0031-6997/80/3101-0019$02.00/0
Pharmacological Reviews

Vol. 31, No. 1
Printed in U.S.A.

The Role of the Nervous System in the Cardiovascular Effects of Digitalis*

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* The authors' own studies cited herein were supported by Grants NB-07651, FR5360, FR5306, HE-13675, and NS-12566 from the U.S. Public Health Service and by the Washington Heart Association.

† Recipient of Research Career Development Award HL-70673 from the National Heart and Lung Institute.

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I. Introduction

NEUROEXCITATORY effects of digitalis drugs have been known for centuries. They were vividly pointed out by Withering in 1785 (881). He described visual disturbances, delusions, confusion, and hallucinations in patients who had been exposed to toxic amounts of these drugs. Experimental studies dealing with neuroexcitatory effects of digitalis were first focused on the parasympathomimetic actions of these drugs (825). Subsequent studies led to the observation that digitalis drugs activated the respiratory center (318). Next, studies were performed that indicated an action of these drugs on "medullary centers" to cause emesis (342). This was then followed by a large series of studies on the effect of digitalis drugs on various reflexogenic sites (355, 356, 362–364). The next major advance came in 1937 with the work of Korth et al. (429), who were the first to emphasize a specific effect of digitalis to increase central sympathetic outflow to the heart. Experiments dealing with the effects of digitalis on efferent peripheral autonomic nerve functions have in general constituted a more recent area of investigation, beginning with the evaluation of these agents on ganglionic transmission (428).

Over the last two decades, there have been many studies on the effect of digitalis drugs on neural activity at all levels of the nervous system. Many of the observed changes in neural function have been implicated in the cardiovascular effects induced by these drugs. As yet, this large body of information has not been integrated into a comprehensive review of how digitalis action on the entire nervous system results in significant changes in cardiovascular function. The only review of this type was published about 20 years ago by Lendle and Mercker (457). Since then, important reviews of this subject have appeared but have dealt only with selected aspects of digitalis action on the nervous system (283, 286, 288, 471, 552, 612, 660, 663, 664, 738).

The purpose of the present review is to assemble the vast amount of information dealing with the effects of digitalis on neural tissue and to describe how these effects are translated into alterations in cardiovascular function. This will be done by pursuing the following four objectives. First, we will describe the known effects of digitalis drugs on neural structures, including reflexogenic areas of the cardiovascular system, central nervous system, and peripheral nerve structures (e.g., ganglia, pre- and postganglionic nerve fibers, and postjunctional sites). Second, we will describe what is known about the mechanisms whereby digitalis drugs elicit these neural effects. This will be discussed from the viewpoint of changes in electrical and chemical activity in neural tissue. Third, and most important, we will discuss the significance of the documented neural effects in mediating or modifying the well-known peripheral effects (i.e., positive inotropic, chronotropic, arrhythmogenic, and smooth muscle vaso-

constrictor activities). Our primary goal will be to assess the relative contribution of the neural and direct cardiac and vascular effects of digitalis in the overall response produced by these agents. Fourth, we will point out areas of research where either controversies currently exist or where new discoveries might occur, with the hope of stimulating future research that will ultimately resolve these controversies and lead to new information about these important drugs.

II. Neuroexcitatory Effects of Digitalis

A. Effects on Afferent Systems

1. Experimental approaches used to evaluate effects of digitalis. It has been demonstrated in many studies that digitalis drugs can activate reflexogenic areas of the cardiovascular system such as arterial baro- and chemoreceptors and other afferent nerve fibers in the nodose ganglion and heart. The following methods have been employed: (a) local administration of digitalis into the reflexogenic area with simultaneous monitoring of afferent nerve activity and/or cardiovascular-respiratory function; (b) systemic administration of digitalis before and after denervation of reflexogenic areas with simultaneous monitoring of afferent or efferent nerve activity and/or cardiovascular-respiratory function; and (c) clinical studies of baroreceptor function before and after systemic administration of digitalis.

2. Baroreceptors. Heymans et al. (364) employed the cross-circulation perfusion technique in anesthetized dogs to study the influence of locally administered digitalis on carotid sinus baroreceptors. The blood of a donor dog was perfused, via the carotid arteries, through the vascularly isolated head circulation of a recipient dog in which one carotid sinus region was intact and the other had been denervated. When strophanthin was injected into the donor dog and allowed to perfuse both of the recipient's carotid sinus regions, bradycardia was observed to occur in the recipient's trunk. The bradycardia was prevented by selectively interrupting the blood flow to the innervated carotid sinus region, whereas it was not altered by interfering with blood flow to the denervated sinus region alone. The results of this study, unfortunately, do not provide good evidence for a direct action of the digitalis preparation on the sensory receptors in the carotid sinus region because strophanthin produced a rise in the blood pressure of the donor dog. Thus, the baroreceptors could have been activated by the rise in pressure rather than by a direct effect of digitalis on carotid sinus baroreceptors.

An excitatory effect of cardiac glycosides on afferent sensory receptors was later shown by Chai et al. (134), who observed that after the local intraarterial administration of 20 to 35 μg of acetylstrophanthidin directly into the carotid sinus regions of anesthetized cats, marked decreases in heart rate and blood pressure occurred in two distinct phases, i.e., immediately after the injection and again several minutes later. The immediate responses were attributed to activation of receptors in the carotid sinus regions since these responses were prevented by sectioning the carotid sinus nerve, while elimination of the blood supply to the nodose ganglion receptors eliminated the delayed responses. However, no attempt was made in this study to distinguish between the effects of digitalis on baroreceptors and chemoreceptors in the carotid artery bifurcations.

Evidence to support a direct action of digitalis drugs on the baroreceptors was provided by the studies of Quest and Gillis (634). In decerebrate unanesthetized cats intracarotid injections of acetylstrophanthidin (1.56 to 25 μg) and ouabain (10 to 20 μg) increased the spontaneously occurring electrical activity of the carotid sinus nerve. The same doses also evoked dose-dependent decreases in arterial blood pressure and myocardial contractile force, which correlated temporally with the augmented neural firing. The cardiovascular changes were prevented by sectioning the carotid sinus nerves. These responses were identical to those evoked by a mechanical increase in pressure applied to the carotid sinus region (481) and suggested an excitatory action of digitalis on the baroreceptors. Acetylstrophanthidin (6.25 μg) was also given to animals with carotid body chemoreceptors destroyed by an intracarotid injection of acetic acid [for technique, see Gernandt (275)]. A marked enhancement of carotid sinus nerve activity still occurred, thus providing direct evidence for an excitation of baroreceptors. In a later study with isolated carotid sinus preparations in anesthetized cats, Quest and Gillis (635) obtained evidence that acetylstrophanthidin and ouabain can directly sensitize baroreceptors. Administration of 1.56 μg of acetylstrophanthidin into the isolated carotid sinus region altered the relationship between an increase in carotid sinus pressure and the subsequent decrease in arterial blood pressure. A given increase in carotid sinus pressure evoked a greater depressor response when the drug was present than when the drug was absent. The administration of 25 μg of ouabain into the isolated carotid sinus region produced a similar alteration in the relationship between an increase in carotid sinus pressure and the subsequent increase in carotid sinus nerve activity. For a given increase in intrasinus pressure there was a greater increase in neural activity in the presence of ouabain. Furthermore, the effect of an increase in intrasinus pressure on carotid sinus nerve activity was mimicked by the administration of digitalis. At a constant carotid sinus pressure, an intracarotid injection of 25 μg of ouabain produced an increase in spontaneous carotid sinus nerve activity that was identical to that produced by raising carotid sinus pressure. These effects of digitalis occurred without a drug-induced change in pressure in the isolated carotid sinus region and demonstrated that acetylstrophanthidin and ouabain directly alter the sensitivity of the baroreceptors and result in an enhanced discharge of the carotid sinus nerve.

In confirmation of this finding, other investigators have also obtained results indicating that digitalis acts directly to sensitize the baroreceptors. Bedynek (60) observed that changes in cardiovascular function characteristic of baroreceptor sensitization occur after the administration of acetylstrophanthidin into the isolated perfused head circulation of anesthetized dogs. Decreases in arterial blood pressure, myocardial contractile force, and heart rate occurred when digitalis, 6 to 12 μg/kg, was injected directly into the perfusion circuit. These responses were prevented by denervation of the carotid sinus regions and occurred in the absence of a rise in perfusion pressure in the isolated cerebral circuit, indicating a direct excitatory effect on the baroreceptors. Ishiko and Fukuda (388) reported that the administration of 10 μg of ouabain into the isolated, perfused aortic arch preparation of anesthetized rabbits, in which intraaortic pressure was held constant, produced a marked increase in the rate of afferent discharge of the aortic depressor nerve. Similar increases in neural activity were produced by ouabain after intravenous injection (5 to 10 μg/kg) and after slow intravenous infusion (2.5 μg/kg/min), and were not associated with pressor effects of the drug. It was concluded that the enhanced electrical activity of the aortic depressor nerve resulted from a direct action of ouabain on the baroreceptors. In support of their conclusion, Ishiko and Fukuda cited that the aortic depressor nerve is predominately composed of baroreceptor fibers, and that an attempt to destroy chemoreceptor fibers with acetic acid in their control studies had no effect on resting aortic depressor nerve activity. Saum et al. (694) examined the effect of ouabain administered into the isolated aortic arch-aortic nerve preparation of the rat on postexcitatory depression elicited by either pressure steps or antidromic stimulation of the aortic nerve. In this preparation, postexcitatory depression occurs immediately after termination of these stimuli. Postexcitatory depression was found to be prevented by perfusion of the aortic arch with ouabain (1×10^{-4} to 1×10^{-3} M).

The only investigators who failed to document an effect of digitalis on baroreceptors were Abdon and Nielsen (3). They isolated and perfused the isolated carotid sinus regions of anesthetized cats and rabbits with Ringer's solution and examined the effects of changes in carotid sinus pressure on heart rate. When pressure in the isolated sinus was raised from 0 to 210 mm Hg, a fall in heart rate of 8% occurred. After perfusion of the sinus regions with 0.5 to 10 μg of ouabain, the bradycardia produced by the same change in carotid sinus pressure was not different from that evoked prior to administration of the drug. From this observation, it was concluded that ouabain did not activate baroreceptors or chemoreceptors. However, Quest and Gillis (634) have shown that larger doses of ouabain are required to produce heart rate responses by an action on the carotid sinus region.

Studies with systemic administration of digitalis in experimental animals support the above findings. McLain (533) examined the effects of ouabain on the spontaneously occurring neural activity of the carotid sinus and aortic depressor nerves of anesthetized cats. He observed that doses of ouabain, 10 to 20 μg/kg, given i.v. or i.m. every 5 to 15 minutes until death, enhanced neural activity in 20 of 25 aortic nerve preparations and in 18 of 21 carotid sinus nerve preparations studied. Although ouabain tended to increase arterial blood pressure under these circumstances, this did not appear to account for the reflex activation since pressure effects were discounted in 84% of the instances reported as neural enhancement (i.e., 32 out of 39 cases). Quest and Gillis (634) administered acetylstrophanthidin, 12.5 and 25 μg/kg i.v., to anesthetized cats and found an increase in the spontaneous discharges occurring in the carotid sinus nerve. The increase in nerve activity was not accompanied by a significant rise in arterial blood pressure. Similar results were also obtained by Pace and Gillis (596) with digoxin (20 μg/kg i.v. every 15 minutes) in anesthetized cats. Baum and Shropshire (56) administered ouabain to anesthetized cats (loading dose of 30 μg/kg i.v. followed by 10 μg/kg i.v. every 10 minutes) and observed an increase in carotid sinus nerve activity that was associated with an increase in blood pressure. In additional experiments, these investigators maintained pressure constant in the carotid sinus region and still observed an increase in carotid sinus nerve activity with i.v. ouabain. From these results, it was concluded that ouabain sensitized the baroreceptor elements.

Further evidence of an indirect nature that digitalis activates baroreceptors has been obtained from denervation studies. Numerous investigators have reported that the bradycardia produced by digitalis in animals is prevented by sectioning the afferent nerves emanating from the reflexogenic areas (5, 6, 11, 290, 355, 356, 362, 363, 556, 894). It has also been demonstrated that denervation of reflexogenic areas prevents digitalis-induced reduction in efferent sympathetic nerve discharge (11, 279, 596, 860).

Data obtained from clinical studies also support the notion that digitalis can activate baroreceptors. These data were obtained by observing changes in heart rate and the occurrence and duration of syncope during the external application of pressure to the carotid sinus regions of patients. In each case, cardiac slowing and syncope produced by pressure applied to the carotid sinus area were exaggerated after the administration of digitalis drugs (227, 295, 422, 494, 507, 571, 572, 579, 813, 868).

3. Chemoreceptors. Schmitt et al. (703, 704) were the first to demonstrate a direct effect of digitalis on the peripheral chemoreceptors. They reported in one of their studies (703) that an intracarotid injection of digipurat (40 μg) increased the action potentials of chemoreceptor fibers originating within the carotid body regions of anesthetized cats. They indicated that the neural activation was due to an effect on chemoreceptors because of: 1) the "anatomic location of the leads" (i.e., electrodes with an 200-μ tip were placed in the region of the carotid body); 2) the observation that neural impulse volleys that

were synchronous with respiration were enhanced after digitalis administration; and 3) the observation that under control conditions, an increase in pCO_2 in the respired air evoked an increase in the recorded action potentials. It was also observed that intracarotid injections of K-strophanthin (30 to 125 μg) produced an increase in both the background activity and the respiratory synchronous volleys derived from the centrally bisected carotid sinus nerve. In their other study (704), similar results were obtained with lower doses (10 to 40 μg) of digipurat.

Other observations of a more indirect nature also indicate that digitalis materials can activate chemoreceptors. Quest and Gillis (634) noted that the marked increase in carotid sinus nerve electrical activity produced by intracarotid administration of 6.25 μg of acetylstrophanthidin in decerebrate cats after destruction of the chemoreceptors with acetic acid was less than that produced by the same dose of drug before chemoreceptor elimination. They suggested that an excitation of chemoreceptors may have contributed to the original response. Carpi et al. (126) reported that small doses of scillaren A (5 to 10 μg) injected directly into the carotid body regions of anesthetized dogs enhanced the respiratory stimulant actions of acetylcholine and nicotine.

Studies of systemic administration of digitalis to animals support the finding of an excitatory effect on baroreceptors. By using electrical recordings of action potentials from the centrally bisected carotid sinus nerve, Schmitt et al. (703) reported that an i.v. injection of 40 μg of digipurat increased chemoreceptor activity in anesthetized cats. In a study performed by Viana (844), the respiratory stimulant effects produced by intracarotid administration of sodium cyanide and acetylcholine in anesthetized dogs were potentiated during an i.v. infusion of 2 μg/kg/min of digoxin, whereas the respiratory stimulant effects produced by i.v. injections of the centrally acting agents bemegride and dimeflin were not altered by digoxin infusion. It was also observed that atropine blocked the respiratory stimulation produced by an intracarotid injection of acetylcholine, and that the acetylcholine response could be restored after a subsequent infusion of digoxin. Viana concluded that digoxin potentiated cholinergic mechanisms at the level of the carotid body chemoreceptors to produce an increase in respiration.

Additional indirect evidence to indicate that systemically administered digitalis can activate chemoreceptors has been obtained from denervation studies. Viana (845) observed that the respiratory stimulation (i.e., respiratory rate and minute volume) produced by intravenously infused digoxin (2 μg/kg/min) in anesthetized dogs was prevented after the carotid body chemoreceptors were eliminated by sectioning the carotid sinus nerves or surgically removing both carotid body regions. Pace and Gillis (596), and later Weaver et al. (861), also demonstrated that the increase in phrenic nerve activity produced by i.v. digoxin (20 μg/kg every 15 minutes) is attenuated by surgical denervation of peripheral chemoreceptor regions.

4. Nodose ganglion receptors. Chai et al. (134) postulated that the nodose ganglion receptors represent one of the principal afferent receptor sites that can be activated by digitalis. Their conclusion was based on a series of experiments in which they examined the effects of sectioning the carotid sinus nerves and interrupting the blood supply to the nodose ganglion on the cardiovascular responses produced by intracarotid and i.v. injections of acetylstrophanthidin. It was observed that the administration of acetylstrophanthidin (20 to 35 μg) directly into the carotid sinus regions of anesthetized cats produced, in some animals, a fall in heart rate and blood pressure in two phases. The first was immediate and brief and the later one was gradual and prolonged. The immediate responses were attributed to an activation of carotid sinus receptors, since they were prevented by sectioning the carotid sinus nerve, whereas the delayed responses were attributed to activation of nodose ganglion receptors, since they were abolished by interrupting blood flow to the nodose ganglion. They also observed similar responses after i.v. acetylstrophanthidin. These responses were decreased either by sectioning both carotid sinus nerves or by bilateral functional exclusion of the nodose ganglia from their blood supply. Based on the effectiveness of the exclusionary procedures, the authors concluded that nodose ganglion receptors were a predominant reflex site of action of digitalis.

To obtain additional evidence that digitalis stimulates the nodose ganglion receptors, Gillis et al. (290) examined the ability of i.v. acetylstrophanthidin to slow cardiac rate in anesthetized cats with all reflexogenic areas denervated except for the nodose ganglion receptors. Under those conditions, cardiac slowing in control preparations was due to sympathetic withdrawal. Gillis et al. found no slowing in heart rate when acetylstrophanthidin was administered to animals with only the nodose ganglion receptors intact. Possible reasons for the discrepancy between this study and that of Chai et al. (134) are as follows: First, Gillis et al. were examining the bradycardia produced by sympathetic withdrawal while Chai et al. were studying bradycardia produced by vagal activation. However, this does not seem to explain adequately the discrepancy because several investigators have reported that activation of nodose ganglion receptors leads to withdrawal of sympathetic tone (88, 797). Second, in most of the animals in the study by Chai et al., a much larger dose of acetylstrophanthidin was employed (56 μg/kg i.v.) than in the study by Gillis et al. (35.5 μg/kg i.v.).

5. Cardiac receptors. Sleight et al. (734) examined the effects of digitalis on cardiac receptors by administering acetylstrophanthidin (25 to 100 μg) directly onto the epicardium of the left ventricle of conscious and anesthetized dogs. This topical administration resulted in marked decreases in blood pressure and heart rate. Similar responses were noted when acetylstrophanthidin (20

and 40 μg) was injected into the anterior descending branch of the left coronary artery of anesthetized dogs. The hypotension and bradycardia were attributed to an action of digitalis on afferent receptors, since these responses were blocked by cooling the cervical vagus nerve or by applying procaine locally to the ventricular epicardium. Electrical stimulation of the vagus nerve above the site of cooling or after the administration of procaine still caused slowing, indicating that efferent fibers in the vagus nerve were still capable of conducting impulses. Furthermore, by recording electrical discharges from single or multifiber preparations of the right recurrent cardiac nerve emanating from several ventricular regions, it was found that the only afferent receptors that responded to epicardially administered acetylstrophanthidin were mechanoreceptors originating in the left ventricle. Sleight et al. indicated that the sinus bradycardia caused by digitalis drugs may result partly from excitation of these receptors. As alluded to by this group of investigators, their findings mimic those produced by the veratrum alkaloids (365, 733) and presumably represent an effect on Bezold-Jarisch receptors. Another interesting and more recent report by Thames (808), wherein renal sympathetic nerve activity of anesthetized dogs was measured after denervation of carotid sinus and aortic depressor nerves, indicated that acetylstrophanthidin administered either into the left circumflex coronary artery (mean dose of 81 μg) or on the epicardial surface of the left ventricle (mean dose of 108 μg) resulted in a decrease in nerve activity, as well as in heart rate and arterial pressure. These responses were abolished by bilateral vagotomy. In another study of this type, Hood et al. (378) reported that no reflex bradycardia or hypotension occurred when an infusion of acetylstrophanthidin (0.25 μg/kg to 1.7 μg/kg) was administered into the left coronary artery of anesthetized dogs. The reason for these negative findings might be related to the lower dose of acetylstrophanthidin employed by Hood et al. as compared to the dose used by Sleight et al. (734) and by Thames (808).

Activation of cardiac receptors has also been reported in studies in which digitalis was administered to animals systemically. Kido (410) examined the effect of i.v. digitamine (0.2 ml/kg, concentration not given) on the spontaneously occurring neural activity of what he believed to be afferent fibers of cardiac nerves. These were neural fibers obtained from the right vagosympathetic nerve trunk at a level just below the inferior cervical ganglion. After the administration of digitamine, an increase in impulse activity was observed that coincided with a "slight decrease" in heart rate. In a later study, Oberg and Thoren (585) recorded the spontaneously occurring electrical activity from afferent nerve filaments in the right vagus nerve of anesthetized cats, which emanated from mechanoreceptors located in the left ventricle. The origin of the neural activity was localized to the left ventricle, by observing augmented neural firing during periods of aortic occlusion and mechanical stimulation of the ventricle. Oberg and Thoren observed that single i.v. injections of 60 to 100 μg of strophanthin did not affect the spontaneous receptor discharge. However, these doses did appear to cause a sensitization of the receptors since aortic occlusion, performed after administration of strophanthin, was found to produce a more pronounced receptor discharge than before administration of the drug. The sensitization effect with aortic occlusion was also seen with low doses (2 to 3 μg) of protoveratrine, which in some experiments did not activate the receptors. It was also observed that if strophanthin was injected repeatedly, resulting in total doses of 100 μg or more, the spontaneous electrical activity of the filaments was increased. Oberg and Thoren concluded that the ventricular receptors sensitized or activated by strophanthin were probably important for the emergence of the Bezold-Jarisch reflex.

Additional support for the concept that systemically administered digitalis can activate cardiac receptors is provided from studies in which cardiovascular changes have been measured before and after denervation of the cardiac reflexes. Fukuda and Kurotsubo (250) administered digitamine (0.4 to 0.5 ml/kg i.v., concentration not given) to anesthetized dogs, presumably with carotid sinus and aortic depressor nerves sectioned, and observed bradycardia. This response was abolished after selective removal of afferent nerve fibers that innervate various portions of the cardiac musculature. The efferent vagal pathways remained intact in their study, since stimulation of the cervical vagosympathetic trunks still produced slowing. Tanakawa (798) obtained confirmatory results in anesthetized cats with i.v. strophanthin (0.02 to 0.03 mg/kg). He found that bradycardia was not entirely abolished by section of the carotid sinus and aortic depressor nerves, but he did not go further to determine the afferent neural pathways mediating the bradycardia. In another study, Melville (542) administered ouabain (0.05 to 2.0 mg/kg) and digitoxin (infusion of 0.00342 mg/kg/min for up to 20 minutes) to anesthetized dogs pretreated with either atropine or flaxedil to block the effects of the efferent vagus nerves. An initial transient rise in blood pressure accompanied by bradycardia was observed, which was followed by a striking and prolonged fall in blood pressure and tachycardia. When the influence of afferent fibers in the vagus nerves was eliminated by cutting the vagus nerves, the only response observed was a marked pressor effect. Melville concluded that the pressor response after vagotomy was "associated with the release of some reflex vasoinhibition originating in the heart under digitalis," and suggested that the "Bezold Reflex" was involved. It should be pointed out, however, that the response he observed with vagotomy could have been due to removal of aortic depressor nerves as well as nerves linked to cardiac receptors. Consistent with Melville's findings are those of Gillis et al. (290) who found that acetylstrophanthidin administered to anesthetized

cats with carotid sinus and aortic depressor nerves denervated, but with cardiac receptors intact, produced bradycardia. This response did not occur when the cardiac receptors were denervated.

Digitalis has also been shown recently to excite atrial receptors in the dog. Gilmore and Zucker (293) recorded vagal afferent activity that arises from activation of receptors that discharge during atrial filling. Administration of ouabain increased the discharge rate of these receptors. These receptors have been shown to decrease sympathetic outflow to the renal bed, thereby resulting in an increase in renal blood flow (480). It has also been suggested that activation of these receptors produces a decrease in the release of antidiuretic hormone as well as an increase in the release of an unknown diuretic substance (480).

In addition to the reports considered above, earlier studies on the effects of digitalis on cardiac reflexes were discussed in the review by Lendle and Mercker (457).

6. Conclusions. Present information permits the conclusion that digitalis drugs cause excitation of baroreceptors in the carotid sinus and aortic arch regions of experimental animals and the carotid sinus region of people. The same conclusion can be drawn from experimental animal studies for carotid body chemoreceptors and mechanoreceptors in the left ventricle and atria. Based on similarities between the reflex actions of digitalis and veratrum alkaloids, it has been suggested that digitalis administration can result in activation of the Bezold-Jarisch reflex (542, 585, 734, 808). Finally, there are some data that indicate that digitalis might be capable of exciting nodose ganglion receptors, but more studies would be needed to establish whether these sites are targets of digitalis action.

B. Effects on the Central Nervous System

1. Experimental approaches used to evaluate effects of digitalis. Several general techniques have been used to examine the effects of digitalis on neural activity of the central nervous system (CNS). These include: 1) monitoring of efferent neural activity during i.v. administration of digitalis; 2) monitoring of either efferent neural activity or end-organ effects during administration of digitalis into the CNS; and 3) monitoring efferent neural activity during electrical stimulation of CNS sites in conjunction with the i.v. administration of digitalis.

2. Sympathetic centers. With preganglionic sympathetic nerve recordings as an indicator of whether digitalis acts on central sympathetic centers, Gillis (279) found that high doses (40 to 100 μg/kg) of ouabain given i.v. produced an increase in cardiac sympathetic nerve activity. This was observed in decerebrate cats with cardiovascular reflexes intact and in animals in which the reflex areas were denervated. The increased nerve activity was observed with recordings from intact sympathetic nerves and from sectioned sympathetic nerves, with the recording electrode placed on the proximal end of the nerve. The sectioning procedure eliminated the problem of recordings being from afferent rather than from efferent nerves. A similar observation had been made a few years earlier by Abiko et al. (10), who found that a subarrhythmic dose of strospeside reduced preganglionic cardiac sympathetic nerve activity in cats whereas continued administration produced an increase in sympathetic nerve discharge that coincided with the appearance of arrhythmias. Pace and Gillis (596), with anesthetized cats and recording from cardiac preganglionic sympathetic nerves, obtained identical results with digoxin. They also observed an increase in activity in preganglionic splanchnic nerves and in preganglionic superior cervical nerves. With the latter two nerves, cardiovascular reflexes were not denervated but increases in activity occurred before arterial blood pressure dropped below the control level.

In another study, Gillis et al. (291) demonstrated that the increase in sympathetic nerve activity did not occur when ouabain was administered to cats with spinal cords transected. These results strongly support the idea that the enhancement of preganglionic sympathetic nerve discharge was due to a neuroexcitatory effect of ouabain on central sympathetic centers.

In contrast to the above findings, Weaver et al. (860) found that i.v. digoxin failed to augment neural activity of preganglionic splanchnic nerves of areflexic cats. Doses used were in the range to produce arrhythmias. Weaver et al. concluded that a primary site of action of digitalis was not in CNS sympathetic centers.

Data obtained from monitoring either efferent sympathetic neural activity or end-organ effects during administration of digitalis into the CNS are consistent with drug stimulation of sympathetic centers. Garvey (263), by recording efferent preganglionic sympathetic nerve activity as an indication of central sympathetic activation, found that administration of 2 to 20 μg of ouabain into the lateral hypothalamus of anesthetized dogs produced increases in sympathetic activity and multifocal ventricular arrhythmias; after doses of 30 to 40 μg, ventricular fibrillation ensued. These effects were not observed when ouabain at these doses was administered into the medulla. Weaver et al. (859), with smaller doses (1 to 1000 ng) of ouabain administered into the hypothalamus and medulla of anesthetized cats, observed no consistent effect on sympathetic activity (i.e., increases, decreases, or no change in activity were recorded from the postganglionic external carotid, preganglionic stellate, and postganglionic inferior cardiac nerves) and no cardiac arrhythmias. With a larger dose of ouabain (20 μg), only a depression of neural activity was observed and no arrhythmias were seen.

By using end-organ responses as an indication of central sympathetic activation, Korth et al. (429) reported that intracerebroventricular administration (the exact ventricle was not indicated) of strophanthidin (20 to 200

μg) to unanesthetized dogs produced sinus tachycardia, ventricular extrasystoles, and ventricular tachycardia. These electrocardiographic (ECG) changes could also be evoked in animals after bilateral vagotomy. Because systemic administration of strophanthidin in doses up to 1.5 mg produced no changes in cardiac rhythm, Korth et al. concluded that strophanthidin exerted an important effect on CNS structures that influence cardiac rhythm. Weinberg and Haley (866) confirmed these results in the same animal preparation. K-strophanthin (0.02 to 0.55 μg) was injected into the third cerebral ventricle and produced ventricular extrasystoles and paroxysmal ventricular tachycardia. Intravenous administration of equivalent and higher doses (up to 1.0 μg) produced no change in cardiac rhythm. In another study, Haley and Weinberg (326) injected lower doses of K-strophanthin and also tryptamine-strophanthidin into the third ventricle of conscious dogs. K-strophanthin produced bigeminy and bradycardia (at 6.4 μg/kg), trigeminy and tachycardia (at 9.4 μg/kg), bigeminal pauses, trigeminy, quadrigeminy, ventricular extrasystole (at 20.7 μg/kg), and paroxysmal ventricular tachycardia and ventricular fibrillation. Similar results were seen with tryptamine-strophanthidin, except that it was more potent than K-strophanthin. Tryptamine-strophanthidin at 3.2 to 4.4 μg/kg was equivalent to K-strophanthin, 6.4 μg/kg, and at 10.9 and 17.2 μg/kg the cardiovascular effects corresponded with those observed with K-strophanthin, 31.4 and 57.4 μg/kg. The same doses given i.v. did not produce ventricular arrhythmias. In a later study, Weinberg and Haley (867) obtained similar results.

Melville and Shister (544) studied the effects of centrally administered digitalis in unanesthetized cats. They administered either lanatoside C, digoxin, or digitoxin (5 to 200 μg) into the lateral ventricle and observed cardiac arrhythmias. When these digitalis preparations were administered along with K^+ into the brain, ventricular fibrillation was occasionally observed. Stickney and Lucchesi (780) administered acetylstrophanthidin (10 μg/kg) into the lateral ventricle of chloralose-anesthetized dogs. They observed a marked pressor response and ventricular tachyarrhythmias that were not prevented by bilateral vagotomy. Basu Ray et al. (50) reported that injections of 20 μg of ouabain every 20 minutes into the ventromedial hypothalamus of chloralose-anesthetized cats produced ventricular arrhythmias that were prevented by surgical sympathectomy. Administration of ouabain into the lateral ventricle produced the same effects but larger doses were required. The authors concluded that the effects seen with lateral ventricular injections were due to the action of ouabain in the ventromedial hypothalamus. Ammar and Afifi (19) studied the effects of ouabain (25 μg/kg) administered into the lateral ventricle of unanesthetized rabbits and observed ventricular fibrillation; this was not prevented by bilateral vagotomy. Saxena and Bhargava, in a series of papers (695–697), reported that ouabain administered into the lateral ventricle of the brain of anesthetized-vagotomized cats (50 to 90 μg) and dogs (75 to 300 μg) produced ventricular arrhythmias. These arrhythmias were abolished by spinal cord transection, bilateral sympathetic chain resection, and systemic administration of a ganglionic blocking agent. The doses employed for central injection did not produce arrhythmias when the ouabain was administered i.v. The effects of local injections of ouabain into different areas of the ventricular system of the brain of the cat indicated that the most sensitive site of action was the posterior hypothalamus (697). Finally, Holloway et al. (375) perfused ouabain (10^{-5} M) through the cerebroventricles of anesthetized dogs and observed bigeminal rhythms, extrasystoles, and ventricular tachycardia. These arrhythmias, since they were not affected by bilateral vagotomy, appeared to be due to activation of central sympathetic centers.

All of the above studies dealt generally with the administration of digitalis drugs into forebrain regions (hypothalamic areas). Investigators have also administered these agents into the fourth cerebroventricle and compared the responses to those obtained by administration into more rostral areas. For example, Bircher et al., in a series of investigations, studied the effect of deslanoside administered into four different regions of the CNS, including the fourth ventricle, of unanesthetized dogs. In the first study (70), these investigators examined the effects of 4 μg/kg administered by 1) direct application to the cortical surface, 2) injection into the lateral ventricle, 3) injection into the third ventricle, and 4) injection into the fourth ventricle. This dose produced arrhythmias only when administered into the third and fourth ventricles. The incidence of arrhythmias (bigeminal rhythm) was highest with injections into the fourth ventricle. In two studies published in 1963 (71, 72), Bircher et al. reported that 4 μg/kg of deslanoside injected into the fourth ventricle produced ventricular extrasystoles whereas injection of this dose into the lateral ventricle did not. Even though the authors reported data suggestive of digitalis-induced activation of sympathetic centers, the vagus nerves seemed to be more important; the investigators alluded to the fact that vagotomy prevented the arrhythmias in a high percentage of the animals studied. An additional important finding was that arrhythmias produced by i.v. deslanoside did not appear to be mediated by the autonomic nervous system (and CNS), since the arrhythmias were unaffected by surgical or pharmacological blockade of the autonomic nervous system.

In a second study of this type, Basu Ray et al. (52) administered microinjections of ouabain into the dorsal nucleus of the vagus and the nucleus tractus solitarius. They observed sinus bradycardia and a junctional rhythm, followed by ventricular tachyarrhythmias, with animals dying in cardiac arrest. This was in contrast to the ventricular fibrillation observed after i.v. administration. Vagotomy abolished the sinus bradycardia and

junctional rhythms but had no effect on the ventricular tachyarrhythmias. The latter were abolished by spinal cord transection. Comparing these results with the data from the study in which ouabain was administered into forebrain areas (50), it could be concluded that the most sensitive area for the production of arrhythmias was the ventromedial portion of the hypothalamus.

The CNS mechanisms whereby centrally administered digitalis drugs act by excitation of sympathetic centers has been evaluated in three of the above studies. Stickney and Lucchesi (780) observed that intracerebroventricular administration of *d,l*-propranolol prevented ventricular tachyarrhythmias elicited by acetylstrophanthidin in three of four dogs, whereas intracerebroventricular administration of *d,l*-propranolol had no effect. Pretreatment with systemic administration of reserpine also prevented the ventricular arrhythmias. On the basis of these results, Stickney and Lucchesi suggested that central administration of acetylstrophanthidin produced arrhythmias by causing the central release of an adrenergic mediator from CNS tissue. Additional evidence for a role of central catecholaminergic mechanisms was obtained by Saxena and Bhargava (696), who employed other drugs known to impair activity of central noradrenergic neurons. For example, when administered centrally, RO4-1284, 6-hydroxydopamine (agents known to reduce the brain norepinephrine concentrations), and bretylium were each found to prevent the cardiovascular responses induced by centrally administered ouabain in dogs and cats. In contrast to the findings of these two groups, Holloway et al. (375) reported that central administration of propranolol or phenoxybenzamine had no effect on centrally induced cardiovascular effects of ouabain in the dog. On the basis of the above information, it may be premature to conclude that a central noradrenergic mechanism is involved in the excitatory effects of digitalis drugs on sympathetic centers.

Two studies have been performed in which i.v. digitalis was tested for its effect on neural responses produced by electrical stimulation of the hypothalamus. Evans and Gillis (221) attempted to define the site of the arrhythmogenic action of digitalis in a cat preparation by using focal stimulation of the brainstem as a method for evoking arrhythmias. They used arrhythmias produced by electrical stimulation of the posterior portion of the hypothalamus and mediated by sympathetic nerves to study the arrhythmogenic action of digitalis. When large but subarrhythmogenic doses of ouabain were administered, electrical stimulation of the hypothalamus that produced no arrhythmias and little enhancement of sympathetic nerve firing during the predrug period was found to produce arrhythmias and intense sympathetic discharge. Evans and Gillis concluded that the arrhythmogenic action of digitalis was related to central sympathetic excitatory actions of the drug. Weaver et al. (860) performed a similar study in cats with monitoring of preganglionic splanchnic nerve activity. They observed no enhancement of evoked nerve activity after the i.v. administration of digoxin. The difference in results between the two studies might be related to the differing intensities of hypothalmic stimulation used. Evans and Gillis employed a stimulus strong enough to evoke significant changes in arterial blood pressure and sinus rate whereas Weaver et al. used a stimulus strength that was insufficient to elicit cardiovascular responses (858).

3. Spinal cord. Osterberg and Raines (593) investigated the influence of digitalis on spinal cord neuronal mechanisms. They recorded action potentials of the ventral spinal cord roots in response to electrical stimulation of spinal cord afferent fibers during the i.v. administration of three digitalis compounds. Polysynaptic responses were increased after digitoxigenin (300 μg/kg) and acetylstrophanthidin (75 μg/kg) in a manner similar to that reported for strychnine. Digitoxigenin (300 μg/kg), acetylstrophanthidin (75 μg/kg), and ouabain (50 to 75 μg/kg) depressed the amplitude of the monosynaptic spikes in a manner similar to that observed for pentylenetetrazol. Digitoxigenin (300 μg/kg), but not acetylstrophanthidin and ouabain, facilitated high frequency transmission through the monosynaptic reflex pathway. In addition, Osterberg and Raines observed enhanced recurrent inhibition with digitoxigenin (300 μg/kg), but not with acetylstrophanthidin or ouabain. Both of these effects have also been observed with pentylenetetrazol. Finally, digitoxigenin (300 μg/kg), but not acetylstrophanthidin or ouabain, reduced presynaptic inhibition in a manner similar to that seen with picrotoxin and pentylenetetrazol. From these results it appears that digitalis drugs exert effects on spinal synaptic processes that resemble those seen with convulsant agents. If these excitatory effects extend to synaptic processes involved in spinal cord sympathetic transmission, the increase in sympathetic outflow seen with digitalis drugs might involve an action on a spinal cord site.

4. Vagus center(s). Three studies employed recordings of neural activity from preganglionic vagal nerve fibers to assess the effects of digitalis on the vagal center(s). McLain (532) found that both ouabain and digitoxin produced an enhancement of vagal activity and that the effect occurred with subarrhythmic amounts of each agent. He reported that in most cases glycoside administration raised systemic arterial pressure, but the correlation between enhanced vagal activity and the change in blood pressure was not consistent enough to explain the augmentation of neural activity by an indirect pressor-induced activation of reflexes. The vagal activity was usually observed to decline once cardiotoxicity developed. Gillis et al. (291) reported an augmentation of preganglionic vagal nerve activity after the administration of both subarrhythmic and cardiotoxic doses of ouabain. These investigators were also unable to discern a correlation between the change in neural activity and changes in arterial pressure produced by the drug. The findings from both of these studies could reflect either a

central parasympathetic effect of digitalis or a reflex activating effect of the drug. This problem was addressed in the study of Pace and Gillis (596). By recording from efferent preganglionic vagal fibers, they observed that administration of subarrhythmic as well as arrhythmic doses of digitalis increased the rate of firing of these neurons. Interestingly, enhancement in activity of parasympathetic preganglionic fibers was prevented by prior sectioning of the afferent nerves linking baro- and chemoreceptors to the CNS. This suggests a lack of a direct action of digitalis on central parasympathetic areas. On the other hand, another interpretation can be given to these findings. That is, sectioning of afferent nerves abolished incoming neural activity, which may have been facilitated at the central level by digitalis, and which would have resulted in an increase in parasympathetic outflow. Thus, prevention of digitalis-induced enhancement of parasympathetic efferent activity by reflex denervation does not rule out a CNS site of action for this response.

