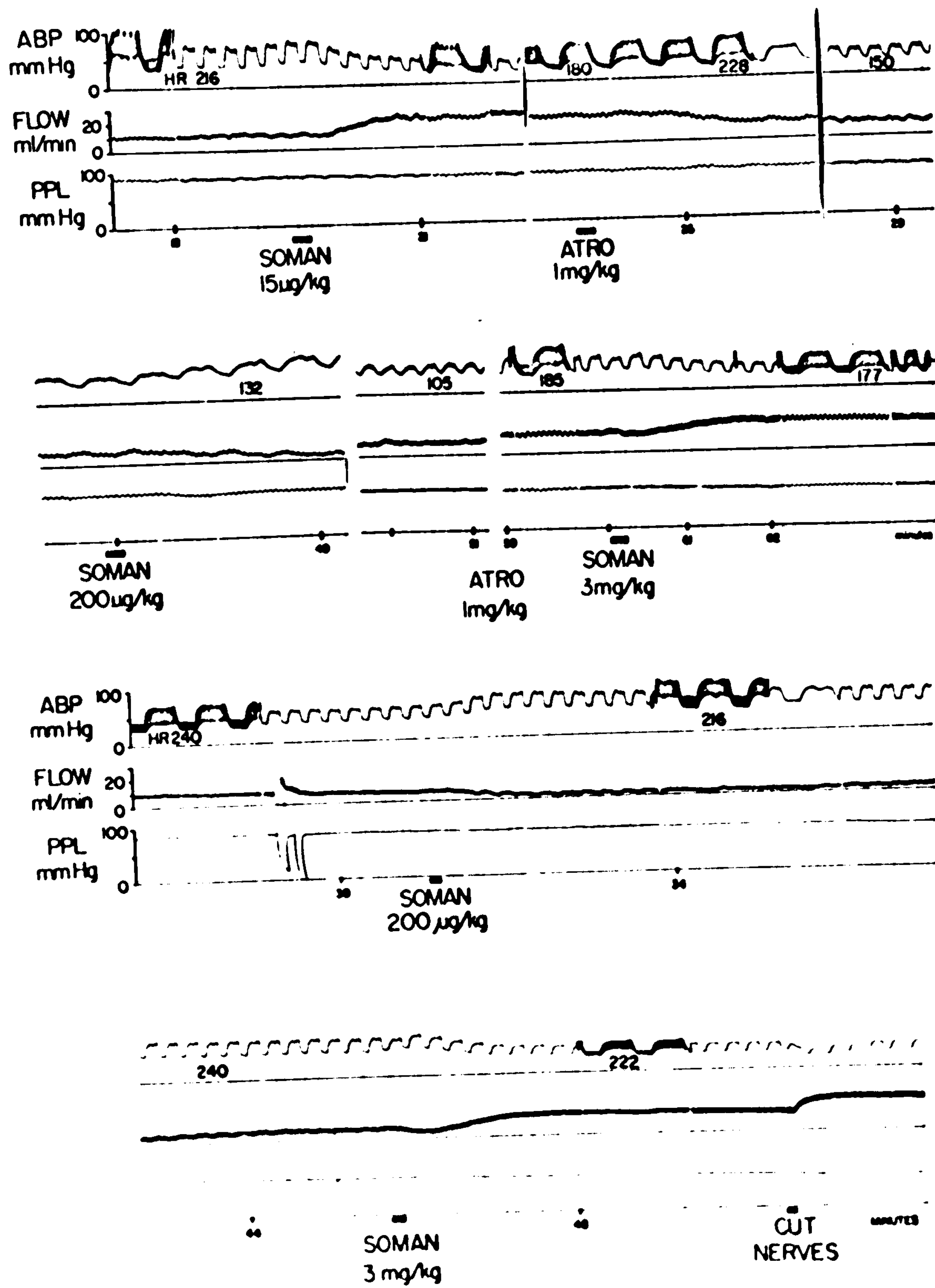


FIGURE 15: EXPERIMENTS H AND J

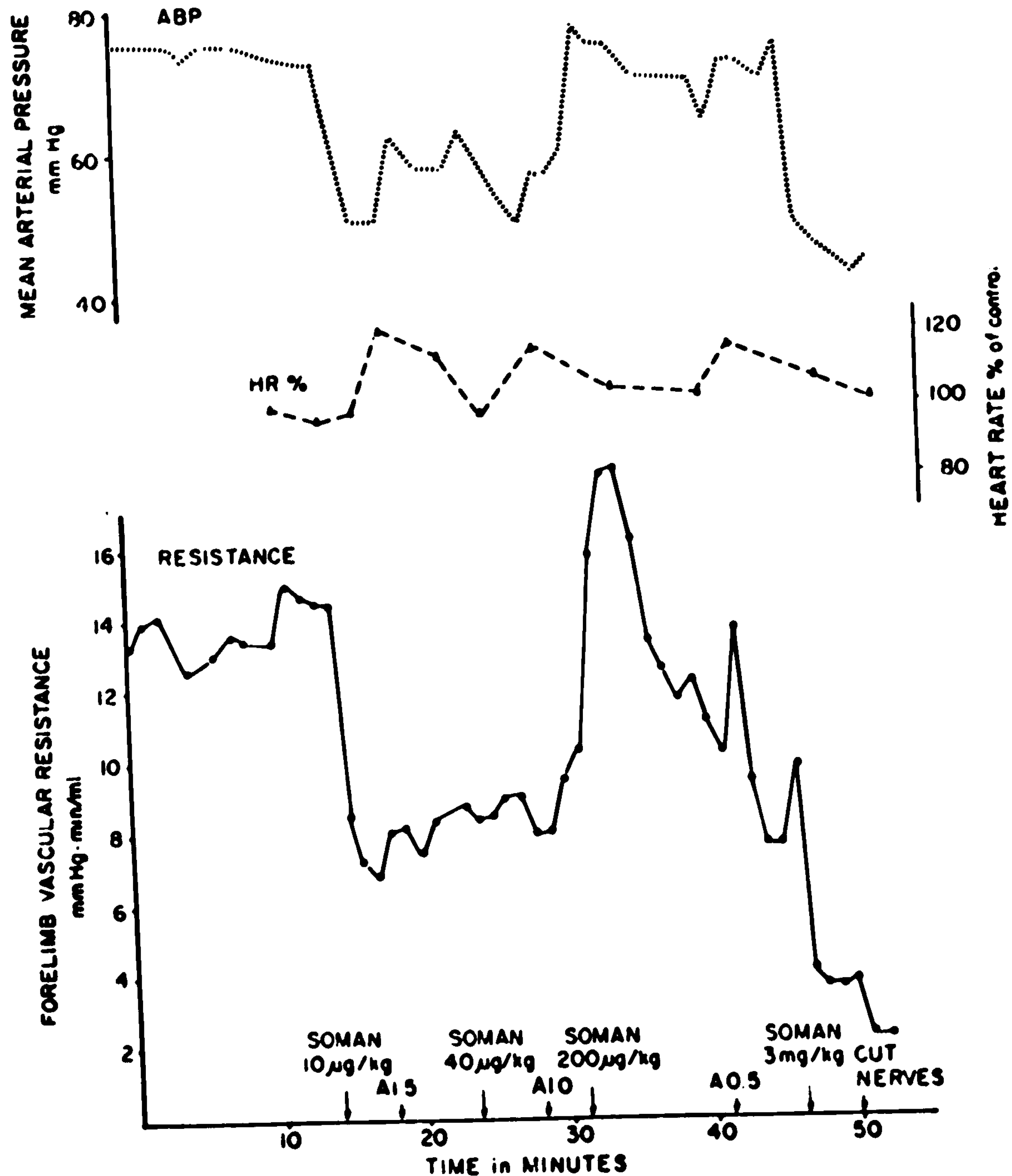


FIGURE 16: CROSS-PERFUSION
EXPERIMENT J, FIG. 15, PLOTTED IN FULL

Experiment shows the pressor effect of 200 µg/kg soman that can be unmasked by a prior state of hypotension and near complete cholinesterase inhibition.

TABLE I. LOWER BRAIN-STEM CHOLINESTERASE ACTIVITY FOLLOWING INTRAVENOUS SOMAN

SOMAN DOSE (μg/kg)	CHOLINESTERASE ACTIVITY* (meq ACh/mg tissue/min) x 10^6 $\bar{x}$	PERCENT OF NORMAL**
20	1.33	11.3
25	1.21	10.3
30	0.23	2.0
40	0.09	0.8
45	0.22	1.8
50	0.02	0.2

* Mean activity measured in five aliquots of a homogenized brain-stem from one rabbit. $SE_{\bar{x}}$ ranged from 0.07 to 0.18 (p.102)

** The mean activity of brain-stems from seven untreated rabbits was (11.76±0.75) x 10^{-6} meq ACh/mg tissue/min (p.101).

Intravertebral Injection Experiments

Control Experiments: In seven ventilated rabbits given no atropine, 5 μg/kg soman injected intravenously had no effect on heart rate or blood pressure. In four of these rabbits the forelimb was isolated and cross-perfused to measure vasomotor output. Soman, 5 μg/kg i.v., had no effect on vasomotor tone, demonstrated in the sample experiment, Figure 17. The effect of this soman dose injected intravertebrally was next investigated.

Injection of 5 μg/kg soman into the vertebral artery caused bradycardia, presumably due to central vagal excitation. In experiment K (Figure 18), following a control injection of saline, soman similarly injected caused heart slowing from 174 to 108 beats per minute. Bilateral vagotomy restored normal heart rate. To more clearly indicate the effect of soman on blood pressure, the rabbits used in experiments L to R (Figures 18 to 21) were bilaterally vagotomized before intravertebral injection.

