CARDIOVASCULAR PHARMACOLOGY[1,2]

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CARDIAC GLYCOSIDES

Considerable attention has been devoted in recent years to the actions of cardiac glycosides on myocardial contractility and the peripheral vascular system. Most of this interest has centered upon descriptions of the positive inotropic action of cardiac glycosides in both the presence and absence of failure, upon the electrolyte composition of heart muscle, and upon the energy sources for muscular contraction. These investigations have led to an increased understanding of the hemodynamic actions of glycosides and of the actions of glycosides on biochemical mechanisms.

Cardiac Actions

It has been repeatedly claimed that glycosides either depress or at least do not stimulate the normal heart. For example, Olson, Roush & Liang (1) concluded that acetyl strophanthidin had a negative inotropic effect in the normal dog, since cardiac output and calculated cardiac work were decreased promptly following injection of approximately 55 per cent of the fatal dose of the glycoside. In dogs with congestive heart failure, on the other hand, the same dose of acetyl strophanthidin increased cardiac output and ventricular stroke work. Regan, Talmers & Hellems (2) also reported that acetyl strophanthidin had a negative inotropic action in the normal dog, since cardiac output and left ventricular stroke work diminished after administration of the glycoside. Mercier and co-workers (3) found that ouabain and digoxin decreased ventricular work despite a rise in arterial pressure in dogs anesthetized with chloralose. Similarly, it had been reported that the papillary muscle of the cat failed to respond to nontoxic concentrations of several glycosides, although the same concentrations of these compounds increased the contractile force of failing muscle [White & Salter (4); Sciarini, Ackerman & Salter (5)]. In contrast to the foregoing papers, numerous recent investigations have shown that cardiac glycosides increase the force of myocardial contraction of normal heart muscle equally as well as that of failing heart muscle. One of the most definitive studies of this problem is that of Sanyal & Saunders (6) who used strips of guinea pig

[1] The survey of the literature pertaining to this review was concluded in August, 1960.

[2] Abbreviations used in this chapter include: DCI (dichloroisoproterenol); DOC (desoxycorticosterone); EDTA (ethylenediaminetetraacetic acid); PC (creatine phosphate); IP (inorganic phosphate); P:O ratio (ratio between the uptake of inorganic phosphate and uptake of oxygen); TMA (tetramethylammonium).

ventricular muscle driven by rhythmic electric stimuli. Ouabain increased the contractile force substantially before any evidence of failure was observed, as well as after failure had been produced by permitting the muscle to contract in the presence of a phosphate buffer. When a bicarbonate buffer was employed, muscle preparations showed no evidence of failure over periods of up to seven hours, and ouabain increased the contractile force substantially whether added promptly after establishing the preparation or after several hours had elapsed. These latter results were confirmed by the investigations of Stewart (7) who showed that the contractile amplitude of guinea pig ventricle strips contracting in the presence of a bicarbonate buffer was increased by cardiac glycosides. The positive inotropic action of cardiac glycosides has also been demonstrated in intact, normal animals, including human beings. In anesthetized normal dogs ouabain, in a dose approximating 40 per cent of the fatal dose, increased the force of ventricular contraction as measured with a strain-gauge arch [Cotton & Stopp (8)]. In these same experiments, ouabain also increased the amount of ventricular stroke work performed at any given ventricular filling pressure over a wide range of filling pressures. These findings confirmed the earlier work of Walton, Leary & Jones (9), who found that a number of glycosides all increased ventricular contractile force of normal dogs to about the same extent when administered in equivalent fractions of their fatal doses. In addition to normal experimental animals, human subjects without clinical evidence of heart failure also respond to acetyl strophanthidin with a marked increase in ventricular contractile force as measured with a strain-gauge arch [Bloodwell *et al.* (10)]. The characteristics of the contractile response in human subjects were the same as those observed in dogs and other mammalian species. Although glycosides stimulate both normal and failing cardiac muscle alike, failing heart muscle may be more sensitive to glycosides. Olson, Roush & Liang (1) and Bliss & Adolph (11) have shown that dogs with experimental heart failure developed cardiac arrhythmias with doses of acetyl strophanthidin having no effect upon the cardiac rhythm of normal dogs. There are no quantitative data available, however, regarding the sensitivity of failing and normal heart muscle with respect to the action of glycosides upon myocardial contractility.

Glycosides increase not only the contractile force of heart muscle, but of skeletal and arterial smooth muscle as well. Faust & Saunders (12) have clearly shown that the contractile force of the unfatigued isolated diaphragm from the guinea pig was increased by ouabain when the muscle was stimulated both directly and indirectly with supramaximal shocks. The increase in the contractile force of the isolated diaphragm occurred with the same concentrations of ouabain used to increase the contractile force of ventricle strips from the same animals. These findings are in contrast to much previous work showing that glycosides do not increase the force of contraction of skeletal muscle, but consideration of the methods employed by Faust & Saunders (12) leaves little doubt that cardiac glycosides augment the contractility of skeletal as well as cardiac muscle. The contractile

strength of strips of carotid artery from the rabbit is also increased by cardiac glycosides [Leonard (13)].

Temperature is an important determinant of the magnitude of the contractile response to glycosides. Lowering the body temperature of anesthetized dogs from 37° to 22 to 25° caused a marked reduction in the positive inotropic action of ouabain as compared with responses obtained in these same animals at normal body temperature [Cotten & Brown (14)]. Under these conditions, sympathomimetic amines such as norepinephrine still produced substantial increments in ventricular contractile force at a time when the response to ouabain was virtually abolished. Saunders & Sanyal (15) found a direct relationship between temperature and the magnitude of the positive inotropic response to ouabain. In these experiments with guinea pig ventricle strips, ouabain increased the contractile force substantially at a bath temperature of 37°, but the contractile response diminished markedly as the temperature was reduced to 27°; at 17° ouabain failed to affect the contractile force. However, the significance of these findings is clouded by the fact that the control level of contractile force increased progressively as the bath temperature was reduced, being approximately 90 mg. at 37° and 250 mg. at 17°. The possibility thus exists that the positive inotropic action of ouabain was simply masked at low temperatures by the rise in the control contractile force to levels far above the maximum force produced by ouabain at 37°. The fact that rate of onset of the positive inotropic action of ouabain was not signficantly affected by lowering the bath temperature led Saunders & Sanyal (15) to suggest that the rate-determining process resulting in the onset of the contractile response to ouabain is of a physical rather than a chemical nature. The rate of onset of action of ouabain is dependent, however, upon the frequency of contraction as has been demonstrated by Sanyal & Saunders (16) using strips of ventricular muscle from guinea pigs. As the frequency of contraction was increased progressively, the time required for onset of action of the positive inotropic effect of the glycoside was progressively shortened. At a contraction frequency of 200/min. the maximum contractile force response to 2.3×10^{-7} *M* ouabain occurred in approximately 10 minutes, while at a contraction frequency of 25/min. the maximum effect required about 40 minutes to develop. Extrapolation of these data led Sanyal & Saunders (16) to the conclusion that ouabain produces the effects leading to an increased force of contraction only in actively contracting muscle. If this conclusion is a valid one, as it now appears, the significance of much previous work done with non-contracting muscle in experiments aimed at elucidating the biochemical actions of glycosides is subject to serious question.

The maximum force achieved in response to ouabain was influenced by the frequency of contraction in the experiments of Sanyal & Saunders (16) described above. At a frequency of 25/min., the control contractile force averaged 45 mg., and ouabain increased the force to an average maximum of 90 mg., an increase of 100 per cent. At a contraction frequency of 100/min., on the other hand, the control contractile force averaged 110 mg.,

and ouabain increased the force to an average maximum of 162 mg., an increase of only 48 per cent. Thus, as the frequency of contraction was increased, the per cent response to ouabain was decreased markedly because of the elevated control level of contractile force, whereas the actual increment in force evoked by ouabain was relatively unaffected.

Peripheral Vascular Effects

Although the preponderance of evidence indicates that cardiac glycosides increase the contractile force of normal and failing heart muscle alike, an explanation is needed for the failure of glycosides to augment the cardiac output despite an increase in contractile strength. This problem has been resolved, at least in part, by the finding that glycosides decrease venous return in normal dogs concomitantly with the increase in myocardial contractility [Cotten & Stopp (8)]. In these experiments ouabain increased myocardial contractility and blood pressure but decreased cardiac output, left atrial pressure, and heart rate of normal anesthetized dogs. When left atrial pressure was kept constant throughout the action of the glycoside by controlled infusion of blood into the left atrium, cardiac output was substantially increased as was left ventricular stroke work. Venous return was decreased by approximately 15 ml./kg. in these experiments as judged by the amount of infused blood required to keep left atrial pressure constant while the effects of ouabain developed. These findings have been confirmed by Ross, Braunwald & Waldhausen (17) who found that acetyl strophanthidin reduced venous return by about 13 ml./kg. in dogs in which the direct cardiac actions of the glycoside were excluded through the use of the cardiopulmonary bypass technique and a constant perfusion rate. The decrease in venous return is apparently caused by venous pooling of blood, especially in the splanchnic bed, since venous return actually increased by about 8 ml./kg. when the splanchnic bed was excluded from the circulation prior to administration of acetyl strophanthidin. The exact mechanisms responsible for the trapping of blood in the splanchnic bed have not yet been elucidated, although venoconstriction probably plays an important role. Ross, Braunwald & Waldhausen (17) noted an increase in pressure in the inferior and superior venae cavae after administration of acetyl strophanthidin at a time when venous return was reduced indicating that venoconstriction had taken place. The increase in venous return when the splanchnic bed was excluded from the general circulation offered a further indication of a generalized venoconstrictor action of the glycoside. A venoconstrictor action of cardiac glycosides has also been demonstrated in human subjects by Horsley & Eckstein (18) who measured venous tone in the forearm veins. Evidence of a venoconstrictor effect of glycosides on hepatic smooth muscle in normal human subjects has been obtained by Baschieri *et al.* (19). In the latter study, digitalization was followed by an increase in hepatic wedge pressure, an increase in the gradient between the hepatic wedge pressure and the inferior vena caval pressure, and a reduction in hepatic blood flow. Cardiac glycosides have also been shown to

have a marked constrictor action on arterial smooth muscle. Ross, Waldhausen & Braunwald (20), using dogs subjected to the cardiopulmonary bypass technique, found that acetyl strophanthidin, ouabain, and lanatoside C each increased arterial pressure substantially when a constant perfusion rate was used. The rise in pressure reflected an increase in peripheral resistance produced by the glycosides. The elevation of the peripheral vascular resistance was not abolished by ganglionic blockade or by adrenalectomy. Studies with human subjects undergoing total cardiopulmonary bypass with a constant perfusion rate have yielded equivalent results upon injection of acetyl strophanthidin [Bloodwell *et al.* (10)]. This increase in total peripheral resistance and consequent rise in blood pressure is, of course, not commonly observed when patients are digitalized slowly, but may be readily demonstrated when a glycoside is given by rapid intravenous injection [Williams, Zohman & Ratner (21)].