A large body of data has been accumulated by injections of digitalis drugs into the CNS and simultaneous monitoring of end organ effects. Greene and Peeler (314) administered digitalis into the perfusate circulating through the isolated head of turtles. The only connections between the head and body were the vagus nerves. Digitalis administration resulted in cardiac slowing and heart block. Section of the right vagus resulted in restoration of normal cardiac rhythm. The doses of digitalis employed were extremely large (1.3×10^{-4} and 1.3×10^{-3} M, respectively). In contrast, Heymans et al. (361, 366, 367, 370) have reported that digalene (0.28 mg/kg), strophantin (0.27 mg/kg), or cymarine (0.10 mg/kg) administered into the isolated perfused dog head produced no bradycardia, indicating absence of central vagal activation. Krayer (432) administered digitoxigenin (0.05 to 0.2 mg) into the perfused head of a dog and observed bradycardia. It is unclear, since bilateral vagotomy was not performed, whether the bradycardia was due to vagal activation. This is an important consideration because of the data of Weaver et al. (859) demonstrating that central administration of digitalis can reduce sympathetic outflow (which in turn could produce the bradycardia). Solti et al. (747) administered strophanthin (0.5 mg) into the circulation of the isolated head of a dog and observed bradycardia and a variety of arrhythmias. These consisted of junctional rhythms and atrial and ventricular extrasystoles. Bilateral vagotomy or systemic administration of atropine prevented these responses.

Several investigators have introduced various digitalis preparations into the lateral or fourth ventricles and observed bradycardia (15, 134, 429, 544). They have concluded from their findings that digitalis was stimulating vagal center(s). However, they did not prove their point by testing the effect of vagal nerve section on the response. Wallace et al. (854) administered deslanoside, 50 μg/kg, into the fourth ventricle of conscious dogs. They observed a period of asystole followed by a slow escape rhythm. Vagotomy was not performed in these animals but the arrhythmia observed is consistent with activation of the parasympathetic nervous system by deslanoside. Bhargava and Gupta (67), Afifi and Ammar (15), Stickney and Lucchesi (780), and Holloway et al. (375) administered digoxin and ouabain into the cerebral ventricles and observed bradycardia. In all studies, bilateral vagotomy prevented the bradycardia. However, the bradycardia did not seem to be caused by a direct effect of digitalis on vagal centers. Instead, it appeared to be associated with a pressor response and, in one study, it was found to be prevented by carotid sinus denervation.

Basu Ray et al. (50, 52) observed bradycardia and bradyarrhythmia when they administered ouabain locally into specific brain nuclei (dorsal nucleus of the vagus, ventromedial hypothalamus). They found that sectioning the vagus nerves reversed these rate and rhythm changes and concluded that digitalis was activating central vagal neurons. A potential criticism of their study is that removal of normal vagal tone will result in an increase in heart rate per se.

5. Respiratory center(s). The effects of i.v. digitalis on phrenic nerve activity have been examined in four studies. In three of these, which were performed in anesthetized cats, only an increase in phrenic nerve activity was observed (291, 596, 861). The dose of digitalis that produced enhancement in nerve activity coincided with the dose that elicited cardiac arrhythmias (291, 596). In the other study, which was performed in anesthetized or decerebrate rats, Dal Ri and Schmidt (171) reported that K-strophantin and digitoxin induced a decrease in phrenic nerve activity and respiratory depression.

Other indices of respiratory activity (e.g., measurements of tidal volume, respiratory rate, and blood CO_2 levels) have also been monitored during the i.v. administration of digitalis. In these studies at least four different patterns of respiratory effects have been observed. These include stimulation followed by depression, depression followed by stimulation, stimulation alone, or depression alone.

Stimulation of respiration followed by depression was observed early by Traube (825), who found that small i.v. doses of digitalis produced an increase and larger doses a decrease in respiratory rate in the dog. Somewhat later, Nestor (577) observed this same pattern of response with digitalis leaf and digitaline nativelle in dogs. He further observed that respiratory arrest preceded the cardiac arrest produced by lethal doses of digitalis in rats, guinea pigs, rabbits, and pigeons. In 1914, Gross (318), using strophanthin and digitoxin, reported an early enhancement of respiration followed by depression in the rabbit. In the case of digitoxin, respiratory arrest was observed to precede cardiac arrest, whereas with strophanthin, respiratory arrest occurred simultaneously with cardiac

arrest. Popova (625) reported that administration of small doses of strophanthin to dogs increased the tidal volume (but decreased the respiratory rate), whereas larger doses reduced respiratory activity leading eventually to respiratory arrest. Sohn et al. (742) administered ouabain by infusion to both spontaneously breathing and mechanically ventilated cats. They observed an initial stimulation of respiration (characterized by an increase in the rate and depth of thoracic respiratory movements), which was followed by respiratory depression (characterized by an increased pCO_2 and a decreased pH) when larger doses of the drug were administered. Finally, Afifi and Ammar (15) reported that LD50 doses of ouabain, digoxin, and digitoxigenin caused hyperpnea, bradypnea, and respiratory arrest in conscious mice.

Depression of respiration followed by stimulation has been reported by a few investigators. Basu Ray et al. (50) noted that small doses of ouabain reduced the respiratory rate in anesthetized cats, while larger doses increased the rate and depth of respiration. Buterbaugh and Spratt (106) reported that digitoxigenin caused respiratory depression, which was followed by stimulation, in conscious rats. In their study, the respiratory stimulation coincided with the appearance of clonic convulsions and was followed by apnea and death. Popova (626) also reported that initial respiratory depression preceded the stimulation evoked by a small dose of strophanthin in dogs. As mentioned previously, they observed that respiratory arrest occurred when larger doses of the drug were employed.

Stimulation of respiration was the only response observed by several investigators. This was true in the study by Usubiaga et al. (836) in anesthetized dogs, in which cardiotoxic doses of ouabain were found to produce respiratory stimulation as reflected by a decrease in pCO_2 and an increased arterial pH. Similar responses were observed in anesthetized cats to deslanoside by Sohn et al. (743) and to ouabain by Levitt et al. (471). Basu Ray et al. (52), in contrast to their first report (50), found only respiratory stimulation (tachypnea) with ouabain in anesthetized cats. Viana (844, 845) reported that digoxin caused respiratory stimulation in anesthetized dogs, manifested as an increased expiratory volume. Yen and Chow (888) reported that ouabain increased respiratory activity (increased amplitude and rate of respiration, reduced pCO_2, and increased pH) in anesthetized dogs. Beller et al. (62, 63) found marked hyperventilation, increases in pH and pO_2, and a decrease in pCO_2 in conscious dogs after a cardiotoxic dose of ouabain.

Depression of respiratory activity, only, was reported in three studies, in which respiratory depression and arrest were observed in anesthetized rats, mice, and cats after i.v. digitoxigenin [Buterbaugh and Spratt (106, 107)] and after i.v. digitoxigenin and ouabain in rabbits [Okada et al. (588)].

In humans, nontoxic and toxic doses of digitalis have been described as having respiratory effects. Both stimulation (330, 891) and depression (228, 229, 240, 539, 725, 243, 546, 732) have been observed.

Studies in which digitalis was administered locally into the CNS indicate the same divergent results as seen after i.v. administration. The respiratory responses observed included stimulation followed by depression (15, 19, 326, 844), depression followed by stimulation (50), stimulation alone (20, 118, 165, 367, 544, 611, 867), or depression alone (52).

The disparity in the results described in the above animal studies may be explained to some extent by the data obtained with phrenic nerve recordings. In cats, only an enhancement in phrenic nerve activity has been shown in the presence of digitalis, which becomes increasingly intense as the lethal dose is approached (291, 596). This enhanced neural activity could result in increased respiratory muscle movement and functional changes such as decreased pCO_2 and increased pO_2 and pH, which are indicative of respiratory stimulation. During the time of continuous phrenic discharge, apparent respiratory depression could occur due to failure of the respiratory muscles to follow the intense phrenic nerve discharges. Such events could account for both the respiratory stimulant effects and the subsequent respiratory depressant effects noted in the above studies. Probably there are also species differences, since rats do not exhibit prominent respiratory stimulation (106, 107) and administration of digitalis to rats produces a decrease in phrenic nerve discharge (171, 281).

There is evidence that part of the stimulating effect on respiration is elicited on a site in the CNS. For example, digitalis produces some enhancement in phrenic nerve discharge in animals with denervated chemoreceptors (596). In addition, Okada and Suga (587) reported that bufogenins (e.g., resibufogenin) isolated from dried toad venom were capable of evoking respiratory stimulation after section of the carotid sinus and vagus nerves in rabbits. It has been suggested that the mechanism of this centrally induced activation of respiration involves potassium. Cameron (118) found that ouabain produced respiratory stimulation and that this was associated with an elevation in the potassium concentration in the CNS. This idea has been countered by the data of Yen and Chow (888), who reported increased respiration but no change in cerebrospinal fluid potassium concentration. There are drugs that have been reported to block the respiratory stimulant effects of digitalis. These include lidocaine (836), diphenylthiohydantoin (743), diphenylhydantoin (471, 844), N-isopropyl-*p*-nitro-phenylethanolamine (INPEA) (471), propranolol (291), hexamethonium (846), and reserpine (846). Findings with these drugs, however, do not provide information as to the mechanism for the respiratory stimulant effect of digitalis drugs.

Digitalis drugs also act on peripheral chemoreceptors

to stimulate respiration. Indeed, this might be the primary site of action (596, 844, 861). Evidence is based on studies in which effects on respiration and phrenic nerve activity of digitalis were compared in animals with and without functioning carotid and aortic bodies. The greatest proportion of the responses was found still present in animals without functional peripheral chemoreceptors. Also, there is evidence that after digitalis administration, there was a reduction in the doses of acetylcholine and cyanide required to excite carotid body chemoreceptors.

In the few studies where digitalis was found to produce an initial decrease in respiratory activity in nontoxic doses (50, 626), the mechanism for the depression may have involved activation of baroreceptors. Stimulation of the baroreceptors has long been implicated in the inhibitory effect on respiration (365). The site of the respiratory depression observed in rats appears to be in part in the CNS (106, 107, 171). The mechanism for the depressant effect appears to involve brain serotonin, since depletion of central serotonin antagonizes the respiratory depressant effect of digitalis (107). There is also some evidence that respiratory depression occurs peripherally as well, since some investigators (171, 312) have observed depressed respiratory muscle activity independent of any changes in phrenic nerve activity.

6. Conclusions. Studies dealing with the effect of digitalis drugs on various CNS "centers" and the spinal cord support the notion that these agents in arrhythmogenic doses can evoke excitatory responses from these sites. Data obtained from studies of CNS centers are most convincing for the sympathetic and respiratory centers of cats, where i.v. digitalis has been shown to increase spontaneously occurring sympathetic and phrenic nerve discharge. Increases occur in animals with baroreceptors and chemoreceptors denervated. In the case of the vagus center(s), animals with reflexogenic areas denervated do not exhibit an increase in parasympathetic nerve discharge but, as indicated above, this finding does not rule out a CNS site of action of these drugs. Finally, in the case of the spinal cord, data from one study indicate an excitatory effect of digitalis but the neural responses examined were designed to elucidate the convulsant effects of digitalis rather than the sympathetically mediated cardiovascular responses. Sympathetic outflow was monitored in one study where the spinal cord was sectioned at the C-1 level. In that study, no enhancement in sympathetic outflow was observed (291). However, as in the case of parasympathetic outflow responses, spinal cord activity may have to be present in order to observe an effect of digitalis.

Data obtained from studies in which digitalis was administered locally into the CNS also strongly indicate that such agents excite each of the "centers." The problem with this type of study is that the extremely high local concentrations of the drug provided may never be attained when the drug is given by systemic administration, and cardiovascular effects of centrally administered digitalis may not be mediated by the same mechanisms as the effects of i.v. digitalis. The latter was shown to be true by Bircher et al. (69), who compared the effects of hexamethonium on centrally induced and intravenously induced responses elicited by deslanoside in dogs.

C. Effects on Efferent Systems

1. Experimental approaches used to evaluate effects of digitalis. Digitalis drugs have produced excitatory effects at several efferent sites, including autonomic ganglia, autonomic nerve fibers and somatic nerves, postganglionic neuroeffector junctions, and the adrenal medulla. The techniques employed for studying these agents varied according to the efferent site examined. For evaluating the effects of digitalis at the ganglia, the following techniques have been employed: 1) recording the contractile response of the nictitating membrane during either electrical stimulation of the preganglionic superior cervical nerve fibers or local administration of acetylcholine; 2) recording electrical potentials from sympathetic ganglia; and 3) recording spontaneous activity from both pre- and postganglionic nerves. For evaluating the effects of digitalis on autonomic nerve fibers and somatic nerves, the following techniques have been employed: 1) recording spontaneous electrical potentials from postganglionic sympathetic neurons; 2) recording evoked electrical potentials from either pre- or postganglionic neurons; 3) measuring release of acetylcholine from preganglionic nerve fibers; 4) recording either contractile activity of skeletal muscle or antidromic nerve firing from single axons in response to electrical stimulation of motor nerves; 5) recording frequency of miniature end plate potentials; and 6) measuring the binding of digitalis to neural tissue.

The largest number of studies carried out with digitalis have been at the autonomic neuroeffector junction. The techniques used consisted of monitoring the activity of either cardiac tissue (e.g., chronotropic and dromotropic function of sinoatrial (S-A) node, atrioventricular (A-V) node, and Purkinje cells) or vascular smooth muscle (e.g., arteries and veins) in response to neural stimulation or to administration of acetylcholine and norepinephrine.

The influence of digitalis drugs on adrenal gland function has been studied with the following techniques: 1) collecting venous blood from adrenal glands of preparations in vivo; 2) collecting the perfusate from isolated adrenal gland preparations; 3) measuring the release and uptake of catecholamines by isolated chromaffin granules; and 4) measuring epinephrine and norepinephrine content of adrenal glands in preparations both in vivo and in vitro.

2. Autonomic ganglia. The first full report of an action of digitalis to excite autonomic ganglia was that of Konzett and Rothlin (428). They perfused the superior cervical ganglia of anesthetized cats with Locke's solution

and tested the ability of acetylcholine to produce contraction of the nictitating membrane in the presence of various cardioactive glycosides. Acetylcholine, when administered alone into the perfusion circuit in doses of 0.05 and 0.1 μg, produced no contraction of the nictitating membrane. However, when these subthreshold doses of acetylcholine were administered after the introduction of digitoxin, lanatoside C, K-strophanthoside, and scillaren A into the perfusion fluid in concentrations approximating 1.2×10^{-6} to 6.0×10^{-6} M or after single injections of these substances (1 to 30 μg) close to the ganglion the contractile responses of the nictitating membrane were enhanced. Pretreatment with these digitalis materials also augmented the contractile responses of the nictitating membrane produced by electrical stimulation of the preganglionic sympathetic nerve fibers. These potentiating effects were often followed by an inhibitory effect, especially when the larger doses of the glycosides were administered. Konzett and Rothlin expressed the opinion that digitalis could both enhance and depress ganglionic transmission.

These findings were confirmed a few years later by Perry and Reinert (613) in anesthetized cats. They observed that perfusion of the superior cervical ganglion with Locke's solution containing 2 μg of acetylcholine produced small contractions of the nictitating membrane. After ouabain (30 μg) was added to the perfusion circuit, the responses to the same dose of acetylcholine were potentiated. In the presence of ouabain, 2 μg of acetylcholine produced a response equivalent to the normal response observed with 5 μg of acetylcholine. Pretreatment with similar doses of ouabain (20 to 30 μg) also potentiated the contractile responses of the nictitating membrane produced by electrical stimulation of the preganglionic sympathetic nerve fibers. When higher doses of ouabain were used, an inhibitory phase often followed these potentiations. The ability of ouabain to enhance ganglionic transmission was abolished if the ganglia was pretreated with hexamethonium or nicotine.

Konzett and Carpi (427) infused the cardiac glycosides scillaren A, K-strophanthoside, and digitoxin, in a concentration of approximately 1.2×10^{-5} M into the normal blood supply of the superior cervical ganglia of anesthetized cats. These drugs were found generally to exert a ganglionic stimulating effect as manifested by a contraction of the nictitating membrane. It was also observed that in those cases in which scillaren A did not produce a contraction of the nictitating membrane, it did enhance the effects produced by submaximal stimulation of the preganglionic cervical sympathetic nerve.

After these studies were performed, additional studies on the physiology of ganglionic transmission revealed the various potentials that occur during electrical stimulation of preganglionic neurons (202). The initial electrophysiological response to acetycholine released from the presynaptic nerve terminals is a fast excitatory postsynaptic potential (fast EPSP), which is responsible for the generation of the action potential at the ganglion. This initial depolarization is accompanied by a subsequent and more prolonged phase of "after-hyperpolarization" (after-HP). If the fast EPSP and after-HP are attenuated or blocked, an inhibitory postsynaptic potential (IPSP) is observed. Finally, a slow excitatory postsynaptic potential (slow EPSP) is observed after the above electrical events have occurred. The slow EPSP is thought to facilitate impulse transmission or trigger after-discharges of ganglion cells and is subject to a modulating influence by the previously occurring IPSP.

The fast EPSP, whose extracellularly recorded counterpart has been termed the N wave, is caused by an action of acetylcholine on nicotinic ganglionic receptors and involves an increased Na^+ and K^+ conductance of the subsynaptic membrane. The after-HP that follows is due to the activity of an electrogenic Na^+-K^+ pump. The IPSP, referred to as the positive or P wave, is due to an action of dopamine on alpha-adrenergic receptors on ganglion cells. It is thought that a dopamine interneuron is activated muscarinically by preganglionic nerve impulses to mediate the production of the IPSP in sympathetic ganglion cells. The ionic mechanism underlying this potential appears to be inactivation of sodium conductance (73, 864). The slow EPSP, referred to as the late negative or LN wave, is caused by an action of acetylcholine on muscarinic receptors on ganglion cells. The ionic basis for the slow EPSP is not clear but it is probably generated by a metabolically based electrogenic mechanism that is different from the electrogenic sodium or chloride pumps (582).

The fast EPSP (N wave), the IPSP (P wave), and the slow EPSP (LN wave) do not appear to be mediated by the activity of an electrogenic sodium pump mechanism. Since processes affected by digitalis appear to be those involving an electrogenic sodium pump (810), it would not be anticipated that digitalis would have a selective effect on any of these events. Indeed, it has been noted in several reports (420, 478, 582) that ouabain has no specific effect on any of these potentials. Large amounts of ouabain (concentrations greater than 10^{-5} M) depress all of these potentials, and this depression is presumably due to the gradual accumulation of Na^+ and loss of K^+ in the presynaptic terminals and the ganglion cells after blockade of the electrogenic pump has already occurred (420, 478). However, the after-HP that follows the initial fast EPSP evoked by nicotinic receptor agonists should be selectively affected by digitalis since the electrical event is mediated by an electrogenic sodium pump. This selective effect of ouabain has been demonstrated in the recent studies of Libet et al. (479) and Smith and Weight (737). In the former study, the after-HP occurring in rabbit sympathetic ganglia was selectively abolished by 10^{-5} M ouabain, whereas the drug was without effect on the dopamine-induced IPSP. In the latter study, the same observation was made using the 9th or 10th paravertebral sympathetic ganglia of the bullfrog and 10^{-6} M

ouabain. Several other studies previously had demonstrated that ouabain could evoke a depression of the after-HP associated with nicotinic activation of the ganglia (103, 269, 454, 657, 851).

Gebber (268), with the superior cervical ganglion of cats and recording surface potentials from the ganglion, also observed a depressant effect of ouabain on the positive afterpotential (i.e., on after-HP). However, this effect required doses of ouabain ranging between 20 and 50 μg administered directly into the blood supply of the ganglion. Most importantly, with lower doses of ouabain (10 to 15 μg), Gebber observed an unmasking of the slow EPSP (LN wave). Gebber referred to this response as the "late response" and pointed out how it is sensitive to blockade by muscarinic receptor antagonists. Unmasking of the atropine-sensitive late discharge was the only ganglionic effect produced by ouabain in the dose range of 10 to 15 μg. When 20 to 50 μg doses of ouabain were used, the late response was further enhanced. Doses of ouabain in this range also had other effects; in addition to the reduction in the positive afterpotential as noted above, a low amplitude early depolarization was observed in association with an increase in the amplitude of the ganglionic spike (corresponding to the N wave). When 100 to 300 μg doses of ouabain were employed, depolarization of the ganglion occurred, leading to an irreversible blockade of transmission. This obliterated both the early (N wave) and the late (LN wave) responses.

The finding that digitalis enhances muscarinic ganglionic transmission (i.e., unmasks the "late response" or slow EPSP) was confirmed by use of i.v. deslanoside in cats and recording from postganglionic cardiac sympathetic nerves. After blockade of nicotinic ganglionic receptors with hexamethonium, large toxic doses of deslanoside evoked firing in postganglionic fibers that was sensitive to blockade by atropine (284).

In summary, it appears that digitalis drugs (as represented by ouabain and deslanoside) do affect potentials recorded from the surface of the ganglion. The most frequently documented effect is a reduction in the positive after-HP following depolarization of nicotinic ganglionic receptors. Abolition of positive after-HP should increase nicotinic ganglionic transmission, since a reduction of this potential has been shown to increase the amplitude of the spike that occurs with early depolarization (268). The significance of this effect for preparations in vivo, however, is open to question because large intraarterial injections to the ganglion are required to produce this response. The important effect of digitalis on ganglionic transmission appears to be the unmasking of muscarinic responses. This effect is important, since it is the primary response evoked by lower doses of ouabain administered intraarterially to the ganglion in vivo (268) and it can be observed upon systemic administration of deslanoside as well (284). Furthermore, the effect appears to be important despite the fact that it may not be mediated by an electrogenic sodium pump and is not selectively influenced by digitalis in studies in vitro (420, 478). The manner by which digitalis produces the enhancement of muscarinic ganglionic transmission and the underlying ionic mechanisms involved in the effect remain to be elucidated.

There are two studies that, in addition to evaluating the effects of digitalis on ganglionic action potentials, describe effects based on recording of spontaneous discharges from pre- and postganglionic sympathetic nerves. Pace and Gillis (596) obtained simultaneous recordings of pre- and postganglionic nerve activity during digoxin intoxication in anesthetized cats. With an arrhythmogenic dose of digoxin, there was a significant increase in sympathetic discharge of the postganglionic nerves. This was also the case for the activity in the preganglionic nerves. This relationship between pre- and postganglionic nerve activity as affected by digoxin was examined more closely by Pace (595). In three experiments performed, the neural discharge pattern observed in the preganglionic nerves during the control period was also present in the postganglionic nerves. Administration of digoxin produced uniform effects in only one experiment. Activity increased simultaneously in both nerves. Nonuniform effects were observed in the other two experiments during the administration of subarrhythmogenic doses of digoxin. In one experiment, there was an initial decrease in activity of the preganglionic discharge with no concomitant decrease in activity of the postganglionic discharge. In the other experiment, the converse was seen, i.e., an initial decrease in activity was seen in the postganglionic discharge with no concomitant decrease in activity in the preganglionic discharge. In arrhythmogenic doses, however, simultaneous increases in both pre- and postganglionic activity were observed. Although only a few experiments were performed, the data obtained suggest that digoxin caused an alteration in ganglionic transmission. Weaver et al. (860) also recorded spontaneous activity from pre- and postganglionic sympathetic nerves of anesthetized cats. Preganglionic activity was recorded from the splanchnic nerve while postganglionic activity was recorded from the inferior cardiac nerve. Digoxin administration resulted in an increase in postganglionic activity of animals with cardiovascular reflexes denervated but did not increase activity in preganglionic nerves. These investigators concluded that the increases in postganglionic sympathetic activity resulted from a stimulant effect of digoxin on the ganglia.

3. Autonomic nerve fibers and somatic nerves. Evidence consistent with an effect of digitalis on efferent autonomic fibers has been obtained in studies in which spontaneous discharges of sympathetic postganglionic neurons were measured. Abiko (7) and Abiko et al. (10) measured nerve activity in anesthetized cats intoxicated with strospeside. They observed an initial decrease in sympathetic activity with subarrhythmic doses and a subsequent increase during the time that ventricular arrhythmias developed. McLain (532) observed that ouabain administered to anesthetized cats enhanced postganglionic sympathetic nerve activity. This enhancement

was seen with cardiotoxic doses of ouabain, and was not preceded by a decrease in activity with lower, nontoxic doses. Weaver et al. (860) also examined the effect of digoxin on postganglionic sympathetic nerve discharge in six anesthetized cats. They observed inconsistent responses; nerve activity increased in two cats, decreased in two cats, was biphasic in one cat, and did not change in one cat. In cats with reflexogenic areas denervated, digoxin administration resulted in only an increase in nerve activity. Weaver et al. described the responses in their study as "similar to those reported by McLain (532)." The conclusion of a similarity in effect between the two studies seems unwarranted because McLain (532) reported an increase in postganglionic sympathetic activity in about 90% of the experiments performed (i.e., 23 of 26 cats exhibited an increase in nerve activity with an arrhythmogenic dose of digitalis).

Roberts and colleagues (446, 447, 664) have obtained evidence indicating that digitalis drugs produce a nonuniform effect on cardiac sympathetic nerve discharge by producing different effects in individual nerve filaments within the same nerve fiber. Neurograms obtained from postganglionic sympathetic nerve fibers (e.g., simultaneous recordings of one to three nerve bundles of the postganglionic trunk) during ouabain intoxication revealed a nonuniform effect; some recordings indicated enhancement, some a decrease, and some no change (446).

The sites of action of digitalis to produce changes in spontaneous nerve activity described above are unclear but it has been speculated that they include pre- and postganglionic nerve fibers as well as baroreceptors, the CNS, and ganglia (251).

Better evidence for a direct action of digitalis drugs on autonomic nerves has been obtained by recording evoked action potentials of autonomic neurons. Studies examining the effect of ouabain on posttetanic hyperpolarization of unmyelinated fibers in the cervical sympathetic trunk of rabbits indicate that the drug abolishes this event (86, 658). Studies examining the effect of ouabain on evoked action potentials of either the cervical sympathetic or vagus nerves indicate enhancement as well as a reduction in the evoked response elicited by submaximal electrical stimulation (806).

Evidence of direct neural effects of digitalis in autonomic neurons has also been obtained from studies in which the release of acetylcholine from cat superior cervical ganglia (74) and the plexus of the guinea-pig ileum longitudinal strip (608, 849) was measured. Data from all three studies indicate that digoxin and ouabain can increase the output of acetylcholine.

An excitatory action of digitalis drugs on somatic nerves has also been indicated. Although the responses would not be manifested by alterations in cardiovascular function, the findings confirm and parallel studies with autonomic nerves.

Evidence of a neuroexcitatory effect in somatic nerves was obtained in studies with neuromuscular junction preparations from frogs, rats, and cats. Fraser (245) demonstrated that strophanthin produced twitching and subsequent blockade of transmission at the neuromuscular junction of the frog. He found that once the nerve stimulation-induced effects were abolished, the muscle still responded to direct stimulation. From these results he proposed that both twitching and paralysis were due to an effect of strophanthin on motor nerve terminals. Katagi (398) reported that contracture produced by strophanthin in the sciatic-gastrocnemius preparation of the frog did not occur in denervated muscle, which indicated that the effect of the drug had been neuroexcitatory. Shigei et al. (726, 727) confirmed these results with digitoxigenin in the frog rectus abdominus and semitendinosis muscles. In addition, these investigators found that tetrodotoxin, the selective inhibitor of sodium movement during rapid depolarization of neural tissue, prevented the digitoxigenin-induced contracture.

Other evidence of neural effects of digitalis on neuromuscular preparations has been obtained from studies in which miniature end-plate potentials (MEPP) were measured. In four studies of this type (39, 74–76), administration of digitalis drugs (ouabain, digoxin, and strophanthidin) increased the MEPP frequency. Since MEPP frequency reflects a change in presynaptic neuronal activity (384), the results indicated a neuroexcitatory effect of digitalis on somatic nerve terminals. In contrast, Gage (253) found that neither ouabain nor digoxin had a significant effect on MEPP frequency recorded from the rat diaphragm preparation. However, Birks and Cohen (75) have pointed out that the failure of Gage to demonstrate an effect of the drugs on MEPP frequency was due to his use of doses of ouabain and digoxin that are too low to produce effects in the rat, a digitalis-resistant species. Indeed, Birks and Cohen cited unpublished data indicating that ouabain in doses 10 to 20 times larger than those employed by Gage did produce neural effects.

Birks and Cohen (75) demonstrated that either large doses of digitalis or long exposure to moderate doses of digitalis produce transmission blockade. The block appears to occur in terminal axons, since bathing the nerve trunk with digoxin did not produce block and the usual size of MEPP continued to occur even after transmission failure, indicating no reduction in sensitivity of the endplate to acetylcholine.

Additional evidence that digitalis drugs can influence activity in somatic nerves is given in the review by Levitt et al. (471). They reported that ouabain administered intraarterially into the soleus neuromuscular junction of the cat enhanced antidromic nerve firing from single axons in the soleus nerve trunk. Results of another study (254) indicated that digoxin and ouabain depressed posttetanic potentiation in the rat phrenic nerve-hemidiaphragm preparation. However, Birks and Cohen (76) indicated that measurements of posttetanic potentiation appeared to have been made in that study only a short time before the onset of digitalis-induced conduction blockade; at such time the sodium gradient across the

membrane would be substantially reduced, the amount of sodium entering the axon per nerve impulse would be diminished, and posttetanic potentiation would not be expected to increase.

Consistent with these effects of digitalis on autonomic and somatic fibers are the observations that ouabain binds to both of these types of nerves (40, 41, 443). In unmyelinated fibers (vagus), ouabain was shown to bind to two sites. One was associated with sodium-potassium pumping, and the ability of this site to bind was dependent upon external potassium concentration. The other site was not associated with pumping and was unaffected by external potassium. An additional finding is that of Birks (73), who reported that ouabain induced structural changes in cat cervical ganglia and frog sartorius myoneural junctions. The changes were observed in cells of the ganglia and in the motor nerve terminals. In the ganglia, the changes consisted of a reduction in the size and an increase in the density of the mitochondria, swelling of the Nissl substance, disorganization and fragmentation of neurofilaments, and an absence of synaptic vesicles. In the motor nerve terminals, the changes consisted of swollen nerve endings, increased density and disorganization of the mitochondria, a reduction in the number and in the swelling of synaptic vesicles, and the appearance of double-walled vacuoles in the nerve terminal cytoplasm. No changes were observed in the nonneural elements of the ganglia and myoneural junctions. The changes seen were thought to be due to intracellular accumulation of sodium secondary to blockade of the sodium pump by ouabain in neural cells. There is also evidence that an important binding site for digitalis is the (Na^+-K^+)-adenosine triphosphatase (ATPase) contained within postganglionic sympathetic nerve endings (722). These endings contain a major fraction of (Na^+-K^+)-ATPase found in the heart, and binding of digitalis appears to be primarily to this enzyme (722).

4. Postganglionic neuroeffector junctions and end-organ responses. A. PARASYMPATHETIC NEUROEFFECTOR JUNCTION AND END-ORGAN RESPONSES. During the late 19th and early 20th centuries, several investigators observed that electrical stimulation of the vagus nerves of frogs and various mammals was more effective in slowing the heart rate when performed in the presence of digitalis (168). One of the earliest studies of this type was performed in 1872 by Boehm (82), who demonstrated in the frog that "the cardiac standstill from vagal stimulation, which in the control period did not last more than five seconds, was increased after digitalization to 30 to 120 seconds." Since the time of these early studies, numerous workers have confirmed the observation that the effects of both vagus nerve stimulation and acetylcholine administration on the heart are enhanced in the presence of digitalis. These effects have been shown to involve both the S-A and A-V nodal regions of the myocardium.

The effect of i.v. digitalis on vagal bradycardia produced by mechanically elevating pressure in the carotid sinus regions was studied by Heymans et al. (362). They isolated and perfused the left carotid sinus region of the dog with Ringer's solution, and observed that when the intrasinus pressure was raised from 75 to 125 mm Hg, the heart rate decreased by 14%. After the i.v. administration of ouabain, 30 μg/kg, the same increase in intrasinus pressure decreased the heart rate by 25%. A few years later, Abdon and Nielsen (3) performed similar experiments in which they isolated and perfused both carotid sinus regions of cats and rabbits with Ringer's solution. They reported that an increase in intrasinus pressure from 0 to 210 mm Hg resulted in a decrease in cardiac rate of 7% to 9%. When the procedure was repeated after ouabain, 30 to 90 μg/kg i.v., the heart rate decreased by 26% to 30%. More recently, Baethke and Schmidt (35) observed that mechanically increasing the pressure in the isolated carotid sinus regions of cats by 200 mm Hg caused a reduction in heart rate of approximately 30%. Subsequent to K-strophanthin, 10 μg/kg, or digitoxin, 20 μg/kg i.v., the same increase in carotid sinus pressure reduced the heart rate by approximately 60%. These investigators further observed that the bradycardia produced by electrical stimulation of the right carotid sinus nerve or the afferent vagus nerve was enhanced following the administration of digitoxin. The potentiation of the reflex bradycardia by digitalis was not observed in preparations in which the vagus nerves were sectioned or in which atropine was administered. The conclusion of all three studies was that digitalis produced bradycardia by potentiating the response to efferent vagal stimulation. Their results, however, do not rule out the possibility that digitalis could have enhanced reflex-induced bradycardia by acting on either central vagal centers, preganglionic vagal fibers, or parasympathetic ganglia.

Studies concerned with electrical stimulation of the peripheral ends of the cut vagus nerves (preganglionic fibers) also indicate that digitalis administration enhances cardiac slowing. Since Boehm made his initial finding in the frog, other investigators have made similar observations in cats and dogs. Gremels (316) reported that the bradycardia produced in the cat by stimulation of the right vagus nerve at a rate of 25 to 40 Hz was markedly enhanced after strophanthin, 10 to 20 μg/kg i.v. Perry and Reinert (613) demonstrated that cardiac slowing produced by peripheral vagal stimulation in the cat was potentiated by 5 μg of ouabain i.v. Gaffney et al. (252) further observed that in open-chest dogs in which the stellate ganglia and the sympathetic chains had been extirpated, the cardiac slowing produced by electrical stimulation of the right vagus nerve at rates of 15 to 40 Hz was increased after ouabain, 20 to 40 μg/kg i.v. When lower frequencies of vagal stimulation were employed, i.e., 10 Hz or less, ouabain failed to exert an effect. In a study performed by Chai et al. (134), low and high intensity stimulation of the peripheral vagus nerves of cats reduced the heart rate by 9% to 13%, and 35% to

46%, respectively. After small doses of acetylstrophanthidin (30 μg/kg) and digoxigenin (15 μg/kg) i.v., which were subthreshold for influencing cardiac rate, the bradycardia produced by vagal stimulation was intensified. The low intensity stimulation reduced heart rate by 46% to 67%, while after high intensity stimulation, the cardiac rate was decreased by 55% to 82%. In addition, the threshold intensity of vagal stimulation required to evoke bradycardia was considerably reduced after digitalis administration.

The potentiating effect of digitalis on peripheral vagal stimulation at the level of the S-A node has also been demonstrated in isolated cardiac preparations. McEwen (530) observed in the isolated heart of the rabbit that bradycardia produced by submaximal stimulation of the vagus nerves at a rate of 23 Hz was increased from 34% to 68% after the addition of ouabain, 0.25 μg/ml, to the preparation. Similar observations were made by Toda and West (821, 822) with rabbit right atrial preparations, in which the negative chronotropic response evoked by stimulation of the preganglionic right vagus nerve at a rate of 20 Hz was enhanced by approximately 11% to 35% in the presence of 3.4×10^{-7} M ouabain. Working with isolated guinea-pig hearts, Seifen and Seifen (715) found that stimulation of the vagus nerve at a frequency of 8 Hz reduced the heart rate by 50%. After the administration of ouabain in a concentration of 2×10^{-7} M, the frequency of vagal stimulation required to produce the same degree of slowing was reduced to 4.5 to 5 Hz.

There are two studies, both performed in vivo, in which administration of digitalis had either a variable or no significant effect on bradycardia induced by preganglionic vagal nerve stimulation. In the study where variable responses were obtained, Franke (243) stimulated the vagus nerves of the turtle and found that the stimulation threshold required to produce bradycardia was reduced in 41% of the experiments, remained unchanged in 49% of the experiments, and increased in the remaining 10%. The reason for the divergent results obtained in this study appears to be that the doses of the digitalis preparations employed, i.e., digitoxin and tincture of digitalis, were in the toxic range and caused arrhythmias and prolongation of conduction in the S-A and A-V nodes. In the other study where no significant effect was observed, Lendle et al. (458) observed no potentiating effect of K-strophanthin (20 to 30 μg/kg, i.v.) and digitoxin (60 μg/kg, i.v.) on the bradycardia induced by peripheral vagal stimulation. The reason for the failure to observe an effect in this study, as noted by Gaffney et al. (252), was that the stimulation frequency employed by Lendle et al. (0.5 to 2 Hz) was too low. Toda and West (821) supported the explanation given by Gaffney et al. for the inability of digitalis to enhance vagal-mediated bradycardia. In the study by Toda and West, ouabain failed to exert an effect on cardiac slowing produced by vagal stimulation when a low frequency (i.e., 5 Hz) was used.

Postganglionic vagal nerve fibers were directly stimulated in one study. Toda and West (821) applied transmural stimulation to the S-A node region of the isolated rabbit right atrium and found the bradycardia evoked by this procedure to be enhanced by ouabain.

In studies where the effects of digitalis on responses to exogenously administered acetylcholine were examined, three types of preparations were employed. These included intact animals, canine heart-lung preparations, and isolated heart preparations of various species.

Danielopolu et al. (174) and Danielopolu (172) reported that bradycardia produced by i.v. acetylcholine in the dog was converted to cardiac arrest after 1 mg of strophanthin i.v.

With the canine heart-lung preparation, Gremels (317) demonstrated that a dose of acetylcholine that was too low to evoke bradycardia when given alone produced marked cardiac slowing when tested in the presence of 0.025 to 0.1 μg doses of strophanthin. A similar result with a subthreshold dose of acetylcholine in the dog heart-lung preparation, after the administration of 30 to 75 μg of ouabain, was reported by Gaffney et al. (252).

In one of the studies with isolated heart preparations, Abdon et al. (2) evaluated the effect of digitalis on the heart of frogs, rabbits, and a stillborn child. In the hearts of the frogs and the child, the bradycardia evoked by acetylcholine was enhanced by the administration of strophanthidin (4.9×10^{-5} M) and K-strophanthin (30 ml of approximately 2.4×10^{-6} M solution), respectively. In the rabbit hearts, concentrations of acetylcholine that were subthreshold for inducing bradycardia were found to produce slowing in the presence of strophanthin (the approximate range of concentrations used was 4.0×10^{-8} to 1.2×10^{-6} M). Mazella (529), with the isolated frog heart, found that strophanthin (approximate range of concentrations, 1.2×10^{-5} to 3.0×10^{-5} M) augmented the chronotropic effect of acetylcholine. Sedef (710), also with the isolated frog heart but employing the amplitude of systolic contraction as an index of cholinergic responsiveness, found that the acetylcholine-induced decrease in this parameter was enhanced by ouabain. Baker (38) studied the interaction of acetylcholine and digitalis on hearts obtained from human fetuses at the time that abortions were performed on pregnant women suffering from advanced tuberculosis or cardiac disease. During perfusion of the hearts by the Langendorff method, the bradycardia produced by acetylcholine was enhanced when ouabain (3.4×10^{-7} M) was added to the preparation. Perry and Reinert (613) observed in isolated hearts from cats, guinea pigs, rabbits, and rats that the slight bradycardia produced by a small dose of acetylcholine was enhanced after the addition of a subthreshold dose of ouabain (0.5 μg). When larger doses of ouabain were given (i.e., 10 to 20 μg), the potentiating effect was less marked and it was often followed by depression. In studies performed in more recent years, Toda and West (821, 822) reported that the negative chronotropic response produced by acetylcholine in isolated and spon-

taneously beating rabbit atrial preparations was enhanced after the administration of subtoxic as well as toxic concentrations of ouabain (8.5×10^{-8} to 1.7×10^{-7} M). Similarly, Seifen and Seifen (715) observed that prior to digitalis administration, a dose of 0.65 μg of acetylcholine was required to reduce the rate of the isolated guinea-pig heart by 50%, whereas in the presence of 4×10^{-7} M of ouabain, the effective dose of acetylcholine was reduced to 0.1 μg.

