Journal of Pharmaceutical Sciences

OCTOBER 1974

VOLUME 63 NUMBER 10

REVIEW ARTICLE

Effects of Prolonged Administration of Certain Antihypertensive Agents

BHAGAVAN S. JANDHYALA ˣ, DAVID E. CLARKE, and JOSEPH P. BUCKLEY

Keyphrases □ Antihypertensive agents—effects of prolonged administration, review □ Reserpine—effects of prolonged antihypertensive therapy, literature review of acute and chronic studies □ Hydrochlorothiazide—effects of prolonged antihypertensive therapy, literature review of acute and chronic studies □ Thiazide diuretics—effects of prolonged antihypertensive therapy, literature review of acute and chronic studies □ Guanethidine—effects of prolonged antihypertensive therapy, literature review of acute and chronic studies

Cardiovascular diseases are, for the most part, chronic conditions and the therapeutic agents used to treat these diseases must be administered over prolonged periods. One prevalent cardiovascular disease is hypertension, and it has been estimated that there are approximately 23,000,000 hypertensive patients[1] in the United States today and that hypertension is responsible for approximately 60,000 deaths per year. Hypertension has been identified as the major contributing factor to the development of atherosclerosis (1) and also appears to be of major importance in the development of congestive heart failure, coronary thrombosis, and uremia.

Most information concerning the pharmacology, toxicology, and mechanisms of action of the antihypertensive compounds has been obtained from acute experiments which, in many instances, utilized anesthetized animals and/or isolated tissues. Since one or more of these compounds might be administered throughout the lifespan of the hypertensive patient, it is possible that the effects of prolonged administration may vary greatly from those observed upon acute administration. The purposes of this paper are: *(a)* to review the available preclinical and clinical data on three of the more widely used antihypertensive compounds (reserpine, hydrochlorothiazide, and guanethidine); *(b)* to identify, wherever possible, disagreements that may have been found between the chronic effects of the compounds and data obtained from acute experiments; and *(c)* to point out the implications of these differences in relation to patient care.

It is feasible that prolonged administration of one or more of these compounds could influence markedly the absorption or metabolism of the compound administered, receptor sensitivity, the biochemical effects or even the known pharmacological actions of the drug, the control of the cardiovascular system by the central nervous system (CNS), the inherent characteristics of the autonomic innervation to the myocardium or the vasculature, and possibly other organ systems. Investigations concerning the prolonged effects of antihypertensive compounds in experimental animals or human patients could provide data that would lead to a better understanding of how the therapeutic agent produces its efficacious and/or toxic effects. This information should aid the practitioner in better utilizing these compounds in the treatment of

[1] See *NIH Record*, 25(3), 1(1973).

hypertensive cardiovascular disease and possibly other cardiovascular diseases, as well as in limiting toxic effects produced by the compounds themselves upon chronic administration. A review of the literature indicates that comparatively little is currently being done to investigate the pharmacological effects of antihypertensive compounds administered to experimental animals for prolonged periods.

RESERPINE

Since Wilkins (1) first introduced the use of rauwolfia serpentina in the United States for the treatment of hypertension, many studies have confirmed the efficacy of this drug and its active constituents in lowering blood pressure in hypertensive patients. Oral administration of large doses of reserpine (3–9 mg) or parenteral administration in a dosage as low as 1 mg can produce a marked reduction in arterial blood pressure in humans. Unfortunately, large doses of reserpine produce a high incidence of undesirable side effects, the more prominent being drowsiness and depression (2).

The hypotensive effect of reserpine is due to a selective interference with transmission of nerve impulses from postganglionic sympathetic nerve endings to smooth muscle cells in the arteriolar and venous beds. Norepinephrine, the chemical transmitter, is depleted by reserpine from its storage sites at postganglionic sympathetic nerve endings (3, 4); as a result, less transmitter is released by nerve impulses and little or no response of smooth muscle cell develops following reserpine administration. Sympathetic nerve blockade develops only after almost complete depletion of tissue norepinephrine has occurred. Lee (5) observed that no significant alterations occurred in the responses to sympathetic nerve stimulation in the right atrium, adrenal glands, and nictitating membrane unless the catecholamine content had fallen below 50% of normal values. On the other hand, measurable responses were still observed when catecholamine content was well below 10%. These studies further indicated that the sensitivity of the right atrium to the catecholamine-depleting action of reserpine was highest and that of the adrenal medulla was lowest. Similar findings concerning differential sensitivity of various organs were reported (6), suggesting that very small amounts of endogenous norepinephrine are capable of maintaining a residual function of the adrenergic nerves (7). These observations are consistent with the view (8) that the degree of depletion of catecholamine stores by reserpine depends not only on the dose of reserpine but also on the rate of turnover of the stores. The adrenal medulla is known to have a low rate of turnover and is very resistant to the depleting action of reserpine; the heart, on the other hand, has a high rate of turnover and is very sensitive to the depleting action of reserpine (9).

Since reserpine produces widespread depletion of peripheral sympathetic transmitters, it is widely believed that the action of reserpine on the sympathetic system is exclusively confined to the postganglionic neuronal sites. During the 1st to 3rd hr after intravenous injection of low and medium doses of reserpine, Bein (10–12) found no impairment of function in the peripheral sympathetic pathways, even though relaxation of the nictitating membrane, a decrease in the arterial pressure, bradycardia, and inhibition of centrally mediated pressor reflexes were fully apparent. Significant impairment of peripheral sympathetic transmission is usually found after a much longer latency period. For example, Bianchi and Fargier (13) noted that depletion of adrenal catecholamines in dogs and a lack of responsiveness to nerve stimulation could not be demonstrated until 8–24 hr after administration of reserpine. Reports concerning the effects of reserpine on preganglionic sympathetic activity are conflicting. Bein (11) observed a decrease in the electrical activity of preganglionic sympathetic cardioaccelerator nerve in the cat together with bradycardia, whereas Iggo and Vogt (14) found no change in the electrical activity of the cervical sympathetic fibers. A slow, progressive, and marked diminution in efferent splanchnic nerve activity was observed in cats and dogs (15), as was a moderate depression of efferent splanchnic nerve activity (16). Plummer (17) demonstrated that when cats are pretreated with reserpine, the nictitating membrane displays a greater hypersensitivity to epinephrine than to norepinephrine, the condition thus resembling preganglionic rather than postganglionic denervation.

The two main features of action of reserpine on which its widespread use in hypertension is based are the lowering of arterial blood pressure associated with bradycardia (which in animals appears to be species dependent) and a sedative effect (18). In general, depletion of the neurohumoral transmitter substances in the adrenergic system results in inhibition or block of adrenergic neurotransmission. Thus, one expects a fall in blood pressure, a reduction in peripheral vascular resistance, and bradycardia accompanied by a reduction in cardiac output. Reserpine, however, has been shown to increase renal plasma flow and the glomerular filtration rate in conscious dogs (19). In humans, the dilation of peripheral vessels is mainly confined to skin (20, 21). The ability of reserpine to diminish concentrations of norepinephrine in atrial appendages of humans has been demonstrated (22).

Whelan and Skinner (20) also found that reserpine exerted a prolonged dilator action on peripheral blood vessels but did not interfere with sympathetic reflex activity or with vasoconstrictor activity of ephedrine and amphetamine. Reserpine elicited its normal response in the chronically sympathectomized limb, whereas the constrictor response of the limb vessels to ephedrine and amphetamine has been shown to be dependent on the integrity of the sympathetic nerves. Thus, if reserpine exerts its effects by depleting tissue norepinephrine, the tissue norepinephrine stores may not play a part in reflex activity but would be involved in mediating tonic influences on the vessels (20). Other studies indicated that, following prolonged reserpine administration, reflex venoconstriction and reflex arteriolar constriction in

the forearm of normal subjects, elicited by leg exercises and by cold stimulation, are inhibited (23).

After prolonged treatment with reserpine, its pattern of activity against various vasoconstrictor agents changes. Therefore, results obtained in acute experiments do not necessarily apply to the more complex pharmacological situations arising after chronic treatment (24).

The usefulness of reserpine used alone or in combination with other hypotensive agents in controlled studies in reducing morbidity and the mortality rate due to hypertension has been demonstrated (25, 26). Moyer *et al.* (27) compared the effects of chronic reserpine therapy in humans to those observed in dogs following acute intravenous administration. Following intravenous administration of the drug to dogs, cardiac output was quite variable and was sometimes reduced during the reduction of blood pressure; however, these alterations were of short duration and usually of little consequence. Renal hemodynamics and electrolyte excretion rates were not altered significantly after intravenous administration of reserpine to dogs. When reserpine was administered either intravenously or orally for 3 months to patients with hypertension, renal blood flow, glomerular filtration rates, and renal excretion of water and electrolytes were not altered, and there was no evidence of renal toxicity or depression of renal function (27). Thus, at least in these studies, no visible differences were noted between acute and chronic studies.

Adams *et al.* (28) investigated certain physiological effects of prolonged oral administration of reserpine to mongrel dogs for a period of 12 months. The doses of reserpine (0.137–0.274 mg/dog/day)($\bar{X}$ = 26 μg/kg/day) selected were comparable to clinical doses used in veterinary medicine. In these studies, while hematologic values of untreated dogs were within normal limits (of canines), the hematocrit and hemoglobin content were consistently and significantly lower in reserpine-treated dogs; in addition, the leucocyte count was consistently lower in the treated population (28). These findings are in contrast with the reports by Earl (29), who failed to observe any effects of chronic reserpine upon hematologic components in dogs, and by West *et al.* (31), who found no effect of reserpine on leucocytosis in leukemic mice. However, in the studies of Earl, reserpine was administered for only 5 days a week in contrast to daily administration in the studies reported by Adams *et al.* (28). Marley and Pare (30) also observed a reduced hemoglobin value and an increased plasma portion of the hematocrit following reserpine treatment in humans, and they suggested that reserpine produced a hemodilution *via* fluid retention; they rejected the possibility of altered hematopoiesis. However, impaired hematopoietic processes could also induce a microcytic condition, and the peripheral edema observed by these investigators may have been a sequela to reserpine-induced cardiotoxicity (or failure), as noted by many investigators, rather than an indication of fluid retention leading to cardiac decompensation, as suggested by these workers. The most significant finding in the studies of Adams *et al.* (28) was the presence of right ventricular dilations in 50% of the reserpine-treated animals but in less than 10% of the placebo group.

Adverse effects of reserpine on myocardium have been the concern of many investigators. Cohen *et al.* (32) examined the cardiac effects of chronic administration of 0.25 and 0.5 mg reserpine/day for 20–48 days in patients with essential hypertension. Their studies indicated that therapeutically administered reserpine may decrease cardiac output, increase atrioventricular (A–V) conduction, and augment second degree heart block during induced tachycardia in a hypertensive population. Similar antihypertensive therapeutic doses of reserpine induced signs of cardiac failure in humans (30, 33). Disruption of normal ECG patterns and premature ventricular contractions likewise were associated with therapeutic dose levels of reserpine (34, 35). Perera (33) reported five cases that, within a week after daily administration of 0.4 mg of reserpine, developed edema accompanied by moderate exertional dyspnea; all symptoms were reversed within a week after discontinuation of the drug. In one patient, when reserpine therapy was reinstituted, the same symptoms reappeared within 1 week.

Histological examinations of the heart and other organs removed from animals pretreated for 1 or several days with 1–1.25-mg/kg total doses of reserpine revealed severe degenerative changes in cardiac muscle, characteristic signs of severe stress in the adrenal cortex, and centrilobular changes in liver indicative of heart failure and increased venous pressure (36). Similar histopathological alterations in myocardial structure of cats treated with large doses of reserpine were also noted (37). Large doses (1 mg/kg) or small doses administered over prolonged periods produced marked changes similar to those conditions in which oxygen supply or oxygen utilization of the myocardium is substantially decreased (38). Other investigators (39) conducted histochemical and electron microscopic studies on the effects of reserpine on the heart muscle of mice. In these studies, a disarrangement in lipid and glycogen metabolism and depression of mitochondrial oxidative enzymes were demonstrated. Ultrastructural studies showed significant changes in mitochondrial architecture in reserpine-treated mice. These findings supported the hypothesis that reserpine could cause damage to the myocardium and provided anatomical evidence of previously reported uncoupled oxidative phosphorylation in reserpine-treated animals. The doses used in these studies were 0.125 mg/mouse (or approximately 6 mg/kg), with two additional doses at the end of the 5th and 10th days. Wilcken *et al.* (40) also demonstrated mitochondrial swelling and fragmentation in dogs following daily administration of 25 μg/kg reserpine.

