# EFFECT OF DRUGS ON THE INOTROPIC PROPERTY OF THE HEART[1,2]

By Bernard H. Marks

*Department of Pharmacology, Ohio State University College of Medicine, Columbus, Ohio*



## Introduction

Cardiovascular pharmacology has been reviewed extensively in *The Annual Review of Pharmacology* (2) and a thorough analysis of the chemical and biochemical bases and the physiological control of heart function is given in the new section of *The Handbook of Physiology* (78). The present reviewer will attempt to describe the recent literature of one specialized function of the heart, that of inotropism. We can accept as a working definition that which Mommaerts & Langer use in their informative review (135): a positive inotropic effect results when "full activation at the same initial fiber length gives rise to a greater force of contraction." Unfortunately, in many studies it may be quite difficult to decide whether the observations are relevant to inotropism or not, particularly those hemodynamic studies in which changes in heart rate and left ventricular end diastolic pressure may not be controlled. The measurements devised by Siegel & Sonnenblick (171) and the functional description of the left ventricle given by Franklin et al. (63) are examples of the precise definitions of terms that would aid in understanding measured responses.

## Digitaloids

In reviewing the evolution of our concepts regarding the positive inotropic action of digitaloids, the last years have seen no sudden "breakthroughs," but rather stepwise increments in our understanding of the response. Each advance in our knowledge of the fundamental processes involved in muscle contraction and the coupling of excitation to the contractile process has been regularly applied to the study of cardiotonic glycosides and their genins. Perhaps as important as any other approach has been the systematic study of such factors as time, frequency, and temperature on contractile tension and the action of digitaloids on these relationships.

Sonnenblick et al. (175) have done a technically difficult study of the electron microscopic fine structure of cat papillary muscle at different points of the length-tension curve which this muscle describes. They showed that the I-band is absent when the tension approaches zero, and increases in length with increasing tension and increasing sarcomere length. The A band and H zone do not change in length with these alterations in tension. They propose

[1] The survey of the literature pertaining to this review was concluded in May 1963.

[2] Personal Research reported in this review supported by Grants HE-07051 and NB-03853, USPHS.

that "folding" of myofilaments offers the best model for contraction, in contrast to the "sliding" model proposed earlier for skeletal muscle. It will be interesting to discover whether ouabain produces any fine-structure changes.

Koch-Weser (99) has studied the frequency-tension relationship and Sonnenblick the velocity-tension relationship (174) of isolated cat papillary muscle. They demonstrated the association of frequency of muscle stimulation with both the peak tension developed and with the time required to develop peak tension. An index of contractility has been devised (171) which is independent of fiber length and which may be very appropriate for evaluating the effect of drugs. This index appears to be applicable to the study of either heart muscle strips or of the intact heart. These studies, and those of Kruta (103 to 105), and Bautovich (14) on frequency vs. contractility of other mammalian heart tissues, point to a number of possible physiological control mechanisms that may be amenable to alteration by drugs. The reduction of inotropic effects of ouabain in cat heart at elevated and reduced temperatures (11, 131) also points the way to other interesting studies. Comparison of length-tension relationships in a number of homeothermic and poikilothermic animal species reveals some rather marked differences (146).

A by-product of these systematic studies of the physiological control of inotropy is that they have permitted the development of indirect indices of inotropy that may be of great value in studies of drug effects in human subjects. For example, the measurement of the systolic ejection time from a peripheral pulse contour may allow an investigator to define a frequency-tension relationship. Weissler has used this approach and demonstrated that it may be used in clinical experimentation to yield interesting data of drug effects on human hearts (198, 199). He showed that the ejection time varied in a regular manner with the heart rate of resting unanesthetized subjects, and devised an "ejection time index" as an indirect estimate of inotropy corrected for changes in heart rate. He has been able to show the time course of deslanoside effects on the myocardium in normal human subjects using this approach, as well as the dose dependence of the effect (197). Other investigators have also demonstrated the positive inotropic effects of digitalis glycosides in intact human subjects with nonfailing hearts (91, 123, 160).

Using more classical pharmacological preparations, Tuttle & Farah (185), and Koch-Weser & Blinks (100) have studied the effect of ouabain and acetylstrophanthidin, respectively, on the frequency-dependent changes in force of contraction of isolated heart muscle, the latter authors covering a much wider range of frequency. They showed that these glycosides altered the frequency-dependent tension-generating mechanism in such a way as to produce positive inotropic effects. This alteration may produce the greatest positive inotropic effect at the lowest point of the force-frequency curve, a point which is different in different regions of the heart and in different species. In hypodynamic hearts, where the frequency-force curve is flattened, digitaloids may restore a more normal relationship. It seems clear that fre-

quency-coupled events provide a key to the understanding of the mechanism of positive inotropic action of digitalis (106). It is interesting that, in high-calcium Tyrode's solution, force of contraction becomes fixed and unresponsive to rate-dependent change (94). One investigator has reported that in isovolumic dog heart preparations ouabain acts as a coronary vasodilator. He suggested that the other hemodynamic and biochemical changes due to ouabain were due to the increased coronary blood flow (68). The reviewer has always considered the reverse to be the case. Areskog has studied the inotropic and electrolyte effects of convallotoxin and acetylstrophanthidin in dog heart-lung preparations. There were suggested interesting changes in positive inotropy with pH which demand more systematic study (4, 5).

The question of whether adrenergic activity modifies the positive inotropic effect of digitalis upon the heart remains a subject of study. Morrow et al. feel that it does not (138), but perhaps a different measure of the contractile response of the heart might yield other results.

The question of how rapidly the heart binding sites for digitaloids are able to come to equilibrium with the blood has been made amenable to experimentation by the use of labeled glycosides. In our laboratory, Dutta et al. (51) have found that in sheep the coronary arterio-venous difference of ouabain-$H^3$ disappears approximately 16 min after drug administration. Marks & Dutta (119) have found that the human coronary arteriovenous difference of ouabain-$H^3$ disappears between 4–8 min after drug injection. Since most of the cardiac uptake of ouabain is accomplished within the first 4 min, this seems to correspond well with the observed onset of hemodynamic events. Bretschneider et al. (29) have shown that the uptake of lanatoside C-$C^{14}$ in the dog heart is completed in about three minutes. It would be interesting to know if thyroid hormone affects the rate of digitalis binding to the heart, since it has been shown that hypothyroid dogs achieve a substantially normal inotropic response to ouabain, but the response is slow to develop (161).

It has been a matter of some concern to surgeons who utilize total cardiopulmonary bypass for open-heart surgery that the large extra-corporeal circulation may extract digitaloids from the heart and might, therefore, render the heart less competent during and after the surgical procedure. In attempting to determine whether the cardiopulmonary bypass circulation should be "digitalized" before connecting it to the patient, several groups have resorted to the use of labeled digitaloids. Removal of digoxin-$H^3$ by the pump was found both in dogs (48) and humans (53), but other measurements of digoxin-$H^3$ content of the myocardium of pretreated patients before and after bypass revealed no significant change (15). Likewise, we found (119) that the ouabain-$H^3$ content of auricle biopsies was not altered by a period of total cardiopulmonary bypass. The glycoside extracted by the pump, therefore, may come from more loosely bound drug from some extra-cardiac tissues. The results of such studies may be influenced by the length of the pretreatment period before the onset of the bypass.

The localization of glycosides within the heart tissue may offer some clue

to their mechanism and site of action. Dutta et al. (51) find substantially less ouabain-$H^3$ uptake in the Purkinje cells than in the contractile cells of sheep heart, though Purkinje cells are much more sensitive to toxic effects of ouabain than are the muscle cells of the same heart (190). The technique described by West (201) may make it possible to study local differences in cardiac glycoside response upon the heart *in situ*.

Autoradiography of labeled glycosides in microscopic sections of heart tissue has been technically difficult because of diffusion. Initial reports based on this technique showed randomly scattered drug, not associated with any particular component of heart muscle cells (193). Smith & Fozzard (173) have published a single photograph of an electron microscopic autoradiograph that shows that radioactivity of digoxin-$H^3$ appears to be associated with the A bands of heart muscle. A similar result was described by Dutta (50) for ouabain-$H^3$ distribution using light microscopy, but it was difficult to be certain that this distribution was unique and related to the pharmacological site of action. It may be relevant that Schaer found by equilibrium dialysis that several digitaloids bind to purified myosin obtained from skeletal muscle, the binding corresponding to one molecule of glycoside per myosin molecule (165). We would like to know what the nature of the binding site and the binding forces are. Japanese workers (132, 133, 141) have shown that effects of ouabain on actomyosin from normal heart muscle, failing heart muscle and skeletal muscle were similar.

## Biochemical Changes in Heart Muscle Associated with Changes in Contractility

Cahn reviewed the relationship of enzymes to myocardial function (36) without reaching any firm conclusions concerning enzymatic pathways which are concerned with positive inotropic response. Bouyard compared various biochemical and pharmacological relationships of cardiac and skeletal muscle (25).