Soman injected intravertebrally in vagotomized rabbits caused hypotension associated with a muscarinic vasodilatation. In experiments L, M and N soman caused an immediate fall in blood pressure while heart rate remained the same or increased. In each instance the isolated foreleg vasodilated (increased flow at the same perfusion pressure), most prominently in experiment N. It was evident that intravenous atropine could cause recovery of blood pressure (experiments M and N) and reverse the vasodilatation in the isolated limb (experiments L and N).

Rabbits given no atropine could recover spontaneously from this hypotensive effect of soman. In experiment O (Figure 20), blood pressure began to recover within four minutes from the onset of hypotension. Spontaneous recovery also occurred in experiment P. In experiment Q a pressor response occurred.

In some experiments effects on vasomotor tone in the isolated leg appeared dissociated from systemic blood pressure effects. Note in experiments P and Q that after soman injection the leg eventually vasodilated and an injection of atropine into the recipient reversed this effect. Systemic blood pressure in the recipient did not reflect the vasodilatation or the recovery of vasomotor tone.

In these experiments each 5 μg/kg dose of soman was washed into the vertebral artery in 40 seconds (7.5 μg/kg/min). In two atropinized rabbits soman was infused continuously into the vertebral artery at 5 μg/kg/min for 30 minutes. In experiment R, Figure 21, following a control intravertebral infusion of saline for 20 minutes, the soman solution similarly infused had no hypotensive effect not reversible by atropine. This was confirmed in the second experiment (not shown).

Intravertebral injection of soman caused hypotension at doses of 2.0, 1.0, 0.5, 0.25 and 0.20 μg/kg (one rabbit tested at each dose). A dose of 0.15 μg/kg soman was ineffective when tested in one rabbit. Two of these experiments are shown in Figure 22, experiments S and T. In experiment S only, the vagi were not previously cut and bradycardia occurred. Since vagotomy restored normal heart rate, central vagal excitation was demonstrated at this low dosage. In both experiments there was a spontaneous recovery from the hypotension.

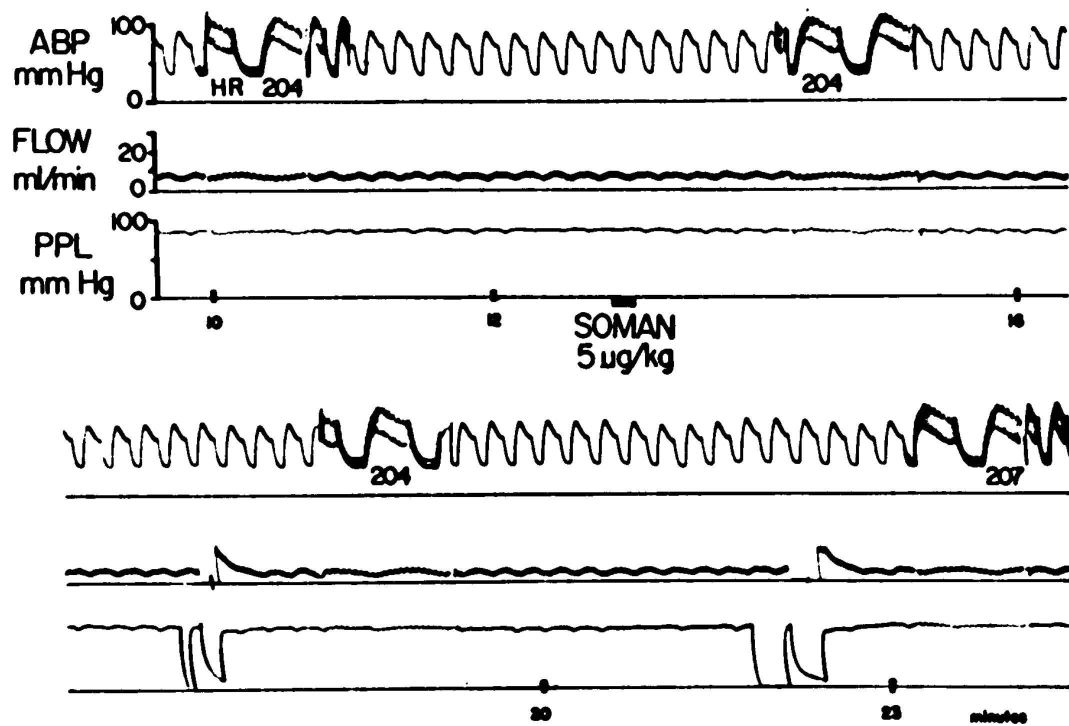


FIGURE 17: CONTROL CROSS-PERFUSION EXPERIMENT

To demonstrate the lack of effect of 5µg/kg soman injected intravenously into the recipient rabbit.

FIGURES 18 - 22: INTRAVERTEBRAL INJECTION EXPERIMENTS

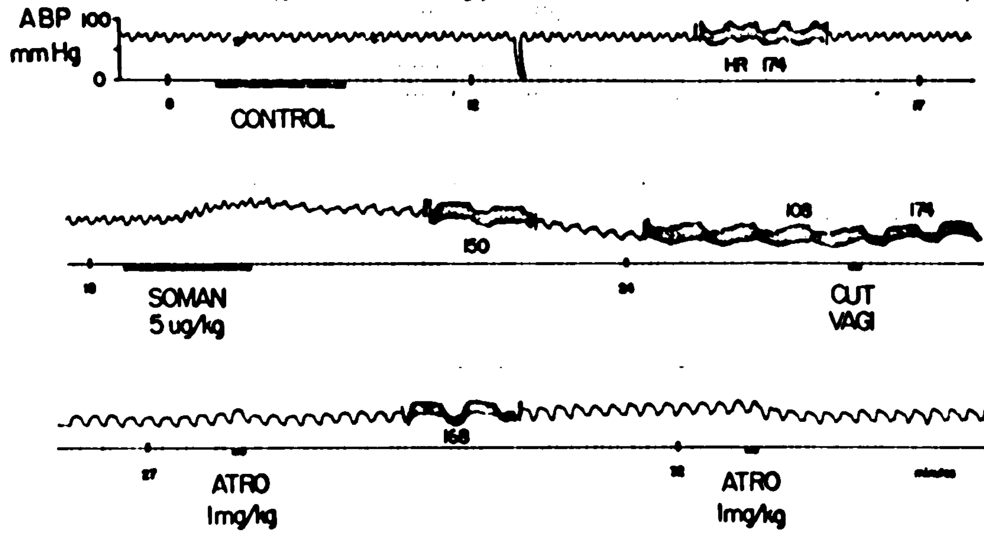
In all experiments soman was injected into the vertebral artery of an artificially ventilated rabbit. Effects on vasomotor tone were measured in the isolated cross-perfused leg. In all experiments a control intravertebral injection of saline was made prior to the soman injection. Atropine (ATRO) was always injected intravenously.

ABP - arterial blood pressure

HR - heart rate

FLOW - blood flow to the isolated leg

PPL - perfusion pressure of the isolated leg



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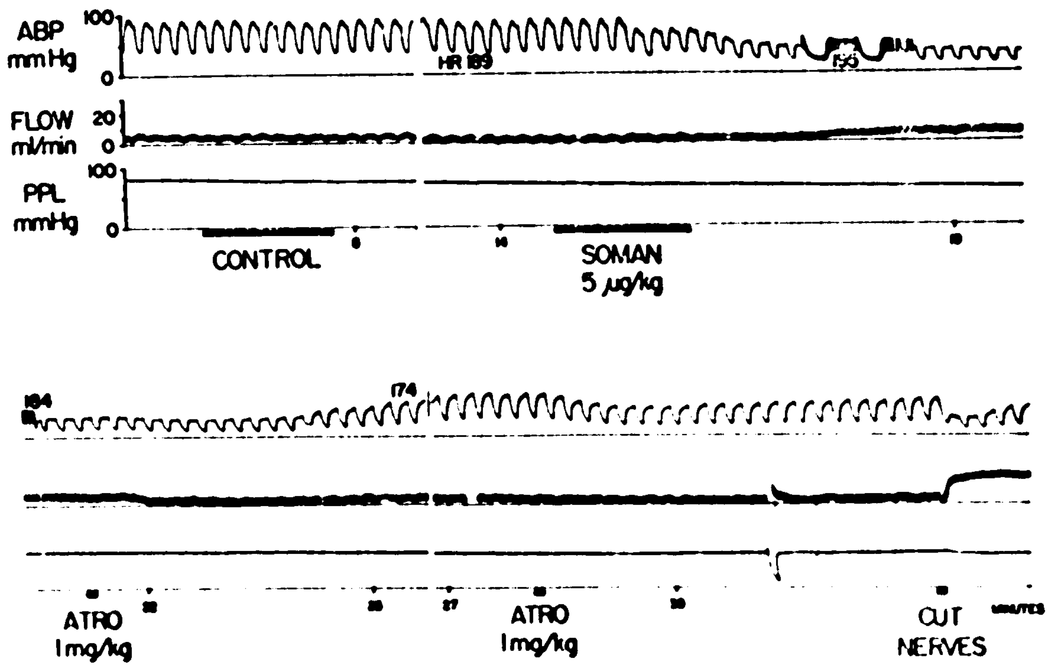
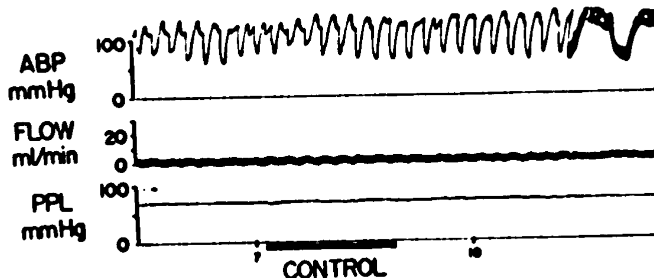
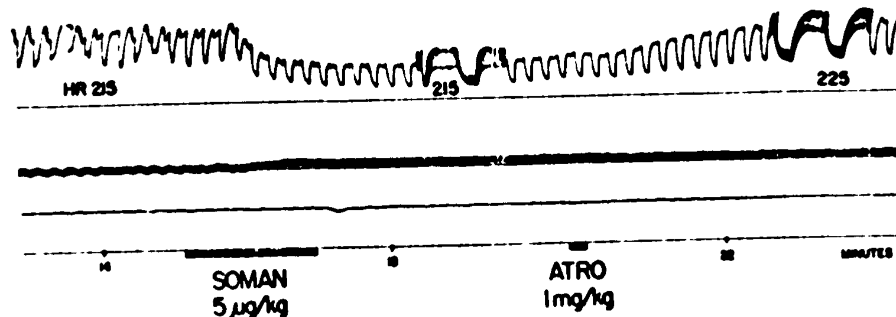


