Short Title of Thesis

CENTRAL CARDIOVASCULAR RESPONSES

Huq, S.
M.B., B.S.

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CENTRAL NERVOUS SYSTEM MEDIATED CARDIOVASCULAR RESPONSES TO DRUGS

by

Shariful Huq, M. B., B. S.

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Department of Pharmacology
McGill University
Montreal.

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This work is dedicated to my parents, who instilled in me the importance of seeking knowledge, to my teacher, Professor Melville, who fostered in me the scientific interest, and to my wife, who has made the search enjoyable and worthwhile.

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ABBREVIATIONS

ADR	Adrenaline
B.P. or B.P. (mm. Hg)	blood pressure in millimeters of mercury.
Bre	bretylium
caff	caffeine
chlor	chloralose
coca	cocaine
ECG	electrocardiogram
ethyb	ethybenztropine
gal.	gallamine (Flaxedil)
H.R. or H.R./min.	heart beats per minute
hexa.	hexamethonium
hemi.	hemicholinium (HC-3)
5-HT	5-hydroxytryptamine
i.v.	intravenous
i.vt.	intraventricular
Ipn	iproniazid
meca.	mecamylamine
NAD	noradrenaline
pento	pentobarbitone
PHE	phenoxybenzamine
RSP	reserpine
SCH	succinylcholine

I. INTRODUCTION*

In earlier reported studies from this laboratory (Share and Melville, 1961; Varma et al, 1962; and Melville and Share, 1963) it was shown that injection of the convulsant drug, picrotoxin, into the lateral cerebral ventricles of cats and rabbits can lead to a rise in arterial blood pressure associated with various types of electrocardiographic ST-T alterations and cardiac arrhythmias (bradycardia, tachycardia, extrasystoles and even ventricular fibrillation in rabbits). It was suggested that these cardiovascular effects of picrotoxin were mediated through central excitation of adrenergic mechanisms, probably involving "release" of catecholamines (noradrenaline mainly) from the hypothalamus and midbrain.

Following subcutaneous injection of picrotoxin in cats, it was also reported by Vogt (1954) that there is a marked reduction (approximately 50%) in the concentration of extractable noradrenaline in the brain. In contrast, following similar injections of other convulsants, such as leptazol, there was no change in brain noradrenaline content. It has long been known, however, that leptazol can induce

*Some preliminary results of this work were presented at the meeting of the Canadian Federation of Biol. Societies (Proc. Can. Federation Biol. Soc. 6, 31, 1963).

cardiovascular changes of central origin (See Literature Survey). It appeared, therefore, that unlike picrotoxin, changes in brain catecholamines may not be important in the centrally-mediated cardiovascular responses induced by leptazol.

While considerable work has been done in analyzing the actions of leptazol on the somatic nervous system, including its striking convulsant actions (See Hildebrandt's Review, 1937), the data in the literature concerning the mechanism of its cardiovascular effects are rather conflicting.

It has long been known that the central nervous system stimulant caffeine can induce changes in the heart and blood vessels through a central action (Sollmann, 1957), and it has also been reported by Vogt (1954) that like leptazol there was no change in brain catecholamine concentration following subcutaneous injections of caffeine. Dikshit (1934) showed that both intravenous and intraventricular injections of caffeine in cats led to cardiovascular changes and speculated that these might be due to hypothalamic excitation. Since the minimum effective intraventricular dose of caffeine was only about one-fifth of the corresponding intravenous dose, it was concluded that these changes were due to central nervous system effects rather than to any direct action on the heart or blood vessels. However, the basic mechanism of this action is still obscure.

With the hope of elucidating further the mechanism of the centrally-mediated cardiovascular effects of leptazol

and caffeine, it was of interest to undertake a systematic investigation of the cardiovascular responses induced by injections of these agents into the lateral cerebral ventricle, presumably limiting the injected agent to central nervous system structures. It is conceivable that a drug might be capable of exerting a strong central action, but when injected subcutaneously or intravenously, this may not be in evidence because the drug cannot reach the nervous structures due to the existence of the so-called "blood-brain barrier" (See Literature Survey). Conversely, when injected intra-ventricularly i.e. into the lateral cerebral ventricle, some substances may not be appreciably absorbed into the general circulation, and would, therefore, act centrally without inducing any significant peripheral effect.

In the initial experiments, attempts were made to assess and compare the quantitative changes in cardio-vascular responses elicited by (a) central (lateral ventricle injections) and (b) systemic (intravenous) administrations of the two agents, using increasing doses in order to establish a 'dose-response effect.' In connection with these studies the influence of different anesthetics and curarizing agents were also compared in both vagotomized and non-vagotomized animals. In other experiments, the influence of spinal section, that is, section of the spinal cord at the second cervical level in order to exclude all efferent sympathetic pathways, was also studied.

Since it has been postulated that mechanisms mediated by noradrenaline or by acetylcholine might be of physiological importance in the central nervous system, the possible significance of these "mediator substances" in the responses to leptazol and caffeine were also investigated, using various types of pharmacological agents known to influence adrenergic and cholinergic mechanisms. It was also hoped that from these studies some further knowledge might be gained concerning the physiologic mechanisms involved in the central nervous system mediation and regulation of cardiovascular functions.

II. LITERATURE SURVEY

A. CARDIOVASCULAR RESPONSES TO LEPTAZOL

Leptazol (B.P.) is chemically pentamethylenetetrazol, and is also known as, pentylenetetrazol (U.S.P.). It was synthesized in 1924, introduced first under the name of Cardiazol and is generally known today under the trade-name of Metrazol. For the sake of uniformity in presentation the drug will be referred to throughout under the name of leptazol, as given in the British Pharmacopoeia (1958), although not included in the 1963 Edition. It should be emphasized that while the drug has been extensively studied, and although it appears to be of questionable therapeutic value, in view of its striking convulsant action, it is still of considerable pharmacological interest.

a) Earlier Observations

The earlier published studies concerning the cardiovascular responses to leptazol led to conflicting results. Schmidt et al (1925) and Sanders (1927) reported that leptazol exerted a direct cardiac stimulant action, increasing amplitude, contractile force and frequency of the heart beats in the isolated perfused frog heart. Indeed, Eismayer and Quincke (1928) suggested that in the frog heart

high concentrations (1:1000 to 1:500) of the drug exerted an action similar to that of strophanthin.

On the other hand, Hildebrandt (1926) and Stross (1926) found that leptazol in such concentrations induced no effect on the normal frog heart, but in high concentrations (1:100) increased the contractions of the heart previously rendered hypodynamic by various experimental procedures. David et al (1929) also reported that with excessive concentrations (1:100 or higher) the drug induced an initial depression of the normal frog heart. It also appeared to improve contractions in the previously depressed heart.

In contrast, Camp (1928) concluded that the drug exerted no demonstrable significant action on the perfused frog's heart. In addition, following subcutaneous or intravenous injections into anesthetized dogs there was no consistent increase in heart rate. Indeed, in anesthetized animals, leptazol often induced a slight fall (10 to 15 mm. Hg) of blood pressure, associated in some experiments with an increase in volume of the splanchnic vessels (kidney and spleen). This vasodilatation did not occur after section of the splanchnic nerves nor after paralyzing doses of nicotine, and, therefore, was presumably due to some central action. Postmortem examinations also revealed marked congestion of all the abdominal viscera. It was, therefore, concluded by this worker, that leptazol had no apparent stimulatory effect on the normal heart, but caused vasodilatation of

the internal organs due to a central action.

Following intravenous injections of leptazol into anesthetized cats or rabbits, Eichler et al (1926) and Stross (1926) previously reported that the drug produced a transient rise in blood pressure associated with constriction of the splanchnic vessels. These effects were also abolished by decapitation or by cutting the spinal cord, and were, therefore, concluded to be of central nervous system origin.

From several other early experimental studies (See Hildebrandt's Review, 1937) it was concluded that leptazol enhanced the excitability of central vasomotor mechanisms in the medulla oblongata. Thus, the pressor response to the drug was also reduced when the vasomotor centre was depressed by various agents (ether, chloroform, chloral hydrate). Von Esveld (1930) also showed that small doses of leptazol enhanced both the respiratory stimulation and the pressor responses following administration of low concentrations (2.5%) of carbon dioxide in decerebrate, vagotomized, un-anesthetized cats. Conversely, when the medulla is depressed both of these responses were reduced in similar manner.

David et al (1929) reported that following injections of leptazol (50 to 100 mg.) in anesthetized animals the effects on blood pressure were variable. Thus, while there was generally only a transient fall in blood pressure, in some cases there was no change and in others a slight rise. Oncometric studies by these workers also showed an increase in the internal organ volumes, indicating dilatation of

splanchnic blood vessels. They, therefore, ascribed the fall of blood pressure to splanchnic vasodilatation. Furthermore, these authors found that perfusion of the blood vessels of the pithed frog with leptazol did not cause any appreciable change in their calibre, thereby excluding any direct action on the peripheral blood vessels. Leyko (1930) also reported no effect of leptazol on the cardiac output of the Starling heart-lung preparation, confirming the absence of any direct action of leptazol on the heart.

In regard to the initial fall in blood pressure observed following intravenous injections of leptazol in anesthetized animals, Maloney et al (1932) reported that this effect could be prevented by vagotomy and, therefore, concluded that it was due to central vagal stimulation.

Gellhorn et al (1939) using cats anesthetized with chloralosane also observed an initial fall of blood pressure with intravenous leptazol and reported that this was followed by a progressive rise. They suggested that the fall of blood pressure involved some action of leptazol on the "buffer nerves" mechanisms regulating blood pressure. In some experiments, they deprived the cats of their "buffer nerves" mechanisms at the start of the experiments and compared the responses observed with those obtained in normal animals. It was concluded that removal of the pressor chemoreceptor mechanisms (carotid sinus denervation) reduced the tendency for leptazol to induce a fall in blood pressure. It was also noted (a) that the initial fall of

blood pressure occurred with leptazol in the absence of convulsions and (b) that following repeated injections of leptazol, the initial fall became less or disappeared entirely. The blood pressure then showed greater tendency to increase during and even after the convulsions. The significance of these findings is not clear. From their experiments on adrenalectomized animals, these workers also concluded that the release of adrenaline from the adrenal medulla was not important for the cardiovascular actions of leptazol in subconvulsant doses.

Further investigations of the circulatory actions of leptazol on dogs and cats by Haury et al (1939) showed that in normal animals, following intravenous injections of small doses (5 mg./kg.), there was almost exclusively a pure dilatation of the visceral organs with a simultaneous fall in blood pressure, whereas with large doses (20 mg./kg.) there was, at first, a dilatation and then a constriction of the organs studied. With the latter doses, the dilatation of the organs was accompanied by a fall in blood pressure and the constriction of the organs by a rise in blood pressure. Using spinal and nicotinized animals, leptazol, however, caused only an increase in the size of the organs and a fall in blood pressure. These effects have, therefore, been attributed by the authors to a peripheral action of leptazol (since similar dilatation of the viscera also ensued in normal animals). In some experiments, the adrenal glands were mass-ligated and it was observed that injections

of leptazol in this preparation still produced the same results as those observed in the intact animals. No significant change in the heart rate was reported by these workers.

According to Haury et al (1939), vagotomy also did not affect the changes in blood pressure and splanchnic volume. It was concluded, therefore, that leptazol has a twofold action:- (a) a peripheral action which produced splanchnic dilatation and a fall in blood pressure and (b) a central action which produced splanchnic constriction and a rise in blood pressure. These workers further concluded that the action of leptazol is independent of the adrenal glands. Whether or not the constriction of the organs and the simultaneous rise in blood pressure are primary effects of the drug or only secondary to an anemia of the vasomotor centre, due to a decrease in the blood flow through the brain, as pointed out by Leibel et al (1938), cannot be concluded from these experiments.

In summary, the conclusions from early observations concerning the cardiovascular actions of leptazol are rather conflicting. Thus, (1) it has been variously concluded by some workers that leptazol exerts a direct stimulant action of the myocardium, while others suggest that the drug does not affect the normal heart but might favourably influence a depressed heart. (2) The initial fall in blood pressure observed with intravenous injections of leptazol

has also been considered to be due, by different workers, (a) to peripheral vasodilatation of central origin, (b) to central vagal stimulation, (c) to changes in the responses of the "buffer nerve" mechanisms and (d) to vasodilatation of peripheral origin (splanchnic dilatation). (3) The pressor response following the initial depressor response has been ascribed to stimulation of central vasomotor mechanisms in the medulla oblongata, since (a) direct vasoconstriction can be excluded by vessel perfusion experiments, and (b) the release of adrenaline from the adrenal medulla appears to be of no importance for the response.

b) Later Observations

Woodbury et al (1941) reported that the intravenous injections in man of convulsant doses of leptazol (10 mg./kg.) markedly increased the arterial pressure (average maximum increase of 100 mm. Hg). These authors, however, considered that direct vasoconstriction played only a minor role. It was concluded that this rise of blood pressure was brought about as a result of the intense contractions of the skeletal muscles, which markedly increased the extravascular pressure in most areas of the body, compressing blood vessels and, thereby, elevating the blood pressure. However, the authors also suggested that in man leptazol excited both parasympathetic and sympathetic nervous systems. The observed effects upon the

arterial pressure and the heart rate were, therefore, mixed and the changes which predominate, depended upon the type of premedication and upon the individual patient. Thus, in the presence of high spinal anesthesia, leptazol produced a dangerous degree of bradycardia, which was decreased by atropine, and in patients with curare, the pressor responses to leptazol were also much reduced.

In experiments on unanesthetized dogs, Woodbury et al (1941) moreover found that both subconvulsant and convulsant doses of leptazol induced intense and prolonged elevations of the arterial pressure. In contrast to man, these pressor responses were, therefore, considered to result from vasoconstriction due to central vasomotor excitation. Parasympathetic excitation was also observed, but was effectively masked, unless the sympathetic system was rendered ineffective by high spinal anesthesia or ergotoxine. Thus, in dogs, after paralyzing doses of curare (3 mg./kg.) convulsant doses of leptazol (15 to 20 mg./kg.) still caused the same prolonged rise in arterial pressure. It was concluded by the authors that convulsions in dogs failed to increase markedly the intrathoracic-abdominal pressures, and, therefore, differences in the blood pressure responses observed in man and in dogs might be due to this factor.

In agreement with the earlier observations of Hildebrandt (1937), Hahn et al (1957) reported that leptazol restores the excitability of the vasomotor centre when it is depressed by narcotics. On the other hand, injections of

high doses of leptazol (1 to 4 mg./kg. i.v.) in dogs, during experimental barbiturate poisoning can cause a fall in blood pressure rather than a rise (Bailey et al, 1953).

Cicardo (1954) reported that in unanesthetized, curarized dogs, leptazol (10 mg./kg. i.v.) produced an immediate rise in blood pressure which returned to normal within 5 to 10 minutes. In sympathectomized animals, leptazol caused a fall in blood pressure. Total adrenalectomy slightly decreased the pressor response to leptazol, although never abolished this response. From these experiments, it was concluded that the pressor response to leptazol was due to stimulation of the medullary vasomotor centre, while stimulation of some vasodilator centre in the central nervous system was responsible for the fall in blood pressure, as seen in the sympathectomized animals. It was also shown by this author that the release of adrenaline from the adrenal medulla did not appear to be important for the pressor response to leptazol. This latter finding confirmed similar earlier observations of Gellhorn (1939) and Haury (1939).

It has also been reported by Romagnoli and Melville (1958) that following intravenous injections of leptazol (10 to 20 mg./kg.) in non-vagotomized, curarized (succinylcholine) dogs, there was a marked rise in blood pressure, slowing of the heart and a decrease in cerebral blood flow. However, following previous intravenous injection of chlorpromazine (10 mg./kg.) the pressor response to leptazol,

in similar doses, was more intense and sustained, but there was no associated bradycardia and a rise rather than a fall in cerebral blood flow. It was concluded that these changes were due to central nervous system actions rather than to any direct peripheral actions of leptazol on blood vessels.

Gatgounis et al (1960) have investigated the cardiovascular effects of leptazol in pentobarbital anesthetized, open chest, vagotomized dogs with intact circulation, as well as in similarly anesthetized dogs in which the head and body circulations were separated, according to the method described by Swiss and Maison (1952). With the intact circulation and continuous recording of arterial pressure and heart force, the cardiovascular stimulant action of leptazol (50 mg./kg. i.v.) was largely abolished by preganglionic sympathetic blockade, which was accomplished by epidural infusions of procaine hydrochloride (0.5%) as previously employed by Brewster et al (1952). During similar recordings of circulatory functions in animals with separated head and body circulations, injections of leptazol into the head circulation produced marked cardiovascular stimulation, while injections into the body circulation were usually without cardiovascular stimulant effects. These experiments demonstrated again clearly the existence of a central nervous system component in the circulatory stimulant actions of leptazol, confirming earlier observations.

Covino et al (1962) have investigated the possibility of a direct cardiac action of leptazol. They compared the

effects of increasing concentrations of the drug on the isolated atria, on the papillary muscle and on the perfused isolated heart of cats. On the atria (90 to 120 mg. percent) the drug induced sinus bradycardia followed by arrhythmias characterized by a bigeminal or trigeminal pattern. Papillary muscle (60 to 250 mg. percent) showed a decrease in conduction velocity, prolongation of refractory period and increase in threshold, prior to the development of spontaneous activity. Finally, the intact isolated heart (150 mg. percent) showed sinus bradycardia, A-V block, ventricular fibrillation or asystole. These workers, therefore, concluded that the drug exerted a direct action upon the heart, but in comparison with the in vivo activity of leptazol, much higher in vitro concentrations were required. These authors also postulated that the arrhythmias associated with the therapeutic uses of leptazol, as an analeptic or for convulsant purposes, were probably the result of a central rather than a direct cardiac action.

Further evidence for a primary central origin of the cardiac arrhythmias and pressor responses to leptazol has recently been presented by Bircher et al (1963), using unanesthetized dogs under succinylcholine. The comparative effects of intravenous, subarachnoid (cortical), lateral ventricle, third ventricle and fourth ventricle administrations of leptazol were studied. It was confirmed that the fourth ventricle was the most sensitive target area for the induction of cardiac arrhythmias (Bircher et al, 1962). Comparisons

of the intravenous and fourth ventricle routes in this regard also showed that the required equieffective dose by the intravenous route was ten times that by the intraventricular route. In other investigations by these workers concerning the effects of leptazol following injection into the fourth ventricle in unanesthetized dogs, it was observed that similar cardiac arrhythmias and pressor responses were induced by intravenous doses of 30 mg./kg., as compared with doses of 5 mg./kg. injected into the fourth ventricle. The arrhythmias consisted of ventricular extrasystoles, A-V block, but more often ventricular tachycardia or bigeminal rhythms. These authors considered that these responses were mediated mainly by vagal and to a lesser extent by sympathetic pathways. Thus, the cardiac arrhythmias could be converted to supraventricular (possibly sinus) tachycardia in half of the cases, either by double vagotomy alone or by administration of the central anticholinergic drug, ethybenztropine (See Discussion). However, by combining these procedures with chronic sympathectomy or low cervical spinal transection or administration of hydergine, the cardiac arrhythmias could be prevented or converted to normal sinus rhythm in all cases. In these experiments, the pressor responses to leptazol were only completely prevented by chronic sympathectomy or low cervical transection. Indeed, in some experiments when the heart rate was reduced, the blood pressure fell. These results were interpreted to indicate that pressor responses to leptazol were due to intense

centrally mediated sympathetic discharges. They also suggested that the cardiac arrhythmic responses were mainly of central origin.

In summary, later observations have shown (1) that leptazol exerts striking effects on the cardiovascular system, primarily due to central nervous system actions; (2) that the pressor response appears to involve some types of central sympathetic component and the release of adrenaline from the adrenal medulla does not appear to be important; and, (3) that the arrhythmias appear to involve both sympathetic and parasympathetic mechanisms. The basic mechanism or mechanisms of action of the drug in producing these changes are, however, still unknown.

B. CARDIOVASCULAR RESPONSES TO CAFFEINE

The effects of caffeine on the cardiovascular system have been the subject of numerous investigations for many years, but the findings and conclusions of different workers have been rather contradictory. The actions of caffeine on the cardiovascular system are complex, and subject to considerable variation, depending apparently on experimental conditions and on the dosages of the drug employed. While it is assumed that variability in the responses to the drug might be due to these factors, the exact mechanism of action of caffeine at the cellular level is still obscure.

In addition to its direct cardiovascular effects, caffeine has also long been known to induce respiratory stimulation (Edsell, 1914). In experimental animals (cats and dogs), Lemessurier (1936) has shown that this effect is not affected by vagotomy or carotid sinus denervation, and therefore, is considered as primarily due to central medullary stimulation. Since changes in respiration can influence circulation, this might be an additional complicating factor in assessing the general cardiovascular responses to the drug.

a) Earlier Observations

As early as 1887, Phillips studied the effects of caffeine on the circulation of curarized animals (dogs, cats

and rabbits). It was observed that the drug induced an initial diminution in the force of the heart beats, followed by either slight or no acceleration of the rhythm. Subsequently, there was an increase in the force of the heart and a distinct slowing of the rhythm, occasionally followed by a slight but persistent acceleration. During the initial stage, there was a fall of arterial pressure, which regained or slightly exceeded its normal level during the second stage. After sectioning the vagi, it was observed that caffeine produced a fall of arterial pressure associated with diminution in the force of the cardiac beats. This fall was more prolonged and frequently greater than the fall produced by the same dose prior to section of these nerves. Although blood pressure returned to its former level, there was no secondary increase in blood pressure observed. These results were interpreted as due to actions of caffeine both on the cardiac muscle and on the vagus centre in the medulla; that is, the diminution of the cardiac beats resulted from a direct action in the heart, while the rise in blood pressure was due to central vagal inhibition.

Caffeine was also early reported, by the same author, to lead to dilatation of the renal vessels in association with its diuretic action in cats, dogs and rabbits. While it was observed that renal vascular dilatation paralleled to some extent the diuresis, it was concluded that the diuretic effect was not entirely due to the vasodilatation. On the other hand, Cushing et al (1921) observed that the

diuresis started earlier and outlasted the renal vaso-dilatation, and therefore concluded that the two actions were not related.

While studying the reaction of surviving arterial strips from animals or man, Cow (1911) demonstrated that caffeine (concentrations not stated) induced relaxation of isolated arterial rings. The experiments also showed that caffeine exerts a more pronounced vasodilatation on the splanchnic vessels than on other systemic vessels.

Sollmann et al (1911) performed experiments on dogs, under morphine-anesthesia, supplemented by ether during the operation and generally with curare. Some cats were also used with morphine-atropine-ethylcarbamate narcosis. In some experiments the vasomotor centre was destroyed while in some experiments the vagi were cut. It was concluded that the vagus centre is stimulated by caffeine and that in small doses (2 to 20 mg./kg.) slowing of the heart ensued. With larger doses, a direct stimulation of the heart with increase in rate, also occurred and predominated. These workers also studied the actions of caffeine on the blood pressure and on the peripheral vessels and observed that on intravenous injection of small doses there was a momentary depression of the myocardium and a consequent fall of blood pressure. This was promptly succeeded by a rise of blood pressure due to stimulation of the myocardium. From oncometric studies, it was observed that the changes in organ volumes usually followed the blood pressure, so that

the rise was mainly cardiac; but, the vasomotor centre was also stimulated and, in some cases, the vasoconstrictor factor was more effective than the cardiac factors. Thus, while direct peripheral vasodilatation occurred simultaneously, the output of the heart increased more than was necessary to compensate for the dilatation of the peripheral vessels, hence the blood pressure rose. With larger doses (20 to 150 mg./kg.) there was a persistent fall of blood pressure owing to direct cardiac depression and vasomotor paralysis. According to these workers, there was also considerable variability in the cardiovascular sensitivity to caffeine.

Pilcher (1911a), while studying the antagonism of alcohol by caffeine observed that when caffeine (2 to 10 mg./kg.) was injected hypodermically or administered through stomach tube to cats, it produced a small and variable effect on the heart rate. Moderate doses (15 to 30 mg./kg.) gave maximal quickening. No arrhythmias were seen with caffeine even with large doses.

Pilcher (1911b) also published other experiments concerning the actions of caffeine on the mammalian heart, using both cardiac-plethysmography and myocardiography in dogs. He found that during the acute fall of blood pressure, following intravenous injections of caffeine in doses below 10 mg./kg., the heart volume and amplitude remained unchanged, but with higher doses the diastolic volume increased and the amplitude lessened. With doses up to 20 mg./kg. a moderate rise of blood pressure and increase in both heart rate and

amplitude occurred. With larger doses there was a progressive fall of blood pressure, an increase of rate and diastolic volume and a decrease in amplitude of contractions.

Means et al (1915) observed the effects of caffeine on the blood flow of a few human subjects, during rest and during muscular work. They found that a single oral dose of 300 mg. of caffeine in normal subjects caused an increase in total blood flow, without a corresponding increase in oxygen absorption. The pulse rate was unchanged, consequently the systolic output was increased. During muscular work, no other action was obtained from caffeine than possibly an increase in pulse rate and consequently a slight diminution in systolic output. It was suggested that during rest caffeine increased the blood flow by increasing the venous supply through an action upon some mechanism outside the heart. When the blood supply becomes adequate no such action was obtained.

Barbour et al (1915) observed that caffeine decreased markedly the response of the frog's heart to faradic stimulation of the vagus nerve. These results were intepreted to mean that caffeine could produce depression of vagal endings, although the probability of increased irritability of the heart muscle due to the drug was not excluded.

Plant (1914) using the Knowlton-Starling heart-lung preparation found that the rate, amplitude and total output of the heart were increased by caffeine (25 mg.).

There was also a rise of pressure in the tube leading to the artificial resistance. The output of the heart per beat was not altered and the relaxation of the heart was slightly reduced.

Bush (1920) investigated the action of caffeine on the isolated medulla of the striped turtle, especially on the cardio-inhibitory centre. He perfused the medulla with frog Locke's solution. The addition of caffeine (0.04%) to the perfusing solution produced no constant changes in the rate or rhythm of the heart.

Heathcote (1921) studied the action of the xanthines on the excised hearts of both frogs and mammals. He demonstrated that caffeine can accelerate and augment the heart beat when perfused in dilutions, ranging from 1 in 1300 to 1 in 5000. He attributed this to a direct action of caffeine on the cardiac musculature rather than to stimulation of the accelerator nerve endings. He further observed that caffeine also exerted an active vasodilator action on the coronary vessels of mammals,- probably of muscular origin.

In summary, (1) the actions of caffeine on the frog and mammalian heart have been investigated by many early workers employing both the isolated heart and the heart <u>in situ</u>. (2) It was considered by these workers that on the excised heart, small doses increased cardiac efficiency while large doses exerted an opposite effect. (3) In the intact animal, intravenous injections of small

doses caused an initial fall of blood pressure (presumably due to myocardial depression) and followed by a slight rise in blood pressure (due to both direct myocardial and vasomotor stimulation). (4) The intense central vasomotor stimulation produced by caffeine can presumably overcome the peripheral vasodilatation produced by the drug, thereby leading to a rise in blood pressure associated with vasodilatation.

b) Later Observations

Beattie et al (1930) produced extrasystoles in anesthetized (Dial) cats by injecting caffeine (35 to 45 mg./kg.) intravenously. They also observed that a transitory effect still ensued in the decerebrate animal, and that section of all nerve connections to the heart and removal of the suprarenal glands had no effect on the production of heart irregularities by caffeine. They concluded, therefore, that caffeine has a direct effect "on the heart itself, either on the myocardium or on the sympathetic nerve endings within the organ."

It should be emphasized that while caffeine has been shown to induce extrasystoles in cases of poisoning in man, cardiac arrhythmias are only induced in experimental animals by excessive doses. However, Sollmann (1957) states that doses of about 1 Gm. of caffeine may produce alarming symptoms in man.

Grollman (1932) using an 'acetylene method' for the

determination of cardiac output, found that oral administration of large doses (0.97 Gm.) in man produced detectable increase in cardiac output, whereas the blood pressure remained unchanged.

Dikshit (1934) later reported that intravenous administration of caffeine (50 mg./kg.) as well as intraventricular injections of doses of 10 to 12 mg./kg. produced cardiac irregularities. He also observed a marked pressor response on intraventricular administration. As already pointed out (See Introduction) since it was found that the minimum effective intraventricular dose of caffeine was only one-fifth of the minimum effective intravenous dose, the author considered this as strong evidence against the drug acting directly on the heart or on any tissue outside the central nervous system.

Cheney (1935) has studied the influence of caffeine on an isolated sino-auricular strip preparation of the frog. He observed that caffeine (0.025 to 0.20 percent) produced an increased amplitude in the sino-auricular strip contraction without any demonstrable effect upon other characteristics of the tissue. Recovery occurred rapidly and completely unless the preparation was subjected to a caffeine percentage exceeding 0.20 percent. Caffeine in higher concentrations than 2.0 percent, caused reduction in amplitude and irregularities in rhythm from which only incomplete recovery occurred.

Starr et al (1937) studied the cardiovascular actions of caffeine on patients suffering from cardiac or circulatory disease but not from congestive heart failure. They observed a 14.8 percent increment in cardiac output with a intramuscular dose of 0.5 Gm. of caffeine although the blood pressure and electrocardiogram were essentially unchanged. They concluded that caffeine can directly stimulate the heart and increase cardiac rate, but in man this is usually balanced by the effect of medullary vagal stimulation.

Using isolated perfused electrically-induced fibrillating hearts of dogs, Lindner et al (1941) demonstrated that caffeine (125 to 500 mg.) caused moderate coronary dilatation. They attributed this to a direct action of caffeine on coronary vessels. This confirmed earlier observations on the coronary circulation.

Krop (1944) using isolated papillary muscle of the cat heart observed that caffeine in concentrations between 1:2000 and 1:5000 produced an increase in systolic tension in less than half the experiments and lower concentrations were without effect. Increases in tension of 50 to 250 percent occurred with concentrations of from 1:1000 to 1:250. The higher concentrations caused inexcitability and rigor in 15 to 20 minutes. He concluded that caffeine is ineffectual on the heart muscle except in high concentrations.

In summary, (1) later studies have confirmed that caffeine acts on the cardiovascular system both peripherally

and centrally. (2) These effects, however, remain complex and vary with dose, route of administration and other experimental conditions employed. (3) It is concluded that both systemic (intravenous) and central (intraventricular) administrations of caffeine can produce cardiac arrhythmias. (4) Following intraventricular injection, there is also an associated intense pressor response. (5) While these latter changes are of central nervous system origin, the underlying mechanism of their production has not been established.

C. MISCELLANEOUS AUTONOMIC RESPONSES TO LEPTAZOL

Apart from the changes in cardiovascular autonomic functions, as outlined above (Section A), the effects of leptazol on other autonomic functions have been extensively studied both in experimental animals and man. Thus, it has been repeatedly reported by many investigators that leptazol can induce salivation, sweating, piloerection, exophthalmus, mydriasis, contraction of the nictitating membrane and of the spleen, inhibition of gastric and intestinal mobility (or a slight increase in tone), bronchial dilatation, contraction (and inhibition) of the bladder, erection and ejaculation, hyperglycemia, increase in metabolic rate and actions on the chromatophores (Camp, 1928; Gellhorn et al, 1939; Hildebrandt, 1937; Leibel et al, 1938; Lowe, 1938; Masserman, 1939; Mautner et al, 1943; Teague, 1939). Leptazol has also been shown to exert an emetic action in pigeons (Chakravati, M., 1939).

Camp (1928) has shown that when introduced into the fourth ventricle of rabbits, leptazol (20 mg.) induced direct stimulation of the respiratory centre, although the response was quite variable. The drug also caused an increase in salivary secretion, which was thought to be due to a central action of leptazol, since the effect was abolished after section of the seventh cranial nerve. Similarly, there was

transient relaxation of the stomach, which did not occur after section of the splanchnic nerves. Kidney volume was increased after leptazol injection, but this was also abolished after splanchnic section, indicating that the vasodilatation is of central origin. In addition, leptazol was also shown (Camp, 1928) to induce pupillary dilatation in the rabbit, although this change was not observed after sympathetic denervation (removal of cervical sympathetic chain), the superior cervical ganglion remaining intact. It was, therefore, evident that leptazol did not induce pupillary dilatation either by a peripheral action in the eye or on the superior cervical ganglia. It was concluded, therefore, that the mydriatic action was clearly of central sympathetic origin. Finally, in regard to effects on the urinary tract, no consistent results were obtained on urinary secretion. It was, however, observed (Camp, 1928) that leptazol induced contractions of the urinary bladder, which were abolished by section of the dorsal cord, whereas pilocarpine still produced contractions. Injection of the drug directly into the urinary bladder was also without effect. From the above findings it was concluded that leptazol can produce multiple non-cardiovascular actions of central nervous system origin, due to activation of sympathetic and parasympathetic functions.

In connection with the therapeutic use of leptazol in schizophrenic patients, as suggested by Von Meduna (1935 and 1937), it has been shown by Gellhorn (1938) that

associated with the improvement of the patients there was some evidence of stimulation of the sympathetic division of the autonomic system. Soloman et al (1939) also concluded that with improvement of psychosis the autonomic balance is shifted increasingly towards the sympathetic side. However, the mechanism of the underlying action of leptazol in these studies has not been elucidated.

In order to follow quantitatively the effects of leptazol on the two divisions (sympathetic and parasympathetic) of the autonomic system, Darrow et al (1938) recorded simultaneously the following parameters:- blood pressure changes; pupillary changes in the normal eye and after sympathectomy of the other eye; contraction of the nictitating membranes both normal and unilaterally denervated; and changes in the galvanic skin reflex from the footpads - a cholinergic sympathetic response. Employing these techniques, using anesthetized cats, Darrow et al (1938) and Gellhorn et al (1939) demonstrated that associated with the convulsions induced by leptazol, there were greatly increased sympathetic discharges in various autonomic mechanisms. It was also shown by these workers that these autonomic effects ensued even when the convulsive action of the drug is completely suppressed by curare.

From the various types of experiments performed along the lines indicated above, it was observed that there is an increased "spontaneous" and reflex excitability of the

sympathetic centres following leptazol, independent of its convulsive somatic effects. In this state of autonomic excitability any slight stimulus produces a strong reaction. The reflex reaction of the normal pupil to stimulation of an afferent nerve was also increased, indicating an increased reflex sympathetic excitation. This was also associated with increased contraction of the normally innervated nictitating membrane. In addition, the galvanic responses to reflex stimulation often appeared, if previously absent, and if present were increased. The picture was, therefore, one of predominantly strong reflex excitability of the sympathetic system. On the other hand, parasympathetic excitatory responses were also demonstrated. Thus, a constriction of the sympathectomized pupil, in contrast with dilatation of the normal pupil, was suggestive of parasympathetic (cholinergic) excitation. The reflex dilatation of the denervated pupil, attributable to inhibition of the parasympathetic constrictor tonus, was ordinarily little changed. In unanesthetized cats during convulsions constriction of the sympathectomized pupil was also of shorter duration than in anesthetized cats, and was also followed by some dilatation. This would indicate that in unanesthetized animals sympathetic excitation lasts longer than the parasympathetic excitation. According to these authors, similar responses to leptazol could be elicited even in decerebrate cats.

It was, therefore, concluded by these workers that

the actions of leptazol on the autonomic nervous system comprise:- 1) excitation of sympathetic mechanisms: 2) increased reflex excitability of the sympathetic system by afferent stimulation; and 3) excitation of parasympathetic or cholinergic mechanisms.

Masserman (1939) studied the effect of intravenous and intradiencephalic injections of leptazol (10% solution) in varying doses, on the responses to bipolar faradic stimulation of the hypothalamus in anesthetized cats. It was observed that intravenous injection of leptazol (1 ml.) produced no change in the response to faradic stimulation of the hypothalamus. However, injection of doses of 0.05 to 0.2 ml. i.e. 5 to 20 mg. into the diencephalon of an acute preparation caused marked lowering of threshold and an increase in the intensity of the responses (salivation, mydriasis, pilo-erection, forced urination, clawing, etc.) to stimulation during a period of ten minutes following the injection. It was, therefore, concluded that leptazol stimulates hypothalamic functions when applied directly to this region of the diencephalon.

Gellhorn (1941) also observed that leptazol (0.2 to 0.8 cc. 10% i.e. 20 to 80 mg. per cat) when given <u>intravenously</u> affects both divisions of the autonomic nervous system, but the sympathetic activation predominated. This is in agreement with the findings of Woodbury <u>et al</u> (1941), (See Literature Survey, Section A, above). The experiments reported by Gellhorn are of interest not only because they established the fact that leptazol markedly increases the excitability

of the central sympathetic, but they also throw light on the interrelation between the parasympathetic and sympathetic nervous system in conditions of central excitation. It was observed that, whereas normal rats react to leptazol with a hyperglycemia, a fall in blood sugar results from leptazol after the adrenals have been removed. This hypoglycemic reaction occurs only when the vagi are intact. This effect seems to indicate that leptazol stimulates not only the sympathetico-adrenal system, but also simultaneously the vagus, leading to increased insulin secretion (Britton, 1925). Similar conclusions were made by Heilbrunn et al (1939) and Lougheed et al (1939).

Gellhorn (1941) also showed that adrenalectomized rats in which the vagi were divided no longer showed a hypoglycemia under leptazol effect. He, therefore, came to the conclusion that leptazol led to an excitation of both divisions of the autonomic nervous system. The excitation of the vagus caused an increased insulin secretion, the stimulation of the sympathetic system an increased secretion of adrenaline in the intact animal. The latter action, however, predominates so that it completely obscured the equally important effects on the vago-insulin system. Using cats and stimulating the hypothalamus while recording the contractions of the nictitating membrane, Gellhorn also found that leptazol (0.2 to 0.8 cc., 10% per cat) when given intravenously markedly increased hypothalamic excitability as measured by the increased

contraction of the nictitating membrane. However, Masserman (1939), as stated above, observed no alteration in hypothalamic excitability following intravenous leptazol, although intradiencephalic injection of leptazol did increase the hypothalamic excitability.

Intrahypothalamic injections of leptazol (10% solution, 0.04 ml.) were also observed by Gellhorn (1955) to increase the sympathetic responsiveness of the hypothalamus and shortened the hypotensive actions of intravenously injected acetylcholine, mecholyl and histamine, leading after an initial hypotensive phase to a secondary hypertension. Simultaneously, the contractions of nictitating membrane were increased.

Onuma (1957) while studying the effects of drugs on the predisposition to convulsions recorded the action potential of autonomic nerves. Rabbits were injected with five percent leptazol at a slow, constant rate of 0.2 c.c. per minute. The action potentials recorded 1 minute and 2.5 minutes after the start of infusion showed a slight increase of intermediate waves (15.5 to 9.8 cycles per second) in the vagus, but a decrease of fast waves (39.2 to 24 cycles per second) and an increase of intermediate waves (5.5 to 9.8 cycles per second) in the sympathetic. These tendencies became more pronounced before the onset of convulsions. The amplitude of the action potentials also showed a tendency to increase over the normal levels. These findings are in agreement with the previous studies of Orihara (1952).

In summary, (1) all observations indicate clearly that leptazol acts on both branches (sympathetic and parasympathetic) of the autonomic nervous system. (2) These autonomic responses to leptazol appear to be of central origin. (3) Effects on sympathetic centres appear to predominate over those on parasympathetic centres. (4) Leptazol appears to increase hypothalmic excitability, especially when injected directly into the hypothalamus.

D. SPECIAL CONSIDERATIONS OF THE DISTRIBUTION AND ABSORPTION OF DRUGS WHEN ADMINISTERED BY THE INTRAVENTRICULAR ROUTE

The method of intraventricular administration of drugs, as described by Feldberg and Sherwood in 1953, has been extensively employed by various workers for studying the central actions of drugs. A detailed account of the distribution and absorption of drugs when this route of administration is employed has been recently compiled by Feldberg (1963).

As previously pointed out (Introduction), it is conceivable that while a chemical agent might be capable of exerting important pharmacological effects in the central nervous system, these may be less evident or absent when the drug is injected subcutaneously or intravenously. This is so, because the drug may not reach its site or sites of action in the central nervous system, either in an effective concentration or not at all, presumably due to the so-called "blood-brain barrier."

According to Feldberg, "the brain can be looked upon as a hollow organ, the cerebral ventricles forming its inside and the subarachnoid space as its outside, both being filled with cerebrospinal fluid." Feldberg also concludes, "there are some holes in the wall of the fourth ventricle through which the inside communicates with the outside.

The arrangement of the ventricular system is essentially the same in all mammals and consists of four communicating ventricles. The lateral ventricles communicate with the third ventricle through the foramina of Monro, and the third with the fourth ventricle through the aqueduct of Sylvius." Hence, when a drug is injected into the lateral ventricle, the whole ventricular system and parts of the subarachnoid space are flooded with it. It is generally stated that a drug acts from the "inside" of the brain, when it acts on structures reached from the cerebral cavities; and from the "outside" of the brain when it acts on structures reached from the subarachnoid space. For studying the central site or sites of action of a drug, either direct injections through an indwelling cannula or perfusion experiments through the ventricular system can be used. Although used by several workers, the results of such drug administrations can only lead to limited conclusions regarding the exact anatomical sites affected, as indicated below.

Before a substance can act on the brain via the cerebral ventricles, it must penetrate the brain tissue. The fact that many drugs exert central actions when applied by this route shows that they are able to do so. Concerning the depth of penetration, there are two extreme views. On the one hand, Von Monakow (1921) believed that the cerebrospinal fluid passed from the ventricles through the whole wall of the brain and on its way carried hormonal substances

to nuclear regions. On the other hand, Spatz (1933) found that after two hours following intraventricular injection of trypan blue (0.1 to 0.3 ml. of 1 to 1.5% solutions), the dye only penetrated a distance of 0.5 mm. into the brain tissue, and that there was no variation between different regions of the brain. It was, therefore, concluded that, with regard to the uptake of substances from the cerebrospinal fluid, the brain behaves rather like a homogenous colloidal mass. Bakay (1956), as a result of his studies with p^{32}, also assumed a diffuse penetration of substances into the brain tissue independent of the type of nervous tissue structure.

