ON THE PHARMACOLOGY OF

PHENOXYBENZAMINE

by

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INTRODUCTION

The concept of antagonism has proved to be a fruitful approach in efforts to gather information on many physiological and pharmacological problems. In the case of the sympathetic system, ergot was used as early as 1906 by Dale, who then supplied an accurate description of what we know as 'adrenergic blockade'. This pharmacological effect may be defined as the blockade of responses to adrenaline, noradrenaline and other sympathomimetic amines and to sympathetic nerve activity. However, it is well known that all responses are not blocked, but the term 'adrenergic blockade' has not been replaced by a more accurate or descriptive expression. This is perhaps partly due to our incomplete knowledge of the locus and mechanism of action of drugs classified as adrenergic blockers. Dale's work was followed by very few important observations for some forty years.

With advances in chemical synthesis, and renewed interest in this problem, a large number of different types of agents, some of which appear to exert a relatively more specific action, have now been introduced. (Bovet and Bovet-Nitti, 1948; Nickerson, 1949).

As a representative of this later group, phenoxybenzamine (dibenzyline) (N-benzyl-N-phenoxyisopropyl-β-chloroethylamine) was selected for detailed pharmacological study. This compound, which has been extensively studied by Nickerson and co-workers (Nickerson and

Goodman, 1947; Nickerson, 1949; Nickerson, Henry and Nomaguchi, 1953) has been claimed to induce a prolonged and complete adrenergic blockade. From these studies of Nickerson and co-workers, it has been concluded that adrenergic blockade by phenoxybenzamine results from a selective stable chemical union between the antagonist and the "adrenergic receptors" to form what has been described as a "non-equilibrium" antagonism. (see section B in Historical).

In earlier studies from this laboratory (Benfey et al., 1958) it was observed that in dogs under phenobarbital anesthesia, following adrenergic blockade induced by piperoxane, chlorpromazine and hydergine, there was an increased urinary excretion of injected adrenaline and noradrenaline. It has also been reported by Brown and Gillespie (1957) that following blockade with dibenamine or phenoxybenzamine, there was an increased venous output of noradrenaline from the spleen in cats when the splenic nerves were stimulated electrically. It would appear from these observations that quantitative changes in the 'adrenergic transmitters' might therefore be involved in adrenergic blockade. These findings would also imply that there is no significant destruction of the transmitter substance or substances during this type of blockade.

In view of the above observations, it was of interest to investigate more thoroughly the problem of the relationship between the excretion of the catecholamines and phenoxybenzamine blockade. In this connection and with the hope of elucidating the possible underlying mechanisms involved, a systematic study was undertaken of the

changes in the urinary excretion of the amines under various experimental conditions, with special reference to the concomitant changes in systemic blood pressure. Comparisons have also been made between the influence of phenoxybenzamine on the excretion of the amines from their endogenous sources or following infusions of adrenaline or noradrenaline.

HISTORICAL

A) Studies on the urinary excretion of adrenaline and noradrenaline during adrenergic blockade.

In 1951, in connection with studies on the metabolism of injected adrenaline, Bacq and co-workers could observe no effect of various blocking agents (F 933, F 883, yohimbine or ergotamine) on the percentage of injected adrenaline recoverable in urine. These authors used dogs anesthetized with chloralose, and large doses of adrenaline (1 mg./kg. of racemic adrenaline stabilized with BAL) were infused during a period of two hours. The blocking agents were injected intravenously in divided doses during the period of infusion. Adrenaline was measured by a biological assay method in cats under dial anesthesia, using the sensitized nictitating membrane (denervation or cocaine) for the test. With improvement in methods for the quantitative estimation of the catecholamines, in 1958, Benfey, Mazurkiewicz, and Melville employed a modification of the method of Euler and Floding (Euler and Floding, 1955; Millar and Benfey, 1958) to estimate the relative quantities of adrenaline and noradrenaline excreted in the urine before and after piperoxan, hydergine, and chlorpromazine. During these experiments,an infusion of 5.4% glucose was used to promote diuresis. These workers observed that "...following adrenaline and noradrenaline injections after previous adrenergic blockade, there is a strikingly increased adrenaline and noradrenaline excretion." When given alone,

the antisympathomimetic drugs piperoxan and chlorpromazine also markedly elevated the urinary adrenaline excretion.

Using a similar chemical method of estimation for the amines, Schapiro reported in 1958 also that over a period of several days, in unanesthetized rats injected intraperitoneally with phenoxybenzamine (10 mg./kg.), there was an increased urinary excretion of noradrenaline but no change in adrenaline. This author also showed that a loss of noradrenaline could be detected in the heart and spleen of such animals at autopsy.

There are no other previous studies in the literature concerning the urinary excretion of the amines during adrenergic blockade, and the underlying mechanism is still obscure.

B) Studies regarding the mechanisms concerned with "adrenergic blockade".

It has been well established that adrenaline and noradrenaline are intimately concerned with the functions of adrenergic neurones and adrenergic activity in general, and that they probably represent the most important "chemical transmitters". (Euler, 1956; Gaddum and Holzbauer, 1957). On the basis of extensive pharmacological and physiological studies, the term "adrenergic blockade" may be conveniently defined as the condition in which responses, either to certain effects of adrenaline, noradrenaline and other sympathomimetic agents or to adrenergic nerve stimulation, are inhibited (blocked) at postganglionic neuroeffector adrenergic sites.

Investigations of mechanisms underlying such a blockade have taken into account the assumption, easily complementing the concept of chemical transmitters, that the latter react with a "receptor" in order to produce an effect. Nickerson (1959) has expressed this concept in the following terms: "we may assume that activation of a cell by adrenaline or noradrenaline involves a primary combination of the stimulant with some cell constituent which then activates a chain of events or reactions of undetermined length and nature, culminating in the measured response."

In 1906, Dale used ergot alkaloids in pithed cats, and differentiated for the first time two distinct types of adrenergic responses: some which were excitatory (e.g. in arteries, uterus and the sphincter of the iris) and could be "paralysed" by ergot; some which could not be paralysed and were inhibitory (e.g. in stomach and intestines, gall-bladder, etc.). This would suggest the existence of what might be called "excitatory receptors" and "inhibitory receptors", although it has been difficult to include adrenergic receptors in the heart in this type of classification.

More recently, Ahlquist (1948) has proposed a classification of receptors, into α-receptors, that is those concerned with stimulation of smooth muscles (blood vessels and nictitating membrane) and of some exocrine glands, but with inhibition of intestinal activity, and β-receptors, that is those concerned with relaxation of smooth muscle

(vascular) and cardiac stimulation. This concept was based on the comparative blocking responses observed with a series of six sympathomimetic amines: 1. l-adrenaline, 2. d,l-adrenaline, 3. noradrenaline, 4. methyl-noradrenaline, 5. methyl-adrenaline, 6. N-isopropyl-noradrenaline. Thus on functions corresponding to α-receptors, i.e. vasoconstriction, contraction of the nictitating membrane and inhibition of the gut those agents showed the following decreasing order of potency: 1-2-3-4-5-6. In contrast, on functions which would correspond to β-receptors, i.e. vasodilation and myocardial stimulation, the order of decreasing potency was 2-4-6-5-3-1-. Furthermore, Ahlquist showed that adrenergic blocking agents such as ergot, dibenamine, priscol and tolazoline could block responses mediated through α-receptors whereas responses mediated through β-receptors remained unaffected.

Dale (1906) had previously shown that ergot selectively blocked the action of adrenaline on the excitatory or "motor" (presumably α) receptors in many vascular smooth muscles, but led to a "reversal effect" i.e. a relaxing effect on blood vessels and fall in blood pressure because of the action of adrenaline on unblocked "inhibitory" (presumably β) receptors.

In 1937, Clark and his co-workers were the first to use the concept of drug-receptor interaction to make quantitative studies of the actions of drugs on tissues. In order to derive an equation which would relate the variables involved in a drug-receptor interaction, Clark

employed a number of basic assumptions which may be briefly summarized as follows: a) the reaction between drugs and specific receptors is reversible and obeys the law of mass action; b) receptors all have the same affinity for drugs; c) the magnitude of the response is directly proportional to the fraction of the total number of receptors combined with the drug eliciting the response, and d) there is an all-or-none action on receptors by drugs combining with them.

On the basis of Clark's postulates, three different types of mechanisms responsible for adrenergic blockade have been reported in the literature (Gaddum, 1957) by workers, using a mathematical approach similar to that employed in deriving equations for the inhibition of enzymes. However, as Furchgott has pointed out (1955), actual experimental evidence is available for only one of these three types (see below).

1. Non-competitive antagonism. A "non-competitive" type of antagonism, as theoretically defined (Gaddum, 1957) would be expected to show that two drugs combine with different parts of the receptor mechanism, so that the presence of either does not exclude the other, but when the antagonist is present the agonist (the active drug, e.g. adrenaline) is ineffective. Chen and Russell, in 1950, made some quantitative studies of the vasopressor effect of adrenaline in dogs under chlorbutanol anesthesia, by comparing the effects of increasing doses of the cardiovascular agent before and after treatment with SY 28 (an antisympathomimetic agent) or after yohimbine.

However, this type of approach has been questioned by Nickerson (1957) who emphasized the complexity of the system used and pointed out that any relation of reactions such as a pressor response to adrenaline in intact animals to theoretical drug adsorption curves, must be fortuitous.

2. Competitive antagonism. A second type of adrenergic blockade has been termed "competitive reversible antagonism" or "classical competitive antagonism". (see Nickerson, 1959). In this blockade, the antagonist interferes with the reaction of agonist with specific receptors, and appears to be in mass-action equilibrium with the latter. There is thus a competition between agonist and antagonist for receptor occupancy.

In this connection, Gaddum, in 1937, was the first to point out, using some of Clark's assumptions, that much of the quantitative data on the response of tissues to agonists in the presence of antagonists, could be explained on the basis of competition between drug and antagonist for the same receptors. The results could then be predicted by a simple application of the mass laws in terms of, for instance, the final concentrations of the drugs in a bath. (Gaddum, 1937). Thus, as early as 1928, Mendez had found that on the isolated rabbit uterus, ergotamine blocked the effects of adrenaline in a constant ratio. On the basis of his evidence, Mendez described the phenomenon as a "mass-equilibrium" blockade and showed that if the concentration of adrenaline needed to produce a given action in the absence of ergotamine be subtracted from the concentration of adrenaline producing the same action

in the presence of ergotamine, then the ratio between this difference and the concentration of ergotamine is constant.

However, this ratio, as pointed out by Nickerson, (1949) may only imply a dynamic equilibrium between the blocking and exciting agent with some specific grouping or locus in the cell. Moreover, in the case of dibenamine (Nickerson, 1949) it could be shown that once an adequate block had been established, it could not be overcome by massive adrenergic stimulation. (see below)

Fleckenstein (1952) investigated the anti-adrenaline potencies of various substances in a comparative study on the perfused vessels of the rabbit's ear, and found that ergot alkaloids, phentolamine and dibenamine were "specific" inhibitors. However, these agents antagonized the vascular constriction produced by both adrenaline and histamine to a similar extent, but when washed out, the response to adrenaline remained reduced for a much longer time than that to histamine.

3. Non-equilibrium competitive antagonism. In 1956, Nickerson using isolated strips of guinea pig ileum, showed that histamine dose-response curves could be shifted along the dose axis without changing the slope, by low concentrations of the antihistaminic agent G-D-127 *, but that high concentrations altered the slope considerably while still further shifting the curve to the right. Similar shifts of

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* N-1-naphthylmethyl-N-ethyl β -chloroethylamine.

adrenaline and histamine dose-response curves have been reported to occur after exposure of tissues to dibenamine (Furchgott, 1955). This type of evidence has prompted the speculation that certain drugs (e.g. the β-haloalkylamines) react with specific receptors in an almost irreversible manner so that they reduce the number of available receptors. But, it may be assumed that a maximal response is elicited by a relatively small number of specific receptors, and as the total number of free receptors is progressively decreased by increasing doses of the blocking agent, larger and larger concentrations of the agonist are needed for any given response. Ultimately (i.e. when the slope flattens out) the total number of free receptors is reduced below that required for a maximal response, even when they may be flooded with very high concentrations of the agonist.

The above studies, have led to the concept of a third type of blockade in which the antagonist combines with the same receptors as the agonist, but then reacts with the receptor or adjacent group to form a relatively stable chemical bond, i.e. a non-equilibrium blockade.

From their detailed studies on dibenamine blockade, Nickerson and Goodman (1947) found that, in cats under pentobarbital anesthesia, dibenamine blocks and reverses the effects of adrenaline <u>over a wide dose range</u>. Thus, unlike the effect obtained with ergot alkaloids, intravenous doses of adrenaline ranging from 0.1 μg/kg. to 10 mg./kg.

(100,000 times) are blocked and reversed.

In 1948, Nickerson and Nomaguchi also reported quantitative studies on the inhibition by dibenamine of the response of the cat nictitating membrane to electrical stimulation of the cervical sympathetic nerve and of the response of the chronically denervated nictitating membrane to various sympathomimetics and other agents. They found that, after administration of adequate blocking doses of dibenamine (15 mg./kg.), the control response of the nictitating membrane can never be reproduced or approached even by maximal nerve stimulation or the concentrations of adrenaline as large as could be tolerated by the intact animal.

Furchgott (1954) used spiral strips of rabbit aorta to test both competitive and non-competitive types of blockade with dibenamine and dihydroergotamine. He found that sensitivity to adrenaline returned at a slow rate, almost identical for the two substances. On the other hand, high concentrations (no figures stated) of adrenaline present during exposure to dihydroergotamine supplied no protection against blockade, whereas, in contrast, a good protection was obtained in similar experiments with dibenamine.

In 1949, Nickerson and Gump also observed in cats (pentobarbital or urethane anesthesia) that <u>during the early stages</u> of development of the dibenamine adrenergic blockade, an "equilibrium" between adrenaline and the blocking agent is readily demonstrated, and the

presence of sympathomimetic agents markedly inhibits the production of blockade. However, once the blockade has become established, it cannot be overcome by massive doses of the agonist or exciting agent (Nickerson and Goodman, 1947; - Nickerson and Nomaguchi, 1948).

This phenomenon has been confirmed by observations of Furchgott on isolated strips of rabbit aorta (Furchgott, 1954). However, in this preparation, the contractile responses to a variety of stimulating drugs (adrenaline, noradrenaline, isopropyl-noradrenaline, 5-HT, histamine, acetylcholine) can be blocked by pre-treatment with dibenamine. The significance of this is not clear in view of the claimed specificity of the dibenamine adrenergic blockade.

It is also evident from the above observations that "adrenergic blockade" is a complex phenomenon which appears to differ with different types of agents, and no definite conclusions can be drawn at present regarding the exact mechanisms involved. The problem clearly requires further study.

METHODS

A. General procedures

Both dogs and cats were used in these experiments. The animals were anesthetized with pentobarbital sodium. A dose of 30 mg./kg. injected intravenously usually sufficed. However, additional small amounts (1 to 3 mg./kg.) were necessary in most experiments to maintain a satisfactory anesthesia. In the cats, preliminary ether anesthesia was employed during the cannulation of a femoral vein (approximately 5 minutes).

In two experiments, the spinal cord was transected between C_1 and C_2 in dogs under pentobarbital anesthesia with artificial respiration. This was done to exclude any central nervous effects.

In the experiments in which analysis of the urine for catecholamines (see below) was done, in order to promote urine secretion, the animals were given a continuous intravenous (femoral) drip of 0.9% saline at the rate of 0.2 ml/kg./min. throughout the experiment. For this purpose, a cannula attached by a short piece of rubber tube to a burette filled with saline, was inserted into a femoral vein.

