CARDIOVASCULAR PHARMACOLOGY[1,2]

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Cardiovascular pharmacology, like other biological disciplines, is not free from controversy. Part of this, which might well be avoidable, is the result of such as the following: free application to the intact animal of results obtained on isolated organs and tissues; underestimation of species differences as well as the effect of anesthetics; insufficient appreciation of the influence of dosage and method of administration of drugs. It is not uncommon to observe one effect at low concentrations and an entirely opposite effect with higher dosages even on the same segment of the cardiovascular system. Mellander (1), for example, found that intravenous epinephrine (E) infusion at the rate of 0.4 μg/kg/min resulted in marked dilatation of the "resistance" vessels of a skin-muscle region, but no change in or even slight constriction of "capacitance" vessels, while with 1 μg there was a definite, and with 5 μg a marked constriction of both resistance and capacitance vessels. These results are in harmony with observations on man where minimal doses of E may reduce the diastolic pressure while large doses raise it. Differences in methodology also account for some of the conflicting reports. It cannot be claimed that all methods currently used for measuring such parameters as blood flow and cardiac output are perfect and a change of 10 per cent may often be within the limits of experimental error. Finally, it is not always realized that drugs usually have actions other than those under which they are classified.

On the other hand, there are difficulties due to our incomplete knowledge of the anatomy and physiology of the peripheral circulatory system. According to one classification [Folkow (2)] the latter consists of "Windkessel" (arteries), "resistance" (including precapillary or arterioles and postcapillary or venules) "sphincter," "shunt," "capacitance" (veins), and "exchange" vessels. No reliable methods are yet available for measuring simultaneously and quantitatively the reactions to drugs of all these segments of the circulation and in all parts of the organism. There is no reason to believe that these different kinds of blood-vessels react always in the same way to any one drug. A drug may constrict the resistance vessels without affecting the capacitance vessels, or constrict or dilate both resistance and capacitance vessels. The terms "vasoconstriction" and "vasodilatation" can thus be quite misleading. For if a drug or stimulus constricted both capacitance and

[1] The survey of the literature pertaining to this review was concluded in May 1962.

[2] Abbreviations in this chapter include: ADP (adenosinediphosphate); AMP (adenosinemonophosphate); ATP (adenosinetriphosphate); CO (cardiac output); E (epinephrine); NE (norepinephrine).

resistance vessels the resulting hemodynamic effect could not be precisely predicted since narrowing of the resistance vessels would decrease while constriction of the capacitance vessels would increase the venous return from the region examined. The situation becomes more complex if it is further remembered that the reaction of vessels in different tissues may vary qualitatively as well as quantitatively.

Let us examine some factors that determine cardiac output (CO). When a drug is observed to change CO, some investigators consider it a primary action on the heart instead of thinking of it as peripheral action that alters venous return. A normal heart can handle a considerable increase in venous return unaided by drugs. It is only the insufficient heart that acts as a bottleneck. Consider a hypothetical substance that has no other action but to increase cardiac contractility. Administration of such a drug would momentarily increase the stroke volume by diminishing the amount of residual blood in the ventricle. It is argued that this could lead to a sustained increase in minute output by one of two ways. (*a*) No pressoreceptor reflex activity is elicited. The increased stroke volume raises arterial pressure without changing peripheral resistance, heart rate or venous pressure. This results in an increased arteriovenous pressure gradient and a blood shift from the arterial to the venous side, decreased circulation time and increased output. (*b*) Pressoreceptor reflexes activated due to increase in stroke volume. Here no rise in arterial pressure is observed owing to dilatation of resistance vessels. If the capacitance vessels are not affected by reflex activity, this condition could also lead to an increased shift of blood from the arterial to the venous side and thus an increased venous return to the heart. Situation (*a*) probably does not occur as it is unlikely that an increased stroke volume would remain without influence on baroreceptor activity. So only situation (*b*) will be considered. The effect of the resulting diminution in sympathetic discharge, however, cannot be predicted. A dilatation of resistance vessels would tend to increase blood flow and venous return, while dilatation of capacitance vessels would have the opposite effect. Since low rates of sympathetic discharge may preferentially influence the capacitance vessels [Mellander (1)] it is not inconceivable that a momentary increase in stroke volume would dilate the capacitance rather than the resistance vessels with a resulting decrease in venous return. Another more serious objection against the arguments put forward in situation (*b*) is that an increase in myocardial contractility can lead to a greater stroke volume of the first systole. Now if the increased stroke volume is to be maintained, a corresponding rise in venous return must be ensured for the next systole. The pressoreceptor reflex system cannot do that since it takes about 3–4 sec for it to act [Wang & Borison (3)]. So, unless it is assumed that the heart can act as a suction pump—a hypothesis that is not universally accepted although there is much evidence to support it [Brecher (4); Guyton, Langston & Carrier (5)]—to increase venous return in the interval before the action of the reflex system comes into play, it is difficult to visualize how a drug could raise minute output merely

by increasing the force of contraction. In reality, there are no known drugs, not even digitalis, that influence cardiac action without affecting the peripheral circulation.

The effect of drugs on veins has been the subject of many recent studies but the interpretation of the results and their bearing on the general hemodynamic situation is not easy. More useful information would be obtained if a simultaneous study were made of the reaction of the segments of the same and parallel circuits. Now digitalis may decrease CO in normal animals and "venoconstriction" has been offered as an explanation for that [Cotten & Moran (6)]. However, along with the venoconstriction a rise in arterial pressure was noted which has been attributed to direct action by digitalis on the resistance vessels [Lendle & Mercker (7)]. It is more reasonable to attribute the digitalis-induced decrease in venous return to the arteriolar rather than the venoconstriction, since the latter alone would increase rather than decrease venous return.

Changes in the diameter of the resistance vessels are more important than those of capacitance vessels in determining regional blood flow and venous return. So-called "vasodilators" like sodium nitrite, nitroglycerine, histamine and carbachol, which are not known to have direct action on the heart, also raise CO [Nakano *et al.* (8)]. The potent "vasodilator" bradykinin increases CO and stroke volume at a time when it lowers systolic and diastolic pressures [Gersmeyer & Spitzbarth (9)]. The same is true of hydralazine [Freis (10)] and the antihypertensive drug dizoaxide [Zitowitz & Rubin (11)]. Isoproterenol, in doses leaving mean arterial pressure unchanged or decreasing it slightly or markedly, also leads to an increase in CO and decrease in total peripheral resistance [Nakano *et al.* (8)]. Although isoproterenol is known to act directly on peripheral vessels Nakano *et al.* discuss the possibility that the increase in CO produced by the direct action of this drug on the heart might also induce a reflex peripheral vasodilatation. Gorten *et al.* (12) also observed an increase in CO after isoproterenol along with decreases in peripheral resistance and central venous pressure.

The above considerations were meant to emphasize wide gaps in our knowledge regarding the pharmacology of peripheral circulation. More progress can be made by calling attention to these gaps rather than attempting to bridge them by speculation. The latter, no matter how ingenious, is often misunderstood, especially by clinicians, and is likely to creep into textbooks to become standard teaching in our medical schools.

CARDIAC GLYCOSIDES

Mechanism of Action

Effect on cation transport.—The suggestion put forward several years ago [Hajdu & Leonard (13)] that the therapeutic action of digitalis might be causally related to the reduction of intracellular K^+ concentration or decrease in K^+ influx has not found general acceptance. It is now realized that this

inhibition of active K^+ and Na^+ transport is an effect of "toxic" concentrations of digitalis, although it is not easy to tell what constitutes a toxic dose particularly when strips of cardiac muscle are used. It is not correct to state that a certain molar concentration of a drug represents the threshold toxic dose. That has to be determined for every preparation and for the particular experimental set-up, since factors such as volume of bath fluid and period of exposure to the drug are not irrelevant. It is often observed that a certain concentration shows a "therapeutic" effect early in the experiment only to end in a toxic effect as shown by irregular rhythm, contracture, or negative inotropic action.

Klaus, Kuschinsky & Lüllmann (14) using isolated guinea pig auricles observed with toxic doses of digitoxigenin a decrease in influx and an increase in efflux of K^+, a rise in intracellular Na and decrease in intracellular K. "Therapeutic" doses produced an almost opposite effect: rise in K^+-influx with no change in efflux, a decrease in Na and no change in K cellular content. Lee *et al.* (15) using cat papillary muscle reported a decrease in K and an increase in Na content with toxic doses but no change with therapeutic doses. Vick (16) used dihydroouabain on guinea pig ventricles and noticed that low doses produced positive inotropic effects, but the K lost was less than that observed in the controls. With higher doses marked inotropic effects were seen before there was appreciable net loss of K. Hagen (17) found that small doses of digilanid C raised the K content of the heart while large doses decreased it. Brown, Acheson & Grupp (18) working with dog heart-lung preparation observed a small steady gain of K by the heart. This net gain is increased with small doses (50 μg) of dihydroouabain, while after larger doses that are still below the irregularity dose, the net gain was changed to a net loss. Kahn (19) concludes that "a mechanism entailing a net K loss from the heart need not be invoked to explain the positive inotropic effects of the glycosides on cardiac muscle." Tuttle, Witt & Farah (20) came to similar conclusions on the basis of *in vitro* studies. They report that only toxic doses of ouabain lead to K loss, but that therapeutic doses may actually decrease the rate of loss of K and decrease intracellular Na concentration in confirmation of earlier workers. Tuttle & Witt (21) observed that therapeutic doses of ouabain do not influence K^+ influx or outflux while toxic doses inhibit influx. Therapeutic doses of ouabain injected into intact rabbits had no effect on intracellular K but decreased Na concentration. Lethal doses of ouabain reduced intracellular K.