Feldberg (1963) has studied the histamine uptake in anesthetized cats, in which the cerebral ventricles were perfused with histamine (1/1000 for one hour) from the cannulated lateral ventricle to the aqueduct. It was found that histamine had penetrated into the ventricular walls. The grey matter had taken up more histamine than the white matter and the hypothalamus was penetrated for at least 2.5 mm. Hess (1955) in his experiments on electrical stimulation of diencephalic structures also obtained responses predominantly from points approximately 3 mm. from the midline; that is, from the regions which can apparently be most easily reached by substances penetrating from the cerebral ventricles. However, when Feldberg perfused the cerebral cavities with bromophenol blue (0.1 to 0.2 percent solution) in the same manner as

histamine, the dye penetrated the brain so deeply from the "inside" that some areas from the "outside" also became stained.

Similar results were obtained by Roth et al (1959), who studied the uptake of S^{35} labelled acetazolamide (Diamox) injected intravenously in cats. The radioactive dose was diluted with unlabelled drug to bring the total acetazolamide administration to 150 mg./kg. Combined radio assay and autoradiographic techniques showed that the drug first appeared in the cerebral ventricles, then penetrated the ventricular walls. Feldberg has also suggested that the essential factor which governs the uptake of substances appears to involve specifically the neuroglia. This idea is in accord with the recent electron microscopic findings by Gershenfeld et al (1959), Luse (1959) and Luse et al (1960).

With regard to systemic absorption, it is known that some substances injected intraventricularly can be absorbed into the blood stream. It is important, therefore, to know to what extent any agent is absorbed, that is to say, what is the contribution of the absorbed fraction to the total observed response when the drug is injected centrally. This problem is relatively simple in the case of drugs whose central effects are opposite from their peripheral effects. For example, it is well-established that tubocurarine when administered intraventricularly leads to convulsions, but if absorption were to occur to any great extent, this would induce neuromuscular blockade and, hence, would mask the

convulsions. It has also recently been shown (Share and Melville, 1963) that intraventricular noradrenaline leads to a fall in blood pressure as opposed to the well-known pressor effect of intravenous injections. Bhawa (1958) has shown that histamine injected intraventricularly into anesthetized cats causes acid gastric secretion as a result of its absorption. However, Feldberg (1963) has demonstrated that the secretion produced by a dose of 500 µg. of histamine injected intraventricularly was less than that produced by an equal dose of histamine injected subcutaneously. Indeed, it was even less than that produced by a dose of 250 µg., subcutaneously. In conclusion, although the degree of absorption may vary with different substances introduced into the cerebral ventricles, the following criteria for differentiating effects due to intraventricular absorption from those due to generalized systemic absorption have been established by Feldberg (1963):- (1) Systemic absorption can be excluded when the effects observed are different from those following intravenous or subcutaneous injection. (2) When these effects are similar systemic absorption may still account for the observed effects but can be excluded if the effects are shown to be abolished by interruption of the appropriate nervous pathways. These criteria have been employed throughout for studying the centrally-mediated cardiovascular effects of leptazol and caffeine.

III. MATERIALS AND METHODS

1. General Procedure

Cats of either sex and weighing between 2.4 to 3.4 kg. were used. A total of four hundred experiments were performed in connection with this investigation. In some experiments the animals were anesthetized with intraperitoneally injected pentobarbitone sodium (25 to 30 mg./kg.) In another series of experiments, the animals were anesthetized with intravenous chloralose (70 mg./kg.), following ether induction. Reserpine pretreated animals were anesthetized with a smaller dose of pentobarbitone sodium (15 to 25 mg./kg.), injected intraperitoneally.

To eliminate any possible changes in the cardiovascular system due to respiratory alterations, following the administration of the drugs all the animals were placed in the supine position on artificial respiration using a Harvard pump. The animals received approximately 15 to 20 ml. of room air per kilogram of body weight at a rate of 15 to 20 times per minute.

For the purpose of intravenous administration of drugs a polyethylene tubing (PE 90), filled with 0.9 percent sodium chloride was inserted into a femoral vein and connected to a 20-gauge hypodermic needle.

In some animals the vagi on both sides were sectioned in the neck. Such animals are referred to throughout as vagotomized. In one series of experiments gallamine was administered intravenously as the curarizing agent, in initial doses of 4 mg./kg. followed every 20 or 30 minutes by an additional 2 mg./kg. Bovet et al (1947, 1949) and Melville (1952) have shown that gallamine has an atropine-like action on the heart, although it does not prevent the depressor response to injected acetylcholine. To avoid this effect on the heart, succinylcholine (1 mg./ml.) was used as the curarizing agent in another series of experiments. It was administered through continuous intravenous infusion (approximately 1 ml./min.) through a polyethylene tubing (PE 90) inserted into a second femoral vein.

Femoral arterial blood pressure was recorded in all experiments. For this purpose a polyethylene tubing (PE 90) filled with heparin (0.1% in 0.9% sodium chloride) was inserted high into a femoral artery. This was connected by a Sanborn pressure transducer to a carrier pre-amplifier and the blood pressure was recorded on a Sanborn Twinviso Recorder. Electrocardiograms were taken concurrently with a standardization of 1 mv. giving a deflection of 10 mm. Lead II (with needle electrodes inserted under the skin of the limbs) was taken at fixed successive intervals in all experiments. Paper speed during recording was 25 mm. per second.

With the cat turned on its abdomen, and to keep the head of the animal in a fixed position for surgical procedures, as well as to maintain uniformity in the posture of all animals, they were maintained in the same postural condition, as follows:- With the animal in the prone position, the head rested on a brass bar (30 inches long) which was placed transversally through the mouth just behind the canine incisors. This brass bar was fixed 6 cm. above the operating table, to which it was firmly held at both ends. The posterior parts of the mandibles rested on two other vertically placed brass rods (6 cm. in length each) which were flattened at the top to support the mandibles.

In order to limit the application of drugs to central nervous system structures and to avoid the peripheral direct activity of substances on the cardiovascular system, central administration of drugs was carried out by injecting them into the right lateral cerebral ventricles through a Collison's cannula using the method of Feldberg and Sherwood (1953), as modified by Melville and Shister (1957). The procedure employed was, as follows:- A longitudinal incision is made over the parietal bone, parallel and close to the midline and extending for 3 to 5 cm. The underlying tissue was cleared away until the junction of the coronal and sagittal sutures is identified. At a point located at 6 to 6.5 mm. posterior to the coronal suture and 3 to 3.5 mm. from the midline, the skull was trephined with a small electric hand drill inclined towards the midline at an angle

of about 30 degrees. Bleeding which usually started at this stage was easily stopped by applying bone wax. The reaming of the orifice also stopped the bleeding promptly and provided the requisite thread for the cannula.* The dura was perforated with a 20-gauge needle and the cannula now screwed into place with a slight inclination towards the midline. While the cannula was firmly held by a spanner, the stylet was removed. Cerebrospinal fluid generally appeared with rhythmic pulsations and the fluid could also be aspirated through the cannula. If blood was seen coming out of the cannula, the animal was discarded.

The amount of drug injected intraventricularly at any one time was contained in a volume of 0.2 ml. Whether or not the cannula reached the lateral cerebral ventricle and the drug was distributed into the ventricular system of the brain was determined at the end of the experiment in the following manner:- 0.2 ml. of a solution of methylene blue (0.1%) was injected intraventricularly and the ventricular system examined. Battacharya and Feldberg (1958) have shown that if methylene blue was injected into the lateral cerebral ventricle it stained the right and left lateral ventricles, the third and the ventral part of the floor of the fourth ventricle, but not the caudal part beyond the lateral recesses. However, the outer surface

*The cannula (with outfit), under the name of "Collison cannula," was obtained from C. F. Palmer Ltd., Brixton, England.

of the brain stem was also deeply stained, and the staining extended to the dorsal surface of the colliculi. When this distribution was not observed the experiment was discarded because either the cannula did not reach the right lateral cerebral ventricle or there was a blockade at the right intraventricular foramen or in the aqueduct of Sylvius.

In experiments requiring intraventricular pre-treatment with drugs for longer periods of time before the animals were used, the cannula was inserted under pento-barbitone anesthesia and the incision closed with Michel wound clips after the cannula was placed in position. These animals received post operatively intramuscular injections of 400,000 i.u. penicillin-G-procaine in aqueous suspension with 0.5 gm. streptomycin sulfate. A period of six to seven days was allowed for the animal to recuperate before they were used for the experiment.

During shorter acute experiments, a period of 30 minutes or longer was allowed to elapse after all the surgical procedures were completed, and before the various recordings were started. Control electrocardiogram and blood pressure were recorded for some period before administration of a drug.

Both systolic and diastolic blood pressures were recorded but for comparative purposes only the means of the systolic and diastolic blood pressures (referred to as the mean blood pressure) were used, as illustrated in the figures.

The heart rates were calculated from the electrocardiographic tracings. When the heart was regular and it was possible to count the sinus rate, three cycles were measured. When arrhythmias were present the heart rates given were obtained by counting the fundamental ventricular rate in a large number of cycles (6 to 10 cycles). The electrocardiograms and arterial blood pressure were taken at fixed successive time intervals before (controls) and following the administration of drugs, depending on the nature of the experiments.

For statistical analysis of the results, the data from several similar experiments were grouped together. The maximum mean changes in heart rate and mean blood pressure were determined. For this purpose, standard errors of the means and tests of significance with probability determinations were worked out according to Snedecor (1955).

No exact quantitative measurements were made for comparison of ST-T segment changes observed in these experiments. On a qualitative basis the results may, however, be expressed, as follows:-

- , none; + , slight; ++ , moderate; +++ , maximum deviation.

Most substances were freshly dissolved in sodium chloride solution (0.9%) prior to their administration. Phenoxybenzamine (100 mg.) was first dissolved in 2 ml. of alcohol (95%) and sodium chloride solution (0.9%) added to make 10 ml. total volume immediately before using. The

dosages of the employed drugs are expressed as weights of their salts, except in cases of leptazol, hemicholinium (HC-3) and chloralose.

2. Spinal Section

For spinal C_2 sections, the general surgical procedures employed were the same as described above. When these were completed, a longitudinal incision was made over the long spine of the second cervical vertabra. The underlying muscle and tissue were cleared to the bone, which was chipped away with bone forceps, exposing approximately 2 cm. of the spinal cord. The spinal cord was then sectioned at the upper level of the second cervical vertebra. Absorbable gelatin sponge, previously dampened with saline was then inserted to control the bleeding. At least one hour elapsed between cutting of the spinal cord and the taking of control records prior to drug administrations.

3. Systemic Drug Pretreatments

All substances used in systemic pretreatments (except reserpine and iproniazid) were injected intravenously in acutely cannulated animals. The time intervals between these pretreatments and the intraventricular injections of leptazol and caffeine are indicated in the results. Reserpine was injected intraperitoneally for two days (2.5 mg./kg. per day) to produce systemic

reserpinization. These animals were used at least 18 to 22 hours after the last reserpine dose. Iproniazid was injected intraperitoneally in a dose of 100 mg./kg. and the animals were used 10 to 14 hours after the injection.

4. Central (Intraventricular) Drug Pretreatments

After the general procedures were completed, all substances other than hemicholinium and reserpine when employed for "central pretreatment" were injected intraventricularly in the acutely cannulated cats. The time intervals between pretreatment or the injection of leptazol or caffeine are indicated in the results.

HC-3 is the compound No. 3 of a series of quaternary bases (Long and Schueler, 1954) designated as "Hemicholiniums" by Schueler (1955). In the case of pretreatment with hemicholinium (HC-3) a dose of 0.5 mg. was injected intraventricularly, and a period of one and a half to 2 hours was allowed to elapse before the leptazol or caffeine was injected intraventricularly. The electrocardiogram and blood pressure was recorded before and after administration of HC-3 to serve as controls. The term "central reserpinization" is used to indicate the administration of a single intraventricular injection of reserpine (500 µg.) in previously cannulated cats. These animals were subsequently used three hours, six hours and eighteen hours following the injection of reserpine.

5. Drugs Used

The following drugs were used in the course of these studies: 1) Adrenaline bitartrate (Suprarenin, Sterling-Winthrop); 2) Cocaine hydrochloride (Merck); Gallamine triethiodide (Flaxedil, Poulenc); 4) Iproniazid (Marsalid, Hoffmann-La Roche); 5) Nicotine bitartrate (British Drug House); 6) Phenoxybenzamine (Dibenzyline, Smith Kline & French); 7) Reserpine (Serpasil, Ciba); 8) Leptazol (Metrazol, Bell-Craig Pharmaceuticals, Montreal); 9) Caffeine citrate (Howards & Sons Ltd., Essex, England); 10) Bretylium tosylate, (Darenthin, B. W. & Co., Lond.); 11) N-ethy-nortropin-benzhydrylether-hydrobromide (Ethybenztropine, Sandoz, Inc. Pharmaceuticals, New Jersey); 12) Atropine sulphate (Merck & Co. Ltd., Montreal); 13) Hexamethonium bromide (Vegolysen, Poulenc, Montreal); 14) Hemicholinium (HC-3)*; 15) Mecamylamine hydrochloride (Inversine, Merck, Sharp & Dohme, Montreal); 16) Succinylcholine Chloride (Anectine, Burroughs Welcome & Co., Montreal); 17) Chloralose (British Drug House).

*Kindly supplied by Professor MacIntosh, McGill University.

IV. RESULTS

SECTION A. STUDIES ON THE CARDIOVASCULAR RESPONSES TO LEPTAZOL

PART I - Cardiovascular Effects of Intraventricular Injections of Leptazol

1. Observations following intraventricular and intravenous injections of leptazol

a) Dose-response effects in non-vagotomized animals following intraventricular injections.

In the first series of experiments, the cardiovascular responses to increasing dosages (10, 30 and 85 mg.) of leptazol (lep.), following intraventricular (i.vt.) injections were compared in 18 normal non-vagotomized cats, under pentobarbitone (pento.) and gallamine (gal.). The results of these studies are summarized in Table 1. In Figures 1A and 1B and 2A and 2B are also shown typical examples of the responses to doses of 30 mg. and 85 mg., respectively. In a general way, as can be seen (Table 1) with increasing doses of lep. there were increasing degrees of pressor responses and ECG changes.

The most striking and consistent cardiovascular changes were, however, observed following the dose of 85 mg. (8 exp.). Thus, the pressor responses following this

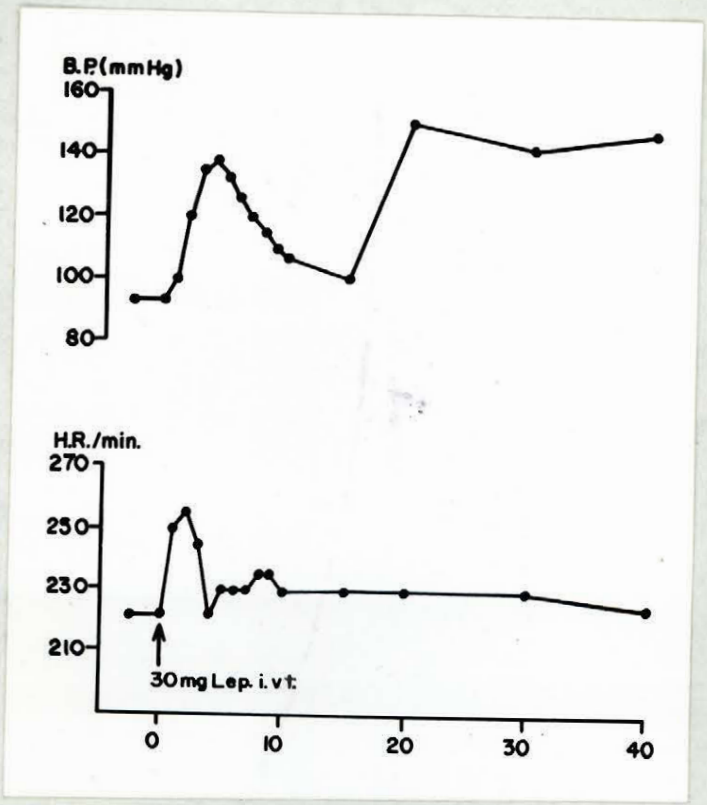
TABLE 1

Comparison of the cardiovascular changes induced by increasing doses of leptazol, following intraventricular injections in cats under pentobarbitone - gallamine (See Methods).

Leptazol Dose	N	B.P. mm. Hg		H.R./min.		ECG changes	
		Control	Maximum Increase	Control	Maximum Increase	E.B.	ST-T
10 mg.	5	110.0 ±21.5	43.8 ±15.5	210.0 ± 9.5	24.6 ±14.1	1	+
30 mg.	5	120.6 ±10.6	68.6 ±14.0	207.0 ±16.0	27.6 ± 3.6	1	++
85 mg.	8	107.5 ±16.2	91.0 ±12.8	203.0 ±26.6	33.6 ± 8.2	6	+++

N = Number of experiments
Mean values of B.P. and H.R. with ± standard errors of the means are given.
E.B. = Number of experiments where ventricular extrasystoles occurred.
ST-T = Comparative degrees of ST-T segments deviations.

IA



IB

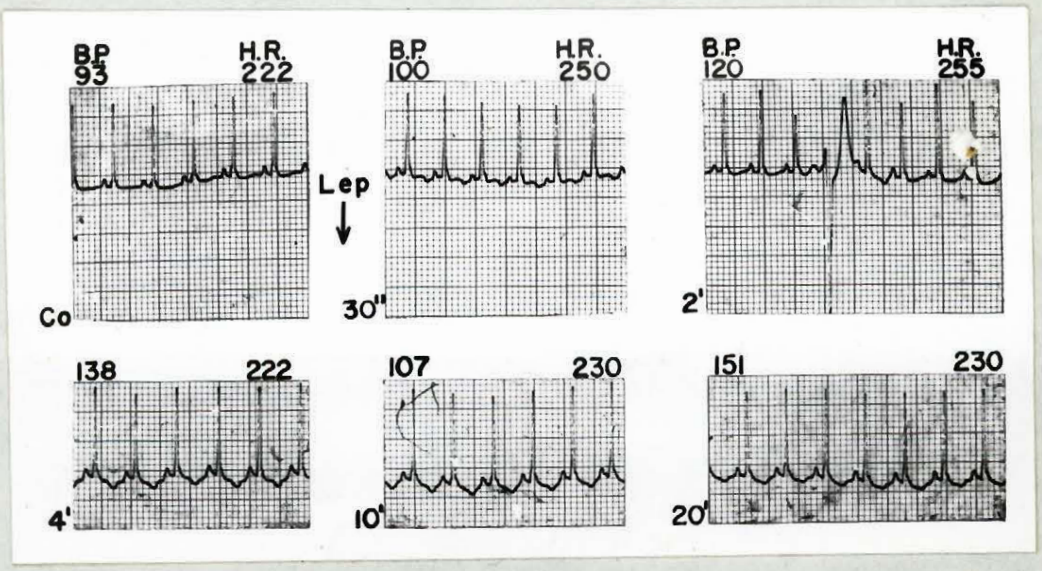
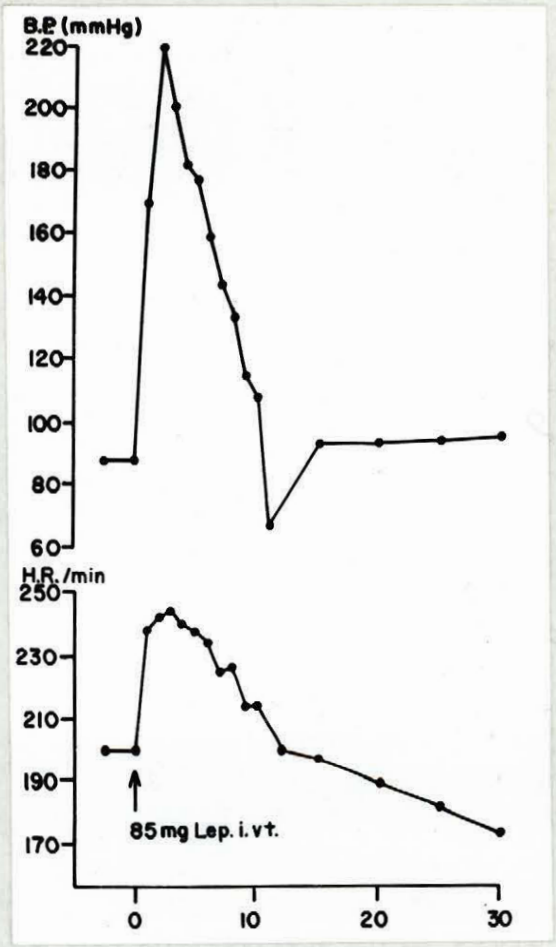


FIGURE 1A: Changes in mean B.P. and H.R. induced by i.vt. lep. (30 mg.) in the non-vagotomized cat (female, 2.7 kg.).
FIGURE 1B: ECG and associated mean B.P. and H.R. changes related to Figure 1A, before (CO) and 30 seconds, 2, 4, 10 and 20 minutes after lep.

2A



2B

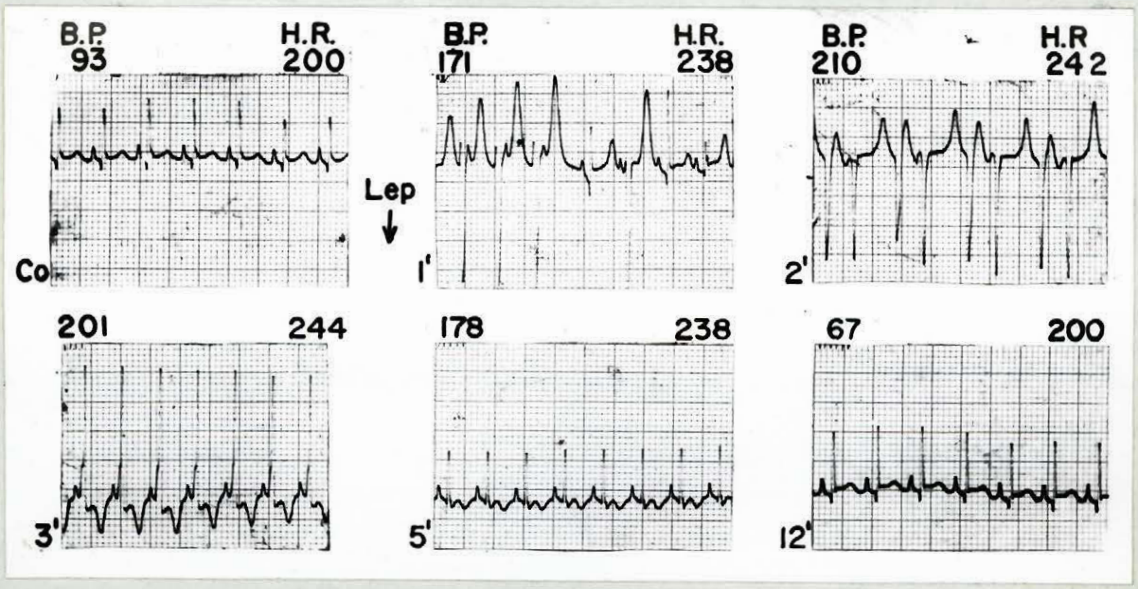


FIGURE 2A: Changes in mean B.P. and H.R. induced by i.vt. lep. (85 mg.) in the non-vagotomized cat (male, 2.6 kg.).
FIGURE 2 B: ECG and associated mean B.P. and H.R. changes related to Figure 2A, before (CO) and 1, 2, 3, 5, and 12 minutes after lep.

dose ensued generally within 30 sec. to 2 min., reaching increased peak values ranging from 79 to 137 mm. Hg (mean, 91.0) over the control levels within 5 to 10 min., and declining to the control levels or slightly below by 30 min. This response, as also illustrated in Figures 2A and 2B, was accompanied by marked cardiac arrhythmias (ectopic beats of multifocal origin, runs of auricular and ventricular tachycardia) and ischemic-like electrocardiographic changes (ST-T alterations), as well as sinus tachycardia, with increases in heart rate, ranging from 12 to 74 per min. (mean, 33.6) over the control values. These changes progressively diminished and returned to the control levels after approximately 30 min.

The above observed responses to lep. were also repeatable and the changes were almost identical following 2 successive injections of the same dose (85 mg.) at 30 min. intervals (5 exp.). Further similar repetitions of the same dose up to 5 times in some of the experiments, also still led to responses, which however were progressively reduced.

Concerning the comparative arterial pressure changes (Table 1), the mean pressor responses to a dose of 10 mg. were significantly less than those to 85 mg. ($P < 0.05$) although not significantly different ($P > 0.2$) from the mean pressor responses to a dose of 30 mg. The pressor responses to doses of 30 mg. and 85 mg. were also not

significantly different ($P > 0.2$).

The increases in heart rate which were observed with increasing doses show only slight differences which were statistically insignificant ($P > 0.8$ - 10 mg. vs. 30 mg.; $P > 0.5$ - 30 mg. vs. 85 mg.; $P > 0.5$ - 10 mg. vs. 85 mg.). These anomalous findings might be due to the higher control heart rates in these experiments (See Discussion).

On the other hand, in regard to the relative incidence of arrhythmias and ischemic (ST-T) changes with a dose of 10 mg., ventricular extrasystoles occurred infrequently and in only 1 out of 5 exp., and the associated ST-T segment deviations were insignificant. Following a dose of 30 mg. extrasystoles also occurred infrequently only in 1 out of 5 exp., but significant ST-T segment changes were evident in all 5 exp.

From the above observations, it is evident that the highest dose of lep. employed (85 mg.), although relatively high, induced (a) the most marked and consistent cardiovascular changes; (b) the highest incidence of ventricular extrasystoles; and (c) responses which were repeatable. For comparative pharmacological purposes, therefore, this dose of lep. was employed throughout.

In summary, (1) it was observed that in normal (non-vagotomized) cats, under pentobarbitone with gallamine, following intraventricular injections of doses of 10, 30 and 85 mg. of leptazol, there were significantly increasing degrees of pressor responses, associated with electrocardiographic changes (ectopic beats, ventricular tachycardia and

ST-T deviations. (2) The degrees of cardioacceleration observed with these doses were not significantly different. (3) Under these conditions, the most consistent cardiovascular changes ensued following doses of 85 mg. which were, therefore, employed for comparative purposes in all subsequent experiments. (4) The responses to this dose were repeatable, following successive injections at 30 minute intervals.

b) Influence of anesthetics and curarizing agents on the responses following intraventricular injections

In regard to the influence of different anesthetics and curarizing agents on the effects of lep., the cardiovascular responses, following i.vt. injections of a dose of 85 mg. of lep. were studied and compared in cats, under the following conditions:- (i) pento. with succinylcholine (SCH); (ii) chloralose (chlor.) with gal.; (iii) chlor. with SCH and (iv) ether induction followed by gal. In Table 2 are summarized the changes which were observed under these different conditions.

(i) Pentobarbitone with succinylcholine (See Table 2)

The injections in cats of doses of 85 mg. of lep. (6 exp.), under these conditions, resulted in similar response to those described above. Pressor responses occurred within 30 sec. to 2 min., reaching increased peak values of 64 to 135 mm. Hg (mean, 105.3) within 5 to 10 min. Tachycardia also ensued, ranging from increases of 30 to 60

TABLE 2

Comparison of the cardiovascular changes induced by i.vt. leptazol (85 mg.) in non-vagotomized cats under various anesthetics and curarising agents (See Methods).

		B.P. mm. Hg		H.R./min.		ECG changes	
	N	Control	Maximum Increase	Control	Maximum Increase	E.B.	ST-T
Pento. & ga.	8	107.5 ±16.2	91.0 ±12.8	203.0 ±26.6	33.6 ± 8.2	6	+++
Pento. & SCH	6	123.3 ± 8.4	105.3 ±11.4	224.6 ± 8.7	42.8 ± 4.7	4	+++
Chlor. & gal.	5	75.0 ± 9.3	105.0 ± 6.5	152.0 ±28.0	48.0 ± 9.7	3	+++
Chlor. & SCH	5	127.6 ± 7.6	108.4 ±14.2	209.4 ±15.8	35.0 ± 9.4	3	+++
Ether & gal.	5	93.0 13.0	126.0 13.0	243.0 18.0	26.0 5.6	3	+++

N = Number of experiments
Mean values of B.P. and H.R. with ± standard errors of the means are given.
E.B. = Number of experiments where ventricular extrasystoles occurred.
ST-T = Comparative degrees of ST-T segments deviations.

per min. The associated ECG changes were also similar to those observed above and extrasystoles occurred in 4 out of 6 exp. Typical examples of these changes are illustrated in Figures 3A and 3B. The pressor changes and the degrees of cardioacceleration recorded in this group of exp. were not significantly different from those recorded in the earlier-observed exp., using pento. and gal. ($P > 0.4$ and $P > 0.3$, respectively).

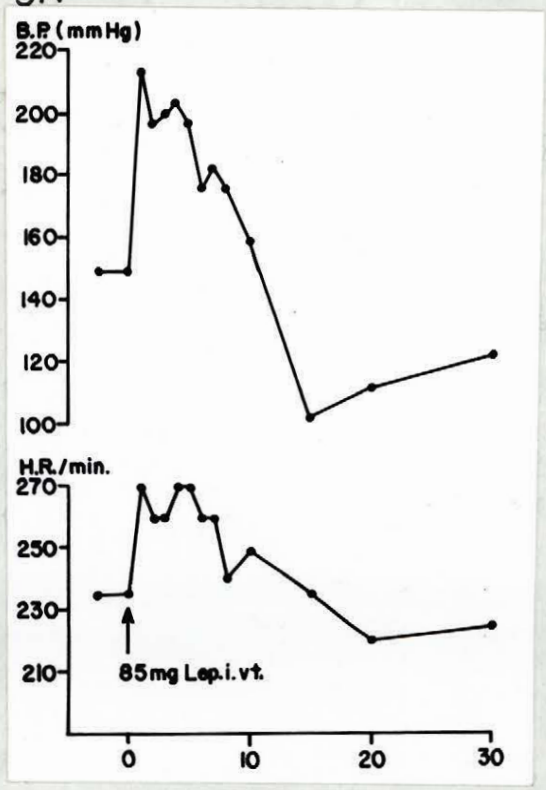
(ii) Chloralose with gallamine (See Table 2)

Under these conditions, the mean pressor response (5 exp.) to i.vt. lep. (85 mg.) was 105.0 mm. Hg, with increased peak values, ranging from 56 to 136 mm. Hg. The cardioacceleration ranged from increases of 26 to 75 per min. (mean, 48). These changes again were insignificantly different from those seen under pento. and gal. ($P > 0.4$ and $P > 0.2$, respectively), as above, and similar ECG alterations were also seen. Extrasystoles occurred in 3 out of 5 exp.

(iii) Chloralose and succinylcholine (See Table 2)

Under these conditions (5 exp.), the increased peak values for the pressor response to lep. varied from 74 to 144 mm. Hg (mean, 108.4), and the cardioacceleration showed increases varying from 10 to 62 per min. (mean, 35). These changes were also insignificantly different, as compared with those observed with the same doses of lep. in cats, under pento. and gal. ($P > 0.3$ and $P > 0.9$,

3A



3B

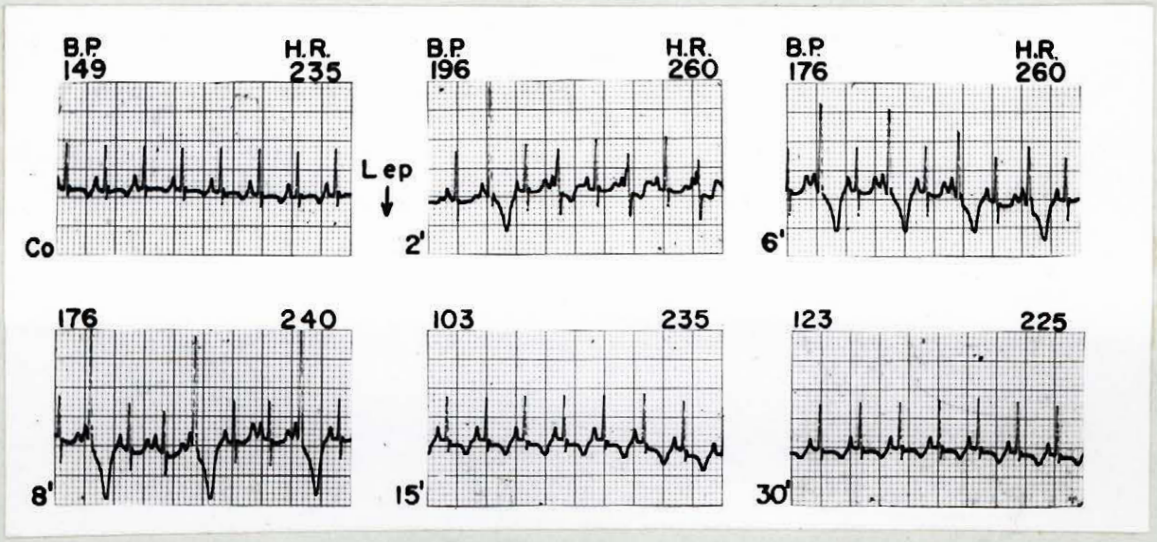


FIGURE 3A: Changes in mean B.P. and H.R. induced by i.vt. lep. (85 mg.) in the non-vagotomized cat (female, 2.9 kg.).
FIGURE 3B: ECG and associated mean B.P. and H.R. changes related to Figure 3A, before (CO) and 2, 6, 8, 15 and 30 minutes after lep.

respectively), as above, and similar arrhythmias were also observed here. Extrasystoles occurred in 3 out of 5 exp.

(iv) Ether induction followed by gallamine (See Table 2)

In this group (5 exp.), i.vt. lep. (85 mg.) led to increases in pressor responses varying from 86 to 153 mm. Hg (mean, 126). These were the most marked pressor responses to i.vt. lep. observed under any of the experimental conditions employed. It was also observed that increases in cardioacceleration ranged from 7 to 40 per min. (mean, 26). Both of these responses, however, were not significantly different from those seen in cats, under pento. and gal. ($P > 0.5$ and $P > 0.5$, respectively), as above, and similar ECG changes were also seen. Extrasystoles occurred in 2 out of 5 exp.

In summary, comparisons of the cardiovascular responses to doses of 85 mg. of leptazol, injected intraventricularly, under different anesthetics (pentobarbitone, chloralose, ether induction only) and curarizing agents (gallamine and succinylcholine) in non-vagotomized cats showed only slight variations, although these were not significantly different.

c) Comparative cardiovascular effects following intravenous injections of leptazol

In order to determine whether or not the above-described responses to lep. were due to any significant degree of systemic absorption, the comparative cardiovascular responses following i.v. injections of the same dose of

lep. (85 mg.) were studied in cats, under (i) pento. with gal. in non-vagotomized animals, and (ii) chlor. and SCH in vagotomized animals.

Following i.v. injections of lep. there was always a transient initial depressor response, followed by a slight pressor response. There was also an associated slight sinus tachycardia. The cardiovascular changes induced by i.v. injections of lep. in these experiments are summarized in Table 3.

In the non-vagotomized animals, a dose of 85 mg. of lep. (5 exp.) resulted in a mean increased pressor response of 20 mm. Hg, ranging from 8 to 44 mm. Hg. These effects were preceded by slight depressor responses in degrees ranging from 5 to 10 mm. Hg below the control levels. The associated cardioacceleration ranged from increases of 4 to 38 per min. (mean, 11.4) over the controls. There were no extrasystoles and only insignificant ST-T changes observed in these experiments.

Following i.v. injections, the pressor responses also occurred later than following i.vt. injections, i.e. within 10 min., reaching peak values also in 10 to 20 min. These responses were significantly ($P < 0.01$) less than those observed following i.vt. administrations, under identical conditions.

The mean (11.4/min.) cardioacceleration following i.v. lep. was much less than that following i.vt. (33.6/min.). However, these values were not significantly different

TABLE 3

Cardiovascular changes induced by intravenous leptazol (85 mg.) in non-vagotomized and vagotomized cats (See Methods)

	N	B.P. mm. Hg		H.R./min.		ECG changes	
		Control	*Maximum Increase	Control	Maximum Increase	E.B.	ST-T
Pento. with gal. Non-vagotomy	5	109.8 ±10.3	20.0 ±6.6	206.0 ± 7.2	11.4 ± 7.2	0	+
Chlor. with SCH Vagotomy	6	119.5 ± 4.7	22.3 ± 7.8	231.1 ±13.8	14.1 ± 5.4	0	+

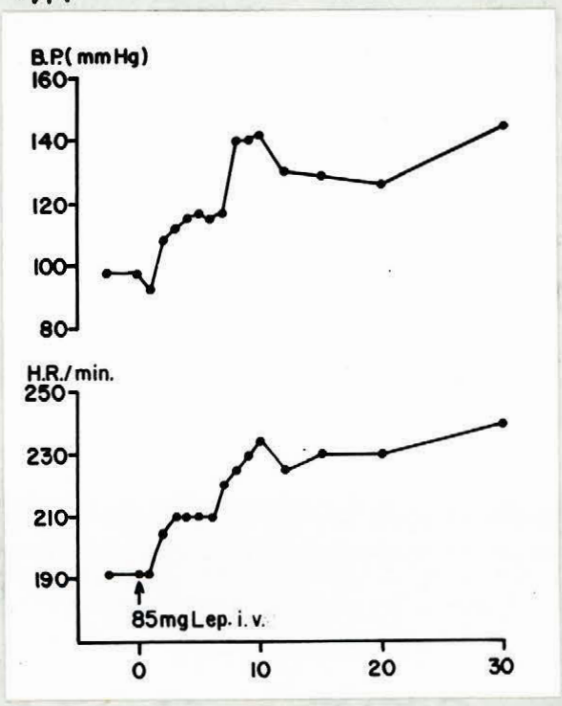
N = Number of experiments
Mean values of B.P. and H.R. with ± standard errors of the means are given.
E.B. = Number of experiments where ventricular extrasystoles occurred.
ST -T= Comparative degrees of ST-T segments deviations.
* = Pressor response following initial depressor response.

($P > 0.05$). Typical examples of the changes recorded in non-vagotomized animals,in such experiments, are shown in Figures 4A and 4B.

In vagotomized animals the effects of i.v. administrations of lep. (6 exp.) led to similar cardiovascular changes. Again, there was always an immediate slight depressor response, ranging in degree from 5 to 10 mm. Hg below the controls, followed by a pressor response, which developed within 10 min. and reached peak values of increases ranging from 5 to 55 mm. Hg (mean, 22.3) within 10 to 20 min. The associated cardioacceleration varied from increases of 5 to 35 per min. (mean, 14.1). In these experiments both the pressor responses and cardioacceleration were significantly less than those observed with i.vt. administrations of the same dose of lep. ($P < 0.001$ and $P < 0.01$, respectively). No extrasystoles were again recorded in these experiments, but insignificant T wave changes were observed in some.

In summary (1) following intravenous injections of leptazol, the general cardiovascular responses to a dose of 85 mg. were always less than those observed with the same dose following intraventricular injections. (2) In contrast to the effects of intraventricular injections, following intravenous administrations, the pressor responses were always delayed, significantly less marked and less sustained, and preceded by slight depressor responses. (3) The degree of cardioacceleration recorded in the non-vagotomized cats

4A



4B

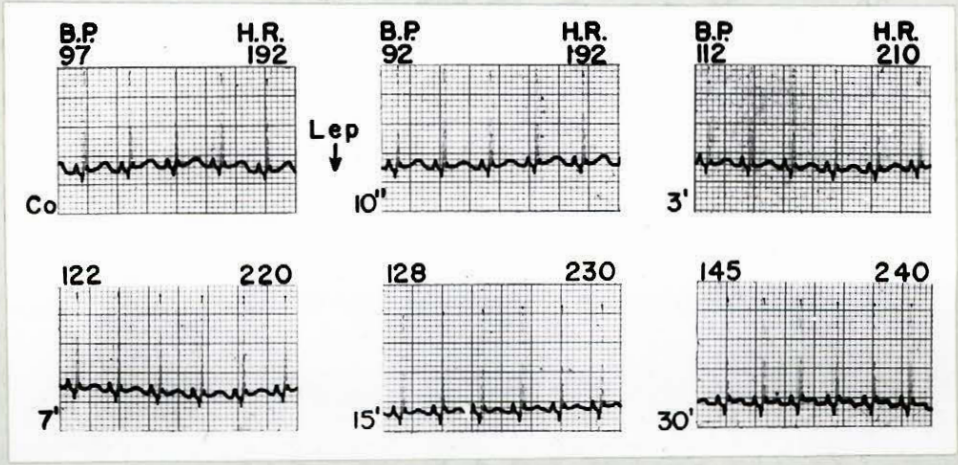


FIGURE 4A: Changes in mean B.P. and H.R. induced by i.v. lep. (85 mg.) in the non-vagotomized cat (male, 2.6 kg.)
FIGURE 4B: ECG and associated mean B.P and H.R. changes related to Figure 4A, before (CO) and 10 seconds, 3, 7, 15 and 30 minutes after lep.

following intravenous leptazol was, however, insignificantly different from that observed with intraventricular injections. (4) Following intravenous injections, cardiac arrhythmias were never seen, although some minor ST-T changes were observed.

2. Effects in Vagotomized Cats

In order to assess the possible involvement of parasympathetic pathways in the responses to i.vt. lep., similar experiments to those described above were performed on vagotomized cats, under the following conditions:- (i) pento. with gal.; (ii) pento. with SCH; and (iii) chlor. with SCH. The changes observed under these different conditions are summarized in Table 4.

