CHEMICAL FACTORS IN HYPERTENSION

A collection of the papers and discussion presented at the Symposium on Chemical Factors in Hypertension held by the Division of Medicinal Chemistry of the American Chemical Society at the 115th national meeting in San Francisco, March 28 to April 1, 1949

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FOREWORD

Hypertension is today one of the major and most complex problems of medical practice. As in all other areas affecting human welfare, chemistry must play a part in its final solution. When the chemist, the physiologist, the pharmacologist, and the clinician can meet on common ground to discuss the factors involved and can join forces in planning new lines of attack, we may confidently expect new advances in a better understanding of, and in methods for, control of this disease.

Grateful acknowledgment is made to William G. Clark, who was primarily responsible for the program and who acted as chairman of the symposium, and to Harry Goldblatt for the introduction to this volume. We particularly appreciate the fine cooperation of the speakers in participating in the symposium and in preparing their manuscripts for publication.

GLENN E. ULLYOT, *Secretary-Treasurer*
Division of Medicinal Chemistry, 1949

Introduction

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This symposium is a timely one, for it demonstrates clearly that it takes a long time to establish beyond question any single advance in medical knowledge. More than 15 years have passed since the successful production of a persistent type of hypertension in animals by interference with the hemodynamics of their kidneys. Confirmation of this finding came quickly, but the exact mechanism of the development, and especially of the maintenance, of this type of hypertension is by no means established and is still the subject of much study.

From what has already been published, and from what is contained in this symposium, it is abundantly clear that at least the early period of the hypertension which develops after constriction of the main renal arteries of animals is of humoral origin. The difficulty has been, and still is, the direct application of what has been learned about experimental renal hypertension to the problem of the pathogenesis of human essential hypertension. Of the greatest importance would be the determination of the exact cause of the relatively long period of experimental hypertension and of human hypertension in which, up to the present time, the existence of a humoral mechanism has not been proved.

Schroeder evidently believes in the chemical mechanism, even of arterial hypertension in man; yet he regards the initial condition as primarily of psychosomatic (sympathetic) origin, with the neurogenic vasoconstriction in the vascular bed of the kidneys resulting, in the initial stages, in the release of the renal humoral pressor mechanism and, in the later stages, the appearance of a variety of pressor substances, probably acting in combination. It is interesting that most of these pressor substances, with the exception of norepinephrine, are also of renal origin. It is clear that much remains to be learned about the interrelationship of these pressor substances which may have a common or similar effector substance.

It is comforting to hear Beyer say that "the various concepts of the etiology of essential hypertension are not mutually exclusive" and that a combination of these theories may be the correct idea. He, too, agrees that the initial vasoconstriction is probably motivated by autonomic impulses, with the release of pressor substances that induce the development of vascular disease in the kidneys and other organs. The weakness of Beyer's concept is that there is no unequivocal proof that hypertension per se produces arteriosclerosis. Even in the hypertension associated with pheochromocytoma, for example, the development of vascular disease in the kidneys or other organs, as a direct result of the hypertension, has not been established.

Role of Adrenergic Blockade

Nickerson also agrees that neurogenic factors may play a part in the origin, mechanism, and maintenance of essential hypertension; yet he admits that the role of adrenergic blockade (chemical sympathectomy) in the therapy of hypertension is still obscure. Even if the renin-hypertensin mechanism could be shown to play an important part, at one stage or another, in the origin of human essential hypertension, yet the nature of the stimulus for the formation or liberation of the renin from the kidney into the blood must still be discovered. This is a very important gap in our knowledge, on the elucidation of which Erwin Haas and the writer have been working for several years.

The participants in this symposium have not dealt to any extent with the subject of

antirenin, but this continues to be an important matter and deserves more consideration than it has received in the past. In our hands it has proved an interesting and important tool. It is highly probable that humoral pressor substances, producing vasoconstriction, are concerned in the initiation of arterial hypertension in man. It may be, as all the authors have indicated, that vasoconstrictor effector substances act in conjunction with neurogenic stimuli to bring about the persistence of the hypertension. Just what part certain hormones of the adrenal cortex play in this phenomenon is certainly not established. The fact that the substances responsible for the elevation of blood pressure are present in such a small amount, and that at least some of them are highly unstable, multiplies the difficulties. As Schroeder has stated, "The identification of the nature of these materials is essential to the elucidation of the mechanism of this common disease or group of diseases."

Although the development of agents capable of specifically and effectively blocking response to sympathoadrenal activity is of the greatest importance, yet their significance in the treatment of clinical hypertension will certainly depend, as Nickerson has stated, "upon the establishment of the part played by the sympathoadrenal system in this type of hypertension." Except in a few rare types of hypertension, this role is still obscure. There is certainly no definite similarity between what is generally recognized as a neurogenic type of experimental hypertension (carotid sinus hypertension) and what is usually called essential human hypertension. Nickerson admits, and the writer, of course, believes, that hypertension resulting from interference with renal hemodynamics resembles closely essential hypertension of man, and he goes so far as to state "that the development of this type of hypertension is completely independent of a nervous mechanism." With this the writer also agrees, and believes with him that the assertion that nervous factors are important in the late stage of renal hypertension is far from being conclusively established and that the crucial experiments or observations to prove this are still to be made.

The results of sympathectomy have not yet clearly defined the extent to which surgical elimination of sympathetic vasoconstriction may be expected to lower the blood pressure in essential human hypertension and there is therefore no good reason for believing that chemical (adrenergic) blockade can accomplish more than surgical excision. The unfortunate thing is that many of the adrenergic blocking agents have side effects that are undesirable.

The participants in this symposium are to be congratulated upon the restraint they have practiced in the fair presentation of their own views and upon their sincere attempt carefully to weigh the available evidence upon which they have based their cautious and altogether reasonable, even if still debatable, conclusions. In this publication those interested in the subject of hypertension, from both the experimental and clinical standpoint, will find much valuable information and food for thought.

Humoral Pressor Substances and Their Relation to Arterial Hypertension

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Arterial hypertension, one of the most important diseases under modern social and economic conditions, presents a major problem to biochemistry: the discovery of specific therapeutic measures for its alleviation. This paper discusses known humoral substances, examines each in the light of its possible relation to hypertension, and considers other evidence of the existence of such substances and their chemical structure. Humoral pressor mechanisms are probably initiated by neurogenic ones and therefore comprise only one link in the chain of events that lead to chronic hypertension.

The discovery of specific therapeutic measures directed at arterial hypertension has become one of the major problems of biochemistry. The prevalence of this condition in the modern social and economic environment has made it one of the most important diseases to which man is heir. It is time, therefore, to examine the progress made during the past few years in the elucidation of the pathogenesis of this disease, especially those factors of biochemical interest.

Arterial hypertension is very common. Approximately 40% of the population over the age of 40 exhibit elevations of blood pressure, more than 140 mm. of mercury systolic and 90 mm. diastolic (*64*). The incidence increases with advancing years. It appears to be greater in negroes (*2, 3, 23*), the obese (*33*), and those exposed to higher degrees of civilization (*16*).

The effects of chronic hypertension on the human organism are, with one exception, of little interest to the investigator studying pathogenesis, although of great import to the sufferer and his physician. That exception is failure of the kidneys. Disease and failure of the heart are probably caused by chronic overstrain, often associated with another metabolic disease, arteriosclerosis of the coronary arteries. They account for about two thirds of the deaths primarily due to hypertension. Strokes of apoplexy, or cerebral vascular accidents, from rupture or thrombosis of a cerebral artery weakened by disease cause another sixth, uremia about one twelfth, and other conditions the remainder (*28*). Except for uremia, these events are usually the result of overwork and increased arterial tension. Only rarely does the heart escape hypertrophy.

Aside from major changes in organs, the only constant pathological findings concern the arterioles; their walls are thickened, the ratios of wall to lumen being increased. Degeneration of muscular coats, intimal thickening, and sometimes (in "malignant" hypertension) necrosis occur. These alterations are most marked in the kidneys, but are found less constantly in most other areas. The evidence that arteriolar disease is secondary to, and is not the primary cause of, chronic hypertension is conclusive, although indirect. Experimentally, similar lesions can often be produced in the kidneys of certain animals (*56, 67*), when renal hypertension is induced. In the case of the dog, lesions ap-

pear less readily, and special techniques must be used (*52*). In almost half of a series of renal biopsies taken from hypertensive patients little or no arteriolar sclerosis was seen (*11*), whereas almost all the kidneys of such individuals show it at autopsy. Certainly, the nature of the lesions themselves suggest that they cause renal ischemia; although a result of hypertension, they probably contribute to its maintenance and further progress.

Therefore, arterial hypertension may begin as a generalized physiological alteration in hemodynamics, producing secondary pathological changes in arterioles. Sustained hypertension leads to cardiac hypertrophy and more renal arteriolar sclerosis. Finally, because of overwork, overstrain, and the frequent association of arteriosclerosis, the heart or brain is permanently damaged and death ensues. The duration of life from onset of hypertension to terminus is extremely variable (6 months to over 40 years).

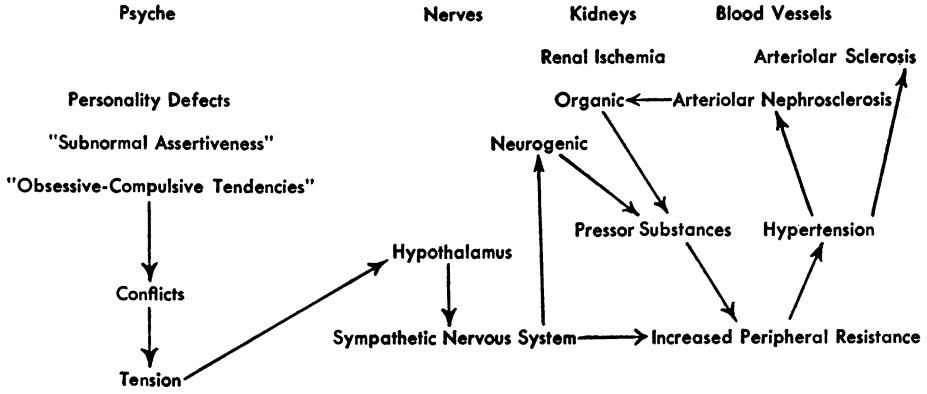

Figure 1. Pathogenesis of Psychoneurogenic Hypertension

Probable sequence of events leading to sustained hypertension when accessory etiological factors are absent. Repressed emotional tension causes discharges of the sympathetic nervous system via the hypothalamus, which lead to generalized vasoconstriction. The kidneys are included in the response. The resultant disturbance of intrarenal hemodynamics causes production of pressor substances. Humoral vasoconstriction lasts longer than the neurogenic type. Repeated discharges eventually lead to sustained nephrogenic hypertension, which is accompanied by changes in the walls of the arterioles, especially those of the kidneys. The organic renal ischemia caused thereby is part of a vicious circle, in which the continued production of pressor substances is predominant. The neurogenic element is superimposed thereon. When organic renal diseases, infectious, destructive, metabolic, or arterial, are present as accessory etiological factors, the course of hypertension is often altered. When the adrenal cortex is hyperactive, the vicious circle may be instituted by direct action of its hormones upon arteriolar muscle.

Effects of Psychic Disturbances

It is becoming increasingly evident that arterial hypertension in man is usually a psychosomatic disease. Therefore, to understand pathogenesis one must examine the psyche and the effects of psychic disturbances upon the soma. It is not within the province of this discussion to consider at length such disorders and their relation to hypertension. For purposes of orientation toward a view of the whole picture, however, we must scrutinize briefly the influences which bear on this condition in order to direct our attention to salient features which deserve further investigation. Suffice it to say, therefore, that disorders or deficiencies of personality exist (*30*); that they antedate the occurrence of hypertension (*8*); and that the emotional tension arising from their presence profoundly affects vegetative functions, especially those concerned with blood flow and blood pressure (*68*).

The pathogenesis of neurogenic hypertension appears to be somewhat as follows:

Emotional tension is discharged via the hypothalamus and the sympathetic nervous system.

Neurogenic (sympathetic) vasoconstriction occurs, with its well-known effects upon heart and blood vessels.

The renal circulation takes part in the generalized vasoconstrictive process.

When renal vasoconstriction is relatively greater than the concomitant rise in blood pressure and increase in cardiac output—i.e., when total renal blood flow is lowered—the

kidneys are stimulated to release into the blood humoral vasoconstrictor substances which have a more prolonged action and maintain the elevation of blood pressure.

Repeated sympathetic discharges lead to repetitions of this phenomenon, causing transient but more prolonged periods of hypertension.

The hypertension itself damages renal arterioles, which become hypertrophied and narrowed. Thus, organic renal ischemia results, in itself causing continuous production of pressor substances.

On this organic change, functional neurogenic vasoconstriction is superimposed.

The whole process may take years to develop, rarely months, and is a dynamic one, with changes constantly occurring (Figure 1). When organic renal or urologic diseases are present concomitantly, or sclerosis of the larger renal arteries develops, the course is likely to be altered by the associated disturbance.

The purpose of this report is to discuss known humoral substances, to examine each in the light of its possible relation to hypertension, and to consider other evidence, both direct and indirect, for the existence of such substances and for their chemical structures. Inquiry into the neurogenic influences of this condition and methods for neutralizing them will be discussed by others (*6*, *42*). It must be remembered, however, that humoral pressor mechanisms are probably usually initiated by neurogenic ones, and therefore comprise only one link—even though the most important one—in the chain of events which lead to chronic hypertension.

Sixteen different substances are known or have been found in body fluids or modifications of them, which either cause acute hypertension or lead to vasoconstriction in the experimental animal (Table I). Three of these substances are proteins, three peptides, six amines, two nicotinelike bases, and one steroid. Other unidentified substances have been detected. The prodigality of Nature in providing so many substances which act in

Table I. Pressor Substances Possibly Related to Hypertension[a]

Name	Source	Accessory Conditions	Chemical Nature	Pharmacologic Action	Obtained Pure	Found in Hypertension	Similarity to *Hochdruckstoff*
Proteins							
Renin	Kidney	Renal ischemia	Protein	Prolonged	No	Acute	Yes
VEM	Kidney	Renal ischemia and anoxia	Protein (?)	Prolonged	No	Yes	Unknown
Prolonged pressor subs.	Kidney and blood	Hypotension (renal ischemia)	Protein	Very prolonged	No	(?)	Unknown
Peptides							
Hypertensin	α_2-Globulin	Renin	Peptide	Acute	No	Acute	Yes
Pepsitensin	α_2-Globulin	Pepsin	Peptide	Acute	No	No	Yes (?)
Serotonin	Blood	Standing	Peptide	Acute	Yes (?)	No	Unknown
Amines							
"Amines"	Blood	Anoxia and ischemia	Amine	Acute	Yes	Yes	Probably
Norepinephrine	Tissue	Nervous stimuli	Amine	Acute	Yes	(?)	Yes (?)
Urosympathin	Urine	Nervous stimuli	Amines	Acute	No	Yes	Unknown
Von Euler's substance I	Blood and tissues	Nervous stimuli	Norepinephrine	Acute	No	No	Yes (?)
Victor's material	Kidney	Anaerobic autolysis	Tyramine (?)	Acute	No	No	Unknown
Pherentasin	Arterial blood	Hypertension (renal)	Aminopeptide (?)	Prolonged	No	Yes	Unknown
Nicotine bases							
Lockett's base	Urine and blood	Renal ischemia	Complex alkaloid	Acute	No	Experimental only	Unknown
Urohypertensin	Urine	Renal ischemia	Nicotine base (?)	Acute	No	Yes (?)	Unknown
Others							
Nephrin	Kidney	Renal ischemia	Unknown	Prolonged	No	Yes	Unknown
Desoxycorticosterone	Adrenal cortex	Salt and hypertension (?)	Steroid	Prolonged	Yes	Certain types (?)	Yes (?)

[a] List taken from literature. Where ? is shown, characteristic of material is in doubt. Chemical identification and pharmacology of most of these substances have been insufficiently studied.

similar ways to maintain blood pressure and blood flow appears unjustifiable even for so vital a function. The possibility therefore exists that the effector substances, the final active principles, of many of these materials may be of similar chemical structure, and that the activity of the materials on the circulation was by chance demonstrated during some stage of their intermediary catabolism.

In order to determine whether each of these substances might or might not be the active agent in chronic hypertension, it is necessary first to postulate a hypothetical material which fulfills the pharmacological requirements to produce the hemodynamic picture seen in hypertension. For purposes of brevity, we will call this material the *Hochdruckstoff*. Its characteristic action is to cause: (1) elevation of blood pressure; (2) generalized and relatively equal vasoconstriction of all vascular beds; (3) no great change in cardiac output or pulse rate; (4) renal vasoconstriction, greater on efferent arterioles; and (5) no visible sympathetic effects (sweating, pallor, spasm of sphincters, etc.). It is not necessary for the substance to have a prolonged action, provided continuous production is assured. When a pressor agent acts in this manner, and can occur naturally in the body, attention is immediately given it as a possible effector substance in hypertension. Unfortunately, we do not know for certain whether peripheral resistance is equally increased in all vascular beds, or whether some are relatively more constricted than others. This criterion (2) therefore cannot be absolute, but at least we can say that epinephrine and epinephrinelike substances are not the principal effector agents, as they do not fulfill requirements (2), (3), and (5).

Nephrogenic Protein Pressor Substances

The three protein pressor substances are renin, a prolonged pressor substance found in shock, and vasoexcitor material. Apparently they are different substances. Renin is the best understood (*10*). Its reaction with a globulin substrate to form hypertensin or angiotonin suggests that it is a proteolytic enzyme. Renin has been considerably concentrated, but has not been purified. The stimulus for its release by the kidney is a reduction of renal blood flow; just how this comes about is unknown.

Certain readjustments take place in the renal circulation when blood flow is acutely reduced by arterial constriction; internal vasodilatation occurs slowly and total blood flow may return toward or to normal, although the actual renal arteriolar resistance must be considerably lowered (*58*). This phenomenon is seen when blood flow is measured by a thermostromuhr, or when oxygen tension in the cortex is estimated by an electrode. One measurable change does occur under these conditions, however. Although oxygen tension and blood flow may be normal while the renal artery is partially constricted, the pH of renal cortical tissue changes in the acid direction (*43*). Further constriction of the renal artery, of course, permanently reduces renal blood flow, the cortex continuing to have a higher hydrogen ion concentration. Although renin is released under these conditions, its relation to lowered pH has not been demonstrated.

The site of formation of renin is not known, although the indirect and circumstantial evidence favors slightly the juxtaglomerular apparatus rather than the tubules as a source (*18*). Crude renin, however, is extracted readily from renal cortex by saline extraction, acidification, and precipitation with ammonium sulfate and sodium chloride. Other active protein substances are likewise extracted, and their separation from renin is often a matter of considerable difficulty. The renin substrate, an α_2-globulin, is found in blood serum and is probably formed by the liver. It can be easily salted out of beef serum as a crude preparation.

Under the proper stimulus renin is released into the circulating blood where it can be identified, especially in that from the renal vein. It acts rapidly upon its specific substrate, splitting the protein into peptides, one or more of which have been called "hypertensin" or "angiotonin." Hypertensin has been concentrated but not obtained in pure form. It is the effector substance of renin, constricting arterioles and raising blood pressure. The action of hypertensin is abolished by "hypertensinase," an enzyme found in blood and renal extracts. Fortunately, the latter is destroyed by heat and alkalinity.

The renal pressor mechanism just described acts in a manner that fulfills the require-

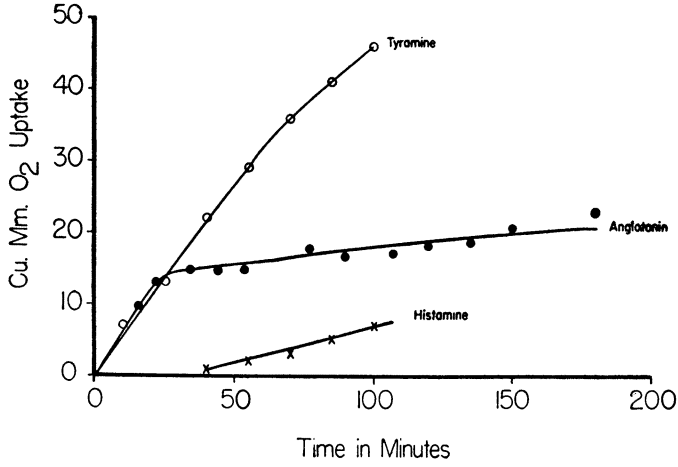

Figure 2. Oxygen Uptake by Amine Oxidase Preparations and Tyramine, Hypertensin (Angiotonin), and Histamine

Concentrations of tyramine and histamine were similar; that of angiotonin is estimated. The preparation, which contained active amine oxidase and no other oxidases, was supplied by Karl Folkers of Merck and Company. Data were obtained from C. C. Stock, to whom the author is indebted.

ments of the *Hochdruckstoff*. Demonstrable amounts of renin, however, have been found in renal venous blood of dogs and human beings only during the acute stage of hypertension. In acute glomerulonephritis, toxemia of pregnancy, and a few other conditions renin is released by ischemic kidneys (*14*); as the hypertension becomes more chronic it disappears to a point lower, if anything, than in renal venous blood of normal individuals. Renin is also demonstrable in other conditions, such as congestive heart failure and shock (*15*). It is found likewise only in the early stages of experimental hypertension and in experimental shock. Either methods for its detection are too crude, or some other mechanism takes over its function in chronic elevation of the blood pressure.

Renin can be antigenic; dogs will produce antibodies to hog renin. Wakerlin (*66*) and his group found that in a certain proportion of experiments the development of "antirenin" was accompanied by a lower blood pressure or protection against experimentally induced renal hypertension. The renin used, however, was impure, and other active or antigenic substances were probably present. Similar effects against hypertension could be caused by renal extracts, the renin of which had been destroyed. Some other antihypertensive substance may have accounted for part of his results. Kidneys are known to contain antihypertensive materials effective in rats, dogs, and probably man, although little attention has been paid them in recent years (*44*).

The available evidence suggests, therefore, that this renal pressor mechanism is an important factor in the maintenance of blood pressure through vasoconstriction, without marked effect upon the output of the heart. There are several such mechanisms. The sympathetic nervous system and chromaffin tissue exert an emergency action by way of neurogenic vasoconstriction and cardiac stimulation. The renal pressor mechanism is called into play under more severe conditions of stress—i.e., when circulation through the kidney is impaired because of central or peripheral circulatory insufficiency (and in local pathological renal conditions). It requires a greater stimulus, acts longer, and disappears more slowly. A third, longer-acting and less easily evoked mechanism of wider implications is that whereby blood volume is increased, probably monitored in part by the adrenal cortex, and its action on salt and water balance. There may be other homeostatic mechanisms which are less well known. In regard to time of action, it appears that the neurogenic one acts in seconds, the renal in minutes and hours, and the adrenal in days. There is no direct evidence, however, that the last two are disturbed in most cases of experimental or clinical hypertension.