There is continued interest in the structure of the contractile proteins of normal and hypodynamic heart muscle. Dog heart and skeletal muscle myosins show great biochemical similarity with small changes in tertiary structure probably being present (28). Intensive physico-chemical study of myosin from normal and failing dog hearts has shown an apparent end-to-end trimerization of the protein in the failing heart (144). No change in the myosin ATPase activity was shown. By contrast, when cardiac muscle myofibrils were isolated, the myofibrillar suspension from failing human heart muscle demonstrated reduced ATPase activity and reduced rate of rise of activity with increase of substrate concentration and with increase of Mg concentration. Fragmentary studies on dog heart actomyosin also point to possible diminished ATPase activity from failing heart muscle (132, 133), when compared to normal heart tissue. These discrepant findings characterize the literature in this field, in which variation in the purity of the preparations and in the conditions for carrying out the enzymatic assays must account for

the diversity of the results. Electron (183) and light (166) microscopic histochemical localization of myofibrillar ATPase to the A bands of rat heart muscle confirm the association of myosin with this fine-structural component.

Though we might expect more attention to be paid to interaction of digitaloids with the contractile elements of the heart, interest is currently still focussed on interaction of digitaloids with the sodium-potassium activated ATPase first described by Skou (172) and later associated in a large number of tissues with the active transport of sodium and potassium in amphibia, birds, and mammals (24). The enzyme was found in 29 of 36 tissues of the cat, highest activities being associated with nerve and secretory cells. In all instances the enzyme is inhibited by ouabain, although the half-maximal inhibiting concentration of ouabain varies from greater than $10^{-6}$ to less than $10^{-7}$ $M$ for various tissues, and for the same tissues from various species. The Na-K-ATPase is usually described as occurring in the same fractions of cells in which "membranes" are observed to occur. Auditore has described the Na-K-ATPase preparation from rabbit heart (6), and Schwartz from rat heart (168). The enzyme is activated by Mg and the further activation by Na-K addition was prevented by $2.7 \times 10^{-6}$ $M$ ouabain. It is of interest that the structural requirements of digitaloids for positive inotropy, for Na-K-ATPase inhibition, and for inhibition of Na-K transport in various tissues appear to be similar, implying some relationships between these three biological effects (24, 157). Portius & Repke also reviewed the relationships between digitalis and Na-K-ATPase from guinea pig heart and concluded from detailed comparison that this enzyme is a digitalis receptor (149). This matter was thoroughly reviewed at the 1st International Pharmacological meeting (154) and in the 1963 *Annual Review of Pharmacology* (2) and continues to be an exciting field for future exploration.

Investigators continue to study the effects of digitaloids on diverse myocardial enzymes and conversely the effects of enzyme substrates on myocardial contractility. Studying enzymes of carbohydrate metabolism (47), marked effects of ouabain on phosphorylase and aldolase are described which scarcely seem to be related to the pharmacological actions of this drug. A calcium-dependent effect of lanatoside C on oxygen consumption in human heart slices is described (43), the pharmacological significance of which again seems doubtful. With the marked dependence of myocardial energy metabolism on fat oxidation, it is interesting that malonate potentiates the positive inotropic effects of ouabain (200), although the mechanism of malonate action is not described. Robb has described complex effects of digitaloids on the pattern of nucleotides extractable from dog heart (158). The importance of these changes to the interpretation of drug action remains somewhat obscure, as do the reported inotropic effects of heart extracts containing various amino acids and nucleotides (109). It is intriguing that considerable increase in inosine and hypoxanthine output in the effluent from hypoxic hearts has been described as evidence for a role of nucleosides in autoregulation of coronary blood flow (20). Haugaard has proposed an interesting cycle of phosphorylase

activation as part of rhythmic changes which help to correlate energy production with the proper stage of the cardiac cycle (81).

## Cations and Contractility

The relationship between calcium movement and the reversible states of contraction and relaxation of striated muscle remains one of the exciting areas of pharmacologic study, since it opens new possibilities for drug mechanisms. Detailed studies of relaxation in skeletal muscle clearly show the importance of sarcoplasmic reticulum particles as physiological relaxing factor. This factor firmly binds calcium, thus removing it from its complex with actomyosin (196). The interpretation of a cycle of contraction and relaxation based on this mechanism is founded on the observation that myofibrils in the presence of ATP will contract only if they have formed a complex with Ca. If the Ca ion concentration is sufficiently lowered, the ATP-Ca-myofibril complex dissociates and the ATP causes a partial reversal of the contracted state (194, 195). Sarcoplasmic reticulum relaxing factor can sufficiently lower the Ca concentration in such a system to effect this relaxation (196). A simplified method of preparing relaxing factor has been described (145).

Calcium flux has been intensively studied in heart muscle by a number of investigators, in order to determine if the general picture of the reversible states of skeletal muscle also applies to heart. Well-designed studies of guinea pig atria demonstrated the Ca compartmentalization of this tissue, the resting flux, and the marked additional influx associated with each beat (202). Also interesting and of even greater interest is the good correlation between the tension per beat and the Ca influx per beat. This suggests that the calcium uptake is one of the important factors that controls the developed tension.

Stam & Honig have studied the preparation and the properties of a soluble cardiac relaxing factor, which would presumably be involved in the control of the rhythmic changes in muscle tension during the cardiac cycle (88, 178). They suggest that while the particulate relaxing factor may be involved with excitation-contraction coupling, soluble relaxing factor may control or modulate the resultant tension development. They view the inotropic effects of catecholamines as inhibiting the tonic restraint on contraction imposed by this calcium-insensitive soluble relaxing factor (88, 177) and this soluble relaxing factor as the inotropic adrenergic receptor.

Hemodynamic effects of Ca infusions continue to be studied, as new experimental heart preparations are devised (58). They continue to document the positive inotropic effect of Ca and the absence of ouabain effect in Ca depleted heart tissues (32). Calcium infusion was shown to increase the toxicity of two glycosides but not to affect that of five other cardenolides (61). No blood calcium measurements were made.

Systematic studies of the effect of ouabain on $Ca^{45}$ influx have been carried out on rabbit atria and ventricles and on guinea pig atria (30, 31, 86,

114). Resting atria show $Ca^{45}$ influx which responds little to 10° C change in temperature. Stimulated atria show much greater Ca influx. The $Q_{10}$ for ouabain effect on Ca influx and on resting tension is the same order of magnitude. Ouabain appears to affect a Ca transport system very different from that in resting muscle. There seems to be a good correlation between the entry of Ca and the increase in resting tension after ouabain. When ouabain is administered in doses which produce a positive inotropic effect, rather than contracture, no pronounced effect on tissue Ca content is noted, but there seems to be reasonable correlation between an exchangeable Ca fraction and the contractile tension that develops. The same general conclusions can be reached from experiments using digitoxigenin on isolated guinea pig atria (95). A toxic concentration of digitoxigenin increases intracellular Ca concentration, perhaps by reducing the outward transport of this ion. Even in isolated rat atria, provided 100–200 times as much ouabain is used as in guinea pigs, there is correlation between positive inotropy and increased $Ca^{45}$ exchange (67). In summary, the role of calcium in excitation-contraction coupling is becoming clarified, and a mechanism of action of digitaloids in producing a redistribution of calcium in intracellular compartments seems likely (156).

The effect of replacing calcium with strontium in perfused frog hearts has been restudied. Instead of merely replacing one ion with the other in the perfusion fluid (41), these authors removed Ca ions with EDTA. Under these conditions Sr induced contracture and reduced the resting membrane potential (143). It was concluded that whereas Sr can apparently participate in the contraction process, it cannot replace Ca in maintaining the membrane potential. The perfused frog heart responds to increased extracellular Mg concentration with marked reduction in the duration of the action potential, until finally the activation of contractile responses no longer occurs (3). Drugs which increase the duration of the action potential, such as epinephrine and quinidine, were able to restore contractile responses. Mg was thought to alter the permeability of the membrane to Na or to occupy some Na sites in the membrane.

Although it seems to be well established that the positive inotropic ("therapeutic") responses to various digitaloids cannot be explained by alteration of intracellular K concentrations or of K flux (34, 75, 96, 97, 186, 187), small losses of K may be associated with the action of various digitaloids (5, 71), as well as with other positive inotropic responses (164). It is interesting that a sharp break in the slope of the dose-response curve for K loss occurs at the point where toxic effects occur. This implies that the mechanisms for these two phases of K loss are different. Potassium infusion markedly slows A-V conduction both in the absence (59) and, even more effectively, in the presence of digitalis (205).

Kahn showed that both ouabain and elevation of Ca concentration increase contractile force and cause a net loss of K from perfused heart muscle (93), but they produce the effect differently. Ouabain reduced K influx, and

Ca increased K efflux. This may be interpreted as being evidence for competition of Ca and K for an intracellular site associated with the contractile mechanism.