(i) Pentobarbitone with gallamine (See Table 4)

The i.vt. injection of the standard dose of lep. (4 exp.) resulted in a mean increase in pressor response of 89 mm. Hg, over the control levels. This was almost identical to that observed in the non-vagotomized animals and was not significantly different ($P > 0.9$). The increases in arterial pressure occurred again within 30 sec. to 2 min., reaching peak values, ranging from 68 to 105 mm. Hg (mean, 89) within 5 to 10 min. These were associated with ventricular extrasystoles in 3 out of 4 exp. and ST-T segment deviations in all exp. Varying degrees of increased tachycardia also ensued, ranging

TABLE 4

Cardiovascular changes induced by i.vt. leptazol (85 mg.) in vagotomized cats under different anesthetic and curarizing agents (See Methods).

		B.P. mm. Hg		H.R./min.		ECG changes	
	N	Control	Maximum Increase	Control	Maximum Increase	E.B.	ST-T
Pento. & gal.	4	114.5 ±18.3	89.0 ± 8.0	210.0 ±30.7	32.5 ± 9.6	3	+++
Pento. & SCH	4	122.2 ± 2.6	98.2 ± 4.6	261.2 ±11.2	27.5 ± 8.4	2	+++
Chlor. & SCH	5	135.4 ± 8.8	117.2 ± 6.1	211.6 ± 7.1	44.4 ± 6.4	3	++

N = Number of experiments
Mean values of B.P. and H.R. with ± standard errors of the means are given.
E.B. = Number of experiments where ventricular extrasystoles occurred.
St-T = Comparative degrees of ST-T segments deviations.

maximally from 10 to 55 per min. (mean, 32.5). These were also insignificantly ($P > 9$) different from those observed in non-vagotomized cats. Typical examples are illustrated in Figures 5A and 5B.

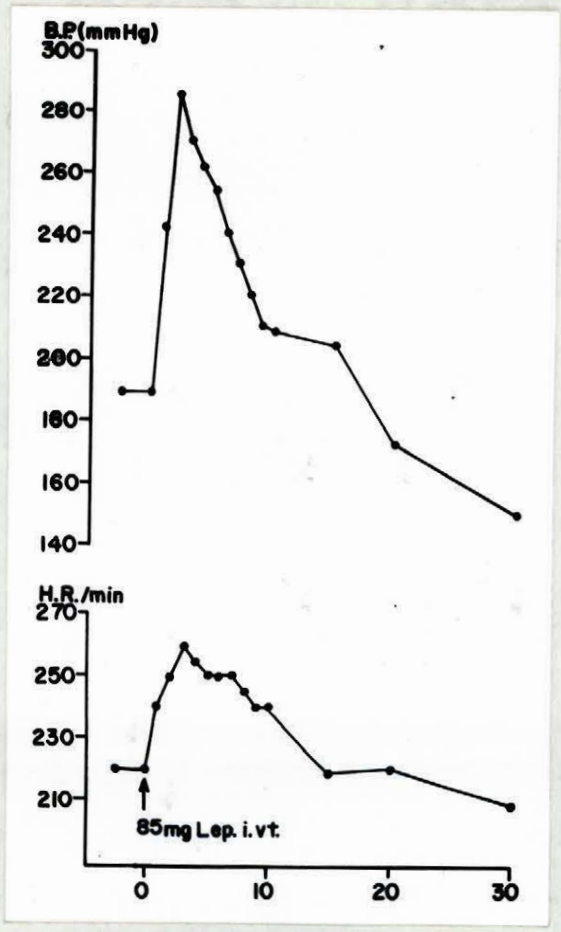
(ii) Pentobarbitone with succinylcholine (See Table 4)

The mean increased pressor response (4 exp.) to i.vt. lep. (85 mg.) was 98.2 mm. Hg, ranging from 92 to 112. The associated cardioacceleration also showed increases ranging from 10 to 45 per min. (mean, 27.5). These changes were insignificantly different from those observed in similar types of exp. in non-vagotomized cats ($P > 0.6$ and $P > 0.1$, respectively). Similar ECG changes also occurred as in the case of non-vagotomized cats - extrasystoles occurring in 2 out of 4 exp. A typical illustration of these changes is shown in Figures 6A and 6B.

(iii) Chloralose with succinylcholine (See Table 4)

The i.vt. injection of 85 mg. lep. (5 exp.) led to increased pressor responses which varied from 105 to 133 mm. Hg (mean, 117.2) and the increased cardio-acceleration ranging from 30 to 60 mm. Hg per min. (mean, 44.4). These were again not significantly different from those observed in similar exp. on non-vagotomized cats ($P > 0.5$ and $P > 0.4$, respectively). Similar types of arrhythmias were also seen - extrasystoles occurring in 3 out of 5 exp. A typical example of these results is shown in Figures 7A and 7B.

5A



5B

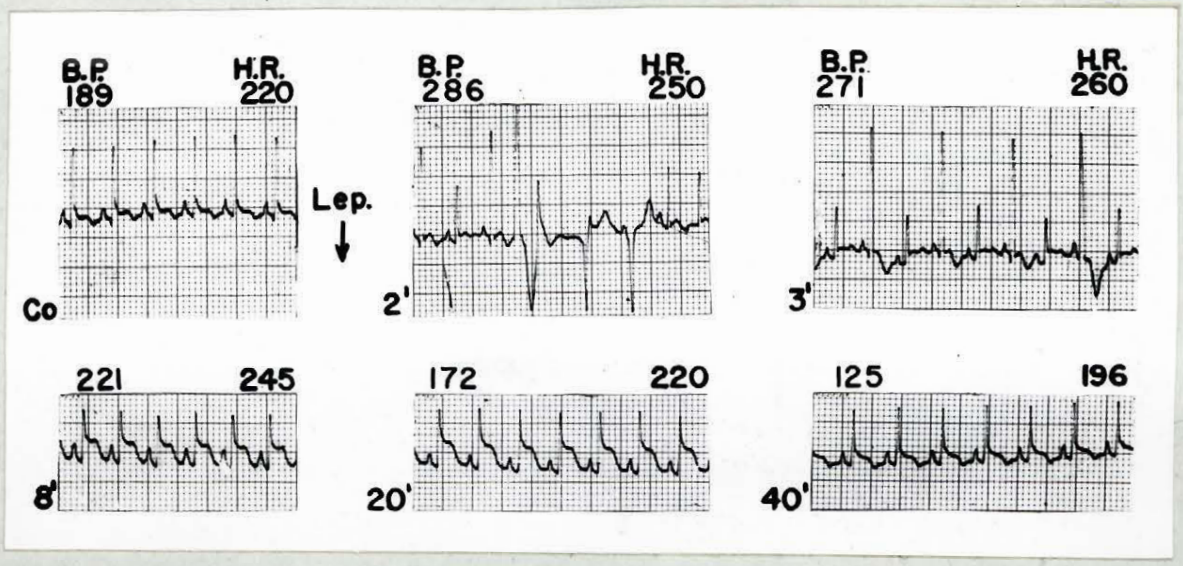
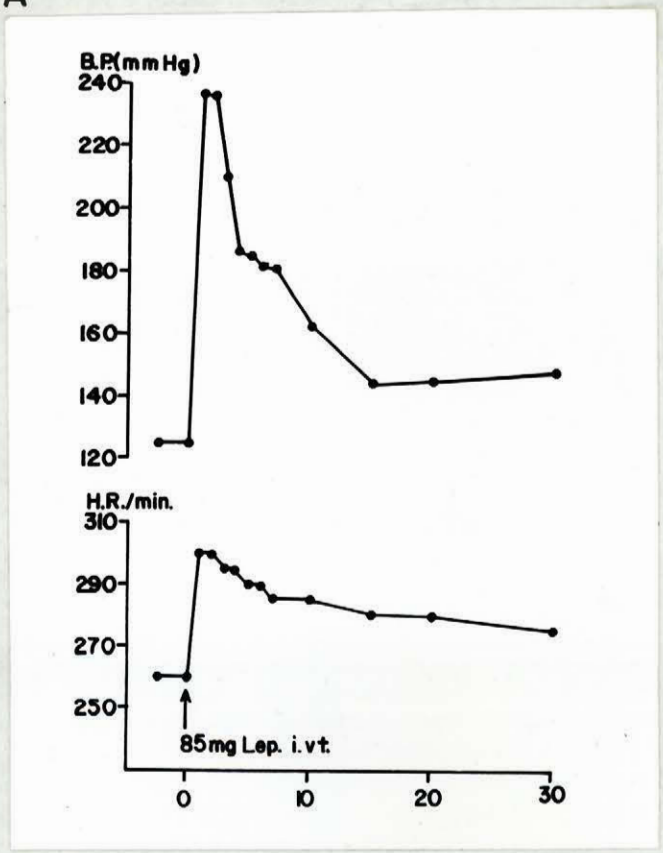


FIGURE 5A: Changes in mean B.P. and H.R. induced by i.vt. lep. (85 mg.) in the vagotomized cat (female, 2.6 kg.).
FIGURE 5B: ECG and associated mean B.P. and H.R. changes related to Figure 5A, before (CO) and 2, 3, 8, 20 and 40 minutes after lep.

6A



6B

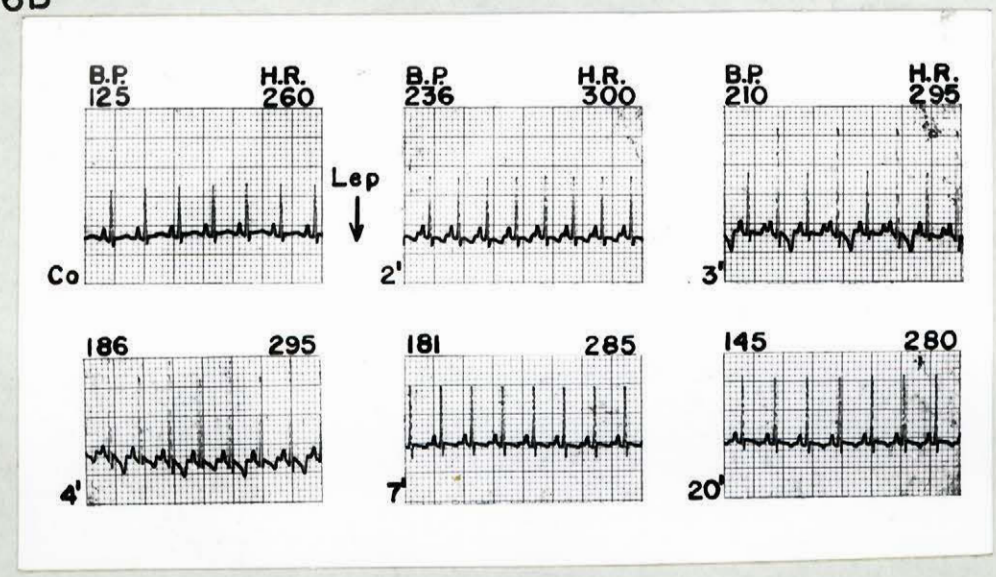
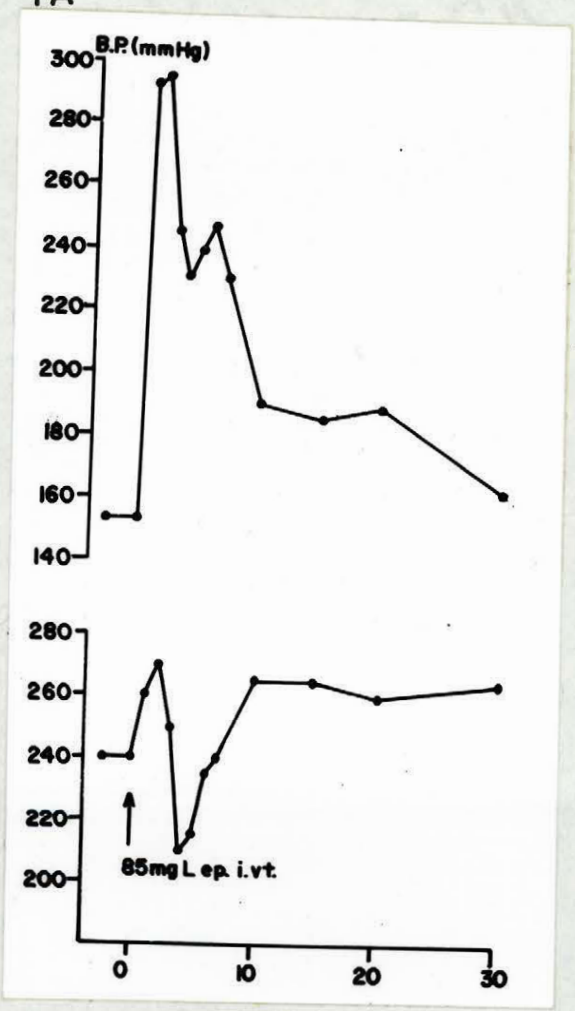


FIGURE 6A: Changes in mean B.P. and H.R. induced by i.vt. lep. (85 mg.) in the vagotomized cat (male, 2.8 kg.).
FIGURE 6B: ECG and associated mean B.P. and H.R. changes related to Figure 6A, before (CO) and 2, 3, 4, 7 and 20 minutes after lep.

7A



7B

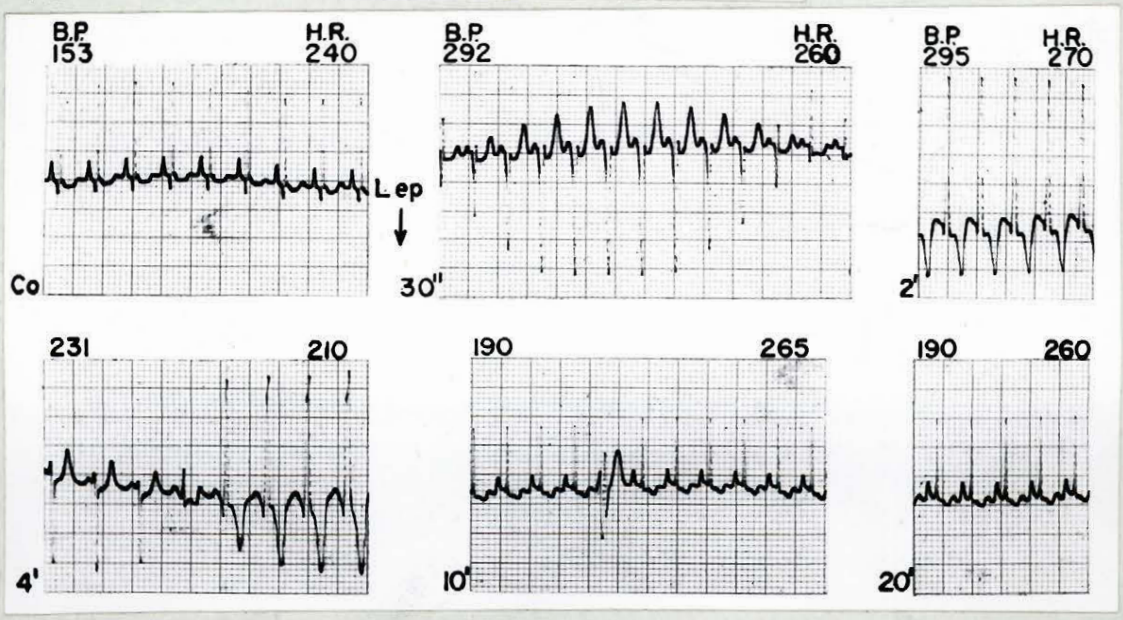


FIGURE 7A; Changes in mean B.P. and H.R. induced by i.vt. lep. (85 mg.) in the vagotomized cat (male, 3 kg.).
FIGURE 7B: ECG and associated mean B.P. and H.R. changes related to Figure 7A, before (CO) and 30 seconds, 2, 4, 10 and 20 minutes after lep.

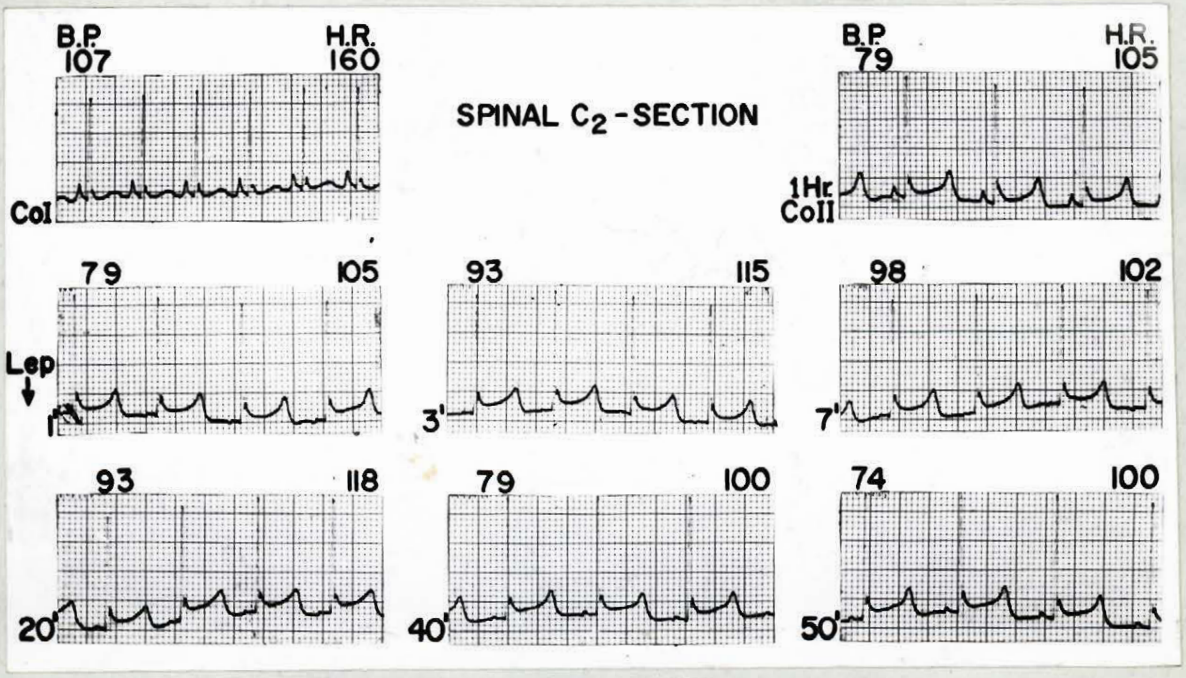
In summary, the cardiovascular responses observed following intraventricular injections of leptazol (85 mg.) in vagotomized cats showed some minor differences from those in non-vagotomized animals, but were not significantly different in the two groups, under comparable experimental conditions (See Discussion).

3. Influence of Spinal Section in Non-vagotomized and Vagotomized Cats

In order to assess the role of efferent sympathetic pathways on the observed responses, spinal sections (See Methods) were performed at the level of the second cervical vertebra (C_2 spinal section) to exclude these pathways. For comparative purposes the results obtained in these experiments are summarized in conjunction with other pertinent data in Tables 5 and 6 (pp. 75 and 76). In non-vagotomized animals (Table 5), one hour following spinal section, the arterial pressure was decreased by 18 to 65 mm. Hg and the heart rate by 20 to 89 per min., in comparision with the initial control levels. There was, however, no significant changes in the ECG pattern.

When the dose of 85 mg. of lep. was administered i.vt. one hour after spinal section (4 exp.), there was only a mild pressor response, occurring within 5 to 10 min. As shown in Table 5, this increase ranged only from 5 to 19 mm. Hg (mean, 10.5). These increases were significantly less than those observed in normal control animals ($P < 0.01$). The blood pressure returned to approximately the control

8A



8B

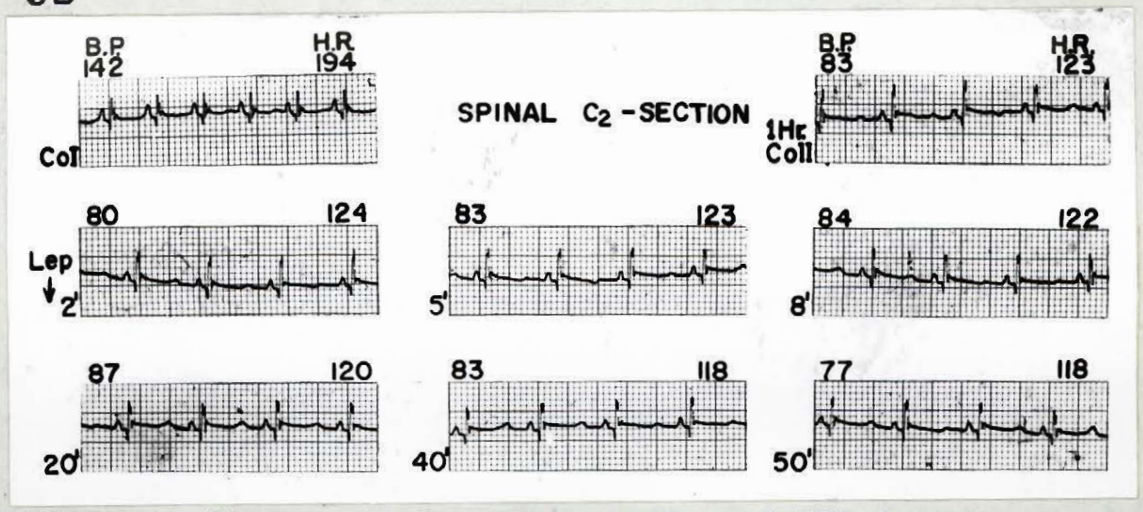


FIGURE 8A: Changes in mean B.P., H.R. and ECG in the non-vagotomized cat (female, 2.7 kg.), before spinal C_2 section (CO I), one hour later and before i.vt. lep. (CO II) and 1, 3, 7, 20, 40 and 50 minutes after lep.

FIGURE 8B: Changes in mean B.P., H.R. and ECG in the vagotomized cat (male, 3 kg.) before spinal C_2 section (CO I) one hour later and before i.vt. lep. (CO II) and 2, 5, 8, 20, 40 and 50 minutes after lep.

levels within 30 min. The tachycardia also varied from increases of 6 to 13 per min. (mean, 8.7), as compared with increases varying from 12 to 74 per min. (mean, 33.6) in non-spinal control animals. These differences were found insignificant ($P > 0.05$). No ECG changes were seen in these exp. Fig. 8A shows typical examples of the findings in this type of experiment.

In vagotomized cats (Table 6), one hour following C_2 spinal section, the blood pressure was decreased by 18 to 69 mm. Hg and the heart rate reduced by 15 to 72 per min., but the ECG pattern was not significantly changed. Following i.vt. lep. (4 exp.) there ensued a slight pressor response, increases ranging from 2 to 11 mm. Hg (mean, 5.7). This pressor response was significantly less than that observed in non-spinal control animals ($P < 0.001$). The cardioacceleration also varied only from increases of 2 to 12 per min. (mean, 5.0) and was again significantly less than that observed in normal animals ($P < 0.01$). ECG changes were completely prevented. Fig. 8B illustrates a typical example of these results.

In summary (1) in both non-vagotomized and vagotomized cats, spinal C_2 section significantly reduced the pressor responses following intraventricular leptazol. (2) In non-vagotomized cats, following spinal C_2 section, cardioacceleration still ensued. (3) All of the electrocardiographic changes normally observed following intraventricular leptazol were completely prevented by previous

spinal C_2 section.

PART II - Influence of Systemic Pretreatments on Cardiovascular Changes Induced by Intraventricular Injections of Leptazol

From the preceding exp. with spinal (C_2 section) animals, it is evident that sympathetic mechanisms predominate in the overall cardiovascular responses to i.vt. lep. In order to obtain a clearer evaluation of the sympathetic responses involved, the cardiovascular changes following various types of pretreatment (Sections 1 to 5, below) were studied in non-vagotomized and vagotomized animals. The results of these studies are summarized in Tables 5 and 6.

1. Effects of "Systemic Reserpinization"

It is now well-known that reserpine can deplete catecholamines from the tissues (Holzbauer et al, 1956 and Carlsson et al, 1956). Since catecholamines are intimately related to the function of the peripheral sympathetic system, an assessment of the dependence of the action of lep. on this system was attempted by observing the effects of lep. (3 exp.) in the systemically reserpinized animals (See Methods). A typical illustration of the results observed in these experiments is shown in Fig. 9A.

During the control periods the heart rates and

TABLE 5

Cardiovascular changes induced by i.vt. leptazol (85 mg.) in control and under various experimental conditions in non-vagotomized cats under pentobarbitone with gallamine.

		B.P. mm. Hg		H.R./min.		ECG changes	
Pretreatment	N	Control	Maximum Increase	Control	Maximum Increase	E.B.	ST-T
None	8	107.5 ±16.2	91.0 ±12.8	203.0 ±26.6	33.6 ± 8.2	6	+++
Spinal C_2-Section	4	79.2 ± 7.9	10.5 ± 3.2	141.5 ±16.8	8.7 ± 1.4	0	-
'Systemic' Reserpinization	3	79.0 ± 7.5	24.6 ± 6.8	93.0 ±15.7	15.0 ± 9.6	0	-
'Systemic' Phenoxybenzamine	3	82.3 ±13.8	*36.0 ±11.2	153.0 ± 7.7	22.6 ± 7.3	0	+
'Systemic' Bretylium	3	152.6 ±39.9	11.3 ± 3.5	142.6 ± 6.4	10.0 ± 4.0	0	-
'Systemic' Cocaine	3	163.3 ±23.7	120.0 ±19.2	223.0 ±10.7	26.6 ±10.9	2	+++
'Systemic' Iproniazid	4	131.0 ±12.5	70.2 ±13.9	190.0 ±22.4	22.5 ± 1.4	0	+++

N = Number of experiments
Mean values of B.P. and H.R. with ± standard errors of the means are given.
E. B. = Number of experiments where extrasystoles occurred.
ST-T = Comparative degrees of ST-T segments deviations.
* = Depressor response

TABLE 6

Cardiovascular changes induced by intraventricular leptazol (85 mg.) in control and under various experimental conditions in vagotomized cats, under chloralose with succinylcholine.

		B.P. mm. Hg		H.R./min.		ECG Changes	
Pretreatment	N	Control	Maximum Increase	Control	Maximum Increase	E.B.	ST-T
None	5	135.4 ± 8.8	117.2 ± 6.1	211.6 ± 7.1	44.4 ± 6.4	3	+++
Spinal C_2-Section	4	50.7 ±11.1	5.7 ± 2.4	121.5 ±17.3	5.0 ± 2.5	0	-
'Systemic' Reserpinization	3	82.3 ±17.3	33.0 ± 8.8	184.0 ±30.3	35.3 ± 7.5	0	-
'Systemic' Phenoxybenzamine	4	81.7 ±11.3	*9.7 ± 2.2	203.0 ±23.8	29.2 ±20.6	0	+
'Systemic' Bretylium	3	104.6 ± 9.1	5.6 ± 2.8	155.0 ±10.4	2.3 ± 1.4	0	-
'Systemic' Cocaine	3	155.3 ±31.9	61.0 ±17.2	176.6 ±43.3	33.3 ±14.5	3	++
'Systemic' Iproniazid	3	129.6 ±20.6	110.6 ± 7.2	210.3 ±10.7	23.0 ±13.0	0	+++

N = Number of experiments.

Mean values of B.P. and H.R. with ± standard errors of the means are given.
E.B. = Number of experiments where ventricular extrasystoles occurred.
ST-T = Comparative degrees of ST-T segments deviations.
* = Depressor response.

blood pressures of the reserpinized cats were reduced and the electrocardiograms often showed some minor defect with T wave. As can be seen in Table 5, the pressor responses to i.vt. lep. were significantly attenuated ($P < 0.01$). Although the cardioacceleration was insignificantly different ($P > 0.2$), the increased mean value (15 per min.) for the reserpinized animals was lower than that observed (33.6 per min.) for the untreated animals. The ECG was essentially unchanged, except for minor T wave changes in these experiments (See Fig. 9A).

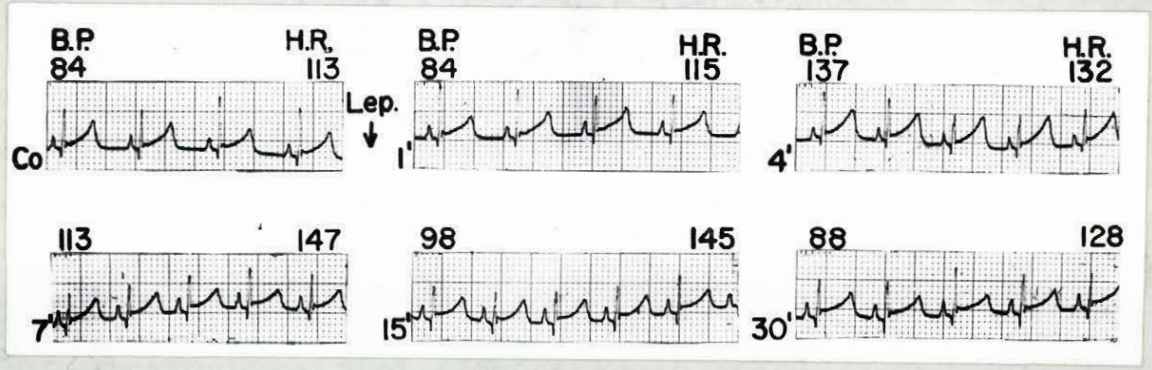
In vagotomized cats (3 exp.), as shown in Table 6, the pressor responses to i.vt. lep. (85 mg.) were also significantly attenuated ($P < 0.001$), although the cardioacceleration was insignificantly different ($P > 0.4$). There were no arrhythmias in any of these experiments, but the ECG showed progressive ST segment depression in 1 exp., as shown in Fig. 9B.

In summary, "systemic reserpinization" markedly reduced most of the cardiovascular changes normally observed following the intraventricular injection of lep. (85 mg.); the resultant mean cardioacceleration, however, was not significantly reduced.

2. Effects of Phenoxybenzamine

Phenoxybenzamine (PHE) is a potent adrenergic blocking agent (McLean et al, 1951; Macko et al, 1951 and Fellows et al, 1954). When a dose of 4 mg./kg. was

9A



9B

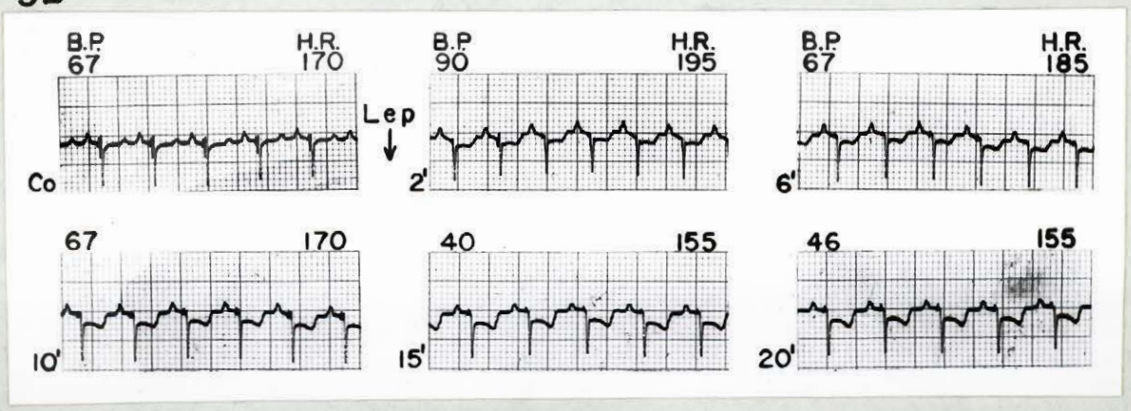


FIGURE 9A; Changes in mean B.P., H.R. and ECG in the "systemically reserpinized," non-vagotomized cat (female, 2.8 kg.), before (CO) and 1, 4, 7, 15 and 30 minutes after i.vt. lep.

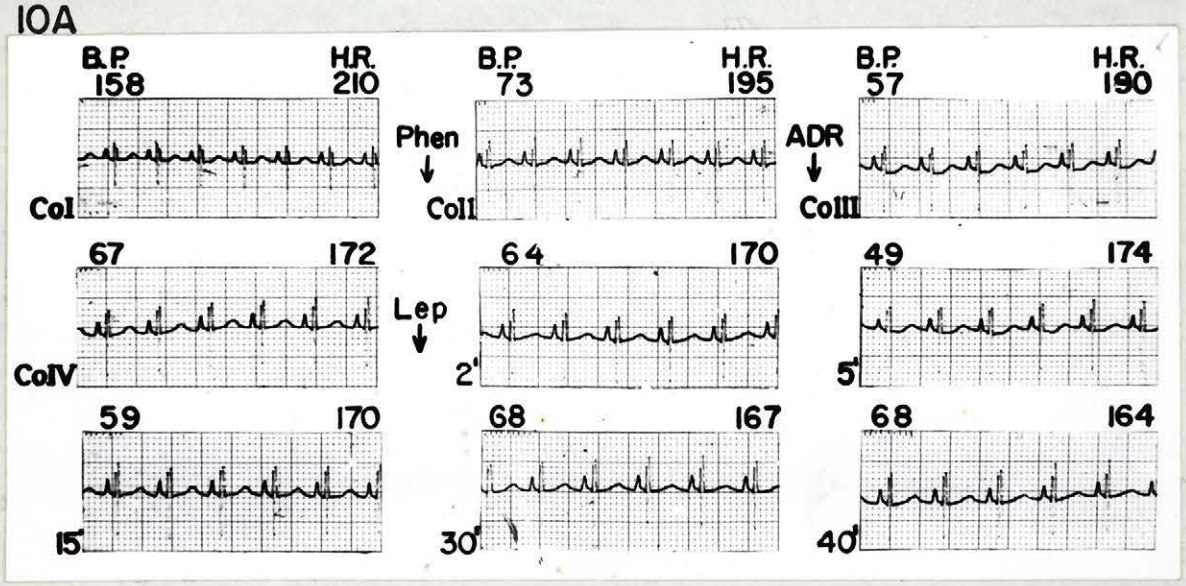
FIGURE 9B: Changes in mean B.P., H.R. and ECG in the "systemically reserpinized," vagotomized cat (female, 3 kg.), before (CO) and 2, 6, 10, 15 and 20 minutes after i.vt. lep.

injected intravenously slowly (during 5 to 15 min.), the arterial pressure was reduced to varying degrees by 28 to 70 mm. Hg, the heart rate also reduced by 5 to 15 per min., below control levels, but the ECG did not appear to be significantly altered at the end of an hour, when lep. was administered intraventricularly (3 exp.). Before injecting lep. the presence of adrenergic blockade was demonstrated by the pressor reversal response resulting from an injection of adrenaline (2.5 ug./kg. i.v.). A typical illustration of the effects of PHE pretreatment upon the responses to lep. is shown in Fig. 10A.

The cardiovascular changes normally following the i.vt. injections of lep. (3 exp.) were markedly attenuated (Table 5). In all experiments lep. induced a depressor response varying from 19 to 57 mm. Hg (mean, 36) below the control levels. There was also an associated tachycardia varying from increases of 8 to 30 per min. (mean, 22.6), which was not significantly different from that observed with lep. in the untreated animals ($P < 0.9$). Cardiac arrhythmias did not occur in any of these experiments, in contrast to that normally seen following the injection of lep. alone. Some ST segment changes, however, were present in 2 experiments.

In vagotomized cats (4 exp.), PHE pretreatment also reduced the normal responses to lep. (Table 6). Fig. 10B illustrates a typical example. Following lep. there was a depressor response varying from 6 to 15 mm. Hg (mean, 9.7)

10A



10B

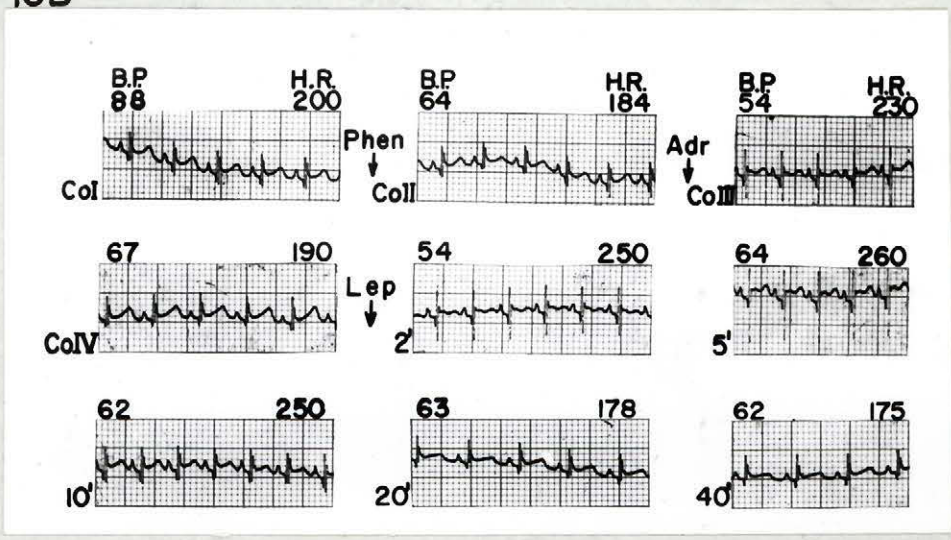


FIGURE 10A: Changes in mean B. P., H. R. and ECG in the systemic PHE-pretreated (4 mg./kg. i.v. 30 min. earlier), non-vagotomized cat (male, 3 kg.), before PHE (CO I), 30 minutes later and before ADR (CO II), 2 minutes after ADR (CO III), 1 hour after PHE and before lep. (CO IV) and 2, 5, 15, 30 and 40 minutes after i.vt. lep.

FIGURE 10B: Changes in mean B.P., H.R. and ECG in the systemic PHE-pretreated (4 mg./kg. i.v. 30 min. earlier), vagotomized cat (female, 3 kg.), before PHE (CO I), 30 minutes later and before ADR (CO II), 2 minutes after ADR (CO III), 1 hour after PHE and before lep. (CO IV) and 2, 5, 10, 20 and 40 minutes after i.vt. lep.

below the control levels. The associated cardioacceleration ranged from increases of 5 to 90 per min. (mean, 29.2), and was insignificantly different from that observed with lep. in the untreated animals ($P > 0.4$). No extrasystoles were observed in any exp., although ST-T changes were again seen in 1 experiment.

In summary, (1) phenoxybenzamine pretreatment markedly reduced the cardiovascular responses to leptazol. (2) Variable depressor responses following intraventricular leptazol were observed. (3) The cardioacceleration recorded was not significantly reduced. (4) Cardiac arrhythmias were completely prevented, although ST segment deviations were seen in some experiments.

3. Effects of Bretylium

Bretylium (Bre) appears to selectively depress adrenergic nerve functions in man (Boura et al, 1956b, 1959a), in dog (Boura and Green, 1959; Aviado et al, 1960) and in cat (Garattini, 1962), and has very little parasympathomimetic action (Boura et al, 1959). It was administered (5 mg./kg.) i.v. 30 min. before lep. i.vt., in order to assess the dependence of the effects of lep. on sympathetic nervous pathways. Thirty min. following the slow (3 to 5 min.) administration of Bre the blood pressure and the heart rate were reduced below control levels.

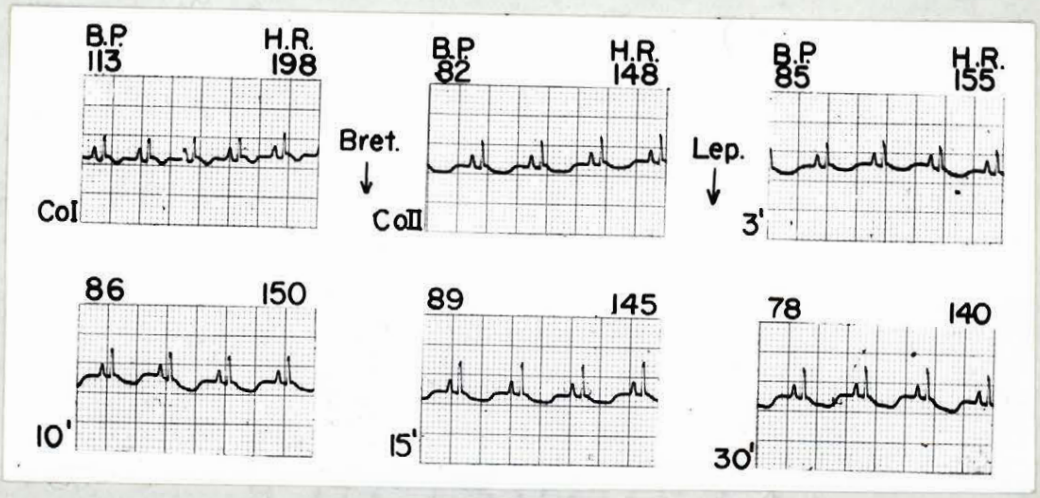
As can be seen in Table 5, following 85 mg. lep.

i.vt. (3 exp.), the arterial blood pressure increased only 6 to 18 mm. Hg (mean, 11.3) over the controls within 5 to 8 min., tending to normalize before termination of the exp. which lasted 30 min. This pressor response is significantly less than that normally seen in untreated animals ($P < 0.01$). The cardioacceleration, however, was insignificantly different ($P > 0.1$) and varied from increases of 5 to 18 per min. (mean, 10) over the controls. No significant ECG changes were seen in any of these exp. Fig. 11A shows a typical illustration of these changes.

In the vagotomized cats (Table 6), the blood pressure and heart rate were also reduced during 30 min. periods, following Bre (5 mg./kg. i.v.). The recorded pressor responses to injection of lep. varied from increases of 4 to 9 mm. Hg (mean, 5.6) and the cardioacceleration from increases of 2 to 5 per min. (mean, 2.3). These changes are significantly reduced from those seen in the untreated animals ($P < 0.001$ and $P < 0.01$, respectively). The ECG was essentially of normal pattern throughout. A typical example of these results is shown in Fig. 11B.

In summary, bretylium pretreatment completely prevented the electrocardiographic changes normally seen after leptazol. The pressor response is significantly reduced, although the cardioacceleration still ensued in non-vagotomized animals.