The second protein material to be considered is a prolonged pressor substance (PPS) first obtained by Shipley, Helmer, and Kohlstaedt (*62*) from the blood of animals dying after a long period of shock, and later obtained from human blood and from kidney tissue. It has not been purified. It is closely allied to, but not identical with, renin; although it can be demonstrated in most renin preparations, it is most difficult to separate. The preparation best suited to test for its presence is a 2-day nephrectomized pithed cat, in which very prolonged elevations of blood pressure can be produced. In this respect it differs from all other known pressor substances. Like renin it is relatively species specific, is not blocked by Dibenamine [*N*-(2-chloroethyl)dibenzylamine], and may be antigenic. Not only does it come from kidney tissue, but is inactivated or destroyed by kidney tissue, remaining in the circulation of nephrectomized animals for some time and capable of being transferred to other animals while retaining its activity. So far, prolonged pressor substance has not been found in human beings suffering from hypertension.

Although not strictly a pressor substance and not definitely established as a protein, the vasoexcitor material (VEM) of Shorr and his group (*69*) can be considered as the third of the nephrogenic protein vasoactive substances, until established otherwise. It probably plays a role in hypertension. Vasoexcitor material is obtained from an anaerobic autolyzate of renal tissue. It can be detected in minute amounts when injected intravenously by observing directly its principal action, which is to potentiate the vasoconstrictor action of topically applied epinephrine. The metarterioles of the rat's mesoappendix are the vessels commonly used for its determination. Vasoexcitor material is opposed in its action by a vasodepressor material (VDM) which produces an opposite type of response in arterioles, and which has been identified by immunological techniques as ferritin or apoferritin (*41*). Vasodepressor material is obtained from an anaerobic autolyzate of liver. Both materials are inactivated under aerobic conditions by the organs in which they are formed, presumably by enzymatic action.

It has not been conclusively demonstrated that potentiation of the vasoconstrictor action of epinephrine is a specific reaction confined only to vasoexcitor material. Other substances, in the authors' hands at least, will produce the same reaction. The action of vasoexcitor material, however, lasts considerably longer than that of hypertensin and other short-acting amines, but only a little longer than that of ephedrine and benzedrine. Therefore, it is impossible to apply this test as one specific for vasoexcitor material; it may be a general reaction common to other compounds, but is extremely valuable in detecting minute amounts of vasoexcitor material and vasoexcitor material-like substances. Similarly, depression of the reaction of arterioles to epinephrine is a phenomenon not confined to the action of vasodepressor material; in this laboratory the authors have seen definite prolonged, although less marked, depression of arteriolar response caused by adenosine triphosphate.

Regardless of the lack of specificity of the test, vasoexcitor material has been demonstrated in increased amounts in the blood of animals under the same conditions as renin is produced—i.e., shock, renal ischemia, and the early stages of experimental hypertension (*63*). It has also been found in the blood of human beings suffering from shock and congestive circulatory failure. When hypertension becomes chronic, however, vasoexcitor material can no longer be detected. During this stage a change in the relative concentrations of vasoexcitor material and vasodepressor material has occurred, so that they have now become equal, vasodepressor material having been formed by the liver in large amounts, eventually masking the action of vasoexcitor material. Fortunately, vasoexcitor material can be destroyed by incubation with renal tissue under high oxygen tensions, leaving the increased amount of vasodepressor material to be assayed. The assumption can therefore be made that, in chronic hypertension, vasoexcitor material is present in greatly increased amounts in the circulating blood.

From these very interesting observations we can speculate with reasonable assurance that blood pressure, or at least vasoconstriction and vasodilatation, is controlled by two oppositely acting substances and that in the hypertension of animals and human beings these two substances are increased in amounts. Most of the work on identification, purification, and characterization, using the rat's mesoappendix as a bioassay, has been done

on vasodepressor material; it is hoped that the chemical identification of vasoexcitor material will be soon forthcoming. There is no doubt now that these two substances play an important role in maintaining vasomotor tone and, furthermore, that the kidney and the liver are intimately identified with their formation and their inactivation. The findings of Shorr and his group have provided a new outlook upon the regulation of blood pressure and of arteriolar reactions to abnormal conditions. It is not difficult to believe that disturbances of these regulatory mechanisms, which are naturally affected by chemical substances, may lead to hypertension and may be important when blood pressure is altered in the opposite direction as in shock. But to be able to understand and to alter these disturbances in the normal direction by influences under our control, we must know their chemical nature and the exact mechanism of their formation.

There are curious similarities among these three nephrogenic pressor proteins which are worthy of mention. All are formed by kidneys under the stimulus of shock, which causes renal ischemia. Prolonged pressor substance has not been identified in renal venous blood of kidneys made ischemic by other means, but could conceivably be present. Prolonged pressor substance and vasoexcitor material are both found closely associated with extracts containing renin (although neither is renin) and can be separated only with considerable difficulty. The pharmacologic action of each is prolonged, suggesting slow continued action and breakdown, which is the case with renin. None has been obtained pure. Vasoexcitor material is found in solutions of hypertensin (*24*). There are certain chemical differences (heat stability, pH sensitivity), however, which suggest that they are different substances. The question to be answered is: Are these materials different aspects of the same general homeostatic mechanism, or do they represent three different mechanisms?

Peptide Pressor Agents

Of the three peptides, hypertensin is the most clearly understood, resulting as it does from the enzymatic action of renin on a specific substrate. It has already been discussed in that connection. Hypertensin is, however, considered separately, for we are not certain that renin (being impure) produces only a single effector substance (nor are we certain that hypertensin is pure). It is not known whether hypertensin is broken down into simpler effector components (such as pressor amines) by specific enzymatic action in smooth muscle or whether the peptide as itself acts upon arterioles. The composite result, however, fulfills the requirements we have set for the *Hochdruckstoff;* the duration of action is much shorter than that of renin, but longer than epinephrine.

Hypertensin is soluble in alcohol, glacial acetic acid, phenol, and water, and insoluble in ether (*61*). Because it is inactivated by tyrosinase it probably contains a catechol or phenol group, and by amine oxidase, an amine group on an α-carbon atom (Figure 2). Hypertensin is inactivated by certain phenolic, catecholic, and amine oxidases, by pepsin, trypsin, chymotrypsin, and carboxypeptidase, and by "hypertensinase" found in plasma. The nature of hypertensinase is unknown, but it is probably not an oxidative enzyme. Because it is heat-labile, hypertensinase can be removed from blood and renin preparations by heating; hypertensin itself is heat-stable. Lack of pure preparations of hypertensin has delayed its further chemical identification.

Consideration must be given to the protective action of hypertensin in congestive failure, shock, and acute hypertensive states. Failure of this mechanism by depletion of the circulating substrate of renin may account for certain irreversible changes. Further investigation into the mode of action of this compound is necessary. Is this renal pressor mechanism one way by which vasoconstrictor substances may be obtained from proteins? Hypertensin is a peptide, one stage in protein breakdown, and on further degradation may produce simpler pressor materials. The problem is of fundamental importance and must be investigated.

A second peptide is pepsitensin, formed by peptic digestion of the substrate of renin (*13*). It represents an interesting type reaction similar to that of renin. Because two proteolytic enzymes, renin and pepsin, act on a protein to produce vasoconstrictor substances, the substrate must have peculiar structural properties conducive to formation of these substances when broken down. The pharmacology of pepsitensin has not been ex-

tensively investigated, nor has it been purified, but evidence available suggests a similarity to hypertensin, although it is not identical. Therefore, we are not concerned with its relation to abnormal vascular states, but only with the suggestion that the renin mechanism may not be a specific one. The nature of the substrate is probably the important specific for the formation of pressor substances.

The third peptide to be examined is serotonin, an indole-containing substance isolated from blood, which is probably identical with "*spätgift*" (*46*). This vasoconstrictor appears in shed blood as a constant source of annoyance to those performing perfusion experiments, and has been recognized for years. Only recently has it been isolated and partially purified, and its chemical constituents partially identified. It differs from hypertensin in containing an indole ring, and its pharmacology and resemblance to the *Hochdruckstoff* have not been thoroughly worked out. There is no evidence that it may be involved in hypertension. Again, we do not know whether it acts directly, or whether it is broken down by cellular enzymes into simpler effector substances such as tryptamine, a much neglected pressor substance.

Amine Pressor Substances

That amines formed from naturally occurring amino acids are partly responsible for chronic hypertension is a rather attractive hypothesis first suggested by the experiments of Holtz (*35*). Besides the normal metabolic enzymes of amino acids, tissues, especially kidney, liver, and brain, contain amino acid decarboxylases, some of them specific for certain amino acids, some less so. These are anaerobic enzymes. After decarboxylation, certain monoamines are deaminated by amine oxidases which are sensitive to oxygen tension. The best known of these oxidases is the enzyme of Blaschko, Richter, and Schlossmann (*9*), which may be a mixture of three or more (*29*), and which is specific for many nonsubstituted vasoactive amines found in the body, with the notable exception of histamine.

Therefore, if the amount of oxygen available for the deamination of amines were cut down sufficiently, decarboxylation would continue but deamination would be incomplete, resulting in an accumulation of amines. The most powerful pressor substances known are amines. If an amino acid, such as *p*-hydroxyphenylalanine, were decarboxylated but not deaminated the result would be *p*-hydroxyphenylethylamine or tyramine, a relatively active pressor substance. If this same disturbance of deamination were prevalent and affected other amines we might expect a mixture in the circulating blood, and their effects upon the circulation might be profound. One could expect to find eight principal vasoactive amines in blood: butylamine, amylamine, isoamylamine, phenylethylamine, tryptamine, tyramine, hydroxytyramine, and histamine. There might be others. Histamine dilates capillaries but constricts arterioles. It has, however, a specific decarboxylase and deaminase. The others, as far as we know, constrict arterioles, although the action of tryptamine is not well known.

Presumably, vasoactive substances act upon smooth muscle cells by intimate association with them, and are destroyed in the process of stimulation or depression. Epinephrine, when injected intravenously, can be recovered in much larger amounts from arterial than from venous blood; minute doses given intraarterially may affect only the local circulation. If amines formed from the incomplete catabolism of amino acids are active in hypertension, one must postulate their formation by ischemic organs in direct venous connection with the heart (kidneys, brain, liver, adrenals, etc.) or in direct arterial connection with the arteriolar bed (heart and lungs). If the former, they must not be destroyed in large amounts by the lungs. Furthermore, arterial blood could be expected to contain larger quantities than venous. Absorption from or formation in the intestinal tract or spleen of amines would not produce vascular effects, as these substances probably would be metabolized by the liver.

Because cerebral and hepatic blood flow is normal in hypertension and renal blood flow is usually reduced, our attention is focused on renal oxidative mechanisms and their susceptibility to disturbances caused by lowered oxygen supply. Fortunately for this hypothesis, renal amine oxidase, although a slowly acting enzyme in vitro, is highly sus-

ceptible to moderate changes in oxygen tension (*61*). Furthermore, Bing and Zucker (*7*) have shown, as have others, that severely ischemic kidneys of dogs will decarboxylate but not deaminate dihydroxyphenylalanine. DOPA [β-(3,4-dihydroxyphenyl)alanine] is a pressor substance for rats (*49*). Whether slight or moderate ischemia will affect renal oxidative deaminases in vivo is not known. If amino acids are at all metabolized by kidneys (which have the highest resting metabolic rate of any organ) a simple calculation will point out the magnitude of changes expected from slight inhibition of deamination.

Taking 3 mg. % of α-amino nitrogen as the normal concentration in blood, and 1000 ml. of blood per minute as the normal total renal blood flow, we can readily see that 30 mg. of amino acid nitrogen pass through the kidneys per minute. To decarboxylate but not deaminate 0.1% of this amount would lead to levels in renal venous blood of 30 micrograms of amine nitrogen per liter, certainly a large amount of vasoactive substance if the nitrogen were part of certain amines. Although the effective dose of phenylethylamine as judged by the rat is approximately 2 mg. per liter of blood, or 100 micrograms per kg. of body weight, even this relatively weak pressor agent could be expected to produce vascular changes if continued accumulation of only slight degree occurred; this dose is about seven times the hypothetical one considered above. Obviously, effects could be caused by much smaller amounts of the more active compounds—for example, one third of this amount of tyramine, one fourth of hydroxytyramine, or one three-hundredths of arterenol (if the last has an amino acid precursor).

We may therefore take for granted that failure of renal deamination of appreciable amounts of tyrosine, tryptophan, and phenylalanine may produce vascular effects, and that disturbances of similar more complex mechanisms for synthesis or metabolism may cause even greater ones. The evidence, however, that amines are actually present in increased amounts in chronic hypertension must be examined. Methods for their measurement are on the whole unsatisfactory. There is a strong suggestion, however, that amines are in general present in higher concentrations in the blood of hypertensives than of normal subjects (*57*, *59*). Occasionally, however, they are found in large amounts in the latter.

In over half of a series of 33 samples of arterial blood of hypertensives, the amine picrates measured in terms of isoamylamine were higher than in twelve of fifteen normal ones, and in nine cases were more than 10 mg. per liter. The normal range was 0 to 7.0 mg., only three being greater than 3 mg. In a second series, the results, expressed in terms of units of color, showed high values in two of twelve normal subjects, seven of fourteen patients with neurogenic hypertension, and eleven of thirteen with renal hypertension. The color-concentration curves obtained from picrates of various amines, however, differed in their slopes; therefore the amount of color found was no true indication of the actual concentration of any one amine.

In spite of the lack of specificity of the method, it is obvious that "amines" which give a color by Richter's method (*48*) are usually increased in chronic hypertension, especially in the more severe forms. Because the more highly active phenolic amines are not measured by this method, and others might be present in too small amounts to measure, these results may be taken as a manifestation of a general disturbance of amino acid metabolism in chronic hypertension which may include the formation of pressor amines; it does not prove their presence.

The pharmacology of these compounds and their similarity to the *Hochdruckstoff* have not yet been investigated thoroughly. It is not known whether a mixture of them will produce the true picture of hypertension, although there are similarities (Table II). One closely related substance, however, *l*-arterenol or noradrenaline, which is the most active pressor amine known, does reproduce the hemodynamic effects of the *Hochdruckstoff* everywhere but on the pulmonary vascular bed (*26*). Infusions of small amounts of this amine will cause elevation of blood pressure in man without affecting cardiac output; the renal hemodynamic picture is also simulated, but the pulmonary vessels, contrary to the state existing in hypertension, are constricted under the conditions of the experiment. It appears very likely that *l*-arterenol is Sympathin E, the effector substance of the sympathetic nervous system, which again calls neurogenic factors to mind. Arterenol is found in the adrenal medulla along with epinephrine, and is present in U.S.P. epinephrine obtained from this source (*25*). It has not been isolated from hypertensive blood.

Table II. Action of "Natural" Pressor Amines on Various Circulatory Functions

Amine	Pressor[a] Ratio	Heart	Lung	Kidney	Vasoconstriction	Ergotoxin Reversal	Cocaine Potentiation	Inhibition of Smooth Muscle
Hochdruckstoff	?	0	0	+	+	0	0	?
Arterenol								
l-	1640	0	+	+ ?	+	±	+	0
d-	50							
Epinephrine								
l-	1000	+	+	+	±	+	+	+
d-	40							
Guanidine	40	..	..	0 ?	+	+	..	+
Hydroxytyramine	20	+ ?	..	..	+	..	..	0
Tyramine	10	+	+	+	+	±	0	+
Phenylethylamine	7	±	+	+ ?	+	..	..	+
Tryptamine	3	±	..	+	+	0	..	0
n-Amylamine	2	..	..	..	+	..	..	..
n-Butylamine	1	..	..	..	+	..	..	..
Isoamylamine	1	±	+	+	±	+	..	+

[a] Pressor ratio was obtained from a study of the literature of Barger and Dale (*5*), Hartung (*32*), and Guggenheim (*31*). The action on other constituents than blood pressure was obtained from these and many other sources.

Heart. + indicates either increase in cardiac output or increase in amplitude of contraction.

Lung. + indicates production of arteriolar constriction.

Vasoconstriction. + indicates constriction in perfusion experiments or an action on smooth muscles of arterioles.

Ergotoxin reversal. + indicates that pressor effect of the agent was inhibited or reversed by the prior injection of ergotoxin into an animal.

Cocaine potentiation. + indicates that pressor effect was increased by the prior injection of cocaine.

Inhibition of smooth muscle. + indicates that an action similar to that of epinephrine—i.e., inhibition—was observed on the isolated muscle of an organ commonly inhibited by epinephrine (virgin cat's uterus, etc.).

Substances Probably of Amine Nature

Four substances have been described as having pressor effects, which are probably amines or aminelike. Urosympathin, described by Holtz, Credner, and Kronberg (*34*), is a substance found in normal urine in amounts per day, giving a pressor response equal to 2 to 3 mg. of hydroxytyramine or 100 to 150 micrograms of epinephrine or arterenol. In cases of essential hypertension the amount is said to be increased three- to fourfold. Because its action was intensified by cocaine and lessened by ergotoxin and yohimbine, they believed that it represented a mixture of hydroxytyramine, epinephrine, and arterenol. The material was recovered by lead acetate precipitation of urine with subsequent acid hydrolysis.

A substance found by von Euler and Schmiterlöw (*21*) in human and bovine blood is believed to be identical with Sympathin E. Although its pharmacology has not been thoroughly studied to identify it with the *Hochdruckstoff*, its similarity to *l*-arterenol, as judged by its action, is striking. Von Euler has also found a similar substance in a variety of organs, spleen being an especially rich source (*20*). While probably representing a normal constituent, the demonstration of its frequent occurrence is of considerable interest to the problem of hypertension. No greater pressor action of hypertensive blood extracts, however, was found than in normal ones (*22*).

A crude pressor substance was obtained from the anaerobic autolysis of renal tissue by Victor *et al.* (*65*), which was inactivated by tyrosinase (*53*) and amine oxidase. It probably contained isoamylamine, phenylethylamine, and tyramine (*17*) released by proteolytic enzymes. Pressor substances were not formed or were destroyed under conditions of aerobiasis. It is difficult to apply these results to in vivo pathological states except under extreme anoxia and necrosis of renal tissue.

Before leaving the matter of amines, we should examine some of the known simpler ones for their effects upon the circulation, especially as to cardiac action and duration of pressor response. The former is noteworthy, as its absence is a necessary prerequisite of the *Hochdruckstoff*. Presumably an extended vasospastic action should also be a prerequisite. Prolonged action can be produced in at least four ways: (1) by some property inherent in the structure of the molecule of the pressor agent which prevents its rapid destruction in situ or in blood; (2) by slow liberation of an effector substance from a larger, more complex molecule or system; (3) by inhibition of the action of an enzyme system which inactivates some naturally occurring pressor agent; and (4) by continuous production from parent sources. Clear-cut examples are not evident at present, although

renin is an example of (2) and ephedrine, by inactivating epinephrine oxidases, may be an example of (3).

Using the rat as a test object, the authors found that of a number of aminelike and other pressor compounds only one, phenylethylamine, had a prolonged action (5 to 30 minutes) on blood pressure (Table III). Of the others renin had only a relatively long action (5 minutes or less), while isoamylamine, tyramine, epinine, arterenol, hypertensin, casein hydrolyzate, epinephrine, and tryptamine had only a short action (2 minutes or less). Phenylethylamine therefore may be an example of either (1) or (3). This was an unexpected result, and suggests further the importance of certain amine pressor agents. DOPA, although not an amine, also had a prolonged action.

There is one further substance of unknown, but probably amine, nature, which has been isolated from arterial blood of hypertensive patients, and therefore probably plays a part in the causation of hypertension, although its pharmacological relation to the *Hochdruckstoff* is unknown (*57*). This for the moment somewhat optimistically has been named pherentasin (φερο = hold; ἔντασις = pressure; name suggested by Henry A. Schroeder, Jr.) until its chemical structure is indicated. Certain assumptions were made in developing methods for its extraction.

That *Hochdruckstoff* is present, and could be demonstrated pharmacologically. The direct approach seemed the most rewarding, although eventually placing a decided burden upon organic chemical methods.

That it is present in very small amounts; otherwise it would have been discovered by the techniques available.

That it is rather rapidly oxidized or otherwise destroyed by blood.

Table III. Pressor Activity of Aminelike Compounds in the Rat

Compound[a]	Dose, γ	Max. Rise in B.P., Mm. Hg.	Time of Rise, Min.	Remarks
Isoamylamine	175	14/20	5	Initial rise. 30/23 <1
	440	8/6	5	Slight initial depression
	440	...	...	No demonstrable effect
	875	28/34	2	
	875	45/40	<1	10/8 at 2 minutes
Tryptamine	100	20/20	<1	
	100	18/20	2	60/48 in <1 minute
	100	39/33	<1	Ether extracted
	300	20/20	<1	Ether extracted
	100	15/12	<1	
	300	36/27	<1	
Epinine	10	45/31	<1	Fall to normal at 2 minutes
	10	52/30	<1	Fall to normal at 2 minutes (ether extracted)
Phenylethylamine	120	22/26	30	Immediate rise
	24	2/0	5	Immediate rise also
	24	8/0	5	Immediate rise also
	60	10/2	5	Immediate rise also
	60	20/14	2	Immediate rise also
	60	48/42	15	Immediate rise also
	60	30/30	15	Immediate rise also
	Mg.			
Casein hydrolyzate	100	35/24	<1	Immediate rise followed by depression
	10	28/28	<1	Immediate rise followed by depression
	90	47/30	<1	Immediate rise followed by depression
	Ml.			
Angiotonin	0.2	10/13	2	Immediate rise
	0.5	15/14	2	Immediate rise also
Renin	0.1	5/14	3	Normal at 5 minutes
	0.1	40/29	5	Normal at 6 minutes
Mixed picrates of blood extracts	1	16/17	<1	14/11 at 20 minutes
Pooled pressor blood extracts	1	45/39	5	

[a] Of compounds tested in the rat, only phenylethylamine produced a prolonged rise in blood pressure. Mixed picrates of blood extracts were a preparation of hypertensive blood extracts purified by the formation of picrates. These gave an immediate pressor response. Pooled pressor blood extracts indicate extracts taken from a number of samples of hypertensive arterial blood and concentrated. Pooled samples from normal subjects gave no such response.

That it comes from the kidney, an unnecessary assumption.

That the materials in blood might be amines and might therefore be unstable.