Forster has correlated the action of therapeutic doses of 15 genins on inhibition of transport of the accumulated Na of cold-stored human erythrocytes with the positive inotropic action on guinea pig atria rendered hypodynamic with phenylbutazone (62). He finds a reasonably good correlation coefficient between these diverse biological effects. The response of quinine-damaged heart to these genins could not be correlated with the erythrocyte transport effect.

After the intracellular compartment of frog heart has been equilibrated with $Na^{24}$, the rate of sodium efflux demonstrated fast and slow exponential components. The presence of epinephrine in the bath markedly increased the slow component (76).

A new volume of *Heffter's Handbuch* reviews extensively the biological role of alkaline earth cations in various types of cells, including a section on cardiac muscle (10).

## Adrenergic Drugs

Interesting dose-response curves of various hemodynamic measurements were presented following norepinephrine infusion into anesthetized dogs (54). It seemed clear from comparison of chlorothiazide pretreated dogs with control dogs that the effects of chlorothiazide treatment were to reduce the baroreceptor regulatory reflexes, since there was less bradycardia, and considerable increase in cardiac output and stroke work in chlorothiazide treated animals. On the other hand, responses of the resistance vessels to norepinephrine infusion were markedly diminished in the thiazide treated dogs, perhaps demonstrating basis for the antihypertensive action of thiazides.

A detailed study of the effect of catecholamines on coronary blood flow has been carried out, using methods in which effects of hemodynamic variables such as heart rate, blood pressure, and cardiac output can be controlled (117). The great importance of myocardial oxygen consumption as a determinant of coronary flow is made very clear in the data accumulated from many experiments. The observed increases in coronary flow are thought to follow the effects of catecholamines on oxygen consumption of heart muscle. By contrast, direct effects of epinephrine and norepinephrine on coronary blood vessels consist of immediate constriction, followed by dilatation due to the metabolic actions on heart muscle.

An interesting contribution relating to epinephrine refractoriness, a not unusual clinical problem, describes the effects of acidosis and hypoxia on epinephrine responses in dogs (203). Acidosis, particularly respiratory rather than metabolic, exerted a profound effect in reducing pressor and positive inotropic responses to epinephrine, as well as reducing the frequency of arrhythmias (189). Hypoxia also reduced pressor responses. The observations remind us that epinephrine-refractory experimental animals and patients

may often be restored to sensitivity by attention to factors such as pulmonary ventilation, reduction of which may create just the proper conditions for refractoriness. Marked circulatory shock, too, would be expected to result in this state. Strangely, hypothermic digitalized dogs show no positive inotropic response to norepinephrine even after they have been rewarmed; they only show cardiac depression (42).

Daniel et al. have re-examined the effects of norepinephrine on the transmembrane potential of rabbit heart, and it is very evident that the positive inotropic effects of norepinephrine cannot be related to increased duration of depolarization (44). Their results may be attractively related to the proposal of Stam & Honig (88, 177) that norepinephrine removes tonic inhibition of myofibrillar ATPase by soluble relaxing factor, thus giving more contraction for a given depolarization. This concept may be difficult to reconcile with the "glycogenolytic" view of the inotropic action of catecholamines. There is no question about the association of changes in per cent phosphorylase A with positive inotropic responses to epinephrine (18, 83, 84, 108, 129), but causal relationships in this system are difficult to prove. Likewise, the observations of increased cardiac hexose-phosphate content after catecholamine and nonadrenergic positive inotropic drugs (17) can be incorporated into our ideas of phosphorylase-activating mechanisms, but do not demonstrate causal relationships between these mechanisms and positive inotropy. Recent observations suggest that positive inotropy, activation of phosphorylase and accumulation of glucose-6-phosphate do not have the same dose-response and temperature relationships (127, 128).

The most provocative and exciting observation which has been made in this field in years is the claim by Uchida & Mommaerts that cyclic 3,5-AMP is identical with relaxing factor (188). If more complete studies can confirm the relationship, this may point the way to reconciling the biochemical and the pharmacological effects of adrenergic mediators. They found that cyclic AMP is formed in relaxing factor preparations, that in the presence of a low-Ca medium cyclic AMP markedly inhibits the contraction of an actomyosin-ATP suspension, and that the inhibition of contraction can be immediately relieved by addition of calcium. As one first considers this evidence, it would appear that adrenergic drugs, by activating the formation of cyclic AMP, should cause negative, rather than positive, inotropy. It is the view of Uchida & Mommaerts, however, that the presence of cyclic AMP can be considered to sensitize actomyosin to small changes in the free Ca concentration, so that the changes in calcium flux which accompany each cardiac cycle serve to generate a greater muscular response. This view has some points of similarity to the concept of Stam & Honig involving their soluble relaxing factor (88, 177). Is it possible that both groups are studying similar substances under somewhat different ionic conditions?

Reserpinized animals continue to be used as a tool for the identification of direct and indirect adrenergic effects upon the heart. Ephedrine has been found to produce considerable augmentation of contractile force in reserpin-

ized dogs (137), whereas the positive inotropic effects of tyramine (65), phenethylamine, amphetamine, norephedrine, and mephentermine were abolished. In papillary muscles from reserpinized cats, a systematic study also showed that ephedrine may have some direct action, although reserpine did produce considerable diminution of response (37). Reserpine pretreatment abolished positive inotropic responses to bretylium and guanethidine, confirming norepinephrine release as the mechanism of this action of these drugs (21, 102), although the norepinephrine-releasing mechanism of guanethidine, reserpine and tyramine may be quite different (22, 64, 80, 150). The addition of $10^{-5}$ *M* reserpine to isolated spontaneously beating rabbit atrium preparations caused these preparations to beat less forcefully and with slower rate and finally to stop. The norepinephrine content of these atria is only slightly reduced when they stop beating. They can be restarted by adding small amounts of norepinephrine or norepinephrine precursors to the bath (125, 181). These acute effects of large doses of reserpine may have some other basis than depletion of amines, as do the negative inotropic effects described by Nayler (142). Considering the heavy investment which pharmacologists have made in reserpine-induced amine depletion as a tool, it is important that we keep an open mind about the possibility that reserpine may have other effects on cells as well.

The uptake and metabolism of norepinephrine-$H^3$ have been described for isolated hearts of rats (101) and dogs (39). The effects of a great many drugs have been studied upon the heart content of norepinephrine-$H^3$ (and normetanephrine-$H^3$) after cats (82) or rats (7, 8, 150) were treated with norepinephrine-$H^3$. Very clear indeed is the marked reduction of heart content of norepinephrine-$H^3$ produced by pre-treatment with amphetamine, tyramine, chlorpromazine, imipramine, reserpine, guanethidine, bretylium, *d*-epinephrine, cocaine and dibenzyline. In order to determine the distribution of the norepinephrine taken up by the heart and other tissues, an autoradiographic method has been developed which may yield interesting and useful information (120, 162). This method cannot be presumed to have quantitative significance, but if we can accept the reasonable conclusion that norepinephrine is taken up and stored in adrenergic nerve endings, then this method may give some idea of the size and shape of adrenergic nerve endings and their distribution in tissues. In the heart, the norepinephrine-$H^3$ was rapidly taken up by long narrow fibers adjacent to muscle cells. These fibers became intensely labeled within 1–2 min and retained the radioactivity as long as four hours. Apparently, the entire length of these fibers in heart muscle became labeled simultaneously, implying that the functional exchanging surface of the adrenergic neurones in the heart was quite sizeable. Pretreatment of animals with reserpine was found to prevent both the uptake and the storage of norepinephrine-$H^3$ in these structures. Pretreatment with cocaine delayed the uptake but did not prevent storage of norepinephrine (57, 163). There was no indication in any of these studies of a second storage form of norepineph-

rine in the heart. Drug effects on norepinephrine turnover in the heart may well be promising future studies (16, 38, 136).

A demonstration of the functional diversity of the norepinephrine stores in the heart was the finding that tyramine infusions, which essentially abolish the chronotropic and inotropic effects of a subsequent tyramine injection, do not appreciably alter the cardiac responses to cardioaccelerator nerve stimulation (79). Thus, although both nerve-releasable and tyramine-releasable norepinephrine must be part of an "available" store, this store in turn must be compartmentalized (150). It is interesting that beta-haloalkylamines, but not other alpha-adrenergic blocking drugs, perfused through an isolated cat heart will prevent the release of norepinephrine by tyramine and also abolish the positive inotropic effect due to tyramine (180). It was contended, but not proved, that this was not an example of beta-adrenergic blockade but was instead a specific effect of beta-haloalkylamines on prevention of norepinephrine release. The action of sympathetic nerve stimuli on norepinephrine release and positive inotropic effect has been correlated in isolated rabbit hearts in the presence of a number of inhibitors (90).