The parasympathetic neuroeffector junction at the atrioventricular node is also affected by digitalis. Essentially the same techniques that have been used to test the effects of digitalis on the S-A node region were employed. With regard to the effects of digitalis on the responses of the A-V node to electrical stimulation of the peripheral cut ends of the vagus nerves (preganglionic fibers), Rand and Stafford (645) reported that the duration of heart block produced by vagal stimulation (100 Hz) in guinea pigs was occasionally enhanced when ouabain was administered at i.v. infusion rates of 3 μg/kg/min or more. Measurements of heart block were derived from standard ECG traces. The total doses of ouabain required to produce an augmentation of vagal effects on the A-V node were close to the doses that caused ventricular ectopic beats. With somewhat lower doses of ouabain, no effect on alterations in A-V conduction mediated by the vagus nerve was observed. Gaffney et al. (252) observed that in open-chest dogs in which the stellate ganglia and the sympathetic chains had been extirpated, the prolongation of the A-V nodal refractory period produced by electrical stimulation of the vagus nerves was enhanced by ouabain. A-V nodal refractory period changes were assessed by determining the atrial rate at which dropped ventricular beats were observed in the electrocardiogram trace. Prior to ouabain administration, dropped beats occurred during vagal stimulation (5 to 10 Hz) at an atrial rate that ranged between 33% and 46% of the control atrial rate necessary for eliciting A-V conduction block. With ouabain (10.7 to 78 μg/kg, i.v.), dropped beats occurred during vagal stimulation at an atrial rate that ranged between 55 and 66% of the control atrial rate necessary for eliciting A-V conduction block. A similar result was obtained by Nadeau et al. (565) after the administration of acetylstrophanthidin directly into the A-V node artery of dogs in which the atrial rate was held constant by artificial pacing. In this study, stimulation of the peripheral end of the sectioned left vagus nerve at a rate of 30 Hz produced, during the control period, only a slight prolongation of the P-R interval. However, when the vagus was stimulated 15 minutes after an intranodal injection of 1 to 10 μg of acetylstrophanthidin, at which time the heart was in 2:1 block, complete A-V block and a slow ventricular escape rhythm were noted. Further vagal stimulation after 30 minutes, when conduction had returned to normal, unmasked a 2:1 A-V block. In another study, Greenspan and Lord (315) demonstrated in dogs with artifically induced atrial fibrillation that the reduction in ventricular rate produced by vagal stimulation (10 to 20 Hz) was progressively enhanced after the i.v. infusion of 25% to 90% of the arrhythmogenic dose of acetylstrophanthidin. They indicated that the ability of digitalis to sensitize A-V conduction to the depressant effect of vagal stimulation was enhanced in almost a linear fashion with subarrhythmic doses of the drug.

Rather than use constant stimulation of the peripheral vagus nerves to assess digitalis-parasympathetic interactions at the A-V node, Pace and Martin (597) examined the interplay between brief bursts (1 to 3 stimuli/burst) of peripheral vagal stimuli placed at different times within the cardiac cycle and periodic i.v. doses of digoxin on A-V conduction in anesthetized dogs. Sympathetic tone to the heart was blocked in their animals either by propranolol or by ligation of both ansa subclavia and the posterior pole of both stellate ganglia. Pace and Martin employed this method of vagal stimulation for the purpose of closely simulating the spontaneous electrical activity normally seen in the vagus nerves under basal conditions. They found that the maximum delay in A-V conduction (i.e., P-R interval), the time after the stimulus at which A-V conduction was maximally inhibited, and the length of time for depressed A-V conduction produced by the discrete single bursts of vagal stimulation placed within the cardiac cycle, were all increased in a dose-dependent fashion by digoxin.

Postganglionic vagal nerve fibers were directly stimulated by Toda and West (823) in isolated rabbit atrial preparations in which A-V node and His'-bundle tissues remained intact. Conduction through the A-V nodal structures was measured in terms of cycle duration as well as alterations in nodal action potentials. They observed that ouabain (3.4×10^{-7} M) augmented the negative dromotropic response produced by transmural electrical stimulation applied at the region of the A-V node. The concentration of ouabain employed was subtoxic, i.e., it had no effect on the spontaneous rate or rhythm of the preparation and exerted no effect on conduction through the A-V node or His' bundle, and the results obtained were considered to be dependent upon a cholinergic mechanism.

Similar enhancing effects of digitalis on A-V node responses to exogenously administered acetylcholine have been observed by several investigators. Rand and Stafford (645) reported that the administration of ouabain (doses ranging from 55 to 120 μg/kg i.v. given by infusion or injection) increased the duration of heart block produced by injection of 3 to 5 μg of acetylcholine directly into the left atria of guinea pigs. The duration of heart block increased from control values of 4 to 6 seconds to periods of 8 to 13 seconds after ouabain administration. Indirect data obtained from studies in patients also indicated an enhancing effect of digitalis on acetylcholine. Pitt and Kurland (620) reported that the ventricular slowing in patients with atrial flutter or fibrilla-

tion normally produced by edrophonium, an agent that prevents the breakdown of acetylcholine, was enhanced in the presence of a therapeutic dose of digitalis. In a study performed in dogs by Nadeau et al. (565), the degree of A-V block produced by injections of acetylcholine in doses of 0.1 and 1.0 μg/ml into the atrioventricular node artery was found to be intensified after the intranodal administration of 1 to 5 μg doses of acetylstrophanthidin.

With the canine heart-lung preparation, Gaffney et al. (252) stimulated the atria at frequencies (220 to 360/min) just high enough to produce regular 2:1 A-V block. When acetylcholine was then added to the preparation, additional beats were dropped, indicating a prolongation of the A-V node refractory period. After the administration of ouabain in doses ranging from 15 to 75 μg, they observed a marked reduction in the doses of acetylcholine that were required to cause additional beats to be dropped. In a less detailed study performed in isolated guinea-pig hearts, Seifen and Seifen (715) reported that digitalis enhanced the depressant effect of acetylcholine on A-V conduction. They found that the decrease in ventricular rate produced by acetylcholine, presumably acting on the A-V node to produce an impairment of A-V conduction, was enhanced in the presence of ouabain.

In opposition to these findings are the early studies of Rall et al. (643) and Luisada and Mautner (501). Rall et al. administered one-twelfth of the estimated lethal doses of lanatoside A, lanatoside B, and lanatoside C i.v. every 10 minutes to dogs, and examined the response of the heart to doses of acetylcholine (0.2 to 2.0 mg) reported to cause A-V block. They observed that "cardiac inhibition" produced by acetylcholine was abolished by lanatoside A in 2 out of 10 experiments at 58% to 60% of the lethal dose, by lanatoside B in 5 out of 10 experiments at 58% to 84% of the lethal dose, and by lanatoside C in 10 out of 10 experiments at 43% to 85% of the lethal dose. In no case was the response to acetylcholine enhanced by the digitalis preparations. A possible explanation for the findings of this study may be that the doses of the digitalis preparations that were reported to abolish the effect of acetylcholine evoked cardiac arrhythmias in virtually every one of their experiments. The appearance of rapid idioventricular rhythms would preclude observations of any interaction of acetylcholine and digitalis on the A-V node. Luisada and Mautner (501) observed that injections of either methacholine or carbachol produced complete A-V block in dogs and rabbits. This was associated with either ventricular escape or cardiac standstill. Administration of ouabain (1.7×10^{-4} M) and K-strophanthin (approximate concentration of 1.7×10^{-7} M) reversed these responses. The reason for the reversal appeared to be the induction of a rapid idioventricular rate by these digitalis preparations. Similar findings were also reported by these latter investigators for the isolated frog heart.

B. SYMPATHETIC NEUROEFFECTOR JUNCTION AND END-ORGAN RESPONSES. Investigations of the effects of digitalis drugs on sympathetic responses at the S-A node have yielded conflicting results. The positive chronotropic responses to adrenergic stimuli have been reported to be either reduced, unchanged, or enhanced by these drugs.

Almost all of the studies in which digitalis has been found to reduce the sensitivity of the S-A node to adrenergic stimuli have been performed in anesthetized dogs and cats with subtoxic doses of digitalis. In 1961, Mendez et al. (545) reported that i.v. acetyldigitoxin and digitoxin in 40% to 60% of the lethal dose reduced the positive chronotropic effect evoked by either stimulation of preganglionic sympathetic fibers of the right stellate ganglion or i.v. epinephrine. These results were obtained in anesthetized dogs subjected to vagal nerve section as well as extirpation of the sympathetic chains. Tuttle and Innes (834) reported that ouabain, 50 μg/kg i.v., reduced the chronotropic response to i.v. isoproterenol in anesthetized dogs. Roberts (661) found that the positive chronotropic response in anesthetized cats produced by either i.v. isoproterenol or stimulation of the right postganglionic sympathetic fibers emanating from the stellate ganglion was depressed by ouabain in doses ranging from 20 to 50 μg/kg and 40 to 50 μg/kg, respectively. Beiser et al. (61) found that the positive chronotropic response evoked by i.v. isoproterenol was reduced by the administration of a subtoxic i.v. dose of ouabain in anesthetized dogs. Nadeau and James (567) reported that acetylstrophanthidin, administered directly into the sinus node artery of anesthetized dogs in a dose of 2.5 μg, reduced the positive chronotropic response of the sinus node to electrical stimulation of the right stellate ganglion and to epinephrine administered directly into the sinus node artery.

There has been only one study with preparations in vitro in which an antiadrenergic effect of digitalis was demonstrated. Seifen (714) observed in the isolated guinea-pig right atria that ouabain (0.5 to 4.0×10^{-7} M) caused progressive inhibition of the positive chronotropic response to norepinephrine. Other studies in vitro indicate that digitalis causes either no change or an enhancement of the chronotropic response of the S-A node to adrenergic stimuli. Toda (819) reported that the increase in sinus rate produced by electrical stimulation of postganglionic sympathetic nerves in the isolated rabbit right atrial preparation was not affected by ouabain (6.85×10^{-8} and 2.75×10^{-7} M). Toda also examined a higher concentration of ouabain (1.37×10^{-6} M) and found an enhancement in the chronotropic response to sympathetic nerve stimulation. Nakashima et al. (569) found that ouabain (7.5×10^{-7} gm/ml) did not alter the chronotropic response to norepinephrine in isolated guinea-pig atria. Tiwari et al. (818) examined the effects of chronic administration of digoxin (100 μg/kg/day i.p. for 10 days) on the isolated right atrial preparation of the rat and reported an increase in the chronotropic response elicited

by norepinephrine. Godfraind and Godfraind-De Becker (298) reported that subtoxic doses of ouabain (1.7×10^{-9} and 1.7×10^{-7} M) did not modify the chronotropic effect of epinephrine in isolated guinea-pig atria. However, a toxic dose (1.7×10^{-6} M) of ouabain suppressed the chronotropic response to epinephrine.

Investigations of the effects of digitalis on sympathetic responses at the A-V node region indicate an antagonism of sympathomimetic effects on the automaticity and refractory period of this tissue. These studies have all been performed in vivo in anesthetized dogs. Mendez et al. (545), with preparations with the sinoatrial node destroyed, reported that acetyldigitoxin and digitoxin at 40% to 60% of the lethal dose administered i.v. countered the increase in A-V nodal rate produced by i.v. epinephrine and stimulation of the left stellate preganglionic sympathetic fibers. These dogs were subjected to vagal nerve section as well as section of the sympathetic chains. Nadeau et al. (565) reported that acetylstrophanthidin, digoxin, and desacetyl-lanatoside C, administered into the A-V node artery in doses ranging from 1 to 5 μg, reduced the degree of A-V nodal tachycardia evoked by left stellate ganglion stimulation. Porlier et al. (627) studied the effect of acetylstrophanthidin administered into the A-V node artery at a dose of 5 μg in two types of preparations. First, they used a preparation wherein A-V nodal pacemaker activity was augmented either by left stellate ganglion stimulation or by administration of norepinephrine and isoproterenol into the A-V node artery, in animals where S-A node function was blocked by the administration of propranolol into the S-A node artery. Acetylstrophanthidin counteracted the A-V nodal tachycardia induced by the sympathetic activating procedures. Second, they used a preparation in which atrial fibrillation was induced by high frequency stimulation of the right atrial wall. Left stellate ganglion stimulation in this preparation increased A-V conduction, as demonstrated by an increased ventricular rate. Acetylstrophanthidin reduced this increase in ventricular rate. Mendez et al. (546), again working in preparations with the S-A node destroyed, reported that i.v. acetyldigitoxin counteracted the decrease in A-V node functional refractory period produced by either left stellate ganglion stimulation or i.v. epinephrine.

Investigations of the effects of digitalis on sympathetic responses of cardiac ventricular tissue indicate an enhancing effect of these agents. Johnson and Gilbert (394) reported that ouabain and digilutea (doses not given) in anesthetized dogs enhanced the arrhythmogenic effect of ephedrine. Seevers and Meek (711) reported that subtoxic doses of digifolin (30 to 75 mg/kg i.v.) and digitalin (1 mg/kg i.v.) increased the incidence and prolonged the duration of arrhythmias induced by ephedrine in the conscious dog. They also found that administration of these agents precipitated arrhythmias in dogs exposed to a subarrhythmic dose of ephedrine. Saito and Shudo (689) reported that subtoxic doses of ouabain (40 to 50 μg/kg i.v.) increased the incidence of premature ventricular contractions produced by i.v. norepinephrine, epinephrine, and isoproterenol in anesthetized dogs. Pearle and Gillis (610) examined the effect of a subarrhythmic dose of ouabain (40 μg/kg i.v.) on ventricular rate increases induced either by sympathetic nerve stimulation or isoproterenol in dogs with heart block. Ouabain had no significant effect on the cardiac rate response but precipitated arrhythmias in the presence of the adrenergic interventions. This latter finding is consistent with augmentation of the arrhythmogenic effects of adrenergic stimulation by digitalis. In a recent study, Lum et al. (503) found that a dose of epinephrine that produced no arrhythmias in anesthetized cats induced ventricular tachycardia after the administration of a subtoxic dose of ouabain (50 μg/kg i.v.). These investigators also obtained similar results in the isolated rabbit heart preparation; a subarrhythmic dose of epinephrine administered during perfusion of the isolated heart with 6.4×10^{-4} M of ouabain was found to produce premature ventricular beats and ventricular tachycardia. This latter observation confirmed the earlier report of Tanabe (795) that ouabain potentiated the induction of automaticity by catecholamines in the isolated guinea-pig papillary muscle preparation.

There are several possible explanations for the above-noted variable effects of digitalis drugs on sympathetic responses in different cardiac regions. One of these appears to be related to a basic difference in the response of cardiac tissue from various areas of the heart (552). This is supported by data showing that supraventricular areas of the dog and cat heart (S-A and/or A-V nodes) exhibit an antagonistic interaction between catecholamines and digitalis (61, 545, 546, 565, 567, 627, 661, 834); whereas ventricular tissues of the dog and cat heart exhibit a synergistic interaction between these two agents (394, 503, 610, 689, 711). Possible explanations for the variable effects of digitalis drugs seen in the S-A node region may involve both species differences and the doses of the digitalis preparations employed. For example, the atria of dogs and cats, as noted above, exhibit a diminished response to sympathetic activating procedures, whereas the atria of rats exhibit only an augmented chronotropic response to these procedures (818). Depending on the dose of digitalis employed, the atria of guinea pigs and rabbits can exhibit multiple responses. In the presence of subtoxic doses of digitalis, the atria of these two species show no change in the chronotropic response to sympathomimetic effects (298, 569, 819). On the other hand, in the presence of near-toxic doses of digitalis, the atria of guinea pigs exhibit a diminished chronotropic response to sympathomimetic procedures (298, 714), whereas atria of rabbits exhibit an augmented response to sympathomimetic procedures (819). Reasons for the differing responses with increasing doses of digitalis may relate to the finding that high doses of these agents can cause multiple effects at the sympathetic

neuroeffector junction. These include a direct effect on postsynaptic membranes as well as indirect effects involving release of norepinephrine and inhibition of uptake of this neurotransmitter (see section III A 2 b).

Investigations of the effects of digitalis on the sympathetic response of vascular tissue also indicate an enhancing effect of these drugs. These investigations have, for the most part, been performed in vitro with isolated vascular strips or segments of arteries and veins. However, in a few cases, results have been reported from studies in vivo.

Those studies performed with arterial strips in vitro have demonstrated that digitalis drugs can augment the constrictor effects of either exogenously administered norepinephrine or transmural electrical stimulation of vascular tissue. Transmural stimulation presumably selectively activates postganglionic sympathetic nerves that innervate the smooth muscle cells. Augmentation has been shown in carotid artery strips from rabbits (234, 465), aortic strips from rabbits (100, 396, 397, 795, 820) and guinea pigs (100), mesenteric artery strips from rabbits (87), and facial artery strips from cows (270) by digitalis drugs (primarily ouabain, but in a few cases, digoxin and strophanthidin) in concentrations generally ranging from 1×10^{-8} to 1×10^{-4} M.

Three studies have been performed in which the responses of venous strips have been studied in vitro. In 1967, Holman and McLean (376) demonstrated that the contractile response of isolated segments of sheep mesenteric veins to transmural stimulation was initially potentiated in the presence of 1.7×10^{-7} M ouabain, whereas with prolonged exposure to ouabain the response became depressed. In the same year, Matthews and Sutter (528) reported that the contraction induced by norepinephrine in isolated rabbit anterior mesenteric veins was augmented in the presence of 10^{-5} M ouabain. In 1969, Brender et al. (96) showed that the contractile responses of isolated canine cutaneous veins evoked by transmural stimulation or exogenously administered norepinephrine were potentiated in the presence of 6.7×10^{-7} to 2.2×10^{-5} M of acetylstrophanthidin.

Information on the effects of digitalis on sympathetic responses of vascular tissue in vivo is somewhat limited. In terms of arterial responses, two groups of investigators reported that the pressor effects of catecholamines were markedly enhanced after digitalization in intact animals, but they did not provide experimental details to support their contentions (687, 795). In a more detailed study, Ozawa and Katsuragi (594) demonstrated that the administration of ouabain (20 μg/kg i.v.) to cats with spinal cord transected potentiated the pressor response of i.v. norepinephrine and tyramine. For venous responses, Brender et al. (96) examined the effect of digitalis on the contractile response of the isolated left lateral saphenous vein of the dog evoked by stimulation of the lumbar sympathetic trunk. The saphenous vein was isolated in vivo and perfused with blood taken from the median sacral artery of the same animal. Doses of acetylstrophanthidin, 12.5 to 15.5 μg/kg, injected either into the right atrium or i.v. into the pump circuit perfusing the left lateral saphenous vein, potentiated the venoconstrictor response to sympathetic stimulation.

The above studies pertaining to vascular tissue indicate that digitalis drugs can enhance the effects of sympathetic stimuli to cause vasoconstriction at the level of the vascular smooth muscle. In addition, results of one study has demonstrated that digitalis can enhance the effect of sympathetic stimuli to cause vasodilation at the level of the vascular smooth muscle. Broekaert and Godfraind (100) found that the relaxation produced by isoproterenol in the isolated guinea-pig aortic strip was potentiated by a low concentration of ouabain (1×10^{-9} M). They further reported that the relaxant effect of isoproterenol was reduced and eventually abolished when higher concentrations of ouabain (up to 1×10^{-5} M) were used.

5. Adrenal medulla. Richards and Wood (656) were the first to study the effects of digitalis on the adrenal gland. They used both dogs and cats. The animals were studied in the decerebrate unanesthetized state and received i.v. doses of strophanthin ranging from 0.034 to 0.62 mg/kg. Catecholamine release was measured by collecting venous blood from the adrenal glands and subjecting it to bioassay on isolated intestinal strips from dogs or cats. Strophanthin, in doses larger than 0.04 mg/kg in dogs and 0.058 mg/kg in cats, was found to enhance the release of amines from the adrenal glands. The effect was not observed in animals with sectioned splanchnic nerves or sectioned spinal cords, leading these investigators to conclude that the drug was acting centrally to cause adrenal catecholamine release. A few years later Stewart and Rogoff (771), by using similar methods in cats anesthetized with ether, were unable to confirm these results with strophanthin. However, in almost all of their experiments the doses of strophanthin employed were smaller than the threshold dose of the drug (i.e., 0.058 mg/kg) required for activity in cats in the study of Richards and Wood. In another study performed much later, Conn et al. (152) also failed to demonstrate an increase in epinephrine and norepinephrine in adrenal venous blood with a borderline toxic dose of acetylstrophanthidin (30 μg/kg) in dogs anesthetized with pentobarbital. From these few studies that have been performed in intact animals, it appears that very large doses of digitalis may be capable of evoking a release of catecholamines from the adrenal glands, whereas smaller and generally subtoxic doses do not.

Banks (42) reported that ouabain (1×10^{-4} M) increased both the spontaneous release and carbachol-induced release of catecholamines by the isolated perfused adrenal gland of the cow. Nishikawa and Tsujimoto (583) confirmed the findings of Banks by perfusing the isolated canine adrenal gland with ouabain (1×10^{-6} to 1×10^{-4} M). Their assay distinguished between epinephrine and

norepinephrine; the release of both amines was increased, that of norepinephrine more than that of epinephrine. Despite the fact that digitalis has been shown to evoke a release of catecholamines from adrenal glands in vitro, the relevance of such studies to preparations in vivo can be questioned because of the large concentrations of digitalis required to produce a response in vitro. Whole animal preparations are unsuitable for testing these large doses because they would be lethal.

The mechanism for the releasing effect seen in the isolated adrenal gland studies appears to involve calcium. According to Banks (42) and Nishikawa and Tsujimoto (583), ouabain acted by increasing calcium entry into cells, and the calcium in turn caused release of catecholamines. Their evidence was that ouabain-induced release was prevented by removing calcium from the medium and that ouabain caused an increase in the influx of ^{45}Ca into the adrenal gland cells. Nishikawa and Tsujimoto suggested that a nicotinic receptor might be involved, based on the findings that hexamethonium caused a slight inhibition of the response and that acetylcholine and physostigmine both blocked the release (presumably because of depolarization blockade of nicotinic receptors). The block by acetylcholine and physostigmine was associated with an inhibition of ^{45}Ca influx.

Studies on the effects of digitalis on release (42) and uptake (122, 123, 415) of catecholamines in isolated chromaffin granules from bovine adrenal glands indicate that neither of these processes are affected. All studies utilized ouabain in concentrations ranging between 1×10^{-4} and 1×10^{-3} M. The lack of effect of ouabain on catecholamine release was unexpected in view of the positive findings obtained with isolated adrenal glands (42, 583). Release of catecholamines in both preparations requires calcium (197), and the difference in the response of these two preparations is, thus, perplexing. The inability of digitalis to affect uptake is consistent with what is known about the transport mechanism of granular uptake. Granular uptake is dependent upon a magnesium-activated ATPase (607, 615) that is not inhibited by digitalis (48, 602).

Studies on the effects of digitalis drugs on adrenal gland catecholamine content have indicated either a decrease, no change, or an increase in content. Those investigators who reported a decrease observed this change in the adrenal gland homogenate of the rat with 10^{-3} M ouabain (320) and in whole animal preparations (rats and rabbits) with, in most cases, toxic doses of ouabain, strophanthin, or convallatoxin (22, 136, 265, 679). Those investigators who reported no change used preparations both in vitro (132) and in vivo (43, 145, 360). Ouabain was used for the study in rats in vitro but the dose employed was not reported. The whole animal preparations included dogs, cats, and rats, and generally the doses of the preparation used (ouabain) were in the toxic range. In two studies in rats, ouabain was reported to cause an augmentation in catecholamine content both in vitro at a low concentration of 1×10^{-10} M (320) and in vivo (132).

6. Conclusions. Data obtained from studies of digitalis drugs on efferent systems indicate that these agents exert primarily excitatory effects on autonomic ganglia, autonomic and somatic nerves, postganglionic neuroeffector junctions (including postsynaptic receptors of cardiac and vascular tissue), and the adrenal medulla. Effects on autonomic ganglia appear to involve unmasking of muscarinic ganglionic receptors and abolition of the after-HP that follows activation of nicotinic ganglionic receptors. Effects on autonomic nerve fibers have been best documented by measuring either evoked responses or acetylcholine release from these nerves during the administration of digitalis drugs. Results from these studies indicate that digitalis abolishes the after-HP of postganglionic sympathetic nerves, enhances evoked responses elicited by submaximal electrical stimulation, and increases the release of acetylcholine produced by electrical stimulation. Consistent with these observations is the finding that spontaneous occurring activity is also altered, but multiple sites of action of digitalis drugs may be involved in producing this response. Also consistent with an excitatory effect on peripheral nerves are findings that digitalis increases the frequency of MEPP and posttetanic repetitive firing of motor nerves. In terms of the neuroeffector junction, the most straightforward findings are with the parasympathetic nervous system. Here digitalis drugs consistently augment end-organ responses to vagal stimulation or to injected acetylcholine. The best evidence of an enhancing effect has been obtained from studies with electrical stimulation of postganglionic parasympathetic nerves along with parallel studies with acetylcholine. The effect of digitalis drugs on the sympathetic neuroeffector junction is more complex and includes augmentation as well as antagonism. Augmentation has been clearly demonstrated for cardiac ventricular tissue and vascular smooth muscle and the latter may involve responses mediated by both alpha- and beta-adrenergic receptors. Antagonism has been demonstrated at the S-A and A-V nodes in studies performed in vivo, whereas inconsistent findings have been reported from studies in vitro. The inconsistency may be due to multiple effects of digitalis occurring as a consequence of the much larger amount of drug used for the studies in vitro. These multiple effects may include release of norepinephrine and prevention of norepinephrine uptake as well as effects on the postsynaptic membrane.

An analysis of the studies dealing with digitalis effects on the adrenal medulla suggests that these drugs release catecholamines. In general, in those cases in which positive effects were seen, the animals were exposed to a toxic dose of digitalis and were studied either in the conscious or lightly anesthetized state (22, 136, 265, 656, 679). In those situations in which no effects were seen,

the doses of digitalis employed were generally in a nontoxic range (43, 152, 771) or the animals were studied under deep anesthesia (132, 145, 360).

III. Mechanisms for Digitalis-Induced Neuroexcitatory Effects

A. Effect on Neurotransmitters

1. Central nervous system. A. INTRODUCTION. Digitalis drugs have been shown to exert important effects on many of the known or putative CNS neurotransmitters. These include norepinephrine, dopamine, serotonin, acetylcholine, and gamma-aminobutyric acid (GABA). The synaptic processes that have been examined include the release, uptake, synthesis, degradation, and storage of the neurotransmitters. In addition, evidence for the action of digitalis on CNS neurotransmitter mechanisms can be found in studies dealing with noncardiac toxic effects on the CNS (e.g., convulsions) of these drugs in animals.

There are data to indicate that each neurotransmitter may be involved in regulating central autonomic and, in some cases, central respiratory activity. Norepinephrine is the neurotransmitter that has been most frequently implicated in the regulation of both central sympathetic and parasympathetic outflow (23, 135, 324, 421). Activation of CNS noradrenergic mechanisms can result in different effects on central sympathetic outflow depending on the areas of the CNS that are studied. Present data suggest that activation of forebrain and spinal cord noradrenergic mechanisms can result in an increase in sympathetic outflow whereas activation of hindbrain noradrenergic mechanisms can result in a decrease in sympathetic ouftlow (324, 804). Activation of hindbrain noradrenergic mechanisms appears to cause enhancement of central vagal outflow (23, 323, 421, 667). Likewise, dopamine, serotonin, acetylcholine, and GABA have been implicated as having important roles in regulating central outflow of both divisions of the autonomic nervous system. Activation of CNS dopaminergic mechanisms has been reported to decrease sympathetic (231, 448) and increase parasympathetic outflow (24, 25). Activation of serotonergic mechanisms also alters autonomic outflow but whether increases or decreases in sympathetic outflow occur is unclear at the present time (24, 55, 135, 389, 390, 791). Recent data suggest that activation of CNS serotonergic mechanisms results in a reduction of parasympathetic outflow (479a, 790). In addition, activation of serotonergic mechanisms results in a depression of respiratory function (28). Activation of CNS cholinergic mechanisms appears to result in an enhancement of both central sympathetic (444) and parasympathetic outflow (453, 680, 837). Finally, activation of CNS GABAergic mechanisms results in inhibition of both sympathetic and parasympathetic outflow (26, 187, 188).

B. NOREPINEPHRINE. The effect of digitalis on norepinephrine release has been examined in only one study. Levi et al. (463) reported that ouabain had a biphasic effect on evoked norepinephrine release in rat cerebral cortex synaptosomes in vitro. Norepinephrine-induced release of [^{3}H]norepinephrine from this preparation was initially increased and then depressed by large concentrations of ouabain (1×10^{-4} and 1×10^{-3} M).

Norepinephrine uptake into CNS tissue has also been reported to be decreased by the administration of ouabain in vitro. Dengler et al. (182) were the first to report ouabain-induced inhibition of norepinephrine uptake into CNS tissue (feline cerebral cortex slices). Ouabain was administered in concentrations of 1×10^{-6} to 1×10^{-5} M. Subsequent studies confirmed the initial findings (180, 181). Concentrations of ouabain ranging from 1×10^{-6} to 1×10^{-3} M have also been shown to inhibit uptake in rabbit and mouse brain slices (675, 724) and in rat and rabbit brain synaptosomes (379, 815, 816, 874). The sensitivity of the transport system to the effects of digitalis drugs varies between different species. Cat and mouse CNS tissues require lower doses (1×10^{-6} to 3.4×10^{-5} M) of ouabain for inhibition of norepinephrine uptake than are necessary in either rabbit (1×10^{-5} to 1×10^{-4} M ouabain) or rat (1×10^{-5} to 1×10^{-3} M ouabain) CNS tissue. Pineal gland norepinephrine uptake is similarly affected by ouabain (1×10^{-6} to 1×10^{-4} M) in vitro (180, 183).

There are two studies in vivo in which the effects of digitalis were studied on transport mechanisms in CNS tissue. In the first, Weil-Malherbe et al. (865) reported that in cats, ouabain, 50 mg/kg i.v. (an extraordinarily high dose for this species—probably a typographical error and the dose was actually 50 μg/kg), had no effect on the uptake of [^{3}H]norepinephrine into the pituitary gland, hypothalamus, cerebral cortex, or medulla. In the second, Helke et al. (350) reported that a lethal arrhythmogenic dose of deslanoside administered to cats had no effect on [^{3}H]norepinephrine uptake into the forebrain, hypothalamus, midbrain, medulla-pons, and area postrema. The reason for the ineffectiveness of digitalis in these uptake studies performed in vivo as compared to those performed in vitro is probably the differences in the drug concentrations employed. The studies in vitro utilized concentrations of digitalis generally in the range of 10^{-6} to 10^{-3} M whereas the studies in vivo were limited by lethal toxicity to plasma concentrations ranging between 1×10^{-8} and 1×10^{-7} M (201, 653).

In two studies by Goldstein's group (21, 304), digitalis was described as inhibiting the synthesis of norepinephrine. These investigators reported that ouabain, administered by the intraventricular route in doses of 25 to 75 μg to rats together with [^{3}H]dopamine, decreased the formation of [^{3}H]norepinephrine in the hypothalamus, brainstem, and cerebellum (21). They also reported that the synthesis of catecholamines from [^{14}C]tyrosine was enhanced, with dopamine being the major product formed. In the second study (304), ouabain (1×10^{-4} M)

increased the synthesis of [^{14}C]dopamine in rat brain cortex slices. Initially, there was an increase in norepinephrine concentration in the slices but this was followed by a decrease below control level. These investigators postulated that the inhibition of norepinephrine synthesis was indirect and due to inhibition of neuronal uptake of catecholamines. Ouabain did not act to inhibit synthesis of norepinephrine by altering the activity of the synthesizing enzyme, tyrosine hydroxylase. The reason suggested for the increase in catecholamine synthesis was an impairment in feedback inhibition of synthesis secondary to ouabain-induced inhibition of catecholamine uptake and drop in neuronal catecholamine stores.

Only one of the two enzymes responsible for the catabolism of norepinephrine, namely monoamine oxidase, has been reported to be influenced by digitalis drugs. Popov and Forster (624) reported that single or repeated administration of large doses of digitoxin and digitoxigenin inhibited monoamine oxidase enzyme activity of guinea-pig, rat, and cat brain in vivo. Similar studies performed with G-strophanthin produced no change in the activity of this eynzme in rats. Roy and Chatterjee (679) also reported that ouabain causes inhibition of monoamine oxidase. However, they cited unpublished data and provided no indication of the species, tissue, or experimental conditions employed. To our knowledge, no studies have been performed on the effects of digitalis on the other catabolic enzyme, catechol-O-methyl transferase.

Studies dealing with the effect of digitalis drugs on norepinephrine concentrations in brain tissue present a confusing picture. Forster et al. (238) found no change in the content of norepinephrine in whole brain of rats or guinea pigs after high doses of either ouabain (100 mg/kg, i.p.), digitoxigenin, or digoxin (3 to 5 mg/kg, i.p.). Angelucci et al. (22) reported that rabbits that died from an infusion of K-strophanthin (13.5 μg/kg/min, i.v. until the onset of ECG abnormalities) had significantly lower brain norepinephrine levels, while rabbits that showed clear-cut but rapidly reversible ECG abnormalities had a less pronounced fall in the norepinephrine content of the CNS. Similarly, Buterbaugh and Spratt (108) showed in rats that the LD50 dose but not the LD25 dose of i.v. digitoxigenin reduced brain norepinephrine content. In 1973, Ladisch (440) demonstrated that the subdural application of ouabain in rats, in a dose (150 μg) that produced tonic and clonic convulsions, decreased the content of [^{3}H]norepinephrine in the brainstem and telencephalon and increased the levels of the norepinephrine metabolites. Of these metabolites, 3-methoxy-4-hydroxyphenethylene glycol (MHPG) and 3-methoxy-4-hydroxymandelic acid were increased in the telencephalon while dihydroxymandelic acid and dihydroxyphenyl glycol were elevated in the brainstem, telencephalon, and cerebellum.

Helke (345) found no effect of deslanoside intoxication on the norepinephrine content of the following eight CNS areas of six chloralose-anesthetized cats: hypothalamus, medulla-pons, amygdala, colliculi, midbrain, caudate nucleus, hippocampus, and spinal cord. Tissues were taken from "saline control" cats and cats exposed to a lethal arrhythmogenic dose of deslanoside. Helke et al. (354) also determined the effect of a lethal dose of deslanoside on the levels of MHPG, the major CNS norepinephrine metabolite in the cat (516, 700), in three brain areas, the hypothalamus, medulla-pons, and caudate nucleus. Deslanoside intoxication resulted in a significant elevation in the MHPG level in the hypothalamus but no change was detected in the other two areas assayed.

Evidence that digitalis influences CNS noradrenergic mechanisms can be obtained from studies on digitalis-induced behavioral, respiratory, and convulsant effects in rodents. High doses of digitalis produce CNS stimulation characterized by convulsions and death, while lower doses induce CNS depression, characterized by loss of locomotor activity and lack of response to external stimuli (194). Lage and Spratt (441) studied the effect of reserpine-pretreatment on digitoxigenin (2.16 mg/kg, i.v.)-induced convulsions and death in mice and found that the animals given reserpine were protected from toxicity. They attempted to restore the catecholamine depletion produced by reserpine by infusion of norepinephrine i.v., and found that the mice were no longer protected from toxicity. However, norepinephrine by the i.v. route would not cross the blood-brain barrier, and hence the protective effect of reserpine must involve peripheral depletion of monoamines. Buterbaugh and Spratt (107, 108) studied the effects of reserpine-, tetrabenazine-, and syrosingopine-pretreatment on the lethal effect of i.v. digitoxigenin in rats. In this species, death is due to respiratory depression. The three agents, known to deplete peripheral and central norepinephrine (and serotonin) to varying degrees (460, 638), all exerted a protective effect. However, the protective effect did not appear to be related to norepinephrine depletion since the time of maximum reduction in norepinephrine levels did not correlate with time of maximal protection observed with these agents. Furthermore, pretreatment with an agent that depletes norepinephrine but not serotonin, namely, α-methyl-*p*-tyrosine, had no protective effect (107). Likewise, Buterbaugh and London (109) reported that the ability of reserpine to block digitoxigenin-induced lowering of the electroshock threshold in rats did not appear to be related to norepinephrine depletion since the time courses of the blocking effect and the norepinephrine depleting effect of reserpine did not correlate. From these studies dealing with the question of whether norepinephrine is involved in mediating the toxic actions of digitoxigenin (convulsive activity and respiratory depression) in mice and rats, the answer appears to be negative.

In contrast to the lack of evidence for an action of digitalis drugs in toxic doses on CNS noradrenergic mechanisms in rodents, there is some indirect evidence that digitalis drugs may depress CNS catecholaminergic func-

tion at lower doses. CNS depression is observed when low doses of ouabain (0.1 to 0.4 μg) are administered directly into the CNS of mice. This depression is reversed by an i.p. injection of either dexamphetamine or desmethylimipramine (194). Both ouabain and digitoxin (0.5 mg/kg, i.p.) produced a marked potentiation of the CNS depressant effects associated with reserpine. These potentiating doses were low enough so as to be devoid of CNS depressant activity when given alone (193). CNS depression produced by these digitalis drugs did not appear to be due to a reserpine-like depleting action, because as mentioned, the depression was reversed by agents (dexamphetamine and desmethylimipramine) that require the presence of catecholamine stores for their action (349, 385). The data from these studies suggest that digitalis may interfere with CNS neuronal function involving catecholamines, but whether norepinephrine, epinephrine, or dopamine is involved is unclear.

In summary, it appears that digitalis drugs do produce significant changes in CNS noradrenergic mechanisms. However, some of these changes documented by the studies in vitro are probably of little consequence because of the high concentrations of digitalis needed to evoke the effects (e.g., effects on release and uptake). The changes evoked in norepinephrine content, norepinephrine synthesis, monoamine oxidase activity, and tissue content of norepinephrine metabolites were performed in vivo and suggest that digitalis can change the activity of CNS noradrenergic systems. Except for the effect seen on synthesis reflecting a reduction in CNS noradrenergic activity and the effect of subtoxic doses on behavior in rodents, the other effects are consistent with digitalis activation of the noradrenergic system. That is, studies in vivo demonstrated a decrease in whole brain norepinephrine content (22) and an increase in MHPG content (354).

Consistent with digitalis activation of CNS noradrenergic function are findings obtained with the CNS administration of drugs that depress noradrenergic mechanisms. Centrally administered 6-hydroxydopamine and piperoxan significantly increased the dose of deslanoside necessary to produce ventricular arrhythmias and ventricular fibrillation (354). In these experiments, the 6-hydroxydopamine was restricted to the CNS by intracerebroventricular injection and the piperoxan was restricted to the regions of the lateral and third cerebral ventricles. When piperoxan was administered i.v. in the same dose employed for central administration, no protective effect was observed. Other investigators have found that drugs that depress noradrenergic function when administered into the CNS counteract the arrhythmias produced by centrally administered digitalis (696, 780).

In the studies of Helke et al. (349, 354), the CNS noradrenergic mechanisms that appeared to be activated were in the hypothalamus. This area exhibited a higher content of MHPG after exposure of animals to a lethal arrhythmogenic dose of deslanoside. In addition, this area was the region that showed the greatest degree of norepinephrine depletion after 6-hydroxydopamine. This fits with the findings that there is a noradrenergic system in the posterior hypothalamus that, when activated, enhances sympathetic outflow (324, 616–618, 633) and that the receptors in this region are of the alpha type (97, 618).