In dogs, urine was collected from the ureters directly. A small (approximately 10 cm.) incision was made through the abdominal wall in the midline, the ureters were then gently dissected out for a distance of about 10 cm. from the bladder, and tied off close to the bladder. A long (about 60 cm.) polyethylene tube with a bevelled

edge was then inserted into the ureters and tied in place. The free ends of the tubes were placed in a cylinder, so that a pooled urine sample from both kidneys was collected.

In similar experiments in cats, the bladder was cannulated with a small glass cannula which was connected to a polyethylene tube.

Following cannulation of the ureters on the bladder, in all experiments, heparin, (5 mg./kg.) was injected intravenously. The blood pressure was also recorded directly on a kymograph using a mercury manometer. In dogs, the pressure was taken from a femoral artery, and in cats from a common carotid.

The surgery and preparation of the animal took about 45 minutes, at the end of which time collection of the first sample of urine was started. Each sample for analysis corresponded to a 30 minute period. Immediately following collection, the urine was acidified to a pH between 3 and 4 with sulfuric acid (1 N). The urine was then filtered and stored in a cold room (4° C).

The first two periods of collection were generally used as "controls", and no drugs administered until 60 minutes after the experiments were set up. However, in some of the experiments with hexamethonium, the drug was given during the initial control periods.

In the course of this investigation, the following drugs were used:

1) Adrenaline bitartrate: (Suprarenin)[1].

2) Noradrenaline bitartrate; (Levophed)[1].

3) Phenoxybenzamine hydrochloride; (Dibenzyline)[2] referred to as phenoxybenzamine.

4) Hexamethonium bromide; (Vegolysen)[3] referred to as Hexamethonium.

5) Methacholine chloride; (Methacholine, Merck).

6) Acetylcholine chloride; (Acetylcholine, Hoffman-Laroche).

7) Atropine Sulfate; (Atropine, Merck).

8) Heparin; (Heparin, sodium salt-110 un. per mg-, Connaught Medical and Research Lab. Toronto).

9) Pentolinium tartrate (Ansolysen)[3] Pentamethylene-1:5-bis-(1 methylpyrrolidinium) tartrate, referred to as pentolinium.

10) Phentolamine Methanesulfonate; (Regitine, Ciba) 2-[N,p'-tolyl-N-(m'-hydroxyphenyl)-amino-methyl] -imidazoline, referred to as phentolamine.

11) Piperoxan HCl ; (Benzodioxane, Poulenc Ltd) 2-Piperidinomethylbenzo-1; 4-dioxane.

12) Tetraethylammonium bromide; (TEA, Eastman Organic Chemicals).

13) Pempidine tartrate; (Pempidine, Ayerst, McKenna, and Harrison, Mtl.) 1, 2,2,6,6-pentamethylpiperidine, referred to as pempidine.

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(1) Kindly supplied by Sterling-Wintrop Research Institute, Rensselaer, N.Y.
(2) Kindly supplied by Messrs. Smith, Kline and French, Montreal.
(3) Kindly supplied by Poulenc Ltd., Montreal.

Adrenaline and noradrenaline were made up in stock solutions of 1mg. base/ml. in 0.2N acetic acid (pH-4). Immediately before use, suitable dilutions were made in 0.9% sodium chloride. All doses and concentrations of catecholamines are expressed in terms of base. All other drugs are referred to, quantitatively, in terms of the salts mentioned in the nomenclature.

Phenoxybenzamine was dissolved in either propylene glycol or 95% alcohol, according to procedures described by Nickerson and Goodman (1947) for dibenamine, as follows:-

1. The required dose of the powder was weighted out and dissolved in 1 ml. of propylene glycol to which a few drops of 1% sulfuric acid were added. Then, slowly, 0.9% saline was added to make 20 ml.; if the solution became cloudy, the addition of a few more drops of diluted sulfuric acid rendered the solution clear again. This volume was slowly injected at the rate of 2 ml. a minute, a stop watch being used. 2. Alternatively, the powder was dissolved in less than 1 ml. of 95% alcohol and the volume was made up to 10 ml. with saline. This gave a stable milky suspension which was injected at the rate of 1 ml. a minute.

Both types of the above solutions were very acidic (pH between 2 and 3). Indeed, phenoxybenzamine alone, when added to propylene glycol or dissolved in 95% alcohol without the addition of acid, also gave an acidic reaction (pH less than 3). The results obtained with

either solvent were identical. All other drugs used were freshly dissolved in 0.9% sodium chloride prior to administration.

B) Assay of Catecholamines.

The procedure employed was a modification of the technique of Euler and Floding (1955) as previously described by Millar and Benfey (1958). This consisted of two operations: a) extraction of adrenaline and noradrenaline from the urine samples; b) differential assay of the mixture by a trihydroxyindole fluorescence method.

a) Extraction Procedure.

The purpose of this preliminary treatment was twofold: (1) to remove interfering substances and (2) to concentrate the catecholamines. This was accomplished by adsorbing the active substances on alumina, after which a concentrated acidic eluate is obtained containing a mixture of adrenaline and noradrenaline. The adsorption was carried out in a glass column (approximately 0.5 cm. in diameter) with a central bulbous section (approximately 5 cm. in diameter). A constriction in the limb below the bulb supported a small cotton plug, above which the alumina collected.

Urine samples obtained during experiments were stored in a cold room and purified within the next 24 hours. All pH measurements were made with a Beckman pH-meter, Model G, with glass electrodes.

To each sample was added one third its volume of 3% sodium thiosulfate and approximately 500 mg. of a chelating agent, disodium ethylenediamine tetra-acetate. This mixture was stirred by an electrically driven glass rod while pH was carefully adjusted to 8.4-8.5 with 2% sodium hydroxide. The sample was then ready for adsorption on alumina.

Approximately 700 mg. alumina (Merck, Acid-washed) were placed in the bulb and 5 ml. of acetate buffer (pH 8.5) added. The alumina was allowed to settle after gentle stirring with a glass rod so that air bubbles would be removed from the alumina. The supernatant fluid was then forced through the column by air pressure.

A urine sample, prepared as described above, was then poured into the top of the column and forced through by air pressure at a constant rate (2 ml./min.). Air was always prevented from entering the column of alumina by discontinuing the pressure before all the fluid above the alumina was removed. The urine which remained in the column was washed through with an additional 5 ml. of the acetate buffer, which was in turn washed with 5 ml. of glass redistilled water.

Elution was then made with 5 ml. of acetic acid (0.2 N) followed by 5 ml. of glass redistilled water. The eluate containing the mixture of catecholamines was made to an exact volume of 10 ml. Prior to the elution, a period not exceeding 15 to 20 minutes elapsed, during which the samples were alkaline. This, as confirmed by other workers (Euler and Floding, 1955; Euler, 1956) does not lead to any significant

loss of catecholamines. If not assayed immediately after extraction, the eluates were stored in a cold room (4° C).

b) Differential Assay of the Eluates.

In this method, adrenaline and noradrenaline are oxidized to adrenochrome and noradrenochrome, respectively. These oxidation products are then rearranged in alkali to strongly fluorescent trihydroxyindoles, adrenolutine and noradrenolutine, respectively (see Lund 1949). Differential estimation of these compounds is performed by using the spectral characteristics.

The general procedure employed was as follows: to 1 ml. of eluate, 2 ml. of sodium acetate (pH 6) and 1 ml. of glass redistilled water were added. Oxidation was achieved by adding 0.1 ml. of 0.25% potassium ferricyanide. After 2 minutes, 1 ml. of a mixture of 9 parts of 20% sodium hydroxide with one part of 2% ascorbic acid was added.

Sample blanks were prepared in the same way, except that no potassium ferricyanide was added. Two "reagent blanks" were used: the one containing all reagents, the other being prepared without potassium ferricyanide. The difference in fluorescence readings obtained from these two "reagent blanks" (i.e. the fluorescence introduced by the addition of potassium ferricyanide) was added to the values obtained from each sample blank. Standard solutions were prepared with 0.1 μg

adrenaline and noradrenaline.

A Beckman DU fluorescence attachment was used for the measurement of fluorescence, which was read 15-60 minutes after development. Differential estimation was achieved by means of the following filter arrangements: (1) Excitation at a primary wavelength of 365 mμ , with secondary interference filter above 400 mμ . This combination gives an approximately equal fluorescence for both amines; (2) Excitation at a primary wavelength of 436 mμ , with secondary interference filter above 500 mμ . This combination gave a fluorescence for noradrenaline which was about 20% of that given by adrenaline. The quantities of the amines were then calculated as described by Millar and Benfey (1958).

Recoveries of known amounts of the amines (1 to 10 μg.) added to the urine of dogs or cats varied between 60% and 75%. When the fluorescence of pure solutions (0.01 to 0.09 mμ g.) was read on the Beckman spectrophotometer, adrenaline read 100%, and noradrenaline 85% of the known concentrations • (Benfey et al., 1958; Millar and Benfey, 1958).

RESULTS

Although no specific attempt has been made to study the origin of catecholamines (adrenaline and noradrenaline) as shown in the urine, for the sake of convenience in presentation, the results are divided into two separate groups: 1.- those concerned with the excretion of the amines as produced in the body, i.e. endogenous, and 2.- those concerned with infused or exogenous amines. In a third section, some specific observations regarding the blood pressure changes obtained in conjunction with these studies, are presented.

A) The action of phenoxybenzamine on the urinary excretion of endogenous adrenaline and noradrenaline.

1) The action of phenoxybenzamine on the urinary excretion of adrenaline and noradrenaline in dogs.

The initial observation, which was at the origin of this work, was the constant effect of an injection of phenoxybenzamine (10 or 20mg./kg.) on the urinary concentrations of amines in dogs as presented in a preliminary report (Benfey et al, 1958).

Tables Ia and Ib show results of six typical experiments in which were studied the effects of phenoxybenzamine on the systemic blood pressure (Ia), the rate of urine secretion (Ia), and the variations of urinary concentrations of the animes (Ib). In all of these experiments, some degree of hypotension was always induced by phenoxybenzamine. On the other hand, it was not possible to establish a clear relationship between rate of urine excretion and concentration of catecholamines. For instance, experiment No I shows no variation in urine secretion rate for a period of 4½ hours, although both adrenaline and noradrenaline are significantly increased for a period of 3½ hours (period of observation). On the other hand, comparing experiments No. 3 and No. 4, the increase in noradrenaline output is higher when the rate of urine excretion is lower, whereas the total amounts of adrenaline are approximately the same in both experiments. Experiments No.4 and No. 5 are also rather similar as far as the rate of urine secretion is concerned, but the outputs of amines are increased to different degrees. However, in all of these experiments, a definite increased excretion of catecholamines is observed except in experiment No. 2, where noradrenaline is not increased over the control levels. In this instance, diuresis was stopped probably due to the fact that the systemic blood pressure had fallen below an effective level for urine formation.

Mean values of changes in blood pressure, urine flow and catecholamine excretion in the experiments summarized in Table Ia and Ib

TABLE I a

MEAN BLOOD PRESSURE AND URINE SECRETION BEFORE AND AFTER PHENOXYBENZAMINE

Blood pressure (B.P.) in mm. Hg and urine secretion in ml./kg./min.

Expt. No.:	1		2		3		4		5		6		Mean (1 to 6)	
Period	B.P.	U	B.P.	U	B.P.	U	B.P.	U	B.P.	U	B.P.	U	B.P.	U
1	166	0.01	104	0.14	160	0.08	138	0.02	130	0.03	160	0.01	143	0.05
2	154	0.01	90	0.06	150	0.10	140	0.02	138	0.04	158	0.02	138	0.04
	Phenoxybenzamine 10 mg./kg.						Phenoxybenzamine 20 mg./kg.							
3	96	0.01	94	0.08	116	0.09	78	0.05	108	0.12	90	0.16	97	0.09
4	78	0.01	82	0.08	112	0.09	98	0.02	88	0.16	70	0.04	88	0.07
5	84	0.01	54	0.06	112	0.14	104	0.05	64	0.07	68	0.01	81	0.06
6	100	0.01	40	0.02	120	0.21	108	0.04	60	0.02	72	0.01	84	0.05
7	118	0.01	38	0	122	0.18	102	0.02	74	0.02	84	0.01	90	0.04
8	130	0.01	38	0	120	0.14	110	0.02	90	0.03	84	0.02	95	0.04
9	140	0.01	-	-	-	-	117	0.03	118	0.04	84	0.02	115	0.03

TABLE I b

URINARY EXCRETION OF ADRENALINE (A) AND OF NORADRENALINE (N) BEFORE AND AFTER PHENOXYBENZAMINE.

Urinary catechol amine estimations are given in ng./kg. min.
No urine secreted after 7th 30 min. period in Expt. No. 2;
9th period sample not analysed in Expt. No. 3.

Expt. No.:	1		2		3		4		5		6		Mean (1 to 6)	
30 min. Period	A	N	A	N	A	N	A	N	A	N	A	N	A	N
1	0	0	0.6	0.7	1.5	1.1	0	0	0	0	0.1	0.5	0.4	0.4
2	0	0	1.1	0.2	2.4	1.5	1.3	0	0	0	0.1	0.5	0.8	0.4
	Phenoxybenzamine 10 mg./kg.						Phenoxybenzamine 20 mg./kg.							
3	0.5	2.5	3.1	0	3.1	0.8	0	0	0.5	1.2	0.1	3.4	1.2	1.3
4	2.0	5.0	3.1	0.4	2.9	0.6	2.6	7.1	2.5	1.7	0	9.4	2.2.	4.0
5	2.5	4.1	4.7	0.3	4.3	1.9	3.0	3.3	6.0	2.2	0	2.6	3.4	2.4
6	2.5	5.4	2.9	0.6	5.8	2.9	3.0	5.4	3.6	1.1	0.6	4.0	3.1	3.2
7	1.1	1.6	-	-	1.9	3.0	2.0	2.6	13.6	5.1	0.6	4.0	3.8	3.3
8	1.2	2.7	-	-	1.1	2.1	1.6	2.3	17.0	9.5	11.0	5.6	6.4	4.4
9	1.2	3.1	-	-	-	-	1.0	1.0	13.6	12.5	11.0	5.6	6.7	5.5

are shown graphically in Fig. I. This shows the course of the hypotension induced by phenoxybenzamine and the fact that urinary catecholamine concentrations were still increasing at the end of the observation period (3½ hours after injection of phenoxybenzamine).

2) The influence of hexamethonium on the action of phenoxybenzamine on the urinary excretion of adrenaline and noradrenaline in dogs.

In view of the above findings, it was of interest to study the effect of ganglionic blockade upon the responses to phenoxybenzamine. It was hoped that from such experiments further information might be gained regarding the importance of the transmitter substance liberated at the postganglionic terminals, in regard to the action of phenoxybenzamine.

In this study three dogs were used and hexamethonium employed as the ganglionic blocking drug. In order to obtain a prolonged action, the drug was infused intravenously at the rate of 0.1 mg./kg./min.

As shown in Fig. 2, the effect of the hexamethonium infusion was an immediate depressor response, and the blood pressure was kept at a lower and steady level, as long as the infusion was maintained.

One hour of hexamethonium infusion was used following the usual one hour of "control", before administration of phenoxybenzamine.

