# RENAL PHARMACOLOGY[1]

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After making a preliminary collection of references in preparation for the organization of this review, it became quite clear that a review of all of the revelant contributions to the general field of renal pharmacology during the past eighteen months would be impossible. Over two thousand papers were indexed. What follows is an arbitrary selection of the subjects we considered to be of special interest at this time. Furthermore, with few exceptions, only papers immediately available to us in our library have been reviewed. Many subjects which might have been included, have been covered elsewhere. References to pertinent reviews have been included.

## Autoregulation of Blood Flow by the Kidney

"The renal circulation has the remarkable capacity to adjust itself quickly in the face of changing arterial pressure so that the renal blood flow tends to remain constant whether the arterial pressure rises or falls" [Smith (1)]. For example, Waugh (2) has shown that, within a normal range of blood pressure, a 50 per cent increase in arterial pressure produced only a 5 per cent increase in renal blood flow. This fact has been appreciated for many years, and the mechanisms involved in the phenomenon of "autoregulation" have been the subject of extensive study. However, considerable controversy still exists concerning the importance of autoregulation in renal function and the role of various factors which may be involved. Hinshaw *et al.* (3) pointed out that the kidney differs from other organs in the mammalian body in ways which influence its vascular bed. It has a semirigid capsule and a profuse structural stroma. Also, its extensive ultrafiltration mechanism which responds to changes in pressure, filters fluid at rates much higher than in other tissues. Extravascular pressures, both in the general tissue space and in the glomerular capsule, can affect the vascular geometry and resistance to blood flow. The tissue pressure is the resultant of a number of factors: (*a*) intravascular pressure and volume; (*b*) volume of the filtered fluid within the nephrons; (*c*) elasticity of the renal capsule; (*d*) volume and elasticity of the Bowman's capsules; and (*e*) stroma rigidity. In the view of Hinshaw *et al.* (3), tissue pressure rises in response to increased arterial perfusion pressure and becomes a major factor in opposing vascular distention. In addition to the structural characteristics, other factors play a role in autoregulation. Suggestions have included neural, humoral, and myogenic

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changes in the tone of renal blood vessels, and changes in viscosity of the perfusing blood as a result of plasma skimming.

Many experimental conditions have been employed to study the phenomenon of autoregulation. It occurs *in situ* and in isolated perfused kidneys. The character and extent of autoregulation that can be demonstrated varies with the methods employed. Reasons for this should be evident from the discussion which follows. With rather specialized techniques, some investigators have recently obtained results which made them question the occurrence and physiological importance of autoregulation.

By cannulating the renal artery via the aorta so that the renal hilus was not damaged, Langston *et al.* (4, 5, 6) showed that the kidney, like many other organs, responded with an increase in blood flow following a stepwise increase in arterial pressure over the range of 60 to 280 mm Hg. The usual picture of autoregulation was apparent only after dissection of the renal artery or after a 5- to 20-min occlusion of it. Hardin, Scott & Haddy (7) criticized the perfusion method of Langston and co-workers by claiming that perfusion of the area supplied by the lumbar arteries occurred with this method. With the lumbar arteries included, the curves of pressure versus flow did not show autoregulation; however, when these vessels were occluded the renal circulation showed good autoregulation. To answer this criticism, Langston, Guyton & Hull (6) repeated their experiments and were again unable to demonstrate autoregulation. They showed that lumbar artery perfusion was not a factor since, after clamping the renal artery close to the kidney, there were unable to detect any extrarenal blood flow. In answer to another criticism of their technique, namely that it predisposes the renal vascular bed to an extremely high resistance to flow which lowers blood flow to below the usual range at moderate perfusion pressure, Langston *et al.* observed only slight autoregulation at the more usual rates of blood flow (2 to 4 ml/min per g of kidney). In these experiments spinal anesthesia was instituted prior to any surgical manipulation. In the previous study without spinal anesthesia, blood flow was only 0.6 to 2 ml/min per g at a perfusion pressure of 120 mm Hg.

Rosenfeld & Sellers (8) observed that the isolated perfused kidneys of rabbits did not show autoregulation when kidneys used were from rabbits anesthetized by intraperitoneal 30 per cent ethanol. Kidneys obtained from animals anesthetized with pentobarbital, or those from spinal animals showed partial autoregulation. These authors studied functional characteristics of the isolated kidneys (urine flow, *p*-aminohippurate and creatinine clearance, *p*-aminohippurate extraction and glucose excretion), and could not correlate the presence or absence of autoregulation with the functions and clearances studied in their preparation. Ohler, Harth & Kreienberg (9) were unable to demonstrate autoregulation in rat kidneys perfused with homologous or heterologous serum.

On the other hand, autoregulation of renal blood flow has been observed and confirmed in a variety of preparations. Weiss, Passow & Rothstein (10)

demonstrated autoregulation in rat kidneys perfused with dextran. Thurau, Kramer & Brechtelsbauer (11) demonstrated autoregulation in the denervated kidneys of dogs anesthetized with morphine-chloralose. In this study, aging of the preparation produced an increase in the smooth muscle tone of the renal vasculature and a partial loss of autoregulation, both of which were produced also by infusions of epinephrine or by perfusion with hypertonic or colloidal fluids. Schmid & Spencer (12) studied the pressure-flow relation in the dog kidney by means of a square wave electromagnetic flowmeter placed around the renal artery. Changes in blood pressure were produced by aortic compression and autoregulation of renal blood flow was observed. Bounous, Shumacker & King (13) also reported autoregulation in anesthetized dogs, and they commented that the vascular resistance of the kidney is normally low. Harvey (14) and Scher, McDonald & Koch (15) reported autoregulation in the isolated perfused kidney.

The controversy concerning the importance of renal autoregulation has not been resolved. The basic tone of the renal blood vessels and the general condition of the kidney during the experiment undoubtedly have an important effect on the results obtained. For example, the type of cannulation of the renal artery (via the descending aorta) employed by Langston *et al.* (4) conceivably could have produced, by means of reflexes, conditions unfavorable for the demonstration of renal autoregulation. Langston and co-workers (5) proposed that damage to the renal lymphatics, or the sensitization of the renal blood vessels to circulating catechol amines, might account for autoregulation in some preparations. However, from the studies of Thurau, Kramer & Brechtelsbauer (11), it follows that vasoconstriction should, if anything, reduce autoregulation. In some older experiments, Forster & Maes (16) denervated kidneys and demedullated adrenal glands several weeks before conducting autoregulation studies on unanesthetized rabbits. In this preparation, good autoregulation was observed in the renal vascular bed when blood pressure changes were produced by initiation of carotid sinus reflexes. Acute damage to renal nerves was not responsible for autoregulation in these experiments.

On the other hand, denervation or damage to renal nerves might induce a state favorable to the demonstration of autoregulation. In Selkurt's (17) experiments, in which autoregulation was demonstrated during compression of the aorta, the effect of possible damage to renal nerves or lymphatics, occurring after cannulation of the renal vein (for determining renal blood flow), must be considered. Langston and co-workers (4, 5, 6), as mentioned above, found that autoregulation was apparent only after the renal artery was dissected or clamped at the hilus, procedures which could break the reflex constriction of renal blood vessels. Experiments in which the renal artery is approached from below and in which the aortic pressure sensitive area is denervated, might provide a new approach to this problem. Langston *et al.* (5) speculated that damage to renal lymphatics might account for autoregulation in some preparations and could contribute to the rise in

renal tissue pressure observed by Hinshaw and co-workers (3, 18) which occurs following an increase in perfusion pressure. Still another mechanism, which might account for apparent autoregulation in some experiments, is a vascular response produced by damage to the vasa vasorum of the renal arteries. Such damage might produce a reactive hyperemia in the arterial wall which could affect the tone of the vessel. Autoregulation can be demonstrated in muscle vascular beds that show reactive hyperemia [Renkin & Stainsby (19)].

Much work has been conducted in attempts to explain autoregulation in the kidney. Active vasomotor changes and passive physical mechanisms have been invoked to explain it. From among the proponents of active vasomotor mechanism a number of different suggestions have been proposed. Thus, DeMuylder (20) proposed that a reflex function of the juxtaglomerular apparatus is related to autoregulation. A neurogenic theory has been proposed by Lamport (21), based on the occasional finding that autoregulation is lost after denervation of the kidney. Page & McCubbin (22) suggested that intrarenal neural reflexes which persist after denervation of the kidney may play a role in autoregulation, and Sellwood & Verney (23) think that baroreceptors situated in Bowman's capsule may be involved. All of these theories have received little attention in recent years. Another postulate, namely, that autoregulation results from a myogenic reaction of renal blood vessels, has received considerable attention and support (2, 11 24, 25, 26).

Opposing views are that renal autoregulation is caused either by viscosity changes produced by intrarenal plasma skimming [Pappenheimer *et al.* (27 to 30)], or by increased interstitial pressure which compresses intrarenal vascular channels (3, 15, 18, 31). These views have been reviewed and discussed by Winton (32), and more recently by Waugh & Shanks (26).

The cell-separation theory of Pappenheimer & Kinter (27) attempts to explain autoregulation in the kidney by proposing that plasma skimming occurs in the interlobular arteries; the blood, poor in red cells, goes via the vasa afferentia to the glomerular capillaries, while the cell-rich fraction remaining in the interlobular arteries passes through short shunts. The cell-poor and cell-rich blood join again on the venous side, and renal venous blood has about the same hematocrit as arterial blood. The high content of red blood cells in the interlobular arteries and shunts makes it possible to vary resistance by changing the cell concentration in these vessels. An increase in arterial pressure should increase plasma skimming, thus increasing the cell-rich fluid and resistance to flow. This would explain the observed relative constancy of renal blood flow in the face of relatively high fluctuations in renal pressure. In favor of this theory are the observations that intrarenal hematrocrit is low; at normal pressure it is about half that of renal arterial blood and varies inversely with arterial pressure (27).

According to this theory, red cell passage time through the kidney should be less than that of plasma, but Ochwadt (33) has found that red cells

and serum albumin have about the same passage time in the kidney. If the production of a high hematocrit controls intrarenal resistance, perfusion with cell-free plasma or a plasma substitute, should eliminate renal autoregulation. According to Pappenheimer & Kinter (27), cell-free perfusion of the cat kidney with dextran eliminated autoregulation, while addition of red blood cells caused the reappearance of the resistance changes. However, dog (2, 24, 34) and rat (10) kidneys perfused with cell-free plasma substitutes showed good autoregulation, especially if 20 per cent of plasma had been added to the perfusion solution [Waugh & Shanks (26)]; this is a crucial point against the cell separation theory. The occurrence of high renal extraction ratios for iodopyracet and *p*-aminohippurate has been used as supporting evidence for this theory. According to it, high extraction ratios are caused by the passage of the plasma-rich blood through the peritubular capillaries. If cell-poor perfusion fluid is used, the extraction ratio should fall since plasma can now pass through the shunts. This has been observed experimentally in the cat kidney [Kinter & Pappenheimer (28)]. However, in the experiments of Thompson *et al.* (34), normal dogs extracted 75 per cent of the plasma *p*-aminohippurate while anemic animals showed only a decrease to 62 per cent in *p*-aminohippurate extraction.

A further indirect line of evidence in favor of plasma skimming is the finding that $O_2$ tension in the urine is low during breathing of air and $O_2$. The oxygen tension in urine has to be in equilibrium with capillary $O_2$ tension. If cell separation occurred, the $O_2$ supply for the tissue would be mainly dependent on the physically dissolved $O_2$ in plasma. This could explain low urinary $O_2$ tensions as well as the constancy of the arteriovenous $O_2$ difference. It has been observed that $O_2$ traverses the renal circulatory system more rapidly than do red blood cells [Levy & Sauceda (35)]. This was interpreted to signify that $O_2$ diffuses from some point upstream to a point further downstream, and bypasses a portion of the vascular bed. The vasae rectae of the renal medulla, with their hairpin loop configuration, were considered to be the most likely candidates as the sites for such $O_2$ diffusion. However, Levy (36) was unable to show a difference between the superficial and deep portions of the kidney with respect to the manner in which $O_2$ consumption was influenced by changes in renal blood flow. In both cortical and medullary areas, arteriovenous $O_2$ differences remained constant in spite of a reduction of renal blood flow. More recent studies by Levy & Imperial (37) have shown that $O_2$ shunting occurs in both cortical and medullary tissues. The capillary structure around the cortical tubules is such that, as in the medullary vasae rectae, the countercurrent diffusion of $O_2$ could also occur in the cortex. The anatomical arrangement of both cortical and medullary capillaries would favor the shunting of $O_2$ from the arterial to the venous side. These considerations could explain the relative constancy of the renal arteriovenous $O_2$ difference, and at the same time explain the low $O_2$ tension in urine which would equilibrate with the oxygen-poor contents of the capillary loop in both cortex and medulla. This alternative explanation of the

low $O_2$ pressure in urine would not require the concept of cell separation.

The fact remains that renal hematocrit is low. This finding has been recently confirmed by the determination of hematocrits and hemoglobin concentrations in vasa recti blood of the papilla in golden hamsters by Ullrich & Stockle (38). The values obtained for hemoglobin content were 50 per cent of those in the systemic arterial blood, while hematocrit varied with the osmotic pressure. This was explained by the shrinkage of red blood cells in hypertonic solutions. These findings show that cell separation occurs at least in the medulla. However, medullary blood flow does not amount to more than 1 per cent of total renal blood flow [Kramer *et al.* (39); Lilienfield *et al.* (40)]. Thus, the finding of a low hemoglobin content and low hematocrit in vasa recti blood cannot explain the low hematocrit values determined with tagged red blood cells and plasma in the total kidney.

A second possibility is that the low hematocrits observed in the renal cortex are only artifacts, since plasma low in protein content can accumulate in the extravascular renal tissue. The movement of this fluid in and out of the vascular bed occurs rapidly with changes in blood pressure [Heimburg & Ochwadt (41)]. Thus, at a high blood pressure, interstitial fluid containing labelled serum protein could accumulate, and this could conceivably simulate a low hematocrit. Swann, Railey & Carmignani (42) estimate that this extravascular fluid is about 28 per cent, and the intrarenal blood volume is about 16 per cent of the kidney weight. The high proportion of extravascular fluid may explain the apparent low hematocrit determined in the kidney. Direct hematocrit and hemoglobin concentrations in blood obtained from cortical blood vessels would be essential for a final clarification of this point.

Recently a second physical explanation for autoregulation of renal blood flow has been extensively discussed by Hinshaw and co-workers (3, 18, 43, 44). In essence, this theory states that changes in extravascular volume and pressure alter the resistance in the small and less rigid postglomerular vessels and in Bowman's capsule. A rise in extravascular volume and pressure thus increases resistance to flow. These investigators have shown that deep venous pressure, tissue pressure, and kidney weight increase in the perfused kidney when blood pressure is raised in the range where autoregulation does occur. Heimburg & Ochwadt (41) have shown that changes in blood pressure cause rapid movements of extravascular fluid. Thus, changes in tissue pressure and deep venous pressure should be proportional to the amount of autoregulation observed. The difficulties of measuring deep venous pressure and tissue pressure have to be kept in mind. The measurements of Thurau *et al.* (11), and the more recent results of Waugh & Shanks (26), indicate that changes in kidney weight and tissue pressure in good preparations are not causally related to the marked adjustments that occur in the renal vasculature following an increase in the perfusion pressure. Changes similar to those observed by Hinshaw *et al.* (3) were seen when perfusion temperature was reduced and in kidneys poisoned with chloral hydrate (26). This controversy has not been resolved, but it seems to us that

published tracings of both the Göttingen and Georgia groups clearly show the secondary nature of the delayed weight and deep venous pressure changes. Hinshaw *et al.* (31), however, object to this interpretation, and claim that intrarenal venous pressure depends upon the position of the catheter in the venous bed. They point out that sudden changes in blood pressure may displace the venous catheter and thus result in low readings.

The last theory to be discussed is that autoregulation results from the constriction of the renal vascular smooth muscle in response to an increase in intravascular pressure. It is known that autoregulation in the kidney involves glomerular filtration as well as blood flow at blood pressures above 90 mm Hg [Selkurt (45); Shipley & Study (46); Thurau & Kramer (47)]. Thurau & Kramer (47) have shown that a sudden increase in perfusion pressure causes, first, an increase followed by a fluctuation in flow which frequently undershoots before it reaches the new steady-state in about 30 to 60 seconds. A sudden reduction in pressure produced tracings which indicated that the increased vascular tone lagged behind the changes in perfusion pressure, thus causing a temporary reduction in blood flow which was followed by a readjustment of flow to its steady-state level. Somewhat similar tracings have been published by Waugh & Shanks (26). Such findings strongly suggest the vascular nature of autoregulation; since the glomerular filtration rate is also involved, the preglomerular nature of this vascular constriction must be considered.