The idea that the hypothalamus may be a target area for digitalis is consistent with the findings of Saxena and Bhargava (695–697). They demonstrated indirectly that ouabain administered into the CNS caused an increase in sympathetic outflow to the heart and vasculature of cats and dogs. Most importantly, they demonstrated that the most sensitive site for eliciting this response was the posterior hypothalamus (696). Similarly, Garvey (263) reported that microinjections of ouabain into the lateral hypothalamus (but not injections into the medulla) induced marked increases in cardiac sympathetic nerve activity and ventricular arrhythmias. In addition, Saxena and Bhargava (695–697) demonstrated by the use of drugs that depress central adrenergic function that CNS noradrenergic mechanisms were involved in mediating the increase in central sympathetic outflow, as reflected by changes in cardiovascular function evoked by intracerebroventricular administration of ouabain.

There are data of an indirect nature against the idea that digitalis drugs activate central noradrenergic mechanisms. Saito et al. (688) and Tanabe et al. (796) administered 6-hydroxydopamine centrally to guinea pigs and dogs and observed no significant effect on the arrhythmogenic dose of digitalis. In addition, Ram and Hesse (644) found that intracisternal 6-hydroxydopamine reduced the arrhythmogenic dose of ouabain in rabbits. These divergent results might be explained on the basis of the CNS sites affected by 6-hydroxydopamine administration. For example, 6-hydroxydopamine-induced interference with noradrenergic function in the hypothalamus results in a depression of sympathetic function. The same is probably true of the spinal cord, since Taylor and Brody (804) have shown that activation of alpha-adrenergic receptors in this area increases sympathetic outflow. In contrast, depression of sympathetic function in the medulla would presumably augment sympathetic activity, since local injections of 6-hydroxydopamine have been shown to increase arterial pressure (190) and activation of alpha-adrenergic receptors decreases sympathetic outflow (324). Thus, the response seen with 6-hydroxydopamine may depend on the area within the CNS at which this agent acts. In the case of the data of Helke et al. (349, 354), this site would be the hypothalamus, whereas in the case of the data of Ram and Hesse (644), the site would be primarily in the hindbrain and spinal cord. In the studies of Saito et al. (688) and Tanabe et al. (796), the predominant site of action is unclear, because these investigators did not specify the site in the CNS where 6-hydroxydopamine was administered.

C. DOPAMINE. The effect of digitalis on the release of dopamine was investigated by Geier et al. (271), who reported that ouabain enhanced the spontaneous release of dopamine from rat corpus striatum synaptosomes in vitro and that the effect did not result from lysis of the synaptosomes.

The ability of digitalis drugs to alter the active transport of dopamine in vitro has been investigated in three studies. Horn et al. (379) reported that ouabain (1×10^{-5} M) inhibited the uptake of [^{3}H]dopamine into rat striatal synaptosomes. These findings were confirmed by Harris and Baldessarini (333) and Holz and Coyle (377), who used the same preparation and ouabain in the concentrations of 1×10^{-4} M and 2×10^{-6} M, respectively. In a study in vivo, Helke et al. (350) found that a lethal arrhythmogenic dose of deslanoside in chloralose-anesthetized cats had no effect on [^{3}H]dopamine uptake into the forebrain, hypothalamus, midbrain, medulla-pons, and area postrema. The reason for the difference between the results of the studies performed in vitro and the studies performed in vivo may again relate to the different doses of digitalis employed.

Information on the inhibitory effect of digitalis on monoamine oxidase, the catabolic enzyme for dopamine, was discussed in the above section on norepinephrine.

Studies dealing with the effect of digitalis drugs on the dopamine content of brain tissue were first reported by Anagnoste and Goldstein (21) who found that administration of ouabain (25 to 75 μg) intraventricularly to rats increased the formation of [^{14}C]dopamine from [^{14}C]tyrosine in the hypothalamus, brainstem, and cerebellum. Similar results were obtained in a later study by Goldstein et al. (304), who found that 1×10^{-4} M ouabain increased the formation of [^{14}C]dopmaine from [^{14}C]tyrosine in rat brain cortex slices. Intracerebroventricular ouabain (0.1 to 0.4 μg) in mice has been reported to increase whole brain dopamine content (195). In contrast, i.v. administration of an LD50 dose of digitoxigenin (1.07 mg/kg), which alters central norepinephrine and serotonin contents, was reported to have no effect on whole brain dopamine content (108).

Helke (345) studied the effect of deslanoside intoxication on dopamine content in the following brain areas of chloralose-anesthetized cats: hypothalamus, medulla-pons, midbrain, and caudate nucleus. These tissues were taken from saline-treated control cats and cats given a lethal arrhythmogenic dose of deslanoside. Deslanoside administered to six animals increased the dopamine content in the hypothalamus (0.64 ± 0.07 to 0.98 ± 0.15 μg/gm) but did not change dopamine content in the other three regions examined. Helke (345) also measured the levels of the two major metabolites of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the hypothalamus, medulla-pons, and caudate nucleus of "saline control" cats and deslanoside-intoxicated cats. Deslanoside intoxication produced a significant elevation of DOPAC in the caudate nucleus. DOPAC concentrations in the hypothalamus and medulla-pons, as well as HVA levels in all three areas, were unchanged by deslanoside.

Digitalis drugs have also been reported to alter the activity of CNS dopamine receptors. Doggett et al. have published a series of papers that suggest that ouabain blocks dopamine receptors. Doggett (192) reported that centrally administered ouabain (1 μg) to rats antagonized the compulsive gnawing behavior induced by the dopamine agonist, apomorphine. In addition, apomorphine reversed catalepsy produced by centrally administered ouabain (0.3 μg) in mice. Furthermore, it was found that the behavioral effects of centrally administered ouabain in mice (ptosis, catalepsy, and reduction in spontaneous motor activity and responsiveness to external stimuli) resembled those seen with the dopamine receptor blocking agent, pimozide (192, 194). Likewise, effects of ouabain on unconditioned and conditioned avoidance behavior resembled those seen with another dopamine receptor antagonist, chlorpromazine (195).

Gaitonde and Joglekar (255) presented evidence to indicate that digitalis drugs activate dopamine receptors. Their data came from studies on the interaction of digitalis and the dopamine-receptor antagonist, haloperidol, on emesis. Haloperidol administration prevented emesis induced by either ouabain (60 μg/kg, i.v.) or peruvoside (125 μg/kg, i.v.) in cats. Gaitonde and Joglekar (256) also evaluated the effect of haloperidol on other neurotoxic effects of these two agents administered into the cerebroventricles. Injections of ouabain (10 to 20 μg) or peruvoside (5 to 20 μg) into cats caused restlessness, rage, convulsions, and death, and these effects were not altered by pretreatment with haloperidol.

In summary, it appears that digitalis drugs can produce significant changes in CNS dopaminergic mechanisms. As in the case of the noradrenergic system, the changes documented by the studies in vitro utilized extremely high doses, which are probably of little clinical significance. The studies in vivo involving measurements of dopamine content and dopamine receptor changes (as indicated by behavioral, respiratory, and convulsant effects) suggest that digitalis drugs might reduce CNS dopaminergic function. There are also contrary data, with emesis and also tissue content of the metabolite, DOPAC, as endpoints, to suggest that digitalis might activate CNS dopamine receptors.

Consistent with data suggesting that digitalis drugs reduce CNS dopaminergic function are recent findings pertaining to the effect of CNS administration of the dopamine receptor agonist drug, apomorphine, on the arrhythmogenic doses of systemically administered deslanoside in cats. Apomorphine administered into the fourth cerebroventricle was found to increase markedly the doses of deslanoside required to produce ventricular arrhythmias and ventricular fibrillation (349). However, no protection was seen when apomorphine was localized to the third and lateral ventricles. Administration of the dopamine receptor blocking agent, haloperidol, counteracted the protective effect of apomorphine. These results

suggest that digitalis may block dopamine receptors that are present in the hindbrain and that this blockade may contribute to the increase in sympathetic outflow produced by digitalis. Consistent with this idea is the finding that when dopamine receptor blocking agents, e.g., haloperidol and pimozide, are administered directly into the brain, increases in sympathetic outflow occur as reflected by increases in both heart rate and blood pressure (178, 872). The findings dealing with the interaction of digitalis, apomorphine, and haloperidol on cardiac rhythm changes in cats also fit with the notion that activation of CNS dopaminergic mechanisms results in a decrease in central sympathetic outflow to the heart (231, 448).

D. SEROTONIN. One study has been performed in vitro that indicated that digitalis may increase the release of serotonin from brain tissue. Segawa et al. (712) observed that ouabain (1×10^{-4} M) increased the spontaneous release of [^{3}H]serotonin from rat brain synaptosomes. These investigators also found that ouabain attenuated the release of [^{3}H]serotonin induced by potassium in this preparation, and attributed this response in part to the marked increase observed in the spontaneous release of serotonin. In a study in vivo with conscious cats, Gaitonde and Joglekar (256) demonstrated that an intracerebroventricular injection of either ouabain (10 to 20 μg) or peruvoside (5 to 20 μg) resulted in a dose-related increase in serotonin collected from the ventricular perfusate 15 minutes after injection of these drugs. The increased release of serotonin correlated with the development of digitalis-induced neurotoxicity, leading the authors to conclude that the "neurotoxicity of digitalis glycosides may be due to massive release of serotonin over widespread areas in the brain."

Uptake studies with digitalis have been performed in vitro with brain slices and synaptosomes. Blackburn et al. (79) reported that ouabain inhibits [^{3}H]serotonin uptake into rat brain slices. This was later confirmed by Shaskan and Snyder (724), who used the same preparation. Numerous investigators (85, 434, 561, 584, 815, 816) have demonstrated that ouabain will inhibit [^{3}H]serotonin uptake into synaptosomes taken from whole brain, brainstem, and raphe of the rat. The concentrations of ouabain employed in these studies ranged from 1×10^{-5} to 1×10^{-3} M. Serotonin uptake processes in cerebral cortex slices appear to be more sensitive to ouabain in the mouse than in the rat, since the ED50 for uptake inhibition in mice has been shown to be 3×10^{-6} M (121). Inhibition of serotonin uptake into CNS tissue has also been demonstrated in vivo. Palaic et al. (599) administered ouabain (1×10^{-5} M) along with radiolabelled serotonin into the lateral ventricle of the perfused rat brain and reported an inhibition of serotonin uptake. Helke et al. (350) examined the effect of a lethal arrhythmogenic dose of deslanoside administered to chloralose-anesthetized cats on the uptake of [^{3}H]serotonin into the forebrain, hypothalamus, midbrain, medulla-pons, and area postrema. Deslanoside administration has a pronounced inhibitory effect (approximately 50%) on the uptake of [^{3}H]serotonin into area postrema homogenates. This striking effect on [^{3}H]serotonin uptake into the area postrema was not observed in any of the other areas tested.

The effect of digitalis on the synthesis of serotonin in CNS tissue has been examined in only one study. Deslanoside was administered to cats in a lethal arrhythmogenic dose and the activity of tryptophan hydroxylase was measured in several brain areas and the spinal cord (353). The activity of this enzyme was reduced in the caudate nucleus, but was unchanged in the hypothalamus, colliculi, amygdala, midbrain, hippocampus, medulla-pons, cerebellum, and spinal cord.

From the reports (624, 679) that digitalis can inhibit the activity of monoamine oxidase, which is the catabolic enzyme for serotonin, norepinephrine, and dopamine, it would appear that digitalis should affect the degradation of serotonin. However, Pletscher et al. (621) found that the formation of [^{14}C]5-hydroxytryptamine metabolites was not diminished by ouabain (1×10^{-6} to 1×10^{-4} M) and concluded that this agent was not a monoamine oxidase inhibitor. In addition, Helke et al. (353) measured the end product of monoamine oxidase action on serotonin, 5-hydroxyindoleacetic acid, and found it to be unchanged after deslanoside intoxication in cats. Furthermore, there was no difference between the 5-hydroxytryptamine to 5-hydroxyindoleacetic acid ratio of several brain areas of control and deslanoside-intoxicated cats.

The effect of digitalis on the CNS tissue content of serotonin has been examined in several studies. Palaic et al. (599) reported that ouabain (1×10^{-5} M) administered concomitantly with serotonin into the perfused rat brain reduced the total brain content of serotonin and 5-hydroxyindoleacetic acid. Buterbaugh and Spratt (108) reported that the LD25 of digitoxigenin in rats decreased the serotonin content of whole brain by 18% without altering norepinephrine or dopamine levels, and that the LD50 reduced both serotonin (35%) and norepinephrine levels (36%) in the brain. The decrease in monoamines was not observed in pargyline-treated rats, indicating that the decline was probably due to an increase in turnover rather than a decrease in synthesis.

In contrast to the effects of high doses of digitalis in rats, intracerebroventricular injections of small doses of ouabain (0.1 to 0.4 μg) into mice evoked marked changes in behavior but did not alter whole brain serotonin content (194).

Helke et al. (353) examined the effect of deslanoside intoxication on brain and spinal cord levels of serotonin and 5-hydroxyindoleacetic acid in anesthetized cats. Analysis of tissues taken at the time of ventricular fibrillation induced by deslanoside revealed no significant effects of this agent on either serotonin content or 5-hydroxyindoleacetic acid content of hypothalamus, midbrain, colliculi, amygdala, caudate nucleus, medulla-pons, hippocampus, or spinal cord.

Evidence that digitalis influences CNS serotonergic

mechanism can also be obtained from studies in rodents and cats dealing with digitalis-induced behavioral, respiratory, and convulsant effects. Digitoxigenin-induced convulsions and lethality in mice after acute i.v. administration were antagonized by reserpine pretreatment (441). In a later study, Buterbaugh and Spratt (107) found that specific depletion of serotonin with *p*-chlorophenylalanine (PCPA), an agent that inhibits the synthesis of serotonin, protected rats against digitoxigenin-induced convulsions, respiratory depression, and death while norepinephrine depletion with the norepinephrine synthesis inhibitor, α-methyl-*p*-tyrosine, had no effect. They concluded that "a digitoxigenin-biogenic amine interaction or, at the least, that intact brain amine levels, have a permissive role in the development of digitoxigenin toxicity and lethality." Buterbaugh and London (109) reported that the ability of digitoxigenin to lower the electroshock seizure threshold in mice was antagonized by serotonin depletion. Serotonin depletion was produced by treating animals with PCPA.

Convincing evidence that CNS serotonergic systems are affected by digitalis toxicity has also been presented by Gaitonde and Joglekar (256). These investigators found that administration of peruvoside or ouabain by intracerebroventricular injection into cats produced labored respiration, restlessness, tremor, rage, convulsions, and death. Prior administration of either 2-bromolysergic acid diethylamide (BOL-148), a serotonin receptor antagonist, or PCPA, reduced the incidence and severity of these neurotoxic effects. In addition, while BOL-148 lowered the incidence of death, PCPA pretreatment completely prevented death from central injections of peruvoside and ouabain.

To summarize, digitalis drugs do produce significant changes in CNS serotonin mechanisms. Many of the observations in vitro with high doses have been confirmed with lower doses in vivo. That is, digitalis administered in vivo enhances release of serotonin, inhibits uptake of this neurotransmitter, inhibits the activity of the synthesizing enzyme tryptophan hydroxylase in the caudate nucleus, and reduces the content of serotonin and 5-hydroxyindoleacetic acid in some studies (with rats) but not in other studies (with cats). In addition, toxicity studies not involving the cardiovascular system in rodents and cats suggest an interaction of digitalis drugs with CNS serotonin mechanisms, leading to activation of this system.

To obtain information on whether CNS serotonin mechanisms are activated by arrhythmogenic doses of digitalis, Helke et al. (347) pretreated cats with the serotonin neurotoxin, 5,7-dihydroxytryptamine (57). Administration of this agent into the cerebroventricles of anesthetized cats, in a dose that caused significant depletion of serotonin in the hypothalamus, midbrain, medulla-pons, and spinal cord, had no effect on the dose of deslanoside required to cause ventricular tachycardia and ventricular fibrillation. In addition, arrhythmogenic doses of deslanoside were found to have no effect on serotonin and 5-hydroxyindoleacetic acid content of brain regions known to be involved in cardiovascular regulation (353). This was also the case for serotonin uptake. The only CNS area affected by arrhythmogenic doses of deslanoside was the area postrema. In this region, deslanoside caused approximately 50% inhibition of tritiated serotonin uptake (350). This effect should result in amplification of serotonergic function at this site but the significance of this enhancement is unclear.

The area postrema is known to be an important site of action for the emetic effect of digitalis (133) and may be involved in the arrhythmogenic effect of these drugs as well. This has been suggested by Schoener et al. (707), Helke et al. (350), and by Somberg and Smith (755, 757). Schoener et al. based their suggestion on observations that high levels of digitalis are concentrated in the area postrema after intravenous administration. Somberg and Smith reached this conclusion from studies showing that exclusion of the area postrema will increase the cardiotoxic dose of digitalis. Based on the biochemical findings of Helke et al. (350), an interaction of digitalis with the area postrema might involve an alteration of serotonin function.

E. ACETYLCHOLINE. The information on the effects of digitalis on cholinergic mechanisms in CNS tissue is very limited. The report of Vizi (849) appears to be the only one to deal with the effect of digitalis on the release of acetylcholine from central neural tissue. He found that ouabain (2×10^{-5} M) enhanced the spontaneous release of acetylcholine from rat brain cortical slices in vitro. There are a few reports on the effect of digitalis on the activity of cholinesterase in the brain, but the findings were inconclusive, demonstrating no change (311, 412, 719, 824, 848), a decrease (728), or an increase (412) in cholinesterase activity of brain tissue with digitalis. They are discussed below in the section on peripheral neurotransmitters. There are no studies that we are aware of on the effects of digitalis on the uptake, synthesis, or content of acetylcholine in the CNS. Evidence that digitalis influences CNS acetylcholine mechanisms can be inferred from a study in which digitalis was administered directly into the brain to evoke changes in cardiac rhythm. Dogs pretreated with agents that block muscarinic receptors in the CNS (i.e., intracerebroventricular administration of *l*-hyoscyamine and ethybenztropine) prevented cardiac arrhythmias evoked by deslanoside (680). When these antagonists to acetylcholine were administered after the onset of cardiac arrhythmia, similar results were obtained.

F. GAMMA-AMINOBUTRYIC ACID (GABA). Levi et al. (463) reported that ouabain (1×10^{-4} M) enhanced GABA-induced release of [^{3}H]GABA from superfused rat brain synaptosomes obtained from cerebral tissue.

G. CONCLUSIONS. As mentioned in the introduction to the section on the effects of digitalis drugs on CNS neurotransmitters, many of these neurotransmitters have been implicated in the regulation of central autonomic outflow. Digitalis drugs have been demonstrated to affect

noradrenergic, dopaminergic, and serotonergic mechanisms. Studies in vivo involving measurements of cardiovascular indices suggest that digitalis drugs may enhance norepinephrine activity in the hypothalamus, enhance serotonin activity in the area postrema, and counteract dopamine activity in the hindbrain. Augmentation of norepinephrine activity in the hypothalamus increases central sympathetic outflow, and blockade of dopamine function in the hindbrain increases central sympathetic outflow. Thus, the interaction of digitalis drugs with these two monoamine systems may comprise part of the mechanisms whereby these agents increase sympathetic outflow and alter cardiovascular function. In addition, deslanoside has been found recently to inhibit selectively the uptake of serotonin in the area postrema, but the significance of this effect for central autonomic neural activity is not yet known. Little information is available on the effects of digitalis drugs on acetylcholine and GABA mechanisms, but since both these neurotransmitters can cause marked alterations in central autonomic outflow (see the introduction to this section), more studies should be carried out on the interaction of digitalis drugs with these neurotransmitter systems.

2. Peripheral nervous system. A. INTRODUCTION. Another mechanism whereby digitalis drugs can produce neuroexcitatory effects is by affecting the neurotransmitters contained in peripheral tissues. The major neurotransmitters that have been studied with digitalis drugs include norepinephrine, serotonin, and acetylcholine. The synaptic processes that have been examined include the release, uptake, synthesis, degradation, and storage of the neurotransmitters.

B. NOREPINEPHRINE. Studies that have been performed to examine the effects of digitalis on norepinephrine release have utilized isolated cardiac, vascular, splenic, iris, or vas deferens tissue. When digitalis drugs were administered in vitro to these preparations, catecholamine release, in general, was found to be enhanced.

Bogdanski and Brodie (83), with cardiac ventricle slices from rats preloaded with tritiated norepinephrine, reported that 1×10^{-3} M ouabain produced a slight increase in the spontaneous efflux of [^{3}H]norepinephrine. Katz and Kopin (401) studied the effect of ouabain on [^{3}H]-norepinephrine release from isolated rat atria preloaded with the radioactive amine. Release was evoked by electrical field stimulation and presumably was due to an effect on sympathetic nerve endings since the process was calcium dependent. Administration of 1×10^{-6} M ouabain into the bathing medium had no effect. Administration of 1×10^{-4} to 1×10^{-3} M ouabain caused a decrease in release. Paton (606) reported that spontaneous release of norepinephrine from atria taken from rabbits pretreated with reserpine and then preincubated with [^{3}H]norepinephrine was enhanced by ouabain (1×10^{-5} to 1×10^{-4} M). He concluded that the observed increase in release was real and not due to inhibition of [^{3}H]norepinephrine uptake induced by ouabain, since drugs known to inhibit uptake (e.g., cocaine and desmethylimipramine) had no effect on the efflux of [^{3}H]-norepinephrine in his preparation. Lindmar and Löffelholz (482) also reported that ouabain in a concentration of 1×10^{-4} M produced an increase in the spontaneous efflux of norepinephrine from the isolated perfused rat heart. This effect required the presence of sodium in the extracellular fluid. Harvey (338) showed that ouabain (5 to 10×10^{-6} M, and 5.5×10^{-5} M) increased spontaneously occurring norepinephrine release from isolated perfused rat and guinea-pig hearts, respectively. Seifen (714) demonstrated a loss of radiolabelled norepinephrine from isolated guinea pig atria preloaded with radioactive norepinephrine. The increase in spontaneous release of norepinephrine was accompanied by a positive chronotropic effect (i.e., a shortening of cyclic length).

In a recent and interesting study, Lorenz et al. (486) reported that superfusion with acetylstrophanthidin (6 μg/min) of strips of dog saphenous veins preloaded with [^{3}H]norepinephrine resulted in an enhancement of both spontaneously occurring and neurally evoked release of the neurotransmitter. They found that the enhancing effect was prevented by pretreatment with either alpha- or beta-adrenergic receptor blockers, and concluded that acetylstrophanthidin was acting at prejunctional sites to enhance release.

Kirpekar et al. (414) examined the effect of 1×10^{-4} M ouabain on the release of norepinephrine from the perfused spleen of the cat. This concentration of ouabain enhanced spontaneous norepinephrine release as well as potassium-induced norepinephrine release. However, 1.3×10^{-5} to 1×10^{-4} M concentrations of ouabain blocked the norepinephrine release caused by electrical stimulation of the splenic nerve. Garcia and Kirpekar (260) incubated spleen slices from cats pretreated with reserpine with [^{3}H]norepinephrine and demonstrated that 1×10^{-5} M ouabain increased spontaneous release of the amine. They concluded that a significant part of the ouabain effect was due to increased release, as a drug known to inhibit both release and uptake (guanethidine) had less effect on [^{3}H]norepinephrine release than did ouabain. In a second study performed in the same year in which spleen slices were incubated with [^{3}H]norepinephrine, Garcia and Kirpekar (261) reported that ouabain in concentrations ranging from 1×10^{-7} to 1×10^{-4} M had very little effect on calcium-induced release of norepinephrine. In a study wherein ouabain was tested on the spontaneous release of norepinephrine from isolated nerve granules of bovine splenic nerves, a concentration of 1.4×10^{-4} M was found to be without effect (218).

Farnebo (223) demonstrated ouabain-induced release of norepinephrine from iris tissue incubated with [^{3}H]-norepinephrine. He examined the effect of a 1×10^{-3} M concentration of the drug on release evoked by field stimulation, and observed an initial increase in neurally evoked release followed by a decrease in efflux.

Katsuragi and Suzuki (399) demonstrated ouabain-induced release of norepinephrine from neuronal as well as

extraneuronal sites. They used the isolated guinea-pig vas deferens and incubated the tissue with high concentrations of norepinephrine as well as with epinephrine (1×10^{-6} to 1×10^{-4} M). Concentrations of the amines in this range are stored extraneuronally as well as neuronally. With the contractile activity of the guinea-pig vas deferens as an index of neuronal (early contraction) and extraneuronal (late contraction) release, 1×10^{-5} M ouabain was found to evoke both contractile phases, suggesting release of amines from both pools.

In summary, the digitalis drugs (ouabain and acetylstrophanthidin) that have been administered in vitro to isolated organ preparations appear to increase the spontaneous release of norepinephrine, as well as the release of this neurotransmitter induced by nerve stimulation or by the administration of potassium. In most of the studies performed it was the release of neuronal norepinephrine that was affected. However, data from one study suggested that extraneuronal release of norepinephrine was also affected (399). In some cases, digitalis was found to reduce and block the release of norepinephrine evoked by nerve stimulation, which fits with the finding that large doses of these drugs can block axonal conduction (258). Norepinephrine release induced by calcium was not affected by digitalis. The mechanism whereby these drugs enhance release is not clear. There are three possibilities 1) inhibition of Na^+-K^+-activated ATPase; 2) direct depolarization of nerve endings, which is probably related to Na^+-K^+-ATPase inhibition; and 3) an increase in intracellular calcium concentration, as suggested by Duncan (200). It would seem that the first two mechanisms are the most plausible because release of norepinephrine from granules is not affected by digitalis, and uptake and release processes in granules are not dependent on Na^+-K^+-activated ATPase as are uptake and release processes of the neuronal membrane.

A large number of experiments have been performed in which the effect of digitalis drugs on the uptake of norepinephrine has been studied. Since uptake of norepinephrine into the neuronal membrane is dependent on membrane Na^+-K^+-activated ATPase, it would be anticipated that digitalis drugs should inhibit the uptake of this amine. This indeed appears to be the case, since studies with heart slices (65, 66, 83, 180, 181, 277, 604, 605, 723, 777, 778, 786, 817), isolated perfused whole hearts (210, 455), spleen slices (180, 181, 723, 777, 778), isolated perfused lungs (391, 580), isolated intestinal smooth muscle (803), and human platelets (13) indicate that digitalis drugs (primarily ouabain but in a few cases, digoxin and digitoxin) administered in vitro in concentrations ranging from 1×10^{-7} to 1×10^{-3} M inhibit the active neuronal uptake of norepinephrine and metaraminol. Metaraminol was employed in some studies in place of norepinephrine because it is not as subject to metabolism during the course of an experiment (65, 66). The wide range of effective digitalis concentrations appears to be related to the differing susceptibilities of various species, as pointed out by Stickney (777, 778). Her studies indicated that uptake of catecholamines into tissues of dogs is affected by concentrations of digitalis as low as 1×10^{-7} M, whereas uptake into tissues of rats requires as much as 1×10^{-3} M; the sensitivity of guinea-pig tissue was intermediate between that of dogs and rats. A similar species sensitivity to the inhibitory effect of ouabain on catecholamine uptake was demonstrated by Sharma and Banerjee (723).

In a few studies, digitalis was shown not to have a significant effect on the uptake of norepinephrine by cardiac tissue (563, 590). These negative results can be attributed to failure to use a large enough dose of digitalis. One of these groups of investigators (590) also reported that under certain experimental conditions, digitalis can increase the uptake of norepinephrine by the heart. By utilizing the guinea-pig heart-lung preparation that was acutely "failed" by mechanically increasing outflow tract resistance and overloading the left ventricle, a marked decrease in the myocardial uptake of norepinephrine was seen. After administering ouabain the uptake of norepinephrine was increased and restored to normal. It appears from these data that in hearts rendered hypodynamic, unlike those from normal animals, the uptake of norepinephrine can be enhanced by digitalis.

In studies in which digitalis was tested on norepinephrine uptake into isolated nerve granules (218) and into extraneuronal sites (211), no inhibition was observed. This fits with the finding that uptake processes into granules and into extraneuronal sites are not dependent on the activity of membrane Na^+-K^+-activated ATPase (607, 615).

Only a few studies have been performed in which the effect of digitalis drugs on catecholamine uptake has been examined in vivo. Carlsson and Waldeck (124) reported that ouabain, 2 mg/kg, administered i.v. to mice had no effect on the accumulation of [^{3}H]metaraminol by the heart. Cotten et al. (158) administered ouabain, 60 to 70 μg/kg i.v., to dogs that previously had been given radiolabelled norepinephrine. Measurement of [^{3}H]norepinephrine in biopsy samples of the right ventricle indicated no effect on uptake by ouabain. In contrast, Eikenburg and Stickney (210) reported that ouabain administered i.v. to guinea pigs in doses of 150 to 212 μg/kg inhibited the uptake of metaraminol by the heart. Only the highest doses tested by these investigators caused arrhythmias. Helke et al. (350) examined the effects of a lethal arrhythmogenic dose of deslanoside on [^{3}H]norepinephrine uptake in atrial and ventricular tissue of anesthetized cats. They observed a significant inhibition of norepinephrine uptake in the left ventricle but not in the right ventricle or in either of the atria.

The mechanism by which digitalis acts to inhibit the uptake of norepinephrine into nerve endings has not been firmly established. Some investigators have attributed the effect to inhibition of Na^+-K^+-activated ATPase (65,

66, 83, 277, 723, 777), whereas others have found no relation between inhibition of uptake and inhibition of the enzyme (455, 563, 874). It is of interest, however, that Stickney (777), and Sharma and Banerjee (723), have drawn attention to the observation that the species-dependent inhibition of norepinephrine uptake by digitalis in adrenergic nerve endings of cardiac and splenic tissue in vitro parallels the ability of digitalis to exert a species-dependent inhibition of Na^+-K^+-activated ATPase enzyme activity in the same tissues.

The recent finding by Sharma and Bannerjee (722) has confirmed an important effect of digitalis on Na^+-K^+-activated ATPase associated with neurones. These investigators demonstrated that [^{3}H]ouabain binds to Na^+-K^+-ATPase of cardiac sympathetic nerve endings. Indeed, over 80% of the binding in cardiac tissue was found to be to the Na^+-K^+-ATPase of nerve endings imbedded in the tissue.

The effect of digitalis on tissue content of catecholamines has been studied by numerous investigators and the results obtained cover the entire spectrum of possible effects. A decrease in catecholamines was reported in cardiac tissue of rats (131, 132, 136, 237, 338, 566), guinea pigs (308, 338, 488, 490), mice (338) dogs (145, 152), and rabbits (22), and for splenic tissue of cats (260, 261, 414). These experiments were performed by administering a wide range of doses of digitalis preparations parenterally and by analyzing excised tissues for content of catecholamines. No change in catecholamine content was reported for cardiac tissue of rats (43, 238), guinea pigs (238), rabbits (679), and cats (360, 787). In these studies the digitalis preparations employed were also administered parenterally. In a few other studies, the digitalis preparations were administered to isolated perfused hearts (423, 455, 801). In regard to other tissues, Hertting et al. (360) demonstrated that digitalis administered in vivo to cats had no effect on either spleen or liver catecholamine content. Some investigators have observed an increase in catecholamine content to occur. These studies all were performed with cardiac tissue and with administration of digitalis in vivo. In one study in dogs, Ciofalo and Treece (145) found that a lethal dose of ouabain produced an increase in the total cardiac content of catecholamines. In another study performed in cats, Helke (345) examined the effect of deslanoside intoxication on cardiac norepinephrine levels. Administration of a lethal arrhythmogenic dose of deslanoside had no significant effect on the norepinephrine content of the atria and ventricles. In two studies performed in rats in which heart failure was present as a result of mechanical constriction of the aorta, digitalis drugs administered parenterally increased the cardiac catecholamine content (43, 137). However, the cardiac catecholamines of these animals had been partially depleted by heart failure, and digitalis administration resulted only in restoration of the amine levels toward normal.

The disparate results obtained with digitalis on tissue catecholamine content might be due to the fact that digitalis exerts several effects that can alter catecholamine content in either direction. For example, monoamine oxidase inhibition has been reported to occur with high doses of digitalis (624), and this could lead to an increase in catecholamine content (679). Similarly, digitalis has been reported to release catecholamines from the adrenal glands (42, 583, 656) and the resultant increase in circulating amine levels could lead to uptake of catecholamines by other tissues. Finally, the effect of digitalis either to release catecholamines or to inhibit their uptake into the various tissues could result in a reduction in content. Hence, the end result of digitalis would depend on the balance of the outcome of the effects of these various actions.

In summary, data obtained from studies dealing with the effect of digitalis drugs on norepinephrine release from peripheral tissues indicate that these agents produce an increase in release. Studies performed in vivo would be needed in order to confirm this effect. In addition, these agents have also been shown to inhibit uptake of norepinephrine into neuronal tissue. This is true of studies both in vitro and in vivo. Finally, the effect of digitalis drugs on tissue content of catecholamines is unclear. The effect of these drugs probably depends on the net effect of digitalis on release, uptake, and degradation in nerve endings, as well as on the adrenal gland.

C. SEROTONIN. The effect of digitalis on serotonin release has been examined in only one study. Okuda and Nemerson (589) reported that ouabain (1×10^{-4} M) administered in vitro to human platelets preloaded with radiolabelled serotonin increased the efflux of the amine.

Most of the studies on the effects of digitalis on the uptake of serotonin have used the platelet as the experimental model, and the digitalis drugs were adminstered in vitro in concentrations from 1×10^{-7} to 1×10^{-3} M. Platelets from rats (739), guinea pigs (621), rabbits (46), and people (119, 589, 764, 765, 869) were used and, in each instance, digitalis inhibited the active uptake of serotonin. In vivo, Godefroy and Weil-Fugazza (297) found that the plasma levels of free serotonin were increased in animals given a 40 μg/kg i.v. dose of ouabain and exposed to a continuous i.v. infusion of serotonin. These investigators attributed the increase in plasma serotonin to an effect of ouabain to prevent the uptake of serotonin into platelets. In another study in vivo, Helke et al. (351) found that a lethal arrhythmogenic dose of deslanoside reduced the uptake of [^{3}H]serotonin into cat platelets.

There are two studies in which ouabain (1×10^{-4} and 1×10^{-3} M) was tested in vitro on serotonin uptake into vesicles of rabbit and human blood platelets (176, 681). In agreement with other studies on vesicular uptake (122, 123, 218, 415), no effect on uptake was observed. The lack of effect of digitalis on the uptake of serotonin into platelet granules has been reiterated in other articles on

subject (622, 740).

Several miscellaneous types of tissue have also been studied. These include the isolated perfused lungs of rats (395) and rabbits (391), the isolated small intestine (276) and the isolated vas deferens of guinea pigs (809), and the isolated subesophageal ganglion of the snail (592, 766). In each study ouabain was used in concentrations ranging from 1×10^{-5} to 1×10^{-3} M and was found to inhibit the uptake of serotonin.

Only two groups have examined the effect of digitalis on the tissue content of serotonin. In a study in vitro, Pletscher et al. (621) reported that ouabain (1×10^{-7} to 1×10^{-4} M) produced no significant decrease in the platelet content of endogenous serotonin in guinea pigs. In a study in vivo, Helke (345) examined the effect of a lethal arrhythmogenic dose of deslanoside on serotonin and 5-hydroxyindoleacetic acid levels in the heart, whole blood, and plasma of anesthetized cats. Deslanoside administration increased the serotonin content of the right atrium without significantly changing the content of serotonin in the left atrium, both ventricles, intraventricular septum, papillary muscle, whole blood, or plasma. Deslanoside had no effect on the 5-hydroxyindoleacetic acid content of cardiac tissue, whole blood, or plasma.

In summary, digitalis drugs have been shown to exert an effect on the release and uptake of serotonin, and these actions occur in platelets. Enhancement of release and prevention of uptake have been demonstrated. These effects can lead to an alteration in sympathetic function since it has been shown that serotonin can cause ganglionic stimulation (829), inhibition of norepinephrine uptake (393), release of norepinephrine from sympathetic nerves (230, 241), and release of catecholamines from the adrenal medulla (649).

D. ACETYLCHOLINE. There is considerable evidence to support the idea that digitalis drugs can enhance the release of acetylcholine from parasympathetic nerve endings. This effect has been demonstrated directly in studies in which the output of acetylcholine from neural tissue has been measured after the administration of digitalis. Birks (74) reported that spontaneous release of acetylcholine from the cat superior cervical ganglion perfused with eserinized choline-Locke's solution was enhanced more than 10-fold in the presence of 6.4×10^{-6} M of digoxin. Similar results were also obtained with ouabain. Paton et al. (608) also reported that the spontaneous release of acetylcholine from the guinea-pig ileum longitudinal strip was enhanced in the presence of ouabain (1.4×10^{-6} to 5×10^{-5} M). Finally, Vizi (849) reported that the spontaneous release of acetylcholine from the guinea-pig ileum longitudinal muscle strip was enhanced by ouabain (2×10^{-5} M). Indirect evidence that digitalis can increase the release of acetylcholine is discussed above in the sections dealing with the effects of digitalis on ganglionic transmission and peripheral and motor nerves.

The effect of digitalis drugs on the activity of the enzyme cholinesterase taken from blood (serum and red cells) and cardiac and neural tissue has been widely studied. The studies have been performed in preparations both in vitro and in vivo. The investigators who used blood or red cell cholinesterase as their test preparation and administered digitalis in vitro have reported either no effect on cholinesterase activity (4, 128, 435, 551, 719, 847, 848) or an inhibition of this enzyme (164, 529, 892). The enzyme used in these studies was taken from the horse, the rat, or from humans. The same picture emerges from the studies in vivo. Some investigators reported no change in cholinesterase enzyme activity (435, 551, 847), while others reported an inhibition of cholinesterase activity (173, 175). All of these studies dealt with serum esterase activity, and the species employed were the cat, rabbit, guinea pig, and dog. In regard to the effect of digitalis on the cholinesterase activity of cardiac and neural (brain extracts or electric organ of the eel) tissue, the results of studies in vitro and in vivo have demonstrated for the most part no change in the activity of this enzyme (311, 412, 551, 719, 824, 848). The finding of Shinohara (728) is an exception. He reported inhibition of cholinesterase activity of both cardiac and brain medullary tissue of cats exposed to a lethal dose of digitoxin. G-Strophanthin and convallatoxin also inhibited the cholinesterase activity from cardiac tissue.

The differences observed in the effects of digitalis on cholinesterase activity cannot be explained on the basis of the species used, the digitalis preparation or dose of digitalis employed, or whether the study was performed by administering digitalis in vivo or in vitro. However, essentially every study performed wherein cholinesterase activity was found not to be inhibited by digitalis utilized biochemical techniques for the measurement of enzyme activity (4, 128, 311, 412, 435, 551, 719, 824, 847, 848). These biochemical techniques consisted of manometric measurement of the amount of CO_2 generated in a system from the acetic acid discharged from the hydrolysis of acetylcholine. The only exception was the study of Zinnitz and Rentz (892), who employed a manometric technique but used extraordinarily high doses of digitalis (up to 20 mg of K-strophanthin administered in vitro) and found a decrease in the activity of cholinesterase taken from human blood. In general, in the other studies reporting an inhibitory effect of digitalis on cholinesterase activity bioassay techniques were used to assess the activity of the enzyme. The bioassay preparations included the isolated frog rectus abdominus muscle (164, 529) and the arterial blood pressure response of intact animals (173, 175). The responses of these systems to acetylcholine were found to be enhanced in the presence of digitalis. This potentiation of the effect of acetylcholine was attributed to inhibition by digitalis of cholinesterase activity. However, these data can be interpreted in another way; the potentiated response could be due to potentiation of the effect of acetylcholine on the postsynaptic receptor.

There are two studies with cardiac and brain tissue in which digitalis was reported to increase the activity of cholinesterase. Govier et al. (311) demonstrated that the administration of barbiturates and cholinesterase inhibitors produced cardiac decompensation and an inhibition of cholinesterase activity in the canine heart-lung preparation. Administration of 100 μg of ouabain reversed the cardiac decompensation and in some cases it increased the activity of cholinesterase. Kimura (412) administered digitoxin, ouabain, and digiglusin i.p. to rats, guinea pigs, and frogs and found an increase in cholinesterase activity of brain medullary tissue.

