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THE SITE AND MECHANISM OF ACTION OF MERCURIAL DIURETICS

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TABLE OF CONTENTS

I. INTRODUCTION

About 7 years have elapsed since Beyer and Baer (12) reviewed diuretics in Pharmacological Reviews. The tempo of research has now created a need for another article, for in the intervening period information on mercurial diuretics has piled up and older studies have acquired new meaning. The primary purpose of this review is to render an orderly account of current concepts of the site and mechanism of action of mercurials. I hope that this account will direct attention especially to those difficult or unexplored problems which can be solved at least in part by application of methods presently in vogue. For convenience the terms "mercurial" and "mercury" will be used interchangeably. Although the organic portion of a mercurial diuretic may alter the distribution and excretion and certain other pharmacodynamic properties, its diuretic activity is always due to the mercury contained. Moreover, in most instances no appreciable quantity of Hg^{++} ion is detectable in tissue or urine after the injection of an organic compound. Exceptions will be noted, and official names of individual mercurials will be mentioned when there is reason to do so.

Pitts (134) defined a diuretic as a substance that increases the net renal excre-

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tion of sodium and water. It is unfortunate that this rather precise definition has been disregarded so often, for it captures the essence of the basic elements—the ability of all such drugs to block sodium reabsorption and the necessity for doing so in order to rid the body of edema fluid. Many investigators speak of diuretics as substances that increase the renal excretion of water and of saluretics as substances that increase the excretion of sodium chloride, as though the two phenomena were unrelated. Except under most unusual circumstances, extra water invariably accompanies extra salt in the urine. For this reason, the imprecise term saluretic will not be used in this review.

As used here, "site of action" refers to a morphological segment of a nephron, or of the cells thereof, where a drug exerts an action that contributes or can contribute to diuresis.[2] The term should not be confused with "site of reaction," the chemical or molecular site with which a drug interacts to bring about a measurable, physiologic change or not as the case may be. This distinction is especially important in the case of mercurial diuretics, for a site of reaction may or may not be a site of action. Since it is not yet possible to identify the biochemical operations of active transport processes, "mechanism of action" will be discussed only in terms of mercury-receptor attachments and how these attachments may interfere with the reabsorptive capacity of renal tubular cells.

A few years hence, the mercurial diuretics may be drugs of the past—remembered but not used. This is fitting, for their successors now equal or exceed them in efficacy and ease of administration. As investigational drugs, however, the mercurials will be valuable for many years to come. This too is fitting, for they helped to establish the field of renal pharmacology, served as models for the development and study of the compounds currently displacing them, and have been exceedingly useful in the evolution of techniques for studying the site and mechanism of action of diuretic drugs. Consequently, there are thousands of papers on mercurials in the scientific literature. A semblance of continuity is established in the following reviews: Pitts and Sartorius (135) and Vogl (176) organized and interpreted the reports up to and including the late forties; Orloff and Berliner (129) and Beyer and Baer (12) included the mercurials in their general reviews of renal pharmacology; Farah and Miller (62) brought us up to date in 1962; and more recently Cafruny *et al.* (24) and Weiner and Farah (183) provided a summation of some current concepts. This selective review is constructed to bring out the factors relating especially to the site and mechanism of action of mercurial diuretics.

II. SITE OF ACTION

A. The "extrarenal hypothesis"

Studies on the pharmacology of mercurial diuretics began in the early twenties soon after the discovery of novasurol, the first useful mercurial. In a series of papers that collectively described the basic actions of novasurol, Saxl and Heilig

[2] Although extrarenal actions of mercurial diuretics may influence renal tubular reabsorptive processes, it is extremely doubtful that these actions augment the diuretic response to any large extent. There will be more discussion of this point in section II A.

(150–152) not only established its value as a diuretic but also offered an explanation for the occurrence of diuresis. They found, in man and in the dog, that the drug often reduced the concentration of protein present in plasma. Accordingly, they postulated that novasurol somehow mobilized the salt and water of the tissues. The fluid mobilized entered the plasma compartment and stimulated flow of urine. Thus the diuresis was extrarenal in origin. A large number of investigators confirmed or supported the observations of Saxl and Heilig, but, within a few years, it became obvious that the "extrarenal hypothesis" was incorrect. The first discordant note was struck by Govaerts (75), who transplanted kidneys of dogs treated with a mercurial into untreated recipients. The transplanted organs continued to produce urine copiously. Then Gremels (77) found that mercurials increase urine flow in heart-lung-kidney preparations. Bartram's (5) subsequent proof of renal action—one-sided diuresis produced by small doses injected into a renal artery—finally scuttled the "extrarenal hypothesis." Scientists had rushed to confirm the results of Saxl and Heilig; a decade later almost everyone who looked (14, 20, 44, 50, 155) denied that mercurials increased plasma volume.

But there are still a few recusant workers committed to a modified "extrarenal hypothesis." Although they acknowledge the primacy of renal sites of action, they decry the tendency to discount *a priori* the existence of extrarenal sites. Their argument is valid, for the importance of any possible extrarenal action of mercurials depends, not on the magnitude of the diuretic response such an action may possibly elicit, but rather on the mechanism by which the response may be evoked. It is only the evidence for extrarenal action that counts, and there is evidence.