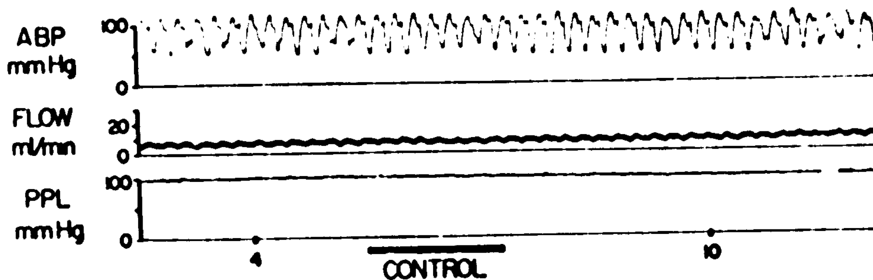
L

FIGURE 18: EXPERIMENTS K AND L



M





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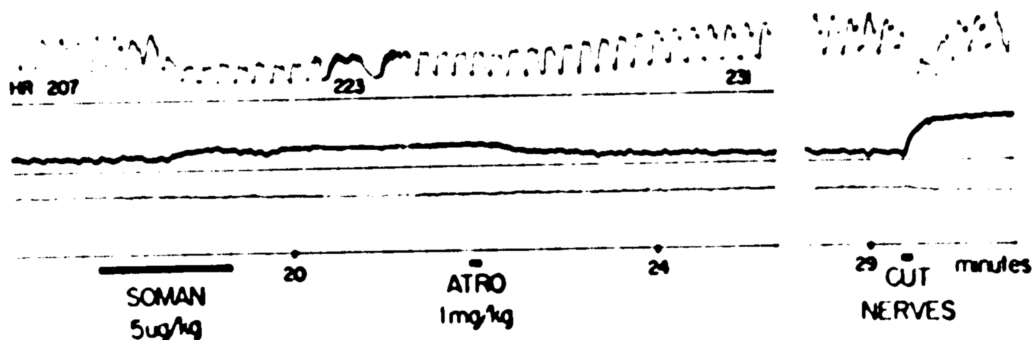


FIGURE 19: EXPERIMENTS M AND N

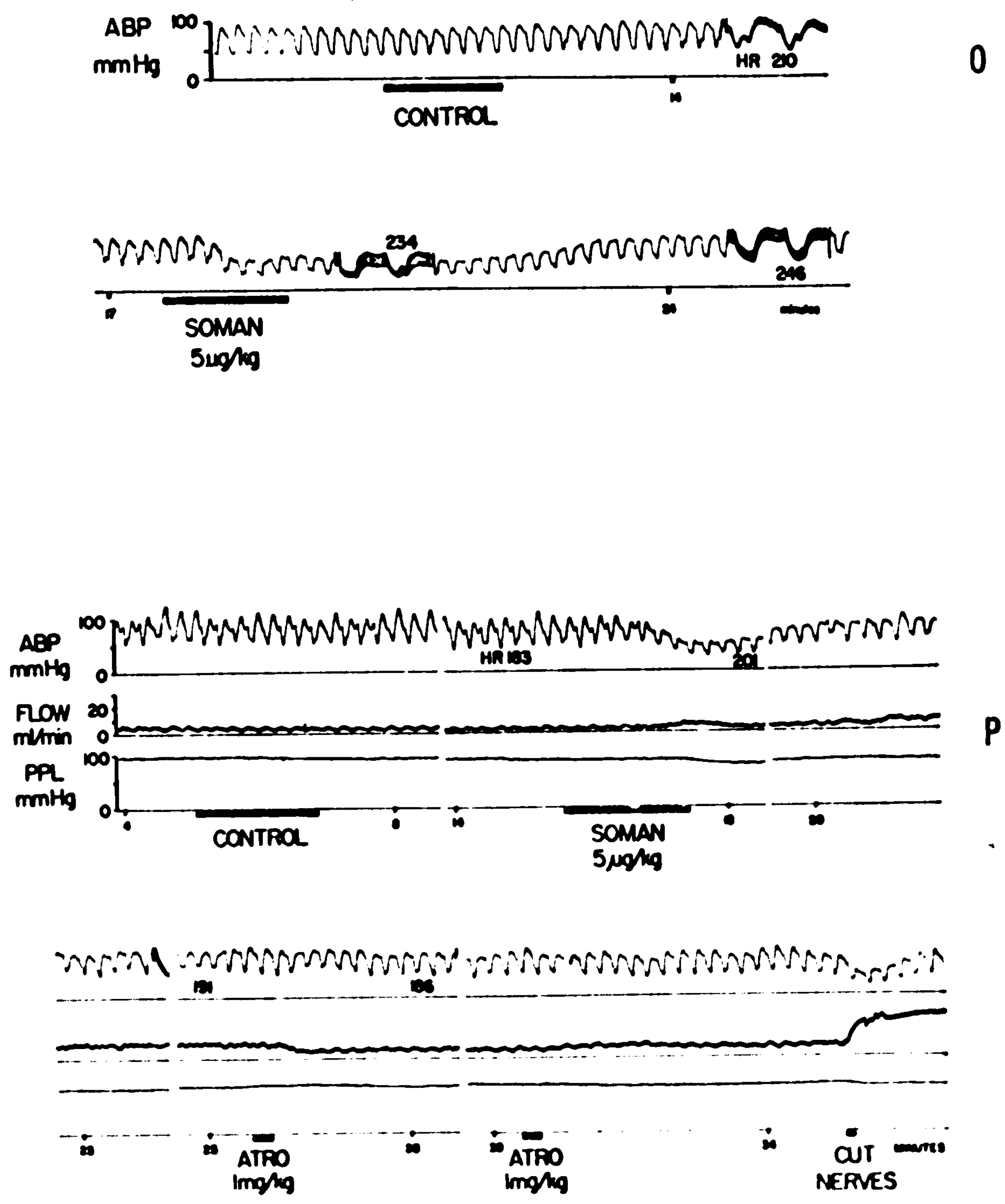


FIGURE 20: EXPERIMENTS O AND P

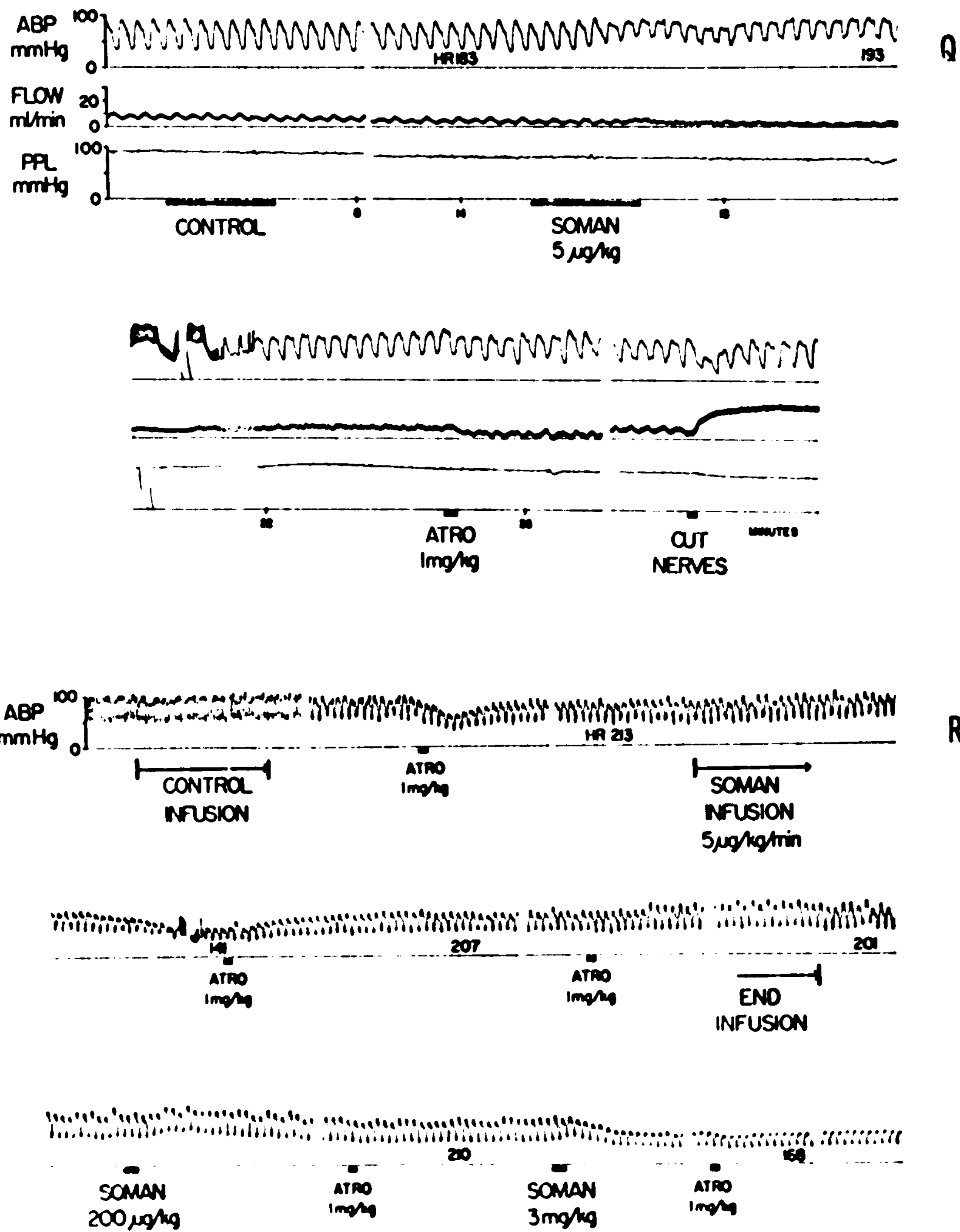
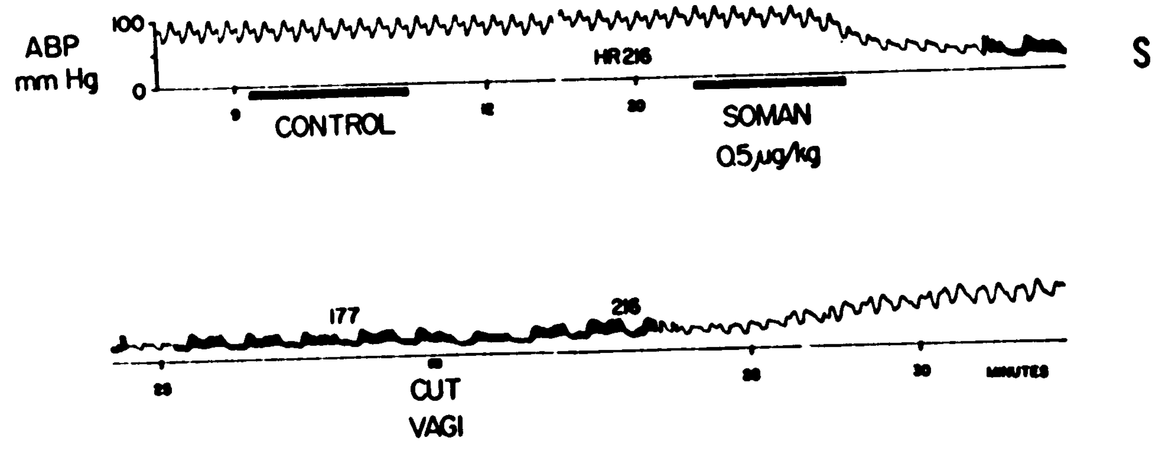