Withrington and Zaimis (41) studied the cardiovascular effects of prolonged administration of small doses of reserpine (10 μg/day for 5–26 weeks). In these studies, mean arterial blood pressure and heart rate were lowered significantly in reserpine-treated animals, and atropine treatment or bilateral vagoto-

my made little difference in these resting lower levels of heart rates. The administration of epinephrine and norepinephrine produced larger positive chronotropic and inotropic responses in reserpine-treated groups. Pressor responses induced by both epinephrine and norepinephrine in reserpine-treated cats were markedly greater than those noted in control groups, while the sensitivity of the hindlimb vasculature to epinephrine, norepinephrine, and isoproterenol was unchanged. Furthermore, epinephrine administration also resulted in various forms of cardiac irregularities, especially ectopic ventricular contractions in reserpinized cats, the hearts of these animals were unable to sustain the prolonged increase in heart rate brought about either by the administration of isoproterenol or by electrical stimulation. The investigators concluded that chronic administration of reserpine resulted in alterations in myocardial function; the greater pressor responses to epinephrine and norepinephrine in reserpine-treated cats were due to a greater increase in cardiac output rather than due to an increase in sensitivity of the vasculature (41). In these studies, no direct measurements of cardiac output were made. The investigators assumed that greater inotropic effects (contractile force) must have resulted in greater output from the heart, despite the occurrence of marked cardiac irregularities following the administration of catecholamines. If increased contractility did result in increased cardiac output to a greater degree than in untreated groups, one could assume that hearts of the treated animals were ejecting blood effectively, which would not be the case if cardiac function was indeed depressed by reserpine treatment. This is not to say that there were no alterations in cardiac function following reserpine treatment, evidence of which has been documented by these and other investigators. Normal relationships between contractility and cardiac output may not exist in a depressed heart, as shown in other studies (42). Administration of epinephrine and norepinephrine to dogs treated chronically with small doses of reserpine resulted in a greater increase in the blood pressure than in control groups (42). Following catecholamine administration, a greater elevation of contractile force did not result in a greater increase in cardiac output. These studies indicated that greater pressor responses to catecholamines in reserpine-treated animals were due to the marked elevation of total peripheral resistance rather than to cardiac output. Also, one cannot assume that the alterations in the responsiveness of the peripheral vasculature to catecholamines is essentially the same in all vascular beds. Investigators (43, 44) have clearly demonstrated that, following chronic reserpine treatment to dogs, there was a twofold increase in sensitivity of α-adrenergic receptor activity in femoral arteries, while no such alteration in the sensitivity was noted in the mesenteric vasculature. Thus, the assumptions made by Withrington and Zaimis concerning peripheral vascular sensitivity to reserpine may not be valid, and in these studies greater pressor responses to catecholamines could be due to greater increases in peripheral resistance in the reserpine-treated cats.

Several other workers (45, 46) disagreed with the opinion that reserpine may induce adverse myocardial changes. These studies demonstrated: *(a)* that reserpine does not change contractile force of the cat heart (46), *(b)* that it affects neither contractility in the papillary muscles of the cat (47) and in the isolated rabbit atria (48) nor the interval strength relationship of the isolated kitten heart (49), and *(c)* that it exercises beneficial influences on the recovery of isolated myocardium from anoxia (50). The oxygen consumption of rabbit atria has been found to undergo no changes after reserpine (48). Several investigators (34, 35, 51, 52) observed various alterations in human and animal ECG's following reserpine treatment. Hensler (51) reported the occurrence of extrasystoles and bigeminy which disappeared when the reserpine therapy was discontinued. Wilson and Wimberley (34) recorded premature ventricular contractions in hypertensive patients receiving reserpine. Schreader and Etzl (35) observed a consistent presence of premature ventricular contractions in patients receiving reserpine and digitalis. The investigators concluded that rauwolfia alkaloids produce premature ventricular contractions in all patients, but more readily in patients receiving digitalis (35). Lown *et al.* (52) demonstrated that reserpine enhanced the toxic effects of acetylstrophanthidin upon the atrium, A–V conduction system, and ventricles. Dick *et al.* (53) observed that simultaneous administration of reserpine and digitalis may induce arrhythmias and that this was not due to low serum potassium. The capacity of reserpine to enhance the toxicity of digitalis has been demonstrated by other investigators both *in vivo* and *in vitro* (54–56). Large doses of reserpine depressed digitalis-induced arrhythmias and this effect of reserpine was due to a nonspecific generalized depression of myocardium by reserpine (57, 58).

Cohen and colleagues (59) studied the effects of reserpine therapy on cardiac output and A–V conduction in patients with essential hypertension. In patients receiving reserpine (0.25–0.5 mg) daily for 20–48 days, there was a prolongation of A–V conduction time. This effect of reserpine may be due to an increased refractory period resulting from catecholamine depletion, as suggested by Gaffney *et al.* (60). These changes in A–V conduction (second degree A–V block) indicate a potential source of clinical difficulty when reserpine is administered to a patient with a preexisting A–V conduction disturbance (52, 60, 61).

Further observations on the adverse effects of chronic reserpine administration on myocardium were made (62). These studies in guinea pigs indicated that reserpine induced ECG changes characteristic of progressive myocardial damage. Similarly, Roberts and Modell (63) reported a high fatality rate from reserpine in dogs in which heart block was induced experimentally. In a series of four animals, two died after two doses of reserpine (0.1 mg/kg) given on successive days. The other two animals survived three such daily doses. Even in the case of a single dose of 0.1 mg, two of the 14 dogs so treated died

within a day of injection. Changes in QRS time and QRS configuration were often seen, and occasionally as many as four distinctly different QRS complexes were observed. Postmortem examination revealed an enlarged heart, ascites, and pulmonary edema, and the investigators concluded that the cause of death was probably heart failure. It was also found that *N*-methylatropine given at the time of the peak effect of reserpine did not alter the auricular and ventricular rates. The authors concluded that vagal mechanism seems to play no role in the bradycardia following reserpine administration (63).

Studies of Withrington and Zaimis (41) also supported this conclusion in that administration of atropine or sectioning of the vagi did not alter the resting heart rate levels in the reserpine-treated animals. These studies supported the view that bradycardia to reserpine involves a direct cardiac depressant component. Withrington and Zaimis (41) also reported that reserpine-treated cats were unable to sustain adequately a marked and prolonged increase in heart rate induced either by electrical stimulation or by the administration of isoproterenol. In the drug-treated animals, isoproterenol produced a marked and prolonged tachycardia, but the positive inotropic responses were less pronounced and were usually followed by a secondary depression of the heart contraction. The cardiac muscles in these animals apparently fatigued more easily under the influence of the abrupt and marked increase in the heart rate. Similar results were obtained when heart rates were increased by electrical stimuli. Possible adverse effects of reserpine on the myocardium received further support from the findings of Taylor (64), who noted that reserpine given to hypertensive patients with myocardial embarrassment may precipitate acute insufficiency if the heart is further subjected to additional stress such as heavy exercise, hypoxia, or anesthesia.

Withrington and Zaimis (41) postulated that catecholamine depletion is only one effect of reserpine and that the progressive deterioration of the myocardium is the result of a biochemical lesion which develops due to continued treatment or an increase in the daily dose. In support of this hypothesis, they indicated certain histochemical and biochemical changes accompanying reserpine-induced myocardial abnormalities. These included accumulation of stainable lipid and of glycogen and an increase in phosphorylase. Furthermore, it was possible to correlate these effects with disturbances in hydrogen transport associated with the Krebs cycle as demonstrated by the mitochondrial enzyme succinic dehydrogenase.

Effects of prolonged administration of reserpine (18–39 μg/kg po) to mongrel dogs for 12–13 months were reported (42). There was a moderate decrease in both systolic and diastolic blood pressures after 2 weeks of treatment; however, these alterations were restored to pretreatment levels during the subsequent 2–3 weeks. There was a gradual and consistent decrease in the heart rate of the treated animal; at the end of 12–13 months of treatment, heart rates remained significantly below control levels. A variety of autonomic tests conducted in these dogs revealed that prolonged reserpine treatment did not affect blood pressure responses to hypoxia, cold pressor test, exercise, and intravenous administration of sodium nitrite and phenylephrine. However, postural hypotension was evident when the animals were subjected to tilt (60° head-up position).

Hexamethonium produced a marked initial increase in heart rate and blood pressure in untreated dogs, followed by a prolonged hypotensive response. However, in the treated dogs, the hypotensive response was absent while the pressor and tachycardia responses of equal magnitude persisted. Since it has been established that tachycardia to hexamethonium in conscious animals is predominantly due to vagal blockade (65, 66), bradycardia to reserpine may not be due to enhanced vagal activity. While pressor responses to phenylephrine were essentially identical in both treated and untreated groups, the accompanying reflex bradycardia was markedly inhibited by reserpine treatment. Based on the pressor responses of phenylephrine, one may conclude that intrinsic α-adrenergic receptor activity was not altered by chronic reserpine treatment. Lack of a hypotensive response to hexamethonium, inhibition of reflex bradycardia to phenylephrine, and inhibition of tyramine pressor responses suggested marked inhibition in sympathetic tone to the cardiovascular system. These conclusions were further confirmed in the studies conducted in the same animals under pentobarbital anesthesia, in which blood pressure, responses to bilateral carotid occlusion, and hexamethonium were markedly inhibited. Furthermore, in the reserpine-treated animals, there was a reduction in cardiac output and left ventricular work in comparison with the untreated dogs. While there was a marked increase in contractile force and rate of tension development in the ventricular myocardium of the reserpine-treated dogs, there was no corresponding increase in stroke volumes. Systemic function curves obtained in these studies indicated that reserpine treatment did not alter the ability of the peripheral vasculature to return blood to the heart (42). Gross pathological examination revealed marked dilation of the right ventricles in these and other reserpine-treated dogs (28). The authors suggested that reserpine-induced ventricular dilation resulted in a stretch of initial ventricular fiber length. As a consequence, there were a greater contractile force and rate of tension development which were associated with increased energy consumption (67). However, this increase in energy consumption was not followed by an increase in work output, since stroke volumes were not altered. The authors suggested that these data indicated diminished efficiency of the right ventricle of the dogs treated with reserpine (42).

In a similar group of dogs that received reserpine for 12 months, there was an attenuation of sympathetic neuronal function in the isolated perfused mesenteric vessels (44). The decrease in sympathetic nerve activity could be restored by infusion of norepinephrine, indicating that chronic reserpine failed to alter neuronal uptake mechanisms. There was no alteration in α-adrenergic receptor activity in the per-

fused mesenteric vessels (44). *In vitro* studies also suggested that, while there was a twofold increase in α-adrenergic receptor activity in the femoral artery, no such alterations were noted in the superior mesenteric artery (43). In conscious reserpine-treated animals, pressor responses induced by phenylephrine were essentially the same as in control animals while pressor responses to norepinephrine were potentiated in the treated dogs under pentobarbital anesthesia. Thus, it is difficult to explain the differences in the responsiveness of reserpine-treated dogs to the pressor effects of phenylephrine and norepinephrine. Pentobarbital anesthesia itself may have some influence on the α-adrenergic receptor activity.

GUANETHIDINE

Guanethidine is an adrenergic neuronal blocking agent (68), which finds considerable use in the management of moderate to severe hypertension. Adrenal medullary release is unaffected (69) and the drug is contraindicated in the treatment of pheochromocytoma.