While these results indicate that a decrease in intracellular K content or K^+ influx is associated with toxic doses of digitalis, the question may be asked whether the opposite is not true; i.e., whether the increase in intracellular K or decrease in Na content with low doses is not causally related to the therapeutic actions of digitalis. Recent studies on membrane ATPases help to throw light on this question. Early work by Schatzmann (22), Glynn (23), and Solomon, Gill & Gold (24) indicated that digitalis inhibits active $Na^+ - K^+$ transfer in RBC and that inhibition of K transfer is counteracted by

raising extracellular K^+. It was suggested that K^+ and digitalis compete for a receptor in the RBC. The discovery by Skou (25) of a Na^+, K^+-activated ATPase in nerve tissue and the application of Skou's methods to RBC, led to the finding that the ATPase of RBC is blocked by doses of digitalis which also inhibit active K^+ transfer and that K^+ counteracts this enzyme inhibition. The conclusion was drawn that the receptor for which K^+ and digitalis were competing was none other than the Na^+, K^+-activated ATPase [Post *et al.* (26); Dunham & Glynn (27); Kahn (19)]. In fact, Dunham & Glynn (27) noted that those changes in the molecular structure of cardiac glycosides that affect cardiac action also influence inhibition of cation pump activity in the same direction.

Repke & Portius (28) compared the inhibitory actions of digitoxin and also of K-strophantoside on membrane ATPases from human and rat RBC with the cardiotoxic actions of these drugs. They concluded that the difference in glycoside sensitivity between man and rat is reflected by a corresponding difference in the susceptibility of the respective ATPases. These authors also found that structural differences in the cardiac glycosides affect both membrane ATPase inhibition and cardiotoxicity in the same manner. For instance, saturation of the double bond in the lactone group of digitoxin decreases its inhibitory activity on ATPase 11 times and its cardiotoxicity 25 times. The corresponding figures for the effect of inversion of the lactone ring in cymarin (from β to α) are 1000 and 500 respectively. For dehydrogenation of the 3-hydroxyl group of digitoxigenin the figures are 7 and 5, and for epimerization of the 3-hydroxyl group of digitoxigenin 16 and 16. Portius & Repke (29) found that the Mg^{++}, Na^+, K^+-activated membrane ATPase obtained from cardiac muscle is stimulated by low glycoside concentrations ($10^{-10}-10^{-7}$ M) and inhibited by higher concentrations. Incubation of the enzyme preparation with a cardiac glycoside could change a stimulatory effect to an inhibitory one. For example, 5×10^{-7} M K-strophanthoside showed an initial stimulation of 43 per cent and an inhibition of 18 per cent after a thirty-minute period of incubation with the enzyme preparation. This action of digitalis on cardiac membrane ATPase is reminiscent of its action on isolated cardiac muscle preparations where a positive inotropic action may be observed at the start of the experiment and a toxic action after a period of incubation. It is unfortunate that Repke did not report experiments where K-strophantoside concentrations below 5×10^{-10} M were used. The latter concentration produced a 30 per cent stimulation (after incubation) while a 5×10^{-9} M gave only 13 per cent, and concentrations above 5×10^{-7} produced progressive inhibition. It is important to know whether in the lower dosage range (below 10^{-10} M) a dose-response curve is obtained for stimulatory activity, in other words, whether an ascending limb of the curve is obtained for the action of digitalis on cardiac membrane ATPase as is the case for the biphasic action of digitalis on isolated cardiac muscle [Farah & Witt (30)]. If an ascending limb of the curve does exist it would be important to determine the ratio stimulatory: inhibitory dose for various naturally

occurring glycosides and see whether this ratio is as constant as and comparable to the ratio therapeutic:toxic dose.

From the foregoing it can be concluded that doses of digitalis which inhibit the Na^+, K^+-activated membrane ATPase are also cardiotoxic. The decrease in intracellular K and or K^+ influx and increase in intracellular Na are consistent with inhibition of ATPase pump activity. That a causal relationship exists between inhibition of ATPase by digitalis and its cardiotoxicity is indicated by the clinical observation that digitalis toxicity can best be counteracted by K^+ administration, just as K^+ blocks inhibition of membrane ATPase by digitalis. On the other hand, the increase in intracellular K and the decrease in Na observed by several investigators after therapeutic doses of digitalis are consistent with an activation of ATPase, actually observed by Portius & Repke (29). Whether, however, there is a causal relationship between the therapeutic action of digitalis and stimulation of ATPase cannot be determined at present.

Digitalis and calcium.—It has been known for a long time that some of the cardiac actions of digitalis can be simulated by calcium ions. Farah & Witt (30) have reviewed the similarities and the differences between the two. The mechanisms of the entry of calcium into, and its exit out of, cardiac cells are still obscure. A cell membrane pump has not been demonstrated for calcium but it has been shown that calcium on the one hand, and sodium and potassium on the other, compete for sites on the membrane or within the cardiac cell [Wilbrandt & Koller (31); Niedergerke (32)]. Hasselbach & Makinose (33, 34)] have described an inwardly directed calcium pump in isolated endoplasmatic reticulum ("relaxing granules"). Simultaneous with the storage of calcium there is a seven to eight fold increase in splitting of adenosinetriphosphate (ATP) and also a marked increase in phosphate exchange between ATP and adenosinediphosphate (ADP). The function of this calcium pump in muscle contraction remains to be determined but Portius & Repke (29) have found that this pump's ATPase activity cannot be inhibited by even high concentrations of digitoxin. These authors conclude that the antagonistic action of ouabain against the "relaxing factor" reported by Lee (35) cannot be related to the ATPase activity of this calcium pump.

Therapeutic doses of ouabain and also of digitoxigenin decrease intracellular calcium in heart muscle [Klaus *et al.* (14); Lee (36)] without affecting the uptake of Ca^{45} [Harvey & Daniel (37); Thomas, Jolley & Grechman (38)]. On the other hand, toxic concentrations of digitalis were observed to increase the intracellular Ca content [Klaus *et al.* (14); Lee *et al.* (15)], and the Ca^{45} uptake [Witt (39); Thomas *et al.* (38); Holland & Sekul (40)]. Repke (41) has reported a stimulatory effect of small concentrations of calcium on the Mg^{++}, Na^+, K^+-activated cardiac membrane ATPase and an inhibitory effect of larger concentrations.

Tuttle & Farah (42), and Farah & Witt (30) studied the effects of ouabain and acetyl-strophanthidin on such rate-dependant phenomena as the "frequency-force relation" and the "post-stimulation potentiation." It was

found that in all species studied, low "therapeutic" doses increased contractility at the high rates of stimulation and also increased post-stimulation potentiation. On the other hand, higher, "toxic" doses abolished the typical *S*-shaped frequency-force curve and reduced post-stimulation potentiation. "*In vivo*" studies also confirmed these results, in that isolated strips from rabbits pretreated with toxic doses of ouabain showed a loss of the frequency-force curve and reduction in post-stimulation potentiation. Farah & Witt (30) consider that the positive inotropic effect of digitalis is related to the mechanism which couples the membrane phenomena to the actual contraction, and that the key substance of this coupling mechanism is the calcium-containing "potentiating substance" which Rosin & Farah (43) surmised to be produced during every depolarization and assumed to be identical with the "activator" of contraction described by Lüttgau & Niedergerke (44). Farah & Witt (30) speculate that digitalis sensitizes the coupling mechanism to calcium or that it promotes the formation of the calcium-containing "activator" without increasing the total calcium content of the heart. The literature on the possible role of calcium in excitation-contraction coupling of heart muscle has been reviewed by Winegrad (45). This author observed a considerable increase in Ca^{45} transfer during contraction which was correlated with the strength of the contraction. This relationship is maintained at different frequencies of stimulation and at different concentrations of external calcium.

It thus appears that Ca^{+} is implicated with the membrane phenomena, the coupling mechanism, and the contractile process. Na^{+} and K^{+} are also directly or indirectly involved in these phenomena. However, as long as the intracellular distribution of these ions and the movements they undergo during activity are not known, any attempts to define the relationship between digitalis and calcium will remain speculative [Kahn (19)].

Digitalis and the contractile proteins.—Olson (46) isolated myosin from ventricles of dogs with experimental congestive failure, and found that it differed in its physico-chemical properties from "normal" myosin. This work has since been extended [Olson (47)]. It was observed that the myosin from failing hearts not only showed a higher molecular weight than that from normal hearts (690,000 vs. 226,000) but that it had a higher amide nitrogen content indicating a decrease in polarity. It was further observed that only normal myosin was obtained from dogs with edema due to inferior vena cava ligation, valvular disease or cardiac hypertrophy but without failure. The observation by Mallov & Robb (48) of a direct action of digitalis on actomyosin has stimulated further work. Kako & Bing (49) reported that Ca^{++} and digoxin improved the contractility of actomyosin bands obtained at autopsy from human failing hearts. A direct action of ouabain on contractile protein has been demonstrated by Lee (35). This author used cat papillary muscle fibers extracted in glycerol-histidine buffer and retaining the "relaxing factor." ATP treatment resulted in the development of only 33 per cent of full tension in the control fibers whereas ouabain-treated fibers developed almost

full tension. Subsequent addition of Ca^{++} raised tension in the control fiber markedly but only slightly in the ouabain-treated fiber. It is noteworthy that only those fibers containing the relaxing factor reacted to ouabain treatment in this manner.