Möller (119) found that mersalyl brought about simultaneously a reduction in the concentration of hemoglobin and an elevation in plasma chloride of nephrectomized rabbits. These effects were not as pronounced in animals with intact kidneys probably because they were offset by renal losses of fluid and chloride. In unanesthetized dogs, van Riezen (174) found that mercaptomerin increased the volume of distribution of Evans blue dye, lowered hematocrit, and induced a small but significant rise in glomerular filtration. Patterson and Ray (131) measured the flow of lymph in the thoracic duct of dogs and found there was a marked increase in rate after the injection of meralluride. This effect preceded the onset of diuresis and lasted for about 30 minutes. It occurred in a nephrectomized animal but, for some strange reason, did not persist for a longer time than in intact dogs. Patterson and Ray postulated that mercury acts by altering water binding in tissues. "Water, mobilized from the interstitial compartment, enters the circulation and thus may augment the primary renal action of mercury."

If, as the above authors suggested, an extrarenal action of mercurials causes expansion of plasma volume before the onset of diuresis, renal plasma flow and glomerular filtration rate should increase. With rare exceptions (see above), most investigators (55, 135, 175) have found either no change or a transient decrease during the lag period preceding the diuretic effect and also during the early phase of diuresis. Perhaps expansion of circulating plasma volume is too small to influence renal plasma flow and glomerular filtration rate, or the renal

vascular constrictor effects of mercurials (55, 175) may prevent expression of the effect. In either event, there is every reason to believe that the concept of extrarenal action as a purely mechanical phenomenon is worthless. If there is any hope of salvaging even a portion of the idea, it is essential to consider that an increase in circulating plasma volume is more likely to augment the action of mercurials by suppressing or releasing a chemical mediator (*e.g.*, aldosterone or a salt-wasting hormone) that subsequently acts directly on the renal tubules.

B. Inferences from the location of renal lesions

Before the separate functions of the various segments of the nephron had been identified and described, there were reasons to suspect that mercurials might interfere with the reabsorptive capacity of some but not all reabsorbing units. The nephrotoxic action of mercury, *i.e.*, the morphologic change it produces, first described in animals by Pavy (132), appeared to be limited to proximal tubules except when large, often nondiuretic doses were used. In fact, in most species lesions were present only in the terminal portion of the proximal tubule (53, 127, 158, 164, 178). Although some of the reported studies were not properly controlled, the recent careful work of Rodin and Crowson (145) substantiated the major points of their predecessors. We do not know whether the injurious effect in the terminal part of the proximal tubule means that the cells involved are exceedingly sensitive to mercury or whether such cells are exposed to higher concentrations because they actively transport mercury. Whatever the reason, it is likely that the normal functions of those cells most susceptible to the damaging effects of mercury are the functions most readily depressed. We may regard functional disturbances as harbingers of structural disturbances (nephrotoxic responses). Strictly speaking, this inference is not demonstrable, but its corollary —all drugs can have morphologic effects—is. Viewed as circumstantial evidence, the data on the nephrotoxic action of mercurials have meaning. They permit us to infer that mercurials block reabsorption of electrolytes in the terminal portion of the proximal tubule. They reveal nothing of functional actions in other parts of the renal tubule.

C. Inferences from actions on the reabsorption of sodium, potassium, and water

Experimental models for studying actions of drugs are based largely on antecedent principles of physiology and biochemistry. Consequently, most of our working hypotheses make use of deductive inference. Our conclusions often are sound although the original assumptions later turn out to be false. There are no better examples of this than some of the very best work on the site of action of mercurials.

Data from early micropuncture experiments (179, 188) indicated that about two-thirds of the filtered sodium was reabsorbed in the proximal tubule. Duggan and Pitts (48) reasoned that if mercurials could block a fraction of sodium reabsorption greater than one-third of the filtered load it would be necessary to consider the proximal tubule as a site of action. When they found that maximal

inhibition did not exceed 20%, they suggested that the distal tubule was the site of action, but were willing to concede that mercury could be blocking reabsorption incompletely in the proximal tubule. Farah *et al.* (57) later found that the infusion of isotonic saline greatly increased the response to mercurials so that as much as 40% of the filtered sodium was excreted. They considered the proximal tubule to be involved in the production of mercurial diuresis but did not rule out actions at other sites. Subsequently, Farah and Koda (59) found that there was a mechanism for reabsorbing sodium that could be inhibited by cyanide but not by mercury. Thus mercurials might be expected to block reabsorption incompletely either in proximal or distal tubule. While these early studies on site of action served as models for later work and stimulated interest in the field, it is clear now, from a stronger vantage point, that they did not supply the information they were designed to supply. We now possess the following additional information: On micropuncture of the first two-thirds of the proximal tubule of the dog, the fractional reabsorption of sodium is found to vary considerably depending on conditions of salt and water balance (9, 47); reabsorption in the last third of the proximal tubule, that critical segment where mercury is apt to produce lesions, may occur at a rate different from that of the rest of the tubule; the state of acid-base balance and the levels of filtered sodium have a marked influence on the diuretic action of mercurials (57, 58, 120); saline infusions depress the fractional reabsorption of sodium in the proximal tubule (46, 47). It is doubtful whether the maximal effect a mercurial may exert on reabsorption of sodium has ever been determined. It is most difficult to do so. Supposing that ideal conditions for the experiment were attainable, how would it be possible to prevent the renal vascular constriction that invariably occurs when large doses of mercury are used?

