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CHAPTER I

INTRODUCTION

Norepinephrine has been utilized as a pressor agent to combat hypotensive states in patients since 1949. The first report of this use, published by Goldenberg et al. (1), indicated that norepinephrine was beneficial in correcting acute hypotension in 22 of 23 patients during and following thoracolumbar sympathectomy. Since that time, norepinephrine has been found useful in reversing the shock-like states which frequently occur in conditions such as peritonitis, myocardial infarction, removal of pheochromocytoma, hemorrhage, epidemic hemorrhagic fever, trauma, surgery of all kinds and spinal anesthesia (2,3,4,5,6,7).

Some interesting phenomena have been noted as a result of the increased therapeutic use of norepinephrine to correct shock-like states. It has been found that some patients who receive norepinephrine over periods of several hours to several days require increasing doses of the agent in order to maintain the blood pressure at a normotensive level. Furthermore, after long-continued infusion of norepinephrine, a number of patients have been noted who do not tolerate abrupt removal of the drug without returning to an hypotensive state. In this event, the dose of norepinephrine must be slowly decreased over a period of time

until it can be safely stopped. This phenomenon has also been repeatedly observed during surgery for removal of the norepinephrine-epinephrine-producing tumor, pheochromocytoma. During ligation and excision of these tumors, the patients develop an acute and profound hypotension in virtually every instance. An immediate infusion of norepinephrine will return the blood pressure to normal in most cases. Here again, a weaning process must be used to discontinue infusion of the drug.

In the present study the vasodepressor aftereffect of norepinephrine was produced by infusing dogs with high doses of norepinephrine over a period of several hours. A long-term infusion in dogs invariably shows two distinct effects: 1) during infusion the blood pressure rises precipitously at first, but in a few minutes gradually declines until it eventually attains or falls below pre-infusion levels; 2) after discontinuing the infusion, the blood pressure falls to an even lower level.

The purpose of this investigation has been to study the mechanism or mechanisms responsible for the depressor effect of norepinephrine both during and following long-term infusion. In the vast literature concerning the physiological and pharmacological actions of both norepinephrine and epinephrine, certain properties have been described which might account for this phenomenon.

This study has attempted to test the plausibility of the following possible mechanisms as a cause for the vasodepressor aftereffects of norepinephrine:

1. Autonomic ganglionic blocking action of norepinephrine.
2. Increased inactivation of norepinephrine.

3. Decreased response of the cardiovascular system to norepinephrine.

4. Elaboration into the circulation of vasodepressor material (VDM) as a response to norepinephrine.

5. Hypoglycemic hypotension as a response to norepinephrine.

CHAPTER II

HISTORY

Norepinephrine was first synthesized by Stoltz (8), in 1904. He also made the first observations concerning some of its biological properties, noting for example, that it was as potent as epinephrine with respect to its pressor effect but less toxic (9). The period from 1904 to 1946 yielded little information regarding the pharmacological and physiological role of norepinephrine. This lack of information was a direct consequence of the fact that most investigators regarded epinephrine to be the sole adrenal medullary hormone. However, there were some intimations that epinephrine was not the sole adrenergic nerve transmittor as early as 1910 when Barger and Dale (10) published their classical paper, "Chemical structure and sympathomimetic action of amines". They realized that the actions of epinephrine deviated in some respects from those obtained by stimulation of sympathetic nerves. The significance of this work was not generally recognized or further pursued until Cannon and Rosenbleuth, in 1933, revealed the appearance of a factor other than epinephrine following sympathetic nerve stimulation (11). In 1934, Bacq (12) suggested that the sympathin E and I of Cannon and Rosenbleuth were identical, respectively, with norepinephrine and epinephrine. Although the terms sympathin E and sympathin I are no

longer used because their connotations are somewhat erroneous, this work provided the impetus for the investigations of Euler who from 1946 to 1948 published papers showing that extracts of adrenergic nerves, and the organs supplied by them, contained appreciable quantities of norepinephrine. From the reports of Euler and others, it is now generally accepted that norepinephrine is the adrenergic nerve mediator, although it is recognized that epinephrine is also present at adrenergic nerve endings. The consensus, at present, is that norepinephrine is the chemical mediator at postganglionic adrenergic nerve endings and epinephrine is the circulating hormone elaborated by the adrenal medulla (13).

Formation of Norepinephrine and Epinephrine

The mechanism of the biosynthesis of norepinephrine and epinephrine is still largely unknown. The most important contributions, to what seems to be the most probable mode of formation of norepinephrine, have come from Holtz, Heise and Ludtke (14) who discovered Dopa-decarboxylase; and Blaschko (15) who proposed a reaction series including norepinephrine. The biosynthesis may tentatively be assumed to take the following course: 1) 3,4-dihydroxyphenylalanine (Dopa) derived from tyrosine or phenylalanine, loses CO_2 by the action of Dopa-decarboxylase, to form hydroxytyramine; 2) hydroxytyramine is oxidized to form norepinephrine; 3) norepinephrine is methylated to form epinephrine. This reaction is supported by a fair amount of evidence, although circumstantial in some regards. Two other precursors of norepinephrine have been suggested; dihydroxyphenylserine by Blaschko, Burn and Langemann (16), in 1950, and Schmiterlow (17), in 1951, and p-hydroxyphenylethanolamine

(octopamine) by Erspamer (18), in 1952.

The question of whether norepinephrine is also a precursor of epinephrine, in addition to its independent biological action, has been answered in the affirmative by Bulbring (19), in 1949; and later by Masuoka, Schott, Akawie and Clark (20), in 1956. Masuoka et al., injected a-C^{14}-arterenol intraperitoneally into rats and found C^{14}-epinephrine in the adrenal glands. The use of radioactive carbon labelled compounds by Udenfriend and Wyngaarden (21), in 1956, has also provided us with good evidence that 3,4-dihydroxyphenylalanine, and 3,4-dihydroxyphenethylamine are precursors of epinephrine and norepinephrine in vivo. Pellerin and D'Iorio, in 1957, studied the metabolism of dl-3,4-dihydroxyphenylalanine a-C^{14} in bovine adrenal homogenate and found good yields of C^{14}-labelled norepinephrine but no epinephrine (22). They were also unable to detect any 3,4-dihydroxyphenylserine which led them to postulate that this compound was not in the metabolic pathway from tyrosine to norepinephrine.

Inactivation and Elimination of Norepinephrine and Epinephrine

The possible routes of inactivation and elimination of norepinephrine appear to be the following:

1. Oxidative deamination by amine oxidase.
2. Oxidation by cytochrome oxidase systems, catechol oxidase, dopa-oxidase, adrenalinedehydrogenase, peroxidase-peroxide systems.
3. Conjugation (ethereal sulphates, glucuronides).
4. Excretion in the free form.

Oxidative Deamination by Amine Oxidase

The effect of amine oxidase is to cause an oxidative deamination of monoamino compounds such as norepinephrine. Norepinephrine is thus oxidized to an aldehyde with the liberation of NH_3. The aldehyde may then be further oxidized to dihydroxyphenylglycolic acid by aldehyde oxidase.

The role of the amine oxidase system as a means of inactivation of norepinephrine in vivo has been a subject of much controversy. Many of the earlier investigators, such as Richter and Tingey (23), have discounted the efficacy of this enzyme because of the slow deamination of norepinephrine in vitro, while recent observations have tended to stress this way of inactivation.

A recent series of papers by Burn and his co-workers has been devoted to the problem of the physiological importance of amine oxidase. These investigations were prompted by the studies of Blaschko, Richter, and Schlossmann (24), in 1937, and Gaddum and Kwiatkowski (25), in 1938, on the effects of ephedrine. Gaddum and Kwiatkowski concluded that the biological activity of ephedrine was due to its inhibition of amine oxidase, thus, potentiating the action of epinephrine.

Schapira, in 1945, demonstrated that amine oxidase was present in every tissue tested (26). Bain and Dickinson, in 1950, showed that liver contained amine oxidase and was capable, in vitro, of the inactivation of epinephrine in various species, including man (27). The presence of amine oxidase in the iris and nictitating membrane of the

cat and rabbit was reported by Robinson (28), in 1952.

Blaschko and Burn, in 1951, reported that amine oxidase has a greater preference for norepinephrine than it does for epinephrine (29). This fact added support to the work of Burn and Robinson, in 1951, who observed that ephedrine added to the fluid perfusing a rabbits' ear increased the vasoconstrictor action of norepinephrine more than it did that of epinephrine (30). In accordance with this theory, ephedrine does not alter the activity of cobefrine (corbasil) which is not a substrate for amine oxidase.

Burn and Robinson, in 1952, studied the amine oxidase content of the intact, and denervated nictitating membrane (31). It was found that 10 to 12 days following denervation, the amine oxidase activity fell to 66 per cent of normal. They also found that the membrane was more sensitive to norepinephrine when there was a reduction of the enzyme activity. The amount of enzyme activity in the denervated membrane gradually returned toward normal after about 10 days; and after 33 days, the enzyme activity attained a normal level. From these experiments, it is inferred that amine oxidase at the adrenergic nerve endings has a function similar to that of cholinesterase at cholinergic nerve endings.

The observations of Innes and Kosterlitz (32), in 1951, on the effect of cocaine, and of denervation, on the positive chronotropic effect of norepinephrine on the heart might be at least partly explained along these lines.

Catechol and Cytochrome Oxidase. Peroxidases

Catechol oxidase, which is a copper proteide, has been reported to inactivate epinephrine. Cytochrome oxidase, dopa-oxidase, and peroxidase-peroxide systems have also been found capable of inactivating epinephrine but most of the work was done in vitro with little evidence that the processes occur in vivo.

Conjugation (Ethereal Sulfates, Glucuronides)

Richter, in 1940, found that after the administration of relatively large quantities of certain catechol amines to human subjects, a substantial amount appeared in the urine in a conjugated form (33). On the basis of this work many considered conjugation to be a major pathway for the elimination of these amines.

Schayer (34), in 1951, studied the urinary excretion pattern of dl-adrenaline labelled in two ways with C^{14}. One compound was C^{14}-labelled in the N-methyl group, and a second was C^{14}-labelled in the B carbon position. After intravenous injections of B-C^{14}-adrenaline in rats, there were no conjugates detected in the urine indicating that conjugation is not a route of inactivation under physiological conditions. When large quantities of B-C^{14}-adrenaline were fed to rats, considerable quantities of conjugated adrenaline were found in the urine. This conjugation presumably occurred in the intestine or liver. Although all of the C^{14} of small quantities of B-C^{14}-adrenaline was excreted in the urine of rats in 4 hours, under similar conditions only slightly more than half of the administered C^{14} from the N-methyl-labelled compound was excreted. Schayer concluded from this work that a reaction involving cleavage of the molecule between the B and N-methyl carbon atoms

constitutes a major pathway of metabolism.

These findings by Schayer add to the theory that destruction by amine oxidase is a major factor in the inactivation of norepinephrine and epinephrine because a splitting of the compounds between the B and N-methyl carbon atoms could be accounted for by the action of this enzyme.

Excretion in the Free Form

Euler (35) states that in his experience only a very small amount of norepinephrine and epinephrine are excreted in the free form. By infusion experiments he could find only 1.5 to 4 per cent of the administered norepinephrine and 0.5 to 1 per cent of the epinephrine in urine in the free form. Acid hydrolysis of the urine increased the quantity recovered to a total of about 6 per cent which indicated that a portion was conjugated. In the presence of a pheochromocytoma where the excretion of norepinephrine and epinephrine is greatly increased, acid hydrolysis does not materially increase the quantity of catechol amines found.

Fluorescent Products of Norepinephrine and Epinephrine

Solutions of norepinephrine and epinephrine will undergo spontaneous oxidation in the presence of oxygen. This is particularly true if the solution is alkaline. The products of norepinephrine and epinephrine oxidation, under these conditions, are fluorescent. The mechanisms of this reaction and the chemical nature of these compounds have been the object of considerable study and have been extensively reviewed by Euler (35). Some very useful information has evolved from

these investigations. The fact that the oxidation by-products of epinephrine and norepinephrine are fluorescent has afforded a method for their quantitative and qualitative determination in urine, blood and tissue (36,37,38,39,40,41).

In an alkaline medium, epinephrine is oxidized progressively to adrenochrome, adrenolutin, and finally melanin. The oxidation of norepinephrine proceeds in the same fashion as that of epinephrine, yielding noradrenochrome and noradrenolutin. Noradrenolutin has been characterized as being 3:5:6-trihydroxyindole. The compound is apparently in equilibrium between the enol and ketone form.

Adrenolutin and noradrenolutin have been shown to condense with ethylenediamine to form condensation products which are as fluorescent but much more stable than the parent compounds. These condensation compounds have been used by Weil-Malherbe and Bone for the quantitative estimation of catechol amines in both blood and urine (42,43,44,45,46).

The quantitative estimation of norepinephrine, epinephrine and other catechol amines by the photofluorimetric methods of Euler (47,48, 49,50), Weil-Malherbe and Bone, Goldenberg (51), Lund, and others, is probably the most accurate now available. However, these methods are still only accurate to about 10 per cent, and are technically difficult to perform. The accuracy is dependent upon the type of fluorimeter used and the optical filters available. Variations in apparatus have led to some differences, quantitatively, between the results obtained by investigators who have used the same method. The same discrepancy of results has been found repeatedly by investigators who use identical apparatus but slight modifications of the same method.