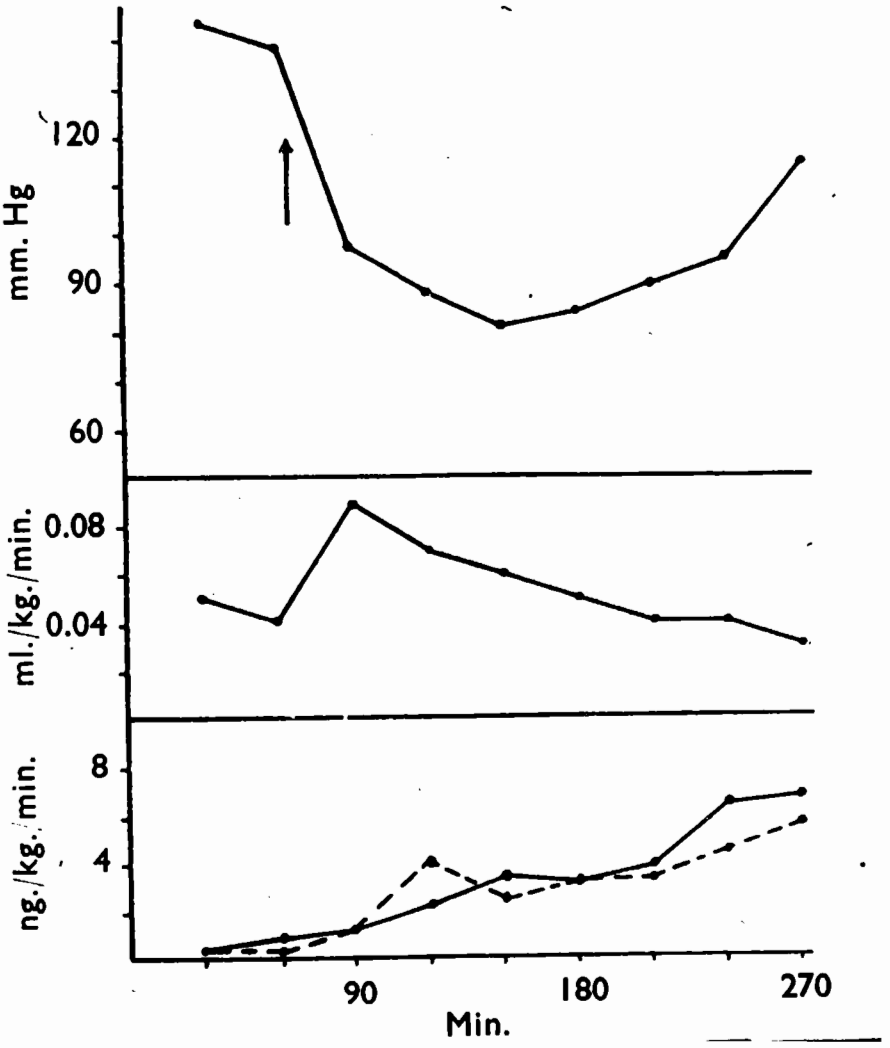


Fig. 1 - Blood pressure (upper curve), urine flow (middle curve) and urinary excretion (lowest two curves) of adrenaline (full line) and of noradrenaline (broken line) before and following administration (at arrow) of phenoxybenzamine (means of observations in Tables Ia and Ib.)

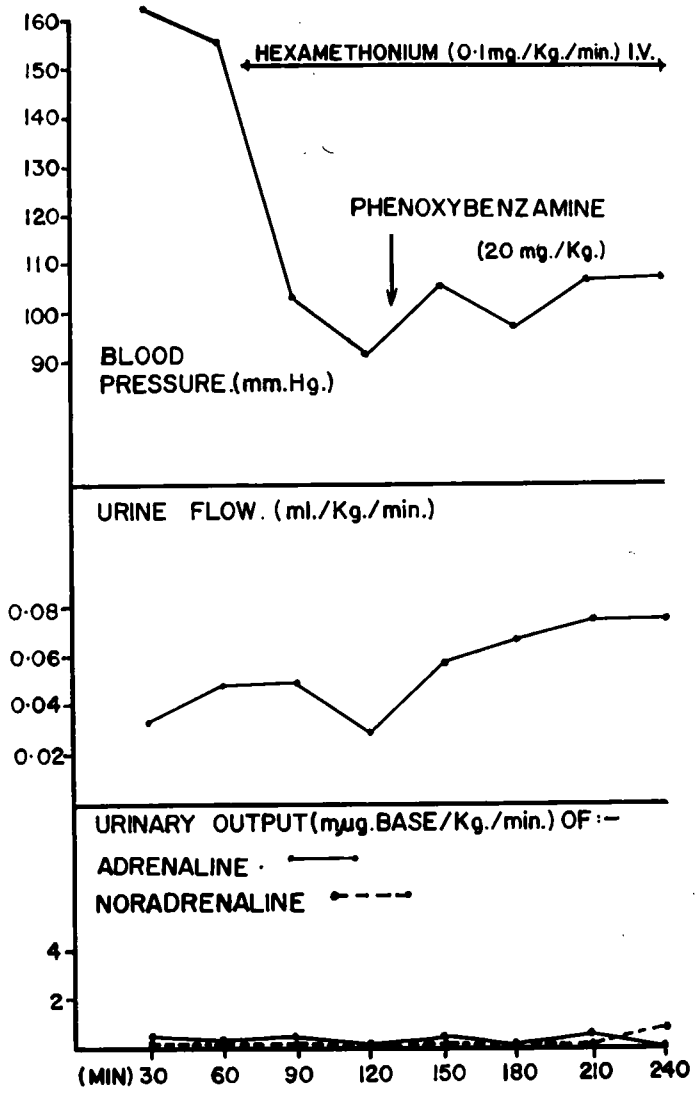


Fig. 2 - Blood pressure, urine flow and urinary output of adrenaline and noradrenaline in dogs (mean of 3 experiments).
Phenoxybenzamine and hexamethonium administration. Blood pressure recorded from femoral artery, urine collected from cannulated ureters. Pentobarbital anesthesia. Saline infusion (0.2 ml./kg./min.)

Throughout the "control" and hexamethonium infusions, there was no change in urine secretion, and no significant increase in catecholamine elimination, despite the slight fall in blood pressure induced by hexamethonium. Outputs of the amines were extremely low throughout as shown in Table II.

When phenoxybenzamine was injected, (20mg./kg. during the infusion of hexamethonium) the output of adrenaline and noradrenaline was not augmented during the 90 minutes of observation. It should also be noted that not only was the effect of phenoxybenzamine on the excretion of amines suppressed, but there was no further fall in blood pressure. (See Section C of Results) Indeed, immediately following the administration of phenoxybenzamine, there was some tendency for the blood pressure to rise before settling down at the level previously established by the ganglionic blockade (Table II).

In summary, as far as catecholamines were concerned, there was no difference between either series of control samples and those samples collected after phenoxybenzamine treatment. It may be concluded therefore that in the presence of hexamethonium blockade, phenoxybenzamine leads to no detectable increased urinary output of the amines.

3) <u>The influence of phenoxybenzamine on the effect of ganglionic stimulation by acetylcholine on the urinary excretion of adrenaline and of noradrenaline in dogs.</u>

TABLE II

MEAN URINARY EXCRETION OF ADRENALINE AND OF NORADRENALINE, RATE OF URINE SECRETION, AND BLOOD PRESSURE BEFORE AND DURING HEXAMETHONIUM INFUSION AND BEFORE AND AFTER PHENOXYBENZAMINE INJECTION.

All values were based on 3 experiments.
Means with range in parentheses.

30 min. Period	Adrenaline (ng./kg./min.)	Noradrenaline (ng./kg./min.)	Urine (ml./kg./min.)	B.P. (mm. Hg)
1	0.3 (0.0–0.7)	0.0 (0.0–0.1)	0.03 (0.01–0.05)	162 (146–178)
2	0.2 (0.0–0.4)	0.1 (0.0–0.1)	0.05 (0.01–0.08)	155 (132–179)
	Hexamethonium (0.1 mg./kg./min.) infusion started			
3	0.4 (0.0–1.1)	0.1 (0.0–0.4)	0.05 (0.01–0.08)	103 (48–130)
4	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.03 (0.00–0.06)	91 (62–120)
	Phenoxybenzamine injection (20 mg./kg.)			
5	0.3 (0.0–0.6)	0.1 (0.0–0.2)	0.06 (0.01–0.09)	105 (68–124)
6	0.0 (0.0–0.1)	0.1 (0.0–0.3)	0.07 (0.01–0.11)	97 (64–120)
7	0.4 (0.0–1.1)	0.1 (0.0–0.4)	0.07 (0.02–0.12)	106 (80–138)

In order to test the effects of phenoxybenzamine on the recovery from the urine of amines released in greater amounts within the body, the following scheme of ganglionic stimulation was adopted: in four atropinized (2mg./kg.) dogs, an injection of 5 mg./kg. of acetylcholine every three minutes was given, so that one collection period would correspond to ten such injections. After two periods consisting of 10 "ganglionic stimulations" each, phenoxybenzamine (10 mg./kg.) was administered in the usual way, and a period of 90 minutes allowed for development of the blockade. Then, the same two periods of stimulation were repeated, giving this time a period of blood pressure "reversals" instead of the pressor responses obtained before phenoxybenzamine.

Table III shows that before phenoxybenzamine, in the two consecutive periods during which acetylcholine was administered, there was an increased excretion of adrenaline, but no significant change in noradrenaline.

Variable pressor responses were observed after each injection of acetylcholine the mean blood pressure during these periods reaching 191 and 175 mm. Hg respectively, as compared with the mean control values of 135 and 149 mm. Hg. (Fig. 3).

Following these two 30 minutes stimulation periods, phenoxybenzamine (10 mg./kg.) induced only a slightly increased output of both amines, which was associated with an increased urine flow and a

TABLE III

URINARY EXCRETION OF ADRENALINE AND OF NORADRENALINE, RATE OF URINE SECRETION, AND BLOOD PRESSURE BEFORE AND AFTER PHENOXYBENZAMINE INJECTION.

During periods 3, 4, 8 and 9 (marked *) atropine (2mg./kg.) followed by 10 injections of acetylcholine (5mg./kg.) at 3 min. intervals were given. All values are based on 4 experiments. Means with range in parentheses.

30 min. Period	Adrenaline (ng./kg./min.)	Noradrenaline (ng./kg./min.)	Urine (ml./kg./min.)	B.P. (mm.Hg)
1	0.2 (0.0-0.5)	0.7 (0.0-1.6)	0.03 (0.01-0.06)	135 (70-197)
2	0.6 (0.0-1.1)	0.4 (0.0-1.6)	0.03 (0.01-0.07)	149 (102-186)
*3	2.6 (0.1-4.6)	0.6 (0.0-1.2)	0.02 (0.01-0.03)	191 (100-270)
*4	4.8 (2.8-6.6)	0.8 (0.0-2.1)	0.02 (0.01-0.03)	175 (110-230)
		Phenoxybenzamine (10 mg./kg.)		
5	1.0 (0.7-1.5)	0.4 (0.1-1.4)	0.05 (0.01-0.11)	87 (70-116)
6	0.7 (0.2-0.9)	1.1 (0.0-2.5)	0.05 (0.03-0.07)	72 (58-92)
7	0.8 (0.0-1.5)	0.9 (0.1-1.9)	0.04 (0.03-0.05)	73 (58-92)
*8	3.9 (1.4-8.3)	2.4 (0.2-4.5)	0.04 (0.01-0.08)	55 (44-64)
*9	2.7 (0.4-5.7)	2.4 (0.5-4.0)	0.04 (0.01-0.12)	51 (43-60)
10	0.4 (0.0-0.7)	0.4 (0.0-0.6)	0.07 (0.01-0.17)	94 (70-134)

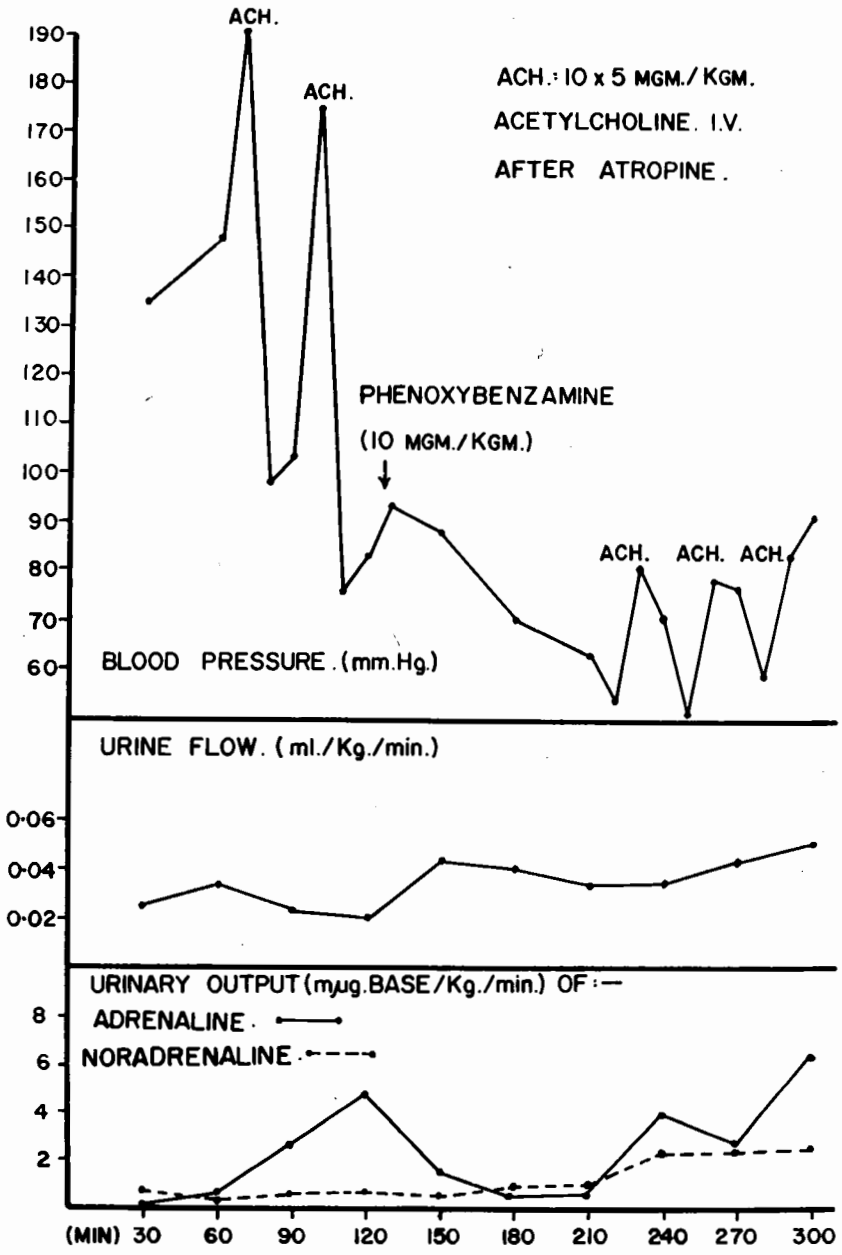


Fig. 3 - Blood pressure, urine flow and urinary output of adrenaline and noradrenaline following administration of acetylcholine (after atropine), before and after phenoxybenzamine in dogs (mean of 4 experiments). Blood pressure recorded from Femoral artery, urine collected from cannulated ureters. Pentobarbital anesthesia. Saline infusion (0.2 ml./kg./min.).

fall of blood pressure. Further acetylcholine injections after phenoxybenzamine again increased the adrenaline excretion, but at this time there was also an increased excretion of noradrenaline. Since identical "stimuli" were applied to release the amines before and after phenoxybenzamine, it would appear that phenoxybenzamine augmented the excretion of endogenously liberated noradrenaline but not that of adrenaline under these conditions.

In connection with these studies on endogenous or "released" catecholamines by ganglionic stimulation, earlier experiments showed that a single injection of acetylcholine (5mg./kg.) after atropinization, although it induced a marked pressor response, led to no significant change in the quantities of the amines excreted in the corresponding 30 minute samples. For this reason, a succession of ten doses of acetylcholine in one period was used, as described. It should also be added that when adrenaline or noradrenaline was injected in amounts which raised the blood pressure to the same extent as that observed with the large dose of acetylcholine, increased excretion of the amines in the urine was readily detectable.