IIA



IIB

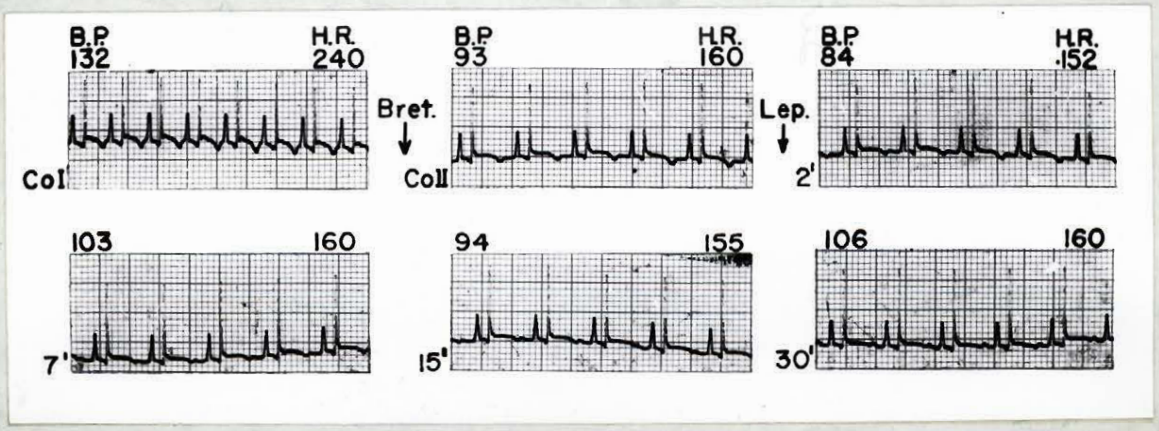


FIGURE 11A: Changes in mean B.P., H.R. and ECG in the Bre-pretreated (5 mg./kg.i.v. 30 min. earlier), non-vagotomized cat (female, 2.6 kg.), before Bre (CO I), 30 minutes later and before i.vt. lep. (CO II) and 3, 10, 15 and 30 minutes after lep.

FIGURE 11B: Changes in meanB.P.,H.R. and ECG in the Bre-pretreated (5 mg./kg. i.v. 30 min. earlier), vagotomized cat (female, 2.9 kg.), before Bre (CO I), 30 minutes later and before i.vt. lep. (CO II) and 2, 7, 15 and 30 minutes after lep.

4. Effects of Cocaine

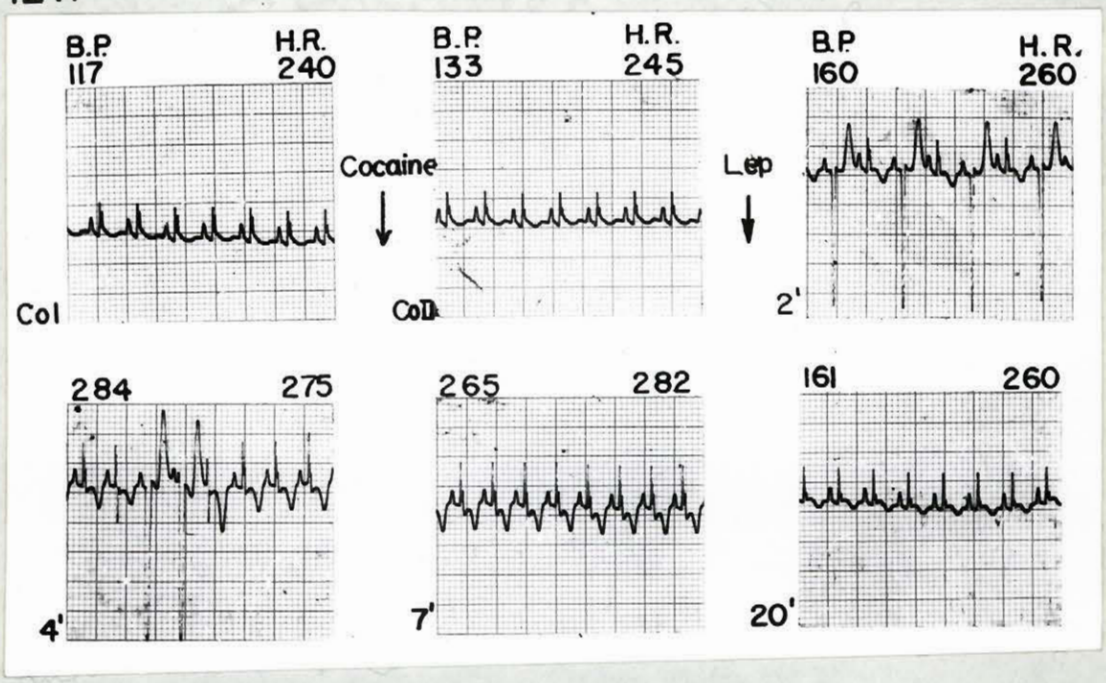
Frolich et al (1910) postulated that cocaine is capable of potentiating catecholamine activity on the cardiovascular system. Since catecholamines are related to the function of the sympathetic system, pretreatment with cocaine was, therefore, studied as an indicator of the degree of sympathetic involvement in the action of lep.

Slow (5 to 10 min.) i.v. injection of cocaine (3 mg./kg.) was followed in 30 min. by an i.vt. injection of lep. (85 mg.). As summarized in Table 5, the resulting pressor responses and cardioacceleration were not significantly different than those observed with lep. alone, ($P > 0.1$ and $P \gg 0.9$, respectively). Similar cardiac arrhythmias were observed as compared to the untreated animals, although the degree of ST-T segment deviations were more marked. Fig. 12A shows a typical example of the changes recorded in these experiments.

In vagotomized animals (Table 6), cocaine pretreatment resulted in a less marked pressor response than that observed in the untreated animals ($P < 0.01$), whereas the cardioacceleration was insignificantly different ($P > 0.4$). The associated cardiac arrhythmias and ST-T alterations were of similar types as in the untreated animals. Fig. 12B shows a typical example.

In summary, cocaine pretreatment neither enhanced nor attenuated the pressor response or cardioacceleration, resulting from i.vt. injection of lep. (85 mg.) in non-

12 A



12 B

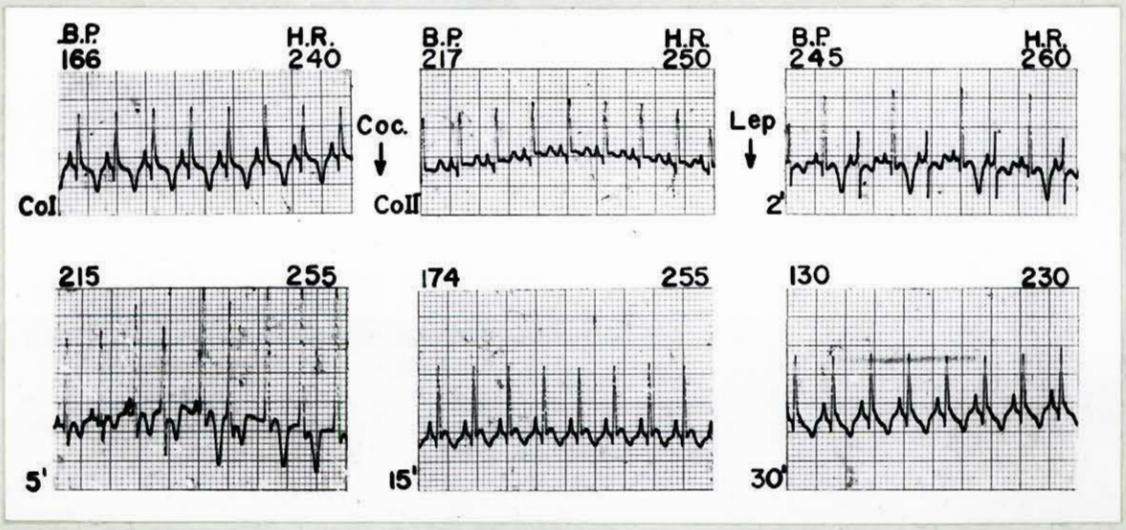


FIGURE 12A: Changes in mean B.P., H.R. and ECG in the cocaine-pretreated (3 mg./kg. i.v. 30 min. earlier), non-vagotomized cat (male, 3 kg.), before cocaine (COI), 30 minutes later and before i.vt. lep. (CO II), and 2, 4, 7 and 20 minutes after lep.

FIGURE 12B: Changes in mean B.P., H.R. and ECG in the cocaine-pretreated (3 mg./kg. i.v. 30 min. earlier), vagotomized cat (male, 3 kg.), before cocaine (CO I), 30 minutes later and before i.vt. lep. (CO II), and 2, 5, 15 and 30 minutes after lep.

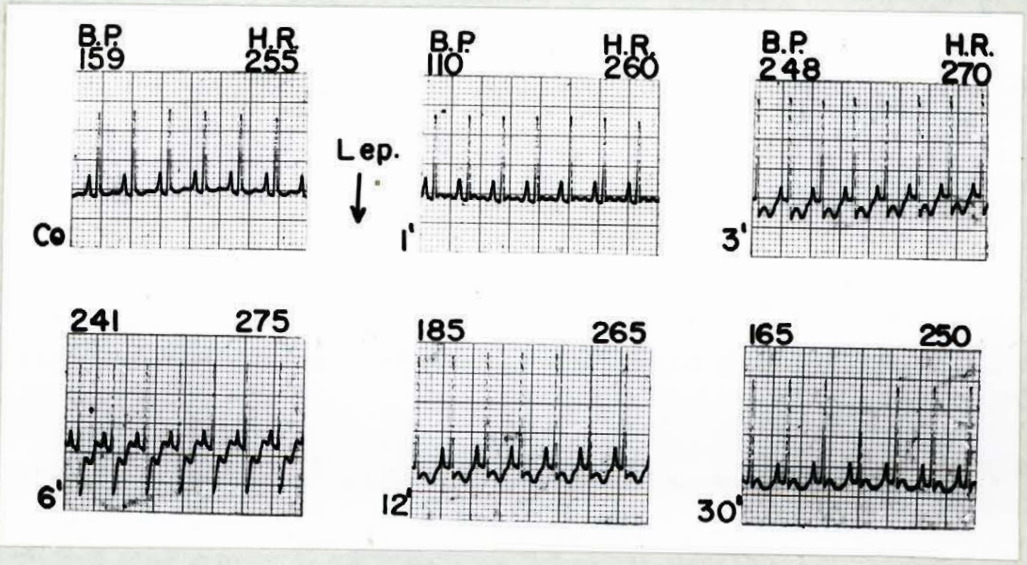
vagotomized cats. The pressor response in vagotomized cats is rather reduced. In general, electrocardiographic changes were similar to those of untreated animals, except that the ST-T segment deviations were more marked in the non-vagotomized cocaine pretreated cats.

5. Effects of Iproniazid

Iproniazid (Ipn), which is a known monoamine oxidase inhibitor (Zeller et al, 1952), is also reported to be capable of elevating brain NAD and 5-hydroxytryptamine levels in rabbits (Spector et al, 1957). Since monoamine oxidase is related to the metabolism of catecholamines, Ipn was used to evaluate the sympathetic activity induced by lep. It was used in a dose of 100 mg./kg. body weight intraperitoneally 10 hours before the i.vt. injection of lep.

Following i.vt. lep., the usual pressor responses and cardioacceleration ensued in both the non-vagotomized (4 exp.) and vagotomized (3 exp.) animals, and there appeared no significant difference from the previously observed types of changes with lep. alone (Tables 5 and 6). Thus, the degree of pressor responses and cardioacceleration were insignificantly different in non-vagotomized ($P > 0.3$ and $P > 0.9$, respectively) and vagotomized ($P > 0.5$ and $P > 0.1$, respectively) cats. However, Ipn pretreatment prevented the appearance of ventricular extrasystoles in all 7 exp., although the occurrence of ST-T segment deviations remained

13A



13B

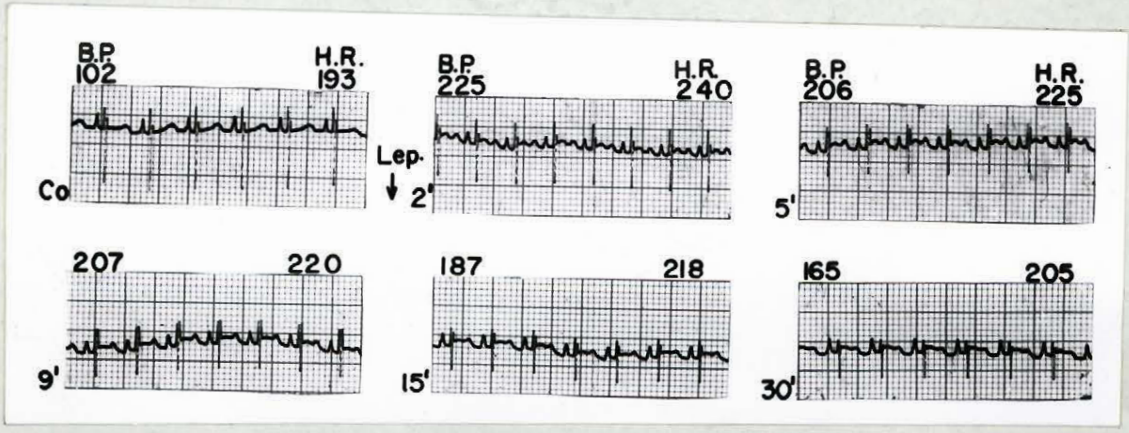


FIGURE 13A: Changes in mean B.P., H.R. and ECG in the Ipn-pretreated (100 mg./kg. i.v. 10 hrs. earlier), non-vagotomized cat (female, 3 kg.), before (CO) and 1, 3, 6, 12 and 30 minutes after i.vt. lep.

FIGURE 13B: Changes in mean B.P., H.R. and ECT in the Ipn-pretreated (100 mg./kg. i.v. 10 hrs. earlier), vagotomized cat (female, 3 kg.), before (CO(and 2, 5, 9, 15 and 30 minutes after i.vt. lep.

unaltered. Figures 13A and 13B illustrate typical examples of these changes. Table 5 shows, comparatively, these results in non-vagotomized and Table 6 shows these changes in vagotomized cats with those observed following various other types of pretreatment prior to lep.

In summary, pretreatment with iproniazid neither enhanced nor attenuated the pressor response or cardio-acceleration, resulting from the intraventricular injection of leptazol, both in non-vagotomized and vagotomized cats, but completely prevented the occurrence of ventricular extrasystoles. The degree of ST-T segment deviations, however, were similar to those seen with leptazol in the untreated animals.

PART III - Influence of Central Pretreatments on Cardio-vascular Changes Induced by Intraventricular Injections of Leptazol

With the hope of elucidating further the central nervous system mechanisms which might be involved in the cardiovascular changes following i.vt. lep., the effects of various types of 'central pretreatments' (See Methods) were carried out in non-vagotomized cats, under pento. and SCH. The results of these studies are summarized in Table 7.

TABLE 7

Comparative cardiovascular changes induced by intraventricular leptazol (85 mg.) alone, and by similar injections following intraventricular pretreatment with various drugs in cats anesthetized with pentobarbitone and curarized with succinylcholine (See Text).

Intraventricular Pretreatment	N	B.P. mm. Hg		H.R./min.		ECG Changes	
		Control	Maximum Increase	Control	Maximum Increase	E.B.	ST-T
None	6	123.3 ± 8.4	105.3 ±11.4	224.6 ± 8.7	42.8 ± 4.7	4	+++
Reserpine (3 hours)	3	97.3 ± 3.9	133.0 ± 2.0	145.3 ±15.7	77.3 ± 3.9	2	++
Reserpine (6 hours)	3	106.0 ± 8.8	132.0 ± 8.5	188.6 ±26.9	76.3 ±21.8	2	+++
Reserpine (18 hours)	4	106.7 ± 3.7	103.5 ±17.6	150.5 ±16.4	57.0 ±11.3	3	+++
Atropine	5	112.2 ±21.8	6.8 ± 2.0	183.0 ±48.1	5.2 ± 0.5	0	-
Ethybenztropine	5	121.6 ±13.8	21.8 ± 3.7	220.0 ±22.7	5.0 ± 1.5	0	+
Hexamethonium	5	110.8 ± 7.4	22.8 ± 6.8	181.0 ±19.4	18.8 ± 6.9	0	-
Mecamylamine	4	86.5 ±10.2	3.5 ± 1.3	210.0 ±19.1	2.0 ± 0.08	0	-
Hemicholinium	5	136.2 ± 9.3	21.0 ± 8.7	186.0 ± 7.1	17.0 ± 5.7	0	+

N = Number of experiments
Mean values of B.P and H.R. with ± standard errors of the means are given.
E.B. = Number of experiments where ventricular extrasystoles occurred.
ST-T = Comparative degrees of ST-T segments deviations.

1. Effects of "Central Reserpinization"

It is known that reserpine is capable of depleting catecholamines from peripheral structures (Carlsson et al, 1956) as well as from brain stem (Holzbauer et al, 1956; Carlsson, 1958). It also depletes 5-hydroxytryptamine (5-HT) from the brain stem (Paasonen and Vogt, 1956). It was thought that local brain depletion of catecholamines and 5-HT by pretreatment with i.vt. reserpine might prevent the centrally-mediated cardiovascular responses to lep., if those brain amines were in any way involved. On the other hand, if these responses to lep. were not significantly influenced by central reserpinization it is evident that the levels of catecholamines and 5-HT in the brain might not be important in the initiation of these responses.

In attempting to limit the "depletion" to central structures, a dose of 500 µg. of reserpine was injected i.vt. (usually 18 hours before the exp.) in previously cannulated cats. However, it was also considered important to determine whether or not the i.vt. injection of reserpine caused peripheral catecholamine depletion. As a general test for systemic reserpine action, a dose of 1.5 mg./kg. of nicotine was injected intravenously 18 hours following i.vt. reserpine. In 8 such exp., nicotine produced the usual pressor responses, tachycardia and ST-T segment deviations. These results suggest strongly that i.vt. reserpine in a dose of 500 µg. did not induce depletion of

catecholamines in the peripheral cardiovascular system.

Following central reserpinization and prior to the i.vt. administrations of lep., it was observed that the cats were drowsy but they were easily aroused, and tended to lie down when left undisturbed. Miosis and relaxation of the nictitating membranes were also evident in varying degrees. During the control period the heart rate and blood pressure of these animals were low and the ECG showed some evidence of conduction defect with T wave changes.

Sourkes et al (1961) have reported that when DL-AMDP (α-methyl-3:4-dihydroxyphenylalanine) is used as the depleting agent for catecholamines in the rat brain, the content of dopamine in the brain was decreased by 50 percent in 3 hours, but was restored to the normal level in 6 hours. In contrast, within 1 hour following the depleting agent, the noradrenaline levels in the brain increased slightly, but thereafter fell more slowly than in the case of dopamine and remained depressed for at least 72 hours. In view of these observations, it was thought that although reserpine can deplete NAD, ADR, dopamine, as well as 5-HT from the brain, the rates of depletion and restoration to normal levels of these different substances might vary with time. In the present exp., therefore, the responses to i.vt. lep. (85 mg.) were studied at time intervals of 3, 6 and 18 hours after central reserpinization, as summarized below in Table 7.

a) Three hours after reserpine

In these experiments, following i.vt. lep. (3 exp.), as shown in Table 7, there occurred increased pressor responses. However, the pressor responses were not significantly different from those seen in normal control animals ($P > 0.1$). The degree of cardioacceleration observed was, however, increased significantly more than in the control group ($P < 0.01$). Cardiac arrhythmias and ST-T changes of the usual types were also observed. Fig. 14A shows a typical example.

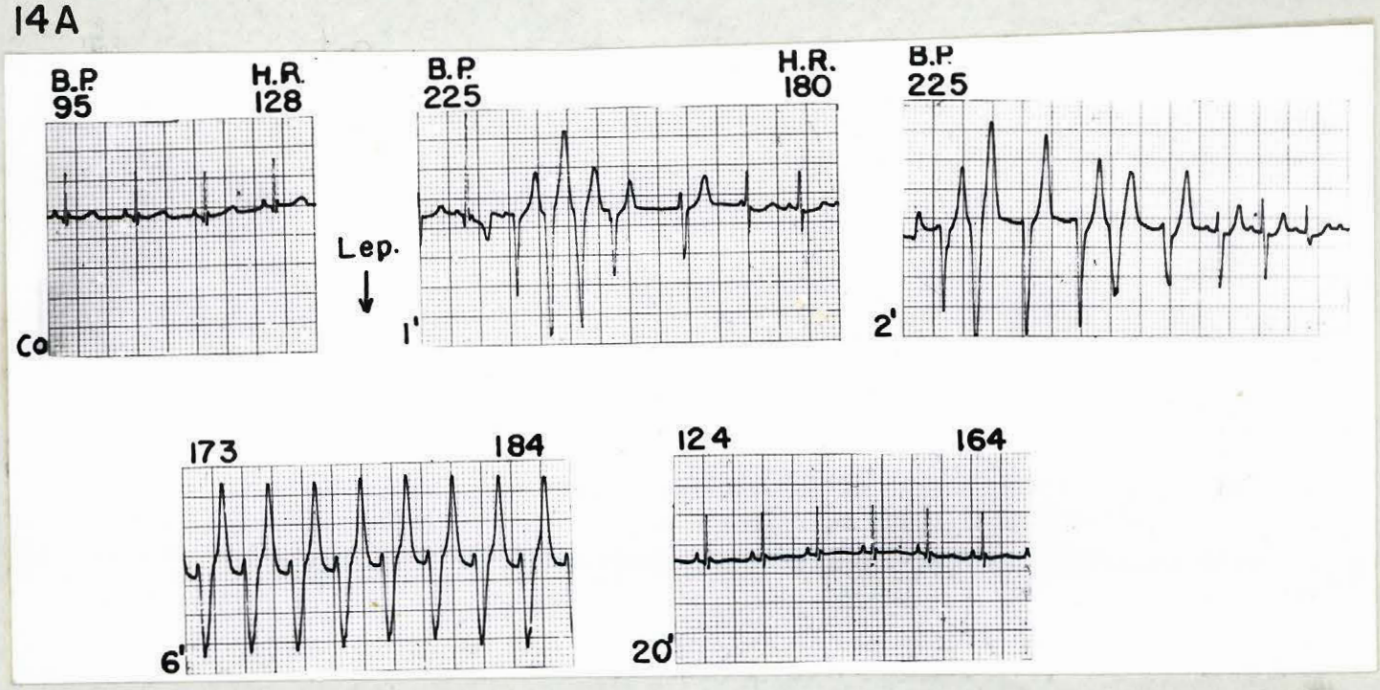
b) Six hours after reserpine

In these experiments, following i.vt. lep. (3 exp.), as shown in Table 7, pressor response and the degrees of cardioacceleration recorded were not significantly different from those seen with lep. alone ($P > 0.1$ and $P > 0.5$, respectively). Fig. 15A illustrates such an example. Extrasystoles occurred in 2 out of 3 exp. and ST-T segment alterations were seen in all three.

c) Eighteen hours after reserpine

In these experiments, following the i.vt. injection of lep. (4 exp.), as shown in Table 7, the resulting pressor responses and cardioacceleration were again not significantly different ($P > 0.9$ and $P > 0.2$, respectively) from those previously observed with lep. alone. The pressor responses showed increases varying from 66 to 122 mm. Hg (mean, 103.5) and the cardioacceleration increases ranging from 26 to 77

14A



14B

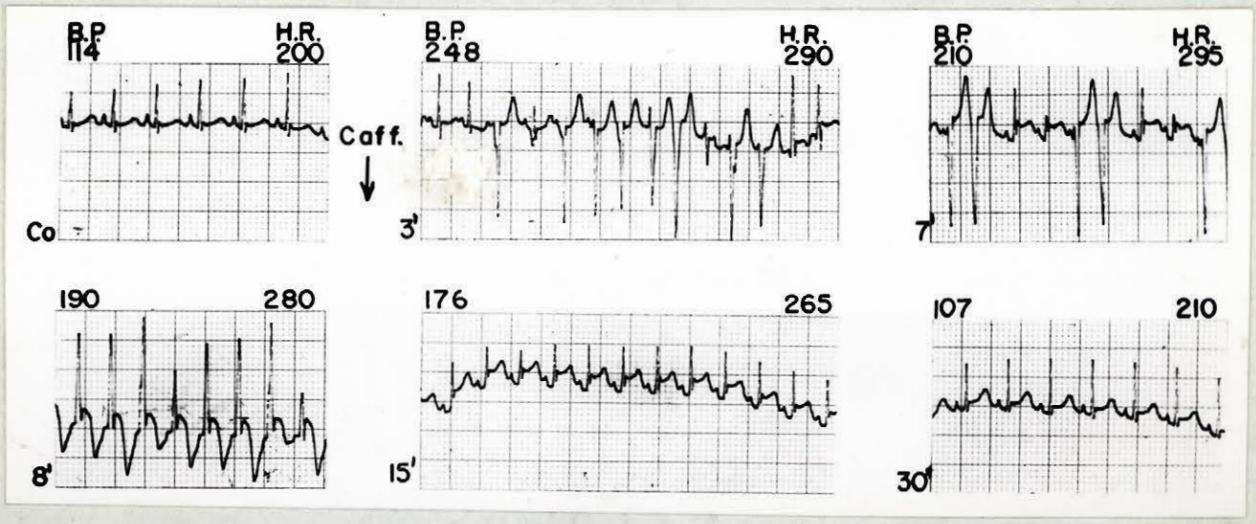
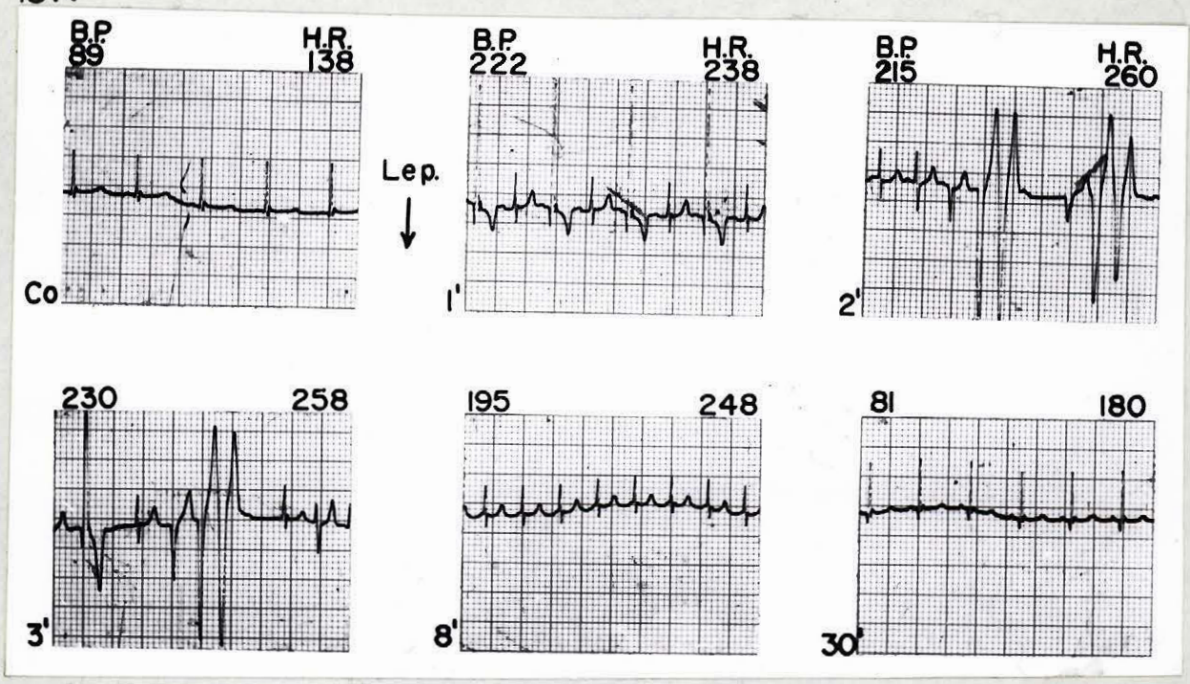


FIGURE 14A: Changes in mean B.P., H.R. and ECG in the "centrally reserpinized" (3 hours), non-vagotomized cat (female, 2.7 kg.), before (CO) and 1, 2, 6 and 20 minutes after i.vt. lep.

FIGURE 14B: Changes in mean B.P., H.R. and ECG in the "centrally reserpinized" (3 hours), non-vagotomized cat (female, 2.8 kg.), before (CO) and 3, 7, 8, 15 and 30 minutes after i.vt. caff.

15A



15B

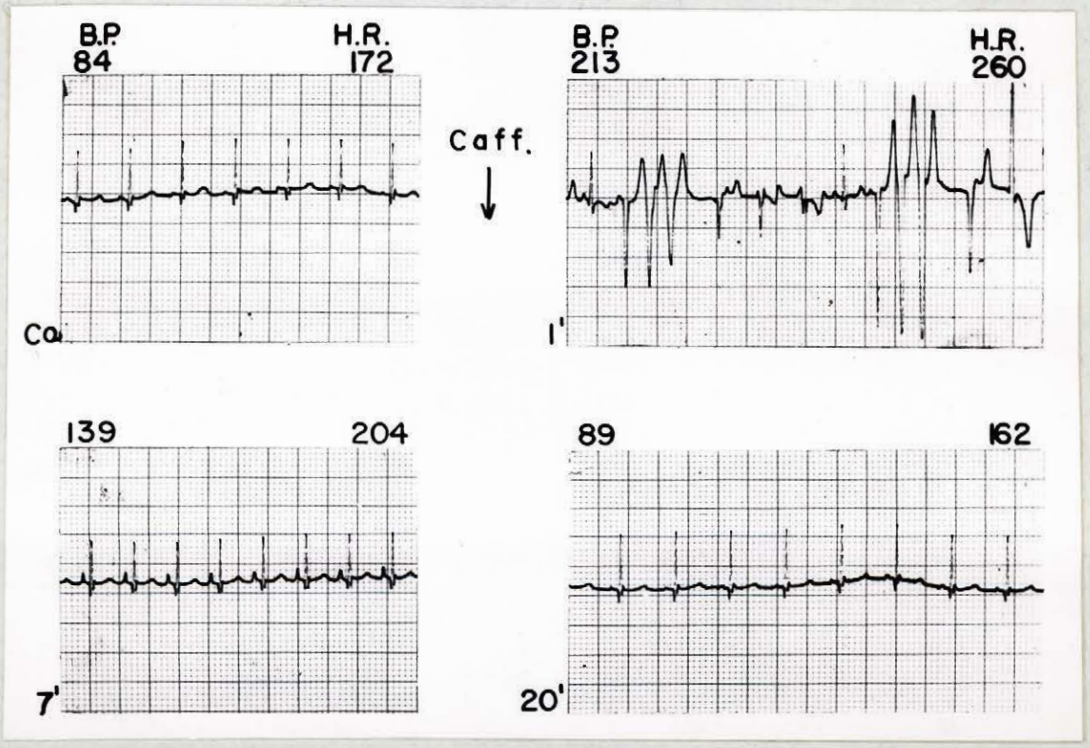


FIGURE 15A: Changes in mean B.P., H.R. and ECG in the "centrally reserpinized" (6 hours), non-vagotomized cat (female, 2.7 kg.), before (CO) and 1, 2, 3, 8 and 30 minutes after i.vt. lep.

FIGURE 15B: Changes in mean B.P., H.R. and ECG in the "centrally reserpinized" (6 hours), non-vagotomized cat (female, 2.7 kg.), before (CO) and 1, 7 and 20 minutes after i.vt. caff.

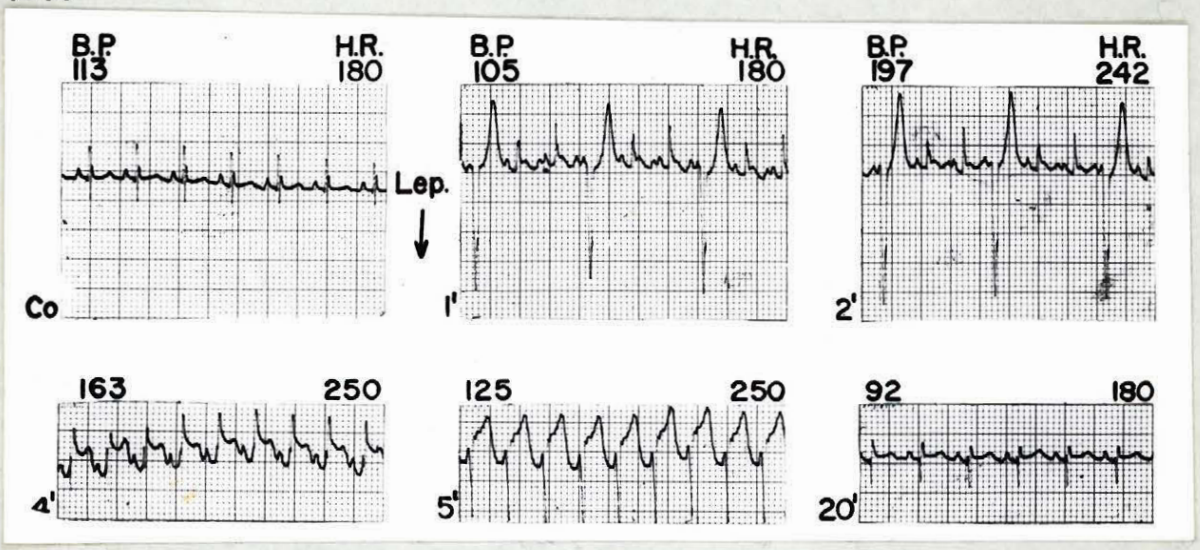
per min. (mean, 57). Fig. 16A shows some examples of these changes. The ECG alterations were also similar to those seen in untreated animals and extrasystoles occurred in 3 out of 4 exp. The intensity of the ECG changes also appeared to be somewhat less in the reserpinized group.

2. Effects of Intraventricular Atropine

It is now well-known that atropine blocks the effects of acetylcholine at the postganglionic parasympathetic neuroeffector junctions. However, it is still uncertain whether or not atropine is capable of similarly blocking or abolishing the central effects of acetylcholine. Some investigators have demonstrated atropine-acetylcholine (or eserine) antagonism in the brain, offering evidence that some central effects of acetylcholine are abolished by atropine (Feldberg, 1945). Brenner et al (1942) also found that atropine applied to either the normal or eserinized cortex produced changes in electrical activity which were very similar. Stone (1957) has demonstrated that atropine prevents the arousal reaction induced by cholinergic excitation in the brain. He, therefore, speculated that atropine competes with acetylcholine for some 'receptive substance' in the brain and might thereby block transmission at the endings of cholinergic neurones.

On the assumption that cholinergic mechanisms are involved in central nervous system functions, experiments were undertaken to assess the influence of i.vt. pretreatment

16 A



16B

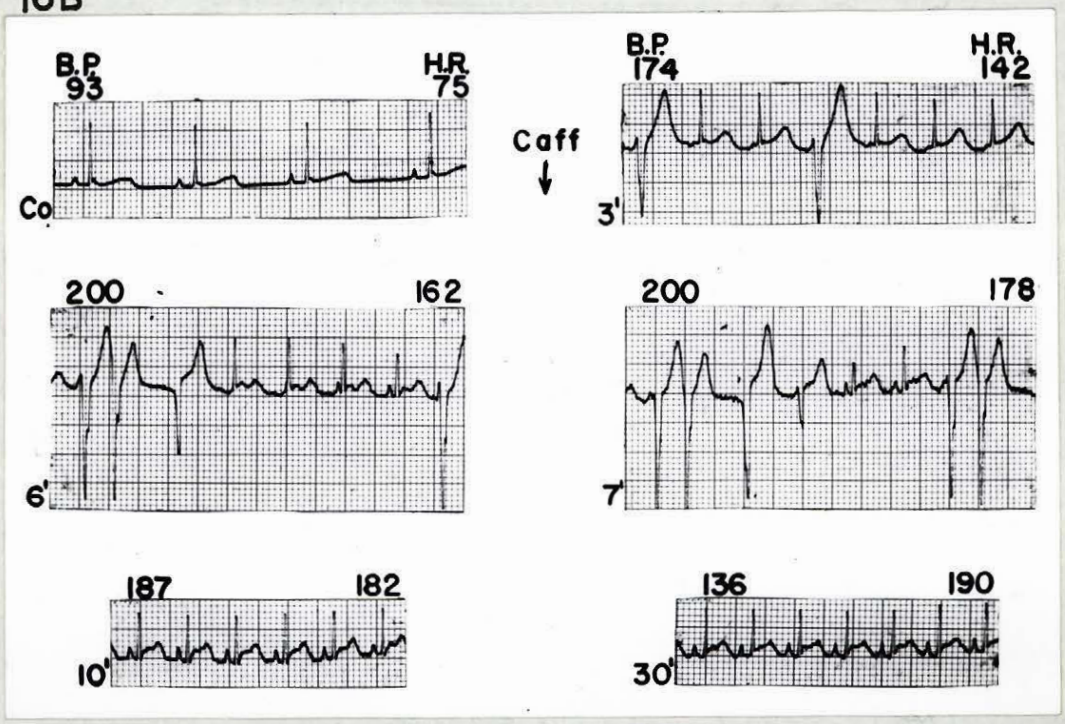


FIGURE 16A: Changes in meanB.P., H.R. and ECG in the "centrally reserpinized," (18 hours), non-vagotomized cat (female, 2.7 kg.), Before (CO) and 1, 2, 4, 5 and 20 minutes after i.vt. lep.
FIGURE 16B: Changes in mean B.P., H.R. and ECG in the "centrally reserpinized," (18 hours), non-vagotomized cat (female, 2.6 kg.), before (CO) and 3, 6, 7, 10 and 30 minutes after i.vt. caff.

with atropine (central atropinization) upon the cardiovascular responses induced by i.vt. lep. Following i.vt. atropine (0.1 mg.), there was no evidence of any peripheral cholinergic blockade and i.v. injections of acetylcholine (5 µg./kg.) 15 min. after i.vt. atropine, still produced normal depressor responses (3 exp.). There was no evidence, therefore, of systemic absorption of atropine.

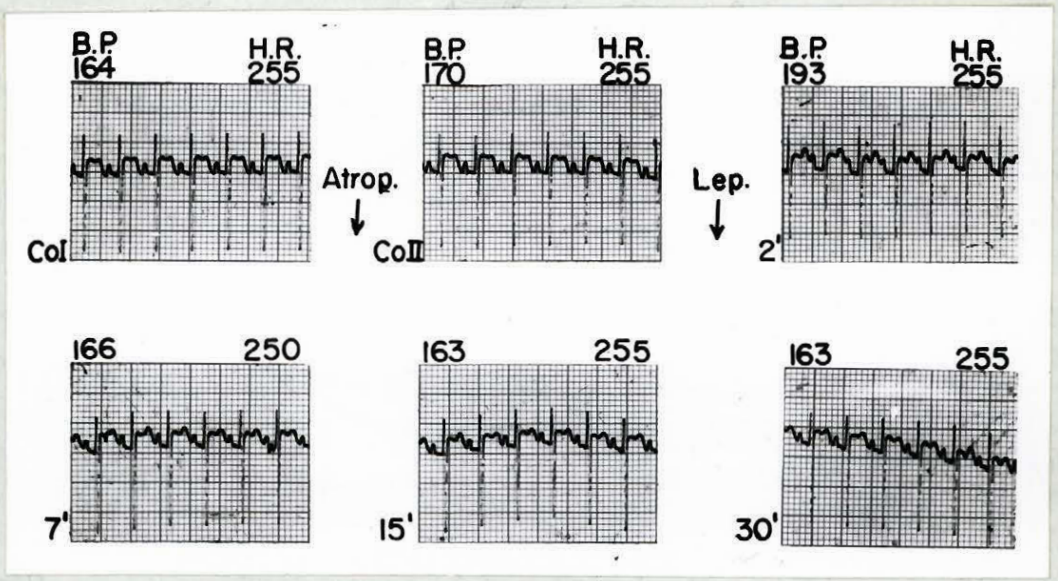
After previous i.vt. atropine, i.vt. lep. (5 exp.) produced, however, insignificant increases in pressor responses ranging only from 4 to 10 mm. Hg (mean, 6.8), associated with increases in cardioacceleration ranging from only 4 to 7 per min. (mean, 5.2). These were both significantly reduced responses ($P < 0.001$ and $P < 0.001$, respectively). No extrasystoles were seen in any of these experiments and ST-T changes were insignificant. Fig. 17A shows an example of these results.

In summary, (1) intraventricular atropine prevented the cardiac arrhythmias which are normally elicited by intraventricular leptazol. (2) Other cardiovascular responses following intraventricular leptazol are also significantly attenuated by central atropine pretreatment.

3. Effects of Intraventricular Ethybenztropine

Ethybenztropine is chemically N-ethy-nortropin-benzhydrylether hydrobromide, an ethyl derivative of benztropine (Cogentin). It has been reported to be a potent central anticholinergic agent (Taeschler et al, 1962).