One of the strongest arguments against the vascular nature of renal autoregulation has been the observation that a reduction in temperature to about 12°C. did not eliminate autoregulation, nor did the treatment of the kidney with 0.5 per cent chloral hydrate (a concentration which eliminated vascular reactivity of the kidney vessels) [Winton (48)]. Waugh & Shanks (26) have restudied the effects of cold and chloral hydrate in isolated perfused dog kidneys. They were able to show that autoregulation occurs in the excised denervated kidney perfused with corpuscle-free perfusion fluid. When the kidney was cooled to 3 to 10°C., so-called genuine autoregulation was abolished. Also removed was the sensitivity of the kidney to epinephrine and to a ganglionic stimulant 1,1-dimethyl-4-phenylpiperazinium. However, a peculiar type of autoregulation appeared which was characterized by an initial increase in blood flow proportional to the increase in pressure. This was followed by a gradual reduction in flow accompanied by increases in kidney weight and intrarenal venous pressure. This type of autoregulation was different from that observed in the warm kidney, and was caused mainly by changes in interstitial pressure. In a like manner, addition of chloral hydrate (0.5 gm per 100 ml. of perfusion fluid) eliminated the reactivity of the renal vessels to epinephrine and 1,1-dimethyl-4-phenylpiperazinium, and the genuine type of autoregulation was replaced by a delayed type of resistance increase which was similar to that seen in the cold kidney. Autoregulation was eliminated rapidly when the kidney was perfused with either polyvinylpyrolidone or dextran. Such perfusion resulted in

a reduction in the responsiveness of the renal vasculature to epinephrine and to the phenylpiperazinium derivative. Addition of 5 to 20 per cent plasma to the perfusion fluid prevented this deterioration, and the kidneys showed good autoregulation of flow. Waugh & Shanks (26) believe that this effect of plasma may explain some of the results of Kinter & Pappenheimer (29) who showed that the addition of red blood cells restored autoregulation in dextran-perfused cat kidneys. They suggest that the addition of plasma trapped between the cells, or some material in the red blood cells, was responsible for the restored autoregulation rather than the red blood cells per se.

Thurau & Kramer (47) have shown that papaverine eliminates autoregulation by the kidney. In the presence of this drug, an increase in arterial pressure caused an increase in blood flow, glomerular filtration, and urine flow. Waugh & Shanks (26) have shown that procaine in low concentrations (0.4 mg per cent) eliminated the effects of the ganglionic stimulant dimethyl phenylpiperazinium, but not the effects of epinephrine. Autoregulation occurred in the presence of this low concentration of procaine. A high concentration of procaine (100 mg. per cent) eliminated reactivity to the phenylpiperazinium derivative and to epinephrine and prevented autoregulation. The adrenergic blocking agents, dibenzyline or yohimbine, in doses which blocked the effects of epinephrine and 1,1-dimethyl-4-phenylpiperazinium, did not block but modified autoregulation. Relatively high concentrations of $\gamma$-aminobutyric acid (500 mg. per cent) did not block autoregulation in the isolated perfused kidney. Anoxic perfusion of the renal vessels did not eliminate, although it impaired, autoregulation to some extent. Preliminary hemorrhage in the dog produced intense vasoconstriction in the kidney, which persisted after the kidney was isolated, and resulted in a marked reduction of blood flow through the kidney and the elimination of autoregulation.

The Göttingen and the Georgia groups have shown that autoregulation occurs in both the blood- and dextran- perfused kidney. The drug effects outlined suggest that no local neurogenic reflex is operative in this phenomenon, and that the main constrictor effect arising from pressure is probably preglomerular. It seems likely that intrinsic myogenic responses to pressure or distention are the cause of this autoregulation. As the evidence stands, autoregulation in the kidney seems to be myogenic in nature, and most probably is related to constriction of the preglomerular vessels. The autoregulation caused by increased interstitial and intrarenal pressures may also play a role under certain conditions. However, this seems to be secondary to the myogenic mechanism championed by Thurau & Kramer and Waugh & Shanks. The evidence presented minimizes the importance of cell separation in autoregulation, especially since most of the indirect evidence presented by Pappenheimer and his colleagues can be explained on a different basis.

Just what causes the constriction of the vessels is not clear. The specially

marked autoregulation observed in the renal vascular bed and the presence of juxtaglomerular apparatus, have tempted some to invoke the release of renin and production of hypertensin II as a causative factor. It is likely that the low intrinsic tone of the renal vessels is important in autoregulation, since any form of constriction seems to impair or eliminate this phenomenon. The observation of Folkow (49) that the hyperemic muscle vascular bed also shows some autoregulation favors the idea that the relatively low tone of the renal vascular bed predisposes these vessels to autoregulation.

## Renal Hemodynamics

Indirect methods for determining renal blood flow are based upon clearance measurements of rapidly excreted substances, such as *p*-aminohippurate and iodopyracet. The usual analytical procedure for *p*-aminohippurate does not determine the acetylated form. If acetylation takes place in the kidney, a considerable error in renal blood flow measurements would be introduced. Setchell & Blanch (50) have shown that *p*-aminohippurate conjugation does occur to a significant extent in the kidneys of a number of species and will thus interfere with the determination of effective renal plasma flow. For those species (guinea pig, mouse, rabbit, cat, cow, pig, and sheep) which conjugate the hippurate in the kidney, a more precise value of effective renal plasma flow would be obtained by determining the clearance of free plus conjugated *p*-aminohippurate or the clearance of acetyl-*p*-aminohippurate. Balint and co-workers (51, 52) have shown that under conditions of hypoxia or hypovolemia, the clearance of *p*-aminohippurate is not a good index of renal plasma flow. Thus, during posthemorrhagic hypotension or after 72 hr of water deprivation, $O_2$ extraction was increased and renal blood flow was decreased. The latter decreased in proportion to the decrease in cardiac output. *p*-Aminohippurate clearance, under these conditions, decreased more than the directly determined blood flow [see also Balint *et al.* (53, 54)]. Since the decrease in renal blood flow was proportional to the reduction in cardiac output, there was no increase in renal resistance. Atik & Gutierrez-Saenz (55) compared direct renal blood flow measurements with those calculated from aminohippurate clearances. Under stable normotensive conditions, the agreement between these two types of measurements was fairly good; but under hypovolemic or hypotensive conditions, discrepancies between the two methods became quite apparent.

Gilmore (56) has shown that carotid sinus occlusion in dogs anesthetized with pentobarbital, produced variable changes in glomerular filtration rate, renal plasma flow, urine flow, and electrolyte excretion. The increased intrarenal resistance, as a result of carotid occlusion, may be caused mainly by an increase in extrinsic sympathetic activity.

McGiff & Aviado (57) measured blood flow in the renal and femoral bed. Carotid occlusion and hypoxia caused a greater increase in femoral than in renal bed resistance. Hemorrhage increased the renal and decreased the femoral bed resistance. Aortic constriction reduced resistance more in the

femoral than in the renal vascular bed. Norepinephrine and hypertensin, on the other hand, increased the resistance of the renal bed more than that of the femoral. The authors suggest that the renal vascular bed is more reactive to hormonal influences, while the femoral bed responds more strongly to chemo- or baroceptor-induced changes. Jontz, Shumacker & Bounous (58), using direct methods for measuring the renal blood flow in both kidneys, showed that denervation of one kidney (by previous sympathectomy and splanchnicectomy) produced an increase in blood flow in the denervated organ. This difference was especially marked when the animal was subjected to the combined stress of anesthesia and hemorrhage. Dieter (59, 60, 61) has published a number of interesting papers on the electrical activity of efferent renal nerves in the frog. Centrifugal action potentials were recorded from renal nerves which arise from the ganglia of the abdominal sympathetic chain. The filling of the vascular bed decreased centrifugal activity, while emptying increased it. It was shown that filling of the auricle decreased renal nerve activity, while a decrease in filling increased it. Vagotomy eliminated the effects on renal nerve activity of filling and emptying of the vasculature. These experiments demonstrated that pressoreceptor stimulation in the auricle of the frog can influence renal nerve activity. Gauer (62) and Henry, Gauer & Reeves (63) demonstrated in dogs that the inflation of a balloon in the left auricle increased urine flow. They eliminated this effect by cooling the vagus nerves. Dieter (60) has studied the effects on renal activity of sectioning the central nervous system of the frog. He concluded that auricular filling activates pressor receptors, and that afferent impulses are carried via the vagi to the central nervous system. These impulses modulate central sympathetic activity which, in turn, controls the spontaneous activity of the renal efferent sympathetic fibers. The central control areas are situated somewhere between the optic lobes and the beginning of the spinal cord. Epinephrine inhibits the activity of the efferent renal nerves by actions on sensory end organs in the region of the heart, in the central nervous system, and in peripheral ganglia [Dieter (61)]. The activity that Dieter recorded is from nerve bundles, and not from single fibers. Thus, little can be said concerning the number of fibers involved and the ratio of active to inactive fibers, or the tonic effect of these impulses on the renal vessels. It is known that under relatively physiological conditions, the renal vessels are under low sympathetic tone [Pappenheimer (64)]; and it is thus not surprising that Dieter (65) could not demonstrate a difference between innervated and denervated dog kidneys in response to epinephrine injections. The problem of the physiology of renal innervation and its central representation, was briefly discussed by Pappenheimer (64).

Barger *et al.* (66, 67) have demonstrated that in experimentally produced heart failure in dogs, there is a reduction in effective renal plasma flow, and an increase in the filtration fraction. The response of these animals to a saline infusion was a sensitive index of the cardiac status. In normal dogs, a saline infusion increased renal plasma flow, glomerular filtration

rate, and sodium excretion, but in the animals with heart failure, the response to saline was depressed approximately in proportion to the severity of the heart lesions produced. The decline in renal blood flow may be related to increased sympathetic tone since intrarenal infusion of an adrenergic blocking agent (dibenzyline) increased renal plasma flow. Furthermore, the response to a saline load was improved by dibenzyline—so much so, that in some instances this drug restored the normal sodium excretion pattern. The concentrations of dibenzyline used had no effect on the kidneys of normal dogs. These effects of dibenzyline could be produced unilaterally on the infused side and, therefore, cannot be explained on a hormonal basis. Barger *et al.* (67) quote a number of other investigations, all of which suggest that variations in sympathetic activity in the kidney may play a role in the adjustment of sodium and water excretion. Whether this is an effect on the renal vasculature or directly on tubular activity is still not clear.

In contrast are some of the findings of Carpenter *et al.* (68) who have shown that in the transplanted cervical kidney, constriction of the thoracic vena cava resulted in virtually complete sodium retention, an effect also seen in the normally located kidney. Transplanted adrenal glands responded to inferior vena cava constriction with an increased secretion of aldosterone, corticosterone, and Porter-Silber chromogens. These results indicate that neither intact renal or adrenal nerves, nor an increased venous pressure, are essential for the hypersecretion of aldosterone, or for the renal retention of sodium which follows thoracic inferior vena cava constriction. Craig *et al.* (69) showed that dogs with experimental ascites had a depressed glomerular filtration rate and renal plasma flow which were further decreased by hypophysectomy. However, sodium excretion, depressed by ascites, was restored to normal by hypophysectomy. The Na/K ratio in the urine was depressed by the experimental ascites and was increased after the hypophysectomy.

Changes occurring in the renal circulation during heart failure have been reviewed by Goodyer (70). The increase in the vascular resistance of the kidney, also observed in heart failure, is not necessarily a result of neurogenic vasoconstriction, since an acute reduction of blood flow increases the resistance in the denervated kidney (71) and spinal anesthesia does not eliminate the increased renal resistance in human heart failure (72). Sympatho-adrenal stimulation in man is probably not a major factor which promotes changes in the renal circulation and edema formation. Goodyer (70) discusses the possible role of hypertension, arterial pulse pressure, renal venous pressure, and humoral and endocrine factors in the etiology of cardiac edema.

Horster, Peters & Brunner (73) have studied in rats the effects of jugular vein ligation on the excretion of an orally administered saline load. They showed that this procedure causes a swelling of the head region, a reduction in the clearance of *p*-aminohippurate and creatinine, and a decrease in sodium excretion. They suggest that the reduced elimination of sodium is

possibly attributable to pressure changes in the head region. It is claimed that ligation of the femoral bed did not produce these effects, and that adrenalectomy did not substantially modify them. Kanter (74) has studied the effects of hyperventilation on glomerular filtration and renal plasma flow in anesthetized dogs. Zoster *et al.* (75) determined the effects of local pH changes on the renal blood flow in dogs. Sheehan & Davis (76) have shown that obstruction of the renal veins causes a localized necrosis, mainly in the outer cortex and the intermediate zone of the kidney. Abbrecht & Malvin (77) studied the effects of changes in renal plasma flow and glomerular filtration rate on urine concentration, and showed that renal blood flow has important effects on the renal concentrating mechanism in addition to its effects on glomerular filtration rate.

Peters (78) studied the clearance of endogenous and exogenous creatinine, as well as inulin and *p*-aminohippurate, in rats receiving different types of diuretics. The effects of hypothermia on kidney function have been studied by Moyer (79), and of hyperthermia by Kanter (80). The data show that the reduction of glomerular filtration rate and renal plasma flow are directly related to the hyperthermia. In addition, Kaufman *et al.* (81) have shown in man that exposure to high environmental temperature causes a decrease in urine volume, sodium, potassium, and chloride excretion, and a reduction in glomerular filtration rate and renal plasma flow; filtration fraction was increased; and arterial pressure had a tendency to be reduced while peripheral venous pressure was increased. All these effects occurred with an increase of rectal temperature of less than 1°C. The authors concluded that the redistribution of blood during exposure to a hot environment is the main cause of the observed effects.

Pabst & Thron (82) have studied cold diuresis in dogs. They conclude that exposure to a cold environment produces an increase in the renal hemodynamics with an increase in urine flow, and often an increase in sodium, potassium, and osmolar excretion. They believe that cold diuresis is mainly caused by renal vascular changes, and that hormonal factors play only a secondary or minor role.

Kramer & Deetjen (83) have studied renal blood flow, arteriovenous $O_2$ differences, and $O_2$ consumption of the kidney. In a range of blood flow between 200 to 800 ml/min per 100 g of kidney, $O_2$ consumption was linearly related to blood flow. Under 200 ml/min $O_2$ consumption changed exponentially with blood flow. In the linear portion of the curve, the arteriovenous $O_2$ difference was constant (1.3 volumes per cent); in the exponential portion of the curve, the $O_2$ difference increased with a decrease in renal blood flow. Oxygen consumption was linearly related to glomerular filtration rate. It was suggested that a causal relationship exists between glomerular filtration rate and renal oxygen consumption because an increase in the former led to an increase in proximal tubular reabsorption of sodium.

A series of papers on the effects of epinephrine and related substances on renal function have appeared. Mills, Moyer & Handley (84) studied the

effects of a series of sympathomimetic amines on renal plasma flow and glomerular filtration rate. No correlation between the rise in blood pressure and the increase in renal vascular resistance could be found. The authors concluded that the changes observed were attributable to a greater reactivity of the renal constrictor adrenergic receptors to certain
to those of the vasculature in general. McQueen & Morrison (85) compared the effects of norepinephrine and angiotensin on blood pressure and renal function. Angiotensin increased blood pressure and depressed renal function more than did norepinephrine. On the other hand, angiotensin produced much less venospasm.

Botting *et al.* (86, 87) studied the effects of subcutaneously administered epinephrine and isoprenaline in rats. These compounds produced antidiuresis, retention of sodium, chloride, and potassium, decreased ammonia production, and induced a rise in urinary pH without a detectable change in glomerular filtration rate or renal plasma flow. Denervation of the kidneys, adrenalectomy, removal of the posterior lobe of the hypophysis, partial hepatectomy, or section of afferent nerves from the injection site, did not appreciably modify this response to these sympathomimetic amines. Worthen *et al.* (88) studied the effects of epinephrine on the isolated perfused dog kidney. Large doses of epinephrine decreased both blood flow and urine flow. No increase in urine flow was observed with the smaller amounts of epinephrine used in this study [see also Nelson, Henry & Lyman (89)]. Blake (90) used indirect methods and suggested that norepinephrine may augment blood flow to the renal medulla at the expense of cortical areas. Heimburg & Ochwadt (41) have shown that epinephrine decreased kidney weight and blood flow in isolated dog kidneys; hemoglobin and plasma protein concentrations in the venous outflow decreased very rapidly. These effects are similar to those seen with a sudden reduction in arterial perfusion pressure. The reductions in hemoglobin and albumin concentration probably result from a rapid movement of extravascular fluid into the intravascular renal bed. The volume of this fluid can be equal to the total blood volume in the kidney. The significance of the renal hematocrit, kidney volume and weight, and intrarenal blood distribution measurements have been pointed out in the section on autoregulation.

Granitsas (91) studied the effects of epinephrine on the excretion of nitrogenous products in the urine of rats. There was an increased excretion of purine metabolites and a marked increase in total nitrogen excretion. This effect of epinephrine was not inhibited by ergot alkaloids, and was not duplicated by the administration of cortisone. Norepinephrine had similar although less pronounced effects. Dihydrogenated ergot alkaloids blocked the effects of epinephrine and renal nerve stimulation on the renal circulation [Kubicek (92)]. Serotonin caused a constriction of the renal vascular bed in the isolated kidney perfused with dextran. The constriction was readily reversed by lysergic acid diethylamide [Passow *et al.* (93)]. By use of a benzidine staining technique and India ink injections, Dolcini *et al.* (94)

showed that relatively large doses of serotonin caused an increased filling of the glomeruli and peritubular capillaries, followed by a marked reduction in filling of the cortical region. The juxtamedullary blood supply appeared to be normal. The authors suggested that serotonin, in the doses used, shunts both red blood cells and plasma away from the cortex. Infusion of serotonin caused a decrease in the renal excretion of electrolytes with no significant change in renal plasma flow and glomerular filtration and rate. Low concentrations of serotonin had an effect mainly on sodium excretion, and the results were interpreted as indicating a specific effect of serotonin on electrolyte reabsorption by the renal tubules of the dog. Frick (95) discussed the possible role of serotonin in changes in the renal circulation observed during the use of heart-lung machines.