The functional activity of dopamine in mammalian hearts continues to be of interest. In dog heart-lung preparations with controlled heart rate, dopamine injection produced prompt increases in isometric contractile tension and cardiac output comparable to those of norepinephrine if given in doses 25 to 100 times as great. The onset of action of dopamine tended to be slightly slower than that of norepinephrine and the duration of action somewhat more prolonged (87). Dopamine has similar effects in open-chest dogs, with not completely explained depressor effects in low doses (130). Although acknowledging that dopamine has some direct effects, we tend to think of this chemical primarily as a substrate for the enzyme in heart tissue which converts it to norepinephrine (40, 176). It raises very interesting possibilities, though, to discover that the distribution of dopamine in mammalian heart tissue does not parallel that of norepinephrine, but that there is a high concentration of this amine in the region of the sinus node (1). This may be in part responsible for high right atrial catecholamine levels (98, 124). Whether this reflects a different rate of norepinephrine turnover in this region, a different amount of beta-hydroxylase enzyme or a different functional role remains to be described. Further study certainly is indicated.

The actions of adrenergic drugs on human subjects remain a continuing challenge as new pharmacologic concepts emerge. Although expected hemodynamic effects were recorded following norepinephrine infusion into normal and labile hypertensive subjects, it is interesting that no correlation existed between infusion rate and norepinephrine blood levels or between hemodynamic responses and blood levels (55). The pressor effect of tyramine injections has been studied in normal human subjects and a pressor dose-response curve described. After moderate doses of reserpine, reduction of pressor activity of tyramine occurred (115, 122). The authors propose a tyramine

pressor test for the adequacy of tissue norepinephrine stores, but in view of the normal responses to cardioaccelerator nerve stimulation in tyramine insensitive animals (79), one wonders about the physiological significance of such a test. The cardiovascular responses to dopamine infusions have been studied in normal subjects; the pressor response is accounted for by increased cardiac output and increased stroke volume, but no change in heart rate accompanied the other hemodynamic changes. It was suggested that dopamine might be useful in supporting the cardiac output of patients in some circumstances (89).

An important new pharmacological tool for studying adrenergic effects on the heart and other tissues has been introduced (23). This is nethalide, Compound 38,174, 2-isopropylamino-1-(2-naphthyl) ethanol. The compound resembles the structure of DCI with the exception that a benzene ring has been fused on to the 3′,4′ positions occupied by Cl in DCI. Nethalide appears to be a much poorer agonist than DCI and probably a better beta-adrenergic antagonist. In concentrations of $10^{-7}$ to $10^{-3}$ g/ml it blocks such typical beta-adrenergic effects of epinephrine as positive inotropic effects in papillary muscle, positive chronotropic effects in perfused heart, relaxation of tracheal chain or fowl cecum, while much higher concentrations do not block uterine contraction or peripheral vasoconstriction. In anesthetized dogs, nethalide prevented positive inotropic responses to epinephrine but not to ouabain (52). The drug is undoubtedly destined for wide use in the pharmacology laboratory, whether or not it will ever see clinical application.

Species difference in the response of cat and rat heart to an MAO inhibitor was described (70). *Trans*-2-phenyl-cyclopropylamine (SKF 385) depletes norepinephrine in cat heart but augments norepinephrine content of rat heart. This drug caused modest positive inotropic effects in both species which were blocked by DCI, by beta TM-10 and by reserpine pretreatment. Data on norepinephrine metabolism by heart tissue supported the conclusion that the major route of biotransformation of norepinephrine in cat and rat heart homogenates differs, the rat utilizing predominantly oxidative (MAO) and the cat conjugative (COMT) routes. The effect of another MAO inhibitor, isocarboxazid, on hemodynamic changes following exercise was studied in three subjects with angina pectoris (69). Reduced pressor responses to exercise were described during periods of isocarboxazid treatment compared with placebo periods. It was felt that possible useful results of MAO inhibitor treatment in patients with angina pectoris should not be overlooked, and that the prevailing opinion concerning lack of effectiveness of this treatment may have been due to the use of doses of drug that were not high enough to accomplish effective MAO inhibition in vivo. Nialamide pretreatment markedly increased the norepinephrine content of guinea pigs heart but may decrease the content in cat heart. It hastened the development of pressor tachyphylaxis to tyramine and reduced the norepinephrine released by nerve stimuli. This MAO inhibitor appears to lessen the "available" norepinephrine store (45).

Alpha-methyl dopa acts as if it reduces the amount of the "available" norepinephrine store (179); a proposal has been made that this drug may act as a precursor for the formation of alpha-methyl norepinephrine, an inactive neurotransmitter, and this, rather than dopa decarboxylase inhibition, may be the basis for antihypertensive effect (46).

## Cholinergic Drugs

The inhibition of cyclic AMP formation that Murad has shown to occur with acetylcholine or with carbachol (140) has been beautifully correlated with pharmacological effects of acetylcholine upon the mammalian heart. Hess et al. described the effect of acetylcholine injections or vagal stimulation upon the active phosphorylase of the rat heart. As might be expected, these treatments resulted in a diminution of the per cent active phosphorylase (85). Subsequently, Vincent & Ellis showed that acetylcholine infusion did not alter cardiac glycogen content, but that it would completely prevent the glycogenolytic effect of epinephrine which was infused simultaneously (191). Atropine was found to block this metabolic effect of acetylcholine upon the heart, as well as to block the reduction of per cent phosphorylase a by acetylcholine (107). In atropinized perfused rabbit hearts acetylcholine causes positive inotropic effects and an increase in the per cent active phosphorylase, as do ganglionic stimulants such as nicotine and DMPP (107). These effects are blocked by DCI. Whether these nicotinic actions are entirely on sympathetic ganglia in the rabbit heart Langendorff preparation, or whether some of the effect is due to catecholamine release by the above drugs is not certain.

The negative inotropic effect of acetylcholine on isolated rat atrium shows frequency dependency, being most pronounced at high frequency of stimulation and absent at very low heart rates. Regardless of the rate of stimulation, the effect of acetylcholine upon intracellular action potentials recorded from these atrial muscles was the same: it consisted in a marked shortening of the duration. There was no correlation between the duration of the action potential and the force of contraction (12). It was proposed that the negative inotropic effect of acetylcholine was due to an interference with the calcium dependent coupling of excitation and contraction (13). It may be pertinent that EDTA potentiates vagal inhibitory effects on frog heart (204). A systematic report of the effects of acetylcholine upon the refractory periods of dog atria includes a nice dose-response curve for the effect of acetylcholine in decreasing the absolute refractory period (184).

In toad atrium, acetylcholine and vagal stimulation reduce the duration of the action potential, as they do in other species. Unusual, though, is the observation that even if considerable care was taken to prevent any possible diffusion of acetylcholine from the atria, vagal stimulation in the toad would also produce shortening of the intracellular action potential in the ventricle. It was concluded that in the heart of this species there is vagal innervation of ventricular muscle, although it was thought that the distribution of vagal

fibers must be sparse (9). It has long been established that in mammalian hearts no such functional vagal innervation exists in the ventricle.

Isolated ventricle, of 39 different species of bivalves were perfused with acetylcholine solutions, and the contractile responses observed. Most species showed initial depressor responses followed by increased contractility at higher doses. One species showed only increased contractility; several species only reduced contractility. Unfortunately, the data is not presented in as quantitative a manner as might be desirable (72).

A stimulating study of the action of sulfhydryl blocking chemicals upon the negative inotropic effect of acetylcholine in isolated frog heart and guinea pig auricle was carried out by Pohle & Matthies (148). A number of metals were inactive, but mercury, iodacetamide, and N-ethylmaleimide were active in inhibiting the negative inotropic effect of acetylcholine. The blockade by mercury was reversible with cysteine, but the blockade by N-ethylmaleimide was not reversible. The dose-response curves for acetylcholine vs. negative inotropy showed a reduced slope and a reduced maximum effect when N-ethylmaleimide was present; it was concluded that the blockade was noncompetitive. This same sulfhydryl blocking agent also inhibits the ATPase activity of myosin. Using N-ethylmaleimide-$C^{14}$, peptide mapping of the ATPase site of myosin is beginning (73, 74).

In normal muscle-relaxing doses *d*-tubocurarine lowers the blood pressure and reduces isometric tension of heart muscle in anesthetized dogs (26). It seems likely that at least a part of this effect may be due to histamine liberation. Acetylcholine or neostigmine was able to antagonize part of the depressor and negative inotropic effect of *d*-tubocurarine. On hypodynamic cat papillary muscle, *d*-tubocurarine has very little effect, but may augment the response to ouabain (27). It is doubtful that this property has any useful significance.