17A



17B

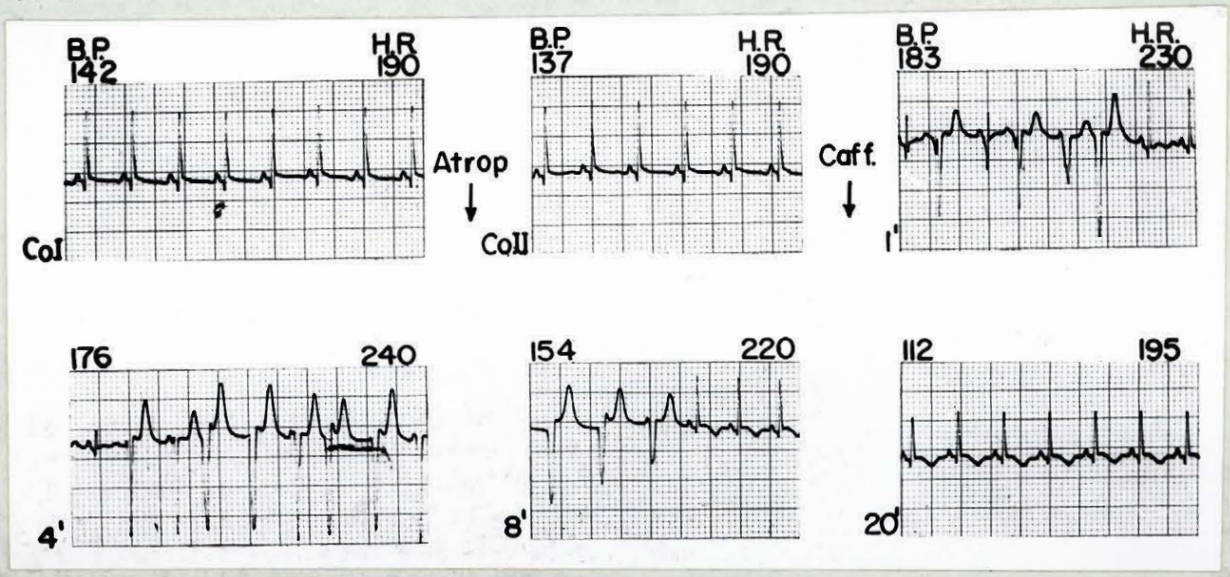


FIGURE 17A: Changes in mean B.P., H.R. and ECG in "centrally atropinized" (0.1 mg. i.vt.), non-vagotomized cat (male, 3 kg.), before Atro. (CO I), 15 minutes later and before i.vt. lep. (CO II) and 2, 7, 15 and 30 minutes after lep.

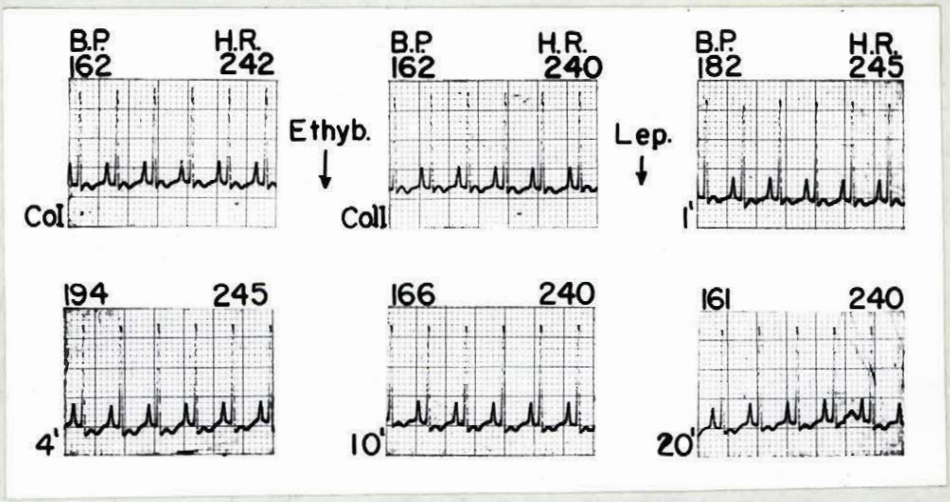
FIGURE 17B: Changes in mean B.P., H.R. and ECG in "centrally atropinized" (0.1 mg. i.vt.), non-vagotomized cat (female, 2.7 kg.), before Atro. (CO I), 15 minutes later and before i.vt. caff. (CO II) and 1, 4, 8 and 20 minutes after caff.

It was, therefore, also investigated in attempts to assess the role of central cholinergic mechanisms in the central actions of lep. For pretreatment, ethyb. was used in a dose of 0.1 mg. injected i.vt. 20 min. before i.vt. lep. (85 mg.). Following the pretreatment the heart rate and blood pressure were not altered appreciably. Furthermore, the treated animals showed no evidence of peripheral cholinergic blockade and in all cases no ECG changes. In order to evaluate the integrity of the peripheral parasympathetic functions, a dose of 5 μg./kg. of acetylcholine was injected intravenously (3 exp.) 20 min. after the i.vt. injection of ethyb., and the usual depressor response was observed in all cases.

In 5 exp., following i.vt. lep. after central pretreatment with ethyb. (Table 7), the observed cardiovascular responses were all markedly attenuated. The pressor response was slight and varied from increases of 10 to 30 mm. Hg (mean, 21.8) and the cardioacceleration ranged from increases of only 2 to 10 per min. (mean, 5). These changes were significantly different from the normal responses ($P < 0.001$ and $P < 0.001$, respectively). Also, there occurred no extrasystoles in any exp. and the ST-T alterations were insignificant in all. Fig. 18A illustrates a typical example of these results.

In summary, intraventricular pretreatment of cats with ethybenztropine significantly attentuated the pressor responses, cardioacceleration, and cardiac arrhythmias

18A



18B

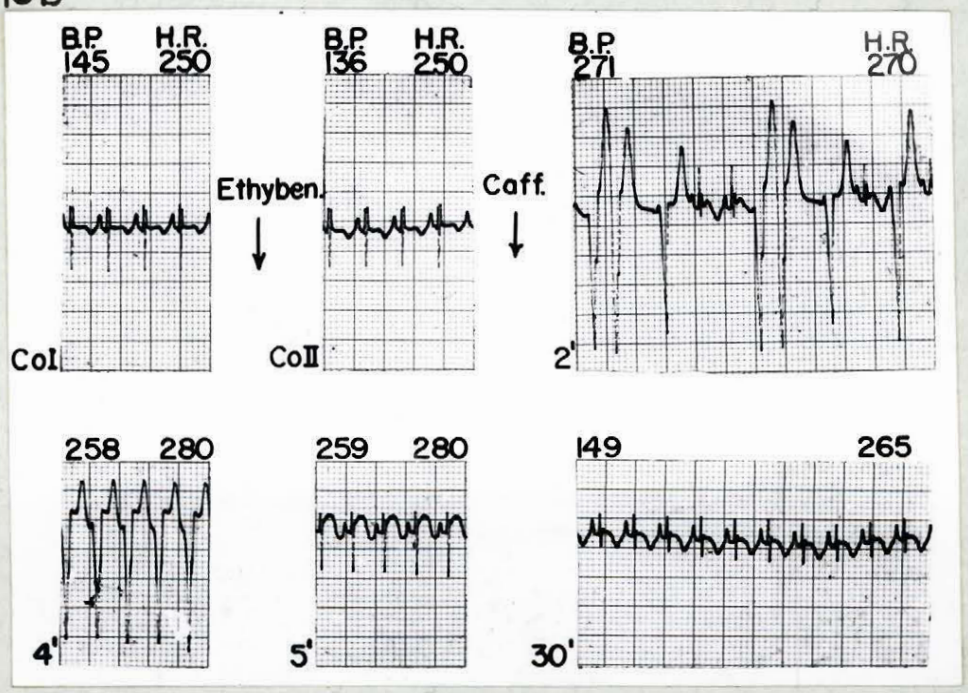


FIGURE 18A: Changes in mean B.P., H.R. and ECG in the non-vagotomized cat (female, 2.8 kg.) under central pretreatment with ethyb. (0. 1 mg. i.vt. 20 min. earlier), before ethyb. (CO I), 20 minutes later and before i.vt. lep. (CO II) and 1, 4, 10 and 20 minutes after lep.

FIGURE 18B: Changes in mean B.P. H.R. and ECGin the non-vagotomized cat (male, 3 kg.), under central pretreatment with ethyb. (0.1 mg. i.vt. 20 min. earlier), before ethyb. (CO I), 20 minutes later and before i.vt. caff. (CO II) and 2, 4, 5 and 30 minutes after caff.

induced by intraventricular leptazol; ST-T changes were insignificant.

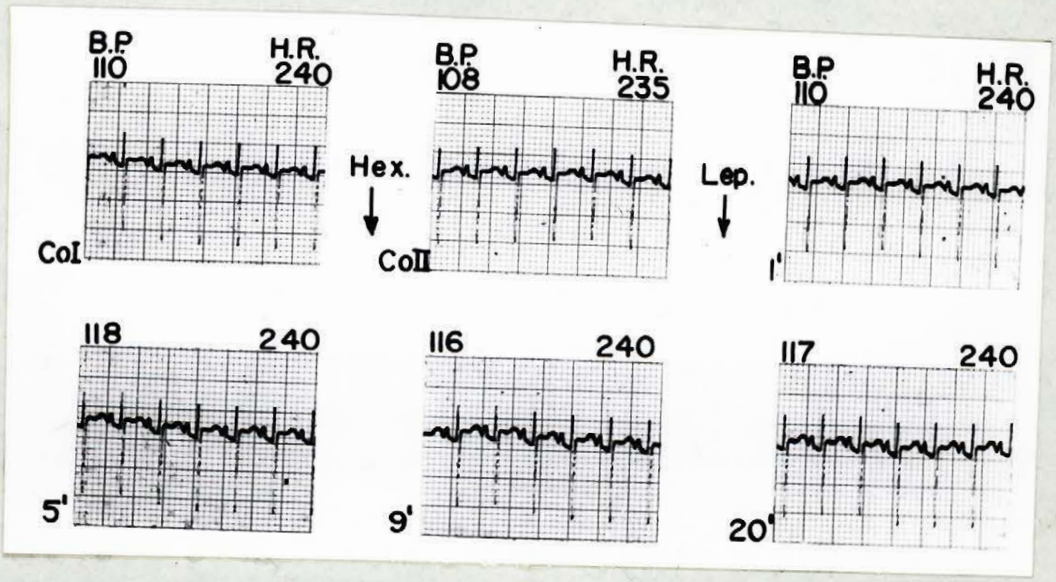
4. Effects of Intraventricular Hexamethonium and Mecamylamine

Hexamethonium (hexa.) and mecamylamine (meca.) are known potent autonomic ganglionic blocking agents, exerting their actions primarily at cholinergic interneuronal sites. By employing these drugs intraventricularly, it was hoped to induce a similar type of central synaptic blockade, and possibly throw some further light upon the nature of the trigger mechanisms inolved in the centrally-mediated cardiovascular actions of lep.

Following i.vt. injection of either hexa. (0.1 mg.) or meca. (0.1 mg.), there was no evidence of any peripheral autonomic ganglionic blockade. This was demonstrated by the use of atropine (1 mg./kg.) which was injected intravenously, 20 min. after i.vt. injection of either agent, and followed 7 min. later by the i.v. injection of a dose of 150 µg./kg. of acetylcholine (4 exp. with each agent). The usual pressor response due to the ganglionic or nicotinic action of acetylcholine was seen in each instance.

After pretreatment with hexa. (Table 7), the pressor responses and cardioacceleration induced by i.vt. lep. (85 mg.) were greatly reduced. The pressor effects observed ranged only from increases of 4 to 43 mm. Hg (mean, 22.8) and the cardioacceleration varied from increases of 2 to 40 per min. (mean, 18.8). These effects were

19A



19B

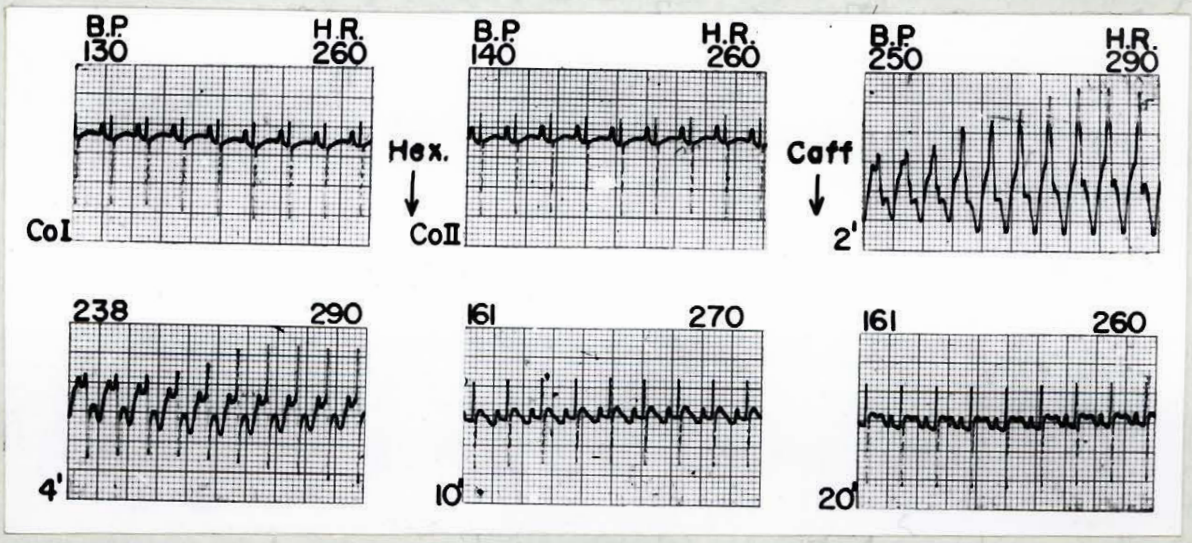


FIGURE 19A: Changes in mean B.P., H.R. and ECG in the non-vagotomized cat (female, 3 kg.) under central pretreatment with hexa. (0.1 mg. i.vt. 20 min. earlier), before hexa. (CO I), 20 minutes later and before i.vt. lep. (CO II) and 1, 5, 9 and 20 minutes after lep.

FIGURE 19B: Changes in mean B.P. H.R. and ECG in the non-vagotomized cat (female, 3 kg.) under central pretreatment with hexa. (0.1 mg. i.vt. 20 min. earlier), before hexa. (CO I), 20 minutes later and before i.vt. caff. (CO II) and 2, 4, 10 and 20 minutes after caff.

significantly different ($P < 0.001$, and $P < 0.02$, respectively) from the control responses to lep. The ECG pattern was essentially normal, except for minor T wave changes. Fig. 19A shows a typical example of the results.

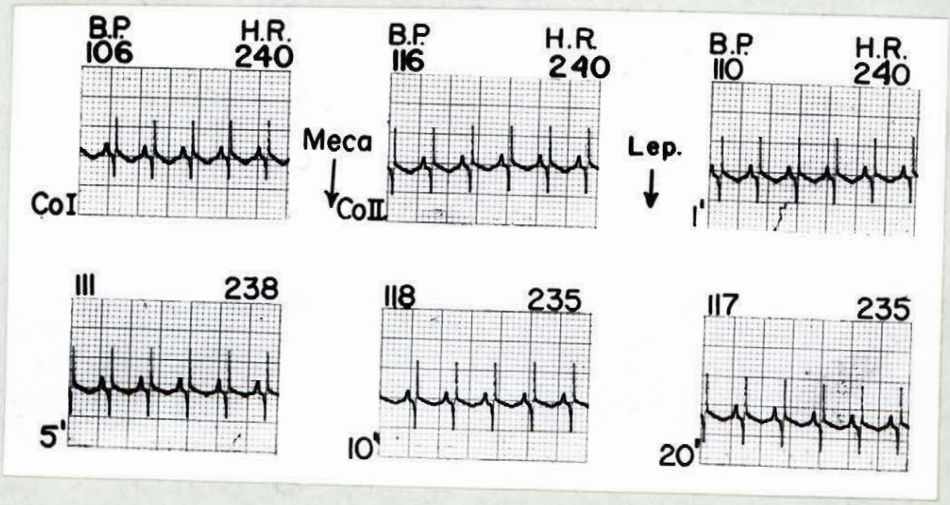
In regard to the cardiovascular responses to i.vt. lep. after pretreatment with meca., the pressor responses observed (Table 7) varied from increases of 2 to 7 mm. Hg (mean, 3.5) and insignificant cardioacceleration with increases of only 1 to 4 per min. (mean, 2). Both of these changes were significantly less ($P < 0.001$ and $P < 0.001$, respectively) than in the control experiments. The usually observed ECG changes were also completely absent, as shown in Fig. 20A.

In summary, central pretreatment with either hexamethonium bromide (0.1 mg.) or mecamylamine (0.1 mg.) prevented the electrocardiographic changes and significantly reduced the pressor response and cardioacceleration following intraventricular leptazol.

5. Effects of Intraventricular Hemicholinium (HC-3)

Following systemic administration, hemicholinium (HC-3) has been shown to inhibit the synthesis of acetylcholine both in peripheral autonomic ganglia and in the brain.(Schueler, 1955; MacIntosh et al, 1956, 1958; Birks et al, 1957, 1961; MacIntosh, 1959, 1961). It has also been shown that HC-3 does not significantly block transmission across autonomic ganglia (Birks and MacIntosh,

20A



20B

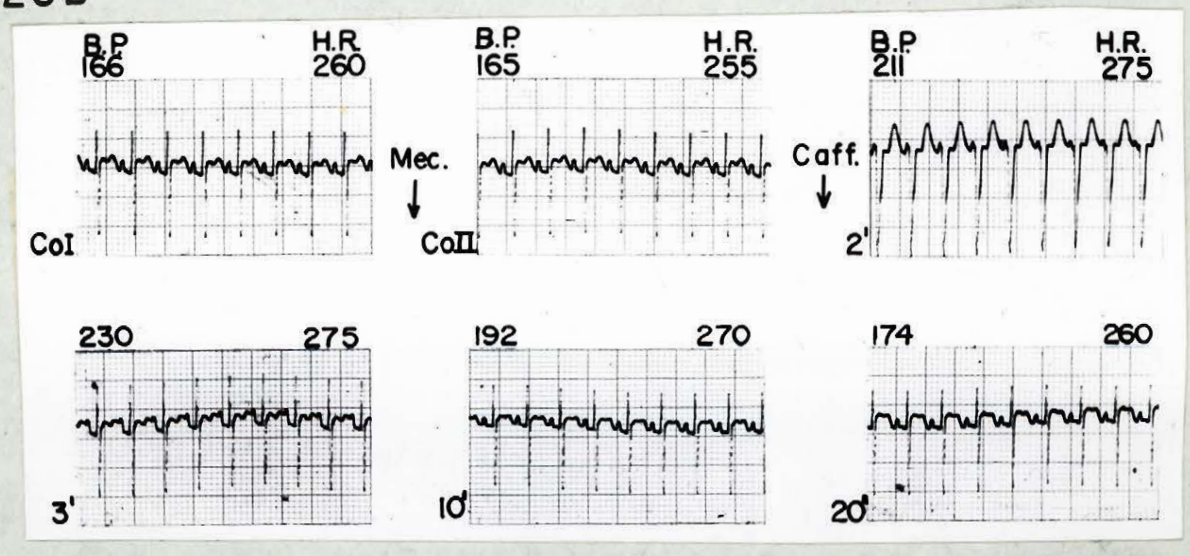


FIGURE 20A: Changes in mean B.P., H.R. and ECG in the non-vagotomized cat (female, 2.6 kg.) under central pretreatment with meca. (0.1 mg. i.vt. 20 min. earlier) before meca. (CO I), 20 minutes later and before i.vt. lep. (CO II) and 1, 5, 10 and 20 minutes after leptazol.

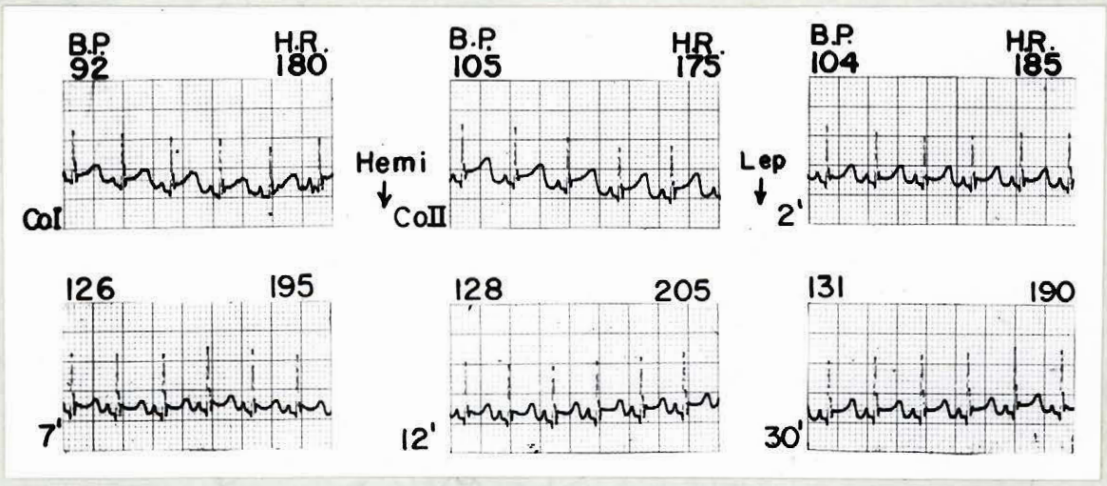
FIGURE 20B: Changes in mean B.P., H.R. and ECG in the non-vagotomized cat (male, 2.9 kg.) under central pretreatment with meca. (0.1 mg. i.vt. 20 min. earlier) before meca. (CO I), 20 minutes later and before i.vt. caff. (CO II) and 2, 3, 10 and 20 minutes after caff.

1961). It was assumed that if acetylcholine were involved in the central actions of lep. in these exp., inhibition of acetylcholine synthesis by the i.vt. injection of HC-3 might prevent or reduce the cardiovascular responses following lep.

Since it is known that the inhibition of acetylcholine synthesis after HC-3 develops slowly, an interval of one and a half to 2 hours was allowed to elapse following the i.vt. injection of HC-3 (0.5 mg.). During this period, it was also observed that arterial blood pressure, heart rate and ECG were not significantly altered. Following the usual i.vt. injection of lep. (85 mg.), it was also observed that the cardiovascular responses were markedly reduced. The cardiac arrhythmias were completely prevented, although there were minor ST-T changes. As shown in Table 7, the pressor responses observed ranged from increases of 13 to 51 mm. Hg (mean, 21) and cardioacceleration varied from increases of 2 to 31 per min. (mean, 17). These changes were significantly less ($P < 0.001$ and $P < 0.01$, respectively). Fig. 21A illustrates a typical example of these results.

In summary, intraventricular HC-3 significantly reduced the pressor response and cardioacceleration and completely prevented the extrasystoles induced by intraventricular leptazol, although there occurred minor ST-T changes in the electrocardiogram.

21A



21B

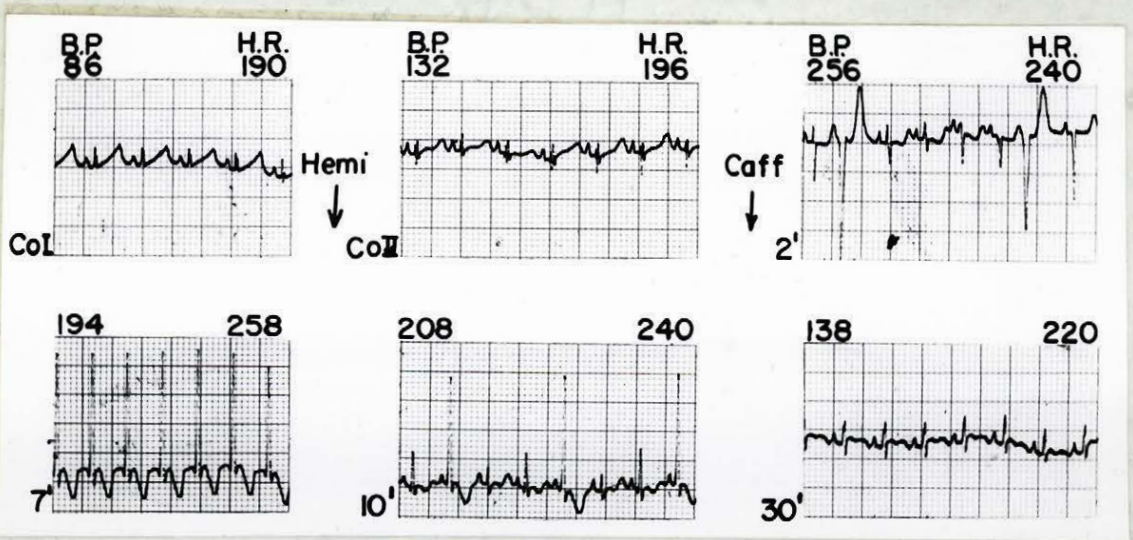


FIGURE 21A: Changes in mean B.P., H.R. and ECG in the non-vagotomized cat (male, 2.8 kg.) under central pretreatment with HC-3 (0.5 mg. i.vt.-1½ hours earlier), before HC-3 (CO I), 1½ hours later and before i.vt. lep. (CO II) and 2, 7, 12 and 30 minutes after lep.

FIGURE 21B: Changes in mean B.P., H.R. and ECG in the non-vagotomized cat (female, 3 kg.) under central pretreatment with HC-3 (0.5 mg. i.vt.-1½ hours earlier), before HC-3 (CO I), 1½ hours later and before i.vt. caff. (CO II) and 2, 7, 10 and 30 minutes after caff.

SECTION B. STUDIES ON THE CARDIOVASCULAR RESPONSES TO CAFFEINE

PART I - Cardiovascular Effects of Intraventricular Injections of Caffeine

1. Observations following intraventricular and intravenous injections of caffeine

a) Dose-response effects in non-vagotomized animals following intraventricular injections

The i.vt. injection of 20 mg. of caffeine (caff.) in non-vagotomized cats (6 exp.), under pento. and gal. (Table 8) was followed by marked cardiovascular changes. The blood pressure started to rise within 30 sec. to 2 min. following the injection and reached increased peak values of 140 to 159 mm. Hg (mean, 149.1) within 5 to 10 min. The associated tachycardia showed increases ranging from 75 to 120 per min. (mean, 94). The ECG showed marked arrhythmias consisting of ectopic beats, bigeminus or trigeminus rhythm, auricular and ventricular tachycardia, as well as ST-T alterations. Typical examples of these changes are shown in Figures 22A and 22B. These changes were repeatable for 2 successive injections but were reduced progressively on further repetitions of administrations of the same dose of caff. (3 exp.).

The cardiovascular responses to doses of 5, 10 and

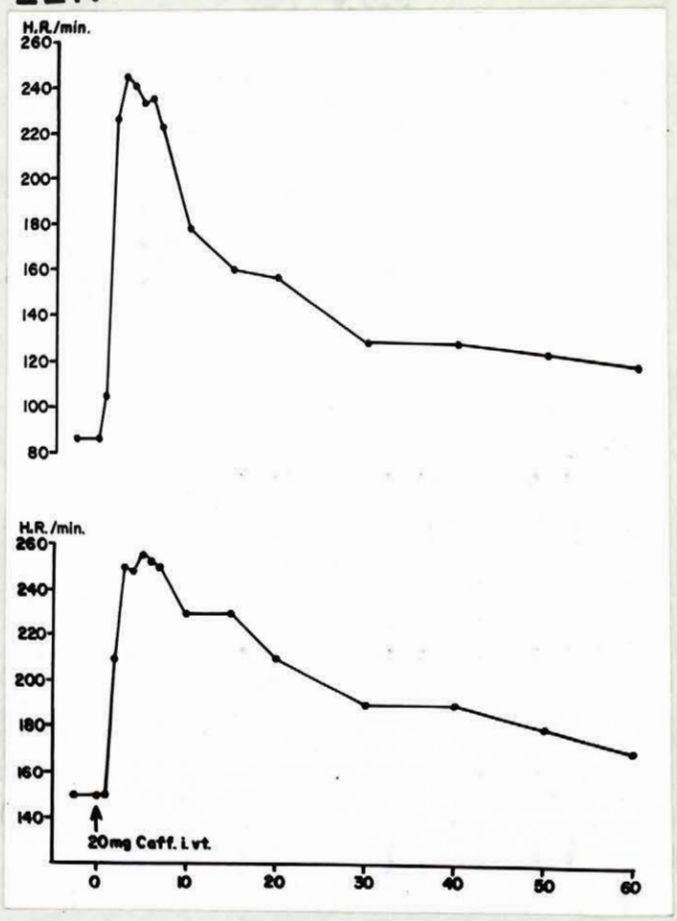
TABLE 8

Comparison of the cardiovascular changes induced by increasing doses of caffeine, following intraventricular injections in cats under pentobarbitone-gallamine (See Methods).

Caffeine Dose	N	B.P. mm. Hg		H.R./min.		ECG changes	
		Control	Maximum Increase	Control	Maximum Increase	E.B.	ST-T
5 mg.	5	130.0 ± 8.3	57.0 ± 5.7	239.0 ± 6.6	13.0 ± 4.0	0	+
10 mg.	5	132.0 ±14.9	72.0 ±15.8	217.0 ±11.0	26.6 ± 9.2	2	++
20 mg.	6	103.0 ± 5.5	149.1 ± 3.3	164.0 ±13.3	94.0 ±10.7	5	+++

N = Number of Experiments
Mean values of B.P. and H.R. with ± standard errors of the means are given.
E.B. = Number of experiment where ventricular extrasystoles occurred.
ST-T = Comparative degrees of ST-T segments deviations.

22A



22B

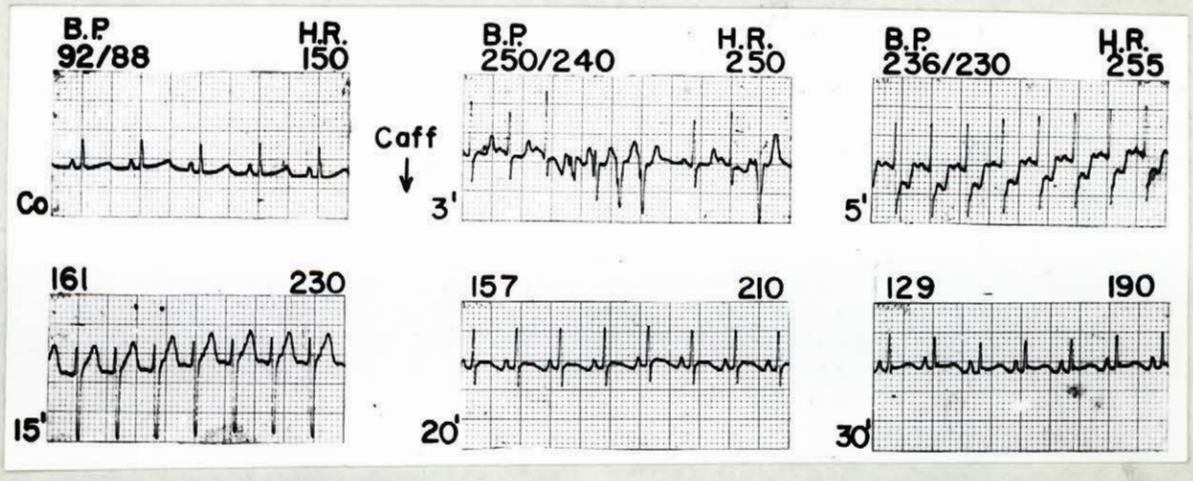


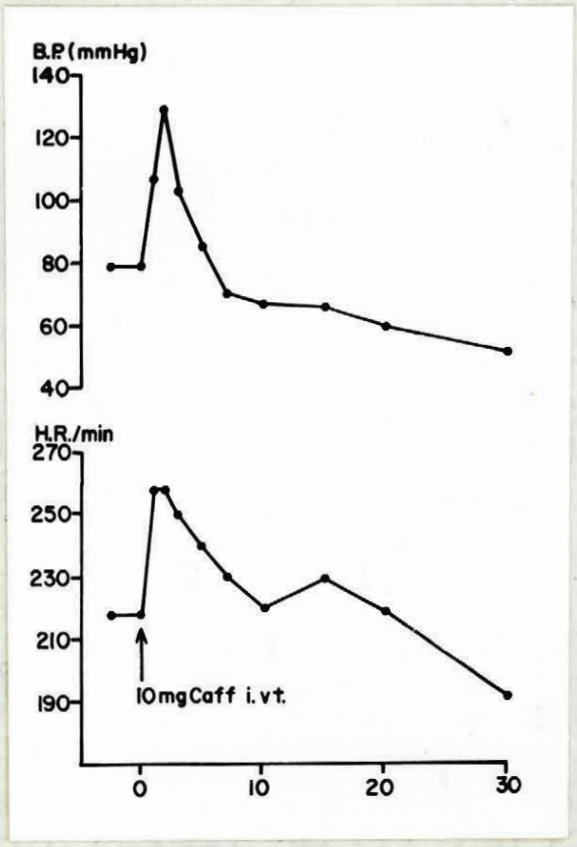
FIGURE 22A: Changes in mean B.P. and H.R. induced by i.vt. caff. (20 mg.) in the non-vagotomized cat (female, 2.4 kg.)
FIGURE 22B: ECG and associated B.P. and H.R. changes related to Figure 22A before (CO) and 3, 5, 15, 20 and 30 minutes after caff.

20 mg. of caff. injected i.vt. were studied in 16 non-vagotomized cats. The changes observed with these doses are summarized in Table 8.

In Figures 23A and 23B are illustrated typical examples of the responses to a dose of 10 mg. of caff. The pressor responses and cardioacceleration resulting from a dose of 10 mg. were found to be significantly less than those from 20 mg. ($P < 0.001$ and $P < 0.001$, respectively). The pressor responses and cardioacceleration resulting from a dose of 5 mg. were also found to be significantly less than that from 20 mg. ($P < 0.001$ and $P < 0.001$, respectively), but these responses to 5 mg. and 10 mg. of caff. were not significantly different from each other ($P > 0.3$ and $P > 0.2$, for pressor response and cardioacceleration, respectively). No ectopic beats were observed with the dose of 5 mg., although there were some minor ST-T changes. With the dose of 10 mg. there were ST-T changes and ectopic beats in 2 out of 5 exp. However, with the dose of 20 mg., ectopic beats occurred most frequently. Since a dose of 20 mg. of caff. was the dose which consistently induced cardiovascular changes rather like those of lep., and these were also repeatable, for comparative pharmacological studies this dose was employed throughout.

In summary, (1) increasing doses of caffeine (5, 10 and 20 mg.) injected intraventricularly into non-vagotomized cats, under pentobarbitone with gallamine, induced increasing degrees of pressor responses and cardioacceleration; pressor responses to doses of 5 and 10 mg. were not significantly different. (2) The incidence of arrhythmias and ST-T changes

23A



23B

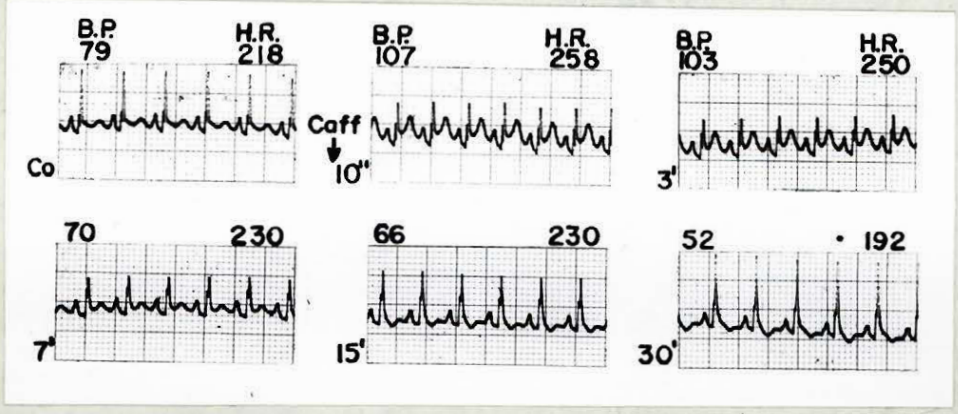


FIGURE 23A: Changes in mean B.P. and H.R. induced by i.vt. caff. (10 mg.) in the non-vagotomized cat (female, 2.8 kg.).
FIGURE 23B: ECG and associated B.P. and H.R. changes related to Figure 23A before (CO) and 3, 7, 15 and 30 minutes after caff.

were significantly increased with increasing doses. (3) The most consistent responses were observed following a dose of 20 mg. (4) The responses to the latter dose were repeatable on successive administration at 30 minute intervals.

b) Influence of anesthetics and curarizing agents on the responses following intraventricular injections.

In Table 9 are summarized the cardiovascular responses observed following i.vt. injection of a dose of 20 mg. caff. in cats, under different anesthetic and curarizing agents. Some typical examples of these responses are shown in Figures 23A and 23B and Figures 24A and 24B. In all of these exp. similar ECG changes were recorded.

(i) Pentobarbitone with succinylcholine (See Table 9)

In this group (6 exp.) the pressor responses to i.vt. caff. varied from increases of 83 to 130 mm. Hg (mean, 112.1) and the cardioacceleration from increases of 60 to 142 mm. Hg (mean, 96.5) over the controls. The pressor responses were significantly less ($P < 0.01$) than those observed under gal., but the cardioacceleration was not significantly different ($P > 0.8$). A typical example of these changes is shown in Figures 24A and 24B.

(ii) Chloralose with gallamine (See Table 9)

In this group (4 exp.) the mean pressor response observed was 125.5 mm. Hg, ranging from increases of 102 to 136 and was significantly less than that under pento. ($P < 0.02$). The mean cardioacceleration observed was 43.7/min.

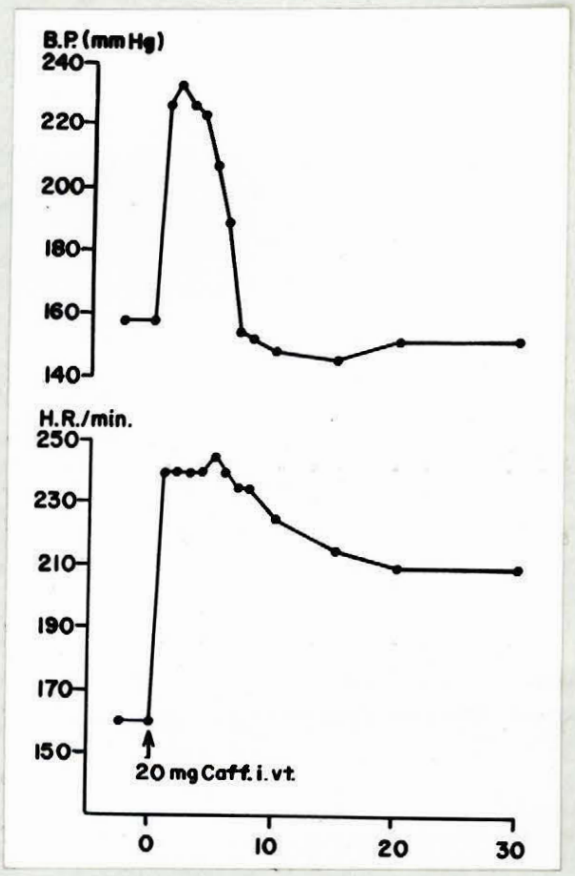
TABLE 9

Comparison of the cardiovascular changes induced by intraventricular caffeine (20 mg.) in non-vagotomized cats under various anesthetics and curarizing agents (See Methods).

		B.P. mm. Hg		H.R./min.		ECG changes	
	N	Control	Maximum Increase	Control	Maximum Increase	E.B.	ST-T
Pento. and gal.	6	103.0 ± 5.5	149.1 ± 3.3	164.0 ±13.3	94.0 ±10.7	5	+++
Pento. & SCH.	6	137.6 ± 7.5	112.1 ± 7.5	174.3 ±10.4	96.5 ±13.1	5	+++
Chlor. & gal.	4	115.2 ± 3.3	125.5 ± 7.9	242.4 ±14.5	43.7 ±15.9	3	+++
Chlor. & SCH	5	127.6 ± 4.1	117.2 ± 8.9	200.0 ±16.0	88.0 ±19.6	4	+++
Ether & gal.	4	136.2 ± 5.9	85.0 ± 6.5	201.7 ±24.3	50.7 ±17.0	3	+++

N = Number of experiments
Mean values of B.P. and H.R. with ± standard errors of the means are given.
E.B. = Number of experiments where ventricular extrasystoles occurred.
ST-T = Comparative degrees of ST-T segments deviations.

24A



24B

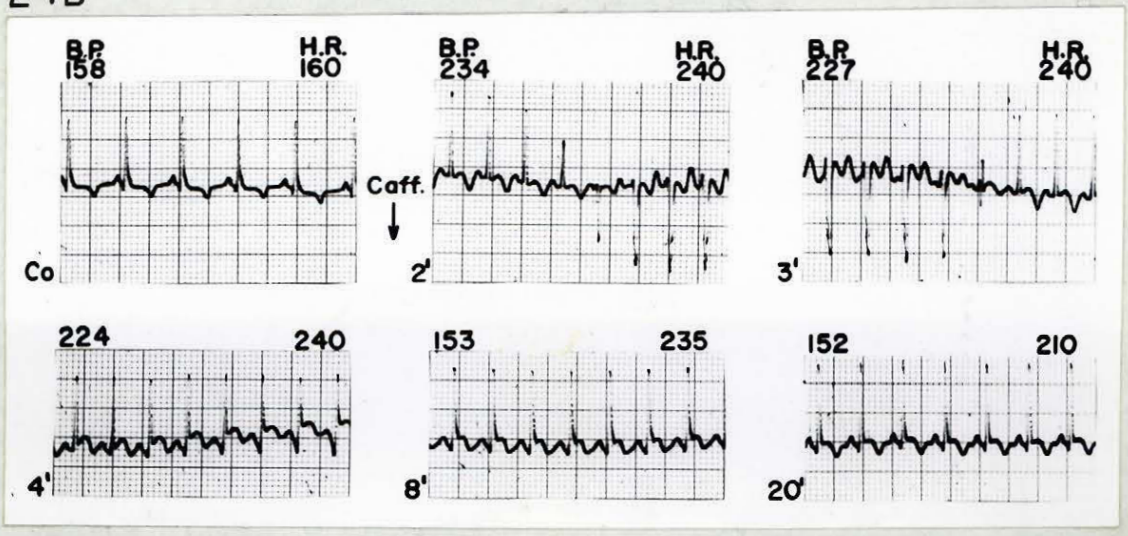


FIGURE 24A: Changes in mean B.P. and H.R. induced by i.vt. caff. (20 mg.) in the non-vagotomized cat (male, 2.8 kg.).
FIGURE 24B: ECG and associated B.P. and H.R. changes related to Figure 24A before (CO) and 2, 3, 4, 8 and 20 minutes after caff.

ranging from increases of 20 to 90 and was also significantly less ($P < 0.05$).