It would appear, then, that "endogenous" and "exogenous" catecholamines differ in that the former may be more active and/or more quickly inactivated than the latter. It was therefore of special interest to study the effects of phenoxybenzamine on the urinary excretion of the amines following their infusion (Section B of Results).

However, before this aspect is reported, two series of experiments are added which showed the significance of the hypotension induced by phenoxybenzamine in connection with increased urinary catecholamines.

4) The action of methacholine on the urinary excretion of adrenaline and noradrenaline in dogs.

In order to see if the increased excretion of catecholamines after phenoxybenzamine resulted from a reflex response to the hypotension produced by the drug, three experiments were performed in which subcutaneous injections of methacholine were given in small repeated doses (100-400 μg.) in order to match the type and degree of hypotension which usually followed an injection of phenoxybenzamine.

Table IV shows that, during methacholine injections, there was a decrease in mean blood pressure from the control readings of 147 and 139 mm. Hg. (first and second period) to 108, 93, and 82 mm. Hg. during the third, fourth, and fifth periods respectively. Concomitant with the depressor response, the excretion of adrenaline was however increased, but that of noradrenaline was not.

Fig. 4 shows graphically data from the mean values in the three methacholine experiments. The type of hypotension is comparable to that shown in Fig. 1, but there was only a peak in urinary adrenaline

TABLE IV

URINARY EXCRETION OF ADRENALINE AND OF NORADRENALINE, RATE OF URINE SECRETION AND BLOOD PRESSURE BEFORE, DURING, AND AFTER METHACHOLINE INJECTED SUBCUTANEOUSLY.

All values are mean values based on 3 experiments.

30 min. Period	Adrenaline (ng./kg./min.)	Nor-adrenaline (ng./kg./min.)	Urine (ml./kg./min.)	B.P. (mm.Hg)
1	0.2 (0.0-0.6)	0.3 (0.0-0.5)	0.11 (0.03-0.14)	147 (143-153)
2	0.6 (0.0-1.2)	0.3 (0.0-0.5)	0.16 (0.13-0.18)	139 (138-140)
		Methacholine injections started		
3	0.4 (0.0-1.1)	0.3 (0.0-0.6)	0.08 (0.02-0.16)	108 (90-120)
4	1.4 (0.1-2.1)	0.2 (0.0-0.6)	0.05 (0.01-0.11)	93 (83-104)
5	1.3 (1.3)	0.1 (0.1)	0.03 (0.00-0.05)	82 (80-84)
		Methacholine stopped		
6	3.6 (0.0-7.2)	0.5 (0.0-1.0)	0.02 (0.00-0.06)	105 (86-118)
7	0.9 (0.1-1.3)	0.2 (0.0-0.4)	0.04 (0.01-0.10)	122 (99-138)
8	0.1 (0.0-0.1)	0.3 (0.0-0.6)	0.03 (0.03)	113 (92-134)
9	0.1 (0.0-0.1)	0.3 (0.0-0.5)	0.05 (0.03-0.06)	119 (100-138)

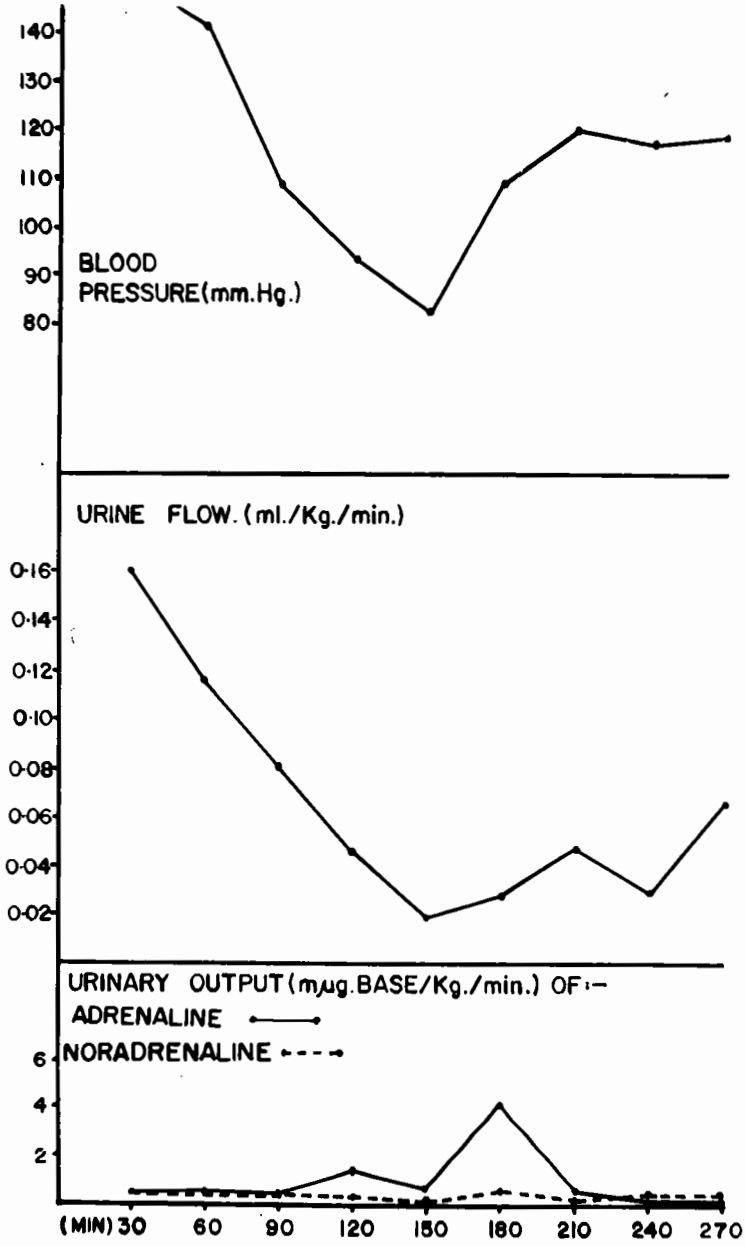


Fig. 4 - Blood pressure, urine and urinary output of adrenaline and noradrenaline before, during and after methacholine administration in dogs. (Mean of 3 experiments).
Blood pressure recorded from femoral artery, urine collected from cannulated ureters.
Pentobarbital anesthesia. Saline infusion (0.2 ml./kg./min.)

occurring at the end of the hypotension, unlike the changes observed with phenoxybenzamine (Fig. 1). It is therefore evident that the hypotension is not entirely responsible for the increased output of the amines following phenoxybenzamine injection.

5) The action of phenoxybenzamine on the urinary excretion of adrenaline and noradrenaline in cats and the effect of vasopressin thereon.

In this series of experiments, an attempt was made to counteract the depressor response induced by phenoxybenzamine by the vasoconstrictor agent, vasopressin.

With the doses of phenoxybenzamine used in dogs, (10 or 20 mg./kg.), the depressor response was however difficult to counteract. After several unsuccessful trials, a procedure was found in which small doses of vasopressin (2 to 10 units), when injected intravenously at intervals of ten minutes (more frequent injections were ineffective), prevented the mean blood pressure from falling too greatly. The effect was however inconsistent in dogs, and was only successful in maintaining the blood pressure at or above 110 mm. Hg. in one out of a group of six animals. On the other hand, more consistent results could be achieved in cats, and in five out of a group of eight such experiments, the urine collected was analysed for catecholamines, in the same way as had been

carried out in the experiments on dogs. The reason for the differences in the two species is unknown.

The results in Table V show that, in cats, while the systemic blood pressure was kept at approximately 110 mm. Hg by the procedure described above, the urinary concentrations of catecholamines were not significantly different from those observed in control experiments. Thus, when 10 mg./kg. of phenoxybenzamine was injected in cats without vasopressin, (and allowing the depressor response to follow its usual course) there was an increase in the urinary excretion of noradrenaline, but the output of adrenaline was not influenced. This is in contrast to the observations made in dogs which showed increases in both noradrenaline and adrenaline outputs. Under the influence of the drug, the blood pressure fell and remained at a level of about 90 mm. Hg for at least 3 hours. (Fig. 5)

It is concluded, therefore, that under the influence of vasopressin which was used to keep the blood pressure at or above 110 mm. Hg, the great increase of noradrenaline excretion after phenoxybenzamine was not obtained. The rates of urine secretion were not affected by vasopressin.

B) <u>The action of phenoxybenzamine on the urinary excretion of infused adrenaline and noradrenaline in dogs.</u>

TABLE V

Mean of 5 experiments on cats anesthetized with Pentobarbital. 30 min. periods. Results before and after Phenoxybenzamine. • Experiments where repeated injections of Vasopressin (2 to 10 units) kept blood pressure around 110 mm Hg. o Experiments where no Vasopressin was used.

Period	B.P. mm Hg		Urine secretion (ml/kg/min.)		Urinary Adrenaline.—Noradrenaline. (ng/kg/min.)			
	o	•	o	•	o	•	o	•
1	132	148	0.07	0.05	1.5	0.1	2.5	2.8
2	132	127	0.12	0.08	1.4	0.1	1.5	1.0
	Phenoxybenzamine injected. (10 mg/kg.)							
3	82	114	0.11	0.11	1.0	0.2	1.6	1.0
4	88	115	0.10	0.12	0.8	0.2	3.6	1.0
5	90	115	0.11	0.13	0.9	0.2	4.0	0.5
6	90	116	0.14	0.13	0.5	0.2	4.9	0.9
7	90	114	0.14	0.15	0.6	1.0	4.0	0.9
8	92	114	0.16	0.16	0.9	0.9	4.1	1.0

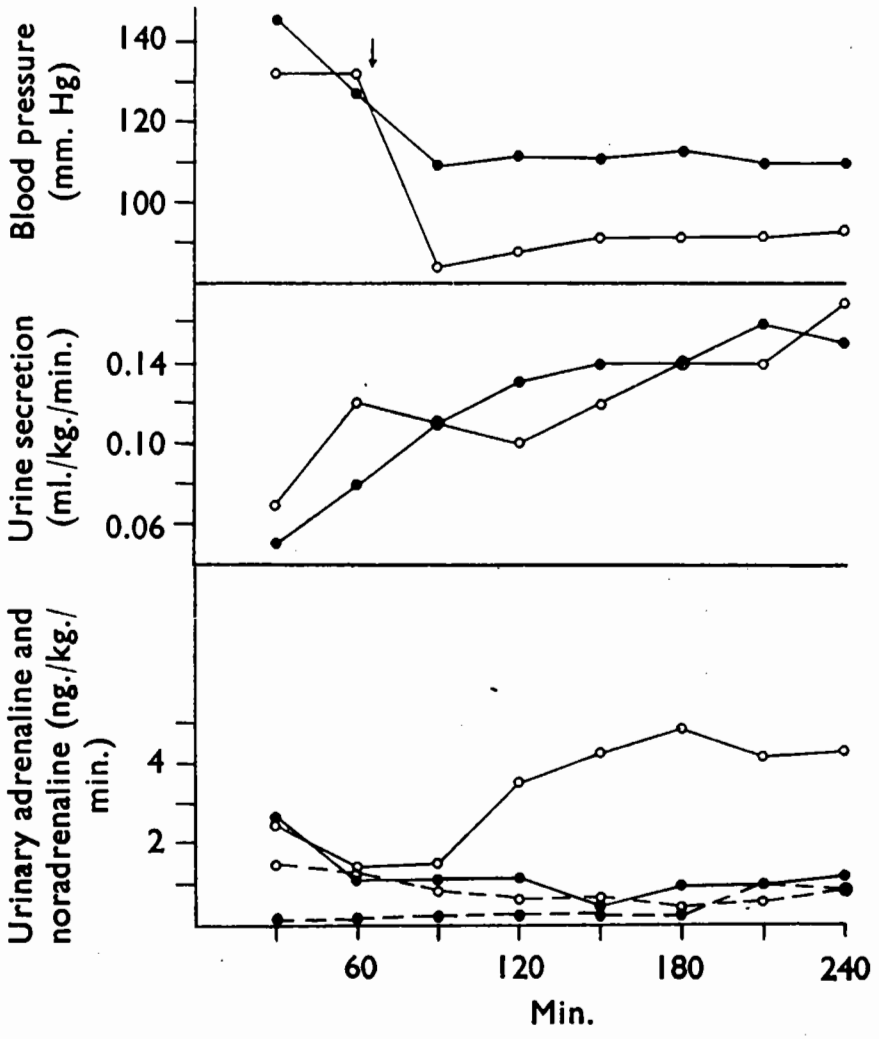


Fig. 5. - Cats anesthetized with pentobarbital. Blood pressure (top), urine flow (middle) and urinary excretion (bottom) of adrenaline (broken line) and noradrenaline (full line). At arrow, phenoxybenzamine (10 mg./kg.). o, without, and ●, with repeated injections of vasopressin (2 to 10 units). Mean of 5 experiments.

Before studying the effects of phenoxybenzamine on infused adrenaline and noradrenaline, it was necessary to establish to what extent these amines could be "recovered" from the urine of the otherwise untreated dog. Although this type of study had been carried out by other workers, (Benfey et al., 1958), it was necessary to repeat such experiments for comparative purposes. The findings would also serve to check the validity of the earlier observations and of the general procedures employed in this study.

1) The urinary recovery of infused adrenaline and noradrenaline.

Table VIa shows the amounts of catecholamines found in the urine when a constant infusion of adrenaline (0.5 μg/kg./min.) was given over a period of four hours. The infusion was started after two control periods and stopped four hours later, following which, two more periods served again as control.

Urine secretion rate and blood pressure were not significantly affected by such infusions, however, urinary concentrations of adrenaline showed from the very first period after the start of the infusion, a considerable rise over control levels. If the latter are subtracted from the amounts found during infusion, the average recovery calculated on the basis of 30 minute periods of excretion was 3 per cent. Moreover, the graph in Fig. 6, shows good agreement between the quantities

TABLE VI a

CHANGES IN URINARY EXCRETION OF ADRENALINE (A) AND OF NORADRENALINE (N), URINE SECRETION (U), BLOOD PRESSURE (B.P.) DURING INFUSIONS OF ADRENALINE WITHOUT PHENOXYBENZAMINE.

The infusion of adrenaline was at the rate of 0.5 μg./kg./min. Estimates of A and N are in ng./kg./min.; urinary secretion in ml./kg./min. and blood pressure in mm. Hg. All values are means of three experiments with ranges in parentheses.

