Catecholamines and Ouabain

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RELATIONSHIP OF CATECHOLAMINES TO THE CARDIAC EFFECTS OF OUABAIN

by

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INTRODUCTION

Since the introduction of foxglove in clinical medicine by Withering in the 18th century, extensive clinical and experimental investigations have been performed to elucidate the mechanism of action of cardiac glycosides. Until recently, it was believed that cardiac glycosides exert fundamentally different actions on normal and failing myocardium. However, it is now generally agreed that the fundamental action of digitalis on the normal and the failing myocardium is the same (Cotten & Moran, 1961). Thus, a study of the mechanism of action of digitalis on normal heart could be considered to apply also to the failing heart.

The mechanism by which the cardiac glycosides exert their action on cardiac contractile force and on cardiac rhythm is still not clear. The effect of cardiac glycosides has been attributed, among others, to a change in cardiac metabolism, phosphorylase activity, Na, K and Ca transport. Comprehensive reviews on this subject are available (Cotten & Moran, 1961; Farah & Will, 1962; Hajdu & Leonard, 1959; Leonard & Hajdu, 1962; Winegrad, 1961).

With the introduction of catecholamine depleting agents and of beta-adrenergic blocking agents, numerous investigations have been

carried out to elucidate the possible role of endogenous catecholamines in the inotropic and arrhythmic action of cardiac glycosides. Unfortunately, the results of these investigations are conflicting. The present study was undertaken to further investigate the role of catecholamines in the cardiac actions of digitalis. An attempt was also made to find out the reason for the discrepancy in the results of different workers.

The following abbreviations are used: CA-Catecholamines;
DCI-Dichloroisoproterenol;
NA-Noradrenaline.

HISTORICAL REVIEW

Relation of Catecholamines to Cardiac Function.

The importance of endogenous catecholamines in regulating the performance of the heart has been recognized for a long time. Only in recent years, however, have a number of studies been performed attempting to elucidate the precise role of catecholamines in the regulation of cardiac dynamics.

Relation of Catecholamines to Cardiac Contractility.

There seems to be no general agreement on the role of cardiac catecholamines in the maintenance of normal myocardial contractility. Lee and Shideman (1959) found that the contractile force of the isolated papillary muscles, obtained from reserpine-treated cats, was reduced. They concluded that the stores of catecholamines in the myocardium are important in maintaining a normal contractility. Burn (1956), Hukovic and Muscholl (1962) and Muscholl (1960) have reported that the catecholamines (noradrenaline and adrenaline in a ratio of 10:1) are released from the isolated spontaneously beating rabbit atria. Kako et al. (1960), Leonard and Hajdu (1962) and Nayler and McCulloch (1960) noted that progressive loss of catecholamines, from the normally beating rabbit heart, was accompanied by a gradual decrease in its contractile force and that this

decrease in the contractile force could be reversed by the addition of noradrenaline. Furchgott et al. (1959) suggested that the contractile response of the isolated guinea-pig papillary muscle to suprathreshold stimulation was due to the release of an adrenergic transmitter substance, since DCI and prior reserpinization reduced this response. Similar conclusion was drawn by Tanz (1960), Rossin and Farah (1955) and Whalen et al. (1958).

On the other hand, Kako et al. (1960) found that although the mechanical efficiency of the isolated dog heart was significantly decreased after one hour, there was no correlation between this decrease and the myocardial catecholamine content. Similarly, Angelakos and Torchiana (1963) found that depletion of catecholamine of the isolated rabbit heart by tyramine was not associated with a decrease in its contractility.

Relation of Catecholamines to Congestive Heart Failure.

Chidsey et al. (1962) observed an augmented sympathetic nervous system (SNS) activity in patients with congestive heart failure (CHF). This augmentation was reflected by an increase in the plasma NA during exercise. Thus, in normal subjects the concentration of NA rose from 0.28 to 0.46 μg/L, while in patients with CHF it rose from 0.63 to 1.73 μg/L. This augmentation in sympathetic activity was also evidenced by an increase in the urinary excretion of NA in heart failure patients during rest

(Chidsey & Morrow, 1964). Thus, the NA excretion of normal subjects was 20.0 ± 2.5 (S.E.), while the NA excretion of patients with heart failure was 49.2 ± 4.3 (S.E.) μg/day. Similar results were obtained by Chidsey et al. (1965, 1966) and Tomomatsu (1963).

It was also observed by Chidsey and Braunwald (1966) that when radiolabelled NA was administered intravenously to normal subjects and patients with heart failure, the proportion of radioactivity excreted as the unmetabolized amine was similar in the two groups. Therefore, the increased urinary NA in heart failure resulted from an augmented rate of secretion from the sympathetic nerves and not from an altered metabolism of NA.

Chidsey et al. (1963) measured the concentration of NA in atrial tissue of patients without heart failure and of patients with heart failure. The respective values of NA were 1.82 ± 0.77 μg/g and 0.53 ± 0.47 μg/g. Similar values were obtained by Chidsey and Morrow (1964).

Chidsey et al. (1966) correlated the NA concentration of the papillary muscle from the failing human heart with the maximum tension developed by these muscles. They found that muscles with the lowest NA concentrations in general developed the smallest force.

Spann et al. (1964, 1965) confirmed the above results in

guinea-pigs with experimental heart failure produced by supravalvular constriction of the aorta. The reduction in NA concentration of the ventricles was related to the degree of heart failure. A decreased cardiac NA formation, as well as binding capacity of the heart, was observed while the net turnover of NA in the left ventricle was unchanged.

Covell et al. (1966) found that if they stimulated the cardiac accelerator nerve, the increase in the myocardial contractile force and heart rate was much smaller in animals with heart failure than in the controls. This observation was taken to support the hypothesis that sympathetic transmission is defective when cardiac norepinephrine stores are decreased in experimental heart failure.

Chidsey and Braunwald (1966) suggested that the decrease in myocardial CA, although secondary to heart failure, would produce a further deterioration of the heart.

Thus, in conclusion, it may be said that congestive heart failure, both in man and experimental animals, leads to an augmented sympathetic nervous system activity with an associated depletion of NA stores of the heart.

Relation of Catecholamines to Cardiac Rhythmicity.

Roberts and Stadter (1960) observed that following reserpinization,

the ventricles could no longer escape from vagal control. Administration of NA reversed this effect. They concluded that catecholamines are important in the intrinsic rhythmicity of the ventricles. Roberts and Modell (1961) found that in dogs with complete heart block the rhythmicity of the ventricular pacemaker was greatly decreased following reserpine and hexamethonium. These workers concluded that catecholamines are of great importance in the rhythmicity of the ventricular pacemaker but are of lesser importance in the rhythmicity of the atrial pacemaker.

Innes and Krayer (1958), Krayer and Fuentes (1958) and Waud et al.(1958), using the dog heart-lung preparation, found that myocardial NA was involved in the maintenance of sinus activity, since depletion of myocardial catecholamines lead to a reduction in the sinus rate. Burn and Rand (1958) drew similar conclusions from their experiments on the isolated atria of reserpine treated rabbits.

Alper and Schmier (1962) using the heart-lung preparation, found a significant decrease in the heart rate of dogs pretreated with reserpine (0.5 mg/kg for two days before the experiment). They concluded that reserpine reduced the heart rate by depleting the myocardium of its NA stores.

On the other hand, no significant difference was observed between the initial heart rate of control hearts and those obtained from previously reserpinized animals by Fawaz (1961) and Fawaz and Siman (1963)

in the dog heart-lung preparation, by Maxwell et al (1964) in the rabbit Langendorff preparation, by Roberts et al. (1965) in the cat atrial preparation, by Tanz and Marcus (1966) in the cat Langendorff preparation and by Trendelenburg et al. (1963) in the isolated guinea-pig atria.

Use of Reserpine and Beta-Adrenergic Blocking Agents in the Study of Cardiac Adrenergic Function.

Both reserpine and beta-adrenergic blocking agents have been extensively employed to study cardiac adrenergic function as well as the mechanism of action of several direct and indirect acting sympathomimetic agents. The possibility that both reserpine and beta-adrenergic blocking agents have a direct effect unrelated to their respective CA depleting and CA antagonizing action, has been investigated in recent years.

Reserpine.

Matsuo and Tachi (1962) found that the atria of rabbits pretreated with reserpine (1.0 mg/kg) continued to contract rhythmically although the NA content was depleted by 90%. On the other hand, application of 10^{-5} M of reserpine to the atrium in vitro abolished the rhythmic contractions in spite of only 20% depletion. From these results it was concluded that the abolition of the rhythmic contractions of the atrium was due to some

unknown effect of reserpine other than its CA depleting action. The addition of 10^{-6} to 2 X 10^{-6} M NA or adrenaline restarted the atria although there was no significant difference in the NA content of the atria arrested by reserpine and the atria restarted by the addition of NA. Tachi et al. (1962) noted a similar depressant effect of reserpine (10^{-5}M) on isolated rabbit atria and Pepeu et al. (1961) observed it on the spontaneously beating guinea-pig atria.

Innes and Krayer (1958) reported that, in the heart-lung preparation, reserpine had a negative chronotropic effect on the heart of dogs depleted of CA by previous reserpinization.

Nayler (1963) reported that while reserpine (1 µg/ml) evoked a positive inotropic response on the isolated toad ventricular muscle, larger doses of reserpine (1.5 to 5 µg/ml) produced a decrease in the contractile response. This decrease could be reversed by the addition of Ca, caffeine and strophanthin-G. From these results he suggested that reserpine may exert a direct depressant effect on the contracility of the toad ventricular muscle apart from that which can be explained in terms of depletion of CA.

Witherington and Zaimis (1961) and Zaimis (1961) also observed a depressant effect of reserpine on the myocardial contractility of cats. This could be reversed by the administration of ouabain. They suggested that the myocardial depressant action of reserpine may be produced by

the depression of either some energy yielding or energy consuming reaction. These workers concluded from their physiological as well as histological investigations that the reserpine treated animal is in a state of heart failure. The heart of these animals appeared to be large and flabby and its capacity to contract was also reduced. Similar observations were made by Nayler (1963) who found that toads that had received reserpine for four days were oedematous and had dilated ventrilces. The tension produced following stimulation of isolated strips of ventricular muscle was small when compared with that of non-reserpinized tissue. From this he concluded that the animals were in a state comparable with that of heart failure. Nayler suggested that this action of reserpine may reflect its effect on the cellular distribution of Ca ions.

Kirpekar and Lewis (1959) have suggested that reserpine has a depressant effect on the oxidative metabolism of tissues.

On the other hand, Fawaz (1961) using the isolated dog heart preparation, found no difference between control and reserpine pretreated animals with respect to heart rate, oxygen consumption, mechanical efficiency or coronary flow. Similarly, Cairoli et al. (1962) observed in intact cats that, although reserpinization decreased the heart rate, the contractile force of the heart was unaffected. These results were confirmed by Moore and Moran (1962) in the open-chest dog.

Roberts et al. (1963) also did not find any significant difference in the amplitude of contraction or the rate of failure of the papillary muscle isolated from cats pretreated with reserpine (1 to 5 mg/kg) and those from untreated preparations. These results were confirmed by Maxwell et al. (1964) in the isolated left ventricle of rabbits and by Tanz and Marcus (1966) in the rabbit Langendorff and isolated cat papillary muscle preparations. Reserpine and guanethidine were used as CA depleting agents in both these studies. Tanz and Marcus (1966) suggested, on the basis of their results, that endogenous CA contribute little to the maintenance of heart rate or force of contraction in the isolated preparation.

Raab et al. (1961) found that although prolonged administration of reserpine (0.1 to 0.2 mg/day) and syrosingopine (2 to 4 mg/day) lowered the blood pressure, reduced the heart rate and exerted a negative inotropic effect, the degree of these effects on the normal heart was directly proportional to the pre-existing sympathetic tone. It was thus concluded that these effects are due to the depletion of myocardial catecholamines produced by rauwolfia alkaloids.

In conclusion it may be said that although in general the effects of reserpine have been attributed to a decrease in tissue CA, a strong possibility exists that reserpine may produce effects apart from those which can be attributed to its ability to deplete endogenous

catecholamines.

Beta-Adrenergic Blocking Agents.

Moran et al. (1962) pointed out that dichloroisoproterenol (DCI) may possess a non-specific antiarrhythmic action. Pronethalol was also shown to have antifibrillatory and quinidine-like effects on the heart by Sekiya and Vaughan Williams (1963) and Vaughan Williams and Sekiya (1963). Lucchesi and Hardman (1961) and Lucchesi (1964) reported that the antiarrhythmic action of DCI and pronethalol cannot entirely be explained by their adrenergic blocking action since the duration of their adrenergic blockade outlasts their antiarrhythmic action. Similar results were obtained by Tuttle and Innes (1964). Benfey and Varma (1966) found that whereas pronethalol and propranolol were equipotent as antiarrhythmic agents, the latter compound was about seventeen times more potent as a beta-adrenergic blocking agent. They concluded that the antifibrillatory and quinidine-like effects of these agents may be separate from their ability to produce beta-adrenergic blockade. Similar conclusions were made by Sekiya and Vaughan Williams (1963) and Lucchesi (1965). Somani and Lum (1965) observed that both DCI and pronethalol had antiarrhythmic effects similar to those of local anesthetics.

Thus, in conclusion, it may be stated that beta-adrenergic

blocking agents produce a non-specific depressant action on the myocardium in addition to their ability to block beta-adrenergic receptors.

THE RELATION OF CATECHOLAMINES TO THE CARDIAC EFFECTS OF OUABAIN.

The Effect of Ouabain on the Uptake and Release of Noradrenaline.

Studies by Dengler et al. (1961) and Wilson, et al. (1962) have shown that the process by which brain and heart slices of cats take up ^{3}H-NA against a concentration gradient is blocked by reserpine and ouabain. Dengler et al. (1962) found that ouabain strongly inhibited the uptake of ^{3}H-NA by the heart, brain and spleen slices of cats incubated in a medium containing both ^{3}H-NA (50 mg/ml) and ouabain (10^{-5}M). These workers concluded that ouabain blocks or inhibits the active transport system for noradrenaline. Similarly, Agrawal (1965) found that ouabain inhibited the uptake of ^{14}C-NA by the isolated rabbit heart. On the other hand, Hertting et al. (1961) did not observe that pretreatment of intact cats with ouabain (50 µg/kg) affected the amount of ^{3}H-NA taken up by the heart.

Cession-Fossion (1962) reported that the CA content of rat heart was significantly lower one hour after the administration of ouabain (0.5 to 1 mg/kg) than it was in normal fresh tissue. From this observation they concluded that ouabain released NA from the tissue. Loubatières et al.

(1965) also observed that in guinea-pigs ouabain in a quantity of 125 µg/kg greatly lowered the total cardiac CA level, especially NA. Experiments with increasing doses of ouabain administered over a five-day period showed the depletion of cardiac catecholamines to be more or less proportional to the dose.