Guanethidine exerts multiple actions at the level of the adrenergic neuron which are of both pharmacological and clinical significance. Thus, the compound inhibits the neuronal uptake of sympathomimetic amines which are injected or endogenously released (70). Pharmacological doses of guanethidine enhance the peripheral sympathomimetic effects of direct-acting amines, whereas the effects of indirect-acting amines are depressed or abolished (69, 71, 72). Sneddon and Turner (73) showed that the local instillation of guanethidine (5% solution) into the human eye produces identical effects. However, following oral therapeutic doses of guanethidine (4–6 weeks), such interactions are less dramatic, indicating only a partial inhibition of the neuronal uptake process (74). Nevertheless, this inhibition is sufficient to potentiate the responses to injected norepinephrine and released adrenal medullary amines and adequately explains the contraindication for guanethidine in the patient with pheochromocytoma.

Guanethidine-induced impairment of adrenergic neuronal uptake arises because of the relative nonspecificity of this active transport process. Guanethidine itself is a good substrate for transport, thereby gaining access to intraneuronal sites (75–77). This fact partially explains why certain inhibitors of the uptake process (tricyclic antidepressants, some monoamine oxidase inhibitors, the amphetamines, *etc.*) attenuate the adrenergic neuronal blocking activity of guanethidine and reduce or prevent its clinical efficacy as an antihypertensive agent (78, 79). Amphetamine and its analogs are particularly potent antagonists of guanethidine-induced neuronal blockade and have been shown to restore readily the neurogenic function even after full inhibition (69, 80). These agents deplete guanethidine from certain intraneuronal storage sites (81), which are believed to provide a constant but slow release of the drug onto an active "receptor" intimately involved with neuronal blockade (82).

The initial entry of guanethidine into the adrenergic neuron induces a transient sympathomimetic effect which includes hypertension and cardiac stimulation. This effect is particularly evident in both humans and animals after intravenous injection and is dose related. This action may be qualitatively described as a tyramine-like effect of guanethidine and, as with tyramine (83), the effectiveness of the released norepinephrine is enhanced by the concomitant inhibition of neuronal uptake. Thus, an intravenous injection of guanethidine is of potential danger in hypertensive patients exhibiting crisis states or in those portraying cardiac arrhythmias. In contrast, the well-known depletion of endogenous norepinephrine by guanethidine occurs more slowly and may be attributed to a "reserpine-like" effect at the level of the intraneuronal vesicles (84, 85). This latter action is interesting from a pharmacological standpoint, since other neuronal blocking agents possessing almost identical guanethidine-like properties (bretylium, bethanidine, chlorobethanidine, and debrisoquin) fail to deplete endogenous norepinephrine (69, 79). All of these latter agents selectively inhibit intraneuronal monoamine oxidase (86–88); guanethidine is the lone exception (89–91). Thus, like guanethidine, other adrenergic neuronal blocking agents may interfere with amine storage mechanisms, but the liberated norepinephrine would be protected from deamination, whereas norepinephrine released by guanethidine is metabolized and lost from the nerve. In this respect, it should be recalled that monoamine oxidase inhibitors, including bretylium, can prevent guanethidine-induced depletion (92, 93). The lack of a monoamine oxidase inhibitory action of guanethidine is of some definite advantage clinically, since in contrast to other adrenergic neuronal blocking agents the responses to injected indirect-acting amines are not vastly exaggerated (94).

The actual mechanism whereby guanethidine blocks adrenergic neuronal function is still debated. Of the theories advanced, membrane stabilization (95), interference with calcium-induced release (96), and local anesthetic action (69, 95) appear the most attractive, although alternative views have been expressed (97–101). The high intraneuronal accumulation of guanethidine (75, 95) could well explain its marked potency and selectivity as a local anesthetic at postganglionic adrenergic neurons as compared with other neuronal elements. In addition, local anesthesia would readily explain membrane stabilization and its inhibitory effect upon calcium-induced release. In fact, the adrenergic neuronal blocking action of guanethidine is reversed by raising extracellular calcium (96, 102, 103). This antagonism should be borne in mind when guanethidine and hydrochlorothiazide diuretics are used concomitantly since overt hypercalcemia has been reported in both dogs (104) and humans (105) with this latter agent. The gross norepinephrine-depleting action of guanethidine plays little, if any, role in its neuronal blocking action. Depletion occurs secondary to inhibition of nerve function (69), even when it is well established. Spriggs (106) showed that the neurogenic function

could be quickly restored with dextroamphetamine. Finally, although it is quite clear that the peripheral adrenergic blocking action of guanethidine is responsible for its potent antihypertensive effect, the possibility of direct additional influences on vascular smooth muscle tone is suggested by experiments in which guanethidine elicited vasodilation in the perfused foreleg of reserpine-pretreated dogs (107).

The properties of guanethidine summarized so far were elucidated primarily from acute or subacute experimentation. By comparison, little detailed information is available concerning the chronic pharmacological and clinical effects of this compound. Recently, however, several publications have dealt with the chronic toxicity of guanethidine upon certain adrenergic neuronal elements in rats.

Gannon *et al.* (108) and Burnstock *et al.* (109) reported that the administration of guanethidine (5 mg/kg/day ip for 18 weeks) resulted in a greater depletion of norepinephrine in the genital organs than in the heart, cerebral arteries, or superior cervical ganglion. Within 2 weeks of discontinuing treatment, the fluorescence intensity of catecholamines in the ganglion, cerebral arteries, and heart appeared normal, whereas the genital organs failed to recover even after 6 months. Likewise, Evans *et al.* (110) demonstrated that chronic guanethidine treatment (10 mg/kg/day ip for 13 weeks) resulted in a marked depletion of norepinephrine from the short adrenergic neurons innervating the vas deferens. The nerves lost their ability to take up norepinephrine and showed markedly diminished responses to electrical stimulation even in the presence of a 15-fold increase in the effectiveness of added norepinephrine. Norepinephrine depletion existed for at least 6 months after the cessation of treatment and, although the sensitivity to this amine declined, an eightfold increase above normal still persisted. The sensitivity to acetylcholine was unaffected, strongly indicating the lack of any postjunctional, nonspecific supersensitivity.

These observations have a decided clinical relevance since there have been frequent reports of impotence and failure of ejaculation in males receiving chronic guanethidine therapy (111–114). In addition, the findings suggest that this inconvenient side effect may persist for long periods following the cessation of treatment due to functional damage (108) of the intramural neurons.

Although the genital organs are particularly susceptible to guanethidine (109), neurotoxicity has been found in certain sympathetic ganglia following prolonged treatment with the same or higher daily doses (usually 10 mg/kg and more). Using histochemical fluorescence techniques and electron microscopic methods, Burnstock *et al.* (109) found a severe reduction in amine fluorescence in the superior cervical ganglion and rat irides (25 or 30 mg/kg/day ip for 6 weeks). Less than 2% of the nerve cell bodies in the superior cervical ganglion remained at this time, and the mitochondria were badly damaged in these cells. This situation persisted for up to 4 months after the cessation of treatment (longest time period examined). Similar observations were made previously (115–119). These studies disclosed clear evidence of ganglionic cellular lysis, loss of specific and nonspecific cholinesterase activity (up to 70%), and marked catecholamine depletion (20 mg/kg/day ip for 2 weeks). A subsequent publication by these investigators (120) confirmed the presence of marked ultrastructural changes in rat sympathetic ganglia treated with guanethidine (4–40 mg/kg/day ip for 7–14 days) which were partially reversible after 26–56 days of discontinued treatment. Maximal alterations were observed with 20 mg/kg, whereas 4 mg/kg failed to produce any definite overt toxicity. The specificity of guanethidine for sympathetic ganglia was established since no changes were observed in the ganglion nodosum or dorsal root ganglia; furthermore, the liver and salivary glands were unaffected.

These observations might suggest that the neurotoxicity of guanethidine results from its ability to accumulate in adrenergic neurons (discussed previously), although this hypothesis has not been tested. On the other hand, the claimed local selectivity for the ganglion, as opposed to the postganglionic adrenergic terminals (120), would not be predicted, since both the neuronal uptake mechanism and the blood supply are relatively poor in the former region. In fact, the relative selectivity of 6-hydroxydopamine for terminal adrenergic fibers has been explained using this same reasoning (121). In view of the marked biochemical and structural changes reported in the rat superior cervical ganglion (122), it is surprising to find that Downing and Juul (122) reported only very slight or no qualitative impairment of ganglionic potentials in the isolated superior cervical ganglion preparation taken from guanethidine-treated rats (20–40 mg/kg ip for 5–21 days). Recorded action potentials were of normal form but did exhibit a diminished amplitude. The authors left their readers with indefinite conclusions but suggested that the majority of ganglion cells may be more susceptible than others to the damaging effects of guanethidine, thus accounting for the reduction in the amplitude of the recorded potentials. Finally, these workers acknowledged surprise at finding the same sensitivity to acetylcholine in guanethidine-pretreated ganglia as in control preparations in view of their previous reports concerning the marked reduction in cholinesterase activity (115–117).

Several other investigators studied the effects of guanethidine upon developing sympathetic ganglia utilizing newborn rats or mice. Eranko and Eranko (123, 124) described changes in the catecholamine fluorescence properties of the superior cervical and coeliac ganglia taken from newborn rats. Three weeks after the cessation of treatment with guanethidine (20 mg/kg/day ip for 8 days), the total number of ganglionic cells was reduced but the population of small, intensely fluorescent cells was increased markedly (300–500%). This latter interesting effect was not evident in adult rats, although an increased number of small, nonfluorescent, infiltrating cells was found. Whether these cells are related to small intensely fluorescent cells is still undecided, but Eranko and Eranko (123) felt that such a relationship is

unlikely. Presumably such cells correspond to proliferating connective tissue cells also seen in ganglia taken from guanethidine-treated newborn rats and mice (125). In cultured sympathetic ganglia derived from newborn rats, guanethidine failed to induce cytotoxic effects, but the increase in small intensely fluorescent cells was retained with a guanethidine concentration of 1 mg/ml (126). The relevance of this observation has been questioned (119) on the grounds that cultured tissues may not be fully representative of *in vivo* states. Although this objection must gain sympathetic support, considerable evidence to the contrary has been presented (127) in regard to many adrenergic aspects of functional ganglionic mechanisms. On the basis of their culture studies, Eranko *et al.* (126) speculated that guanethidine may evoke indirect cytotoxic effects *in vivo,* possibly by initiating an autoimmune response, by being converted to a toxic metabolite, or by initiating the endogenous formation of a toxic substance. Any or all of these suggestions might explain why guanethidine appears particularly toxic to ganglionic nerve cells as compared with the axons or nerve terminals. Experiments on cultured ganglia with tissue extracts or serum obtained from guanethidine-treated rats should aid in clarifying the possibilities. However, the guanethidine-induced increase in the population of small intensely fluorescent cells would appear to involve a direct component; the lack of such an increase in intact adult rats remains to be explained.

Guanethidine is not the only adrenergic neuronal blocking agent shown to exert ganglionic damage. Bretylium (128), 6-hydroxydopamine (129), debrisoquin, and, particularly, guanacline (130, 131) have also been implicated. Whether the cytotoxic actions of guanethidine and the other compounds occur therapeutically has not been established, but the persistence of postural hypotension after the withdrawal of treatment is a known complication of guanacline therapy (132). Many investigators are reluctant to translate effects seen with high daily doses of guanethidine in rodents to the clinical situation. While this caution is understandable, it should be recalled that very high therapeutic doses of guanethidine are used clinically and that rats are generally far more resistant to pharmacological and toxicological manipulations than are humans. However, even if ganglionic damage does occur in humans, the work of Downing and Juul (122) might suggest little resultant impairment of functional ganglionic transmission.