30 Min. Period	A	N	U	B.P.
1	4.8 (2.5–8.9)	0.2 (0–0.4)	0.07 (0.03–0.1)	106 (98–114)
2	6.5 (3.0–9.7)	0.3 (0–0.8)	0.08 (0.01–0.17)	98 (68–146
		Adrenaline infusion started		
3	18.7 (16.5–21.5)	0.3 (0–0.8)	0.11 (0.01–0.27)	111 (88–138)
4	23.5 (20.5–28.2)	0	0.10 (0.02–0.21)	111 (90–136)
5	20.8 (16.8–24.0)	0	0.09 (0.02–0.16)	114 (98–138
6	21.0 (17.6–25.4)	0	0.09 (0.03–0.13)	115 (100–138)
7	18.1 (17.0–19.5)	0.1 (0–0.3)	0.08 (0.03–0.13)	118 (100–146)
8	20.9 (17.3–23.8)	0	0.09 (0.04–0.13)	124 (106–146)
9	18.8 (18.6–19.2)	0	0.11 (0.05–0.16)	122 (94–146)
10	21.5 (19.9–23.2)	1.3 (0–3.8)	0.12 (0.08–0.16)	119 (90–146)
		Adrenaline stopped		
11	9.0 (4.1–13.6)	0.5 (0–0.9)	0.11 (0.07–0.15)	109 (80–146)
12	3.7 (0–8.9)	0	0.10 (0.06–0.16)	104 (70–146)

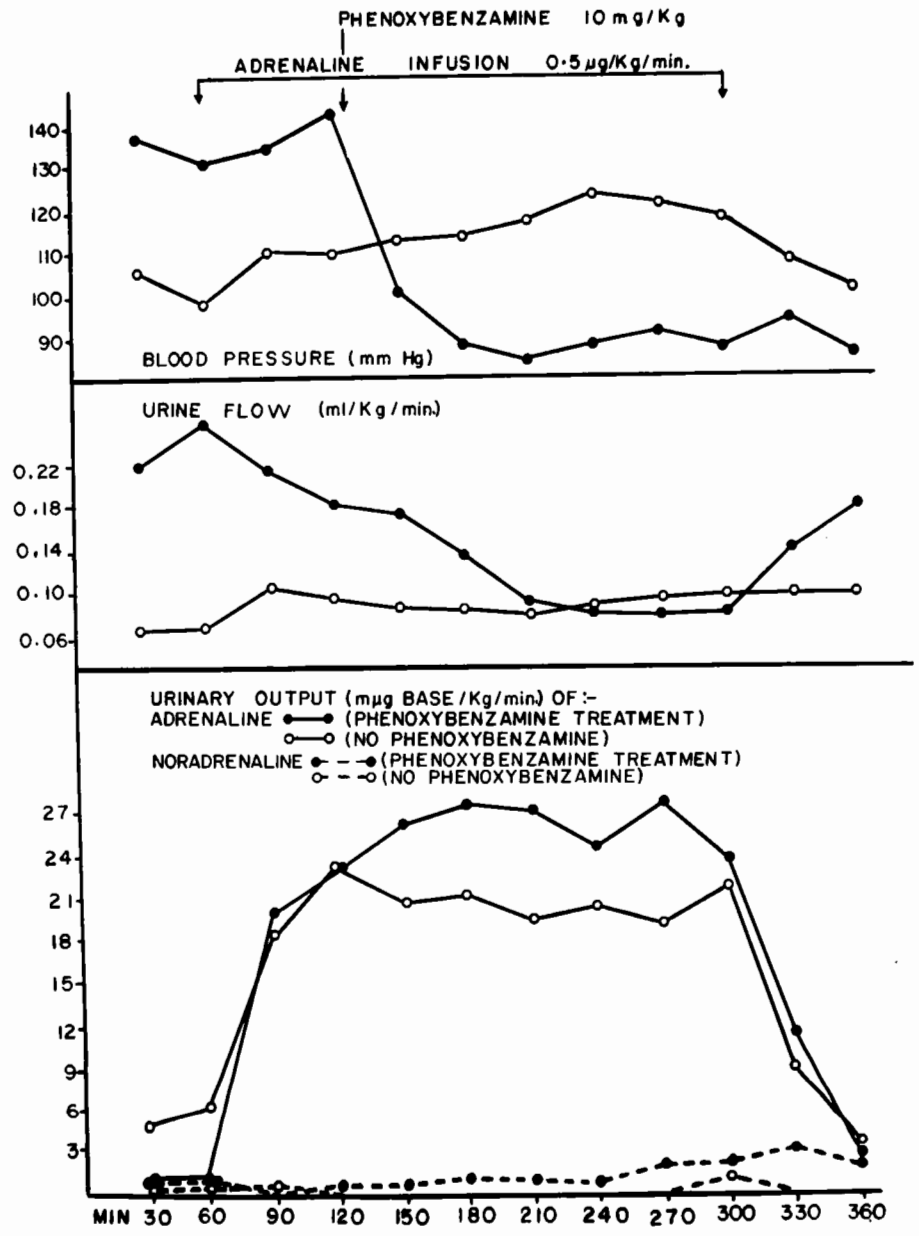


Fig. 6. - Dogs anesthetized with pentobarbital. ● Experiments where phenoxybenzamine was injected during the infusion. o No phenoxybenzamine.

of the amines recovered during each of the eight periods of observation. During the infusion of adrenaline, urinary concentrations of noradrenaline, for all practical purposes, were not detectable.

When noradrenaline (0.5 μg./kg./min.) was infused according to the procedure mentioned above, recoveries were also quite uniform (see Table VIIa), but somewhat lower (1.8 per cent) than in the case of adrenaline. Minor alterations of blood pressure and urine secretion were also observed. It is to be noted that during the infusion of noradrenaline, urinary excretion of adrenaline took place steadily, but never exceeded the levels of the controls.

When these infusions were stopped, an immediate drop in urinary catecholamines was evident, the latter returning to original control levels after an hour. (Tables VIa and VIIa)

2) <u>The effect on the urinary recovery of infused adrenaline and noradrenaline, of phenoxybenzamine given during the infusions</u>.

The infusions of adrenaline or noradrenaline as described in the previous section were repeated, and after the first hour of infusion, phenoxybenzamine (10 mg./kg.) was injected. (Table VIb).

Fig. 6 and 7 represent graphically the data tabulated in Tables VI and VII. The curve of the urinary adrenaline after phenoxybenzamine indicates an increase in the recovery which rose to 4.3 per cent

TABLE VII a

CHANGES IN URINARY EXCRETION OF ADRENALINE (A) AND NORADRENALINE (N), URINE SECRETION (U), BLOOD PRESSURE (B.P.), DURING INFUSIONS OF NORADRENALINE WITHOUT PHENOXYBENZAMINE.

Estimates of A and N are given in ng./kl./ml.; urinary secretion in ml./kg./min. and blood pressure in mm. Hg. The infusion of noradrenaline was at the rate of 0.5 μg./kg./min. All values are the means of three experiments with phenoxybenzamine with ranges in parentheses.

30 Min. Period	A	N	U	B.P.
1	1.9 (0–2.9)	0.7 (0–2.2)	0.14 (0.04–0.29)	123 (102–148)
2	3.5 (0–6.1)	0.1 (0–0.4)	0.16 (0.09–0.25	115 (94–141)
	Noradrenaline infusion started			
3	3.0 (0–5.7)	7.5 (4.3–13.2)	0.16 (0.10–0.22)	115 (94–141)
4	2.9 (0.1–6.4)	10.0 (7.7–13.5)	0.11 (0.07–0.15)	118 (94–140)
5	2.2 (0–3.9)	10.0 (6.3–17.5)	0.11 (0.07–0.17)	125 (98–148)
6	1.3 (0.8–1.7)	9.5 (7.0–10.8)	0.13 (0.08–0.20)	133 (106–154)
7	1.8 (0.9–2.9)	9.6 (7.1–10.9)	0.15 (0.10–0.20)	145 (118–164)
8	1.6 (0.9–2.3)	10.0 (5.6–13.2)	0.14 (0.11–0.17)	141 (120–164
9	1.7 (1.6–1.9)	8.0 (6.1–10.0)	0.13 (0.11–0.15)	126 (118–134
10	1.5 (1.4–1.5)	7.8 (5.2–10.5)	0.14 (0.13–0.14)	117 (104–130)
	Noradrenaline stopped			
11	0.9 (0.2–1.6)	2.1 (0.6–2.9)	0.13 (0.10–0.14)	113 (102–126)
12	0.8 (0–1.6)	1.2 (0–2.8)	0.12 (0.08–0.17)	110 (98–120)

of the amount infused, as compared with 3 per cent without phenoxybenzamine treatment. As the experiments progressed, noradrenaline excretion also appeared after phenoxybenzamine in steadily increasing amounts, even after the infusion periods. Blood pressure was significantly decreased by phenoxybenzamine.

During an infusion of noradrenaline (0.5 μg/kg./min.), again a tendency toward an increased recovery was indicated (Fig. 7). However, the amounts of adrenaline excreted were also significantly increased during the infusion. Again, the blood pressure was markedly decreased by phenoxybenzamine. (Table VIIb).

It is concluded therefore that recovery of infused noradrenaline did not appear to be significantly augmented by the influence of phenoxybenzamine. Why this was so is not clear, and in an attempt to clarify the question a second procedure was adopted in which the administration of phenoxybenzamine was not made during the infusion of the amines. The findings are presented in the next section.

3) <u>The urinary recovery of noradrenaline infused before and after treatment with phenoxybenzamine</u>.

In these experiments, after a control period of one hour, dogs received an intravenous infusion of 0.2 μg./Kg./min. noradrenaline for one hour. One hour after the infusion had been stopped, phenoxybenzamine was injected (10 mg./kg.), and an hour later a second

TABLE VI b

CHANGES IN URINARY EXCRETION OF ADRENALINE (A) AND OF NORADRENALINE (N), URINE SECRETION (U), BLOOD PRESSURE (B.P.) DURING INFUSIONS OF ADRENALINE WITH PHENOXYBENZAMINE.

The infusion of adrenaline was at the rate of 0.5 μg./kg./min. Estimates of A and N are in ng./kg./min.; urinary secretion in ml./kg./min. and blood pressure in mm. Hg. All values are means of four experiments with ranges in parentheses.

30 Min. Period	A	N	U	B.P.
1	1.1 (0.5–2.1)	1.2 (0.7–1.9)	0.22 (0.07–0.28)	138 (90–170)
2	1.4 (0.8–1.9)	1.1 (0.3–1.9)	0.27 (0.08–0.48)	131 (98–162)
	Adrenaline infusion started			
3	19.3 (12.6–24.1)	0	0.21 (0.10–0.34)	135 (100–166)
4	23.5 (17.0–27.8)	0.9 (0–3.6)	0.18 (0.12–0.29)	144 (112–166)
	Phenoxybenzamine (10 mg./kg.) injected			
5	26.2 (21.1–30.0)	0.7 (0–2.6)	0.17 (0.11–0.27)	101 (90–126)
6	27.7 (19.2–36.6)	1.1 (0–4.2)	0.13 (0.07–0.21)	89 (70–116)
7	27.2 (17.3–39.1)	1.2 (0–4.9)	0.09 (0.04–0.16)	85 (62–106)
8	23.0 (15.0–33.2)	1.3 (0–5.2)	0.08 (0.03–0.14)	88 (66–108)
9	27.7 (22.7–31.6)	2.4 (0–9.4)	0.09 (0.04–0.14)	91 (70–108)
10	23.5 (21.8–27.6)	2.7 (0–9.4)	0.10 (0.09–0.14)	88 (66–104)
	Adrenaline stopped			
11	11.0 (6.6–16.4)	3.5 (0.4–6.1)	0.14 (0.06–0.18)	99 (90–112)
12	3.3 (1.9–6.5)	2.8 (0.2–4.7)	0.18 (0.04–0.25)	89 (78–104)

TABLE VII b

CHANGES IN URINARY EXCRETION OF ADRENALINE (A) AND NORADRENALINE (N), URINE SECRETION (U), BLOOD PRESSURE (B.P.), DURING INFUSIONS OF NORADRENALINE WITH PHENOXYBENZAMINE.

Estimates of A and N are given in ng./kl./ml.; urinary secretion in ml./kg./min. and blood pressure in mm. Hg. The infusion of noradrenaline was at the rate of 0.5 μg./kg./min. All values are the means of four experiments with phenoxybenzamine with ranges in parentheses.

30 Min. Period	A	N	U	B.P.
1	0.4 (0–0.6)	0.7 (0–1.9)	0.07 (0.04–0.10)	129 (121–140)
2	1.4 (0–3.8)	0.3 (0–1.2)	0.10 (0.05–0.18)	119 (88–140)
	Noradrenaline infusion started			
3	1.1 (0.3–1.9)	7.0 (1.6–11.2)	0.08 (0.04–0.14)	126 (100–150)
4	0.8 (0–1.5)	13.1 (9.1–19.0)	0.09 (0.04–0.12)	124 (92–148)
	PHENOXYBENZAMINE (10 mg./kg.) injected			
5	0.7 (0–1.6)	11.3 (4.1–18.7)	0.08 (0.05–0.13)	91 (68–128)
6	3.0 (0–4.6)	12.5 (10.7–15.8)	0.04 (0–0.09)	67 (45–97)
7	6.1 (0–11.2)	15.7 (13.9–18.6)	0–04 (0–0.07)	70 (50–104)
8	3.3 (0–9.4)	13.0 (6.2–17.1)	0.06 (0.03–0.08)	77 (56–121)
9	2.0 (0–6.2)	12.4 (5.6–15.9)	0.05 (0.03–0.07)	66 (60–73)
10	2.4 (1.5–3.4)	12.7 (8.6–16.9)	0.08 (0.08)	70 (64–76)
	Noradrenaline stopped			
11	1.0 (0–2.2)	9.2 (3.7–17.1)	0.08 (0.04–0.13)	106 (84–135)
12	0.5 (0.1–0.6)	2.1 (0–5.7)	0.10 (0.05–0.13)	104 (84–138)

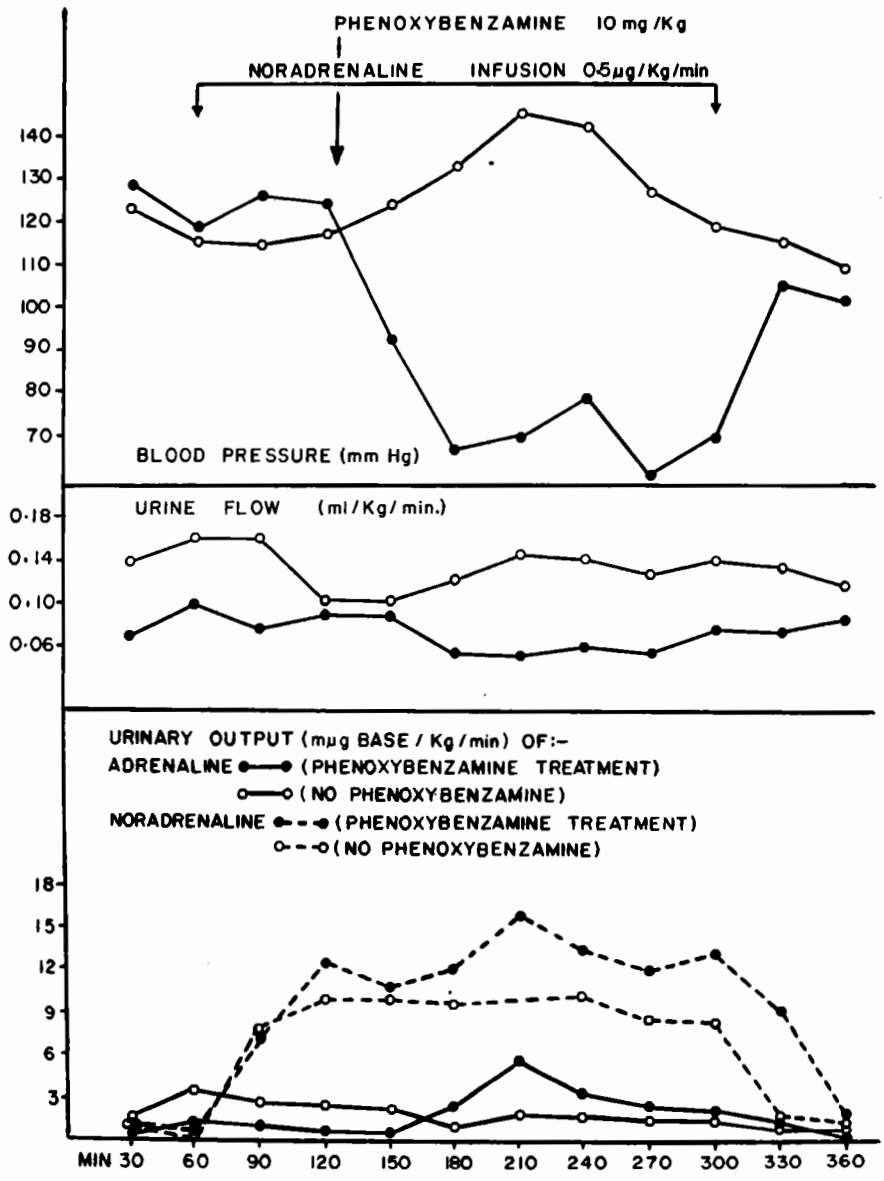


Fig. 7.- Dogs anesthetized with pentobarbital. ● Experiments where phenoxybenzamine was injected during the infusion. ○ No phenoxybenzamine.

infusion, similar to the first one, was begun. Throughout the experiment, 0.1 mg./kg./min. hexamethonium was given in order to eliminate an action of phenoxybenzamine on the excretion of endogenous noradrenaline (see section A2 in this chapter on results).