The effects of guanethedine on renal circulation were determined by Bonomini *et al.* (96), Vercillo & Giraldi (97), Richardson *et al.* (98), and Wagle & Plummer (99). Some reduction in renal blood flow was observed. Atropine injections in dogs usually caused some antidiuresis accompanied by a fall in urinary sodium chloride concentration. Choline increased urinary pH and decreased urinary sodium concentration; and at high rates of infusion, it seemed to act as an osmotic diuretic [Solomon, Davies & Boone (100)]. Granitsas (101) showed that, in contrast to epinephrine, acetylcholine under certain conditions can produce a decrease in nitrogen and creatinine excretion in rats. This effect of acetylcholine was comparable but not identical to that of growth hormone. Carter & Atkinson (102) showed that the administration of physostigmine and prostigmine to hydrated rats, induced a five- to ten-fold increase in the amount of sodium excreted during a 3- to 4-hr period of observation. Atropine inhibited these effects [see also Kuschinsky & Langecker (103)]. Greeff & Contzen (104) determined the mechanism of dextran antidiuresis and edema. Histamine probably plays an important role in the production of the former, while the edema formation may be caused by the release of serotonin.

Kuschinsky & Vorheer (105) utilized the benzedine method in kidney slices to study the effects of osmotic and theophylline diuresis on the distribution of blood in the kidney and on the size of tubules [see also Marchetti *et al.* (106)]. The effects of infusions of magnesium sulfate in normal and hypertensive patients were studied by Kelly *et al.* (107).

## Diuretics

A number of general reviews on diuretic drugs have been published (108 to 116). It is inevitable, therefore, that the following will be somewhat repetitious. From the very large number of papers dealing with the pharmacology and clinical use of diuretics, only those papers which, in our opinion, have contributed to the understanding of the action of these drugs have been reviewed. While restricting ourselves in this sense, we have nevertheless attempted a reasonably comprehensive review of recent developments.

Screening methods for determing diuretic activity in various species have been published (117 to 121). Gold *et al.* (122) have developed rapid methods for comparing the clinical efficacy of diuretics, and have suggested that these methods may become useful in establishing the relative efficacy of these drugs.

*Benzothiadiazine diuretics.*—The literature on chlorothiazide and related compounds has been voluminous. No new information concerning the mechanism or site of action of these diuretics has been published, and little can be added to the discussion of Orloff & Berliner (123) which appeared in the first volume of these *Annual Reviews*. There have been no suggestions about the possible chemical interactions of this group of drugs with a renal receptor responsible for their chloruretic action. Additional structure-activity studies may eventually provide a clue.

Experimental evidence obtained from the application of different techniques has led to opposing views concerning the locus of action along the nepthron. Vander *et al.* (124) and Kessler *et al.* (125) using the stopflow technique concluded that the major action of chlorothiazide on sodium chloride reabsorption was localized in the proximal tubule. The evidence, a decrease in free water clearance ($C_{H_2O}$), obtained by Earley *et al.* (126) in dogs undergoing water diuresis, led them to conclude that chlorothiazide acts at sites in the distal nephron which are responsible for urinary dilution. In addition, failure of the drug to interfere with the process of concentration of the urine at high rates of solute excretion (no change in net free water reabsorption), led them to suggest that the predominant action occurs in the distal convoluted tubules. Similar findings of a lack of effect of chlorothiazide on the concentrating ability at high rates of solute excretion were reported by Au & Raisz (127). However, chlorothiazide, like the mercurials, has been found to increase urine flow with little or no change in $C_{H_2O}$ in dogs [Blackmore (128)] and in human subjects [Heinemann *et al.* (129)]. The increase in urine flow is evidence for a proximal action. Therefore, the possibility that chlorothiazide and related compounds produce a diuretic effect by acting at a number of sites along the renal tubule must be considered.

The recent clinical literature confirms the studies of Edmonds & Wilson (130) and Liddle (131) which showed that repeated administration of a benzothiadiazine caused a progressive increase in potassium excretion, and that this effect was related to an increased secretion of aldosterone. Their interpretation is supported by the observation that aldosterone antagonists enhance the natriuresis and suppress the kaluresis induced by benzothiadiazine diuretics [Edmonds (132)].

The search for more active benzothiadiazine diuretics has continued, and some of the newer compounds are more active milligram for milligram than are the original benzothiadiazine derivatives (111). The increase in potency is usually associated with a reduction in the carbonic anhydrase inhibiting action of the compounds, and, under standard conditions, the newer derivatives also produce a less pronounced urinary loss of potassium. However, it

is quite clear that not only the compound but, also, the condition of the subject determine the effect of these compounds on potassium excretion. This is probably related to their ability to inhibit a mechanism for sodium reabsorption which increases the load of sodium delivered to the distal sites where potassium-sodium exchange takes place. Any factor that increases the efficiency of this exchange mechanism, such as high aldosterone production, might alter the Na/K ratio in the urine, following the use of a diuretic which acts at a site proximal to that of the sodium-potassium exchange mechanism. The hypersecretion of aldosterone in some disease states may explain the decreased Na/K ratio of the urine following a benzothiadiazine diuretic. The combination of spirolactones and benzothiadizine diuretics should thus increase the Na/K ratio in the urine. The effect of such combinations has been observed (132 to 135). Gantt & Synek (136) have studied the kaluretic effects of hydrochlorothiazide in adrenalectomized rats. To achieve the maximal effects of this drug on K excretion, deoxycorticosterone had to be administered. These studies demonstrate that potassium loss during a thiazide diuresis is partly dependent upon the aldosterone content of body fluids. Pitts *et al.* (137) have shown that chlormerodrin, a mercurial,counteracts the kaluretic effects of chlorothiazide. More recently, Cafruny *et al.* (138) proposed that a nondiuretic mercurial, *p*-hydroxymercuribenzoate, also blocks the kaluretic action of hydrochlorothiazide. However, according to McBride *et al.* (139), *p*-chloromercuribenzoate even in high doses has no effect on the renal tubular secretion of potassium. Our own studies (140) confirmed the findings of McBride and his colleagues. In our opinion, additional studies of the effects of this agent on the kaluretic action of benzothiadiazine drugs are in order.

Haenze (141) concluded from short-term experiments in man, that chlorothiazide, hydrochlorothiazide, and mercurials increased the excretion of magnesium and calcium. Acetazolamide increased calcium excretion, but to a lesser extent than did the other diuretics, and decreased magnesium excretion. It was concluded that the excretion of magnesium is not dependent upon carbonic anhydrase, and that different tubular mechanisms must be responsible for the renal tubular reabsorption of calcium and magnesium (141). However, Kiil (142) reported that chlorothiazide and hydroflumethiazide had no significant effects on calcium or magnesium excretion.

Confirming earlier observations, Crosley *et al.* (143) observed that the intravenous administration of chlorothiazide produced a reduction in glomerular filtration rate and renal plasma flow and which was related to a fall in cardiac output and a decrease in central venous pressure. This probably was the result of a decrease in venous return or venous pooling. These effects of chlorothiazide are probably related to the hypotensive effects of the drug. However, the mechanism of the hypotensive effect continues to be a controversial problem. Several investigators have shown that the initial hypotensive effect of benzothiazide diuretics was related to an acute reduction in plasma volume resulting from the diuresis (144 to 147). However,

chlorothiazide produced a fall in blood pressure in the hypertensive, but not in the normotensive, patient although the saluretic response, weight loss, decrease in plasma volume, and the decrease in reactivity to norepinephrine were the same in both groups. Plasma volume replacement tended to restore blood pressure and responsiveness to norepinephrine [Freis *et al.* (147)]. Similar experiments conducted by Hollander *et al.* (148) have shown that replacement of plasma volume produced only a partial restoration of the blood pressure in hypertensive patients treated with chlorothiazide. Conway & Lauwers (146, 149) observed that, following one or two weeks of therapy with chlorothiazide, plasma volume and cardiac output fell and total peripheral resistance was increased. After one month or more of therapy, plasma volume and cardiac output were back to pretreatment levels while the fall in blood pressure was maintained. This is evidence for a reduction in peripheral resistance. Total body water was decreased, probably because of a decrease in intracellular water, since there were no consistent changes in exchangeable sodium, plasma volume, or extracellular fluid volume. In rats, Gessler & Heinze (150) have shown that 24 hr after hydrochlorothiazide administration there was a reduction in intracellular water volume and an increase in extracellular fluid volume. This was accompanied by a shift of potassium out of the cell compartment. A return to normal was observed after 48 hr. Beavers (151) claimed that chlorothiazide and hydrochlorothiazide produced a reduction of serum potassium in the nephrectomized dog. This experiment was repeated by Blackmore (152) who did not confirm this finding.

Changes in the sensitivity of the vascular system to pressor drugs, following benzothiadiazine diuretics, have been described in man (153 to 157) and in lower animals (158, 159, 160). These changes were not a specific effect of chlorothiazide since mercaptomerin and acetazolamide also decreased the reactivity of the vascular bed to pressor substances [Blackmore & Beavers (161)]. Mercaptomerin and spironolactone produced a reduction in blood pressure in hypertensive patients. Restoration of blood volume by infusion of plasma and correction of the negative sodium balance by means of mineralocorticoid administration did not restore blood pressure to pretreatment levels [Hollander *et al.* (157)]. However, Doleery *et al.* (162) have shown that the drop in blood pressure produced by pentolinium, a ganglionic blocking agent, was increased by short-term chlorothiazide therapy. The blood pressure lowering properties of pentolinium were directly related to a reduction of plasma volume in these patients. It follows that diuretic therapy, at least in its early stages, reduces plasma volume and influences reactvity of the blood vessels to some hypotensive and hypertensive drugs. However, from clinical findings, this does not seem to be the sole factor in the reduction of blood pressure produced by chlorothiazide since plasma volume was restored during prolonged diuretic therapy but the reduction in blood pressure persisted. Exchangeable sodium also returned to normal during extended therapy [Hollander *et al.* (157)]. That a reduction in serum potas-

sium is not related to this phenomenon is clear from the finding that spironolactone has similar blood pressure effects but does not cause a negative potassium balance. The well-known blood pressure lowering properties of a low sodium diet, seen in some hypertensive patients, strongly suggests that negative sodium balance and the possible concomitant changes in intracellular ion concentrations following prolonged diuretic therapy may also play a role in producing the fall in blood pressure (163).

The contention that benzothiadiazines have a specific antagonist effect to certain constrictor substances which might be related to human hypertension, is not supported by any reliable evidence. A recent report by Taylor *et al.* (164) states that 3-methyl-7-chloro-1,2,4,-benzothiadiazine-1,1-dioxide reduces blood pressure of normal and hypertensive animals, but has no diuretic or natriuretic effects. It is suggested by these authors that the antihypertensive and diuretic actions of the benzothiadiazine group of drugs may have been separated. Further detailed studies will be necessary before such a separation of actions can be accepted.

Increases in the intracellular sodium and water content of arterial tissue obtained from hypertensive animals have been reported [Tobian & Binion (165, 166)], and Vick *et al.* (167) found that aortic strips obtained from rats fed on a high salt diet showed increased reactivity to epinephrine and norepinephrine. However, Bohr *et al.* (168) found that high sodium concentrations in the bathing fluid decreased, and low sodium increased, the reactivity of rabbit aortic strips to epinephrine. Mallov (169) showed a decrease in sensitivity to epinephrine and norepinephrine of rat aortic strips obtained from hypertensive as compared to normotensive animals. More recently, Mallov (170) showed that aortic strips from hypertensive rats when exposed to hypertonic sodium chloride or sucrose contracted slowly, while strips from normal rats did not contract in the hypertonic solutions. These findings may imply that aortic strips from hypertensive animals are structurally and functionally so changed that they respond to increased sodium ion concentration or hypertonic solutions in a manner different from strips obtained from normotensive rats. This may be a primary factor or may be secondary to the general vascular damage arising from the hypertension.

From the data available, it is impossible at this time to make any definitive statements concerning the mechanism of the hypotensive effects of diuretics. The fact that a number of different diuretics have this antihypertensive effect, and have in common the ability to reduce plasma volume and produce a negative sodium balance, points to these factors as being of major importance. The changes in the plasma and the extracellular fluid volumes, and the concomitant increase in sensitivity to hypotensive drugs can be rationally explained on the basis of increased vasoconstrictor tone. The problem of late hypotensive effects of chlorothiazide where blood volume is restored is not well understood but may be related to electrolyte changes in the smooth muscle of the vascular system.

There is good agreement that diuretic doses of chlorothiazide and its

congeners reduce urine flow and increase urine concentration in diabetes insipidus (171 to 178). In laboratory studies on rats with severe diabetes insipidus, Crawford & Kennedy (171) observed a reduction in daily water intake to less than one-half of pretreatment levels with large doses of chlorothiazide and related compounds. A similar effect has been observed in patients treated with diuretic doses. An initial diuresis is observed in which sodium and chloride excretion is markedly increased. On continued treatment, the excretion of these ions returns to pretreatment levels and the daily urine volume declines. The effect is distinct from the control afforded by posteriorpituitary extracts, since the urine is seldom more concentrated than the plasma, and the daily urine volume remains higher than is normal. However, the effect achieved is clinically useful since it can be maintained by continuous administration.

There is disagreement about the cause of the reduced water requirement. Crawford and co-workers (173, 179) have suggested that the thiazide diuretics exert a specific action at the site of free-water formation in the nephron, and hence lead to water conservation by means of a renal effect which increases the excretion of solute in a smaller urine volume. Upon the basis of careful clinical study, Havard & Wood (177) and Gayer & Kaufmann (180) explained the antidiuretic effect as resulting from a net loss of extracellular sodium with a consequent reduction in plasma volume and osmolality, and a large decrease in glomerular filtration rate. Cutler *et al.* (181) agree. They prevented the antidiuretic response to chlorothiazide in studies on nephrogenic diabetes insipidus, by administering sodium chloride in amounts sufficient to prevent negative salt balance. Furthermore, salt restriction has long been known to reduce urine volume in diabetes insipidus [Beaser (182)]. Shannon (183) showed, in dogs with diabetes insipidus, that urine volume varied with salt and water intake and with time after feeding and drinking. Furthermore, the reduced glomerular filtration rate observed in dogs with diabetes insipidus is increased by high rates of salt intake which also markedly increases urine flow. Earley & Orloff (184) have shown that once the antidiuretic effect of hydrochlorothiazide has been achieved, it can be maintained without further drugs if salt intake is rigidly restricted.

An analogous effect has been reported to occur following injection of a mercurial diuretic. Kourilsky *et al.* (185) observed a decrease in daily urine volume to one-half of pretreatment levels which lasted nearly one week after a single administration. A good saluretic response is very likely required since this effect of mercurials has been denied by Crawford *et al.* (173) who gave no data on the mercurial action. The effect of saluretic therapy and salt restriction to reduce urine volume in diabetes insipidus is most likely related to one or more results of these treatments. A chronic decrease in the filtered load of water and salt may account for the increased urinary concentration, but just as important, perhaps, is the decrease in thirst produced by a dilution of the extracellular water and the accompanying increased cellular hydration. Thus, during treatment with saluretics, patients with diabetes insipidus still

require an increased water intake to satisfy their thirst, but the time elapsed between drinks is increased compared to their pretreatment status.

While the reviewers disagree with the conclusions of Kennedy & Crawford (288) regarding the mechanism of the decrease in water intake produced by thiazide drugs, the concept of an antagonism between mineralocorticoids and the diuretic agents is intriguing. Their story would be more convincing if some measurements of plasma volume and glomerular filtration rate were included to rule out the importance of these parameters in adrenal insufficiency, and during thiazide treatment of diabetes insipidus.

Chlorothiazide is secreted by a renal mechanism similar to the one used by *p*-aminohippurate, iodopyracet, phenol red, and other organic acids. The rate of secretion of chlorothiazide was reduced by probenecid (109, 186, 187), and stop-flow analysis localized the site of chlorothiazide secretion to the proximal tubule (188). Chlorothiazide can inhibit the accumulation of *p*-aminohippurate in renal slices of the rabbit (189, 190). The fact that chlorothiazide and a number of related substances inhibit *p*-aminohippurate transport, possibly by a competitive mechanism, makes it unlikely that covalent linkages are involved in this transport. The possibility that ionic linkages are involved was studied by Essig (190). He could show that a number of substances related to chlorothiazide inhibit *p*-aminohippurate uptake by renal slices. A correlation between pK values and inhibition of aminohippurate uptake could be shown. However, variations in the pH of the buffer medium had little influence on the inhibition of aminohippurate uptake produced by chlorothiazide or dihydroflumethiazide, although ionization of the drugs varied between 4 and 85 per cent. According to Foulkes & Miller (191), the uptake of *p*-aminohippurate by tissue slices involves at least four steps, and any further analysis of these drug effects will depend on experimental procedures which can distinguish between these various steps.