FIGURE 21: EXPERIMENTS Q AND R



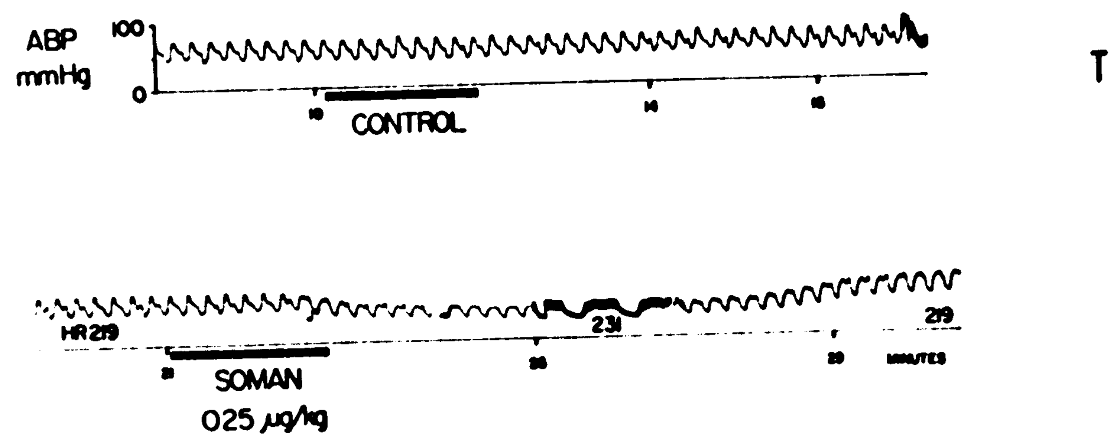


FIGURE 22: EXPERIMENTS S AND T

Cholinesterase Measurements

Table II shows lower brain-stem cholinesterase activities measured in these experiments. Mean cholinesterase activity was below detectable levels in six rabbits given 5 µg/kg intravertebral soman (original data are in Table V, page 103). This dose given intravenously caused a mean 20 percent cholinesterase inhibition in four rabbits (original data are in Table IV, page 102).

In the group of individual small-dose experiments (2.0-0.15 µg/kg soman) the degree of cholinesterase inhibition was related to the dose injected. The minimally effective dose which substantially reduces cholinesterase activity and induces hypotension appears to be in the range of 0.15 to 0.25 µg/kg soman.

TABLE II. LOWER BRAIN-STEM CHOLINESTERASE ACTIVITY FOLLOWING SOMAN INJECTION

	SOMAN DOSE (μg/kg)	CHOLINESTERASE ACTIVITY (meq ACh/mg tissue/min) x 10^6 $\bar{x} \pm SE_{\bar{x}}$	PERCENT OF NORMAL**
GROUP EXPERIMENTS	5.0 (intravenous)	9.44±0.87	80 (4 rabbits)
	5.0 (intravertebral)	0	0 (6 rabbits)
SINGLE EXPERIMENTS (intravertebral injection)	2.0	0.10*	0.8
	1.0	0.25	2.1
	0.5	0.66	5.6
	0.25	1.36	11.6
	0.20	1.80	15.3
	0.15	6.31	53.7

* The mean activity measured in four or five aliquots of a homogenized brain-stem from one rabbit. $SE_{\bar{x}}$ ranged from 0.08 to 0.22 (p.103)

** The mean activity of brain-stems from seven untreated rabbits was (11.76±0.75) x 10^{-6} meq ACh/mg tissue/min (p. 101).

DISCUSSION

It is without doubt that hypotension is a direct pharmacological effect of organophosphate poisons. Classical investigations into this phenomenon have attributed the hypotension primarily to a muscarinic effect on the heart, which results in bradycardia and therefore low cardiac output. The other major determinant of blood pressure, peripheral resistance, has been shown to act as a compensating factor that may sustain blood pressure until the animal dies the usual asphyxial death. In several investigations it was clear that atropine, by antagonizing the cardiac effects, enabled the severely poisoned, ventilated dog to maintain his normal blood pressure. The development of these general concepts has been presented in the Literature Review, pages 13 to 17; and the reader is referred specifically to discussion of work by Holmstedt, 1951; Daly and Wright, 1956; Polet and Schaepdryver, 1959; Fukuyama and Stewart, 1961; and Lubash et al., 1960.

In the rabbit the organophosphates induce hypotension which cannot be prevented by atropine and artificial ventilation. In the experiments in Figure 4 it was characteristic that blood pressure fell within a minute or two of intravenous soman injection. The small

decrease in heart rate was not sufficient to cause the degree of hypotension that occurred, and a second injection of atropine was ineffective in improving blood pressure.

These findings were confirmed in the blood pressure measurements grouped in Figure 5. The decrease in heart rate following constant infusion of sarin, soman or DFP did not account for the severity of hypotension; and failure of repeated atropine injections to restore blood pressure implicated other causative factors of a non-muscarinic nature.

These infusion experiments established that hypotension was related to the systemic presence of organophosphate. Control rabbits did not become hypotensive when exposed to the same regimen of atropine and anesthesia, infused with saline alone and made completely dependent on the the artificial-ventilation system.

The characteristics of recovery from the hypotension, i.e. the rapid blood pressure increase when sarin and DFP infusion stopped, indicate that a direct organophosphate effect was involved, apart from cholinesterase inhibition. It is extremely unlikely that the pressure increase reflected a rapid regeneration of functional cholinesterase. In the sarin experiments an increase in heart rate contributed somewhat to the pressure recovery. This was not so in the three DFP experiments, and other factors are implicated, i.e. increased peripheral resistance or myocardial contractility.

The results of these experiments, taken together with the concepts briefly mentioned at the start of this discussion, formed the basis for further experimental work. Since bradycardia was not the sole cause of hypotension, the two remaining considerations were effects on myocardial contractility and on the vasomotor system.

Work with the Langendorff rabbit heart (Figure 6) showed that depression of myocardial contractility played no significant role in non-muscarinic hypotension. During three hours exposure of the isolated, atropinized heart to soman (about 25 μg/ml in the perfusate) there was no serious effect on contractility. In order to achieve a concentration of 25 μg/ml free soman in the blood system of a rabbit, a dose of 2.5 mg/kg would have to be instantaneously present in the blood stream (based on a blood/body weight ratio of 10 %). Hypotension occurred at far smaller soman dosage (Figure 4).

The results from isolated hearts showed no evidence that soman had a cardiotoxic effect, such as that reported for DFP in rats (Wolthuis and Meeter, 1968). They found that DFP (25 μg/ml) in the perfusate of Langendorff rat hearts diminished contractile force by more than 60 percent in three hours. Despite extensive therapeutic measures, intact rats died of heart failure in two hours following subcutaneous injection of 56 mg/kg DFP. A gradually diminishing ECG voltage was observed.

In the Langendorff rabbit heart DFP (50 μg/ml) caused less than 10 percent depression in contractility during this time (Preston and Heath, 1970). The three DFP-infused rabbits (115-125 mg/kg, Figure 5) showed constant ECG voltage amplitude and no sign of cardiac failure. These data on rabbits generally concur with reports of organophosphate effect on Langendorff guinea-pig hearts (Wolthuis and Meeter, 1968) and cat papillary muscle (Murtha and Wills, 1964).