The clearance of p-aminohippuric acid and creatinine before and after phenoxybenzamine were also determined in these experiments by Mr. M. Segal. As seen in Table VIII and Fig. 8, the control values excretion of noradrenaline (periods Nos. 1, 2 and 6 to 8) were low. Before phenoxybenzamine, the mean noradrenaline excretion in periods Nos. 3 to 5 was 290$\pm$ 40 (S.E.) ng./kg. and after phenoxybenzamine in periods Nos. 7 to 9 it was 454$\pm$ 49 (S.E.) ng./kg. The difference was statistically significant ($P<0.05$). Taking into account the control urinary noradrenaline output of 0.4 ng./kg./min., the urinary recovery of infused noradrenaline was calculated as 2.1 per cent before and 3.5 per cent after phenoxybenzamine. The urine output was 10.0 ml./kg. during periods Nos. 3 to 5, and 10.6 ml./kg. during periods Nos. 9 to 11. The mean p-aminohippuric acid clearance was 12.2 ml./kg./min. in periods Nos. 3 and 4, and 10.7 in periods Nos. 9 and 10. The creatinine clearance was also somewhat reduced from 5.7 ml./kg./min. during periods 3 and 4 to 4.7 during periods Nos. 9 and 10. A change in "kidney function" was therefore not responsible for the effect of phenoxybenzamine. There was no evidence that the infusion of noradrenaline raised the urinary excretion of adrenaline, so there was no indication that part of the infused noradrenaline might have been converted to

TABLE VIII

THE EFFECTS OF PHENOXYBENZAMINE ON THE URINARY EXCRETION OF ADRENALINE AND NORADRENALINE, BLOOD PRESSURE, URINE SECRETION, CLEARANCE OF P-AMINOHIPPURIC ACID, AND OF CREATININE, AND FILTRATION FRACTION IN DOGS DURING INFUSION WITH NORADRENALINE.

Intravenous infusions of 0.9% NaCl, and 0.05% Hexamethonium were given throughout the experiment (0.2 ml./kg./min.). A, indicates Adrenaline (ng./kg./min.); N, Noradrenaline (ng./kg./min.); B.P., blood pressure in mm. Hg; U, urine secretion (ml./kg./min.); C_{pah} clearance of p-aminohippuric acid (ml./kg./min.); C_{cr}, clearance of creatinine (ml./kg./min.); FF, filtration fraction. Noradrenaline infusion, 0.2 μg/kg./min. Phenoxybenzamine injection 10 mg./kg. Mean of four experiments. 30 min. periods.

Period	A	N	B.P.	U	C_{pah}	C_{cr}	FF
1	1.5	0.2	112	0.08	13.2	6.0	0.444
2	1.0	0.4	105	0.10	13.1	5.6	0.419
		Noradrenaline infusion started.					
3	1.3	3.4	110	0.11	12.9	5.6	0.442
4	1.0	4.7	110	0.10	10.5	4.6	0.451
		Noradrenaline infusion stopped.					
5	0.9	1.6	101	0.09			
6	0.9	0.3	104	0.09			
		Phenoxybenzamine injection.					
7	0.8	0.5	100	0.09	7.2	3.5	0.503
8	1.2	0.4	93	0.10	8.4	3.9	0.469
		Noradrenaline infusion started.					
9	1.1	4.8	81	0.11	9.5	4.1	0.454
10	1.1	7.7	85	0.10	9.4	3.8	0.416
		Noradrenaline infusion stopped.					
11	0.5	2.2	93	0.09			

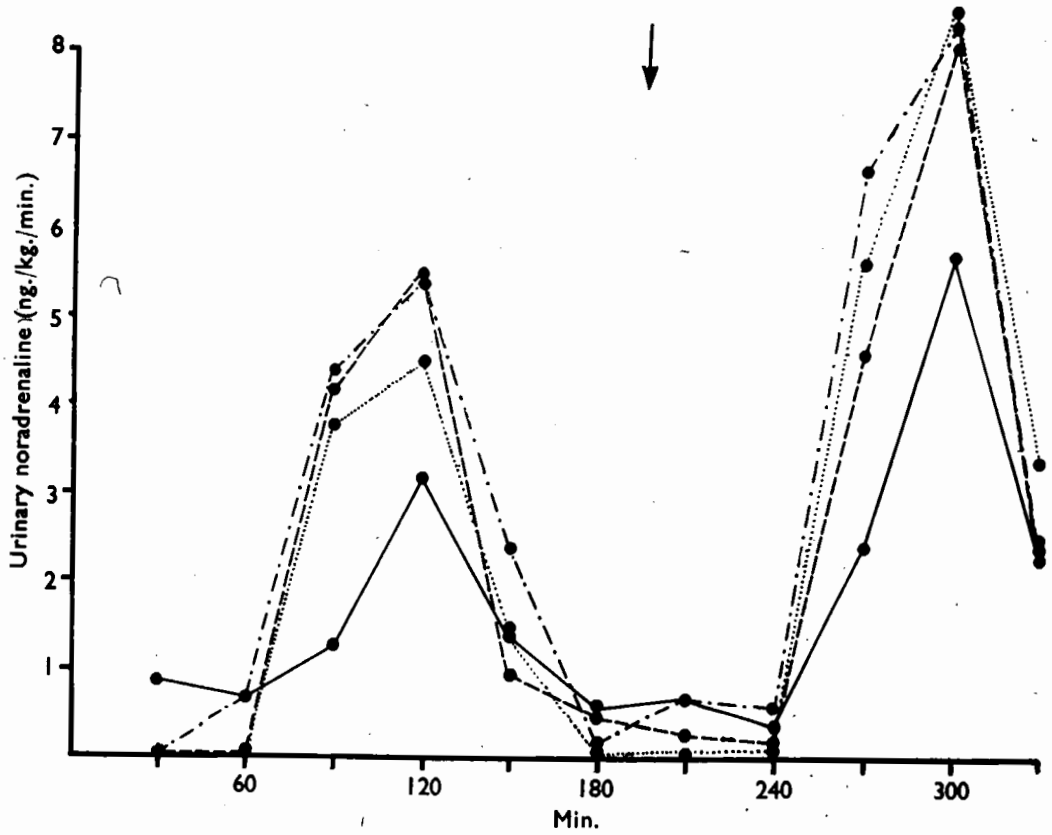


Fig. 8. - Urinary excretion of noradrenaline before and following administration (at the arrow) of phenoxybenzamine (10 mg./kg.). Each line represents a record from an individual dog. Noradrenaline (0.2μg./kg./min.) was infused from 61 to 120 and 241 to 300 min.

adrenaline.

It would appear from these experiments that phenoxybenzamine can also increase the urinary excretion of noradrenaline under these conditions.

C) The action of phenoxybenzamine on the blood pressure.

In the first two sections (A, B) of this chapter, the tables and graphs show data on the systemic blood pressure. In fact, (see A^2, A^4, B2) the effect of phenoxybenzamine on the urinary excretion of adrenaline and noradrenaline appeared to be connected with, or partly dependent on a depressor effect on the systemic blood pressure. As already pointed out, when hexamethonium was used to establish ganglionic blockade, an injection of phenoxybenzamine did not lower the blood pressure further.

In a later series of experiments, it was found that an injection of phenoxybenzamine (20 mg./kg.) could actually induce a pressor response after pretreatment with ganglionic blocking drugs. Thus, Fig. 9 illustrates the marked pressor effect of phenoxybenzamine after two doses of tetraethylammonium (30 mg./kg.). It is also shown that with TEA itself a pressor effect occurs with the second injection, as reported by Page et al (1949). In addition, after the pressure had started to decline or to level off following phenoxybenzamine, similar

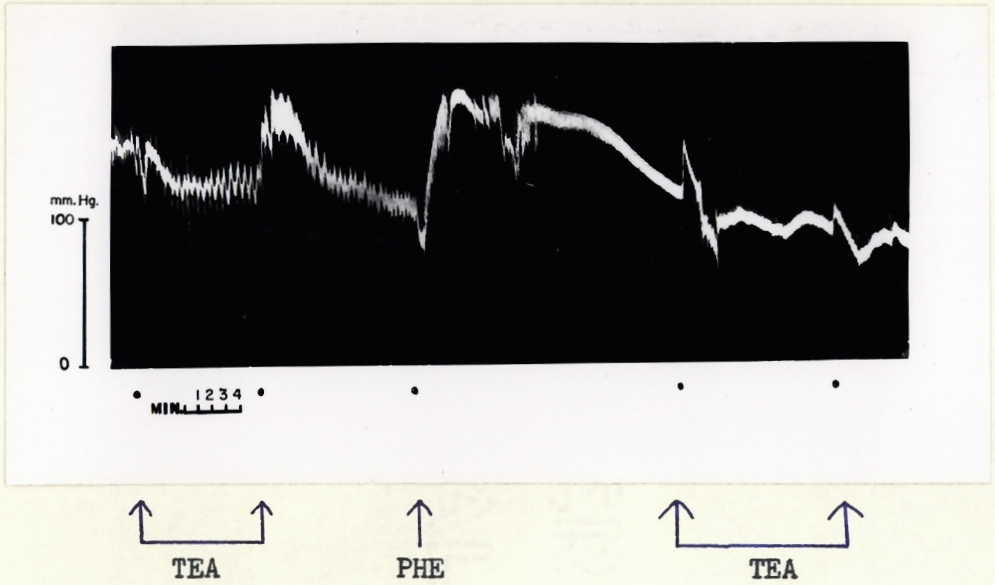


Fig. 9 - Blood pressure recording from a dog (26.0 kg.), anesthetized with sodium pentobarbital (30 mg./kg.). Time in minutes. TEA: 30 mg./kg. tetraethylammonium; PHE: 20 mg./kg. phenoxybenzamine.

doses of TEA showed only a slight initial pressor effect, followed by a depressor response. In this and subsequent blood pressure records shown, similar results have been observed in two or three experiments of the same type.

In Fig. 10, three increasing (5, 10, 15 mg./kg.) doses of hexamethonium reduced the mean pressure to approximately the same level, confirming a previous observation (Mantegazza et al., 1958) that repeated doses of hexamethonium produce decreasing responses on the blood pressure, and this time, phenoxybenzamine again induced an initial transient depressor effect followed by a sustained pressor response.

In Fig. 11, it can also be seen that with two repeated injections of pentolinium there were decreasing effects of the blocking agent (Mantezza et al., 1958). As in the previous cases, phenoxybenzamine now led to a marked pressor response and the blood pressure, in this case, remained at a relatively high level.

Using the new ganglionic blocking agent pempidine (2 injections of 2.5 mg./kg.), as shown in Fig. 12, a subsequent injection of phenoxybenzamine raised the pressure, but did this after an initial and sudden drop. In fact, in all of these blood pressure experiments,

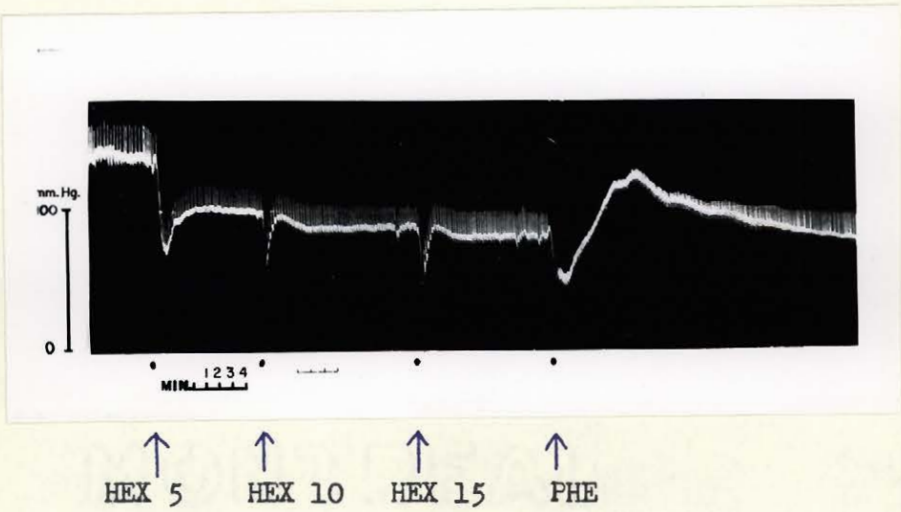


Fig. 10 - Blood pressure recording from a dog (11.3 kg.), anesthetized with sodium pentobarbital (30 mg./kg.). Time in minutes. HEX 5, HEX 10, and HEX 15: 5, 10, and 15 mg./kg. hexamethonium; PHE: 20 mg./kg. phenoxybenzamine.

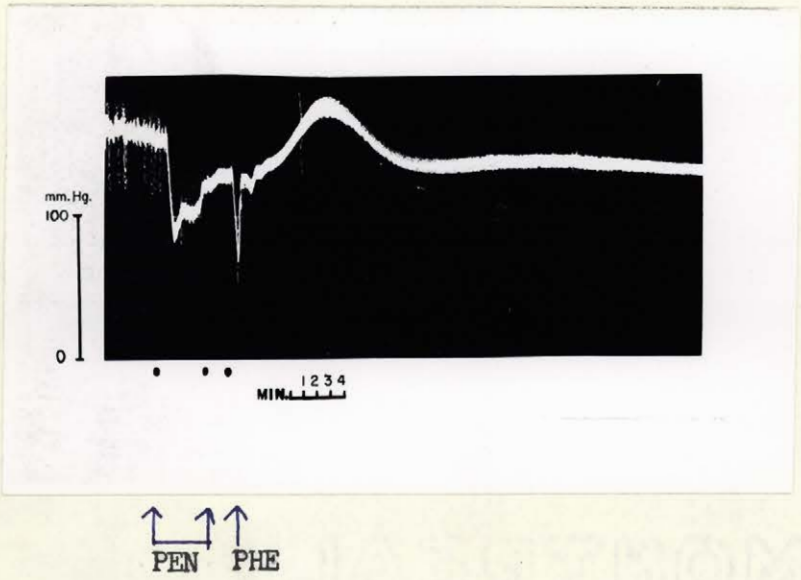


Fig. 11 - Blood pressure recording from a dog (6.1 kg.), anesthetized with sodium pentobarbital (30 mg./kg.). Time in minutes. PEN: 5 mg./kg. pentolinium; PHE: 20 mg./kg. phenoxybenzamine.