*The mercurial diuretics.*—From a number of studies recently published, it is apparent that there is still considerable disagreement concerning the locus of action of mercurials along the nephron. The results obtained with the method of stop-flow analysis indicate that mercurial diuretics produce their effect by an action on the proximal convoluted tubule (125, 192). However, studies of the effects of mercurials on free-water clearance ($C_{H_2O}$) and on renal concentrating ability, as well as a number of other studies, have made it appear unlikely that the effects of these drugs can be explained on the basis of this single site of action.

Orloff *et al.* (193) have shown that $C_{H_2O}$ in hydrated animals is increased by the infusion of mannitol. Their interpretation of this observation is based upon the concept that in the absence of antidiuretic hormone, solutes, but not water, are reabsorbed in the distal tubules. This reabsorption of solute is responsible for a positive $C_{H_2O}$. Any increase in the load of tubular fluid presented to the distal tubules should increase $C_{H_2O}$. Thus, if mercurials were to act only on the proximal tubules to inhibit the isosmotic reabsorption, then $C_{H_2O}$ should be increased. Heinemann *et al.* (129, 194, 195), as well as Black-

more (128), have shown that preparations of mercurial diuretics which contain theophylline produced an increase in $C_{H_2O}$. On the other hand, Wesson & Anslow (196) and Miller & Riggs (197) report that pure organic mercurial diuretics produced no significant change in $C_{H_2O}$.

Some of the figures published in the papers of Capps *et al.* (198) showed that meralluride temporarily increased urine flow with minimal changes in sodium excretion. This effect was followed by a decrease in urine flow to about control levels, a marked increase in sodium excretion and, thus, a reduction in $C_{H_2O}$. Similar observations have been made by Ladd (199) and more recently by Goldstein *et al.* (200). The latter group showed that meralluride (a mercurial preparation that contains theophylline) caused a biphasic response. The first phase was characterized by a rapid increase in urine volume, $C_{H_2O}$, and osmolar clearance ($C_{osm}$) which lasted 45 to 60 min. The second or delayed response was much larger and more sustained. It was characterized by an increase in urine volume and sodium excretion while $C_{H_2O}$ did not change, or was decreased, and remained fixed at the lower level. Aminophylline was shown to increase $C_{H_2O}$, and Goldstein *et al.* (200) suggest that any increase in $C_{H_2O}$ caused by a mercurial preparation must be ascribed to the simultaneously injected theophylline, especially so, since theophylline-free mercurial preparations do not cause the temporary increase in $C_{H_2O}$. These findings explain the discrepancies in the literature, since all those authors who observed an increase in $C_{H_2O}$ used preparations containing theophylline, while those who did not find an increase in $C_{H_2O}$ used theophylline-free preparations. (It is rather surprising and disturbing that so much work on the renal pharmacology of mercurials has been conducted with a mixture of two potent drugs.) It is, therefore, likely that the true effect of a mercurial diuretic is either no change or a small decrease in free-water clearance.

Reabsorption in the proximal tubule is isosmotic (201, 202), and it follows that most of the water reabsorbed there leaves passively as the result of the reabsorption of sodium chloride. Any block of proximal sodium chloride reabsorption should increase the volume of fluid which reaches the more distal reabsorption apparatus. From the data of Orloff *et al.* (193) one might expect an increase in $C_{H_2O}$ from a drug which blocks the reabsorption of solute in the proximal tubules. This is not the case with mercurials, and a single site of action in the proximal convoluted tubule is unlikely. There is other evidence in agreement with this contention. Raeser & Burch (203) and Farah & Koda (204, 205) have shown that under certain conditions, small doses of mercurials can increase sodium excretion independently of a change in urine volume. This would suggest a decrease in $C_{H_2O}$. Here again, the increase in solute excretion with no change in urine volume is not compatible with a single proximal tubular site of action for mercurial diuretics.

The concentrating ability of the kidney is a reflection of distal tubular processes. Attempts to demonstrate a change in response to vasopressin during mercurial diuresis have been made by Capps *et al.* (198) and by Farah *et al.* (206). Both groups found that mercurials do not interfere with the ac-

tion of vasopressin in dogs or rats. However, the experiments in the dog were not a quantitative comparison, and the experiments in rats were not too useful since mercurials produce little if any diuresis in the rat. More recently, Au & Raisz (127) and Lambie & Robson (207) have conducted carefully controlled experiments which have shown that in man mercurial diuretics decrease the concentrating ability of the human kidney at high urine flows by decreasing the tubular capacity to reabsorb solute-free water. It was suggested in both of these papers that this effect is attributable to an effect of mercurial diuretics on the sodium transport mechanism in the loop of Henle.

Histochemical studies have shown that mercurial diuretics produce changes which can be demonstrated in the terminal part of the proximal tubule, in Henle's loop, and in the collecting ducts; but no effect could be demonstrated in cells of the proximal tubules close to the glomeruli or in the distal convoluted tubules [Cafruny *et al.* (208, 209, 210)]. These histochemical data support the contention that mercurials act at several sites along the nephron.

Which sites are involved? Evidence for a proximal site of action includes the following: Mercurials can inhibit the reabsorption of over 40 per cent of the glomerular filtrate. The histochemical studies referred to above indicate that mercury is present in proximal tubules during mercurial diuresis. This has been demonstrated more directly by dark field microscopy [Timm & Arnold (211, 212)]. In man, mercurial diuretics interfere with the proximal tubular processes of glucose reabsorption and *p*-aminohippurate secretion. Miller & Riggs (197) suggest that the increase in urine flow produced by mercaptomerin in dogs with diabetes insipidus during maintained water diuresis is evidence for an action in the proximal tubules. Giebisch (213) has shown that the potential measured across proximal tubules is reduced by mercurial diuretics. White *et al.* (214) demonstrated a mercurial-induced increase in permeability to radioactive sodium in proximal tubules by analysis of urine samples collected by the stop-flow method. And, finally, the stop-flow studies cited above all tend to implicate the proximal tubular apparatus in mercurial-induced diuresis.

What other sites may be involved? Free water is probably formed in the ascending limb of Henle and in distal convoluted tubules. Inhibition of solute reabsorption at these sites could explain the effects of mercurials on $C_{H_2O}$ referred to above. Since histochemical methods indicate an action in cells of the ascending limb of Henle's loop but not in distal convoluted tubules, it seems more likely that mercurials act on the former. An action on the ascending limb could also account for the reduction of concentrating ability of the human kidney as compared to that observed during mannitol diuresis. However, an increase in blood flow through the medullary tissue would also account for a reduction in concentrating ability. No data are available on the effects of mercurials on blood flow in this region. If mercurials had such an effect, and if increasing the load of reabsorbable solute to the

distal tubules increases $C_{H_2O}$, the lack of an increase during mercurial diuresis would indicate an action at a site more distal than Henle's loop. The collecting ducts are a good possibility. The inhibition of potassium secretion by mercurial diuretics has been well established. Hierholzer (215) has shown that potassium secretion occurs in the collecting tubules of the rat, and histochemical observations have shown that mercurials produce a slight but reproducible reduction in protein-bound sulfhydryl in the collecting ducts.

The data currently available do not permit the choice of a definite locus for the action of mercurials in the distal nephron. However, it is clear that the action of the mercurials cannot be explained on the basis of a single site of action; and it is not unreasonable to suggest that, in an organ as complex as the kidney, these agents alter the function of various segments of the nephron.

The nature of the process of sodium chloride reabsorption which is blocked by mercurial diuretics has been considered, but no definitive studies on this are available as yet. Malvin *et al.* (216) suggested that proximal sodium and water reabsorption are passive processes. However, the recent micropuncture experiments of Windhager & Giebisch (202) conclusively show that proximal tubular sodium reabsorption cannot be quantitatively accounted for by colloid osmotic forces, and, thus, an active sodium transport in the proximal tubule must be operative although some passive contribution to overall sodium transfer cannot be excluded. The studies of White *et al.* (217) indicate that, in addition to active sodium transport, a process of exchange diffusion or simple sodium diffusion exists, since labelled sodium can move from plasma into the luminal fluid of the proximal tubules. Mercuhydrin increased the sodium flux across proximal tubule walls (214). Two possibilities were considered which explain this action of mercuhydrin. One is an increase in exchange diffusion with a reduction in pump activity, and the other is an increase in passive leak. The data do not allow a differentiation between these possibilities. A mercurial-induced increase in the passive leak of sodium through the tubular membrane has been proposed by Kleinzeller & Cort (218). This work was conducted on renal cortex slices, and it is clear that passive diffusion changes do occur when excessively high concentrations of mercurial are added to the medium. As Maizels & Remington (219) pointed out, the concentration of mercury used in the incubation medium cannot be compared to those observed in the renal cortex *in vivo*.

It is well known that monothiols counteract certain effects of the mercurials but do not significantly reduce the diuresis. The data of Maizels & Remington (219) clearly show that at a low temperature mercaptomerin, a preparation containing sulfhydryl, is much less effective in promoting the leak of sodium than is Esidron (N-($\gamma$-hydroxymercuri-$\beta$-methoxy) propylquinolinamide), a preparation that does not contain a monothiol. Furthermore, the nondiuretic compound, *p*-chloromercuribenzoate, produces similar effects *in vitro*. More recently, Dzurik & Krajci-Lazary (220) measured the sodium and potassium content of rat renal cortex before and after the injec-

tion of 10 and 20 mg of mersalyl mercury per kilogram. Both doses increased the sodium and water content of cortical tissue. The finding was offered as support for the Kleinzeller & Cort hypothesis. The concentration of mercury used was two to four times the diuretic dose, and in our experience 10 mg of mercury per kg, administered as mersalyl, can produce antidiuresis and severe renal damage. Therefore, the above findings are by no means convincing evidence for the passive diffusion hypothesis.

Mudge & Weiner (221) have reaffirmed the old hypothesis that organic mercurial diuretics act after releasing inorganic mercury. They showed that the magnitude of the diuretic effect of organic mercurials is influenced by acid-base changes, but that inorganic mercuric cysteine is a potent diuretic even in alkalosis. Confirming evidence has been presented by Farah *et al.* (222, 223) who demonstrated that acid-base changes influenced alterations in protein-bound sulfhydryl groups produced by diuretic organic mercury compounds. The effect of nondiuretic, acid-stable mercury compounds was not altered by acid-base changes.

The evidence that pH changes in renal cells or urine are important for determining the extent of diuresis produced by organic mercurial diuretics has been reviewed by Mudge & Weiner (221) and more recently by Orloff & Berliner (123). The experiments that seem to throw some doubt on this interpretation are those of Axelrod & Pitts (224) and Kessler (225), which showed that respiratory acidosis produced by $CO_2$ inhalation did not increase the response of dogs to mercurials. Even 12 per cent $CO_2$ in inspired air did not increase the diuretic response to chlormerodrin, although the plasma pH was down to 7.1 to 7.2 (225). Riggs & Friedman (226) found that during the infusion of sodium bicarbonate, 10 per cent $CO_2$ in $O_2$ did not lower the urine pH below 7.4, and that little or no response to the administration of mercaptomerin could be elicited. Although the plasma pH and the plasma chloride level in these experiments were low, the authors suggested that their findings could be explained on the basis of urine pH. It should be noted that the pH of the urine in the studies referred to above was also little affected by the high concentration of $CO_2$ in the inspired air. If the pH of the tubular fluid or of certain cells of the renal tubule is the critical factor, perhaps the extent of the respiratory acidosis achieved in these experiments was not sufficient to produce the conditions required for organic mercurial diuresis. Using 15 per cent $CO_2$, Heidenreich & Schneider (227) were able to elicit a good diuresis with an organic bismuth salt (see below) during the infusion of sodium bicarbonate. The urine pH remained alkaline, but plasma pH dropped to 7.0. A study of the action of an organic mercurial under the same conditions would be of great interest.

It has been assumed repeatedly that mercurials act by inhibiting an essential sulfhydryl-dependent process in renal cells. The observation that certain nondiuretic organic mercury compounds also reduce the sulfhydryl content of kidney cells was difficult to explain on this basis (223). Recently,

Miller & Farah (228) have shown that *p*-chloromercuribenzoate, an acid-stable compound, can prevent or even counteract the diuretic action of a number of organic mercurial diuretics or mercury bichloride. Furthermore, in doses which counteracted the diuretic effect of chlormerodrin, it displaced from the kidney a fraction of radioactive mercury injected as labelled chlormerodrin (229). From the data, it was clear that only a small fraction of the total amount of mercury fixed in the kidney can be related to the mercurial diuresis. Weiner *et al.* (230) reached the same conclusions in studying the reversal of mercurial diuresis produced by dimercaprol. Compounds such as methyl and ethyl mercury chloride did not counteract or prevent a mercurial diuresis, and ethyl mercury chloride did not displace mercury from the kidney (229). The data are compatible with the suggestion that mercuric ion requires a two-point attachment to an essential receptor to produce a diuresis, one site of attachment being a sulfhydryl group. The second group might be another sulfhydryl, an amino, a carboxyl, or an imidazole group. However, no specific data are available on the nature of this second site in the receptor.

Jamison (231) showed that chlormerodrin ($1.25–1.5 \times 10^{-4} M$) depressed the sodium current and electrical potential of the isolated toad bladder. The permeability of this membrane to chloride was either unchanged or increased. The author suggested that these findings are consistent with the hypothesis that mercurials interfere with salt reabsorption in the renal tubule by a direct depression of active sodium transport, and not by decreasing permeability to chloride. The effects of *p*-chloromercuribenzoate in Jamison's preparation were an increase in both sodium current and membrane potential, which was followed by a steady decline. Since the effects of a mercurial in the kidney are not prevented by monothiols, it would be of interest to see whether the effects on the toad bladder are also resistant to monothiol reversal.[4]

Bickers *et al.* (233) found that the activity of five renal tubular enzyme systems could be modified by meralluride. However, enzyme changes occurred only with doses of the mercurial that produced definite histological signs of nephropathy. Shore & Shore (234) found that rats maintained on a sucrose diet developed a tolerance to mercuric chloride. The activity of the renal tricarboxylic acid cycle and those of a number of other enzyme systems in these animals were not depressed by mercuric chloride administration as much as those from chow-fed rats. Hamamoto (235) studied the effects of mercury compounds on enzymes of carp kidney. Scott & Gamble (236) showed that low concentrations of organic mercurials stimulate the rate of exchange of potassium between mitochondria and the incubation medium. Oxidative phosphorylation was depressed and the total amount of bound potassium was decreased. Monothiols exerted a protective action, and ethylenediaminetetraacetic acid was partially effective in preventing these effects

[4] Dr. B. Rennick (232) found that in the presence of sufficient amounts of monothiol and with mercaptomerin, the effects of mercurial diuretics on the sodium current described by Jamison (231) are not observed.

of mercury. Weed (237) studied the interaction of mercury with red blood cells and correlated mercury binding with potassium loss and fragility changes.

Miscellaneous observations include the following: Munck *et al.* (238) showed that prolonged administration of chlormerodrin did not produce any signs of kidney damage, although there was a considerable amount of mercury in the kidneys. Calesnick *et al.* (239) showed that over 90 per cent of an injected dose of $Hg^{203}$-labelled mercaptomerin appeared in the rat kidney within the first hour. That most of the mercury was located in the cortex was evident from radioautographs.

Differences in the renal distribution of various organic mercury compounds were pointed out by Miller *et al.* (240) and by Bergstrand *et al.* (241). Methyl mercury, after chronic administration, was localized throughout the renal cortex, while mercury bichloride was found mainly in the intermediate zone.

Campbell (242, 243, 244) observed that inhibitors of *p*-aminohippurate transport, such as probenecid and bromcresol green, can prevent the tubular secretion of certain mercurial diuretics and prevent a unilateral diuresis in the chicken. If the inhibitor was administered after the mercurial, the diuresis was not inhibited, which indicates that probenecid and related substances interfere with the secretion of the mercurial. Studies with a number of mercurial diuretics showed that this inhibitory effect could be demonstrated for mercaptomerin, diurgin (N-succinyl-N-($\gamma$-carboxymethylmercaptomercuri-$\beta$-methoxy)propylcarbamide), and chlormerodrin. The diuretic effects of mercuric chloride were not prevented by probenecid, and the effects of mercumatilin could be only partially inhibited. Campbell suggested, on the basis of his findings with mercuric chloride and mercumatilin, that simple diffusion into cells of the renal tubules may be involved in transporting mercury to its site of action. Species differences in the effect of probenecid no doubt exist, since the lack of an effect in the dog has been reported (245).

*Bismuth.*—Bismuth salts produce a diuresis which is similar to that of the mercurials. Heidenreich *et al.* (227, 246, 247) studied the diuretic properties of some organic bismuth compounds (bismuth ammonium citrate, bismuth colamine citrate, and bismuth-sodium complex of $\alpha$-oxy-$\alpha$-phosphonopropionic acid). A diuresis occurred in dogs following the administration of relatively small doses (2 to 3 mg bismuth per kg of body weight). The diuretic effects of the bismuth compounds, like those of organic mercurials, were sensitive to changes in acid-base metabolism. This diuresis was suppressed by the administration of sodium bicarbonate, but the inhalation of 15 per cent $CO_2$ caused an increase in the response. These findings suggested that the refractoriness to bismuth compounds during the infusion of sodium bicarbonate, resulted from the lowered hydrogen ion concentration, perhaps in cells of the renal tubule, rather than to a reduction in chloride load to the kidney.