Relation of Catecholamines to the Inotropic Effect of Ouabain.

Much evidence has been accumulated in recent years both for and against the hypothesis that the inotropic and arrhythmic actions of ouabain are related to the presence or release of myocardial catecholamines.

Thus, Tanz (1960, 1962, 1964) and Tanz and Marcus (1966) have emphatically stated that the cardiac actions of digitalis depend either on the release of CA from storage sites located in the heart and/or on the presence of a certain level of myocardial CA. They based their conclusion on the fact that in both the isolated cat papillary muscle and cat Langendorff preparation, taken from cats pretreated with reserpine or guanethidine or after beta-adrenergic blocking agent DCI, the positive inotropic effect of ouabain was significantly reduced. These authors did not find that the chronotropic action of ouabain was significantly affected by reserpine or guanethidine pretreatment.

Levy and Richards (1965a) confirmed the above result. They observed that the positive inotropic response of the electrically driven rabbit atria to non-toxic amounts of ouabain was attenuated by pretreatment

of these animals with reserpine. Toxic amounts of ouabain, however, produced an almost identical percent increase in the contractile force of both normal atria and atria of reserpine pretreated rabbits. They concluded that CA are involved in the contractile response produced by non-toxic concentrations of ouabain. The same workers, Levy and Richards (1965b), did not find that pretreatment with or the presence in the bath of pronethalol or propranolol modified the positive inotropic effect of ouabain on the rabbit left atria. Denis et al. (1963) noted a reduction in the ouabain-induced augmentation of the contractile force of the auricular strips of rabbits pretreated with reserpine or guanethidine. They could not, however, directly relate the reduction in myocardial NA to a decrease in the ouabain induced augmentation. Similar results were obtained by Förster and Stolzenburg (1963) in the guinea-pig auricular preparation and by Loubatières et al. (1965) in the open-chest dog. Cession-Fossion (1962) concluded that since ouabain administration reduced the CA content of the rat heart, the action of cardiac glycosides to increase cardiac contractility would depend, at least in part, on NA liberated from the tissue. They also found that both the pretreatment of the tissue with reserpine or guanethidine produced a decrease in the inotropic action of ouabain.

Dhalla and McLain (1964) observed that a non-depressant dose of pronethanol (1 μg/ml) reduced the positive inotropic effect of ouabain to

56% of the control. From this they concluded that the inotropic action of ouabain is at least partially mediated through the activation of beta-adrenergic receptors.

On the other hand, Yelnosky and Ervin (1961) found no significant difference in the inotropic action of ouabain in normal dogs and dogs pretreated with reserpine (0.5 mg/kg for two days) or with DCI. They concluded, therefore, that this action of ouabain is not dependent upon the release of catecholamines from the heart, adrenal glands or stores which mediate the responses of certain adrenergic nerves. Similarly, Eckstein (1961) observed no significant difference in the inotropic response of control and reserpinized (0.05 - 0.1 mg/kg/day for five days) dogs to acetylstrophanthidin.

Zaimis (1961) observed that in untreated, intact cats a dose of ouabain (50 to 100 μg) produced very little change in the cardiac contractile force while in animals pretreated with reserpine (1 mg/kg/day for one day), ouabain produced a gradual increase in the amplitude of contraction. Morrow et al. (1963) studied the cardiovascular effects of ouabain in open-chest dogs. CA depletion was produced by reserpine (0.1 mg/kg/day for two days) or by chronic cardiac denervation. They found that CA depletion did not reduce the positive inotropic effect of ouabain. They concluded that the inotropic action of ouabain is independent of autonomic innervation or of myocardial CA stores. Similar

results were obtained by Daggett and Weisfeldt (1965).

The above results were confirmed by Spann et al. (1965b) using the isolated cat papillary muscle. They observed that NA depletion of the cardiac tissue by reserpinization or cardiac denervation did not depress the basic contractile state of the myocardium. However, in the presence of acetylstrophanthin (1.0 mg/kg), the maximum inotropic response of the reserpinized muscle was much lower than that of the papillary muscle removed from hearts depleted of CA by prior cardiac denervation. The latter group displayed the same degree of augmentation as did the untreated controls. They concluded that the reduced inotropic response which is shown by the reserpine pretreated papillary muscle was due to a direct depressant effect of reserpine, unrelated to its CA depleting action.

Boyajy and Nash (1963, 1965) found no evidence that reserpine (0.1 mg/kg) pretreatment influenced the positive inotropic response of isolated dog trabecular strips to ouabain.

Moran and Perkins (1958) also observed no reduction in the force of digitalis induced contraction by DCI in the intact dog or rabbit Langendorff preparation.

Relation of Catecholamines to the Toxic and Arrhythmic Effects of Ouabain.

Roberts et al. (1963) reported that the incidence of ouabain

induced automaticity was reduced in the papillary muscles taken from cats pretreated with reserpine (1 and 5 mg/kg). Furthermore, reserpine pretreatment decreased the incidence of ventricular tachycardia induced by acetylstrophanthin in combination with vagal stimulation in cats. They also observed that trimethyl-ammonium chloride (β TM_{10}), an agent which has been reported by McLean et al. (1960) to prevent the release of CA, markedly reduced the response of the ventricular pacemaker of dogs with A-V block to acetylstrophanthin (100 µg/kg). Since the arrhythmias produced by larger doses (150 µg/kg) were not affected by reserpine, they concluded that another mechanism, other than CA release, is involved in the arrhythmic action of large doses of cardiac glycosides. Repletion of CA stores with NA or isoproterenol restored the arrhythmic effect of ouabain. Similar results were obtained by Méndez et al. (1961).

Levitt et al. (1966) also reported that reserpine (5 mg/kg) increased the threshold dose of ouabain which produces ventricular arrhythmias in combination with vagal stimulation. Since reserpine did not depress ventricular reactivity to CA, they suggested that the antidigitalis action of reserpine is related to a decrease in adrenergic activity. Cairoli et al. (1961) concluded that since reserpine pretreatment reduced the positive inotropic action of ouabain and abolished ouabain induced automaticity in the isolated cat papillary muscle, CA were involved in both of these action of ouabain. Cairoli et al. (1962) further reported that pretreatment with reserpine increased the dose of acetylstrophanthin required to produce

ventricular arrhythmias in dogs with surgically induced atrio-ventricular block but did not influence the incidence of ventricular fibrillation.

Takagi et al. (1965) reported that reserpinization produced a consistent increase in the lethal dose of digoxin in dogs. Erlij and Mendez (1964) found that exclusion of the adrenergic influences on the heart by (a) reserpinization (1.5 mg/kg daily for two days), (b) acute sympathectomy and adrenalectomy and (c) slow and repeated injection of a beta-adrenergic blocking agent, pronethalol, resulted in an increase in the lethal dose of digoxin. The animals died of cardiac standstill rather than ventricular fibrillation which is the usual cause of death in digitalis intoxication. They attributed the modification in digitalis toxicity to a reduction in adrenergic influences on the heart. However, since acute sympathetic denervation does not deplete CA stores, but modified digitalis intoxication, liberation of CA or the level of CA in the heart cannot be the only factor involved.

Using the beta-adrenergic blocking agents DCI and pronethalol, Vaughan-William and Sekiya (1963) found that both agents prevented ventricular fibrillation produced by ouabain.

On the other hand, Yelnosky and Ervin (1961) did not find that the mean dose of ouabain required to produce ventricular tachycardia was affected by the pretreatment of dogs with DCI or reserpine. Similar results were obtained by Morrow et al. (1963) in the intact reserpinized

or chronic cardiac denervated dogs. Levy and Richards ((1965a) observed that the toxic effects of ouabain as well as the time required to produce ventricular arrhythmias in rabbit atria was affected by reserpine pretreatment.

Boyajy and Nash (1963, 1965) suggested that since both reserpine and ajmaline, a non-catecholamine depleting rauwolfia alkaloid, provided protection against the arrhythmic effects of ouabain, CA depletion does not seem to be an essenial component in the antifibrillatory effect of reserpine.

Schmid and Hanna (1966) and Somani and Lum (1966) concluded that since the beta-adrenergic blocking agent, MJ—1999, antagonized the arrhythmias produced by adrenaline but not those produced by ouabain, the arrhythmic effect of ouabain is not exerted through a release of catecholamines. Similarly, Lucchesi and Hardman (1961), Lucchesi (1964), Lucchesi et al. (1966), Moran et al. (1962), Tuttle and Innes (1964) and Benfey and Varma (1966) showed that the antagonism by beta-adrenergic blocking agents of the ouabain toxicity is independent of adrenergic receptor blockade.

One interesting point has been made by Sziegoleit and Förster (1964). These authors suggest that there may be a difference in the mechanism by which different cardiac glycosides are influenced by DCI.

They found that DCI increased the toxic dose of ouabain and lanatoside-C in guinea-pigs but did not affect the toxicity of digoxin and digitoxin.

METHODS AND MATERIALS

A. IN VIVO STUDIES.

Cats of either sex and weighing between 2.5 and 5 kg were used. Each animal was anaesthetized with an intraperitoneal injection of chloralose (80 mg/kg) and pentobarbitone sodium (10 mg/kg). The trachea was cannulated and the animal was respired by a positive pressure Harvard Respiratory Pump utilizing room air. The respiratory rate was about 18/min. and the volume of air was adjusted to the need of each animal (approx. 20 ml/kg).

The chest was opened by a mid-sternal incision. The heart was exposed by incising the pericardium above the right ventricle. In order to measure the cardiac contractile force, a strain gauge arch was attached to the right ventricle by cotton sutures. Care was taken not to occlude any major branch of coronary artery. The tissue between the feet of the arch was stretched to 50% more than the diastolic length. The use of strain gauge arch has been shown by Cotton and Bay (1956) to be a safe and practical method for assessing the inotropic effect of drugs. The inotropic response to ouabain was expressed as percent change from the control.

The jugular vein and the carotid artery were exposed in the neck. A cannula was inserted into the jugular vein and was pushed gently into

the right atrium. Its position was ascertained visually as well as by the pressure recording. The carotid artery was cannulated for recording arterial pressure. Right atrial pressure was recorded by means of Stathum pressure transducer P23 BC (for low pressure) and the arterial pressure by means of Statham pressure transducer P23 AA. Recordings were made on a four channel Gilson Polygraph. The zero pressures were determined at the end of the experiment by opening the right atrium and incising the aorta with the tip of the cannulae in place. The femoral vein was cannulated for making injections. Heparin (1 mg/kg) was injected I.V. to prevent clotting of blood in the cannula.

The electrocardiogram Lead II was recorded throughout the experiment in order to determine the effect of ouabain on heart rate and cardiac rhythm. The criteria for the onset of ventricular arrhythmias was taken to be the appearance of at least three consecutive ectopic ventricular contractions. The end point of the experiment was irreversible ventricular fibrillation or cardiac standstill. At this point the arterial pressure was nearly zero.

The heart rate, myocardial contractile force and arterial pressure were allowed to reach a steady state before the infusion of ouabain was started (usually 1 hour). Ouabain was administered by slow intravenous infusion into the femoral vein at a rate of 1 μg/kg/min. using a Harvard Infusion Pump. With this rate of infusion, various effects of ouabain

could be easily analyzed.

At the end of the experiment, the heart was excised from the animal and weighed. The heart was quickly frozen and stored in a frozen state for two to eight weeks at which time it was used for catecholamine determinations. The abdomen was opened by a mid-line incision and the peritoneal fluid aspirated and measured.

Relation of Catecholamines to the Cardiac Effects of Ouabain.

In order to study the relation of catecholamines to the cardiac effects of ouabain, some cats were pretreated with 0.1 mg/kg of reserpine s.c., one day before they were used for the experiment. This dose of reserpine is known to reduce the cardiac catecholamines to approximately 4% of the control (Trendelenburg & Weiner, 1962). A higher dose of reserpine (2.5 mg/kg) was injected in some cats to further ensure that the tissue catecholamines are maximally depleted.

The catecholamines of the adrenal medulla are less sensitive to the depleting action of reserpine (Carlsson et al. 1957; Trendelenburg & Weiner, 1962). In order to exclude the possible mediation of adrenal catecholamines in the cardiac effects of ouabain, acute bilateral adrenalectomy was performed in some cats pretreated with 2.5 mg/kg of reserpine one day before. This was done by opening the abdomen and exposing the adrenals. The tissue and vessels surrounding the adrenal glands were carefully cleaned and ligated. The adrenal glands were excised.

The infusion of ouabain was started after the preparation had stabilized from the effect of adrenalectomy (approx. 1 hour).

In order to ascertain the role of autonomic reflexes in the effects of ouabain, mecamylamine in a dose of 2 mg/kg was injected I.V. in some of the animals. The effectiveness of ganglionic block was ascertained by comparing the response of the nictitating membrane to preganglionic sympathetic stimulation before and after the injection of mecamylamine. Ouabain infusion was started after the heart rate, arterial pressure and myocardial contractile force had stabilized.

Experimental Congestive Heart Failure.

Since it was found that pretreatment with reserpine did not reduce the effect of ouabain in normal animals, it was of interest to see if the same would hold true for animals with experimentally produced heart failure. Healthy male or female cats weighing between 3 to 5 kg were selected. Food was withdrawn for one day prior to the operation. Anaesthesia was induced by intraperitoneal injection of 35 mg/kg pentobarbitone sodium. Surgery was performed under aseptic conditions. The trachea was intubated and the animal was respired with room air. EKG Lead II was monitored during the operation.

An incision was made along the fifth left intercostal space. This exposed the trunk of the pulmonary artery and the aorta. The

pulmonary artery was freed from the adjoining tissue. The ligature was passed around the trunk of the pulmonary artery. The vessel was constricted slowly and progressively to approximately one-third of its original diameter. Guyton et al. (1961) has suggested that constriction of the pulmonary artery to one-third results in heart failure. This was confirmed in these studies. The degree of constriction was determined visually. A 2% solution of xylocaine was applied topically whenever ventricular arrhythmias developed during the surgery. Approximately thirty minutes after producing the constriction of the pulmonary artery, the chest was closed. The animal was treated with intramuscular injections of streptomycin and penicillin daily for three days following the operation.

Of the 17 animals that were operated, 2 died within twenty-four hours after the operation, 3 died within three to seven days after the operation. The probable cause of death was pulmonary oedema. The surviving animals were divided into two groups. One group served as control and the other was pretreated one day before the experiment with 0.1 mg/kg of reserpine.

B. IN VITRO STUDIES

Isolated Rabbit Papillary Muscle.