Waser (50) summarized his investigations on the *in vitro* interaction between digitalis and contractile proteins. He found that the formation of a glycoside-myosin complex is accompanied by a marked increase of the binding power of myosin for K^+ and also for Ca^{++}. The author believes that digitalis acts not only on the membrane but enters the sarcoplasm to enhance the contractile mechanism. In autoradiographs of interventricular muscle bundles of beef hearts (perfused through a small artery) tritiated digitoxin was found outside the membrane and inside the muscle cells at the moment of maximal inotropic action [Waser (51)]

Digitalis and Energy Metabolism

A considerable number of papers dealing with the metabolic aspects of heart failure and the effects of digitalis have been published during the last ten years. The present state of knowledge has been reviewed by Furchgott & Lee (52), Lee (36), Bing (53), and Olson (47). It is now generally agreed that low-output "congestive" failures as are produced surgically in dogs are not associated with a diminished content of high-energy phosphorus compounds, or with a change in myocardial oxygen consumption or even a significant change in the nature of the substrates metabolized. Work is calculated from the product of pressure and output, and since in congestive failure there is a decreased output without a change in oxygen consumption the "mechanical efficiency" diminishes. Therapeutic doses of digitalis, by increasing contractility, raise the output without changing the oxygen consumption. On the basis of these experimental results it has been postulated that the biochemical lesion in congestive failure is not a deficiency in producing high-energy phosphorus compounds—the ultimate sources of energy for contraction—but in utilizing them. It has even been suggested that the function of digitalis is to increase the efficiency of the conversion of chemical into mechanical energy. This may well be the case, but there is no experimental basis for this assumption. It can just as well be assumed that digitalis corrects some fault in the complex machinery of the contraction process thus restoring a normal systole and a normal output. Increased mechanical efficiency would thus be a by-product of digitalis action rather than its primary function. The situation can be likened to running a car with the hand-brakes on. Here one gets less mileage per unit volume of gasoline just as in the case of an engine where there is inefficient conversion of thermal into mechanical energy in the combustion chamber. In both cases there is decrease in mechanical efficiency. Sarnoff *et al.* (54) raised the output of a heart-lung preparation 500 per cent, keeping aortic pressure constant, and observed only a 50 per cent increase in oxygen consumption. A common demonstration experiment in the reviewer's laboratory is to increase volume work of a heart-lung preparation by 30 per

cent and find no measurable increase in oxygen consumption. These observations do not conflict with the first law of thermodynamics. The mechanical efficiency of a conventional dog heart-lung preparation is much less than that of the heart *in situ*. The output in the intact dog is also correspondingly higher. One obtains the impression that at a given aortic pressure almost the same amount of oxygen is consumed per stroke whether the output is low as in the isolated heart or failing intact heart, or high as in the normal intact heart. A 10 per cent increase in the output of a normal heart-lung preparation with no change in pressure is no more likely to raise the oxygen consumption than the addition of an extra baby to a half-loaded Boeing 707 is likely to raise its fuel bill. In contrast to therapeutic doses, toxic concentrations of digitalis do increase the myocardial oxygen consumption. This has been demonstrated by Fawaz & Tutunji (55) and Lee, Yu & Burstein (56). Lee *et al.* (56) attribute this rise in oxygen consumption to an increased turnover of high-energy phosphate nucleotide initiated by digitalis, since toxic doses were accompanied by a decrease of ATP and especially phosphocreatine. It is possible, on the other hand, that the increased oxygen consumption initiated by digitalis, by producing a relative hypoxia, was the cause of the decrease in phosphocreatine and ATP in Lee's experiments, and not vice versa. Myocardial phosphocreatine is extremely sensitive to hypoxia. In fact, the extent of the latter can often be gauged by the diminution in the level of phosphocreatine. Thin segments of myocardial tissue suspended in oxygenated aqueous solutions may suffer no hypoxia as long as the oxygen consumption is low, but a relative hypoxia may develop when the oxygen demand rises, since here, unlike the situation *in vivo*, the increased oxygen demand cannot be met by a rise in coronary flow, nor can an aqueous solution store as much oxygen as blood. Fawaz & Tutunji (55) observed no decrease in the phosphocreatine level of a dog heart-lung preparation during ouabain-induced ventricular tachycardia, although the oxygen consumption had risen to 75 per cent compared to 25 per cent when the untreated preparation was stimulated through the right auricle. It is important to estimate the oxygen content of coronary venous blood in such studies to be certain that no relative hypoxia develops. The suggestion put forward by Lee *et al.* (57) that toxic doses of digitalis uncouple phosphorylation and oxidation deserves further investigation. Actually, dinitrophenol, a known uncoupler, has been found to decrease the myocardial phosphocreatine content even when the oxygen content of coronary venous blood was within normal limits [Fawaz, Hawa & Tutunji (58)]. However, for a heart whose oxygen consumption is double or triple the normal, a "normal" oxygen content of coronary venous blood does not exclude relative hypoxia of the myocardium.

Metabolism and Structure-Activity Relationship

Reviews on this subject have been written by Wright (59), Chen (60), and Repke (41). Brown, Stafford & Wright (61) studied the relationship between the chemical structure and pharmacological activity of some 15

derivatives of digitoxigenin and digoxigenin, including the parent glycosides digitoxin, digoxin and lanatosides A and C. They concluded that there are three centers in a cardiac aglycone molecule that are linked with cardiac activity. These are: (*a*) an OH at carbon-3 which can be combined as a glycoside thus enhancing activity or which can be esterified or oxidized thereby diminishing it; (*b*) a 14 β-OH associated with a *cis*-junction of rings C and D, alteration of which abolishes activity; (*c*) an unsaturated lactone ring, reduction of which greatly decreases activity. These authors further concluded that the order of relative potency of the compounds remained the same whether they were tested for toxicity or positive inotropic activity, and emphasized the generally accepted close relationship existing between the latter. The data of Brown *et al.* (61) contain much that is instructive. The authors measure toxicity using cats, guinea pigs and isolated hearts of 48 hr chick embryos, and inotropic activity by the use of isolated cat papillary muscles and guinea pig Langendorff hearts. Now, depending on the preparation used to measure toxicity or inotropic action there are considerable variations in the potency ratios of the derivatives when the activity of the corresponding aglycone is taken as one. For example, the toxicity ratios of lanatoside A, digitoxin, lanatoside C and digoxin are 1.4, 0.7, 1.4, 1.9 respectively when the cat method was used, and 6.6, 4.4, 23, 23 respectively when guinea pigs were used. The figures for the inotropic ratios of these same compounds are 0.8, 4.1, 0.3, 1.5 with papillary muscle, and 3.6, 5.1, 8.9, 10 with the Langendorff heart. Cattel & Gold (62) called attention long ago to the discrepancies observed when different animal preparations are used to measure the therapeutic index of digitalis compounds. For instance, they found that ouabain and digitoxin have the same potency when tested on papillary muscle, but, using intact cats, they found ouabain to be four times as potent as digitoxin and, by the frog method, eight times as potent. The lanatosides were observed to be one-tenth as potent as digitoxin when the papillary muscle was used, but in the intact animal lanatoside C was more potent than digitoxin.

The failing heart-lung preparation of a dog offers the possibility of measuring both inotropic and toxic actions of cardiac glycosides on the same specimen. This has been done for ouabain, digoxin, digitoxin, oleandrin, lanatoside B, [Farah & Maresh (63)]; gitalin [Gruhzit & Farah (64)], and scilliroside [Fawaz & Meyer (65)]. It was found that the ratio of the so-called "minimal therapeutic dose" to the minimal lethal dose was the same for all these glycosides, and is around 1:5 if the beginning of a decline in right atrial pressure is taken as the start of therapeutic action. The time required to produce a therapeutic or a toxic effect varies inversely, but the doses required to produce these effects vary proportionately with the rate of administration [Farah (66)]. Yet, regardless of the rate of administration, a heart-lung preparation can take up only one minimal lethal dose, the excess remaining in the blood [Weese (67, 68)].

The so-called "latent period" for the action of cardiac glycosides in the heart-lung preparation may be considered as the time required for the drug

to reach the effective receptor site. The data of Farah & Maresh (63) do not permit a comparison among the latent periods of the glycosides studied, since the maximum rate of administration used was not the same fraction of the minimal lethal dose for each drug. For ouabain, the maximum rate per min was 0.38 of the minimal lethal dose and the latent period was 2.5 min; for digoxin, the maximum rate was 0.12 of the minimal lethal dose and the latent period was 7 min. It thus cannot be inferred that ouabain has a shorter latent period than digoxin. Now, for a compound that had no latent period, the "irregularity dose" (the dose at which irregularities start and which also produces maximal therapeutic effect) would be a smaller fraction of the lethal dose than in those compounds that show a latent period, since with the conventional glycosides the "irregularity dose" includes the "minimal therapeutic dose." Whether or not drugs with no latent periods are considered to be "safer," is an academic question, but such drugs do exist. Vick, Kahn & Acheson (69) studied the effect of dihydroouabain on the failing dog heart-lung preparation and found that the minimal therapeutic dose varied from less than one per cent to 3.5 per cent of the lethal dose, which is tantamount to saying that a latent period does not exist. It would be highly desirable to study the effect of the aglycones on failing heart-lung preparations and see whether these compounds, which presumably show no latent period, would also exhibit a lower ratio irregularity: lethal dose than their corresponding glycosides.