The Histamine - Antihistamine Relationship to Epinephrine and Norepinephrine

Staub reported, in 1946, that an injection of 0.2 mg. of adrenaline, in man, caused an increase in blood histamine (52). If 0.2 gm. of Antistine (N-phenyl-N-benzylaminomethylinidazoline) was given 3 to 9 minutes before adrenaline, the rise of blood histamine was prevented. Baur and Staub compared the effects of some sympathomimetic agents on blood histamine levels in man and rabbit. They found that norepinephrine increases blood histamine levels in the rabbit but not in man (53).

Euler (54) reported that histamine was a specific constituent of certain autonomic nerve fibers. The highest amount of histamine was found in the postganglionic sympathetic fibers of the splenic, splanchnic and mesenteric nerves. The splenic nerves of the ox were richest in norepinephrine and also histamine. This and other work by Rexed and Euler (55), supports the idea of a definite correlation between norepinephrine and histamine in adrenergic nerves. This may, in some way, be related to the fact that histamine has the ability to release catechol amines from chromaffin cells.

Loew, MacMillan and Kaiser studied the pharmacological activity of the antihistamine, Benadryl (B-dimethyl-aminoethylbenzhydrylether HCl), and reported that it augmented the pressor effect of epinephrine (56). At about the same time Yonkmann and his group, noted that the antihistaminic agent, Pyribenzamine HCl (N'-pyridyl-N'benzyl-N-dimethyl-ethylenediamine), also augmented the pressor effect of epinephrine (57).

LaBelle and Tislow reported the potentiating effect of Trimeton (prophenpyridamine) on the pressor effect of epinephrine (58). Loew and Konrad showed that some antihistaminics inhibited epinephrine-induced hyperglycemia in rabbits (59).

Tickner (60), while searching for an explanation of the antihistamine-augmenting effect on epinephrine, showed that many of the antihistaminics were amine oxidase inhibitors. Piperoxan (933F) and dibenamine, which are adrenergic blocking agents, were also shown to be slightly endowed with this property.

The literature, unfortunately, does not contain adequate data on the relative antihistaminic potency of the antihistaminic agents which are known. Those papers which report relative potencies of a few of these compounds are not always in agreement (61,62,63). The discrepancies seem to be due to the varied techniques which are used to determine antihistaminic properties. No reports have been found which relate antihistaminic action or potency, and epinephrine augmenting effect.

The Autonomic Ganglionic Blocking Action of Epinephrine and Norepinephrine

Marrazzi has studied the action of epinephrine and other sympathomimetic amines on transmission processes in sympathetic ganglia (64-72). His investigations have provided evidence that epinephrine can reduce the action potentials in the postganglionic fibers of adrenergic nerves following faradic stimulation of the preganglionic nerves. The same phenomenon has also been shown to occur with ephedrine. In addition to the ganglionic blocking effect of ephedrine, his studies reveal an

increased potential in preganglionic nerves, indicating that perhaps ephedrine activates sympathetic cell bodies in the central nervous system. Ephedrine, however, appears to have less effect as a ganglionic blocker than does epinephrine. The depression of ganglionic transmission by epinephrine has been discussed by Marrazzi as a mechanism having a beneficial effect, whereby epinephrine can check excessive sympathetic activity. In other words, this mechanism would afford the adrenergic system with its own "brake".

Bülbring and Burn found that small doses of epinephrine augmented ganglionic transmission while large doses depressed it. They theorized, in the discussion of their work, that a decrease of sympathetic ganglionic transmission caused by the infusion of epinephrine might well be the cause of the fall in blood pressure seen following cessation of the infusion (73).

Konzett has reported that norepinephrine produces reduced transmission across the superior cervical ganglion in the cat (74). Lundberg, who used the stellate ganglion of the cat as his test preparation, concluded that norepinephrine was four times less potent than epinephrine in regard to its ganglionic blocking effect (75).

The Metabolic Effects of Epinephrine and Norepinephrine

Epinephrine has been known, for many years, to produce an increase in basal metabolic rate. The work of Goldenberg et al., showed that norepinephrine, in contradistinction to epinephrine, had relatively little effect on the metabolic rate in man (76). Most investigators now agree that norepinephrine is approximately 5 to 10 times less active

than epinephrine in this regard.

Sahyun and Webster, in their studies on rats, observed that norepinephrine tended to cause glycogenesis in the skeletal muscle while epinephrine caused glycogenolysis (77). From the data compiled by the many investigators who have studied the effects of norepinephrine and epinephrine on glycogenolysis and glycemia, all authors agree that the actions of norepinephrine are only 1/5 to 1/10 that of epinephrine. Norepinephrine seems to have all of the metabolic actions which epinephrine has, but to a much lesser degree. These actions include production of hyperglycemia, increase in blood lactic acid, decrease in adrenal ascorbic acid, and decrease in blood amino acids.

Prolonged Infusion of Epinephrine and Norepinephrine

The literature contains many more studies of the long-term infusion of epinephrine than of norepinephrine.

Freeman, Freedman, and Miller (78) produced shock in unanesthetized dogs following the continuous infusion of 0.0034 to 0.0164 mg./kg./min. epinephrine, continued for 1 to 1.5 hours. They noted a decrease in plasma volume.

Blacket et al., infused rabbits with varying amounts of norepinephrine for 5 to 8 days. They found no significant hemoconcentration but did find massive gas pockets in the intestines. They suggested that the reason for shock, produced by norepinephrine, was a release of some vasodilator substance into the circulation (79).

Vigran and Essex observed shock and death in dogs who were given large doses of epinephrine by infusion. They came to no conclusions

regarding the mechanism involved (80). However, Essex reported intensive constriction of hepatic veins, an increase in portal venous pressure and occlusive spasm of the secondary branches of the pulmonary arteries (81).

Guyton and Gillespie, using dogs, reported a decrease in blood volume of 17 per cent during the first 10 minutes of infusion with epinephrine. However, the blood volume decreased no further during the rest of the infusion (82).

Pekkarinen and Hortling (83) infused epinephrine, 0.2 mcg./kg./min., into human subjects for 52 minutes. Blood studies revealed a 67 per cent increase in blood sugar, a 33 per cent increase in blood lactic acid, a 13 per cent increase in serum cholesterol, and a 22 per cent decrease in serum potassium.

Muirhead et al., reported a decrease in plasma sodium and an increase in plasma potassium in dogs following the infusion of 1 to 7 mg. norepinephrine/kg. for 20 to 50 minutes. There were no changes in plasma chlorides (84).

Vasodepressor Material (VDM) and its Relation to Epinephrine and Norepinephrine

Chambers et al., found two substances in the blood of animals in which shock had been induced which they called vasopressor and vasodepressor material (85). Early in shock, the blood of the shocked animals showed a vasopressor action on the rat mesoappendix; late in shock there was a vasodepressor response. Shorr and his co-workers found that liver and skeletal muscle formed considerable quantities of vasodepressor material, but that other organs such as cardiac muscle,

smooth muscle, spleen and kidney did not (86). Shorr later reported that the renal tubules formed vasopressor material, and liver was the principal site for the formation of vasodepressor material (87). Since 1947, Shorr and his associates have published a series of papers supporting the theory that vasopressor and vasodepressor material were regulatory hepato-renal factors involved in circulatory homeostasis.

Mazur and Shorr (88) characterized hepatic vasodepressor material (VDM) as being identical with ferritin and apoferritin. Baez, Mazur and Shorr showed also that ferritin and vasodepressor material were antidiuretic (89).

Baez, Srikantia and Shorr (90) reported that Dibenzyline and GD-131 were beneficial in protecting rats from both traumatic and hemorrhagic shock. Dibenzyline is an adrenergic blocking agent (91). GD-131 is similar to Dibenzyline chemically but has little or no adrenergic blocking ability (92). Both compounds exhibited a blocking action on the formation of vasodepressor material in rat liver in vitro.

Mazur, Green and Shorr (93), and Green, Mazur and Shorr (94), have reported that Fenton's reagent ($Fe^{++}+H_2O_2$) oxidizes adrenaline to adrenochrome at pH 4.5. Ferritin plus hydrogen peroxide also catalyzes the oxidation of adrenaline to adrenochrome. The iron utilized in this process is only a fraction of the total iron found in ferritin.

Shorr's findings that vasodepressor material is formed most readily in the liver following marked ischemia of the organ have provided evidence that VDM might be a factor in the depressor aftereffects of norepinephrine. Norepinephrine has been shown to cause massive constriction of hepatic vessels (81). This could lead to hepatic ischemia and formation of VDM.

CHAPTER III

PROCEDURES

Mongrel dogs were anesthetized with pentobarbital sodium (30 mg./kg. I.V.) followed by doses of 3 mg./kg. as was necessary to maintain the desired degree of anesthesia. The femoral vein was cannulated with polyethylene tubing. The tubing was attached to a Luer-Lok-Coupler which adapts to any standard syringe. A syringe filled with saline was inserted into the coupler. Injections of the various drugs used were made through this tubing and then washed in with a few ml. of saline. The trachea was cannulated to maintain an open airway and to facilitate the use of a respiration pump if necessary. The right carotid artery was cannulated and the blood pressure recorded using a mercury manometer (capable of recording 350 mm. Hg.) attached to the cannula by means of heavy wall rubber tubing. The recording system was filled with 10 per cent sodium citrate to inhibit clotting. For long-term experiments, 3 to 10 mg. of heparin were given I.V., depending upon the size of the animal, as an additional precautionary measure to prevent clotting in the cannula.

In the experiments which involved vagal stimulation, the right vagus was sectioned and a shielded electrode was attached to the nerve distal to the point of section. Square wave stimulation was accomplished

by an American Electric Laboratory Model 751 Electronic Stimulator.

For long-term infusion experiments, a constant rate infusion apparatus (95) (Fig. 1), using a 100 cc. syringe, was attached to the cannula in the femoral vein. The syringe was filled with the desired amount of norepinephrine in isotonic saline and the apparatus adjusted for the desired rate of infusion.

Glucose was determined in the venous blood, in some experiments, by the micro-method of Horvath and Knehr (96), using a Bausch and Lomb Spectronic 20 Colorimeter.

Some experiments were designed to test the augmenting action of ephedrine and certain antihistaminics on the pressor response to single doses of norepinephrine or epinephrine. Since neither height nor duration of the pressor response were considered to be dependable indices of augmentation, a planimeter was employed to determine the area under the blood pressure curves. Thus, a ratio was established between the pressor response to an injection of norepinephrine or epinephrine, before and after the injection of the augmenting agent.

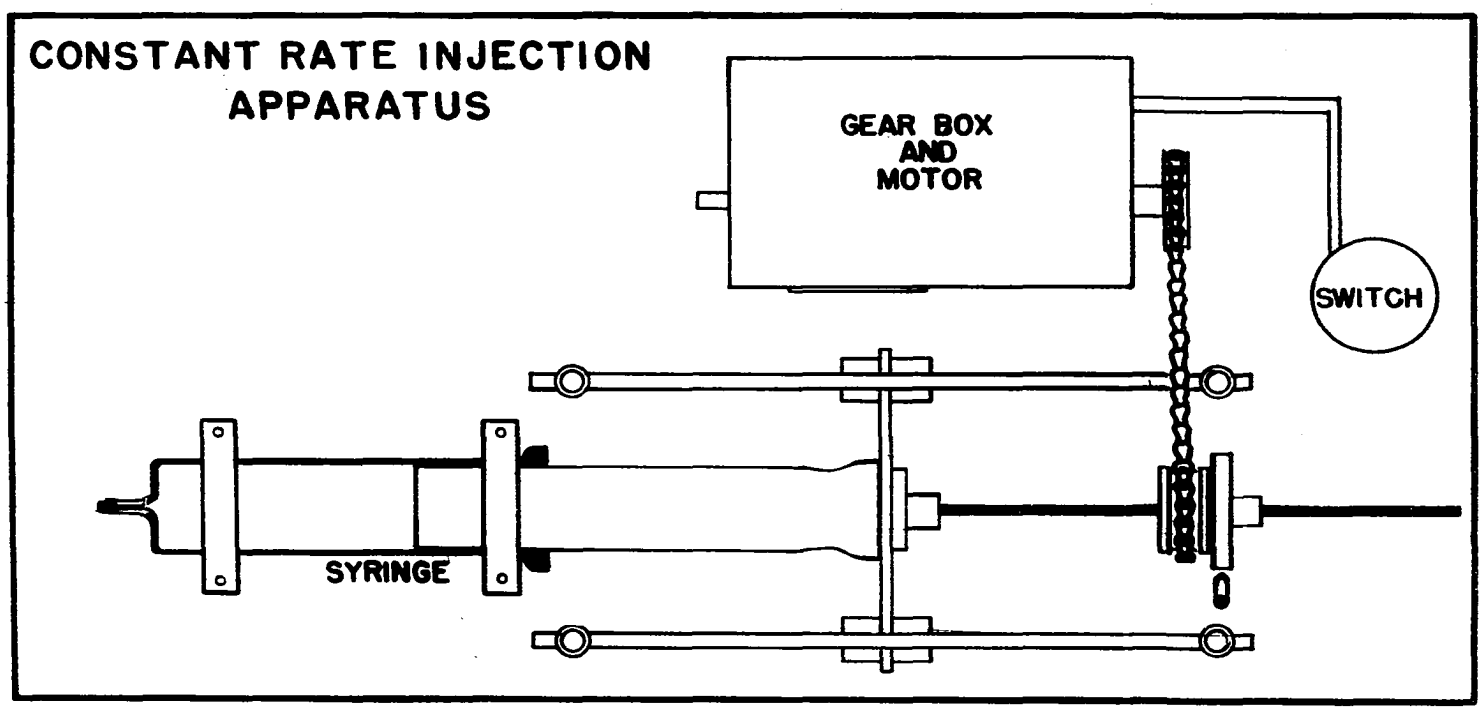
CONSTANT RATE INJECTION
APPARATUS
GEAR BOX
AND
MOTOR
SWITCH
SYRINGE

Fig. 1

CHAPTER IV

EXPERIMENTS AND DISCUSSION

The Effects of Continuous Intravenous Infusion of Norepinephrine

Six dogs were prepared to record blood pressure as described in Chapter III. Blood pressure was recorded until it attained a stable level and then constant-rate infusion of norepinephrine was started. Two animals were given by intravenous infusion a total dose of 0.5 mg./kg. of norepinephrine over a period of 5 hours. Three animals were infused with increasing doses of norepinephrine each hour for 4 hours: 0.1 mg./kg. norepinephrine was infused during the first hour; 0.15 mg./kg., the second hour; 0.20 mg./kg., the third hour; and 0.25 mg./kg., the fourth hour. One animal was infused with 1 mg./kg. in 5 hours.