Albino rabbits of either sex and weighing between 1 and 2 kg. were used. The animals were stunned by a blow on the head and the heart rapidly excised. Two papillary muscles were dissected from the left

ventricle. The muscles were suspended in a 100 ml organ bath containing Krebs-Henseleit solution. The composition of the solution was as follows: (g/L) NaCl - 6.78; KCl - 0.43; $CaCl_2.2H_2O$ - 0.37; $Mg\ SO_4.7H_2O$ - 0.29; KH_2PO_4 - 0.162; $NaHCO_3$ - 2.18 and glucose -2.0. The solution was oxygenated by a mixture of 95% oxygen and 5% carbon dioxide. The temperature of the bath was maintained at 37.5°C. One end of the muscle was clamped to a fixed lever. The tendenous end was connected to a Grass FT-03 Force Transducer by means of a cotton thread. The preparation was stimulated by a Tektronix stimulator at a rate of 1/sec. using square wave stimuli of 2 msec. duration. Supramaximum voltage was used. Contractions were recorded isometrically on a Gilson Polygraph. The tension on the muscle was adjusted to give a maximum contraction (approx. 1.5 g). Addition of ouabain in the bath was started 60 to 90 minutes after setting up the preparation. The muscle had stabilized during this period. Ouabain was added to one preparation while the other muscle served as the control.

Cumulative Concentration Response to Ouabain.

The inotropic response to cumulative concentration of ouabain was recorded. The concentration in the bath was increased by a factor of two starting with an initial concentration of 0.005 μg/ml. The higher concentration of ouabain was added after the maximum response to the preceding concentration had been attained. The maximum concentration of

ouabain tolerated by the muscle was 1.28 to 2.56 µg/ml. The end point of the experiment was cessation of contractions.

Effect of a Single Optimal Concentration of Ouabain.

Since it was observed that pretreatment of rabbits with reserpine did not reduce the positive inotropic response of the papillary muscle to cumulative concentrations of ouabain, the response of some preparations to a single concentration (0.32 ug/ml) of ouabain was tested and the response followed as a function of time. This dose of ouabain was selected since it was observed to produce approximately 50% of the maximum contraction.

The inotropic response was calculated as percent change from the control. It was observed during the period of stabilization (i.e. before the addition of ouabain into the bath) that the time course and the magnitude of the percent changes in the two papillary muscles were identical. Therefore, during the period of addition of ouabain to one preparation, any change in the contractility of the control preparation was used to correct the contractility of the experimental muscle.

In order to find out the influence of catecholamine depletion on the inotropic and arrhythmic action of ouabain, some rabbits were treated with reserpine. The dose schedule of reserpine pretreatment was as follows: single s.c. injection of 1/mg/kg; daily injections of 3 mg/kg for 2 days

for 3 days.
and daily injections of 3 mg/kg/ Experiments were performed 18 to 24 hours after the last dose of reserpine. According to the studies of Carlsson et al. (1957), Bertler et al. (1956), Higuchi (1962) and Higuchi et al. (1962), this schedule of reserpinization will be expected to produce nearly complete depletion of cardiac catecholamines. The mortality of rabbits receiving three daily injections of reserpine was 30%.

Isolated Electrically-Driven Left Atria of Rats.

This preparation was selected for two reasons. Firstly, it was possible to produce catecholamine depletion by "immunosympathectomy", thus avoiding the use of a drug. Secondly, the effect of ouabain could be tested in a different preparation and in a different species of animal. Levi-Montalcini and Angeletti (1962) showed that when an anti-sera to the nerve growth factor was injected in rats at birth, the amount of catecholamines in the peripheral sympathetic nerve endings was greatly reduced. In these studies "immunosympathectomy" was produced by injecting sympathetic Nerve Growth Factor-Bovine anti-serum (the anti-sera was kindly supplied by Dr. A.I. Cohen of Abbot Laboratories, Chicago) in rats one to two days after birth. A single injection of 180 µl of 61000 anti-units/ml of the anti-sera was made. This treatment has been shown by Iversen et al. (1966) to reduce the cardiac noradrenaline to 4% of the

control. The animals were used in these studies after they were approximately three months old. The untreated litter mate served as the control. Some of the normal rats were injected subcutaneously with two daily injections of 3 mg/kg of reserpine, the last injection of reserpine was given 18 to 24 hours before the experiment. Bhagat et al. (1964) found that a single injection of 1.5 mg/kg of reserpine reduced the cardiac noradrenaline in rats to about 6% of the control.

It was found in the initial studies that the rat papillary muscle was highly insensitive to the inotropic effect of ouabain. The response of the spontaneously beating right and left atria to ouabain was also very small. As much as 80 µg/ml of ouabain hardly produced any effect. Finally, the left atrium was selected for the study. The rat was killed by a blow on the head. The left atrium was dissected and mounted in a 100 ml organ bath containing Krebs-Hanseleit solution of the same composition as was used for rabbit papillary muscle. Almost every left atrium showed spontaneous regular contraction. It was, however, possible to eliminate the spontaneous contraction by surgically removing part of the atria. This was done by trial and error. The atria were driven electrically at a rate of 2/sec. with square wave pulses of 5 msec. duration and supramaximum voltage. Tektronix stimulator was used. The setup was similar to that used for the rabbit papillary muscle. The tension on the muscle was approximately 1g. Since this preparation was also less sensitive to the effects of ouabain, higher concentrations of ouabain were used.

The effect of cumulative concentration of ouabain on the rat atria was determined by increasing the concentration by a factor of two starting with a dose of 0.32 µg/ml. The final concentration was 164 µg/ml. As in the case of papillary muscle, the higher concentration was added after the maximum effect of the preceding concentration had been reached. The end point of the experiment was the cessation of the contraction.

Myocardial Catecholamine Determination.

The catecholamines were determined according to the method described by Anton and Sayer (1962). Approximately 2 g of the left ventricular tissue of cat was used for the determination. Catecholamines were extracted with 0.4N perchloric acid and adsorbed on acid-washed alumina (Woelm neutral activity, Gradel). A concentration of 0.05N perchloric acid was used to elute the catecholamines. Potassium ferricyanide (0.25%) was used as the oxidizing agent. Fluoroscence of noradrenaline and adrenaline was read at pH 7 and of adrenaline at pH 2. Fluoroscence of samples were read in Aminco-Bowman Spectrofluorophotometer using slit arrangement #5. The activation and fluoroscence wave lengths for noradrenaline and adrenaline at pH 7 and for adrenaline were 409-510 and 422-529, respectively. Internal standards were used for each sample. Calculations were made according to the method described by Crout (1961). Recovery of catecholamines was approximately 80%. The values are corrected

for the blank but not for the recovery.

The following drugs were used: Mecamylamine; Reserpine (Serpasil, Ciba - courtesy of Dr. C. Walter Murphy, Ciba, Dorval, Quebec); Ouabain (Nutritional Biochemical Ltd.).

Statistical procedures employed throughout were based on Mainland's (1952) "Elementary Medical Statistics". In order to test the significance between two sample means, the student "t" Test of Significance was applied. The value of P was obtained from Fisher's table of "Probabilities of t". At 5% levels of confidence (or $P < 0.05$), the results were taken as significant in all of the experiments.

RESULTS

Effect of Pulmonary Stenosis and of Reserpine.

Of the five out of seventeen animals which died as a result of pulmonary artery constriction, death was attributed to heart failure. An autopsy performed on some of these animals showed that the lungs were oedematous. Varying degrees of fluid accumulation were noted in the pleural and abdominal cavities. The heart appeared to be grossly enlarged. Table 1 (p. 34) summarizes these findings. It was found that the mean heart weight of thirty-seven unoperated animals, with and without reserpine pretreatment, was 3.8 ± 0.03 g/kg body weight while that of ten operated animals was 4.6 ± 0.03 g/kg body weight. The difference in the heart weight of the two groups of animals was significant ($P < 0.01$). Animals with experimental heart failure had an average of 10 ± 1.1 ml/kg body weight of ascitic fluid as compared to 2.1 ± 1.5 ml/kg body weight found in normal animals. The difference was significant ($P < 0.01$). The average right atrial pressure of animals with pulmonary stenosis was 65.3 ± 15 mm H_2O. This pressure was significantly higher ($P < 0.01$) than the atrial pressure of 10.2 ± 3.4 mm H_2O which was found in normal control animals. Pretreatment of normal animals with reserpine 0.1 mg/kg and reserpine 2.5 mg/kg significantly

TABLE I

HEART RATE, MEAN ARTERIAL AND RIGHT ATRIAL PRESSURE, HEART WEIGHT AND ASCITIS OF DIFFERENT GROUPS OF CATS.

Group	Number	Control Heart Rate Beats/Min.	Control Mean Pressure Arterial mm Hg	Control Mean Pressure Right Atrial mm H_2O	Heart Weight g/kg Body Wt	Ascitis ml/kg Body Wt	
Untreated Control	12	168 ± 7.8	74 ± 6.3	10.2 ± 3.4		1.5	
R 0.1 mg/kg	9	118[b] ± 4.7	51[a] ± 4.8	42.2[b] ± 5.2		1.0	
R 2.5 mg/kg	6	151 ± 6.9	66 ± 7.8	35.4[a] ± 8.8	3.8 ± 0.03	3.0	2.1 ± 1.
R 2.5 mg/kg + Adrenalectomy	6	137 ± 15	51[a] ± 5.2	26.3 ± 9.4		2.6	
Mecamylamine 2 mg/kg I.V.	4	160 ± 10	46[b] ± 3.9	20.4[b] ± 2.8		3.0	
Pulmonary Stenosis	5	207 ± 25	57 ± 8.6	65.3[b] ± 15		11.0	
Pulmonary Stenosis + R 0.1 mg/kg	5	115[b] ± 6.3	59 ± 2.8	32.6 ± 9.5	4.6[b] ± 0.03	9.0	11[b] ± 1.1

R Reserpine injected (s.c.) 18 to 24 hours before the experiment. Pulmonary stenosis performed 10 to 14 days before the experiment. Adrenalectomy (bilateral) was performed acutely.

[a] P 0.05 different from untreated control;

[b] P 0.01 different from untreated control.

elevated the mean atrial pressure to 42.2 $\pm$ 5.2 and 35.4 $\pm$ 8.8 mm H_2O, respectively. Pretreatment with reserpine (0.1 mg/kg) of animals with pulmonary stenosis reduced the atrial pressure to 32.6 $\pm$ 9.5 mm H_2O. Both the heart rate and atrial pressure were elevated in animals with pulmonary stenosis when compared with the control. Pretreatment of these animals with reserpine (0.1 mg/kg) decreased the heart rate as it did in the normal animals.

The effect of reserpinization on the heart rate and arterial pressure of normal animals and animals with pulmonary stenosis will be described again later.

Catecholamine Content of the Myocardium.

The effect of reserpinization and of experimental pulmonary stenosis on the catecholamine content of the left ventricle of cats is summarized in Table II, p.36 . The NA content of the left ventricle of normal cats was 1.28 $\pm$ 0.3 µg/g of tissue. The adrenaline content was 0.15 µg/g of tissue. Pretreatment of the cat with reserpine (0.1 mg/kg) reduced the NA content to 0.06 $\pm$ 0.02 µg/g. Thus, pretreatment of normal animals with reserpine 0.1 mg/kg reduced the CA concentration to less than 4% of the control.

Pretreatment of normal cats with reserpine (2.5 mg/kg) reduced cardiac noradrenaline to 0.03 $\pm$ 0.01 µg/g (i.e. less than 2% of the normal

TABLE II

CATECHOLAMINE CONTENT OF THE LEFT VENTRICLE OF DIFFERENT GROUP OF CATS

Group	Number	Noradrenaline µg/g Tissue	Adrenaline µg/g Tissue
Untreated Control	4	1.28 ± 0.3	0.15
R 0.1 mg/kg	3	$0.06^{a} \pm 0.02$	0.01
R 2.5 mg/kg	3	$0.03^{a} \pm 0.01$	0.03
Pulmonary Stenosis	5	$0.52^{a} \pm 0.17$	0.06
Pulmonary Stenosis + R 0.1 mg/kg	4	$0.04^{a} \pm 0.02$	0.02

R Reserpine was injected (s.c.) approximately 24 hours before the hearts were excised. Pulmonary stenosis was performed 10 to 14 days earlier.

a $P < 0.01$: Different from untreated control.

value) and adrenaline to 0.03 µg/g (i.e. 18% of the normal control value). The CA depletion produced by reserpine 2.5 mg/kg was not significantly greater than that produced by reserpine 0.1 mg/kg, The mean ventricular NA content of animals with pulmonary stenosis was 0.53 ± 0.17, while the adrenaline content was 0.056 µg/g. Thus, pulmonary stenosis reduced the ventricular CA content to less than 40% of the control. These values are comparable with those reported by Chidsey et al. (1963) who observed that in patients with congestive heart failure, the myocardial NA content was reduced from 1.82 ± 0.77 µg/g to 0.53 ± 0.47 µg/g or to 30% of the control. Similarly, Spann et al. (1965) reported a decrease in the left ventricular NA content of guinea-pigs with left-sided heart failure from a control value of 1.82 ± 0.10 µg/g to 0.48 ± 0.08 µg/g (30% of the control). In animals with pulmonary stenosis, pretreatment with reserpine (0.1 mg/kg) further reduced the NA content to 0.04 ± 0.02 µg/g and the adrenaline content to 0.02 µg/g. These values are 9% and 30% respectively of the concentration found in the ventricular myocardium of control animals with pulmonary stenosis. The degree to which reserpine 0.1 mg/kg depletes the tissue is similar in normal animals and in animals with pulmonary stenosis.

Relation of Catecholamines to the Inotropic Effect of Ouabain.