From the foregoing account, it is clear that the effects resulting from chronic guaethidine treatment can be expected to vary from those seen following single acute doses. However, with the exception of neural toxicity, little is known about such time-dependent variations. One early significant study concerning the chronic pharmacology of guanethidine was conducted by Boura and Green (133). They found that chronic guanethidine treatment could produce highly cumulative effects. For instance, subcutaneous doses of 2.5 mg/kg/day failed to cause nictitating membrane relaxation in cats after 1 or 2 days, but relaxation became progressively more pronounced between 3 and 12 days. Similarly, the responses to nerve stimulation to the same organ were much less after 2 weeks than after a single injection (2.5 mg/kg/day). The same pattern of events is seen clinically after oral administration. The blood pressure response often requires 3–4 days to become apparent and is cumulative in effect, requiring several additional days to attain maximal hypotensive effects (134). Conversely, Boura and Green (133) also found that when guanethidine was administered for longer periods (*e.g.,* 5 mg/kg/day sc for 5 weeks), pronounced tolerance developed, such that the mean frequency–response curve to nerve stimulation in the nictitating membrane was shifted markedly to the left of that found with the same dose given for only 3 days and approached control values. In the main, tolerance was attributed to the accompanying marked hypersensitivity to transmitter responses, and subsequent studies (135) reaffirmed this conclusion. However, in the femoral vascular bed of both cats and guinea pigs, the extent of the hypersensitivity to injected norepinephrine and epinephrine was considerably less than in the nictitating membrane. Stocks and Robertson (136) reported that tolerance to guanethidine was a major drawback to the long-term clinical management of severe hypertension (35 patients studied for 5 years); but in other investigations (137, 138), tolerance has been considered to occur only rarely or not at all. This lack of tolerance might be related to the use of concurrent therapy with other antihypertensive agents and/or to shorter periods of study. However, Stocks and Robertson (136) found that the addition of chlorothiazide medication to five tolerant guanethidine patients failed to produce beneficial effects on blood pressure control and a further increase in guanethidine dosage was required in three of the five subjects.

The successful use of guanethidine in the control of hypertension depends upon careful dosage adjustment (134). The effective dosage regimen can vary considerably from one individual to another, but the factors that give rise to this difference are not fully understood. Clinical observations on the ratio of the effective intramuscular and oral doses suggest that the variation in absorption is not the only factor responsible. In fact, although guanethidine absorption exhibits considerable biological variation, it has been reported to remain constant within a particular individual even during chronic therapy (139). However, other workers (140) refuted the claimed variability of patient absorption. McMartin *et al.* (141) pointed out that renal clearance, metabolism, and tissue uptake of guanethidine also play very important roles in determining the body's content of guanethidine. In addition, other factors that might account for variations in patient response are the severity and etiology of the hypertension and the development of hypersensitivity.

Clinical evidence suggests that the antihypertensive effect of chronic guanethidine therapy results primarily from a reduction in peripheral resistance rather than cardiac output (142–144), although this latter factor seems to be of importance during the

first few days of treatment. Thus, after 4–10 days of oral guanethidine therapy, the cardiac output in hypertensive patients was lower, even in the recumbent position; the decrease became more marked after tilting, but at this time the systemic resistance was only moderately decreased. Virtually opposite effects were seen after longer periods of treatment (10–32 days), since neither supine nor standing cardiac output was significantly lower, despite the presence of bradycardia, but systemic vascular resistance was decreased (143). Mason *et al.* (145) reported that reflexogenically induced arterial and venous constriction in the forearm of humans was completely inhibited in 10 of 17 subjects treated with guanethidine (30–50 mg/day for 26–43 days). No persistent impairment resulted, however, since normal reflexogenic regulation returned following the withdrawal of treatment. The authors suggested that reflexogenic impairment would be expected to reduce both venous return and cardiac output and thereby contribute to the antihypertensive properties of the compound. In fact, many workers have noted a pronounced postural and exercise hypotension in guanethidine-treated patients, which is of sufficient severity to be classified as a major complication inherent in the therapeutic management of hypertension with this agent (136, 146). Because of this effect, Stocks and Robertson (136) strongly recommended that all patients taking guanethidine should be adequately exercised while monitoring blood pressure to determine an effective and safe dose level. Exercise hypotension with guanethidine seems to be due to a greater than normal decrease in peripheral resistance and can be seen frequently even in the presence of a raised cardiac output (142). In this latter respect, it should be pointed out that although bradycardia may be marked with guanethidine, stroke volume is increased (142).

The effect of guanethidine on electrolyte excretion has received attention but has yielded conflicting data. Oral guanethidine in human subjects produced an enhanced natriuresis to saline loads and less salt retention in response to sodium-retaining steroids (147) and angiotensin (148). In another study (149), these investigators administered guanethidine to normal human subjects deprived of dietary sodium and obtained a marked sodium loss accompanied by a decreased creatinine clearance. The investigators suggested that these results were due to adrenergic neuronal blockade which gave rise to an inhibition of the tubular reabsorption of sodium. This conclusion is compatible with the previous observation of Wagner (150), who reported that patients with autonomic insufficiency excreted enhanced amounts of sodium during saline infusions. In a carefully thought-out acute study, Williams *et al.* (151) infused guanethidine directly into a renal artery of anesthetized dogs to divorce the systemic effects of the drug from direct renal actions. They found a unilateral increase in sodium, chloride, calcium, potassium, and water loss with no significant change in glomerular filtration rate or renal plasma flow. They were able to attribute these effects to local adrenergic neuronal blockade. However, the diuretic action of guanethidine, at least in dogs, may be short lived (152). In contrast with these studies, it has been shown that clinical treatment of hypertension with guanethidine may cause fluid retention (137, 153), reduced renal plasma flow, reduced glomerular filtration rate (154), and decreased excretion of sodium, potassium, and water. Such effects may account for guanethidine-induced weight gain (155). In a study on 10 patients treated with guanethidine for 7–21 days, Villarreal *et al.* (143) noted that the altered electrolyte excretion pattern was similar to that resulting from aldosterone release and suggested this or the lowering of renal perfusion pressure as a possible explanation. Thus, guanethidine would seem to exert both direct and indirect actions on the kidney and, although the precise nature of these actions requires clarification, it would seem wise to be cautious in treating hypertensive patients with accompanying renal insufficiency.

Two important studies sum up the side effects most likely to occur during the long-term use of guanethidine. Moser (134) reported on 9 years of clinical experience with guanethidine in treating more than 250 patients. Dizziness and muscular weakness were the most frequently noted side effects followed by diarrhea, bloating, "gas pains," and "indigestion." Occasionally, these latter symptoms persisted and were severe enough to warrant drug withdrawal. They are believed to be due to excessive parasympathetic activity in the face of sympathetic impairment. Muscular weakness is prominent on awakening and tends to disappear later in the day, suggesting alterations in fluid distribution overnight with early morning venous pooling (134). In addition, guanethidine exhibits neuromuscular blocking properties (69, 156) which could be extensive enough to account for this troublesome side effect. Tolerance, impotence, and failure of ejaculation were discussed earlier. Stocks and Robertson (136) encountered frequent depression, especially in female patients, but this symptom responded well to imipramine. Using this antidepressant, one patient experienced loss of blood pressure control (discussed previously). Out of 35 patients studied for up to 5 years, approximately 50% failed to complete the trial due in the main to severe side effects. Others suffered strokes (four patients), myocardial infarctions (four patients), and tolerance so that only 10 patients were still attending at the conclusion of the study, two of which experienced significant hypotensive symptoms. Stocks and Robertson (136) concluded that "despite its potency, guanethidine used alone is unlikely to be a satisfactory agent in the long-term therapy of more than one-third of young patients [average age was 46 years] with severe hypertension."

Recently, a time course study concerning the effects of guanethidine on the cardiovascular and peripheral autonomic nervous systems using purebred beagle dogs was completed (157, 158). The oral administration of 2.5 mg/kg of guanethidine failed to inhibit the chronotropic responses to cardiac sympathetic stimulation after 2 days, but continued administration of the same dose for 7 days significantly depressed cardiac sympathetic nerve activity. However,

following chronic administration of the same dose for 6–8 months, cardiac sympathetic nerve function returned completely to predrug levels, demonstrating the presence of a pronounced tolerance to sympathetic nerve activity with guanethidine (157, 158). A similar phenomenon was also noted in the hindlimb vasculature since the neurogenic tone and the vasoconstrictor responses to lumbar sympathetic stimulation (which were depressed after 7 days of treatment) were restored to placebo levels in dogs treated with guanethidine for 6–8 months (158). In addition, a progressive potentiation of the sympathetic cholinergic vasodilator activity was found as the duration of treatment increased. In this study, increased tolerance to guanethidine was not accompanied by any alteration in adrenergic receptor activity either in the heart or in the hindlimb vasculature, although a minor prejunctional increase in sensitivity to norepinephrine was found in the heart after both 7 days and 6 months of treatment. Thus, the mechanism responsible for tolerance differs from that reported in other studies (69, 133, 135), since it seems to be largely independent of transmitter sensitivity changes (157).

In contrast, no evidence for tolerance was found in the isolated perfused mesenteric arteries (157). The vasoconstrictor response to periarterial nerve stimulation was abolished after 7 days and 2, 6, and 8 months of treatment. Treatment for 1 day failed to alter significantly the frequency–response curve to periarterial nerve stimulation, but a twofold shift to the left in the dose–effect curve to injected norepinephrine was obtained. This twofold shift was evident throughout the study. However, the responses to nerve stimulation, as well as to norepinephrine, approached control values when guanethidine treatment was suspended for 2 months. At this time the nerves were fully susceptible to acute blockade by guanethidine when injected into the perfusing fluid. Other data revealed that, whereas dextroamphetamine could reverse the acute effects of guanethidine on neuronal transmission, it exerted little or no effect after 8 months of treatment[2]. There appeared to be a direct correlation between the duration of treatment and the inability of dextroamphetamine to reverse the neuronal blocking action of guanethidine. Although the mechanism of this effect has not been identified, the result reveals alterations in the normal pharmacology of the neuron upon chronic treatment, which, in turn, must reflect an increasingly deepening impairment of an important physiological process. Thus, these studies showed a much more persistent neuronal blocking action on the sympathetic innervation to the mesenteric arteries than on the myocardium and hindlimb vasculature. Functional transmission in the stellate ganglion and lumbar sympathetic chain was unaffected, as were both the monoamine oxidase activity and the catecholamine content as judged by the histochemical fluorescence technique. In accordance with previous observations, no alteration was observed in the population of small, intensely fluorescent cells. Heart norepinephrine was depleted by about 80% after 7 days of treatment and failed to recover even at 6 and 8 months. Therefore, tolerance to the inhibitory effect of guanethidine in the heart is not accompanied by a return in myocardial norepinephrine levels.

Despite these marked time-dependent changes in the functional responsiveness of sympathetic neurons, no detectable alterations in the neural regulation to the cardiovascular system was detected in conscious animals. Chronic administration of guanethidine (2.5 mg/day orally) over 8 months did not produce any significant alteration in body weight, blood volume, or blood pressure. There was, however, a slight reduction in heart rate, which was more prominent and significant in males than in females. The cardiovascular responses to tilt (head-up), mild exercise (on a treadmill, 12° inclination at 5 miles/hr for 4–5 min), ganglionic blockade, sodium nitrite, tyramine, angiotensin, phenylephrine, and norepinephrine did not differ significantly in treated animals compared with control dogs[2].

THIAZIDES

It is generally agreed that thiazide diuretics are moderately effective in the treatment of hypertension and are reported to have a relatively low incidence of side effects (159, 160). Frequently recognized toxic effects of these compounds are hypokalemia, infrequent hyperuricemia, possible exacerbation of previously controlled diabetes, prolongation of hypoglycemia even in normal patients, jaundice, skin eruptions, photosensitivity, thrombocytopenia, neutropenia, and pancreatitis. Although the compounds do initially decrease plasma volume and electrolytes, the mechanisms by which diuretics produce their antihypertensive effects (161–164) are still not understood.