These experiments were designed as controls for ensuing experiments and to determine whether the cardiovascular response would be markedly different by using different dosage regimens.

These experiments pointed out two main facts concerning the cardiovascular response to constant-rate infusion of large amounts of norepinephrine regardless of the regimen used. First, during the early minutes of infusion the blood pressure rises precipitously, and then falls gradually to or below pre-infusion levels. Second, the blood pressure falls precipitously at the conclusion of an infusion. Following

this fall, the blood pressure may remain relatively stable at this hypotensive level (Table I: Dogs 3, 4, 13); it may slowly climb toward the pre-infusion level (Table I: Dogs 2, 12); or it may fall to zero with death resulting (Table I: Dog 1).

Observation revealed other consistent effects. These animals had episodes of paroxysmal hyperventilation. Heart rates were extremely erratic, ranging from marked tachycardia to severe bradycardia. "Gallop rhythm" and other arrhythmias were manifest in these experiments. Most of the animals salivated profusely.

General findings at autopsy showed by gross inspection ischemic viscera, acute passive congestion of the liver, edema of lungs and limbs, and distention of the stomach by gas. The lungs of most of the animals displayed recent focal hemorrhages.

Fig. 2 (Dog 12, Table I) is a typical example of those experiments in which the infusion dose of norepinephrine was increased by a fixed increment each hour during the infusion. At the beginning of the infusion the blood pressure rose dramatically. This rise was accompanied by a marked bradycardia, probably vagal in origin. Within five minutes the blood pressure began to drop and the heart rate changed toward the control-period level. At the end of each hour, during the time in which the syringe was being filled with norepinephrine at the new dosage level, the blood pressure dropped markedly. When the infusion was resumed, the same cycle recurred.

Fig. 3 (Dog 13, Table I) is an example of those experiments in which a single dose level of norepinephrine was infused at a constant rate for five successive hours without stopping. It can be seen that

TABLE I

CONTROL INFUSION EXPERIMENTS

DOG-SEX-WEIGHT	DOSE OF NOREPINEPHRINE	MEAN BLOOD PRESSURE IN MM. HG. PRE-INFUSION	3 MIN. POST-INFUSION	1 HR. POST-INFUSION	REMARKS
1-male-13.6 kg.	0.5 mg./kg./5 hrs.	160	108	0	died 1 hr. post-infusion
2-male-10 kg.	0.5 mg./kg./5 hrs.	146	70	110	killed 1 hr. 48 min. post-infusion; B. P. 114
3-male-6.8 kg.	0.1 mg./kg.,1st hr. 0.15 mg./kg.,2nd hr. 0.20 mg./kg.,3rd hr. 0.25 mg./kg.,4th hr.	138	46	30	killed 1 hr. 10 min. post-infusion; B. P. 30
4-male-7.3 kg.	same as dog 3	150	68	60	killed 1 hr. 5 min. post-infusion; B. P. 60
12-female-9 kg.	same as dog 3	114	40	64	killed 1 hr. 32 min. post-infusion; B. P. 72
13-female-7 kg.	1 mg./kg./5 hrs.	140	66	56	killed 1 hr. 39 min. post-infusion; B. P. 64
* female-24 kg.	30 mg./21 hrs. 15 min.	---	--	---	----------

* See Fig. 4 and text

Fig. 2

Dog 12
Female 9 kg.

Upper Marker: 0 Blood Pressure
Lower Marker: Time: 10 seconds

1. Norepinephrine 10 mcg. I.V.
2. Kymograph: stopped.
3. Norepinephrine 0.1 mg./kg./hr. I. V. infusion started.
4. Kymograph: 1 min. on, 10 min. off.
5. Norepinephrine 0.1 mg./kg./hr. I. V. infusion stopped.
6. Norepinephrine 0.15 mg./kg./hr. I. V. infusion started.
7. Kymograph: 1 min. on, 10 min. off.
8. Norepinephrine 0.15 mg./kg./hr. I. V. infusion stopped.
9. Norepinephrine 0.2 mg./kg./hr. I. V. infusion started.
10. Kymograph: 1 min. on, 10 min. off.
11. Norepinephrine 0.2 mg./kg./hr. I. V. infusion stopped.
12. Norepinephrine 0.25 mg./kg./hr. I. V. infusion started.
13. Kymograph: 1 min. on, 10 min. off.
14. Norepinephrine 0.25 mg./kg./hr. I. V. infusion stopped.
15. Kymograph: 1 min. on, 10 min. off.
16. Killed animal with ether I. V.

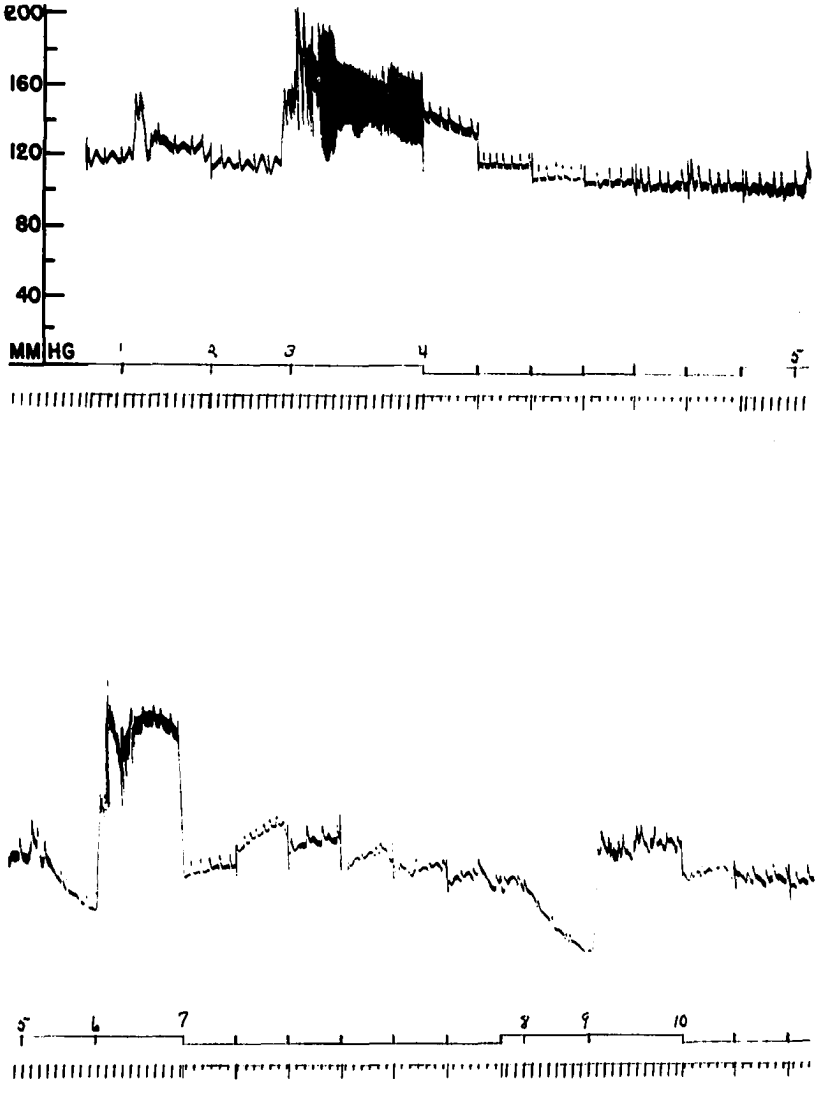

Fig. 2

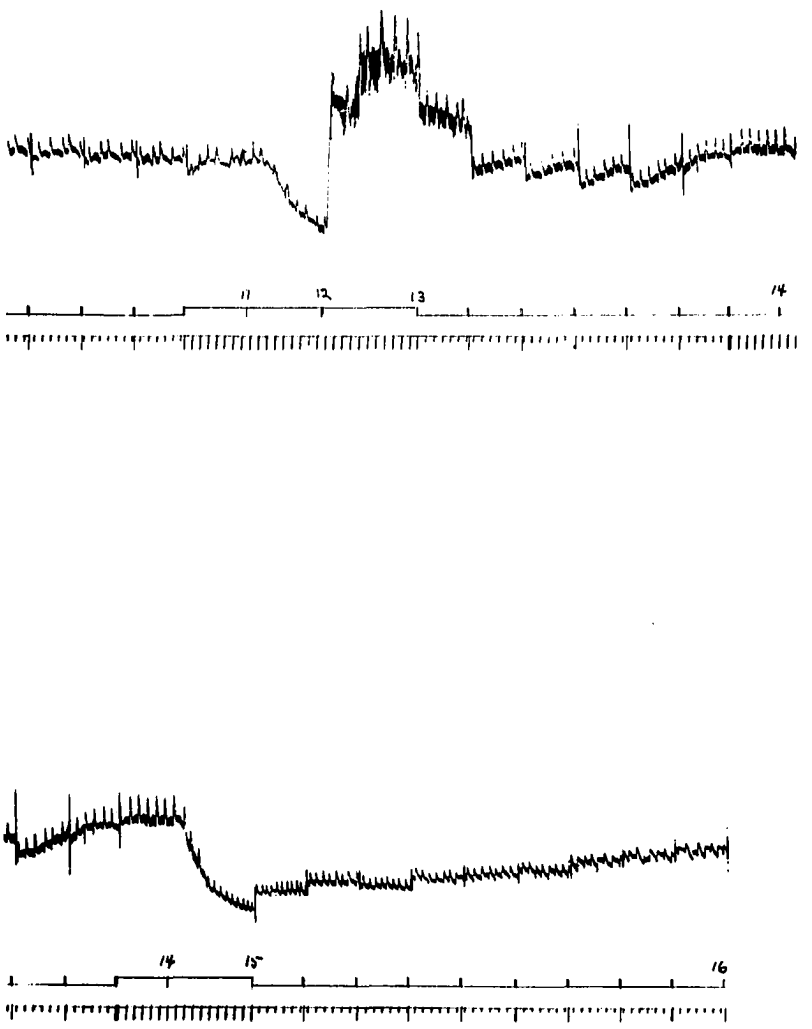

Fig. 2 cont.

Fig. 3

Dog 13
Female 7 kg.

Upper Marker: 0 Blood Pressure
Lower Marker: Time: 10 seconds

1. Norepinephrine 1.0 mg./kg./5 hrs. I. V. infusion started.
2. Kymograph: 1 min. on, 10 min. off.
3. Norepinephrine 1.0 mg./kg./5 hrs. I. V. infusion stopped.
4. Kymograph: 1 min. on, 10 min. off.

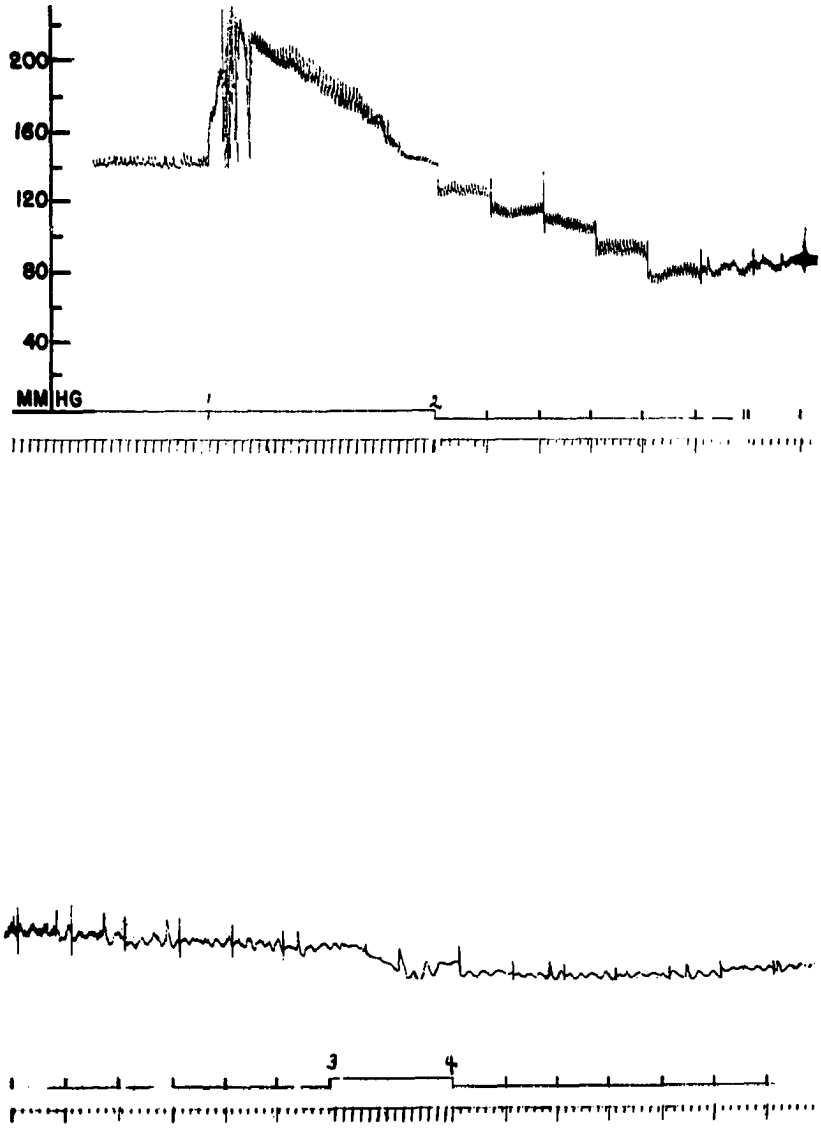

Fig. 3

essentially the same phenomenon occurred as was seen in Fig. 2.