There are a few studies in which the effects of digitalis on the tissue content of acetylcholine were studied. The tissue used in each case was the heart, and preparations both in vivo and in vitro were employed. Kull (435) reported that 0.05 mg of strophanthin increased the acetylcholine content of isolated frog heart muscle. However, no increase was reported for the isolated cat heart. Similar results were reported by Staub and Kull (769) for frog and cat heart muscle with strophanthin. Madan et al. (514) reported that 1×10^{-5} M of deslanoside increased the acetylcholine content of isolated rabbit atria. Torsti (824) demonstrated that G-strophanthin (40 to 60 μg/kg given i.v. over 5.5 to 16 hours) increased the right atrial acetylcholine content of rabbits that previously had been depleted of acetylcholine in both atria by experimentally induced heart failure.

The importance of digitalis-induced changes in acetylcholine release from artificially perfused preparations of peripheral nerves is difficult to evaluate in terms of the total effect of digitalis action on parasympathetic nervous system function. The doses of digitalis demonstrated to have an effect are larger than those that are achieved by administering the drug in vivo. Furthermore, enhancement of parasympathetic activity by digitalis could be explained in terms of enhancement of the postsynaptic receptor response to this neurotransmitter (821, 822). The importance of digitalis-induced changes in acetylcholine content as a mechanism for enhancement of parasympathetic nervous system function is also unclear.

B. Effect on the Electrogenic Sodium Pump

Data describing an important effect of digitalis on the electrogenic sodium pump have been obtained from studies in which investigators have examined these agents on the neural activity of baroreceptors. For example, Saum et al. (694) demonstrated that ouabain prevented postexcitatory depression (PED) in an aortic arch-aortic nerve preparation in vitro of the rat. PED occurred in this preparation after removal of a pressure step or termination of antidromic stimulation of the aortic nerve. The increase in sodium in the nerve ending resulting from baroreceptor excitation stimulates an electrogenic sodium pump that results in hyperpolarization. Digitalis, because of its ability to inhibit the electrogenic sodium pump (810), will prevent PED. Saum et al. (694) also demonstrated that ouabain could lower the pressure threshold for both transient and steady state discharge of the aortic nerve. In addition, a more prolonged exposure to ouabain (5 to 10 minutes as opposed to 2 to 5 minutes for the above described effects) produced spontaneous activity in the aortic nerve fibers. These effects were also attributed to an action of ouabain on the electrogenic sodium pump in the nerve ending. The change in pressure threshold could occur as a consequence of an alteration in resting membrane potential. This is consistent with the finding that inhibition of the electrogenic sodium pump will reduce the resting membrane potential of sensory neurons (744). Furthermore, a reduction in resting membrane potential can change the performance of a neuron if the resting potential lies close to the critical firing threshold (744). This change would be manifested as spontaneous discharge of the neuron.

In the study of Quest and Gillis (635), it appeared that administration of digitalis altered the adaptative properties of baroreceptors. It was found that the gradual reduction in neural activity that occurred after a step increase in carotid sinus pressure was reduced in the presence of ouabain. However, Saum et al. (694) found no evidence that ouabain could alter the adaptation that occurred in their preparation. The use of multifiber preparations by Quest and Gillis and single fiber preparations by Saum et al. probably accounts for the difference in findings between the two laboratories. It is possible that recruitment of fibers was responsible for the effects seen with the multifiber preparations.

In summary, the ability of digitalis to sensitize baroreceptors as shown in studies in vivo appears to be due to inhibition of an electrogenic sodium pump. Digitalis has also been observed to excite peripheral chemoreceptors but whether this is due to inhibition of an electrogenic sodium pump is not known.

Wherever an electrogenic sodium pump exists in neural tissue, digitalis drugs have the potential to produce an excitatory effect. Small nerve fibers are described as having this pump (646), and hence inhibition of this pump by digitalis drugs could explain the neuroexcitatory effects seen in efferent autonomic nerves (806) and in ganglia (479, 737). The motor nerve terminal also has an electrogenic sodium pump, and inhibition of this pump could explain the neuroexcitatory effects seen to occur with studies in vivo (471) as well as the enhanced release of acetylcholine that has been described in isolated preparations (74, 75).

C. Effect on Specific Receptor Mechanisms

There are some data suggesting that digitalis might act specifically on receptors and either augment or counteract the action of a particular neurotransmitter. Studies have been reported in which an enhancement of the response of the muscarinic receptor to acetylcholine was demonstrated. This has been shown for the responses of

the S-A and the A-V nodes (715, 821, 822) and sympathetic ganglia to acetylcholine (268, 284). Both augmentation and blockade of beta receptors have been described for digitalis. Broekaert and Godfraind (100) described enhancement of isoproterenol-induced relaxation of guinea-pig aorta with ouabain. Pearle and Gillis (610) observed an apparent enhancement of isoproterenol-induced chronotropic effects in ventricular tissue. Mendez et al. (545, 546) described studies in which digitalis was found to antagonize epinephrine-induced chronotropic and dromotropic effects on the S-A node and A-V conduction system, respectively. This effect was described by Ten Eick and Hoffman (807) as an apparent beta-adrenergic blocking effect. Finally, Tuttle and Innes (834) reported data that suggested a direct pharmacological antagonism between the beta-adrenergic blocking agent, pronethalol, and ouabain in the heart. They found that ouabain enhanced the cardioaccelerator action of isoproterenol on the S-A node in the presence of beta-adrenergic blockade with pronethalol.

There is also some evidence to suggest that digitalis drugs will block dopamine receptors in the brain. These data have been obtained in rodents with behavioral changes as endpoints (192) and in cats with cardiac rhythm changes as endpoints (349).

D. Effect on Postsynaptic Membranes

Digitalis drugs, as indicated in section II C 4 b, augment postsynaptic vascular effects of norepinephrine. The mechanism for this effect appears to involve calcium storage and release at the vascular level (233, 820). According to Flaim and DiPette (233), digitalis causes calcium release from sarcolemmal storage sites in arterial smooth muscle and this results in potentiation of norepinephrine-induced vasoconstriction. Their evidence was based on the finding that the calcium antagonist, verapamil, could counteract the norepinephrine-potentiating effects of digoxin.

IV. Role of the Nervous System in the Cardiovascular Effects of Digitalis

A. Cardiac Contractile Force

Probably the most important action of digitalis drugs from a clinical viewpoint is the positive inotropic effect. The sympathetic nervous system has been implicated as being important in this action. Some studies, with isolated cardiac atrial and ventricular tissues or the heart-lung preparation, have indicated that part of the inotropic action of digitalis may be due to the release of catecholamines. In these studies prior treatment with beta-adrenergic receptor blocking drugs (64, 185, 236, 789), reserpine (114, 127, 184, 239, 473, 762, 799, 800, 802, 827, 828), or guanethidine (184, 800, 801) reduced the contractile force effect of digitalis. On the other hand, numerous investigators have been unable to confirm these observations. They reported that pretreatment of similar isolated cardiac preparations with beta-blocking drugs (12, 78, 224, 246, 423, 473, 554, 591, 690, 741, 827), reserpine (91, 224, 239, 246, 573, 662, 785, 873), and 6-hydroxydopamine (828) did not reduce the positive inotropic action of digitalis. Furthermore, studies in vitro with papillary muscles from chronic cardiac denervated and catecholamine-depleted cat hearts (762), atria from immunosympathectomized rats (873), and chick embryo hearts obtained prior to the development of the sympathetic nervous system (456) demonstrated no diminution of the positive inotropic action of digitalis. In a few instances, the inotropic effect of digitalis was enhanced in the presence of beta blockade (8, 343, 763) or reserpine (127). An examination of the data obtained from the isolated heart studies leads us to the same conclusion reached by others, namely, that the inotropic action of digitalis is not mediated by catecholamines but is due to a direct effect of the drug on the heart (206, 423, 762, 814).

There are several possible reasons why some investigators have observed that drugs that interfere with cardiac adrenergic mechanisms counteract the inotropic effects of digitalis. One reason relates to the direct negative inotropic effect of the agents themselves on the heart, which could offset the inotropic effect of digitalis. Direct myocardial depressant effects have been reported for the beta-adrenergic blocking drugs (185, 236, 576, 581), reserpine (573, 789, 799), and guanethidine (581). With the beta-blocking drugs and reserpine this effect may be due to alterations in the transport of myocardial calcium ions in directions that impair normal contractile mechanisms (573, 574, 576). Tremblay et al. (827, 828) have also indicated that the ability of 6-hydroxydopamine to modify the inotropic action of digitalis is related to its ability to reduce the basal level of contractile force in isolated cardiac preparations. They found that when 6-hydroxydopamine reduced the basal level of contractile force in isolated rat hearts, it also reduced the positive inotropic effect of ouabain. In contrast, when the drug did not reduce base-line contractility in isolated guinea-pig hearts, it did not alter the positive inotropic effect of ouabain. Since 6-hydroxydopamine depleted cardiac catecholamines in both preparations, it would appear that its sympatholytic effect per se was not important for the depressant effects on the inotropic activity of ouabain. Another reason why drugs such as the beta blockers and reserpine may have acted to reduce the positive inotropic effect of digitalis relates to their ability to inhibit the uptake of digitalis drugs into cardiac tissue (247, 518). Despite the cogency of such explanations, we nevertheless feel some misgivings about our conclusion in view of the findings that digitalis can release catecholamines from the heart (83, 338, 401, 482, 606, 713), block the reuptake of catecholamines from the heart (e.g., 181, 455), and produce a significant reduction in the level of catecholamines in the heart (e.g., 22, 131, 145, 338, 488).

In the more relevant situation of studies in vivo, drugs

or surgical denervation procedures that counteract the activity of the sympathetic nervous system either have no effect on the contractile force action of digitalis (105, 155, 204, 329, 450, 554, 559, 586, 736, 877, 879, 882, 887, 889) or enhance the inotropic response (9, 327, 787, 873). This again indicates that the positive inotropic effect of digitalis is not mediated through catecholamines. There are, however, three studies in which drugs that reduce sympathetic function were found to counteract the positive inotropic action of digitalis (90, 489, 730). These drugs are reserpine, propranolol, 6-hydroxydopamine, and α-methyl-p-tyrosine. Again, the reason for this effect may have nothing to do with the ability of these agents to interfere with sympathetic function. Instead, impairment of the inotropic effect of digitalis may be related to an alteration in the base-line contractility (827, 828), direct myocardial depressant actions of these agents (576), or interference with digitalis uptake into the myocardium (247, 518).

A difficulty in assessing whether the sympathetic nervous system (i.e., catecholamines) mediates the positive inotropic effect of digitalis in intact animals is that these drugs can induce changes in baroreceptor function (56, 634). With reflexes intact in the control situation, the administration of digitalis might diminish sympathetic tone and invalidate the employment of these animals as controls. That is, since sympathetic tone might be changed by positive inotropic doses of digitalis, it may not be possible to use these animals as controls for the study of drugs or procedures that might interfere with sympathetic tone. If animals with intact reflexes are used, a comparison will be made between two groups of animals that are alike in that both have lost their sympathetic tone; in the control group this is brought about reflexly by the cardiac glycoside, and in the test group it is brought about by drugs that suppress the efferent sympathetic activity (e.g., reserpine). Under these circumstances it would be difficult to evaluate the role of the sympathetic nervous system in the inotropic response of digitalis. The meaningful control would be an animal in which reflexes are not active and in which a constant background sympathetic tone exists throughout the experiment. In this case, the effects of procedures in the test group that depress the adrenergic nervous system can be assessed against a proper comparison group.

With these kinds of animals as controls, data obtained from one study (636) suggest that the positive inotropic effect of ouabain may be mediated in part by activation of the sympathetic nervous system. Ouabain (40 μg/kg) was administered i.v. to cats with reflexogenic areas of the cardiovascular system denervated but with an intact central nervous system and increased cardiac contractile force (with strain gauge arch recordings from the right ventricle) by 32%. Animals with reflexogenic areas denervated and subjected to midcollicular decerebration exhibited only a 7% increase in cardiac contractile force. Midcollicular decerebration per se had no significant effect on base-line arterial pressure or heart rate. These results indicate that ouabain may increase contractile force in cats by stimulating forebrain areas, resulting in an increase in sympathetic neural tone to the heart. In support of a positive inotropic action of digitalis arising from the central nervous system is the finding that acetylstrophanthidin, in doses from 6 to 12 μg/kg, administered into the isolated circulation of the head of the dog produced an increase in contractile force in animals subjected to deafferentation (60). These observations are relevant in terms of possible mechanisms whereby digitalis drugs can increase cardiac contractile force but may not be relevant in terms of the clinical use of these drugs. The reasons are: First, baroreceptor reflexes are normally present and would counteract any stimulating effects of digitalis on forebrain-stimulating areas. Second, the dose of ouabain used to demonstrate a sympathetically mediated positive inotropic effect was high and might not be encountered with clinical use of these drugs. Third, with improvement in cardiac function produced by digitalis in subjects exhibiting heart failure, there should be a decrease in efferent sympathetic nerve traffic and catecholamine liberation.

The importance of the role of reflexes in modifying the positive inotropic effect of digitalis in animals with hearts not in failure was first realized by Daggett and Weisfeldt in 1965 (170). They administered acetylstrophanthidin to pentobarbital-anesthetized dogs and observed no change in myocardial contractility, with left ventricular function curves as indices of contractile function. However, after either pharmacological or surgical removal of the efferent sympathetic nervous system, an increase in myocardial contractility was seen after acetylstrophanthidin administration. Daggett and Weisfeldt concluded that the direct inotropic effect of digitalis was obscured by reflex-induced withdrawal of cardiac sympathetic tone. Reflexes were said to be activated by an acetylstrophanthidin-induced increase in arterial pressure. However, inspection of the data indicates that no significant increase in arterial pressure occurred in this study. Bussman et al. (105) reported similar results with proscillaridin A in morphine-chloralose anesthetized dogs. They observed a greater increase in left ventricular V_{max} after blockade of cardiac efferent sympathetic neural effects. The difference between the findings of Bussman et al. (105) and Daggett and Weisfeldt (170) was that an increase in contractile force occurred when proscillaridin A was administered to dogs prior to pharmacological blockade of the sympathetic nervous system. Halloran and Downing (327) found that administration of acetylstrophanthidin to pentobarbital-anesthetized puppies produced a greater inotropic response (i.e., increase in left ventricular dP/dt) after beta-adrenergic blockade with practolol than before pretreatment with this agent. They concluded that in the normal heart, reflex-withdrawal of sympathetic activity partially offsets the positive inotropic effect of digitalis. In a second study by this group

(328), digoxin was administered to pentobarbital-anesthetized dogs. In control animals, digoxin increased left ventricular dP/dt and stroke volume. When autonomic blockade was instituted with practolol, atropine, and tetraethylammonium, greater increases in these indices of myocardial contractility were observed.

The findings of Kim and colleagues (411) are in contrast to the above findings indicating that dogs with efferent sympathetic tone blocked exhibit a greater inotropic response to digitalis. These investigators examined the effects of combined propranolol and atropine administration on the inotropic effect of acetylstrophanthidin in anesthetized dogs. They found that acetylstrophanthidin increased contractility in these animals to values attained by the dogs with an intact autonomic nervous system. One problem with their study is that the two groups of animals differed with respect to the anesthetic agent employed. The control group was anesthetized with morphine-chloralose and the autonomic-blocked group was anesthetized with pentobarbital. It may be that the anesthetic itself affected the magnitude of the inotropic effect of acetylstrophanthidin, resulting in equivalent inotropic responses in both groups.

Experiments performed in which the afferent limb of the autonomic nervous system, rather than the efferent limb, was removed also indicate an important effect of reflex-induced modification of the inotropic action of digitalis. For example, denervation of the sinoaortic reflex areas as well as the cardiac sensory areas in chloralose-anesthetized cats resulted in a significant increase in contractile force (with strain gauge arch recordings from the right ventricle) after the administration of either ouabain or acetylstrophanthidin, whereas a negative inotropic effect was observed when these areas were intact (634). Similar results were obtained with decerebrate cats in another study (636). Reing et al. (651) observed no positive inotropic effect (with strain gauge arch recordings from the left ventricle) of acetylstrophanthidin in chloralose-anesthetized dogs with cardiovascular reflexes intact. This was also the case when dogs were placed on left ventricular bypass. When the reflex areas were denervated in both groups of dogs, acetylstrophanthidin produced an increase in contractile force. The left heart bypass experiments were performed to rule out increases in arterial pressure as being responsible for activating the reflexes. Instead, this as well as other studies (56, 289, 633, 636) indicate that digitalis causes sympathetic withdrawal by directly sensitizing baroreceptors.

Results contrary to the above were reported in a study recently performed by Basu Ray et al. (51). Pentobarbital-anesthetized dogs were given ouabain by i.v. infusion at rates of 1.5 to 2 μg/kg/min. Animals that received cumulative doses from 17 to 51 μg/kg exhibited a significant increase in contractile force (with left ventricular dP/dt as an index of myocardial contractility). Animals that were debuffered, i.e., that had undergone sinoaortic denervation plus vagotomy, exhibited no increase in contractility. Only four animals were studied, and the inotropic responses were extremely variable, as indicated by the large standard errors.

Recently, McRitchie and Vatner (540) have obtained data to indicate that reflexes play no role in modifying the contractile force effects of digitalis. They administered ouabain to conscious dogs and measured left ventricular dP/dt/P and isolength ventricular velocity as indices of myocardial contractility. They observed the same inotropic responses in control animals and animals subjected to sinoaortic denervation. The conclusion was reached that reflex-mediated sympathetic withdrawal induced by ouabain played only a minor role in blunting the inotropic effect of the drug in the conscious dog. Two reasons were given for the minor role. First, conscious dogs with sinoaortic nerves sectioned do not exhibit a very great increase in contractility. The reason given for this is that the heart is not depressed by anesthetics. Supportive evidence was provided by data from an earlier study by this group (840, 841). However, there is one study providing data that disagree with this proposal. In this study, a substantial inotropic effect of digitalis occurred in unanesthetized animals with reflexogenic areas of the cardiovascular system denervated (636). These investigations were performed in animals subjected to midcollicular decerebration. It should be noted that the animals in this study differed from those employed by McRitchie and Vatner (540) in that all cardiovascular reflexes were removed. That is, receptors not only from the carotid sinus and aortic arch were denervated, but also the receptors from the cardiac chambers. That these receptors are an important site of action for digitalis drugs has been amply demonstrated (290, 410, 585, 734, 808). The second reason for the minor role is that the carotid sinus baroreceptor reflex appears to exert only a minimal effect in the control of myocardial contractility (540, 839). This may not be the case for animals under anesthesia or subjected to midcollicular decerebration, but this point needs to be tested. In addition, since digitalis drugs appear to affect reflexes other than the carotid sinus baroreceptors, i.e., the Bezold-Jarisch reflex (216, 290, 585, 734, 808), the effect of this reflex on control of myocardial contractility needs to be evaluated in conscious dogs.

B. Cardiac Output

With respect to the role of the nervous system in the changes in cardiac output seen with digitalis drugs, no studies, to our knowledge, have been performed to determine whether the increase in cardiac output that is observed in subjects with congestive heart failure (16, 68, 80, 226, 525, 772, 773) is due to augmentation of sympathetic influences on the heart. However, it has been postulated in several instances that part of the increase in cardiac output seen upon administration of digitalis to subjects with congestive heart failure may be attributable to a reflex-mediated reduction in arterial and venous tone (524–526). That is, digitalis acts directly on the heart

to increase contractile force. This in turn causes a reflex-induced reduction in sympathetic tone to arterioles, resulting in a reduction in afterload (94, 337, 521–526). Such a reduction in afterload would increase the ejection fraction and the stroke volume, hence resulting in an increase in cardiac output (149). Mason et al. have also reported that digitalis causes venodilation when administered to patients with congestive heart failure (94, 521–526). This venodilation was postulated to be derived from the direct inotropic effect of digitalis to cause a reflex-induced decrease in sympathetic tone. The venodilator effect occurs predominantly in the hepatic veins, resulting in a shift of blood from the splanchnic bed to the systemic venous bed. The end result, according to Mason et al., is an increase in venous return to the heart. It is difficult to accept this analysis, because an increase in venous return in the setting of acute heart failure would precipitate additional failure (381, 536). More likely, digitalis would cause generalized venodilation in congestive heart failure due to withdrawal of sympathetic tone, and this effect would result in an initial decrease in venous return. A decrease in venous return during acute heart failure would initially lead to an increase in cardiac output, as shown by Howarth et al. (381). In addition, since the effect of digitalis on venous pressure in some instances of heart failure has been described to occur quickly after i.v. administration of digitalis and precedes any increase in cardiac output (537), it should be considered that venodilation might occur secondarily to a direct sensitizing effect of digitalis on baroreceptors.

In the case of subjects that are not in heart failure, digitalis may produce either no change in cardiac output (16, 199, 307, 668, 709, 717) or a decrease in cardiac output (68, 104, 148, 159, 191, 336, 400, 538, 772, 876). A reflex action of digitalis has also been suggested to be responsible for these cardiac output responses (170, 525, 634, 651, 870). However, there is only one study in which this postulate was purportedly tested. McRitchie and Vatner (541) administered ouabain (17.5 μg/kg, i.v.) to conscious dogs with carotid sinus and aortic arch baroreceptors intact or denervated. In animals with intact reflexes, ouabain decreased cardiac output, whereas no change in cardiac output occurred in animals with baroreceptors denervated. The mechanism for the decrease in cardiac output was reported to be due to a ouabain-induced reflex slowing in heart rate, since constant pacing of the heart prevented the decrease in cardiac output. Again, the problem with this study is that animals were employed in which not all the cardiovascular reflex areas had been denervated. That is, the animals undergoing denervation of their reflexes had intact vagus nerves, hence the Bezold-Jarisch reflex mechanism was still preserved.

C. Vascular Tone

1. Arterial. Vascular changes on the arterial side of the circulatory system caused by digitalis include both increases (93, 159, 166, 191, 335, 400, 523, 674, 856, 876) and decreases (1, 134, 146, 207, 449, 523, 665) in tone, and in some cases, no change in tone was observed (16, 104, 199, 205, 289, 505, 512, 629, 717, 718, 736, 772). The nervous system has been shown to play an important role in digitalis-induced decrease in tone. Four mechanisms have been proposed to explain how a decrease in tone is mediated by the nervous system. These include reflex sympathetic withdrawal (60, 94, 134, 521, 522, 524–526, 542, 634, 734, 768, 808), cholinergic vasodilation (257, 258, 290, 368, 436, 634), non-reflex-mediated sympathoinhibition (696, 859), and enhancement of beta-adrenergic receptor responses (100).

Reflex sympathetic withdrawal has been reported to be involved in the decrease in vascular tone produced by digitalis by numerous investigators. An early report of this finding was by Melville (542) in 1952. He administered ouabain i.v. to morphine-pentobarbital-anesthetized dogs pretreated with either flaxedil or atropine to block the effects of efferent vagal tone and observed an initial transient rise in arterial pressure that was followed by a striking and sustained fall in pressure. Removal of vagal afferent tone by sectioning both vagus nerves during the ouabain-induced vasodepression evoked a marked and sustained pressor response. Melville's results indicated that ouabain was activating the Bezold-Jarisch reflex to withdraw sympathetic tone to the vasculature. Other evidence that the Bezold-Jarisch reflex and/or cardiac receptors are activated by digitalis, resulting in vasodilation, can be obtained from the data of Sleight et al. (734) and Thames (808) with anesthetized dogs. Sleight et al. reported that either epicardial application of acetylstrophanthidin or intracoronary injection of this agent produced a fall in arterial pressure, which was blocked by cooling the vagus and by epicardial application of procaine. Cooling the vagus did not abolish efferent vagal effects (i.e., cardiac slowing produced by electrical stimulation of the vagus nerve), indicating the reflex nature of the acetylstrophanthidin response. Thames obtained similar results in that intracoronary injections of acetylstrophanthidin to sinoaortic deafferentiated dogs produced decreases in mean arterial pressure and renal sympathetic nerve activity. These responses were not seen in similar preparations after vagotomy, indicating an effect of acetylstrophanthidin on cardiac vagal afferent pathways.

Chai et al. (134) reported that small doses of acetylstrophanthidin administered into the carotid sinus region of anesthetized cats produced an initial fall in arterial pressure followed by a return to normal and a later drop in pressure. The early response was antagonized by sectioning the carotid sinus nerve, whereas the later response was abolished by interrupting the blood supply to the nodose ganglion. Chai et al. also observed that larger i.v. doses of acetylstrophanthidin produced a fall in arterial pressure. This response was also dependent on the presence of intact carotid sinus nerves and nodose ganglia. Consistent with a hypotensive effect of digitalis

mediated by an action on the carotid sinus baroreceptors are the findings of Quest and Gillis (634). They administered both ouabain and acetylstrophanthidin into the carotid sinus regions of decerebrate cats and observed a marked hypotensive effect that was blocked by sectioning the carotid sinus nerves.

Stark et al. (768) have obtained data from anesthetized dogs that indicate that reflex-mediated withdrawal of sympathetic tone is responsible for vasodilation occurring in a skeletal muscle bed after i.v. acetylstrophanthidin. This was demonstrated by observing no decrease in resistance of the separately perfused (flow was maintained constant) gracilis muscle preparation after denervation of the carotid sinus, aortic arch, and cardiac receptors, and in the autoperfused preparation of the denervated muscle. Other data that indicate that digitalis acts through arterial baroreceptors to cause vasodilation was obtained by Bedynek (60). Bedynek administered acetylstrophanthidin into the isolated head circulation of the dog and observed a fall in systemic arterial pressure. After denervation of the carotid sinus regions, no systemic hypotension was observed.

It has also been postulated by Mason and colleagues (94, 521, 522, 524–526) that reflex withdrawal of sympathetic tone is responsible for the decrease in forearm vascular resistance (calculated as the ratio of mean arterial pressure to blood flow, measured indirectly with a plethysmographic technique) that occurs in heart failure patients receiving digitalis. Reflex activation was stated to occur indirectly as a consequence of the direct inotropic action of these agents.

Evidence for cholinergic vasodilation has been obtained from studies in which arterial blood pressure was measured before and after atropine administration. Kumar et al. (436) reported that an i.v. infusion of acetylstrophanthidin failed to increase either arterial pressure or systemic vascular resistance in intact conscious dogs, whereas this agent produced marked increases in pressure and resistance in animals pretreated with atropine. These results indicate that the potential rise in blood pressure produced by digitalis may be prevented by an increased parasympathetic tone in the conscious dog. Quest and Gillis (634) and Gillis et al. (290) also demonstrated that greater systemic pressor responses occurred with acetylstrophanthidin in anesthetized cats after atropine administration. Since heart rate was not controlled in any of these studies, the greater pressor response observed with acetylstrophanthidin might have been due to an absence of acetylstrophanthidin-induced cardiac slowing in the atropine-treated group. However, this does not seem to be the case because, in one study, cardiac slowing was of a similar magnitude in both control and atropine-pretreated animals, yet the pressor response was greater in the atropine-pretreated group (634). Higgins et al. (368) demonstrated that the decrease in resistance (calculated as the ratio of mean arterial pressure to blood flow, measured directly with flow probes) induced by ouabain in the mesenteric bed of conscious dogs was prevented by pretreatment with atropine. Powell's group reported that vasodilation induced by either acetylstrophanthidin (257) or digoxin (258) in the isolated perfused (flow was maintained constant) gracilis muscle bed of anesthetized dogs with an initially high sympathetic tone was prevented by local administration of atropine.

Vasodilation (as suggested by decreases in mean arterial pressure) due to non-reflex-mediated sympathetic withdrawal has been reported in two studies. Weaver et al. (859) reported that microinjections of ouabain (1 to 1000 ng) into medullary vasoconstrictor sites of anesthetized cats, with cardiovascular reflexes denervated, produced decreases in arterial pressure that paralleled decreases in sympathetic nerve activity. The decreases in pressure could not be related to decreases in heart rate. Saxena and Bhargava (696) also described vasodepressor responses in vagotomized, anesthetized cats and dogs after administering ouabain into the lateral cerebral ventricle. These investigators concluded that the hypotension was the result of inhibition of sympathetic nerve activity.

An interesting observation that may reflect either cholinergic vasodilation or vasodilation due to non-reflex-mediated sympathetic withdrawal has been reported by Garan et al. (257). They found that the administration of submicrogram amounts of digoxin into the cerebral ventricles of hemorrhaged dogs exhibiting a high sympathetic tone evoked vasodilation in the innervated, separately perfused (flow was maintained constant) gracilis muscle vascular bed. Although, in another study (258), these investigators reported that the vasodilation produced by i.v. digoxin was prevented by atropine in dogs with high sympathetic tone, they did not examine the effect of cholinergic blockade on the vasodilation produced by centrally administered digoxin and consequently the precise mechanism for this effect remains to be determined.

Arterial vasodilation may also occur because of an enhancing action of digitalis on mechanism(s) mediating isoproterenol-induced relaxation of vascular smooth muscle. This has been demonstrated by Broekaert and Godfraind (100) with the isolated guinea-pig aortic strip.

There are many instances where no change in vascular tone has been observed after digitalis administration, as reflected (perhaps incorrectly) by no change in either systemic arterial pressure or systemic total peripheral resistance. We make the qualification because if no change in pressure occurs, it may merely reflect vasoconstriction and vasodilation occurring simultaneously in different vascular beds, with the net result being no observable change in systemic pressure or resistance. Nevertheless, from studies showing no change in systemic pressure after digitalis administration, an important role for the nervous system in the response has been postulated for the reason that digitalis drugs are known to have direct vasoconstrictor actions (99, 162, 270, 409, 510, 555, 820, 850, 855) and the anticipated response should be a rise in pressure. The reason a rise in pressure

does not occur is presumably because digitalis induces a reflex decrease in sympathetic outflow, as suggested by the study of Daggett and Weisfeldt (170). They observed no change in systemic peripheral resistance after acetylstrophanthidin administration to anesthetized dogs, whereas an increase in resistance was observed in dogs pretreated with mecamylamine. In the presence of this ganglion blocking agent, peripheral efferent sympathetic tone was eliminated and this unmasked the direct vasoconstrictor effect of acetylstrophanthidin. Daggett and Weisfeldt concluded that a digitalis-induced initial rise in blood pressure activated the baroreceptors, leading to sympathetic withdrawal to the vasculature, which offset the vasoconstrictor effect of the drug. However, examination of their blood pressure data revealed no rise in arterial pressure after acetylstrophanthidin administration in their non-ganglion-blocked dogs. A similar effect was observed in either decerebrate or anesthetized cats and anesthetized dogs with digitalis, but with denervation of baroreceptors rather than ganglionic blockade (289, 634, 636, 650, 651). In addition, evidence was provided in all of these studies, plus one other study (635), that digitalis-induced withdrawal of sympathetic tone was due to sensitization of baroreceptors. Consistent with an action of digitalis on reflexogenic areas to hold down arterial pressure are the findings of Barron and Bishop (45) in conscious dogs. With reflexes intact they observed a small rise in arterial pressure after ouabain administration, but with all reflexes denervated a marked rise in pressure occurred.

McRitchie and Vatner (540) also reported that denervation of the baroreceptors resulted in greater increases in the arterial pressure and total peripheral resistance effects of ouabain in conscious dogs. In control animals, however, a significant rise in both indices of vascular tone was observed. These results are thus consistent with the concept that baroreceptor-mediated effects of digitalis are offsetting the direct vasoconstrictor effect of these agents. McRitchie and Vatner also make the important point that ouabain did not sensitize the baroreceptors and cause a withdrawal of sympathetic tone from the vasculature. Their conclusion was based on the findings that equivalent rises (i.e., 3-fold) in arterial pressure were seen with both ouabain and methoxamine in debuffered dogs as compared to intact dogs. If sensitization of baroreceptors was important with ouabain, then a greater difference in the arterial pressor response should have been observed with ouabain. We (287) have criticized their findings because they left intact part of the reflexogenic sites where ouabain can act, i.e., cardiac receptors. However, upon repeating their experiments in animals with all reflexogenic sites denervated, McRitchie and Vatner still obtained equivalent pressor responses to ouabain and methoxamine (541). Their data are thus at odds with data we have obtained, indicating that animals with cardiovascular reflexes denervated exhibited different arterial pressure responses to ouabain and norepinephrine (289). An explanation for this discrepancy may be related to the differences in base-line pressures of the debuffered animals in the two studies. The base-line pressures of the debuffered animals used in our study were similar to the base-line pressures of nondebuffered animals. Debuffering initially causes a very large rise in pressure but, with time, the pressure stabilizes at a level close to the predenervation level. Under these circumstances, we found a much greater pressor response with ouabain than with norepinephrine in debuffered animals, suggesting that ouabain was sensitizing baroreceptors. In the study by McRitchie and Vatner (541), base-line pressures of the completely debuffered animals were not given. However, extrapolating from data of Bishop and Peterson (77) in the completely debuffered conscious dog, base-line blood pressure in these animals is extremely high (i.e., mean blood pressures in the range of 160 mm Hg), and the degree to which ouabain can change pressure in animals with a high base-line pressure would be much less than in an animal with a lower base-line pressure. McRitchie and Vatner (541) criticized our studies by stating that we utilized anesthetized animals. This criticism does not hold for one of our studies where decerebrate unanesthetized animals were used, and we demonstrated that ouabain-induced sensitization of baroreceptors was responsible for withdrawing sympathetic tone and masking the direct vasoconstrictor effect of this agent (289).

The role of the nervous system in the arterial vasoconstrictor action of digitalis drugs is controversial. The controversy stems from the fact that many forerunning studies both with isolated arterial strips (99, 162, 510, 850) and intact animal preparations (94, 521, 523, 674, 852, 853) indicated that digitalis drugs have a direct constricting action on arterial smooth muscle, while data obtained more recently indicate that the sympathetic nervous system is also important in mediating the constrictor effects of these drugs (100, 257, 259, 289, 331, 430, 637, 686, 729, 768). The earlier results with isolated strips of arterial smooth muscle may be criticized from the point of view of newer data using these preparations. Thus, it is now known that in addition to acting directly on vascular smooth muscle cells, digitalis drugs can cause vasoconstriction in isolated arteries by releasing catecholamines. For example, Broekaert and Godfraind in 1973 (100) reported that ouabain (1×10^{-5} M)-induced contractions in isolated rabbit aortic strips were prevented by the alpha-receptor blocking agent, phentolamine. They also found that ouabain (1×10^{-8} and 1×10^{-7} M) enhanced the contractile response to norepinephrine and concluded that the ouabain-induced contraction of rabbit aorta was mainly due to release of endogenous catecholamines. Some of the earlier data with preparations in vivo also suggested that the sympathetic nervous system might play a role in the arterial vasoconstriction induced by digitalis. For example, Ross et al. (674) mentioned that although ganglion blockade and adrenalectomy did not prevent the pressor effects of ouabain and acetylstrophanthidin, there appeared to be

a reduction in the magnitude of the response.

More recent findings strongly implicate a role for the sympathetic nervous system in the arterial constriction produced by digitalis. Gillis et al. (289) reported that blockade of alpha-adrenergic receptors with either i.v. phenoxybenzamine or phentolamine significantly attenuated the pressor effect of ouabain in decerebrate, unanesthetized cats in which all cardiovascular reflexes had been denervated. Similar results were obtained in cats with spinal cords transected; in these animals neither phentolamine nor pithing the spinal cord produced a further attenuation of the pressor response, suggesting that the site of action of ouabain to induce sympathetically mediated vasoconstriction was located at a supraspinal level. Kumar et al. (436) reported that blockade of alpha-adrenergic receptors with dibenzyline prevented the increases in arterial pressure and systemic vascular resistance produced by acetylstrophanthidin in atropine-treated conscious dogs. Atropine was required in their study to bring out the vasoconstrictor effect of acetylstrophanthidin (see above section on cholinergic vasodilation and digitalis-induced decrease in vascular tone). Stark et al. (768) reported that blockade of alpha-adrenergic receptors with intraarterially administered phenoxybenzamine prevented the vasoconstriction induced by acetylstrophanthidin in the isolated perfused (flow was maintained constant) canine gracilis muscle vascular bed. Similar results were obtained in their study by sectioning the sympathetic nerves innervating the gracilis muscle vascular bed. Although some constriction was observed after phenoxybenzamine administration or surgical denervation, the major portion of the response was prevented. Stark et al. concluded that the predominant mechanism whereby acetylstrophanthidin increased vascular resistance was via the sympathetic nervous system. Garan et al. (257) tested the effects of phenoxybenzamine administration on the transient vasoconstriction induced by acetylstrophanthidin in the separately perfused (flow was maintained constant) gracilis muscle of anesthetized dogs exhibiting a high sympathetic tone. Sympathetic tone was high because of the induction of hemorrhage. Garan et al. found that the vasoconstriction induced by acetylstrophanthidin was prevented after local administration of the alpha-blocking agent into the circulation of the perfused limb. Quest et al. (637) reported similar findings with ouabain in the isolated, perfused (flow was maintained constant) hindlimb of the anesthetized dog. Pretreatment with the alpha-blocking agent phentolamine attenuated the vasoconstrictor effect of this agent. In addition, phentolamine abolished the hindlimb vasoconstriction produced by dihydroouabain. It was concluded that both agents caused contraction of vascular smooth muscle by activating alpha-adrenergic receptors.

Hamlin et al. (331) tested the effects of alpha-adrenergic blockade with phenoxybenzamine and ganglionic blockade with mecamylamine on coronary vasoconstriction induced by acetylstrophanthidin and digoxin in anesthetized dogs. Prior alpha blockade reduced the acetylstrophanthidin-induced constriction and prevented the digoxin-induced response. Ganglionic blockade was also effective in preventing the digoxin-induced constriction. These investigators concluded that the coronary constriction was primarily neurogenic and largely mediated by alpha-adrenergic receptor stimulation. In a later preliminary study by this group (686), administration of acetylstrophanthidin or digoxin produced "erratic increases" in coronary vascular resistance of anesthetized dogs during acute global ischemia. This response was associated with a rise in left ventricular end diastolic pressure and ventricular fibrillation. Prior alpha-adrenergic receptor blockade with phenoxybenzamine prevented the increases in coronary vascular resistance. It was concluded that digitalis increases coronary vascular resistance in the ischemic heart by activating alpha-adrenergic receptors.

Perhaps one of the most convincing studies indicating that digitalis produces arterial constriction by a neural mechanism is that by Garan et al. (259). They observed that an i.v. injection of digoxin into a donor dog perfusing the isolated head of a recipient animal caused an increase in coronary resistance in the recipient animal. This response was not observed when the same dose of digoxin was administered into the systemic circulation of the recipient dog. In addition, when digoxin was administered i.v. to intact dogs, a rise in cerebrospinal fluid concentration of digoxin occurred that closely followed the time-action curve for the coronary vasoconstrictor effect of the drug. Finally, these investigators found that digoxin, when administered into the lateral cerebral ventricle in one-tenth of the systemic dose, produced an increase in coronary vascular resistance. They concluded that the central nervous system is a major site of action of digitalis in producing neurogenic vasoconstriction in the coronary vasculature. Consistent with an effect of digitalis to produce arterial constriction by acting in the central nervous system are the findings of numerous investigators who observed arterial constriction after administering digitalis drugs locally into various regions of the central nervous system (19, 50, 52, 69–72, 118, 165, 249, 263, 375, 654, 696, 780, 844, 859).

Shudo et al. (729) concluded that peripheral sympathetic nerves and the adrenal glands were involved in the pressor response obtained by arrhythmogenic doses of ouabain in anesthetized dogs. They reported that α-methyl-*p*-tyrosine administration in combination with bilateral adrenalectomy prevented the pressor response of ouabain. However, centrally administered 6-hydroxydopamine had no significant effect. Consistent with digitalis exerting neurogenic vasoconstriction via a peripheral sympathetic mechanism is the recent preliminary data of Kosinski et al. (430). These investigators reported that the highly polar cardiac glycoside ASI-222 (a semisynthetic aminosugar digitoxin derivative with undetectable penetration of the blood-brain barrier) causes an increase in peripheral vascular resistance in anesthetized

dogs. Part of the increase in resistance was found to be prevented by pretreatment with phenoxybenzamine. Kosinski et al. concluded that the alpha-adrenergic vasoconstrictor effect of ASI-222 was mediated either peripherally or by areas of the central nervous system without an effective blood-brain barrier.