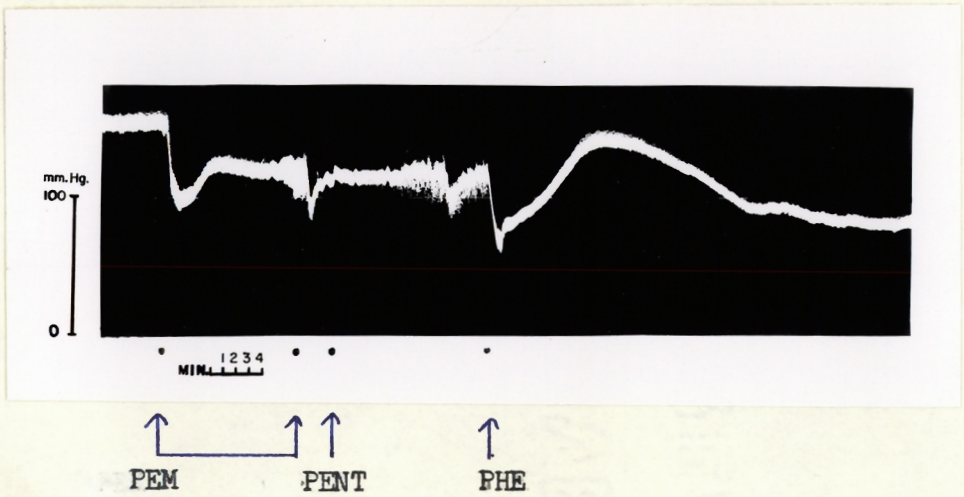


Fig. 12 - Blood pressure recording from a dog (14.0 kg.), anesthetized with sodium pentobarbital (30 mg./kg.). Time in minutes. PEM: 2.5mg./kg. pempidine; PENT: 6 mg./kg. pentobarbital; PHE: 20 mg./kg. phenoxybenzamine.

there was invariably a very brief fall of blood pressure after which, the pressure gradually rose to a maximum of 125-200 mm. Hg within 3-8 minutes. Thereafter, it declined slowly, sometimes dropping below 100 mm. Hg. This hypertensive phase lasted from 15 to 25 minutes. During the rise of pressure following phenoxybenzamine, there was no increase in heart rate as recorded electrocardiographically.

It was also observed that following repeated injections of atropine (2mg./kg.), as shown in Fig. 13, in a dog in which the vagi were cut, phenoxybenzamine again led to a brief depressor effect, followed by a pressor response similar to that observed after the ganglionic blocking drugs.

Fig. 14 shows at the beginning of the tracing the usual depressor response after a small dose (1 mg./kg.) of hexamethonium. This dose was not sufficient to prevent the depressor response to phenoxybenzamine. Following phenoxybenzamine, repeated small doses of hexamethonium induced only a pressor response, in contrast to depressor effects normally occurring in the absence of phenoxybenzamine.

When, as previously shown in preceding sections, phenoxybenzamine was given without pretreatment as above, the blood pressure fell fairly

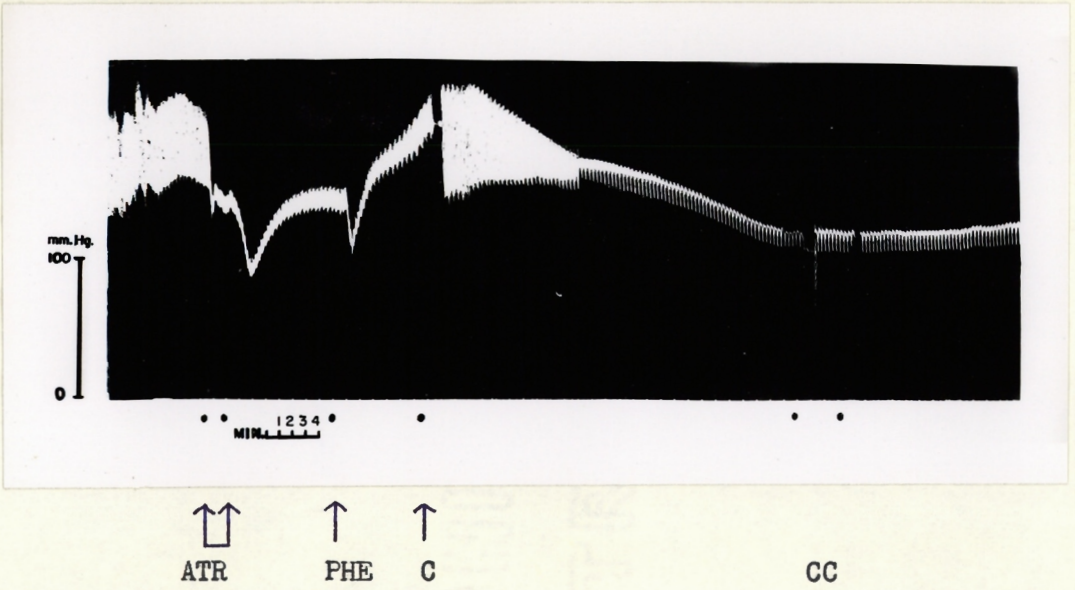


Fig. 13 - Blood pressure recording from a dog (9.0 kg.), anesthetized with sodium pentobarbital (30 mg./kg.), and vagi cut. Time in minutes. ATR: 2 mg./kg. atropine; PHE: 20 mg./kg. phenoxybenzamine. Interruptions of tracing are due to clotting (C)

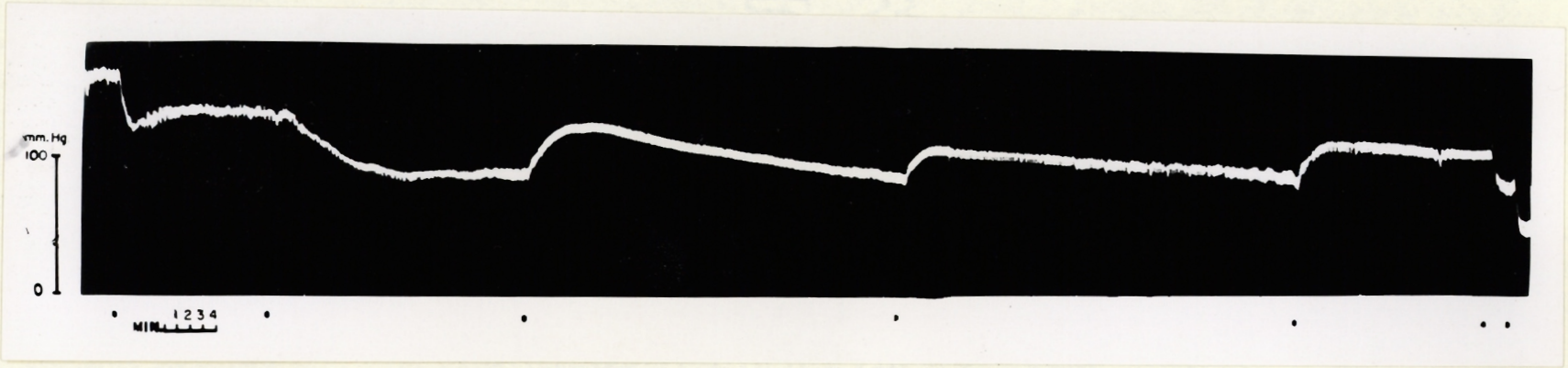


Fig. 14 - Blood pressure recording from a dog (11.6 kg.), anesthetized with sodium pentobarbital (30 mg./kg.). Time in minutes. HEX: 1 mg./kg. hexamethonium; PHE: 20 mg./kg. phenoxybenzamine. NOR 0.5: 0.5 ug./kg. noradrenaline; NOR 5: 5 ug./kg. noradrenaline.

rapidly (5-10 min.) to 80-90 mm. Hg. and it required three to four hours for it to return to its original level which was never exceeded (Fig. 1, 15, 16).

The possibility of a peripheral action of phenoxybenzamine in connection with the observed pressor responses was therefore checked in the spinal dog. In such a preparation, as shown in Fig. 17, only a sustained fall of blood pressure was observed following phenoxybenzamine.

As mentioned in the METHODS, phenoxybenzamine was always injected in a highly acidic form. Neutralization of this solution by sodium hydroxide appeared to render the treatment ineffective (no adrenaline-reversal obtained) even when the animal received the drug within one minute of the pH alteration.

Therefore, the solvent or vehicle, in which phenoxybenzamine was administered (see METHODS) was also tested after treatment with pentolinium (5 mg./kg.) (Fig. 18) and it induced only small depressor responses. Following these, the usual pressor response to phenoxybenzamine was obtained. In addition, in the same experiment, it can be seen that a dose of 1 mg./kg. phentolamine, when injected during the phenoxybenzamine rise, led to a further increase in blood pressure. Such a dose of phentolamine normally lowers the blood pressure briefly.

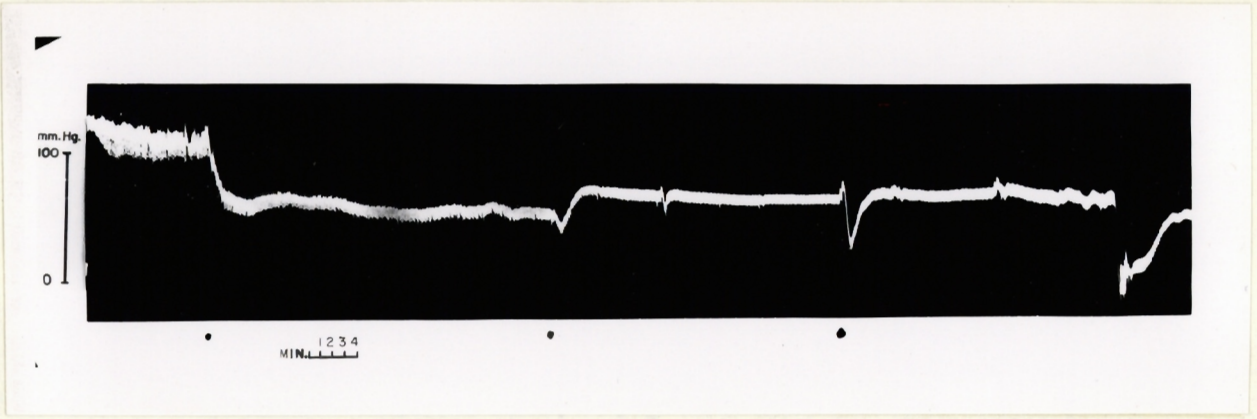


Fig. 15 - Blood pressure recording from a dog (5.0 kg.), anesthetized with sodium pentobarbital (30 mg./kg.). Time in minutes. PHE: 20 mg./kg. phenoxybenzamine; HEX: 1 mg./kg. hexamethonium; TEA: 10 mg./kg. tetraethylammonium.

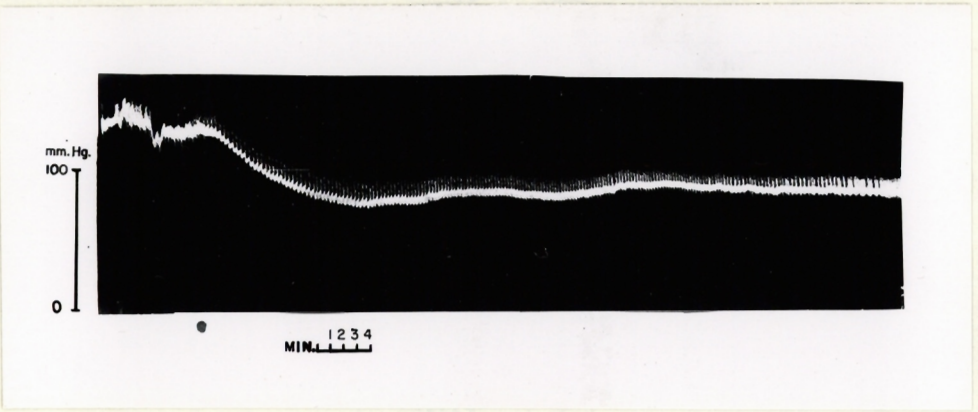
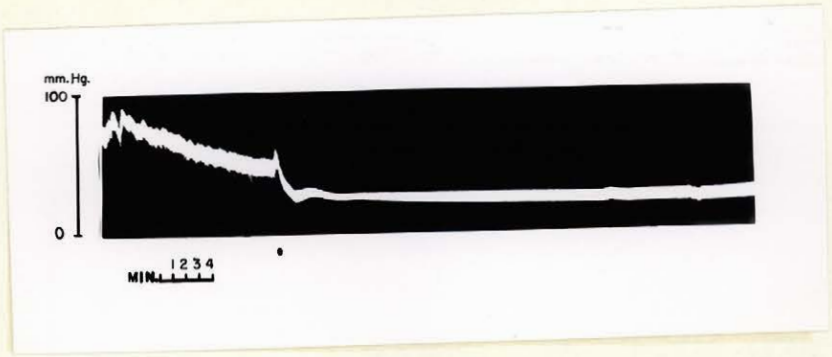


Fig. 16 - Blood pressure recording from a dog (11.9 kg.), anesthetized with sodium pentobarbital (30 mg./kg.). Time in minutes, PHE: 20 mg./kg. phenoxybenzamine.



PHE

Fig. 17 - Blood pressure recording from a dog (9.0 kg.), spinal cord transected between C_1 and C_2. Time in minutes. PHE: 20 mg./kg. phenoxybenzamine.

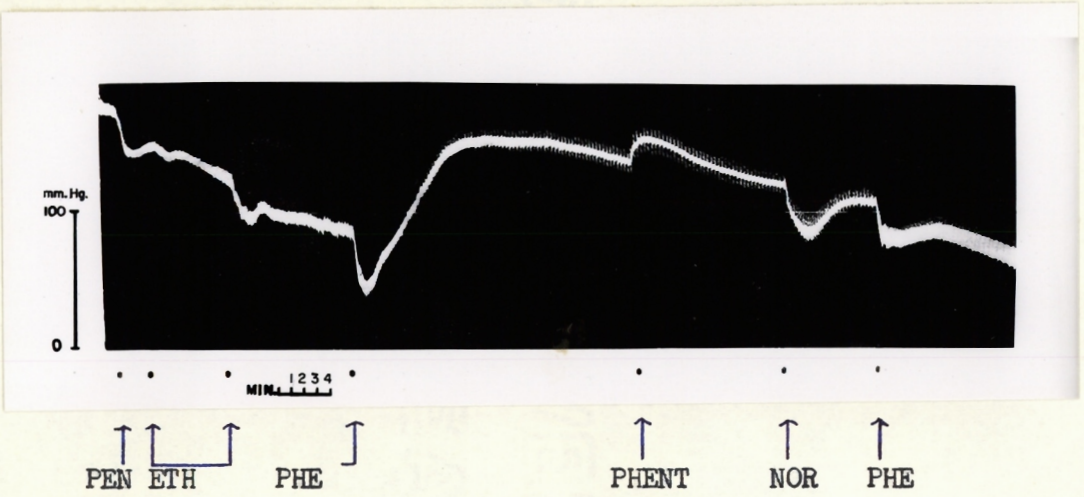
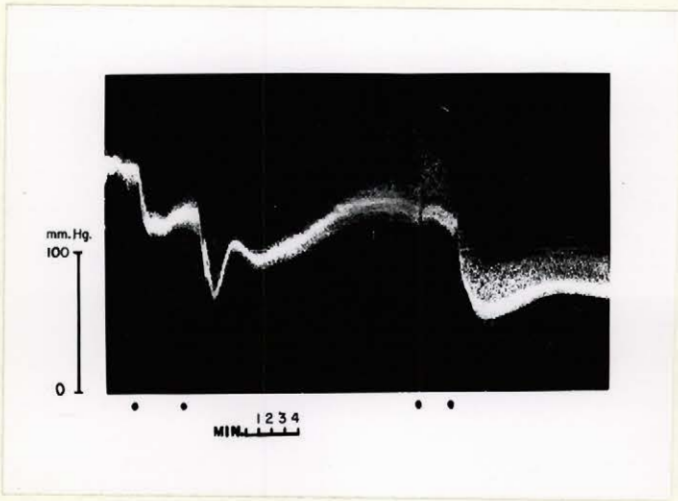


Fig. 18 - Blood pressure recording from a dog (11.0 kg.), anesthetized with sodium pentobarbital (30 mg./kg.). Time in minutes. PEN: 5 mg./kg. pentolinium; ETH: 10 ml. 20% ethanol pH 2; PHE: 20 mg./kg. phenoxybenzamine; PHENT: 1 mg./kg. phentolamine; NOR: 0.5 ug./kg. noradrenaline.