In Fig. 1, p.38 the average percent increase in the cardiac contractile force is shown graphically as a function of cumulative dose

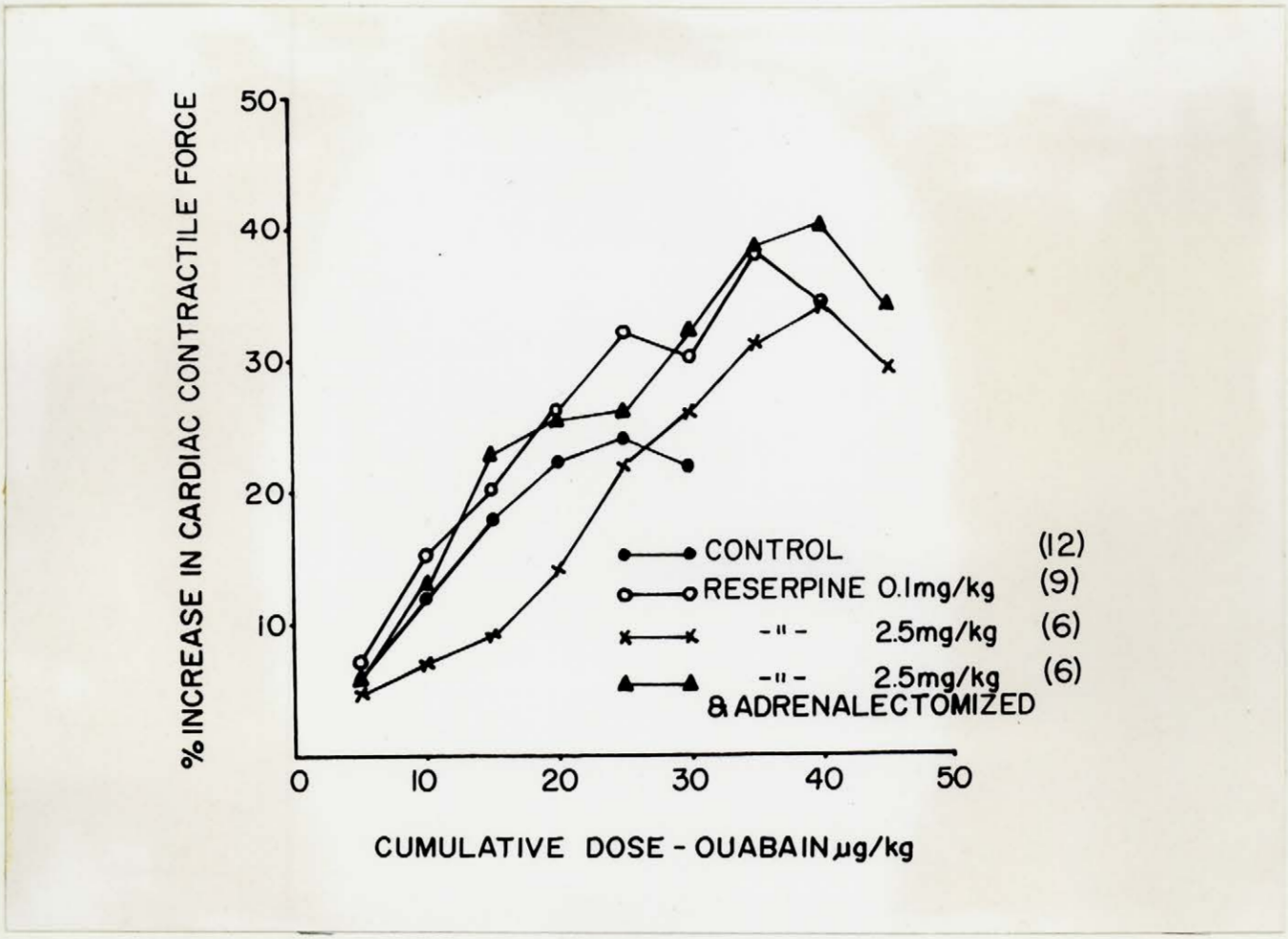

Fig. 1 The mean percent increase in the myocardial contractile force produced by continuous intravenous infusion of ouabain into different groups of open-chest cats. Experiments were performed 18 to 24 hours after the injection of reserpine. Adrenalectomy (bilateral) was performed acutely. The numbers in parentheses denote the number of animals in each group.

of ouabain. The increment in the contractile force produced by lower doses of ouabain (up to 20 μg/kg) was similar in all groups of animals except the one which was pretreated with reserpine 2.5 mg/kg. In these animals, lower doses of ouabain produced a smaller increment in contraction although the difference was not significant ($P < 0.1$). As the dose of ouabain increased above 25 μg/kg, the contractile force of the reserpine pretreated animals continued to increase while that of the control animals was already beginning to decrease. Thus, ouabain in a concentration of 25 μg/kg produced a maximum increase in the contractile force of 24% in the normal control animals. While doses of ouabain of 35 μg/kg, 40 μg/kg and 50 μg/kg produced maximum increases of 38%, 34% and 40% in animals pretreated with reserpine 0.1 mg/kg, reserpine 2.5 mg/kg and reserpine 2.5 mg/kg and adrenalectomized. None of these values are significantly ($P < 0.1$) different from each other.

The increase in the contractile response produced by ouabain in animals with ganglionic blockade is presented in Fig. 2, p. 40 . Ouabain produced an inotropic response in these animals which was greater than that of normal control animals and similar to the response of animals pretreated with reserpine. Thus, 45 μg/kg of ouabain produced a maximum increase (51%) in the contractile force.

The effect of increasing cumulative concentrations of ouabain on the contractile force of animals with pulmonary stenosis is shown

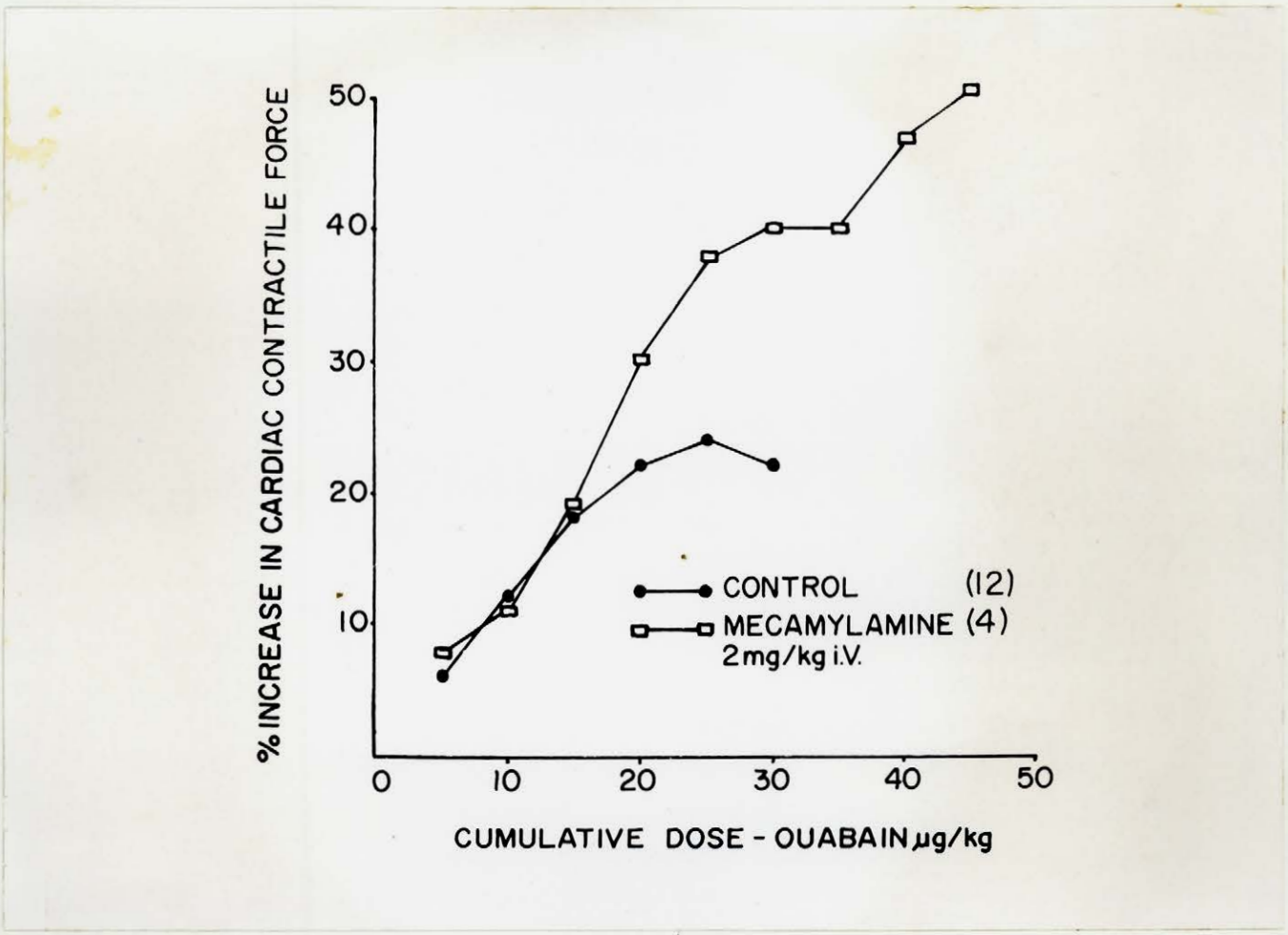

Fig. 2 The mean percent change in the myocardial contractile force produced by continuous intravenous infusion of ouabain into normal and mecamylamine treated open-chest cats. The numbers in parentheses denote the number of experiments in each group. Vertical bars represent the SE.

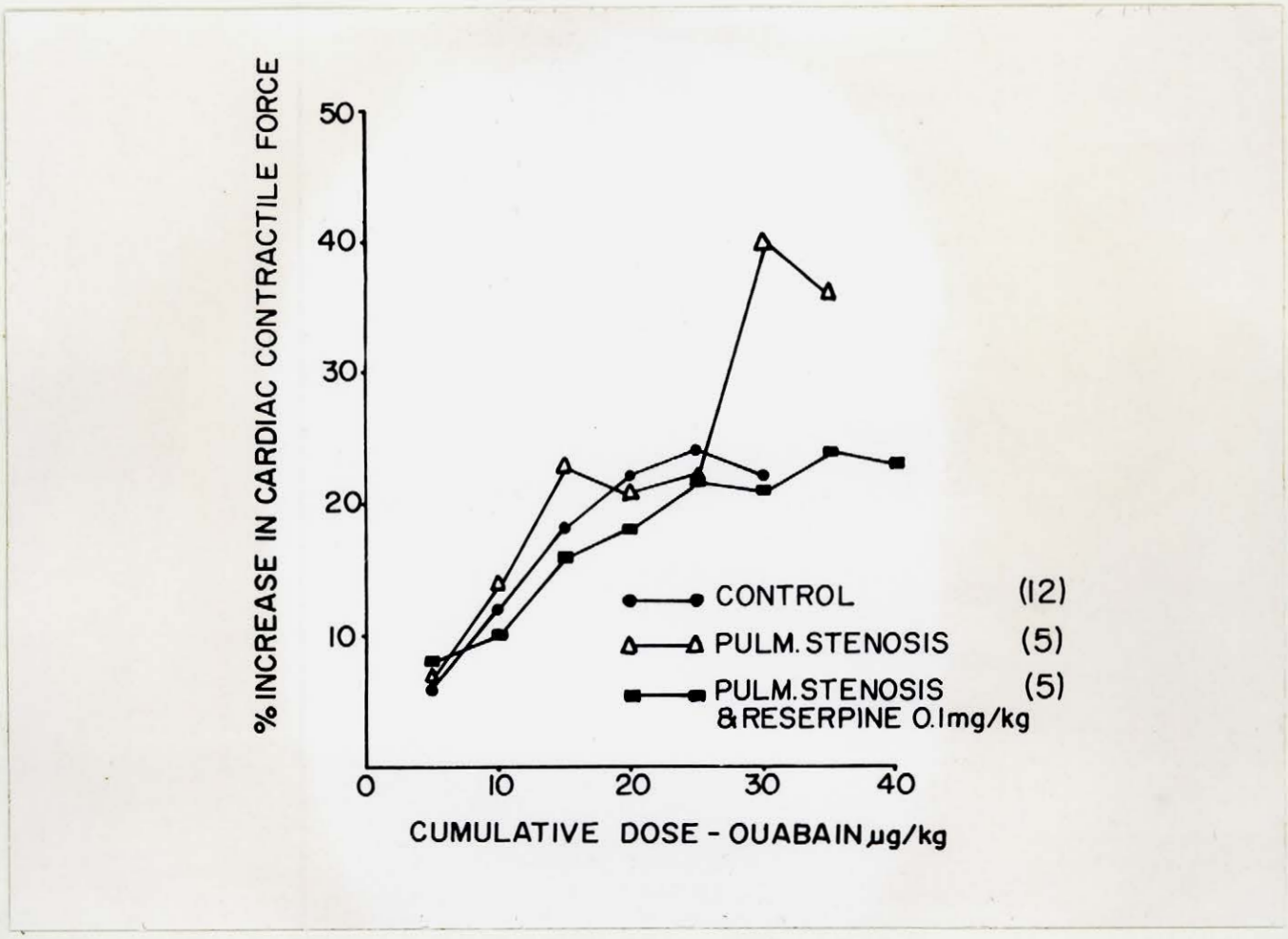

Fig. 3 The mean percent change in the myocardial contractile force produced by continuous intravenous infusion of ouabain into open-chest normal cats and cats with chronic pulmonary stenosis (performed 10 to 14 days before the experiment). Experiments were performed 18 to 24 hours after the injection of reserpine. Numbers in parentheses denote the number of experiments in each group.

graphically in Fig. 3, p. 41. Pretreatment of these animals with 0.1 mg/kg of reserpine slightly reduced the positive inotropic effect of ouabain. This difference, however, was not signficant.

The cumulative dose/response curve as shown in Fig. 1, 2 and 3 does not present an accurate picture of the maximum inotropic effect of ouabain in different groups of animals. The maximum inotropic effect was not produced by the same dose in all the animals of one group. Therefore, the maximum inotropic effect of ouabain in individual animals of the same group was averaged. The results obtained in this way are presented in Table III, p. 43. In this table are also included the inotropic effects of 25 μg/kg of ouabain, the dose which produced the maximum inotropic effect in normal control animals.

From the data presented in Table III, it can be seen that the maximum inotropic effect of ouabain in reserpine and mecamylamine treated animals was greater than the maximum inotropic effect of ouabain in untreated controls. Pretreatment with reserpine, of animals with pulmonary stenosis, reduced the maximum positive inotropic effect of ouabain. This difference was not significant.

The mean dose of ouabain which produced the maximum inotropic effect in animals pretreated with 2.5 mg/kg of reserpine is significantly greater than the mean dose of ouabain required to produce the maximum inotropic effect in untreated controls. Thus in the animals pretreated

TABLE III

EFFECT OF CONTINUOUS INTRAVENOUS INFUSION OF OUABAIN ON THE MYOCARDIAL CONTRACTILE FORCE (C.F.) OF ANAESTHETIZED OPEN-CHEST CATS

Group	Number	Maximum % Increase in C.F. Mean ± SE	Dose of Ouabain (µg/kg) For Maximum Increase in C.F. Mean ± SE	% Increase in C.F. by 25 µg/kg of Ouabain Mean ± SE
Untreated Control	12	29.2 ± 6.8	24.6 ± 2.3	24.0 ± 6.2
R 0.1 mg/kg	9	48.0[a] ± 5.9	33.3 ± 4.2	32.0 ± 5.4
R. 2.5 mg/kg	6	49.1 ± 7.7	46.0[b] ± 4.9	22.0 ± 5.8
R 2.5 mg/kg + Adrenalectomy	6	51.1 ± 9.1	41.8[b] ± 3.9	33.0 ± 5.7
Mecamylamine 2 mg/kg I.V.	4	51.2 ±11.0	33.8 ± 4.4	37.0 ± 6.3
Pulmonary Stenosis	5	63.6 ±21.0	31.0 ± 5.7	22.0 ± 13.0
Pulmonary Stenosis + R 0.1 mg/kg	5	42.6 ± 8.1	32.0 ± 8.3	22.0 ± 8.1

R Reserpine injected (S.C.) 18 to 24 hours before the experiment. Pulmonary stenosis was performed 10 to 14 days before the experiment. Bilateral adrenalectomy was performed acutely.

a $P < 0.05$; different from untreated control;

b $P < 0.01$; different from untreated control.

with 2.5 mg/kg reserpine, both the maximum inotropic effect of ouabain and the average dose of ouabain required to produce this effect are greater than the respective figures for untreated controls. The average inotropic effects of 25 μg/kg of ouabain in various groups of animals were not different from each other.