2. *Venous.* Vascular changes on the venous side of the circulatory system with digitalis include changes reflecting both increases (94, 378, 380, 521–525, 673, 835) and decreases (94, 521–525, 538, 745) in tone. In some cases, the response obtained indicated that no change in tone had occurred (198, 199, 214, 296, 336, 875). The least controversial role for the nervous system is in digitalis-induced changes reflecting a decrease in "venous tone." "Venous tone" (as reflected by pressure-volume characteristics of the capacitance vessels) has been reported to decrease because of digitalis-induced inhibition of sympathetic outflow to these vessels. This was demonstrated by Solti and Iskum (745) in patients with heart failure. Ouabain administered i.v. produced a decrease in forearm venous tone. However, when ouabain was administered directly into an isolated venous segment of the forearm, there was no change in venous tone. As soon as the drug was allowed to enter the systemic circulation, a reduction in forearm venous tone was observed. In addition, infiltration of the isolated venous segment with procaine prevented ouabain administered by the i.v. route from decreasing venous tone in that segment. These investigators concluded that ouabain had no direct effect on the tone of the venous wall but acted probably by inhibiting sympathetic nervous activity to the veins.

These results were later confirmed by Mason and Braunwald (523), who measured forearm vascular tone in patients with heart failure. Administration of ouabain i.v. resulted in an immediate decrease in forearm venous tone (as reflected by pressure-volume characteristics of the capacitance vessels). Since digitalis had been shown to produce direct constriction of isolated venous strips (244, 513), Mason et al. (525) postulated that the decrease in venous tone was due to a reflex withdrawal of sympathetic tone to these vessels. Mason et al. (525) further postulated that withdrawal of sympathetic tone was derived from the large rise of cardiac output in the heart failure patients with consequent activation of the baroreceptors.

A decrease in venous tone has also been postulated to occur from studies in which either venous return or inferior vena cava pressure was measured. Reing et al. (650) observed a decrease in venous return (suggestive of venodilation) in dogs given acetylstrophanthidin and that were not in heart failure. These experiments were performed in dogs with left ventricular bypass and with measurement of blood volume in an extracorporeal reservoir. Denervation of reflexogenic areas of the cardiovascular system prevented the decrease in venous return normally seen with acetylstrophanthidin. In addition, the effect of acetylstrophanthidin on the reflexes occurred independently of a rise in arterial pressure and was postulated to be due to a direct sensitizing effect on baroreceptors. Solti et al. (747) reported a decrease in venous pressure within the inferior vena cava in dogs with heart failure after either administering ouabain i.v. or into the circulation of an isolated, perfused head. These investigators concluded on the basis of the isolated, perfused head data that the decrease in central venous pressure produced by digitalis was due to an interaction of the drug with the nervous system, and specifically to a decrease in sympathetic activity to the veins. Since the carotid sinus baroreceptors appeared to be present in their isolated, perfused head preparation, it could be postulated that digitalis was acting to sensitize these receptors, thereby producing a decrease in sympathetic activity.

Concerning the venous bed that is most affected by digitalis, Mason et al. (525) postulate a predominant effect on the hepatic vein. According to these and other investigators (523, 745, 759, 761), a high sympathetic tone is present in heart failure, and release of this tone, especially in the hepatic veins, results in an important change in venous return (531).

A change in the indices used that presumably reflects an increase in venous tone with digitalis administration has been demonstrated in numerous studies but the role of the nervous system in this response is controversial and not well studied. In general, venoconstriction has been observed in patients not in heart failure. This has been demonstrated by Mason and Braunwald (523), who used pressure-volume characteristics of the capacitance vessels as a measure of venous tone. Pretreatment of normal subjects with guanethidine was reported to have no significant effect on the ability of ouabain to increase forearm venous tone. Brender et al. (96), however, observed that acetylstrophanthidin enhanced the contractile response of the lateral saphenous vein of the dog produced by stimulation of the lumbar sympathetic trunk. Acetylstrophanthidin alone, in the dose used to show enhancement, had no significant effect on venous tone. These data suggest that venoconstriction could occur with digitalis because of enhancement of existing sympathetic tone to the veins. This suggestion is consonant with data obtained with other sympathetically innervated systems such as arterial smooth muscle and the heart, where digitalis has been shown to augment the end organ response to sympathetic stimuli (see section II C 4 b).

In spite of these data, suggestive of venoconstriction evoked by digitalis, the only effect that, to our knowledge, has been clearly demonstrated is a decrease in venous return. This has been described as being due to either an increase in vascular capacity or a decrease in circulating blood volume (159). The latter has been ruled out in studies in which measurements of plasma volume and total blood volume were performed in dogs given ouabain and digitoxin and did not change after administration of these drugs (160). Concerning an increase in vascular capacity, numerous investigators have postulated that

this does occur and that it results primarily from a direct action of digitalis to constrict hepatic veins and cause portal venous pooling (47, 191, 267, 400, 668, 669, 673, 684, 792, 793, 811). However, this contention seems unlikely in view of more recent data showing that pooling in the splanchnic regions after administration of digitalis is insignificant (64, 335, 568, 720, 826). In addition, many investigators have indicated that hepatic venous constriction is not an important effect of digitalis (94, 147, 301, 506, 535, 884). The contention also seems unlikely in view of the finding that baroreceptor denervation prevents digitalis from decreasing venous return (650). If an increase in vascular volume is due to expansion of the splanchnic bed secondary to hepatic vein constriction, denervation of the baroreceptors should not abolish the response. The explanation for the increase in vascular capacity based on the results obtained with baroreceptor denervation is that digitalis produces sensitization of baroreceptors and a reduction of sympathetic nerve activity to venous smooth muscle.

The importance of digitalis action on venous tone for the beneficial hemodynamic effects seen in congestive heart failure is unclear. One reason for this is that, to our knowledge, the effect of digitalis on venous return has not been measured in patients with heart failure. Mason et al. (522, 525, 526) postulate that digitalis will increase venous return in these patients. The mechanism for the increase was described as being through the nervous system, i.e., due to reflex withdrawal of sympathetic tone to the hepatic veins, resulting in a shift of blood from the portal system to the systemic system. This seems unlikely because an increase in venous return would be deleterious to patients exhibiting congestive heart failure (538). Indeed, one therapeutic objective in these patients is to administer a drug that decreases the venous return (242). It would seem more likely that digitalis administration would cause an immediate decrease in venous return in patients with congestive heart failure, since these drugs would sensitize baroreceptors, causing withdrawal of sympathetic tone to the veins. Consistent with this postulation are the findings that, in patients with congestive heart failure, digitalis decreases right atrial and pulmonary wedge pressures (16, 208, 209, 226, 332, 381, 382, 406, 538, 770, 882) and, in dogs in heart failure, digitalis decreases inferior vena cava pressure (747).

D. Heart Rate

Neural actions of digitalis drugs can explain entirely the slowing in sinus rate that is produced by these agents. The first studies to demonstrate this effect were performed by Abiko et al. (5, 6, 10), who administered strospeside i.v. to anesthetized cats with an intact nervous system and observed a slowing in cardiac rate. When the strospeside was administered to cats with denervated hearts, produced by sectioning both vagus nerves and either transecting the spinal cord, extirpating the stellate ganglia, or administering the beta-blocking agent dichloroisoproterenol, no slowing in cardiac rate was observed. When the strospeside was administered to cats subjected only to bilateral vagotomy alone, or to one of the above surgical or pharmacological interventions to remove cardiac sympathetic innervation alone, the effect of the drug to produce cardiac slowing was reduced in magnitude but not abolished. The doses of strospeside explored in their studies ranged from 10% to 100% of the lethal dose of that agent. The results of Abiko et al. suggest that the ability of strospeside to slow the cardiac rate depends upon the presence of an intact autonomic innervation to the heart. Abiko et al. concluded that the strospeside-induced bradycardia was mediated by activation of the vagus nerves and inhibition of the sympathetic nerves to the myocardium. Their findings suggest that each of these neural mechanisms contributed equally to the action of strospeside on the S-A node.

In another study that convincingly demonstrated that cardiac slowing produced by digitalis is entirely neural, Ten Eick and Hoffman (807) determined the negative chronotropic effects of acetylstrophanthidin and ouabain in anesthetized cats, dogs, and rabbits, and in the isolated rabbit right atrium. They explored the effects of a wide range of subtoxic doses of these digitalis drugs, as well as a dose that was just threshold for producing arrhythmias, and found that sinus slowing was prevented by either chronic cardiac denervation or by the use of autonomic blocking agents (i.e., atropine, propranolol, or MJ-1999). In studies in vivo and in vitro, simultaneous blockade of each division of the autonomic nervous system was necessary for total prevention of the negative chronotropic action of digitalis. This indicates that activation of the parasympathetic nervous system and inhibition of the sympathetic nervous system were responsible for the effects on heart rate. The data from their study indicate that the primary mechanism involved in the cardiac slowing was inhibition of the sympathetic nervous system. Pace et al. (598) examined the role of the nervous system in the cardiac slowing produced by digoxin and digitoxin in anesthetized cats. In the case of digoxin, no slowing of sinus rate was observed after spinal cord transection, even though the vagus nerves were intact. In the case of digitoxin, sinus rate slowing was observed after spinal cord transection. Bilateral vagotomy markedly reduced but did not abolish this response. Pretreatment of the cats with atropine did abolish the response. The doses of the digitalis drugs explored in this study consisted of a wide range of subtoxic doses as well as a dose that was just threshold for producing arrhythmias. These findings indicate that the sinus rate slowing produced by both drugs is mediated by the nervous system and that the role of each division of the autonomic nervous system in cardiac slowing depends on the digitalis drug being studied. In these experiments, sympathetic withdrawal was responsible for the entire effect of digoxin, whereas with digitoxin parasympathetic activation exerted a significant effect in the response. Gillis et

al. (290) examined the effect of three subarrhythmic doses of acetylstrophanthidin on cardiac slowing in control, atropine-pretreated, propranolol-pretreated, and combined atropine-propranolol-pretreated animals. Each blocking agent resulted in a significant reduction in acetylstrophanthidin-induced bradycardia. With the lowest dose of acetylstrophanthidin tested, atropine had the greatest effect in reducing the heart rate response. At the higher doses of acetylstrophanthidin, pretreatment with either atropine or propranolol produced about equivalent reductions in the heart rate response. Combined blockade with atropine and propranolol totally abolished the sinus rate slowing normally seen with acetylstrophanthidin. Interestingly, denervation of carotid sinus, aortic arch, and cardiac sensory afferent receptors also totally prevented the response in animals pretreated with atropine. This indicates that the component of sinus rate slowing abolished by propranolol was a reflex-induced sympathetic withdrawal caused by acetylstrophanthidin.

Consistent with the observations in experimental animals are findings in patients with transplanted hearts given digoxin. No significant slowing in sinus rate was noted in these studies (305, 306, 450, 655).

There are numerous other studies in which the role of the autonomic nervous system in the sinus rate slowing produced by digitalis drugs was examined. It was concluded from these studies that a direct effect of digitalis on the heart, in conjunction with a neurally mediated action, was responsible for sinus bradycardia (167, 169, 392, 433, 534, 666, 863). However, these studies were performed with only one division of the autonomic nervous system removed. The only direct chronotropic effect of digitalis on the sinus node appears to be an enhancement of automaticity (341, 807, 823) that occurs with near toxic doses. A significant portion of this positive chronotropic response is due to activation of the sympathetic nervous system (290, 713, 714, 807).

E. Atrial Muscle Refractory Period

Changes in the refractory period of atrial muscle produced by digitalis drugs consist of two effects, one due to a neural action and the other due to a direct action, which occur in opposite directions. They were demonstrated by Farah and Loomis (222), who used G-strophanthin, digitoxin, and lanatoside C in anesthetized dogs. With the technique of Rosenblueth and Garcia Ramos (671), atrial flutter was produced in neurally intact dogs and in dogs subjected to cardiac denervation by sectioning the vagus nerves and removing the sympathetic chains from the stellate to the sixth thoracic sympathetic ganglia. When the digitalis drugs were administered to the neurally intact animals, they converted the atrial flutter to atrial fibrillation. In contrast, when these drugs were administered to the neurally deprived dogs, they reduced the rate of the atrial flutter and eventually caused the restoration of normal sinus rhythm. In addition, by using a modification of the technique of Dawes (177), i.e., rapid stimulation of atrial tissue to assess refractory period changes, these investigators also found that digitalis reduced the maximum rate of stimulation that the atria of neurally deprived animals would follow. These results were interpreted to indicate that digitalis shortens the atrial refractory period of neurally intact animals and lengthens the atrial refractory period of neurally deprived animals. The mechanism for the shortening of the atrial refractory period was studied either by administering atropine or by performing bilateral vagotomy. Both of these procedures resulted in abolition of the digitalis-induced atrial fibrillation. Consistent with this explanation were the findings that vagal nerve stimulation and i.v. infusions of acetylcholine and physostigmine mimicked the effect of digitalis in neurally intact dogs. The authors concluded that the mechanism for the shortening of the atrial refractory period (i.e., conversion of flutter to fibrillation) was due to enhancement of the effect of the vagus nerve on atrial tissue by digitalis. The lengthening of the atrial refractory period was interpreted as a direct action of digitalis on atrial muscle tissue.

These results were confirmed by Mendez and Mendez (549), who measured the functional refractory period of atrial muscle tissue of anesthetized dogs by using paired electrical stimuli to determine the shortest interval that would give a propagated atrial response. In morphine-chloralose-anesthetized dogs, digitoxin shortened the functional refractory period. In animals subjected to bilateral vagotomy, digitoxin produced the opposite effect, i.e., it lengthened the atrial refractory period. In a more detailed study, Mendez and Mendez (547) examined the effect of lanatoside C on atrial absolute and relative refractory periods. They found that in anesthetized neurally intact dogs the lanatoside C shortened the refractory period, and this effect was primarily due to abbreviation of the absolute refractory period rather than to abbreviation of the relative refractory period. The effect in dogs subjected to bilateral vagotomy was to increase the atrial refractory period, and this was reflected primarily as an increase in the relative refractory period. Thus, the results of the above three studies can be summarized by stating that digitalis shortens the absolute refractory period of the atrial muscle tissue by activating the vagus and lengthens the relative refractory period of this tissue by a direct effect.

These effects on atrial refractory period have been difficult to demonstrate in the few clinical studies performed in patients with transplanted hearts. Leachman et al. (450) have data from a limited number of patients to indicate that digoxin in therapeutic doses exerts a direct effect on the atrial muscle refractory period. In this study they reported that digoxin converted atrial flutter to normal sinus rhythm rather than to atrial fibrillation. Goodman et al. (305) reported that the same dose of digoxin administered to patients with denervated hearts exhibited no significant change in either the atrial

functional refractory period or the effective refractory period. However, in the setting of atrial flutter in patients with neurally intact hearts, many studies indicate that digitalis drugs do exert an important effect on atrial refractory period. Both shortening (i.e., conversion of atrial flutter to atrial fibrillation) and lengthening (i.e., conversion of atrial flutter to sinus rhythm) of the refractory period occur (325, 475, 476, 500, 603). It should be noted that doses of digitalis given to patients with atrial flutter are near the toxic dose (248) and this may be the reason that Goodman et al. (305) failed to see an effect on refractory period in their study.

Very large doses of digitalis, i.e., doses that can be tolerated only by preparations in vitro, produce a shortening of the atrial refractory period by a mechanism independent of the vagus. Govier (310) demonstrated that ouabain (1×10^{-6} M) administered to the isolated rabbit atrium shortened the functional refractory period. This response was prevented by pretreatment with either pronethalol or reserpine. In the presence of these agents that depress sympathetic function, ouabain increased the functional refractory period. When the catecholamine stores of the reserpinized atria were repleted with norepinephrine, ouabain was found to produce the usual decrease in functional refractory period. The ability of ouabain to decrease the functional refractory period was not affected by atropine pretreatment. Govier concluded that ouabain has two opposing actions on the atrial functional refractory period. The first is a release of norepinephrine that tends to decrease functional refractory period, and the second is a direct action to increase functional refractory period. The usual response observed in isolated cardiac tissue with digitalis is an increase in the atrial refractory period (515, 549). It should be emphasized that, to our knowledge, no role for the involvement of adrenergic mechanisms has been indicated for the atrial refractory period changes that occur in vivo with digitalis.

F. Atrioventricular Conduction

The parameter used by investigators to study the effects of digitalis drugs on A-V conduction is A-V node refractoriness. It must be kept in mind that this does not refer to the refractory period of A-V nodal cells (since this is probably decreased by digitalis) but rather represents the behavior of the entire A-V node region. The effect of digitalis is, of course, to slow A-V conduction, and this is usually described as resulting from the effect of such drugs to increase the refractory period of the A-V node. A better description of this effect is that digitalis enhances decremental conduction in the A-V node.

The role of neural factors in digitalis-induced changes in A-V conduction is most prominent. There are both experimental and clinical studies to indicate that the entire effect of digitalis on A-V conduction is mediated through the nervous system. This was first shown by Wallace et al. (854) who administered digoxin to normal, conscious dogs and found a prolongation of the functional refractory period of the A-V node (i.e., the shortest V_1-V_2 interval that could be achieved by reducing the A_1-A_2 interval). When the same dose of digoxin was administered to conscious dogs with hearts chronically denervated by the mediastinal ablation technique of Cooper et al. (154), no prolongation of the functional refractory period of the A-V node was observed. When this dose of digoxin was administered to conscious dogs subjected only to bilateral vagotomy, the effect of digoxin on the functional refractory period of the A-V node was reduced in magnitude but not abolished. Wallace et al. concluded that "the effects of digitalis on the A-V node are dependent upon an intact nerve supply." The following year these investigators (Schaal et al., 698) expanded their findings. Digoxin administered to normal, conscious dogs increased the functional refractory period of the A-V node by 65 ± 25 msec. This dose of digoxin (0.15 mg/kg, i.v.) produced no change (0 ± 8 msec) in conscious animals with chronically denervated hearts. The lack of effect of digoxin could not be explained by an alteration of the base-line functional refractory period of the A-V node by the denervation procedure itself, since base-line values for this index were essentially the same in chronically denervated and intact control animals. Bilateral vagotomy alone attenuated the digoxin-induced prolongation of the functional refractory period but did not abolish it (33 ± 13 msec). Vagotomy per se had no effect on the base-line value for the functional refractory period of the A-V node. In addition, Schaal et al. studied doses of digoxin that were just threshold for producing ventricular arrhythmias (i.e., doses up to 0.21 mg/kg), and again found no effect of digoxin on the functional refractory period of the A-V node of conscious dogs with chronically denervated hearts. The authors concluded that "since total cardiac denervation essentially abolishes the effect of digitalis on the functional refractory period of the A-V node, it seems unlikely that a direct effect of digitalis contributes importantly to the changes of the functional refractory period observed in normal dogs." They also concluded that the mechanisms for the increase in the functional refractory period consisted of vagal activation and an antiadrenergic effect of digitalis. Based on their data, it would appear that each neural mechanism contributed equally to the action of digitalis on the functional refractory period of the A-V node.

Hirshfeld et al. (371) also observed that denervation of the heart, by vagotomy, and propranolol prevented the effect of digitalis on the functional refractory period of the A-V node. They used anesthetized cats and observed that pretreatment with propranolol prevented ouabain from prolonging the A-V nodal functional refractory period. The doses of ouabain used were just threshold for inducing ventricular arrhythmias. Kim et al. (411) studied the effect of pharmacological denervation of the heart

on A-V conduction time and the A-V nodal refractory period of anesthetized dogs before and after acetylstrophanthidin administration. A-V conduction time was measured by determining the interval between an atrial stimulus and the first rapid deflection of the bipolar His electrogram. This interval, designated as S-H time, was described as approximating the conduction time through the A-V node since the interval between the atrial stimulus and the initial deflection of atrial activation recorded from an area adjacent to the A-V node (i.e., atrial conduction) was unaffected by digitalis. A-V nodal refractory period was determined by pacing the right atrium with a continuous train of single successive stimuli for less than 6 seconds at a fixed rate and reducing the cycle lengths between stimuli until 1:1 A-V conduction was no longer present. The shortest His-His spike interval was defined as the measure of the A-V nodal refractory period. Kim et al. found that acetylstrophanthidin in doses up to 100% of the arrhythmic dose increased the A-V nodal refractory period in control animals but not in animals pretreated with propranolol plus atropine. Surprisingly, no significant increase in conduction time in the A-V node was observed in control animals. As expected, no increase was observed in animals with pharmacologically induced blockade of the autonomic nervous system. Atropine given alone failed to counteract the increase in A-V nodal refractory period. Indeed, the increases in A-V nodal refractory period of control and atropine-treated animals were similar, suggesting that the lack of response in animals with total autonomic blockade was mainly due to an acetylstrophanthidin-induced inhibition of sympathetic activity.

Consistent with a predominant role for digitalis-induced reduction in sympathetic tone in digitalis action at the A-V node are the findings by Ogden et al. (586), who assessed the effect of acetylstrophanthidin on A-V conduction in anesthetized dogs by determining the ventricular rate response produced by electrically induced atrial fibrillation. In control animals, acetylstrophanthidin slowed the ventricular rate during atrial fibrillation by 32.7%. Approximately the same degree of ventricular slowing was produced by acetylstrophanthidin in animals given atropine. However, in animals pretreated with the beta-blocking agent sotolol, acetylstrophanthidin produced no slowing of ventricular rate during atrial fibrillation. Ogden et al. concluded that sotolol per se slowed the ventricular response so much (by itself) that the acetylstrophanthidin was incapable of producing any further delay in A-V conduction. This might be the case in the study by Ogden et al. (586), but it does not appear to be true for the study by Kim et al. (411), who used atrial pacing to demonstrate that changes in A-V node function were not maximal after beta-adrenergic blockade.

There are two animal studies in which interruption of neural influences on the heart largely but not entirely prevented the effect of digitalis on A-V node conduction. In the first study, Mendez et al. (546) reported that in anesthetized dogs, vagotomy plus acute cardiac sympathectomy (i.e., extirpation of the stellate ganglia and upper three thoracic sympathetic ganglia) and adrenalectomy prevented the increase in the functional refractory period of the A-V node produced by acetyldigitoxin in doses less than 60% of the lethal dose. A dose larger than this produced a slight prolongation and this was attributed to a direct effect of acetyldigitoxin. However, myocardial stores of catecholamines are still intact in this preparation (559). Therefore, the slight prolongation of the A-V nodal functional refractory period could be due to antagonism by acetyldigitoxin of the effect of norepinephrine that is released from the myocardial tissue on the A-V node. This explanation is tenable since digitalis releases norepinephrine from myocardial stores (713) and counteracts the direct effects of catecholamines on the A-V node (545). In the second study, Morrow et al. (559) reported that ouabain produced a slight prolongation in the A-V nodal functional refractory period of anesthetized dogs subjected to chronic cardiac denervation (154) and vagotomy. This residual effect was described as a direct action of ouabain on the A-V node. In control animals, ouabain in a maximal nontoxic dose increased the functional refractory period of the A-V node by 46 ± 9 msec. After bilateral vagotomy, the response was reduced to 21 ± 2 msec. With combined vagotomy and cardiac denervation, the response was only 10 ± 3 msec. These data suggest that vagal activation played a greater role in the response than the antiadrenergic effect of digitalis. The remaining response could be due to antagonism of catecholamines released from the adrenal glands by ouabain. This fits with the results of Mendez et al. (546), who demonstrated that adrenalectomy per se can counteract part of the effect of digitalis to prolong the functional refractory period of the A-V node. In addition, Morrow et al. (559) reported in the same type of study that after vagotomy and reserpinization, ouabain prolonged the functional refractory period by 9 ± 6 msec. Although the increment was similar to that seen in vagotomized and chronic cardiac denervated animals, it was not a statistically significant increase in the A-V nodal functional refractory period. Thus, it would appear from these data that complete abolition of autonomic influences does indeed prevent the A-V nodal effects of digitalis.

Clinical studies indicating that digitalis-induced changes in A-V conduction are entirely due to neural effects have been reported by Leachman et al. (450) and by Harrison's group (305). Patients with transplanted hearts were used in both studies. Leachman et al. found that digoxin administered to two cardiac transplant patients with atrial flutter and atrial fibrillation did not produce any change in ventricular response, indicating no effect of digoxin on the A-V node. Goodman et al. (305) measured both A-V node effective (longest A_1-A_2

interval in which A_2 does not conduct to His' bundle) and functional (shortest H_1-H_2 interval achievable by atrial stimulation) refractory periods in patients with transplanted hearts and in patients with neurally intact hearts. Digoxin increased both the effective and functional refractory periods of the A-V node of the neurally intact patients but had no effect in the patients with transplanted hearts. This was true when the hearts were not paced as well as when the hearts were paced at a rate averaging about 125 beats/minute.

In a later study by Harrison's group (655), these investigators employed different techniques for assessing the effects of digoxin on A-V conduction in cardiac transplant patients. They measured the P-R interval, the duration from an atrial pacing stimulus to the onset of the QRS complex (S'R interval), and the cycle length at which Wenckebach block occurred during periods of rapid atrial pacing. They also administered digoxin differently from the method used in their earlier study (305). Here the drug was given chronically over a period of 37 days whereas before the drug had been given as a single i.v. bolus injection. The results obtained with digoxin under basal conditions (i.e., spontaneous sinus rhythm) and atrial pacing at a rate of 95 beats/minute indicated no statistically significant effect of digoxin on A-V conduction. However, with atrial pacing at rates of 110 beats/minute and higher (up to 155 beats/minute), there was a significant increase in A-V conduction with digoxin. The effect of atropine and propranolol was studied in a few of these patients, and it was found that atropine given to two patients had no effect on the digoxin response; likewise, when propranolol was also administered to one of the above patients, the effect of digoxin was not changed. It was concluded that digoxin exerted a direct depressant effect on A-V conduction in man, although the stress of tachycardia was necessary to demonstrate the effect. It was also stated that "whether this finding has any clinical significance is unknown."

There are numerous other investigations in which neural and direct effects of digitalis on A-V conduction have been studied. However, the data obtained are hard to evaluate because either only one division of the autonomic nervous system was blocked (167, 169, 300, 302, 303, 370, 402, 431, 477, 565, 628, 678, 823, 863) or when combined blockade of both divisions was instituted, blockade of the sympathetic nervous system was inadequate, i.e., adrenal glands were left intact or cardiac catecholamine stores were not depleted (549, 559).

In summary, neural effects of digitalis appear to be entirely responsible for the increase in A-V conduction. The only exception to this is when the stress of tachycardia is present (655). The neural mechanisms involved include augmentation of vagal influence and inhibition of sympathetic influence on the heart. The degree to which each is involved is unclear at the present time. According to data of Schaal et al. (698), each component is represented equally. According to Morrow et al. (559) and Carleton et al. (120), the primary component is vagal activation. However, the data of Kim et al. (411) indicate that the response is mediated primarily by inhibition of the sympathetic nervous system.

G. Cardiac Arrhythmias

1. Role of the sympathetic nervous system. A. INTRODUCTION. It has long been known that digitalis drugs can cause ventricular arrhythmias by acting directly on cardiac tissue. However, direct effects on cardiac tissue do not appear to be the only action by these drugs. There are several types of studies in which data have been obtained that suggest that activation of the sympathetic nervous system by digitalis drugs is in part responsible for cardiac arrhythmias evoked by these agents. These types of studies include: 1) recording pre- and postganglionic sympathetic nerve activity during intoxication with digitalis; 2) determining the doses of digitalis drugs required to produce ventricular arrhythmias after surgical extirpation of cardiac sympathetic nerves; and 3) assessing the influence of drugs known to interfere with sympathetic nervous system function on ventricular arrhythmias induced by digitalis drugs. In addition, there appear to be specific actions of digitalis drugs that lead to augmentation of sympathetic neural function. These have been described earlier (section III A) and some of these effects have been evaluated in assessing the role of the sympathetic nervous system in digitalis-induced arrhythmias. These specific actions consist of inhibition of norepinephrine uptake in nerve endings, increase in cardiac norepinephrine content, and interaction with CNS dopamine receptors. Finally, there are data that suggest that doses of digitalis drugs lower than those required for eliciting an increase in the activity of the sympathetic nervous system exert an antiarrhythmic effect (81, 221, 493). The sympathetic nervous system has also been demonstrated to play a role in this effect.

B. STUDIES IN WHICH CHANGES IN SYMPATHETIC NERVE DISCHARGE WERE CORRELATED WITH CHANGES IN CARDIAC ELECTRICAL ACTIVITY. As indicated in sections II B and II C, digitalis drugs have been demonstrated to alter activity in both pre- and postganglionic sympathetic nerves. The role of this effect on cardiac sympathetic nerves in the development of digitalis-induced ventricular arrhythmias has been shown in several ways. First, a correlation between digitalis-induced increases in sympathetic activity and the occurrence of ventricular arrhythmias has been noted (279, 291, 532, 596). Lathers et al. (446, 447, 453) also correlated digitalis-induced changes in postganglionic sympathetic discharge with the development of ventricular arrhythmias. However, in their studies with digitalis, recordings of activity from several nerve bundles within the accelerator nerve were made rather than from the whole trunk. Ouabain either caused enhancement or depression or no change in the activity in these nerve bundles, and these nonuniform changes were associated with the development of ven-

tricular arrhythmias. To determine whether digitalis-induced increases in sympathetic activity and the occurrences of ventricular arrhythmias were related, the effect of counteracting this hyperactivity on arrhythmias was studied. Hyperactivity in cardiac sympathetic nerves can be depressed by diphenylhydantoin (701), propranolol (279, 474), clonidine (706), and chlordiazepoxide (699). Administration of these drugs results in a reduction of neural activity and a concomitant abolition of the associated cardiac arrhythmias (279, 282, 285, 291, 292, 596).

Another method that has been used to determine whether digitalis-induced sympathetic neural effects contribute to the development of arrhythmias is focal stimulation of the central nervous system in anesthetized cats. A site in the posterior hypothalamus was defined from which arrhythmias could be reproducibly obtained. These arrhythmias were mediated by the sympathetic nervous system, since they could be prevented by pretreatment with propranolol but not with atropine, and direct recording from cardiac sympathetic nerves revealed continuous activity coincident with the arrhythmias (220). The stimulus intensity to the posterior hypothalamus was then reduced to a degree insufficient to elicit arrhythmia but intense enough to increase sympathetic nerve activity, blood pressure, and heart rate (220), and ouabain was administered as a continuous infusion of 2 μg/kg/min with hypothalamic stimulation repeated at 5-minute intervals. After infusion of large doses of ouabain (50 to 70 μg/kg), arrhythmias occurred during hypothalamic stimulation and these arrhythmias were associated with significant augmentation of sympathetic nerve activity. The neural discharge evoked by hypothalamic stimulation was considerably greater than that seen during hypothalamic stimulation before administration of ouabain. Restoration of sinus rhythm occurred within 1 minute after termination of hypothalamic stimulation. The results indicate that ouabain and hypothalamic stimulation, each applied at a dose or intensity subthreshold for arrhythmias, in combination can be arrhythmogenic. In summary, these observations strongly suggest that digitalis-induced changes in sympathetic nerves play an important role in the genesis of ventricular arrhythmias.

As mentioned in the introduction to this section, doses of digitalis drugs lower than those required for eliciting an increase in the activity of the sympathetic nervous system exert an antiarrhythmic effect (221, 443). The mechanism for the antiarrhythmic effect was examined in one study and appeared to be related to depression of efferent sympathetic activity (221). This was demonstrated by first eliciting sympathetically mediated arrhythmias by electrically stimulating the posterior hypothalamus of anesthetized cats. This resulted in alterations of cardiac rhythm, and recordings from cardiac sympathetic nerves revealed continuous activity during the time of arrhythmia. Intravenous administration of small doses of ouabain (i.e., 10 to 30 μg/kg) prevented both the arrhythmias and the associated hyperactivity of the sympathetic nerves. The antiarrhythmic effect was not present in animals with denervated baroreceptors, indicating that the effects of ouabain were probably related to activation of these reflexogenic areas.

C. STUDIES WITH SURGICAL DENERVATION. The effects of surgical sympathetic denervation of the heart on the capacity of digitalis drugs to produce cardiac arrythmias has been widely studied. The denervation techniques that have been employed include: 1) decerebration at the midcollicular level; 2) spinal cord transection between C-1 and C-6; 3) spinal cord pithing; 4) acute cardiac denervation; 5) chronic cardiac denervation; 6) cardiac autotransplantation; and 7) adrenalectomy.

Helke et al. (354) found that midcollicular decerebration, performed in chloralose-anesthetized cats, increased the dose of deslanoside required to evoke ventricular arrhythmias and ventricular fibrillation. This interesting observation suggests that an action of digitalis on forebrain sites may be involved in the cardiotoxic effects of these drugs. In contrast, Somberg and Smith (757) observed no difference in the dose of ouabain required to produce ventricular arrhythmias when midcollicular decerebration was performed in pentobarbital-anesthetized cats. The difference in these findings might be related to the different types of anesthetic agents employed.

The earliest studies in which the technique of spinal cord transection was utilized were performed primarily for the purpose of developing a more sensitive bioassay for digitalis drugs, rather than for assessing the role of the nervous system in their cardiotoxic effects. All of these studies had the common feature of comparing lethal doses of digitalis preparations in spinal cord-transected cats and in cats anesthetized with ether. In the first reported study of this type, Macdonald and Schlapp (509) observed that there was less variation between the i.v. lethal doses of four different tinctures of digitalis in spinal cord-transected cats compared to ether-anesthetized cats. Analysis of their data also indicates that the lethal doses of the digitalis materials were larger in the animals with spinal cord transected. Macdonald sought to confirm and extend this observation a few years later by comparing the lethal doses of a commercial preparation of strophanthin in spinal cord-transected cats and in cats anesthetized either with ether, dial, or chloralose (508). As was the case in his earlier study, he found that spinal cord-transected cats were more resistant to the effect of digitalis than were ether-anesthetized cats. However, Macdonald found no difference in the lethal dose of strophanthin when the spinal cord-transected cats were compared to animals anesthetized with dial or chloralose. Two explanations of this unexpected finding were offered. The first was that the technique of sectioning the spinal cord involved ligating the jugular veins and carotid arteries and clamping the vertebral arteries, thereby reducing the circulating blood volume and tissue mass. Thus, the observed lethal dose of strophanthin in the cats with

their spinal cord transected was lower than expected and would have to be corrected for the loss in circulating volume and tissue mass. Second, Macdonald suggested that the use of ether alone increased the susceptibility of the test animals to the lethal effects of strophanthin. This suggestion was based on the findings that the lethal dose of strophanthin was lower in ether-anesthetized cats than in dial- or chloralose-anesthetized cats and that the lethal dose of strophanthin became progressively greater with time as the level of ether anesthesia was allowed to dissipate. This second suggestion fits with observations that ether can enhance sympathetic neural activity in animals (550, 578, 732). Hence, ether could enhance the known stimulating effect of digitalis on sympathetic neural activity (279, 532). Because of the reduced blood volume and tissue mass and because of the use of ether, it is difficult to determine whether spinal cord transection per se was responsible for reducing digitalis toxicity in the earlier studies of Macdonald (508, 509). This difficulty in interpretation may also apply to the study of Mendez in 1942 (548), who reported that larger doses of digitoxin and lanatosides B and C were required to produce death in cats with spinal cord transected than in cats anesthetized with ether.

Despite the potential problems in interpreting the earlier studies, it is clear from numerous and more recent studies performed in dogs and cats by using a variety of anesthetic agents that transection of the spinal cord is indeed capable of conferring protection against the cardiotoxic effects of digitalis (92, 111–113, 282, 291, 292, 465, 466, 596, 640, 641, 753, 754, 756). For example, Popova (626) reported that "high level" transection of the spinal cord prevented the tachycardia and extrasystoles evoked by i.v. strophanthin in vagotomized, morphine-ether-anesthetized dogs. Similarly, Boyajy and Nash (92) found that spinal cord-transection at the C-6 level increased the lethal dose of ouabain and changed the mode of death with this agent from ventricular fibrillation to cardiac arrest in pentobarbital-anesthetized dogs. In studies performed in vagotomized, dial-urethane-anesthetized cats, Raines et al. (640) observed that spinal cord-transection at the C-1 level increased the dose of ouabain required to produce ventricular tachycardia and ventricular fibrillation. In addition, after spinal cord transection, more animals died from cardiac arrest than from ventricular fibrillation in their study Gillis et al. (291) obtained similar results in dial-urethane-anesthetized cats with spinal cords and vagus nerves sectioned. These investigators found that the doses of ouabain necessary to produce ventricular tachycardia and ventricular fibrillation in animals with spinal cord and vagus nerves sectioned were nearly twice those required to produce these same arrhythmias in animals with spinal cord and vagus nerves intact. In addition, they further reported that spinal cord transection prevented ouabain from enhancing preganglionic cardiac sympathetic nerve activity. Many additional studies, performed in anesthetized cats, have demonstrated that the procedure of spinal cord transection can increase the dose of various digitalis preparations (ouabain, deslanoside, digitoxin, and digoxin) required to produce ventricular arrhythmias and death (111–113, 282, 292, 465, 466, 596, 641, 753, 754, 754). In all of these studies the conclusion was reached that the sympathetic nervous system plays an important role in the arrhythmogenic actions of digitalis drugs. There is one study, however, in which spinal cord transection produced negative data. Hashimoto et al. (340) reported that this procedure had no effect on the dose of ouabain required to produce ventricular tachycardia and death in anesthetized dogs. Although no clear explanation can be given for this negative finding, it should be pointed out that the ability of a surgical denervation technique to modify the doses of digitalis drugs required to cause cardiac arrhythmias depends on the rate of infusion of the digitalis preparation employed (112).

In addition to removing sympathetic innervation from the heart, spinal cord transection also produces several cardiovascular changes that could account for the protective effects of this procedure against digitalis-induced cardiotoxicity (465). For example, the marked reduction in blood pressure that accompanies spinal transection results in a diminished perfusion of tissues and could impair the delivery of digitalis drugs to the heart. Similarly, in view of reports that both the onset of action of digitalis and its uptake into the heart correlate directly with the rate of cardiac beating (553, 692, 784), the profound bradycardia normally seen after sectioning the spinal cord could result in an altered sensitivity of the heart to digitalis. The possibility that these events might be responsible for altering the cardiotoxic dose of digitalis has been studied extensively by Levitt's group (111, 465, 466, 753). They administered [^{3}H]ouabain and [^{3}H]digitoxin by i.v. infusion to neurally intact and spinal cord-transected cats, and determined the doses, serum levels, and myocardial tissue contents of these drugs necessary to produce ventricular tachycardia and ventricular fibrillation. These investigators found that at the time of occurrence of these arrhythmias, both the doses and the serum levels of these drugs were significantly higher in the spinal cord-transected animals. Most importantly, the myocardial content of digitalis at the time these toxic events occurred in the spinal cord-transected animals were higher than the levels measured in the intact animals at the time of ventricular tachycardia and ventricular fibrillation. This was true even though the spinal cord-transected animals had lower blood pressures and heart rates than did the intact animals. From these data they concluded that the reduced heart rates and arterial pressures observed in the spinal cord-transected animals were not important factors in contributing to the reduced sensitivity to digitalis. Rather, they indicated that neural factors were probably critical for the induction of arrhythmias in the intact cat and that the spinal cord-transected animals were protected from the toxic rhythm

disorders by neural ablation. An examination of the role of reduced heart rate per se in several studies elicited data to suggest that this fact was unimportant in digitalis-induced toxicity. This was shown in the study of Ciofalo et al. (143), wherein bradycardia induced by continuous vagal stimulation or by the administration of hexamethonium did not alter the ability of ouabain to induce arrhythmias in vagotomized cats, and again in the study of Kelliher and Roberts (404), wherein hexamethonium-induced bradycardia failed to alter the doses of digoxin required to produce arrhythmias and death in cats. More recent data (113), however, indicate that heart rate does play some role in the reduced sensitivity of spinal cord-transected animals to digitalis-induced ventricular arrhythmias. Pacing the hearts of "spinal" animals to a rate comparable to that observed in nonspinal animals partially reversed the protective effect of cord sectioning (as reflected by dose and time to develop cardiac toxicity). However, the cardiac content of digitalis required to produce lethal arrhythmias was higher in spinal cord-transected animals that were paced than in animals with spinal cord intact, indicating that loss of sympathetic drive was also a factor in decreasing the sensitivity of these animals to digitalis arrhythmias.