The behavior of the dihydro compounds as compared with the parent drugs may be understood if it is assumed that the conventional glycosides combine in the cardiac cell with three sites that show decreasing avidity for the glycosides. Saturation of the first site marks the end of the latent period and the beginning of therapeutic action, while saturation of the second and third sites ushers the onset of irregularities and ventricular fibrillation respectively. Receptor one is not associated with pharmacological activity, and it can be assumed that the dihydro-compounds which show no latent period do not react with this receptor. This interpretation is not in harmony with the observations of Farah and Maresh (63) that the "irregularity" dose of a digitalis glycoside bears a constant proportion to the lethal dose irrespective of the rate of administration. A better explanation to fit these findings is to assume that in the course of "therapeutic" action by a cardiac glycoside a new digitalis product is slowly formed which begins to produce cardiac irregularities when a threshold concentration is reached.

Taeschler & Cerletti (70) working with isolated, spontaneously beating guinea pig auricles obtained results similar to those of Vick *et al.* (69) and in line with the above discussion. Dihydroouabain showed no latent period regardless of the concentration, whereas ouabain exhibited a latent period which was inversely proportional to the concentration. As a result, the ratio of the minimal effective concentration showing a positive inotropic effect to the toxic concentration eliciting arrhythmia and contracture was greater for ouabain than for dihydroouabain.

The clinical observation of Lown *et al.* (71), that in the presence of

reserpine, digitalis toxicity appears in patients with atrial fibrillation before therapeutic activity is well under way, deserves further experimentation on animals and heart-lung preparation. On the other hand, Cairoli *et al.* (72) found that reserpine pretreatment significantly increases the dose of acetyl-strophanthidin necessary to produce arrhythmia in dogs with experimental heart-block.

Repke complains that the metabolism of cardiotonic steroids has not received the same attention as that of other steroids. In a review (41) containing much of his own work, he concludes that biotransformations of the cardiac glycosides are not related to their mechanisms of action. These biotransformations include: (*a*) reduction of the lactone ring, dihydrodigitoxin having been detected in the liver in minute quantities after digitoxin administration. (*b*) hydroxylation at C_{12} as for example, the formation of digoxin from digitoxin. The purpose of this reaction is to increase the polarity of the steroid and facilitate its excretion. (*c*) cleavage of desoxysugar residues with formation of aglycones. The latter cannot be detected unchanged as they are rapidly metabolized. Ten minutes after injection of equimolar doses of digitoxin and digitoxigenin, most of the digitoxin could be recovered in the liver whereas only traces of digitoxigenin could be found, the latter being transformed to the 3-epi isomer; and (*d*) epimerization at C-3. The mechanism is the same as for other steroids and apparently involves the same enzymes. The first step is an oxidation to the ketone followed by reduction of the latter yielding the α-derivative. In this process the aglycone acts like a transhydrogenase coenzyme. However, this activity is not related to biological potency, since compounds that are biologically inactive show high activity as coenzymes and vice versa.

All these metabolic reactions appear to take place in the liver and not in the heart. Although the aglycone is the active part of the glycoside there is every reason to believe that the glycoside is active as such. The sugars influence the solubility and binding power, and according to Repke (41) "protect" the aglycone from rapid inactivation.

In the light of all the information regarding the metabolism of cardiac steroids it is difficult to subscribe to the view that the aglycone is less potent than its glycoside, particularly when intact animals are used. Work on heart-lung preparations would be more instructive but even here the sugars, by affecting solubility, permeability and binding power would render a comparison between glycoside and aglycone difficult.

ADRENERGIC DRUGS; ADRENERGIC BLOCKADE

Catecholamines

A new approach to the study of the interaction between sympathomimetic amines and receptor sites has been made by Belleau (73, 74) who believes that this interaction cannot be likened to an enzyme-substrate reaction in which the agonist is the substrate. The triggering of the response is figured to

be due to an electrostatic interaction between the ammonium ion of the sympathomimetic amine and a nucleophilic site at the receptor which is postulated to be a phosphate anion. The search for antagonists with groups isosteric with the ammonium ion, showed that neither oxygen nor sulfur nor phosphorus would do since at physiological pH they do not add a proton to yield oxonium, sulfonium or phosphonium ions. However, an isosteric antagonist was found in dibenamine which contains a carbonium ion, and a plausible explanation for the blocking action of dibenamine was offered which involves an esterification reaction of the anionic site on the receptor which normally binds the agonist. Similarly, an explanation of the action of epinephrine in catalyzing the enzymatic formation of cyclic adenosine monophosphate (AMP) from ATP is offered which includes a metal linking the catechol group of the amine with the two terminal phosphate residues of ATP. The interpretations of Belleau may not be complete but they point the way for further progress. There is no reason to believe that the reaction between agonist and receptor site is different from any other reaction in plain organic chemistry, and the ion-pairing mechanism suggested by Belleau may well explain the initiation of the triggering process.

The precise mechanism of action of the catechol amines on cardiac and smooth muscles remains a mystery. Early in the embryological development of the heart all cardiac cells possess the properties of automaticity, conduction and contractility. Later on, these functions are taken over by the pacemaker, the bundle of His and the musculature respectively. Now the catecholamines enhance all three functions. It is reasonable to assume that these amines act on some basic or primitive process which is common to these structures. The role of catechol amines in activating phosphorylase has been established, but it is very doubtful whether the almost instantaneous actions of catechol amines on the heart can be explained on the basis of phosphorylase activation. Steps which precede glycogen breakdown in the contraction process should be investigated. Stam & Honig (75) found that a relaxing substance prepared from cardiac muscle granules caused significant inhibition of cardiac myofibrillar ATPase activity. This inhibition was counteracted by E, NE and isoproterenol in concentrations between 10^{-8} and 10^{-6} M, isoproterenol being more effective than the first two. The significance of this finding remains to be determined.

Mellander (1) studied the effects of catechol amines and sympathetic stimulation on consecutive vascular sections of a muscle-skin preparation. In this preparation, venous outflow is determined mainly by the resistance vessels while the capacitance vessels determine tissue volume. Sympathetic stimulation and norepinephrine (NE) decrease outflow by constricting resistance vessels and decrease tissue volume by constricting capacitance vessels as well as by producing an intracapillary influx of tissue fluid. The latter is due to decrease in hydrostatic capillary pressure as a result of greater constriction of precapillary than of postcapillary resistance vessels. The frequency-response curve for capacitance vessels is placed well to the left of that

for resistance vessels. Thus low or moderate rates of sympathetic discharge constrict the capacitance vessels and also increase heart rate [Folkow, Löfving & Mellander (76)] and myocardial contractile force [Sarnoff (77)] without constricting resistance vessels. The implications of these findings as seen by Mellander are as follows: slight increases in reflex sympathetic activity, as occurs during hemorrhage, by simultaneously mobilizing blood from capacitance vessels and increasing heart rate and contractile force, increase venous return which helps maintain a normal CO before the resistance to flow is changed. Only when mobilization of intravascular blood is not sufficient to maintain cardiovascular equilibrium is there mobilization of extravascular fluid. This occurs as a result of increased sympathetic discharge and constriction of resistance vessels as described above. Folkow *et al.* (78) using the same muscle-skin preparation found that the constrictor action of angiotensin on the resistance vessels is greater than that of NE. On the other hand, NE constricts the capacitance vessels far more than angiotensin. Folkow (79), again using the same skin-muscle preparation injected isoproterenol, acetylcholine and E intra-arterially in doses that produce equal dilator effects on resistance vessels. It was found that while isoproterenol and acetylcholine dilate the capacitance vessels, E constricts them. Sharpey-Schafer (80) described a method for measuring changes in tone of forearm vessels. He found that while E and NE were powerful venoconstrictors, isoproterenol dilated the venous system. These findings are not in harmony with the conclusions of Eckstein & Hamilton (81) that isoproterenol is a venoconstrictor.

The "calorigenic" action of sympathomimetic amines on the heart—also known as the "oxygen-wasting" effect—is the increase in oxygen consumption which cannot be accounted for by the rise in heart-rate and increase in work performed. The inotropic effect of sympathomimetic amines has not been put into clinical use, for fear that any benefits derived therefrom would be more than outweighed by the "oxygen-wasting effect," and when NE was first used in cardiogenic shock it was with the idea that, unlike E, it acted mainly peripherally. E and NE, however, have similar chronotropic and intropic actions [Krayer & Van Maanen, (82); Goldberg *et al.* (83); Rushmer & West (84); Goldberg *et al.* (85)]. Fawaz & Tutunji (86) utilizing dog heart-lung preparations found that NE was not only as effective as E in its chronotropic, inotropic and coronary dilator effects but was even more potent regarding its calorigenic action. It was also found, that small doses of E which had a definite inotropic action caused no measurable increase in cardiac oxygen consumption. These authors raised the question as to whether the efficacy of NE in some cases of cardiogenic shock might not be due to its cardiac (inotropic) action. The doses of NE used clinically may increase the force of myocardial contraction without raising cardiac oxygen consumption. The same question had been asked previously by Sarnoff *et al.* (87), who, on the basis of experiments with metaraminol, criticized the view that the drug of choice in the treatment of cardiogenic shock should act solely on the peripheral vascular bed. The shock that often accompanies extensive myocardial

infarction may have a complex etiology involving cardiac as well as peripheral factors which can combine to produce a vicious circle [Selzer & Rytand (88)]. However, recent years have witnessed a more extensive use of cardiotonic drugs, including digitalis, in the treatment of cardiogenic shock. It must be remembered, however, that in cases of extensive infarction the remaining healthy tissue cannot be expected to carry a normal work load. Attempts to extract from it the last "ounce" of energy by using cardiotonic drugs may hasten the onset of failure. Drug treatment should be supplemented by measures that decrease the general body metabolism as by antithyroid drugs and, in urgent cases, even by using moderate hypothermia.