Fig. 4 (Dog *, Table I) shows a record taken in one of six experiments in which the animals were subjected to an infusion of norepinephrine at a dose and rate necessary to keep the blood pressure elevated (95). All of these animals died during infusions which lasted from 14 to 21+ hours. The record illustrates the fact that as the experiment progressed the rate of infusion had to be increased in order to keep the blood pressure elevated. A second point to be noted is that at the beginning of each 2 mg. dose of norepinephrine the blood pressure attained quite high levels but gradually lowered even though the infusion was proceeding at a constant rate. These experiments indicate that there is a progressively decreasing cardiovascular response to norepinephrine given over a long period of time.

The Effects of Dibenzyline and GD-131

These experiments were designed to determine whether vasodepressor material (VDM) might be a contributing factor in producing the vasodepressor aftereffect of norepinephrine. Since the materials necessary to determine VDM in liver were not available, it was decided to investigate the matter pharmacologically using Dibenzyline and GD-131. The rationale concerned with the use of these drugs in the analysis of this factor has been discussed in Chapter II, page 17.

Control experiments were performed to determine the amount of Dibenzyline necessary to block the pressor effect of a given dose of norepinephrine and epinephrine. It was found that 5 mg./kg. of Dibenzyline would block the pressor response to epinephrine but 10-15 mg./kg. were

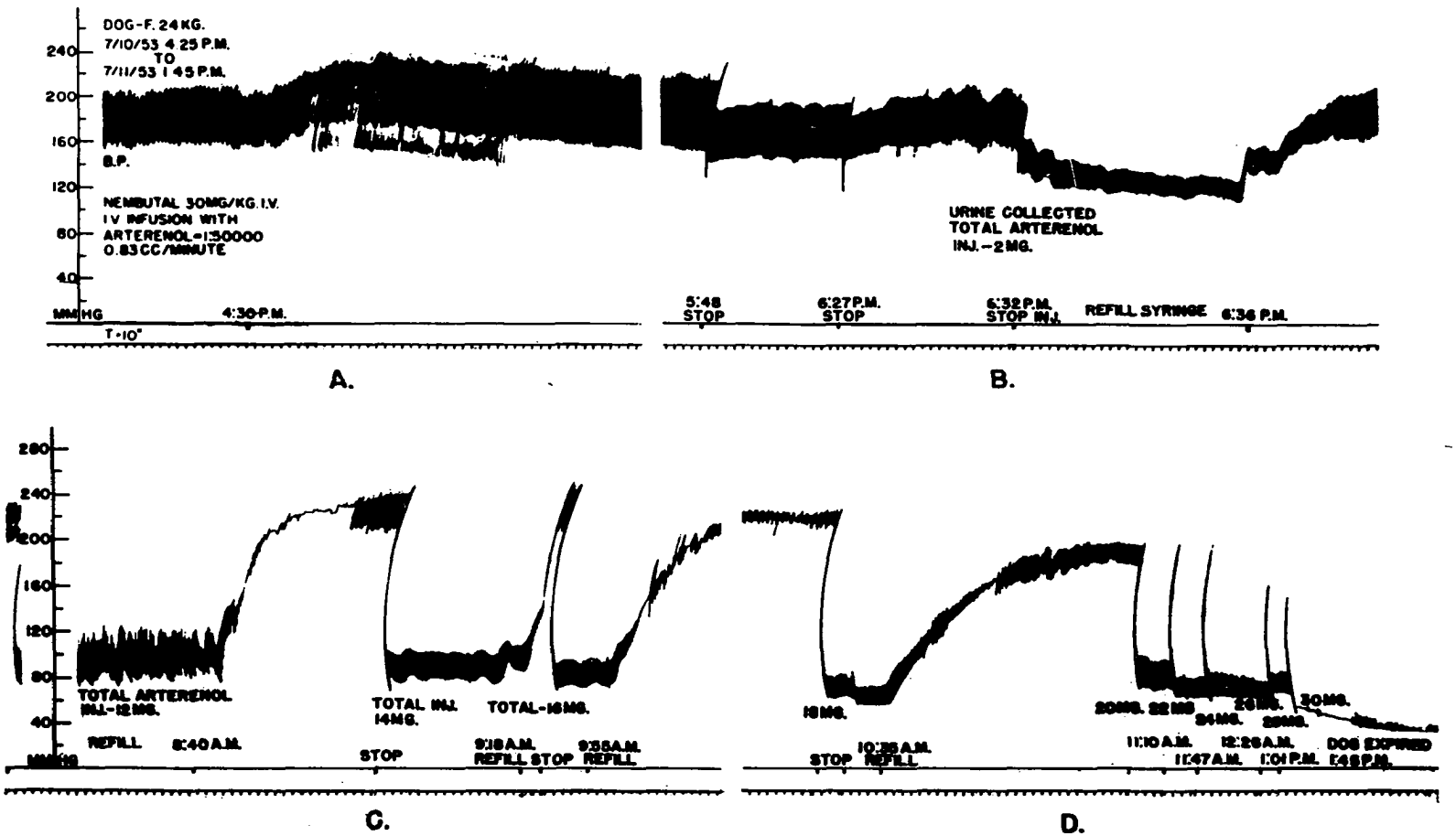
DOG-F. 24 KG.
7/10/53 4 25 P.M.
TO
7/11/53 1 45 P.M.
240
200
160
120
80
40
B.P.
NEMBUTAL 30MG/KG. I.V.
I.V. INFUSION WITH
ARTERENOL-1:30000
0.83 CC/MINUTE
MM HG
4:30 P.M.
T-10°
A.
URINE COLLECTED
TOTAL ARTERENOL
INJ.-2 MG.
5:48
STOP
6:27 P.M.
STOP
6:32 P.M.
STOP INJ.
REFILL SYRINGE
6:36 P.M.
B.
280
240
200
160
120
80
40
TOTAL ARTERENOL
INJ.-12 MG.
REFILL
8:40 A.M.
TOTAL INJ.
14 MG.
STOP
TOTAL-16 MG.
9:18 A.M.
REFILL STOP
9:36 A.M.
REFILL
C.
18 MG.
10:28 A.M.
STOP REFILL
20 MG.
24 MG.
28 MG.
30 MG.
11:10 A.M.
11:47 A.M.
12:26 A.M.
1:01 P.M.
DOG EXPIRED
1:48 P.M.
D.

Fig. 4

necessary to block that of norepinephrine. A disturbing feature was that Dibenzyline at these dose levels produced hypotension. Fig. 5 (Dog 6, Table II) shows that Dibenzyline produced reversal of the pressor effect of epinephrine and norepinephrine. "Epinephrine reversal" is a well known phenomenon. However, norepinephrine reversal is not well recognized. The theory of this phenomenon is that adrenergic blocking agents inhibit the pressor effect (vasoconstrictor action) but do not inhibit the depressor effect (vasodilator action) of epinephrine. Norepinephrine, according to most investigators, is supposed to be purely vasoconstrictor and to have little, if any, vasodilator properties. Nickerson, Henry and Nomaguchi (92) have reported that norepinephrine does have a slight depressor effect in cats, following Dibenzyline in doses of 2-5 mg./kg.

Fig. 6 (Dog 7, Table II) is the record of an experiment which shows the effect of constant-rate infusion of norepinephrine following Dibenzyline. The pressor effect of norepinephrine was almost completely blocked and the reversal (depressor effect) was so pronounced that it led to the death of the animal.

It soon became evident that this method could not be used to protect against the norepinephrine depressor effect because it actually complemented the hypotension. It did, however, make clear that norepinephrine is not a pure vasoconstrictor agent. This adds support to the growing opinion that norepinephrine is not as different qualitatively from epinephrine as many investigators have indicated. For many years clinicians have been warned against the use of epinephrine in combating shock-states because of its ability to sensitize heart muscle, and the

TABLE II

DIBENZYLINE EXPERIMENTS

Dog 5-male-12.3 kg.

Dibenzyline: 15 mg./kg. I. V. in divided doses, in 40 minute period.

Norepinephrine: 20 mcg. I. V. as single injections.

Epinephrine: 20 mcg. I. V. as single injections.

Remarks: Norepinephrine and epinephrine reversal. Normal B. P. 110 mm. Hg.; at end of experiment B. P. 36 mm. Hg.

Dog 6-male-7 kg.

Dibenzyline: 10 mg./kg. I. V. in divided doses, in 29 minute period.

Norepinephrine: 7 mcg. I. V. as single injections.

Epinephrine: 7 mcg. I. V. as single injections.

Remarks: Norepinephrine and epinephrine reversal. Normal B. P. 120 mm. Hg.; at end of experiment B. P. 54 mm. Hg.

Dog 7-female-9 kg.

Dibenzyline: 10 mg./kg. I. V. in divided doses, in 25 minute period.

Norepinephrine: Infusion
0.1 mg./kg., 1st hour
0.25 mg./kg., 2nd hour
0.25 mg./kg., 3rd hour

Remarks: Animal died 43 minutes in 3rd hour of infusion. Norepinephrine reversal from the start of infusion.

TABLE II (cont.)

DIBENZYLINE EXPERIMENTS

Dog 8-male-6.4 kg.

Dibenzyline: 5 mg./kg. I. V.

Norepinephrine: Infusion
0.1 mg./kg., 1st hour
0.15 mg./kg., 2nd hour
0.20 mg./kg., 3rd hour
0.25 mg./kg., 4th hour

Remarks: Normal B. P. 104 mm. Hg.; after Dibenzyline B. P. 30 mm. Hg. Wyamine SO_4 (1 mg./kg. I. V.) was given before and after Dibenzyline. The pressor action of Wyamine was diminished by Dibenzyline. Norepinephrine infusion did not elevate B. P. above 30 mm. Hg.

Dog 9-male-10 kg.

Dibenzyline: 10 mg./kg. I. V. in divided doses, in 20 minute period.

Norepinephrine: Infusion
0.1 mg./kg., 1st hour
0.15 mg./kg., 2nd hour
0.20 mg./kg., 3rd hour

Remarks: Normal B. P. 140 mm. Hg.; after Dibenzyline B. P. 76 mm. Hg. The animal died 13 minutes in 3rd hour of infusion.

Fig. 5

Dog 6
Male 7 kg.

Upper Marker: 0 Blood Pressure
Lower Marker: Time: 10 seconds

3. Norepinephrine 7 mcg. I. V.

4. Epinephrine 7 mcg. I. V.

5, 6, 7, Dibenzyline 10 mcg. I. V.
Total dose was 70 mg. or 10 mg./kg. Record does not show total amount administered.