The myocardial content of digitalis appears to be a valid measure for assessment of toxicity in these spinal cord-transected animals, as shown by Somberg et al. (756). These investigators assessed the activity of cardiac Na^+-K^+-ATPase and found significantly greater inhibition of this enzyme in spinal cord-transected animals than in neurally intact animals. They concluded that the protection observed with spinal cord transection could not be explained by a reduced interaction of digitalis with its myocardial cellular site of action.

Other investigators have focused on the role of the reduced arterial pressure per se and have concluded that it does not seem to be important in altering the cardiotoxic effect of digitalis. Erlij and Mendez (215) reported that the dose of digitoxin required to produce ventricular fibrillation in vagotomized dogs was not changed when arterial pressure was reduced by the administration of phenoxybenzamine. Likewise, Somani and Lum (751) found that the arrhythmogenic dose of oubain in dogs was not altered in the presence of a reduction in arterial pressure induced by nitroglycerin.

In summary, it appears that there are two mechanisms whereby spinal transection confers protection against digitalis-induced cardiotoxicity; one is by eliminating sympathetic input to the heart and the other is by reducing the heart rate.

Consistent with the finding that spinal cord transection can modify the arrhythmogenic actions of digitalis are the results of studies showing that spinal anesthesia can depress digitalis cardiotoxicity. Wilson et al. (878) found that pretreatment of anesthetized dogs with epidural lidocaine delayed the onset of acetylstrophanthidin-induced ventricular tachycardia and altered the mode of death produced by this digitalis drug from ventricular fibrillation to cardiac arrest. In addition, when spinal anesthesia was induced by epidurally-administered lidocaine during an established acetylstrophanthidin-induced ventricular tachycardia, they observed a conversion of the arrhythmia to normal sinus rhythm in 7 out of 11 dogs tested and death due to asystole rather than ventricular fibrillation in all of the 11 dogs tested. Boyajy and Nash (92) reported that subdurally administered lidocaine in anesthetized, vagotomized dogs increased both the arrhythmogenic and lethal doses of ouabain and also changed the mode of death produced by ouabain from ventricular fibrillation to cardiac arrest.

The effect of pithing the spinal cord on digitalis toxicity has been studied in anesthetized dogs by Hashimoto et al. (340). They employed an isolated papillary muscle from the dog and perfused it with the arterial blood from a donor dog. The contractility and rhythm of the papillary muscle and the heart rate and blood pressure of the donor dog were measured simultaneously. Ouabain was administered i.v. to the donor dog and the doses required to produce ventricular tachycardia and death were determined. When each of the endpoints was reached, the changes occuring in the blood-perfused isolated papillary muscle were examined. In control animals, a dose of ouabain that produced ventricular tachycardia in the donor dog produced arrhythmic contractions in the isolated papillary muscle. In animals with spinal cords pithed, the dose of ouabain required to produce ventricular tachycardia was increased and no arrhythmic contractions were observed in the papillary muscle preparation in five of six dogs studied. Similar findings were observed in adrenalectomized dogs. Furthermore, pithing the cord was found to prevent largely the occurrence of ventricular fibrillation, since only one of five animals exhibited this response; death in the other four animals was due to ventricular standstill. The investigators suggested that ouabain causes excitation of sympathetic preganglionic neurons in the spinal cord, which in turn results in catecholamine secretion from the adrenal glands.

With the exception of two studies, acute cardiac denervation has also been found to increase the doses of digitalis drugs required to cause cardiac arrhythmias. This was first shown by Rothberger and Winterberg in 1913 (677), who used extirpation of the thoracic sympathetic chains. Mendez et al. (545) reported that bilateral extirpation of the stellate ganglia plus the upper three thoracic ganglia in vagotomized, anesthetized dogs resulted in an increase in the lethal dose of acetyldigitoxin (0.48 mg/kg vs. 0.55 mg/kg). They also indicated that fewer denervated animals died in ventricular fibrillation than did the control animals (3 of 5, vs. 6 of 6). This group reported later (215) that the same sympathetic denervation procedure plus functional exclusion of the adrenal glands from the circulation increased the lethal doses of ouabain and digitoxin and changed the mode of

death by these agents from ventricular fibrillation to cardiac arrest. Cagin et al. (110) compared the type of arrhythmia produced by ouabain and the arrhythmogenic concentration of ouabain in guinea-pig hearts under conditions both in vivo and in vitro. They found that the lethal event in vivo was ventricular fibrillation while that in vitro was asystole. In addition, the myocardial concentrations of ouabain needed to produce these lethal effects were lower in vivo than in vitro. This finding is consonant with acute removal of the nervous system exerting an antiarrhythmic effect. One of the studies in which acute sympathectomy (i.e., bilateral excision of the stellate ganglia plus the upper four thoracic ganglia) was found to have no effect was that of Morrow et al. (559). They found that the mean dose of ouabain required to produce multiple premature ventricular contractions (i.e., three consecutive premature ventricular contractions) in anesthetized dogs was not affected by denervation. It is not clear why disparate data were obtained by Morrow et al., but it should be noted that their endpoint for an arrhythmic effect was different from the endpoint in the above studies that showed a positive effect of acute denervation. In the other study where acute denervation was tested and found to have no protective effect, George et al. (274) reported that bilateral stellate ganglionectomy rendered the canine heart more sensitive (relative to animals with intact sympathetic reflex mechanisms) to the effects of electrically induced ventricular fibrillation in the presence of ouabain. These data are surprising in view of all the accumulated findings to indicate that removal of the sympathetic nervous system makes the heart less sensitive to arrhythmogenesis. A possible explanation for these surprising findings is that bilateral stellate ganglionectomy removes the efferent limb of the baroreceptor reflex arc. When this reflex is intact, administration of digitalis actually increases ventricular fibrillation threshold (101, 274). In spite of the interesting findings obtained with digitalis on ventricular fibrillation threshold, there is some question as to the validity of data obtained with this technique. The problems of using this technique to assess cardiac irritability have been discussed by Zipes (893).

Chronic cardiac denervation, as performed by the method of Cooper et al. (154), has in most cases been found to protect animals from the arrhythmogenic effects of digitalis. This was demonstrated by Solti et al. (746) for i.v. strophanthin. They reported that 0.75 mg of this drug produced arrhythmias in control dogs but not in animals subjected to cardiac denervation. Wallace et al. (854), who used conscious dogs intoxicated with ouabain, reported a similar finding. These investigators found that cumulative doses of ouabain that consistently produced ventricular extrasystoles and ventricular tachycardia in neurally intact dogs produced no evidence of ventricular irritability in the denervated dogs. They concluded that denervation of the heart inhibits the cardiac manifestations of an overdose of digitalis. Cavoto and Kelliher (130) examined the effects of ouabain on Purkinje fibers obtained from the hearts of cats previously subjected to chronic cardiac denervation. They found that doses of ouabain required to produce premature complexes were larger in sympathectomized Purkinje fibers than in control Purkinje fibers. In other studies from Roberts's group (251), who measured left ventricular repolarizations in the cat heart, it was demonstrated that denervation increased the amount of ouabain necessary to produce nonuniform changes in refractory period. Denervation also delayed the time to onset of arrhythmias evoked by ouabain. Morrow et al. (559) did not observe a protective effect of chronic denervation on the capacity of ouabain to induce three consecutive multiple premature ventricular contractions.

Cardiac autotransplantation has also been used as a technique for producing cardiac denervation and assessing the role of the sympathetic nervous system in the arrhythmogenic effects of digitalis. Willman et al. (877) used baboons (whether anesthesia was employed was not stated) and found no effect of autotransplantation on the capacity of ouabain to elicit ventricular extrasystoles, tachycardia, or fibrillation. Smith et al. (736) reported similar findings in anesthetized dogs. They found no difference between the autotransplant group and the control group with respect to the dose of ouabain required to produce ventricular automaticity. One criticism of both of these studies is that the control animals did not undergo a sham operation. This would seem necessary because the extensive surgery involved for the autotransplant procedure might itself increase the sensitivity of the animals to the arrhythmogenic effects of digitalis.

The role of the adrenal glands in the arrhythmogenic effects of digitalis has been evaluated under several conditions. Adrenalectomy alone appears to exert either a slight or no protective effect against digitalis-induced arrhythmias. Hermansen (358) reported that bilateral adrenalectomy in anesthetized guinea pigs slightly but significantly increased the dose of ouabain required to produce ventricular fibrillation. Hashimoto et al. (339, 340) found that isolated canine papillary muscles perfused with blood from a donor dog exhibited no arrhythmic contractions when ouabain was administered to the donor dog after extirpation of both adrenal glands. In contrast to these positive findings, numerous other investigators have observed no protective effect of adrenalectomy against several digitalis preparations (ouabain, digitoxigenin, and digoxin) either in conscious mice or anesthetized cats (143, 418, 441, 596). The circumstances under which adrenalectomy does appear to have a protective effect are when it is combined with other procedures that interfere with the function of the sympathetic nerves, reserpine pretreatment (143, 358), acute extirpation of the cardiac nerves (546), or pretreatment with 6-hydroxydopamine (418). In some of these studies, adrenalectomy enhanced the already protective effect of

surgical or pharmacological denervation of the sympathetic nervous system (143, 358, 546), while in other studies none of the procedures alone had a significant effect but the combination of the two procedures produced a significant increase in the doses of digitalis to cause ventricular irritability (418).

In summary, most surgical denervation procedures that exclude components of the sympathetic nervous system, including decerebration at the midcollicular level, spinal cord transection, pithing the spinal cord, and acute and chronic cardiac denervation, appear to reduce the sensitivity of the heart to digitalis-induced ventricular arrhythmias. These results are consistent with the idea that ventricular arrhythmias caused by digitalis overdosage are mediated in part through digitalis-induced enhancement of cardiac sympathetic nerve function. Negative results have been obtained with cardiac autotransplantation but the proper control experiments have not been performed. Finally, adrenalectomy appears to have a "small" protective effect in some studies, but more importantly, when combined with denervation of the heart, adrenalectomy results in a significant reduction in the capacity of digitalis drugs to evoke ventricular arrhythmias.

D. STUDIES IN WHICH DRUGS TO MODIFY SYMPATHETIC NERVOUS SYSTEM FUNCTION WERE USED. Evidence that the sympathetic nervous system has a significant role in causing ventricular arrhythmias after overdosage with digitalis drugs has been obtained by testing drugs known to interrupt efferent sympathetic neuronal function at each point in the transmission process (i.e., from the central nervous system to the postsynaptic cardiac adrenergic receptors). These agents include clonidine (an agent that depresses central sympathetic outflow), ganglionic blocking drugs, drugs that interfere with storage and release of norepinephrine at the postganglionic sympathetic nerve ending, and drugs that block beta- and alpha-adrenergic receptors on cardiac tissue. These drugs have been tested for their capacity either to alter the dose of digitalis required to cause ventricular arrhythmias or to convert digitalis-induced ventricular arrhythmias to sinus rhythm.

There are two studies in which the effect of the centrally acting sympathetic neurodepressant agent, clonidine, on digitalis-induced ventricular arrhythmias was examined. Gillis et al. (282) administered a toxic dose of deslanoside to anesthetized cats and, with recordings of preganglionic cardiac sympathetic nerve activity, found that the deslanoside produced an increase in neural discharge at the time that ventricular tachycardia was observed. When clonidine was administered i.v., it abolished the enhanced neural activity and at the same time counteracted the ventricular rhythm disorder. No antiarrhythmic activity was seen when clonidine was administered to deslanoside-intoxicated cats with transected spinal cords. From these observations, it was concluded that clonidine was effective against deslanoside-induced cardiotoxicity because of a sympathetic neural depressant action. In addition, pretreatment with clonidine was found to increase significantly the doses of deslanoside necessary to produce ventricular arrhythmias. A few years later, Pace and Gillis (596) reported similar results, that i.v. clonidine abolished simultaneously the increase in sympathetic neural activity produced by digoxin and converted the ventricular tachycardia produced by digoxin to normal sinus rhythm.

The influence of ganglionic blockade on the capacity of digitalis materials to induce disorders of cardiac rhythm has been the subject of several investigations (92, 143, 284, 404, 662, 676, 843). These studies have not yielded uniform results. In some of the studies performed, ganglionic blockade was reported to provide protection against digitalis-induced cardiac rhythm disorders. Veselova (843) found that the administration of either hexamethonium or mecamylamine to urethane-anesthetized cats increased the dose of G-strophanthin necessary to produce cardiac arrest. He concluded that the toxicity of G-strophanthin was reduced by blockade of the autonomic ganglia. There were, however, wide variations in the lethal doses of G-strophanthin among the "ganglion-blocked" animals in his study, and it is difficult to determine whether the lethal doses of the cardiac glycoside in the blocked and control animals were significantly different. In a later study, Boyajy and Nash (92) did observe a significant increase in the lethal dose of ouabain in pentobarbital-anesthetized dogs pretreated with chlorisondamine in comparison to control animals. However, chlorisondamine administration failed to alter the dose of ouabain required to produce ventricular arrhythmias (i.e., three consecutive ventricular ectopic beats) in their study. Rothaus and Powell (676) studied the effect of mecamylamine (5 mg/kg, i.v.) on tachyarrhythmias induced by digoxin in dogs anesthetized with chloralose-urethane and reported that mecamylamine transiently and consistently converted the tachyarrhythmias to sinus rhythm. On the other hand, ganglionic blockade has also been reported to confer no protection against digitalis-induced rhythm disorders in several other studies. Roberts et al. (662) observed that the administration of hexamethonium to pentobarbital-anesthetized dogs with experimentally induced A-V block did not alter the ability of acetylstrophanthidin to produce ventricular tachycardia. Ciofalo et al. (143) found that the administration of hexamethonium to dial-urethane-anesthetized, vagotomized cats did not influence the time required for ouabain to produce ventricular extrasystoles, ventricular tachycardia, or death. Similarly, Kelliher and Roberts (404) reported that hexamethonium administration to dial-urethane-anesthetized cats did not change the dose of digoxin necessary to evoke premature ventricular contractions, ventricular tachycardia, or ventricular fibrillation.

A possible explanation for the nonuniform results obtained in the above studies may be the employment of

agents (e.g., hexamethonium, mecamylamine, chlorisondamine) that block only nicotinic receptor sites on ganglia. It has been demonstrated that autonomic ganglia of both cats and dogs also possess muscarinic receptor sites (232, 830). That is, to achieve complete interruption of ganglionic transmission in these animals, the administration of atropine would be required along with a nicotinic ganglionic blocking agent. Furthermore, it was shown that the use of a nicotinic blocking drug alone impaired ganglionic transmission to a variable extent, depending on the degree of functional muscarinic transmission present in ganglia (232). Thus, the degree to which muscarinic receptors are capable of participating in ganglionic transmission could therefore explain the variable results of ganglionic blockade on digitalis toxicity in those studies in which only nicotinic ganglionic blocking agents were employed.

Gillis et al. (284) examined the role of both nicotinic and muscarinic blockade of autonomic ganglia on the disorders of cardiac rhythm produced by deslanoside in dial-urethane-anesthetized cats. In confirmation of the results of others (143, 404, 662), they found that blockade of ganglionic nicotinic receptor sites alone with hexamethonium failed to alter the dose of deslanoside needed to produce ventricular tachycardia or ventricular fibrillation. They also found that blockade of ganglionic muscarinic receptor sites alone with atropine also failed to alter these indices of cardiotoxicity. However, combined nicotinic and muscarinic blockade, achieved by combined administration of hexamethonium and atropine, was found consistently to produce an increase in the dose of deslanoside required to induce the cardiac rhythm disorders. The observation was also made in this study that administration of atropine to hexamethonium-pretreated cats intoxicated with deslanoside decreased deslanoside-induced postganglionic sympathetic nerve activity without affecting the glycoside-induced increase in preganglionic nerve activity. The results of this study indicate that digitalis can cause activation of both nicotinic and muscarinic receptor sites on ganglia, and that blockade of both of these receptors is necessary in order to assess accurately the role of ganglionic blockade in digitalis toxicity. These results also point to the conclusion that the central nervous system is an important site of digitalis action in the induction of cardiac rhythm disorders.

A number of drugs that interfere with storage and release of norepinephrine at the postganglionic sympathetic nerve endings alter the doses of digitalis drugs required to produce cardiac arrhythmias. These include reserpine, guanethidine, bretylium, B-TM-10, 6-hydroxydopamine, and α-methyl-*m*-tyrosine.

Nearly all of the studies in which chronic pretreatment with reserpine was used show a protective effect of this agent, as exhibited by a change in either the dose of digitalis required to produce arrhythmias and death (33, 91, 92, 106, 107, 127, 141–143, 193, 215, 272, 273, 358, 472, 557, 566, 570, 609, 614, 652, 662, 691, 741, 780, 787, 794, 846), or a change in the mode of death (i.e., cardiac arrest rather than ventricular fibrillation) (215, 472, 662, 774, 781, 873). Only two animal studies that we are aware of have failed to report that chronic reserpine pretreatment caused an alteration in the arrhythmogenic effect of digitalis drugs; these are the studies of Yelnosky and Ervin (887) and Morrow et al. (559). Statistical evaluation of the raw data of Yelnosky and Ervin revealed that the arrhythmogenic dose of ouabain in the small number of reserpine-treated dogs tested was actually significantly higher ($P < .05$) than in control animals. The data of Morrow et al. might be explained by the point raised by Roberts (660). That is, when small doses of reserpine such as those employed by Morrow et al. are used, the catecholamine content of the adrenal medulla is not affected. Roberts suggests that noradrenergic neurons in the heart that are depleted of norepinephrine by reserpine will take up catecholamines released from intact adrenal glands. This fits with other findings demonstrating that digitalis drugs will release catecholamines from the adrenal glands (42, 340). It also fits with the finding that restoration of reserpine-depleted cardiac stores are dependent mostly on catecholamines secreted from the adrenal medulla (660).

As stated by Roberts (660), "the effect of reserpine on digitalis-induced ventricular arrhythmia is probably related to its catecholamine-depleting action on the heart." Evidence for this is that repletion of catecholamine stores of reserpine-pretreated animals restores to normal the sensitivity of the animal to digitalis-induced arrhythmic effects (22, 662, 794). In addition, the time-course of the antiarrhythmic effect of reserpine parallels the time-course for depletion of catecholamines (660).

It is not entirely clear whether the protective effect of reserpine is due to depletion of catecholamines in the periphery, in the CNS, or in both areas. Evidence that peripheral depletion is important is derived from the repletion data. Repletion of norepinephrine stores and restoration of normal sensitivity to digitalis is most likely due to repletion of peripheral stores, because norepinephrine does not cross the blood-brain barrier. Other evidence that peripheral catecholamine depletion may be involved are findings obtained with drugs that deplete peripheral stores, but not CNS stores, of monoamines. These include systemically administered 6-hydroxydopamine (403, 566, 688) and syrosingopine, which is a reserpine analogue with a selective action to deplete peripheral catecholamine stores (107). Guanethidine is another agent that, in addition to blocking release of norepinephrine from postganglionic nerve endings, depletes peripheral catecholamine stores and increases the dose of digitalis required to cause arrhythmias (642).

There also is evidence that a CNS catecholamine-depleting effect might be responsible for the antiarrhythmic action of reserpine. This is indicated by the studies of Buterbaugh and Spratt (106) with tetrabenazine. This agent is an analogue of reserpine that

exerts a selective action to deplete CNS catecholamine stores. Pretreatment of cats and rats with tetrabenazine increased the doses of digitoxigenin required to produce lethal arrhythmias. Nadeau and de Champlain (566), in their comparative studies with systemically administered reserpine and 6-hydroxydopamine, also concluded that there was an important role for CNS catecholamine depletion. Greater protection against ouabain-induced cardiotoxicity was observed with reserpine, which depleted both CNS and peripheral catecholamine stores, than with 6-hydroxydopamine, which depleted only peripheral catecholamine stores. However, it is difficult to accept these findings in view of other data demonstrating that depletion of monoamines in the CNS with reserpine, or reserpine plus α-methyl-p-tyrosine, has either no effect or even increases spontaneously-occurring sympathetic neural discharge (324, 705). In addition, pretreatment with reserpine does not counteract the increase in sympathetic discharge evoked by either hypothalamic or medullary stimulation (324, 705). Thus, it would appear that any CNS antiarrhythmic action of reserpine or tetrabenazine might occur independent of CNS catecholamine depletion.

In summary, the data obtained with reserpine strongly implicate the sympathetic nervous system in the arrhythmogenic effects of digitalis. In addition, the site of action of the protective effect of reserpine as related to catecholamine depletion appears to be in the periphery, specifically on noradrenergic nerve terminals in the heart.

As in the case of reserpine, depletion of peripheral catecholamine stores with 6-hydroxydopamine results in a protective effect against digitalis-induced arrhythmias. Nadeau and de Champlain (566) found that pretreatment of pentobarbital-anesthetized rats with 100 mg/kg i.v. of 6-hydroxydopamine 18 hours before ouabain reduced the incidence of ventricular fibrillation caused by this digitalis drug. However, pretreatment with 6-hydroxydopamine did not increase the survival rate of ouabain-intoxicated animals in their study. Saito et al. (688) found that pretreatment of urethane-anesthetized guinea pigs with 6-hydroxydopamine for a period of up to a week increased the dose of ouabain required to produce both ventricular arrhythmia and death. In these studies, cardiac norepinephrine concentrations were measured after 6-hydroxydopamine administration and were reduced markedly. The results of these investigations indicate that depletion of cardiac norepinephrine with 6-hydroxydopamine is sufficient to attenuate the cardiotoxic effects of ouabain. Consistent with the findings obtained from rats and guinea pigs are the preliminary data of Kelliher (403), which indicated that pretreatment with 6-hydroxydopamine delayed the cardiotoxic action of ouabain in anesthetized cats. Interestingly, pretreatment with 6-hydroxydopamine did not alter the cardiotoxic action of digoxin in this species (403). In addition, subsequent studies in cats have also shown that pretreatment with 6-hydroxydopamine does not protect against ouabain-induced ventricular arrhythmias and death (659). Results of a preliminary study performed by Klepser et al. (418) indicate that depletion of cardiac catecholamine stores alone with 6-hydroxydopamine has no influence on arrhythmogenic or lethal doses of digoxin in anesthetized cats, unless this procedure is also combined with adrenalectomy. That is, neither pretreatment with 6-hydroxydopamine alone nor adrenalectomy alone influenced digoxin cardiotoxicity in their studies, but when these procedures were combined the arrhythmic and lethal doses of digoxin were significantly increased. It thus appears that the presence of both cardiac and adrenal catecholamines are involved in the arrhythmias produced by this digitalis preparation.

The ability of guanethidine, an agent that acts to prevent the release of norepinephrine from peripheral adrenergic nerve terminals and also to cause depletion of norepinephrine from peripheral nerve endings with time, to alter digitalis-induced cardiotoxicity has been examined in two studies. In the first study of this type, Raines et al. (642) reported that pretreatment of dial-urethane-anesthetized, vagotomized cats with 20 mg/kg of guanethidine 24 hours prior to ouabain administration significantly increased the dose of that glycoside required to produce ventricular tachycardia and ventricular fibrillation. Wilkerson and Glenn (875) were unable to confirm those results. They found that pretreatment of pentobarbital-anesthetized dogs with guanethidine, 10 mg/kg, 24 hours prior to ouabain administration did not influence the dose of ouabain required to produce premature ventricular beats, ventricular tachycardia, or death. They did report, however, that guanethidine pretreatment changed the mode of death in ouabain-intoxicated dogs from ventricular fibrillation to cardiac arrest. Wilkerson and Glenn pointed out that the reason they failed to observe an antiarrhythmic effect with guanethidine may have been due to the use of pentobarbital as an anesthetic, because this anesthetic depresses sympathetic tone and could negate the sympathoinhibitory effect of guanethidine. This appears to have been the case in their study, since guanethidine did not reduce the heart rate in their pentobarbital-anesthetized dogs, whereas in the study by Raines et al. guanethidine did produce bradycardia in animals anesthetized with dial-urethane, an agent that may be less depressant to the sympathetic nervous system.

Bretylium, like guanethidine, exerts an antiadrenergic action by interfering with norepinephrine release from sympathetic postganglionic nerve endings (89). Studies in which bretylium has an antiarrhythmic effect in animals have been performed with chronic administration of the drug (as opposed to acute administration where other actions of bretylium prevent an evaluation of the antiadrenergic property of this agent in digitalis-induced arrhythmias; see below). Papp and Vaughn Williams (600) were the first to study the effect of long-term bretylium administration on digitalis-induced arrhyth-

mias. They found that pretreatment of urethane-anesthetized guinea pigs with bretylium, 30 mg/kg, at 24 hours and 4 hours before ouabain administration increased the dose of ouabain required to induce the first sign of atrial arrhythmia (unequal intervals between atrial beats) and reduced the incidence of these arrhythmias as well as the incidence of ventricular fibrillation. These investigators also reported that bretylium produced no "quinidine-like" actions on isolated rabbit atria or local anesthetic effects on frog sciatic nerve at clinically relevant concentrations, and concluded that "it is possible that the anti-arrhythmic action of bretylium could be due to depression of activity in sympathetic nerves." A protective effect of bretylium, when given as a long-term pretreatment, was also demonstrated by Gillis et al (280). They reported that pretreatment of dial-urethane-anesthetized cats with bretylium, 30 mg/kg, at 24 and 4 hours before administering deslanoside significantly increased the dose of this digitalis material required to produce ventricular tachycardia. Gillis et al. also concluded that the antiarrhythmic activity of bretylium was due to its ability to block release of norepinephrine from cardiac sympathetic nerves. Support for this concept was provided by Bassett and Hoffman (49), who indicated in their review of antiarrhythmic drugs that the antiarrhythmic activity of bretylium may be exerted "through depression of neural function." They noted that the antiarrhythmic efficacy of bretylium in laboratory studies does not appear to be due to any intrinsic direct electrophysiological effects, since it does not decrease cardiac automaticity nor does it resemble either the quinidine-like drugs or diphenylhydantoin and lidocaine in its effect on the relative magnitude of changes in action potential duration and effective refractory period.

The forerunner of both bretylium and guanethidine was B-TM-10. Roberts et al. (662) evaluated this agent against ventricular arrhythmias induced by acetylstrophanthidin. Specifically, they administered a large single dose of acetylstrophanthidin (100 μg/kg) to pentobarbital-anesthetized dogs with surgically induced A-V block and observed that it increased the ventricular rate by 175 beats/min. After the effects of this dose had disappeared, they administered B-TM-10, 20 mg/kg i.v., and ½ to 3 hours later readministered the same dose of acetylstrophanthidin. They observed that acetylstrophanthidin increased the ventricular rate by only 21 beats/min after the administration of B-TM-10. Since B-TM-10 is known to prevent the release of catecholamines from sympathetic nerve terminals, Roberts et al. concluded that the acetylstrophanthidin-induced ventricular tachycardia was related to the release of catecholamines from postganglionic adrenergic neurons.

The effect of α-methyl-*m*-tyrosine (an inhibitor of norepinephrine synthesis) on digitalis toxicity in urethane-anesthetized, vagotomized rabbits has been studied by Ciofalo and Treece (144). They reported that pretreatment of animals at various intervals with α-methyl-*m*-tyrosine (α-MMT), ranging from 48 hours to 2 hours prior to ouabain administration, prolonged the time required for ouabain to produce ventricular extrasystoles, ventricular tachycardia, and death. The conclusion was reached that catecholamine depletion, resulting from the conversion of α-MMT to metaraminol and subsequent displacement and loss of norepinephrine from the adrenergic nerve terminal, plays a role in the protective effect of this drug against digitalis-induced cardiac arrhythmias.

In summary, drugs known to interfere with the function of the postganglionic sympathetic nerve terminal by interfering with either storage, release, or synthesis of norepinephrine, and with the integrity of the neuron (6-hydroxydopamine), have all been demonstrated to interfere with the capacity of digitalis drugs to cause ventricular arrhythmias. However, it should be pointed out that most of these agents exert an initial sympathomimetic effect, and that this action can alter the capacity of digitalis drugs to cause arrhythmias in the opposite direction. That is, arrhythmogenic effects of reserpine have been found in some clinical studies in which this amine-depleting agent was administered to patients already receiving digitalis (186, 491, 708). A reserpine-induced enhancement of digitalis toxicity occurred in these studies. This was probably because of the administration of reserpine against a background of chronic digitalis therapy, where an initial release of catecholamines by reserpine enhanced the arrhythmogenic potential of digitalis. This idea is supported by the findings in two animal studies in which digitalis was given simultaneously with reserpine and an enhanced cardiotoxicity was noted (91, 186). Consistent with this is the finding that exogenously administered catecholamines can exacerbate cardiac arrhythmias induced by digitalis. This has been shown for norepinephrine (22, 557, 558), epinephrine (503, 531, 575, 652, 831, 871, 895, 896), isoproterenol (59, 272, 273, 648, 716), and ephedrine (394, 711). However, Lown et al. (491) found that the arrhythmias caused by reserpine in the presence of acetylstrophanthidin consisted of heart block, escape beats, and junctional rhythm, which may have been due in part to the vagal stimulating effect of reserpine (84) and resulting ventricular escape rhythms.

As in the case of reserpine, 6-hydroxydopamine is capable of aggravating digitalis-induced arrhythmias. These effects accrue from the ability of 6-hydroxydopamine to release norepinephrine from adrenergic nerve terminals when administered in the presence of subtoxic doses of digitalis. Kelliher and Roberts (403a) demonstrated that 6-hydroxydopamine can enhance digitalis cardiotoxicity in dial-urethane-anesthetized cats. They administered this agent 20 minutes after the start of an i.v. infusion of ouabain, at a time when animals were in normal sinus rhythm, and observed a marked reduction in the subsequent doses of ouabain required to produce premature ventricular contractions, ventricular tachycar-

dia, and ventricular fibrillation in comparison to animals treated with the ouabain alone. They concluded that the initial catecholamine-releasing action of 6-hydroxydopamine was responsible for the enhanced cardiac toxicity of ouabain.

Bretylium and guanethidine also produce an initial release of norepinephrine from the adrenergic nerve terminal (229, 413) and potentiate the effect of circulating catecholamines on adrenergic receptors (359, 413). Papp and Vaughan Williams (600) reported that an i.v. injection of bretylium, 20 mg/kg, given 10 minutes before initiation of an infusion of ouabain in urethane-anesthetized guinea pigs, increased ouabain toxicity. Their conclusion was based on the somewhat unimpressive finding that 13 of 13 acutely pretreated animals developed ventricular fibrillation whereas 18 of 20 control animals fibrillated. Papp and Vaughn Williams indicated that the early sympathomimetic action of bretylium could account for the enhancement of ouabain toxicity. At the same time, Reynolds and Horne (652) reported similar but more convincing results. They found that the s.c. administration of bretylium, 10 mg/kg, given 10 to 20 minutes before initiation of a ouabain infusion in pentobarbital anesthetized cats, reduced the dose of ouabain required to produce ectopic rhythm and death. This was not the case, however, when bretylium was given in combination with pronethalol prior to the ouabain administration. In the presence of beta-adrenergic receptor blockade with pronethalol, bretylium no longer enhanced ouabain cardiotoxicity, thus implicating a role for bretylium-induced norepinephrine release in the response. In several additional studies acutely administered bretylium enhanced digitalis toxicity. Proctor et al. (632) administered 5 mg/kg of bretylium i.v. to dogs intoxicated with ouabain and found it to increase the ventricular rate. Kleiger and Shander (417) administered bretylium, 10 to 20 mg/kg i.v., to dogs that had been allowed to recover for 4 minutes from the effects of acetylstrophanthidin-induced ventricular tachycardia. They found that the bretylium caused a reinduction of the ventricular tachycardia and that the rate of the ventricular rhythm disorder was 30 beats/min faster than that previously induced by the acetylstrophanthidin alone. Lipski et al. (484) administered bretylium, 5 mg/kg i.v., to anesthetized dogs 1 to 1½ hours after the signs of acetylstrophanthidin-induced toxicity had disappeared. Bretylium increased automaticity, as indicated by a decrease in the time required for a ventricular escape beat to occur after vagally-induced cardiac arrest, and also produced brief spontaneous bursts of premature ventricular premature systoles and ventricular tachycardia, leading to death in some of the animals. The arrhythmias occurred during the period when the ventricular escape time was decreased. Gillis et al. (280) reported that administration of bretylium (2 to 100 mg/kg i.v.) to anesthetized cats intoxicated with deslanoside enhanced the cardiac toxicity produced by this digitalis preparation. When increasing doses of bretylium were given for the purpose of converting deslanoside-induced ventricular tachycardia to sinus rhythm, they increased the rate of ventricular tachycardia and in some animals produced ventricular fibrillation. When a fixed dose of bretylium was given for the purpose of shortening the duration of an established ventricular tachycardia, it caused death due to ventricular fibrillation or hypotension. No animal survived more than 11 minutes after bretylium was administered, whereas animals treated in the same way with deslanoside but not given bretylium survived for an average of 72 minutes with eventual return to sinus rhythm occurring in more than half of the animals studied. These investigators also found that bretylium no longer resulted in the production of deleterious effects when it was administered to animals pretreated with propranolol. Instead, in the presence of beta-adrenergic blockade, bretylium exerted an antiarrhythmic effect that was manifest as a reduction in the duration of ventricular tachycardia in comparison to the animals treated with bretylium alone.

The results of the above studies indicate that the ability of bretylium to release catecholamines from the adrenergic nerve terminal may be responsible for its arrhythmogenic activity when administered just before or during the establishment of digitalis-induced ventricular rhythm disorders. This sympathomimetic effect of bretylium may also explain other findings that short-term pretreatment or acute administration of bretylium exerted no effect on digitalis-induced arrhythmias. For example, Reynolds and Horne (652) reported that bretylium, 30 mg/kg s.c., in pentobarbital-anesthetized dogs 4 hours prior to ouabain administration had no effect on the dose of ouabain required to produce cardiac arrhythmia and death. Similarly, Kleiger and Shander (417) observed that pretreatment of dogs with bretylium, 10 to 20 mg/kg i.v., 2 hours prior to acetylstrophanthidin administration had no effect on the dose of digitalis required to produce ventricular tachycardia. There is a possibility that, in these studies, the pretreatment time intervals of 4 and 2 hours were not sufficient to allow for the norepinephrine-releasing action of bretylium to subside, an effect that would tend to mask an antiarrhythmic effect of the drug. Baum et al. (54) and Kniffen et al. (419) also reported that bretylium, 10 to 20 mg/kg i.v., to ouabain-intoxicated dogs anesthetized with pentobarbital had no effect on ventricular tachycardia induced by the glycoside. That is, bretylium neither aggravated nor prevented the arrhythmia in their studies. Although no clear explanation is available to explain their findings, it may be postulated that although the amount of catecholamine released by bretylium may have been insufficient to enhance the arrhythmia, it nevertheless may have been adequate to offset any antiarrhythmic effect of bretylium.

There have been numerous studies in several species in which beta-adrenergic receptor blocking agents were used to evaluate the role of sympathetic nervous system

activity in digitalis-induced arrhythmias. The majority of studies in guinea pigs indicate a positive role for the sympathetic nervous system in the cardiotoxicity produced by digitalis drugs. Vaughn Williams (842) and Sekiya and Vaughan Williams (716) reported that guinea pigs pretreated with the beta-adrenergic blocking agents dichloroisoproterenol and pronethalol required significantly larger doses of ouabain to produce extrasystoles, ventricular arrhythmias, and cardiac arrest. These drugs also prevented the occurrence of cardiac arrest. In addition, they reported that pronethalol administered during the time that there was fibrillation restored the heart to normal sinus rhythm. Stanton et al. (767) reported that pretreatment with either pronethalol or MJ-1999 increased the dose of ouabain required to produce ventricular arrhythmias and reduced the incidence of ventricular fibrillation. Hermansen (357) treated guinea pigs with propranolol, Ph-QA-33, and INPEA, and found that these agents in beta-adrenergic blocking doses converted ventricular fibrillation to normal sinus rhythm. Animals treated with these agents all required larger doses of ouabain to produce cardiac arrest.

Papp and Vaughan Williams (601) examined the effect of ICI 50172, a beta-adrenergic blocking agent lacking local anesthetic properties, on ouabain-induced arrhythmias. Treatment with this agent reverted ventricular fibrillation to normal sinus rhythm. Pretreatment increased the dose of ouabain required to produce cardiac arrhythmias. Dohadwalla et al. (196) compared the ability of *d,l*-propranolol with *d*-propranolol to counteract ouabain-induced arrhythmias. The *d,l*-propranolol was significantly more effective in countering arrhythmias than the *d*-isomer. Since the racemic mixture possessed greater beta-adrenergic blocking activity but an equal degree of local anesthetic effect, the authors concluded that beta-blocking activity of the racemic mixture contributed to the protective effect. Papp and Vaughan Williams (601) next studied the effects of the *l*-isomer of propranolol and found that it too, in beta-adrenergic blocking doses, protected guinea pigs against ouabain-induced arrhythmias. Singh and Vaughan Williams (731) reported that antiarrhythmic activity of INPEA and LB-46 correlated more closely with their beta-blocking activities than with their direct membrane activities. They concluded that beta-adrenergic blocking activity of these drugs contributed to their protective effect against digitalis cardiotoxicity. Marmo (519) found that beta-blocking drugs that do not possess significant "non-specific myocardial effects" increase the time to onset of K-strophanoside-induced arrhythmias, ventricular fibrillation, and death. Finally, Cheymol et al. (138) observed that beta-adrenergic blocking doses of Kö 1266 and Kö 1313 were effective in increasing the doses of ouabain required to produce ventricular fibrillation and death. These investigators also made the interesting observation that doses far in excess of the beta-blocking dose were required to produce a similar antiarrhythmic effect in the dog. They concluded that an adrenergic component was involved in the production of digitalis arrhythmias in the guinea pig but not in the dog.

Experiments in cats with the beta-adrenergic receptor blocking drugs indicate that a sympathetic component is involved in digitalis-induced arrhythmias. The first study wherein cats and dogs were pretreated with pronethalol was reported by Erlij and Mendez (215). It was found that cats, but not dogs, required significantly greater amounts of digitoxin and ouabain to produce death. In both species, pronethalol changed the mode of death of digitoxin from ventricular fibrillation to cardiac arrest. Raper and Wale (647) found that beta-adrenergic blocking doses of propranolol, MJ-1999, and Ciba 39089 abolished a ouabain-induced ventricular tachycardia in 20% of the cats studied. Evans et al. (219) reported that pretreatment of cats with beta-adrenergic blocking doses of either nadolol or propranolol increased the dose of ouabain required to produce ventricular arrhythmias. Kelliher and Roberts (404) observed that pretreatment with sotalol (MJ-1999) in beta-adrenergic blocking doses increased the dose of digoxin to produce premature ventricular beats, ventricular tachycardia, and ventricular fibrillation. Finally, Somberg et al. (754) reported that surgical removal of the sympathetic nervous system abolished the antiarrhythmic effect of propranolol. They concluded that the antiarrhythmic action of propranolol to antagonize ouabain-induced arrhythmias was dependent on an intact nervous system.