The element of cardiac failure in hypovolemic shock is also receiving more attention [Walton *et al.* (89)]. Greenfield *et al.* (90) measured myocardial contractile force during acute hemorrhage and concluded that myocardial depression occurs at an early stage in acute bleeding and probably results from a critical diminution in coronary perfusion pressure. Crowell & Guyton (91) studied the changes that take place during the transition period between reversible and irreversible shock and obtained results that suggested to them that irreversible hemorrhagic shock is due to acute cardiac failure.

The use of sympathomimetic amines in the treatment of congestive heart failure is a field worthy of exploration. There is no reason to believe that the inotropic actions of digitalis and sympathomimetic amines have the same mechanism, and it is conceivable that digitalis-resistant patients may respond to sympathomimetic amines. Much basic experimental work, however, is needed before proceeding with clinical trials. In the first place, a thorough quantitative study should be made of the calorigenic and inotropic effects of the various amines. The inotropic effect should be studied not only by the strain-guage arch technique but also by Krayer's competence tests [Krayer (92)] using failing hearts. Frye, Kahler & Braunwald (93) tried mephentermine on four patients with congestive failure. Two patients failed to improve and two patients got worse. These results should not be discouraging. It is possible that mephentermine was not the right drug to choose. Experiments on the heart-lung preparation [Fawaz (94)] showed that its calorigenic and chronotropic effects are much greater than was previously seen in the intact animal. The extreme tachyphylaxis shown by mephentermine in animal experiments might also have been responsible for its failure in the clinical tests. Perhaps a combination of isoproterenol with a peripherally-acting drug like phenylephrine or methoxamine to counteract its vasodilating effect would do. Isoproterenol is the most potent cardiotonic sympathomimetic amine but its calorigenic action on the heart as well as its effect on general basal metabolism remain to be explored.

A review of the physiology, pharmacology, and clinical use of sympathomimetic amines has been written by Eckstein & Abboud (95).

The Norepinephrine "Stores"

Organs with sympathetic innervation like heart, spleen, and blood vessels contain varying amounts of NE which can be extracted and estimated

chemically or biologically. Treatment with reserpine for about 24 hr may reduce the NE content to almost zero. Such preparations no longer respond to non-catechol amines like tyramine unless the "stores" are first "repleted" by treatment with NE [Burn & Rand (96)]. Two problems connected with these stores remain unsolved: their location and whether there is one or more stores. Anatomists have given us little aid and we are dependent on indirect evidence. Brown (97) believes that NE released by nerve stimulation may be stored at the receptor sites. Brown, Davies & Ferry (98) reported that treatment with β-haloalkylamines increases the amount of NE liberated into the blood stream after stimulation of the splenic nerve and that a period of neuronal "rest" produced by decentralization increases the amount of NE liberated by stimulation as well as that taken up by these receptor sites. Paton (99) suggested that the uptake of released NE may involve the nerve endings themselves. NE, being the dominant intracellular cation in the nerve endings, as in the adrenal medulla, is released when the membrane potential is reduced and "sucked back" into the store when the events of excitation are over. This suggestion which has been described as "heretical" by Brown (99) helps explain certain findings as will be seen later.

There is much to support von Euler's original suggestion that the main NE stores are located inside the nerve terminals, or in the words of Furchgott & Kirpekar (100) structures neurotropically dependent on them. Denervation depletes the stores and so does reserpinization, but there is an important difference between both procedures. "Repletion" of prereserpinized preparations with NE is possible while that of denervated organs is not. Burn & Rand (101) showed that tyramine, a NE releaser, is unable to contract the denervated iris or diminish blood flow of the denervated foreleg even after pretreatment with NE, whereas in prereserpinized preparations NE treatment restored the action of tyramine. Furchgott & Kirpekar (100) obtained similar results with isolated atria when the NE releasing agent was bretylium. This findings suggest that the stores are found in the nerve endings which are destroyed by denervation but not by reserpinization. Hertting *et al.* (102) showed that sympathetically denervated organs do not take up tritiated NE from the blood. Hertting & Axelrod (103) found that tritiated NE which had been taken up from the circulation by the spleen, could be released into the blood by stimulating the splenic nerve according to the technique of Brown *et al.* (98). After each stimulation there was a concomitant release of the tritiated metabolic product normetanephrine, the amount of which was directly related to the amount of tritiated NE released. Hertting & Axelrod (103) thus concluded that NE liberated by stimulation reacts with the receptor and a part is discharged into the blood stream, a part o-methylated and a part bound again at sympathetic nerve endings.

Axelrod, Hertting & Patrick (104) observed that the spontaneous release of H^3-NE from the heart is rapid at first and becomes progressively slower. This suggested to the authors that H^3-NE is taken up by a store with rapid

turnover rate and then gradually enters stores with lower turnover. The problem of whether there is one or more stores has been tackled in a more direct manner by Potter, Axelrod & Kopin (105). Rats were given 10 μc of H^3-NE per 100 gm. In Experiment I the animals were given tyramine 30 min after the H^3-NE injection in order to release NE; in Experiment II tyramine was given 24 hr after H^3-NE. In both experiments the animals were sacrificed 30 min after the tyramine injection and the hearts assayed for H^3-NE and endogenous catecholamines. Control rats received H^3-NE but no tyramine. It was found that in Experiment I the control rats had 419±17 mμc H^3-NE per gm heart and a specific activity of 438±8; whereas, the tyramine-treated rats had 243±10 mμc and a specific activity of 468±8. The corresponding figures for Experiment II are: control rats 19.5±0.9, specific activity 22.1 ±1.0; tyramine-treated rats 16.2±1.6, specific activity 29.9±2.9. Since after 48 hr, tyramine treatment increased the specific activity of heart NE as compared to the controls, it was concluded that the catechol amines released must have originated from the stores that are turning over more rapidly and have a smaller percentage of the remaining H^3-NE. In view of the importance of the problem and the fact that such a small percentage of radioactivity remains after 48 hr it would be highly desirable to perform a time-study to know what happens between 30 min and 48 hr.

Paasonen & Krayer (106) observed positive chronotropic and inotropic actions on the dog heart-lung preparation after the acute administration of reserpine. This effect was attributed to a release of NE since there was a substantial fall in the NE content of the heart after reserpine and, furthermore, this action could not be observed in preparations from dogs pretreated with reserpine. Gaffney (107) using the general procedure of Paasonen & Krayer (106) made similar observations with guanethidine, except that the maximum chronotropic effect produced by the latter was much greater than that of reserpine and was reached in a much shorter period of time. The same results with regard to the chronotropic actions of guanethidine in the heart-lung preparation were obtained by Krayer, Alper & Paasonen (108), and by Simaan & Fawaz (109). Krayer *et al.* (108) reported no great difference between the NE-depleting effects of maximum doses of reserpine and guanethidine after 2–3 hr. They attributed the difference between the chronotropic effects to a potentiation by guanethidine of the action of NE, whereas reserpine, if anything, has an antiaccelerator, veratramine-type of effect. From this one could conclude that reserpine and guanethidine act by a similar mechanism and perhaps on the same NE-store. However, the results of Simaan & Fawaz (109) do not lend themselves to such a simple explanation. These authors found that if guanethidine was administered to a heart-lung preparation after the acute effects of reserpine had subsided, the maximum heart rate reached was not different from that observed if guanethidine had been given alone. The reduction in the NE content of the right atrium was greater after combined treatment than after either reserpine or guanethidine alone, but the magnitude of the reduction was less than that reported by

Krayer *et al.* (108). Simaan & Fawaz (109) using large numbers of animals, observed considerable variation in the NE content of the right atria of normal dogs (from 0.4 μg/gm to 1.2), and since one animal cannot serve as its control it is obviously difficult to determine percentage reductions in NE content with any degree of accuracy. The same authors also observed that repletion of a heart-lung preparation from reserpine-pretreated animals (which always contained no measurable amounts of NE) by the infusion of 500 μg NE in the course of 30 min results in an uptake of NE equal to about 10 per cent of that contained in normal atria. Nevertheless, treatment of this "repleted" preparation with guanethidine raises the heart-rate almost to the same extent as in preparations from normal animals. It is tempting to speculate that two stores of NE exist: one situated adjacent to the effector cells (e.g., the store filled by the "repletion" experiment), and another store where released NE would have to cover a longer distance, perhaps through the blood stream, to reach its target.

In spite of all these indications regarding the existence of more than one store it will be assumed in the forthcoming discussion that there is one store of NE which is distinct from the receptor site. Sympathetic nerve stimulation would release NE from the store to act on the receptor site.