Arterial blood was used for the reasons outlined previously, about 500 ml. being obtained from each individual directly into 95% ethyl alcohol. After acidification, filtration, concentration, and further extraction with alcohol and with petroleum ether, a crude extract was obtained. All the various fractions were tested and discarded if inactive, the test animal used being the anesthetized rat. Because the active material may have been present in very small amounts, concentrated crude extracts were injected intravenously while the rat's blood pressure was being continuously measured by an optical manometer, the needle of which was inserted into the femoral artery. Blood extracts from normal individuals were used as controls and the differences compared.

As this work progressed it soon became obvious that there were differences in action on blood pressure between normal and hypertensive blood extracts. Both types of extracts contained depressor materials, but on the average those from hypertensives contained less; these resembled adenyl compounds, both pharmacologically and spectrophotometrically. The extracts from hypertensive patients appeared to exert prolonged pressor effects which lasted 10 to 15 minutes, sometimes as long as 1 to 3 hours. Therefore, a test was made available by which the active principle could be followed through various purifications. The work was interrupted by the war; after it the results were confirmed in a second series of crude extracts. By further purification, formation of picrates, and the use of specific solutes, the authors have obtained active extracts of hypertensive blood which contain roughly from 10 to 20 micrograms of active material per liter of original blood. Inability to get sizable quantities, due to its extremely low concentration in blood, has held up its chemical characterization. Although highly purified, 10 to 20 micrograms per liter of hypertensive blood may or may not be the total amount originally present and may not be pure. The authors believe, however, that this material is aminelike in nature; its pressor action is destroyed by incubation with amine oxidase, oxygen being taken up in the reaction. Other chemical inactivation procedures have failed to show that it is not an amine.

In order to integrate further some of the various reactions mentioned, and to detect its presence by other methods, the vessels of the rat's mesoappendix were employed as a test object (Chambers-Zweifach preparation, *12*). A good correlation between the presence of a vasoexcitor material-like substance in the extracts and the presence of hypertension was found. When the whole rat was used for assay, a much cruder index, sizable quantities of active pressor material were isolated from the blood only of those patients showing at least a degree of renal impairment (lessened ability to concentrate urine, etc.). In general it may be stated unequivocally that patients with severe hypertension have in their arterial blood extractable substances which are pressor for the rat; there are less or undemonstrable amounts in blood of less severe or neurogenic hypertensive patients; there is little or none in blood of normotensive subjects; a vasoexcitor material-like activity is exerted by blood from most hypertensive patients; adenyl compounds, having a depressor action, present in extracts of blood are less prevalent in those from hypertensive patients; the active rat pressor material (pherentasin) is probably aminelike in nature, is not a protein, but may be a simple peptide or an amine.

Amine Oxidase and Amines

Various tissues contain amine oxidases, the liver, kidney, and intestines usually having the highest concentrations depending on species, but brain and muscle containing large amounts. That in brain may be different from that in kidney. These oxidases act upon amines to give ammonia or methylamine and aldehydes. If one assumes that amine oxidase is in the body for a purpose, and examines the amines coming from naturally occurring amino acids, one discovers that there is a remarkable correlation between pressor action and deamination by amine oxidase. This enzyme acts only when the amino group is on the end of a carbon chain, and when there is no substituent on this carbon atom (carboxyl, methyl groups, etc.). It does not act upon diamines, triamines, or tetramines. Histamine is not attacked, there being a specific histaminase in tissues.

Although the list is incomplete, Table IV shows the relative pressor effect and susceptibilities to oxidation of some "natural" amines coming indirectly or directly from the amino acids which are considered "building blocks" of proteins. It is obvious that all the pressor amines are so inactivated, whereas most of the inert or depressor amines are not attacked. Little is known of the pharmacological actions of amines resulting from the decarboxylation of isoleucine, serine, threonine, iodogorgoic acid, or thyroxine, but by analogy amine oxidase may well inactivate them. If it does, this group forms an exception, as all of them, except possibly the iodine-containing compounds, are vascularly inert. The other eight amines are probably not acted upon, although this is not known for certain. Of course, many other aminelike compounds are synthesized in the body; some of them may have vascular effects, but it is curious that amine oxidase appears to have a predilection for pressor amines rather than for others. Specific decarboxylases are known only for histidine, DOPA, tyrosine, and tryptophan (*61*).

Table IV. Pressor and Amine Oxidase Ratios of Naturally Occurring Amines

Amine	Precursor	Oxidase Ratio	Pressor Ratio[a]
Hydroxytyramine	Dihydroxyphenylalanine	140	20
Isoamylamine	Leucine	105	1
Tyramine	Tyrosine	100	10
Tryptamine	Tryptophan	87	3
l-Epinephrine	Dihydroxyphenylserine ?	65	1000
Butylamine	Norvaline	54	2
l-Arterenol	Dihydroxyphenylserine ?	51	1640
d-Arterenol	Dihydroxyphenylserine ?	51	50
d-Epinephrine	Dihydroxyphenylserine ?	45	40
Amylamine	Norleucine	19	2
Phenethylamine	Phenylalanine	11	7
Methylamine	Glycine	0	0
Ethylamine	Alanine	0	0
Choline	Glycine	0	0
Ethanolamine	Serine	+	0
β-Hydroxypropylamine	Threonine	+	0
β-Aminopropionic acid	Aspartic acid	?	0
γ-Aminobutyric acid	Glutamic acid	?	0?
γ-Amino-β-hydroxybutyric acid	Hydroxyglutamic acid	?	0?
β-Methylbutylamine	Isoleucine	+	+?
Taurine	Cysteine	0	?
β-Aminoethyl sulfide	Cysteine	?	?
Di-(β-aminoethyl)disulfide	Cystine	?	?
3-Methylmercaptopropylamine	Methionine	?	?
Putrescine	Arginine	0	...
Cadaverine	Lysine	0	...
Guanidine	Arginine	0	40
Ornithine	Arginine	0	0
4-Hydroxy-3,5-diiodophenethylamine	Diiodotyrosine	+	?
3,5-Diiodo-4-(4-hydroxy-3,5-diiodophenoxy)-phenethylamine	Thyroxine	+	?
Pyrrolidine	Proline	0	0?
3-Hydroxypyrrolidine	Hydroxyproline	0	0?
Histamine	Histidine	0	...
Glucosamine	Glucose	0	0

[a] Pressor ratios were obtained as noted in footnote to Table II. Amine oxidase ratios were obtained from Blaschko, Richter, and Schlossmann (*9*). Where + is given under oxidase ratio, it is assumed that the amine would be oxidized according to the general characteristics of the enzyme as described by Blaschko *et al.*, although this has not been studied, as far as can be determined. ? indicates that the action of the enzyme is doubtful in view of the presence of accessory groups or sulfur-containing compounds which might be inhibitory. The pressor effect of β-methylbutylamine is not known but theoretically should be similar to butylamine. It is not known whether the sulfur- and iodine-containing amines and those with a pyrrolidine ring are active on the vascular system. Guanidine is the only pressor substance not acted upon by amine oxidase.

Nicotine Bases

Pressor bases, closely allied to *l*-nicotine, have been isolated from urine.

Lockett (*36*, *37*) has described two compounds, base A and base B. Base A occurred in the urine of men (who smoked), women (who did not smoke), and bitches. There was approximately three times as much in the urine of men and bitches as of women. The base was somewhat more active pharmacologically than *l*-nicotine, and was found to exert its pressor effect through the mediation of the corpus striatum and thalamus, stimulating the sympathetic nervous system (*38*). Base A could be converted to *l*-nicotine by drying in a high vacuum, with alteration of its action from a central to a peripheral one; therefore it must be very similar in structure to nicotine. Base B was a different compound, also with pressor activity; both were obtained by steam distillation from strongly alkaline urine. Furthermore, another compound, which she called base x, appeared in the

urine only after the production of unilateral renal ischemia (*39*); it was excreted mainly by the contralateral kidney, and was also detected in blood. Chronic canine hypertension was accompanied by this base in blood and urine, disappearing when it regressed. Base x increased in blood and disappeared in urine when hypertension was made worse by the administration of salts, especially potassium. Lockett believed that base A and base x were similar substances coming from a single compound which was transformed into the latter by renal ischemia and into the former by alkaline hydrolysis (*40*). The fact that they are pressor is of considerable interest. Although their action is depressed by ergotoxine, these bases differ from epinephrine in certain characteristics. Lockett's work has not been confirmed.

Urohypertensin (*1*) was isolated from urine many years ago, and resembles in some respects Lockett's base A, except that it was not, like base A, precipitated with mercuric chloride. Bain (*4*) thought that isoamylamine, which he found in human urine, was urohypertensin. Lockett was unable to find isoamylamine in urine, although von Euler and Sjostrand (*22*) have stated that it is present in decreased amounts in essential hypertension. These pressor bases are worth further study, especially their relation to amines.

Other Substances

An unidentified substance, named nephrin, was described by Enger (*19*). Nephrin is a pressor material having a prolonged action, obtained from extracts of renal cortex, but not present in any other tissues. It was also found in urine and in blood and was said to have been present in increased amounts both in hypertensive dogs and in patients with hypertension, eclampsia, nephritis, and other hypertensive conditions. Its action was not intensified by cocaine nor affected by ergotoxin and it did not act upon the guinea pig uterine muscle. As it was dialyzable, it probably was not renin. If this work is substantiated, nephrin represents yet another renal pressor substance probably of nonprotein nature. Its relation to hypertension has not been substantiated.

Many other unidentified pressor materials have been isolated from organs and fluids (see von Euler, *20*, for discussion). Some of them were probably simple amines, others epinephrinelike. They have been given a variety of names. Because of their nonspecific nature and the manner of their extraction it seems unlikely that they are concerned in the development or maintenance of chronic human hypertension.

An interesting reaction to intradermal histamine has been described (*55*), which, while not a pressor substance, appears to reproduce certain symptoms and signs encountered in hypertensive subjects. Histamine has a renal action similar to epinephrine (*47*), constricting efferent arterioles. In neurogenic hypertensive patients it also produces the "hypertensive diencephalic syndrome." Whether this reaction is direct, or indirect through some other mechanism, is obscure.

Adrenal Cortical Hormones

One hormone, desoxycorticosterone, which comes from or is closely allied to an adrenal cortical steroid, has the probably unique characteristic of raising blood pressure in hypertensive human beings (*27*, *45*) not shared by similar steroid substances. As the acetate or more soluble glucoside it also acts as a pressor agent in normal dogs and rats. Whether it sensitizes vessels to the vasoconstrictor action of other circulating agents, or acts directly of itself, is not known; in chronic experiments, at least, salt is necessary for its hypertensive action. Desoxycorticosterone acetate also produces chronic hypertension and renal vascular disease in rats when given in large doses (*60*). In the majority of cases of human hypertension there is no evidence of excessive adrenal cortical activity; in a certain clinical group, however, sodium concentration of sweat is low, and other signs of endocrine disturbances are present (*54*). The response of the blood pressure to diets low in salt is often dramatic. In such cases the authors believe that a different pathogenesis is operating, governed mainly by the adrenal cortex. Probably the output of salt-retaining hormone is excessive; hypertension may have been initiated on this basis, leading to secondary changes in the renal vascular bed and the institution of a renal pressor mechanism.

Indirect Methods for Identifying the *Hochdruckstoff*

By the use of specific enzymes, studies have been made in an attempt to classify the pressor substances in experimental hypertension. If a specific enzyme lowered blood pressure in hypertensive animals and not in normal ones, it was assumed that the substrate of that enzyme was attacked, and therefore contributed to the hypertension. Such assumptions, while not wholly valid, nevertheless pointed to certain substances as possibly concerned in hypertension.

Tyrosinase obtained relatively pure was tested for its effect in rats, dogs (*53*), and man (*50*), and was found to exert a specific depression of blood pressure only in hypertensive animals. Tyrosinase contains catecholase and cresolase, and has the property of

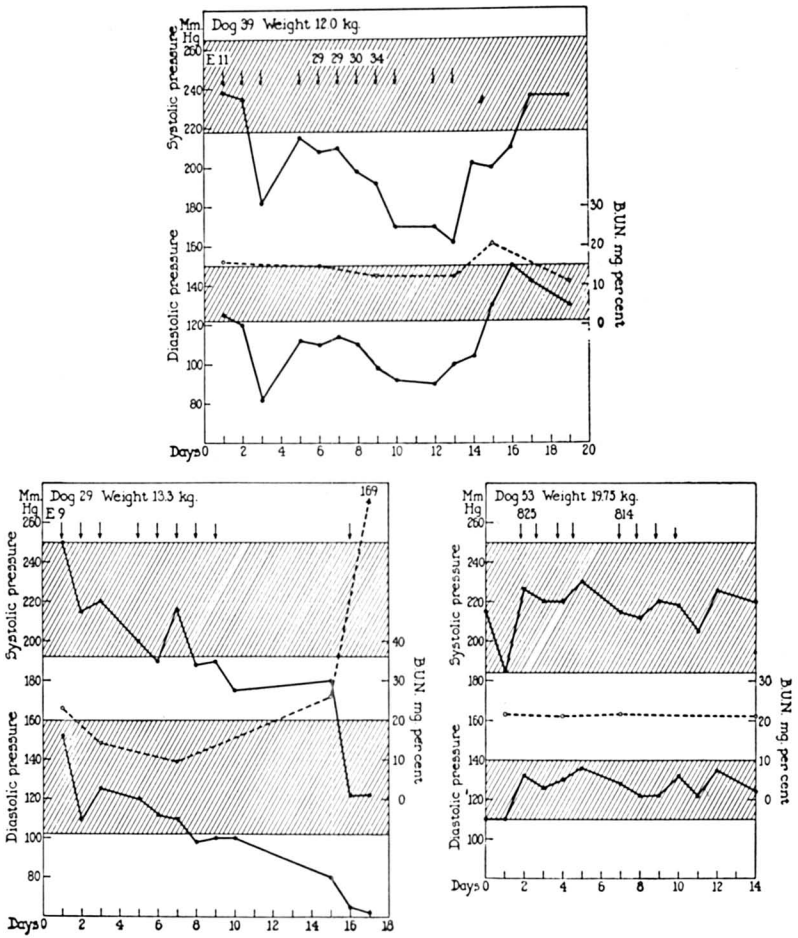

Figure 3. Effect of Intravenously Administered Amine Oxidase on Blood Pressure and Blood Urea Nitrogen of Hypertensive Dogs

Dogs were made hypertensive by the Goldblatt method. The hatched area represents the extremes of the control values for 3 to 12 months of hypertension prior to the experiment.

Dog 39. Daily injections of active preparations (E 11, 29, 30, 34) resulted in a fall of blood pressure to normal levels. After discontinuance of the enzyme, blood pressure soon returned to former values. Blood urea nitrogen was little affected.

Dog 29. A similar result was obtained with another active preparation (E 9), the effect lasting for a week after stopping administration of the enzyme, without elevating blood urea nitrogen. Another larger injection, given on the 16th day, caused further hypotension, uremia, and death.

Dog 53. Two preparations, of which the enzyme had been inactivated by alkaline hydrolysis, did not affect blood pressure or blood urea nitrogen.

Amine oxidase was supplied through the courtesy of Karl Folkers, Merck and Company.

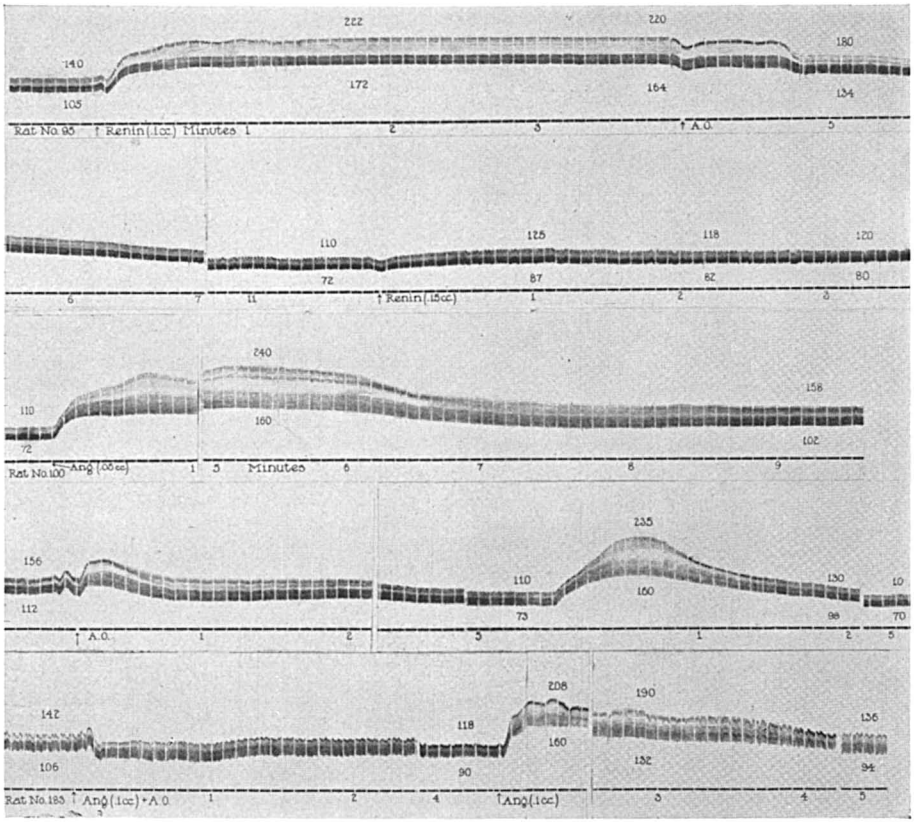

Figure 4. Prevention of Pressor Action of Renin and Hypertensin (Angiotonin) by Amine Oxidase

Photokymographs of the blood pressure of rats, using a Hamilton optical manometer. The upper figures represent the systolic, the lower the diastolic pressures in mm. of mercury.

Upper two curves (rat 95). Effect of a large intravenous dose of renin (0.1 ml.). After 4 minutes, at A.O. a solution of amine oxidase was injected intravenously, which slowly lowered the blood pressure to normal. After 12 minutes, a larger dose of renin (0.15 ml.) was given, with little effect on blood pressure. Rat was hypertensive.

Middle two curves (rat 100). Effect of a single dose of angiotonin. At A.O. amine oxidase was injected, which returned the moderately elevated blood pressure to control values. After 5 minutes the same dose of angiotonin was given, with a modified response. Rat was normotensive.

Lower curve (rat 183). Effect of a double dose of angiotonin mixed with amine oxidase and shaken for 30 minutes at room temperature. Angiotonin was completely inactivated. After 4 minutes the same dose alone produced a modified pressor response. Rat was hypertensive.

oxidizing mono- and orthodihydroxyphenols to quinones. Unfortunately, the quinones thus formed are active oxidizing agents, and therefore the effects cannot be attributed to any specific phenolic pressor substance. The least that can be said, however, is that easily oxidizable pressor substances are present in hypertension; possibly they are catechol or cresol derivatives. When injected intravenously into hypertensive rats, no effect was observed until 5 to 15 minutes later, when blood pressure invariably fell to normal and did not rise again, even for as long as 2 weeks. In dogs effects were more transient, but hypertension could be controlled by daily injections. Tyrosinase acted as a parasympathomimetic agent in dogs, its action being observed for an hour or two after administration, and consisting of vomiting, diarrhea, and bradycardia, which were abolished by atropine. It is probable, from the nature of the enzyme, that tyrosinase produced a true chemical, or "enzymatic" sympathectomy. Specific enzymatic activity was detected in blood for 24 hours following injection. Furthermore, urea nitrogen in blood was lowered, and urea clearance unchanged in the face of a lowered blood pressure. Similar effects were observed in man, which were usually not correlated with febrile episodes.

The course of malignant hypertension in man was reversed readily by this agent, and blood pressure in benign cases lowered. No hypotensive effect was noted when the enzyme was inactivated.

Tyrosinase had other interesting actions. Preparations of old renin contained a substrate for the enzyme and pressor activity was abolished. This probably occurred through quinonic oxidation, for the substrate could be dialyzed from the renin. Freshly dialyzed renin, not affected by the enzyme alone, was inactivated by tyrosinase plus a catechol-hydroquinone substrate. One sample of hypertensin (angiotonin) was inactivated directly by tyrosinase; another was inactivated only when serum was added as well. Prior injections of tyrosinase modified the pressor actions of angiotonin, tyramine, and epinephrine, but did not abolish them. Although these amines are rapidly oxidized in vitro, the relative rates of vasoactivity after injection (rapid) and oxidation in vivo (less rapid) probably accounted for the results; very slow infusions of epinephrine were without effect on the blood pressure of animals having circulating enzyme. Victor's renal pressor substance (*65*) was completely inactivated by tyrosinase. Furthermore, renal blood flow in dogs was increased, and epinephrine was seen to act as a renal vasodilator after tyrosinase had been given. A foreign (plant) protein, tyrosinase was a good antigen, precipitins being formed which inactivated the enzyme only when it was in an insoluble state; it did not cause anaphylactic shock. In man, however, it could not be used for more than a few weeks because of its antigenicity.

This phenolic oxidase therefore seems to be a true antihypertensive substance, affecting blood pressure without deleterious effects on kidneys. There is a strong presumption, therefore, that some phenolic substrate important for the maintenance of hypertension

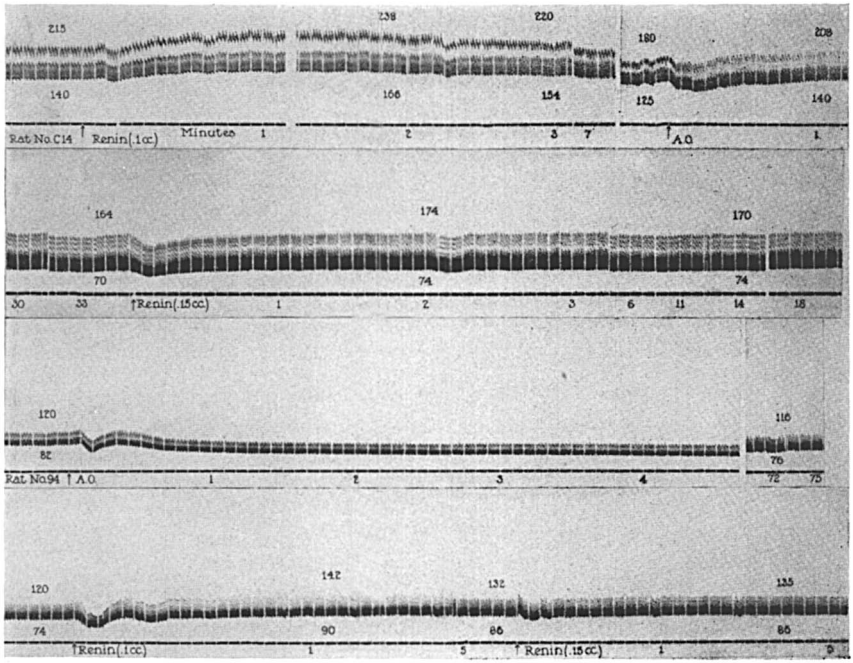

Figure 5. Prevention of Pressor Action of Renin and Hypertensin by Amine Oxidase

Upper two curves (rat C14). Effect of injection of renin (0.1 ml.) upon blood pressure of a markedly hypertensive rat. After 8 minutes at A.O. amine oxidase was injected intravenously, which was followed by little immediate effect but a slow fall of blood pressure to much lower levels. After 33 minutes a larger dose was given, with little effect on blood pressure.