The Effect of Ouabain on the Mean Right Atrial Pressure.

The effect of ouabain on the atrial pressure of normal untreated and reserpine treated animals is shown in Fig. 4, p. 45 . The control atrial pressure in reserpine treated animals was found to be higher than in untreated control animals. The statistical significance of these differences is shown in Table 1, p. 34 . Ouabain up to 15 μg/kg dose level did not change the atrial pressure of untreated controls. With higher doses of ouabain (> 15μg/kg), a slight progressive increase in the atrial pressure was noted. The rise in the atrial pressure was found to coincide with a decline in the positive inotropic effect of ouabain.

Smaller doses of ouabain slightly decreased the atrial pressure in reserpine treated,normal animals. The magnitude of the decrease appeared to be related to initial atrial pressure. In general, the higher the control atrial pressure, the greater was the decrease due to ouabain. It was generally noted that the dose of ouabain which produced the maximum

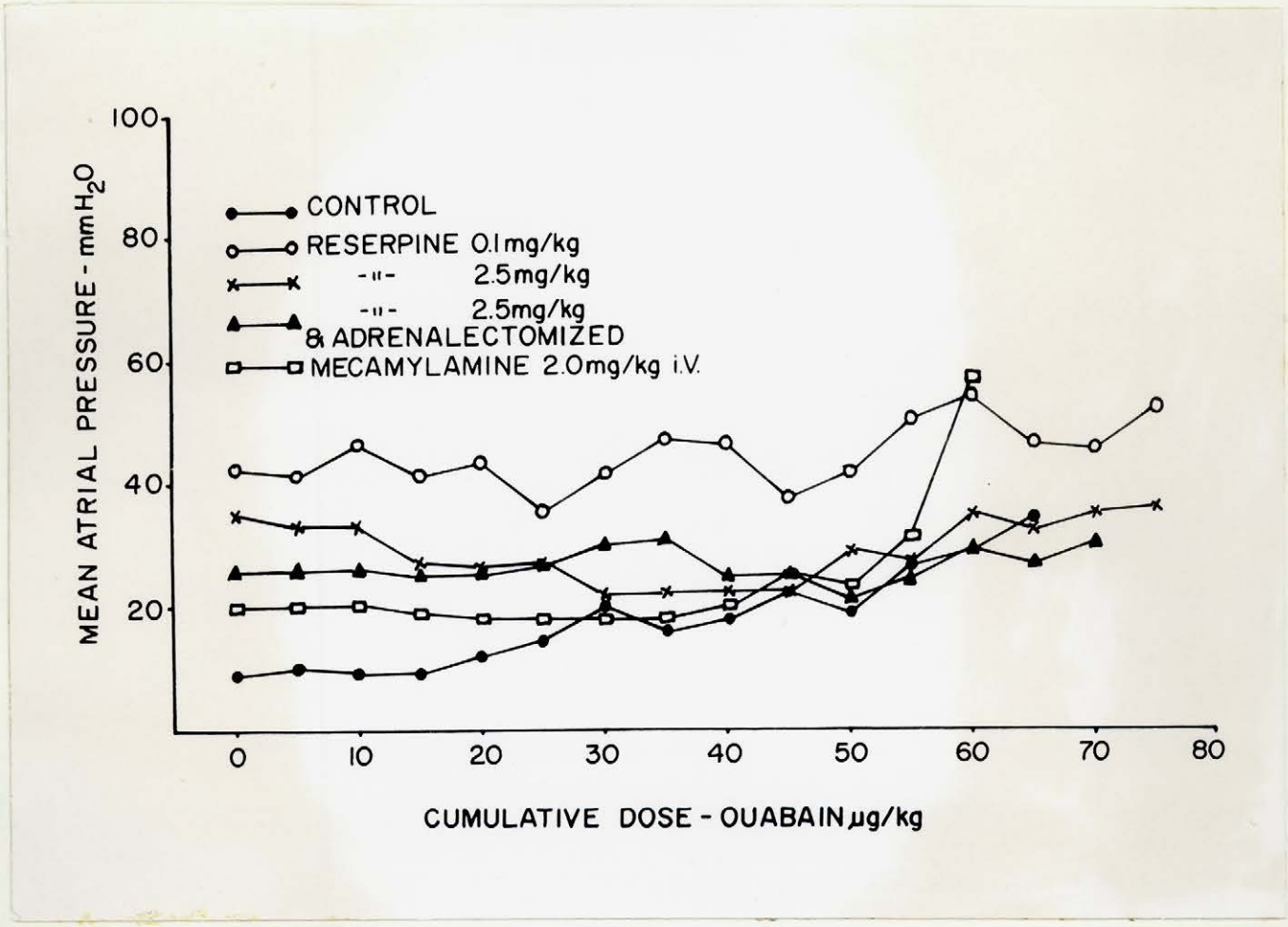

Fig. 4 The mean changes in the mean right atrial pressure produced by continuous intravenous infusion of ouabain into open-chest cats. The data are obtained from the same animals as in Figures 1 and 2.

decrease in the atrial pressure was very near the dose which produced the maximum positive inotropic effect. As the dose of ouabain was increased further, the atrial pressure began to rise. The highest atrial pressure was noted as the heart went into ventricular fibrillation or cardiac standstill.

The effect of ouabain on the atrial pressure of animals with pulmonary stenosis is shown in Fig. 5, p.47 . The control atrial pressure in animals with pulmonary stenosis was 65 mm H_2O. This was significantly greater than the control atrial pressure in normal animals. Indeed, this pressure was close to, or higher than, the highest pressure recorded in normal animals during ventricular fibrillation or cardiac standstill. In these animals, unlike in the normal, pretreatment with reserpine resulted in a decrease in the control atrial pressure. The decrease in the atrial pressure produced by ouabain seemed to be related to the initial atrial pressure rather than to the pretreatment. In untreated animals, as the dose of ouabain was increased beyond 30 µg/kg, the atrial pressure progressively increased until it reached a maximum of 90 mm H_2O with a dose of 50 µg/kg of ouabain. In the animals with pulmonary stenosis and pretreated with reserpine, a dose of ouabain greater than 50 µg/kg produced a steep, sudden rise in atrial pressure.

When the dose of ouabain which produced the maximum inotropic

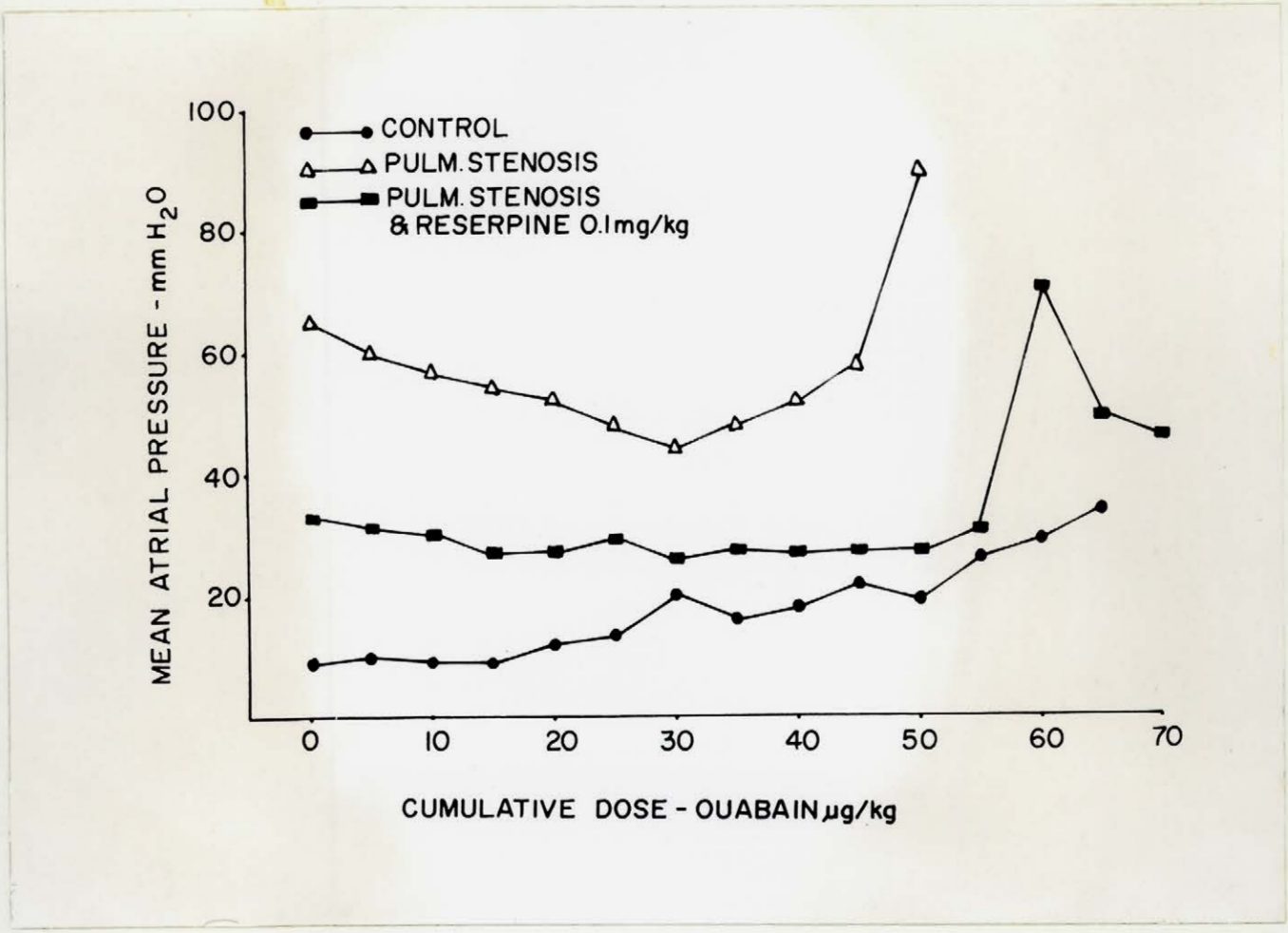

Fig. 5 The mean changes in the mean right atrial pressure produced by continous intravenous infusion of ouabain into open-chest cats. The data are obtained from the same animals as in Figure 3.

was
effect/correlated with the dose of ouabain just before the atrial pressure began to increase, it was found that it was the same (30 μg/kg) in untreated animals. In the reserpine treated animals, the maximum inotropic effect was produced by 40 μg/kg of ouabain. The atrial pressure abruptly increased after an injection of 55 μg/kg of ouabain.

The Effect of Ouabain on the Heart Rate.

The effect of ouabain on the heart rate of normal untreated and reserpine treated animals is shown in Fig. 6, p. 49 . Pretreatment of animals decreased the control heart rate. The lowest control heart rate of 118 beats/min was observed after pretreatment with 0.1 μg/kg of reserpine. The statistical significance of the difference in the control heart rate of different groups of animals is presented in Table 1, p. 34 .

Ouabain produced a slight but progressive bradycardia in all animals. With higher doses of ouabain an increase in the heart rate occurred shortly before the onset of ventricular arrhythmias. Pretreatment with reserpine did not appear to influence the effect of ouabain on the heart rate.

The effect of ouabain on the heart rate of animals with pulmonary stenosis is presented in Fig. 7, p. 50 . The control mean heart rate of untreated animals was 207 beats per minute. Pretreatment with

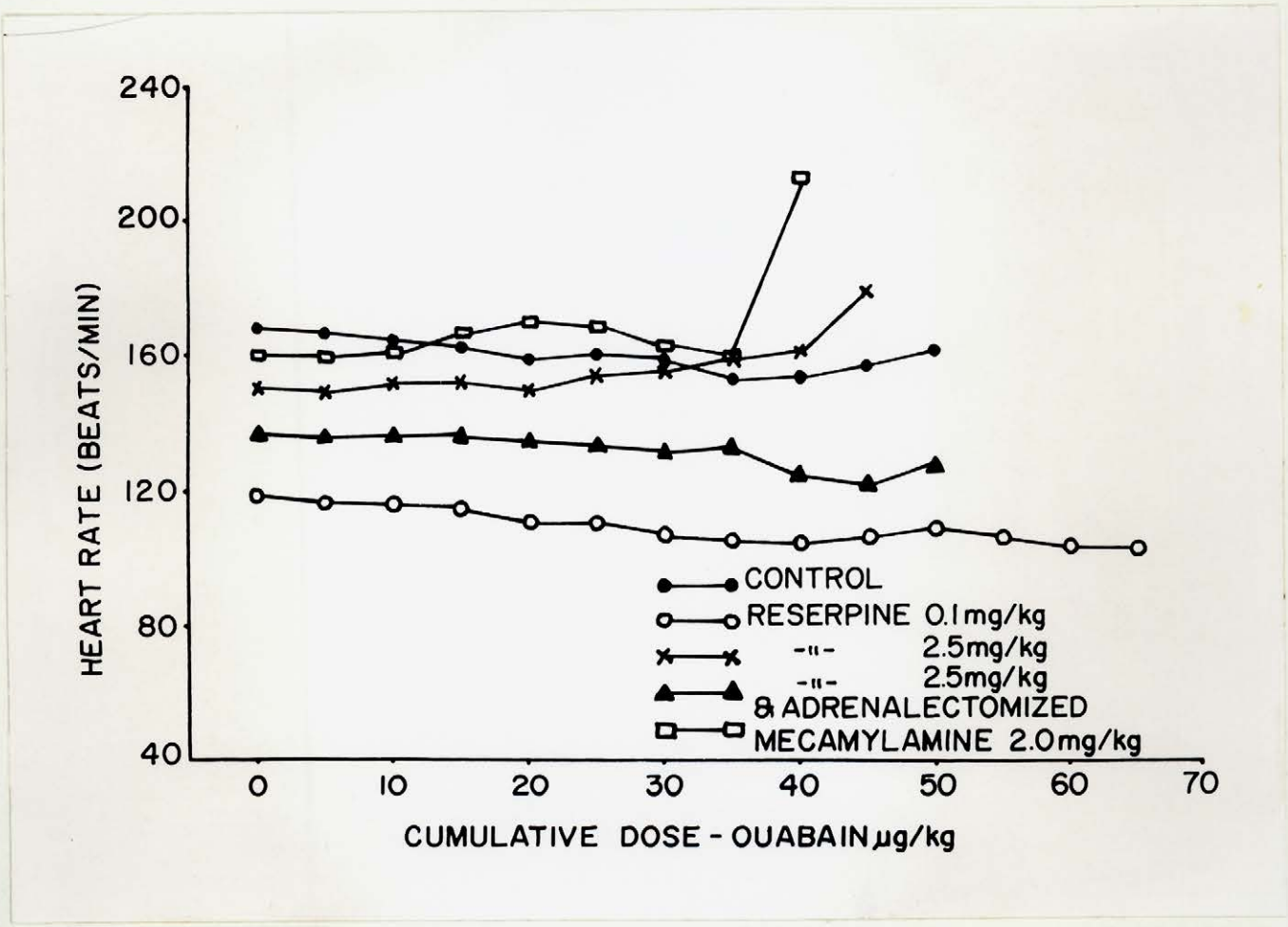

Fig. 6 The mean changes in the heart rate (beats/min) produced by continous intravenous infusion of ouabain into open-chest cats. The data are obtained from the same animals as in Figures 1 and 2.