The autoperfusion experiments (Figures 8 and 9) confirmed the hypothesis that non-muscarinic hypotension resulted from loss of peripheral

vascular resistance. The vasodilator (and concomitant hypotensive) effect of soman was particularly well demonstrated in experiments A, B and D. It was also clear from experiments A and C that a muscarinic vasodilatation could contribute to the hypotension. A possible reason for this was a muscarinic vasomotor depression at the lower brain-stem level as demonstrated by Stewart and Anderson (1964). In experiment A the vasodilatation following soman injection was greater than that which followed denervation later in the experiment. This implied that the reduction of vascular smooth muscle tone was due to local factors, in addition to decreased sympathetic drive. It is pertinent that Daly and Wright (1956) suggested that increased blood concentrations of acetylcholine in the poisoned animal would have a direct relaxant effect on vascular smooth muscle. Stewart (1952) and Barnes and Duff (1954) demonstrated substantial blood levels of acetylcholine in organophosphate-poisoned rats and rabbits.

Autoperfusion experiments in the denervated subclavian bed (Figures 10 and 11) tested whether the vasodilator actions of soman were mediated at the vascular smooth muscle level. Muscarinic depression of intrinsic, basal vascular tone was found to be a major contributor to vasomotor depression. In experiments E, F and G the recovery of basal resistance following atropine was associated with partial recovery of blood pressure. Nevertheless, in all four experiments systemic hypotension remained despite near or complete restoration of basal vascular resistance. It was proposed from these results that the site of non-muscarinic action of soman was in the neuronal elements of the vasomotor pathway.

It should be mentioned at this point that the influence of atropinesterase was dramatically evident in some of these experiments. Despite prior atropinization, further large doses of atropine were necessary to antagonize the loss of basal vascular resistance and the bradycardia following soman injection (Figures 10 and 11). This factor was not evident in earlier experiments (Figures 4 and 5) where 1 or 2 mg/kg atropine doses could offer a relatively sustained protection against bradycardia.* Atropinesterase could be a complicating factor in the interpretation of experiments. Therefore, the criterion accepted for adequate atropinization of any rabbit was the absence of bradycardia following soman injection. In applying this criterion, it had to be taken into consideration that a slight heart slowing which was not atropine reversible, often followed high soman dosage. This was seen in the constant-infusion experiments, Figure 5 (text).

Cross-perfusion experiments (Figures 12-15) supported the hypothesis that soman had a non-muscarinic depressant action in the neuronal vasomotor pathway. In experiments A, B and C, a reduction in sympathetic vasomotor output to the isolated leg explains the simultaneous loss of systemic blood pressure. Atropine could not reverse this effect. In experiment A the vasomotor depression was almost as great as in complete denervation (the brachial plexus was severed at the end of experiment A).

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* It was advantageous that these rabbits came from only a few breeding pairs, apparently lacking the enzyme (experiments were done at Suffield). This inherited enzyme-trait does not occur in all rabbits. Glich and Glaubach (1941) observed atropinesterase in 1 of 4 rabbits of 68 animals studied. Hobbiger and Lessin (1955) reported the enzyme in 14 of 39 rabbits.

The non-muscarinic depressant effect is due to a mechanism other than cholinesterase inhibition. This is supported by the following facts. An intravenous dose of 50 μg/kg soman inhibits more than 98 percent of lower brain-stem cholinesterase activity (Table I). This degree of cholinesterase inhibition causes a marked pharmacological effect. Metz (1958) found that 89-92 percent inhibition of medullary cholinesterase activity coincided with central respiratory failure in dogs. It had been observed earlier that 25 μg/kg soman was a lethal intravenous dose in five conscious rabbits. Nevertheless, in cross-perfusion experiments F and G, 50 or 100 μg/kg soman caused only a small loss of vasomotor tone or systemic pressure. In the autoperfusion experiments, 100 μg/kg soman had no effect on pressure or resistance in two of three atropinized rabbits. If the non-muscarinic effect was due solely to cholinesterase inhibition, it would have occurred predictably at 50 or 100 μg/kg soman, especially since it did so at higher dose levels.

Conclusive evidence that a mechanism exists other than cholinesterase inhibition comes from cross-perfusion experiments E, G, H and J. These rabbits received $\geq$ 215 μg/kg soman, which is more than sufficient to cause complete cholinesterase inhibition. In spite of this, 2 or 3 mg/kg soman induced a severe degree of vasomotor depression and concomitant hypotension. Atropine injected before or after soman had no important influence on the effect.

Kamijo and Koelle (1952) and Holaday et al. (1954) observed in cats a direct depressant effect of DFP on sympathetic ganglionic transmission, a high-dose effect unrelated to cholinesterase inhibition. Although no study has correlated such an action with vasomotor depression,

this mechanism may be related to the effects observed in the cross-perfusion experiments.

Both of the above studies also demonstrated that a dose of 5 mg/kg atropine inhibits ganglionic transmission for several hours. It must be allowed that the large atropine doses given in the cross-perfusion experiments might have facilitated the depressant effects of soman. Nevertheless, the latter occurred in rabbits already well equilibrated with large doses of atropine. Furthermore, hemodynamic effects of atropine, when seen, were much smaller than those due to soman.

It is difficult to assess what role cholinesterase inhibition itself may have played in the induction of non-muscarinic hypotension. There is little doubt that the direct mechanism was operative at very high (mg/kg) doses. However, the relative importance of either mechanism in the non-muscarinic effects of lower doses, 50 μg/kg soman (Figure 4), remains unassessed.

These experiments give little information about the direct mechanism by which soman exerts its vasomotor-inhibiting action. One might consider, however, the alternatives of (a) a specific drug effect involving soman interaction with a pharmacological receptor or (b) a non-specific drug effect not involving receptors (as in general anesthesia). The fact that soman is effective in relatively low doses, 2-3 mg/kg or less, favors the former suggestion. If a receptor is involved and therefore organophosphate structure is important, it raises two questions: (i) What is the relationship of structural differences amongst soman, sarin and DFP to the apparently slower recovery from soman- vs sarin- or DFP-induced hypotension i.e. the rapid pressure

recovery phenomena (Figure 5)? (ii) Is the mechanism of prolonged, central, non-muscarinic respiratory paralysis that Meeter and Wolthuis (1968, Lit. Rev. page 12) observed with soman, as opposed to sarin or DFP, based on a structure-activity principle similar to that operative above?

The cross-perfusion experiments E, G and J (also see Figure 16) showed that a prolonged pressor effect of 200 μg/kg soman was unmasked if the rabbit was previously in a state of hypotension and near-complete cholinesterase inhibition ($\geq$50 μg/kg soman was previously injected). Two possible mechanisms one may suggest for this are: (a) the inhibition of a small but highly functional store of cholinesterase at some vasomotor site (<2 % of normal levels, from data of Table I) or, more likely, (b) a pharmacological action other than cholinesterase inhibition. Certain literature reports are relevant to the latter suggestion. Cohen and Posthumus observed that DFP sensitizes the isolated frog rectus abdominus muscle (1955) and the rat phrenic nerve-diaphragm preparation (1957) to cholinergic agonists not hydrolyzable by cholinesterase. This effect was attributed to organophosphate binding to the esteratic 'B' group of end-plate receptors and sensitization of the anionic 'A' site to agonist compounds. The possibility that acetyl- or pseudocholinesterase is a functional component of the cholinergic receptor has been supported (Zupancic, 1953; Belleau, 1964; Ehrenpreis, 1967). Ehrenpreis suggested that inhibitors such as DFP sensitize a tissue to acetylcholine by partial disruption of a cholinesterase-lecithin receptor complex. Complete disruption of the complex by acetylcholine permits ion movement. Such a transmitter sensitization at some vasomotor synapse may explain the pressor effect described above.

It is possible that the pressor effect of 200 μg/kg soman is related to the fleeting pressor phase that followed injection of 2 or 3 mg/kg soman in experiments E, F and J. Ultimately one might wonder if both the pressor and depressor effect of soman which overrides it stem from a concentration-dependent mechanism at the same receptor site. However, a much firmer foundation of experiments must be established before any such hypothesis can be intelligently forwarded.

It is likely that cholinesterase inhibition at some interneuronal synaptic region mediated the vasomotor depression that is antagonized by atropine in experiments D and H. It cannot be said that this also accounted for the vasomotor depression that atropine antagonized at the end of experiment F. The initial dose of 50 μg/kg should have been more than adequate to cause acetylcholine buildup, especially since 15 μg/kg soman was effective in experiment H. No further work was done to validate the observation in experiment F.

The objective of the intravertebral injection experiments (Figures 18-22) was to demonstrate the possible muscarinic and non-muscarinic actions of soman at the lower brain-stem level. Although 5 μg/kg soman had no intravenous effect, it was expected that this dose might be effective if injected into the brain-stem arterial supply.

The demonstration that soman caused central vagal excitation (experiments K and S) confirmed the report by Stewart and Anderson (1964). Such an effect would intensify the bradycardia caused by peripheral actions of soman at vagal ganglia and nerve endings. The degree of bradycardia at a given dosage of sarin was substantially less in rabbits which were previously vagotomized (W.C. Stewart, personal communication).

The nature of the probable central cholinergic mechanism influencing vagal output in the rabbit is unknown. Although a systemic injection of atropine alleviates the bradycardia, it is not known whether it antagonizes the central vagal excitation as well. It is of interest that no such effect was observed in dogs when sarin was injected intracisternally into the fourth ventricle (Brown, 1960) or into the blood supply of the cross-perfused isolated head (Polet and Schaepdryver, 1959).

The work reported herein leaves little doubt that soman affects a brain-stem cholinergic mechanism which influences vasomotor output. The main support for this comes from observation that intravertebral soman in doses from 5 to 0.2 μg/kg caused hypotension paralleled by an unchanged or slightly increased heart rate in vagotomized rabbits (experiments L, M, N, O, S and T). Because such doses are ineffective if given systemically, there seems to be no other explanation than that hypotension was due to vasodilatation caused by a brain-stem effect of soman. Further support for such a conclusion is that in experiment N, vasodilatation occurred in the vascularly isolated foreleg, concomitant with the fall in blood pressure.