(iii) Chloralose and succinylcholine (See Table 9)

In this group (5 exp.) the increased peak values for the pressor responses observed varied from increases of 92 to 133 mm. Hg (mean, 117.2) and were significantly less than under pento. and gal. ($P < 0.01$). The mean cardioacceleration observed was 88.0/min., ranging from increases of 35 to 140, and was insignificantly different ($P > 0.7$).

(iv) Ether induction followed by gallamine (See Table 9)

In this group (4 exp.) the mean increased pressor responses was 85.0 mm. Hg ranging from 66 to 95, and was less than that under pento. with gal. ($P < 0.001$). The mean cardioacceleration was 50.7/min., ranging from increases of 23 to 100, but was not significantly different ($P > 0.05$).

In summary, (1) the pressor responses to a dose of 20 mg. of caffeine, injected intraventricularly were significantly less in animals under pentobarbitone with succinylcholine, as compared to those under pentobarbitone with gallamine; the cardioacceleration was, however, insignificantly different. (2) The pressor responses and cardioacceleration were both significantly less under chloralose with gallamine, as compared to those under pentobarbitone with gallamine. (3) The pressor responses under chloralose with succinylcholine were less marked than under

pentobarbitone with gallamine. (4) Similarly, the pressor response with ether induction followed by gallamine was less than that observed under pentobarbitone with gallamine, whereas the cardioacceleration was not significantly different in these two groups. (5) The electrocardiographic changes under all of the conditions employed were similar.

c) Comparative cardiovascular effects following intravenous injections of caffeine

In Table 10 are summarized the cardiovascular responses observed following intravenous injections of caff. (20 mg.), (i) in non-vagotomized cats, under pento. with gal. and (ii) in vagotomized cats, under chlor. with SCH.

(i) Non-vagotomized cats under pentobarbitone with gallamine

In this group (6 exp.), i.v. caff. (20 mg.) led to an immediate slight depressor response ranging from decreases of 5 to 12 mm. Hg, below the control levels and followed by a pressor response. The pressor response ensued within 5 to 10 min. and reached increased peak values of 4 to 12 mm. Hg (mean, 5.8) within 10 to 20 min. The degree of cardioacceleration varied from increases of 5 to 40 per min. (mean, 12.8). Both the pressor response and cardio-acceleration were significantly less than those observed with i.vt. administration of the same dose (20 mg.) of caff. ($P < 0.001$ and $P < 0.001$, respectively). No extra-systoles were observed in any of these exp. although there

TABLE 10

Cardiovascular changes induced by intravenous caffeine (20 mg.) in non-vagotomized and vagotomized cats (See Methods).

		B.P. mm. Hg		H.R./min.		ECG changes	
	N	Control	*Maximum Increase	Control	Maximum Increase	E.B.	ST-T
Pento. with gal. Non-vagotomy	6	102.5 ±12.6	5.8 ± 1.8	180.6 ±26.7	12.8 ± 5.7	0	+
Chlor. with SCH. Vagotomy	5	106.6 ±11.6	3.2 ± 0.7	238.8 ±14.6	3.2 ± 1.9	0	+

N = Number of experiments
Mean values of B.P. and H.R. with ± standard errors of the means are given
E.B. = Number of experiments where ventricular extrasystoles occurred.
ST-T = Comparative degrees of ST-T segments deviations.
* = Pressor response following initial depressor response.

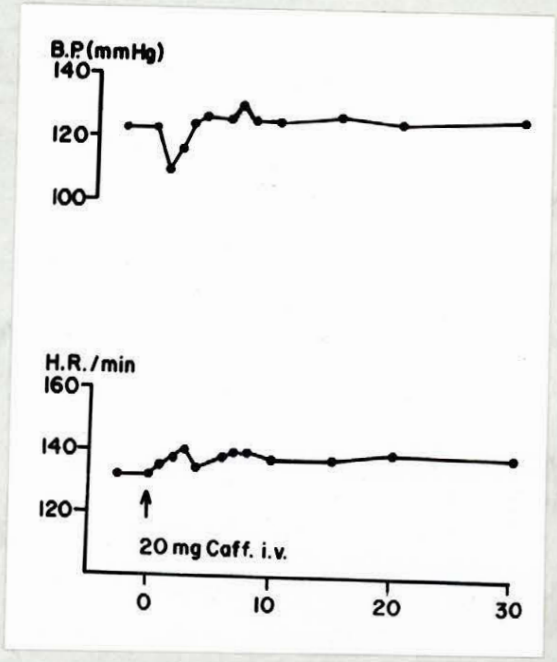
were minor T wave changes in 4 out of the 6 exp. Figures 25A and 25B show typical examples of these changes.

b) Vagotomized cats under chloralose with succinylcholine

In this group (5 exp.) intravenous injections of 20 mg. of caff. resulted in pressor responses preceded by slight depressor responses (5 to 10 mm. Hg below controls). The peak values of the pressor responses varied from increases of only 2 to 5 mm. Hg (mean, 3.2). The cardioacceleration also ranged from increases of only 2 to 10 per min. (mean, 3.2). The pressor responses ensued within 5 to 10 min. and reached peak values within 10 to 20 min. Both the pressor responses and cardioacceleration were significantly less compared with those seen with i.vt. injection of the same dose (20 mg.) of caff. ($P < 0.001$ and $P < 0.001$, respectively). There were no associated ECG changes observed in any of these experiments.

In summary, (1) the cardiovascular responses to a dose of 20 mg. of caffeine injected intravenously were significantly less than those observed following intraventricular injections of the same dose of caffeine in both non-vagotomized and vagotomized animals. (2) The pressor responses and cardioacceleration were markedly less with intravenous caffeine than with intraventricular caffeine. (3) Intravenous caffeine also led to an initial transient depressor response not observed with intraventricular caffeine. (4) In contrast to the striking electrocardiographic changes seen with intraventricular caffeine, following intravenous

25A



25B

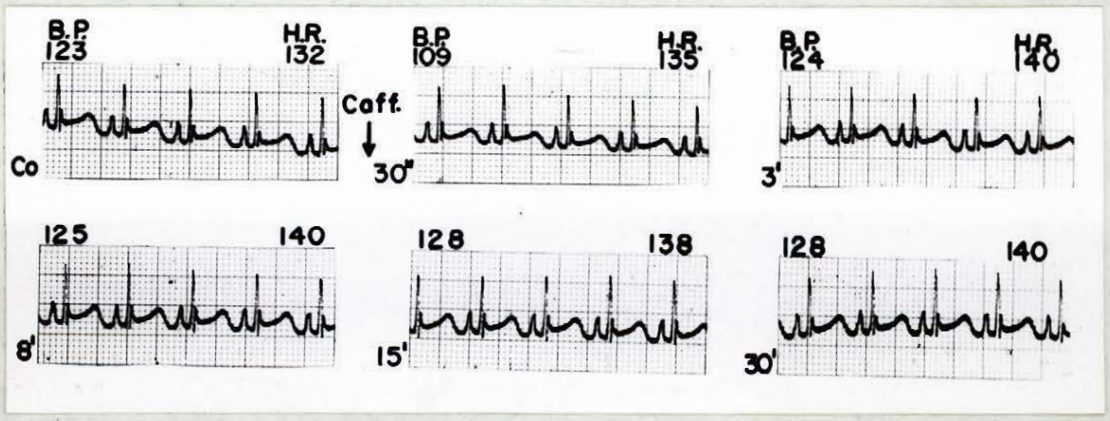


FIGURE 25A: Changes in mean B.P. and H.R. induced by i.vt. caff. (20 mg.) in the non-vagotomized cat (female, 2.7 kg.).
FIGURE 25B: ECG and associated B.P. and H.R. changes related to Figure 25A before (CO) and 30 seconds, 3, 8, 15 and 30 minutes after caff.

caffeine there were only minor ST-T changes.

2. Effects in Vagotomized Cats

In Table 11 are summarized the responses to i.vt. caff. (20 mg.), in vagotomized cats, under different experimental conditions. Some typical examples of these changes are shown in Figures 26A, 26B, 27A, 27B and 28A and 28B. In all of these experiments the electrocardiographic changes were similar.

i) Vagotomized cats under pentobarbitone with gallamine (See Table 11)

In this group (4 exp.) the mean pressor response was 97 mm.. Hg, occurring in 30 sec. to 2 min. and reaching increased peak values of 72 to 117 mm. Hg within 5 to 10 min. These were significantly ($P < 0.001$) less than were observed in the non-vagotomized animals. The degree of tachycardia varied from increases of 15 to 64 per min. (mean, 37) and was also significantly less than observed in non-vagotomized animals ($P < 0.01$). A typical example of these results is shown in Figures 26A and 26B.

ii) Vagotomized cats under pentobarbitone with succinylcholine (See Table 11)

In this group (4 exp.) the mean pressor response was 94.2 mm. Hg with increased peak values ranging from 49 to 121 mm. Hg. The cardioacceleration varied from increases of 20 to 60 per min. (mean, 41.2) over the controls.

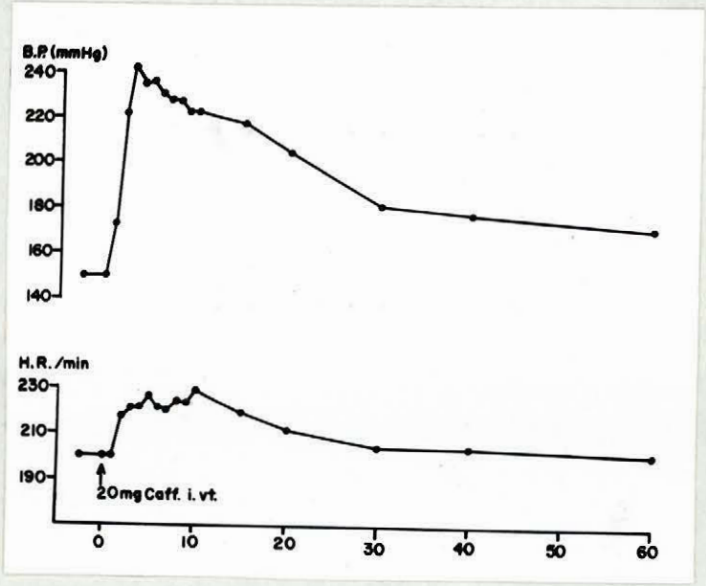
TABLE 11

Cardiovascular changes induced by intraventricular caffeine (20 mg.) in vagotomized cats under different anesthetic and curarizing agents (See Methods).

	N	B.P. mm. Hg		H.R./min.		ECG changes	
		Control	Maximum Increase	Control	Maximum Increase	E.B.	ST-T
Pento. & gal.	4	124.7 ±13.4	97.0 ±10.6	202.7 ±29.8	37.0 ±10.2	3	+++
Pento. & SCH	4	130.2 ± 9.1	94.2 ±18.8	240.0 ± 7.1	41.2 ± 9.0	2	+++
Chlor. & SCH	5	122.0 ± 9.2	122.8 ±19.4	214.6 ±10.8	41.8 ± 6.4	3	++

N = Number of experiments
Mean values of B.P. and H.R. with ± standard errors of the means are given.
E.B = Number of experiments where ventricular extrasystoles occurred.
ST-T = Comparative degrees of ST-T segments deviations.

26 A



26 B

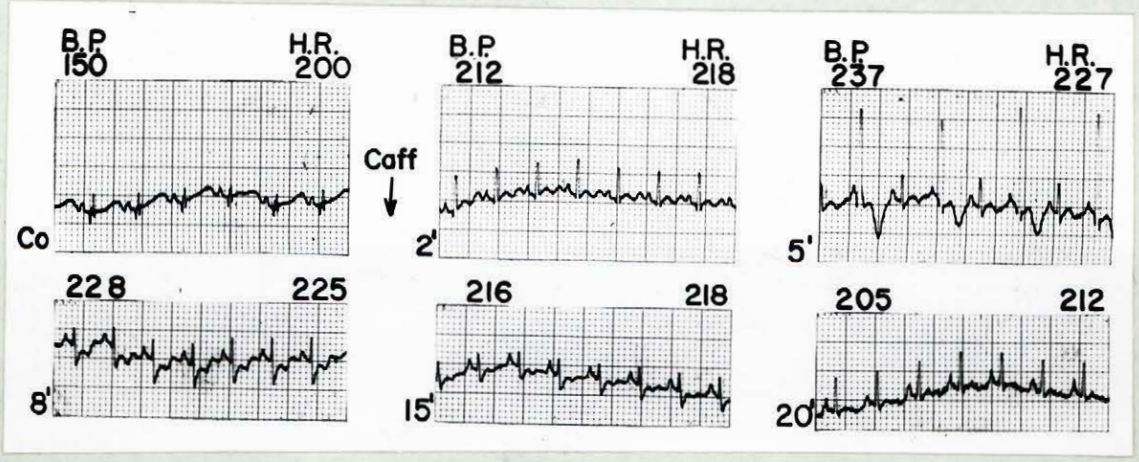


FIGURE 26A: Changes in mean B.P. and H.R. induced by i.vt. caff. (20 mg.) in the vagotomized cat (female, 3 kg.).
FIGURE 26B: ECG and associated B.P. and H.R. changes related to Figure 26A before (CO) and 2, 5, 8, 15 and 20 minutes after caff.

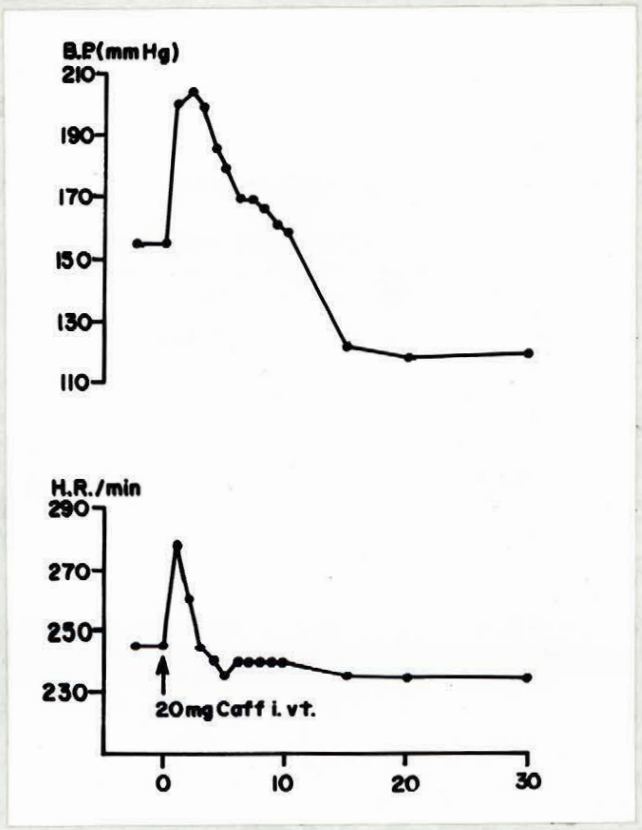
The pressor response was insignificantly different ($P > 0.3$) from that observed in non-vagotomized animals, but the cardioacceleration was significantly less ($P < 0.02$). A typical example of these results can be seen in Figures 27A and 27B.

(iii) Vagotomized cats under chloralose with succinylcholine (See Table 11)

In this group (5 exp.) the pressor responses varied from increases of 56 to 167 mm. Hg (mean, 122.8)and was not significantly different from those observed in the non-vagotomized animals ($P > 0.8$). The degree of tachycardia ranged from increases of 25 to 57 per min. (mean, 41.8) over the controls and was also insignificantly different ($P > 0.05$). Figures 28A and 28B show typical examples of these findings.

In summary, (1) the pressor responses and cardioacceleration to intraventricular caffeine (20 mg.) in vagotomized cats, under pentobarbitone and gallamine, were significantly less than those observed in non-vagotomized cats. (2) Under pentobarbitone and succinylcholine the pressor responses to caffeine in vagotomized cats were similar to those observed in the non-vagotomized animals under similar situation, but the degree of cardioacceleration was less. (3) Under chloralose with succinylcholine the pressor responses and cardioacceleration to caffeine in non-vagotomized and vagotomized cats were not significantly

27 A



27 B

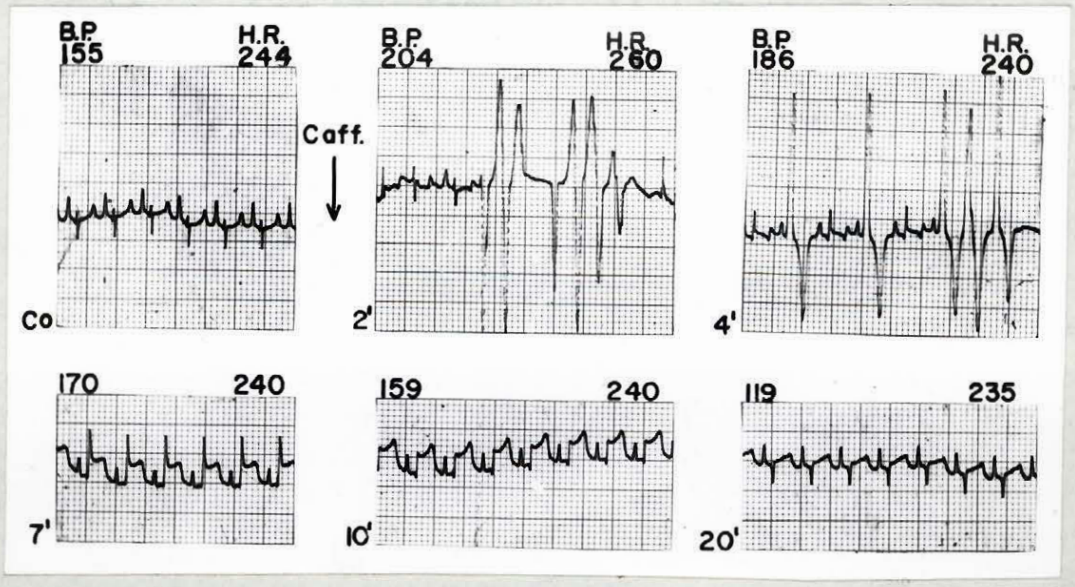
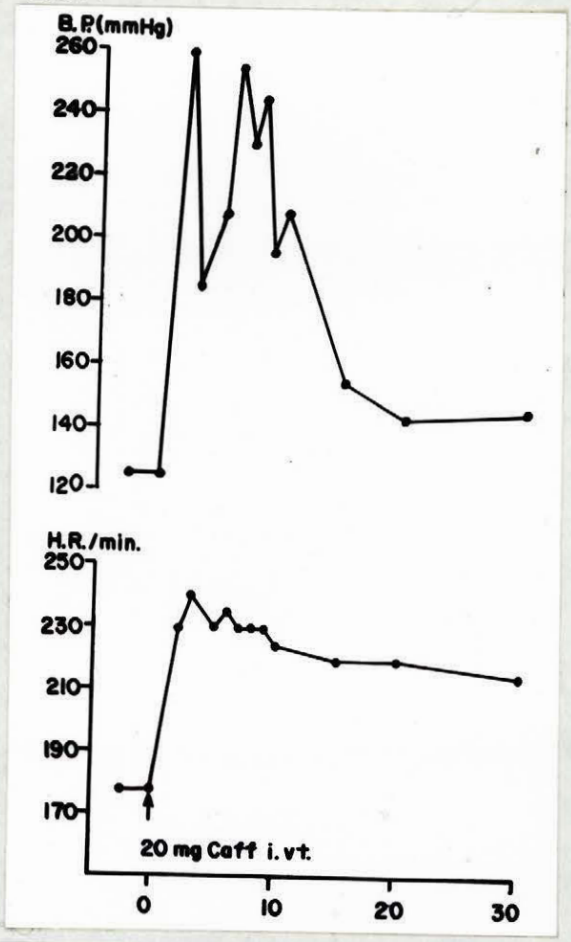


FIGURE 27A: Changes in mean B.P. and H.R. induced by i.vt. caff. (20 mg.) in the vagotomized cat (male, 2.8 kg.).
FIGURE 27B: ECG and associated B.P. and H.R. changes related to Figure 27A before (CO) and 2, 4, 7, 10 and 20 minutes after caff.

28A



28B

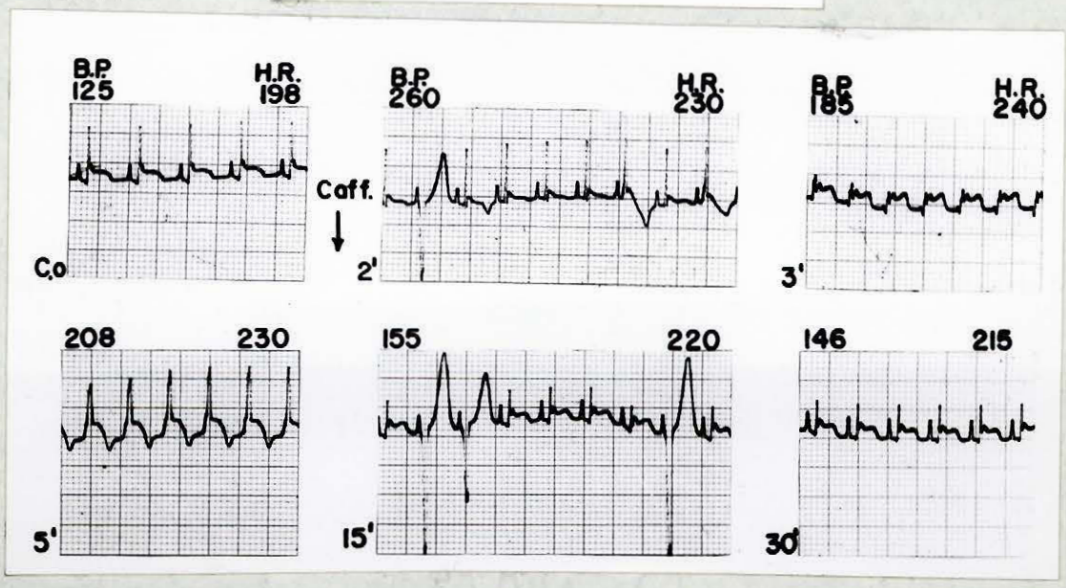


FIGURE 28A: Changes in mean B.P. and H.R. induced by i.vt. caff. (20 mg.) in the vagotomized cat (female, 3 kg.).
FIGURE 28B: ECG and associated B.P. and H.R. changes related to Figure 28A before (CO) and 2, 3, 5, 15 and 30 minutes after caff.

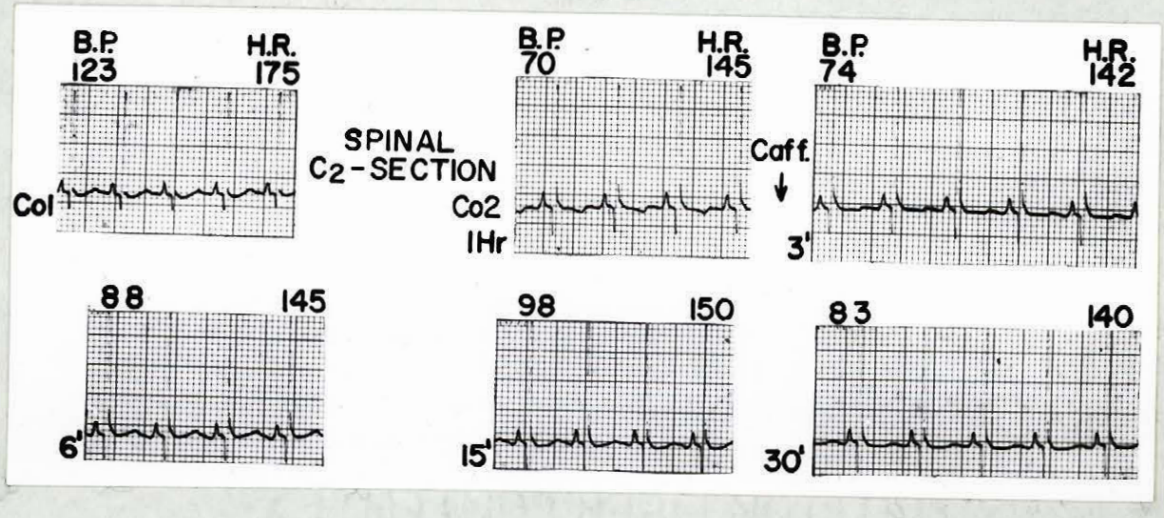
different. (4) Similar electrocardiographic patterns were observed following caffeine both in non-vagotomized and vagotomized animals.

3. Influence of Spinal Section in Non-vagotomized and Vagotomized Cats

In Table 12 (see below) are summarized the responses observed after i.vt. injections of caff., 1 hour following spinal sections at the second cervical vertebra (3 exp.) in non-vagotomized cats. Prior to the injection of caff. the blood pressure was reduced by 19 to 124 mm. Hg, the heart rate decreased by 30 to 128 per min., below the controls and the ECG showed only minor T wave changes. Following the i.vt. injection of caff. (20 mg.) there was only a slight pressor response, which varied from increases of 5 to 17 mm. Hg (mean, 13) and the degree of tachycardia ranged from increases of 3 to 6 per min. (mean, 5.3) above control levels. These changes were significantly reduced ($P < 0.001$ and $P < 0.001$, respectively), as compared to the responses of caff. in normal animals. The characteristic ECG changes observed following i.vt. caff. were completely absent. An example of the changes is also seen in Fig. 29A.

In the vagotomized animals, as shown in Table 13, (3 exp.) the pressor responses to i.vt. caff. following C_2 section were also considerably reduced, varying from increases of 5 to 24 mm. Hg (mean, 13.6), and the cardio-acceleration ranged from increases of 2 to 28 per min.

29A



29B

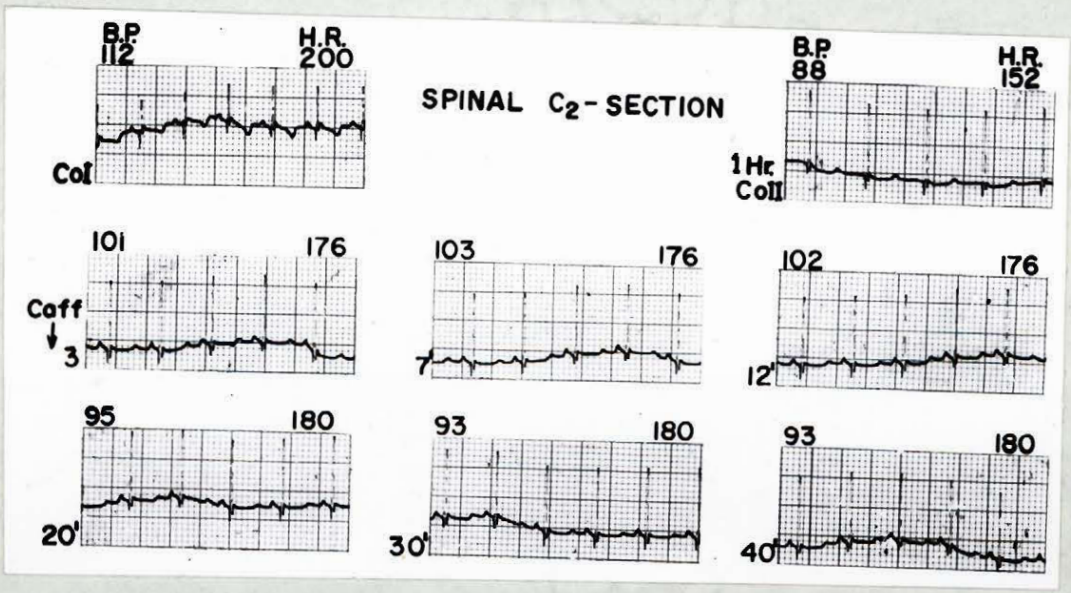


FIGURE 29A: Changes in mean B.P. H.R. and ECG in the non-vagotomized cat (female, 2.8 kg.), before spinal C_2 section (CO I), one hour later and before i.vt. caff. (CO 2) and 3, 6, 15 and 30 minutes after caff.

FIGURE 29B: Changes in mean B.P., H.R. and ECG in the vagotomized cat (female, 3 kg.), before spinal C_2 section (CO I), one hour later and before i.vt. caff. (CO II) and 3, 7, 12, 20, 30 and 40 minutes after caff.

(mean, 12). Pressor response and cardioacceleration were significantly reduced ($P < 0.01$ and $P < 0.05$, respectively). Again the characteristic ECG changes following i.vt. caff. were completely absent. An example of these results is shown in Fig. 29B.

In summary, in non-vagotomized and vagotomized cats, spinal C_2 section (1) completely prevented the electrocardiographic changes normally seen with intraventricular caffeine, and (2) the pressor and cardioacceleration were significantly attenuated.

PART II - Influence of Systemic Pretreatments on Cardiovascular Changes Induced by Intraventricular Injections of Caffeine

The general cardiovascular changes which were observed in the various groups of these experiments are summarized in Tables 12 and 13.

1. Effects of "Systemic Reserpinization"

In the reserpinized animals (Table 12), the pressor responses and cardioacceleration (3 exp.) following i.vt. caff. (20 mg.) were reduced. Pressor responses showed increases ranging from 71 to 87 mm. Hg (mean, 84) and the cardioacceleration varied from increases of 21 to 40 per min. (mean, 33). These responses were significantly attenuated as compared with those seen in non-vagotomized control animals ($P < 0.001$ and $P < 0.01$, respectively).

TABLE 12

Cardiovascular changes induced by intraventricular caffeine (20 mg.) in control and under various experimental conditions in non-vagotomized cats under pentobarbitone with gallamine.

		B.P. mm. Hg		H.R./min.		ECG changes	
Pretreatment	N	Control	Maximum Increase	Control	Maximum Increase	E.B.	ST-T
None	6	103.0 ± 5.5	149.1 ± 3.3	164.0 ±13.3	94.0 ±10.7	5	+++
Spinal C_2-Section	3	60.3 ± 8.6	13.0 ± 7.5	116.0 ±15.6	5.3 ± 1.5	0	-
'Systemic' Reserpinization	3	91.0 ± 1.6	84.6 ± 8.1	129.0 ±16.6	33.0 ± 6.3	0	++
'Systemic' Phenoxybenzamine	3	82.6 ± 8.7	*31.3 ±22.8	195.6 ± 6.2	19.0 ±13.5	0	+
'Systemic' Bretylium	3	160.0 ±49.8	20.3 ± 8.8	146.3 ± 3.6	11.0 ± 5.1	0	-
'Systemic' Cocaine	3	151.0 ±10.1	96.3 ±29.7	215.0 ± 5.0	35.0 ±25.0	2	+++
'Systemic' Iproniazid	4	115.2 ±12.4	131.0 ±10.2	178.0 ±29.8	19.0 ±2.6	0	+++

N = Number of experiments
Mean values of B.P. and H.R. with ± standard errors of the means are given.
E.B. = Number of experiments where extrasystoles occurred.
ST-T = Comparative degrees of ST-T segments deviations.
* = Depressor response.

TABLE 13

Cardiovascular changes induced by intraventricular caffeine (20 mg.) in control and under various experimental conditions in vagotomized cats, under chloralose with succinylcholine.

		B.P. mm. Hg		H.R./min.		ECG changes	
Pretreatment	N	Control	Maximum Increase	Control	Maximum Increase	E.B.	ST-T
None	5	122.0 ± 9.2	122.8 ±19.4	214.6 ±10.8	41.8 ± 6.4	4	+++
Spinal C_2-Section	3	90.3 ±13.0	13.6 ± 5.5	140.6 ± 5.9	12.8 ± 8.0	0	-
'Systemic' Reserpinization	3	65.6 ±16.3	24.0 ±22.5	166.6 ±14.5	12.6 ± 2.9	0	+
'Systemic' Phenoxybenzamine	3	81.0 ±18.0	* 24.0 ± 9.5	199.6 ± 0.1	25.6 ±11.9	0	+
'Systemic' Bretylium	3	94.0 ±10.9	4.6 ± 8.4	155.0 ±10.4	0.0	0	-
'Systemic' Cocaine	3	122.0 ±15.5	120.0 ± 7.2	171.0 ±41.6	47.3 ±16.9	2	++
'Systemic' Iproniazid	3	152.3 ±15.5	58.3 ±28.6	215.0 ±17.2	2.6 ± 1.2	0	+++

N = Number of experiments
Mean values of B.P. and H.R. with ± standard errors of the means are given.
E. B. = Number of experiments where ventricular extrasystoles occurred.
ST-T = Comparative degrees of ST-T segments deviations.
* = Depressor response.

No extrasystoles were observed in any instance, but in 2 exp. there were some ST-T alterations. Fig. 30A shows typical examples of the changes recorded in these experiments.

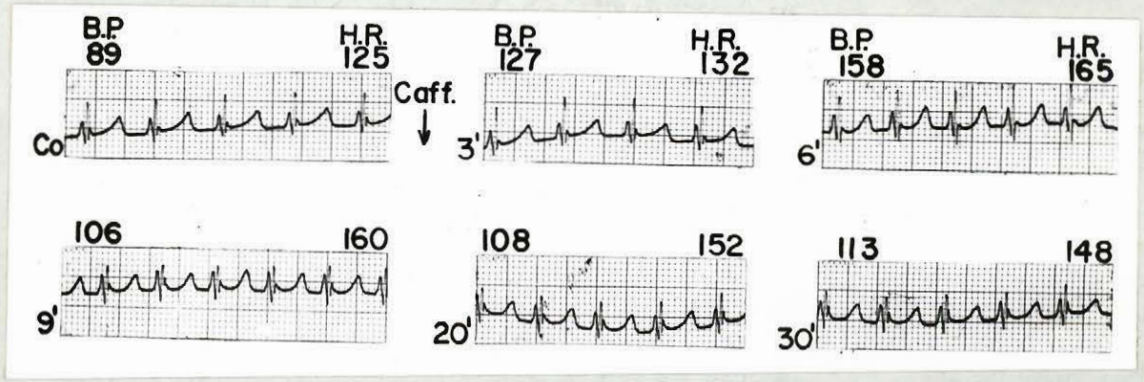
In vagotomized reserpinized (Table 13) cats (3 exp.) the observed changes in pressor responses ranged from increases of 3 to 69 mm. Hg (mean, 24) and the cardio-acceleration from increases of 8 to 18 per min. (mean, 12.6). These were significantly attentuated responses as compared with those of the non-reserpinized vagotomized animals ($P < 0.02$ and $P < 0.02$, respectively). The ECG pattern was also unchanged except for some minor T wave changes. A typical example is shown in Fig. 30B.

In summary, systemic reserpinization completely prevented the cardiac arrhythmias and significantly attenuated the other cardiovascular responses normally seen following intraventricular caffeine, in both non-vagotomized and vagotomized untreated (non-reserpinized) animals; some associated minor ST-T changes ensued in both groups.

2. Efffects of Phenoxybenzamine

In this group (Table 12) following PHE (4 mg./kg.) intravenously, the arterial pressure was reduced by 37 to 64 mm. Hg and the heart rate decreased by 30 to 33 per min. over the controls, but the ECG was of normal pattern. Adrenergic blockade was demonstrated in each experiment by the pressor reversal effect, resulting from an injection of adrenaline (2.5 ug./kg.) intravenously. One hour after

30A



30B

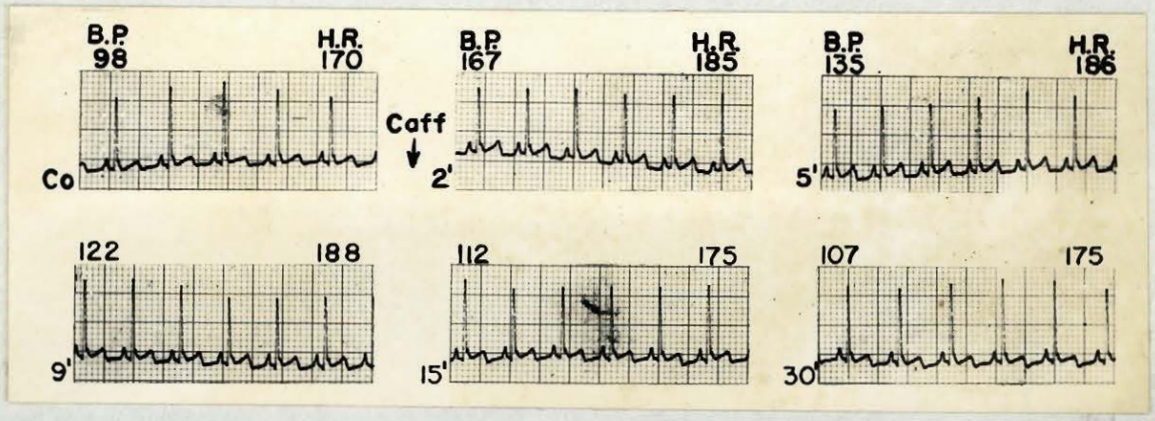


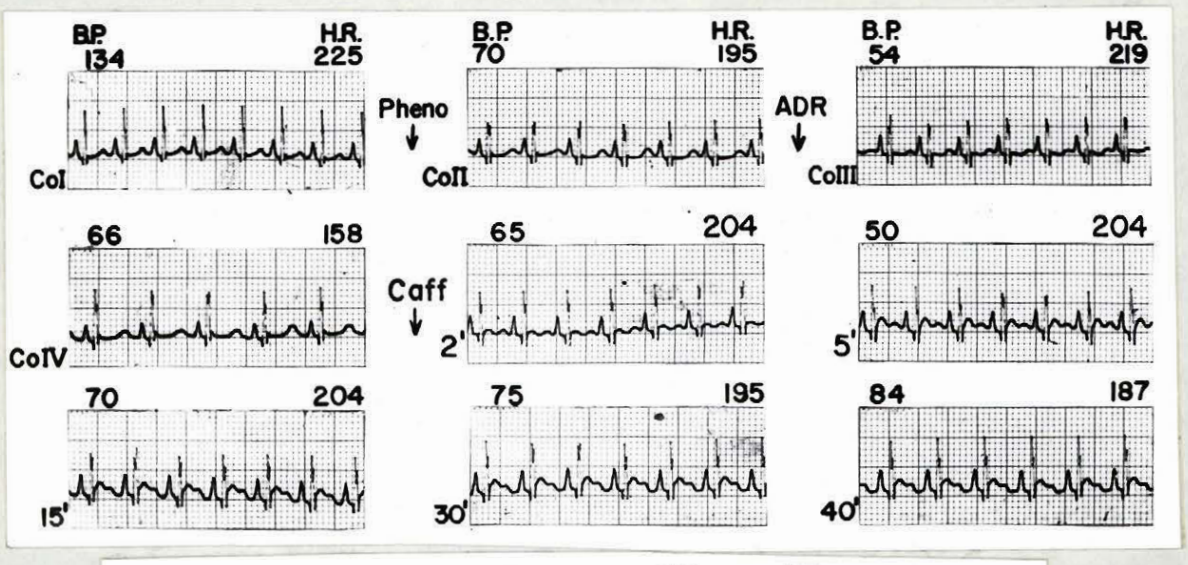
FIGURE 30A: Changes in mean B.P., H.R. and ECG in the "systemically reserpinized," non-vagotomized cat (male, 3 kg.), before (CO(and 3, 6, 9, 20 and 30 minutes after i.vt. caff.
FIGURE 30B: Changes in mean B.P., H.R. and ECG in the "systemically reserpinized," vagotomized cat (female, 2.8 kg.), before (CO) and 2, 5, 9, 15 and 30 minutes after i.vt. caff.

the PHE administration i.vt. caff. (20 mg.) (3 exp.) induced depressor responses ranging from 16 to 41 mm. Hg (mean, 31.3) in contrast to the usual pressor responses. The associated cardioacceleration varied from increases of 6 to 46 beats per min. (mean, 19), and was significantly reduced as compared to the response in the untreated control animals ($P < 0.05$). No extrasystoles were seen in any exp., although ST-T changes were observed in 2 out of 3 exp. Fig. 31A illustrates these changes.

In vagotomized cats (3 exp.), after PHE pretreatment (Table 13) i.vt. caff. (20 mg.) also induced depressor responses varying from 7 to 40 mm. Hg (mean, 24). The associated cardioacceleration ranged from increases of 12 to 40 beats per min. (mean, 25.6), which were insignificantly different from those seen in the responses to caff. alone ($P > 0.02$). Cardiac arrhythmias did not occur in any of these exp., but ST-T changes were present in 2 out of 3 exp. Fig. 31B illustrates these changes.

In summary, (1) following phenoxybenzamine pretreatment, intraventricular caffeine led to variable degrees of depressor responses in both vagotomized and non-vagotomized cats. (2) The degree of associated cardioacceleration was reduced significantly only in non-vagotomized animals. (3) Cardiac arrhythmias were completely prevented, although ST segment deviations were seen in some experiments in both vagotomized and non-vagotomized cats.