## Anesthetic Agents

An informative very short review of the effect of anesthetic agents on the isometric systolic tension of ventricle muscle summarized very well the basic information available on that topic (33). The graphical representation of the effect of ethyl ether demonstrated the increased force of contraction during the induction period and during emergence from anesthesia. These responses can be correlated with the catecholamine release that occurs during these periods. During the stages and planes of deepening anesthesia, ether shows rather small effects on inotropy. Even at the time of respiratory arrest (Stage IV) the contractile force was still 50 per cent of control. Divinyl ether contractile force was 63 per cent of control in Stage IV; cyclopropane was 51 per cent of control in Stage IV; chloroform reduced contractile force to only 26 per cent of control at the end of Stage III. Animals could not be taken to Stage IV because they would almost invariably die.

Recently, halothane has seen extensive use as an anesthetic, and considerable interest has developed in studying the effects of this gas on the contrac-

tile activity of the heart. Direct measurement of contractile force using a strain gage arch showed diminution of developed tension during halothane anesthesia in dogs (116) and humans (116, 139, 169). In intact closed chest dogs, 0.5 per cent halothane anesthesia did not produce any effects, but 1 per cent to 2 per cent halothane produced graded reduction in a number of different ventricular function measurements (170). Even when dogs were premedicated with ouabain, these concentrations of halothane produced graded reduction in the slope of ventricular function curves, although the reductions were less than in nondigitalized dogs. While the cardiac output usually fell after halothane, it was well maintained or even increased in the digitalized halothane anesthetized dog (169). In the dog heart-lung preparation, too, halothane reduced the competence when administered in concentration of 1 per cent or more (60). Norepinephrine infusion could to some extent antagonize this reduction in contractility produced by halothane.

It has been a common clinical observation that halothane produces gradual bradycardia and lowering of blood pressure. In a study of the circulatory responses to respiratory acidosis, it is interesting that halothane reduces quite considerably the hypertension and the tachycardia associated with induced respiratory acidosis ($CO_2$ inhalation). By comparison, cyclopropane had a similar though much less profound effect (153).

Halothane was able to reduce the rate of dog heart-lung preparations in a concentration of 1 per cent or greater and the decline in rate was proportional to the dose. This effect on heart rate is not due to adrenergic blockade (60). Gardier finds that when acetylcholine is administered to atropinized dogs, the administration of halothane will prevent the nicotinic effects on contractility of the myocardium but will not prevent the positive chronotropic effects (66). It seems likely that the effects of halothane on the heart may be a combination of some direct negative inotropic effects on the myocardium with, perhaps, a reduction in sympathetic outflow mediated through some central nervous mechanism.

In contrast to the depressor activity of halothane, cyclopropane has long been recognized to produce pressor responses which are known to be mediated by norepinephrine liberation (152). Observations have now been reported which show that if cyclopropane is perfused into the isolated head of a dog, pressor responses occur in the body which is maintained with a separate circulation but with nervous connections to the head intact. No pressor effects occur if the cyclopropane is introduced only into the body circulation of the dog but is kept out of the head circulation. Lesion experiments show that the central regulatory mechanisms affected by cyclopropane could be localized to the medulla (151).

Another indication that the pressor effect of cyclopropane is not due to direct effects upon the heart is the report on the study of isolated rabbit atria, in which it is clearly demonstrated that cyclopropane in concentrations ranging from 1 per cent to 12 per cent produces a linear fall in contractile tension. Intracellular electrode recordings in these same preparations show that cyclo-

propane causes more rapid repolarization, especially in the early phases. The negative inotropy of cyclopropane could not be accounted for on the basis of reduced oxygen content of the perfusion fluid, although it is admitted that this could play a role (111).

## Miscellaneous

Some years ago, during the heyday of the antihistamines, some similarity of their effects to those of anti-arrhythmic drugs such as quinidine and procaine was noted. Some recurrence of interest in the cardiac effects of antihistamines has led to re-evaluation of their effects on inotropy as well as other cardiac functions. In a brief report (192), the comparative effects of ten drugs on the rate and contractility of isolated rabbit atria were described at each of three concentrations. All of the antihistamines reduced contractility rather considerably without affecting spontaneous rate. Curiously, marked variability in the concentration-negative inotropic response relationships were evident; no explanation was offered. The antihistamine, antazoline, was studied in intact dogs and humans, and in addition to marked effect on arrhythmias, it seemed probable that this drug also produces negative inotropic effects, although direct measurement in the dog experiments would have been preferred (49). The negative inotropic effect of quinidine and procaine is but one aspect of their effect on heart muscle, but in studies of toxicity of these anti-arrhythmic agents, some attention has been given to the effects of *tris* buffer (THAM) and other drugs on contractility (113, 159), although the studies have been anything but systematic. Attempts to define the biochemical basis for the negative inotropy produced by quinidine are intriguing, but observed inhibitory effects on granular and myofibrillar ATPases require such high quinidine concentrations that it seems difficult to reconcile them with observed biological activity (56). Other anti-arrhythmic drugs, RO 2-5803 (134) and ajmaline (147), also reveal characteristic negative inotropic effects.

Rall & West studied the effect of the methylxanthines, theophylline and caffeine, on contractile force of isolated rabbit atria (155). They explain the observed positive inotropic effects by the inhibition of phosphodiesterase with consequent preservation of cyclic AMP (35). They demonstrated increase of the positive inotropic effects of norepinephrine by methylxanthines, in accord with this theory. The systematic study of this relationship to the activation of phosphorylase may be another means of subjecting the "cyclic AMP" theory of norepinephrine inotropy to experimental test. New xanthines which produce positive inotropic responses continue to be introduced (118, 126).

Aldosterone was reported to produce positive inotropic effects on cat papillary muscle preparations in very low concentrations (182). It is curious that isolated stimulated rabbit atria do not show this response at all, and higher concentrations of either *d,l*- or *d*-aldosterone, if anything, show slight reduction of contractility. Likewise, aldosterone produces no effect on sodium

or potassium content of these preparations (110, 112). Are these differences between cat and rabbit heart only due to species variation?

A series of pyridine derivatives and their N-oxides showed that the positive inotropic response to the pyridine compounds was probably due to catecholamine release, but the N-oxides, though less potent, remained equally effective on catecholamine-depleted tissue. The positive inotropic effect of the N-oxides was not accompanied by any important action on heart rate (167).

The interesting drug, dipyridamole (Persantin), despite much study of its biochemical and pharmacological effects (2), is still looking for a disease to cure. A recent study shows this drug to produce inconstant fall in contractile force of dog heart associated with changes in coronary flow, but, most important, the essential absence of any cardiac effects when administered by the intragastric route (19). Failure of absorption may explain many of the clinical failures with this drug.

Some years ago, Hajdu described a positive inotropic response from a lipid present in blood serum. The lipid, a lysolecithin, was presumably derived from plasmalogen hydrolysis (77). This was of interest because the heart contains a large amount of this particular phospholipid. Recently, two groups have restudied the inotropic effects of purified beta-palmitoyl lysolecithin in relationship to that of digitaloids. This lipid was found to have positive inotropic effects in frog heart along with increased potassium exchange (121). In mammalian heart and erythroctyes, however, Kahn & Schindler (92) could detect neither positive inotropy nor inhibition of potassium transport.

*Effect of Drugs on the Inotropic Property of the Heart.*—Two recent reports (135a, 181a) make it clear that the preliminary experiments suggesting a role of cyclic 3,5-AMP as a relaxing factor in striated muscle have not been repeatable in the same nor in an independent laboratory.