Drugs Which Release NE from "Stores"

Many compounds with varied chemical structures have been reported to exhibit sympathomimetic actions dependent on the release of NE from tissue stores. The evidence offered for the NE liberation includes: depletion of NE in tissues; detection of NE in blood or perfusate; lack of action on reserpinized or denervated preparations; specific inhibition of action, as by dichlorisoproterenol in heart tissue. These compounds include, in addition to reserpine:

(*a*) *Guanethidine:* [Cass, Kuntzman & Brodie (110); Butterfield & Richardson (111); Gaffney (107); Krayer, Alper & Paasonen (108); Philippu & Schümann (112); Bogaert, De Schaepdryver & De Vleeschhouwer (113)]. (*b*) *Bretylium:* [Green (114); Siegel & Gilmore (115); Philippu & Schümann (112); Furchgott & Kirpekar (100)]. (*c*) *Noncatechol amines such as tyramine, amphetamine and phenylethylamine:* [Burn & Rand (96); Lindmar & Muscholl (116); Axelrod, Hertting & Potter (117); Philippu & Schümann (112); Chidsey, Harrison & Braunwald (118); Strömblad (119); von Euler & Lishajko (120)]. (*d*) *Acetyl choline, Carbachol, nicotine and ganglionic stimulants including DMPP (1,1-dimethyl-4-phenyl piperazinium)*: [Burn & Rand (121); Richardson & Woods (122); Lee *et al.* (123); de la Mata *et al.* (124); Lindmar & Muscholl (116); Philippu & Schümann (112); Lindmar (125)]. (*e*) *β-Haloalkylamines such as Phenoxybenzamine:* [Furchgott & Kirpekar (100); Benfey & Varma (126); Farrant, Harvey & Pennefather (127); Axelrod, Hertting & Potter (117)]. (*f*) *α-Methyl dopa:* [Sjoerdsma & Udenfriend (128)]. (*g*) *Pyridine derivatives:* [Schoepke & Shideman (129)]. (*h*)

Monoamineoxidase inhibitors: [Lee, Shin & Shideman (130)]. (*i*) *Thiopental:* Burn & Hobbs (131)]. (*j*) *Succinylcholine:* [Conway (132)]. (*k*) *Aldosterone:* [Tanz (133)].

Non-Catechol Amines and Their Mechanism of Action

Fleckenstein *et al.* (134, 135, 136) divided sympathomimetic amines into three groups depending on whether their actions were potentiated, inhibited or unaffected by cocainization or chronic denervation. Group I included the "direct acting" catechol amines and phenylephrine; Group II the "indirect acting" non-catechol amines, such as tyramine and amphetamine. Group III included the amines with mixed actions and differing from Group II in having an hydroxyl group on the β-carbon atom e.g., ephedrine. The knowledge that chronic reserpine treatment like denervation depletes tissue catechol amine stores stimulated further experimentation. Liebman (137) studied dose-response curves using heart-lung preparations from normal and reserpine-pretreated dogs. Reserpinization did not affect the dose-response curve of the first group (synephrine), while it reduced the curve maximum of the second group (tyramine and amphetamine), and caused a shift to the right with some depression of the maximum in the third group (phenylpropanolamine). Fleming & Schmidt (138) studied the sensitivity of the isolated rabbit ileum to sympathomimetic amines following reserpine pretreatment. Here the inhibitory actions of the amines were studied, yet the results upheld the original classification of Fleckenstein. Later in the review, this classification will be discussed in the light of recent knowledge regarding the mechanism of sensitization and the effect of drugs on the uptake of NE by and its release from the stores. At present, the mechanism of action of sympathomimetic amines in the second category of Fleckenstein's classification, such as tyramine, amphetamine and mephentermine will be discussed. There is at present no doubt that non-catechol amines like tyramine are able to release NE from tissue stores, but as has been seen, this is a property shared by many other compounds. Reserpine, guanethidine, and nicotine are structurally quite different from the sympathomimetic amines and their sympathomimetic properties may be explained solely on the basis of the amount of NE they release. Not so with the non-catechol amines, and here the question arises as to whether they do not have an action of their own, i.e., act directly on the receptor sites with the released NE serving merely as a catalyst. If the non-catechol amines require catecholamines for their action and if the NE stores are geographically separated from the receptor sites it is difficult to visualize how NE inside the store can catalyse a reaction at such a distance. Release of a small amount of NE from the store would be a prerequisite for such a reaction. This problem can be settled only on the basis of quantitative studies.

Kuschinsky *et al.* (139) reported that pretreatment with reserpine does not alter quantitatively the reaction of isolated rat atria to E or NE, but it

abolishes the reaction to non-catechol amines like tyramine, phenylethylamine, and methamphetamine. The action of non-catecholamines in the reserpinized preparations could be restored by subthreshold doses of E and especially NE. This may be taken as evidence that the catechol amines acted as catalysts. Fawaz (94) used modified dog heart-lung preparations to study the effects of E, mephentermine, and a combination of both drugs on heart rate, oxygen consumption, and coronary flow. The dose of E chosen had less effect on heart rate and oxygen consumption than that of mephentermine. In the preparations from reserpine pretreated dogs the action of mephentermine was very small, but addition of E on top of the mephentermine caused a rise in heart rate, oxygen consumption, and coronary flow which almost duplicated the additive effect of both amines on the preparation not pretreated with reserpine. The action of E alone on reserpinized preparations was not different from that on the normal ones except for a slightly exaggerated chronotropic effect. It was concluded that E added after mephentermine in the reserpinized preparation acted on its own and in addition restored the action of mephentermine. Clearly, we are dealing here with a catalytic action of E and not with a situation where mephentermine is releasing catechol amines from stores, since the latter are depleted. Burn & Rand (101) observed that an infusion of NE often restored the action of tyramine in a reserpinized animal to a greater extent than it restored the effect of sympathetic stimulation. This again is in harmony with the concept that tyramine acted on its own and needed only small amounts of NE for its activation, whereas stimulation depended for its activity solely on the amount of NE liberated from the repleted stores. It is known that "repletion" of reserpinized preparations with NE leads to deposition of only small amounts of NE in the stores. Similar results were obtained by McCubbin, Kaneko & Page (140) who used dogs pretreated with guanethidine. Here NE infusions restored the pressor actions of tyramine but not the pressor effect of carotid occlusion or electrical stimulation of sympathetic nerves. Lindmar & Muscholl (116) working with Langendorff hearts of rabbits observed that tyramine liberated 16 μg of NE per min and increased the heart rate 61 per cent, while the ganglionic stimulant DMPP (dimethyl phenylpiperazinium) liberated 107 μg of NE to increase the heart rate 67 per cent. Again these results may be interpreted to mean that DMPP acted through the NE released while tyramine acted mainly on its own. Another experiment that can be interpreted in the same way is that of Lindmar (125) who found that the sympathomimetic action of tyramine (but not that of nicotine) on the auricles of guinea pigs pretreated with reserpine could be restored by the presence of small concentrations of NE in the bath fluid. Nasmyth (141) in an investigation of the action of tyramine and its interrelationship with the effects of other sympathomimetic amines, concludes that his results are inconsistent with the view that the sympathomimetic effects of tyramine are produced entirely by the release of catechol amines. So while more quantitative experiments are needed, the above results are in harmony with the

concept that the indirect-acting sympathomimetic amines require for their action small amounts of catechol amines as catalysts.

Drugs That Affect the Uptake and Release of Catecholamines by the Stores

The potentiating effect of denervation and cocainization upon the activity of exogenous catechol amines has been known for a long time. Reserpine treatment has two properties in common with denervation: viz., depleting the NE stores and increasing the activity of injected catechol amines. This supersensitivity after reserpinization is usually not as pronounced as that observed after denervation and furthermore does not affect all the actions of the catechol amines. For instance, Moore & Moran (142) found that reserpine treatment augments the pressor and chronotropic, but not the inotropic effects of E and NE, while Fawaz (94) observed no potentiation of the calorigenic action of E on the heart after reserpine pretreatment. The view expressed by Burn & Rand (143) that the sensitivity of an organ is inversely proportional to its NE content, was not supported by the data of Fleming & Trendelenburg (144). These authors made quantitative studies on the hypersensitivity to NE following reserpinization, and found no direct relation between depletion of NE stores and supersensitivity of the nictitating membrane. Kirpekar, Cervoni & Furchgott (145) found that decentralization of the nictitating membrane did not decrease its NE content yet increased its sensitivity to NE even more than reserpinization (but less than denervation).

The view that has been gaining ground recently is that the so-called supersensitivity to catechol amines following denervation or cocainization is not due to an increased reactivity of the receptor sites but to an increased concentration of the catechol amines at these sites. Injected NE or NE liberated from the stores is disposed of in two ways. (*a*) inactivation by catechol-o-methyl transferase or monoaminoxidase depending on the organ and the species [Shideman & Goldberg (146)]; (*b*) uptake by the stores. It appears that the first method of disposal—unlike the situation with acetylcholine—is not as important as the second. Crout (147) reported no marked potentiation of the cardiovascular action of injected NE after simultaneous inhibition of catechol-o-methyl transferase and monoamine oxidase. Decreased uptake by the stores would make more NE available at the receptor sites and could explain supersensitivity after denervation (disappearance of the stores) or cocainization (blockage of NE uptake by stores). This explanation has been suggested by several authors: Whitby, Hertting & Axelrod (148); Hertting, Axelrod & Whitby (149); Muscholl (150, 151); Dengler, Spiegel & Titus (152); Kirpekar, Cervoni & Furchgott (145); Kukovetz & Lembeck (153); [see also Koelle (154)]. Whether sensitization can be explained solely on this basis remains to be seen. Supersensitivity after decentralization still awaits an interpretation. No experiments have been performed yet to indicate whether in non-reserpinized preparations decentralization inhibits NE uptake by the stores. In the light of this concept,

Fleckenstein's classification of the sympathomimetic amines would be interpreted as follows: Group I compounds, including the synthetic catechol amines and phenylephrine are taken up by the stores in order to be stored. This postulate can be tested by the use of radioactive phenylephrine according to Axelrod's techniques; Group II compounds like tyramine enter the stores less readily, perhaps by diffusion, and release an equivalent amount of NE to activate the non-catechol amines at the receptor sites; Group III compounds like ephedrine, also enter the stores with difficulty. The NE which they release need not contribute to their activity since they require no activation.