Effect of Glycosides on Electrolyte Composition of Heart Muscle

Increasing interest is developing in the influence of glycosides on the electrolyte composition of cardiac muscle. These investigations deserve particular attention in view of the marked influence which alterations in ionic content and composition exert on the contractile process. Glycosides increase the influx of calcium[45] into isolated, rhythmically contracting rabbit atria, but have no apparent effect on its efflux [Holland & Sekul (22)]. The influx of calcium was markedly increased by ouabain when the concentration of potassium in the medium was low, but was severely reduced when potassium concentration was elevated. These results suggest that at least part of the positive inotropic action of glycosides may be related to the effects of increased calcium either at the cell membrane or in the myoplasm. Such an hypothesis is consonant with a considerable body of earlier work showing that the magnitude of glycoside action upon myocardial contractility of isolated cardiac muscle can be enhanced by increasing the concentration of calcium in the medium, and decreased in the presence of a calcium deficiency [for discussion and reference see (23)].

In addition to promoting an increase in the influx of calcium, glycosides also cause a negative potassium balance in heart muscle (24 to 27). Rayner & Weatherall (24) have shown that digoxin and ouabain reduced the influx of potassium into isolated, beating rabbit auricles, but had no appreciable effect on the efflux of potassium, leading to a net decrease in intracellular potassium content. The loss of potassium was accompanied by a gain of sodium. Similar changes in intracellular potassium of the guinea pig heart were obtained by Vick & Kahn (25) and by Vick (26) who also observed that the loss of potassium was small at concentrations of ouabain producing a positive inotropic action, but increased as the concentration of ouabain was increased. Loss of potassium from heart muscle after administration of cardiac glycosides to intact animals is a well-established phenomenon [see Cairns, Love & Burch (27)]. Glycosides also inhibit the influx of potassium into human red cells bringing about a net fall in intracellular

potassium [Glynn (28); Gill & Solomon (29)]. This effect may be attributable to an action of the glycosides on the red cell transport system for potassium rather than the cell's energy supply, since there were no changes observed in ATP, ADP, or 2,3-diphosphoglyceric acid [Kunz & Sulser (30)]. The action of potassium on cell membranes of heart muscle and red blood cells may involve similar mechanisms, but the validity of this assumption remains to be proved.

The exact relationship of the reduction in intracellular potassium to the positive inotropic action of cardiac glycosides remains obscure. Hajdu (31) has suggested that the positive inotropic action of glycosides is caused by reduced re-entry of potassium into cardiac cells during relaxation providing a more optimal intracellular environment for contraction of the contractile proteins. This hypothesis was based upon Hajdu's finding that reduction in intracellular potassium by 3 m.eq./l. of fiber water from a level of 107 m.eq./l. changed the contractile force from zero to maximum values. However, it would appear from the work of Vick (26) that potassium loss from myocardial cells per se may not be a causative factor in the glycoside-induced augmentation of contractile force, since several lactones having a negative inotropic action also caused a loss of intracellular potassium. In these same experiments ouabain caused a similar loss of potassium but had a positive inotropic action.

The development of contracture in isolated heart muscle after exposure to toxic amounts of cardiac glycosides may be related, at least in part, to the loss of potassium, as had been suggested by Vick & Kahn (25) and by Vick (26). In their experiments the isolated guinea pig heart lost potassium when contracture was progressing. With the disappearance of contracture, on the other hand, the heart gained potassium. Recent work by Thomas (32) demonstrated the role of calcium in the production of contracture. Development of contracture in the frog heart treated with ouabain was associated with an increased uptake of calcium[45]. Administration of ethylenediaminetetraacetic acid (EDTA) abolished the contracture induced by ouabain, but not that induced by iodoacetate. Thomas (32) interprets his findings to mean that contracture induced by ouabain is chiefly attributable to altered handling of calcium by the cells and not primarly to a shift in the sodium or potassium concentration or to gross interference with intracellular energy metabolism.

Metabolic Actions of Glycosides

The possibility that the positive inotropic response to cardiac glycosides might be explained in terms of associated metabolic changes has led to numerous studies. Most of these investigations have dealt with the actions of glycosides upon labile energy-rich phosphate compounds and upon carbohydrate metabolism. Only a few of the more recent papers are discussed in detail below.

Furchgott & de Gubareff (33) have demonstrated that concentrations of K-strophanthin inducing a maximum increase in contractile amplitude of

the electrically driven, hypodynamic left atrium from the guinea pig have no demonstrable effect on the concentration of ATP, ADP, and creatine phosphate (PC) in the muscle. High concentrations of K-strophanthin inducing manifestations of cardiac toxicity, on the other hand, resulted in significant reductions in the concentration of ATP and PC, while increasing the concentrations of ADP, AMP, and inorganic phosphate (IP). These observations have been confirmed and extended by Lee, Yu & Burstein (34) who simultaneously measured changes in contractile force, oxygen consumption, and concentrations of ATP and PC in the isolated cat papillary muscle. In this study a relatively high concentration of ouabain (1.37×10^{-6} *M*) increased the contractile force some seven or eight minutes before oxygen consumption began to increase. At this time there were no significant changes in the concentrations of ATP or PC. The increase in contractile force without a concomitant change in oxygen consumption led to a substantial increase in the mechanical efficiency of the papillary muscle. The maximum increase in contractile strength was obtained within approximately 40 minutes after addition of ouabain to the bath. At this time the oxygen consumption had increased to nearly the maximum value obtained in any given experiment, but the concentrations of ATP and PC were decreased only slightly. Some 80 minutes after addition of ouabain to the bath contracture developed, and little or no contractile tension was detectable. Oxygen consumption continued at the maximum value during contracture despite the loss of individual contractions, and the concentrations of ATP and PC were significantly reduced. Using a lower concentration of ouabain (2.74×10^{-7} *M*), Lee, Yu & Burstein (34) were able to show a marked temporal separation of about 20 minutes between the time of onset of the positive inotropic effect and the time of onset of the increase in oxygen consumption. The increased oxygen consumption seen after administration of ouabain to the cat papillary muscle apparently is not the result of the increased contractile force per se, because experiments performed by Lee and co-workers (34) with both resting and contracting cat papillary muscles demonstrated that approximately the same changes in oxygen consumption and concentrations of ATP and PC occurred upon the addition of 1.37×10^{-6} *M* of ouabain. The times required for attainment of the maximum increases in oxygen consumption and decreases in concentrations of ATP and PC were longer for the resting muscle than for the contracting muscle.

The observation that cardiac glycosides increase the oxygen consumption of heart muscle, but not that of other tissues has been reconfirmed recently by Peschel & Schlayer (35). The basis for the increased oxygen consumption by heart muscle after exposure to a glycoside remains uncertain. One possible explanation has been suggested by Lee and his co-workers (34), but the evidence is, at present, circumstantial. An increase in the content of ADP and IP has been shown to accelerate oxidation steps coupled with phosphorylation when these substances are limiting [Lardy & Wellman (36); Hock & Lipmann (37)]. Since it has been shown that the content of

ADP and IP were increased when guinea pig atrial muscle was exposed to excessive amounts of a cardiac glycoside (33), part of the increased oxygen consumption may be related to these changes. Another possible explanation for the increased oxygen consumption evoked by glycosides has been suggested by Lee, Schwartz & Burstein (38) who have presented some evidence for a mild glycoside-induced uncoupling of oxidative phosphorylation. As has been amply demonstrated, glycosides have no effect upon the oxygen uptake or the ratio between the uptake of inorganic phosphate and uptake of oxygen (P:0 ratio) of mitochondria from heart muscle either when the glycoside is added *in vitro* or given *in vivo* [Lee, Schwartz & Burstein (38); Plaut, Gertler & Plaut (39)]. However, in the presence of a deficiency of IP or phosphate acceptor (ADP), oxygen uptake of mitochondria from atrial muscle of guinea pigs pretreated with a large dose of ouabain or digitoxin was significantly greater than that of mitochondria from untreated animals [Lee, Schwartz & Burstein (38)]. Further, in the presence of excess IP the addition of hexokinase and glucose, to increase the amount of available ADP, increased the oxygen uptake of mitochondria from the untreated animals but had no effect upon the already elevated oxygen uptake by the mitochondria from the animals pretreated with a glycoside. The increased oxygen consumption of mitochondria from the treated animals was found not to be attributable to an activation of ATPase. These data permit the tentative conclusion reached by Lee, Schwartz & Burstein (38) that glycosides induce mild uncoupling of oxidative phosphorylation. The increased oxygen uptake seen with heart mitochondria from animals pretreated with a glycoside is presumed to be related to an undemonstrated increase in intramitochrondrial IP or ADP, or both. However, final proof of an uncoupling action by glycosides will require a demonstration of a reduced P:0 ratio in the presence of an IP or ADP deficiency, and failure of this deficiency to increase upon addition of hexokinase and glucose in the presence of excess IP. Whatever the explanation for the increased oxygen consumption, it does not appear to be the result of the increased contractility per se, since oxygen consumption increases whether the muscle is contracting or not (34). Neither does an increased oxygen consumption per se lead necessarily to an increased vigor of contraction. Addition of 20 m*M* succinate to a substrate-free medium containing a contracting papillary muscle increased oxygen consumption to approximately the same extent as produced by 2 m*M* of pyruvate, but had no effect on the contractility [Lee, Yu & Burstein (34)]. Similarly, increasing the concentrations of ATP and PC by the addition of succinate did not lead to an increase in contractile force. It would thus appear that the positive inotropic action of glycosides is not dependent upon an increase in the content of energy-rich phosphates in the heart, a conclusion upon which there is general agreement [see Furchgott & de Gubareff (33) and Wollenberger (40)]. Further, the increased contractile force and augmented oxygen consumption produced by glycosides does not seem to be dependent upon increased substrate uptake caused by altered membrane permeability, since the characteristic effects of ouabain on force