## LITERATURE CITED


1. Angelakos, E. T., and Torchiana, M. L., *Fed. Proc.*, **22**, 447 (1963)
2. *Ann. Rev. Pharmacol.*, **3** (1963)
3. Antoni, H., Engstfeld, G., and Fleckenstein, A., *Arch. Ges. Physiol.*, **275**, 507 (1962)
4. Areskog, N. H., *Acta Physiol. Scand.*, **55**, 139 (1962)
5. Areskog, N. H., *Acta Physiol. Scand.*, **55**, 264 (1962)
6. Auditore, J. V., *Proc. Soc. Exptl. Biol. Med.*, **110**, 595 (1962)
7. Axelrod, J., Hertting, G., and Patrick, R. W., *Brit. J. Pharmacol.*, **18**, 161 (1962)
8. Axelrod, J., Hertting, G., and Potter, L., *Nature*, **194**, 297 (1962)
9. Azuma, T., Hayashi, H., and Matsuda, K., *Science*, **138**, 895 (1962)
10. Bacq, Z. M., *Handbuch der Experimentellen Pharmakologie*, **17**, part 1, *Ions Alcalino-Terreux Systemes Isoles* (Springer, Berlin, 1963)
11. Bassett, A. L., and Hoffman, B. F., *Am. J. Physiol.*, **203**, 206 (1962)
12. Baumann, F., *Experientia*, **17**, 1 (1961)
13. Baumann, F., and Waldvogel, F., *Helv. Physiol. Acta*, 19, 53 (1961)
14. Bautovich, G., Gibb, D. B., and Johnson, E. A., *Australian J. Exptl. Biol. Med. Sci.*, **40**, 455 (1962)
15. Beall, A. C., Johnson, P. C., Driscoll, T., Alexander, J. K., Dennis, E. W., McNamara, D. G., Cooley, D. A., and DeBakey, M. E., *Am. J. Cardiol.*, **11**, 194 (1963)
16. Beaven, M. A., Costa, E., and Brodie, B. B., *Life Sciences*, 241 (1963)
17. Belford, J., and Feinleib, M. R., *Biochem. Pharmacol.*, **11**, 987 (1962)
18. Belford, J., and Feinleib, M. R., *J. Pharmacol.*, **127**, 257 (1959)
19. Ben, M., Boxill, G. C., Scott, C. C., and Warren, M. R., *Arch. Intern. Pharmacodyn.*, **142**, 228 (1963)
20. Berne, R. M., *Am. J. Physiol.*, **204**, 317 (1963)
21. Bhagat, B., and Shideman, F. E., *Brit. J. Pharmacol.*, **20**, 56 (1963)
22. Bisson, G. M., and Muscholl, E., *Arch. Exptl. Pathol. Pharmakol.*, **244**, 185 (1962)
23. Black, J. W., and Stephenson, J. S., *Lancet*, II, 311 (1962)
24. Bonting, S. L., Caravaggio, L. L., and Hawkins, M., *Arch. Biochem. Biophys.*, **98**, 413 (1962)
25. Bouyard, P., *Actualites Pharmacol.*, **14**, 25 (1961)
26. Bouyard, P., *Therapie*, **15**, 320 (1960)
27. Bouyard, P., and Sassine, A., *Therapic*, **17**, 609 (1962)
28. Brahms, J., and Kay, C. M., *J. Biol. Chem.*, **238**, 198 (1963)
29. Bretschneider, H. J., Doering, P., Eger, W., Haberland, G., Kochsiek, K., Mercker, H., Scheler, F., and Schulze, G., *Arch. Exptl. Pathol. Pharmakol.*, **244**, 117 (1962)
30. Briggs, A. H., and Holland, W. C., *Nature*, **195**, 294 (1962)
31. Briggs, A. H., and Holland, W. C., *Am. J. Physiol.*, **202**, 641 (1962)
32. Broadbent, J. L., *Brit. J. Pharmacol.*, **19**, 183 (1962)
33. Brown, J. M., *Anesthesia Analgesia*, **39**, 487 (1960)
34. Brown, T. E., Acheson, G. H., and Grupp, G., *J. Pharmacol.*, **136**, 107 (1962)
35. Butcher, R. W., and Sutherland, E. W., *J. Biol. Chem.*, **237**, 1244 (1962)
36. Cahn, J., *Revue Agressologie*, **3**, 421 (1962)
37. Cairoli, V. J., Reilly, J. F., and Roberts, J., *Brit. J. Pharmacol.*, **18**, 588 (1962)
38. Chidsey, C. A., and Harrison, D. C., *J. Pharmacol.*, **140**, 217 (1963)
39. Chidsey, C. A., Kahler, R. L., Kelminson, L. L., and Braunwald, E., *Circ. Res.*, **12**, 220 (1963)
40. Chidsey, C. A., Kaiser, G. A., and Braunwald, E., *Science*, **139**, 828 (1963)
41. Coraboeuf, E., Guilbault, P., Breton, D., and DuMont, M., *Compt. Rend. Soc. Biol.*, **155**, 1251 (1961)
42. Cotten, M. deV., and Cooper, T., *J. Pharmacol.*, **136**, 97 (1962)
43. Crevasse, L., and Wheat, M. W., *Circ. Res.*, **11**, 721 (1962)
44. Daniel, E. E., Johnston, P. K., and Foulks, J. G., *Arch. Intern. Pharmacodyn.*, **138**, 267 (1962)
45. Davey, M. J., Farmer, J. B., and Reinert, H., *Brit. J. Pharmacol.*, **20**, 121 (1963)
46. Day, M. D., and Rand, M. J., *J. Pharm. Pharmacol.*, **15**, 221 (1963)
47. Dmitrieva, N. M., and Rubechinskaia, K. I., *Pharmacol. Toxicol.*, **6**, 719 (1961)
48. Doherty, J. E., Masauki, H., Lincoln, B. M., Perkins, W. H., *Am. Heart J.*, **64**, 376 (1962)
49. Dreifus, L. S., McGarry, T. F., Watanabe, Y., Kline, Ronald S.,

Waldman, M., and Likoff, W., *Am. Heart J.*, **65**, 607 (1963)
50. Dutta, S. (Doctoral thesis, Ohio State University, 1962)
51. Dutta, S., Marks, B. H., and Smith, C. R., *J. Pharmacol.* (In press)
52. Dutta, S. (Unpublished observations)
53. Ebert, P. A., Morrow, A. G., and Austen, W. G., *Am. J. Cardiol.*, **11**, 201 (1963)
54. Eckstein, J. W., Abboud, F. M., and Pereda, S. A., *J. Clin. Invest.*, **41**, 1578 (1962)
55. Eich, R. H., Cuddy, R. P., Barry, J. A., and Smulyan, H., *Am. J. Cardiol.*, **10**, 819 (1962)
56. Ells, H. A., and Faulkner, P., *Arch. Intern. Pharmacodyn.* **CXLI**, 75 (1963)
57. Farrant, J., *J. Brit. Pharmacol*, **20**, 540 (1963)
58. Feinberg, H., Boyd, E., and Katz, L. N., *Am. J. Physiol.*, **202**, 643 (1962)
59. Fisch, C., Feigenbaum, H., and Bowers, J. A., *Am. J. Cardiol.*, **11**, 487 (1963)
60. Flacke, W., and Alper, M. H., *Anesthesiology*, **23**, 783 (1962)
61. Forster, W., and Lindenau, M., *Klin. Wochschr.*, **41**, 339 (1963)
62. Forster, W., and Sziegoleit, W., *Experientia*, **18**, 573 (1962)
63. Franklin, D. L., Van Citters, R. L., and Rushmer, R. F., *Circ. Res.*, **11**, 702 (1962)
64. Gaffney, T. E., Chidsey, C. A., and Braunwald, E., *Circ. Res.*, **12**, 264 (1963)
65. Gaffney, T. E., Morrow, D. H., and Chidsey, C. A., *J. Pharmacol.*, **137**, 301 (1962)
66. Gardier, R. W., Richards, A. B., Stoelting, V. K., and White, N., *Brit. J. Pharmacol.*, **20**, 586 (1963)
67. Gersmeyer, G., and Holland, W. C., *Circ. Res.*, **12**, 620 (1963)
68. Goksel, F., Katz, L. N., and Feinberg, H., *Am. J. Physiol.*, **204**, 21 (1963)
69. Goldberg, L. I., Horwitz, D., and Sjoerdsma, A., *J. Pharmacol.*, **137**, 38 (1962)
70. Goldberg, N. D., and Shideman, F. E., *J. Pharmacol.*, **136**, 142 (1962)
71. Greeff, K., Von Meng, K., and Moog, E., *Arch. Exptl. Pathol. Pharmakol.*, **244**, 270 (1962)
72. Greenberg, M. J., and Windsor, D. A., *Science*, **137**, 534 (1962)
73. Groschel-Stewart, U., and Turba, F., *Biochem. Z.*, **337**, 104 (1963)
74. Groschel-Stewart, U., and Turba, F., *Biochem. Z.*, **337**, 109 (1963)
75. Grupp, G., *Fed. Proc.*, **22**, 184 (1963)
76. Haas, H. G., and Trautwein, W., *Nature*, **197**, 80 (1963)
77. Hajdu, S., Weiss, H., and Titus, E., *J. Pharmacol.*, **120**, 99 (1957)
78. *Handbook of Physiology, Sect. 2: Circulation*, 1 (Hamilton, W. F., Ed., Williams and Wilkins, Baltimore, 1962)
79. Harrison, D. C., Chidsey, C. A., and Braunwald, E., *Proc. Soc. Exptl. Biol. Med.*, **112**, 37 (1962)
80. Harrison, D. C., Chidsey, C. A., Goldman, R., and Braunwald, E., *Circ. Res.*, **12**, 256 (1963)
81. Haugaard, N., *Nature*, **197**, 1072 (1963)
82. Hertting, G., Axelrod, J., and Whitby, G., *J. Pharmacol.*, **134**, (1961)
83. Hess, M. E., and Haugaard, N., *J. Pharmacol.*, **122**, 169 (1958)
84. Hess, M. E., Shanfeld, J., and Haugaard, N., *Biochem. Pharmacol.*, **11**, 1031 (1962)
85. Hess, M. E., Shanfeld, J., and Haugaard, N., *J. Pharmacol.*, **135**, 191 (1962)
86. Holland, W. C., and Lullmann, H., *Arch. Exptl. Pathol. Pharmakol.*, **243**, 495 (1962)
87. Holmes, J., and Fowler, N., *Circ. Res.*, **10**, 1 (1962)
88. Honig, C. R., Stam, A. C., Jr., and Mahan, P. E., *Am. J. Physiol.*, **203**, 137 (1962)
89. Horwitz, D., Fox, S. M., and Goldberg, L. I., *Circ. Res.*, **10**, 237 (1962)
90. Hukovic, S., and Muscholl, E., *Arch. Exptl. Pathol. Pharmakol.*, **244**, 81 (1962)
91. Kahler, R. L., Thompson, R. H., Buskirk, E. R., Frye, R. L., and Braunwald, E., *Circulation*, **27**, 397 (1963)
92. Kahn, J. B., Jr., and Schindler, R., *Experientia*, **18**, 79 (1962)
93. Kahn, J. B., Jr., Eakin, E., and Levi, D. E., *Am. J. Physiol.*, **203**, 1130 (1962)
94. Kavaler, F., *Nature*, **196**, 1104 (1962)
95. Klaus, W., and Kuschinsky, G., *Arch. Exptl. Pathol. Pharmakol.*, **244**, 237 (1962)
96. Klaus, W., Kuschinsky, G., and Lullman, H., *Arch. Exptl. Pathol. Pharmakol.*, **2442**, 480 (1962)
97. Klaus, W., Kuschinsky, G., and Lullmann, H., *Klin. Wochschr.*, **16**, 823 (1962)