Most studies with beta-adrenergic blocking agents have been performed in the dog. Interestingly, in this species, pretreatment with beta-adrenergic blocking doses of this class of drugs does not seem to exert a protective effect (27, 29, 215, 235, 685, 887). Only one group of investigators, to our knowledge, observed an increase in the dose of digitalis required to produce arrhythmias. Abiko and Ito (9) reported that pretreatment with pronethalol, 3 mg/kg, increased the dose of strospeside to produce arrhythmia. Pronethalol pretreatment did not alter the lethal dose but changed the mode of death from fibrillation to arrest. Alteration of the mode of death was also observed by Erlij and Mendez (215) and Stickney (774). They attributed this effect to reduction of adrenergic influences on the heart.

For treatment of digitalis-induced arrhythmias, there are several studies that indicate that blockade of beta-adrenergic receptors will restore the abnormal rhythm to sinus rhythm. This has been most dramatically demonstrated in the guinea pig wherein administration of these drugs counteracts ventricular fibrillation (cardiac arrest occurs as the endpoint) and increases the dose of ouabain required to produce arrhythmias (196). Raper and Wale (647) have reported that administration of three different beta-adrenergic receptor blocking agents will revert a digitalis-induced ventricular tachycardia to sinus rhythm in 20% of the cat experiments performed. Barrett and Cullen (44) reported that the *l*-isomer of propranolol was consistently more effective than the *d*-isomer in reversing ouabain-induced ventricular tachycardia in cats and

dogs. Zeft et al. (890) reported that beta-adrenergic receptor blocking doses of MJ-1999 and propranolol shortened the duration of ventricular tachycardia in conscious pigs intoxicated with acetylstrophandin. Pearle and Gillis (610) demonstrated that beta-adrenergic receptor blocking doses of propranolol abolished ouabain-induced ventricular tachycardia in four of six dogs with complete heart block. Finally, Apantaku et al. (27) reported that oxprenolol, a beta-adrenergic receptor blocking agent devoid of nonspecific quinidine and/or local anesthetic activity, reverted an established ventricular arrhythmia to sinus rhythm in about one-half of the dogs studied.

Some of the best evidence that activation of cardiac beta-adrenergic receptors are involved in mediating digitalis-induced arrhythmias comes from studies in patients. Except for one report (383), these studies demonstrate that beta-adrenergic blocking doses of pronethalol and propranolol counteract both atrial and ventricular arrhythmias induced by digitalis (53, 278, 334, 386, 387, 504, 735, 782, 783, 788, 805, 833, 854, 857, 883). This is also true of pindolol (32), tolamidol, and tolamolol (30, 31). In addition, practolol, a beta-adrenergic agent that has relatively little direct nonspecific membrane effects, was found to be effective in counteracting digitalis-induced arrhythmias (683). The observation that beta-adrenergic receptor activity is involved in digitalis-induced arrhythmias in humans has been made by others (151, 610, 721, 880).

One explanation for the divergent findings with beta-adrenergic blocking agents, that is, the inability of these agents in beta-blocking doses to counteract digitalis-induced arrhythmias in animals (e.g., 499), has been the use of anesthesia, since the sympathetic nervous system may not be very active in animals with a depressed CNS (880). Consistent with this suggestion is the conclusion stated by Epstein and Braunwald (213), that "it is clear from clinical observations that arrhythmias produced by digitalis are often accompanied by a high level of sympathetic activity." Another explanation is that there are species differences in the role of beta-receptor activation in mediating arrhythmias produced by digitalis. The sympathetic nervous system may be more involved in the generation of these arrhythmias in some species (i.e., guinea pig, cat, and man) than in other species (i.e., dog). Finally, many of the studies that demonstrated an inability of beta-blocking doses of the various adrenergic antagonists to counteract digitalis-induced arrhythmias have been performed in isolated hearts (8, 64, 1, 224, 246, 343, 423, 424, 438, 439, 442, 495–498, 748–750, 752, 763), wherein a functional adrenergic innervation of the heart has been excluded.

Recent data suggest that activation of alpha-adrenergic receptors on cardiac tissue and/or on the coronary vasculature can result in changes in the transmembrane action potential of cardiac cells that lead to cardiac arrhythmias. Ledda and colleagues (294, 451, 452) observed an increase in refractory period and action potential duration when an alpha-receptor agonist was administered into a medium bathing isolated sheep Purkinje fibers. These investigators observed the same effects with norepinephrine when administered after blocking beta receptors on the Purkinje fibers with propranolol. The occurrence of this effect in some cells but not in others could theoretically lead to arrhythmias. Ledda and coworkers also reported that a high concentration of an alpha-receptor agonist enhances automaticity and occasionally induces automatic activity in quiescent Purkinje fibers. Alpha-adrenergic receptors are also present on coronary vessels and when activated result primarily in vasoconstriction (672). Recent evidence suggests that alpha-adrenergic-induced constriction of these arteries may be responsible for coronary artery spasm in Prinzmetal's variant form of angina (461, 520, 886). This syndrome is characterized by ventricular arrhythmias and by S-T segment elevation and depression in reciprocal leads of the ECG (462, 630, 631).

The fact that a drug that acts in the CNS to increase sympathetic outflow to the heart can elicit ventricular arrhythmias mediated by alpha-adrenergic receptors on either the heart or coronary vessels has been demonstrated recently by DiMicco et al. (189). In their study, arrhythmias induced by centrally administered picrotoxin and mediated by the sympathetic nervous system were either prevented or abolished by administering an alpha-adrenergic receptor blocking agent. Pertinent to this review are findings that alpha-adrenergic receptors on the heart and coronary vasculature also appear to be activated by digitalis drugs, presumably due to their action to increase sympathetic neural activity in toxic doses. Evidence for this has been obtained with alpha-adrenergic receptor blocking agents to treat digitalis-induced ventricular arrhythmias. Ettinger et al. (217) reported that phentolamine administered to tranquilized dogs intoxicated with ouabain resulted in reversion of ventricular tachycardia to sinus rhythm in four of five animals tested. An antiarrhythmic effect of phentolamine also was reported in patients exhibiting extrasystoles induced by digitalis (309). Alps et al. (18) reported that indoramin counteracted ouabain-induced ventricular arrhythmias in anesthetized cats. Consistent with these findings are the results of Rothaus and Powell (676), who reported that phenoxybenzamine converted digoxin-induced ventricular arrhythmias to normal sinus rhythm in anesthetized dogs. They suggested that alpha-adrenergic receptor stimulation might contribute to digitoxin tachyarrhythmias. Finally, Hamlin et al. (331) documented that both ouabain and acetylstrophanthidin activate alpha-adrenergic receptors on the coronary vasculature. They observed that increases in coronary vascular resistance occurred in anesthetized dogs and that this effect was prevented by pretreatment with phenoxybenzamine.

Some investigators did not observe an antiarrhythmic effect of alpha-adrenergic blocking agents when they were administered to animals intoxicated with digitalis

drugs (215, 543). The reason for this may be related to the fact that the alpha-adrenergic blocking agents were given as a pretreatment rather than as a treatment, and it is known that some agents work under one circumstance but not under the other circumstance (470). Another reason may relate to the fact that these agents lower blood pressure and cause a reflex increase in sympathetic tone (543), an effect that could increase beta-receptor-mediated effects on the heart and offset the protective effect of the alpha-adrenergic blocking agents.

In summary, drugs such as the centrally acting neurodepressant agent clonidine, the ganglionic blocking agents, agents that inhibit the storage, release, and synthesis of norepinephrine in the postganglionic nerve terminal, and agents that block postsynaptic beta- as well as alpha-adrenergic cardiac receptors all antagonize the arrhythmogenic effects of digitalis. Thus, the plethora of positive data obtained with drugs that interfere with efferent cardiac sympathetic neural activity at each point in the transmission pathway strongly suggest an important role for the sympathetic nervous system in ventricular arrhythmias caused by digitalis.

E. STUDIES IN WHICH THE ROLE OF OTHER SPECIFIC ACTIONS OF DIGITALIS ON THE SYMPATHETIC NERVOUS SYSTEM WAS EVALUATED. It is clear that digitalis drugs produce significant changes in norepinephrine release, uptake, and tissue content. The specific role of these changes in the arrhythmogenic actions of digitalis has been explored to some extent. Although the effects of digitalis on noreprinephrine release have been studied only with preparations in vitro, effects on norepinephrine uptake and tissue content have been studied both in vitro and in vivo. The role of inhibition of uptake in vivo has been evaluated in four studies (124, 158, 210, 350). In two of the studies (124, 158), no effect of ouabain was observed on the uptake of catecholamines by the heart. In a study by Eikenburg and Stickney (210), inhibition of uptake did occur but was described as not playing a role in the genesis of ouabain-induced arrhythmias. This conclusion was based on the findings that inhibition of uptake occurred with a subarrhythmogenic dose of ouabain in vivo and that no inhibition of uptake occurred in vitro with isolated hearts, although arrhythmias did occur in the latter preparation. In a study performed by Helke et al. (350), inhibition of norepinephrine uptake was observed in the left ventricle of cats with a lethal arrhythmogenic dose of deslanoside. From this finding it was concluded that inhibition of norepinephrine uptake could play a role in cardiotoxicity of digitalis. The conclusion of Eikenburg and Stickney (210) can be questioned because their isolated hearts were not subjected to the excessive sympathetic tone that occurs when arrhythmogenic doses of digitalis are administered in vivo. In addition, even though inhibition of uptake did occur with subarrhythmogenic doses of ouabain, the time during which inhibition of uptake becomes significant and would most likely contribute to the development of arrhythmias is the period when digitalis is increasing sympathetic outflow, i.e., during exposure to arrhythmogenic doses. The role of digitalis-induced alteration in cardiac catecholamine content in arrhythmias produced by these agents was inferred from a study by Ciofalo and Treece (145). They observed an increase in cardiac catecholamine content with a lethal dose of ouabain in dogs and suggested that this alteration in content contributed to the lethal arrhythmias occurring in this species.

It appears from several studies (see section III A 1 c) that digitalis drugs interact with CNS dopamine receptors and it has been postulated that this interaction results in an increase in central sympathetic outflow (349). Evidence that this interaction might be involved in the generation of digitalis-induced ventricular arrhythmias has been inferred from studies with the dopamine receptor agonist drugs apomorphine and piribedil. Both of these agents confer marked protection against the ventricular tachyarrhythmias that occur in anesthetized cats (349), and the site of this protective effect appears to involve dopamine receptors in CNS tissue surrounding the fourth cerebral ventricle. Because the protective effect was so pronounced relative to the magnitude of protection seen with standard antiarrhythmic drugs, it is conceivable that CNS dopaminergic mechanisms, via their control over sympathetic outflow, may be important for the generation of arrhythmias by digitalis drugs. Confounding this conclusion is the recent finding of Briggs and Delallo (98), who used isolated rabbit hearts and the dopamine receptor blocking agent, haloperidol. This agent exerted a marked protective effect against ouabain toxicity in these isolated hearts. A protective effect was also observed in vivo in anesthetized rabbits exposed to toxic doses of ouabain. This beneficial effect of haloperidol may be due to a direct cardiac action of the drug that may mask the deleterious effect of digitalis on central dopamine receptors.

2. Role of the parasympathetic nervous system. Less interest has been shown in evaluating the role of the parasympathetic nervous system than that of the sympathetic nervous system in the arrhythmogenic effects of digitalis drugs (i.e., the induction of ventricular arrhythmias). It would be anticipated that the parasympathetic nervous system would exert a role for several reasons. One is that digitalis drugs in toxic doses produce simultaneous activation of both the vagus and the sympathetic nerves (291). Simultaneous activation of both nerves predisposes to cardiac arrhythmias more than excitation of either nerve alone (517). Activation of parasympathetic nerves results in suppression of the sinus node, which is richly innervated by cholinergic fibers. While the normal impulse-forming mechanism is being depressed, the impulse-forming mechanism of Purkinje cells in the ventricle is being enhanced by the augmented sympathetic discharge. Because of sparse cholinergic innervation to the ventricle, there is little acetylcholine available to oppose the sympathetic influence. Thus, the

ventricular pacemaker escapes and gains control over the impulse-forming activity of the heart. Without parasympathetic depression of sinus node pacemaker cells, an unopposed sympathetic influence in this area of the heart would provide an "overdrive" and mask the enhanced automaticity occurring in the latent pacemaker cells of the ventricle.

Another reason relates to the newer finding that parasympathetic activity does exert an antiarrhythmic influence on the ventricle (157). Thus, it has been shown recently with histological techniques that both human and canine hearts exhibit parasympathetic innervation to the Purkinje conduction network (407). Evidence that an antiarrhythmic effect of the vagus does take place in ventricular tissue has come from studies with arrhythmias produced by coronary occlusion (156, 408, 564). The mechanisms for the antiarrhythmic effect may involve either a direct effect of acetylcholine on the automaticity and resting membrane potential of Purkinje cells (36, 163, 832) or an indirect effect to prevent release of norepinephrine from sympathetic nerves (425, 426, 485, 527, 639).

Either bilateral vagotomy or atropine administration has been used to evaluate the role of the parasympathetic nervous system in digitalis-induced ventricular arrhythmias. The data obtained are about equally divided between the vagus having a protective role (405, 467, 469, 487, 511, 531, 626, 855, 895, 896) and the vagus having no effect (92, 212, 416, 467, 469, 534, 559, 560, 596, 598, 648, 844). Perhaps the most comprehensive studies performed in terms of the number of drugs evaluated are those of Levitt et al. (467, 469) who examined the role of the vagus in the toxic effects of five digitalis preparations (digoxin, deslanoside, acetylstrophanthidin, ouabain, and digitoxin). They reported that bilateral vagotomy as well as atropine pretreatment decreased the dose required of four of these agents to evoke ventricular arrhythmias. Digitoxin was unaffected by removal of vagal tone. Another interesting finding made by Levitt et al. was that the protective effect of the vagus nerves became apparent only at a specific rate of infusion of the digitalis drug studied. For example, vagotomy lowered the dose of digoxin to produce arrhythmias when the infusion rate was 2 μg/kg/min, but not when the infusion rate was either 1 or 3 μg/kg/min (468). Thus, from their findings, it appears that both a protective effect of the vagus as well as no effect of the vagus can be observed. The reason for these disparate findings appears to be related both to the speed at which the animal is intoxicated with digitalis and to the digitalis drug employed.

The mechanism for the protective effect of the vagus appears to be due to the presence of efferent rather than afferent vagal tone. That is, exclusion of vagal influence either by sectioning the whole nerve or by blocking the efferent component with atropine resulted in the same alteration in toxic dose (469, 531, 855, 895, 896). The mechanism for the protective effect of efferent vagal tone appears to be antagonism of sympathetic influence on the ventricle. Evidence for this was obtained by Pace and Martin (597); bilateral vagotomy failed to alter the arrhythmogenic effects of digoxin (2 μg/kg/min) in animals with sympathetic nervous system function excluded by spinal cord transection.

In two studies, exclusion of vagal tone by vagotomy or atropine was reported to protect against digitalis-induced arrhythmias (433, 666). In addition, Epstein (212) reported a similar effect with both vagotomy and atropine pretreatment. However, our statistical analysis of his data indicates that there was no significant effect of these procedures on digitalis-induced arrhythmias. The reason for the disparity in the findings of the above two studies showing a deleterious effect of the vagus and the rest of the studies showing either a protective effect or no effect is unclear. However, on a mechanistic level, it would not be unexpected to see both a deleterious and a protective effect of the vagus. In the former case vagal stimulation would suppress the normal pacemaker and might unmask activity of subsidiary pacemakers. This in fact may be the case since, in the two studies where the vagus nerves enhanced toxicity (433, 666), the occurrence of ventricular abnormal beats, but not death, was reported to be the endpoint that was altered by exclusion of the vagus. In the latter case, vagal stimulation would suppress the activity of the sympathetic nervous system and hence protect the heart against the arrhythmogenic influence of digitalis. In most of these studies (405, 467, 469, 487, 531, 855, 895, 896), the presence of the vagus nerves conferred protection against the lethal effects of digitalis.

3. Role of reflexogenic areas of the cardiovascular system. In several studies the effect of surgically denervating the cardiovascular reflex areas on the ability of digitalis drugs to produce cardiac arrhythmias has been examined. In essentially all of the studies performed, denervation of reflexogenic areas arising from carotid sinus, aortic arch, and cardiac regions (via section of the carotid sinus and vagus nerves, or the glossopharyngeal and vagus nerves) has increased the cardiotoxic action of digitalis drugs. This observation was first reported by Gillis et al. (289). Both the lethal dose of ouabain and the time required for this agent to induce ventricular fibrillation were found to be significantly reduced in decerebrate cats with reflexes denervated when compared to decerebrate cats with reflexes intact. Stickney (775) reported similar results with both ouabain and acetylstrophanthidin in pentobarbital-anesthetized cats. Lower doses of these digitalis drugs were required to produce ventricular tachycardia and death in animals with reflex areas denervated than in control animals. This observation has since been confirmed in several other studies in anesthetized cats and dogs with ouabain (51, 264, 776), acetylstrophanthidin (776), and digoxin (596, 860, 861). The mechanism whereby reflexes function to protect against the cardiac rhythm disorders induced by digitalis was shown in the study of Gillis (279). He

monitored spontaneous preganglionic efferent sympathetic neural activity to the cat heart and found it to be inhibited by ouabain. When the cardiovascular reflex areas were denervated the sympathoinhibition was no longer observed. Rather, marked increases in preganglionic sympathetic neural activity, indicative of an action of ouabain in the CNS, were observed in the reflex denervated animals. This increased neural activity correlated temporally with the development of cardiac arrhythmias. These findings suggest that digitalis can act on baroreceptors in the neurally intact animal, thereby depressing sympathetic neural influences on the heart (634) to render the animal less prone to the development of arrhythmias. When the baroreceptor input is removed by denervation, the sympathoinhibitory effect of digitalis is eliminated. This leaves the central sympathetic excitatory effect of digitalis unopposed, and ventricular arrhythmias develop with lower doses.

Insofar as chemoreceptors are concerned, Viana (845) evaluated the role of carotid body chemoreceptors in the toxic arrhythmogenic effects of digoxin in anesthetized dogs. Chemoreceptors were excluded from the system either by denervation or by carotid body glomectomy. These procedures resulted in an alteration in the dose of digoxin required to produce ventricular arrhythmias. These animals required a larger dose of digoxin, suggesting that carotid body stimulation by this agent was contributing to the generation of arrhythmias. Since increased chemoreceptor activity results in an increase in central sympathetic outflow (179), it is presumed that it was this augmented neural activity directed to ventricular tissue that was responsible for contributing to the development of cardiac arrhythmias.

4. Role of peripheral serotonergic mechanisms. It is clear that digitalis drugs produce significant changes in the release, uptake, and content of serotonin in peripheral tissues. The significance of these changes induced by digitalis drugs on serotonergic function was studied by Helke et al. (346–348, 352) in relation to the arrhythmogenic actions of these agents. The approach taken was to examine the effects of drugs that either reduce or enhance the functional activity of the serotonergic system in the periphery. Procedures tested that reduce serotonergic function (i.e., pretreatment with either *p*-chlorophenylalanine or methysergide) increased the doses of deslanoside and ouabain to produce ventricular arrhythmias (348, 352). In addition, administration of the serotonin receptor blocking agents methysergide, cyproheptadine, and cinanserin converted deslanoside and ouabain-induced ventricular arrhythmias to normal sinus rhythm (14, 348, 352). The site of action of these agents was interpreted to be in the periphery, because in the case of *p*-chlorophenylalanine, depletion of CNS serotonin had no effect on digitalis-induced arrhythmias (347). In the case of methysergide, administration of this agent by intraarterial routes into the brain did not decrease the dose (as determined by administering methysergide i.v.) of this agent to counteract deslanoside-induced arrhythmias (352). The findings of Stickney and Ball (779) are in support of a peripheral site of action of these drugs. They reported that methysergide, cinanserin, and cyproheptadine counteracted the arrhythmogenic effect of ouabain in isolated guinea-pig hearts. One possible mechanism for the antiarrhythmic effect of methysergide, cyproheptadine, and cinanserin could be antagonism of serotonin receptors. Evidence for this is that two effects that all of these drugs have in common are antagonism of serotonin receptors and reversal of digitalis-induced arrhythmias. In addition, the ability of a representative agent, methysergide, to counteract digitalis-induced arrhythmias is reduced by prior depletion of serotonin with *p*-chlorophenylalanine (352).

A procedure tested that enhances the effect of peripheral serotonin, i.e., administration of serotonin, i.v., increased the susceptibility of the heart to digitalis-induced ventricular arrhythmias. This was demonstrated by infusing serotonin into anesthetized cats exposed to a subarrhythmogenic dose of deslanoside, determining doses of deslanoside required to produce ventricular tachycardia and ventricular fibrillation, and determining the ventricular pacemaker rate during periods of vagal-induced sinus node suppression. It was found that animals receiving serotonin plus deslanoside exhibited a greater increase in ventricular rate during sinus node suppression than with infusion of serotonin alone. In addition, the dose of deslanoside to produce ventricular fibrillation in these animals was significantly correlated with the increase in ventricular pacemaker rate seen during the serotonin infusion in the presence of deslanoside. Studies were also performed to determine whether the arrhythmogenic interaction of serotonin with deslanoside was associated with alterations in either cardiac tissue, blood, or plasma levels of serotonin and 5-hydroxyindoleacetic acid. The results obtained revealed a significant correlation between serotonin content in the left ventricle and the dose of deslanoside required to produce ventricular fibrillation. These results suggest that exogenous serotonin interacts with deslanoside to enhance the arrhythmogenic action of deslanoside (346). These findings are consistent with those obtained by Godefroy and Weil-Fugazza (297), who observed that cardiac arrhythmias could occur in the dog when serotonin was infused at a time when the animal was exposed to a subarrhythmogenic dose of ouabain.

Thus, the available data suggest that peripheral serotonin may be a factor in the production of cardiac arrhythmias by digitalis drugs. Peripherally, serotonin is synthesized in the enterochromaffin cells in the intestinal tract (812) and is continuously released into splanchnic veins (103), where it is either taken up into blood platelets for storage (740) or transported to the liver and lungs where it is metabolized (812). In the presence of high doses of digitalis, uptake of serotonin into platelets is reduced (351) and the resulting "free" serotonin then would be available to interact with digitalis on the heart. This interaction could be on cardiac Na^+-K^+-activated

ATPase, since serotonin has been shown to cause inhibition of this enzyme (262), or on peripheral sympathetic neural structures that might be involved in the arrhythmogenic effect of digitalis and that have also been shown to be affected by serotonin (see 352).

V. Summary

A. Role of the Nervous System in the Therapeutic Cardiovascular Actions of Digitalis

Table 1 summarizes the important cardiovascular effects of digitalis when used in the therapy of congestive heart failure and cardiac arrhythmias, and lists the neural sites of action that may be important in mediating these effects. As can be seen, the nervous system is of primary importance in mediating the effects of therapeutic doses of digitalis on the electrical activity of the heart. The slowing in supraventricular automaticity, the shortening of the atrial refractory period, and the decrease in the speed of atrioventricular conduction are all due to a digitalis-induced increase in parasympathetic nerve activity and decrease in sympathetic nerve activity. The primary neural site of action appears to be the reflexes, and in particular, stretch receptors in the carotid sinus, aortic arch, and the heart. In addition, digitalis-induced activation of peripheral chemoreceptors may contribute to these effects insofar as they may increase parasympathetic activity to the heart. These neurally mediated changes in electrical activity are responsible for the following therapeutic actions of digitalis: slowing in sinus rate seen in patients with congestive heart failure; restoration of sinus rhythm in patients with paroxysmal atrial tachycardia; slowing in ventricular rate in patients with atrial flutter and atrial fibrillation; and development of atrial fibrillation in patients with preexisting atrial flutter.

With respect to beneficial effects on the electrical activity of ventricular tissue, recent data indicate that digitalis exerts an antiarrhythmic effect in some patients exhibiting ventricular arrhythmias (81, 493). In one of the studies (493), 65 out of 142 patients exhibited an antiarrhythmic effect with acetylstrophanthidin administration. In the remaining patients, acetylstropanthidin either had no effect (37 patients) or exacerbated the arrhythmia (40 patients). The beneficial effect of digitalis may well be due to a reflex-induced decrease in sympathetic nerve activity to the ventricle. Evidence for this as a mechanism can be found in the experimental study of Evans and Gillis (221). In that study ouabain was found to prevent sympathetic nervous system-mediated arrhythmias evoked by hypothalamic stimulation in cats with intact reflexes but not in cats with denervated reflex areas. This protection was associated with prevention of sympathetic hyperactivity during the period of hypothalamic stimulation. Lown et al. (493) attribute this antiarrhythmic effect to activation of the parasympathetic nervous system, which could either antagonize norepinephrine release from cardiac nerves or antagonize norepinephrine at the receptor site on ventricular tissue.

In contrast to the important role of the nervous system in mediating the alterations in cardiac electrical activity, the nervous system has little if any role in mediating the positive inotropic effects of digitalis. This effect appears to be due to a direct effect of digitalis on cardiac muscle (129, 301, 762), probably involving an alteration in calcium flux (37, 319, 374, 445, 502).

Neural actions of digitalis do appear to play an important role in the vascular effects of these agents in patients with congestive heart failure. Both arterial and venodilation occur, and these changes are probably mediated by digitalis-induced sympathetic withdrawal from the arteries and veins. These changes in vascular tone would decrease cardiac afterload, and presumably would also decrease the cardiac preload. Decreases in each of these hemodynamic variables would be beneficial to the heart in failure. Furthermore, a decrease in afterload could also

TABLE 1

Summary of neurocardiovascular effects of digitalis when used in the therapy of congestive heart failure and cardiac arrhythmias

Cardiovascular Effect	Neural Mechanism(s)	Site of Neural Action	Contribution of Neural Effect
1. Decrease in sinus rate	Activation of parasympathetic and inhibition of sympathetic nervous system	Peripheral reflexogenic sites; postsynaptic cholinergic cardiac receptors	Major
2. Decrease in automaticity of supraventicular tissue (excluding S-A node)	Activation of parasympathetic and inhibition of sympathetic nervous system	Peripheral reflexogenic sites; postsynaptic cholinergic cardiac receptors	Major
3. Decrease in refractory period of atrial muscle cells	Activation of parasympathetic nervous system	Peripheral reflexogenic sites; postsynaptic cholinergic cardiac receptors	Major
4. Decrease in speed of atrioventricular conduction	Activation of parasympathetic and inhibition of sympathetic nervous system	Peripheral reflexogenic sites; postsynaptic cholinergic cardiac receptors	Major
5. Antagonism of ventricular arrhythmias	Inhibition of sympathetic and activation of parasympathetic nervous system	Peripheral reflexogenic sites; postsynaptic cholinergic cardiac receptors	Not determined
6. Increase in inotropic state of the heart	Probably none		None
7. Decrease in arterial and venous tone	Inhibition of sympathetic nervous system and possibly activation of cholinergic vasodilator fibers	Peripheral reflexogenic sites	Major

have an important effect on cardiac output. That is, arteriolar dilation would increase ejection fraction, thereby enhancing stroke volume.

B. Role of the Nervous System in the Toxic Cardiovascular Actions of Digitalis

The data reviewed above demonstrated clearly that the sympathetic nervous system plays a role in the ventricular arrhythmias induced by digitalis drugs. The exact role is difficult to assess because digitalis drugs have direct effects on cardiac tissue that result in electrophysiological changes consistent with the development of arrhythmias (372, 373, 670). Different sites in sympathetic neural pathways have been postulated as being affected by digitalis. These include the CNS, ganglia, postganglionic nerve fibers, and postsynaptic autonomic receptors. One body of evidence stresses the importance of the CNS (see section II B 2) while another stresses the importance of peripheral sites (251, 664, 859, 860).

Evidence for the importance of the CNS as a site of action is: 1) the increase in cardiac preganglionic sympathetic nerve activity produced by digitalis (221, 278, 596); 2) that selective removal of forebrain (354) or hindbrain, i.e., area postrema (757), sites increases the doses of digitalis required to cause ventricular arrhythmias; 3) that digitalis in arrhythmogenic doses can cause significant changes in CNS monoaminergic neurotransmitter function (350, 354); and 4) that local application of drugs that alter CNS monoaminergic neurotransmitter function can alter the doses of digitalis required to cause arrhythmias (349, 354).

Points raised against the importance of the CNS as a site of action of digitalis include: 1) that digitalis drugs do not accumulate significantly in CNS tissues (201); 2) that CNS Na^+-K^+-ATPase is not altered by arrhythmogenic doses of digitalis (17, 862); 3) that digitalis drugs do not increase spontaneously occurring or evoked preganglionic sympathetic nerve discharge (859, 860); and 4) that a derivative of digitalis that presumably would not cross the blood-brain barrier produces ventricular arrhythmias similar to those seen with digoxin (562). There are data available that can be used to counter each of these points. For example, evidence that digoxin does accumulate in the CNS has been obtained by measuring digoxin concentration in the cerebrospinal fluid in dogs (259) and in people (266). In addition, CNS areas outside the blood-brain barrier known to have an important influence on cardiovascular function (e.g., area postrema) accumulate considerable amounts of digitalis after i.v. administration (707). The finding that systemic administration of digitalis has no effect on brain ATPase activity is of interest, but can be criticized from the standpoint that the CNS areas where ATPase activity was measured may not be the sites where digitalis exerts its neuroexcitatory effect. The areas that have been evaluated for ATPase effects include the medulla, midbrain, thalamus, preoptic area, cortex, pyriform area, and cerebellum of cats, and brainstem and cerebral cortex of dogs. The area postrema, which may be the important CNS site of action of digitalis (757), has not been specifically examined for changes in ATPase activity. Two other reasons for emphasizing that the area postrema should be considered as an important CNS site where digitalis acts to increase sympathetic outflow are 1) vomiting elicited by these drugs is known to be initiated at this site (88a, 256a), and 2) electrical stimulation of this site results in an increase in sympathetic outflow (43a). Another criticism can be raised in regard to the data obtained on ATPase activity in the dog study. These animals were not given a large enough dose of digoxin to cause ventricular tachyarrhythmias. Instead, they were given doses of digoxin that produced heart block. This type of arrhythmia is most likely due to excessive activity in vagus nerves caused primarily by digoxin acting at a peripheral neural site rather than a CNS neural site (596).

The inability to confirm an excitatory effect of digitalis on spontaneous preganglionic sympathetic nerve discharge by Weaver et al. (859, 860) might be related to the preparation employed and the dose of digitalis used. Most of the studies demonstrating an enhancement in nerve activity examined effects of digitalis on cardiac preganglionic sympathetic nerve activity. Weaver et al. examined the effects of digitalis on splanchnic nerve activity. In addition, the inability to observe an effect of ouabain on preganglionic activity when this agent was administered into specific CNS areas might be due to the fact that the dose of ouabain was not sufficient to cause ventricular arrhythmias (859). It might also be due to the possibility that ouabain was not injected into CNS areas where it evokes an action when given systemically. An example of such an area is the area postrema (757). Finally, Weaver et al. (860) were unable to observe an enhancement of hypothalamically evoked sympathetic activity with ouabain. This differs from the data obtained by Evans and Gillis (221). However, a basic difference between the two studies was that the former tested ouabain against submaximal hypothalamic stimulation, which by itself produced no heart rate and blood pressure responses (858), whereas the latter tested ouabain against submaximal hypothalamic stimulation that by itself produced significant increases in heart rate and blood pressure.

The ability of a digitalis preparation with a primary amino group (and therefore presumably unable to cross the blood-brain barrier) to cause arrhythmias similar to those produced by digitalis preparations that do cross the blood-brain barrier can be explained by the recent findings of Somberg et al. (755, 757) and Helke et al. (350) that CNS areas outside the blood-brain barrier (e.g., area postrema) may be important sites of action of digitalis in producing ventricular arrhythmias.

Peripheral neural sites have also been proposed as of

importance in the arrhythmogenic effects of digitalis drugs. The evidence for this has been accumulated by Roberts et al. (251, 446, 447, 664) as well as by others (284, 610, 859–861). Based on the available evidence, it is most probable that all of these neural sites can participate in the genesis of ventricular arrhythmias produced by digitalis.

Another action of digitalis that can result in toxic effects in patients is the effect to increase arterial vascular tone. This can be a serious problem when digitalis is administered to some patients and can lead to hypertension and stroke (437), heart failure (58, 150), increased left ventricular work (483), mesenteric infarction (619), and possibly angina (95). There is good evidence that sympathetic neural activation contributes significantly to the vasoconstrictor response (257–259, 289, 331, 464, 637, 768).

C. Directions for Future Research

To date, the role of neural actions of digitalis drugs has been studied primarily in experimental preparations that are devoid of the deranged physiological states exhibited by patients requiring digitalis therapy. In addition, neural actions and important roles of the nervous system have been documented primarily in experimental preparations requiring anesthesia. An evaluation is needed of neural effects and the role of neural actions of digitalis in experimentally induced disease states resembling those seen in people (e.g., in subjects exhibiting congestive heart failure) and in preparations not requiring anesthesia. This is important because the autonomic nervous system appears to be significantly altered by the disease states requiring digitalis therapy and anesthesia. For example, it has been shown that impairment of the parasympathetic component of the baroreceptor reflex mechanism occurs in heart failure (203, 369, 838). The site of impairment appears to be either on the afferent side or in the CNS, since stimulation of efferent vagal fibers produces normal cardiac responses (203). Impairment of the sympathetic component of the baroreceptor reflex mechanism has also been shown to occur (369). This may represent a defect in the afferent limb and CNS but, in addition, a defect in the efferent limb has been definitely established. This occurs in the heart and is reflected by a reduction in cardiac norepinephrine content (139, 140, 758–761) and reduced cardiac responses produced by stimulation of efferent sympathetic nerves (161). The decrease in tissue content appears to be due to a decrease in the synthesis and in the uptake or binding of norepinephrine (623, 682). In addition, an alteration in sympathetic neural control of vascular resistance may also occur, and may differ according to whether right or left heart failure predominates (702). Examples of anesthetic-induced alterations in autonomic nervous system function are numerous and have been described extensively by other investigators (114a, 611a, 629a). The point here is to recognize that when digitalis is studied in anesthetized preparations, neural control of cardiovascular function has already been altered and that effects that occur may differ from effects occurring when these agents are given to subjects with normal neural control of cardiovascular function. In summary, although data from studies with subjects with nonfailed hearts and under anesthesia have provided important information regarding neuroexcitatory actions of digitalis drugs, there is a need to evaluate the neural actions of these agents under conditions in which the state of the nervous system resembles more closely those states in which digitalis drugs are utilized.

An evaluation of the role of the nervous system in the overall cardiovascular responses produced by digitalis drugs in patients with congestive heart failure may prove fruitful in gaining additional understanding of the way these agents exert their effects, because some of the effects that occur with digitalis (e.g., fluid loss, veno- and arterial dilation) can occur solely by an alteration in neural activity to vascular beds and kidneys. Patients with congestive heart failure, in addition to having a dampened baroreceptor reflex (as mentioned above), exhibit fluid retention and veno- and arterial constriction. Sensitization by digitalis of receptors in the vasculature and heart leading to reflex inhibition of sympathetic tone could result in fluid loss and veno- and arterial dilation, thereby leading to overall improvement in the cardiac status of these patients. Additionally, diuresis may also occur because of an interaction of digitalis with hypothalamic structures, since it has been reported that ouabain when localized to the lateral and medial hypothalamic areas causes an increase in urine volume and sodium excretion (321, 322).

We would like to suggest that reflex activation by digitalis drugs might be worth considering in the therapy of hypertension where sympathetic outflow is elevated. Although not extensively documented, it has been reported that these agents produced a hypotensive effect in patients with hypertension (1) and in hypertensive animals (35). In one unpublished study, the hypotensive effect of ouabain in spontaneously hypertensive rats was prevented by denervating the baroreceptors (693). Reflex effects of digitalis may be important in the treatment of ventricular arrhythmias. It is reported in two studies that acetylstrophanthidin is effective in counteracting ventricular premature contractions and malignant ventricular arrhythmias in patients (492, 493).

Considering the toxic effects of digitalis drugs, another area for future research would be to design a digitalis drug that has a distinctly different spectrum of action on the nervous system than presently available agents. That is, it would appear from our current knowledge that digitalis-induced increases in sympathetic activity can result in deleterious effects on the heart and vasculature. A goal would be to develop a digitalis preparation that is

devoid of a sympathetic neuroexcitatory effect. That this might be feasible is indicated by the recent observations from the laboratories of Caldwell et al. (116, 117, 153), who reported that 4-amino sugar cardenolides, e.g., ASI-222 and ASI-254, possess greater cardiotonic potency and therapeutic indices than ouabain or digoxin in dogs. In addition, the mode of death seen with ASI-254 is cardiac arrest rather than ventricular fibrillation as seen with ouabain and digoxin. Most importantly, it appears that ASI-254 may not activate the sympathetic nervous system and that this may be the reason for the improved therapeutic index and its inability to produce ventricular fibrillation. The evidence for a lack of interaction with the sympathetic nervous system is indirect and based on the finding that beta-adrenergic blockade does not protect dogs against ASI-254-induced ventricular arrhythmias whereas beta blockade does protect dogs against digoxin-induced ventricular arrhythmias. Additionally, ASI-254 is devoid of a respiratory stimulant effect (115). Respiratory stimulation is characteristic of presently available digitalis preparations and is due both to peripheral chemoreceptor stimulation and to stimulation of central neural structures (596). It is known that stimulation of peripheral chemoreceptors results in increased sympathetic activity, and it is also known that increased activity of the CNS respiratory center is associated with an increase in central sympathetic outflow (291). The reason for the lack of sympathetic activation with ASI-254 may be related to the fact that this substance exists primarily in the ionized form at physiological pH. In this form, the drug would be unable to cross the blood-brain barrier and excite CNS areas mediating sympathetic outflow. This fits with the observation that when a 4-amino sugar cardiac glycoside was injected directly into the CNS, there was increased cardiac sympathetic nervous system activity, resulting in arrhythmias that could be blocked by sympatholytic drugs (117).

While the concept that 4-amino sugar cardiac glycosides such as ASI-254 may not activate the sympathetic nervous system and thus provide an improved therapeutic index is exciting, it should be noted that at least one of these compounds, which reportedly does not cross the blood-brain barrier, does interact with the sympathetic nervous system. The interesting study by Somberg et al. (755, 757) demonstrated that ASI-222, which is also ionized at physiological pH, interacts with a CNS area outside the blood-brain barrier (i.e., area postrema) to cause activation of the sympathetic nervous system and cardiac arrhythmias. Whether or not this action is specific for only some of the charged digitalis derivatives remains to be determined.

It may also be possible to develop digitalis drugs that are more vagomimetic than existing compounds. This appears tenable on the basis of recent findings that the vagomimetic properties of existing digitalis preparations may differ (598). An agent that is more vagomimetic, i.e., exerts a vagal-activating effect at a lower dose than presently available agents, could be advantageous in the treatment of supraventricular arrhythmias. It is known that the dose of presently available agents required to activate the vagus nerves and impair A-V conduction is close to the arrhythmogenic dose (411), and by having a more active cholinomimetic agent, slowing in ventricular rate may occur without the threat of toxicity.

In conclusion, the data accumulated from the time of Withering (881) until now point to a powerful effect of digitalis drugs on neural tissue. Since these agents have been used for treating problems associated with the heart, they were always referred to as "cardiac" glycosides. In view of our present knowledge about the important actions of digitalis drugs on neural function resulting in alterations in cardiovascular function, these drugs might just as well be described as "neural" glycosides.

Acknowledgments. The authors wish to acknowledge the help of Dr. Cinda J. Helke in the preparation of certain aspects of this review pertaining to the actions of digitalis drugs on neurotransmitter systems, and to thank Donna Weigle, Carolyn Greenfield, Joan Jones, and Linda Ebner for their assistance in the preparation of this manuscript.

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