and oxygen consumption are evoked in a substrate-free medium [Lee, Yu & Burstein (34)]. There is some evidence, however, that ouabain may increase glucose utilization, since the positive inotropic effect of glucose on rat ventricle strips is enhanced in the presence of ouabain [Berman, Masuoka & Saunders (41)]. Further, Kien & Sherrod (42) have reported that "therapeutic" doses of digoxin (.065 mg./kg.) increased the rate of C^{14}-labelled glucose utilization by the myocardium by about 60 per cent, and increased the contribution of glucose to the total myocardial metabolism which, however, was unchanged. The increased turnover of the labelled glucose was reflected in a marked increase in the production of $C^{14}0_2$ by the myocardium. The significance of these observations in relation to the stimulant action of glycosides on myocardial contractility is obscure, however, since Kien & Sherrod (42) also reported that the dose of digoxin which they employed had no demonstrable effect upon the cardiovascular system. The absence of changes, especially in systemic arterial pressure and cardiac output, in the normal dogs used is suprising in view of the ample demonstrations that glycosides (including digoxin) have distinct cardiovascular effects when given in the same fraction of the fatal dose used by Kien & Sherrod (42). In any event, the possibility that glycosides correct a specific metabolic lesion in failing heart muscle, as discussed by Kien & Sherrod (42), appears to be remote judging from the excellent studies of Furchgott & deGubareff (33) and Lee, Yu & Burstein (34) on energy metabolism, and from the ample demonstrations that glycosides augment myocardial contractile force whether the heart muscle is normal or in failure.

Structure-Activity Relationships

The cardiotonic effects of various substances having structural similarities to cardiac glycosides have been examined by several groups of investigators. The simple lactones, α-angelicalactone and pulvinic acid dilactone, both have been shown to exert a negative inotropic effect upon the hypodynamic isolated guinea pig ventricle [Vick (26)]. This finding is in keeping with the earlier work by other investigators [for references see (23)]. On the other hand Vick (26) found that Patulin, a steroid lactone consisting of an α, β-unsaturated γ lactone attached to a single ring, increased briefly the amplitude of contraction when high concentrations were employed. In these same experiments both ouabain and dihydro-ouabain had positive inotropic effects, the magnitude of which were proportional to the concentrations used. The data suggest that lactones per se do not possess the same pharmacological properties exhibited by cardiac glycosides, but that coupling a simple lactone to a ring structure confers cardiac stimulant properties upon the molecule. Much additional work is required to validate this hypothesis, however.

Bennett *et al.* (43) studied the effects of a large series of conjugated carbonyl compounds on the contractility of the cat papillary muscle. These compounds are closely related to the opened lactone ring of the cardiac glycosides. Acrolein, methylvinylketone, and 4-acetoxy-4-hydroxy-2-pen-

tenoic acid γ lactone were among the more active compounds examined. These substances were capable of restoring the contractile amplitude of the hypodynamic papillary to control levels. The action of some substances persisted for long periods, and contracture could be produced by a sufficiently high concentration. The significance of the data is partially obscured by the finding that the compounds produced a positive inotropic action only in the hypodynamic cat papillary muscle, and not in the nonfailing muscle. This is in contrast to the action of cardiac glycosides such as ouabain.

Various steroids have also been found to evoke changes in the contractile activity of heart muscle. For example, desoxycorticosterone (DOC) and progesterone are effective in abolishing the staircase phenomenon in the isolated beating frog heart, an action similar to that evoked by strophanthidin [Hajdu (44)]. Hajdu also found that corticosterone had an action similar to that evoked by DOC, but was much weaker. Aldosterone had no effect on the staircase phenomenon, whereas steroids such as cortisone, hydrocortisone, and 17-hydroxy progesterone, all having hydroxyl groups in the 17 position, had an effect opposite to that of DOC and strophanthidin. The steroid 9-α-fluorohydrocortisone has a positive inotropic effect associated with a loss of intracellular potassium and a gain of sodium [Tanz, Whitehead & Weir (45)]. In high concentrations, however, 9-α-fluorocortisone depresses myocardial contractility, and the muscle gains potassium and loses sodium. Cortisone also exerts a marked action on the isolated failing heart when added in small quantities, restoring the amplitude of contraction to control levels and protecting the histologic integrity of the muscle [Tanz (46)]. Estrogen [King *et al.* (47)] and testosterone, norethandrolone, and progesterone [Van Arman & Drill (48)] all appear to have negative inotropic actions on isolated heart muscle.

In addition to the direct actions of certain steroids on myocardial contractility some evidence exists to show that certain steroids may modify the actions of glycosides. Grinnell & Smith (49) demonstrated that normal female dogs in anestrus or estrus and castrate female dogs treated with estrogen were much more resistant to the development of cardiac arrhythmias produced by a large dose of digoxin (0.15 mg./kg.) as compared with male dogs or nontreated castrate females. These experiments involved only recordings of cardiac arrhythmias so that no information is currently available concerning a possible interaction of glycosides and estrogen on myocardial contractility. Adrenalectomized rats appear to be more sensitive to ouabain than normal rats with respect to the development of conduction disturbances [Unterman, de Graff & Kupperman (50)], but the basis for the increased sensitivity is unkown. It is also unknown whether adrenalectomy alters the sensitivity of the contractile mechanism to cardiac glycosides.

DRUGS WHICH AFFECT THE CARDIOVASCULAR SYSTEM BY ACTING ON THE SYMPATHETIC NERVOUS SYSTEM

The discovery of new types of pharmacological antagonists of adrenergic stimulant substances, and studies aimed at correlating the physiologi-

cal effects of adrenergic stimulant agents with their metabolic actions have provided new insight into the physiological functions of the sympathetic nervous system and the interaction of adrenergic stimuli on biochemical processes. This portion of the review will be limited to an examination of a few significant contributions in these areas. A more general review of the cardiovascular effects of sympathomimetic amines is that by Aviado (51).

Adrenergic Drugs

The concept of adrenotropic receptors.—The classification of adrenotropic (adrenergic) receptors first proposed by Ahlquist (52) has received substantial verification through the discovery of the special adrenergic blocking properties of dichloroisoproterenol (DCI) by Powell & Slater (53). Ahlquist (52, 54) had proposed that receptors in the adrenergic system be classified as *alpha* and *beta, alpha* receptors subserving primarily excitatory effects of adrenergic stimuli except for those on the heart, and *beta* receptors subserving inhibitory functions as well as cardiac stimulation. This classification seems a valid one as far as the heart and blood vessels are concerned. Dichloroisoproterenol appears to antagonize selectively the cardiac stimulant and vasodilator effects of adrenergic stimuli, but not the vasoconstrictor actions [Moran & Perkins (55)]. On the other hand, conventional adrenergic blocking drugs such as phentolamine, phenoxybenzamine, and certain ergot alkaloids readily block the vasoconstrictor actions, but not the vasodilator or cardiac stimulant effects of adrenergic stimuli. It has been proposed that adrenergic blocking drugs be classified as either *alpha* or *beta* adrenergic antagonists, depending upon the particular type of receptor they block. The terms *alpha* and *beta* adrenergic antagonists will be used here with the understanding that this classification does not explain the nature of the receptors, but only provides a tentative heuristic framework for descriptions of drug action. Specific details of the newer aspects of adrenergic blockade are contained in following sections.

Effects of sympathomimetic amines on the heart. (a) Ventricular function and heart rate.—Although it has been adequately demonstrated that norepinephrine increases myocardial contractility [see (23)], interest still exists in the fact that norepinephrine often does not increase the cardiac output in normal subjects. It is now generally agreed that this is the result of reflex cardiac slowing caused by the rise in blood pressure produced by norepinephrine. Raab and his co-workers (56) have analyzed the effects of epinephrine and norepinephrine on the human heart, estimating changes in myocardial contractility from changes in the duration of the isometric contraction period of the cardiac cycle. They concluded that the usually observed differences in the actions of epinephrine and norepinephrine are attributable to a reflex-induced cholinergic pattern produced by norepinephrine. This reflex-induced pattern is abolished by atropine, revealing the primary adrenergic action of norepinephrine. The dependence of the pattern of the cardiovascular response to agents such as norepinephrine, epi-

nephrine, and isoproterenol upon the magnitude of the associated reflex alterations in heart rate has been nicely demonstrated by Rushmer & West (57, 58) who employed a variety of techniques in both conscious and anesthetized dogs to analyze the complex cardiovascular changes evoked by these drugs. In the absence of opposing reflex bradycardia, each of the three amines increased cardiac output and heart rate, actions in keeping with the fact that all three drugs augment myocardial contractility and automaticity. Isoproterenol increases cardiac output and heart rate even in the presence of fully intact barostatic reflexes, since the mean arterial pressure does not increase as a result of the vasodilator properties of the drug. Such effects have been observed in both dogs (57) and man [Stack *et al.* (59); Weissler *et al.* (60)].