As a rule, thiazides do not produce hypotension on acute administration and do not lower the arterial pressure of normotensive patients or experimental animals. Enhanced natriuresis, decreased blood volume, reduced extracellular fluid volume, and alteration in electrolyte balance are reported to play a role in the antihypertensive effects of these compounds (165–169). Winer (170) suggested that chlorothiazide has no effects on the CNS or on peripheral blood vessels. Based on their studies with normotensive as well as renal hypertensive rats receiving 0.5 mg/kg methyclothiazide for 14 days, Aoki and Brody (171) recorded electrical activity in the lumbar sympathetic nerves and measured the vascular responses in the perfused hindlimbs of the same animal. Treated renal hypertensive rats exhibited a higher basal nerve activity and greater changes in activity in response to asphyxia, carotid occlusion, and intravenous norepinephrine than did treated normotensive and untreated hypertensive and normotensive rats. The reflex increases in blood pressure produced by asphyxia and carotid occlusion were similar in all groups. Hypertensive rats showed greater vasoconstrictor re-

[2] Unpublished observations.

sponses to intraarterial angiotensin and tyramine, while responses to norepinephrine and epinephrine were similar in all rats. Thiazide treatment reduced the increased responsiveness to angiotensin in hypertensive rats to normal levels, and tyramine responses were unchanged. These data suggest that chronic renal hypertension is associated with increased vascular responsiveness to lumbar sympathetic nerve stimulation and intraarterial angiotensin and tyramine. Methyclothiazide treatment produced increased basal sympathetic nerve activity and greater reflex changes in nerve activity unaccompanied by blood pressure increases of similar magnitude and decreased vascular responsiveness to nerve stimulation and intraarterial angiotensin. The authors concluded that methyclothiazide may exert its hypotensive effect by interfering with norepinephrine liberation from sympathetic nerve terminals (171). Other studies indicated that renal hypertension is associated with larger than normal amounts of tissue sodium (172, 173), and thiazide treatment was ineffective in lowering blood pressure of rats with renovascular occlusive hypertension (172, 173). Willard (174) considered whether high tissue sodium levels interfered with the antihypertensive properties of thiazide. In these studies, hypertension was induced by deoxycortone acetate and sodium chloride in normal rats as well as in rats concomitantly treated with cyclothiazide (10 mg/kg/day for 16 weeks). Hypertension developed after 3–6 weeks of treatment with steroid and salt. The concomitant treatment with cyclothiazide did not alter the time for product or degree of hypertension. When the animals were returned to a normal diet, the blood pressure fell in both groups, but the reduction was significantly greater in animals treated with cyclothiazide. In another group, when thiazide treatment started 13 weeks after induction of the hypertensive state, there was a gradual reduction in blood pressure over 14 weeks. When the treatment stopped, the blood pressure returned to the original hypertensive level. The contrast between the slow hypotensive response and relatively fast recovery of hypertension corresponds to the results obtained clinically. These results seem to indicate that a high sodium diet is responsible for the slow onset of action. Therefore, it appears that in several other studies the failure of thiazide to lower blood pressure in renal hypertensive rats could be due to too short a duration of treatment employed (172, 175, 176). In these studies, since a high sodium diet appeared to decrease the effectiveness of thiazide therapy, sodium depletion did play a role in the antihypertensive properties of thiazides.

Decreased responsiveness of the vasculature to catecholamines was suggested as the antihypertensive mechanism for chlorothiazide (177–182). Eckstein *et al.* (177) reported that pressor responses to norepinephrine were due to increased cardiac output in dogs chronically treated with chlorothiazide. In untreated dogs in the same study, similar pressor responses to norepinephrine were due to increased peripheral vascular resistance since no essential changes occurred in cardiac output. These differences in the norepinephrine responses appeared to be due to the ability of chlorothiazide to reduce the effectiveness of cardio-inhibitory reflexes by reducing the responses of peripheral vasculature to the vasoconstrictor effect of norepinephrine. This effect was apparent both before and after ganglionic blockade, suggesting that modification of resting neurogenic vasomotor tone did not play a major role in the reduced responsiveness (177).

Feisal *et al.* (183) investigated the effects of graded intravenous infusions of norepinephrine on heart rate, mean blood pressure, forearm blood flow, and forearm vascular resistance in normal human subjects before and at the end of 7 days of chlorothiazide treatment. In these studies, chlorothiazide reduced the vasoconstrictor response to norepinephrine. The bradycardia accompanying the rise in blood pressure during norepinephrine infusions also appeared to be reduced. Forearm blood flow decreased with increasing doses of norepinephrine before chlorothiazide and increased with increasing doses after chlorothiazide. These results could also be explained on the basis of decreased responsiveness of the vasculature and of cardio-inhibitory reflexes of human subjects after chlorothiazide (183).

Zsoter *et al.* (184) studied the effects of prolonged administration of hydrochlorothiazide in rabbits and dogs for 6–8 weeks. In these studies, hydrochlorothiazide administration depressed mesenteric venoconstrictor responses to norepinephrine in rabbits but failed to alter the responses of acetylcholine, barium chloride, angiotensin, papaverine, and ATP. Hydrochlorothiazide also markedly reduced vasoconstrictor responses in the dog hindlimb to lumbar sympathetic stimulation and to intraarterial norepinephrine. These effects were not accompanied by any significant alteration in water, sodium, potassium, and calcium content of iliac artery or veins. The investigators concluded that diminished responses of the vessels to norepinephrine may be responsible for the antihypertensive effects after prolonged administration of hydrochlorothiazide.

Preziosi *et al.* (182) studied the pharmacological properties of chlorothiazide in rats, rabbits, and dogs. In these studies, chlorothiazide reduced pressor responses to carotid occlusion, epinephrine, norepinephrine, and angiotensin and slightly enhanced the responses to acetylcholine on the vasculature and on the isolated intestines, suggesting that chlorothiazide exerted a depressant action on smooth muscle fibers of the vasculature. The authors concluded that this depressant action might contribute to the chlorothiazide's antihypertensive effects as well as to its ability to potentiate other antihypertensive agents. These data do not support the view that chlorothiazide sensitizes carotid sinus buffer mechanisms (180). Preziosi *et al.* (188) also investigated acute and chronic effects of hydrochlorothiazide in mice and dogs. These studies demonstrated that hydrochlorothiazide had no influence upon carotid sinus baroreceptors, chemoreceptors, or vasomotor centers. Smaller doses inhibited the vasopressor responses to stimulation of adrenergic vasomotor fibers without influenc-

ing the vascular responses to catecholamines or angiotensin. Higher doses of hydrochlorothiazide were found to have adrenolytic, sympatholytic, and direct myolytic properties. Administration of hydrochlorothiazide (10 mg/kg acutely or for 7 days) also decreased catecholamine content of various tissues including the heart.

According to most investigators, the alterations in *fluid* volume or electrolyte balance do not play a major role in the chronic antihypertensive effects of thiazides. Daniel (185) found that hydrochlorothiazide did not significantly diminish inulin space, decrease concentrations of intracellular sodium, or increase the ratio of extracellular to intracellular sodium. Weller and Haight (186) administered 50 mg/kg chlorothiazide to rats for 6 weeks and found no consistent alterations in the electrolyte or water content of plasma or tissue of normotensive or hypertensive rats. On the contrary, administration of hydrochlorothiazide together with potassium chloride to hypertensive patients for 3 months resulted in a significant fall in total blood volume, exchangeable sodium, and blood pressure within 1–2 weeks and after 3 months of treatment (187). However, a fall in serum sodium and body weight was only significant after 1–2 weeks of treatment. Thus, in these studies, hypovolemia may be the explanation for the antipressor effect of thiazide even after 3 months of treatment (187).

Kusumoto *et al.* (189) administered 3.75 mg/kg/day hydrochlorothiazide chronically for 45 days to dogs, and the hydrochlorothiazide had no significant effect on blood pressure, blood and plasma volumes, and hematocrit. A significant decrease in plasma magnesium and potassium persisted throughout treatment, while plasma sodium and calcium remained unchanged. The potassium content of arterial tissue was significantly depressed and the calcium content was significantly enhanced in the left ventricular myocardium. Norepinephrine content was markedly reduced in all of these tissues. The juxtaglomerular index in four dogs examined was significantly elevated. Menard *et al.* (190) also reported an increase in plasma renin activity and juxtaglomerular index in normotensive rats treated with hydrochlorothiazide and lower sodium diet. The clinical and pharmacological significance of these findings has yet to be clarified, although it can be postulated that any compound decreasing plasma sodium content and/or renal blood flow would be expected to increase plasma renin levels.

Bourgoignie *et al.* (191) found elevated plasma renin levels during early phases of thiazide treatment only if the sodium intake was concurrently reduced and renin levels returned to normal levels with continued thiazide treatment. However, Tarazi *et al.* (192) noted that the blood pressure reduction during long-term thiazide therapy was associated with persistent plasma volume reduction, which occurred despite increased peripheral renin activity. There was no evidence for chronic intercellular dehydration, and variations in peripheral renin activity were related to changes in plasma volume and not to serum sodium concentration (192). The finding by Tobian *et al.* (175) of higher juxtaglomerular counts in normal rats given chlorothiazide for 5 weeks also seemed to indicate continued renin stimulation by these diuretics. The higher plasma renin activity and chronic weight reduction with continued thiazide therapy, followed by rapid extracellular water expansion with discontinuance as well as the continued potentiation of other antihypertensive agents by thiazides (193) and the reversal of their effects by higher sodium chloride intake (194), all correlate naturally with chronic reduction in plasma volume, extracellular fluid, and total exchangeable sodium as demonstrated in many studies (187, 195–197). It is by no means conclusive that this chronic hypovolemia is solely responsible for long-term hypotensive effect of thiazides in essential hypertension, but this volume-reducing effect may play a role in potentiating other antihypertensive agents. Despite numerous reports indicating a persistent decrease in fluid volume, the reduction in cardiac output appeared to play only a minor role in the antihypertensive effects of thiazides (169, 192, 198, 204). Conway and Lauwers (198) demonstrated the value of long-term therapy with chlorothiazide as the sole antihypertensive drug. In these studies, a significant fall in blood pressure occurred in 66% of the patients during the initial 2 weeks. There was a decrease in cardiac output accompanied by an increase in total peripheral resistance. However, after about 4 weeks, cardiac output was restored to predrug levels while the fall in blood pressure persisted due to reduction in total peripheral resistance. Although this initial decrease in cardiac output was usually related to a reduction in plasma volume, Frohlich *et al.* (199) noted a reduction in cardiac output unaccompanied by an alteration in fluid volume during acute infusion of chlorothiazide to anesthetized mongrel dogs. This reduction in cardiac output was associated to decreased venous return resulting from venodilation (200, 201). Few investigators, if any, considered the possibility that the reduction in cardiac output may be due to an alteration in contractility of myocardium induced by thiazides.

Studies on the prolonged administration of hydrochlorothiazide (10 mg/kg po for 12 months) to beagle dogs failed to produce any significant alterations in plasma volume, hematocrit, extracellular fluid, electrolyte balance (sodium, potassium, calcium, and magnesium), arterial blood pressure, and heart rate at any time during or at the end of the treatment period (202, 203). In conscious animals, hydrochlorothiazide treatment also did not affect cardiovascular alterations to tilt (60° head-up position) or exercise and blood pressure responses to sodium nitrite, angiotensin, tyramine, phenylephrine, and ganglionic blockade with mecamylamine[2]. Reflex tachycardia to sodium nitrite or reflex bradycardia to angiotensin, tyramine, and phenylephrine was not affected by chronic hydrochlorothiazide administration; however, while the depressor responses to mecamylamine were not influenced, tachycardia to ganglionic blockade was significantly inhibited by hydrochlorothiazide administration. Since it has been demonstrated that ganglionic blocking drugs induce tachycardia in

conscious dogs by essentially interfering with vagal tone (65), it appears from these studies that chronic hydrochlorothiazide may modify the inhibitory effects of ganglionic blocking drugs on vagal tone. These results may also explain the ability of thiazides to potentiate antihypertensive effects of several ganglionic blocking drugs. Prolonged hydrochlorothiazide administration for 10–12 months resulted in a shift of the pressure flow curves to the right, indicating a significant reduction in the neurogenic tone to the hindlimb vasculature (202). This inhibition was not due to any alteration in peripheral adrenergic mechanisms since vasoconstrictor responses to lumbar sympathetic stimulation and to intraarterial norepinephrine were essentially similar in treated animals to those obtained in placebo groups. Based on these findings, the authors postulated that hydrochlorothiazide inhibited neurogenic tone by an action mediated *via* the CNS and that these central effects may contribute to the antihypertensive properties of thiazide diuretics (202).