*Aldosterone antagonists, steroidal compounds.*—The clinical effectiveness of spironolactone has been confirmed by a number of studies (248 to 251), and the effectiveness of spirolactone in the treatment of cardiac edema has been demonstrated (252). Combinations of spirolactone with a mercurial or thiazide diuretic have been found to be especially useful in some cases of refractory edema (132, 134, 135, 253).

Spirolactone administration increases the excretion of sodium and may either decrease or cause no change in potassium excretion. The effects of spironolactone occur only in the presence of endogenous or exogenous aldosterone or like substances, and a competitive mechanism is reasonably well established. Hellman & Faloon (254) showed that, in man, the effect of 3-(3-oxo-17$\beta$-hydroxy-19 nor-4-androsten 17$\alpha$-yl) propionic acid y-lactone (SC 8109) on sodium excretion was reduced by a decrease in the intake of potassium, and that the effect could be increased by increasing intake of potassium. In another study, the administration of spirolactone did not reduce the ability of the kidney to excrete urine containing a low sodium concentration when the subject was given a low-sodium diet [Ross & Winternitz (255)]. Although this study showed that normal adaptation to sodium restriction does not occur when spirolactone inhibits the activity of aldosterone, the kidney still retained an ability to conserve sodium, apparently independent of the presence of sodium-retaining adrenocortical hormone. This suggests that aldosterone only modulates an inherent renal mechanism for sodium reabsorption.

Stop-flow experiments by Vander *et al.* (256) indicated that the steroidal aldosterone antagonists prevented a distal tubular mechanism for sodium reabsorption from producing a maximal concentration gradient of sodium across the tubule. The authors also suggested that in the dog aldosterone did not alter the proximal reabsorption of sodium. Williamson *et al.* (257) applied the stop-flow analysis method to rats. In this preparation, adrenalectomy produced a defect in proximal sodium reabsorption which was followed about 21 days later by a defect in the distal tubular reabsorption of sodium. Data collected shortly after adrenalectomy suggested an increase in the proximal tubular reabsorption of potassium. Penny *et al.* (258) studied the effects of aldosterone and aldosterone antagonists on the frog skin. They observed that aldosterone increased, and that a spirolactone caused a considerable decrease in sodium transport.

D'Amico (259) observed that an increase in the sodium content of human erythrocytes occurred in several edematous conditions. This was confirmed by Riecker & Bubnoff (260). Because of the possible role of aldosterone in the etiology of these disease states, D'Amico & Cesana (261) studied the effects of aldosterone and a spirolactone on the high-sodium and low-potassium content of cold-stored human red blood cells, but no effect of either aldosterone or its inhibitor could be demonstrated.

Aldosterone antagonists may cause an increase in aldosterone production

[Garst *et al.* (262)]. However, the clinical findings on the effects of the spirolactones on aldosterone excretion are not uniform. This is not too surprising since aldosterone excretion must be under the influence of a number of controls.

It is clear that neither the renal site nor the mechanism of action of aldosterone and its antagonists are, as yet, clearly defined. The classical clearance and the stop-flow methods have a limited use for such studies. However, the more precise studies utilizing micropuncture and microcatheterization techniques may give more reliable answers concerning the site of action of these drugs.

Mokler (263) found that a number of steroidal spirolactones had anti-arrhythmic properties when they were tested on experimentally produced atrial arrhythmias in the dog. Anti-arrhythmic and anti-aldosterone effects were shared by all but one of the active compounds.

Studies on the aldosterone-antagonizing effects of progesterone have been made by Koczorek (264), and Kagawa & Brown (265) studied the activity of some isopregnenolone-21-carboxylates in preventing the renal activity of deoxycorticosterone and aldosterone in adrenalectomized rats. The compound 3-oxo-9$\alpha$-fluoro-11$\beta$,17$\beta$-dihydroxy-17$\alpha$-pregn-4-ene-21-carboxylic acid was found to be the most effective of compounds studied.

*Nonsteroid aldosterone antagonists.*—A highly effective aldosterone antagonist which is not a steroid derivative was reported by Wiebelhaus *et al.* (266). The compound, a pteridine derivative (2,4,7-triamino-6-phenyl pteridine) (SKF 8542), was a potent oral diuretic in the rat. In rats fed on a low-sodium diet, it produced a marked natriuresis with a reduction or no change in potassium excretion. In the adrenalectomized rat, the agent antagonized the effects of aldosterone on sodium and potassium excretion. In dogs receiving either aldosterone or 9$\alpha$-fluorohydrocortisone, the compound produced a diuresis with an increase in sodium and chloride excretion. Potassium excretion and urinary pH remained relatively unchanged. The effects of this pteridine derivative were reported to be reversed by dimercaprol, but the significance of this finding is not clear. Clinical reports suggest that it possesses anti-aldosterone properties and is more potent than the clinically useful spironolactone (267, 268). A reversible granulocytopenia was noted in about 20 per cent of the subjects of one series [Laragh (267)]. More recently, Wiebelhaus *et al.* (269) observed that this compound also produced an increase in sodium excretion in the adrenalectomized dog, and that the natriuresis was not inhibited by previous potassium loading. These findings showed that SKF 8542 is not only an aldosterone antagonist, but that it also may act directly on the renal sodium and potassium exchange system by a mechanism which is different from that affected by the cardiac glycosides (SKF 8542 acts in the presence of a high-potassium concentration).

*Heparin.*—A delayed increase in sodium excretion has been observed following the administration of heparin to human subjects. This effect was produced by various heparin-like substances and was not directly related to

the anticoagulant properties of heparin since congeners of heparin with practically no anticoagulant properties had a similar effect on sodium excretion [Schlatmann *et al.* (270)]. The ionic composition of the urine was similar to that observed in Addison's disease or after the administration of amphenone or spironolactone. Heparin diuresis probably resulted from inhibition of aldosterone production since aldosterone excretion was markedly reduced [Cejka *et al.* (271)]. Muldowney & Banks (272) described the acute effects of heparin in the dog. The main effect was an increase in the excretion of potassium, but no change in sodium excretion was observed. These investigators did not study the delayed effects of heparin. The Dutch investigators (270, 271) stressed the fact that the effect of heparin on sodium excretion was seen only about 36 hr after the administration of relatively large amounts of the drug. These are very interesting observations, and more data on these effects will undoubtedly appear. The acute effect of heparin—the increase in potassium excretion described by Muldowney & Banks (272)—may in some way be related to the observation of Hashish (273) who reported that the exchange of $K^{42}$ in rat diaphragm at 1°C. is markedly increased by heparin.

*Cardiac glycosides.*—Recent findings indicate that the positive inotropic effect produced by cardiac glycosides in the heart is not correlated with a reduction of intracellular potassium concentration (274 to 277). A dose of ouabain that produces positive inotropic, but no toxic, effects will actually increase the intracellular potassium concentration and decrease the rate of potassium loss from the isolated heart as compared to untreated control preparations. Toxic concentrations of the glycosides will reduce the concentration of intracellular potassium and decrease the uptake of this ion by cardiac muscle. In regard to the effects of cardiac glycosides on potassium transport, it may be that extra-cardiac tissue is more sensitive than the myocardium to the action of cardiac glycosides [Steward (275)]. The relative sensitivity of the renal tubules to these drugs is not known. If it can be shown that the ion transport mechanisms of the kidney are more sensitive to cardiac glycosides than are those of the heart, then the direct renal effects of the cardiac glycoside may play a role in the overall therapeutic effects of these drugs.

The original observations of Farber *et al.* (278) and Hyman *et al.* (279) that cardiac glycosides have direct renal effects, have been confirmed and extended by more recent studies. Orloff & Burg (280) showed that in the chicken strophanthidin interferes with the transport of sodium, potassium, and hydrogen ion by the renal tubule in a way which is quite different from that observed with carbonic anhydrase inhibitors. Potassium excretion was diminished by strophanthidin when the rate of excretion of this ion was elevated by prior infusion of a potassium salt. Wilde & Howard (281) utilized the stop-flow method and a so-called "slow flow" technique for studying the renal effects of ouabain. They concluded that ouabain acts to depress the distal tubular mechanism for potassium reabsorption. Cade *et al.* (282) showed, in dogs loaded with saline, that intra-arterial infusions of strophan-

thidin produced a prompt fall in glomerular filtration rate and a delayed diuretic and saluretic response. Hydrogen ion secretion was little affected except under conditions in which a large concentration gradient of hydrogen ion was being maintained. Loading with potassium salts inhibited the diuretic effects of strophanthidin, but only partially blocked the depression of the glomerular filtration rate. Similar effects were observed in adrenalectomized dogs. Kupfer & Kosovsky (283, 284) observed an increase in the excretion of sodium, potassium, chloride, calcium, magnesium, and phosphate following the intra-arterial injection of a cardiac glycoside. This pattern of ion excretion was quite different from that produced by either a mercurial or osmotic diuretic. Strickler & Kessler (285) demonstrated that renal activity of a series of digitalis steroids could be correlated with their cardiac activity, and Machova (286) showed a similar relation between cardiac activity and potassium transport in red blood cells. All of these findings would seem to indicate that the effects of cardiac glycosides on the renal tubule are similar to those observed in other tissues, and are the result of an interference with a linked sodium-potassium exchange across the peritubular cell membrane with possibly an additional effect on a linked exchange occurring at the luminal surface.

The suggestion of Wilbrandt (287) that the effect of cardiac glycosides was produced by a competitive action with mineralo-corticoids, was not supported by the findings of Glynn (289) who showed that effects of cardiac glycosides could not be reversed by mineralo-corticoids.

The effects of cardiac glycosides on red blood cell adenosine triphosphatase was studied by Portius & Repke (290). The enzyme was activated by sodium, potassium, and magnesium ions. A correlation between cardiac activity and ability to inhibit the enzyme could be shown. A role for the enzyme in ion transport has been suggested (291). A "membrane" adenosine triphosphatase activity which requires sodium and potassium has been also demonstrated in guinea pig kidney (292) and in brain (293, 294). Tosteson *et al.* (295) suggested that the alkaline phosphatase found in many animal cell membranes may be related to the membrane adenosine triphosphatase, and may play a role in ion transport across these membranes.

*Other diuretics and miscellaneous reports.*—Extracts of juniper produce a diuretic response, and a diuretic substance called junen has been isolated. Recent chemical and pharmacological studies (296) showed that junen was a mixture of monoterpenes. The diuretic activity of juniper oil has now been ascribed to the oxygen-containing component of junen, and the most active compound was found to be terpenenol-4(1-manthenol-4). This substance increased glomerular filtration rate, urine flow and the excretion of sodium, potassium, and chloride. According to Janku *et al.* (296), terpenenol-4 produced a brisk diuretic response by increasing the glomerular filtration rate, and by decreasing the tubular reabsorption of electrolyte.

Van Arman *et al.* (297, 298) studied the effects of the aminouracil diuretic amisometradine. In contrast to other diuretics studied, amisometradine in-

creased the urinary bromide to chloride ratio without changing that of the plasma.

Reubi *et al.* (299, 300) showed that in man, a permanent reduction in glomerular filtration over a wide range had little influence on the diuretic and saluretic properties of meralluride or chlorothiazide. However, glomerular filtration rates below 20 ml/min were accompanied by a sharp decrease in the diuretic efficacy of these drugs. A maximal fraction of about 40 per cent of the glomerular filtration rate was blocked by the diuretics, whether given singly or in combination. Summation of diuretic effects of meralluride and chlorothiazide was demonstrated in patients with a high but not in those with a low glomerular filtration rate.

Domenet *et al.* (301) showed that the combination of intravenous aminophylline and intramuscular mercaptomerin was successful in a number of cardiac patients resistant to usual diuretic therapy.

The monohydrochlorides of L-arginine and L-lysine were found to be useful acidifying agents (302, 303, 304). Bosley *et al.* (305) studied the effect of ammonium chloride acidosis and mercurial diuretics on calcium and phosphorus excretion.

Schteingart *et al.* (306) observed that administration of mercurials, hydrochlorthiazide, or acetazolamide failed to increase the excretion of iodide and had no effect on the 24 hr thyroidal uptake of $I^{131}$ in normals and in patients with congestive heart failure.

The effects of diuretics on the electrophoretic patterns of rabbit serum proteins were studied (307), and Gottlieb & Coye (308) determined the effect of mercury poisoning on serum protein metabolism of rabbits.

## The Antidiuretic Hormone

There are four naturally occurring neurohypophyseal hormones. These are oxytocin, arginine vasopressin, lysine vasopressin, and arginine vasotocin (309). In the mammal, all of the known effects of neurohypophyseal hormones are accounted for by three polypeptides: the antidiuretic-vasopressor substances (arginine vasopressin and lysine vasopressin) and the oxytocic-milk-ejection factor (oxytocin). Arginine vasotocin, with the ring structure of oxytocin and the side chain of arginine vasopressin, is probably the most active natural principle in nonmammalian vertebrates, most of whose posterior lobes also contain oxytocin.

The biological activities of these polypeptides are shared to some extent by the natural hormones and by many synthetic analogues, although remarkable specificities exist. The relation between structure and biological activity was discussed recently in an excellent review by Sawyer (309) and reference to his paper is suggested for those interested in a complete discussion of the neurohypophyseal hormones.

Probably the most popular concept of the mechanism of action of the antidiuretic hormone is that which holds that either the size or the number of pores or channels through which water moves across the tubular epithe-

lium is increased by the presence of antidiuretic hormone. Permeability to water (moving along an osmotic pressure gradient) is increased. This concept is based upon an analogy to *in vitro* systems, the isolated frog skin and toad bladder preparations of Koefoed-Johnsen & Ussing (310), Leaf (311), and others (312, 313).

Studies of these membrane systems showed that when antidiuretic polypeptides were added to the solution bathing the serosal surface of the bladder or skin but not the mucosal surface, the net flux of water increased. Furthermore, the passive movement of urea and acetamide increased as well. Permeability to thiourea and various other nonelectrolytes and ions was not increased [Leaf (311)]. Antidiuretic hormones stimulated the active transport of sodium, and this stimulation was accompanied by an increased oxygen consumption. However, passive movement of water can occur independently of sodium transport (313).

A suggestion concerning hyaluronidase and the mode of action of the antidiuretic hormone was proposed by Ginetzinsky (314). Proceeding on the assumption that hyaluronidase might play a role in regulating intercellular permeability, Ginetzinsky *et al.* (315) attempted to elucidate the function performed by hyaluronidase in the kidney by studying hyaluronidase activity in the urine. They used a viscosimetric assay which involved measuring the decrease in viscosity of a buffered hyaluronic acid solution. An arbitrary unit was defined as a 1 per cent decrease in viscosity during a 20-min period. No studies with purified hyaluronidase were reported, and no attempts to recover added enzyme were undertaken. In healthy human subjects they observed that the activity of urine decreased with increasing urine flow. Maximum activity, about 30 units in 0.5 ml of urine, was observed when urine flow was 0.2 ml/m² min. Studies on 12 healthy individuals showed that enzyme activity in the urine varied with urine flow in a regular manner. Similar observations were made in dogs. In patients with glomerular nephritis, higher levels of hyaluronidase activity were observed during the early stages of the disorder, but in chronic patients the activity was very low at very low urine flows. Hyaluronidase appeared again at urine flows of 0.2 ml/m² min, and reached a constant maximum concentration of about 15 units/0.5 ml at urine flows of 0.8 ml/m²/min. Only traces of activity were observed in patients with amyloid nephrosis. In attempting to determine the origin of hyaluronidase in urine they studied the hyaluronidase content of extracts of kidney tissue. No data were presented, but they reported that the activity of extracts from dog kidney exceeded the activity of testicular extracts. Ivanova (316) extended these studies by attempting to rule out the possibility that dilution of the urine accounted for the decrease in hyaluronidase activity in urine at high rates of flow. By diluting concentrated urine, she was able to observe relatively large amounts of activity when the urine volume corresponded to that observed during water diuresis. Only when considerably greater dilutions were made did the activity decrease to zero. After concentrating, *in vacuo*, urine obtained during water diuresis,

she was unable to show any increase in activity. The effect of salt was eliminated by dialysis. When osmotic diuresis was produced by urea loading, the hyaluronidase activity of the urine was high even at high urine flows. Pituitrin P, containing antidiuretic hormone, increased the hyaluronidase activity of the urine of dogs from zero during water diuresis to values which fell on the curve of activity versus urine flow observed in the control experiments. Combined water and urea diuresis was characterized by little or no hyaluronidase activity in the urine. Administration of antidiuretic hormone increased the activity under these conditions as well.