16. Norepinephrine 7 mcg. I. V.

17. Kymograph: off

18. Epinephrine 7 mcg. I. V.

19. Kymograph: 1 min. on, 10 min. off.

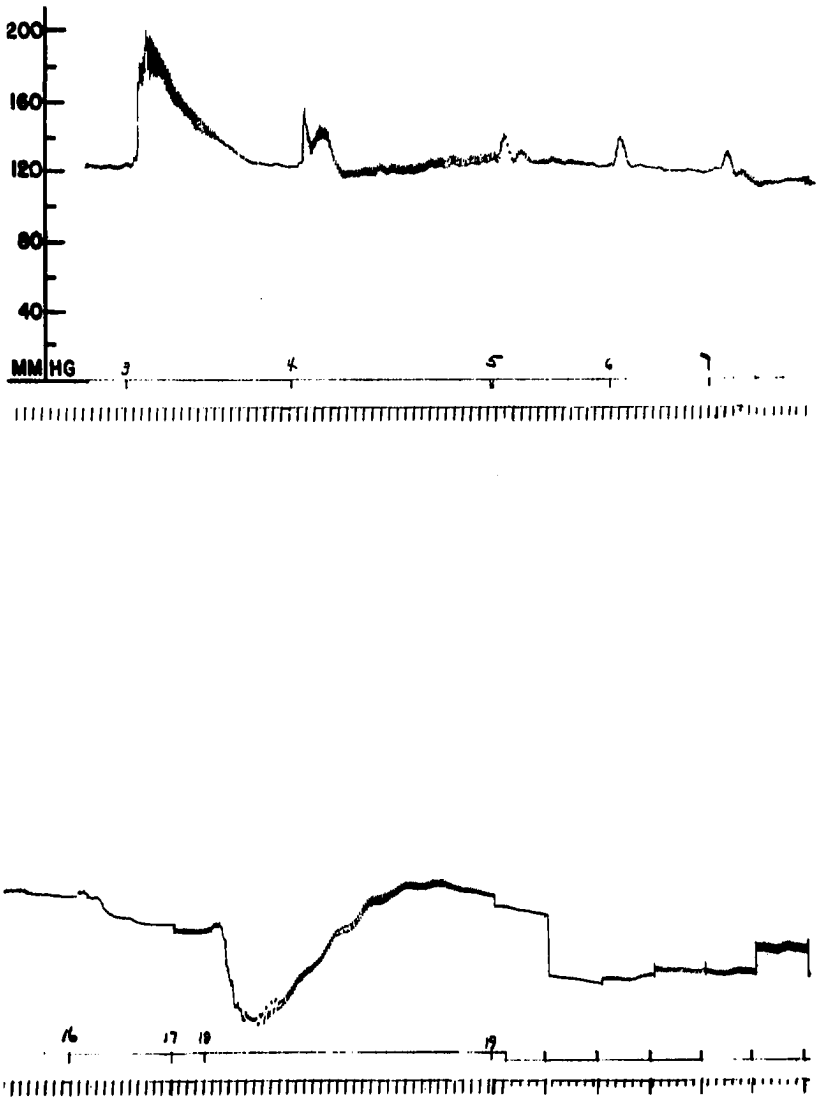

Fig. 5

Fig. 6

Dog 7
Female 9 kg.

Upper Marker: 0 blood pressure
Lower Marker: Time: 10 seconds

1. Norepinephrine 20 mcg. I. V.
2. Dibenzyline 5 mg./kg. I. V.
3. Kymograph: 1 min. on, 10 min. off.
4. Dibenzyline 5 mg./kg. I. V.
5. Norepinephrine 20 mcg. I. V.
6. Started constant rate injection machine.
7. Norepinephrine 0.1 mg./kg./hr. I. V. infusion started.
8. Kymograph: 1 min. on, 10 min. off.
9. Norepinephrine 0.1 mg./kg./hr. I. V. infusion stopped.
10. Norepinephrine 0.25 mg./kg./hr. I. V. infusion started.
11. Kymograph: 1 min. on, 10 min. off.
12. Norepinephrine 0.25 mg./kg./hr. I. V. infusion started.
13. Norepinephrine 0.25 mg./kg./hr. I. V. infusion started.
    Animal died 43 minutes later.

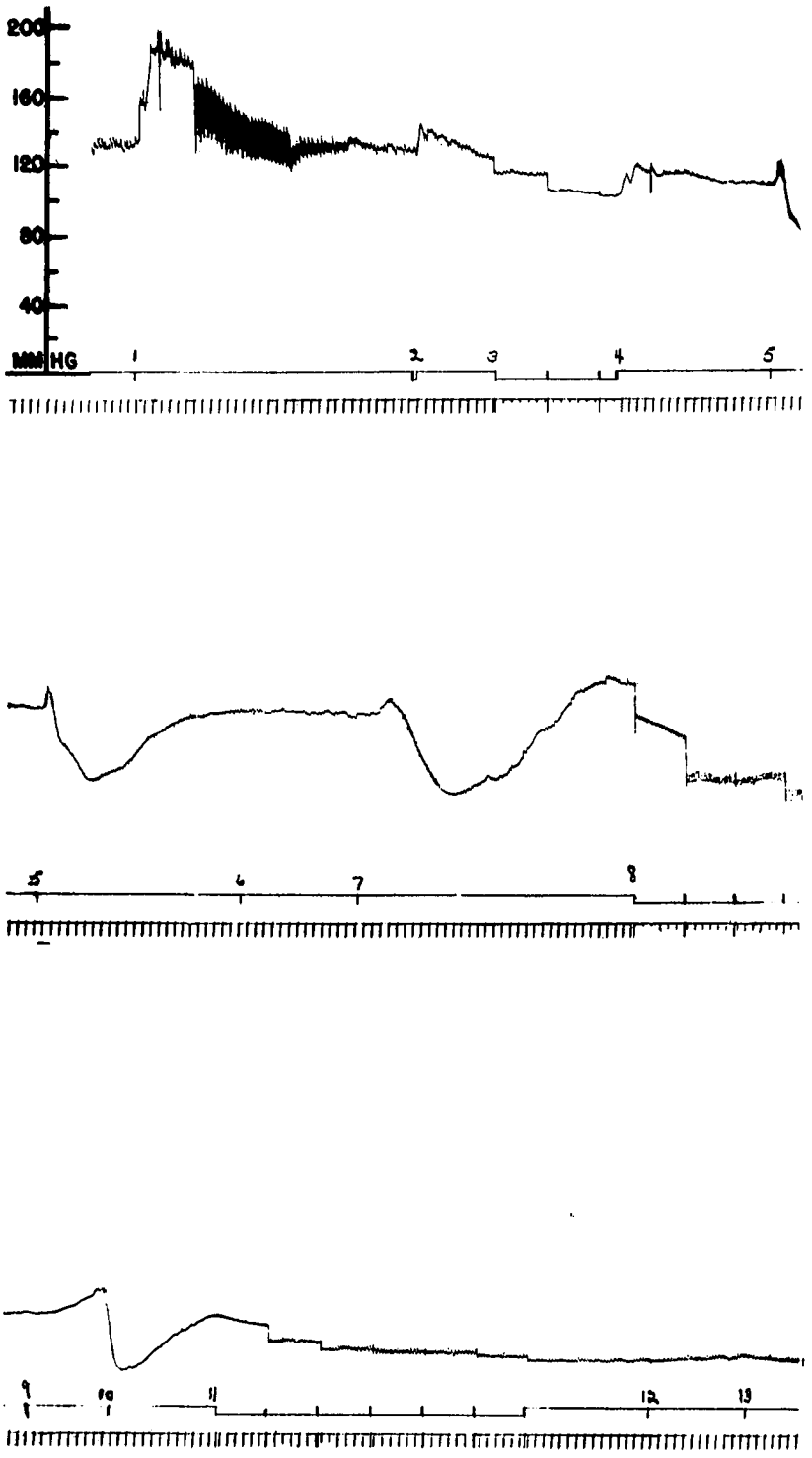

Fig. 6

vasodepressor component of its action. The argument has been that during shock, normal reflex mechanisms promote vasoconstriction and tachycardia; therefore, epinephrine would not be indicated. In addition, there was some concern that the vasodepressor effect would persist after discontinuing epinephrine and thus augment an already hypotensive state. Norepinephrine, on the other hand, has been denoted as having little effect on cardiac sensitivity or rate, and little vasodilator action. These experiments indicate that norepinephrine can cause tachycardia, arrhythmias and vasodepression, although the dose of norepinephrine necessary to produce these phenomena is much higher than that of epinephrine.

GD-131, at a dose level of 10 mg./kg. I. V., produced some blocking effect on the pressor response of norepinephrine, Fig. 7 (Dog 10, Table III). It did not reverse the effect of norepinephrine as did Dibenzyline at the same dose level. It did not protect against the post-infusion hypotension, and the animal died as did some of the control animals.

Fig. 8 (Dog 11, Table III) shows the effects of GD-131 (1 mg./kg. I. V.) given before norepinephrine infusion. The results are not materially different from those seen in the control experiment, Fig. 2.

The record of Dog 14, Table III, was very similar to the control experiment pictured in Fig. 3. In this experiment the dose of GD-131 was 5 mg./kg. I. V. followed by an infusion (1 mg./kg./5 hrs.) of norepinephrine.

These experiments tend to indicate that GD-131 does not protect

TABLE III

GD-131 EXPERIMENTS

DOG-SEX-WEIGHT	DOSE OF GD-131	DOSE OF NOREPINEPHRINE	REMARKS
		INFUSION	
10-female-8 kg.	10 mg./kg. I. V. in divided doses, in 5 min.	0.1 mg./kg. 1st hour 0.15 mg./kg. 2nd hour 0.20 mg./kg. 3rd hour 0.25 mg./kg. 4th hour	B. P. 120 before GD-131; B. P. 122 before infusion with norepinephrine B. P. 120 at point of end of infusion. Dog died 1 hr. 37 min. following end of infusion.
11-female-6 kg.	1 mg./kg. I. V.	same as dog 10	Record not significantly different from control animals. B. P. fall after each dose. Pre-infusion B. P. 114; B. P. 100, 2 hrs. 20 min. post-infusion when killed.
14-male-10 kg.	5 mg./kg. I. V.	1 mg./kg./5 hrs.	Pre-infusion B. P. 112; B. P. 48, 1 hr. 49 min. post-infusion when killed.

Fig. 7

Dog 10
Female 8 kg.

Upper Marker: 0 blood pressure
Lower Marker: Time: 30 seconds

1. Norepinephrine 10 mcg. I. V.
2. Kymograph: off.
3. Norepinephrine 10 mcg. I. V.
4. Kymograph: off.
5. GD-131 5 mg./kg. I. V.
6. GD-131 5 mg./kg. I. V.

6A. Kymograph: 1 min. on, 10 min. off.

7. Norepinephrine 10 mcg. I. V.
9. Not shown, Norepinephrine 0.1/mg./kg./hr. I. V. infusion started.
10. Norepinephrine 0.1 mg./kg./hr. I. V. infusion stopped.
11. Norepinephrine 0.15 mg./kg./hr. I. V. infusion started.
12. Kymograph: 1 min. on, 10 min. off.
13. Norepinephrine 0.15 mg./kg./hr. I. V. infusion stopped.
14. Norepinephrine 0.2 mg./kg./hr. I. V. infusion started.
18. Kymograph: 1 min. on, 10 min. off.
19. Norepinephrine 0.25 mg./kg./hr. I. V. infusion stopped.
20. Kymograph: 1 min. on, 5 min. off.