Lower two curves (rat 94). Induction of tachyphylaxis to renin by amine oxidase without prior injection of renin. Amine oxidase was injected intravenously, followed 75 minutes later by renin (0.1 ml.) and 80 minutes later by renin (0.15 ml.). There was only a slight renin pressor response, never to hypertensive levels.

is altered. All suspected phenolic pressor amines and other pressor substances were inactivated by the enzyme. The evidence thus becomes more suggestive that some catecholic or phenolic pressor compounds may act in hypertension. Enzymes capable of attacking the guanidine linkage in such compounds as arginine, creatinine, and guanidine acted as pressor—not depressor—substances.

Amine oxidase prepared from hog liver and kidney acted similarly if less dramatically (*51*). The preparation used contained no other oxidase activity, but was only partially purified. It had a strong, but slowly developed hypotensive action on the blood pressures of rats, much greater on elevated than on normal ones. Experimental Goldblatt hypertension in dogs was controlled by injection of the enzyme usually at no expense of renal function (Figure 3). The level of blood urea nitrogen was lowered by the therapeutic dose. Overdoses, however, caused enough depression of blood pressure to produce renal insufficiency and death, probably lessening the head of pressure through the clamped artery. Ten inactivated preparations were without effect. The blood pressure of normal dogs was not lowered appreciably by the enzyme. Its impurity prevented its parenteral use in man; by mouth it had no effect. So far little work by qualified organic chemists has been done on the characterization and purification of this interesting enzyme.

Amine oxidase also was found to have the ability of diminishing or abolishing the pressor action of renin. When an active preparation of the enzyme was injected intravenously into rats, subsequent injections of renin had little effect (Figures 4 and 5). Hypertensin, however, showed only a modified response in these preparations, probably because of the relative slowness of action of the enzyme. Similar results were found with other pressor agents.

Characteristics of True Antihypertensive Substances

Merely because a substance depresses blood pressure does not make it a true antihypertensive substance. The level of blood pressure is the resultant of several factors: the viscosity of the blood, the cardiac output, the volume of circulating blood, and the state of the arterial and arteriolar bed, which determines the peripheral resistance to blood flow, other factors being equal. This discussion has indicated that peripheral resistance through arteriolar constriction may be affected by renal blood flow and the production of circulating pressor agents. Therefore, a definition of a true antihypertensive substance is necessary, in order that we be not misled by depressor substances which lower blood pressure at a detriment to the body's economy.

An antihypertensive substance is one which does not affect blood volume, blood viscosity, or the function of the heart; it lowers blood pressure to normal levels in hypertensive states by generalized arteriolar dilatation, including those of the kidneys. Vasodilators (such as histamine), which lower arterial pressure at the expense of renal blood flow, are not antihypertensive. Furthermore, an ideal substance should affect the blood pressure in normal states to little or no extent. It can be predicted, however, that when true antihypertensive substances are found, they will not increase or maintain renal blood flow in the face of lowered arterial pressure when renal arterioles have lost the ability to dilate because of pathological changes (see Figure 3).

Tyrosinase and amine oxidase appear to be true antihypertensive substances; they are useless at the present time for clinical application. Many cardiotoxic and depressor agents are known which will lower blood pressure at the expense of kidneys, heart, or blood volume. A few newer compounds, however, are now being studied which on preliminary trial appear to fit the definition of antihypertensive substances. It is believed that, in the not too distant future, a practical method for the control of this prevalent condition will be found.

Summary and Conclusions

From the evidence now available it appears that arterial hypertension in man is usually a psychosomatic disease, and that the effects of psychic disturbances manifest

themselves upon the soma by way of sympathetic nervous pathways. Discharges of these nerves cause neurogenic vasoconstriction, and include the renal vascular bed. Renal vasoconstriction which is not wholly compensated by elevation of blood pressure causes the kidneys to release pressor substances into the blood. Sixteen different pressor substances or types of substance have been discovered; many others have been detected which may or may not be similar.

The renal pressor mechanism—renin and hypertensin—acts in acute hypertension and in acute renal ischemic states, but apparently not in chronic hypertension. The other mechanisms shown to be active in chronic hypertension are: vasoexcitor-vasodepressor material relationship; pherentasin, a pressor substance found only in human hypertension; amines resulting from the insufficient oxidation of amino acids, which are increased in human hypertension; and norepinephrine (Sympathin E), which largely reproduces the hemodynamic picture of chronic hypertension. Most of the known pressor substances, with the notable exception of norepinephrine, come from disturbances of, or are extracted from, the kidneys. The large number of pressor substances which have been obtained suggests that many may represent different stages of metabolism of certain parent substances, and that their effectors may be fewer in number and simpler in structure. The chemical identification and purification of most of these substances leave much to be desired, and their pharmacology has in most cases been inadequately studied. The whole problem, however, may soon become simplified.

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Discussion of Paper on Humoral Pressor Substances and Their Relation to Arterial Hypertension

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Schroeder has very adequately reviewed the known humoral agents that in the past have been implicated in experimental or clinical hypertension. With the exception of his own substance isolated from the blood of hypertensive patients, these all have one or more serious deficiencies when considered as the causative agent of hypertension. Schroeder's substance is too new to have been given rigid examination in his own and in other laboratories, although it promises interesting possibilities.

There is no dearth of chemical compounds that will cause a rise in blood pressure when injected into the experimental animal. Extracts of plant and animal tissues yield several, and enzymes present in the tissues will often produce pressor substances as a result of autolysis. For a chemical agent then to be proved as a cause of hypertension it must be found as such in the animal and in greater amount in the hypertensive than in the normal animal. The substance must be capable of producing a continued elevation of blood pressure when administered continuously to the normal animal. The substance must be of such a nature that the body does not make corrective or adaptive responses to it. In this fashion tachyphylaxis or immunological reactions may reduce the action of certain agents if given repeatedly.

Recent experimental work indicates that factors in experimental hypertension make the picture more complicated than that of a pressor substance being produced in greater than normal amounts. In early experimental renal hypertension the conclusion is practically inevitable that a humoral agent coming from the ischemic kidney is implicated in the production of the increased arterial pressure. In later stages of the hypertension, however, another mechanism must become operative, since in an animal hypertensive from unilateral renal ischemia the causative kidney may be removed, and the high blood pressure will usually persist for weeks or months thereafter. A humoral mechanism may be involved in the production of this residual or self-perpetuating hypertension, and it is even possible that this substance might be produced by the remaining unmanipulated kidney. And so the pressor substance found by Schroeder in the blood of hypertensive patients might be the result of a long continued condition, rather than the original cause. The fact that he does not find it in all cases gives some support to this notion.

The mechanism of this residual hypertension has been assumed by many to be neurogenic. The evidence for this is not conclusive, and even if the central nervous system is involved (it is involved in most things going on in the body), it is not improbable that humoral factors in addition may act in this self-perpetuating hypertension.

Role of Sympathetic Blockade in the Therapy of Hypertension

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Considerable progress has been made in the development of agents capable of producing a specific and effective blockade of responses to sympatho-adrenal activity. Three groups of compounds show particular promise—the β-haloalkylamines, the dihydro ergot alkaloids, and the imidazolines. However, lack of information regarding the role of sympatho-adrenal factors in the etiology of essential hypertension prevents a definitive evaluation of their potential usefulness in the therapy of this condition.

During the past few years considerable progress has been made in the development of agents capable of producing a specific and effective blockade of responses to sympatho-adrenal activity. Research on several series of blocking agents has progressed to the point where it is now possible to produce a clinically useful "chemical sympathectomy." Such a chemical sympathectomy has obvious uses in the clinical evaluation and treatment of conditions in which a large component of sympathetically mediated smooth muscle spasma is involved. However, any assessment of the place which these agents may ultimately occupy in the therapy of hypertension depends upon a more specific delineation of the role of the sympatho-adrenal system in human hypertension. In spite of extensive laboratory and clinical investigation and elaborate speculation, this role is still obscure.

In his Janeway lecture of 1941 (*78*), Page listed 52 types of human hypertension. These were classified with respect to etiology with two notable exceptions: essential hypertension and malignant hypertension. Unfortunately, about 95% of all cases of human hypertension fall into these two poorly defined groups. Indeed, it is possible that neither of these categories is homogeneous. Inasmuch as the etiology of most cases of human hypertension is still unknown, no rational basis has yet been established for the use of adrenergic blockade in their treatment.

Neurogenic Hypertension

Experimental neurogenic hypertension has been known and studied for many years. Some of its more prominent features are listed in Table I. Even a casual appraisal of these characteristics indicates that human essential hypertension and uncomplicated neurogenic hypertension have little in common. Hypertension induced by infusion of epinephrine or norepinephrine is included for comparison. It is clear that infusion of epinephrine causes hemodynamic changes which are different from those seen in essential hypertension and which resemble those observed in neurogenic hypertension. Infusion of norepinephrine (*36*), on the other hand, induces changes comparable to those observed in essential hypertension. However, the similarity has not been proved to be of etiological significance. Hypertension with these characteristics may be duplicated by the infusion of any agent—

e.g., angiotonin—which produces a generalized peripheral vasoconstriction which predominates over cardiac stimulation.

Because of the many dissimilarities between human essential hypertension and experimental neurogenic hypertension, studies of the latter have been relegated to the background in recent years in favor of work on experimental renal hypertension, which much more closely resembles essential hypertension (Table I; *11, 35, 64, 77*). Nevertheless, a careful analysis of neurogenic hypertension is important as a basis for the recognition or exclusion of neurogenic factors in human hypertension.

Many clinical observations indicate that neurogenic factors in some way influence the development and maintenance of essential hypertension. It has long been recognized that stressful situations may induce marked increases in both systolic and diastolic pressures which persist for varying periods of time (*21, 38*), and that hypertensives tend to have a characteristic type of personality (*2, 99*). Such individuals usually exhibit important components of repressed antagonism and anxiety. They do not find emotional outlets in overt acts, but rather their emotions are expressed through an increased activity of the sympatho-adrenal system with a consequent increase in blood pressure. Relief of psychic tension frequently produces salutary effects in these patients. Individuals who show hyperactive sympathetic vasomotor reflexes (as measured by the cold pressor test) are much more prone than the average individual to develop hypertension in later life (*57*).

Figure 1 illustrates the principal nervous pathways involved in the maintenance of blood pressure. Under normal conditions the afferent pathways from the carotid sinus and aortic arch areas carry tonic impulses which depress the activity of the vasomotor centers. Consequently, section of these moderator nerves in animals (*51, 56*) or man (*103*) brings about a sustained hypertension. Of more importance to an analysis of clinical hypertension, however, is the fact that increased activity of the sympatho-adrenal system may arise on a central basis. In animals such hypertension may be induced by electrical stimulation of or injury to the hypothalamus (*59, 98*); in both animals (*17, 26, 39*) and man (*16*) it may result from an increased intracranial pressure, at least in part because of the cerebral ischemia that results (*16, 48*).

Neurogenic renal vasoconstriction, with consequent activation of the renin-angiotonin mechanism, is not a major factor in most cases of neurogenic hypertension; evidence for this is seen in the limited fall in blood pressure which follows renal denervation (*41, 54*) and the failure of prior nephrectomy to alter the pressor response to moderator nerve section (*95*). However, neurogenic renal vasoconstriction may be adequate to produce a sustained hypertension after other body structures have been sympathetically denervated (*42, 44*), and it is possible that neurogenic renal vasoconstriction may play a significant role in the development of essential hypertension.

Table I. Cardiovascular Characteristics of Various Types of Hypertension

Indexes	Mechanism[a]				
	"Essential" hypertension	Renal hypertension	Neurogenic hypertension	Epinephrine infusion	Norepinephrine infusion
Pulse rate	N	N	↑	↑	N or ↓
Cardiac output	N	N	↑	↑	N or ↓
Total peripheral resistance	↑	↑	N	↓	↑
Blood flow in extremities	N	N	↑	↑	↓
Pressure fluctuations	Marked early Limited late	Limited	Marked	Controlled	Controlled

[a] N. Normal. ↑. Increased. ↓. Decreased.

One of the distinguishing characteristics of uncomplicated neurogenic hypertension is its dramatic response to sympathectomy or to chemical blockade of the sympathetic nervous system. Complete sympathectomy results in an immediate reduction in the blood pressure to normal or to subnormal levels with a gradual return to normotensive or slightly higher levels over a period of 1 to 2 months (*41, 42, 54*). Moderator nerve section or increased intracranial pressure usually fails to increase the blood pressure in completely sympathectomized animals and if a rise is elicited it is relatively slight and develops slowly (*5, 27, 41*).

Consistent lowering of the blood pressure had been observed in dogs with neurogenic hypertension to which ergotamine, 883F (diethylaminomethylbenzodioxan), or 933F (piperidylmethylbenzodioxan) had been administered (*9, 52, 53, 59, 60*). In some of these cases central inhibition of vasomotor activity as well as adrenergic blockade by the drug was undoubtedly involved. However, this does not detract from the fact that the reduction or elimination of sympatho-adrenal activity always induces a dramatic reduction in blood pressure.

Factors involved in the regulation of the blood pressure of normal (*5, 13, 46, 65*) and neurogenic hypertensive animals after complete removal of the sympathetic nervous system have not been clearly defined. However, these factors may include renal humoral (*3, 28, 55, 97*), extrarenal humoral (*6*), extrasympathetic neural (*4, 5, 42, 46*), and local (*22*) components; all are important in any evaluation of the blood pressure response to sympathectomy or to sympathetic blocking agents. The degree of fall in blood pressure after these procedures may depend not only upon the extent to which the sympatho-adrenal system was involved in the maintenance of the initial pressure, but also upon the rapidity and the extent of compensation by other factors. Figure 2 illustrates a possible sequence of adjustments during the development of neurogenic hypertension and its subsequent "cure" by sympathectomy or sympathetic blockade. Few quantitative data are available regarding most of these factors. However, it is clear that some such over-all adjustment does occur.

Renal Hypertension

In contrast to neurogenic hypertension, the role of adrenergic factors in experimental renal hypertension is obscure. The sequence of events by which interference with renal hemodynamics leads to elevation of the systemic blood pressure has been carefully studied and has been shown to be independent of nervous mechanisms (see *11, 35*). Sympathectomy does not prevent the development of renal hypertension and induces only slight and irregular reductions in pressure in renal hypertensive animals (*3, 28, 55, 97*).

Prolonged administration of adrenergic blocking agents may produce a significant, but highly variable, reduction in systemic arterial pressure in animals with experimental renal hypertension. This has been observed after the oral administration of yohimbine to dogs (*58*), the oral administration of Dibenamine [*N*-(2-chloroethyl)dibenzylamine] to rats (*71*), and the intravenous administration of Dibenamine to dogs (*100*). In none of these experiments involving chronic administration of adrenergic blocking agents were the pressures consistently reduced to the normotensive range. Single injections of 883F and 933F produce little or no vasodepression in dogs with chronic renal hypertension (*9, 20, 61*), a response very similar to that seen in normotensive controls. It has been reported that single injections of pentobarbital, yohimbine, and 883F, but not 933F, produce a greater depressor response in rats which have been hypertensive for more than 2 months than in those with a shorter duration of hypertension (*84, 86*); on this basis it has been suggested that neurogenic factors are of importance in late but not in early renal hypertension (*76, 84, 86*). This differential response with regard to the duration of the renal hypertension was not observed with Dibenamine in chronic experiments on rats (*71*), or with various anesthetics and other procedures to reduce vasomotor activity in experiments on dogs (*68*). In general, the significance of those observations which indicate a correlation between the duration of experimental renal hypertension and the magnitude of the adrenergic component seems to be very limited (see *70* and *71* for further discussion).

Other evidence which has been adduced to support the significance of neurogenic factors in renal hypertension is the observation that the arterial pressures of normal and renal hypertensive dogs and rabbits were reduced to essentially the same level after complete destruction of the central nervous system (*18, 19*). However, interpretation of the results obtained with this drastic procedure is difficult. Other workers have observed a sharp fall in pressure when the spinal cord was destroyed below C_5 (the level of the fifth cervical vertebra) in renal hypertensive dogs, but the pressures returned to hypertensive levels as the acute effects of the operation wore off (*32*). Other experiments involving elimination of the central connections of the sympathetics by cervical cord section in the region of C_7

have demonstrated that the pressures of early and late neurogenic hypertensive dogs may fall below those of normal dogs after cord section. This is probably due to a reduction in nonsympatho-adrenal pressor factors in these animals (see Figure 2, *B*). However, under the same conditions pressures of renal hypertensive animals are maintained significantly above those of the normals (*40*). In addition, it has been demonstrated that chronic destruction of the spinal cord below C_5 does not prevent the development of typical chronic renal hypertension (*33*). It appears that the less trauma involved in the surgical elimination of the sympathetic nervous system in animals with renal hypertension, the less effect the procedure has on the blood pressure.

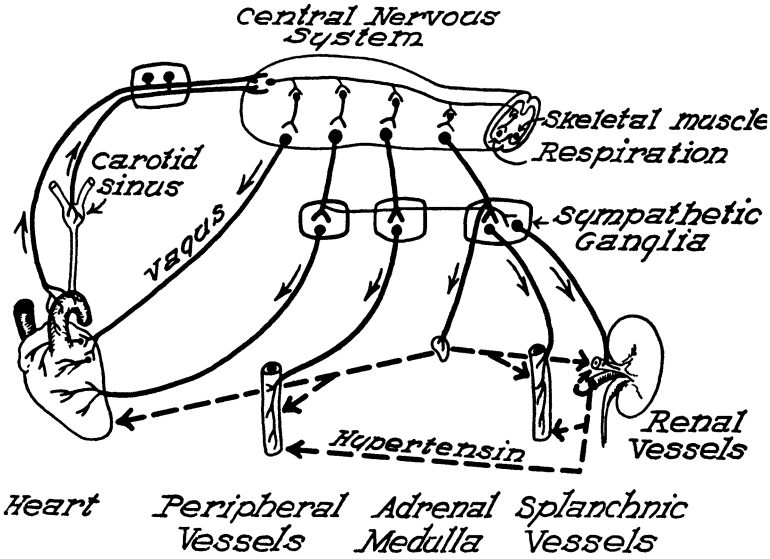

Figure 1. Principal Nervous and Humoral Pathways Involved in Maintenance of Systemic Arterial Pressure

Solid lines depict nervous pathways and broken lines humoral agents

Essential Hypertension

In human essential hypertension also, sympathectomy or blockade of the sympatho-adrenal system brings about an equivocal response. There is little doubt that various degrees of surgical sympathectomy may produce a prolonged reduction in blood pressure in certain selected cases (*43, 81, 82, 89*), but the response is highly variable and the degree of benefit attributed to the procedures employed may depend to a considerable extent upon an evaluation of the natural course of the disease (see *79*).

In the work of Goldenberg and co-workers (*37*), who employed members of the Fourneau series of adrenergic blocking agents to detect pheochromocytoma, it was observed that most cases of essential hypertension actually responded with an increase in the resting blood pressure. This increase was undoubtedly due to the central nervous system stimulant effects of these drugs (see *70*), but failure to respond by a fall in pressure sharply distinguished cases of essential hypertension from those in which the rise in blood pressure was largely due to an excessive secretion of epinephrine or norepinephrine. Clinical treatment of hypertension with the more effective adrenergic blocking agents has also produced highly variable results, which are discussed below in connection with the individual compounds involved.

Factors in Blood Pressure Regulation

The highly variable blood pressure responses to adrenergic blockade in cases of experimental renal hypertension and human essential hypertension, particularly those

responses obtained with single injections of blocking agents, are extremely difficult to interpret. However, their marked irregularity, when compared with the consistent depressor response to adrenergic blockade seen in neurogenic hypertension, argues against derangement of sympatho-adrenal function as a major factor in experimental renal hypertension and most cases of established essential hypertension. In addition, it must be concluded that the contention that nervous factors are of importance in late but not in early renal hypertension has not received convincing support from experiments employing sympathetic denervation or adrenergic blocking agents.

Although blood concentrations of vasoexcitor material (VEM) are increased in both experimental renal and human essential hypertension (see *88*), studies of this material have not progressed to the point where its role in maintaining the elevated blood pressure can be adequately evaluated. VEM appears to have little direct effect on smooth muscle and produces its primary effects not directly, but by sensitizing vessels to the action of epinephrine and probably to that of sympathin. Failure of such powerful adrenergic blocking agents as Dibenamine consistently to reduce to normal the pressure in renal and human essential hypertension argues against VEM playing a major role in maintaining the elevated blood pressure in these conditions.

None of the specific adrenergic blocking agents inhibits vascular responses to angiotonin (see *70*), and the mechanism by which adrenergic blockade or sympathectomy brings about even a partial reduction in blood pressure in renal or human essential hypertension has not been clearly established. However, it has been definitely demonstrated that vasomotor reflexes are still active in the presence of acute or chronic renal hypertension in animals (*7*, *19*, *68*, *97*) and man (*83*) and in human essential hypertension (*31*, *83*). Consequently, it may be assumed that at least part of the observed decrease in pressure is due to the elimination of sympatho-adrenal factors. If a significant element of neurogenic renal vasoconstriction is involved in human essential hypertension, a second factor in the hypotensive effect of adrenergic blockade may be the increase in renal blood flow which could be induced by the blockade. Inhibition of sympathetic vasoconstrictor tone by high spinal anesthesia has been shown to cause an increase in renal blood flow in both human essential hypertension and experimental renal hypertension in dogs (*94*), but the relation of this observation to the etiology of the blood pressure elevation is not clear. Sympathectomy may fail to alter renal blood flow and filtration in at least some cases of essential hypertension (*15*). As pointed out above, the fall in pressure elicited by the elimination of neurogenic factors may not be directly proportional to the magnitude of these components, but may also be dependent upon the extent to which other factors compensate for the deficiency.

Possible interrelationships of various hypertensive factors in renal (and perhaps essential) hypertension are diagramed in Figure 3. The highly variable response of renal and essential hypertension to sympathectomy or sympathetic blockade makes it impossible to present any diagram adequately covering all cases.