The reflex bradycardia induced by a vasoconstrictor sympathomimetic amine has been studied by Aviado & Wnuck (61) using methoxamine, a potent vasoconstrictor drug having no stimulant action upon the heart [Goldberg *et al.* (62)]. Aviado & Wnuck thus avoided the complications which would arise from the use of an amine having a direct stimulant effect upon the automaticity of the sinoatrial node. They concluded that the bradycardia induced by methoxamine was not the result of an action on the central nervous system, since there was no effect after injection of the drug into the vertebral artery. There was also no evidence that methoxamine affected the coronary and pulmonary receptors responsible for the Bezold-Jarisch reflex, or that methoxamine had direct negative chronotropic action. Slowing of the heart by methoxamine was substantially reduced, however, by denervation of the carotid and aortic stretch receptors suggesting that the bradycardia induced by methoxamine was mediated through these structures. As Aviado & Wnuch pointed out, their experiments did not exclude an action of methoxamine on cardiac stretch receptors as a possible mechanism of the cardiac slowing. Stormorken, de Schaepdryver & de Vleeschhouwer (63) also concluded that the bradycardia produced by methoxamine was the result of reflex slowing by way of the carotid and aortic baroreceptors, but, in addition, they also found evidence for an effect of methoxamine on coronary and pulmonary receptors as well as a direct negative chronotropic action. Preziozi and his associates (64) also observed a slight negative chronotropic action of methoxamine on the perfused heart of the guinea pig.

A careful examination of the effect of mephentermine sulfate (Wyamine) has been made in dogs by Welch *et al.* (65) utilizing techniques developed by Sarnoff and associates for the evaluation of ventricular function. They found that mephentermine elevated the ventricular function curve without changing peripheral resistance and concluded that this drug is primarily a cardiac stimulant with little or no vasoconstrictor actions. They also noted that myocardial oxygen consumption increased and efficiency decreased with low filling pressures (nondilated hearts). Under conditions of high filling pressures yielding dilated hearts, on the other hand, oxygen consumption decreased and efficiency increased. These authors

aptly point out that the "oxygen-wasting" effect of sympathomimetic amines is not invariable, that amines may exert an "oxygen-conserving influence" under appropriate conditions such as cardiac dilation. They also emphasize that these two effects of mephentermine on oxygen consumption do not imply two types of metabolic effects, but that the direct metabolic effect to increase oxygen consumption may be altered by such factors as change in duration of systole, the initial filling pressure and cardiac radius.

(*b*) *Arrhythmias.*—Dresel & Nickerson (66) were unable to find evidence for a causal relationship between epinephrine-induced hyperkalemia and ventricular arrhythmias in pentobarbital-anesthetized dogs. Such a relationship had been postulated previously by O'Brien and his associates (67). The conclusion reached by Dresel & Nickerson was based upon several observations, among them a lack of quantitative correlation between the hyperkalemic response to injected epinephrine and the induction of arrhythmia, the lack of increased ability of isoproterenol to induce arrhythmias during intravenous infusion of potassium, and the lack of protection against arrhythmias by exclusion of the liver from the circulation, the latter abolishing the epinephrine-induced hyperkalemia.

Moore & Swain (68, 69) have described the properties of a new compound [U-0882 (α-phenoxy-α-dimethylaminomethyl propiophenone hydrochloride)] which sensitized the heart of the dog to ventricular fibrillation in a manner similar to that of amarine. U-0882 prolongs the refractory period of ventricular myocardium without depressing intracardiac conduction. Intravenous injections of epinephrine, norepinephrine, or isoproterenol in appropriate doses two to five minutes after the injection of U-0882 produced ventricular fibrilation. Moore & Swain postulate the initiation of a continuous conduction in the ventricles to explain the fibrillation.

(*c*) *Metabolic effects.*—Ellis (70) has reviewed the literature up to late 1958 concerning the relation of the biochemical effects of epinephrine to its effects on muscle. In addition, he presented the main lines of evidence which support his hypothesis that hexosemonophosphate may play an important role in muscle contraction, and that the actions of epinephrine on muscle are not dependent on either oxidative metabolism or on the energy produced through the anaerobic glycolytic pathway. His hypothesis places the activation of glycogenolysis in an important position.

The demonstration by Cori (71) and Rall, Wosilait & Sutherland (72) that heart muscle contains enzymes which are analogous to the phosphorylase *a* and *b* of skeletal muscle, and the observation of Ellis (73) that the phosphorylase-*a* activity of rat ventricle slices was increased by incubation with epinephrine led Hess & Haugaard (74) to evaluate the influence of epinephrine on the phosphorylase activity of myocardium. They found that addition of epinephrine to the perfusion fluid in concentrations which augmented the force of contraction of isolated rat hearts led to increased activity of phosphorylase *a* in extracts of myocardium when measured in the absence of adenylic acid, but produced no change in the activity of the total enzyme (*a* and *b*) measured in the presence of AMP. That is, epi-

nephrine stimulated the conversion of inactive phosphorylase *b* to active phosphorylase *a* simultaneously with the increased contractile activity.

Kukovetz and his associates (75) observed that other sympathomimetic amines which also have a positive inotropic effect on isolated perfused rat hearts increased phosphorylase *a* activity without changing total enzyme activity. These amines included norepinephrine, isoproterenol, epinine, synephrine, and phenylephrine. Contractile activity and enzyme activation increased in parallel with increasing doses of epinephrine and norepinephrine. On the other hand, amines such as methoxamine which had no positive inotropic effect did not activate the enzyme.

Mayer & Moran (76) compared the effects of drugs and electrical stimulation of the cardiac sympathetic nerves on the contractile force of the heart *in situ* in anesthetized open-chest dogs. Ventricular contractile force was measured directly with a strain-gauge arch, and phosphorylase activity was assayed on small samples of myocardium which were rapidly excised from the beating heart. They found, as did Hess & Haugaard (74), that epinephrine increased phosphorylase *a* activity without altering total activity of the enzyme. They also confirmed the observation of Kukovetz *et al.* (75) that only those sympathomimetic amines which increased contractile force increased the activity of phosphorylase *a*. In addition, Mayer & Moran (76) found that electrical stimulation of the cardiac sympathetic nerves augmented both ventricular contractile force and phosphorylase *a* activity, both of these responses being abolished by pretreatment of dogs with reserpine. Offering further support to the concept that contractile force augmentation and enzyme activation are parallel actions of adrenergic stimuli was the observation that the *beta* adrenergic blocking drug, DCI, antagonized both types of responses to epinephrine and nerve stimulation. However, the *alpha* adrenergic blocking agent, phenoxybenzamine, anatognized neither of these responses to epinephrine. Nonadrenergic cardiac stimulants such as ouabain, theophylline, and small doses of calcium chloride increased contractile force without augmenting phosphorylase *a* activity. These authors concluded that activation of myocardial phosphorylase is an action intimately associated with the augmentation of contractile force produced by adrenergic stimuli and that nonadrenergic cardiac stimulants do not directly influence phosphorylase activity.

The latter conclusion concerning nonadrenergic drugs is not in agreement with the observations of other workers. Hess & Haugaard (74) found that aminophylline increased both contractile force and phosphorylase *a* activity in the isolated perfused rat heart. Belford & Feinleib (77) reported activation of phosphorylase in isolated atria of guinea pigs in response to calcium chloride and K-strophanthin as well as to epinephrine. However, they observed no activation of the enzyme in hearts from intact cats which had been given K-strophanthin. The conflicting findings regarding cardiac glycosides remain to be resolved.

The question arises whether there is a common effect of epinephrine and related substances which results in increased glycogenolysis, on the one

hand, and increased muscle contraction, on the other. The demonstration by Rall & Sutherland (78) of a cyclic mononucleotide (adenosine-3′,5′-phosphoric acid; 3′,5′-AMP) in particulate fractions of several tissues such as heart, brain, liver, and skeletal muscle provides a framework for such a unitary hypothesis. These investigators found that 3′,5′-AMP promoted the formation of phosphorylase *a* from phosphorylase *b*, and that epinephrine and related amines increased the formation of this nucleotide, thus indirectly activating phosphorylase. Rall & Sutherland have suggested the possibility that 3′,5′-AMP may have other actions than activation of the kinase, i.e., it may influence muscle contraction more directly. The work in this field has been the subject of a recent review by Sutherland & Rall (79).

The new observations concerning the possible role of 3′,5′-AMP in the catechol amine-induced activation of myocardial glycogenolysis are not incompatible with the hypothesis of Ellis (70) that hexosemonophosphate accumulation is important in the action of the amines. If, in the absence of adrenergic stimuli, the reaction catalyzed by phosphorylase *a* (glycogen → glucose-1-PO_4) is a rate-limiting step in the glycogenolytic sequence, the activation of the enzyme by catechol amines should remove this limitation with some other step becoming rate limiting. If the latter is at the level of phosphofructokinase, the accumulation of hexosemonophosphate would result.

Effects of sympathomimetic amines on blood vessels.—Many papers have appeared in the past few years concerning the actions of sympathomimetic amines on blood pressure, blood flow, and on the contraction of isolated-vessel strips. Only a few of these papers will be surveyed below.