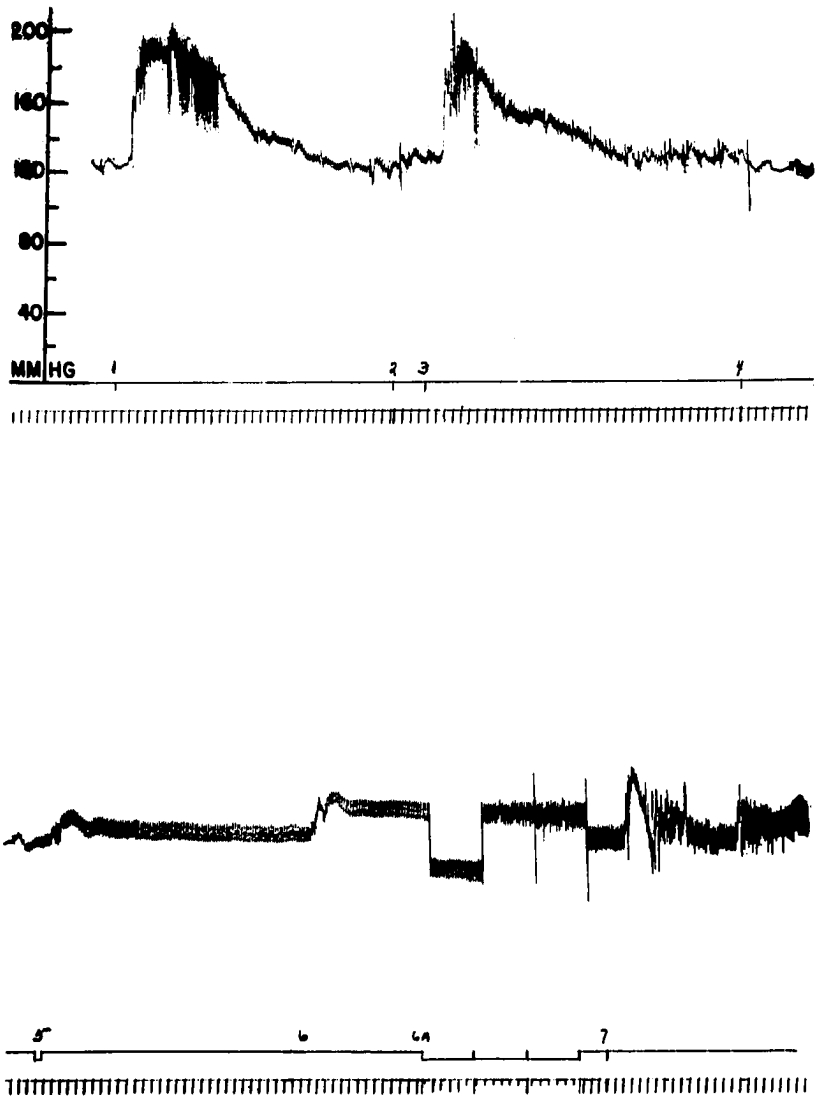

Fig. 7

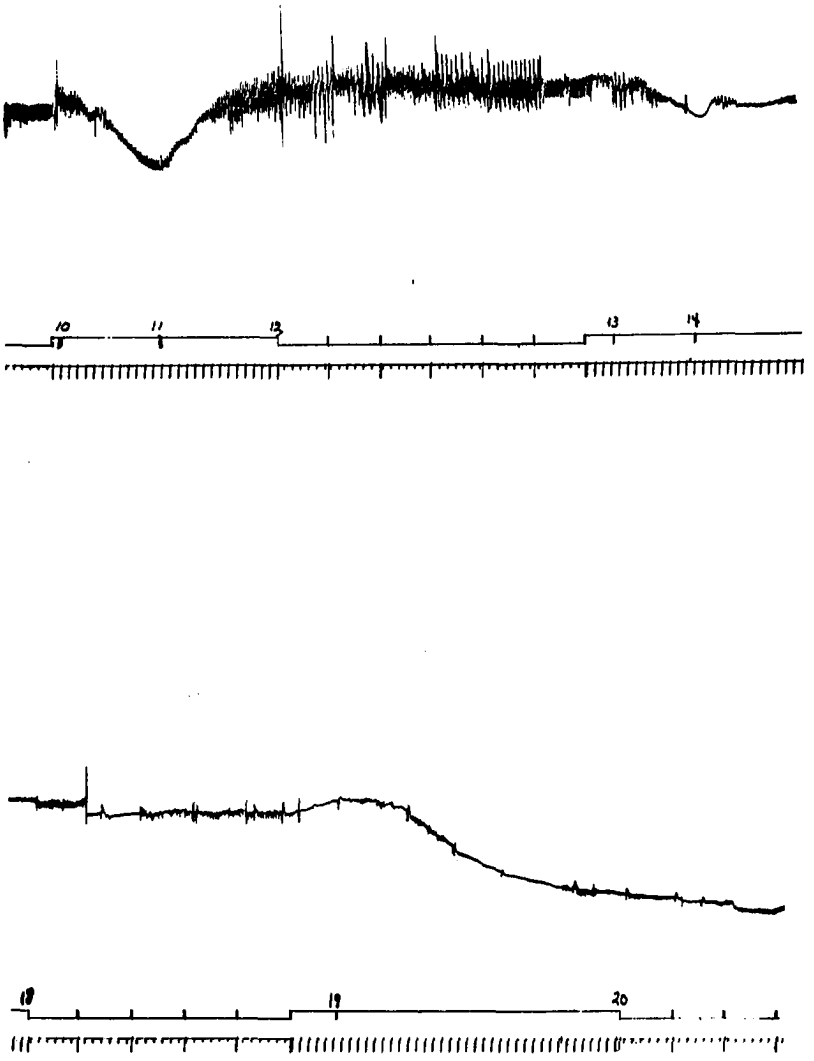

Fig. 7 cont.

Fig. 8

Dog 11
Female 6 kg.

Upper Marker: 0 blood pressure
Lower Marker: Time: 10 seconds

1. Norepinephrine 10 mcg. I. V.
2. GD-131 1 mg./kg. I. V.
3. Kymograph: off.
4. Nembutal 30 mg. I. V.
5. Norepinephrine 0.1 mg./kg./hr. I. V. infusion started.
6. Kymograph: 1 min. on, 10 min. off.
7. Norepinephrine 0.1 mg./kg./hr. I. V. infusion stopped.
8. Norepinephrine 0.15 mg./kg./hr. I. V. infusion started.
9. Kymograph: 1 min. on, 10 min. off.
14. Norepinephrine 0.25 mg./kg./hr. I. V. infusion started.
15. Kymograph: 1 min. on, 10 min. off.
16. Norepinephrine 0.25 mg./kg./hr. I. V. infusion stopped.

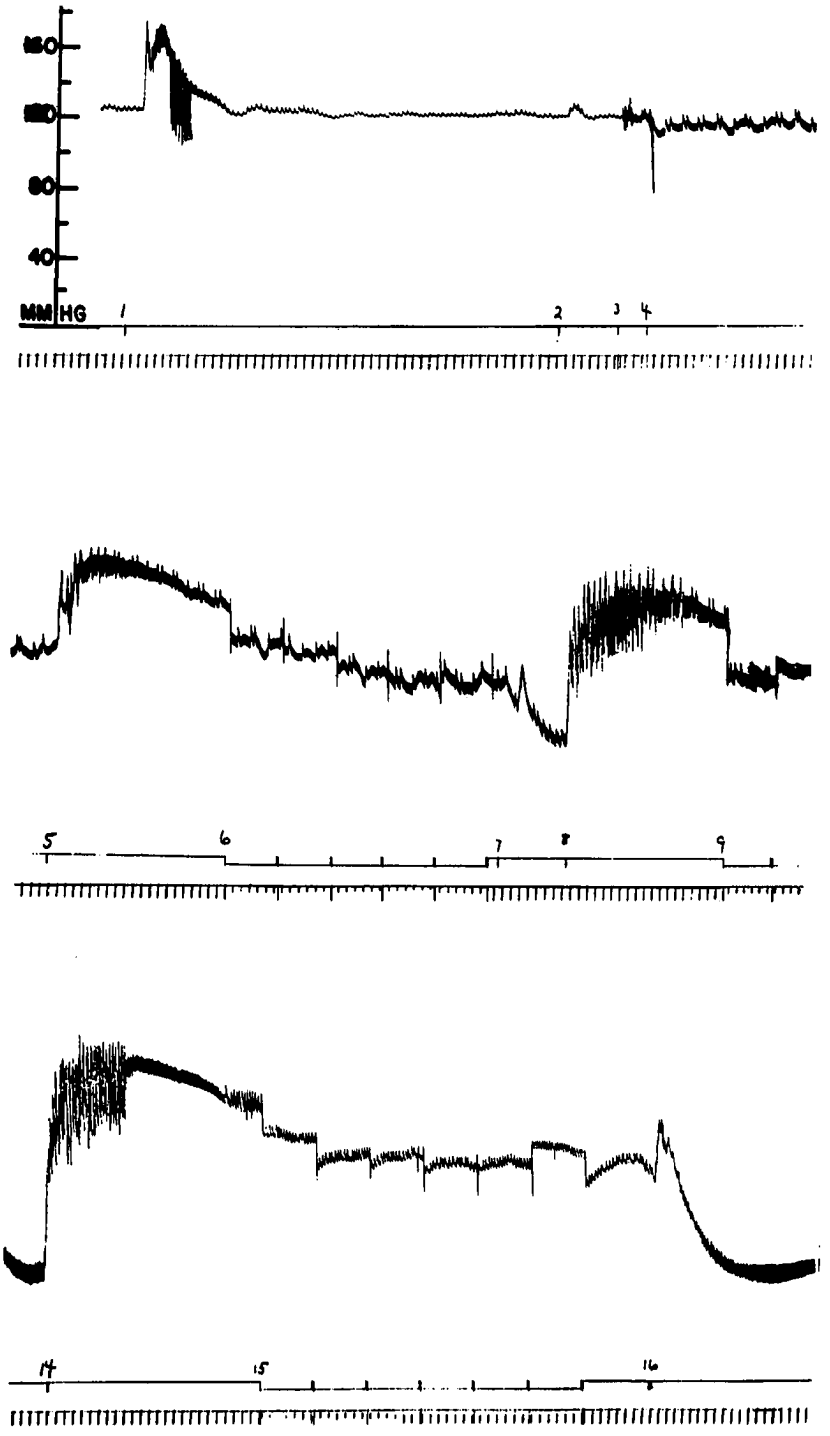

Fig. 8

against the depressor aftereffect of norepinephrine.

Experiments on the Ganglionic Blocking Effect of Norepinephrine

As stated in Chapter II, page 13, epinephrine and norepinephrine have been found to produce some degree of ganglionic blockade in the superior cervical and stellate ganglia of the cat. Ganglionic blockade causes marked hypotension because of inhibition of the normal passage of impulses to postganglionic autonomic nerves. Adrenergic nerves provide for the general tonus of peripheral blood vessels and stabilize peripheral resistance and blood pressure. If blood vessel tonus is reduced by ganglionic blockade, peripheral vasodilation ensues, peripheral resistance is lowered, and the result is hypotension or shock.

The aim of these experiments was to determine if ganglionic blockade was present both during and following infusion with norepinephrine (1 mg./kg./5 hrs.).

Vagal stimulation was chosen as a means of studying ganglionic transmission. There is ample evidence in the literature that parasympathetic ganglia react materially the same as sympathetic ganglia. It was felt, therefore, that failure of the blood pressure to decrease as a response to vagal stimulation would indicate vagal ganglionic blockade. It must be recognized that the effects of vagal stimulation might be somewhat diminished by the positive chronotropic and inotropic effects of norepinephrine on the myocardium. Thus, a decrease in the blood-pressure-lowering effect of vagal stimulation would not necessarily denote ganglionic blockade. However, any drop in blood pressure obtained by vagal stimulation would tend to substantiate the absence of blockade

since vagal action is opposed by the effect of norepinephrine on the sympathetic innervation of the heart.

Four animals were prepared for these experiments as discussed in Chapter III. Vagal stimulation was accomplished by using a 6 volt, 60 impulses/sec., 10 millisecond duration square wave stimulation. A vagal stimulation of 2 to 3 seconds was found adequate to produce a fall in blood pressure of 40 to 100 mm. Hg. prior to infusion. Vagal stimulation was then repeated each hour during and following infusion. Acetylcholine (10 mcg. I. V.) was given before and following the end of infusion. This provided a means of determining the ability of the animal to react to vagal stimulation. As in the control experiments the blood pressure gradually fell while the infusion progressed. The possibility of obtaining a decreased response to vagal stimulation under these circumstances is quite evident because it is difficult to further depress an action which is already depressed or stimulate one which is already stimulated. Therefore, the administration of acetylcholine also provided an indication of the ability of the animal to respond to a depressor influence when the blood pressure was depressed.

Fig. 9 (Dog 16) shows that the peripheral ganglia of the vagus were functioning both during and following infusion, since a fall in blood pressure was elicited by vagal stimulation throughout the experiment. One animal (Dog 17) showed some vagal block but also lacked the ability to react very strongly to acetylcholine. The intact vagus was stimulated in Dog 18 and the blood pressure was recorded from the right femoral artery. This was done in order to keep carotid reflexes intact. An adequate response to vagal stimulation was accomplished by stimulating

Fig. 9

Dog 16
Female 6.4 kg.

Upper Marker: 0 blood pressure
Lower Marker: Time: 10 seconds

2. Vagal stimulation.

3. Vagal stimulation.

4. Acetylcholine 10 mcg. I. V.

5. Norepinephrine 1 mg./kg./5 hrs. I. V. infusion started.

6. Kymograph: 1 min. on, 15 min. off

7. Vagal stimulation.

14. Vagal stimulation 4 hrs. after infusion started.

15. Kymograph: 1 min. on, 15 min. off.

16. Vagal stimulation 4 hrs. 55 min. after infusion started.

17. Norepinephrine 1 mg./kg./5 hrs. I. V. infusion stopped.

18. Vagal stimulation.

19. Vagal stimulation.

20. Acetylcholine 10 mcg. I. V.

21. Dog killed with ether I. V.

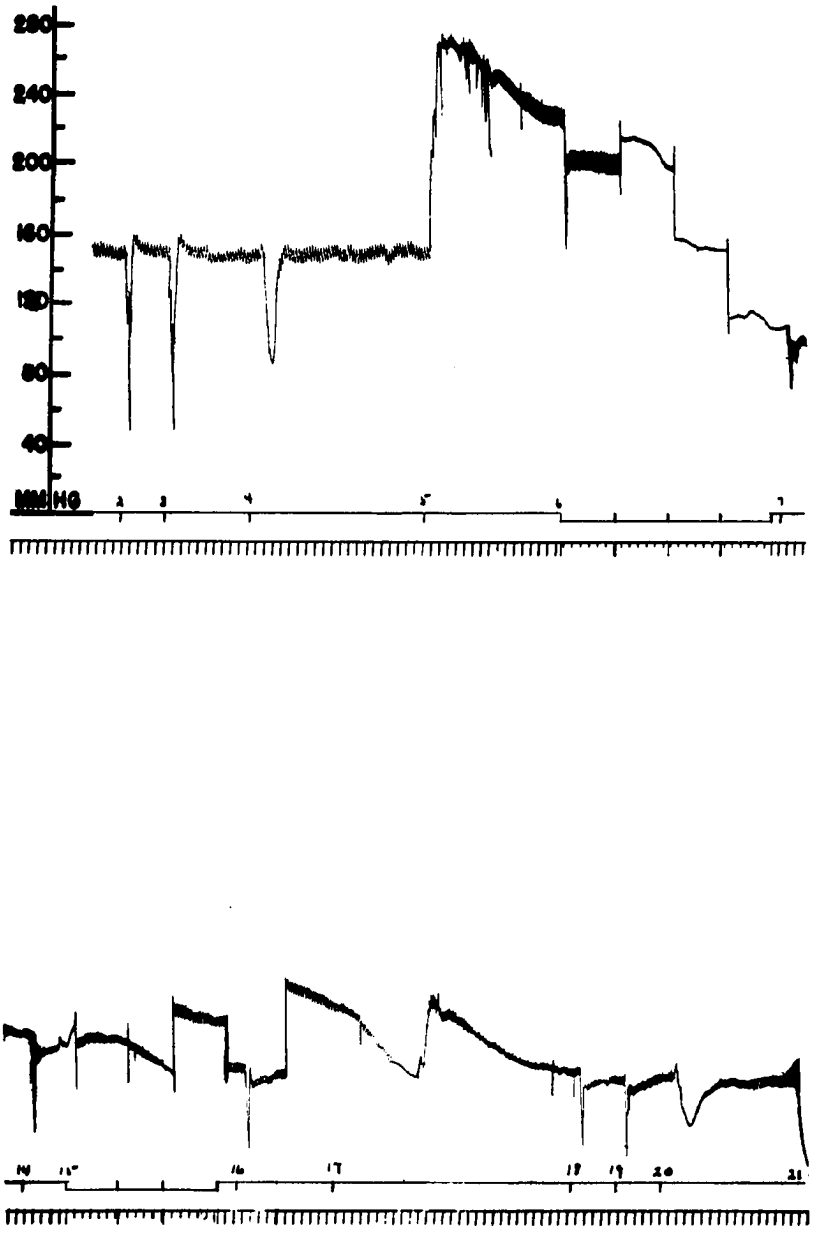

Fig. 9

for 5 seconds and advancing square wave duration to 20 milliseconds. In this experiment the response to stimulation was diminished during infusion. The animal died 12 minutes following the end of the infusion. Autopsy revealed massive pulmonary hemorrhage which was thought to be the immediate cause of death.