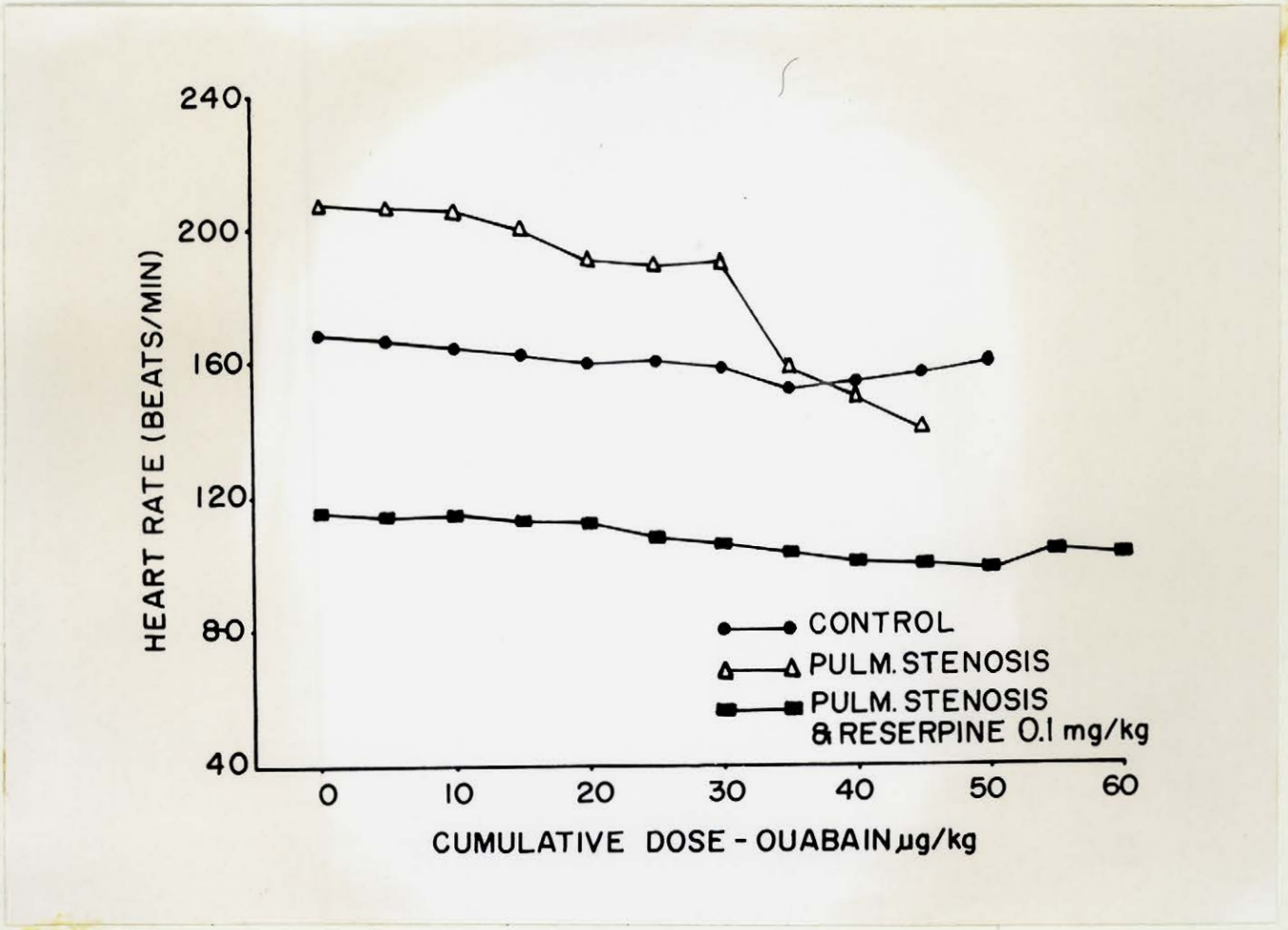

Fig. 7 The mean changes in the heart rate (beats/min) produced by continuous intravenous infusion of ouabain into open-chest cats. The data are obtained from the same animals as in Figure 3.

reserpine reduced the control heart rate to 115 beats/min. This difference was significant ($P < 0.01$).

Ouabain produced a progressive decrease in the heart rate. This decrease was more pronounced in untreated animals presumably because their control heart rate was high. Maximum decrease in the heart rate of untreated animals was produced with 45 µg/kg of ouabain. Ouabain did not, at any dose level, increase the heart rate of animals with pulmonary stenosis. This is in contrast to the effect of ouabain in normal animals.

Effect of Ouabain on Mean Arterial Pressure.

The effect of ouabain on the arterial pressure of normal animals is presented in Fig. 8, p. 52. Ouabain produced only a slight effect on the arterial pressure of normal animals regardless of the pretreatment. Failure of ouabain to increase the arterial pressure in these animals was unexpected.

The effect of ouabain on the arterial pressure of animals with pulmonary stenosis is shown in Fig. 9, p. 53. Reserpine pretreatment did not lower the arterial pressure of these animals. Ouabain progressively increased the arterial pressure of these animals. This increase was somewhat greater in untreated animals.

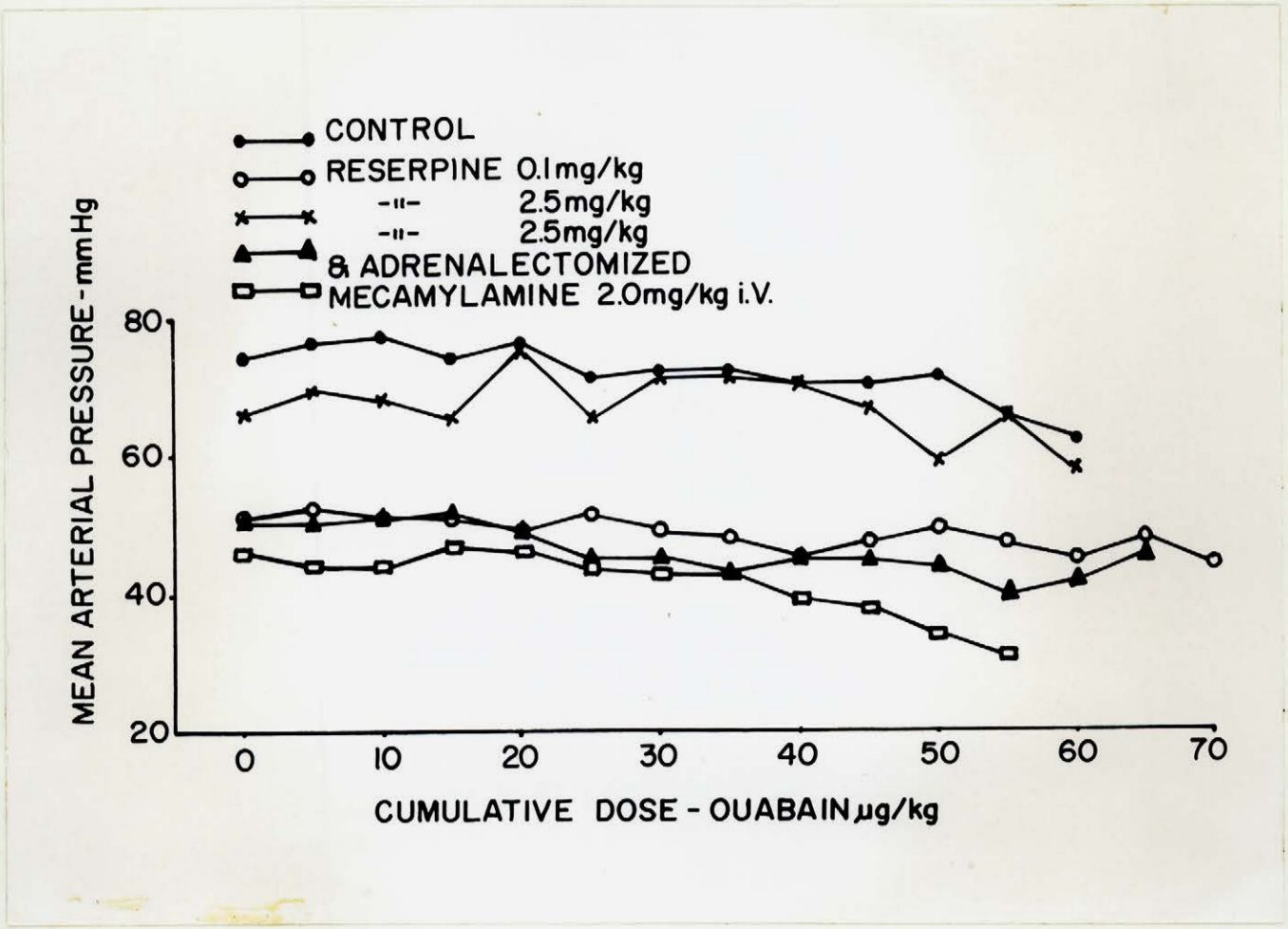

Fig. 8 The mean changes in the mean arterial pressure produced by continuous intravenous infusion of ouabain into open-chest cats. The data are obtained from the same animals as in Figures 1 and 2.

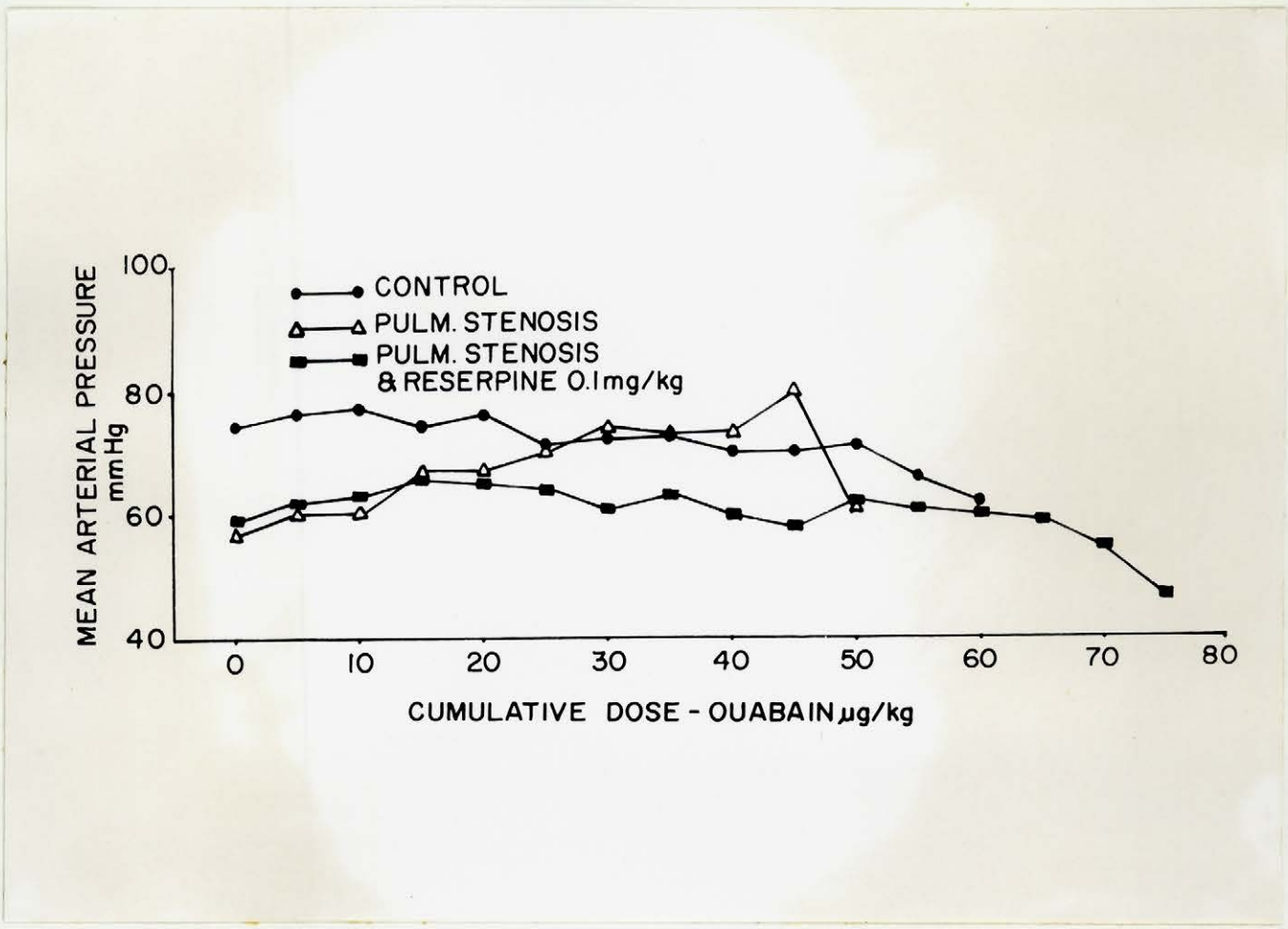

Fig. 9 The mean changes in the mean arterial pressure produced by continuous intravenous infusion of ouabain into open-chest cats. The data are obtained from the same animals as in Figure 3.

As the dose of ouabain was increased, the arterial pressure declined. This coincided with the onset of consistent arrhythmias. With the onset of ventricular fibrillation or cardiac standstill, the arterial pressure fell to zero. These changes are not shown in the figures.

The Arrhythmic Effect of Ouabain.

A summary of the mean cumulative dose of ouabain required to produce ventricular arrhythmias, ventricular fibrillation, cardiac standstill and death is shown in Table IV, p. 55 .

In the normal controls, the dose of ouabain required to produce ventricular arrhythmias was 48.1 $\pm$ 3.6 µg/kg. In the animals treated with 0.1 mg/kg of reserpine, this dose was increased to 62 $\pm$ 7.1 µg/kg. This difference was not significant. In the animals pretreated with 2.5 mg/kg of reserpine, 55 $\pm$ 2.7 µg/kg of ouabain produced ventricular arrhythmias. This value was also not significantly different from the arrhythmic dose of ouabain in untreated controls.

Similarly, pretreatment of animals with 2.5 mg/kg of reserpine and excision of both adrenals failed to significantly increase the arrhythmic or lethal dose of ouabain.