In the presence of phenoxybenzamine, the depressor response to phentolamine is therefore abolished or reversed under these conditions. Fig. 18 shows that an injection of a small dose (0.5 μ g./kg.) of noradrenaline also induced a fall of blood pressure when it had been elevated following pentolinium and phenoxybenzamine. A similar reversal is also shown in Fig. 19.

It may be concluded therefore that ganglionic blocking drugs appear to enhance the "noradrenaline reversal effect."

It has been reported that during continuous infusions of adrenaline, there is evidence of ganglionic blockade (Stehle and Melville, 1943). It was therefore of interest to compare the effect of TEA followed by phenoxybenzamine, as previously shown in Fig. 9, during infusion of a low concentration of adrenaline. Fig. 20 shows an example of the results obtained. As can be seen during such an infusion (0.5 μg./kg./min. of adrenaline), two repeated doses of TEA induced only pressor responses. A subsequent injection of phenoxybenzamine, however, led to a marked and sustained depressor response, in contrast to the pressor effect usually observed without the adrenaline infusion. Even after the infusion was stopped, the blood pressure remained at a low level (80 mm.).



PEN PHE NOR NOR
0.5 5

Fig. 19 - Blood pressure recording from a dog (10.5 kg.), anesthetized with sodium pentobarbital (30 mg./kg.). Time in minutes. PEN: 5 mg./kg. pentolinium; PHE: 20 mg./kg. phenoxybenzamine; NOR 0.5: 0.5 ug./kg. noradrenaline; NOR 5: 5 ug./kg. noradrenaline.

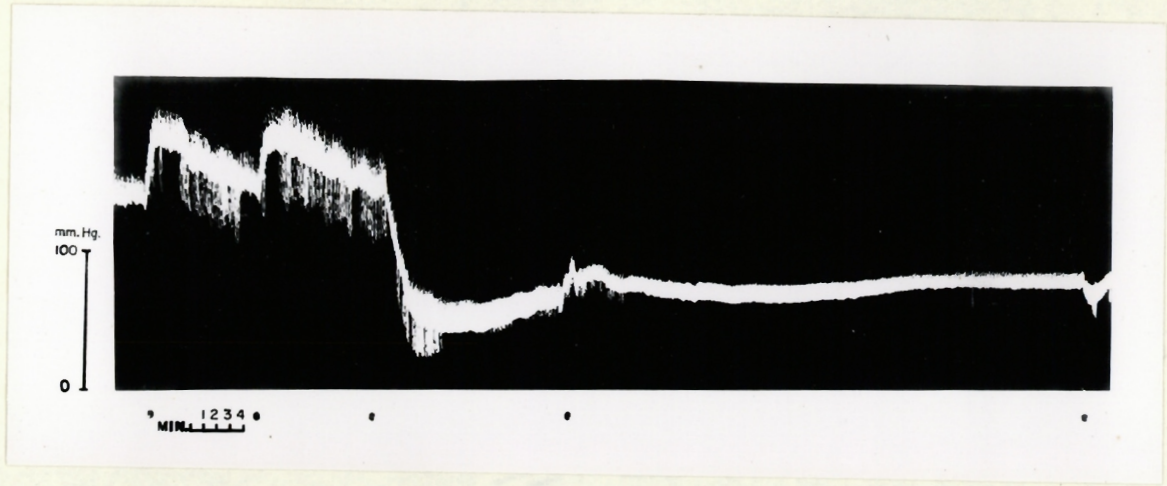


Fig. 20 - Blood pressure recording from a dog (6.5 kg.), anesthetized with sodium pentobarbital (30 mg./kg.) and infused with 0.5 ug./kg./min. adrenaline. Time in minutes. TEA: 30 mg./kg. tetraethylammonium; PHE: 20 mg./kg. phenoxybenzamine; STOP: adrenaline infusion stopped; NOR: 0.5 ug./kg. noradrenaline.

DISCUSSION

The observation in the first group of the above experiments, namely that phenoxybenzamine raises the urinary excretion of adrenaline and noradrenaline (Benfey, Ledoux and Melville, 1958) has been supported by the work of Benfey and co-workers (1959) who also found that phenoxybenzamine raised the arterial concentrations of adrenaline and noradrenaline in dogs under light thiopental anesthesia.

This effect of phenoxybenzamine on the urinary amines was found to be constantly associated with a hypotensive phase. Furthermore, when this hypotension could be prevented, the effect was suppressed. There seems to be, therefore, in the action of phenoxybenzamine some relationship between increased urinary concentrations of catecholamines and the state of hypotension. Such a relationship would imply an increase of circulating neuro-hormones responsible for the higher excretion.

Several lines of evidence point to an increased activity of some parts of the adrenergic system during a state of hypotension. Electrical splanchnic activity, for instance, is greatly increased during hypotension (Dontas and Nickerson, 1957). Generally, the suprarenal medulla releases catecholamines from its stores when stimulated by various reflex mechanisms. Heymans (1929) showed that blood pressure levels influenced the output of medullary amines. Kaindl and Euler (1951) showed that carotid occlusion increased liberation of suprarenal

catechols in the cat, (4- fold increase), and that the noradrenaline percentage was virtually unchanged from the "resting" secretion, assuming values of 60-89%.

Although experiments reported in this work offer no direct evidence as to the origin of the amines excreted, the possible significance of the medulla might be suggested by the following: 1) Referring to some of the work mentioned above, Euler has stated that the "... relatively high proportion of noradrenaline in the supra-renal venous plasma of the cat during carotid occlusion... points at [sic] a predominating liberation of noradrenaline, which, in the cat, as in most animals, is particularly active in maintaining or raising the blood pressure; 2) the work of Millar, Keener and Benfey (1959) has shown that in adrenalectomized dogs, plasma concentrations of noradrenaline after phenoxybenzamine were lower than those of normal animals. The authors note that the increased noradrenaline in normal dogs after phenoxybenzamine may therefore come from an "extra-adrenal component". The present work gives no direct information as to the role played by other types of chromaffin tissue. The ganglionic blocking drug hexamethonium suppresses both responses of the phenoxy-benzamine action, which would fit in with the speculation that some reflex adrenergic activity is "blocked" and that increased liberation of amines from sympathetic nerve endings and other sites has been prevented. The above effects of ganglionic blockade would not favor

the possibility of a direct action of phenoxybenzamine at the periphery in releasing catecholamines.

On the other hand, methacholine-induced hypotension had little influence on the excretion of noradrenaline, and only elevated urinary adrenaline slightly. It has been shown in dogs that hypotension in the early stages of hemorrhage raises mainly the urinary output and plasma level of adrenaline (1958). While the increased urinary amines after phenoxybenzamine might be due in part to the state of hypotension (which would lead to an increased adrenergic activity) it is evident that the finding of such high concentrations of the amines in the urine requires the association of the hypotension with adrenergic blockade. In fact, since the phenoxybenzamine effect was discovered it has been observed that other hypotensive adrenergic blocking drugs such as piperoxane and phentolamine, could also provoke a high urinary excretion of catecholamines (Ledoux et al., 1959).

One of the very few reports in the literature connected with this aspect of the action of phenoxybenzamine (Brown and Gillespie (1957) suggested that "... the mechanism for the destruction of noradrenaline at the nerve endings is linked to the receptors for this substance and can be inactivated with them". This speculation was based on the fact that endogenous noradrenaline could be recovered from a cat's spleen at low stimulation rates (splenic nerve) only after treatment of the cat with phenoxybenzamine. This implied that,

(1) without phenoxybenzamine, the transmitter could not be detected in the venous blood and could possibly be assumed to have been destroyed in some way or other; (2) with phenoxybenzamine, amounts of the transmitter were found, due to overflow, presumably into the circulation not having been destroyed by sites now occupied by phenoxybenzamine. Hence, contact of the transmitter with the site where it acts, would constitute a physiological mechanism of disposal of the transmitter (see Folkow, 1952).

Strong evidence suggests that phenoxybenzamine acts at "receptor sites" and reacts with them to form a prolonged blockade due to some relatively irreversible action on the blocked tissue (Nickerson, 1957). Furchgott, in 1954, used rabbit aorta strips in vitro, to show that a contact of 5 minutes with phenoxybenzamine produces a blockade of the tissue which lasts several days. This blockade is of a special type and differs from other antagonisms in that the "difference is small between a dose which produces no block of adrenaline or sympathin and one which gives a complete or almost complete block over a very wide range of concentrations". (see Nickerson and Nomaguchi, 1948).

The type of antagonism just described does not imply that increased circulating amines would be destroyed or inactivated by any other than normally operating mechanisms. Therefore, a second factor can be postulated to account for the higher excretion of amines, i.e.

decreased "inactivation", presumably along with an increased liberation. This might, therefore, constitute part of the mechanisms involved in the observed responses to phenoxybenzamine. When atropinized dogs were given high doses of acetylcholine, the urinary output of noradrenaline was higher during adrenergic blockade. Phenoxybenzamine is a long acting substance, and high urinary excretion of noradrenaline has been observed for several days in rats (Schapiro, 1958).

The concept of decreased inactivation has also received support from the experiments done with exogenous noradrenaline (Results, B 3). If phenoxybenzamine blockade was allowed to develop in the absence of exogenous sympathomimetic amines, the increased recoveries of infused noradrenaline after blockade would strongly suggest the same kind of mechanism as that postulated by Brown and Gillespie. This similarity would be qualitative only, since it appears that endogenous material cannot be quantitatively compared to injected material in terms of activity (A 3).

On the other hand, if the blockade is developed during an infusion of the exogenous "transmitters", differences in recoveries before and after blockade are not significant, for noradrenaline. The reason for this is probably on the mode of action of phenoxybenzamine which has been classified as a "non-equilibrium competitive" blocking drug (Nickerson, 1956; Furchgott, 1955). Nickerson and Gump (1949) have observed with dibenamine that in the early stages (" labile phase")

of development of the adrenergic blockade, an equilibrium existed between agonist and antagonist, and that blockade could thus be interfered with.

There were great variations in the urinary excretion of adrenaline and noradrenaline in the control as well as in the experimental periods. It is possible that anesthesia and the general condition of the animals were responsible for these variations. As it has been mentioned before (A 1), it was not possible to relate the blood pressure to the output of catecholamines during the experiment. This was also impossible to do for control periods.

In the dose range used, phenoxybenzamine lowered the blood pressure but by giving high doses of ganglion-blocking drugs previously, it was found that phenoxybenzamine could induce a marked pressor response. This was confirmed with several types of ganglion-blocking agents. It was also pointed out from the spinal dog experiment that phenoxybenzamine could not be assumed to raise the systemic pressure by a peripheral mechanism of action. This is in contrast to reserpine which has been reported by Maxwell et al., (1957) to elevate the blood pressure after ganglion-blocking drugs, and also in the spinal dog. The two drugs therefore appear to increase the free adrenaline and noradrenaline by quite different mechanisms.

The concept of "release" of catecholamines by phenoxybenzamine has been brought forward in a recent communication of Furchgott (1959).

This author suggested the possibility that β-haloalkylamines, in some way, caused a great release of transmitter from the sympathetic nerve ending; the evidence was that all of four β-haloalkylamines tested caused increases in force and rate of isolated guinea pig atria, "as a result of their liberation of catecholamines within the atria". This would suggest a reserpine-like action, but the above findings do not support this view.

Whatever the mechanism involved, (and this is difficult to assess because of the fact that we are at present in complete ignorance of the real nature of adrenergic receptors), treatment with phenoxybenzamine increases plasma concentrations (Millar et al., 1959) and urinary excretion of catecholamines. This marked increase may not be assumed to be present in the experiments where phenoxybenzamine raised the systemic blood pressure because of the high concentrations of ganglion-blocking drugs administered beforehand. However, the method used to measure adrenaline and noradrenaline cannot be claimed to indicate clearly a complete absence of amines. Moreover, Spinks and others (1958) have shown that ganglion-blocking drugs cannot paralyse sympathetic ganglia completely.

No evidence is available to explain the pressor responses to phenoxybenzamine. One might speculate that it is due to a sympathetic response. However during these experiments, heart rate was counted routinely using an E.C.G. recording machine, and an increased rate

could never be observed.

Ganglionic-blocking drugs have been known to sensitize various tissues to the action of sympathomimetic drugs or adrenergic stimulation (Bartorelli et al., 1954; Mantegazza et al., 1958). A residual concentration of catecholamines, whether a physiological amount or a small increase indetectable by assay methods, could conceivably lead to the observed rise in blood pressure and, in this sense, the antisympathomimetic action of phenoxybenzamine would be overcome. Further studies are evidently needed which would supply more information in the locus of action of both the adrenergic transmitters and the blocking drugs.

It appears that, when the protective action of phenoxybenzamine combines with the sensitizing effect of ganglionic blocking drugs, and the concentration of free adrenaline and noradrenaline in the body is low, then the antisympathomimetic action of phenoxybenzamine is overcome and an adrenergic rise in blood pressure occurs. An antisympathomimetic drug thus has a sympathomimetic action.

SUMMARY

In dogs anesthetized with sodium pentobarbital, phenoxybenzamine greatly increased the urinary excretion of adrenaline and noradrenaline. This was associated with a fall in blood pressure. Hexamethonium prevented the effect on the urinary amines and on the blood pressure. Methacholine hypotension induced an increased adrenaline excretion, but no change in noradrenaline. The excretion of noradrenaline released following ganglionic stimulation by acetylcholine was increased by phenoxybenzamine. In cats anesthetized with sodium pentobarbital, phenoxybenzamine reduced the blood pressure and increased the urinary excretion of noradrenaline. When the fall of blood pressure after phenoxybenzamine was prevented by repeated injections of vasopressin, the urinary excretion of noradrenaline did not rise.

In dogs, the infusion of small amounts of noradrenaline led to a significantly higher urinary recovery of the amine after phenoxybenzamine than before. It is concluded that phenoxybenzamine interferes with the destruction in the body of noradrenaline, whether released reflexly in hypotension or by ganglionic stimulation or injected.

The injection of phenoxybenzamine in dogs under pentobarbital anesthesia following treatment with ganglion-blocking drugs caused a marked and prolonged rise of blood pressure. During this phase,

injection of phentolamine or piperoxan produced a further brief rise in pressure, but the noradrenaline reversal effect was very strong. Hexamethonium given after phenoxybenzamine elevated the blood pressure. There was no rise of pressure when phenoxybenzamine was injected in the spinal dog. The results suggest that ganglion-blocking drugs sensitize vascular excitatory adrenergic receptors to the actions of adrenaline and noradrenaline which circulate in larger amounts due to the blocking action of phenoxybenzamine. The antisympathomimetic action of phenoxybenzamine is thus overcome. Without pretreatment phenoxybenzamine lowers the blood pressure and greatly raises the urinary adrenaline and noradrenaline excretion.

CLAIMS OF ORIGINAL WORK

The actions of phenoxybenzamine on the urinary excretion of endogenous and exogenous adrenaline and noradrenaline and on the blood pressure have not been described before. All of the results presented are, therefore, original observations and have been published in three papers. (Benfey, Ledoux and Melville, 1959; Benfey, Ledoux and Segal, 1959; Benfey and Melville, 1960).

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