In another aspect of the same study, Clarke *et al.* (205) reported that chronic oral treatment of dogs with hydrochlorothiazide (10 mg/kg/day for 6 months) enhanced the frequency–response curve to periarterial nerve stimulation of mesenteric blood vessels *in vitro* without affecting vasoconstrictor responses to norepinephrine. However, this effect was no longer observable after an additional 6 months of treatment. Hydrochlorothiazide failed to affect the pharmacological interaction of certain agents (angiotensin, bethanidine, atropine, phentolamine, hexamethonium, and cocaine) with sympathetic neuronal receptor mechanisms. Furthermore, the *in vitro* accumulation of tritium, after incubation with ^{3}H-norepinephrine in atria, ventricle, and femoral arterial slices was unaltered by hydrochlorothiazide. These workers also demonstrated that acute infusion of hydrochlorothiazide (2.7 mg/min for 90 min) directly in mesenteric arteries *in vitro* failed to alter adrenergic mechanisms. These studies were in complete disagreement with previous reports that thiazide diuretics possess antiadrenergic properties (177, 184, 188). It may be that the adrenolytic effects of thiazide can only be demonstrated after a limited treatment period, as observed by Zsoter *et al.* (184) with hydrochlorothiazide. On prolonged treatment, peripheral sympathetic neural function may be restored to predrug levels.

The cardiovascular effects of prolonged hydrochlorothiazide administration (10 mg/kg/day for 12 months) in beagle dogs have been reported (203). Hydrochlorothiazide treatment for 12 months resulted in a significant reduction in cardiac output, stroke volume, and left ventricular stroke work without any accompanying changes in mean blood pressure and heart rate. Arterial pressures were maintained by an elevated total peripheral resistance. The reduction in cardiac output was not due to any alteration in plasma volume. Ventricular function curves of the treated animals were significantly depressed to the right, indicating marked diminution in the contractility and capacity of the myocardium to handle external loads. Thus, the decrease in cardiac output can be explained on the basis of reduced contractility. These effects cannot be related to alterations in the electrolyte pattern, either in serum or in the heart, since no such changes occurred. These cardiac effects of chronic hydrochlorothiazide can be reproduced by administering large doses (100 mg/kg/day po for 2 days).

Despite the widespread use of these compounds, little attention has been paid to their cardiac effects. As early as 1959, Barrett *et al.* (206) reported that small doses of hydrochlorothiazide, then a newly discovered diuretic, possessed negative inotropic properties in isolated heart preparations. Later, Preziosi *et al.* (182) observed similar effects with higher doses of chlorothiazide. Herbert and Buxton (208) warned against the danger of sensitization of the myocardium to the action of digitalis in obstetrical patients receiving concurrent treatment with hydrochlorothiazide. Such a toxicity was related to hypokalemia induced by thiazides, to the presence of cardiac decompensation, and to postpartum diuresis. Daniel (185) reported that the administration of hydrochlorothiazide to deoxycortone acetate-hypertensive rats resulted in marked alterations in the left ventricular function and suggested that more attention should be paid to the action of hydrochlorothiazide on the myocardium. More recently, Naylor *et al.* (207) demonstrated that diazoxide, a nondiuretic thiazide, displaced left ventricular function curves to the right in dogs, suggesting a diminution in the contractility of the left ventricle. Many of these reports clearly warrant a careful reevaluation of extensive use of thiazides in cardiovascular therapy.

SUMMARY AND CONCLUSIONS

The obvious conclusion that one can reach in reviewing the chronic pharmacological properties of antihypertensive agents is that the available literature is extremely limited. The dimension of time as an important variable in drug-induced effects has not been considered by most investigators, even in clinical studies; consequently, knowledge of these agents is largely restricted to their acute pharmacological properties. However, of the drugs discussed in this review, all have recorded chronic effects which were not readily observable from acute or even subacute studies. Thus, it cannot be assumed that the acute pharmacological spectrum of a drug will necessarily reflect or even predict its chronic pharmacological effects. Instead, investigators would be well advised to consider the opposite thesis, namely that drug-induced effects may, and probably will, vary with the duration of treatment. This contention is biologically sound, since it may be argued that drug challenges to physiological and pathological states will be resisted by prevailing homeostatic mechanisms. Such autoregulation requires time to develop and may be generalized or restricted to specific organ systems, depending upon the deposition of the drug and the biological limits of the system for adaptive change. The studies conducted by Jandhyala *et al.* (158) and

Clarke *et al.* (157) on guanethidine may well illustrate this point. The lack of persistence of adrenergic neuronal blockade in the heart and hindlimb vasculature compared with the mesentric blood vessels might be due to regional differences in the neurons to undergo adaptive change.

In addition to tolerance, toxicity may be subtle in onset and may involve indirect mechanisms. Alterations in metabolism, absorption, and cumulative effects of a drug may require time to become manifest. Indeed, certain findings in these respects have been discussed. The toxicities of the thiazide diuretics and reserpine on myocardial ventricular function are important findings which are now documented to occur following the repeated administration of relatively low daily doses of these drugs to otherwise healthy animals. However, as indicated earlier, the neuronal toxicity of guanethidine has yet to be discerned under similar circumstances.

Another significant factor, which appears to be almost entirely overlooked by pharmacologists, is the importance of studying the mechanisms of drug action in conscious animals. Perhaps it is considered that this area of study is adequately fulfilled by clinical studies; however, by necessity, these investigations are limited in scope and are mostly descriptive rather than conceptual in their conclusions. However, it should have become apparent to the reader that findings obtained in conscious animals often differed markedly from those obtained following general anesthesia. In general, the observed changes were far less dramatic and, in many instances, gave little or no indication of altered cardiovascular mechanisms. These discrepancies are unsettling and necessitate that due caution be observed when translating observations made in anesthetized animals or isolated tissue preparations to the clinical condition. However, it should not be construed that observations made in conscious animals are necessarily more valid. Under these conditions, the available technology for precise measurement is less well developed and it is extremely difficult to construct dose–effect relationships and elicit precise responses in specific vascular areas. In addition, studies using conscious normotensive animals under sheltered laboratory conditions may not reveal marked alterations in hemodynamic and/or autonomic parameters. Detailed studies involving conscious hypertensive animals subjected to rigorous exercise tests are urgently required.

Beside the general points raised, the literature revealed certain specific information that requires additional research for full comprehension. For instance, the time-dependent increase in cholinergic vasodilator activity in skeletal muscle blood vessels following chronic guanethidine treatment would not be predicted on the grounds of homeostatic readjustment. Neither can it be explained on the basis of an overactive cholinergic response in the face of adrenergic impairment, since the function of the latter neurons were restored to control levels. Thus, exertional hypotension in patients receiving guanethidine may be cholinergic in origin, and clinical studies using atropine or an atropine-like agent to seek further evidence for this contention would seem highly justified. Similarly, the bradycardia noted in clinical and experimental studies with guanethidine cannot be explained as an indirect result of adrenergic neuronal blockade. Although a central action of guanethidine has been generally ruled out with regard to sympathetic tone (209), it cannot be overlooked with respect to cholinergic activity. In this respect, chronic treatment with this agent has been shown to depress brain monoamine levels (210), indicating either direct or indirect effects on the CNS.

Certain other findings promote the possibility that physiological differences may exist with regard to the peripheral adrenergic innervation to various organs and vascular beds. In fixed-dose chronic studies (43, 44), the sensitivity of the femoral arterial smooth muscle to norepinephrine was increased by reserpine, whereas no sensitivity change occurred in the mesenteric vasculature. The opposite situation prevailed with guanethidine (157, 158). Neural toxicity with low doses of guanethidine was largely restricted to the genital organs and only became more diverse with increasing dosage (108, 109). It is apparent that these observations cannot be adequately explained on the basis of available information, and the need for more detailed chronic studies is further emphasized.

The mechanism of the antihypertensive property of the thiazide diuretics remains debatable. Certain findings suggest that at least part of their action may be mediated through an inhibition of peripheral adrenergic mechanisms, particularly at the level of the vasculature. However, chronic studies with hydrochlorothiazide have failed to reveal such effects with regard to the vascular responsiveness of injected norepinephrine. Instead, evidence from these studies indicates a possible central hypotensive effect of hydrochlorothiazide which appears to be mediated through a reduction in neurogenic tone to the vasculature. This latter effect was not noted in any reported acute or subacute studies.

In conclusion, it is apparent that chronic pharmacological studies are essential and exceedingly important, especially for compounds used for the control of chronic disorders. Based upon the available literature, one must conclude that chronic pharmacological investigations, employing relatively low dose levels of drugs, are likely to reveal important differences in regional physiological mechanisms and organ susceptibility to toxic effects and a deeper insight into the mechanism of action of the compounds themselves.

REFERENCES

(1) R. L. Wilkins, *Ann. Intern. Med.*, **37**, 1144(1952).

(2) T. E. Gaffney, *Heart J.*, **15**, 96(1966).

(3) E. Muscholl and M. Vogt, *J. Physiol. (London)*, **141**, 132(1958).

(4) A. Bertler, A. Carlsson, and E. Rosengren, *Naturwissenschaften*, **43**, 521(1956).

(5) F. L. Lee, *J. Pharmacol. Exp. Ther.*, **156**, 137(1967).

(6) A. Carlsson, E. Rosengren, A. Bertler, and J. Nilsson, in "Psychotropic Drugs," S. Garattini and V. Ghetti, Eds., Elsevier Publishing Co., Amsterdam, The Netherlands, 1957, p. 363.

(7) J. R. Crout, in "Standard Methods in Clinical Chemistry,"