The effect of reserpine pretreatment appeared to reduce the incidence of ventricular fibrillation produced by ouabain. Thus, in

TABLE IV

THE ARRHYTHMIC AND LETHAL DOSE OF OUABAIN IN DIFFERENT GROUPS OF ANAESTHETIZED OPEN-CHEST CATS

Group	Number	Cumulative Dose of Ouabain (μg/kg)			
		Ventr. Arrh.	Ventr. Fibril.	Cardiac Standstill	Death
Untreated Control	12	48 ± 3.6	74 ± 4.5 (11)	45	72 ± 4.5
R 0.1 mg/kg	9	62 ± 7.1	76 ± 5.2 (6)	94 ± 13.3 (3)	84 ± 5.8
R 2.5 mg/kg	6	55 ± 2.7	75 ± 6.0 (4)	78 ± 24 (2)	76 ± 7.3
R 2.5 mg/kg + Adrenalectomy	6	59 ± 4.1	81 ± 9.7 (5)	100 (1)	85 ± 9.1
Mecamylamine 2 mg/kg I.V.	4	45 ± 3.5	60[a] ± 4.2 (4)		63 ± 4.9
Pulmonary Stenosis	5	41 ± 3.2	58 ± 6.1 (5)		59 ± 6.6
Pulmonary Stenosis + R 0.1 mg/kg	5	62[b] ± 1.7	85[a] ± 3.2 (3)	83 ± 2.3 (2)	84[b] ± 2.0

R Reserpine injected (s.c.) 18 to 24 hours before the experiment. Pulmonary stenosis was performed 10 to 14 days before the experiment.

Ventr. = Ventriclular; Arrh. = Arrhythmia; Fibril. = Fibrillation.

[a], $P > 0.05$; [b]. $P > 0.01$: Different from untreated control.

case of 12 untreated controls, 92% of the animals died of ventricular fibrillation. Of the 9 animals treated with 0.1 mg/kg of reserpine, and of 6 animals treated with 2.5 mg/kg of reserpine, 66% died of ventricular fibrillation and the rest died of cardiac standstill. The dose of ouabain required to produce ventricular fibrillation was not significantly altered by pretreatment with reserpine.

Pretreatment with reserpine of animals with chronic pulmonary stenosis significantly reduced the toxic effect of ouabain. This was in contrast to the findings in the normal animals. Thus, administration of a single injection of 0.1 mg/kg of reserpine in the animals with pulmonary stenosis, increased the dose of ouabain required to produce ventricular arrhythmias from 41 ± 3.2 µg/kg to 62 ± 1.7 µg/kg, that required to produce ventricular fibrillation from 58 ± 6.1 µg to 85 ± 3.2 µg/kg and the lethal dose of ouabain from 59 ± 6.6 µg/kg to 84 ± 2.0 µg/kg. All these differences are statistically significant. All the five untreated animals and three out of five reserpine pretreated cats died of ventricular fibrillation.

The Effect of Ouabain on the Contractile Force of the Isolated Rabbit Papillary Muscle.

The effect of cumulative concentration of ouabain on percent change in the contractile force of papillary muscle of untreated

and reserpine pretreated rabbits is shown in the left half of Fig. 10, p. 58 . Rabbit papillary muscle was found to be a satisfactory preparation in these studies. The inotropic response of the preparation was dose dependent. The response was stable and hence the determination of a cumulative concentration/response curve was satisfactory. On the right half of the firure is presented the effect of a single concentration of ouabain. The response of the papillary muscle to 0.32 μg/ml of ouabain was comparable whether it was added to the bath in single injection or was attained by gradual increments.

The initial contractile force of the papillary muscle of reserpine treated rabbits was not different from the contractile force of control papillary muscles. Pretreatment of rabbits with reserpine in dosage of 1.0 μg/kg for 1 day, 3 mg/kg daily for 2 days or 3 mg/kg daily for 3 days, did not reduce the positive inotropic response of the papillary muscle to cumulative concentration of ouabain. Indeed, the inotropic effect of ouabain on the papillary muscle of reserpine treated rabbits was somewhat greater, although the difference was not significant. The effect of 1.28 μg/ml of ouabain on the papillary muscle of rabbits pretreated with 3 mg/kg of reserpine daily for 3 days is not presented in Fig. 10, since the lower concentration had produced the maximum effect in three out of four preparations. A concentration of 2.56 μg/ml of ouabain completely stopped

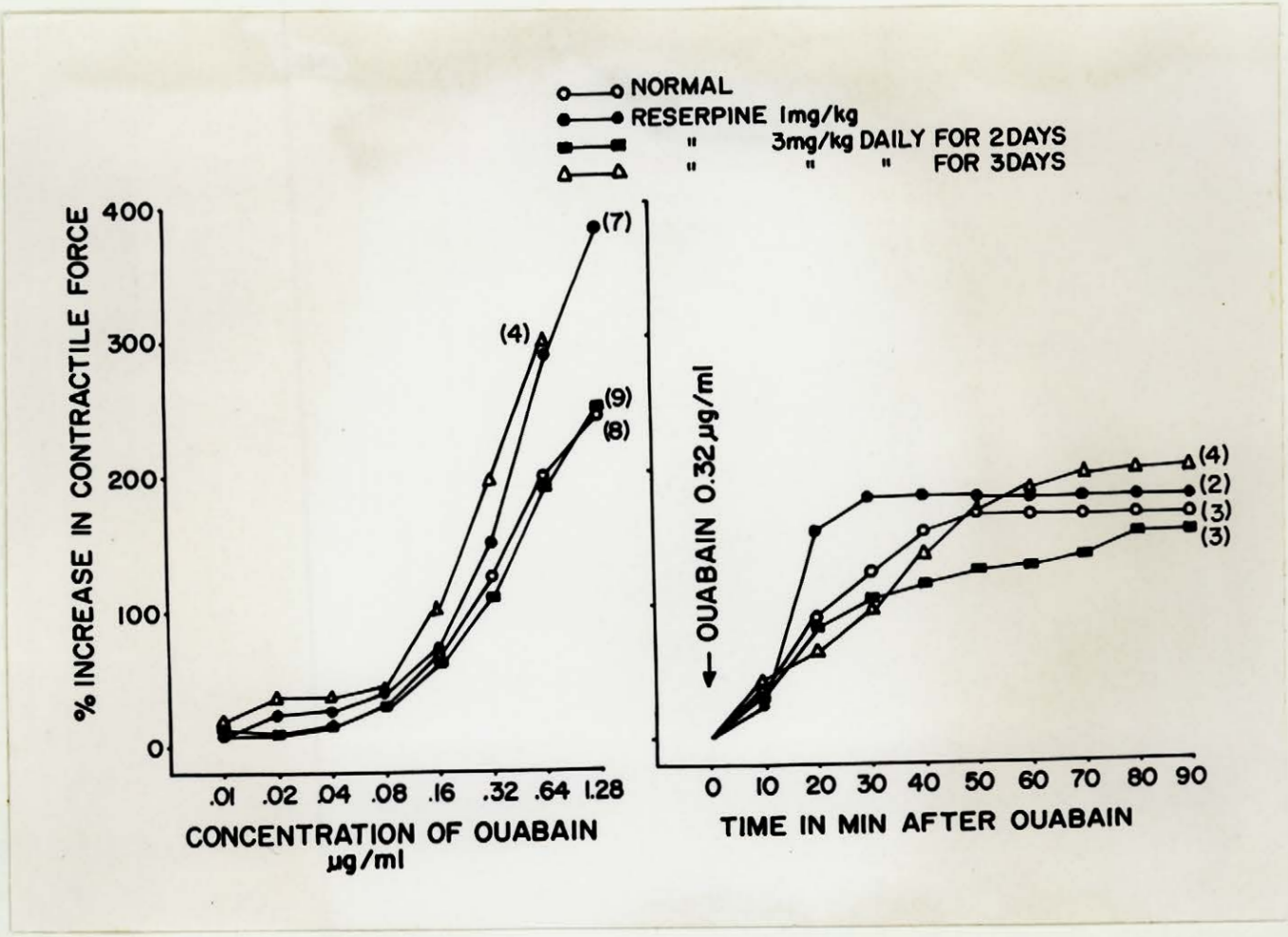

Fig. 10 Left half: The effect of cumulative concentration of ouabain on the contractile force of electrically-driven isolated papillary muscle of different groups of rabbits.
Right half: The percent increase in the contractile force of the rabbit isolated papillary muscle produced by the addition of 0.32 ug/ml of ouabain in the bath. The changes are plotted as a function of time. The numbers in parentheses denote the number of experiments in each group. The vertical lines represent one half the SE.

the contraction of all muscles.

It is also clear from the curve shown on the right half of Fig. 10 that the magnitude of the inotropic response of the papillary muscle to a single concentration of ouabain was also not reduced by prior reserpinization of rabbits.

The time taken for the development of the maximum inotropic effect of ouabain was variably affected by prior reserpinization of the animals. Maximum increase in the contractile force was observed 45 minutes after the addition of ouabain to the control preparation. On the papillary muscle of rabbits pretreated with 1 mg/kg of reserpine, ouabain (0.32 µg/kg) produced a maximum increase in contraction in 30 minutes. The difference in the time taken for producing maximum effect in the two groups of preparations was significant. The maximum positive inotropic effect of ouabain on the papillary muscles of rabbits treated with a dose of 3 mg/kg of reserpine for two and three days was produced in 80 minutes. This difference was significant.

The average concentration of ouabain which produced automaticity was 0.4 ± 0.08 µg/ml in control preparations, 0.62 ± 0.11 µg/ml in preparations from rabbits injected with 1 mg/kg of reserpine, 0.64 ± 0 µg/ml in preparations from rabbits treated with 3 mg/kg of reserpine daily for three days. Fifty percent of normal preparations, 20% of the preparations

from rabbits given 1 mg/kg reserpine, 66% of the preparations from rabbits given 3 mg/kg x 2 of reserpine and 50% of the preparations from rabbits injected with 3 mg/kg x 3 of reserpine, did not show automaticity to any concentration of ouabain. The contractile force slowly decreased in these preparations until complete cessation of contraction. In the remaining preparations, it was frequently noted that the automaticity was induced by a lower concentration and regular contractions were restored with the next higher concentration of ouabain. Comparison of the toxic concentrations of ouabain in different preparations is only a rough assessment, since the concentrations were increased by a factor of 2 rather than in small increments.

Effect of Ouabain on the Contractile Force of Isolated Left Atria of Rats.

The control contractile force of the atria of these rats was not different from each other. It is clear from the Figure 11 that the response of the rat atria to ouabain was not marked and there was no concentration dependent response up to 10.2 µg/ml. The slight positive inotropic response (up to 10.2 µg/ml) probably was due to spontaneous changes in the contractile force of the preparations. With concentrations of ouabain greater than 10.2 µg/ml, the contractile force of control atria increased in concentration dependent manner until the maximum increase

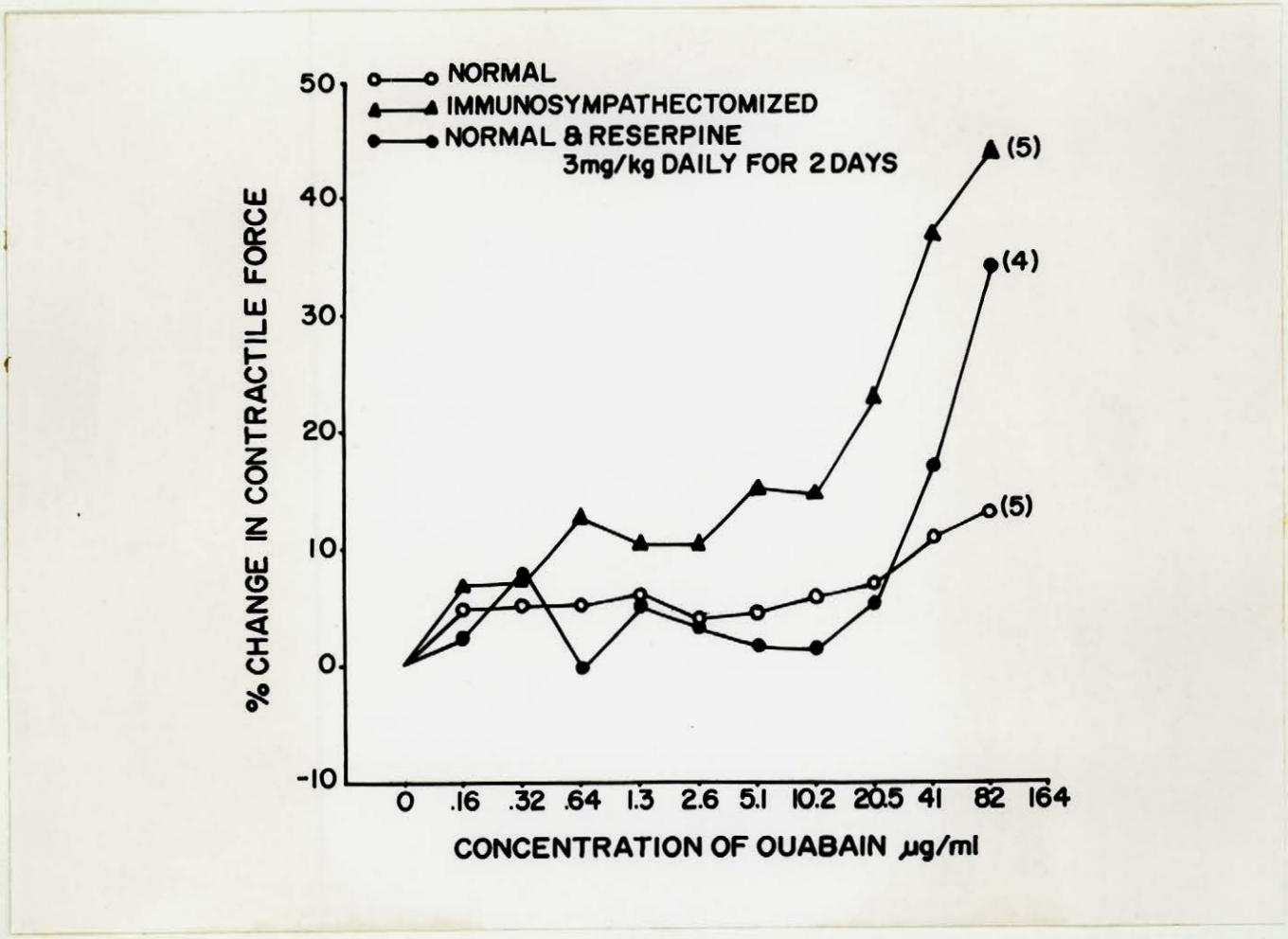

Fig. 11 The effect of cumulative concentration of ouabain on the contractile force of electrically-driven isolated left atria of rats. Experiments were performed 18 to 24 hours after the last injection of reserpine. The numbers in parentheses denote the number of experiments in each group. The vertical bars represent one half the SE.

of 13% was attained with a concentration of 82 μg/ml. The inotropic effect of ouabain on rat atria was not reduced by depletion of tissue catecholemine either by reserpinization or by "immunosympathectomy" of the animals. On the contrary, the inotropic response of the atria from the latter two groups of rats to ouabain was greater, though not significantly so. The maximum positive inotropic response was produced with a concentration of 82 μg/ml of ouabain.