It is concluded from these experiments that ganglionic blockade is not a major factor in the production of the vasodepressor aftereffects of norepinephrine.

The Effect of Norepinephrine on Blood Glucose

Blood glucose was determined in Dogs 12 and 13 (Table I) and Dogs 11 and 14 (Table III). A control sample of blood was collected before infusion. Blood samples were then taken each hour during infusion. The control blood glucose values ranged from 44.4 to 55.6 mg. per cent. The samples taken at the end of each infusion ranged from 60.8 to 89.6 mg. per cent. In every instance the blood glucose level at the completion of infusion was well above control levels. Dog 11 (Fig. 8) was given 100 cc. of 5 per cent glucose intravenously after the infusion of norepinephrine was completed. It did not materially alter the blood pressure of the animal.

In conclusion, norepinephrine in the amount given causes a moderate degree of hyperglycemia with no evidence of hypoglycemia.

The Effects of Ephedrine and Antihistamines on Norepinephrine and Epinephrine

In preliminary experiments an attempt was made to determine the catechol amine concentrations in the blood of dogs receiving

norepinephrine by continuous infusion. The fluorophotometer (Klett) available, contains barrier-layer photocells and was found to have insufficient sensitivity to determine catechol amine concentrations under the conditions of the Weil-Malherbe and Bone method. Therefore, this work was not further pursued.

Since catechol amine determinations could not be made, it was decided to explore the destruction of norepinephrine by amine oxidase. This was done by administering a dose of norepinephrine to a dog and observing the effect on the blood pressure. An amine oxidase inhibitor was then given and when the blood pressure had stabilized the original dose of norepinephrine was repeated. A rise in blood pressure greater than the original was considered indicative of amine oxidase inhibition and a decrease in destruction of norepinephrine.

Animals 19, 20 and 21 (Table IV) were given the same pressor dose of norepinephrine before and following the administration of a dose of ephedrine sulfate (0.05 mg./kg. I. V.). All three of the animals showed an increased blood pressure response to norepinephrine following the ephedrine. The animals were then infused with norepinephrine (1 mg./kg./5 hrs.). At the end of each hour during the infusion, 0.05 mg./kg. of ephedrine was given intravenously. Fig. 10 (Dog 19, Table IV) shows that ephedrine given during the infusion augments the effect of norepinephrine as indicated by an immediate rise in pressure which is a much greater pressor effect than that obtained before infusion by the same dose of ephedrine. This effect was reproduced in all three animals tested although the augmentation diminished somewhat with time. The end result of norepinephrine infusion was not changed

TABLE IV

AUGMENTATION OF NOREPINEPHRINE AND EPINEPHRINE

Dog-Sex-Wt.	Augmenting Drug and Dose	Dose of Norepinephrine	Dose of Epinephrine	Area in Sq. In. Before Augmentor	Area in Sq. In. After Augmentor	Ratio After/Before
19-male-11 kg.	EPHEDRINE SO_4 0.05 mg./kg. I.V.	20 mcg. I.V.		1.030	1.290	1.252
20-male-9 kg.	EPHEDRINE SO_4 0.05 mg./kg. I.V.	10 mcg. I.V.		0.715	0.925	1.294
21-male-8 kg.	EPHEDRINE SO_4 0.05 mg./kg. I.V.	10 mcg. I.V.		0.320	0.565	1.766
23-male-10.9 kg.	PYRIBENZAMINE 1 mg./kg. I.V.	10 mcg. I.V.		0.400	1.410	3.525
34-male-8 kg.	PYRIBENZAMINE 1 mg./kg. I.V.	10 mcg. I.V.		0.440	0.990	2.250
		0.1 mcg. I.V.		0.030	0.020	0.667
			10 mcg. I.V.	0.250	0.510	2.040
35-male-6.4 kg.	PYRIBENZAMINE 1 mg./kg. I.V.	10 mcg. I.V.		0.570	2.890	5.070
		0.1 mcg. I.V.		0.040	0.030	0.750
			10 mcg. I.V.	0.170	0.800	4.706
24-male-10 kg.	PYRIBENZAMINE 0.5 mg./kg. I.V.	10 mcg. I.V.		0.210	1.815	8.643
		10 mcg. I.V.		0.285	1.815	6.368
			10 mcg. I.V.	0.105	0.540	5.143
			10 mcg. I.V.	0.105	0.540	5.143
27-male-12 kg.	PYRIBENZAMINE 0.5 mg./kg. I.V.	10 mcg. I.V.		0.180	0.430	2.389
			10 mcg. I.V.	0.100	0.250	2.500
	0.5 mg./kg. I.V., 1 hr. after initial dose	10 mcg. I.V.		0.200	0.520	2.600
			10 mcg. I.V.	0.180	0.190	1.056
25-male-12 kg.	TRIMETON 0.5 mg./kg. I.V.	10 mcg. I.V.		0.345	0.650	1.884
			10 mcg. I.V.	0.090	0.210	2.333

TABLE IV cont.

AUGMENTATION OF NOREPINEPHRINE AND EPINEPHRINE

Dog-Sex-Wt.	Augmenting Drug and Dose	Dose of Norepinephrine	Dose of Epinephrine	Area in Sq. In. Before Augmentor	Area in Sq. In. After Augmentor	Ratio After/Before
26-male-8.6 kg.	TRIMETON	10 mcg. I.V.		0.860	0.890	1.035
	0.5 mg./kg. I.V.		10 mcg. I.V.	0.055	0.185	3.364
28-male-12 kg.	BENADRYL	10 mcg. I.V.		0.190	0.190	1.000
	0.5 mg./kg. I.V.		10 mcg. I.V.	0.110	0.100	0.909
	0.5 mg./kg. I.V.,	10 mcg. I.V.		0.200	0.300	1.500
	1 hr. after initial dose		10 mcg. I.V.	0.210	0.200	0.952
29-female-12 kg.	BENADRYL	10 mcg. I.V.		0.360	0.630	1.750
	0.5 mg./kg. I.V.		10 mcg. I.V.	0.080	0.090	1.125
	0.5 mg./kg. I.V.,	10 mcg. I.V.		0.410	0.350	0.854
	1 hr. after initial dose		10 mcg. I.V.	0.050	0.070	1.400
22-male-11 kg.	BENADRYL 1 mg./kg. I.V.	10 mcg. I.V.		0.120	0.315	2.625
30-male-10 kg.	THEPHORIN	10 mcg. I.V.		0.670	0.660	0.985
	0.5 mg./kg. I.V.		10 mcg. I.V.	0.180	0.210	1.167
	0.5 mg./kg. I.V.,	10 mcg. I.V.		0.900	0.550	0.611
	1 hr. after initial dose		10 mcg. I.V.	0.240	0.140	0.583
31-male-9.5 kg.	THEPHORIN	10 mcg. I.V.		0.530	0.420	0.792
	0.5 mg./kg. I.V.		10 mcg. I.V.	0.080	0.030	0.375
	0.5 mg./kg. I.V.,	10 mcg. I.V.		0.420	0.170	0.405
	1 hr. after initial dose		10 mcg. I.V.	0.050	0.040	0.800

TABLE IV cont.

AUGMENTATION OF NOREPINEPHRINE AND EPINEPHRINE

Dog-Sex-Wt.	Augmenting Drug and Dose	Dose of Norepinephrine	Dose of Epinephrine	Area in Sq. In. Before Augmentor	Area in Sq. In. After Augmentor	Ratio After/Before
32-male-10.9 kg.	ANTISTINE	10 mcg. I.V.		0.390	0.330	0.846
	0.5 mg./kg. I.V.		10 mcg. I.V.	0.100	0.100	1.000
	0.5 mg./kg. I.V.,	10 mcg. I.V.		0.270	0.290	1.074
	1 hr. after initial dose		10 mcg. I.V.	0.070	0.090	1.286
33-female-10 kg.	ANTISTINE					
	0.5 mg./kg. I.V.	10 mcg. I.V.		0.390	0.290	0.744
			10 mcg. I.V.	0.200	0.150	0.750
	0.5 mg./kg. I.V.,	10 mcg. I.V.		0.180	0.130	0.722
	1 hr. after initial dose		10 mcg. I.V.	0.100	0.050	0.500

Fig. 10

Dog 19
Male 11 kg.

Upper Marker: 0 blood pressure
Lower Marker: Time: 10 seconds

5. Norepinephrine 20 mcg. I.V.
6. Ephedrine SO_4 0.05 mg./kg. I.V.
7. Norepinephrine 20 mcg. I.V.
8. Norepinephrine 1 mg./kg./5 hrs. I.V. infusion started.
9. Kymograph: 1 min. on, 15 min. off.
10. Ephedrine SO_4 0.05 mg./kg. I. V. 2 hrs. after infusion started.
12. Ephedrine SO_4 0.05 mg./kg. I. V. 3 hrs. after infusion started.
13. Kymograph: 1 min. on, 15 min. off.
14. Ephedrine SO_4 0.05 mg./kg. I. V. 4 hrs. after infusion started.
15. Kymograph: 1 min. on, 15 min. off.
16. Norepinephrine 1 mg./kg./5 hrs. I. V. infusion stopped.
17. Ephedrine SO_4 0.05 mg./kg. I. V.
18. Kymograph: 1 min. on, 15 min. off.