Two papers dealing with the effects of amines on the pulmonary bed are particularly noteworthy. In both, the authors carefully avoided many of the problems peculiar to this vascular bed that have beset less sophisticated investigators in the past. Borst, Berglund & McGregor (80) replaced the right ventricle of open-chest dogs with a mechanical pump and perfused each lung separately with blood. Epinephrine and norepinephrine both produced consistent, but moderate, vasoconstriction when injected into the arterial supply of one lung at a time. Aviado & Schmidt (81) measured pulmonary artery pressure in the inflow circuit to one lobe of a lung of a vagotomized, open-chest dog in which the lobe was perfused at a constant flow rate by a mechanical pump. They also measured venous outflow from a cannulated pulmonary vein of one lobe in other experiments. Their results revealed nine patterns of action of sympathomimetic drugs which varied from pulmonary constriction and increased blood flow through the lungs after administration of epinephrine or norepinephrine to vasodilation and decreased blood flow through the lungs after administration of a new drug [Compound 45-50 : β-hydroxyl-β-(2,5-diethoxyphenyl) isopropylamine] having pulmonary hypotensive but systemic hypertensive properties. Isoproterenol elicited vasodilation and increased blood flow in the lungs, while methoxamine increased both pulmonary and systemic blood pressures. Although Aviado & Schmidt (81) do not discuss in detail the complex factors

contributing to the varied patterns of response, they have presented a valuable description of the pulmonary vascular effects of some 80 sympathomimetic amines, many of which are not commercially available. Aviado (82) has also critically and comprehensively reviewed the pharmacology of the pulmonary circulation.

A similar careful evaluation of the effects of 23 sympathomimetic drugs on the renal circulation of dogs was performed by Aviado, Wnuck & De Beer (83). Renal artery inflow was measured directly with a rotameter, and the responses to intraarterial injections of the amines were compared to those elicited by intravenous injections. They found four patterns of action. Type-A drugs constricted renal vessels when injected either intraarterially or intravenously (epinephrine, norepinephrine, phenylephrine, metaraminol, methoxamine, and naphazoline). Type-B drugs constricted blood vessels when injected intraarterially, but not consistently when injected intravenously (ephedrine, phenylpropanolamine, hydroxyamphetamine, and compound 45-50). Type-C amines had no important effects when injected intraarterially but increased renal blood flow when injected intravenously as a result of their hypertensive properties (amphetamine, metamphetamine, mephentermine, and others). Type-D amines increased flow when injected intraarterially but decreased flow when given intravenously as a result of their systemic vasodepressor effect (isoproterenol, isopropheneamine, and others).

Solution of the problem of the actions of sympathomimetic amines on cerebral circulation was approached by McClure & Green (84) by employing extensive ligation of the blood vessels in the necks of dogs to eliminate intra- and extracranial vascular connections. Blood flow in the carotid artery was measured with an electromagnetic flowmeter. They were unable to elicit changes in carotid artery blood flow in response to intraarterial injections of sympathomimetic amines and concluded that apparent constrictor responses obtained by other investigators using other techniques may have been caused by constriction of extracranial vessels. Whether the prolonged and extensive surgery required in this technique altered cerebral vascular reactivity has not been adequately evaluated.

The question of whether sympathomimetic amines have direct actions on coronary vessels has also engendered some controversy. Here a clear approach to the problem is complicated by extravascular effects of the drugs such as their ability to increase myocardial contractility. Berne (85) examined this problem and attempted to evaluate the extent to which the contractile actions of epinephrine and norepinephrine affected coronary flow by using dogs in which coronary blood flow and the oxygen concentration in coronary sinus blood were measured under three different conditions: (*a*) intact beating hearts, (*b*) fibrillating hearts perfused from a donor dog, and (*c*) hearts arrested with potassium and perfused from a donor dog. He concluded that the basic action of the amines on the coronaries is vasoconstriction and that secondary vasodilator responses are caused by metabolic stimulation and relative hypoxia. Denison & Green (86) found that the mean

coronary artery blood flow in dogs was increased by norepinephrine, but that the end-diastolic coronary resistance was virtually unchanged. They concluded that norepinephrine has little direct effect on arterioles of the coronary bed, and that the change in flow can be accounted for by the effect of norepinephrine on lengthening of diastole relative to the cycle length of the heart and by more rapid increase in flow to maximum values during isometric relaxation of the ventricles.

The mechanism of action of adrenergically induced vasodilatation has been studied by the Swedish workers Lundholm and Mohme-Lundholm in a series of several papers [Mohme-Lundholm (87); Lundholm (88, 89, 90)]. They present evidence favoring the view that the vasodilating effect of epinephrine is attributable to the accumulation of lactic acid as a result of the amine-induced activation of glycogenolysis in skeletal muscle. Their evidence consists, in part, of (*a*) the similarity of responses to epinephrine and lactic acid on blood flow in animals and on the relaxation of isolated artery strips, (*b*) the increase in concentration of lactic acid in blood vessels following the administration of epinephrine, and (*c*) the antagonism of the vasodilator effect of epinephrine by substances, such as copper and sodium fluoride, which block the epinephrine-induced enhancement of lactic acid production. Furchgott (91) briefly reviews some evidence which is not compatible with this hypothesis.

One final aspect of the vascular responses to sympathomimetic amines can be reviewed briefly, namely, the influence of the amines on veins. Shadle, Zukof & Diana (92) utilized a technique involving perfusion of the hind limb of a dog with constant arterial inflow and measurement of limb weight, arterial and venous pressures, and venous outflow. They found that an infusion of norepinephrine brought about initial increases in limb weight and arterial pressure which were attributed to arteriolar constriction with impounding of blood in the arteries. This was followed by a marked loss in weight and increase in small vein pressures which, they asserted, was a result of constriction of veins. From results of experiments involving the introduction of P^{32} tags into the circulation, Shadle, Zukof & Diana (92) concluded that little of the weight change was caused by a transcapillary shift of blood. Glover and co-workers (93) concluded that infusions of epinephrine and norepinephrine intraarterially in human subjects in which forearm or hand volumes were measured with plethysmographs reduced the capacity of both high-pressure (arteries) and low-pressure (veins) vessels. Eckstein & Hamilton (94) utilized the plethysmographic method with venous occlusion to obtain venous pressure-volume curves in human forearms. Infusions of epinephrine and norepinephrine increased venous pressure and decreased venous distensibility, larger changes being obtained when the arms were held in a dependent position. The *alpha* adrenergic blocking drug, phentolamine, effectively antagonized this response. The same investigators [Eckstein & Hamilton (95)] found a similar action for isoproterenol on the distensibility of forearm veins. They further demonstrated that intravenous infusions of isoproterenol reduced

atrial transmural pressure as reflected by a decreased antecubital vein pressure with no change in esophageal pressure. They concluded that isoproterenol, through a venoconstrictor effect, causes a shift of blood centrally and that the slight hyperventilation and decreased end-expiratory CO_2 tension in alveolar air were not adequate to explain the circulatory changes. In this respect Guyton and his associates (96), using a method for measuring total venous return in dogs under total spinal anesthesia, found that epinephrine increased mean circulatory filling pressure which results in increased venous return. They concluded that under normal conditions changes in cardiac output brought about by sympathomimetic amines are determined far more by increased venous return than by the heart's ability to pump blood. These five papers dealing with the effect of amines on veins leave little doubt that such amines as epinephrine and norepinephrine are capable of constricting veins, and that this venoconstriction may play an important part in the augmentation of cardiac output produced by these compounds. The finding that isoproterenol constricts veins is at variance with most of the previous studies on this compound which indicate that it is primarily a vasodilator. However, most previous work did not directly consider the action of this amine on veins. The possibility that isoproterenol may constrict veins and yet dilate arterioles, at least in skeletal muscle vascular beds, must be considered in future work.

Adrenergic Antagonists

From the work of many investigators in recent years it has become apparent that antagonism of adrenergic activity by pharmacological means can no longer be thought of in the simple terms of competitive or noncompetitive blockade. One must also consider other types of actions, such as depletion of the transmitter substance at the nerve terminals, prevention of release or synthesis of the transmitter substance at the nerve endings, and inhibition of the central outflow of sympathetic impulses. All but the last of these types of adrenergic antagonism will be considered as they relate to the cardiovascular system. The tentative classification of the various types of adrenergic antagonism used has been adopted on the basis of currently assumed modes of action of the various drugs. This classification will probably become obsolete as more information becomes available, but it is hoped that it will provide a suitable structure for the present. The classification of adrenergic receptors into *alpha* and *beta* types has been discussed above.

Drugs which antagonize adrenergic responses at the "receptors." (*a*) *alpha-type adrenergic blockade.*—Brown & Gillespie (97) have presented evidence linking the mechanism for destruction of norepinephrine and epinephrine to the functional integrity of the receptors. These investigators measured the output of "sympathin" from the splenic veins of cats in response to stimulation of the splanchnic nerves. They found that little or no sympathin could be detected in the splenic-vein blood when the nerve was stimulated at less than 10 impulses/sec., but with increasing frequencies

of stimulation from 10 to 50/sec. significant amounts of sympathin appeared. After the administration of phenoxybenzamine, and inactivation of the *alpha* receptors, the sympathin output was increased in response to nerve stimulation, and stimulation frequencies of between 1 and 30/sec. now produced large and equal outputs. Brown and his associates (98) obtained similar results using the colon and small intestine of cats. In keeping with these findings are the observations of Benfey and co-workers (99) that adrenergic blocking drugs increased the urinary excretion of epinephrine and norepinephrine, and of Millar *et al.* (100) that the concentration of catechol amines in the plasma of dogs increased after administration of phenoxybenzamine. Such data do not necessarily imply that phenoxybenzamine interferes with the enzymatic processes involved in the breakdown of catechol amines, since diminished binding of the amines to the inactivated receptors may in itself permit escape of larger amounts of intact amines into the blood stream.