A concentration of 164 μg/ml of ouabain progressively decreased contractile force to zero in all preparations. Only one atria in each of the three groups developed arrhythmias. In all three preparations the concentration of ouabain that produced arrhythmias was 82 μg/ml.

DISCUSSION

The Relation of Catecholamines to the Positive Inotropic Effect of Ouabain.

Pretreatment of cats with reserpine did not reduce the positive inotropic effect of ouabain in intact animals (Fig. 1, p. 38). These results confirm the earlier findings of Eckstein et al. (1961), Witherington and Zaimis (1961), Yelnosky and Ervin (1961), Zaimis (1961) and Morrow et al. (1963). Furthermore, reserpinization of animals with experimental heart failure also failed to reduce the positive inotropic effect of ouabain. Since reserpine depleted the cardiac noradrenaline to less than 4% of control, it is concluded that the positive inotropic effect of ouabain does not depend on the release or presence of myocardial catecholamines.

This conclusion is contrary to that of Tanz (1960, 1962, 1964), Tanz and Marcus (1966), Cairoli et al. (1961), Cession-Fossion (1962), Denis et al. (1963), Förster and Stolzenburg (1963) and Levy and Richards (1965a). Levy and Richards (1965b) found that the positive inotropic effect of ouabain on the isolated rabbit atria was not antagonized by beta-adrenergic blocking agents. The same workers (1965a) were able to show that the positive inotropic effect of non-toxic doses of ouabain on the isolated atria of reserpinized rabbits was smaller than on the control atria.

It is difficult to explain the discrepancy in the results of

these two groups of workers. Tanz (1960) suggested that insufficient myocardial catecholamine depletion may be the cause of the conflicting results. According to him, a single dose of reserpine (1 mg/kg) as was used by Witherington and Zaimis (1961) is unlikely to deplete the myocardial catecholamines to less than 10% of the control. This criticism of Tanz is not justified. It was early reported from this laboratory (Varma et al., 1964) that a single injection of 0.25 mg/kg or 1 mg/kg of reserpine reduced the cardiac noradrenaline content of cats to less than 4% of the control. It was observed in the present study that a smaller single dose of reserpine)0.1 mg/kg) was effective in reducing the noradrenaline content of the left ventricle of the cat to less than 4% of the control. Trendelenburg and Weiner (1962) found that the noradrenaline content of the cat heart after a single injection of 0.1 mg/kg of reserpine was approximately 4% of the control. Indeed under the experimental conditions of Tanz and Marcus (1966), pretreatment of cats with guanethidine reduced the cardiac catecholamine to only 9% of the control, yet significantly inhibited the positive inotropic effect of ouabain.

Since administration of reserpine does not lead to complete loss of tissue catecholamines (Muscholl & Vogt, 1958; Lee & Shideman, 1959), the possibility that the remaining catecholamines are involved in

the inotropic action of ouabain can never be ruled out by the use of depleting agents.

Strong support for our conclusions comes from the studies of Morrow et al. (1963) and Spann et al. (1965b). Morrow et al. (1963) found that chronic cardiac denervation in dogs reduced the myocardial catecholamine to an undetectable level but did not reduce the positive inotropic effect of ouabain. Similar findings were made by Spann et al. (1965b) using the papillary muscle of chronically cardiac denervated cats.

Since the adrenal catecholamines are less sensitive to the depleting action of reserpine, it may be argued that after depletion of cardiac catecholamines, the positive inotropic effect of ouabain in intact animals is exerted through a release of the remaining adrenal catecholamines. The present experiments rule out this possibility, since a combination of pretreatment with reserpine (2.5 mg/kg, single dose) and acute bilateral adrenalectomy failed to reduce the positive inotropic effect of ouabain (Fig. 1, p. 38).

Additional evidence that the inotropic effect of ouabain is not produced by a release of endogenous catecholamines is provided by studies on animals with experimental heart failure. The noradrenaline content of the left ventricle of these animals was less than 50% of the control, yet the positive inotropic effect of ouabain was more pronounced (Fig. 3, p. 41). Treatment of these animals with reserpine reduced the

cardiac catecholamines as in the normal cats while no significant reduction in the positive inotropic effect of ouabain was observed. The finding that experimental heart failure, produced by chronic pulmonary stenosis, reduced the CA content of the heart is in agreement with the observations of Spann et al. (1965a) who found that experimental heart failure produced in guinea-pigs by aortic constriction, resulted in a reduction of myocardial catecholamine level. Chidsey et al. (1963, 1965, 1966), Chidsey and Morrow (1964) and Chidsey and Braunwald (1966) observed that heart failure patients have a lower atrial catecholamine content than did normal subjects. Since it is well known that the failing myocardium is quite responsive to the inotropic effect of cardiac glycosides, it would seem improbable that cardiac glycosides act through the release of CA.

It is also clear from the results shown in Fig. 1 (p.38) and Table I (p. 34) that following the treatment of animals with reserpine, the maximum positive inotropic effect of ouabain was greater than in the normal animals. Witherington and Zaimis (1961) and Yelnosky and Ervin (1961) also observed that reserpine-treated cats displayed a greater inotropic effect to ouabain than did the unreserpinized controls. Higher doses of ouabain which failed to further increase the inotropic response in the normal controls, continued to augment the response of the reserpine-treated animals.

The absence of intact sympathetic reflexes in the reserpine treated animals may be a contributing factor to the greater inotropic effect of ouabain. This suggestion is supported by the observation that the maximum positive inotropic effect of ouabain following ganglionic blockade was similar to that following reserpinization. Morrow et al. (1963) found that the positive inotropic effect of ouabain was greater after acute cardiac denervation. Daggett and Weisfeldt (1965) made similar observations following ganglionic blockade. The exact mechanism by which the absence or presence of reflexes would affect the inotropic response cannot be ascertained from our experiments.

The conclusion that the inotropic effect of ouabain is independent of catecholamine release is also supported by the results obtained on the isolated tissue. Thus, the inotropic effect of ouabain on the isolated papillary muscle of rabbits was not reduced by pretreatment with reserpine (Fig. 10, p. 58). On the contrary, the inotropic effect of ouabain on the papillary muscle of reserpine-treated rabbits appeared to be greater than on the muscle of non-reserpinized controls. Also the response of these muscles to a single dose of ouabain (32 μg/ml) was not reduced by pretreatment of animals with reserpine. These results are in disagreement with those reported by Tanz (1960, 1962, 1964), Tanz and Marcus (1966) on the cat papillary muscle, of Cairoli et al. (1961), Denis et al. (1963) and Levy and Richards (1965a) on the rabbit atria. The cause for the

discrepancy in the results is not clear. Insufficient depletion of CA by the different doses of reserpine is not a likely explanation, since similar dosage schedules were also used by these authors. Furthermore, according to the studies of Bertler et al. (1956), Carlsson et al. (1957), Higuchi et al. (1962) and Higuchi (1962), these doses of reserpine produce marked depletion. It is, however, possible that under different experimental conditions, a non-specific depressant effect of reserpine may result in the reduction of the inotropic effect of ouabain. Indeed, such a non-specific depressant effect has been attributed to reserpine by Innes and Krayer (1958), Kirpekar and Lewis (1958), Witherington and Zaimis (1961), Nayler (1963), Boyajy and Nash (1965) and Spann et al. (1965b). Such a depressant effect of reserpine may account for the reduced inotropic effect of ouabain observed by some workers in reserpine pretreated preparations.

The inotropic effect of ouabain on the isolated left atria taken from rats pretreated with reserpine or from "immunosympathectomized" rats, was somewhat greater than on the normal atria (Fig. 11, p. 61). Bhagat et al. (1964) have shown that a single dose of 1.5 mg/kg of reserpine reduced the cardiac noradrenaline to less than 6% of the control. Thus, it can be safely assumed that the dose schedule (3 mg/kg daily for 2 days) of reserpine treatment employed in the present study will produce maximum depletion of cardiac noradrenaline. Iversen et al. (1966) found

that "immunosympathectomy" of rats reduced cardiac noradrenaline to about 4% of the control. Since the procedure employed by Iversen et al. (1966) was also followed in the present study, it is inferred that the cardiac noradrenaline of the "immunosympathectomized" rats was greatly reduced.

It is clear from these studies as well as the earlier observations of Reiter (1956) that rat atria are relatively insensitive to the effects of ouabain. Since rat atria are quite responsive to the inotropic action of noradrenaline (Bhagat et al. 1964), it would seem unlikely that ouabain acts through a release of noradrenaline.

In summary, it may be stated that reserpinization of the normal intact cat and of cats with experimental heart failure does not reduce the inotropic effect of ouabain. Results obtained from the isolated papillary muscle of rabbits pretreated with reserpine and from the isolated left atria of reserpinized and immunosympathectomized rat are in agreement with those obtained from the intact animals. It is concluded that the positive inotropic action of ouabain is not mediated through the release of myocardial catecholamines nor does it depend upon the level of myocardial catecholamines.

The Relation of Catecholamines to the Toxic Effects of Ouabain.

The results obtained in our studies on the arrhythmic and toxic actions of ouabain did not clearly illustrate whether or not this effect

of ouabain is mediated through a release of endogenous myocardial catecholamines.

Although pretreatment of cats with 0.1 mg/kg and 2.5 mg/kg of reserpine increased the dose of ouabain which produced persistent ventricular arrhythmias in the normal control cat, this difference was not significant ($P < 0.1$ - Table IV). Since cardiac catecholamines were markedly reduced in both groups, it would appear that the production of ventricular arrhythmias by ouabain is not critically releated to a release of cardiac catecholamines. However, since reserpinization (2.5 mg/kg) followed by acute adrenal extirpation was also not effective in modifying digitalis intoxication, it would seem that CA's released from the adrenal medulla do not contribute to the production of arrhythmias by ouabain. The tendency of reserpine pretreatement to protect against the arrhythmic action of ouabain cannot be ignored, since it reduced the incidence of ventricular fibrillation (Table IV, p.55). The lethal dose of ouabain was not affected by prior reserpinization of normal animals. This indicates that the toxicity of higher doses of ouabain is independent of catecholamine release.

Pretreatment with reserpine of animals with heart failure resulted in a significant reduction in the arrhythmic as well as the lethal dose of ouabain (Table IV, p. 55). It would seem that catecholamines are involved in the toxic effect of ouabain in animals with heart failure.

The following points, however, are against such a conclusion. First of all the catecholamine content of the failing heart was reduced but the toxic effect of ouabain in these animals appeared to be greater. Olson et al. (1955) and Bliss and Adolph (1959) also observed that the dogs with experimental heart failure were more sensitive to the toxic effect of cardiac glycosides. Cotten and Moran (1961) also suggested that the failing heart is more sensitive to the action of ouabain. It would seem unlikely that reserpine which reduces the CA content of the normal heart and the heart in failure to the same extent, should significantly reduce ouabain toxicity only in cats with heart failure. Thus, although the possibility that reserpine reduces the toxicity of ouabain in heart failure by depletion of CA cannot be ruled out, it seems quite likely that a non-specific antiarrhythmic action of reserpine may also be involved. Such an antiarrhythmic action of reserpine, which is not related to CA depletion, was also suggested by Arora and Madan (1958) and Boyajy and Nash (1965).

Our conclusion that the arrhythmic and toxic action of ouabain are to a large extent independent of myocardial catecholamines was further supported by studies on the isolated tissue. Both in the isolated rabbit papillary muscle and on the left atria of rats, the incidence of appearance arrhythmias as well as the dose of ouabain which produced these arrhythmias

was not significantly affected by reserpine pretreatment. This discrepancy between in vivo and in vitro studies was also found by Boyajy and Nash (1965). They suggested that reserpine induced CA depletion contributes to the protection against digitalis toxicity only to the extent that it removes the centrally mediated component of digitalis arrhythmias. This does not seem to be a likely explanation for our results, since the toxic dose of ouabain was not increased in intact cats following ganglionic block.

It is difficult to explain why CA depletion should antagonize ouabain toxicity in vivo, but should fail to influence it in vitro. Cushny (1925) found a relationship between the heart rate and the rate of development of digitalis toxicity. Wilbrandt et al. (1953), Sanyal and Saunders (1958) and Blinks and Koch-Weser (1963) have shown that the time required for the development of the maximum positive inotropic effect of ouabain is dependent on the heart rate. Thus it is possible that the slower heart rate in the intact reserpine treated cats may account for slower development of the inotropic and arrhythmic effect of ouabain. Since the rate factor is eliminated in the electrically driven isolated preparation, ouabain toxicity would not be expected to vary in the different groups used in this study. Our results are in agreement with those of Levy and Richards (1965a) who found no influence of reserpine pretreatment on the toxicity of ouabain in the electrically driven

isolated rabbit atria.

In view of the fact that reserpine did not consistently reduce ouabain toxicity in intact cats and pretreatment with reserpine or "immunosympathectomy" failed to affect ouabain toxicity when tested in isolated preparations (rabbit papillary muscle, rat atria), it is concluded that any effect of reserpine against ouabain toxicity is largely non-specific. A small dependence of ouabain-induced toxicity on the release or presence of endogenous catecholamine, however, cannot be ruled out.

SUMMARY

The object of the present study was to elucidate the possible relationship of endogenous catecholamines to the cardiac effects of ouabain. The influence of catecholamine depletion by reserpine on the inotropic and toxic effects of ouabain was studied in intact normal cats and in cats with chronic experimental pulmonary stenosis. In addition, the inotropic and arrhythmic effect of ouabain was studied on isolated papillary muscle of untreated and reserpine treated rabbits and on electrically driven isolated left atria of untreated, reserpine treated and "immunosympathectomized" rats.

It was observed that the positive inotropic effect of ouabain in intact animals or in isolated preparations was not reduced by catecholamine depletion. On the contrary, catecholamine depletion resulted in somewhat greater positive inotropic effect of ouabain. It is concluded that the positive inotropic effect of ouabain is not mediated through a release of endogenous catecholamines.

Chronic experimental pulmonary stenosis was found to produce congestive heart failure. A significant reduction in myocardial catecholamines and a small increase in the inotropic and toxic effect of ouabain was also observed in these animals.

Depletion of myocardial catecholamines did not reduce the

toxic effects of ouabain in isolated preparations (rabbit papillary muscle and rat atria). The effect of pretreatment with reserpine on the toxic effect of ouabain in intact animals was variable. Reserpine pretreatment significantly reduced the toxic effects of ouabain in cats with experimental pulmonary stenosis but not in normal animals. Since reserpine consistently produced depletion of tissue catecholamines and only variably affected the ouabain toxicity, it is concluded that observed reduction in ouabain toxicity by reserpine is largely non-specific. A facilitatory role of endogenous catecholamines in ouabain toxicity cannot be ruled out.

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