31A



31B

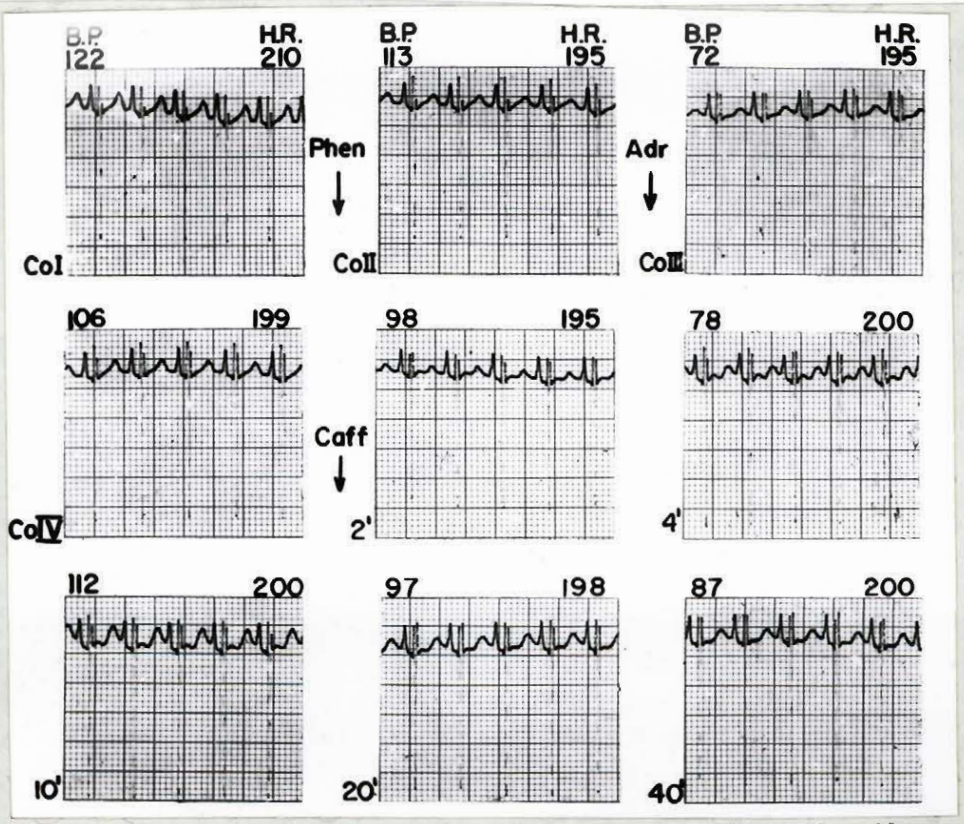


FIGURE 31A; Changes in mean B.P., H.R. and ECG in the systemic PHE-pretreated (4 mg./kg. i.v. 30 min. earlier), non-vagotomized cat (male, 2.8 kg.), before PHE (CO I), 30 minutes later and before ADR (CO II), 2 minutes after ADR (CO III), 1 hour after PHE and before caff. (CO IV) and 2, 5, 15, 30 and 40 minutes after i.vt. caff.

FIGURE 31B:Changes in mean B.P., H.R. and ECG in the systemic PHE-pretreated (4 mg./kg. i.v. 30 min. earlier), vagotomized cat (female, 2.6 kg.), before PHE (CO I), 30 minutes later and before ADR (CO II), 2 minutes after ADR (CO III), 1 hour after PHE and before caff. (CO IV), and 2, 4, 10, 20 and 40 mins. after i.vt. caff.

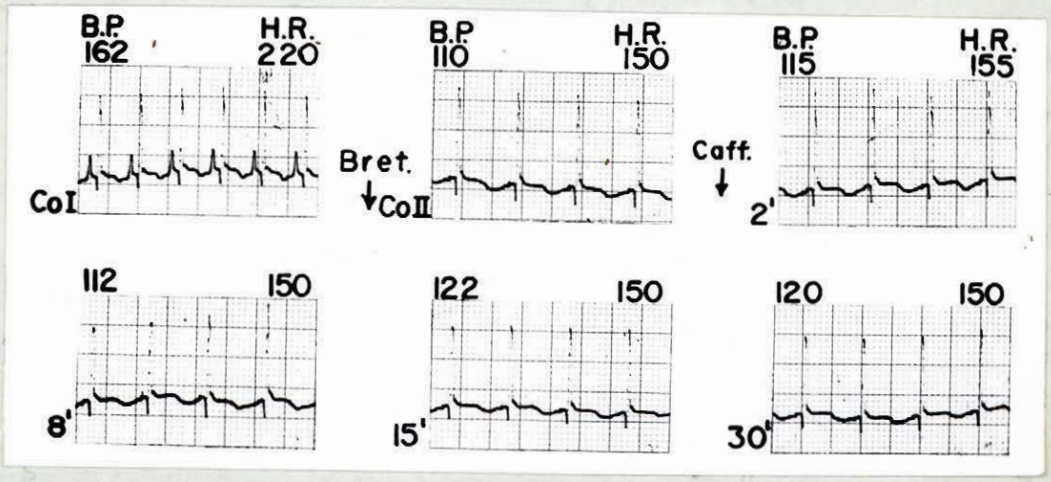
3. Effects of Bretylium

In this group (Table 12), 30 min. following intravenous injections of Bre (5 mg./kg.), the blood pressure and the heart rate were slightly reduced. Following subsequent i.vt. caff. (20 mg.) in 3 exp. the arterial pressure showed increases from 10 to 38 mm. Hg (mean, 20.3) within 5 to 8 min., normalizing within 30 min. The cardioacceleration ranged from increases of 4 to 21 per min. (mean, 11). Both of these changes were significantly reduced, as compared to untreated control animals ($P < 0.001$ and $P < 0.01$, respectively). No significant electrocardiographic changes were seen at any time in these experiments. An example of these responses can be seen in Fig. 32A.

In vagotomized cats (3 exp.), after Bre pretreatment (Table 13) i.vt. caff. led to no significant changes in blood pressure or heart rate. The ECG was also essentially unaltered throughout. Fig. 32B illustrates these changes.

In summary, (1) following intravenous bretylium pretreatment the pressor responses and cardioacceleration induced by intraventricular caffeine were significantly reduced in non-vagotomized cats and completely prevented in vagotomized animals. (2) All electrocardiographic changes normally seen after caffeine were completely prevented.

32A



32B

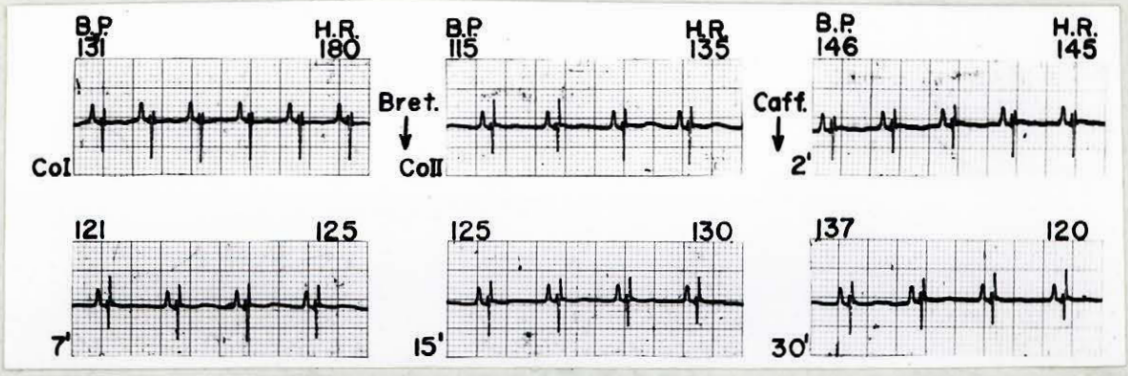


FIGURE 32A; Changes in mean B.P., H.R. and ECG in the Bre-pretreated (5 mg./kg. i.v. 30 min. earlier), non-vagotomized cat (female, 3 kg.), before Bre (COI), 30 minutes later and before i.vt. caff. (CO II) and 2, 8, 15 and 30 minutes after caff.

FIGURE 32B: Changes in mean B.P., H.R. and ECG in the Bre-pretreated (5 mg./kg. i.v. 30 min. earlier), vagotomized cat (female, 2.9 kg.), before Bre (CO I), 30 minutes later and before i.vt. caff. (CO II) and 2, 7, 15 and 30 minutes after caff.

4. Effects of Cocaine

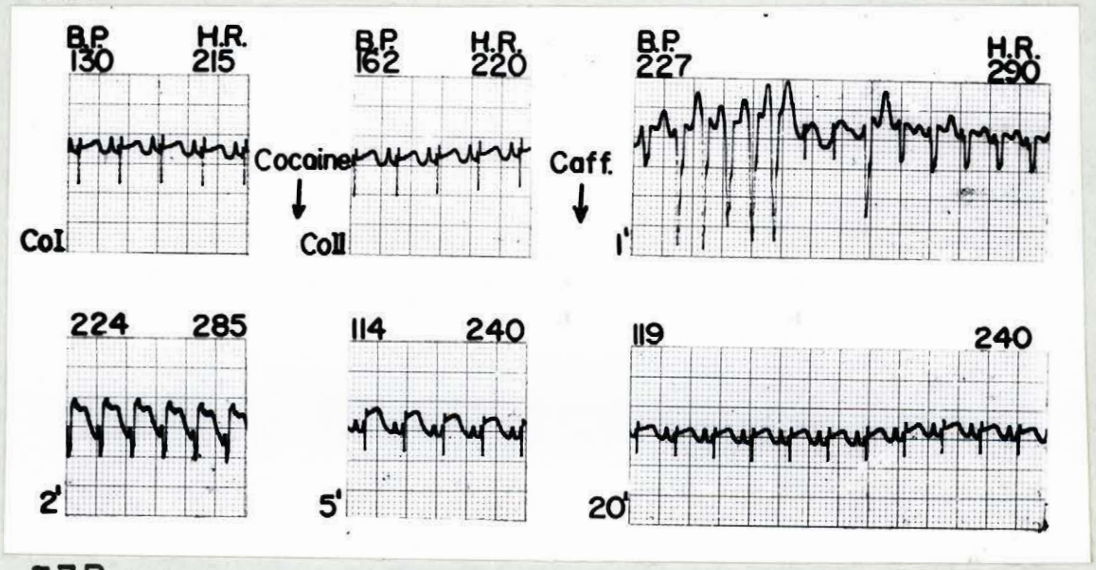
In both non-vagotomized (Table 12) and vagotomized (Table 13) cats, slow (5 to 10 min.) i.v. injections of cocaine (3 mg./kg.) were followed in 30 min. (3 exp. of each type) by i.vt. caff. (20 mg.). The resulting pressor responses and cardioacceleration were not significantly different from those observed in untreated control animals, ($P > 0.1$ and $P > 0.1$ (non-vagotomized) and $P > 0.9$ and $P > 0.7$ (vagotomized), respectively). Similar degrees of cardiac arrhythmias and ST-T alterations were also seen here, as in the untreated control animals. Figures 33A and 33B show typical examples of these changes.

In summary, cocaine pretreatment did not significantly influence the cardiovascular responses normally seen following intraventricular caffeine, both in non-vagotomized and vagotomized cats.

5. Effects of Iproniazid

In both non-vagotomized (Table 12) and vagotomized (Table 13) cats (4 exp. each) following pretreatment with iproniazid (Ipn) (100 mg./kg.) intraperitoneally 10 hours previous to i.vt. caff. (20 mg.), induced the usually observed pressor responses which were not significantly different from those seen in the untreated animals ($P > 0.05$ - non-vagotomized; $P > 0.1$ - vagotomized). The degrees of associated cardioacceleration were, however, significantly reduced ($P < 0.01$ - non-vagotomized and $P < 0.01$ - vagotomized). In both groups of experiments

33A



33B

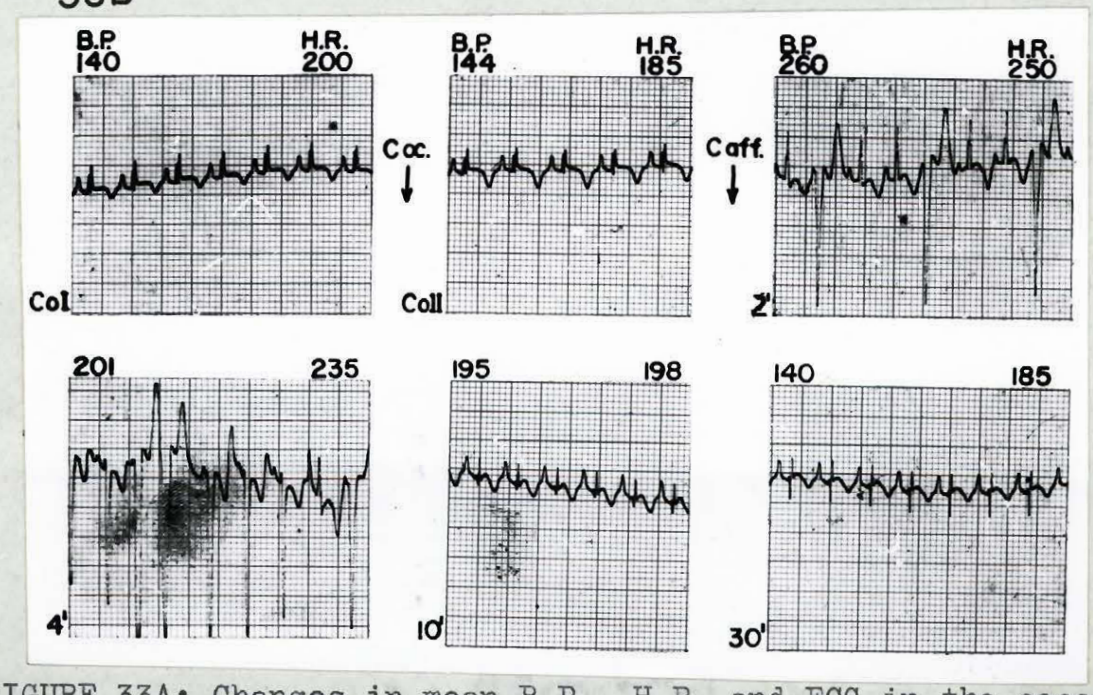


FIGURE 33A: Changes in mean B.P., H.R. and ECG in the cocaine-pretreated (3 mg./kg. i.v. 30 min. earlier), non-vagotomized cat (female, 3 kg.), before cocaine (CO I), 30 minutes later and before i.vt. caff. (CO II), and 1, 2, 5 and 20 minutes after caff.

FIGURE 33B: Changes in mean B.P., H.R. and ECG in the cocaine-pretreated (3 mg./kg. i.v. 30 min. earlier), vagotomized cat (male, 3 kg.), before cocaine (CO I), 30 minutes later and before i.vt. caff. (CO II), and 2, 4, 10 and 30 minutes after caff.

ventricular extrasystoles were absent, although ST-T alterations remained unaltered. Figures 34A and 34B show typical examples of these changes.

In summary, iproniazid pretreatment did not significantly influence the pressor response, but significantly attenuated the degree of cardioacceleration normally seen following intraventricular caffeine in both non-vagotomized and vagotomized animals. Extrasystoles were also completely prevented, although ST-T alterations remained unaltered in both groups.

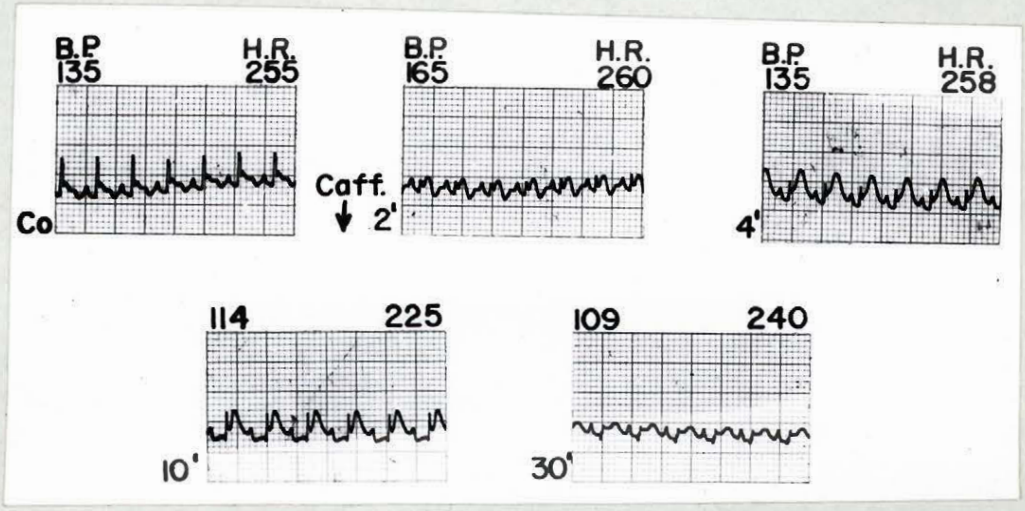
PART III - Influence of Central Pretreatments on the Cardiovascular Changes Induced by the Intraventricular Injections of Caffeine

All of the experiments referred to in this section were carried out on non-vagotomized cats, under pento. with SCH. In Table 14 are summarized the results obtained under the different conditions employed.

1. Effects of "Central Reserpinization" (See Table 14)

The general concepts and procedures employed in these exp. were similar to those observed above under lep. (Section A, Part III). As in the earlier-observed exp. i.v. injections of nicotine (1.5 mg./kg.) after i.vt. reserpine were also observed to produce the usual pressor responses and increases in heart rate, indicating that i.vt. reserpine did not cause significant loss of peripheral

34A



34B

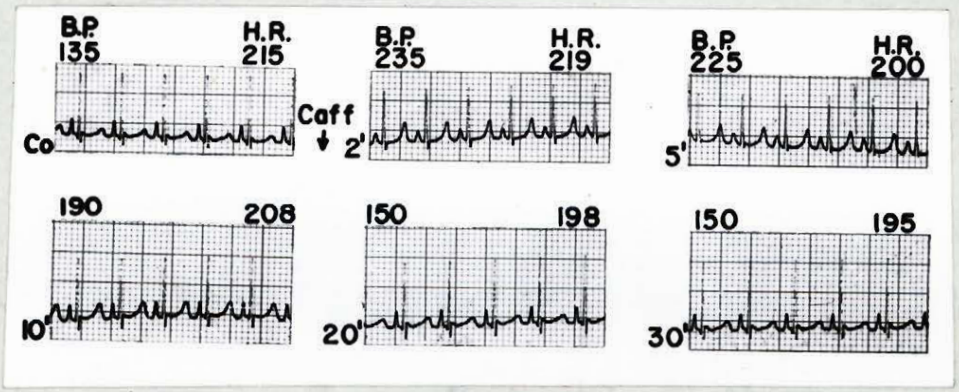


FIGURE 34A: Changes in mean B.P., H.R. and ECG in the Ipn-pretreated (100 mg./kg. i.v. 10 hrs. earlier), non-vagotomized cat (female, 3 kg.), before (CO) and 2, 4, 10 and 30 minutes after i.vt. caff.

FIGURE 34B: Changes in mean B.P., H.R. and ECG in the Ipn-pretreated (100 mg./kg. i.v. 10 hrs. earlier), vagotomized cat (male, 3 kg.), before (CO) and 2, 5, 10, 20 and 30 minutes after i.vt. caff.

TABLE 14

Comparative cardiovascular changes induced by intraventricular caffeine (20 mg.) alone, and by similar injections following intraventricular pretreatment with various drugs in cats anesthetized with pentobarbitone and curarized with succinylcholine (See Text).

Intraventricular Pretreatment	N	B.P. mm. Hg		H.R./min.		ECG changes	
		Control	Maximum Increase	Control	Maximum Increase	E.B.	ST-T
None	6	137.6 ± 7.5	112.1 ± 7.5	174.3 ±10.4	96.5 ±13.1	5	+++
Reserpine (3 hours)	3	134.0 ±10.7	125.0 ±13.9	180.6 ±11.5	95.0 ±21.3	3	+++
Reserpine (6 hours)	3	99.3 ± 7.6	137.6 ± 4.6	153.3 ±31.8	91.6 ± 7.2	3	++
Reserpine (18 hours)	4	79.7 ± 5.6	124.5 ±14.7	104.2 ±17.8	88.5 ± 7.5	2	+++
Atropine	4	143.2 ± 4.5	97.5 ±19.8	211.2 ± 8.2	37.5 ± 7.4	3	+++
Ethybenztropine	4	120.7 ±16.2	75.0 ±23.0	218.7 ±29.6	36.2 ± 9.6	3	++
Hexamethonium	4	127.7 ±12.7	105.5 ± 2.3	227.5 ±16.8	23.7 ± 3.1	3	+++
Mecamylamine	4	114.2 ±20.8	83.5 ±15.3	205.0 ±21.1	26.2 ± 2.3	3	++
Hemicholinium	4	120.7 ±16.2	75.0 ±23.0	218.7 ±29.6	36.2 ± 9.6	3	+++

N = Number of experiments
Mean values of B.P. and H.R. with ± standard errors of the means are given.
E.B. = Number of experiments where ventricular extrasystoles occurred.
ST-T = Comparative degrees of ST-T segments deviations.

catecholamines. The responses to caff. have also been tested 3, 6 and 18 hours after i.vt. reserpine, as indicated below:

a) Three hours after reserpine

In this group (3 exp.) following i.vt. caff. (20 mg.) increases in arterial pressure, ranging from 98 to 143 mm. Hg (mean, 125.0) ensued; tachycardia ranging from increases of 58 to 132 per min. (mean, 95) were also recorded. These were not significantly different from those seen in control experiments ($P > 0.3$ and $P > 0.9$). As shown in Fig. 14B, the ECG changes observed were similar to those usually recorded following i.vt. caff. alone.

b) Six hours after reserpine

In this group (3 exp.) i.vt. caff. (20 mg.) again produced pressor responses showing increases of 129 to 145 mm. Hg (mean, 137.6) and tachycardia with increases of 80 to 105 per min. (mean, 91.6). These were also insignificantly different from those of untreated animals ($P > 0.05$ and $P > 0.8$, respectively). The ECG changes were similar to those observed in the untreated controls. Fig. 15B shows a typical example.

c) Eighteen hours after reserpine

In this group (4 exp.) again it was observed that after local reserpinization the cardiovascular changes induced by i.vt. caff. (20 mg.) were rather similar to those

seen in the untreated controls. The pressor responses varied from increases of 95 to 161 mm. Hg (mean, 124.5) and the tachycardia ranged from increases of 81 to 110 per min. (mean, 88.5). These changes were not significantly different from those observed with caff. alone ($P > 0.4$ and $P > 0.6$, respectively). Fig. 16B shows an example of these changes. The ECG alterations were also similar to those seen in the untreated animals, but the intensity of ECG changes were somewhat less in these animals.

In summary, intraventricular reserpine pretreatment in non-vagotomized cats neither attenuated the pressor responses and cardioacceleration, nor prevented the occurrence of ectopic beats, following intraventricular caffeine (20 mg.), but the associated electrocardiographic changes appeared to be less marked in the reserpinized animals.

2. Effects of Intraventricular atropine and ethybenztropine

(See Table 14)

Following i.vt. injections of either atropine or ethyb. (0,1 mg. doses - 4 exp. each) there was no evidence of peripheral cholinergic blockade, as judged by the observed depressor responses to i.v. acetylcholine (5 µg./kg. - 3 exp. each). However, i.vt. caff. (20 mg.) still induced pressor responses which were not significantly different from those seen with caff. alone in both groups of experiments ($P > 0.4$, atropine; $P > 0.1$, ethyb.). The associated tachycardia was, however, significantly attenuated ($P < 0.02$,

atropine; $P < 0.02$, ethyb.). Again ECG changes observed were similar to those seen in untreated control animals. Figures 17B and 18B illustrate typical examples of these changes.

In summary, intraventricular pretreatment with either atropine or ethybenztropine in non-vagotomized cats did not attenuate the cardiovascular responses normally seen following intraventricular caffeine (20 mg.), but the degree of cardioacceleration was significantly reduced.

3. Effects of Intraventricular Hexamethonium and Mecamylamine

(See Table 14)

After i.vt. pretreatment with either of these ganglionic-blocking agents, hexa. (0.1 mg.) or meca. (0.1 mg.) following i.vt. caff. (20 mg.) in 4 exp. of each type, there occurred the usual pressor responses, which were not significantly different from those seen in untreated controls ($P > 0.5$, hexa; $P > 0.1$, meca.). The degrees of cardioacceleration were, however, significantly reduced ($P < 0.01$, hexa.; $P < 0.01$, meca.). The ECG changes were also similar to those usually observed following i.vt. caff. in untreated controls as illustrated in Figures 19B and 20B.

In summary, pretreatment with intraventricular injections of either hexamethonium or mecamylamine in non-vagotomized animals did not significantly alter the blood pressure and electrocardiographic changes induced by intraventricular caffeine, but the degree of cardioacceleration was significantly reduced.

4. Effects of Intraventricular Hemicholinium (HC-3)

(See Table 14)

As stated earlier, evidence exists that HC-3 does not significantly block the transmission across the peripheral autonomic ganglia (Birks and MacIntosh, 1961). In these experiments following i.vt. HC-3 (0.5 mg.), 1 to one and a half hours later it was observed (4 exp.) that i.vt. caff. (20 mg.) injected induced pressor responses which were not significantly different from those observed in untreated control animals ($P > 0.8$). The associated cardioacceleration, however, was significantly reduced ($P < 0.05$). The ECG changes were also similar to those seen in untreated control animals. Fig. 21B shows a typical example of these findings.

In summary, pretreatment with intraventricular HC-3 in non-vagotomized animals did not significantly alter the subsequent blood pressure and electrocardiographic changes induced by intraventricular caffeine, but the cardioacceleration was significantly reduced.

V. GENERAL DISCUSSION

1. Comparative Cardiovascular Effects Following Intraventricular Injections of Leptazol and Caffeine

The above-described results show clearly that following i.vt. administrations of lep. or caff. striking cardiovascular effects are discernible. These confirm the earlier observations of several different investigators (See Literature Survey).

In general, the intensity of the cardiovascular changes are dose-dependent and the arterial pressure increases, following injections of doses of 10 mg. of lep. in non-vagotomized cats under pento. and gal., were found to be significantly less than those following doses of 85 mg. However, the average pressor response to doses of 30 mg. was less than that to 85 mg., although this difference was not statistically significant. On the other hand, the degrees of cardioacceleration resulting from doses of 10 mg. and 30 mg. were not significantly different, from that resulting from doses of 85 mg.

In regard to the occurrence of arrhythmias, doses of 10 and 30 mg. both led to similar occasional isolated ventricular extrasystoles, but these occurred most frequently with doses of 85 mg. With the latter doses

there were also runs of ventricular tachycardia and ectopic beats of multifocal origin. In regard to changes in the ST-T segment, these were progressively increased with increasing doses.

As already pointed out, the pressor responses which were observed following doses of 30 mg. and 85 mg. were not significantly different. It is also noteworthy that there was no significant difference in the degrees of cardio-acceleration recorded at the different dose levels. However, the degree of arrhythmias was strikingly greater with the higher dose. The significance of these quantitative cardiovascular differences is not clear. It is conceivable, however, that the marked increase in the occurrence of arrhythmias with the highest dose (85 mg.) might be a factor in impairing the cardiac output (due to the arrhythmias), and hence preventing further significant increase in the pressor responses. The importance of reflex vagal control of the heart as a factor in the observed heart rate changes cannot be assessed from these experiments. It is, however, clear that in the evaluation of the cardio-vascular responses in these experiments, these interrelated changes must be considered.

In regard to the cardiovascular changes observed following i.vt. injections of caffeine under similar conditions to those described above, these were clearly dose-dependent. The pressor responses and the degrees of cardioacceleration resulting from doses of 5 and 10 mg.

were both significantly less than those following doses of 20 mg. The incidence of arrhythmias and the degree of ST-T segment deviations were also both less marked with the smaller dose (5 to 10 mg.) than with the highest dose (20 mg.).

The cardiac arrhythmias and ST-T changes following doses of 85 mg. of lep. and those following 20 mg. of caff. under these conditions were rather similar. However, both the heart rate and blood pressure increases following caff. were clearly more marked than with lep. It would, therefore, appear that dose for dose caff. induces more marked responses. These differences between the effects of caff. and lep. might be due to differences in their actions on reflexly controlled homeostatic mechanisms involving the central nervous system. It has previously been reported that the initial depressor response observed when lep. is injected systemically is abolished by cutting the "buffer nerves" (Gellhorn et al, 1939). On the other hand, there is no evidence that a similar effect occurs with caff. Indeed, it has been reported by various workers that caff. can also induce an initial fall in blood pressure following systemic administration, but this is not abolished by vagotomy or destruction of the medulla. A central depressant action of caff. on the vagus centre has also been postulated (See Literature Survey).

(a) Effects of Intravenous Injections

It should be emphasized that following intravenous injections of lep. (85 mg.) or caff. (20 mg.), the general cardiovascular responses observed were always less marked than those recorded following i.vt. injections of the same doses of the drugs. Unlike the responses observed after i.vt. injections, following i.v. injections the pressor responses were always delayed and preceded by a slight depressor response in both non-vagotomized and vagotomized cats. Moreover, cardiac arrhythmias were never seen with i.v. administrations of either lep. or caff. However, some minor ST-T changes ensued. These might possibly be due to some absorption of the drug into the central nervous system.

It is clear from the above that the marked pressor responses and associated arrhythmias induced by i.vt. injections of lep. and caff. are undoubtedly due to a central action of the drugs rather than to any direct peripheral effects following systemic absorption. These findings confirm earlier observations of Eichler et al, (1926); Stross (1926); Morin et al (1957) and Gatgouris et al (1960); Covino et al (1962) and Bircher et al (1963). For details see Literature Survey.

(b) Effects of Different Anesthetics and Curarizing Agents

In the non-vagotomized animals, the results presented above showed that there were no significant differences in the cardiovascular responses observed following i.vt. injections of doses of 85 mg. of lep. under different anesthetics and curarizing agents. It is, therefore, apparent that the different anesthetics and curarizing agents employed in these experiments do not exert any significant influence upon the cardiovascular responses induced by i.vt. lep.

On the other hand, following i.vt. caff. (20 mg.), it was observed that under chloralose both the pressor responses and cardioacceleration were significantly less than under pento. Under initial ether followed by gal. the pressor responses were also still less than under pento., but the degree of cardioacceleration was not changed. Under SCH the pressor responses were less marked than under gal., but the degree of cardioacceleration was not significantly different. It is concluded, therefore, that the pressor responses observed with caffeine were more marked with the use of gal. than with the use of SCH. This is presumably due to the atropine-like action of gal. on the heart (See Methods). Moreover, the pressor responses following caff. were also more marked under pento. with gal. than under chlor. with SCH, and it is, therefore, possible that this might be due to the atropine-like action of gal. rather than the

anesthetic agent.

It should also be emphasized that there was no significant difference in the pressor responses to lep. under gal. and under SCH. As already pointed out lep. itself can interfere with reflexly-controlled homeostatic mechanisms and this might, therefore, explain these observations.

(c) Effects of Vagotomy

Bilateral cervical vagotomy did not appear to significantly alter the cardiovascular responses induced by i.vt. injections of lep. (85 mg.) in cats under the different experimental conditions employed; although the intensity of the occurrence of the cardiac arrhythmias was less marked in some experiments in vagotomized cats than in non-vagotomized animals. It would appear, therefore, that the centrally-mediated cardiovascular responses to lep. are not dependent to any significant degree on vagal pathways. These findings on anesthetized cats are not in agreement with those of Bircher (1963), who injected lep. (5 mg./kg.) into the fourth ventricle of unanesthetized dogs. He observed that following either double vagotomy alone or by systemic administration of the central anticholinergic drug, ethybenztropine (0.1 to 0.2 mg./kg.) these cardiac arrhythmias could be converted to supraventricular (possibly sinus) tachycardias in approximately 50 percent of his experiments. He also found that the cardiac arrhythmias could be completely prevented or converted to sinus rhythm in all cases by

combining the above procedures with either chronic sympathectomy or low cervical transection or systemic administration of Hydergine (0.1 to 0.3 mg./kg.). They concluded, therefore, that these responses were mediated mainly by vagal and to a lesser extent by sympathetic pathways. The differences between our findings and those of Bircher et al might be due to a species difference. However, it is also possible that different central mechanisms are affected following an injection of lep. into the fourth ventricle than are affected following lateral ventricular administration. It should also be emphasized that the mere observation that a central anticholinergic agent reduces or blocks the effects from an i.vt. injection of lep., does not necessarily mean that the central responses to lep. are mediated by vagal pathways, nor does it necessarily mean that a central cholinergic mechanism is involved in exciting the central parasympathetic representations. On the other hand, it may be equally true that a central cholinergic mechanism might be involved in stimulating the central sympathetic representations similar to that existing in peripheral sympathetic ganglia. Hence, it is conceivable that a central anticholinergic agent, as used by Bircher et al, might also block the central sympathetic stimulation.

Both the pressor responses and cardioacceleration following i.vt. injections of caff. (20 mg.) were less marked after vagotomy in cats under pento. with gal.

but were not significantly altered under chlor. with SCH. This difference in responses was not observed in animals under pento. with SCH or chlor. with SCH, and therefore, appears to be due to the atropine-like action of gal. The control heart rates are, however, in general, higher in the vagotomized animals than in the non-vagotomized. This might also, in part, account for the reduced tachycardia observed in the vagotomized animals. The arrhythmias following caff. were also not significantly different in the vagotomized animals. It may be concluded, therefore, that peripheral vagal pathways are not directly involved in the centrally-mediated cardiovascular responses to caffeine.

(d) Effects of Spinal Section

After spinal C_2 section, as already pointed out, the pressor responses following i.vt. injections of both lep. and caff. were significantly attenuated and all associated ECG changes were completely prevented in both non-vagotomized and vagotomized cats. The cardioacceleration following caff. was also significantly reduced in vagotomized cats, although cardioacceleration following lep. in the non-vagotomized cats was not significantly different from that seen in control (non-spinal) animals.

The mechanism of the residual cardioacceleration observed following lep. in spinal C_2 sectioned non-vagotomized cats is not clear. It might, however, be due to release of some hormonal substance. Further experiments along these lines are, therefore, necessary before final conclusions on this question can be drawn.

The above data, however, indicate that the vagal pathways are not directly involved in the cardiovascular responses to lep. and caff., since in the spinal C_2 section non-vagotomized animals there was no bradycardia observed.

The spinal cord is the only connecting link between the central and peripheral sympathetic representations. Therefore, when the efferent sympathetic pathways are excluded by spinal C_2 section, this would prevent or attenuate the cardiovascular response to lep. and caff., if these drugs exert their actions by stimulating central sympathetic mechanisms. Indeed, the experiments showed that spinal C_2 section significantly reduced or prevented the cardiovascular responses normally seen with lep. and caff. The findings confirm (1) that the observed cardiovascular responses to these drugs are of central origin and would rather suggest (2) that they might involve stimulation of central sympathetic mechanisms.

In view of the high doses of lep. (85 mg.) and caff. (20 mg.) employed in these experiments, the question arises whether or not the observed cardiovascular responses involve normal physiological changes. It is, however, evident that following i.vt. injections of these agents, striking and

intense arrhythmias and ischemic-like ST-T changes can be consistently induced in cats under these conditions. Irrespective of the basic central nervous system mechanism involved, these experiments would appear to offer a new experimental approach for the production of cardiac arrhythmias and ischemic changes without involving complicated direct changes in the heart and blood vessels. The procedure might, therefore, be useful in studying problems concerned with cardiac arrhythmias and ischemic changes.

2. Influence of Drug Pretreatments on the Cardiovascular Responses to Intraventricular Leptazol and Caffeine

The results presented above show that the cardiovascular changes induced by i.vt. lep. and caff. can be strikingly influenced by systemic pretreatment of the animals with various pharmacological agents which are known to interfere with or to block peripheral adrenergic mechanisms (reserpine, phenoxybenzamine, bretylium). The observed cardiovascular changes, following i.vt. lep. and caff., were also variably influenced by agents, which have been shown to interfere with catecholamine metabolism and even to potentiate adrenergic responses under certain conditions (cocaine and iproniazid).

While it is well recognized that following systemic administration, these drugs may also be absorbed into the central nervous system and could possibly, thereby,

influence central nervous system responses to lep. and caff., there is no clear evidence that this is involved in these studies. As indicated earlier in the case of reserpine with i.vt. injection (central reserpinization), there was no demonstrable influence upon these cardiovascular responses to i.vt. lep. and caff. In the case of the other agents, it is conceivable that even if they were absorbed to some degree into the central nervous system (cocaine and iproniazid) there is no indication that they exert any significant influence upon central adrenergic mechanisms under these conditions. The effects of i.vt. (central) injections of cocaine and iproniazid were not investigated. In any event, none of these agents when injected systemically have been shown to affect brain catecholamines to the same degree as systemic reserpine. Moreover, it is well-established that both phenoxybenzamine and bretylium exert their effects primarily upon peripheral adrenergic mechanisms, and hence were not tested by i.vt. (central) injections.

(a) Influence of Systemic and Central Reserpinization

It has been shown that reserpine is capable of depleting catecholamines and 5-HT from peripheral tissues as well as from brain tissues (Carlsson et al, 1956, 1958; Holzbauer et al, 1956 and Paasonen et al, 1956).

Comparing the influences of 'systemic' and 'central' reserpinization, it has been shown that following systemic

reserpinization the cardiovascular changes induced by i.vt. lep. and caff. are markedly reduced. On the other hand, central reserpinization did not significantly alter these responses. In connection with these findings it is also noteworthy that the degrees of cardioacceleration following i.vt. lep. after systemic reserpinization were not significantly different from that observed in the normal (non-reserpinized) animals, but this was not so following i.vt. caff. These differences in responses might be due to some basic difference in the actions of lep. and caff. in affecting centrally-mediated mechanisms, controlling heart rate or to differences in the degrees of reserpinization in the two groups of experiments. It has previously been shown that the degree of increased cardioacceleration produced by caff. (20 mg.) was more marked than that produced by lep. (85 mg.), following i.vt. injections and also that lep. appears to exert some action on reflexly-controlled homeostatic mechanisms. To what extent differences in the degrees of reserpinization were involved in these experiments could not be assessed, although it is well-known that such differences can occur. In regard to central reserpinization, the experiments would indicate that "central reserpinization" does not produce peripheral depletion of catecholamines to any significant degree. Although reserpine is capable of depleting noradrenaline, adrenaline, dopamine and 5-hydroxytryptamine in the brain, the rate of depletion and restoration to normal levels of

these different substances might vary according to the time interval after reserpine administration. Indeed, Sourkes et al (1961) employing Dl-AMDP (α-methyl-3:4 dihydroxyphenylalanine) as the depleting agent showed that the dopamine level decreases in the brain with 3 hours after Dl-AMDP administration, but returned to the normal level in 6 hours. Noradrenaline, however, remains low for 72 hours in the brain. Considering these differences in depletion and restoration of these brain amines after reserpine, the responses to lep. and caff. were studied 18, 6 and 3 hours after "central reserpinization."

The experiments described above show clearly that central reserpinization neither prevented the occurrence of ECG changes, nor attenuated the pressor responses and cardio-acceleration normally seen following lep. and caff., under these conditions.

As in the experiment using systemic reserpinization, as pointed out earlier, the cardioacceleration induced by lep. following central reserpinization was increased in some experiments (See Table 7). However, the intensity of the associated ECG changes appeared to be less marked in some experiments.

From the above observations, it is clear that changes in brain catecholamines (noradrenaline, adrenaline and dopamine) or 5-HT do not appear to be important in the centrally-mediated cardiovascular responses induced by lep. and caff.

As previously indicated, Vogt (1954) has shown that subcutaneous injections of lep. (50 to 60 mg./kg.) and caff. (90 to 292 mg./kg.) do not decrease the brain catecholamine levels. Thus, it was shown that after lep. and caff. the amounts of extractable noradrenaline from brain were 1.57 and 1.52 µg. per Gm. respectively, the normal extractable amount being 1.38 µg. per Gm.

In conclusion, the above findings show (1) that the cardiovascular responses observed following intraventricular leptazol and caffeine involved peripheral adrenergic mechanisms, (2) but that neither the catecholamines (noradrenaline, adrenaline, dopamine) nor 5-hydroxytryptamine appeared to be involved in central nervous system initiation of these effects.

(b) Influence of Systemic Phenoxybenzamine. From the results described earlier it is apparent that following PHE pretreatment in both non-vagotomized and vagotomized cats with lep. and caff. when injected intraventricularly induced pressor reversal effects and markedly attenuated the other cardiovascular responses to these agents. Thus, ventricular extrasystoles were completely abolished although ST-T changes were still seen in some experiments. The depressor responses observed following both lep. and caff. in these experiments resemble the characteristic "reversal effect" of adrenaline in the presence of adrenergic blockade. The cardioacceleration following both lep. and caff. was not significantly reduced in vagotomized animals.

However, the cardioacceleration following caff., but not lep., was significantly reduced in non-vagotomized cats. This reduced tachycardia might be due to some direct myocardial depressant effect of phenoxybenzamine, as previously reported by Acheson et al (1949).

In conclusion, it is evident from these results that the centrally induced cardiovascular responses to lep. and caff. are mediated through peripheral sympathetic pathways, confirming the findings obtained with systemic reserpinization.

(c) Influence of Systemic Bretylium. In all of these experiments the pressor responses to both lep and caff., injected intraventricularly were either reduced or completely antagonized, and all of the characteristic ECG changes prevented. Cardioacceleration following caff. was markedly reduced, although that to lep. still ensued in non-vagotomized animals.

In conclusion, these results again confirm the role of peripheral sympathetic pathways in the centrally-mediated cardiovascular responses to both lep. and caff.

(d) Influence of Systemic Cocaine and Iproniazid (monoamino oxidase inhibitors). After pretreatment with both of these agents, as already pointed out, the cardiovascular changes following i.vt. lep. or caff. could not be shown to be markedly altered. In general, these responses were either unaffected or reduced rather than increased. Since both of these agents are readily absorbable into the

central nervous system, it is difficult to separate central and peripheral changes induced by these drugs. High doses of cocaine can depress ganglionic transmission and central sympathetic mechanisms. (Lewis, 1962 and Krantz and Carr, 1961). The degree of cocainization is, therefore, an important factor, but cannot be assessed from these experiments. In addition, Gertner (1961) has shown that iproniazid can reversibly block transmission in the superior cervical ganglion of the cat, when added to the perfusion fluid. Some inhibition of ganglionic transmission might, therefore, be involved in these experiments. Further observations with the use of these agents would, therefore, be necessary before any final interpretation of these actions can be made.

In conclusion, the results obtained with cocaine and iproniazid pretreatment did not indicate any significant potentiation of the cardiovascular responses to lep. and caff. under these conditions.