Ginetzinsky, Zaks & Titova (317) employed a histochemical approach to investigate the role of hyaluronidase in the renal concentrating mechanism. By use of the Hess & Hollander (318) technique for metachromatic staining with toluidine blue, they observed intense staining of mucopolysaccharides in the vicinity of the collecting ducts in rats sacrificed during water diuresis. During dehydration, or following administration of antidiuretic hormone the appearance of the collecting ducts was markedly changed. The intercellular substance which, in rats undergoing water diuresis, stained intensely with the metachromatic red-violet color, had largely disappeared. The cells of the collecting ducts were described as being reduced from high prismatic cells with centrally located nuclei, to cells in which the nuclei protruded, and from which the apical cytoplasm had been lost. Such changes, reported to occur within 15 to 20 min after "introduction of the hormone" (route not specified), led to the conclusion that hyaluronidase was released from cells of the collecting ducts by the action of antidiuretic hormone. The enzyme, according to Ginetzinsky, acted upon the hyaluronic complexes of the intercellular cement and rendered the ducts water-permeable.

Dicker & Eggleton (319) confirmed Ginetzinsky's observations by showing that in man, hyaluronidase was present in urine secreted at a low rate of flow, but that it disappeared during water or alcohol diuresis and reappeared when the diuresis was suppressed by the injection of vasopressin. Their assay method consisted of measuring the decrease in viscosity at room temperature after a 90 min incubation at 34°C. of a mixture of hyaluronic acid, citrate buffer containing salt, and reconstituted urine. Urine was concentrated by dialysis against dry polyethylene glycol and rediluted with water to represent a urine flow of 0.1 to 0.15 ml/min. A standard curve prepared from assays under the same conditions with 0.5, 1.0, and 2.0 units of hyaluronidase (Light and Co. 500 units/mg) was employed in expressing urinary hyaluronidase activity. The antidiuretic activity of urine was also determined, and the lack of a regular quantitative relation between the excretion rates of antidiuretic activity and hyaluronidase was explained by the suggestion that the vasopressin was destroyed during its action. In a subsequent paper, Dicker & Eggleton (332) compared the antidiuretic activity of six structural analogues in man, and confirmed their previous findings that the antidiuretic response is accompanied by the excretion of hyaluronidase. Hyaluronidase excretion was observed to decrease with increasing urine flow above 1 ml/min, but no

increase in rate of excretion was observed at flows below 1 ml/min. Concentration (units/ml) did vary with urine flow.

Criticisms of this work are the following: Although dialysis was employed to remove urinary solutes, no measure of the effectiveness of the treatment was carried out. Addition of buffer containing 8 per cent sodium chloride might not have adjusted ionic strength to optimum levels. Although they reported a recovery of enzyme activity from dialyzed standards or dilute urine, they did not report on the recovery of enzyme added to concentrated urine. Since many of the samples studied were concentrated urine, this additional check of the method might be important.

Berlyne (320) criticized Ginetzinsky's studies on the basis of his assay method and the way in which he expressed the results as concentrations rather than rates of excretion of enzyme. He also observed that "hyaluronidase" was excreted in the urine, but could show no consistent relationship between hyaluronidase excretion and urine volume, total solute, or electrolyte excretion. Berlyne (321) presented data showing that the excretion of hyaluronidase occurs during water diuresis, while in Ginetzinsky's experiments no enzyme activity was detected under similar conditions.

Although the identity of the material which stained metachromatically was not established, it disappeared when kidney slices were treated with pneumococcal hyaluronidase, and was therefore referred to by Ginetzinsky *et al.* (322) as hyaluronic acid. The material was not observed in cyclostomes, teleosts, or newborn rats, none of which show antidiuretic responses. Adult rats, amphibians, and birds show antidiuretic responses and possess the metachromatic intercellular substance. Ginetzinsky *et al.* considered these observations as phylogenetic and ontogenetic evidence for the hypothesis.

Heller & Lojda (323) repeated and supplemented the histological parts of Ginetzinsky's studies but were unable to confirm the histological differences between hydrated and dehydrated rats. They saw very little structural or staining difference and implied that artifacts may have been responsible for much of the picture observed by Ginetzinsky.

Meyer (324) failed to extract from kidney any hyaluronic acid fraction. He suggested that while there is in urine an agent, or agents, which decreases slowly the viscosity of isolated hyaluronate solutions, the agent is probably not any of the known hyaluronidases. He further objected to Ginetzinsky's thesis on the basis that it is highly improbable that the action of hyaluronidase could be reversed, or that the presumed hyaluronic acid could be resynthesized in the 15 to 20 min it takes to abolish the effect. In skin, reconstitution takes between 24 to 48 hr.

In view of the contradictory findings thus far reported, the hypothesis presented by Ginetzinsky must be subjected to more critical evaluation. Recovery and characterization of the urinary material which has viscosity-reducing activity will be necessary if this hypothesis, concerned with the enzyme hyaluronidase, is to be taken seriously. Some comparison of the

assay procedures used by the various investigators is also necessary. A check of the validity of the finding of large amounts of hyaluronidase in dog kidney [Ginetzinsky *et al.* (315)] will also help. Care must be taken in these experiments to prevent contamination of urine specimens by seminal fluid or by microorganisms which release hyaluronidase.

Fong *et al.* (325) have obtained evidence that antidiuretic hormone interacts with a sulfhydryl-containing renal receptor. They studied the binding of tritiated vasopressins to kidney protein in rats. From the observation that mild treatment of the labelled protein with thiol compounds resulted in the loss of about 50 per cent of the label, they concluded that cleavage of a disulfide bond was involved. They proposed that the disulfide bond of the polypeptide hormone takes part in a disulfide-thiol interchange reaction with a free sulfhydryl of a renal cell receptor.

The same group showed that the permeability change produced by neurohypophyseal hormones in the isolated amphibian bladder was not dependent upon oxidative metabolism. A number of reagents (N-ethyl-maleimide, methyl mercury bromide, *p*-hydroxymercuribenzoate, etc.) which combine with free sulfhydryl groups were shown to inhibit the effect of arginine vasopressin when added to both serosal and mucosal surfaces in concentrations of $10^{-3}$ and $10^{-4}$ *M*. If added to the mucosal surface in concentrations of $10^{-4}$ to $10^{-5}$ *M*, the reagents themselves induced a permeability change which, however, was irreversible [Rasmussen *et al.* (313)].

In a third report, Schwartz *et al.* (326) showed that tritium-labelled arginine vasopressin exerted the characteristic effect on the toad bladder. The bladder was labelled with considerable amounts of tightly bound radioactive hormone following a number of treatments which removed loosely bound label. As with the kidney protein, mild treatment with 0.1 *M* cysteine removed 50 to 80 per cent of the remaining radioactivity, and this finding indicated that a loss of radioactivity occurred because a disulfide bond between the hormone and bladder had been split. When tritiated vasopressin was added to bladders which were then washed repeatedly, the natural state of relative impermeability was regained. When bound radioactivity was determined at this time, no cysteine-releasable radioactivity could be demonstrated. Pretreatment of the bladder with sulfhydryl reagents produced a striking reduction in the covalently bound tritium label. An effect of pH on covalent bonding was also demonstrated. At a pH below 7.0 in the serosal bath the hormone action was considerably reduced from that at pH 7.4, and at the low pH ranges the disulfide-bound label could not be demonstrated. The cysteine-resistant radioactivity was of a similar order of magnitude regardless of the treatment, while the normally larger cysteine-sensitive fraction varied according to whether or not the hormone was active.

Schwartz *et al.* suggested that their observations explained the reports which indicated that reduction of the hormone disulfide bond with cysteine or thioglycollate caused the loss of biological activity, and that reoxidation

restored the activity. They discussed a number of possibilities for explaining the permeability changes produced by the hormone, and suggested that conformational changes in the protein of the permeability barrier induced by a sulfhydryl-disulfide interchange reaction could be responsible.

Additional evidence for a role of receptor sulfhydryl in the action of antidiuretic hormone was obtained by Molina *et al.* (327). Earlier studies by Cafruny *et al.* (328) had shown that the concentration of protein-bound thiol in cells of the renal tubule of the hypophysectomized rat, studied by means of a quantitative histochemical procedure, was lower than in the normal rat. After the injection of vasopressin, the protein-bound sulfhydryl concentration was still further decreased. Other hormones had no such effect. In extending these studies, Molina *et al.* (327) observed that the injection of vasopressin, or 24 to 48 hr of dehydration, caused a decrease to about 75 per cent of control levels in the concentration of protein-bound sulfhydryl in cells of the renal tubules. After rehydration or after the effects of vasopressin were gone, the concentration of sulfhydryl returned to normal. Changes were observed in proximal convoluted tubules, proximal terminal tubules, the thick portion of the ascending limb of Henle's loop, distal convoluted tubules, and collecting tubules. During recovery from dehydration, the concentration of protein-bound disulfide decreased to very low levels. In subsequent studies, Molina *et al.* (329) observed that these characteristic changes followed the time course of action of vasopressin administered subcutaneously. However, preliminary attempts to demonstrate the dose-dependency of histochemical changes were not successful, since the lowest dose used, 1 mμ of vasopressin (Pitressin®) per rat, produced maximal effects. Oxytocin, on the other hand, injected subcutaneously in a dose of 100 mμ per rat produced no significant change in protein-bound sulfhydryl or disulfide. Molina *et al.* (329) argue that the magnitude of these changes makes it impossible to propose a direct stoichiometric reaction between hormone disulfide and receptor sulfhydryl groups. Such changes may be accounted for on the basis of a disulfide-sulfhydryl interchange chain reaction discussed by Schwartz and his collaborators and considered in some detail by Jensen (330). Although most investigators consider the action of antidiuretic hormone to be restricted to sites along the distal nephron, Molina *et al.* (329) suggest that an action may be exerted all along the nephron.

Another interesting suggestion concerning the mode of action of vasopressin was published by Orloff & Handler (331). Proceeding from the observation that vasopressin stimulates the release of hydrocortisone from the adrenal, and that the effects of other hormones on this process involve the intermediation of cyclic 3′,5′-adenosine monophosphate and phosphorylase activation, they set about to study whether vasopressin might alter the permeability of the isolated toad bladder by stimulating the production of cyclic 3′,5′-adenosine monophosphate from adenosine triphosphate. They observed that vasopressin, the cyclic monophosphate and theophylline

(which inhibits the conversion of the cyclic adenosine monophosphate to inactive 5′-adenosine monophosphate) all produced an increase in water permeability, current flow, and potential difference across the bladder. The effect of theophylline was additive with the effects of the cyclic monophosphate and with those of submaximal amounts of vasopressin, and the cyclic monophosphate increased the effect of submaximal doses of vasopressin. The results suggested that perhaps the effects of the neurohypophyseal hormones on the bladder and in the kidney resulted from a stimulating effect of the hormone on the production and accumulation of cyclic adenosine monophosphate.

## LITERATURE CITED


1. Smith, H. W., *The Kidney Structure and Function in Health and Disease*, 418 (Oxford Univ. Press, New York, N. Y., 1049 pp., 1951)
2. Waugh, W. H., *Circulation Research*, **6**, 363 (1958)
3. Hinshaw, L. B., Day, S. B., and Carlson, C. H., *Am. J. Physiol.*, **197**, 309 (1959)
4. Langston, J. B., Guyton, A. C., and Gillespie, W. J., Jr., *Am. J. Physiol.*, **197**, 595 (1959)
5. Langston, J. B., Guyton, A. C., and Gillespie, W. J., *Am. J. Physiol.*, **199**, 495 (1960)
6. Langston, J. B., Guyton, A. C., and Hull, C. G., *Federation Proc.*, **20**, 108 (1961)
7. Hardin, R. A., Scott, J. B., and Haddy, G., *Am. J. Physiol.*, **199**, 1192 (1960)
8. Rosenfeld, S., and Sellers, A. L., *Am. J. Physiol.*, **199**, 494 (1960)
9. Ohler, W., Harth, O., and Kreienberg, W., *Arch. ges. Physiol.*, **269**, 274 (1959)
10. Weiss, C., Passow, H., and Rothstein, A., *Am. J. Physiol.*, **196**, 1115 (1959)
11. Thurau, K., Kramer, K., and Brechteisbauer, H., *Arch. ges. Physiol.*, **268**, 188 (1959)
12. Schmid, H. E., Jr., and Spencer, M. P., *Federation Proc.*, 20, 109 (1961)
13. Bounous, G., Shumacker, H. B., Jr., and King, H., *Ann. Surg.*, **151**, 47 (1960)
14. Harvey, R. B., *Federation Proc.*, **20**, 108 (1961)
15. Scher, A. M., McDonald, P., and Koch, A. R., *Federation Proc.*, **20**, 108 (1961)
16. Forster, R. P., and Maes, J. P., *Am. J. Physiol.*, **150**, 534 (1947)
17. Selkurt, E. E., *Am. J. Physiol.*, **147**, 537 (1946)
18. Hinshaw, L. B., Ballin, H. M., Day, S. B., and Carlson, C. H., *Am. J. Physiol.*, **197**, 853 (1959)
19. Renkin, E. M., and Stainsby, W. N., *Am. J. Physiol.*, **187**, 625 (1956)
20. DeMuylder, C. G. [quoted from Waugh & Shanks (26)] *Neurility of the Kidney: Monograph on Nerve Supply to the Kidney*. (Charles C Thomas, Springfield, Ill., 1952)
21. Lamport, H., *Cardiology*, **1**, 2–230 (Luisada, A. A., Ed., McGraw-Hill Book Co., New York, N. Y., 1959)
22. Page, I. H., and McCubbin, J. W., *Am. J. Physiol.*, **173**, 411 (1953)
23. Sellwood, R. V., Verney, E. B., *Phil. Trans. Roy. Soc. London*, **238**, 361 (1955)
24. Thurau, K., and Kramer, K., *Arch. ges. Physiol.*, **268**, 43 (1959)
25. Semple, S. J. C., and deWardener, H. E., *Circulation Research*, **7**, 643 (1959)
26. Waugh, W. H., and Shanks, R. G., *Circulation Research*, **8**, 871 (1960)
27. Pappenheimer, J. R., and Kinter, W. B., *Am. J. Physiol.*, **185**, 377 (1956)
28. Kinter, W. B., and Pappenheimer, J. R., *Am. J. Physiol.*, **185**, 391 (1956)
29. Kinter, W. B., and Pappenheimer, J. R., *Am. J. Physiol.*, **185**, 399 (1956)
30. Rennie, D. W., Reeves, R. B., and Pappenheimer, J. R., *Am. J. Physiol.*, **195**, 120 (1958)
31. Hinshaw, L. B., Hakin, A. A., Worthen, D. M., and Anderson, Q. N., *Federation Proc.*, **20**, 108 (1961)
32. Winton, F. R., *Arch. Internal. Med.*, **103**, 495 (1959)
33. Ochwadt, B., *Arch. ges. Physiol.*, **265**, 112 (1957)
34. Thompson, D. D., Kavaler, F., Lozano, R., and Pitts, R. F., *Am. J. Physiol.*, **191**, 493 (1957)
35. Levy, M. N., and Sauceda, G., *Am. J. Physiol.*, **196**, 1336 (1959)
36. Levy, M. N., *Am. J. Physiol.*, **197**, 1111 (1959)
37. Levy, M. N., and Imperial, E. S., *Am. J. Physiol.*, **200**, 159 (1961)
38. Ullrich, K. J., and Stockle, H., *Arch. ges. Physiol.* (In press) [quoted from Ullrich, K. J., Kramer, K., and Boylan, J. W., *Progr. Cardiovascular Research*, **3**, 395 (1961)]
39. Kramer, K., Thurau, K., and Deetjen, P., *Arch. ges. Physiol.*, **270**, 251 (1960)
40. Lilienfield, L. S., Maganzini, H. C., and Bauer, M. H., *Circulation Research*, **9**, 614 (1961)
41. Heimburg, P., and Ochwadt, B., *Arch. ges. Physiol.*, **273**, 62 (1961)
42. Swann, H. C., Railey, A. B., and Carmignani, A. E., *Am. Heart. J.*, **58**, 608 (1959)
43. Hinshaw, L. B., Flaig, R. D., Logemann, R. L., and Carlson, C. H., *Am. J. Physiol.*, **198**, 891 (1960)