3rd ed., D. Seligson, Ed., Academic, New York, N.Y., 1961, p. 62.
(8) A. Carlsson, in "Mechanisms of Release of Biogenic Amines," U. S. von Euler, S. Rosell, and B. Uvinas, Eds., Pergamon Press, Oxford, England, 1966, p. 331.
(9) W. R. Burack and P. R. Draskoczy, *J. Pharmacol. Exp. Ther.*, **144**, 66(1964).
(10) H. J. Bein, *Experientia*, **9**, 107(1953).
(11) H. J. Bein, *Ann. N.Y. Acad. Sci.*, **61**, 4(1955).
(12) H. J. Bein, in "Psychotropic Drugs," S. Garattini and V. Ghetti, Eds., Elsevier Publishing Co., Amsterdam, The Netherlands, 1957, p. 325.
(13) M. Bianchi and M. C. Fargier, *C. R. Soc. Biol.*, **156**, 1797(1962).
(14) A. Iggo and M. Vogt, *J. Physiol. (London)*, **150**, 114(1960).
(15) J. W. McCubbin and I. H. Page, *Circ. Res.*, **6**, 816(1958).
(16) A. S. Dontas, *J. Pharmacol. Exp. Ther.*, **116**, 17(1956).
(17) A. J. Plummer, in "Hypertension," A. N. Brest and J. H. Moyer, Eds., Lea & Febiger, Philadelphia, Pa., 1961, p. 399.
(18) H. J. Bein, *Pharmacol. Rev.*, **8**, 435(1956).
(19) G. L. Wagle and A. J. Plummer, *Arch. Int. Pharmacodyn. Ther.*, **151**, 1(1964).
(20) R. F. Whelan and S. L. Skinner, *Brit. Med. Bull.*, **19**, 121(1963).
(21) K. D. Bock and H. Muller, *Klin. Wochenschr.*, **34**, 318(1956).
(22) C. A. Chidsey, E. Braunwald, A. G. Morrow, and D. T. Mason, *N. Engl. J. Med.*, **269**, 653(1963).
(23) D. T. Mason and E. Braunwald, *J. Clin. Invest.*, **43**, 1449(1964).
(24) J. Tripod, A. Studer, E. Wirz, and R. Meier, *Arch. Int. Pharmacodyn. Ther.*, **126**, 126(1960).
(25) F. W. Wotf and R. D. Lindeman, *J. Clin. Dis.*, **19**, 227(1966).
(26) H. Shubin, *Clin. Med.*, **73**, 43(1966).
(27) J. H. Moyer, G. Hughes, and R. Huggins, *Amer. J. Med. Sci.*, **227**, 640(1954).
(28) H. R. Adams, H. H. Smookler, D. E. Clarke, B. S. Jandhyala, B. N. Dixit, R. J. Ertel, and J. P. Buckley, *J. Pharm. Sci.*, **60**, 1134(1971).
(29) A. E. Earl, *J. Amer. Vet. Med. Ass.*, **129**, 227(1956).
(30) E. Marley and C. M. B. Pare, *Brit. Med. J.*, **1**, 267(1956).
(31) W. L. West, G. M. Baird, J. D. Steward, and S. N. Pradham, *J. Pharmacol. Exp. Ther.*, **131**, 171(1961).
(32) S. I. Cohen, M. W. Young, S. H. Lau, J. I. Haft, and A. N. Damato, *Circulation*, **37**, 738(1968).
(33) G. A. Perera, *J. Amer. Med. Ass.*, **159**, 439(1955).
(34) B. N. Wilson and N. A. Wimberley, *ibid.*, **159**, 1363(1955).
(35) C. J. Schreader and M. M. Etzl, *ibid.*, **162**, 1256(1956).
(36) E. Zaimis, *Nature (London)*, **192**, 521(1961).
(37) M. G. Scott and E. Zaimis, *J. Physiol. (London)*, **162**, 8P(1962).
(38) P. Withrington and E. Zaimis, *Brit. J. Pharmacol.*, **17**, 380(1961).
(39) S. Chien-sun, R. S. Sohal, H. L. Colcolough, and G. E. Burch, *J. Pharmacol. Exp. Ther.*, **161**, 210(1968).
(40) D. E. L. Wilcken, D. Brender, C. D. Shorey, and G. J. Macdonald, *Science*, **157**, 1332(1967).
(41) P. Withrington and E. Zaimis, *Cardiovasc. Res.*, **1**, 52(1967).
(42) B. S. Jandhyala, I. Cavero, H. R. Adams, H. H. Smookler, B. N. Dixit, and J. P. Buckley, *Eur. J. Pharmacol.*, **16**, 261(1971).
(43) I. Cavero, B. S. Jandhyala, and J. P. Buckley, *J. Pharm. Pharmacol.*, **23**, 989(1971).
(44) D. E. Clarke, H. R. Adams, and J. P. Buckley, *Eur. J. Pharmacol.*, **12**, 378(1970).
(45) R. Hess, *Proc. Eur. Soc. Study Drug Toxicity*, **5**, 130, Excerpta Medica Foundation, Amsterdam, The Netherlands, 1965.
(46) R. D. Tanz and St. M. Marcus, *J. Pharmacol. Exp. Ther.*, **151**, 38(1966).
(47) V. J. Cairoli, J. F. Reily, and J. Roberts, *Brit. J. Pharmacol.*, **18**, 588(1962).
(48) J. V. Levy and V. J. Richards, *J. Pharmacol. Exp. Ther.*, **147**, 205(1965).
(49) J. Koch-Weser, *ibid.*, **150**, 184(1965).
(50) R. G. Penn, *Brit. J. Pharmacol.*, **24**, 253(1965).
(51) L. Hensler, *Schweiz. Med. Wochenschr.*, **83**, 1162(1953).
(52) B. Lown, L. Ehrlich, B. Lipschultz, and J. Blake, *Circulation*, **24**, 1185(1961).
(53) H. L. H. Dick, E. L. McCawley, and W. A. Fisher, *Arch. Intern. Med.*, **109**, 503(1962).
(54) J. Fawaz, *Brit. J. Pharmacol. Chemother.*, **29**, 302(1967).
(55) M. Takagi, D. Zanuttini, E. Khalil, and S. Bellet, *Amer. J. Cardiol.*, **15**, 203(1965).
(56) J. Roberts, R. Ito, J. Reily, and V. J. Cairoli, *Circ. Res.*, **13**, 149(1963).
(57) B. Levitt and J. Roberts, *J. Pharmacol. Exp. Ther.*, **156**, 159(1967).
(58) L. D. Buyajy and C. B. Nash, *ibid.*, **148**, 193(1965).
(59) S. I. Cohen, M. W. Young, S. H. Lau, J. I. Haft, and A. N. Damato, *Circulation*, **37**, 739(1968).
(60) T. E. Gaffney, C. A. Chidsey, and E. Braunwald, *Circ. Res.*, **12**, 264(1963).
(61) J. W. Linhart, E. Braunwald, and J. Ross, Jr., *J. Clin. Invest.*, **44**, 883(1965).
(62) A. Marino, *Rass. Med. Sper.*, **9**, 1(1962).
(63) J. Roberts and W. Modell, *Circ. Res.*, **9**, 171(1961).
(64) S. H. Taylor, in "Evaluation of New Drugs in Man," Proc. 2nd. Int. Pharmacol. Meeting, Prague, E. Zaimis, Ed., Pergamon Press, Oxford, England, 1965, p. 76.
(65) M. F. Lokhandwala, I. Cavero, J. P. Buckley, and B. S. Jandhyala, *Eur. J. Pharmacol.*, **24**, 274(1973).
(66) J. L. Morrison, H. A. Walker, and A. P. Richardson, *Arch. Int. Pharmacodyn. Ther.*, **82**, 53(1950).
(67) S. J. Sarnoff, E. Braunwald, G. H. Welch, Jr., R. B. Case, W. N. Stainsby, and R. Macruz, *Amer. J. Physiol.*, **192**, 148(1958).
(68) R. A. Maxwell, A. J. Plummer, H. Povalski, and A. I. Daniel, *J. Pharmacol. Exp. Ther.*, **128**, 22(1960).
(69) A. L. A. Boura and A. F. Green, *Ann. Rev. Pharmacol.*, **5**, 183(1965).
(70) L. L. Iversen, in "The Uptake and Storage of Noradrenaline in Sympathetic Nerves," Cambridge University Press, Cambridge, England, 1967, p. 168.
(71) I. H. Page and H. P. Dustan, *J. Amer. Med. Ass.*, **170**, 1265(1959).
(72) D. E. Clarke, *Arch. Int. Pharmacodyn. Ther.*, **182**, 78(1969).
(73) J. M. Sneddon and P. Turner, *J. Physiol. (London)*, **192**, 23P(1967).
(74) J. M. Sneddon and P. Turner, *Clin. Pharmacol. Ther.*, **10**, 64(1969).
(75) J. R. Mitchell and J. A. Oates, *J. Pharmacol. Exp. Ther.*, **172**, 100(1970).
(76) J. A. Oates, J. R. Mitchell, O. T. Feagin, and J. S. Kaufmann, *Ann. N.Y. Acad. Sci.*, **179**, 302(1971).
(77) B. K. Koe and J. W. Constantine, *Arch. Int. Pharmacodyn. Ther.*, **195**, 71(1972).
(78) F. O. Simpson and J. V. Hodge, in "Antihypertensive Agents," vol. 7, E. Schlittler, Ed., Academic, New York, N.Y., 1967, p. 459.
(79) R. Laverty, *Brit. Med. Bull.*, **29**, 152(1973).
(80) R. Cass and T. L. B. Spriggs, *Brit. J. Pharmacol.*, **17**, 442(1961).
(81) D. G. Shand, D. H. Morgan, and J. A. Oates, *Fed. Proc.*, **29**, 478(1970).
(82) S. M. Kirpekar and R. F. Furchgott, *J. Pharmacol. Exp. Ther.*, **180**, 38(1972).
(83) E. Muscholl, *Pharmacol. Rev.*, **18**, 551(1966).
(84) I. J. Kopin and E. K. Gordon, *J. Pharmacol. Exp. Ther.*, **140**, 207(1963).
(85) P. Turner, in "Clinical Aspects of Autonomic Pharmacology," Lippincott, Philadelphia, Pa., 1969, p. 57.
(86) A. Giachetti and P. A. Shore, *Biochem. Pharmacol.*, **16**, 237(1967).
(87) D. E. Clarke and G. D. H. Leach, *Brit. J. Pharmacol.*, **32**, 392(1968).
(88) D. E. Clarke, *ibid.*, **38**, 1(1970).
(89) K. Kadzielawa, *ibid.*, **19**, 74(1962).
(90) D. Dvornik, M. Kraml, J. Dubuc, H. Tom, and T. Zsoter, *Biochem. Pharmacol.*, **12**, 229(1963).
(91) R. Kuntzman and M. M. Jacobson, *J. Pharmacol. Exp. Ther.*, **141**, 166(1963).
(92) E. Costa, R. Kuntzman, G. L. Gessa, and B. B. Brodie, *Life Sci.*, **3**, 75(1962).