Column *C* represents a case in which sympathetically mediated renal vascular tone is assumed to be a significant factor in maintaining the elevated pressure, and column *D* depicts possible changes in the few cases of human essential hypertension in which a continued fall in pressure is noted for some time after sympathectomy, perhaps also on the basis of altered renal blood flow. Column *E* represents a common result of sympathectomy or adrenergic blockade in renal and essential hypertension; this result may or may not be preceded by some early fall in pressure such as illustrated in column *C*.

Sympatho-adrenal Factors in Development of Hypertension

On the basis of the above discussion it would appear that the role of adrenergic blockade in the treatment of hypertension, with the exception of isolated cases clearly due to sympatho-adrenal factors, is negligible. However, the possibility remains that neurogenic factors may be involved in the early stages of human essential hypertension. Certain psychic components are known to be involved in the development of hypertension and it is possible that emotionally activated neurogenic factors may cause repeated episodes of renal vasoconstriction and ischemia, which finally lead to the development of local organic

changes capable of permanently altering renal hemodynamics. The experimental basis for such a conclusion is as yet incomplete, but certain points of evidence are of interest in this connection.

It has been observed that reflex activation (*42*, *44*) or electrical stimulation (*62*, *63*) of sympathetic nerves to the kidney may cause sufficient vasoconstriction to produce a marked hypertension. However, in experiments which involved stimulation for 20 to 22 hours per day for as long as 45 days, the hypertension persisted only during and for a few hours after the end of stimulation. It has also been frequently observed that experimental hypertension may itself bring about marked changes in the renal vessels (*30*, *34*, *50*, *87*, *101*). In cases of unilateral compression of the renal artery or kidney parenchyma, vascular changes in the contralateral kidney may alter its hemodynamics to such an extent that it becomes capable of maintaining the hypertension after surgical removal of the kidney initially involved. It is not surprising that many workers have noted a persistence of hypertension after removal of a single ischemic kidney (*30*, *47*, *80*, *101*).

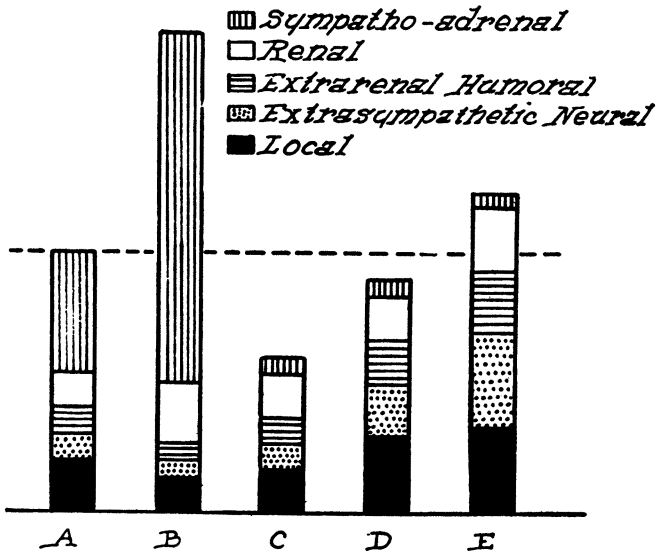

Figure 2. Possible Contributions to Maintenance of Systemic Arterial Pressure during Neurogenic Hypertension and Its Subsequent "Cure" by Sympathectomy or Adrenergic Blockade

A. Normal
B. Neurogenic hypertension
C to E. Sequential stages in recovery of blood pressure after sympathectomy. Pressure may stabilize at either D or E

A similar sequence of renal changes has not been demonstrated in connection with neurogenic hypertension. However, highly suggestive evidence for the development of persistent hypertension on the basis of intermittent neurogenic vasoconstriction is found in observations on rats subjected to repeated audiogenic stimuli (*24*, *66*). The blood pressures of young control and stimulated rats were found to be essentially the same, but it was noted that a large percentage of the experimental animals became hypertensive after they were one year of age. Hypertension was noted particularly among those which had consistently responded vigorously to the stimuli. It is of particular interest that these responses are characterized by a marked sympatho-adrenal discharge including mydriasis, piloerection, etc. (*23*). Although blood pressure was not determined in these studies, it is reasonable to assume that a temporary elevation accompanied each response.

One may speculate that a similar process occurs in some humans. During early stages of the development of hypertension the individual may be subjected to repeated

episodes of increased pressure and renal vasoconstriction on a purely neurogenic (psychic) basis and over a period of years he may secondarily develop sufficient renal hemodynamic changes to sustain a relatively stable, renal hypertension. Such a sequence of events would explain the lability of early and the stability of late hypertension as well as the observed correlation between psychic and sympatho-adrenal factors and the final development of a largely nonneurogenic hypertension.

A sequence of events by which renal hemodynamic alterations induced by either mechanical or neural factors might lead to a persistent, self-perpetuating renal hypertension is depicted in Figure 4. The hemodynamic changes in the kidney might be dependent upon organic changes in a majority of renal vessels or simply upon a redistribution of renal blood flow leading to a relative cortical ischemia (*96*).

In summarizing evidence for the participation of deranged sympatho-adrenal ("neurogenic") factors in human hypertension, it must be concluded that such factors have been conclusively demonstrated only in cases of pheochromocytoma, central nervous system trauma, and increased intracranial pressure. There is presumptive evidence that neurogenic factors may be important during the early, labile phases of essential hypertension and that the effects of this early sympatho-adrenal activity may lead to a persistent hypertension on a renal basis later in life. However, the development of hypertension through this or any other mechanism occurs only in individuals predisposed by some completely unknown, but probably hereditary, influence.

Locus of Blockade

In the treatment of neurogenic factors in hypertension, peripheral vascular disease, etc., it is necessary to inhibit the excitatory, vasoconstrictor effects of sympatho-adrenal activity. Blockade of inhibitory effects of the sympatho-adrenal system and of other nervous activity is not only unnecessary but frequently undesirable. Chemical blockade may occur at many points along the reflex arcs controlling the activity of the sympatho-adrenal system (Figure 1). However, in order to achieve a desirable degree of specificity it is necessary to produce the blockade at the efferent neuro-effector junction. Blockade within the central nervous system, along peripheral nerves, or at autonomic ganglia inevitably affects nervous functions other than excitatory activity of the sympatho-adrenal system. Blockade within the central nervous system alters many vital regulatory reflexes, respiratory activity, and particularly vagal activity, even when consciousness is not impaired. Blockade at autonomic ganglia indiscriminately interrupts all efferent impulses passing over both the sympathetic and parasympathetic pathways.

Adrenergic Blocking Agents

Agents which block responses of effector cells to sympatho-adrenal stimuli may be termed adrenergic blocking agents. It is only at these effector cells that adrenergic mediators are involved in transmission of the nerve impulse. For a number of reasons, the frequently used terms "adrenolytic" and "sympatholytic" agents are ambiguous and undesirable (see *70*).

Efforts to develop pharmacological agents capable of preventing excitatory responses to sympatho-adrenal activity have led to the study of a wide variety of compounds. The search for new agents has been spurred by the fact that none of the currently available agents is wholly satisfactory. Until very recently their use in both research and therapy was seriously limited by lack of specificity, imcompleteness of blocking action, and high toxicity. The characteristics of an adrenergic blocking agent capable of producing a useful "chemical sympathectomy" are a high specificity, a blockade effective against strong stimuli, a prolonged and uniform action, and a high therapeutic index. Much has been said and written about the potency of various blocking agents, but this property seems to be of little importance in comparison with a high therapeutic index. Some of the most potent compounds available at the present time have the lowest therapeutic indexes, particularly because of their stimulating effects upon the emetic center (*70*).

Dibenamine. The β-haloalkylamines, of which Dibenamine may be considered the

prototype, are the most recently discovered and also the most effective, specific, and persistent of the known adrenergic blocking agents (*70, 72, 73, 75*). These compounds apparently block by a direct chemical combination with some substance in the effector cell and thereby prevent responses to adrenergic mediators (*74*). Because of the stable nature of this bond, these agents have a very prolonged action. Certain members of the group block responses of smooth muscle cells to histamine (see *70*). Except for this action, the blockade produced seems to be limited almost entirely to the excitatory effects of adrenergic stimuli. These agents produce a transient stimulation of the central nervous system, but this effect wears off much more rapidly than the adrenergic blockade and is primarily associated with a high concentration of the drug in the blood stream, such as may occur after rapid intravenous injection (*69*). Central nervous stimulation can be almost completely eliminated by slow administration or prior sedation—e.g., the LD_{50} for mice is about 50 mg. per kg. when Dibenamine is administered intravenously within a few seconds, but animals may survive doses as high as 300 mg. per kg. when the injection is made over a period of 0.5 hour. The drugs are effective by all routes of administration, but when administered subcutaneously, intramuscularly, or intraperitoneally their local irritant action may produce tissue necrosis.

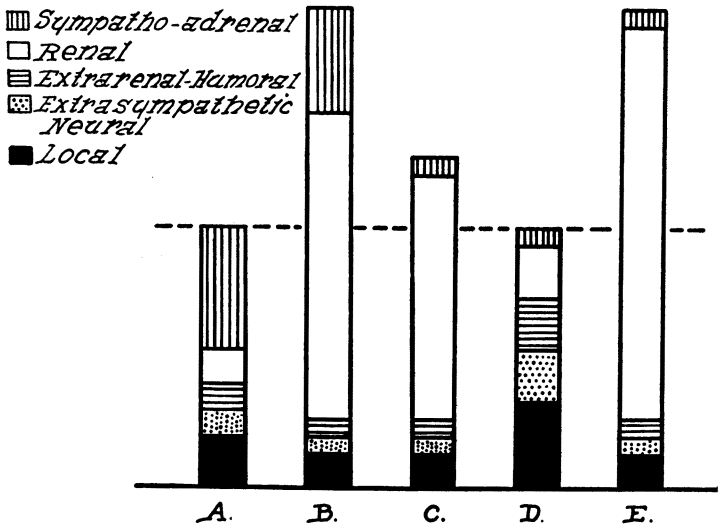

Figure 3. Possible Contributions to Maintenance of Systemic Arterial Pressure during Renal (and Perhaps Essential) Hypertension

- **A.** Normal
- **B.** Renal (and perhaps essential) hypertension
- **C.** Immediately after extensive sympathectomy
- **D.** Case of essential hypertension in which pressure continued to drop after sympathectomy, presumably because neurogenic alteration of renal blood flow was a significant factor in the original hypertension
- **E.** Common end result of sympathectomy, which may or may not be preceded by an initial fall in pressure such as that depicted in C

Dibenamine has been employed with excellent results in the diagnosis and preoperative therapy of pheochromocytoma (*90, 91*). The pressor response evoked by the histamine test in these patients is completely blocked and reversed, and the injection of Dibenamine at 72-hour intervals has been found to provide complete symptomatic relief. In human essential hypertension, therapy with Dibenamine produces a very significant fall in both systolic and diastolic pressures in some patients (see *12*). In severe hypertension, particularly the malignant form, the drug has been found to lower the blood pressure significantly in most cases, but rarely to return it to the normotensive range. However,

striking relief of sequelae such as hypertensive encephalopathy and impaired renal function was noted in most cases (*102*), probably due to the release of local vascular spasm.

Other workers have observed a significant depressor response to Dibenamine lasting 24 to 72 hours in many patients with early or moderately advanced benign hypertension, but not in patients with more advanced organic cardiovascular changes (*49*). On this basis it was suggested that the response to Dibenamine should be determined for prognostic purposes prior to sympathectomy, as a measure of the role of the sympatho-adrenal system in a given case of hypertension (*49, 102*). On theoretical grounds, an agent with the specificity of Dibenamine would be expected to be ideal for this purpose. Indeed, attention has been called to the similarity between the over-all effects of Dibenamine medication and those of surgical sympathectomy (*100*). It has also been reported that Dibenamine is superior to tetraethylammonium as a test for predicting the results of sympathectomy in acute peripheral vascular conditions, but the same investigator questioned its prognostic value in hypertension (*14*). Lack of knowledge regarding the etiology of human essential hypertension and the multiplicity of factors determining the extent of the fall in blood pressure after excision or blockade of the sympathetic system makes the interpretation of any prognostic test extremely hazardous. Results of tests with Priscoline (2-benzyl-2-imidazoline), the ergot alkaloids, tetraethylammonium, spinal anesthesia, and barbiturates, in which factors other than peripheral blockade of the sympatho-adrenal system are involved, would seem to have even less diagnostic specificity than tests with Dibenamine.

Significant toxicity, primarily central nervous system excitation, and the necessity for slow intravenous administration or prior sedation have considerably limited the study of Dibenamine in the therapy of hypertension. However, recent reports have indicated that other members of this series possess up to ten times the potency of Dibenamine without being more toxic (*70, 74*). This increased therapeutic index, perhaps coupled with the increased oral absorption of some congeners (*25*), may largely eliminate the present difficulties and disadvantages of the β-haloalkylamines. It is possible that within the next few years a truly satisfactory clinical agent will be found within this series of adrenergic blocking compounds.

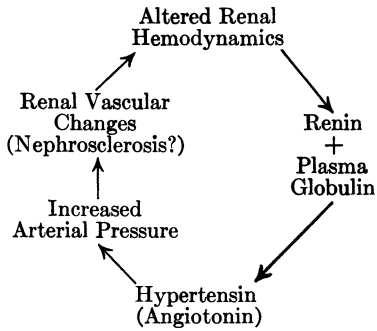

Figure 4. Possible Sequence of Events Leading to Renal Hypertension

Sympatho-adrenal factors could enter this cycle by increasing the arterial pressure or directly altering renal hemodynamics

Ergot Alkaloids. Recently, significant advances have resulted also from the study of adrenergic blocking agents of the ergot alkaloid series. Stoll in Basle has demonstrated that "ergotoxine" is really a complex of three alkaloids: ergocornine, ergocristine, and ergokryptine (*92*). He has also succeeded in producing dihydro derivatives of all these compounds (*93*). Pharmacological tests indicate that all members of the ergotoxine complex are more potent adrenergic blocking agents than the commonly employed ergotamine, and that hydrogenation also markedly increases the potency of all

these alkaloids (*70, 85*). Members of the ergotoxine complex differ from one another only in possessing different amino acids in the polypeptide side chain of the lysergic acid nucleus. No unnatural polypeptide containing ergot alkaloids has yet been reported, but the synthesis of alkaloids containing different amino acids would seem to be a promising line of approach in this field.

Although the ergot alkaloids are true adrenergic blocking agents, they unfortunately also have very potent effects upon the central nervous system (see *70*). All known natural and dihydrogenated members of this group act on the central nervous system to depress reflexes in concentrations lower than those required to produce true adrenergic blockade. Another expression of their central action is the vomiting which they produce in humans in total doses as low as 0.3 mg. (*10, 29*); hypertensive patients are more sensitive than normal individuals to the emetic action of the dihydro alkaloids (*10*). Because of the serious limitation in dosage imposed by stimulation of the emetic center, production of significant adrenergic blockade in humans with any of the natural or dihydrogenated ergot alkaloids has not yet been demonstrated.

However, even in the absence of adrenergic blockade, the ergot alkaloids are capable of inducing some reduction in blood pressure in human essential hypertension (*10, 29*). This fall in pressure appears to have two components: (1) the central stimulant effect of the alkaloid on the vagal center to decrease heart rate and cardiac output, and (2) a central depression of the cardiovascular reflexes which normally compensate for changes in body position. Because of this second factor, the orthostatic hypotension caused by the ergot alkaloids cannot be considered as evidence of a "sympatholytic" action—i.e., the production of adrenergic blockade. In hypertensive patients, reduction in arterial pressure by these agents has been found not to parallel the suppression of experimentally induced vasomotor reflexes, and in many cases an increase in the dose of an ergot alkaloid causes an increase rather than a decrease in pressure (*10*). The pulse pressure is markedly decreased during the fall in blood pressure induced by the dihydro ergot alkaloids in hypertensive patients; an unaltered or increased pulse pressure would be expected if the fall were primarily due to adrenergic blockade with resultant peripheral arteriolar dilatation.

Imidazolines. A third group of adrenergic blocking agents which has recently received attention is the imidazolines. These drugs are moderately effective in causing adrenergic blockade, but they exhibit many important side effects (see *70*). Priscoline, the most thoroughly studied member of the series, in addition to producing adrenergic blockade, stimulates almost all smooth muscles and its effects are comparable in one way or another to those of sympathomimetic, parasympathomimetic, and histaminergic agents. In the few cases of human hypertension given Priscoline, little reduction in blood pressure was observed (*1, 45*), although the agent is capable of producing significant adrenergic blockade and direct peripheral vasodilatation in doses tolerated by humans (*45*). The basis for the failure of the drug to reduce blood pressure is probably that Priscoline is a potent cardiac stimulant; the increase in cardiac output may balance or more than compensate for the vasodilatation produced. Even a massive dose of Priscoline taken with suicidal intent caused no lowering of blood pressure (*67*). Indeed Priscoline may actually cause an alarming increase in blood pressure in some patients (*8*).

Summary

The role of adrenergic blockade in the therapy of hypertension is still obscure. Pharmacological advances during the past few years have led to the development of agents capable of producing a clinically useful "chemical sympathectomy." Such agents are very effective in lowering the blood pressure in cases of neurogenic (sympatho-adrenal) hypertension.

At the present time, pheochromocytoma and intracranial lesions are the only causes of human hypertension which are definitely known to involve overactivity of the sympatho-adrenal system. However, presumptive evidence is accumulating to indicate that neurogenic factors may be involved in early essential hypertension, and it is possible that adequate adrenergic blockade early in the course of such hypertension may be effective in aborting its development. Only additional evidence regarding the etiology of essential

hypertension and further clinical trial of adrenergic blockade can define the extent to which this possibility may be realized.

Of the compounds currently employed for the production of adrenergic blockade, the β-haloalkylamines appear to be the most promising. They are the most specific, effective, and persistent of the available agents. The dihydro ergot alkaloids are much more effective than their nonhydrogenated congeners. However, at present much of the experimental work on these compounds is complicated by the marked depressant effects which they exert upon vasomotor reflexes and the vasomotor center and by their stimulant action on the vagal nuclei. The clinical administration of the dihydro compounds has been limited by their very potent emetic action. Significant specific adrenergic blockade has not yet been produced in humans by members of the ergot series. A third group of adrenergic blocking agents to receive recent attention, the imidazolines, appear to cause so much cardiac stimulation that no consistent lowering of the blood pressure is observed when they are administered to either animals or man.

Research in the field of adrenergic blockade need not be limited to members of series now known to be active. The three groups of compounds mentioned above are structurally unrelated, and only 4 years ago β-haloalkylamines were completely unknown as adrenergic blocking agents. The cooperation of synthetic chemists and pharmacologists in study of series of compounds unrelated chemically to those listed may provide the solution in the search for an agent producing a fully satisfactory "chemical sympathectomy."

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Discussion of Paper on Role of Sympathetic Blockade in the Therapy of Hypertension

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Nickerson and his co-workers are to be highly congratulated for their contribution to the field of biochemorphology in addition to other fields related to chemistry and medicine. They have provided chemists and biologists with knowledge of a new parent compound which possesses pharmacologic properties that were otherwise unpredictable from its structure and physicochemical properties. This lends further support to the often repeated statement that it still is practically impossible to predict pharmacologic effects for a given new chemical structure. Nickerson is to be commended for his modesty and honesty in stating that the discovery of the adrenergic blocking properties of Dibenamine was purely accidental.

In considering paths to follow in the synthesis of agents which will be useful in the therapy of hypertension, one is faced with the necessity of knowing more about the etiology of what is now considered a homogeneous group—viz., the individuals considered to be "essential hypertensives." The essential hypertension group may very well be physiologically heterogeneous, but as yet a satisfactory delineation of subgroups is not forthcoming other than a classification based on the grade or degree of hypertension and associated sequelae. It still remains as a problem in which the cooperative efforts of clinicians, chemists, physiologists, and pharmacologists must be integrated, so that a reasonable solution will be forthcoming.

Because it is considered that an important part of the symptomatic or "curative" therapy of essential hypertension is concerned with vasodilation, a number of mechanisms, by which such an end may be attained, immediately suggest themselves. These and examples of agents which act by such mechanisms are as follows:

1. "Direct" depression of vascular musculature which is nonspecific in character—nitrites and papaverinelike compounds.
2. Cholinergic facilitation—e.g., postganglionic cholinergic stimulants, such as acetylcholine, other choline derivatives, and pilocarpine.
3. Depression of response to excitatory sympathetic nervous system stimulation at postganglionic adrenergic endings ("adrenergic blockade")—Dibenamine, yohimbine, 933F.
4. Depression of autonomic ganglionic transmission—certain quaternary ammonium compounds, such as tetraethylammonium salts.
5. Depression of the vasomotor center or possibly stimulation of the vasodilator center—certain of the ergot alkaloids, primarily the dihydrogenated ergot alkaloids. (Some of the ergot alkaloids are considered also to possess adrenergic blocking properties, but in nontoxic amounts in man; it is difficult to demonstrate such action.)
6. Cerebral cortical depression—depression of transmission in association pathways which may be of importance in initiating central sympathetic stimulant effects, such as hypnotics, primarily the barbiturates.

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Biosynthesis and Metabolism of Phenylethyl (Pressor) Amines

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This report presents the likely pathways in the biosynthesis of phenylethyl (pressor) amines from their amino acid precursors, discusses the relative significance of the various modes by which they may be inactivated in the body, and considers the interplay of these factors as they may relate to hypertension.

Physiologically, hypertension may be defined as an elevation of blood pressure above the normal limits of variability. There are at least five basic factors involved in the maintenance of blood pressure. Aberrations of any one or a combination of these factors could produce an elevation affecting principally systolic or diastolic pressure, or influencing both more or less equally. These factors include peripheral resistance, elasticity of the arteries, cardiac output (heart rate and stroke volume), blood volume, and viscosity of the blood. The central nervous system, the endocrine glands, and the kidney must exert their influence on blood pressure through the above factors.

A clinical diagnosis of essential hypertension usually carries no connotation as to the etiology of the condition, and yet the preponderance of literature directing attention to the etiological role of one or another organ in the production of hypertension very often influences our everyday thoughts on the subject.

Predominantly, the immediate visceral response to stress is mediated through the adrenergic components of the autonomic nervous system. The adrenergic components are those thoracolumbar autonomic nerves whose postganglionic fibers elaborate adrenaline, or arterenol, together with the adrenal medulla whose chromaffin cells are analogous embryologically and functionally to the adrenergic postganglionic neurones (*107*). The most obvious manifestation of this overfunction, immediately and progressively, will depend generally on the individual. Manifestations of one or another imbalance of the autonomic nervous system may take the familiar form of a colitis, peptic ulcer, hypertension, or certain other aberrations of function depending on the individual.