44. Hinshaw, L. B., and Carlson, C. H., *Proc. Soc. Exptl. Biol. Med.*, **103,** 373 (1960)
45. Selkurt, E. E., *Klin. Wochschr.*, **33,** 359 (1955)
46. Shipley, R. E., and Study, R. S., *Am. J. Physiol.*, **167,** 676 (1951)
47. Thurau, K., and Kramer, K., *Arch. ges. Physiol.*, **269,** 77 (1959)
48. Winton, F. R., *Harvey Lectures*, **21,** (Academic Press, Inc., New York, 1951)
49. Folkow, B., *Acta Physiol. Scand.*, **27,** 99 (1952)
50. Setchell, B. P., and Blanch, E., *Nature*, **189,** 230 (1961)
51. Balint, P., and Fekete, A., *Arch. ges. Physiol.*, **270,** 575 (1960)
52. Balint, P., Fekete, A., Gomori, P., and Nagy, Z., *Arch. ges. Physiol.*, **271,** 705 (1960)
53. Balint, P., and Sturcz, J., *Acta Med. Acad. Sci. Hung.*, **13,** 9 (1959)
54. Balint, P., Kiss, E., and Sturcz, J., *Acta Med. Acad. Sci. Hung.*, **15,** 249 (1959)
55. Atik, M., and Gutierrez-Saenz, R., *Federation Proc.*, **20,** 109 (1961)
56. Gilmore, J. P., *Federation Proc.*, **20,** 407 (1961)
57. McGiff, J. C., and Aviado, D. M., *Federation Proc.*, **20,** 109 (1961)
58. Jontz, J., Shumacker, H. B., Jr., and Bounous, G. *Surg., Gynecol. Obstet.*, **110,** 622 (1960)
59. Dieter, E., *Arch. ges. Physiol.*, **270,** 215 (1960)
60. Dieter, E. *Arch. ges. Physiol.*, **271,** 64 (1960)
61. Dieter, E. *Arch. ges. Physiol.*, **273,** 29 (1961)
62. Gauer, O. H., *Verhandl. deut. Ges. Kreislaufforsch.*, **22,** 61 (1955)
63. Henry, J. P. H., Gauer, O. H., and Reeves, J. L., *Circulation Research*, **4,** 85 (1956)
64. Pappenheimer, J. R., *Physiol. Revs.*, **40,** Suppl. 4, 35 (1960)
65. Dieter, E., *Arch. ges. Physiol.*, **273,** 39 (1961)
66. Barger, A. C., Liebowitz, M. R., and Muldowney, F. P., *J. Chronic Diseases*, **9,** 571 (1959)
67. Barger, A. C., Yates, F. E., and Rudolph, A. M., *Am. J. Physiol.*, **200,** 601 (1961)
68. Carpenter, C. C. J., Davis, J. O., Holman, J. E., Ayers, C. R., and Bahn, R. C., *J. Clin. Invest.*, **40,** 196 (1961)
69. Craig, R. L., Wetzel, N., and Bianchi, R. G., *Am. J. Physiol.*, **200,** 890 (1961)
70. Goodyer, A. V. N., *Progr. Cardiovascular Diseases*, **3,** 483 (1961)
71. Berne, R. M., and Levy, M. N., *Am. J. Physiol.*, **171,** 558 (1952)
72. Mokotoff, R., and Ross, G., *J. Clin. Invest.*, **27,** 335 (1948)
73. Horster, F. A., Peters, G., and Brunner, H., *Arch. exptl. Pathol. Pharmakol.*, **238,** 221 (1960)
74. Kanter, G. S., *Am. J. Physiol.*, **200,** 878 (1961)
75. Zoster, T., Banderman, L., and Chappel, C. I., *Am. Heart J.*, **61,** 777 (1961)
76. Sheehan, H. L., and Davis, J. C., *J. Pathol. Bacteriol.*, **79,** 347 (1960)
77. Abbrecht, P., and Malvin, R. L., *Federation Proc.*, **20,** 405 (1961)
78. Peters, G., *Arch. exptl. Pathol. Pharmakol.*, **235,** 113 (1959)
79. Moyer, J. H., *Ann. N. Y. Acad. Sci.*, **80,** 424 (1959)
80. Kanter, G. S., *Am. J. Physiol.*, **198,** 1044 (1960)
81. Kaufman, W., Nieth, H., and Schlitter, J. G., *Arch. ges. Physiol.*, **272,** 31 (1960)
82. Pabst, K., and Thron, H. L., *Arch. ges. Physiol.*, **270,** 585 (1960)
83. Kramer, K., and Deetjen, P., *Arch. ges. Physiol.*, **271,** 782 (1960)
84. Mills, L. C., Moyer, J. H., and Handley, C. A., *Am. J. Physiol.*, **198,** 1279 (1960)
85. McQueen, E. G., and Morrison, R. B. I., *Brit. Heart. J.*, **23,** 1 (1961)
86. Botting, R., Farmer, J. B., and Lockett, M. F., *Arch. intern. physiol. et biochim.*, **69,** 203 (1961)
87. Botting, R. M., and Lockett, M. F., *Arch. intern. physiol. et biochim.*, **69,** 36 (1961)
88. Worthen, D. M., Hinshaw, L. B., and Anderson, Q. N., *Federation Proc.*, **20,** 409 (1961)
89. Nelson, R. M., Henry, J. W., and Lyman, J. H., *Surgery*, **50,** 115 (1961)
90. Blake, W. D., *Federation Proc.*, **20,** 407 (1961)
91. Granitsas, A. N., *Am. J. Physiol.*, **198,** 603 (1960)
92. Kubicek, W. G., *J. Appl. Physiol.*, **15,** 109 (1960)
93. Passow, H., Schniewind, H., and Weiss, C., *Arch. exptl. Pathol. Pharmakol.*, **240,** 179 (1960)
94. Dolcini, H. A., Zaidman, I., Lictenberg, F., and Gray, S. J., *Am. J. Physiol.*, **199,** 1153 (1960)

95. Frick, M. H., *Nature*, **187**, 609 (1960)
96. Bonomini, V., Zucchelli, P., and Dalmastri, G., *Minerva med.*, **51**, 4183 (1960)
97. Vercillo, L., Giraldi, A., *Minerva med.*, **51**, 4190 (1960)
98. Richardson, D. W., Wyso, E. M., Magee, J. H., and Cavell, G. C., *Circulation*, **22**, 184 (1960)
99. Wagle, G. L., and Plummer, A. J. *Federation Proc.*, **20**, 109 (1961)
100. Solomon, S., Davies, B. J., and Boone, O. R., *Am. J. Physiol.*, **198**, 233 (1960)
101. Granitsas, A. N., *Am. J. Physiol.*, **198**, 811 (1960)
102. Carter, M. K., and Atkinson, S. L., *Federation Proc.*, **20**, 412 (1961)
103. Kuschinsky, G., and Langecker, H., *Arch. exptl. Pathol. Pharmakol.*, **204**, 699 (1947)
104. Greeff, K., and Contzen, C., *Arch. exptl. Pathol. Pharmakol.*, **239**, 35 (1960)
105. Kuschinsky, G., and Vorheer, H., *Arch. exptl. Pathol. Pharmakol.*, **238**, 281 (1960)
106. Marchetti, G., Nava, S., and Giordano, M., *Boll. soc. ital. biol. sper.*, **36**, 421 (1960)
107. Kelly, H. G., Cross, H. C., Turton, M. R., and Hatcher, J. D., *Can. Med. Assoc. J.*, **82**, 866 (1960)
108. *Diuresis und Diuretics, An International Symposium* (Buchborn, E., and Bock, K. D., Eds., Springer-Verlag, Berlin-Göttingen-Heidelberg, 382 pp. 1959)
109. Beyer K. H., and Baer, J. E., *Progr. in Drug Research*, **2**, 9 (Yucker, E., Ed., Interscience Publ., Inc., New York, 636 pp., 1960)
110. Pitts, R. F., *The Physiological Basis of Diuretic Therapy* (Charles C Thomas, Springfield, Ill., 332 pp., 1959)
111. *New Diuretics and Hypertensive Agents*, **88**, Art. 4 (Ann. N. Y. Acad. Sci., 771–1020, 1960)
112. Gaunt, R., Reuzi, A. A., and Chart, J. J., *Chemotherapia, (Basel)* **1**, 238 (1960)
113. Buchborn, E., *Verhand. deut. Ges. Kreislauforsch.*, **26**, 47 (1960)
114. Merrill, J. P., *Med. Clin. North Am.*, **44**, 1155 (1960)
115. Romani, J. D., *Progr. Med. (Paris)*, **87**, 403 (1959)
116. Lapiere, C. L., *Farmaco* (Practica Ed.) **15**, 547 (1960)
117. Cummings, J. R., Haynes, J. D., Lipchuck, L. M., and Ronsberg, M. A., *J. Pharmacol. Exptl. Therap.*, **128**, 414 (1960)
118. Aceto, M. D., and Ichniowski, C. T., *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 647 (1960)
119. Nielsen, O. E., *Acta Pharmacol. Toxicol.*, **18**, 23 (1961)
120. Brunner, H., Horster, F. A., Kuschinsky, G., and Loennecke, I., *Z. ges. exptl. Med.*, **133**, 455 (1960)
121. Kuschinsky, G., *Arch. exptl. Pathol. Pharmakol.*, **238**, 195 (1960)
122. Gold, H., Kwit, N. T., Messeloff, C. R., Kramer, M. L., Golfins, A. J., Greiner, T. H., Goessel, E. A., Hughes, J. H., and Warshau, L., *J. Am. Med. Assoc.*, **173**, 745 (1960)
123. Orloff, J., and Berliner, R. W., *Ann. Rev. Pharmacol.*, **1**, 287 (1961)
124. Vander, A. J., Malvin, R. L., Wilde, W. S., and Sullivan, L. P., *J. Pharmacol. Exptl. Therap.*, **125**, 19 (1959)
125. Kessler, R. H., Hierholzer, K., Gurd, R. S., and Pitts, R. F., *Am. J. Physiol.*, **194**, 540 (1958)
126. Earley, L. E., Kahn, M., and Orloff, J., *J. Clin. Invest.*, **40**, 857 (1961)
127. Au, W. Y. W., and Raisz, L. G., *J. Clin. Invest.*, **39**, 1302 (1960)
128. Blackmore, W. P., *J. Pharmacol. Exptl. Therap.*, **125**, 303 (1959)
129. Heinemann, H. O., Demartini, F. E., and Laragh, J. H., *Am. J. Med.*, **26**, 853 (1959)
130. Edmonds, C. J., and Wilson, G. M., *Lancet*, **I**, 505 (1960)
131. Liddle, G. W., *Arch. Internal Med.*, **102**, 998 (1958)
132. Edmonds, C. J., *Lancet*, **I**, 509 (1960)
133. Farrelly, R. O., Howie, R. N., and North, J. D., *Brit. Med. J.*, **5195**, 339 (1960)
134. Morrison, R. S., and Chalmers, T. C., *Ann. N. Y. Acad. Sci.*, **88**, 907 (1960)
135. Thomas, J. P. and Bartter, F. C., *Brit. Med. J.*, **I, 5233**, 1134 (1961)
136. Gantt, C. L., and Synek, J. H., *Proc. Soc. Exptl. Biol. Med.*, **106**, 27 (1961)
137. Pitts, R. F., Kruck, F., Lozano, R., Taylor, D. W., Heidenreich, O. P. A., and Kessler, R. H., *J. Pharmacol. Exptl. Therap.*, **123**, 89 (1960)
138. Cafruny, E. J., Ross, C., and Davitt, J., *Federation Proc.*, **20**, 410 (1961)
139. McBride, W. O., Weiner, I. M., and Mudge, G. H., *Federation Proc.*, **17**, 107 (1958)
140. Farah, A., and Frazer, M. (Unpublished)

141. Haenze, S., *Klin. Wochschr.*, **38,** 1168 (1960)
142. Kiil, F., *Circulation*, **21,** 717 (1960)
143. Crosley, A. P., Jr., Cullen, R. C., White, D., Freeman, J. F., Castillo, C. A., and Rowe, G. C., *J. Lab. Clin. Med.*, **55,** 182 (1960)
144. Freis, E. D., Wanko, A., Wilson, I. M., and Parrish, A. E., *Ann. N. Y. Acad. Sci.*, **71,** 450 (1958)
145. Tapia, F. A., Dustan, H. P., Schneckloth, R. A., Corcoran, A. C., and Page, I. H., *Lancet*, **II,** 831 (1957)
146. Conway, J., and Lauwers, P., *Circulation*, **21,** 21 (1960)
147. Freis, E. D., Wanko, A., Schnaper, H. W., and Frohlich, E. D., *J. Clin. Invest.*, **39,** 1277 (1960)
148. Hollander, W., Chobanian, A. V., and Wilkins, R. W., *Ann. N. Y. Acad. Sci.* **88,** 975 (1960)
149. Lauwers, P., and Conway, J., *J. Lab. Clin. Med.*, **56,** 401 (1960)
150. Gessler, V., and Heinze, V., *Z. ges. exptl. Med.*, **133,** 522 (1960)
151. Beavers, W. R., *Proc. Soc. Exptl. Biol. Med.*, **103,** 711 (1960)
152. Blackmore, W. P., *Proc. Soc. Exptl. Biol. Med.*, **106,** 681 (1961)
153. Wanko, A., and Freis, E. D., *Circulation*, **18,** 792 (1958)
154. Aleksandrow, D., Wysnacka, W., and Gajewski, J., *New Engl. J. Med.*, **261,** 1052 (1959)
155. Merrill, J. P., Guinand-Baldo, A., and Giordano, C., *Clin. Research*, **6,** 230 (1958)
156. Mendlowitz, M., Naftchi, N., Gitlow, S. E., Weinreb, H. L., and Wolf, R. L., *Ann. N. Y. Acad. Sci.*, **88,** 964 (1960)
157. Hollander, W., Chobanian, A. V., and Wilkins, R. W., *Diuresis und Diuretics, An International Symposium*, 297 (Buchborn, E., and Bock, K. D., Eds., Springer-Verlag, Berlin-Göttingen, Heidelberg, 382 pp., 1959)
158. Beavers, W. R., and Blackmore, W. P., *Proc. Soc. Exptl. Biol. Med.*, **98,** 133 (1958)
159. Preziosi, P. A., Bianchi, A., Loscalzo, B., and Schaepdryver, A. F., *Arch. intern. pharmacodynamie*, **118,** 467 (1959)
160. Bock, K. D., and Gross, F., *Arch. exptl. Pathol. Pharmakol.*, **238,** 339 (1960)
161. Blackmore, W. P., and Beavers, W. R., *Proc. Soc. Exptl. Biol. Med.*, **101,** 128 (1959)
162. Dollery, C. T., Harrington, M., and Kaufman, G., *Lancet*, **I,** 1215 (1959)
163. Losse, H., and Wehmeyer, H., *Diuresis und Diuretics, An International Symposium*, 313 (Buchborn, E., and Bock, K. D., Eds., Springer-Verlag, Berlin, Göttingen, Heidelberg, 382 pp., 1959)
164. Taylor, R. M., Milton, R. M., Powers, M. J., and Winbury, M. M., *The Pharmacologist*, **3,** 58 (1961)
165. Tobian, L., and Binion, J., *Circulation*, **6,** 469 (1952)
166. Tobian, L., and Binion, J., *J. Clin. Invest.*, **33,** 1407 (1954)
167. Vick, J., Ederstrom, H. E., and Vergeer, T., *Proc. Soc. Exptl. Biol. Med.*, **93,** 536 (1956)
168. Bohr, D. F., Brodie, D. C., and Cheu, D. H., *Circulation*, **17,** 746 (1958)
169. Mallov, S., *Circulation Research*, **7,** 196 (1959)
170. Mallov, S., *Am. J. Physiol.*, **198,** 1019 (1960)
171. Crawford, J. D., and Kennedy, G. C., *Nature*, **183,** 891 (1959)
172. Reerink, H., Brongers, E. E., and Shouten, H.; cited by Crawford, J. D., and Kennedy, G. C., *Nature*, **183,** 1368 (1959)
173. Crawford, J. D., Kennedy, G. C., and Hill, L. E., *New Engl. J. Med.*, **262,** 737 (1960)
174. Nitschke, U., and Goetzke, D., *Deut. med. Wochschr.*, **85,** 2246 (1960)
175. Plaza De Los Reyes, M., Gomez, R., and Bradford, I., *Lancet*, **I,** 650 (1960)
176. Lefebvre, J. F., and Schumacher, O. P., *Cleveland Clinic. Quart.*, **28,** 122 (1961)
177. Havard, C. W. H., and Wodd, P. H., *Brit. Med.* **I, 5182,** 1306 (1960)
178. Calesnick, B., and Brenner, S. A., *J. Am. Med. Assoc.*, **176,** 1088 (1961)
179. Kennedy, G. C., and Crawford, J. D., *J. Endocrinol.*, **22,** 77 (1961)
180. Gayer, J., and Kaufman, W., *Deut. Med. Wochschr.*, **86,** 1256 (1961)
181. Cutler, R., Kleeman, C. R., Dowling, J. T., and Maxwell, M. H., *J. Clin. Invest.*, **39,** 980 (1960)
182. Beaser, S. B., *Am. J. Med. Sci.*, **213,** 441 (1947)
183. Shannon, J. A., *J. Exptl. Med.*, **76,** 371 (1942)
184. Earley, L. E., and Orloff, J., *Clin. Research*, **8,** 382 (1960)
185. Kourilsky, R., Kourilsky, S., Laudat, M., and Regaud, J., *Bull. mém. soc. méd. hôp. Paris*, **91,** 58 (1942)