98. Klouda, M. A., *Proc. Soc. Exptl. Biol. Med.*, **112**, 728 (1963)
99. Koch-Weser, J., *Am. J. Physiol.*, **204**, 451 (1963)
100. Koch-Weser, J., and Blinks, J. R., *J. Pharmacol.*, **136**, 305 (1962)
101. Kopin, I. J., Hertting, G., and Gordon, E. K., *J. Pharmacol.*, **138**, 34 (1962)
102. Kroneberg, G., and Schumann, H. J., *Arch. Exptl. Pathol. Pharmakol.*, **243**, 16 (1962)
103. Kruta, V., *Arch. Intern. Physiol.*, **70**, 443 (1962)
104. Kruta, V., and Braveny, P., *Proc. I.U.P.S., XXII, Intern. Congr.*, 137 (1962)
105. Kruta, V., and Braveny, P., *Nature*, **197**, 905 (1963)
106. Kruta, V., Braveny, P., Hlavkova-Stejskalova, J., and Husakova, B., *Scripta Medica*, **36**, 1 (1963)
107. Kukovetz, W. R., *Arch. Exptl. Pathol. Pharmakol.*, **243**, 391 (1962)
108. Kukovetz, W. R., and Hess, M. E., Shanfeld, J., and Haugaard, N., *J. Pharmacol.*, **127**, 122 (1959)
109. La Barre, J., *Rev. Belge Pathol.*, **28**, 333 (1961)
110. Levy, J. V., and Richards, V., *Proc. Soc. Exptl. Biol. Med.*, **112**, 225 (1963)
111. Levy, J. V., Ichiyanagi, K., and Frederickson, E. L., *Anesthesiology*, **24**, 185 (1963)
112. Levy, J. V., and Richards, V., *Proc. Soc. Exptl. Biol. Med.*, **112**, 225 (1963)
113. Luchi, R. J., Helwig, J., Jr., and Conn, H. L., Jr., *Am. Heart. J.*, **65**, 340 (1963)
114. Lullmann, H., and Holland, W., *J. Pharmacol.*, **137**, 186 (1962)
115. Mahon, W. A., and Mashford, L., *J. Clin. Invest.*, **42**, 338 (1963)
116. Mahaffey, J. E., Aldinger, E. E., Sprouse, J. H., Darby, T. D., and Thrower, W. B., *Anesthesiology*, **22**, 982 (1961)
117. Marchetti, P. G., Maccari M., and Merlo, L., *Cardiologia*, **42**, 1 (1963)
118. Marchetti, G., and Nava, S., *Arch. Ital. Sci. Farmacol.*, **10**, 3 (1960)
119. Marks, B. H., and Dutta, S. (Unpublished observations)
120. Marks, B. H., Samorajski, T., and Webster, E. J., *J. Pharmacol.*, **138**, 376 (1962)
121. Marro, F., and Capraro, V., *Arch. Ital. Biol.*, **99**, 413 (1961)
122. Mashford, M. L., Bion, J., Wolochow, D. A., and Mahon, W. A., *Proc. Soc. Exptl. Biol. Med.*, **3**, 308 (1962)
123. Mason, D. T., and Braunwald, E., *Clin. Res.*, **10**, 176 (1962)
124. Matsuo, T., *Japan. J. Pharmacol.*, **12**, 62 (1962)
125. Matsuo, T., and Tachi, S., *Japan. J. Pharmacol.*, **12**, 191 (1962)
126. Maxwell, G. M., Elliott, R. B., and Kneebone, G. M., *Australian J. Exptl. Biol. Med., Sci.*, **40**, 335 (1962)
127. Mayer, S. E., *Biochem. Pharmacol.*, **12**, 183 (1963)
128. Mayer, S. E., Cotten, M. deV., and Moran, N. C., *J. Pharmacol.*, **139**, 3 (1963)
129. Mayer, S. E., and Moran, N. C., *J. Pharmacol.*, **129**, 271 (1960)
130. McDonald, R. H., Jr., and Goldberg, L. I., *J. Pharmacol.*, **140**, 60 (1963)
131. Meyer, H. F., and Kukovetz, W. R., *Arch. Exptl. Pathol. Pharmakol.*, **242**, 409 (1962)
132. Miyahara, M., *Japan. Circ. J.*, **26**, 8 (1962)
133. Miyahara, M., Yokoyama, M., Nagasaki, Y., and Kinjo, K., *Japan. Heart J.*, **3**, 46 (1962)
134. Moe, R. A., *Arch. Intern. Pharmacodyn.*, **132**, 295 (1961)
135. Mommaerts, W. F., and Langer, G. A., *Ann. Rev. Med.*, **14**, 261 (1963)
135a. Mommaerts, W. F. H. M., Seraydarian, K., and Uchida, K., *Biochem. Biophys. Res. Commun.*, **13**, 58 (1963)
136. Montanari, R., Costa, E., Beaven, M. A., and Brodie, B. B., *Life Sciences No. 4*, 232 (1963)
137. Moore, J. I., and Moran, N. C., *J. Pharmacol.*, **136**, 89 (1962)
138. Morrow, D. H., Gaffney, T. E., and Braunwald, E., *J. Pharmacol.*, **140**, 236 (1963)
139. Morrow, D. H., and Morrow, A. G., *Anesthesiology*, **22**, 537 (1961)
140. Murad, F., Chi, Y. M., Rall, T. W., and Sutherland, E. W., *J. Biol. Chem.*, **237**, 1220 (1962)
141. Nagasaki, Y., *Japan. Circ. J.*, **26**, 137 (1962)
142. Nayler, W. G., *J. Pharmacol.*, **139**, 222 (1963)
143. Nayler, W. G., and Emery, P. F., *Am. J. Physiol.*, **203**, 844 (1962)
144. Olson, R., Ellenbogen, E., and Iyengar, R., *Circulation*, **24**, 471 (1961)
145. Parker, C. J., Jr., and Yun, J., *Biochem. Biophys. Res. Commun.*, **11**, 88 (1963)
146. Penefsky, Z. J., and Hoffman, B. F.,