The immediate response to stress in a normotensive person may be considered to fall in the alarm reaction stage of what Selye (*145, 147*) has elected to call a general adaptation syndrome, whose manifestations are essentially independent of the specific nature of the stress. The development of clinically sustained hypertension has been considered by him to fall into a second stage of resistance to a prolonged exposure to stress. Similarly, Wolf *et al.* (*166*) have presented recently an interesting discussion of hypertension as a reaction pattern to stress. The very readable article by White (*164*) also stresses the importance of the neurogenic aspects of early hypertension as a major factor that must be dealt with in the management of this disease.

What Selye described as the immediate response to stress in a normotensive individual graduates in the likely candidate into what Corcoran, Taylor, and Page (*51, 52*) have termed early essential hypertension. This state is characterized by moderate, widely fluctuant, sometimes remitting, increases of arterial pressure. It is accompanied

by no change or minimal evidences of vascular or renal damage (*44, 83*) when the patient is at rest. Outwardly, these patients may or may not appear to be unstable emotionally, but Jacobsen (*101*) has stated that under stress the action potential measurements of even their skeletal muscles become extremely high.

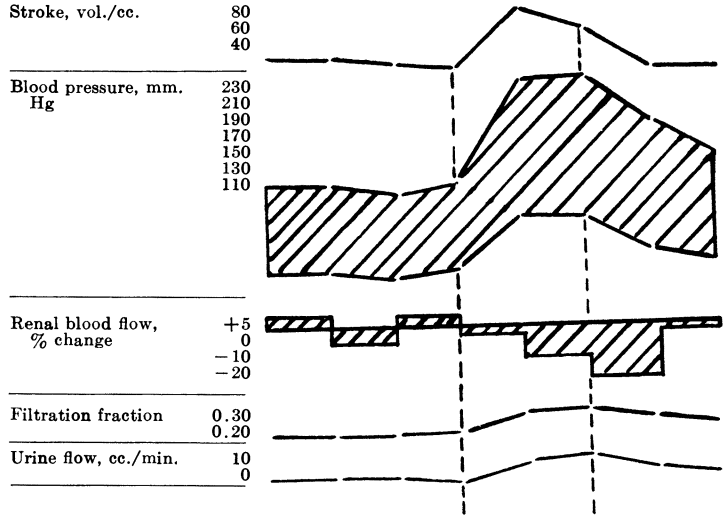

Figure 1. Effect of Unpleasant Interview on Cardiac Stroke Volume, Blood Pressure, Renal Blood Flow, Filtration Fraction, and Urine Flow of Patient (*166*)

Although there may be no demonstrable alteration in the function of the cardiovascular system or the kidneys of such patients, other than the sustained elevation of blood pressure when they are at rest, the extreme responsiveness of these systems to emotional stress is remarkable. There may be no alteration of the inherent ability of an organ or tissue to respond to a stimulus. Instead, their greater reactivity is to an increased transmission of nerve impulses in response to what may be a psychogenic stimulus. Wolf, Pfeiffer, Ripley, Winter, and Wolff (*166*) have demonstrated such changes in their patients admirably, and two of their figures from a recent publication illustrate this point.

Figure 1 shows the effect of "traumatic" or unpleasant interview on blood pressure, cardiac stroke volume, renal blood flow, and the fraction of the renal blood flow that was filtered at the glomeruli (filtration fraction). In response to the interview both the systolic and diastolic blood pressure and the stroke volume of the heart increased. There was a decrease in the flow of blood through the kidney, and this decrease was due predominantly to an efferent arteriolar constriction since the filtration fraction was increased somewhat.

Figure 2 shows the effect of reassurance and sedation on the blood pressure and renal function and the promptness with which this effect could be reversed by introducing a topic of significant conflict into the discussion. The reassurance and sedation decreased blood pressure, increased renal blood flow and glomerular filtration rate, and also increased the filtration fraction; this would indicate that afferent as well as efferent arteriolar constriction was present in the kidneys. At the onset of the unpleasant part of the interview these effects were reversed on the whole, and in the last portion of the interview a very interesting change in renal hemodynamics may be seen to have occurred. At this point there was an increase in renal plasma flow and a decrease in the filtration fractions. To be sure, this change may be thought to be within analytical error, and yet it is most tempting to interpret the increase in blood flow as being attributable to the opening of subcortical renal shunts as described by Trueta *et al.* (*157*). Wilkins *et al.* (*165*) studied the effects of sympathectomy on the function of various organs and concluded that the reduction or abolition of reflex vasopressor overshoots of arterial pressure (after blood pressure–lower-

ing procedures) and their substitution by a more stable, or gradual, homeostatic mechanism may be a very important hemodynamic effect of the operation.

Following repeated insults to the vascular system mediated through or at least initiated in the nervous system the histologic "fractures" of the integument of the blood vessels result in thickening and loss of elasticity. Since the arterioles are the vessels principally involved in the alteration of arterial pressure they are apt to bear the brunt of the injury. Thus the condition gradually progresses to a second phase that Corcoran and Page (*51*) have called established essential hypertension. During this stage the blood pressure is more sustained and at a higher level, is less susceptible to sedation and other therapeutic or surgical measures, and is accompanied by definitive evidence of cardiovascular and renal damage. In the instance of malignant hypertension these various phases are passed through in a matter of months frequently, instead of years.

If one is to admit both neurogenic and nephrogenic as well as other factors in the pathogenesis of hypertension, then the neurogenic element probably plays its dominant role in the initial stages of the disease. Thus it would be anticipated that medical (*164*) or surgical (*72, 149, 150*) approaches to therapy on the neurogenic basis should be the more effective the sooner the disease is recognized. Indeed any nephrogenic component of the disease may be thought to follow changes in the renal blood flow usually, rather than to initiate them. In substantiation of this point, renal function studies indicate that demonstrable alterations of function in the cells lining the renal tubules follow rather than

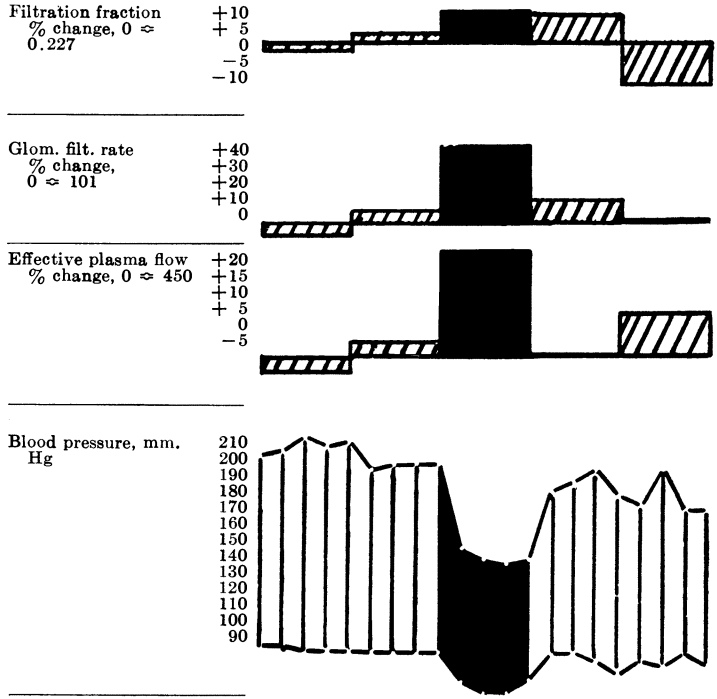

Figure 2. Effect of Reassurance and Sedation on Blood Pressure and Renal Functions of Hypertensive Patient

Following the period of reassurance, indicated in black, a topic of emotional conflict to the patient was introduced into the interview (*166*)

precede the alterations of renal blood flow (*44, 51, 83*). In this connection the review by Smith (*148*) of the evidence relating urologic disease to hypertension could hardly be cited to substantiate the nephrogenic factor as a dominant one in the early etiology of essential hypertension in humans. Presenting the subject in a different perspective, Goldblatt

(*80*) has published an interesting review of the literature pertaining to the renal origin of hypertension.

The chemical mediator or mediators of the neurogenic element in hypertension may be considered to be noradrenaline (synonym: arterenol, norepinephrine), adrenaline, or both. Within recent years a certain orderliness has evolved in this controversial field so that the problems that remain seem better defined and approachable from an experimental standpoint. The uncertainty as to the nature of the chemical mediator of adrenergic nerves is evident if one recalls that from a textbook standpoint adrenergic nerves are supposed to mediate their effects through the liberation of sympathin. Whereas adrenaline is capable of eliciting both excitatory and inhibitory effects, both a Sympathin E (excitatory) and a Sympathin I (inhibitory) were postulated, and much experimentation has been carried out to support the sympathin hypothesis. The evidence up to 1937 has been summarized by Cannon and Rosenblueth (*48, 49*) who were the earlier exponents of this view. Opposing the sympathin theory of chemical mediation were the contemporary data of Loewi (*113*) and of Gaddum and his associates (*76–79*) that indicated adrenaline was the chemical mediator.

It had been anticipated by Barger and Dale (*13*) in their classic (1910) article that adrenaline probably was not the chemical mediator, for this theory "involves the assumption of a stricter parallelism between the two actions than actually exists. Adrenine has, in common with other methylamino bases of its catechol group, the property of exaggerating inhibitor as compared with motor effects. The action of some other bases, particularly of the amino and ethylamino bases of the catechol group, corresponds more closely

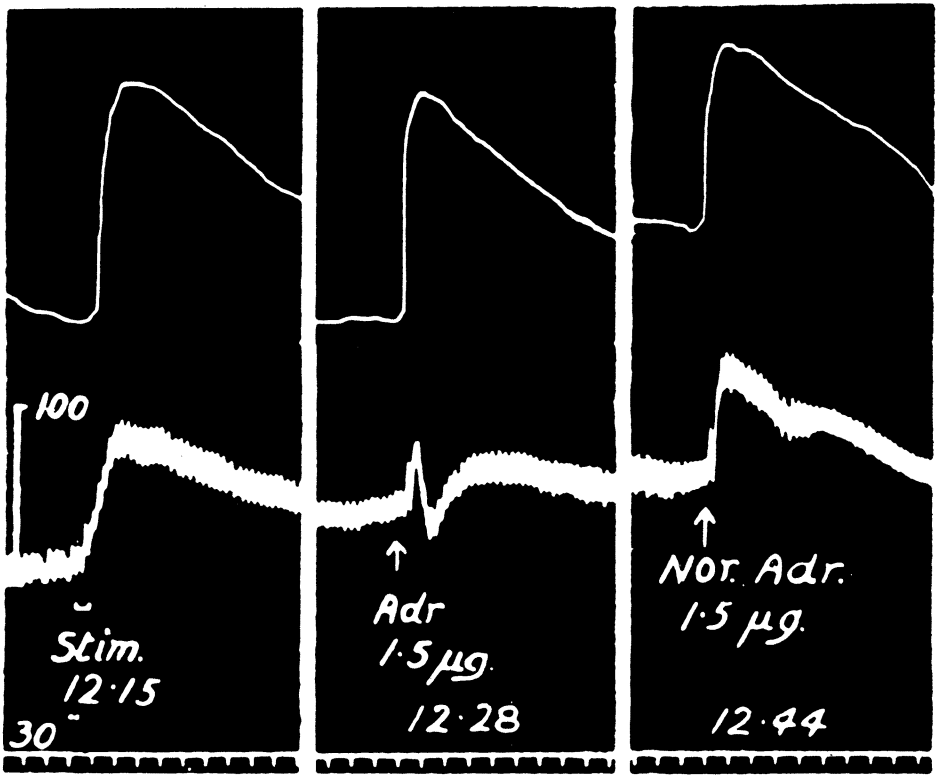

Figure 3. Effect of Hepatic Nerve Stimulation (15 Seconds) and Intravenous Injections of Adrenaline and Noradrenaline on Arterial Blood Pressure and Denervated Nictitating Membrane of Anesthetized Cat

Previous injection of cocaine (*75*)

with that of sympathetic nerves than does that of adrenine." They also anticipated, as in the above quotation, the hypothesis proposed by Bacq (*10*) and reiterated by Stehle and Ellsworth (*153*) and by Greer, Pinkston, Baxter, and Brannon (*84*) that noradrenaline (arterenol) more closely simulated the properties of Sympathin E as released following hepatic nerve stimulation. Barger and Dale (*13*) gave the principal biological methods for distinguishing between the action of adrenaline and its nonmethylated counterpart. Within the past 2 or 3 years many of these differences have become reconciled. The availability of *l*-arterenol, following its resolution in 1948 by Tullar (*158*), has already contributed to the progress in this difficult field.

The present status of the adrenaline against sympathin mediation of nerve impulses would indicate that both adrenaline and arterenol are liberated. In the previous evidence of Gaddum *et al.* (*74, 76–79*) adrenaline clearly was liberated on stimulation of certain nerves. More recently Gaddum and Goodwin (*75*) confirmed Cannon's (*48*) early experiments that the pressor response to hepatic nerve stimulation probably was not adrenaline. Where comparisons were made, the effect of liver sympathin resembled those of noradrenaline more closely than of adrenaline. They conclude that there is no evidence against the theory that liver sympathin is noradrenaline (*75*). One of their illustrations comparing the effect of hepatic nerve stimulation with that of adrenaline and noradrenaline on blood pressure and the denervated nictitating membrane of the cat is reproduced in Figure 3. The character of the blood pressure curve and the relative pressor effect and membrane contraction following nerve stimulation were quite similar to those caused by the intravenous injection of noradrenaline. West (*163*) confirmed the previous observation of Dawes (*57*) that the relation between doses of adrenaline producing equal rises of blood pressure by the jugular and by the portal routes of administration differed according to the amount injected. This jugular/portal equipressor ratio remained constant for *dl*-noradrenaline. It would appear that this indicates a difference in the inactivation of the two compounds by the liver where the arterenol acts as the principal nerve mediator.

This same qualitative difference between adrenaline and noradrenaline obtains for the splenic artery/splenic vein equipressor dosage-response ratios as well, and both observations quite possibly may find their explanation in the recent work of Euler (*64–70*). He has found that the pressor substance isolated from the heart, blood, liver, and spleen has predominantly the characteristics of noradrenaline. Thus, he has considered Sympathin E to be identical with *l*-noradrenaline.

Figure 4 is one of the illustrations from a recent publication by Euler. After the administration of an adrenolytic agent, Dibenamine, the pressor effect of adrenaline was in large measure reversed whereas the pressor response to corresponding increments of *l*-noradrenaline and to extracts of spleen or splenic nerve remained upright and monophasic. In general the effect of hepatic nerve stimulation is not affected by amounts of adrenolytic agents that will reverse the response to epinephrine (*48, 75, 163*).

Bacq and Fischer (*11*) have reported that extracts of mammalian spleen contained only noradrenaline, extracts of mammalian coronary nerves and arteries only adrenaline, but extracts of splenic nerves and sympathetic chains yield a mixture of adrenaline and noradrenaline. They have interpreted this variation as being due to the probability that the synthesis of adrenaline is through the transmethylation of noradrenaline as a final step and that this takes place slowly or not at all in some tissues.

Synthesis of Pressor Amines

The synthesis of pressor amines in the body may convincingly be considered to begin with the essential amino acid, phenylalanine as illustrated in Table I. Gurin and Delluva (*86*) have reported that phenylalanine labeled with tritium, or containing C^{14} located both in the carboxyl group and in the position adjacent thereto, was converted in the rat to radioactive adrenaline. Only one C^{14} atom was present in the side chain and it was in the terminal group bearing the secondary amine (*86*). However the intermediate steps in the synthesis are by no means so certain, nor does this necessarily preclude other paths of synthesis.

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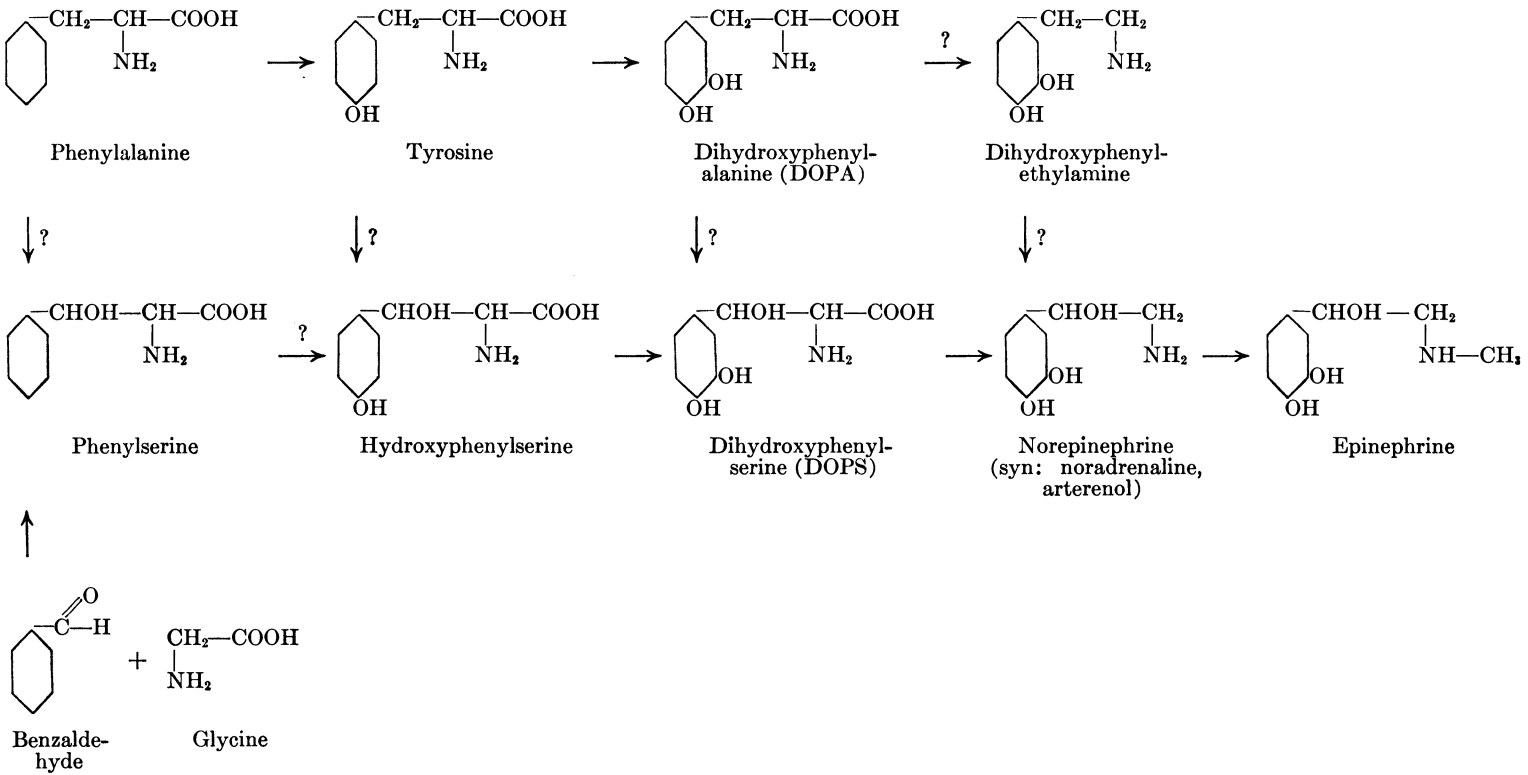
Table I. Alternative Schemes for Synthesis of Epinephrine in the Body



The biological conversion of phenylalanine to tyrosine has been demonstrated by Moss and Schoenheimer (*118*) who administered phenylalanine-*d* to rats and recovered tyrosine containing deuterium from their bodies. In vitro this conversion could be accomplished in the presence of iron and hydrogen peroxide (*130*). The decarboxylation of tyrosine to yield the pressor agent tyramine probably is not a step in direct line to the synthesis of epinephrine, but the compound long has played a role in investigators' thoughts about hypertension. There is a decarboxylase in various mammalian tissues, including kidney and pancreas, capable of converting tyrosine to tyramine (*37, 62, 63, 71, 93, 96*).

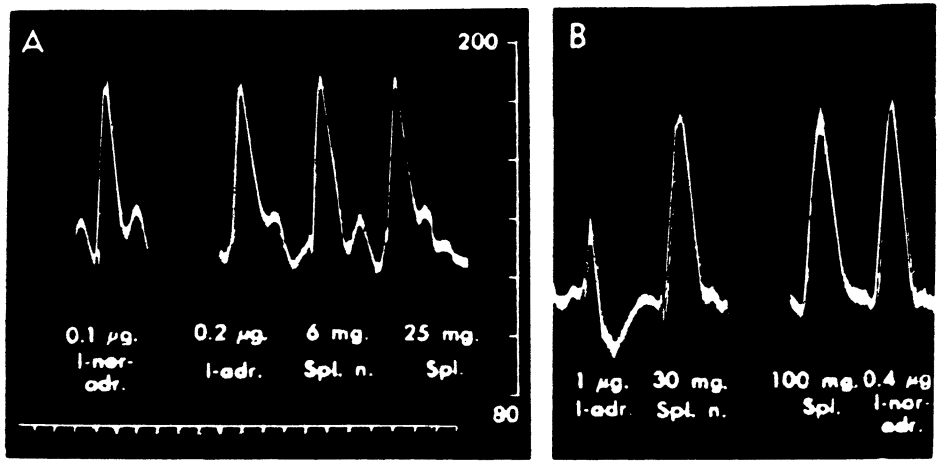


Figure 4. Blood Pressure Records from Anesthetized Cat before and following Administration of Adrenolytic Agent

A = Before Dibenamine; *B* = after 5 mg. Dibenamine per kg.; time = 0.5-minute intervals (*67*)

The oxidation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) can be shown to take place in vitro both enzymatically and by noncatalytic measures (*7, 8, 91, 131, 138*), but the precise in vivo mechanism is less certain. The ascorbic-dehydroascorbic acid system is capable of bringing about this oxidation in vitro (*20*), and it has been shown to stabilize epinephrine in the adrenal gland (*92*). In unpublished work it was found that potato phenol oxidase is capable of bringing about the oxidation of the phenolic to the catechol nucleus, as it does in the case of tyramine. However, Bhagvat and Richter (*31*) have surveyed a number of animal species and they could not demonstrate a phenol oxidase in mammalian tissues by the conventional methods. Probably the most direct in vivo evidence is that of Medes (*117*) who reported the recovery of *l*-3,4-dihydroxyphenylalanine from urine when large amounts of tyrosine were given to a patient diagnosed as having tyrosinosis.