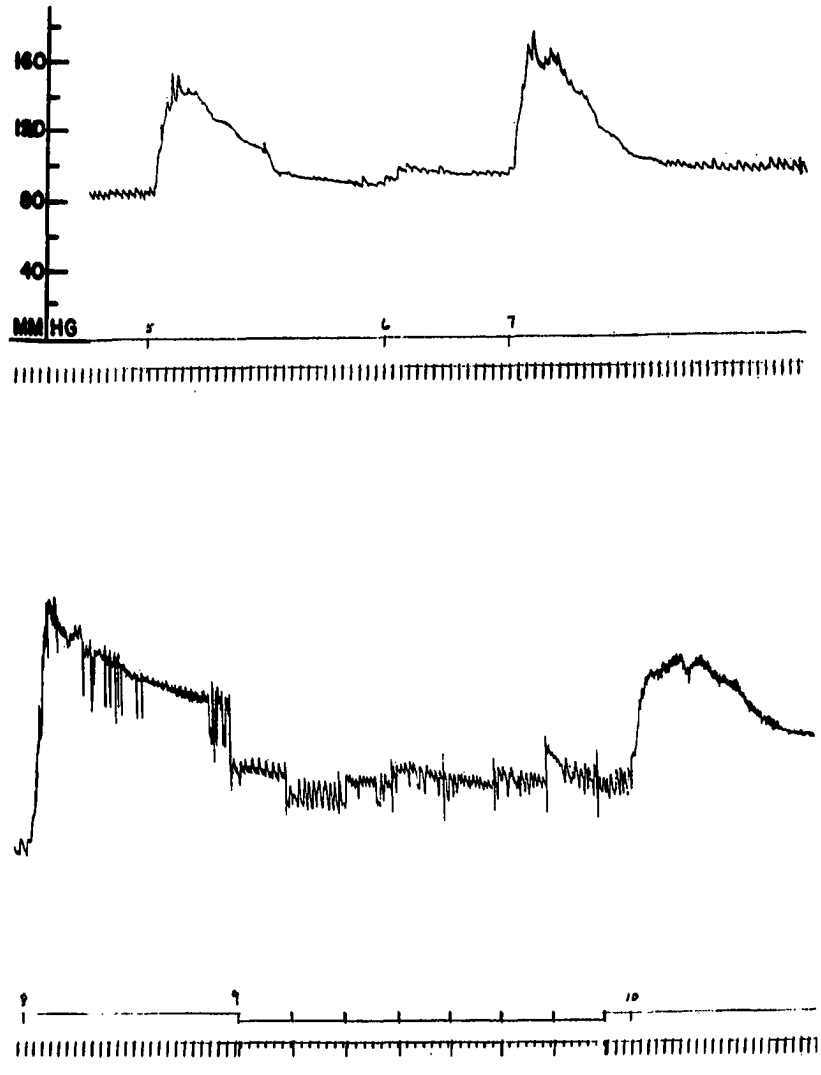

Fig. 10

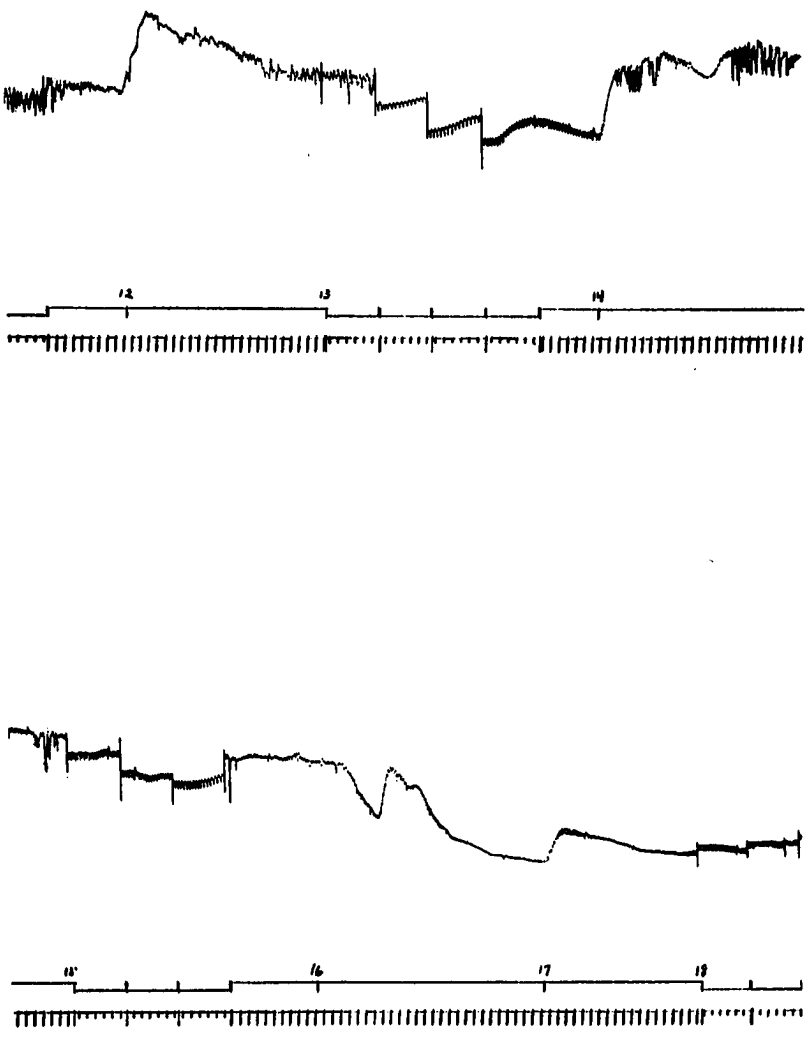

Fig. 10 cont.

significantly from that in the control animals. Dog 20, died 32 minutes after completion of the infusion and Dog 21, had a very low blood pressure 1 hr. and 41 min. after the end of infusion when killed.

Benadryl was given to Dog 22 at a dose level of 1 mg./kg. I. V. It proved to be a good augmentor at this dose level during the first hour of infusion. However, the augmenting effect decreased during the ensuing hours of infusion and failed to protect the animal against post-infusion hypotension. The animal was in profound shock when killed.

The same type of experiment was performed on Dog 23 using Pyribenzamine (1 mg./kg. I. V.) as the augmenting agent. Essentially the same picture developed as was found with Benadryl. The animal died 27 minutes following the end of infusion.

A number of experiments were performed to determine if other antihistaminic compounds were capable of augmenting the pressor effect of norepinephrine and epinephrine. The results are tabulated in Table IV. In those experiments in which a second 0.5 mg./kg. dose of the augmenting agent was given, the "Before" readings of the areas under the blood pressure curves were calculated from curves produced 45 minutes following the first dose of the augmenting drug. The "After" readings were taken five minutes following each dose of the antihistaminic compound.

Table IV reveals that ephedrine, Pyribenzamine, and Trimeton are very good augmenting agents. Benadryl appears to be a relatively weaker augmenting agent. Thephorin and Antistine seem to have little, if any, augmenting effect. In most cases, they inhibit to some degree the pressor action of norepinephrine and epinephrine.

A point worth noting is that those antihistamines which augment, usually augment the pressor effect of norepinephrine to a greater degree than that of epinephrine (Fig. 11). This would tend to conform with the report of Blaschko and Burn (29) which states that amine oxidase has a greater effect on norepinephrine than on epinephrine. Very small doses of norepinephrine (0.1 mcg.) do not appear to be augmented by Pyribenzamine (Fig. 11). This fact would seem to be a paradox. If amine oxidase is the mechanism by which augmentation takes place, small doses of norepinephrine should be augmented to the same degree as larger doses. This paradox might be explained on the basis that not all of the amine oxidase has been inhibited. Thus, very small doses of norepinephrine would be destroyed rapidly and no augmentation take place.

These experiments show that the antihistaminic properties of these compounds are unrelated to their augmenting effect on norepinephrine. It has been stated previously that norepinephrine increases blood histamine, which would tend to reduce the response to the pressor amine. Pyribenzamine and Thephorin are both potent antihistamine compounds and should theoretically have approximately equal ability to block the effect of the histamine released by norepinephrine. However, Pyribenzamine produces augmentation of the norepinephrine pressor effect while Thephorin does not.

Augmentation of the pressor effect of norepinephrine by Pyribenzamine (0.5-1 mg./kg.) suggested that animals so treated might be useful as test subjects for biological assay.

Patients who harbor pheochromocytoma, for example, excrete free epinephrine and norepinephrine in amounts ranging from 200 to above

Fig. 11

Dog 35
Male 6.4 kg.

Upper Marker: 0 blood pressure
Lower Marker: Time: 10 seconds

1. Norepinephrine 10 mcg. I. V.
2. Epinephrine 10 mcg. I. V.
3. Norepinephrine 0.1 mcg. I. V.
4. Pyribenzamine 1 mg./kg. I. V.
5. Norepinephrine 10 mcg. I. V.
6. Epinephrine 10 mcg. I. V.
7. Norepinephrine 0.1 mcg. I. V.
8. Norepinephrine 0.05 mcg. I. V.

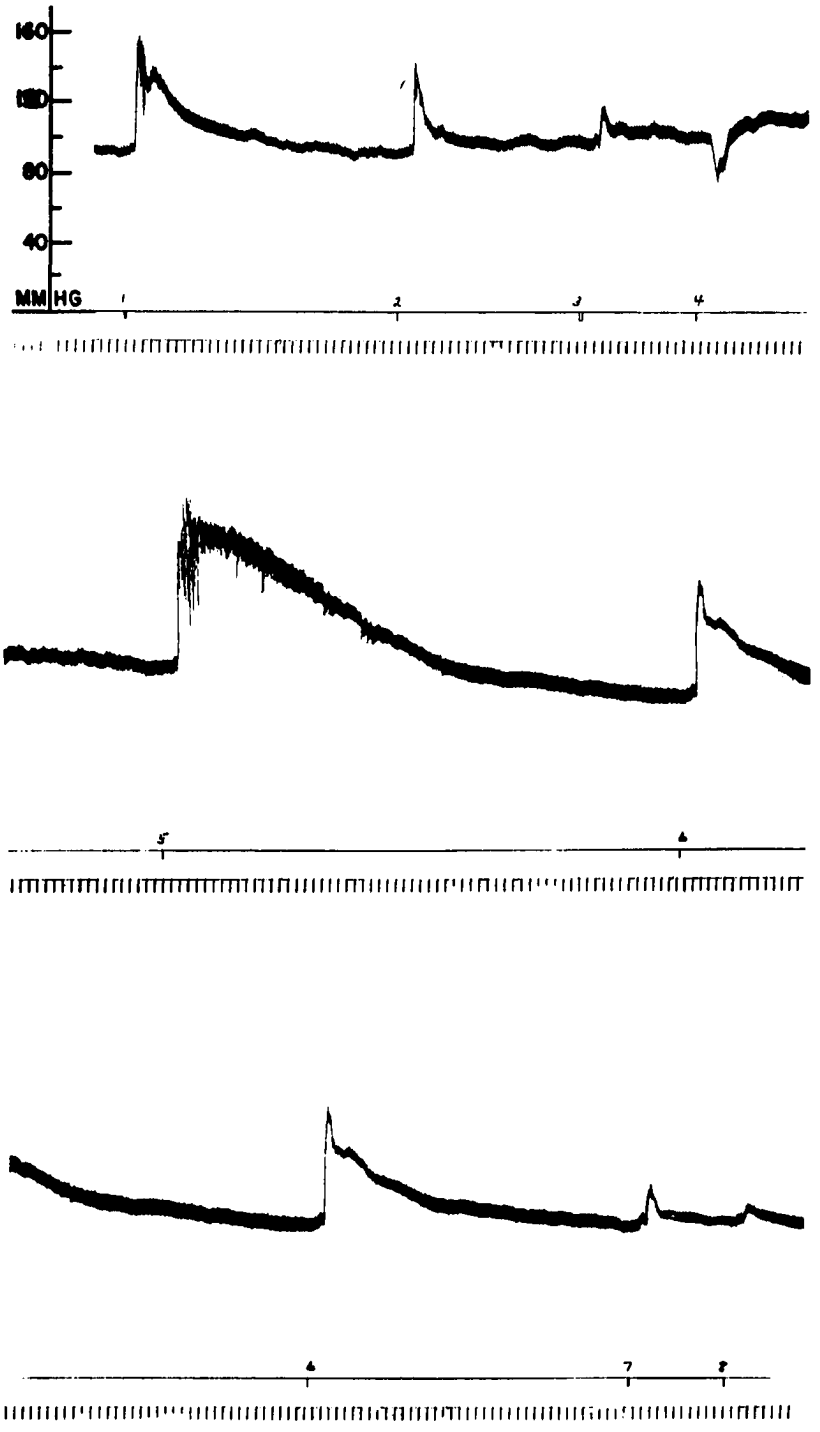

Fig. 11

3000 mcg./24 hours in the urine. A successful method for the detection of pheochromocytoma, based on the change in blood pressure produced by injection of untreated urine into test animals, has been described (95). The present experiments show that sensitization of the test animal with Pyribenzamine improves the capabilities of this assay procedure, since such treatment makes it possible to "titrate" the concentration of pressor amines in the urine in amounts as low as 0.01 mcg./cc. A number of urine specimens taken from patients suspected of having pheochromocytoma have been assayed by this method and found to contain from 10 to 50 mcg. of pressor substances per 1000 cc. of urine. This is well within the normal range of patients who have essential hypertension or other hypertensive disorders but not pheochromocytoma.

CHAPTER V

SUMMARY AND CONCLUSIONS

1. Long-term infusion of norepinephrine in dogs invariably shows two distinct effects: 1) the blood pressure rises precipitously at first, but in a few minutes gradually declines until it eventually attains or falls below pre-infusion levels; 2) after discontinuing the infusion, the blood pressure falls to an even lower level.
2. Most of the animals which were infused with norepinephrine at the dosage levels used in this study showed by gross inspection ischemic viscera, acute passive congestion of the liver, edema of lungs and limbs, distention of the stomach by gas, and recent focal hemorrhages of the lungs.
3. The results of long-term infusion of norepinephrine indicate a progressively decreasing cardiovascular response to the pressor effect of this agent.
4. Dibenzyline in doses of 5-10 mg./kg. I. V. did not protect against the depressor effect of the infused norepinephrine. To the contrary, it caused a reversal of the pressor effect of norepinephrine and epinephrine.
5. GD-131 caused some reduction of the pressor effect of norepinephrine but did not cause norepinephrine reversal as did Dibenzyline at the

same dose level. GD-131 did not protect against the post-infusion hypotension due to norepinephrine. No conclusions can be drawn from these studies concerning vasodepressor material (VDM) as a contributing factor in producing the vasodepressor aftereffect of norepinephrine.

6. The ganglionic blocking effect of norepinephrine is not thought to be a major factor in the development of the vasodepressor effects of norepinephrine.

7. Blood glucose determinations in animals infused with norepinephrine revealed a moderate degree of hyperglycemia. This confirms previous reports in the literature.

8. Ephedrine, Pyribenzamine, Trimeton and Benadryl in doses of 0.5 mg./kg. I. V. augment the pressor effect of single doses of norepinephrine and epinephrine. Ephedrine, Pyribenzamine and Benadryl produce an augmenting effect in the first hour of an infusion of norepinephrine (1 mg./kg./5 hrs.). This effect diminishes with time. None of the agents materially change the end result of norepinephrine infusion from that of the control experiments. However, the results of these experiments are compatible with the theory that norepinephrine is being destroyed by amine oxidase during and following infusion.

9. Animals treated with Pyribenzamine (0.5 -1 mg./kg. I. V.) are very sensitive to pressor substances found in the urine of normotensive and hypertensive patients. It is suggested that the sensitization of dogs by Pyribenzamine will improve the bioassay method for the determination of pressor substances found in the urine of patients suspected of having pheochromocytoma.

ADDENDUM

Following the completion of the preliminary draft of this dissertation, a paper entitled "Secondary fall in blood pressure following noradrenaline infusion in the cat", by H. Duner and U. S. von Euler, appeared in Acta Physiol. Scandinav., 38: 355 (1957).

These authors have concluded that the fall in blood pressure after noradrenaline is partly due to a blocking effect on transmission within the vasomotor system. This interpretation is based on the following observations: 1) after buffer nerve section, when no counter-regulation is effective on the blood pressure, the post-infusional fall is enhanced and prolonged, 2) after administration of a ganglionic blocking agent no further block would be effective and the fall is reduced to a short-lasting fraction.

The second statement appears to be an erroneous assumption, for theoretically, ganglionic blockade should give the same end result as sectioning of the buffer nerves. It is felt that the conclusions presented in this paper are not warranted by the data presented. To the contrary it would seem more probable that their data support more fully the conclusions of this dissertation on this matter.

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