The experiments showed that brain-stem cholinesterase inhibition did not directly cause non-muscarinic hypotension. In experiments M and N the systemic injection of atropine during hypotension was followed by rapid recovery of blood pressure and, as seen in the latter experiment, vasomotor tone. In two experiments, atropine apparently protected the rabbit from intravertebral infusion of 150 μg/kg soman over thirty minutes (experiment R). These results indicate that the brain-stem vasomotor depression involves a muscarinic receptor mechanism at this level. This hypothesis is supported by Stewart and Anderson's observation

(1964) that systemic atropine injection alleviated the hypotension caused by sarin microinjection into the medullary reticular formation.

An observation extremely important to the interpretation of these experiments was that unatropinized rabbits could recover spontaneously from the hypotension e.g. experiments O and P. In experiment Q no hypotension but rather a pressor response occurred. Nevertheless, in each of the above experiments, the lower brain-stem showed no cholinesterase activity (original data shown in Table V, page 103). Stewart and Anderson (1964) did not report any such spontaneous recovery from the hypotension following sarin microinjection. This difference may be based on the different conditions under which either type of experiment was carried out.

The mechanism of rapid, spontaneous blood pressure recovery following intravertebral soman remains obscure. It appears to be a compensatory mechanism (possibly a baroreflex) that varied from one rabbit to the next. This would explain why I could see no correlation between intravertebral dosage and the hypotensive effect e.g. 0.5 and 0.25 μg/kg soman were more effective in experiments S and T than 5 μg/kg in experiments P and Q. The degree and duration of hypotension seem dependent, therefore, on other factors beside cholinesterase inhibition. These might be defined as the dynamic-vasomotor state and vasoreflex capability at the moment, as modified by stresses of artificial ventilation, anesthesia and surgery. In some rabbits these stresses rendered the vasomotor system susceptible to muscarinic depression by soman and, in effect, unmasked the disturbance of some central cholinergic mechanism. It appears to be a "resistant" vasomotor system that was operative in experiments where the depressant effects were fleeting or did not occur.

This is the type of system one would expect in the conscious animal. By the above argument, therefore, brain-stem cholinesterase inhibition per se is of minor importance in the vasomotor depression of untreated systemic poisoning. However, its effect can be important when amplified by extrinsic factors such as those mentioned above.

This is supported by evidence from dogs. Large doses of sarin injected into the blood supply of the isolated dog's head caused only vasomotor excitation, observed as hypertension in its isolated trunk (Polet and Schaepdryver, 1959). Injection of sarin into the dog's fourth ventricle confirmed this observation (Brown, 1960).

The demonstration of a muscarinic depressor effect originating in the brain-stem would have conveniently explained the muscarinic vasomotor depression observed in the cross-perfusion experiments. However, the rapid spontaneous recovery of rabbits from this effect casts some doubt on this explanation. Certain of the intravertebral injection experiments demonstrated that a second muscarinic site of action was likely involved. In experiments P and Q (Figures 20 and 21) the depressant effect of soman on vasomotor output to the isolated leg and its antagonism by atropine were dissociated from any effect on blood pressure. A substantial hypotension should have paralleled such a vaso-dilatation. One explanation is that the intravertebral injection affected a synaptic region important to the isolated leg but not the whole circul-ation, that is, the spinal cord vasomotor synapses. McDonald and Potter (1951) found that a dye injected into the vertebral artery of rabbits stained the spinal cord from the medulla to the first thoracic segment (T1). The remainder of the cord is mainly perfused from spinal

branches of the intercostal and lumbar arteries.* Most of the vasoconstrictor fibres supplying the upper limb emerge from segments T2 and T3 of the cord.** In several experiments segments T1+T2 and T3+T4 were analyzed for cholinesterase activity (see Table VI, page 104 for data). It was found that intravertebral soman could sometimes substantially inhibit cholinesterase at this level. It is regrettable that cord cholinesterase activity was not measured in experiments P and Q.

The above reports on dogs (Polet and Schaepdryver, 1959; Brown, 1960), the rabbit microinjection experiments of Stewart and Anderson (1964) and the rabbit intravertebral injection experiments are of considerable physiological interest. Little is known concerning possible cholinergic relationships among brain-stem and spinal vasomotor structures. Sinha et al. (1967) observed that acetylcholine or carbachol injected by intracerebroventricular (i.c.v.) injection caused a pressor response reversed by i.c.v. atropine. Administration of hemicholinium i.c.v. depressed the carotid occlusion reflex and i.c.v. choline antagonized this effect. It was concluded that acetylcholine plays an excitatory transmitter role in the medullary vasomotor center. The medullary vasomotor effect of sarin in dogs complements these observations.

In experiments K and Q (Figures 18 and 21) a pressor effect of intravertebral soman was observed. The excitatory mechanism here may be analagous to that demonstrated in the dog. However, this study together with that of Stewart and Anderson shows a qualitative difference in the

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* Information obtained from Grays Anatomy (Davies, D.V. and Davies, F., editors) 33rd edition, Longman's Green and Co., Ltd., 1962, p. 975

** Ibid., p. 1232

brain-stem pharmacological disturbance caused by organophosphate in the two species.

It has been said that asphyxia is the immediate cause of death in anticholinesterase poisoning (de Candole et al., 1953; Holmstedt, 1959). The pharmacological actions of organophosphates which depress spontaneous respiration have been clearly defined i.e. central and neuromuscular paralysis, bronchoconstriction and bronchosecretion. The fatal respiratory effects are drawn into a much more complex scheme when one attempts to relate them with the pharmacological effects on cardiovascular function. Bradycardia and low cardiac output aggravate tissue oxygen deprivation because of decreased flow, "*poor circulation*", as Holmstedt called it (1951). Krop and Kunkel (1954) suggested the following:

> "*the blood might not be circulated through the lung at a rate adequate to maintain a degree of hemoglobin oxygenation required to prevent cyanosis and tissue hypoxia*".

Holmstedt (1959) in a review discussion made the following comment:

> "*Study of the part played by the cardiovascular system confirms what every author who has worked with cholinesterase-inhibiting drugs has noted, namely, that when death comes quickly, the circulation is relatively unimpaired when respiration fails*".

Although he gave no reference nor further qualified this statement, he perhaps had in mind the general observation that compensating vasoconstriction parallels bradycardia in untreated poisoning in rabbits (Holmstedt, 1951), rats (Fukuyama and Stewart, 1961) and dogs (Daly and Wright, 1956).

Holmstedt goes on to say,

> "*when death is delayed it is impossible to make such a distinction, since mounting depression and final failure then involve both systems equally. Death appears to be primarily asphyxial in some instances and primarily cardiovascular in others; and in some cases, failure of both seems to coincide*".

A further distinction may be made which, in the rabbit at least, is based on dosage. The reader is referred to Figure 23, page 109, Appendix. In the upper graph an untreated rabbit was given a small but lethal dose of soman, and the symptoms that occurred have been classically described. There was hypotension, bradycardia and a compensating vasoconstriction which preceded death by asphyxia, five minutes later. However, note in the lower graph that at 500 μg/kg soman (over 20 lethal doses) the cardiovascular system of a second rabbit collapsed within two minutes of poisoning. This was a cardio-vascular death. It is attributable to the host of muscarinic and non-muscarinic vasomotor depressant actions described in this thesis. This repeatable experiment indicates that in the rabbit or any species which reacts similarly, rapid chemotherapeutic measures would be essential even before artificial ventilation, to prevent a fatal cardiovascular collapse following a sudden, overwhelming organophosphate exposure.

SUMMARY AND CONCLUSIONS

1. Soman, 50 to 5000 μg/kg i.v., caused up to sixty percent decrease in blood pressures of anesthetized rabbits despite artificial ventilation and atropine sufficient to prevent bradycardia. Blood pressure similarly fell when sarin, soman or DFP were infused intravenously at a constant rate. Control rabbits exposed to similar conditions of anesthesia, atropinization and artificial ventilation were normotensive. It was hypothesized that the non-muscarinic (atropine-insensitive) hypotension was due to organophosphate-induced depression of myocardial contractility or of vasomotor tone.

2. Contractile performance of the atropinized Langendorff rabbit heart was not notably impaired by soman (about 25 μg/ml) in the coronary perfusate for three hours. It was concluded that myocardial impairment played no significant role in non-muscarinic hypotension.

The autoperfused forelimb vasodilated following systemic soman injection, and blood pressure fell simultaneously. Atropine partly alleviated these effects, indicating the existence of both muscarinic and non-muscarinic vasodilator factors.

Vasodilatation occurred in the denervated, autoperfused forelimb after soman poisoning; but this was antagonized by atropine while hypotension still remained profound. This suggested that the atropine

insensitive hypotension was due to an effect located within the neuronal vasomotor pathway. However, loss of locally mediated, basal vascular resistance is an important factor in muscarinic inhibition of vasomotor tone.

4. To test the proposal that the site of action was neuronal, vascular resistance was measured in a foreleg that was innervated but vascularly isolated from a ventilated rabbit given intravenous soman.

Low doses of soman (<100 μg/kg) caused vasomotor depression which usually was antagonized by atropine. Higher doses caused depression that was not atropine reversible. It is therefore concluded that soman has both muscarinic and non-muscarinic inhibitory actions in the neuronal pathway.

A large dose of soman (2 or 3 mg/kg) greatly augmented the vasomotor inhibition and hypotension which had occurred in atropinized rabbits already given 200 μg/kg. A dose of 50 μg/kg soman was shown to inhibit more than ninety-eight percent of lower brain-stem cholinesterase activity. The non-muscarinic, neuronal depressant action of very large, i.e. mg/kg, doses of soman is considered due to a mechanism other than cholinesterase inhibition. Other evidence supporting this claim is discussed. The relative importance of both this mechanism and cholinesterase inhibition per se in the non-muscarinic hypotension of low soman dosage remained unclarified.