There is a long list of compounds that inhibit the uptake of NE by the stores. It is interesting that most of the compounds that have been listed above as NE releasers also inhibit the uptake of NE and potentiate its action. There are also compounds that inhibit the release of NE. All such compounds will be discussed, particularly as they bear upon the mechanism of action of such antihypertensive drugs as bretylium, reserpine, guanethidine, and α-methyl dopa.

Dengler *et al.* (152) studied the effect of various drugs upon the active uptake of radioactive NE by slices of brain cortex, spleen, and heart. The drugs that inhibit NE uptake include reserpine, chlorpromazine, ergotamine, cocaine, guanethidine, bretylium (guanethidine was three times as active as bretylium) dichlorisoproterenol, non-catechol amines like tyramine, ephedrine, and amphetamine. Cocaine was found to be the most effective of the group. Hertting *et al.* (149), Axelrod, Hertting & Potter (117) and Hertting *et al.* (155) used radioactive NE and whole animals. They include in their list of drugs that inhibit NE uptake: dichlorisoproterenol, cocaine, chlorpromazine, imipramine, tyramine, amphetamine, reserpine, guanethidine, bretylium, and phenoxybenzamine. Bisson & Muscholl (156) reported an inhibitory action of guanethidine on the uptake of circulating NE by the heart.

It is obvious that drugs which block the entry of NE into the stores vary in their effectiveness, but in addition, some drugs in the same group appear to inhibit each other's entry. For instance, Kroneberg & Schümann (157) found that the sympathomimetic action of a large dose of guanethidine on the isolated guinea pig atrium (due to NE release) was abolished by cocaine. This finding may be interpreted to mean that cocaine prevented the entry of guanethidine. Guanethidine in turn blocks the action of tyramine on the isolated atrium in an acute experiment at a time when the NE content was diminished by only 30 per cent. The action of tyramine was restored by washing out the guanethidine. Here again guanethidine may have blocked the entry of tryamine. The same authors found that 10 min after the intravenous injection of guanethidine to rats, tyramine action was inhibited. At this time the catechol amine content of the heart, brain, and adrenals was unchanged. Bartlet (158) found that the pressor action of small doses of

guanethidine is inhibited by previous treatment with larger doses of the same drug. The author suggests that guanethidine may inhibit its own action as a catechol amine releaser. Lindmar & Muscholl (116) using Langendorff hearts observed that NE release by tyramine was inhibited by cocaine which itself does not release NE, and by guanethidine at a time when the NE stores are not yet depleted. This can be interpreted as blockage of tyramine entry by cocaine and guanethidine. A similar interpretation can be given to the findings of Benfey & Greeff (159) that phenoxybenzamine and guanethidine inhibited tyramine but potentiated NE action on the isolated guinea pig atrium. Bejrablaya, Burn & Walker (160) also found that cocaine added to the heart-lung preparation potentiated NE and inhibited tyramine action. They interpreted this as a blockage by cocaine of NE release by tyramine. The alternative explanation that cocaine prevents the entry of tyramine in order to release NE is just as plausible since cocaine has not been reported to block the release of NE. Philippu & Schumann (112) studied the effects of bretylium and guanethidine on the liberation of catechol amines from perfused adrenals. Guanethidine inhibited the catechol amine release by carbachol, nicotine, and phenylethyl amine. Bretylium was less effective and did not inhibit the effect of phenylethyl amine. Guanethidine and bretylium themselves released NE but only when applied in large doses. The same authors found that cocaine and tetracaine also abolished the NE releasing effects of carbachol, nicotine and phenylethyl amine, thus confirming earlier observations of Eichholtz & Roesch (161) on intact cats. The sympathomimetic effects of nicotine and tyramine on the isolated atrium are also inhibited by guanethidine and cocaine. The finding by de la Mata *et al.* (124) that bretylium blocks the sympathomimetic action of DMPP on the heart-lung preparation may also be explained on the same basis. Cocaine has been described by Philippu & Schümann (112) as "membrandichtend." Guanethidine also appears to have this property and so does bretylium, but to a lesser extent.

The actions of guanethidine and bretylium are quite complex and depend on the dose, period of administration, and the kind of preparation on which they are tested. Both compounds inhibit the effects of post-ganglionic sympathetic stimulation [Boura & Green (162); Maxwell *et al.* (163)], and both block the release of radioactive NE when sympathetic nerves are stimulated [Hertting, Axelrod & Patrick (155)]. Day & Rand (164) found that when guanethidine and bretylium were added to the bath they abolished the inhibitory response of segments of small intestine to stimulation of sympathetic nerves. In isolated atria of cats guanethidine also blocked accelerator response to sympathetic nerve stimulation. In the experiments on intestinal segments, the effect of guanethidine can be removed by wasing the bath, indicating that guanethidine did not act by depleting catechol amines from the intestine. The authors quote unpublished experiments of Cass where guanethidine added to an organ bath abolished adrenergic response to sympa-

thetic stimulation without altering the NE content of the segment. Addition of larger amounts of guanethidine to the bath potentiated the inhibitory response to E or NE. In experiments with intestinal segments taken from rabbits pretreated with guanethidine for four days the inhibitor response to sympathetic stimulation was not different from that of normal controls indicating that the intestinal catechol amines had not been depleted. Bretylium has been included above among the NE-releasers. However, it is not as effective a NE-releaser as reserpine or guanethidine either in acute or chronic experiments. Bretylium was reported by Hertting *et al.* (155) to block spontaneous and reserpine-induced release of radioactive NE like some monoamine oxidase inhibitors, while guanethidine caused release and partially antagonized the reserpine-induced release.

To summarize: Both guanethidine and bretylium inhibit the uptake of NE by the stores, guanethidine being more effective. Both can release NE, guanethidine again being more effective, and both can block the NE release due to stimulation. The observation that the action of tyramine is inhibited by guanethidine and not by bretylium may be explained as follows: guanethidine blocks the entry of tyramine into the stores so the latter cannot release NE to catalyse its own action. Bretylium blocks the entry of tyramine to a lesser extent than guanethidine, and although it inhibits the release of NE, very small quantities of the latter are needed to catalyse tyramine action. This explanation is not incompatible with the fact that both compounds inhibit the effects of stimulation since here, in contrast to tyramine action, relatively large amounts of NE would have to be liberated from the stores to be effective at the receptor sites. The findings that guanethidine releases NE on the one hand, and on the other prevents its release after stimulation [Hertting *et al.* (155)] may appear contradictory. However, we know so little about the mechanism of uptake and release, and furthermore, release by stimulation may not be the same as release by guanethidine when the nerve is at rest. We have heretofore assumed that there is an NE store which is located within the nerve endings and which is released by nerve impulses. If more than one store is found to exist then the possibilities of speculative manoeuvering are greatly increased.

Hemodynamic Effects of Guanethidine and Bretylium

These two drugs will be considered together since they appear to differ mainly quantitatively, although it has been shown that guanethidine has a direct negative inotropic action and bretylium positive inotropic and negative chronotropic actions [Gaffney (107); Krayer, Alper & Paasonen (108)]. The response of the intact animal to intravenous administration of these drugs is variable [Aviado & Dil (165); Boura & Green (162); Maxwell *et al.* (166); McCubbin *et al.* (140); Gaffney, Braunwald & Cooper (167)]; but, in general, there is first a sudden drop in blood pressure which lasts a few minutes, then a rise above normal lasting up to two hr and finally a more

prolonged fall. There is reason to believe that the initial and the final depressor effects are not due to different mechanisms except that the more prolonged effect, especially if treatment is continued, is contributed to by depletion of NE stores as in the case of reserpine. The pressor effect, however, appears to be dependent on intact NE stores particularly in the heart since pretreatment with reserpine or guanethidine [McCubbin *et al.* (140)] or cardiac denervation [Gaffney *et al.* (167)] markedly reduces it. Gaffney *et al.* (167) used animals with denervated hearts to study the contribution of the heart to this pressor effect. They found that in the normal animal guanethidine and bretylium increase the myocardial contractile force, heart rate, CO and blood pressure. These effects are greatly reduced after cardiac denervation except for an unchanged inotropic effect of bretylium. The authors conclude that the acute circulatory effects of guanethidine, and to a much lesser extent those of bretylium, are largely due to sudden release of myocardial catechol amines. They did not analyse the mechanism of the circulatory effects of guanethidine save to state that since the relative increase in CO exceeded the relative rise in arterial pressure, the pressor effect was probably due to increased CO rather than any vasoconstriction.

A closer look at the data of Gaffney *et al.* (167) shows that after guanethidine there was a considerable rise in CO, up to 250 per cent. A substantial increase in output, up to 100 per cent or more was observed even when the arterial pressure was still below normal or just returned to normal. Abboud, Eckstein & Pereda (168) also observed an increased CO with a fall in systemic arterial pressure during the first minute after intra-venous administration of guanethidine. The initial fall in blood pressure must be due to a primary dilatation of resistance vessels with a resulting increase in venous return and hence CO. This cannot be due to a peripheral effect of released NE which is known to constrict arterioles, but to the direct action of guanethidine. The situation here is not unlike that which obtains in exercise, where dilatation of the resistance vessels increases blood flow and venous return. The heart can cope with this increased venous return by virtue of catechol amine release, as by sympathetic stimulation, which increases rate and contractile force [Rushmer (169); Sarnoff *et al.* (170)]. Guanethidine dilates peripheral arterioles thus increasing venous return and releases catechol amines to allow the heart to cope with it. In this scheme, the peripheral action of guanethidine is of prime importance and no allowance was made for any possible suction effect by the heart. The latter, if it did exist, would lessen the importance of the vasodilating action of guanethidine as a contributor to the increased CO. The clinical observation that exercise can lead to hypotension in patients chronically treated with guanethidine may be explained as follows: in normal individuals, the massive arteriolar dilatation after exercise due to activation of sympathetic cholinergic vasodilatory fibres [Folkow, Mellander & Öberg (171)] and local production of metabolites does not lead to a fall in arterial pressure because it is counteracted by an increased CO.