Although it is uncertain as to just what step comes next, it is probable that either decarboxylation or the introduction of the hydroxyl group on the carbon atom adjacent to the ring must occur, with transmethylation of arterenol to adrenaline being the last step in the synthesis (*37*). Certainly the anaerobic decarboxylation of DOPA to the corresponding *l*-3,4-dihydroxyphenylethylamine occurs smoothly in the presence of kidney, liver, and other organs, as was described by Holtz *et al.* (*100*). Both he and Blaschko (*35–37*) have proposed that this step takes place in the synthesis of *l*-adrenaline. Regarding the alternative pathway involving *N*-methylation before decarboxylation, Heard and Raper (*91*) reported that the action of tyrosinase (phenol oxidase) on *N*-methyl DOPA in vitro yielded adrenalone, the ketone of adrenaline, but the perfusion of the ketone through the adrenal gland did not result in its reduction to adrenaline. In addition, Blaschko (*35*) reported that DOPA decarboxylase did not decarboxylate *N*-methyl-3,4-dihydroxyphenylalanine. Whether or not the introduction of an hydroxyl group into the side chain of DOPA, as by beta oxidation, occurs before or after decarboxylation is uncertain. It is this reviewer's

opinion that the hydroxyl group will be found, eventually, to be introduced into the synthesis of epinephrine at some point before decarboxylation of the amino acid precursor.

Vinet (*161, 162*) has claimed that the adrenal medulla is capable of converting 3,4-dihydroxyphenylethylamine, formed from the decarboxylation of DOPA in the kidney, to epinephrine in vitro, which in effect completes the synthesis. According to him, the adrenal medulla cannot decarboxylate DOPA. Similarly, Schapira (*140*) reported that whereas guinea pig kidney could decarboxylate DOPA, the adrenal medulla did not contain the decarboxylase and that adrenaline inhibited the decarboxylation of 3,4-dihydroxyphenylalanine. Just how the hydroxyl group is introduced into the side chain is not clear, if this is the principal course of the synthesis, but it seems certain that this precedes methylation (*37*). Recently du Vigneaud and his associates (*160*) fed C^{14} radiomethylmethionine to rats and succeeded in isolating adrenaline bearing the radioactive group

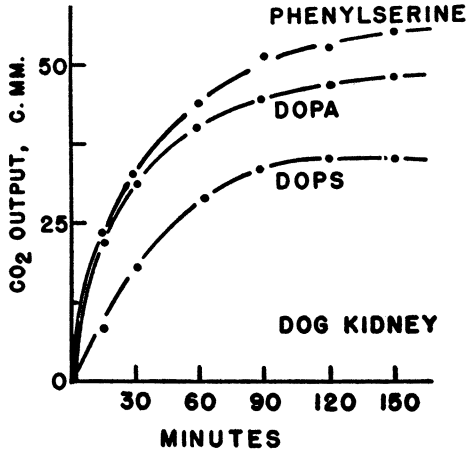


Figure 5. Anaerobic Decarboxylation of Phenylserine, Dihydroxyphenylalanine (DOPA), and Dihydroxyphenylserine (DOPS) by Supernatant from 20% Homogenate of Dog Kidney in 0.1 M Phosphate Buffer

Final concentration of substrates = 0.02 mM.; pH adjusted to 6.5

from their adrenal glands. Thus the transmethylation reaction in the synthesis of adrenaline is indicated. This would seem to complement nicely the aforementioned concepts of Euler (*65, 70*) and of Bacq and Fischer (*11*) that noradrenaline is the principal mediator of adrenergic impulses and is released from those tissues that are not capable of transmethylating it to adrenaline.

In spite of the plausibility of the foregoing hypothesis for the synthesis of adrenaline and arterenol there is a certain attractiveness to the old proposal of Rosenmund and Dornsaft (*137*) that has lost ground by neglect and by the strengthening of the alternative scheme just presented. It was their view that through the condensation of benzaldehyde, or *p*-hydroxybenzaldehyde or phenylglyoxylic acid with *N*-methylglycine (sarcosine), *p*-hydroxy-*N*-methylphenylserine or its *p*-phenolic analog would be formed. They synthesized β-3,4-dihydroxyphenylserine. The synthesis was repeated by Guggenheim (*85*) and more recently by Mann and Dalgliesh (*115*). Hartung (*90*) prepared the series of phenylserine, *p*-hydroxyphenylserine, and 3,4-dihydroxyphenylserine.

If the initial aldol condensation between benzaldehyde and glycine or sarcosine takes place in the body to form phenylserine and/or *N*-methylphenylserine, and it is not an unlikely reaction, then it seems possible for the nucleus to undergo the same phenolic oxida-

tion that has been discussed in the conversion of phenylalanine ⟶ tyrosine ⟶ DOPA. Actually it is conceivable that phenylalanine is a precursor of phenylserine, or that tyrosine is converted by beta oxidation to hydroxyphenylserine. In either instance the introduction of the hydroxyl group would be into a relatively stable compound, as compared to those bearing a dihydric nucleus. In addition this step would make the pattern of synthesis of epinephrine through the phenylserines consistent with the strongest evidence cited for the eventual formation of that pressor agent from phenylalanine.

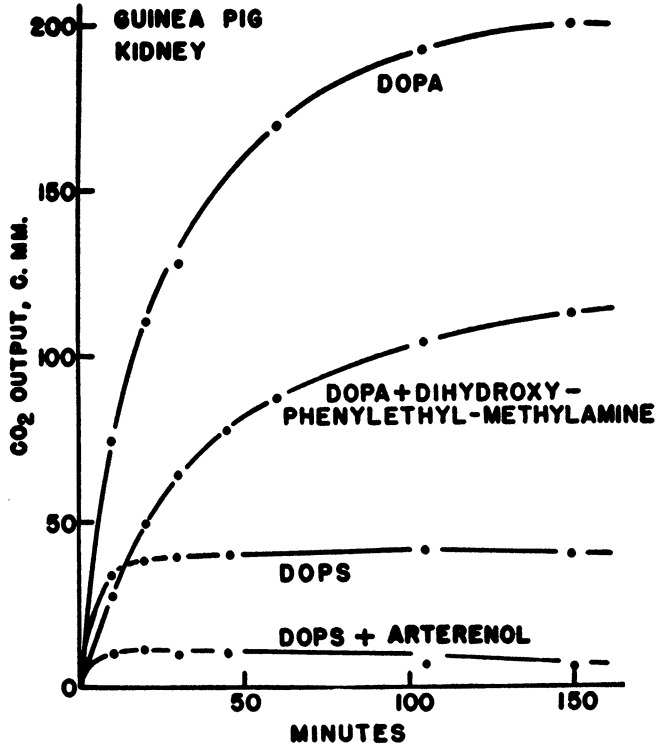


Figure 6. Inhibitory Effect of Amines on Anaerobic Decarboxylation of DOPA and DOPS by Supernatant from 20% Homogenate of Guinea Pig Kidney in 0.1 M Phosphate Buffer

Final concentration of substrates = 0.02 mM.; pH adjusted to 6.5

In experiments not published heretofore, it was found that the oxidation of *p*-hydroxyphenylserine to 3,4-dihydroxyphenylserine occurs rapidly in the presence of phenol oxidase. Contrary to the findings of Blaschko and his associates (*39*), this laboratory found that 3,4-dihydroxyphenylserine and its phenyl and monophenolic precursors were decarboxylated by the kidney of guinea pigs, cats, and dogs. (When this seeming discrepancy was called to Blaschko's attention he was kind enough to repeat his experiment. In correspondence with the author he indicated that in the retest of dihydroxyphenylserine with fresh extract of guinea pig liver there was a very slow formation of carbon dioxide under anaerobic conditions, which was scarcely significant after 1 hour, but which continued with time. Bioassay of the resulting material indicated that *l*-noradrenaline was formed.) An experiment involving the decarboxylation of these three compounds and DOPA by the anaerobic kidney of the dog is illustrated in Figure 5. In all three species the rate of decarboxylation of the phenylserines does not seem to exceed that for DOPA. The decarboxylation of 3,4-dihydroxyphenylserine was least in guinea pig kidney, as may

be seen in Figure 6. In this experiment it was found that addition of the corresponding amine to the vessels decreased or abolished the decarboxylation of both DOPA and dihydroxyphenylserine. This was in keeping with the observation of Schapira that epinephrine inhibited the decarboxylation of DOPA (*140*). Since it is possible that different stereoisomers were present in the racemic mixtures with which Blaschko and this laboratory have worked, there may be no real discrepancy between the two observations, although from an interpretative standpoint the difference is qualitatively significant. Probably the absolute values for carbon dioxide evolution in the illustrations have no definitive significance other than that they may indicate the relative concentration of the single optical form of the phenylserine derivative in the racemic mixture that was susceptible to decarboxylation by the enzyme. One of the principal attractions of this theory is that on decarboxylation of 3,4-dihydroxyphenylserine, noradrenaline is formed. This avoids the awkward necessity of accounting for the introduction of a hydroxyl group into such a compound as dihydroxyphenylethylamine. Here, again, transmethylation of noradrenaline would be the final step in the synthesis of adrenaline, as was anticipated by Blaschko (*35–37*).

The pressor amines have been implicated also in the nephrogenic theories of hypertension. In 1910 Ewins and Laidlaw (*73*) commented that the formation of tyramine from tyrosine in the intestine has quite recently been regarded as playing a part in certain pathological states in which a high blood pressure is the most prominent symptom. Contemporarily, Bain (*12*) reported that tyramine was excreted in the urine of hypertensive patients but to a less extent than in patients having normal blood pressure, the implication being that the elevation in blood pressure was due to the retention of tyramine. The urohypertensin of Abelous and Bardier (*1*) contained isoamylamine.

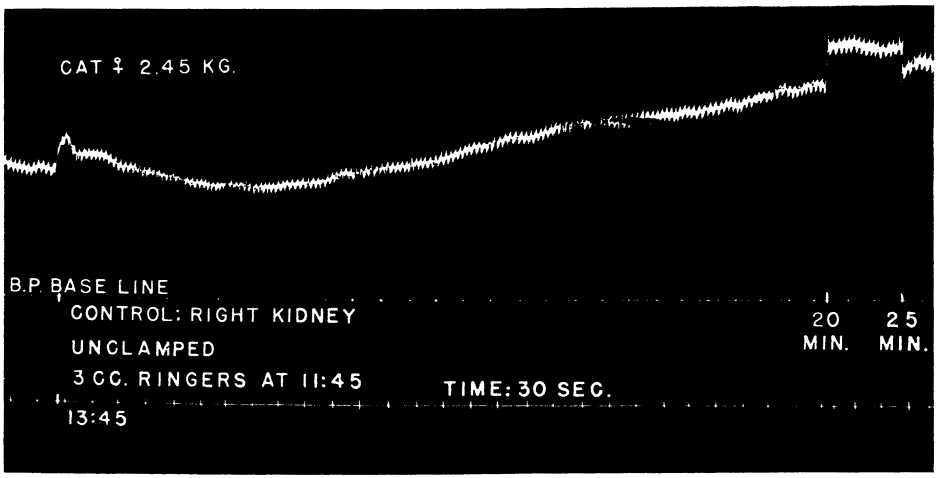


Figure 7. Pressor Response in Anesthetized Cat following Re-establishment of Circulation

Following the initial reports by Holtz (*100*) and Blaschko (*35–37*), Bing (*32, 33*) began a series of experiments on dogs that brought into perspective the possible relationship between renal hypertension and the decarboxylation of DOPA in the kidney. He demonstrated that if DOPA was injected into a partially or totally ischemic kidney of an anesthetized cat and was allowed to remain there for awhile, the re-establishment of circulation through that organ was accompanied by an elevation of blood pressure. The logical interpretation of this observation finds its basis in Holtz' observation (*100*) that in vitro decarboxylation was demonstrable only when the kidney and substrate were maintained under anaerobic conditions. In the presence of air or oxygen, oxidative deamination of the amine occurred simultaneously with decarboxylation so that the carbon dioxide lib-

eration was not readily demonstrable. When DOPA was injected into the ischemic liver or other organs and released to the general circulation of the cat, hypertension did not occur. Neither did a rise in blood pressure follow the injection of DOPA into the unimpaired kidneys of this animal. The interpretation of Bing's experiment was that DOPA was decarboxylated in the ischemic kidney under conditions that would not support the aerobic deamination of the resulting pressor compound by the amine oxidase present in the tissue. Thus there was an outpouring of, presumably, 3,4-dihydroxyphenylethylamine when blood was allowed to circulate through the kidney.

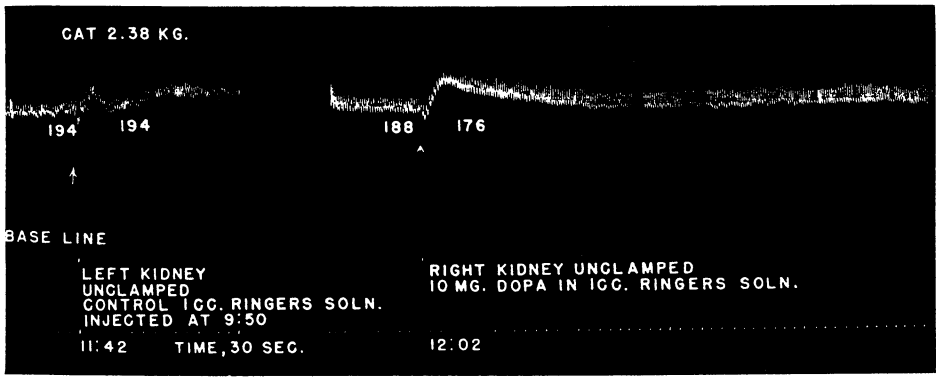


Figure 8. Blood Pressure Response Following Re-establishment of Circulation through Kidney of Anesthetized Cat

Renal artery and vein had been clamped 2 hours previously

The basic experiments by Bing have been confirmed. Schroeder *et al.* (*144*) have reported the isolation of a dialyzable pressor agent from the blood of hypertensive patients that was not present in the blood of normotensive individuals and which had certain biological and chemical characteristics that would relate it to epinephrine or a closely similar compound. Drill (*62*) also has expressed the opinion that the pressor effects of anaerobic kidney extracts are due to the presence of tyramine and other pressor amines. Holtz and Credner (*98*) found that when DOPA was administered parenterally to man and to several animal species they could isolate 3,4-dihydroxyphenylethylamine as such and in a conjugated form from the urine. Page (*124*) has demonstrated the presence of DOPA decarboxylase activity in the kidneys of man, guinea pigs, monkeys, and rabbits, but none was found in the rat. Oster and Sorkin (*123*) made some interesting observations on the effect of intravenous injections of DOPA on the blood pressure of normotensive and hypertensive cats and patients. In both instances the pressor effect of a given injection of DOPA was greater in the hypertensive subject. This they interpreted in the light of Bing's work as attributable to impaired metabolism of the decarboxylated pressor amine in the kidneys of the subjects. None of the conventional renal function studies were mentioned by them as having been performed on the subjects to substantiate this view, and the observation has not provoked substantiation elsewhere.

Although the evidence presented to this point has been reasonably consistent, Page and Reed (*125*) observed that the intraperitoneal or intravenous injection of DOPA into the rat caused a marked and sustained rise in blood pressure. This would not have been anticipated, for Page (*124*) reported that rat tissues do not contain DOPA decarboxylase. Indeed, Page and Reed (*125*) have claimed that the blood pressure response of rats to DOPA cannot be used as a measure of the decarboxylase content of the tissues, nor as an indicator of decreased intrarenal oxygen tension.

A few experiments were conducted in barbiturate anesthetized cats and dogs wherein DOPA, phenylserine, *p*-hydroxyphenylserine (DOPS), and 3,4-dihydroxyphenylserine were injected intravenously or into a kidney to which the blood supply was clamped tem-

porarily. The results of these experiments are shown in Figures 7 through 11. Figure 7 illustrates the effect of unclamping the left kidney into which 3.0 ml. of Ringer's solution had been injected 2 hours previously as a control. Here, as in almost all such experiments, re-establishment of circulation was followed by a rise in blood pressure. When the blood supply to the other kidney into which 10 mg. of DOPA had been injected was re-established, a greater and reasonably sustained rise in blood pressure followed. Figure 8 is the first of three illustrations taken from a single experiment on a cat. In this case, both kidneys were clamped immediately following the intrarenal arterial injection of 3 ml. of Ringer's solution on one side and 3 ml. containing 10 mg. of dihydroxyphenylserine into the kidney on the other side. When the control kidney was released, the blood pressure rose slowly over a period of about half an hour. When the circulation was released through the kidney into which DOPS was injected, the rise in blood pressure was dramatic and was sustained well over an hour, as is illustrated in Figure 9. Figure 10 shows the effect of the intravenous injection of dihydroxyphenylserine into the same cat after the blood pressure had returned to a low level. The rise in blood pressure was greater than when the drug was incubated in the kidney before its release.

In Figures 11 and 12 the pressor effect of intravenously injected phenylserine, *p*-hydroxyphenylserine (DOPS), and DOPA are compared with epinephrine in the dog. DOPA was inactive whereas all phenylserines caused a rise in blood pressure. This would seem to be good presumptive evidence that the rise in blood pressure was due to decarboxylation of the several phenylserines. Although the decarboxylation of the sample of 3,4-dihydroxyphenylserine by the kidney of the dog and cat in vitro has been demonstrated, even a very small percentage contamination of the amino acid with the corresponding pressor amine (noradrenaline) would give rise to a considerable elevation of blood pressure, though probably not as sustained a duration of effect as was present in this instance. However, it would be desirable to have a comparison of the pressor effect of DOPA and dihydroxyphenylserine repeated in another laboratory using other samples of the drugs to check the point.

Convincing as the preceding evidence may seem, there is just basis for a certain reservation concerning their significance. They tend to imply that the balance between the decarboxylation of pressor amine precursors and the elimination of the amines is so delicately maintained that an otherwise undetectable alteration in renal function would permit the formation of such amines to exceed their destruction or excretion, resulting in an elevation of blood pressure. There is evidence to the contrary that must be considered.

The kidney is neither the only nor even the major source of amine oxidase. Bhagvat, Blaschko, and Richter (*30*) and other investigators (*109*, *139*) have found the enzyme to be widely distributed in the body. Richter, Lee, and Hill (*134*) determined that the human body was capable of deaminating phenethylamine at a rate of 26 mg. per kg. of body weight per hour. The fact that β-phenyl-*n*-propylamine, which was deaminated by amine oxidase, was not excreted as such when administered orally unless the liver was injured by an hepatoxic agent, suggests that organ as a principal source of amine oxidase (*25*). In the rat, which is so widely used for studies in hypertension, decreased deamination of pressor amines in damaged kidneys does not contribute to the elevation of blood pressure, for there is no amine oxidase in the kidney of that animal (*122*). Brown and Maegraith (*45*) found no reduction in the amine oxidase content of other organs of hypertensive rats. For that matter, there is good reason to believe that deamination probably does not play a dominant role in the inactivation of phenolic pressor amines (*105*, *136*).

Elimination and Inactivation of Pressor Amines

The elimination of phenolic pressor amines combines inactivation and excretion. This field has been reviewed in recent years by Bernheim (*14*), De Meio (*59*), Hartung (*89*), and Beyer (*23*, *26*).

Transmethylation as a step in the "deactivation" (from a cardiovascular standpoint) of noradrenaline in the body is suggested by the recent report by Goldenberg, Pines, Baldwin, Greene, and Roh (*82*). Although they give no enzymologic studies to substantiate their hypothesis they suggest that essential hypertension might be considered to be a met-

abolic disease of deficient transmethylation—that is, of norepinephrine to epinephrine. Their concept arose from experiments wherein they injected *l*-epinephrine and *l*-norepinephrine into normotensive patients and others with essential hypertension. Direct measurements were made simultaneously of cardiac output, systemic arterial pressure, and pulmonary arterial pressure. Epinephrine, in doses sufficient to cause significant hypertension, was found to act as an over-all vasodilator as well as a powerful cardiac stimulant. The response of hypertensive patients to adrenaline was increased but was qualitatively the same as in normal subjects. The primary action of noradrenaline was an intense generalized vasoconstriction without significant cardiac effects in the dose range studied and this response was greater in hypertensive than in normotensive individuals. The vasoconstrictor action of *l*-arterenol was blocked completely by the synchronous administration of equal doses of epinephrine. They conclude that their findings are compatible with the concept that norepinephrine is a sympathetic mediator of over-all vasoconstriction and suggest that a disturbed balance between both sympathetic transmitters could be concerned in the production of hypertension.

Regardless of the untested merits of the above work, methylation as a first step in the deactivation of noradrenaline in the body is just as plausible as is the evidence that methylation is the final step in the synthesis of adrenaline. The evidence for and against this route of synthesis has been discussed previously in this review. Tainter *et al.* (*155*) reported that in dogs under phenobarbital anesthesia *l*-arterenol had a pressor activity 1.7 times that of *l*-epinephrine. In this sense then, methylation might be considered a process of inactivation. However, they found in contrast that the acute toxicity of *l*-epinephrine (LD_{50}) was about four times that of *l*-norepinephrine (*114, 155*).

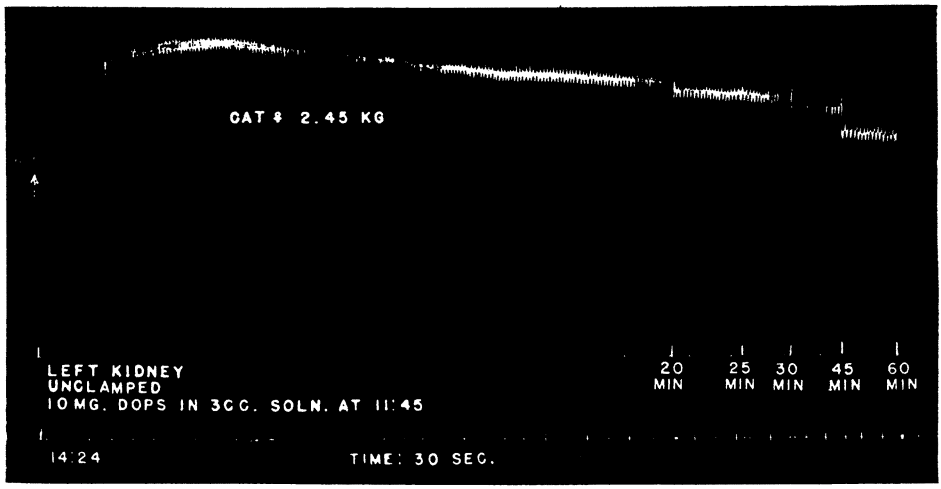


Figure 9. Blood Pressure Response to Unclamping of Opposite Kidney in Same Cat Used for Figure 8

Ten mg. of dihydroxyphenylserine had been injected into kidney at time renal pedicle was clamped, 2 hours preceding this record

The role of amine oxidase in the inactivation of sympathomimetic amines rests on a much firmer basis. The enzymatic oxidative deamination of tyramine was described first by Hare (*87*). Kohn (*105*) partially purified it but the enzyme is widely distributed in mammalian tissues (*30, 109*), is cyanide insensitive (*15*), and has resisted isolation. The name monamine oxidase has been suggested for the enzyme (*154*) referred to in the literature as tyramine oxidase, adrenaline oxidase (*40*), and aliphatic amine oxidase (*128*).