These considerations provide a tentative explanation for some unpublished experiments of Moran in which it was noted that *alpha* adrenergic blocking drugs including phenoxybenzamine, phentolamine, dihydroergotamine, and chlorpromazine increased the heart rate and cardiac contractile force in open-chest dogs, but depressed isolated heart preparations. If it is assumed that these blocking drugs react only with *alpha* receptors, and since the adrenergic receptors in the heart are *beta,* not *alpha,* the increased amount of circulating catechol amines resulting from *alpha* blockade would stimulate the heart *in vivo.* In *in vitro* preparations, where there is no continual neural stimulus for the release of sympathin, no stimulant actions are observed upon addition of the *alpha*-blocking drugs. The hypothesis of Brown and his associates (97, 98) might also explain the observation of Huković (101) that stimulation of the nerve in an isolated rabbit atria-sympathetic nerve preparation produced a greater increase in heart rate after administration of phenoxybenzamine than before.

It has long been suspected that β-haloalkylamines, such as dibenamine, are metabolically altered to an active form [Nickerson *et al.* (102)]. This subject has been examined by Graham (103) who concluded that 2-haloethylamine compounds, except for fluorenyl compounds, form an ethylenimonium ion in neutral solution, and that this represents the pharmacologically active species. The chemical requirements for this type of activity have been discussed by Graham (103). Ferguson (104) also studied the adrenergic blocking properties of two haloalkylamine compounds, their ethylenimonium derivatives, and the hydrolysis products of the latter. He also considers the imonium-ring species to be the active form, but, in contrast to Graham (103), could find no relationship between the duration of the pharmacological effects and the *in vitro* life of the ethylenimonium forms.

(*b*) *beta-type adrenergic blockade.*—Powell & Slater (53) have described many of the pharmacological properties of DCI, finding that it antagonized the vasodilator actions of epinephrine. Moran & Perkins (55) observed that initial intravenous doses of DCI in dogs increased both heart

rate and force of ventricular contraction but that subsequent doses depressed the heart. Following administration of DCI, the positive inotropic response to intravenous injections of epinephrine, norepinephrine, and isoproterenol and to electrical stimulation of the cardiac sympathetic nerves was markedly reduced or abolished. The positive chronotropic response to isoproterenol in the dog was also antagonized by DCI. Specificity of antagonism was demonstrated by the failure of DCI to inhibit the cardiac stimulant effects of ouabain, calcium chloride, and theophylline. Similar results were obtained in isolated perfused rabbit hearts. Confirmation of the cardiac adrenergic antagonism by DCI has been demonstrated on isolated rabbit atria by Furchgott (91) and on the cat papillary muscle by Dresel (105). Reference has been made above to the finding of Mayer & Moran (76) that DCI also antagonizes the activation of myocardial phosphorylase induced in dogs by epinephrine and cardiac sympathetic nerve stimulation.

The work of Moran & Perkins (55) and that of Dresel (105) suggested a competitive or surmountable type of blockade by DCI on the heart. Fleming & Hawkins (106), on the basis of studies with the dog heart-lung preparation, have proposed that the antagonism by DCI should be considered as a case of competitive dualism [Ariens *et al.* (107)] and not a true example of competitive blockade. However one considers it, specificity of the antagonism lends support to the concept that the cardiac adrenergic receptors are of the *beta* type, particularly when it is recognized that conventional *alpha* adrenergic blocking drugs such as phenoxybenzamine and phentolamine lack cardiac adrenergic blocking properties [Mayer & Moran (76); Furchgott (91); Nickerson (108); and Moran & Perkins (unpublished observations)].

Drugs which antagonize adrenergic responses by preventing release or synthesis of the mediator substance, or both.—In 1957 Exley (109) published an analysis of some pharmacological effects of choline-2:6-xylyl ether bromide (compound TM-10) and concluded that it had a specific effect of either preventing the release or the synthesis of the mediator substance at the terminals of adrenergic nerves. Subsequently, similar actions were described for bretylium (N-*o*-bromobenzyl-N-ethyl-N,N-dimethyl ammonium) by Boura & Green (110), for a series of 2,6-disubstituted phenoxyethylammonium bromides by McLean and associates (111, 112), and for guanethidine [(2-[octahydro-1-azocinyl] ethyl) guanidine sulfate] by Maxwell *et al.* (113, 114). The main type of evidence for the postulation regarding the site of action of all of these drugs is the demonstration that they inhibit the response of an end organ (e.g., nictitating membrane) to electrical stimulation through either the pre- or postganglionic fibers innervating the organ, but do not inhibit axonal or ganglionic transmission or the response of the end organ to injection of epinephrine. The cardiovascular effects of these various substances are reduction in blood pressure, postural hypotension, and reduced heart rate. Maxwell *et al.* (113) feel that guanethidine is more effective as a hypotensive agent in experimental hypertensive dogs than in normotensive animals. Confirmation of the proposed mechanism of action

of these compounds must wait upon more detailed experiments as well as increased understanding of the biosynthesis and release of sympathetic neurohumoral transmitters at nerve endings.

Drugs which antagonize adrenergic responses by depleting nerve endings or effector organs of the mediator substances, or both—Much has been written about the ability of reserpine to reduce greatly the stores of catechol amines in the heart [Bertler *et al.* (115); Paasonen & Krayer (116); Waud *et al.* (117); and Lee & Shideman (118)] and blood vessels [Burn & Rand (119)]. This reduction in myocardial catechol amine content leads to a diminished responsiveness of the heart to nerve-induced adrenergic stimuli as has been shown by Trendelenberg (120) who found that the positive chronotropic response to electrical stimulation of the accelerans nerve was markedly reduced in dogs pretreated with reserpine. Similarly, pretreatment of dogs with reserpine completely abolished both the positive inotropic and chronotropic responses to stimulation of the cardiac sympathetic nerves [Mayer & Moran (76)]. Although the responses to sympathetic nerve stimulation were completely abolished by pretreatment with reserpine, the responses of the heart to injected catechol amines remained unimpaired. Lee & Shideman (118) found that isolated papillary muscles from cats pretreated with reserpine exhibited lower contractile amplitudes and catechol amine concentrations than those found in muscles taken from normal cats. Similar reductions in cardiac contractility and catechol amine concentrations were found in papillary muscles from cats subjected to chronic cardiac sympathectomies. These authors proposed that normal cardiac contractility is influenced by endogenous myocardial catechol amine stores.

As a natural consequence of the impairment by reserpine or related analogues of the sympathetic nervous system control over cardiovascular functions, hypotension, bradycardia, and reduced or abolished pressor reflexes occur [de Schaepdryver (121); Orlans *et al.* (122)]. For example, the pressor responses to carotid artery occlusion, stimulation of the splanchnic nerves and central end of the cut vagus nerve, asphyxia, and injection of TMA and nicotine are greatly reduced after pretreatment of animals with reserpine. The depression of the pressor responses has been shown to be accompanied by a reduction in catechol amine content of the adrenal glands, heart, liver, and spleen [de Schaepdryver (121)]. Further evidence that the reduction in sympathetic activity is a result of depletion of peripheral stores of norepinephrine by reserpine-like substances rather than to an effect on the central nervous system has been provided by Orlans *et al.* (122) who observed that all of the peripheral actions of such drugs were evident after injection of syrosingopine, a semisynthetic reserpine alkaloid, in the absence of signs of a tranquilizing effect or depletion of serotonin or catechol amines from stores in the brain.

The acute release of catechol amines by reserpine is accompanied by a positive chronotropic and inotropic action in the dog heart-lung preparation [Paasonen & Krayer (116); Krayer & Fuentes (123)]. The positive chron-

otropic effect of reserpine has been shown to be accompanied by a depletion of myocardial catechol amine stores [Paasonen & Krayer (116)]. Plasma levels of catechol amines did not increase while the myocardial stores were being depleted, but the release may have been sufficiently slow so that the amines were destroyed as rapidly as they were released. Once the myocardial catechol amine stores were depleted by pretreatment of a dog with reserpine, further administration of reserpine when a heart-lung preparation was made did not evoke a positive chronotropic response.

Administration of reserpine to intact animals also produces evidence of sympathetic stimulation including a positive inotropic action [Cotten & Moran (unpublished observations)] and a rise in blood pressure [Domino & Rech (124); Maxwell *et al.* (125); Horita (126)]. The pressor response develops within five to 20 minutes after injection of 1 mg/kg. of reserpine in unanesthetized dogs [Domino & Rech (124)]. In these experiments ganglionic blockade enhanced the response, pentobarbital reduced it, phenoxybenzamine abolished it, and bilateral adrenalectomy did not prevent it. Maxwell *et al.* (125) also noted that intravenous injection of reserpine produced pressor responses in anesthetized dogs that had received large doses of ganglionic blocking drugs. The response was reduced by phentolamine. Lysergic acid diethylamide or spinal cord section at C-1 or C-2 had no effect on the response. Horita (126) made similar observations, finding that intravenous injections of reserpine into anesthetized dogs produced pressor responses if the dogs had been pretreated with cocaine. This response was antagonized by adrenergic blocking agents but not by 2-brom-lysergic acid diethylamide. The response was decreased but not abolished by adrenalectomy. The most obvious mechanism for this pressor response would appear to be an elevation of free catechol amines in plasma and tissue as a result of the acute release of the bound amines, but none of these papers report determinations of plasma levels of amines. However, Muscholl & Vogt (127) found that intravenous injections of reserpine in rabbits raised adrenaline concentration in plasma by 54 to over 1000 per cent. Norepinephrine concentration was too low to determine any change.