186. Beyer, K. H., *Ann. N. Y. Acad. Sci.*, **71**, 363 (1958)
187. Taggart, J. V., *Am. J. Med.*, **24**, 774 (1958)
188. Kessler, R. H., Hierholzer, K., Gurd, R. S., and Pitts, R. F., *Am. J. Physiol.*, **196**, 1346 (1959)
189. Flanagan, C. L., and Earle, D. P., *Federation Proc.*, **17**, 47 (1958)
190. Essig, A., *Am. J. Physiol.*, **201**, 303 (1961)
191. Foulkes, E. C., and Miller, B. F., *Am. J. Physiol.*, **196**, 86 (1959)
192. Vander, A. J., Malvin, R. L., Wilde, W. S., and Sullivan, L. P., *Federation Proc.*, **17**, 166 (1958)
193. Orloff, J., Wagner, H. N., and Davidson, D. G., *J. Clin. Invest.*, **37**, 458 (1958)
194. Heinemann, H. O., and Becker, E. L., *J. Appl. Physiol.*, **12**, 51 (1958)
195. Laragh, J. H., Heinemann, H. O., and Demartini, F. E., *J. Am. Med. Assoc.*, **166**, 145 (1958)
196. Wesson, L. G., Jr., and Anslow, W. P., Jr., *Am. J. Physiol.*, **170**, 255 (1952)
197. Miller, T. B., and Riggs, D. S., *J. Pharmacol. Exptl. Therap.*, **132**, 329 (1961)
198. Capps, J. M., Wiggins, W. S., Axelrod, D. R., and Pitts, R. F., *Circulation*, **6**, 82 (1952)
199. Ladd, M., *J. Appl. Physiol.*, **4**, 603 (1951)
200. Goldstein, M. H., Levitt, M. F., Hauser, A. D., and Polimeros, D., *J. Clin. Invest.*, **40**, 731 (1961)
201. Gottschalk, C. W., and Mylle, M., *Am. J. Physiol.*, **196**, 927 (1959)
202. Windhager, E. E., and Giebisch, G., *Am. J. Physiol.*, **200**, 581 (1961)
203. Raeser, P. B., and Burch, G. E., *Proc. Soc. Exptl. Biol. Med.*, **63**, 543 (1946)
204. Farah, A., and Koda, F., *J. Pharmacol. Exptl. Therap.*, **110**, 361 (1954)
205. Farah, A., and Koda, F., *J. Pharmacol. Exptl. Therap.*, **113**, 256 (1955)
206. Farah, A., Cobbey, T. C., and Mook, W., *Proc. Soc. Exptl. Biol. Med.*, **81**, 601 (1952)
207. Lambie, A. T., and Robson, J. S., *Clin. Sci.*, **20**, 123 (1961)
208. Farah, A., Cafruny, E. J., and Di Stefano, H. S., *J. Histochem. and Cytochem.*, **3**, 271 (1955)
209. Cafruny, E. J., Farah, A., and Di Stefano, H. S., *J. Pharmacol. Exptl. Therap.*, **115**, 390 (1955)
210. Cafruny, E. J., and Farah, A., *J. Pharmacol. Exptl. Therap.*, **117**, 101 (1956)
211. Timm, F., and Arnold, H., *Arch. exptl. Pathol. Pharmakol.*, **235**, 478 (1959)
212. Timm, F., and Arnold, H., *Arch. exptl. Pathol. Pharmakol.*, **239**, 393 (1960)
213. Giebisch, G., *Circulation*, **21**, 879 (1960)
214. White, H. L., Rolf, D., Bisno, A. L., Kasser, I. S., and Tosteson, D. C., *Am. J. Physiol.*, **200**, 885 (1961)
215. Hierholzer, K., *Am. J. Physiol.*, **201**, 318 (1961)
216. Malvin, R. L., Wilde, W. S., Vander, A. J., and Sullivan, L. P., *Am. J. Physiol.*, **195**, 549 (1958)
217. White, H. L., Rolf, D., and Tosteson, D. C., *Am. J. Physiol.*, **200**, 591 (1961)
218. Kleinzeller, A., and Cort, J. H., *Biochem. J.*, **67**, 15 (1957)
219. Maizels, M., and Remington, M., *J. Physiol., (London)*, **143**, 283 (1958)
220. Dzurik, R., and Krajci-Lazary, B., *Arch. intern. pharmacodynamie*, **131**, 450 (1961)
221. Mudge, G. H., and Weiner, I. M., *Ann. N. Y. Acad. Sci.*, **71**, 344 (1958)
222. Farah, A., Bender, C. H., Kruse, R., and Cafruny, E., *J. Pharmacol. Exptl. Therap.*, **125**, 309 (1959)
223. Farah, A., and Kruse, R., *J. Pharmacol. Exptl. Therap.*, **130**, 13 (1960)
224. Axelrod, D. R., and Pitts, R. F., *J. Clin. Invest.*, **31**, 171 (1952)
225. Kessler, R. H., *Clin. Pharmacol. Therap.*, **1**, 723 (1960)
226. Riggs, D. S., and Friedman, G. (Personal communication)
227. Heidenreich, O., and Schneider, W., *Arch. exptl. Pathol. Pharmakol.*, **238**, 258 (1960)
228. Miller, T. B., and Farah, A., *J. Pharmacol. Exptl. Therap.* (In press)
229. Miller, T. B., and Farah, A., *J. Pharmacol. Exptl. Therap.* (In press)
230. Weiner, I. M., Garlid, K., Sapir, D., and Mudge, G. H., *J. Pharmacol. Exptl. Therap.*, **127**, 325 (1959)
231. Jamison, R. L., *J. Pharmacol. Exptl. Therap.*, **133**, 1 (1961)
232. Rennick, B. (Personal communication)
233. Bickers, J. N., Bresler, E. H., and Weinberger, R., *J. Pharmacol. Exptl. Therap.*, **128**, 283 (1960)
234. Shore, V., and Shore, B., *Am. J. Physiol.*, **198**, 187 (1960)
235. Hamamoto, Y., and Seikaga Ku, J., *Seikagaku, (J. Japan. Biochem. Soc.)*, **33**, 81 (1961)
236. Scott, R. L., and Gamble, J. L., Jr., *J. Biol. Chem.*, **236**, 570 (1961)
237. Weed, R. I., *Federation Proc.*, **20**, 62 (1961)

238. Munck, O. J., Pedersen, J. G., Ringsted, J., and Speggers, F., *Acta Pharmacol. Toxicol.*, **16**, 6 (1959)
239. Calesnick, B., Wase, A., and Altarelli, V. R., *Proc. Soc. Exptl. Biol. Med.*, **103**, 22 (1960)
240. Miller, V. L., Klavano, P. A., and Csonka, E., *Toxicol. and Appl. Pharmacol.*, **2**, 344 (1960)
241. Bergstrand, A., Friberg, L., Mendel, L., and Obeblad, E., *Acta Pathol, Microbiol. Scand.*, **51**, Suppl. 144. 115 (1961)
242. Campbell, D. E., *Acta Pharmacol. Toxicol.*, **16**, 151 (1959)
243. Campbell, D. E., *Acta Pharmacol. Toxicol.*, **17**, 137 (1960)
244. Campbell, D. E., *Acta Pharmacol. Toxicol.*, **17**, 213 (1960)
245. Weiner, I. M., cited by Orloff, J., and Berliner, R. W., *Ann. Rev. Pharmacol.*, **1**, 287 (1961)
246. Heidenreich, O., and Schneider, W., *Arch. exptl. Pathol. Pharmak.*, **238**, 245 (1960)
247. Heidenreich, O., Reus, E., and Schneider, W., *Arch. exptl. Pathol. Pharmakol.*, **238**, 270 (1960)
248. Gantt, C. L., *N. Y. State J. Med.*, **61**, 756 (1961)
249. Slater, J. D., *Postgrad. Med. J.*, **36**, 399 (1960)
250. Siegenthaler, W., and Truninger, B., *Deut. Med. Wochschr.*, **86**, 213 (1961)
251. Coppage, W. S., Jr., and Liddle, G. W., *Ann. N. Y. Acad. Sci.*, **88**, 815 (1960)
252. Cejka, V., Vries, L. A. de, and Borst, J. G., *Lancet*, **I**, 312 (1960)
253. Truninger, B., Siegenthaler, W., *Klin. Wochschr.*, **38**, 385 (1960)
254. Hellman, S., and Faloon, W. F., *J. Lab. Clin. Med.*, **55**, 872 (1960)
255. Ross, E. J., and Winternitz, W. W., *Clin. Sci.*, **20**, 143 (1961)
256. Vander, A. J., Wilde, W. S., and Malvin, R. L., *Proc. Soc. Exptl. Biol. Med.*, **103**, 525 (1960)
257. Williamson, H. E., Skulan, Th. W., and Shideman, F. E., *J. Pharmacol. Exptl. Therap.*, **131**, 49 (1961)
258. Penney, D. E., McAfee, R. D., and Locke, W., *Federation Proc.*, **20**, 413 (1961)
259. D'Amico, G., *Am. J. Med. Sci.*, **236**, 156 (1958)
260. Riecker, G., and Bubnoff, M. v., *Klin. Wochschr.*, **36**, 556 (1958)
261. D'Amico, G., and Cesano, A., *Experientia*, **17**, 111 (1961)
262. Garst, J. B., Shumway, N. P., Schwartz, H., and Farrell, G. L., *Federation Proc.*, **20**, 180 (1961)
263. Mokler, C. M., *Proc. Soc. Exptl. Biol. Med.*, **105**, 257 (1960)
264. Koczorek, K. R., *Klin. Wochschr.*, **38**, 826 (1960)
265. Kagawa, C. M., and Brown, E. A., *Proc. Soc. Exptl. Biol. Med.*, **105**, 648 (1960)
266. Wiebelhaus, V. D., Weinstock, J., Brennan, F. T., Sosnowski, G., and Larsen, T. J., *Federation Proc.*, **20**, 409 (1961)
267. Laragh, J. H., Reilly, E. B., and Stites, T. B., *Federation Proc.*, **20**, 410 (1961)
268. Crosley, A. P., Jr., Ronquillo, L., and Alexander, F., *Federation Proc.*, **20**, 410 (1961)
269. Wiebelhaus, V. D., Weinstock, J., Brennan, F. T., Sosnowski, G., Larsen, T., and Gahagen, K., *The Pharmacologist*, **3**, 59 (1961)
270. Schlatmann, R. J., Prennen, H., Jansen, A. P., and Majoor, C. L., *Lancet*, **I**, 314 (1960)
271. Cejka, V., Vries, L. A. de, Daatselaar, J. J. van, Borst, J. G., and Majoor, C. L., *Lancet*, **I**, 317 (1960)
272. Muldowney, F. P., and Banks, P., *Lancet*, **I**, 548 (1960)
273. Hashish, S. E., *Acta. Physiol. Scand.*, **43**, 189 (1958)
274. Tuttle, R. S., Witt, P. N., and Farah, A., *J. Pharmacol. Exptl. Therap.*, **133**, 281 (1961)
275. Steward, G. A., *Biochem. Pharmacol.*, **8**, 14 (1961)
276. Klaus, W., Kuschinsky, G., and Lüllman, H., *Biochem. Pharmacol.*, **8**, 37 (1961)
277. Tuttle, R. S., Witt, P. N., and Farah, A. (In press, 1961)
278. Farber, S. J., Alexander, J. D., Pellegrino, E. D., and Earle, D. P., *Circulation*, **4**, 378 (1951)
279. Hyman, A. L., Jaques, W. E., and Hyman, E. S., *Am. Heart J.*, **52**, 592 (1956)
280. Orloff, J., and Burg, M., *Am. J. Physiol.*, **199**, 49 (1960)
281. Wilde, W. S., and Howard, P. J., *J. Pharmacol. Exptl. Therap.*, **130**, 232 (1960)
282. Cade, J. R., Shalhoub, R. S., Canessa-Fischer, M., and Pitts, R. F., *Am. J. Physiol.*, **200**, 373 (1961)
283. Kupfer, S., and Kosovsky, J. D., *Am. Heart J.*, **61**, 284 (1961)
284. Kosovsky, J. D., and Kupfer, S., *Federation Proc.*, **20**, 412 (1961)
285. Strickler, J. C., and Kessler, R. H., *J. Clin. Invest.*, **40**, 311 (1961)
286. Machova, J., *Experientia*, **16**, 553 (1960)
287. Wilbrandt, W., *Helv. Physiol. et Pharmacol. Acta*, **16**, 31 (1958)

288. Kennedy, G. C., and Crawford, J. D., *Lancet*, **I**, 866 (1959)
289. Glynn, I. M., *J. Physiol. (London)*, **136**, 148 (1957)
290. Portius, H. J., and Repke, K., *Biochem. Pharmacol.*, **8**, 36 (1961)
291. Sen, A. K., and Post, R. L., *Federation Proc.*, **20**, 138 (1961)
292. Kinsolving, C. R., and Post, R. L., *The Physiologist*, **3**, 94 (1960)
293. Jarnefelt, J., *Biochim. et Biophys. Acta*, **48**, 104 (1961)
294. Jarnefelt, J., *Biochim. et. Biophys. Acta*, **48**, 111 (1961)
295. Tosteson, D. C., Blaustein, M. P., and Moulton, R. H., *Federation Proc.*, **20**, 138 (1961)
296. Janku, K., Hava, M., Kraus, R., and Motl, O., *Arch. exptl. Pathol. Pharmakol.*, **238**, 112 (1960)
297. VanArman, C. G., Dettelbach, H. R., and Kagawa, C. M., *Arch. intern. pharmacodynamie*, **126**, 400 (1960)
298. Kagawa, C. M., and VanArman, C. G., *J. Pharmacol. Exptl. Therap.*, **129**, 343 (1960)
299. Reubi, F., Schmidt, E., and Cottier, P., *J. urol. méd. et chir.*, **66**, 145 (1960)
300. Reubi, F. C., and Cottier, P. T., *Circulation*, **23**, 200 (1961)
301. Domenet, J. G., Evans, D. W., and Brenner, O., *Brit. Med. J.*, **I**, **5233**, 1130 (1961)
302. Lasser, R. P., Schoenfield, M. R., and Friedberg, C. K., *New England J. Med.*, **263**, 728 (1960)
303. Rubin, A. L., Spritz, N., Mead, A. W., Herrman, R. A., Braverman, W. S., and Luckey, E. H., *Circulation*, **21**, 332 (1960)
304. Ogden, D. A., Scherr, L., Spritz, N., Rubin, A. L., and Luckey, E. H., *Am. Heart. J.*, **61**, 16 (1961)
305. Bosley, L., Spencer, H., Rogne, M., and Samachson, Y., *Federation Proc.*, 20, 76 (1961)
306. Schteingart, D. E., Perlmutter, M., and Numeroff, M. S., *Am. J. Med. Sci.*, **239**, 571 (1960)
307. Bolechowski, F., Mazur, M., and Lukasiak, H., *Acta Physiol. Polon.*, **11**, 663 (1960)
308. Gottlieb, L. I., and Coye, R. D., *Federation Proc.*, **20**, 379 (1961)
309. Sawyer, W. H., *Pharmacol. Revs.*, **13**, 225 (1961)
310. Koefoed-Johnsen, V., and Ussing, H. H., *Acta Physiol. Scand.*, **42**, 298 (1958)
311. Leaf, A., *J. Gen. Physiol.*, **43**, 175 (1960)
312. Bentley, P. J., *J. Endocrinol.*, **17**, 201 (1958)
313. Rasmussen, H., Schwartz, I. L., Schoessler, M. A., and Hochster, G., *Proc. Natl. Acad. Sci. U. S.*, **46**, 1278 (1960)
314. Ginetzinsky, A. G., *Nature*, **182**, 128 (1958)
315. Ginetzinsky, A. G., Broitman, A. I., Ivanova, L. N., *Bull. Exptl. Biol. Med.*, **38**, 37 (1954)
316. Ivanova, L. N., *Bull. Exptl. Biol. Med.*, **45**, 281 (1958)*
317. Ginetzinsky, A. G., Zaks, M. G., and Titova, L. K., *Doklady Akad. Nauk, S.S.S.R.*, **120**, 216 (1958)*
318. Hess, M., and Hollander, F., *J. Lab. Clin. Med.*, **32**, 905 (1947)
319. Dicker, S. E., and Eggleton, M. G., *J. Physiol.*, **154**, 378 (1960)
320. Berlyne, G. M., *Clin. Sci.*, **19**, 619 (1960)
321. Berlyne, G. M., *Nature*, **185**, 389 (1961)
322. Ginetzinsky, A. G., Krestinskaya, T. V., Natochin, J. V., Sax, M. G., and Titova, L. K., *Physiol. Bohemosloven.*, **9**, 166 (1960)
323. Heller, J., and Lojda, Z., *Physiol. Bohemosloven.*, **6**, 504 (1960)
324. Meyer, D. (Personal communication)
325. Fong, C. T. O., Silver, L., Christman, D. R., and Schwartz, I. L., *Proc. Natl. Acad. Sci. U. S.*, **46**, 1273 (1960)
326. Schwartz, I. L., Rasmussen, H., Schoessler, M. A., Silver, L., and Fong, C. T. O., *Proc. Natl. Acad. Sci. U. S.*, **46**, 1288 (1960)
327. Molina, G., Kruse, R., and Farah, A., *Federation Proc.*, **20**, 407 (1961)
328. Cafruny, E. J., Carhart, E., and Farah, A., *Endocrinology*, **61**, 143 (1957)
329. Molina, G., Kruse, R., and Farah, A. (Unpublished observations)
330. Jensen, E. V., *Science*, **130**, 1319 (1959)
331. Orloff, J., and Handler, J. S., *Biochem. Biophys. Research Communs.*, **5**, 63 (1961)
332. Dicker, S. E., and Eggleton, M. G., *J. Physiol.*, **157**, 351 (1961)

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