In patients treated with oral guanethidine, depletion of cardiac NE stores is expected to occur and the heart cannot pump enough blood to counteract the depressor effect of dilatation of resistance vessels. There is thus no need to ascribe the hypotension in these patients to a direct deleterious effect of guanethidine on the myocardium. The observation by McCubbin *et al.* (140) that pretreatment with phentolamine inhibits the acute pressor effects of guanethidine requires explanation. It has been stated above that this pressor effect was due not to vasoconstriction but to increased CO. Phentolamine which inhibits the action of catechol amines on α-receptors according to Ahlquist's terminology is not expected to inhibit NE action on the heart [Moran & Perkins (172)]. One possible explanation for the inhibitory action of phentolamine is that it might inhibit the entry of guanethidine into the NE stores, just as cocaine abolished the sympathomimetic action of guanethidine on isolated guinea pig atria [Kroneberg & Schümann (157)].

A hypertensive phase after intravenous guanethidine (10 to 40 mg) has not been noted in humans by Taylor *et al.* (173). However, Imhof *et al.* (174) observed a brief pressor response after guanethidine, 0.5 mg/kg. It can be seen that this dose is much less than that used in animals (10 mg/kg). Taylor *et al.* (173) studied their patients for two hr following the intravenous administration of guanethidine and they reported no change in the heart rate of resting individuals but a relative bradycardia during exercise in both normal and hypertensive patients. There was also a small but definite reduction in pulmonary vascular resistance without change in pulmonary wedge pressure. CO was not changed in contrast to ganglionic blocking agents where there is a reduction in output. Bretylium on the other hand was found to increase CO slightly but significantly [Taylor & Donald (175)]. Rokseth *et al.* (176) administered 10 mg guanethidine through a catheter into the pulmonary artery of patients with pulmonary hypertension. There was a mean reduction in CO of 12 per cent and a parallel reduction of pressure in the pulmonary arteries and capillaries and in systemic arteries. Calculated pulmonary and peripheral resistance remained unchanged. Richardson *et al.* (177) studied CO in patients 5 to 7 days following guanethidine treatment and they reported a 10 per cent decrease in the supine and 33 per cent decrease in the standing position. Calculated peripheral resistance did not change. They concluded that guanethidine, like the ganglionic blocking agents, may reduce CO by inhibiting sympathetic vasoconstrictor mechanisms with resultant venous pooling of blood. Page *et al.* (178) believe that guanethidine lowers arterial pressure chiefly by reducing CO, apparently a result of blocking sympathetic nerves to the veins with consequent dilatation. This causes pooling of blood especially in the standing position and failure of return to the heart. Numerous reports have been published on the efficacy of guanethidine in controlling hypertension. While the results are in general favorable, clinicians differ in their enthusiasm for the drug. The least enthusiastic among clinicians claim it is not better than the benzothiadiazines.

α-METHYL-DOPA (α-METHYL-3,4-DIHYDROXY-PHENYL ALANINE; ALDOMET)

Clinical studies on this new hypotensive drug have been reported by Gillespie *et al.* (179); Irvine *et al.* (180); Dollerty & Harington (181), and Bayliss & Harvey-Smith (182). The drug appears to reduce both the supine and standing blood pressure, and has minimal unpleasant side-effects. Its sedative and tranquilizing properties may be advantageous to some patients. The drug is supposed to bridge the gap between guanethidine and Rauwolfia. The mechanism of its hypotensive action is not entirely clear. It is probably not related to inhibition of decarboxylation of certain aromatic amino-acids. Hess *et al.* (183) and Porter *et al.* (184) found that α-methyl-dopa and its analogue α-methyl meta-tyrosine caused marked depletion of NE from the brain and other tissues. However, NE depletion persisted several days while decarboxylase inhibition lasted only several hours. Gillespie *et al.* (179) studied two other decarboxylase inhibitors: α-methyl-5-hydroxytryptophane and the hydrazine analogue of α-methyl-dopa. Both compounds inhibited decarboxylation in hypertensive patients but without producing sedation or lowering arterial pressure. These authors observed that only those decarboxylase inhibitors that sedate also produce hypotension and consider this as an indication that the site of action of α-methyl-dopa or one of its metabolites lies within the central nervous system. Carlsson & Lindqvist (185) injected α-methyl dopa and α-methyl meta-tyrosine into mice and rabbits and noted a transient decrease in 5-hydroxytryptamine and dopamine but a prolonged and more marked decrease in NE in the brain. They also found that the α-methyl-aminoacids underwent decarboxylation and subsequent β-hydroxylation. The authors believe that the resulting α-methyl amines probably displace NE and dopamine from the stores and possibly take over the functions of the physiological amines. One reason why the α-methyl amines might compete so successfully with the latter is that they are not attacked by monoamine oxidase. Stone *et al.* (186) found that the α-methyl aminoacids decrease the catechol amine content of the brain-stem, heart, and spleen but not the adrenal gland. These authors also observed that α-methyl dopa and α-methyl tryrosine and their respective α-methyl-dihydroxy-phenethyl amines antagonized the cardiovascular actions of phenylethyl amine and amphetamine but not NE. However, the α-methyl-aminoacids and their amines, unlike reserpine and guanethidine, were unable to block the pressor response due to stimulation of the central end of the vagus.

MISCELLANEOUS DRUGS

DIPYRIDAMOLE (PERSANTIN)

Dipyridamole (Persantin) (RA 8) [2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido-(5,4-*d*)-pyrimidine] was introduced by Kadatz (187) in 1959 as a coronary vasodilator. A considerable number of publications dealing with its pharmacology and clinical use have appeared in the German litera-

ture. For references see Hochrein *et al.* (188). The enthusiasm, shown by the German clinicians was not shared by their British colleagues [Kinsella *et al.* (189)]. Kinsella *et al.* (189) confirmed the coronary vasodilator action of dipyridamole in man as evidenced by increased oxygen content of coronary venous blood. On the other hand, dipyridamole proved ineffective in angina in that it failed to prevent ischemic changes in the electrocardiogram on effort, while nitroglycerin prevented these changes although it had been reported by Gorlin *et al.* (190) not to be a true coronary dilator in patients with coronary artery disease. Kinsella *et al.* (189) suggest that the mechanism of action of nitroglycerin be reviewed. Wendt *et al.* (191) studied dipyridamole on patients free of cardiovascular disease. They report a decrease in coronary vascular resistance and an increase in myocardial oxygen consumption which was not always related to changes in the work of the heart. Hochrein *et al.* (188) report a positive inotropic effect by dipyridamole on healthy patients, cardiac-compensated but not digitalized patients, and on decompensated patients. In these patients there was a 25 per cent increase in CO, no change in heart rate, blood pressure or blood volume, and a decrease in circulation time and venous pressure. These effects of dipyridamole were not observed in digitalized compensated patients and those with valvular stenosis. West *et al.* (192) noted a marked increase in coronary blood flow and a decrease in coronary resistance after dipyridamole in normal dogs as well as animals with experimental coronary insufficiency. The rise in coronary flow was not associated with increased cardiac contractility or cardiac work. Siess (193), and Kunz & Siess (194) ascribe to dipyridamole the role of a hydrogen acceptor. Dipyridamole was reported by these authors to hasten the recovery of overloaded guinea pig atria driven to exhaustion by ouabain. Recovery in the presence of E or ouabain is accompanied by restitution of high-energy phosphorus compounds. Arrest due to anaerobiosis is also reported to be counteracted in the same way by dipyridamole.

Angiotensin

The effect of angiotensin on pulmonary circulation has been studied by various authors but with conflicting results [Chimoskey *et al.* (195)]. Special interest in this problem arises from the observation that essential hypertension is usually not accompanied by pulmonary arterial hypertension. If angiotensin does not elevate pulmonary resistance it cannot be excluded as a causal factor in essential hypertension. Chimoskey *et al.* (195), working on dogs, observed an elevated systemic and pulmonary pressure with no change in CO after single injections and intravenous infusions of angiotensin. The pulmonary resistance was calculated to decrease. The central blood volume was found to increase, presumably due to blood being forced into the right heart and pulmonary system by systemic vasoconstriction. Yu *et al.* (196) also noted an increase in systemic and pulmonary pressure and a slightly decreased or unchanged CO. However, these authors observed no

alteration in central blood volume and their calculated pulmonary resistance was found to be significantly increased. Page & Olmsted (197) studied the effect of angiotensin infusions on unanesthetized dogs and observed a sharp fall in CO, heart rate and stroke volume as peripheral resistance rose. Thiopental anesthesia increased the pressor response slightly and decreased the bradycardia. Bock & Gross (198) found that angiotensin, renin and NE produce a considerable rise in central venous pressure of unanesthetized dogs. This rise in venous pressure, like the bradycardia, can be diminished or abolished by atropine or thiopental anesthesia, while the rise in arterial pressure is enhanced. The same authors [Bock & Gross (199)] studied renin and angiotensin tachyphylaxis on the conscious dog. They found that large but not medium or small doses of renin or angiotensin produce tachyphylaxis. Cross tachyphylaxis between renin and angiotensin has also been demonstrated. The authors conclude that the so-called renin tachyphylaxis is due to diminished response to angiotensin and consider this as an indirect proof that renin liberates angiotensin *in vivo* as well as *in vitro*.

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