5. Soman caused bradycardia and hypotension when injected into the vertebral arterial supply of the brain-stem in doses shown to be too small to have an effect when injected intravenously (5 μg/kg or less). Vagotomy restored heart rate, showing that the bradycardia was due to central vagal excitation.

The hypotension occurred in vagotomized rabbits following intravertebral doses of soman ranging from 5 to 0.2 μg/kg, with blood pressure falling as much as sixty-five percent while heart rate remained normal or increased. Hypotension was due to vasodilatation, demonstrated in the isolated cross-perfused leg, and is thought to result from cholinesterase inhibition in the vasomotor centers of the medulla.

This vasomotor effect of soman was antagonized by intravenous atropine and, in unatropinized rabbits, by an intrinsic compensatory mechanism that could restore blood pressure within a few minutes despite complete lower brain-stem cholinesterase inhibition. The severity and duration of hypotension appeared unrelated to intravertebral dosage, although the degree of cholinesterase inhibition increased with the latter.

It is concluded that soman disturbs a brain-stem cholinergic mechanism influencing vasomotor tone and involving central muscarinic receptors. Some compensatory physiological mechanism antagonizes this effect. Vasomotor stresses such as artificial ventilation, surgery and anesthesia govern the effectiveness of compensation and therefore the degree and duration of hypotension.

It is suggested that lower brain-stem cholinesterase inhibition is of minor importance in the muscarinic vasomotor depression of untreated systemic poisoning, except when its effect is amplified by extrinsic stresses such as mentioned above. Lower brain-stem cholinesterase inhibition does not directly cause the non-muscarinic hypotension seen in systemic poisoning. However, the depressant effect of soman unrelated to cholinesterase inhibition may be mediated at this central level.

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APPENDIX

Calculation of Cholinesterase Activity

The mean total acid release measured in a group of tissue aliquots reflected the sum of the following events.

A. acetate from enzymatic ACh hydrolysis

B. non-specific acid release (nature unknown)

C. acetate from non-enzymatic ACh hydrolysis plus acid contained in 0.4 ml ACh solution added at zero time

D. phenol red plus water (PR + H_2O) acid release

i.e. Total Acid Release = A + B + C + D
OR ACh enzymatically hydrolyzed (A) = Total* - (B+C+D)

Along with total acid it was necessary to have values for B, C and D. Factors B and D were measured together (measurement a, Methods, p. 35) and the value entered into the above equation thus:

A = [Total* - (B+D)*] - C   units µℓ NaOH

THEREFORE rate A* = [apparent rate*] - rate C   units - meq ACh/mg/min

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* values are entered in tables following

Rate C was obtained by determining (i) the mean value for apparent non-enzymatic ACh hydrolysis and acid added at zero time, (measurement b, Methods, p. 35), and subtracting from this (ii) the mean 10 minute value for PR + H_2O acid release (measurement c, p. 35). The values for these were:

(i) 12.82±0.44 μℓ NaOH/10 min ($\bar{x}$ ± $SE_{\bar{x}}$ from 12 different AChBr solutions, 4 aliquot titrations each)

(ii) 3.32±0.13 μℓ NaOH/10 min ($\bar{x}$ ± $SE_{\bar{x}}$ from 8-30 minute titrations)

C = 9.50±0.57 μℓ NaOH/10 min (subtract means)

Conversion to rate gave a value of 0.52±0.03 meq ACh/mg tissue/min. This value for rate C was accepted as a correction constant for all cholinesterase activity measurements shown in the following tables.

Cholinesterase Activity Measurements

Tables III - VI

		TOTAL ACID PRODUCTION ($\mu\ell$ NaOH neutralized in 10 minutes)						NON-SPEC + PR-H_2O ACID RELEASE $\mu\ell$ NaOH per 10 min.	CHOLINESTERASE ACTIVITY (meq ACh/mg tissue/min) x 10^6	
		Aliquot No. 1	2	3	4	5	$\bar{x} \pm SE_{\bar{x}}$		APPARENT $\bar{x} \pm SE_{\bar{x}}$	CORRECTED $\bar{x} \pm SE_{\bar{x}}$
LOWER BRAIN STEM	Oct. 22	217.30	216.30	253.72	263.18	213.58	232.82±11.84	5.56	12.45±0.64	11.93±0.67
	Oct. 27	272.76	247.04	250.06	279.40		262.34±9.32	7.52	13.96±0.51	13.44±0.54
	Nov. 9	236.44	237.42	248.50	261.66	262.78	249.36±6.33	9.40	13.15±0.35	12.63±0.38
	Nov. 24	204.1	209.0	225.4	211.0		212.37±5.28	8.32	11.18±0.29	10.66±0.32
	Dec. 7	269.20	277.16	241.66	249.80		269.45±11.76	10.82	14.17±0.64	13.65±0.67
	Feb. 10	217.04	212.8	228.4	241.6		224.96±7.45	7.17	11.93±0.41	11.41±0.44
	Feb. 22	182.48	165.74	181.80	162.10		173.03±6.13	6.88	9.10±0.34	8.58±0.37
							group $\bar{x} \pm SE_{\bar{x}}$		12.28±0.72	11.76±0.75
SPINAL CORD (T_1+T_2)	Dec. 16	112.04	106.38	94.82	111.66		106.22±4.63	6.53	5.46±0.25	4.94±0.28
	Dec. 19	108.98	100.00	109.70	115.54		108.55±3.70	6.44	5.59±0.20	5.07±0.23
	Feb. 1	137.52	135.92	119.80	125.18		129.60±4.92	5.85	6.78±0.27	6.26±0.30
	Feb. 22	110.80	104.80	104.80	108.38		107.15±1.72	5.16	5.59±0.09	5.07±0.12
							group $\bar{x} \pm SE_{\bar{x}}$		5.86±0.36	5.34±0.39

TABLE III MEASUREMENTS OF NORMAL CHOLINESTERASE ACTIVITY OF SPINAL CORD AND LOWER BRAIN-STEM

SOMAN DOSE µg/kg	TOTAL ACID PRODUCTION (µℓ NaOH neutralized in 10 minutes) Aliquot No. 1	2	3	4	5	$\bar{x} \pm SE_{\bar{x}}$	NON-SPEC + PR-H_2O ACID RELEASE µℓ NaOH per 10 min.	CHOLINESTERASE ACTIVITY (meq ACh/mg tissue/min) x 10^6 APPARENT $\bar{x} \pm SE_{\bar{x}}$	CORRECTED $\bar{x} \pm SE_{\bar{x}}$
50	10.56	13.38	22.60	15.68	23.26	17.10±2.81	7.20	0.54±0.15	0.02±0.18
45.	23.82	20.16	18.60	20.08	22.10	20.95±1.01	7.52	0.74±0.06	0.22±0.09
40	21.86	16.86	19.72	14.80	20.00	18.65±1.40	7.50	0.61±0.08	0.09±0.11
30	20.64	21.08	23.88	23.30	22.40	22.26±0.69	8.52	0.75±0.04	0.23±0.07
25	45.00	36.72	36.56	35.74	33.66	37.53±2.17	6.02	1.73±0.12	1.21±0.15
20	42.90	38.98	47.40	41.04	41.92	42.45±1.56	8.70	1.85±0.09	1.33±0.12
5	217.8	213.2	215.6			215.53±1.62	15.30	10.97±0.09	10.45±0.12
5	160.3	159.0	152.2	144.4		153.97±4.22	5.35	8.14±0.23	7.62±0.26
5	214.8	218.0	220.5	203.1		214.11±4.43	7.66	11.31±0.24	10.79±0.27
5	178.98	177.82	176.90	175.72		177.35±0.79	5.25	9.43±0.04	8.91±0.07
							group $\bar{x} \pm SE_{\bar{x}}$	9.96±0.84	9.44±0.87

TABLE IV LOWER BRAIN-STEM CHOLINESTERASE ACTIVITY FOLLOWING INTRAVENOUS SOMAN

Expt	SOMAN DOSE µg/Kg	TOTAL ACID PRODUCTION (µℓ NaOH neutralized in 10 minutes) Aliquot No 1	2	3	4	5	$\bar{x} \pm SE_{\bar{x}}$	NON-SPEC + PR-H_2O ACID RELEASE µℓ NaOH per 10 min.	CHOLINESTERASE ACTIVITY (meq ACh/mg tissue/min) x 10^6 APPARENT $\bar{x} \pm SE_{\bar{x}}$	CORRECTED $\bar{x} \pm SE_{\bar{x}}$
K	5.0	14.40	14.30	14.30	14.76		14.44±0.11	5.17	0.51±0.01	(-)0.01±0.04
L		15.70	15.88	14.44	12.04		14.51±1.02	6.53	0.44±0.06	(-)0.08±0.09
N		14.20	16.30	16.54	20.10	22.70	17.97±1.69	8.01	0.55±0.09	0.03±0.12
O		21.26	19.40	27.70	16.28		21.16±2.78	11.48	0.53±0.15	0.01±0.18
P		12.92	19.80	12.72	14.30		14.94±1.92	7.52	0.41±0.11	(-)0.11±0.14
Q		15.04	15.80	16.82			15.89±0.63	7.46	0.46±0.03	(-)0.06±0.06
							group $\bar{x} \pm SE_{\bar{x}}$		0.48±0.02	(-)0.04±0.05
	2.0	28.10	17.50	13.80	18.02	19.52	19.39±2.65	8.08	0.62±0.15	0.10±0.18
	1.0	18.98	16.04	20.32	26.02		20.34±2.41	6.24	0.77±0.13	0.25±0.16
S	0.5	28.00	26.84	30.60	27.00		28.11±1.00	6.63	1.18±0.05	0.66±0.08
T	0.25	46.74	53.82	38.80	38.44	38.42	43.24±3.45	8.87	1.88±0.19	1.36±0.22
	0.20	50.90	46.32	45.60	46.20	54.00	48.60±1.84	6.30	2.32±0.10	1.80±0.13
	0.15	136.22	129.86	138.50	126.98	137.24	133.76±2.52	9.00	6.83±0.14	6.31±0.17