(93) B. B. Brodie, C. C. Chang, and E. Costa, *Brit. J. Pharmacol.*, **25,** 171(1965).
(94) W. A. Pettinger and W. D. Horst, *Ann. N.Y. Acad. Sci.*, **179,** 310(1971).
(95) G. Haeusler, W. Haefely, and A. Huerliman, *Naunyn-Schmiedebergs Arch. Pharmakol.*, **264,** 241(1969).
(96) S. M. Kirpekar, A. R. Wakade, W. Dixon, and J. C. Prat, *J. Pharmacol. Exp. Ther.*, **165,** 166(1969).
(97) J. H. Burn and M. J. Rand, *Advan. Pharmacol.*, **1,** 1(1962).
(98) M. J. Rand and J. Wilson, *Circ. Res., Suppl. III,* **21,** 89(1967).
(99) C. C. Chang, F. M. Lai, and C. C. Chiveh, *Arch. Int. Pharmacodyn. Ther.*, **190,** 34(1971).
(100) D. J. Boullin, E. Costa, and B. B. Brodie, *Life Sci.*, **6,** 803(1966).
(101) E. T. Abbs, *Brit. J. Pharmacol.*, **26,** 162(1966).
(102) J. H. Burn and F. Welsh, *ibid.*, **31,** 74(1967).
(103) S. D. Jafanus, E. Miele, and R. P. Rubin, *ibid.*, **33,** 560(1968).
(104) J. R. Pickleman, F. H. Straus, II, M. Forland, and E. Paloyan, *Metabolism,* **18,** 867(1969).
(105) C. G. Duarte, J. L. Winnacker, K. L. Becker, and A. Pace, *N. Engl. J. Med.*, **284,** 828(1971).
(106) T. L. B. Spriggs, *Brit. J. Pharmacol.*, **26,** 271(1966).
(107) F. M. Abboud and J. W. Eckstein, *Circ. Res.*, **11,** 788(1962).
(108) B. J. Gannon, T. Iwayama, G. Burnstock, J. Gerkins, and M. L. Mashford, *Med. J. Aust.*, **2,** 207(1971).
(109) G. Burnstock, B. Evans, B. J. Gannon, W. J. Heath, and V. James, *Brit. J. Pharmacol.*, **43,** 295(1971).
(110) B. Evans, T. Iwayama, and G. Burnstock, *J. Pharmacol. Exp. Ther.*, **185,** 60(1973).
(111) G. R. Bauer, F. J. T. Croll, R. B. Goldrick, D. Jeremy, J. Raftos, H. M. White, and A. A. Young, *Brit. Med. J.*, **2,** 410(1961).
(112) S. E. Rosenbloom, R. P. Shadera, R. S. Boldbloom, M. C. Sheps, and A. P. Shapiro, *N. Engl. J. Med.*, **268,** 797(1963).
(113) J. H. Sah, P. P. T. Say, and G. S. A. Peoples, *Arzneim.-Forsch.*, **16,** 199(1966).
(114) V. Vejlsgaard, M. Christensen, and E. Clausen, *Brit. Med. J.*, **2,** 598(1967).
(115) J. Jensen-Holm and P. Juul, *Brit. J. Pharmacol.*, **34,** 211P(1968).
(116) J. Jensen-Holm and P. Juul, *Acta Pharmacol. Toxicol.*, **28,** 270(1970).
(117) *Ibid.*, **28,** 283(1970).
(118) *Ibid., Suppl. 1,* **28,** 38(1970).
(119) P. Juul and R. L. McIsaac, *Acta Pharmacol. Toxicol.*, **32,** 382(1973).
(120) P. Jensen-Holm and P. Juul, *ibid.*, **30,** 308(1971).
(121) J. P. Tranzer and J. G. Richards, in "6-Hydroxydopamine and Catecholamine Neurons," T. Malmfors and H. Thoenen, Eds., American Elsevier, New York, N.Y., 1971, p. 15.
(122) O. A. Downing and P. Juul, *Acta Pharmacol. Toxicol.*, **32,** 369(1973).
(123) L. Eranko and O. Eranko, *ibid.*, **30,** 403(1971).
(124) L. Eranko and O. Eranko, *Histochem. J.*, **3,** 451(1971).
(125) P. U. Angeletti, R. Levi-Montalcini, and F. Caramia, *Brain Res.*, **43,** 505(1972).
(126) L. Eranko, C. Hill, O. Eranko, and G. Burnstock, *ibid.*, **43,** 501(1972).
(127) I. J. Kopin and S. D. Silberstein, *Pharmacol. Rev.*, **24,** 245(1972).
(128) F. Caramia, P. U. Angeletti, R. Levi-Montalcini, and L. Carratelli, *Brain Res.*, **4,** 237(1970).
(129) P. U. Angeletti and R. Levi-Montalcini, *Proc. Nat. Acad. Sci. USA,* **65,** 114(1970).
(130) G. Burnstock, A. E. Doyle, B. J. Gannon, J. F. Gerkens, T. Iwayama, and M. L. Mashford, *Eur. J. Pharmacol.*, **13,** 175(1971).
(131) G. Burnstock, B. Gannon, and T. Iwayama, *Circ. Res., Suppl. II,* **26,** 5(1970).
(132) J. K. Dawborn, A. E. Doyle, A. Ebringer, J. Howgua, G. Jerums, C. I. Johnston, M. L. Mashford, and J. D. Parkin, *Pharmacol. Clin.*, **2,** 1(1969).
(133) A. L. A. Boura and A. F. Green, *Brit. J. Pharmacol.*, **19,** 13(1962).
(134) M. Moser, *Amer. Heart J.*, **77,** 423(1969).
(135) A. F. Green and R. D. Robson, *Brit. J. Pharmacol.*, **25,** 497(1965).
(136) A. E. Stocks and A. Robertson, *Amer. Heart J.*, **73,** 569(1967).
(137) C. T. Dollery, D. Emslie-Smith, and M. D. Milne, *Lancet,* **2,** 381(1961).
(138) G. E. Bauer, F. H. Croll, R. B. Goldrick, D. Jeremy, J. Raftos, H. M. Whyte, and A. A. Young, *Brit. Med. J.*, **2,** 410(1961).
(139) C. McMartin and P. Simpson, *Clin. Pharmacol. Ther.*, **12,** 73(1970).
(140) K. H. Rahn and L. I. Goldberg, *ibid.*, **10,** 858(1969).
(141) C. McMartin, R. K. Rondel, J. Vinter, B. R. Allan, P. M. Humberstone, A. W. D. Leishman, G. Sandler, and J. L. Thirkettle, *ibid.*, **11,** 423(1970).
(142) D. A. Chamberlain and J. Howard, *Brit. Heart J.*, **26,** 528(1964).
(143) H. Villarreal, J. E. Emilio, V. Rubio, and H. Davila, *Amer. J. Cardiol.*, **14,** 633(1964).
(144) R. Sannerstedt and J. Conway, *Amer. Heart J.*, **79,** 122(1970).
(145) D. T. Mason, E. Braunwald, D. D. Kruger, C. V. King, and R. B. Karsh, *J. Clin. Invest.*, **43,** 1449(1964).
(146) B. N. C. Pritchard, *Proc. Roy. Soc. Med.*, **62,** 84(1969).
(147) J. R. Gill, Jr., D. T. Mason, and F. C. Bartter, *J. Clin. Invest.*, **43,** 177(1964).
(148) J. C. McGiff, *Circ. Res.*, **20,** 664(1967).
(149) J. R. Gill and F. C. Bartter, *N. Engl. J. Med.* **275,** 1466(1966).
(150) H. N. Wagner, Jr., *J. Clin. Invest.*, **36,** 1319(1957).
(151) R. L. Williams, J. E. Maines, III, and J. E. Pearson, *J. Pharmacol. Exp. Ther.*, **177,** 69(1971).
(152) J. C. Murphy, J. B. Justice, and O. Carrier, Jr., *Arch. Int. Pharmacodyn. Ther.*, **194,** 56(1971).
(153) A. W. D. Leishman, H. L. Mathews, and A. J. Smith, *Lancet,* **2,** 4(1961).
(154) A. N. Brest, G. Onesti, C. Swartz, R. Seller, K. E. Kim, and J. Chinitz, *J. Amer. Med. Ass.*, **211,** 480(1970).
(155) V. Rønnov-Jessen, *Acta Med. Scand.*, **174,** 307(1963).
(156) B. N. Dixit, O. D. Gulati, and S. D. Gokhale, *Brit. J. Pharmacol.*, **17,** 372(1961).
(157) D. E. Clarke, B. S. Jandhyala, I. Cavero, B. N. Dixit, and J. P. Buckley, *Can. J. Physiol. Pharmacol.*, **52,** 641(1974).
(158) B. S. Jandhyala, I. Cavero, and J. P. Buckley, "Abstracts of the APhA Academy of Pharmaceutical Sciences," APhA Academy of Pharmaceutical Sciences, Washington, D.C., 1973.
(159) A. N. Brest and J. H. Moyer, in "Hypertension, Recent Advances," Lea & Febiger, Philadelphia, Pa., 1961, p. 250.
(160) N. A. David, *Curr. Ther. Res.*, **5,** 93(1963).
(161) E. D. Fries, *N. Engl. J. Med.*, **266,** 607(1962).
(162) L. E. Early and J. Orloff, *Ann. Rev. Med.*, **15,** 149(1964).
(163) S. W. Hoobler, J. M. Weller, and P. Blaquier, in "Hypertension," J. H. Moyer, Ed., Saunders, Philadelphia, Pa., 1959, p. 581.
(164) M. A. Greene, A. J. Boltax, E. S. Scherr, and M. Niv, *Amer. J. Med.*, **36,** 87(1964).
(165) W. Hollander, A. V. Chobanian, and R. W. Wilkins, *Ann. N.Y. Acad. Sci.*, **88,** 975(1960).
(166) P. J. Talso and A. J. Carballo, *ibid.*, **88,** 822(1960).
(167) K. H. Beyer, J. E. Baer, H. F. Russo, and A. Haimbach, *Fed. Proc.*, **16,** 282(1957).
(168) R. W. Gifford, Jr., V. R. Mattox, A. L. Orvis, D. A. Sones, and J. W. Rosewear, *Circulation,* **24,** 1197(1961).
(169) P. Lauwers and J. Conway, *J. Lab. Clin. Med.*, **56,** 401(1960).
(170) B. M. Winer, *Circulation,* **23,** 211(1961).
(171) V. S. Aoki and M. J. Brody, *Arch. Int. Pharmacodyn. Ther.*, **177,** 423(1969).
(172) L. Tobian and K. Coffee, *Proc. Soc. Exp. Biol. Med.*, **115,** 196(1964).
(173) P. D. Redleaf and L. Tobian, *Circ. Res.*, **6,** 343(1958).
(174) P. W. Willard, *J. Pharm. Pharmacol.*, **21,** 408(1969).
(175) L. Tobian, J. Janacek, J. Foker, and D. Ferreira, *Amer. J. Physiol.*, **202,** 905(1962).
(176) A. A. Renzi, J. J. Chart, and R. Gaunt, *Toxicol. Appl. Pharmacol.*, **1,** 406(1959).
(177) J. W. Eckstein, M. G. Wendling, and F. M. Abboud, *Circ. Res., Suppl. 1,* **28 and 29,** 1(1966).

(178) F. A. Khalil, J. W. Eckstein, A. W. Horsley, and H. H. Keasling, *J. Appl. Physiol.*, **16**, 549(1961).
(179) M. F. Loevett and T. E. Nicholas, *Brit. J. Pharmacol.*, **33**, 136(1968).
(180) W. R. Blavers and W. P. Blackmore, *Proc. Soc. Exp. Biol. Med.*, **98**, 123(1958).
(181) R. I. Ogilvie and M. D. Schlieper, *Clin. Pharmacol. Ther.*, **11**, 589(1970).
(182) P. Preziosi, A. Bianchi, B. Loscalzo, and A. F. DeSchaepdryver, *Arch. Int. Pharmacodyn. Ther.*, **118**, 467(1959).
(183) K. A. Feisal, J. W. Eckstein, A. W. Horsley, and H. H. Keasling, *J. Appl. Physiol.*, **16**, 549(1961).
(184) T. T. Zsoter, F. Hart, and I. C. Radde, *Circ. Res.*, **27**, 717(1970).
(185) E. E. Daniel, *ibid.*, **11**, 941(1962).
(186) J. M. Weller and A. S. Haight, *Proc. Soc. Exp. Biol. Med.*, **112**, 820(1963).
(187) J. Hansen, *Acta Med. Scand.*, **183**, 317(1968).
(188) P. Preziosi, A. F. DeSchaepdryver, E. Marmo, and E. Miele, *Arch. Int. Pharmacodyn. Ther.*, **131**, 209(1961).
(189) M. Kusumoto, G. Constantopoulos, J. M. Rojo-Ortega, R. Boucher, and J. Genest, *Proc. Soc. Exp. Biol. Med.*, **143**, 1077(1973).
(190) J. Menard, J. M. Rojo-Ortega, R. Boucher, and J. Genest, *Pathol. Biol.*, **19**, 821(1971).
(191) J. J. Bourgoignie, F. J. Catanzaro, and H. M. Perry, Jr., *Circulation*, **37**, 27(1968).
(192) R. C. Tarazi, H. P. Dustan, and E. D. Frohlich, *ibid.*, **41**, 709(1970).
(193) F. A. Tapia, H. P. Dustan, and R. A. Schneckloth, *Lancet*, **2**, 831(1957).
(194) B. M. Winer, *Circulation*, **24**, 788(1961).
(195) *Ibid.*, **23**, 211(1961).
(196) I. M. Wilson and E. D. Freis, *Circulation*, **18**, 800(1958).
(197) *Ibid.*, **20**, 1028(1959).
(198) J. Conway and P. Lauwers, *Circ. Res.*, **21**, 21(1960).
(199) E. D. Frohlich, A. E. Thurman, M. A. Pfeffer, G. F. Brobmann, and E. D. Jacobson, *Proc. Soc. Exp. Biol. Med.*, **140**, 1190(1972).
(200) H. Villarreal, J. E. Exaire, A. Revollo, and J. Soni, *Circulation*, **26**, 405(1962).
(201) M. A. Greene, A. J. Boltax, and E. S. Scheer, *Amer. Heart J.*, **62**, 659(1961).
(202) B. S. Jandhyala, I. Cavero, and J. P. Buckley, *Eur. J. Pharmacol.*, **17**, 357(1972).
(203) B. S. Jandhyala, I. Cavero, H. H. Smookler, and J. P. Buckley, *Proc. Soc. Exp. Biol. Med.*, **144**, 935(1973).
(204) J. Conway and P. Lauwers, *Amer. J. Cardiol.*, **8**, 884(1961).
(205) D. E. Clarke, R. J. Ertel, R. Adams, and J. P. Buckley, *Eur. J. Pharmacol.*, **19**, 380(1972).
(206) W. E. Barrett, R. A. Rutledge, H. Sheppard, and A. J. Plummer, *Toxicol. Appl. Pharmacol.*, **1**, 333(1959).
(207) W. G. Naylor, I. McInnes, J. B. Swann, D. Race, V. Carson, and T. E. Loewe, *Amer. Heart J.*, **75**, 223(1968).
(208) E. Herbert and B. H. Buxton, Jr., *Obstet. Gynecol.*, **17**(6), 674(1961).
(209) T. Baum, A. T. Shropshire, and L. L. Varner, *J. Pharmacol. Exp. Ther.*, **182**, 135(1972).
(210) R. Dagirmanjian, *J. Pharm. Pharmacol.*, **15**, 518(1963).

ACKNOWLEDGMENTS AND ADDRESSES

Received from the *Department of Pharmacology, College of Pharmacy, University of Houston, Houston, TX 77004*

The authors are grateful for the excellent assistance of Barbara M. Lewis and Marie L. Steenberg in preparing this manuscript.

[x] To whom inquiries should be directed.