*Am. J. Physiol.*, **203**, 433 (1963)
147. Petter, A., and Zipf, K., *Arch. Exptl. Pathol. Pharmakol.*, **243**, 566 (1962)
148. Pohle, W., and Hansjugren, M., *Acta Biol. Med. Germ.*, **8**, 167 (1962)
149. Portius, H. J., and Repke, K., *Arch. Exptl. Pathol. Pharmakol.*, **243**, 335 (1962)
150. Potter, L. T., and Axelrod, J., *J. Pharmacol.*, **140**, 199 (1963)
151. Price, H. L., Cook, W. A., Jr., Deutsch, S., Linde, H. W., Mishalove, R. D., and Morse, H. T., *Anesthesiology*, **24**, 1 (1963)
152. Price, H. L., Linde, H. W., Jones, R. E., Black, G. W., and Price, M. L., *Anesthesiology*, **20**, 563 (1959)
153. Price, H. L., Lorie, A. A., Black, G. W., Sechzer, P. H., Linde, H. W., and Price, M. L., *Ann. Surg.*, **152**, 1071 (1960)
154. *Proc. Intern. Pharmacol. Meeting, 1st Meeting* (Pergamon, London, 1962)
155. Rall, T. W., and West, C., *J. Pharmacol.*, **139**, 269 (1963)
156. Reiter, M., *Arch. Exptl. Pathol. Pharmakol.*, **242**, 497 (1962)
157. Repke, K., and Portius, H. J., *Arch. Exptl. Pathol. Pharmakol.*, **245**, 59 (1963)
158. Robb, J., *J. Pharmacol.*, **135**, 323 (1962)
159. Rodensky, P. L., Sierra, A., Keyes, M. H., and Wasserman, F., *Am. J. Cardiol.*, 11, 368 (1963)
160. Rodman, T., and Pastor, B. H., *Am. Heart J.*, **65**, 564 (1963)
161. Rosen, A., and Moran, N. C., *Circ. Res.*, **12**, 479 (1963)
162. Samorajski, T., and Marks, B. H., *J. Histochem. Cytochem.*, **10**, 392 (1962)
163. Samorajski, T., Marks, B. H., and Webster, E. J., *J. Pharmacol.* (In press) (1963)
164. Sarnoff, S. J., Gilmore, J. P., Mitchell, J. H., and Remensynder, J. P., *Am. J. Med.*, **34**, 440 (1963)
165. Schaer, Von H., *Cardiologia*, **40**, 48 (1962)
166. Schevill, W. E., Watkins, A., and Ray, C., *Science*, 141, 47 (1963)
167. Schoepke, H. G., and Shideman, F. E., *J. Pharmacol.*, **135**, 358 (1962)
168. Schwartz, A., *Biochem. Biophys. Res. Commun.*, **9**, 301 (1962)
169. Shimosato, S., and Etsten, B., *Anesthesiology*, **24**, 41 (1963)
170. Shimosato, S., Li, T. H., and Etsten, B., *Circ. Res.*, **12**, 63 (1963)
171. Siegel, J. H., and Sonnenblick, E. H., *Circ. Res.*, **12**, 597 (1963)
172. Skou, J. C., *Biochim. Biophys. Acta*, **23**, 394 (1957)
173. Smith, J. R., and Fozzard, H. A., *Nature*, **197**, 562 (1963)
174. Sonnenblick, E. H., *Am. J. Physiol.*, **202**, 931 (1962)
175. Sonnenblick, E. H., Spiro, D., and Cottrell, T. S., *Proc. Natl. Acad. Sci. U. S.*, **49**, 193 (1963)
176. Spector, S., Sjoerdsma, A., Zaltzman-Nirenberg, P., Levitt, M., and Udenfriend, S., *Science*, **139**, 1299 (1963)
177. Stam, A. C., and Honig, C. R., *Biochim. Biophys. Acta*, **58**, 139 (1962)
178. Stam, A. C., and Honig, C. R., *Biochim. Biophys. Acta*, **60**, 259 (1962)
179. Stone, C. A., Ross, C. A., Wenger, H. C., Ludden, C. T., Blessing, J. A., Totaro, J. A., and Porter, C. C., *J. Pharmacol.*, **136**, 80 (1962)
180. Swaine, C. R., *Proc. Soc. Exptl. Biol. Med.*, **112**, 388 (1963)
181. Tachi, S., Matsuo, T., Fujiwara, M., and Toda, N., *Japan. J. Pharmacol.*, **12**, 197 (1962)
181a. Takauji, M., and Nagai, T., *Biochem. Biophys. Res. Commun.*, **13**, 54 (1963)
182. Tanz, R., *J. Pharmacol.*, **135**, 71 (1962)
183. Tice, L. W., and Barrnett, R. J., *J. Cell Biol.*, **15**, 401 (1962)
184. Torchiana, M. L., and Angelakos, E. T., *J. Pharmacol.*, **137**, 193 (1962)
185. Tuttle, R. S., and Farah, A., *J. Pharmacol.*, **135**, 142 (1962)
186. Tuttle, R., Witt, P., and Farah, A., *J. Pharmacol.*, **133**, 281 (1961)
187. Tuttle, R. S., Witt, P. N., and Farah, A., *J. Pharmacol.*, **137**, 24 (1962)
188. Uchida, K., and Mommaerts, W. F. H. M., *Biochem. Biophys. Res. Commun.*, **10**, 1 (1963)
189. Ueda, I., Fukishima, K., Ballinger, C. M., and Loehning, R. W., *Anesthesiology*, **23**, 342 (1962)
190. Vassalle, M., and Karis, J., and Hoffman, B. F., *Am. J. Physiol.*, **203**, 433 (1962)
191. Vincent, N. H., and Ellis, S., *J. Pharmacol.*, **139**, 60 (1963)
192. Wanebo, H., Katsh, S., and Bromberger-Barnea, B., *Arch. Intern. Pharmacodyn.*, **137**, 115 (1962)
193. Waser, P. G., *Experientia*, **18**, 1 (1962)
194. Weber, A., and Herz, R., *J. Biol. Chem.*, **238**, 581 (1963)

195. Weber, A., and Herz, R., *J. Biol. Chem.*, **238,** 599 (1963)

196. Weber, A., Herz, R., and Reiss, I., *J. Gen. Physiol.*, **46,** 679 (1963)

197. Weissler, A. M., Gamel, W. G., Grode, H. E., Cohen, S., and Schoenfeld, C. D., *J. Clin. Invest.* (In press)

198. Weissler, A. M., Harris, L. C., and White, G. D., *J. Appl. Physiol.* (In press)

199. Weissler, A. M., Peeler, R. G., and Roehill, W. H., Jr., *Am. Heart J.*, **62,** 367 (1961)

200. Wenzel, D. G., and Siegel, I. A., *J. Pharm. Sci.*, **51,** 1074 (1962)

201. West, J. W., *Am. J. Physiol.*, **203,** 1145 (1962)

202. Winegrad, S., and Shanes, A. M., *J. Gen. Physiol.*, **45,** 371 (1962)

203. Wood, W. B., Manley, E. S., Jr., and Woodbury, R. A., *J. Pharmacol.*, **139,** 238 (1963)

204. Young, W., and Upham, F., *Am. J. Physiol.*, **202,** 947 (1962)

205. Zimmerman, H. B., Gentsch, K. W., and Gale, A. H., *Diseases Chest*, **43,** 377 (1963)

# CONTENTS

OUTLINES OF A PHARMACOLOGICAL CAREER, *Ernst Rothlin* . . . . ix
BIOCHEMICAL MECHANISM OF DRUG ACTION, *Jack R. Cooper* . . . 1
RECEPTOR MECHANISMS, *Robert F. Furchgott* . . . . . 21
MODERN CONCEPTS IN RELATIONSHIP BETWEEN STRUCTURE AND BIOLOGICAL ACTIVITY, *F. N. Fastier* . . . . . 51
MECHANISMS OF DRUG ABSORPTION AND EXCRETION, *Ruth R. Levine* and *Edward W. Pelikan* . . . . . 69
METABOLIC FATE OF DRUGS, *R. T. Williams and D. V. Parke* . . . 85
ANTIBACTERIAL CHEMOTHERAPY, *Mary Barber and E. B. Chain* . . 115
CARDIOVASCULAR PHARMACOLOGY, *Domingo M. Aviado* . . . . . 139
EFFECT OF DRUGS ON THE INOTROPIC PROPERTY OF THE HEART, *Bernard H. Marks* . . . . . 155
PHARMACOLOGY OF REPRODUCTION AND FERTILITY, *Louis Fridhandler and Gregory Pincus* . . . . . 177
EFFECT OF DRUGS ON CONTRACTIONS OF VERTEBRATE SMOOTH MUSCLE, *E. E. Daniel* . . . . . 189
TOXICOLOGY: ORGANIC, *Horace W. Gerarde* . . . . . 223
TOXICOLOGY: INORGANIC, *George Roush, Jr., and Robert A. Kehoe* . . 247
DRUG ALLERGY, *Max Samter and George H. Berryman* . . . . . 265
KININS—A GROUP OF ACTIVE PEPTIDES, *M. Schachter* . . . . . 281
COMPOSITION AND MODE OF ACTION OF SOME INVERTEBRATE VENOMS, *John H. Welsh* . . . . . 293
NEW SUBSTANCES OF PLANT ORIGIN, *T. A. Geissman* . . . . . 305
EXCERPTS FROM THE PHARMACOLOGY OF HORMONES AND RELATED SUBSTANCES, *José Ribeiro do Valle* . . . . . 317
EFFECTS OF DRUGS ON THE CENTRAL NERVOUS SYSTEM, *Harry Grundfest* . . . . . 341
PHARMACOLOGY OF THE AUTONOMIC NERVOUS SYSTEM, *Eleanor Zaimis* 365
REVIEW OF REVIEWS, *Chauncey D. Leake* . . . . . 401
AUTHOR INDEX . . . . . 411
SUBJECT INDEX . . . . . 431
CUMULATIVE INDEXES, VOLUMES 1–4 . . . . . 450