TABLE V LOWER BRAIN-STEM CHOLINESTERASE ACTIVITY FOLLOWING INTRAVERTEBRAL SOMAN

	SOMAN DOSE µg/kg	TOTAL ACID PRODUCTION (µℓ NaOH neutralized in 10 minutes) Aliquot No. 1	2	3	4	$\bar{x} \pm SE_{\bar{x}}$	NON-SPEC + PR-H_2O ACID RELEASE µℓ NaOH per 10 min.	CHOLINESTERASE ACTIVITY (meq ACh/mg tissue/min) x 10^6 APPARENT $\bar{x} \pm SE_{\bar{x}}$	CORRECTED $\bar{x} \pm SE_{\bar{x}}$	PERCENT OF NORMAL*	CORD SEGMENT
Expt K	5.0	25.50	26.68	25.22	24.22	25.40±0.58	8.81	0.91±0.03	0.49±0.06	9.2	$T_1 + T_2$
		61.26	52.58	57.20		57.01±3.07	12.55	2.44±0.17	1.92±0.20	36.0	$T_3 + T_4$
Expt L	5.0	76.00	71.26	71.28	77.12	73.91±1.78	6.60	3.69±0.10	3.17±0.13	59.4	$T_1 + T_2$
		77.82	64.06	60.44	59.66	65.47±4.88	6.73	3.22±0.27	2.70±0.30	50.6	$T_3 + T_4$
	2.0	37.68	30.64	30.40	32.50	32.80±1.95	4.42	1.55±0.11	1.03±0.14	19.3	$T_1 + T_2$
		124.50	121.36	128.12	144.30	129.66±5.83	9.04	6.61±0.32	6.09±0.35	114.0	$T_3 + T_4$
	1.0	103.82	102.00	99.68	98.86	101.09±1.29	6.76	5.17±0.07	4.65±0.10	87.1	$T_1 + T_2$
	0.5	56.58	49.50	54.46	54.82	53.84±1.75	7.41	2.54±0.10	2.02±0.13	37.8	$T_1 + T_2$
		133.74	120.62	137.14	122.78	128.57±4.67	3.87	6.83±0.26	6.31±0.29	118.2	$T_3 + T_4$

* Based on the normal activity for cords, $T_1 + T_2$: (5.34±0.39) x 10^{-6} meq ACh/mg tissue/min (from Table III)

TABLE VI SPINAL CORD CHOLINESTERASE ACTIVITY FOLLOWING INTRAVERTEBRAL SOMAN

Langendorff Rabbit Heart Data

Tables VII and VIII

Pulse Pressure Amplitude over Four Hours

	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
	cm H_2O								
Expt. I	42.5	32.5	34.4	38.2	39.4	40.0	40.0	42.5	46.3
II	75.0	75.0	65.0	67.5	65.0	60.0	60.0	55.0	55.0
III	45.0	45.0	51.3	52.6	51.3	50.0	51.3	37.5	35.0
IV	34.4	31.9	32.5	28.8	26.3	26.9	23.8	23.8	28.8
V	27.8	31.5	36.9	29.4	26.9	29.6	30.7	30.7	32.5
VI	50.0	51.3	62.5	55.0	55.0	60.0	55.0	52.5	60.0

Percent of Initial Value at 0.0 Hours

	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
	100.0	76.5	80.9	89.9	92.7	94.1	94.1	100.0	108.9
		100.0	86.7	90.0	86.7	80.0	80.0	73.3	73.3
		100.0	114.0	116.9	114.0	111.1	114.0	83.3	77.8
		92.7	94.5	83.7	76.5	78.2	69.2	69.2	83.7
		113.3	132.7	105.8	96.8	106.5	110.4	110.4	116.9
		102.6	125.0	110.0	110.0	120.0	110.0	105.0	120.0
$\bar{x}$	100.0	97.5	105.6	99.4	96.1	98.3	96.3	90.2	96.8
$SE_{\bar{x}}$		5.0	8.7	5.4	5.8	7.0	7.5	7.1	8.5

TABLE VII LANGENDORFF CONTRACTILITY DATA

Heart Rate Measured Over Four Hours

	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
	beats per minute								
Expt. I	144	132	132	128	124	120	124	124	120
II	164	112	128	108	136	128	128	136	136
III	160	144	112	144	144	136	128	180	180
IV	156	144	148	144	136	140	136	132	136
V	104	104	104	108	104	104	108	104	104
VI	136	124	136	140	128	128	132	132	132
	Percent of Initial Value at 0.0 Hours								
	100.0	91.7	91.7	88.9	86.1	83.3	86.1	86.1	83.3
		68.3	78.0	65.9	82.9	78.0	78.0	82.9	82.9
		90.0	70.0	90.0	90.0	85.0	80.0	112.5	112.5
		92.3	94.9	92.3	87.2	89.7	87.2	84.6	87.2
		100.0	100.0	103.8	100.0	100.0	103.8	100.0	100.0
		91.2	100.0	102.9	94.1	94.1	97.1	97.1	97.1
$\bar{x}$	100.0	88.9	89.1	90.6	90.1	88.4	88.7	93.9	93.8
$SE_{\bar{x}}$		4.4	5.1	5.6	2.5	3.2	4.1	4.7	4.7

TABLE VIII LANGENDORFF HEART RATE DATA

Autoperfusion Experiment

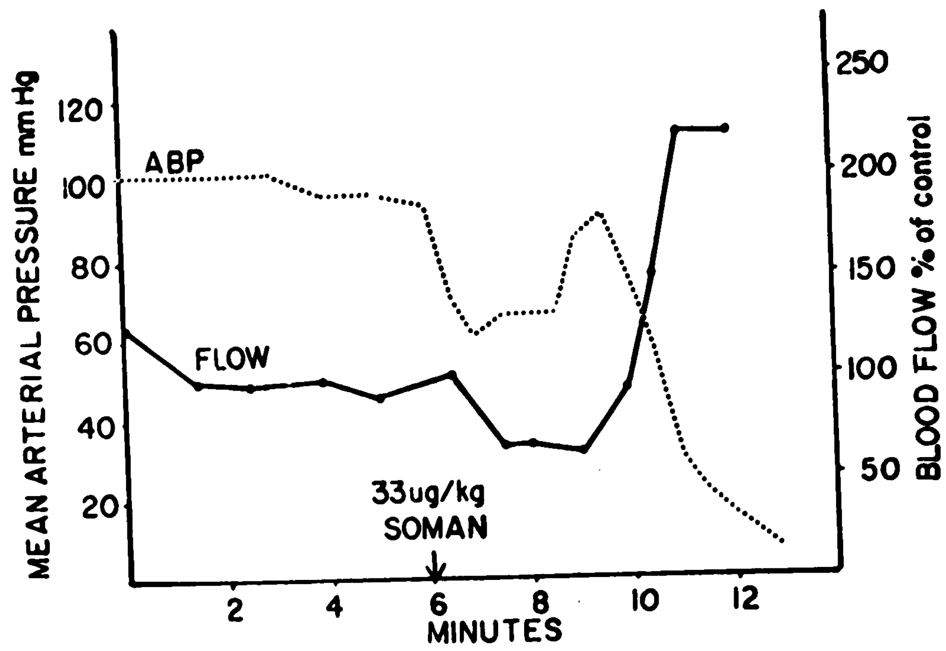
Figure 23

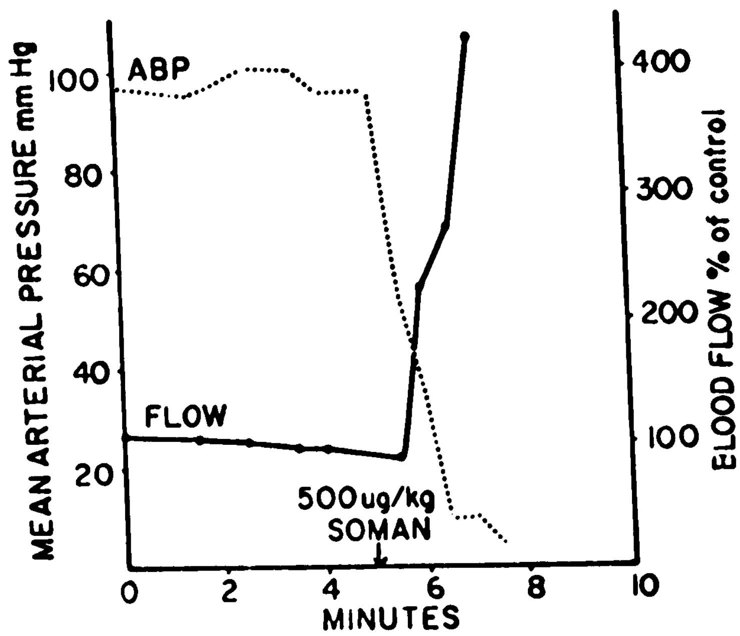
FIGURE 23: EFFECT OF INCREASED SOMAN DOSAGE ON THE TIME TO CARDIOVASCULAR COLLAPSE IN THE RABBIT

Each graph shows the arterial pressure and forelimb vascular resistance in a rabbit given intravenous soman. No atropine or artificial ventilation was provided. The forelimb was autoperfused and increased FLOW represents a proportional vasodilatation.

Heart Rate Changes:

	before soman	2 min after	3 min after
upper	264	128	97
lower	256	54	no pulse


MEAN ARTERIAL PRESSURE mm Hg
120
100
80
60
40
20
ABP
FLOW
33ug/kg
SOMAN
2
4
6
8
10
12
MINUTES
250
200
150
100
50
BLOOD FLOW % of control


MEAN ARTERIAL PRESSURE mm Hg
100
80
60
40
20
ABP
FLOW
500ug/kg
SOMAN
0
2
4
6
8
10
MINUTES
400
300
200
100
BLOOD FLOW % of control