Drugs Which Produce Cardiovascular Sympathomimetic Effects Through Indirect Actions

Brief mention should be made of an increasingly important aspect of drug action: the indirect production of effects through the release of endogenous substances. Evidence is accumulating that cardiovascular responses to a number of drugs may be mediated, at least in part, through the release of catechol amines, either from the adrenal medulla or from tissue stores. Burn & Rand (119) have proposed that the blood vessels contain stores of norepinephrine and that certain drugs can cause either a temporary or a prolonged release of the amine. They also proposed that the stores, once depleted, can be replenished by exposure to norepinephrine. They have found, for instance, that the vasopressor effect in spinal cats,

the vasoconstrictor effect in perfused dog hind limbs and rabbit ears, and the contraction of isolated strips of rabbit aorta produced by tyramine and other noncatechol amines are greatly reduced or abolished by pretreatment of the animals with reserpine. The responses to catechol amines, on the other hand, are potentiated. The action of tyramine and related amines can be restored in the reserpinized preparation by infusion of norepinephrine.

Burn & Rand (128) have also found that the vasoconstriction produced by nicotine and acetylcholine (in the presence of atropine) in the perfused rabbit ear is abolished by reserpine. Reserpine also reduces the amount of a norepinephrine-like substance in the skin of the rabbit ear as well as apparently diminishing the chromaffin granules in the skin.

Richardson & Woods (129) have demonstrated a release of norepinephrine from the isolated perfused rabbit heart in response to acetylcholine when atropine is present in the perfusion fluid. The release of the catechol amine corresponded in time with the increased contractile force resulting from administration of acetylcholine.

Ganglionic stimulants, such as tetramethylammonium (TMA), nicotine, and acetylcholine (in the presence of atropine), produced increased force of contraction of papillary muscles from cats [Lee & Shideman (130)]. This effect was blocked by ganglionic blocking drugs, compound TM-10, and DCI. The effect of epinephrine on the papillary muscle, however, was not blocked either by ganglionic blocking drugs or compound TM-10. The muscle became refractory to repeated administration of the ganglionic stimulant drugs. In papillary muscles from reserpine-treated cats, in which the catechol amine concentration was reduced, TMA and nicotine had little or no effect, but the muscle was fully responsive to epinephrine or norepinephrine. Histological examination of the papillary muscles revealed no ganglion cells. Lee & Shideman concluded that the cardiac stimulation is either mediated through postganglionic sympathetic nerve endings in the heart or through elements that can be classified pharmacologically as ganglia but which do not meet the morphological criteria for such a classification. At any rate, these drugs appear to act through release of catechol amines from the stores in the myocardium. Lee & Shideman (131) subsequently conducted a detailed analysis of the structure-activity relationships of quaternary ammonium compounds regarding the actions on the heart. They found that all of a large series of quaternary ammonium compounds had positive inotropic actions, although in some compounds this action was masked by prominent muscarinic activity. Presumably, these agents act through the same mechanism utilized by acetylcholine, TMA, and nicotine.

LITERATURE CITED

1. Olson, R. E., Roush, G., and Liang, M. M. L., *Circulation*, **12**, 755 (1955)
2. Regan, T. J., Talmers, F. N., and Hellems, H. K., *J. Clin. Invest.*, **35**, 1220 (1956)
3. Mercier, F., Gavend, M. R., Gavend, M., and Mercier, J., *Compt. rend. soc. biol.*, **151**, 356 (1957)
4. White, W. F., and Salter, W. T., *J. Pharmacol. Exptl. Therap.*, **88**, 1 (1946)
5. Sciarini, L. J., Ackerman, E. M., and Salter, W. T., *J. Pharmacol. Exptl. Therap.*, **92**, 432 (1948)
6. Sanyal, P. N., and Saunders, P. R., *Proc. Soc. Exptl. Biol. Med.*, **95**, 156 (1957)
7. Stewart, G. A., *J. Pharm. and Pharmacol.*, **10**, 741 (1958)
8. Cotten, M. deV., and Stopp, P. E., *Am. J. Physiol.*, **192**, 114 (1958)
9. Walton, R. P., Leary, J. S., and Jones, H. P., *J. Pharmacol. Exptl. Therap.*, **98**, 346 (1950)
10. Bloodwell, R. D., Goldberg, L. I., Braunwald, E., Gilbert, J. W., Ross, J., Jr., and Morrow, A. G., *Surg. Forum*, **10**, 532 (1959)
11. Bliss, H. A., and Adolph, R. J., *Am. Heart J.*, **57**, 886 (1959)
12. Faust, R. M., and Saunders, P. R., *Proc. Soc. Exptl. Biol. Med.*, **94**, 351 (1957)
13. Leonard, E., *Am. J. Physiol.*, **189**, 185 (1957)
14. Cotten, M. deV., and Brown, T. G., Jr., *J. Pharmacol., Exptl. Therap.*, **121**, 319 (1957)
15. Saunders, P. R., and Sanyal, P. N., *J. Pharmacol. Exptl. Therap.*, **123**, 161 (1958)
16. Sanyal, P. N., and Saunders, P. R., *J. Pharmacol. Exptl. Therap.*, **122**, 499 (1958)
17. Ross, J., Jr., Braunwald, E., and Waldhausen, J. A., *J. Clin. Invest.*, **39**, 937 (1960)
18. Horsley, A. W., and Eckstein, J. W., *J. Lab. Clin. Med.*, **54**, 827 (1959)
19. Baschieri, L., Ricci, P. D., Mazzuoli, G. F., and Vassalle, M., *Cuore e circolazione*, **41**, 103 (1957)
20. Ross, J., Jr., Waldhausen, J. A., and Braunwald, E., *J. Clin. Invest.*, **39**, 930 (1960)
21. Williams, M. H., Jr., Zohman, L. R., and Ratner, A. C., *J. Appl. Physiol.*, **13**, 417 (1958)
22. Holland, W. C., and Sekul, A. A., *Am. J. Physiol.*, **197**, 757 (1959)
23. Moran, N. C., and Cotten, M. deV., *Structure and Function of Muscle*, **3**, Chap. 2, (Academic Press Inc., London, England, 482 pp., 1960)
24. Rayner, B., and Weatherall, M., *Brit. J. Pharmacol.*, **12**, 371 (1957)
25. Vick, R. L., and Kahn, J. B., Jr., *J. Pharmacol. Exptl. Therap.*, **121**, 389 (1957)
26. Vick, R. L., *J. Pharmacol. Exptl. Therap.*, **125**, 40 (1959)
27. Cairns, A. B., Jr., Love, W. D., and Burch, G. E., *Am. Heart J.*, **59**, 404 (1960)
28. Glynn, I. M., *J. Physiol.*, **136**, 148 **(1957)**
29. Gill, T. J., and Solomon, A. K., *Nature*, **183**, 1127 (1959)
30. Kunz, H. A., and Sulser, F., *Experientia*, **13**, 365 (1957)
31. Hajdu, S., *Am. J. Physiol.*, **174**, 371 (1953)
32. Thomas, L. J., Jr., *Am. J. Physiol.*, **199**, 146 (1960)
33. Furchgott, R. F., and Gubareff, T. de, *J. Pharmacol. Exptl. Therap.*, **124**, 203 (1958)
34. Lee, K. S., Yu, D. H., and Burstein, R., *J. Pharmacol. Exptl. Therap.*, **129**, 115 **(1960)**
35. Peschel, E., and Schlayer, C., *J. Lab. Clin. Med.*, **52**, 417 (1958)
36. Lardy, H. A., and Wellman, H., *J. Biol. Chem.*, **195**, 215 (1952)
37. Hock, F. L., and Lipmann, F., *Proc. Natl. Acad. Sci. U. S.*, **40**, 909 (1954)
38. Lee, K. S., Schwartz, A., and Burstein, R., *J. Pharmacol. Exptl. Therap.*, **129**, 123 (1960)
39. Plaut, K. A., Gertler, M. M., and Plaut, G. W. E., *Circulation Research*, **5**, 226 (1957)
40. Wollenberger, A., *Pharmacol. Revs.*, **1**, 311 (1949)
41. Berman, D. A., Masuoka, D. T., and Saunders, P. R., *Science*, **126**, 746 (1957)
42. Kien, G. A., and Sherrod, T. R., *Circulation Research*, **8**, 188 (1960)
43. Bennett, D. R., Andersen, K. S., Andersen, M. V., Jr., Robertson, D. N., and Chenoweth, M. B., *J. Pharmacol. Exptl. Therap.*, **122**, 489 (1958)
44. Hajdu, S., *J. Pharmacol. Exptl. Therap.*, **120**, 90 (1957)
45. Tanz, R. D., Whitehead, R. W., and Weir, G. J., Jr., *Proc. Soc. Exptl.*