(e) Influence of Intraventricular Atropine. Whether or not atropine can abolish the central effects of ACH is not yet settled. However, Brenner et al (1942), Feldberg (1945) and Stone (1957) have furnished evidence that some central activity of acetylcholine can be abolished by atropine. Thus, it has been shown to block the arousal reaction initiated by anticholinesterases. It is, therefore, speculated that atropine can compete with ACH for some

type of "receptive substance" involved in synaptic transmission and would, therefore, block central cholinergic interneuronal transmission.

Following the i.vt. administration of atropine (0.1 mg.), as already pointed out, there was no evidence of any peripheral cholinergic blockade. In all of these experiments, however, following subsequent i.vt. lep., no arrhythmias were seen. Other cardiovascular changes were also significantly attenuated. On the other hand, similar pretreatment with atropine did not influence the overall cardiovascular responses (blood pressure and ECG changes). following i.vt. caff. However, the degree of cardio-acceleration induced by caff. was also reduced.

These results would rather suggest that the effects of i.vt. lep. on the central nervous system involve cholinergic mechanisms. On the other hand, it has already been clearly established that the peripheral responses resulting from i.vt. lep. involve sympathetic pathways. It is, however, clear that although the peripheral cardio-vascular responses following i.vt. caff. are also mediated by sympathetic pathways, central cholinergic mechanisms do not seem to be involved in the actions of caff.

(f) Influence of Intraventricular Ethybenztropine (N-ethy-nortropin-benzhydrylether hydrobromide). This is claimed to be a potent anticholinergic agent, exerting only a central action (Taeschler et al, 1962). In general, similar results were obtained. Following administration of ethyb. there was again no evidence of peripheral cholinergic blockade and subsequent i.vt. lep. failed to induce the usual characteristic pressor responses, cardioacceleration and ECG changes. On the contrary, ethyb. pretreatment did not influence either the pressor response or characteristic ECG changes induced by caff. The intensity of ST-T alterations were, however, less marked and the degree of cardioacceleration significantly reduced. These findings confirm, in general, those obtained with atropine. Pretreatment with i.vt. atropine or ethyb. antagonized the centrally-mediated cardiovascular changes induced by lep. but not those induced by caff.

It is concluded, that some type of cholinergic mechanism is, therefore, involved in the central mediation of the cardiovascular changes elicited by leptazol but not by caffeine, although the peripheral responses are mediated through sympathetic pathways.

(g) Influence of Intraventricular Hexamethonium and Mecamylamine (ganglion-blocking agent). As already pointed out following pretreatments with these agents the pressor responses, the cardioacceleration and ECG changes following i.vt. lep. were reduced or completely abolished. It is,

therefore, evident that these responses involve central synaptic functions. On the contrary, these ganglionic blocking agents did not prevent the usual cardiovascular responses observed following caff. except that the degree of cardioacceleration was reduced. The results would suggest that the major cardiovascular changes resulting from i.vt. caff. do not involve central synaptic functions.

Since it has previously been shown that the local central actions of lep. involve cholinergic mechanisms, these results would suggest, therefore, that the mechanisms might be located in brain synapses, although the exact nature or site of such synapses are still unknown. On the contrary, the central nervous system effects of caff. involved appear to be due to some type of unknown non-synaptic central action.

In conclusion, these experiments with hexamethonium and mecamylamine suggest again involvement of some type of central cholinergic mechanisms in the centrally-mediated cardiovascular responses to leptazol, but not to caffeine.

(h) Influence of Intraventricular Hemicholinium (HC-3). In all these experiments, HC-3 (0.5 mg. injected i.vt. 1 to 1½ hours previously) markedly reduced all cardiovascular responses to i.vt. lep. No extrasystoles were seen and the pressor response and cardioacceleration to lep. were significantly reduced.

On the other hand, similar HC-3 pretreatment did not attenuate the cardiovascular responses to caffeine to

any significant degree, although the cardioacceleration to caff. was reduced in these animals.

These findings also confirm the concept that acetylcholine is important in the central synaptic processes, which are triggered by leptazol. On the contrary, those central processes triggered by caffeine do not appear to involve acetylcholine. These results also throw no light on the exact nature or sites of the action of leptazol and caffeine. However, in view of the striking differences between basic central nervous system actions of the two agents, further studies on this problem would be highly desirable.

3. Current Concepts of the Role of Acetylcholine and Noradrenaline in Central Autonomic Cardiovascular Control

Both cholinergic and adrenergic mechanisms have been described as of physiological importance in the central autonomic cardiovascular control. Chang and Gaddum (1933) were the first to demonstrate the presence of acetylcholine in brain. Dikshit (1934) and Kaviatkowski (1935) showed the presence of acetylcholine and its uneven distribution in the central nervous system. According to Dikshit, the cerebral cortex contained little, and the cerebellum much less acetylcholine than the basal ganglia, Kaviatkowski found more in the thalamus region than in the cortex.

The acetylcholine content of different parts of the central nervous system has been reported by several

different workers, including Barsoum (1935); Bacq (1935); Dikshit (1938); Haas (1939) and MacIntosh (1939). MacIntosh (1941) concluded that (1) acetylcholine is found in varying amounts both in grey and white matter of the cerebral hemispheres, medulla and spinal cord; and (2) it also occurs in fairly high concentration in the basal ganglia and in the midbrain. However, its presence in any part of the central nervous system does not necessarily signify the presence there of cholinergic neurones, as was also pointed out by MacIntosh (1941), nor does its absence definitely exclude these.. It is conceivable that low store of acetylcholine with a great ability to synthesize it may be a characteristic feature of a tissue which like the central nervous system exhibits continuous activity. Evidence exists that ACH might be continuously released in the brain; and it can be shown to be present in the cerebrospinal fluid of dogs and cats after intravenous injections of eserine, or when the ventricular system is perfused with eserinized solutions (Feldberg et al, 1936; Chang et al, 1938; Adam et al, 1938). Chute et al (1940) observed the output of only small amounts of ACH when they perfused the almost completely isolated cat's brain with 50 percent defibrinated eserinized blood and Bulbring and Burn (1941) made a similar observation when perfusing the spinal cord of dogs.

Feldberg (1945), in his review on the role of cholinergic transmissions in the central nervous system summarized the problem, as follows:- the presence of ACH

and of cholinesterase in central nervous tissue; the ability of such tissue to synthesize acetylcholine and to release it under certain conditions; the central effects of acetylcholine and eserine, all provide strong evidence in favour of the concept that some central synaptic transmission is mediated by acetylcholine. On the other hand, there is little evidence in favour of the theory that acetylcholine is the universal central transmitter, as there are many facts which are at present difficult to reconcile with this theory.

Acetylcholine has also been shown to excite the hypothalamus (Emmelin et al, 1945) and many different investigators have reported that cholinergic transmissions occur at certain central synapses (Pickford, 1947; Richter et al, 1949; Marazzi, 1953 and Varma, 1964).

According to Chatfield et al (1954), there may be two sets of central ACH-sensitive neurones, one inhibitory and the other excitatory. The inhibitory neurones are easily blocked by atropine, while the excitatory neurones are less sensitive. Charles et al (1963) also postulated that the "reticular activating system" either consists of or is driven by two neurone pools, - one adrenergic, the other cholinergic and which have mutually inhibitory components.

Stone (1957) has also postulated a hypothetical neuronal pattern involving both cholinergic and non-cholinergic mechanisms in the convulsive response to

leptazol. These authors presume that the convulsant action of leptazol might involve excitation of certain interneurones (B), as illustrated in Fig. 35. They speculated that the apical dendrites are stimulated either directly or through other interneurones which are noncholinergic (D), as shown in the figure. It is further postulated that collaterals which would activate cholinergic interneurones (C), as shown in the figure, also exist. The axon terminals of the latter would release acetylcholine and the free acetylcholine would then be destroyed by cholinesterase. When the convulsive activity of leptazol is blocked by pentobarbitone, these workers consider that these cholinergic interneurones could still be activated.

In regard to the role of adrenergic mechanisms in the central nervous system, the presence of noradrenaline and adrenaline in the brain has been demonstrated by Raab, (1943a, 1943b); Von Euler (1946) and Holtz (1950) and the presence of 3-hydroxytyramine has also been demonstrated by Montague, K. A. (1957); Weil-Malherbe et al (1957); Carlsson et al (1958); Bertler et al (1959a) and Bertler et al (1959b). Whether or not catecholamines have a functional role in the control of central autonomic system is also still unknown.

As reported by Vogt (1954), the distribution of adrenaline and noradrenaline in the dog and cat brain showed that the catecholamines are unevenly distributed in brain tissue and did not run parallel with the brain vascularity.

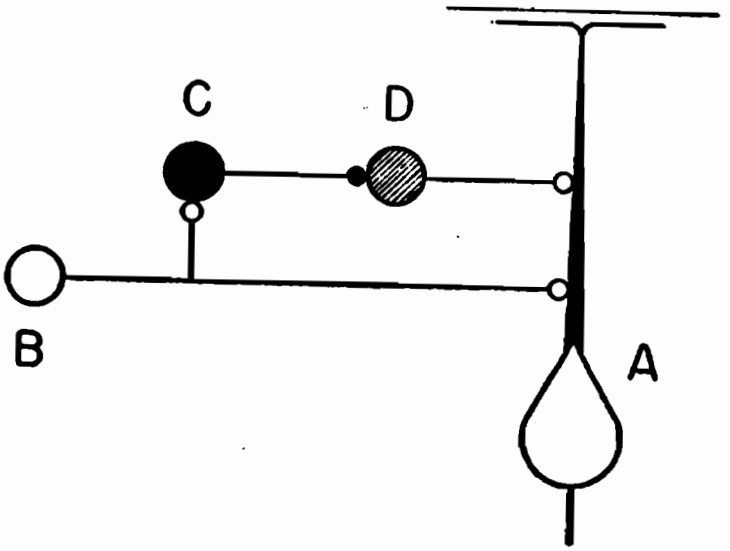


FIGURE 35: Hypothetical neuronal pattern taken from Stone, W. E. (Am. J. Physical Medicine 36, 222-255, 1957). A. pyramidal cell; B, non-cholinergic interneuron sensitive to pentylenetetrazol; C, cholinergic interneuron; D, non-cholinergic interneuron sensitive to acetylcholine.

Highest concentrations appeared in the hypothalamus and area postrema, lesser amounts being present in the midbrain, medulla and medial parts of the thalamus.

It has been reported that various cardiovascular changes could also be induced by central nervous system effects of the catecholamines (Gayet et al, 1927; Tournade (1927), Heymans (1928); Tournade et al (1933); Taylor and Page (1951) and McCubbin et al, 1960). More recently, Share and Melville (1963) studied the cardiovascular responses to intraventricular administration of noradrenaline, adrenaline and 5-hydroxytryptamine in normal animals and in animals previously injected with intraventricular reserpine. The experiments described showed that intraventricular injections of noradrenaline (40 and 80 µg.) in pentobarbitone anesthetized cats produced bradycardia in both non-vagotomized and vagotomized preparations, and that there was an associated depressor response, when the degree of bradycardia was marked. In centrally reserpinized vagotomized cats, intraventricular injections of noradrenaline induced marked pressor responses, cardioacceleration and, at times, ischemic-like ST-T alterations in the ECG. Similar significant changes were not induced by epinephrine or 5-hydroxytryptamine administration. Using 'centrally-reserpinized' preparations, it was found that spinal C_2 section did not alter the arterial blood pressure and heart rate significantly, that following intraventricular norepinephrine

in this preparation, the cardiovascular responses were significantly less, compared with those observed in non-spinal reserpinized animals. From these experiments it was postulated that norepinephrine might be involved as a transmitter substance in central sympathetic excitation at the brain stem level.

Finally, it is well-known both adrenergic and cholinergic peripheral responses can be induced by electrical stimulation of the central nervous system (Karplus et al, 1909 and 1918; Houssay et al, 1925; Kabat et al, 1935; Beattie et al, 1930; Himwich et al, 1930; Uvnas, 1947, and Eliason et al, 1951; Melville et al, 1963). The question, therefore, arises whether or not in the central nervous system these two types of responses are interrelated, that is to say, whether or not cholinergic trigger mechanisms can initiate adrenergic responses, and conversely, whether or not adrenergic trigger mechanisms can initiate cholinergic responses. At the present time no conclusions on this point can be drawn. It is, however, abundantly clear that both cholinergic and noncholinergic mechanisms might be physiologically involved in central nervous system functions and can be influenced by drug agents. Further studies concerning the influence of leptazol and caffeine on the brain-contents of either acetylcholine or the catecholamines would be necessary before final conclusions can be drawn concerning their basic mechanisms of action.

VI. SUMMARY AND CONCLUSIONS

Experiments were performed to study the centrally-mediated cardiovascular responses induced by leptazol and caffeine in cats, using intraventricular injections of these agents and comparing the responses (a) in non-vagotomized and vagotomized animals (b) after spinal C_2 sections and (c) following systemic and central pre-treatments with various types of pharmacological agents known to influence adrenergic and cholinergic mechanisms. The results may be summarized, as follows:-

1. (a) It was observed that in normal (non-vagotomized cats, under pentobarbitone with gallamine, following intraventricular injections of doses of 10, 30 and 85 mg. of leptazol, there were significantly increasing degrees of pressor response, associated with electrocardiographic changes (ectopic beats, ventricular tachycardia and ST-T deviations). (b) The degrees of cardioacceleration observed with these doses were not significantly different. (c) Under these conditions, the most consistent cardiovascular changes ensued following doses of 85 mg. which were, therefore, employed for comparative purposes in all experiments. (d) The responses to this dose were repeatable, following successive injections at 30 minute intervals.

2. Comparisons of the cardiovascular responses to doses of 85 mg. of leptazol, injected intraventricularly under different anesthetics (pento., chlor., ether induction only) and curarizing agents (gal. and SCH) showed some slight variations, although these were not significantly different.

3. (a) Following intravenous injections of leptazol, the general cardiovascular responses to a dose of 85 mg. were always less than those observed with the same dose following intraventricular injections. (b) In contrast to the effects of intraventricular injections, following intravenous administrations, the pressor responses were always delayed, significantly less marked and less sustained, and preceded by slight depressor responses. (c) The degree of cardioacceleration recorded in the non-vagotomized cats following intravenous leptazol was, however, insignificantly different from that observed with intraventricular injections. (d) Following intravenous injections, cardiac arrhythmias were never seen, although some minor ST-T changes were observed.

4. The cardiovascular responses observed following intraventricular injections of leptazol (85 mg.) in vagotomized cats showed minor differences which, however, were not significant from those observed in non-vagotomized animals under comparable experimental conditions.

5. (a) In both non-vagotomized and vagotomized cats, spinal C_2 section significantly reduced the pressor

responses following intraventricular leptazol. (b) In non-vagotomized cats, following spinal C_2 section, cardio-acceleration still ensued. (c) All of the electrocardio-graphic changes normally observed following intraventricular leptazol were completely prevented by previous spinal C_2 section.

6. "Systemic reserpinization" markedly reduced most of the cardiovascular changes normally observed following the intraventricular injection of leptazol (85 mg.); the resultant mean cardioacceleration, however, was not significantly reduced.

7. (a) Phenoxybenzamine pretreatment markedly reduced the cardiovascular responses to leptazol and variable depressor responses following intraventricular leptazol were also observed. (b) The cardioacceleration recorded was not significantly reduced, but cardiac arrhythmias were completely prevented, although ST segment deviations were seen in some experiments.

8. Bretylium pretreatment completely prevented the electrocardiographic changes normally seen after leptazol. The pressor response is significantly reduced, although the cardioacceleration still ensued in non-vagotomized animals.

9. (a) Cocaine pretreatment neither enhanced nor attenuated the degree of the pressor response or cardio-acceleration resulting from intraventricular injections of leptazol (85 mg.) in non-vagotomized cats. The pressor response in vagotomized cats is rather reduced. (b) The

electrocardiographic changes were similar to those of untreated animals, except that ST-T segment deviations were more marked in the non-vagotomized cocaine-pretreated cats.

10. Pretreatment with iproniazid neither enhanced nor attenuated the pressor response or cardioacceleration resulting from the intraventricular injection of leptazol, both in non-vagotomized and vagotomized cats, but completely prevented the occurrence of ventricular extrasystoles. The degrees of ST-T segment deviations, however, were similar to those seen with leptazol in the untreated animals.

11. Intraventricular reserpine pretreatment in non-vagotomized cats did not influence the pressor response and the electrocardiographic changes induced by intraventricular leptazol, but cardioacceleration was rather increased in some experiments.

12. (a) Intraventricular atropine prevented the cardiac arrhythmias which are normally elicited by intraventricular leptazol. (b) Other cardiovascular responses following intraventricular leptazol are also significantly attenuated by the central atropinization.

13. Intraventricular pretreatment of cats with ethybenztropine significantly attenuated the pressor response, cardioacceleration and cardiac arrhythmias induced by intraventricular leptazol; ST-T changes were insignificant.

14. Central pretreatment with either hexamethonium bromide (0.1 mg.) or mecamylamine (0.1 mg.) prevented the electrocardiographic changes and significantly reduced the

pressor response and cardioacceleration following intraventricular leptazol.

15. Intraventricular hemicholinium significantly reduced the pressor response and cardioacceleration and completely prevented the extrasystoles induced by intraventricular leptazol, although there occurred minor ST-T changes in the electrocardiogram.

16. (a) Increasing doses of caffeine (5, 10 and 20 mg.) injected intraventricularly into non-vagotomized cats, under pentobarbitone with gallamine, induced increasing degrees of pressor responses and cardioacceleration; pressor responses to doses of 5 and 10 mg. were not significantly different. (b) The incidence of arrhythmias and ST-T changes were significantly increased with increasing doses. (c) The most consistent responses were observed following a dose of 20 mg. (d) The responses to the latter dose were repeatable on successive administration at 30 minute intervals.

17. (a) The pressor responses to a dose of 20 mg. of caffeine, injected intraventricularly were significantly less in animals under pentobarbitone with succinylcholine, as compared to those under pentobarbitone with gallamine; the cardioacceleration was, however, insignificantly different. (b) The pressor responses and cardioacceleration were both significantly less under chloralose with gallamine, as compared to those under pentobarbitone with gallamine. (c) The pressor responses under chloralose with succinylcholine were less marked than under pentobarbitone with

gallamine. (d) Similarly, the pressor response with ether induction following gallamine was less than that observed under pentobarbitone with gallamine, whereas the cardio-acceleration was not significantly different in these two groups. (e) The electrocardiographic changes under all of the conditions employed were similar.

18. (a) The cardiovascular responses to a dose of 20 mg.of caffeine injected intravenously were significantly less than those observed following intraventricular injections of the same dose of caffeine in both non-vagotomized and vagotomized animals. The pressor responses and cardio-acceleration were markedly less with intravenous caffeine than with intraventricular caffeine. (b) Intravenous caffeine also led to an initial transient depressor response not observed with intraventricular caffeine. (c) In contrast to the striking electrocardiographic changes seen with intra-ventricular caffeine, following intravenous caffeine, there were only minor ST-T changes.

19. (a) The pressor responses and cardioacceleration to intraventricular caffeine (20 mg.) in vagotomized cats, under pentobarbitone with gallamine, were significantly less than those observed in non-vagotomized cats. (b) Under pentobarbitone with succinylcholine the pressor response to caffeine in the vagotomized cats were similar to those observed in the non-vagotomized animals, under similar situation, but the degree of cardioacceleration was less. (c) Under chloralose with succinylcholine the pressor

responses and cardioacceleration to caffeine in non-vagotomized and vagotomized cats were not significantly different. (d) Similar electrocardiographic patterns were observed following caffeine both in non-vagotomized and vagotomized animals.

20. (a) In non-vagotomized and vagotomized cats, spinal C_2 section completely prevented the electrocardiographic changes normally seen with intraventricular caffeine. (b) The pressor response and cardioacceleration were significantly attenuated.

21. "Systemic reserpinization" completely prevented the cardiac arrhythmias and significantly attenuated the other cardiovascular responses normally seen following intraventricular caffeine, in both non-vagotomized and vagotomized untreated non-reserpinized animals; some minor associated ST-T changes ensued in both groups.

22. (a) Following phenoxybenzamine pretreatment intraventricular caffeine led to variable degrees of depressor responses in both vagotomized and non-vagotomized cats. (b) The degree of associated cardioacceleration was reduced significantly only in non-vagotomized animals. (c) Cardiac arrhythmias were completely prevented, although ST segment deviations were seen in some experiments in both vagotomized and non-vagotomized cats.

23. (a) Following intravenous bretylium pretreatment, the pressor response and cardioacceleration induced by intraventricular caffeine were significantly

reduced in non-vagotomized cats, and completely prevented in vagotomized animals. (b) All electrocardiographic changes normally seen after caffeine were completely prevented.

24. 'Systemic' cocaine pretreatment did not significantly influence the cardiovascular responses normally seen following intraventricular caffeine in both non-vagotomized and vagotomized cats.

25. Iproniazid pretreatment did not significantly influence the pressor response, but significantly attenuated the degree of cardioacceleration normally seen following intraventricular caffeine in both vagotomized and non-vagotomized animals. Extrasystoles were also completely prevented, although ST-T alterations remained unaltered in both groups.

26. Intraventricular reserpine pretreatment in non-vagotomized cats neither attenuated the pressor responses and cardioacceleration, nor prevented the occurrence of ectopic beats, following intraventricular caffeine (20 mg.), but the associated electrocardiographic changes appeared to be less marked in the reserpinized animals.

27. Unlike the responses to leptazol, intraventricular pretreatment with either atropine or ethybenztropine in non-vagotomized cats did not attenuate the cardiovascular responses normally seen following intraventricular caffeine (20 mg.), but the degree of cardioacceleration was significantly reduced.

28. Unlike the responses to leptazol, pretreatment

with intraventricular injections of either hexamethonium or mecamylamine in non-vagotomized animals did not significantly alter the blood pressure and electrocardiographic changes induced by intraventricular caffeine, but the degree of cardioacceleration was significantly reduced in both groups.

29. Unlike the responses to leptazol, pretreatment with intraventricular HC-3 in non-vagotomized animals did not significantly alter the subsequent blood pressure and electrocardiographic changes induced by intraventricular caffeine, but the cardioacceleration was significantly reduced.

The above findings have been discussed, and on the basis of these observations the following main conclusions may be drawn:-

(a) Intraventricular injections of leptazol or caffeine can induce marked pressor responses associated with marked electrocardiographic changes (ventricular extrasystoles, auricular and ventricular tachycardia, bigeminal or trigeminal rhythm), ST-T segment deviations as well as tachycardia. These responses are all of central nervous system origin.

(b) The various cardiovascular changes induced by leptazol or caffeine are mediated through peripheral sympathetic pathways.

(c) Peripheral vagal pathways are not directly involved in the centrally-mediated cardiovascular responses to leptazol or caffeine.

(d) Neither the catecholamines (noradrenaline, adrenaline, dopamine) nor 5-hydroxytryptamine are involved in the central nervous system initiation of these effects of leptazol or caffeine.

(e) The cardiovascular responses induced by leptazol or caffeine involve stimulation of central autonomic mechanisms, which appear to be mainly cholinergic in the case of leptazol, but noncholinergic in the case of caffeine. The exact sites of action of these agents have not been determined.

(f) Irrespective of the basic mechanisms involved, intraventricular leptazol (85 mg.) or caffeine (20 mg.) in cats appears to offer a new approach to the experimental production of cardiac arrhythmias and ischemic changes as a basis for pharmacological studies on these problems.

VII. CLAIMS OF ORIGINAL WORK

No systemic experimental studies covering the basic purpose and approach of this investigation have been previously carried out.

The important new findings of these studies are considered to be:-

1. Cerebral lateral ventricular injections of leptazol or caffeine can induce various cardiovascular changes through their central nervous system effects.

2. These responses to leptazol and caffeine involve peripheral adrenergic mechanisms.

3. Leptazol activates central sympathetic representations through some type of unknown cholinergic mechanism.

4. Caffeine activates central sympathetic representations through some type of unknown noncholinergic mechanism.

5. The technique of intraventricular leptazol (85 mg.) or caffeine (20 mg.) offers a new approach to the experimental studies of cardiac arrhythmias and ischemic changes.

VIII. BIBLIOGRAPHY

Acheson, G. H., Farah, A. and French, G. N.: J. Pharmacol. Exper. Therap. 97, 455, 1949.

Adam, H. M., McKail, R. A., Obrador, S. and Wilson, W. C.: J. Physiol. 93, 45p, 1938.

Aviado, D. M. and Dil, A. H.: J. Pharmacol. Exper. Therap. 129, 328, 1960.

Bacq, Z. M.: Arch. int. de Physiol. 42, 42, 43, 1935.

Bailey, H. A., Goth, A. and Lackey, R. W.: Curr. Res. Anesth. 32, 274 - 279, 1953.

Bakay, K.: The Blood Brain Barrier. Charles C. Thomas, Springfield, U. S. A., 1956.

Barbour, H. G. and Kleiner, S. H.: J. Pharmacol. Exper. Therap. 7, 541 - 545, 1915.

Barsoum, G. S.: J. Physiol. 84, 259, 1935.

Battacharya, B. K. and Feldberg, W.: Brit. J. Pharmacol. 13, 156, 1958.

Beattie, J., Brow, G. R. and Long, C.N.H.: The Vegetative Nervous System, Baltimore, William and Wilkins, p. 249, 1930.

Beattie, J., Brow, G. R. and Long, C.N.H.: Proc. Roy. Soc. B. 106, 253-275, 1930.

Bertler, A. and Rosengren, E.: Acta. Physiol. Scand. 47, 350, 1959a.

Bertler, A. and Rosengren, E.: Experim. 15, 10, 1959b.

Bhawa, W. B.: J. Physiol. 149, 169 - 189, 1958.

Bircher, R. P., Kanai, T. and Wong, S. C.: Electroenceph. clin. neurophysiol. 14, 256, 1962.

Bircher, R. P., Kanai, T. and Wong, S. C.: J. Pharmacol, Exper. Therap. 141, 6, 1963.

Birks, R. I. and MacIntosh, F. C.: Fed. Proc. 16, No. 1 March, 1957.

Brown, G. L. and Feldberg, W.: J. Physiol. 88, 265, 1936.

Birks, R. I. and MacIntosh, F. C.: Can. J. Biochem. & Physiol. 39, 787, 1961.

Boura, A. L. A., Copp, F. C. and Green, A. F.: Nature 184, B. A. 70, 1959a.

Boura, A. L. A., Green, A. F., McCoubrey, A., Laurence, D. R., Moulton, R. and Rosenheim, M. L.: Lancet. 11, 17, 1959b.

Boura, A. L. A. and Breen, A. F.: Brit. J. Pharmacol. 14, 536, 1959.

Bovet, D., Depierre, F. and de Lestrange, Y.: C. R. Acad. Sci. Paris, 225, 74, 1947.

Bovet, D., Depierre, F., Courvoisier, S. and de Lestrange, Y.; Arch. int. pharmacodyn. 80, 172, 1949.

Brenner, C. and Merritt, H. H.: Arch. Neurol. and Psychiat. 48, 382, 1942.

Brewster, W. R. Jr., Bunker,J, P. and Beecher, H. R.: Amer. J. Physiol. 171, 37, 1952.

Britton, S. W.: Amer. J. Physiol. 74, 291 - 308, 1925.

Bulbring, E. and Burn, J. H.: J. Physiol. 100, 337, 1941.

Bush, A. D.: J. Pharmacol. Exper. Therap. 14, 313 - 317, 1920.

Camp, W. J. R.: J. Pharmacol. Exper. Therap. 33, 81 - 92, 1928.

Carlsson, A. and Hillarp, N. A.: Klg. Fysiograf. Sallskap. Lund. Forth. 26, n, 8, 1956.

Carlsson, A., Lindquist, M., Magnusson, T., and Waldeck, B.: Science, 127, 471, 1958.

Chakravati, M.: J. Pharmacol. Exper. Therap. 67, 135 - 174, 1939.

Chang, H. C. and Gaddum, J. H.: J. Physiol. 79, 255, 1933.

Chang, H. C., Lim, R. K. S. and Lu, Y. M.: Chinese J. Physiol. 13, 33, 1938.

Charles, D., Barnes and Meyers, F. H.: Pharmacologists 5, 242, 1963.

Chatfield, P. O. and Purpura, P. P.: EEG Clin. Neurophysiol. 6, 287, 1954.

Chute, A. L., Feldberg, W. and Smyth, D. H.: Quart. J. Exper. Physiol. 30, 65, 1940.

Cicardo, V. H.: C. R. Soc. Biol., Paris, 148, 1639 - 1640, 1954.

Covino, Benjamin G., and H. William Gillen: J. Pharmacol. Exper. Therap. 135, p. 136, 1962.

Cow, D.: J. Physiol. 42, 124 - 143, 1911.

Cushny, A. R., and Lambie, C. G.: J. Physiol. 55, 276 - 286, 1921.

Darrow, C. W. and Gellhorn, E.: Proc. Amer. Physiol. Soc. 1938.

David. J. C. and Vareed, C.: Indian J. Med. Res., 16, 920 - 924, 1929.

Dikshit, B. B.: J. Physiol. 80, 409; 81, 382, 1934.

Dikshit, B. B.: J. Physiol. 81, 382 - 394, 1934.

Dikshit, B. B.: Quart. J. Exper. Physiol. 28, 243, 1938.

Edsall, D. L. and Means, J. H.: Arch. Int. Med. 14, 897 - 910, 1914.

Eichler and Hildebrandt: Arch. f. exper. path. u. pharmacol. 116, 100, 1926.

Eismayer G. and Quincke, H.: Arch. fur. exp. path. u. pharmacol. 137, 362, 1928.

Eliason, S., Folkow, B., Lindgren, P. and Uvnas, B.: Acta. Physiol. Scand. 23, 333 - 351, 1951.

Emmelin, N. and Jacobson, D.: Acta. Physiol. Scand. 9, 97 - 111, 1945.

Feldberg, W., Physiol. Rev. 25, 596 - 642, 1945.

Feldberg, W.: J. Physiol. 103, 367, 1945.

Feldberg, W.: A Pharmacological Approach to the Brain from its Inner and Outer Surface; Edward Arnold, London, 1963.

Feldberg, W. and Schriever, H.: J. Physiol. 86, 277, 1936.

Feldberg, W. and Sherwood, S. L.: J. Physiol. 120, 3 P., 1953.

Fellows, E. J., McLean, R. A., Macko, E., Kerwin, J. F., Hall, G. E., Milnes, F. J., Witt, I. H. and Ullyot, G. E.: J. Pharmacol. Exper. Therap. 110, 463, 1954.

Frolich, A. and Loewi, O.: Arch. exp. path. Pharmakol., 62, 159, 1910.

Garattini, S. and Share, P. A.: Advances in Pharmacology. 1, 189, 1962.

Gatgouris, J. and Wallan, R. P.: J. Pharmacol. Exper. Therap. 128, 363 - 371, 1960.

Gayet, R., Gayet, J. and Guillamie, M.: C. R. Soc. Biol. Paris, 97, 1145, 1927.

Gellhorn, E.: Arch. neur. and psychiat., 40, 125 - 146, 1938.

Gellhorn, E.: J. Amer. Med. Assn. 110, 1433, 1938.

Gellhorn, E. and Darrow, C. W.: Arch. int. pharmaco. 62, 114 - 128, 1939.

Gellhorn, E.: Amer. J. Phsychiat. 97, 944, 1941.

Gellhorn, E. and Redgate, E. S.: Arch. Int. Pharmaco. 102, 162 - 178, 1955.

Gerschenfeld, H. M., Wald, F., Zadunaisky, J. A. and De Robertis, E. P. D.: Neurology 9, 412 - 425, 1959.

Gertner, S. B.: J. Pharmacol. Exper. Therap. 131, 223, 1961.

Grollman, A.: The Cardiac Output of Man in Health & Disease, Springfield, Ill., Charles C. Thomas, p. 156, 1932.

Haas, H. T. A.: Arch. Exper. Path und Pharmakol. 19, 119; 192, 117, 350, 1939.

Hahn, F. and Oberdorf, A.: Arch. Exp. path. pharmak. 231, 298 - 310, 1957.

Haury, V. G. and Gruber, C. M.: J. Pharmacol. Exper. Therap. 65, 227 - 234, 1939.

Heathcote, R. S. A.: J. Pharmacol. Exper. Therap. 16, 327 - 344, 1921.

Heilbrunn, G. and Liebert, E.: Endocrinology 25, 354- 358, 1939.

Hess, W. R.: Quoted from Feldberg, W. and Sherwood, S. L., Brit. J. Pharmacol. 10, 374, 1955.

Heymans, C.: Arch. Int. Pharmacodyn, 35, 307, 1928.

Hildebrandt, F.: Arch. f. exper. path. 116, 1, 1926.

Hildebrandt, F.: Handb. Exp. Pharm. 5, 151 - 183, Springer, Berlin , 1937.

Himwich, H.E. and Keller, A. D.: Amer. J. Physiol. 93, 658 - 1930.

Holtz, P.: Acta. Physiol. Scand. 20, 354 - 362, 1950.

Holzbauer, M. and Vogt, M: J. Nerochem. 1, 8, 1956.

Houssay, B. A. and Molinelli, E. A.: C. N. Soc. Biol. Pan's 93, 1454 - 1455, 1925.

Kabat, H., Anson, B. J., Magoun, H. W. and Ranson, S. W.: Amer. J. Physiol. 112, 214 - 230, 1935.

Karplus, J. P. and Kreidl, A.: Pflug. Arch. Ges. Physiol. 129, 138 - 144, 1909.

Karplus, J. B. and Kreidl, A.: Pflug. Arch. Ges. Physiol. 171, 192 - 200, 1918.

Kaviatkowski, H.: Arch. F. Exper. Path. und Pharmak. 177, 154, 1935.

Krantz, J. C. and Carr, C. J.: Pharmacological Principles of Medicinal Practice, William and Wilkins, Baltimore, p. 347, 1961.

Krop, S.: J. Pharmacol. Exper. Therap. 82, 48 - 62, 1944.

Leibel, B. S. and Hall, G. E.: Proc. Soc. Exp.Biol., New York, 38, 894 - 896, 1938.

Lemessurier, D. H.: J. Pharmacol. Exper. Therap. 57, 458 - 463, 1936.

Leyko, E.: J. Pharmacol. Exper. Therap., 38, 31, 1930.

Lewis, J. J.: Introduction to Pharmacology, Livingstone, London, p. 448, 1962.

Lindner, E. and Katz, L. N.: J. Pharmacol. Exper. Therap. 72, 306 - 310, 1941.

Loewe, S.: Arch. Int. Pharmacodyn. 60, 37 - 47, 1938.

Long, J. P. and Schueler, F. W.: J. Amer. Pharm. Assoc. (Sc. Ed.) 43, 79, 1954.

Lougheed, D. W.: Am. J. Phsychiat. 96, 657 - 671, 1939.

Luse, Sarah A.: Biology of Myelin, ed. Korey, S. R., Harper, New York, pp. 59 - 80, 1959.

Luse, Sarah A., and Harris, B.: J. Neurosurg. XVII, 439 - 446, 1960.

McCubbin, J. W., Kaneko, Y., and Page, I. H.: Circulation Re., 8, 849, 1960.

MacIntosh, F. C.: J. Physiol. 96, 6P, 1939.

MacIntosh, F. C.: J. Physiol. 99, 436, 1941.

MacIntosh, F. C.: Can. J. Biochem. & Physiol. 37, 343, 1959.

MacIntosh, F. C.: Fed. Pro. 20, 562, 1961.

MacIntosh, F. C., Birks, R. I. and Sastry, P. B.: Nature 178: 1181, 1956.

MacIntosh, F. C., Birks, R. I. and Sastry, P. B.: Neurology, 8, Suppl. 1, 90, 1958.

Macko, E., McLean, R. A., Fellows, E. J., Kerwin, J. F., Hall, G., Milnes, F. J., Witt, I. H. and Ullyot, G. E.: J. Pharmacol. Exper. Therap. 101, 24, 1951.

McLean, R. A., Fendrick, A. J., Macko, E. and Fellows, E. J.: J. Pharmacol. Exper. Therap. 101, 26, 1951.

Maloney, A. H. and Tatum, A. L.: Arch. int. pharmacodyn. 42, 200 - 211, 1932.

Marrazzi, A.: S. Science 118, 367, 1953.

Masserman, J. H.: Arch. neurol. psychiat. Chicago, 41, 504 - 510, 1939.

Mautner, H. and Yetwin, I. J.: J. Amer. Pharm. Assn., Sci. Ed., 32, 17 - 20, 1943.

Means, J. H. and Newburg, L. H.: J. Pharmacol. Exper. Therap. 7, 449 - 465, 1915.

Melville, K. I.: J. Pharmacol. Exper. Therap. 106, 208, 1952.

Melville, K. I. and Shister, H. E.: Amer. Heart Journal 53, 425 - 438, 1957.

Melville, K. I. and Share, N. N.: Rev. Can. Biol. 22, 265 - 273, 1963.

Melville, K. I., Blum, B., Shister, H. E. and Silver, M. D.: Amer. J. Cardiology, 12, 781 - 791, 1963.

Montague, K. A.: Nature, 180, 244, 1957.

Onuma, T.: Tohoku J. Exp. Med,, 65, 121 - 129, 1957.

Orihara, O.: Tohoku J. Exp. Med., 57, 43 - 55, 1952.

Paasonen, M. K. and Vogt, M.: J. Physiol. 131, 617, 1956.

Phillipes, C. D. F. and Bradford, Rose, J.: J. Physiol. 8, 117 - 132, 1887.

Pickford, M.: J. Physiol. 106, 264, 1947.

Pilcher, J. D.: J. Pharmacol. Exper. Therap. 3, 267 - 298, 1911a.

Pilcher, J. D.: J. Pharmacol. Exper. Therap. 3, 609 - 624, 1911b.

Plant, P. H.: J. Pharmacol. Exper. Therap. 5, 603, 1914.

Raab, W.: Biochem. J. 37, 471, 1943a.

Raab, W.: Experim. Med. Surg. 1, 188, 1943b.

Richter, D. and Crossland, J.: Am. J. Physiol. 159, 247, 1949.

Romagnoli, A. and Melville, K. I.: Can. Anaes. Soc. J., 5, 137, 1958.

Roth, L. J., Schooler, J. C. and Barlow, C. F.: J. Pharmacol. Exper. Therap. 125, 138 - 136, 1959.

Sanders, R.: Arch. Exp. Path. Pharmak., 75, 358, 1927.

Schmidt, Hildebrandt and Krehl: Klin. Wochenschr., IV, 1678, 1925.

Schueler, F. W.: J. Pharmacol. Exper. Therap. 115, 127, 1955.

Share, N. N. and Melville, K. I.: Can. Fed. Proc. 4, 58, 1961.

Share, N. N. and Melville, K. I.: J. Pharmacol. & Exper. Therap. 141, 15 - 21, 1963.

Snedecor, G. W.: Statistical Methods, Iowa State College Press, Ames, Iowa, 1955.

Sollmann, T. and Pilcher, J. D.: J. Pharmacol. Exper. Therap. 3, 19 - 92, 1911.

Swiss, E. D. and Maison, G. L.: J. Pharmacol. Exper. Therap. 105, 87 - 95, 1952.

Sollmann, T.: Manual of Pharmacology, W. B. Saunders Company, p. 288, 1957.

Soloman, A. P., Darrow, C. W. and Blanrock, M. F.: Psychosomatic Medicine, 1, 118 - 137, 1939.

Sourkes, T. L., Murphy, G. F., Beatriz Chavez and Marie Zielinska: J. Neurochem. 8, 109 - 115, 1961.

Spatz, H.: Arch. psychiat., 101, 267 - 358, 1933.

Spector, S., Prockop, D., Shore, P. A. and Brodie, B. B.: Science 127, 704, 1958.

Starr, I., Gamble, C. J., Margolies, A., Donald, J. S. Jr., Joseph, N. and Engle, E.: J. Clin. Investigation 16, 799 - 823, 1937.

Stone,W.E:Am. J. Physical. Med. 36, 222 - 255, 1957.

Stross, W.: Arch. f. exper. path. 114, 177, 1926.

Taeschler, M., Weidman, H. and Perletti, A.: Schweiz. Med. Wschr. 92, 1542, 1962.

Taylor, R. D. and Page, I. H.: Circulation 4, 563, 1951.

Teague, R. S., Norjin, R. A. and Geiling, E. M. K.: J. Pharmacol. Exper. Therap. 115,- 127, 65, 1939.

Tournade, A.: C. R. Soc. Biol. Pan's. 97, 1143, 1927.

Tournade, A. and Malmejae, J.: C. R. Soc. Biol. Paris, 114, 1247, 1933.

Uvnas, B.: Proc. Soc. Exp. Biol., N. Y., 64, 181 - 185, 1947.

Varma, D. R., Share, N. N., Melville, K. I.: Int. J. Neuropharmacol. I, 203 - 206, 1962.

Varma, D. R.: Can. J. Physiol. & Pharmacol. (In Press) 1964.

Vogt, K.: J. Physiol.123, 451, 1954.

Von Esveld, L. W.: Arch. F. Exper. Path. 149, 348, 1930.

Von Euler, U. S.: Acta. Physiol. Scand. 12, 73 - 97, 1946.

Von Meduna: Ztschr. f.d. ges. neurol. u. psychiat. 152, 235 - 262, 1935.

Von Meduna: Schweiz. Arch. f. narol. u. psychiat. (Supp) 39, 32, 1937.

Von Monakow, C.: Schweiz. Arch. neurol. u. psychiat. 8, 233 - 234, 1921.

Weil-Malherbe, H. and Bone, A. D.: Nature, 180, 1050, 1937.

Woodbury, R. A., Hamilton, W. F., Cleckley, H. M. and Volpitto, P. P.: J. Pharmacol. Exper. Therap. 73, 431 - 440, 1941.

Zeller, E. A. and Barsky, J.: Proc. Soc. Exper. Biol. & Med., 81, 459, 1952.