This enzyme rapidly deaminates certain primary and secondary unsubstituted *p*-hydroxy- and 3,4-dihydroxyphenylethylamines wherein the amino group is on the terminal carbon atom of the side chain (*4, 18, 22, 40, 128*).

It seems established that the presence of amine oxidase in the body, and especially that in the liver, determines the oral efficacy of β-phenylethylamines. In general those compounds of this nature having an alpha carbon atom adjacent to that bearing the amine—that is, β-phenylisopropylamine—are not deaminated by amine oxidase (*134*), are active on oral administration, and are excreted in the urine as such following oral or parenteral administration (*28, 102, 132, 151*).

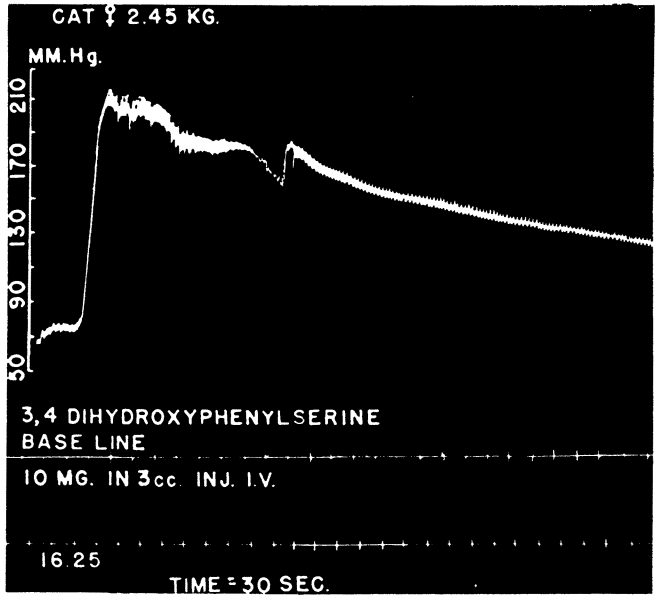


Figure 10. Pressor Response to Intravenous Injection of 10 Mg. of 3,4-Dihydroxyphenylserine into Cat following Record Obtained in Figure 9

Principally because amine oxidase was so abundantly present in the body and could be shown to deaminate certain pressor amines in vitro, it has been thought by some to play a role in the in vivo inactivation of adrenaline and in the etiology of hypertension. The administration of crude amine oxidase has been reported to have decreased the blood pressure of normal and hypertensive rats (*142*), but this has not provoked substantiation. Croxatto and Croxatto (*53, 54*) have shown that renal hypertensinase and amine oxidase were different enzymes. Also, Bing, Zucker, and Perkins (*34*) found angiotonin and amine oxidase to be fundamentally different. Thus the hypertensin and the phenylethyl (pressor) amine approaches to hypertension seem quite dissimilar on these grounds.

However, it seems unlikely that amine oxidase plays a fundamental role in the inactivation of the phenolic pressor amines even though it is of great importance in determining the fate of the β-phenylethylamines.

The phenol oxidases probably play no important role in the elimination of phenolic pressor amines, in spite of the importance that has been attached to the oxidation of the catechol nucleus in the past. The names phenolase and cresolase, polyphenol oxidase, and catechol oxidase serve to identify the enzyme with its mono- or diphenolic substrate, but they usually occur together and are difficultly separated. The enzymes have been purified and their characteristics have been described (*56, 104, 106, 156*). Beyer (*21*), Alles (*3*), and Randall and Hitchings (*129*) have described the relationship of structure of the phenolic pressor amines to the rate of oxidation of their nucleus in the presence of these enzymes.

The functional significance and even the normal presence of other than a DOPA oxi-

dase in the mammalian body have not been well established (*31*). Bloch (*43*) described the "DOPA reaction" of skin, in which case melanin was formed when DOPA was incubated with skin, and this has been confirmed by Pugh (*127*) and Charles (*50*). Cadden and Dill (*47*) reported a polyphenolase to be present in kidney, but this did not oxidize phenolic pressor amines. Hogeboom and Adams (*94*) have reported the presence of a phenolase in a mouse melanoma that oxidized tyramine, and this is probably the most authentic representation of a phenol oxidase that could oxidize the phenolic nucleus of pressor amines in the body.

In spite of the fact that phenol oxidase probably plays no important role in the inactivation of pressor amines in the body, it has been reported that the injection of the enzyme into hypertensive rats led to a reduction in their blood pressure (*141*, *143*). It is difficult to assess the value of these experiments because of the nonspecific depressor effects of crude protein preparations on blood pressure. For example, Prinzmetal *et al.* (*126*) found that their tyrosinase preparations inactivated by boiling decreased the blood pressure of hypertensive patients as well as did their enzymically active preparations.

There are other modes of inactivation of pressor amines that probably are not highly significant factors from our present standpoint. These include the effect of the cytochrome C–cytochrome oxidase oxidation of catechol derivatives to the corresponding orthoquinone (*41*, *103*), the oxidation of the phenolic nucleus by the ascorbic-dehydroascorbic acid system (*18*), and the deamination of pressor amines in the presence of aldehydes (*120*, *121*, *152*). One may refer to the reviews by Hartung (*89*) and by Beyer (*23*) for discussions of these systems.

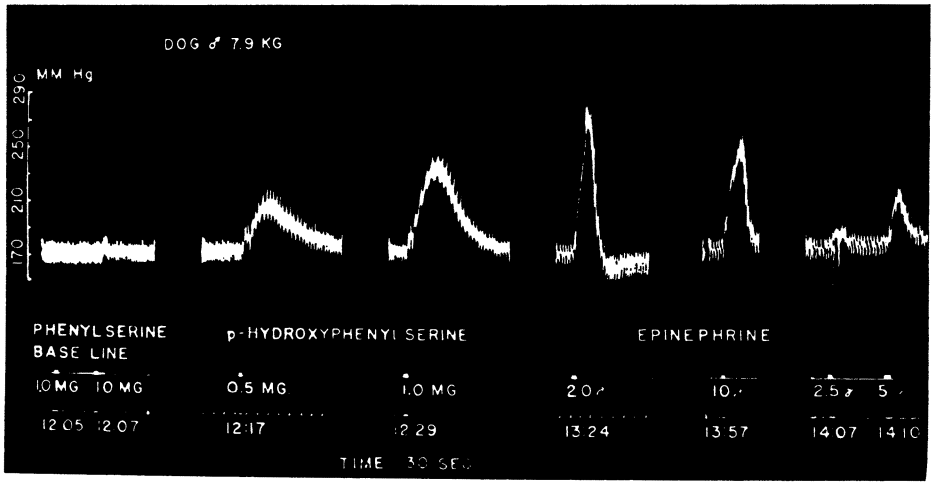


Figure 11. Effect of Intravenously Injected Phenylserine, *p*-Hydroxyphenylserine, and *l*-Epinephrine on Blood Pressure of Anesthetized Dog

Conjugation appears to be the principal mode of inactivation of phenolic pressor amines in the body. In the author's experience the administration of phenolic pressor amines conjugated with organic or inorganic acid radicals on either the hydroxyl group of the ring or the aliphatic amino group reduces or abolishes activity. This is also true for the acetylation of *p*-aminophenylethylamines. Barger and Dale (*13*) found acetoxyphenylethylamine inactive on intravenous injection. Loeper (*112*) reported the synthesis of the sulfuric ester of tyramine and found it inactive as a pressor agent unless it was hydrolyzed to tyramine (*111*).

The in vivo conjugation of sympathomimetic amines having a catechol nucleus seems well established. The identity of the conjugate seems clear but is not certain. Richter (*133*) and Richter and MacIntosh (*135*) reported the excretion of conjugated epinephrine

after the parent compound was administered orally. No free compound was excreted. After acid hydrolysis the epinephrine was recovered in the urine by the iodoadrenochrome reaction. Also, the pressor effect of the hydrolyzed compound was demonstrated. They believed the conjugation to be with sulfuric acid, and proposed that it was mediated through a sulfosynthase. However, they did not definitely identify the conjugate as being with sulfuric acid. Beyer and Shapiro (*27*) quantitated the iodoadrenochrome reaction and reported that the 3,4-dihydroxyphenylethylamine derivatives, cobefrin and epinine, were excreted to the extent of about 65 and 85%, respectively, as a hydrolyzable conjugate in urine following their oral administration to man. Both compounds and epinephrine were excreted as conjugates by dogs and this qualitative observation was not affected by the route of administration of the drugs. The pressor effect of the hydrolyzed urine containing epinephrine was confirmed. Holtz and Credner (*98*) reported that when human subjects were given DOPA orally they excreted both free and conjugated 3,4-dihydroxyphenylethylamine. They presumed the conjugation to be with sulfuric acid.

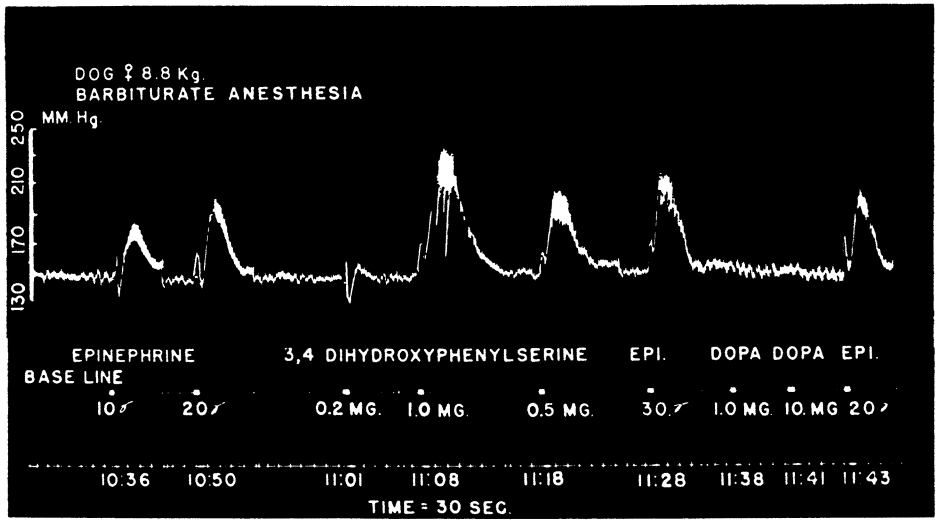


Figure 12. Effect of Intravenously Injected *l*-Epinephrine, Dihydroxyphenylserine, and Dihydroxyphenylalanine (DOPA) on Blood Pressure of Anesthetized Dog

Although no free epinephrine is excreted under the conditions of those experiments just described, it is permissible to question whether they are representative of the fate of epinephrine as it is secreted in the body, or whether conjugation is a mode of inactivation of catechol pressor amines administered as drugs. The relatively minute amounts of adrenaline or noradrenaline secreted normally and the limitations of present analytical methods definitely handicap a direct approach to the problem. However, it has been possible to study the fate of adrenaline secreted by the body in a more or less physiological type of experiment.

There is a tumor of the adrenal medulla that has been described as physiologically malignant but histologically benign (*119*). That is to say, the symptoms of hypertension are rapidly progressive although the tumor cells are benign, within the meaning of that term to the pathologist. These cells secrete adrenaline in large amounts that are at first released only intermittently but later the hypertension may be sustained. The paraganglioma is a similar type of tumor of the sympathetic chain ganglia that gives rise to clinically identical symptoms. Muntz (*119*) and his associates reported the removal of a pheochromocytoma that, on the basis of Chen's assay, was estimated to contain 2.3496 grams of adrenaline. They estimated that it would take a herd of 31 cattle to yield the same amount of adrenaline. Others have reported the isolation of adrenaline from such tumors,

although Bulbring and Burn (*46*) and Holton (*95*) have reported recently that both normal adrenal gland and the pheochromocytoma contain noradrenaline in addition to adrenaline.

The author (*24*) has had an opportunity to study the pheochromocytomas removed from three patients, and their urine specimens obtained between and during hypertensive attacks (*116*). In general, only free adrenaline and noradrenaline were found in the tumor and only conjugated drug in the urine at the time of the hypertensive attacks. Actually the differentiation between epinephrine and arterenol was made only in two of the cases for the iodoadrenochrome method (*27*) does not differentiate between the two compounds. Auerbach and Angell (*9*) developed a method to estimate arterenol in the presence of epinephrine. Using this method they found arterenol to be present in U.S.P. samples of epinephrine from biological source to the extent of 10.5 to 17.5%, in confirmation of the work of Goldenberg *et al.* (*81*). Indeed, Tullar (*159*) was able to isolate *l*-arterenol from natural U.S.P. epinephrine. Thus it seems incontrovertible that noradrenaline as well as adrenaline is a normal constituent of the adrenal medulla. This would seem to be consistent with the view previously expressed herein that norepinephrine is a precursor that can be converted into epinephrine by transmethylation.

The conjugation of epinephrine in the body is with both sulfuric and glucuronic acids, although there seems to be some difference of opinion as to which is the more important route. Arnolt and De Meio (*5, 60*), Bernheim (*17*), and Lipschitz and Beuding (*110*) have reported that the conjugation of phenols in the intestine, liver, and spleen is an enzymatic process. Apparently it is a coupled oxidative system. In sulfate conjugation, inorganic sulfate serves as the precursor (*108*). Deichmann (*58*) fed epinephrine to rabbits and found a marked increase in the excretion of organic sulfates without an increase in the excretion of glucuronates. He concluded that epinephrine conjugation was principally with sulfuric acid. Conversely, Dodgson, Garton, and Williams (*61*) conducted a similar investigation wherein *d*-epinephrine was administered orally. No free drug was excreted, but in this case the conjugation was with glucuronic acid. One would be led to believe that probably both sulfuric and glucuronic acids play a role in the conjugation of catechol pressor amines.

The fate of monophenolic pressor amines is not so certain. Ewins and Laidlaw found that when large amounts of tyramine were fed to dogs, up to 25% was excreted in the urine as *p*-hydroxyphenylacetic acid. They found that this occurred when tyramine was perfused through liver and uterus, but when the drug was perfused through the heart it was destroyed (*73*). Similarly, Bernheim and Bernheim (*16*) found that the heart was capable of opening the phenolic ring in tyramine.

Present evidence indicates that monophenolic pressor amines, such as tyramine, may be excreted partially as such and in a conjugated form with and without deamination and oxidation to the corresponding acid. Beyer and Stutzman (*29*) administered tyramine and β-(*p*-hydroxyphenyl)isopropylamine to both man and dog. Although a physiological effect of the agent was not demonstrable, the urine was found to contain a compound that appeared to be identical with the drug that was administered. This was judged by the fact that it was oxidized by phenol oxidase, deaminated by amine oxidase in the case of tyramine, and possessed pressor properties. The amount of the material excreted was not determined, but since then the results have been repeated and confirmed qualitatively. Undoubtedly this does not account for all the drug that was given, and it is quite possible that the drug was conjugated loosely during its passage through the body. Hartles and Williams (*88*) studied the detoxication of *p*-hydroxybenzylamine and *p*-hydroxybenzylmethylamine administered to rabbits. They found in both instances that the compounds were excreted as the sulfates, the sulfate conjugation being greater for the primary amine. Both compounds also were deaminated and excreted as *p*-hydroxyphenylacetic acid and the corresponding glucuronide.

Comment

Although it has been beyond the province of this review to discuss the several theories of the etiology of essential hypertension, it is the opinion of this author that the various

concepts are not mutually exclusive and that to hold one or another theory wholly accountable for the clinical picture of essential hypertension is misleading and unwarranted at present. The probability that in many or even most instances hypertension is neurogenic in origin seems most attractive. Since the autonomic impulses that initiate vasoconstriction are adrenergic, Sympathin E (noradrenaline), and to a lessor extent adrenaline, undoubtedly play a major role in the earlier phases of the disease.

Vasoconstriction brought about in this manner through psychic stimulation has been shown to decrease renal blood flow. It is attractive to hypothesize that this may initiate a more severe renal cortical ischemia than is apparent from over-all blood flow measurements, if the cortical vasoconstriction should be accompanied by the opening of subcortical arteriovenous shunts such as have been described by Trueta (*157*). Such a circumstance would set up the conditions requisite for the elaboration of renin, or the decarboxylated precursors of adrenaline or noradrenaline that require adequate oxygenation for their inactivation, or the establishment of the endocrine kidney of Selye (*146*). Over the course of time it is possible that these nephrogenic agents may perpetuate the hypertension and the conditions for their continued function. The pathogenesis of the degenerative arteriolitic lesions would be initiated, on this basis, as reparative and compensatory responses to the traumatic effect of the early wide fluctuations in pressure and the later continued insult from that source. In the case of the pheochromocytoma, that mimics so closely the clinical picture of essential hypertension of other origin, the initiation and progress of the disease are attributable to an exaggeration of the normal functions of the adrenal medulla.

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Discussion of Paper on Biosynthesis and Metabolism of Phenylethyl (Pressor) Amines

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As stated by Beyer, it now does appear that both noradrenaline and adrenaline are implicated in the humoral mediation of adrenergic nerve impulses. The hypothesis that adrenoxine as produced by any action of a catechol oxidase in the body acts under appropriate conditions as the vasodilator substance presently appears to be very doubtful, although some of the evidence along this line presented by Bacq (*1*) and by Heirman and Bacq (*7*) appeared to be reliable. Shortly after their reports appeared Carroll Handley and the author in the pharmacology laboratory of the University of California Medical School tried to confirm the apparent reversal of net vasomotor effects of catechol oxidase oxidation of adrenaline solutions but failed to observe any effects beyond those that could be ascribed to the destruction of a part of the adrenaline activity.

In some recent comparisons of the pressor responses of adrenaline and noradrenaline, the appreciably longer duration of pressor action of the nor compound was noticed, and this is evidenced in the figures shown by Luduena and co-workers (*10*) in their recent careful quantitative studies on the relative activities of the two compounds as estimated by various methods on different animals and organs of the body. This longer duration of action of noradrenaline indicates that the over-all inactivation rate in the body is indeed slower. This is in agreement with the indications from the work of West (*15*) on jugular/portal and splenic artery/vein equipressor ratios that the two compounds are apparently inactivated differently by the liver and spleen.

It may indeed be, as Bacq and Fisher (*2*) and Goldenberg and co-workers (*6*) suggest, that the synthesis of adrenaline is through the transmethylation of noradrenaline as a final step and that the deactivation of noradrenaline is through the transmethylation of noradrenaline as an initial step. In this connection it is of interest that Shaw (*13*) found in his studies on the oxidation of adrenaline and noradrenaline by an arsenomolybic reagent that the former catechol amine was about twelve times more rapidly oxidized and that an increase of about five times was from some phenomenon associated with the addition of alkali. Just what the phenomena noted by Shaw are due to is not clear but they do demonstrate that an order of five to twelve times difference in oxidation rates between noradrenaline and adrenaline may be on some simple chemical reaction basis.

The thought that transmethylation of noradrenaline may be an initial step in its deactivation in the body makes one wonder whether further transmethylation of adrenaline is also not a common biological process. As a result of studies of the various alkaloids of plants of the *Ephedra* species, it was noted that not only *l*-ephedrine and its stereoisomer *d*-pseudoephedrine are present but also that *l*-norephedrine, *d*-norpseudoephedrine, *l*-methylephedrine, and *d*-methylpseudoephedrine (*8*) are present. The quaternary trimethylammonium compounds corresponding to these were not reported but very

probably were not looked for and would be difficult of isolation in pure form because of their high base strength.

The question as to whether all of the possible *N*-methylation compounds of noradrenaline are indeed commonly present in storage places in the body such as the adrenal medulla, or the carotid body, or other ganglia or peripheral synapses will probably not be answered soon, for the intensity of the pharmacological activity of *N*-dimethylnoradrenaline and noradrenaline trimethylammonium ion is not great as reported by Stehle, Melville, and Oldham (*14*) who unfortunately do not give any of the chemical details regarding the identity or purity of the preparations they used.

However, the question as to whether the first methylation step, the conversion from noradrenaline to adrenaline, is an active process over-all in the body should be susceptible of fairly easy study by repeating the experiments of Richter and MacIntosh (*12*) and of Beyer and Shapiro (*4*) but administering noradrenaline instead of adrenaline. The urine-excreted compounds could be bioassayed after suitable hydrolysis and the relative amounts of noradrenaline and adrenaline in the hydrolyzate determined by using the differential activities of the two compounds on rabbit intestine and rat uterus as first reported by West (*16*). The differences between normal persons and those in successive stages of essential hypertension with regard to their abilities to conjugate noradrenaline and adrenaline and with regard to their abilities to transform the former into the latter surely should be a subject of precise chemical study in the near future.

Although interest and knowledge today is rapidly increasing with respect to noradrenaline and adrenaline as humoral mediators in the functioning of the sympathetic nervous system, the possibilities should not be overlooked that less intensely active compounds may be implicated in the normal or pathological functioning of this nervous system in man. Along this line the vasoconstrictor material recently isolated from beef serum by Rapport, Green, and Page (*11*) is of considerable interest. This substance in its purest state as reported has a vasoconstrictor activity of about that of tyramine though its various color reactions in relation to its activity indicate it to be considerably different from this compound.

Tyramine has appeared to be ordinarily quite rapidly inactivated in the body, possibly by the amine oxidase mechanism that is active on aliphatic and phenylaliphatic amines. However, this should perhaps be reinvestigated in relation to hypertension in man, for Bain (*3*) apparently found some isoamylamine in the urine in man, and although Lockett (*9*) was not able to find this compound in her studies, there are indications of some unknown variables being involved. Lockett did find pressor bases in urine that were more active than isoamylamine and on further study a close correspondence between one of the bases and nicotine was established. However, her preparations of male urine which included some tobacco smokers corresponded to but 50 to 70 micrograms of nicotine per liter in physiological pressor activity and those of female nonsmokers' urine, to but 17 micrograms of nicotine per liter.

The reinvestigation of von Euler (*5*) of urine of nonsmokers indicated up to about 10 mg. per liter of ether-soluble bases and a pressor activity corresponding to about 500 micrograms per liter. Piperidine was isolated and found to be the principal base present in the ether-soluble bases and comparison between the pressor assay and colorimetric piperidine determinations showed a close correspondence. It was further reported that piperidine was present in the urines of the horse, pig, cat, and rabbit as well as that of man. The meaning of its presence or its possible relation to the metabolism of other amines or the functioning of the sympathetic nervous system in normal or hypertensive man has not yet been developed.

In closing, the excellent presentation of Beyer is recommended for close study by biological, medical, and organic chemists. It is very well balanced, up-to-date, and the result of much thinking and working along the lines he has talked about.

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