Biol. Med., **94**, 258 (1957)
46. Tanz, R. D., *J. Pharmacol. Exptl. Therap.*, **128**, 168 (1960)
47. King, T. M., Whitehorn, W. V., Reeves, B., and Kubota, R., *Am. J. Physiol.*, **196**, 1282 (1959)
48. Van Arman, C. G., and Drill, V. A., *J. Pharmacol. Exptl. Therap.*, **124**, 59 (1958)
49. Grinnell, E. H., and Smith, P. W., *Proc. Soc. Exptl. Biol. Med.*, **94**, 524 (1957)
50. Unterman, D., Graff, A. C. de, and Kupperman, H. S., *Circulation Research*, **3**, 280 (1955)
51. Aviado, D. M., Jr., *Anesthesiology*, **20**, 71 (1959)
52. Ahlquist, R. P., *Am. J. Physiol.*, **153**, 586 (1948)
53. Powell, C. E., and Slater, I. H., *J. Pharmacol. Exptl. Therap.*, **122**, 480 (1958)
54. Ahlquist, R. P., In *Pharmacology in Medicine*, 2nd ed., 378–407 (Drill, V. A., Ed., McGraw-Hill Book Co., New York, N. Y., 1243 pp., 1958)
55. Moran, N. C., and Perkins, M. E., *J. Pharmacol. Exptl. Therap.*, **124**, 223 (1958)
56. Raab, W., Paulae Silva, P. de, and Starcheska, Y. K., *Cardiologia*, **33**, 350 (1958)
57. Rushmer, R. F., and West, T. C., *Circulation Research*, **5**, 240 (1957)
58. West, T. C., and Rushmer, R. F., *J. Pharmacol. Exptl. Therap.*, **120**, 361 (1957)
59. Stack, M. F., Rader, B., Sobol, B. J., Farber, S. J., and Eichna, L. W., *Circulation*, **17**, 526 (1958)
60. Weissler, A. M., Leonard, J. J., and Warren, J. V., *J. Lab. Clin. Med.*, **53**, 921 (1959)
61. Aviado, D. M., Jr., and Wnuck, A. L., *J. Pharmacol. Exptl. Therap.*, **119**, 99 (1957)
62. Goldberg, L. I., Cotten, M. deV., Darby, T. D., and Howell, E. V., *J. Pharmacol. Exptl. Therap.*, **108**, 177 (1953)
63. Stormorken, H., Schaepdryver, A. F. de, and Vleeschhouwer, G. R. de, *Arch. intern. pharmacodynamie*, **120**, 386 (1959)
64. Preziosi, P., Vleeschhouwer, G. R. de, Schaepdryver, A. F. de, and Bianchi, A., *Arch. intern. pharmacodynamie*, **121**, 506 (1959)
65. Welch, G. H., Jr., Braunwald, E., Case, R. B., and Sarnoff, S. J., *Am. J. Med.*, **24**, 871 (1958)
66. Dresel, P. E., and Nickerson, M., *J. Pharmacol. Exptl. Therap.*, **125**, 142 (1959)
67. O'Brien, G. S., Murphy, Q. R., and Meek, W. J., *J. Pharmacol. Exptl. Therap.*, **112**, 374 (1954)
68. Moore, J. I., and Swain, H. H., *J. Pharmacol. Exptl. Therap.*, **128**, 243 (1960)
69. Moore, J. I., and Swain, H. H., *J. Pharmacol. Exptl. Therap.*, **128**, 253 (1960)
70. Ellis, S., *Pharmacol. Revs.*, **11**, 469 (1959)
71. Cori, C. F., *Enzymes: Units of Biological Structure and Function*, 573–83 (Gaebler, O. H., Ed., Academic Press, New York, N. Y., 624 pp., 1956)
72. Rall, T. W., Wosilait, W. D., and Sutherland, E. W., *Biochim. et Biophys. Acta*, **20**, 69 (1956)
73. Ellis, S., *Pharmacol. Revs.*, **8**, 485 (1956)
74. Hess, M. E., and Haugaard, N., *J. Pharmacol. Exptl. Therap.*, **122**, 169 (1958)
75. Kukovetz, W. R., Hess, M. E. Shanfeld, J., and Haugaard, N., *J. Pharmacol. Exptl. Therap.*, **127**, 122 (1959)
76. Mayer, S. E., and Moran, N. C., *J. Pharmacol. Exptl. Therap.*, **129**, 271 (1960)
77. Belford, J., and Feinleib, M. R., *J. Pharmacol. Exptl. Therap.*, **127**, 257 (1959)
78. Rall, T. W., and Sutherland, E. W., *J. Biol. Chem.*, **232**, 1065 (1958)
79. Sutherland, E. W., and Rall, T. W., *Pharmacol. Revs.*, **12**, 265 (1960)
80. Borst, H. G., Berglund, E., and McGregor, M., *J. Clin. Invest.*, **36**, 669 (1957)
81. Aviado, D. M., Jr., and Schmidt, C. F., *J. Pharmacol. Exptl. Therap.*, **120**, 512 (1957)
82. Aviado, D. M., Jr., *Pharmacol. Revs.*, **12**, 159 (1960)
83. Aviado, D. M., Jr., Wnuck, A. L., and De Beer, E. J., *J. Pharmacol. Exptl. Therap.*, **124**, 238 (1958)
84. McClure, C., Jr., and Green, H. D., *Am. J. Physiol.*, **197**, 1183 (1959)
85. Berne, R. M., *Circulation Research*, **6**, 644 (1958)
86. Denison, A. B., Jr., and Green, H. D., *Circulation Research*, **6**, 633 (1958)
87. Mohme-Lundholm, E., *Acta Physiol. Scand.*, **38**, 255 (1957)

88. Lundholm, L., *Acta Physiol. Scand.*, **39,** Suppl. 133 (1957)
89. Lundholm, L., *Acta Physiol. Scand.*, **40,** 344 (1957)
90. Lundholm, L., *Acta Physiol. Scand.*, **43,** 27 (1958)
91. Furchgott, R. F., *Pharmacol. Revs.*, **11,** 429 (1959)
92. Shadle, O. W., Zukof, M., and Diana, J., *Circulation Research,* **6,** 326 (1958)
93. Glover, W. E., Greenfield, A. D. M., Kidd, B. S. L., and Whelan, R. F., *J. Physiol. (London),* **140,** 113 (1958)
94. Eckstein, J. W., and Hamilton, W. F., *J. Clin. Invest.*, **36,** 1663 (1957)
95. Eckstein, J. W., and Hamilton, W. F., *J. Clin. Invest.*, **38,** 342 (1959)
96. Guyton, A. C., Lindsey, A. W., Abernathy, B., and Langston, J. B., *Am. J. Physiol.*, **192,** 126 (1958)
97. Brown, G. L., and Gillespie, J. S., *J. Physiol. (London),* **138,** 81 (1957)
98. Brown, G. L., Davies, B. N., and Gillespie, J. S., *J. Physiol. (London),* **143,** 41 (1958)
99. Benfey, B. G., Ledoux, G., and Segal, M., *Brit. J. Pharmacol.*, **14,** 380 (1959)
100. Millar, R. A., Keener, E. B., and Benfey, B. G., *Brit. J. Pharmacol.*, **14,** 9 (1959)
101. Huković, S., *Brit. J. Pharmacol.*, **14,** 372 (1959)
102. Nickerson, M., Nomaguchi, G., and Goodman, L. S., *Federation Proc.*, **5,** 195 (1946)
103. Graham, J. D. P., *Brit. J. Pharmacol.*, **12,** 489 (1957)
104. Ferguson, F. C., Jr., *Proc. Soc. Exptl. Biol. Med.*, **99,** 362 (1958)
105. Dresel, P. E., *Can. J. Biochem. Physiol.*, **38,** 375 (1960)
106. Fleming, W. W., and Hawkins, D. F., *J. Pharmacol. Exptl. Therap.*, **129,** 1 (1960)
107. Ariens, E. J., Rossum, J. M. van, and Simonis, A. M., *Pharmacol. Revs.*, **9,** 218 (1957)
108. Nickerson, M., *Pharmacol. Revs.*, **11,** 443 (1959)
109. Exley, K. A., *Brit. J. Pharmacol.*, **12,** 297 (1957)
110. Boura, A. L. A., and Green, A. F., *Brit. J. Pharmacol.*, **14,** 536 (1959)
111. McLean, R. A., Geus, R. J., Mohrbacher, R. J., Mattis, P. A., and Ullyot, G. E., *J. Pharmacol. Exptl. Therap.*, **129,** 11 (1960)
112. McLean, R. A., Geus, R. J., Pasternack, J., Mattis, P. A., and Ullyot, G. E., *J. Pharmacol Exptl. Therap.*, **129,** 17 (1960)
113. Maxwell, R. A., Plummer, A. J., Schneider, F., Povalski, H., and Daniel, A. I., *J. Pharmacol. Exptl. Therap.*, **128,** 22 (1960)
114. Maxwell, R. A., Plummer, A. J., Povalski, H., and Schneider, F., *J. Pharmacol. Exptl. Therap.*, **129,** 24 (1960)
115. Bertler, A., Carlsson, A., and Rosengren, E., *Naturwissenschaften,* **43,** 521 (1956)
116. Paasonen, M. K., and Krayer, O., *J. Pharmacol. Exptl. Therap.*, **123,** 153 (1958)
117. Waud, D. R., Kottegoda, S. R., and Krayer, O., *J. Pharmacol. Exptl. Therap.*, **124,** 340 (1958)
118. Lee, W. C., and Shideman, F. E., *Science,* **129,** 967 (1959)
119. Burn, J. H., and Rand, M. J., *J. Physiol. (London),* **144,** 314 (1958)
120. Trendelenburg, U., and Gravenstein, J. S., *Science,* **128,** 901 (1958)
121. Schaepdryver, A. F. de, *Arch. intern. pharmacodynamie,* **124,** 45 (1960)
122. Orlans, F. B. H., Finger, K. F., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.*, **128,** 131 (1960)
123. Krayer, O., and Fuentes, J., *J. Pharmacol. Exptl. Therap.*, **123,** 145 (1958)
124. Domino, E. F., and Rech, R. H., *J. Pharmacol. Exptl. Therap.*, **121,** 171 (1957)
125. Maxwell, R. A., Ross, S. D., Plummer, A. J., and Sigg, E. B., *J. Pharmacol. Exptl. Therap.*, **119,** 69 (1957)
126. Horita, A., *J. Pharmacol. Exptl. Therap.*, **22,** 474 (1958)
127. Muscholl, E., and Vogt, M., *Brit. J. Pharmacol.*, **12,** 532 (1957)
128. Burn, J. H., and Rand, M. J., *Brit. Med. J.*, **1,** 903 (1958)
129. Richardson, J. A., and Woods, E. F., *Proc. Soc. Exptl. Biol. Med.*, **100,** 149 (1959)
130. Lee, W. C., and Shideman, F. E., *J. Pharmacol. Exptl. Therap.*, **126,** 239 (1959)
131. Lee, W. C., and Shideman, F. E., *J. Pharmacol. Exptl. Therap.*, **127,** 219 (1959)

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