The application of stop-flow analysis to the study of the site of action of mercurials represents another example in which sound conclusions were based on questionable assumptions. From stop-flow results, Vander *et al.* (173) and Kessler *et al.* (94) argued that mercurials depressed sodium and water reabsorption in the proximal tubule but not in the distal tubule. The questionable assumption in these studies was discovered quickly by a number of investigators, who pointed out that the composition of the urine of distal samples does not necessarily reflect the functional status of the distal tubule because, during stop-flow, there is time for the distal tubule to lower the concentration of sodium in its lumen to the minimal level possible even though the rate of absorption of sodium may be impaired. Moreover, it was argued that late (proximal) samples of stop-flow urine are altered as they pass through distal channels on the way to the urine collector. This latter objection has been softened somewhat by the report of Cafruny and Ross (31), who found that thiazides significantly elevated the distal minimum for sodium but had little effect on the proximal limb of the stop-flow pattern; mercurials, on the other hand, produced a decided elevation in the proximal limb but had no discernible effect on the distal minimum. The latest, and perhaps most definitive, stop-flow paper on mercurial diuretics is the one published by Schmidt and Sullivan (154). Because of the large amounts of man-

nitol infused during a typical stop-flow experiment, the concentration of sodium in plasma is always reduced to levels of about 110 mEq per liter. Schmidt and Sullivan infused sodium chloride in order to prevent this reduction. They then proceeded to show that mercurials raised the entire stop-flow pattern for sodium. Thus it appeared that mercury inhibited transport of sodium all along the nephron. These studies are suggestive but not conclusive, especially in regard to action on the distal tubule. The height of the distal minimum varies directly with the amount of sodium that enters the distal tubule (30), and large amounts must surely have entered if mercurials block reabsorption in the proximal tubule as all stop-flow studies, including this one, indicate. Needless to say, the stop-flow procedure has not and probably cannot settle the question of the site of action of mercurials, and Orloff's view (128), that its usefulness for studying substances transported at multiple loci (*e.g.*, sodium) is limited, prevails.

The effects of mercurials on the excretion of potassium can be explained only if the distal tubule represents a site of action. Mudge *et al.* (121) noted that mercurials were capable of increasing the excretion of potassium when its rate of excretion was low initially or of decreasing excretion when the rate of excretion was elevated before the administration of a mercurial. Since the distal tubule is a major site where potassium is added to urine (11, 88, 170), this portion of the nephron must be one of the sites of action of mercurials. The recent work of Giebisch *et al.* (71) and Malnic *et al.* (111, 112) on the transport of potassium in the distal tubule provides a basis for this dual action of mercurials. These workers found that the electrical gradient across the luminal membranes of the distal tubule was sufficient to effect net entry of potassium into urine. The concentration of the ion in distal tubular urine was lower than that expected from the measured electrical potential. Thus two pumps must be operative—one carrying potassium into cells from the blood side and one from the urinary side (70). If mercurials reduced the capacity of both pumps, urinary potassium should increase when excretion of the ion was low to begin with or decrease when excretion was initially high.

Since diuretics increase the excretion of water, it was predictable that investigators would begin to apply techniques for studying the transport of water to the analysis of the site of action of mercurials. It is important to recognize that this idea does not impose the requirement that mercurials act directly on the reabsorption of water; the site of water transport being known, the interpretation of the result would rest entirely on whether interference in ion transport at specific loci would alter water movements at predictable sites and, of course, also on numerous underlying assumptions. To appreciate the work done, it is necessary only to consider the mechanisms involved in the formation of free water and the reabsorptive transport of solute-free water. Theoretical details and substantiating data have been published (130, 188). Free water is found only in urine hypoosmotic relative to plasma water and is defined as that fraction of the water of hypoosmotic urine which must be subtracted in order to make the urine isoosmotic. It is usually determined as the free-water clearance (C_{H_2O}) in milliliters per minute. It is formed at the two sites in the nephron—the ascending limb of

Henle's loop and the distal tubule—where sodium and an accompanying anion can be reabsorbed without dragging water along with them. The amount of free water generated can be increased by forcing large amounts of isoosmotic urine to flow through the ascending limb and the distal tubule, the diluting sites. This allows more sodium to be abstracted from the urine, and this in turn leaves more free water behind. Thus drugs that block reabsorption of sodium in the proximal tubule should increase C_{H_2O}. Conversely, drugs that block reabsorption of sodium in the ascending limb, the distal tubule, or both, should reduce C_{H_2O}. For elucidation of the site of action of diuretics it is absolutely necessary to study C_{H_2O} under well controlled conditions in which there is no circulating antidiuretic hormone. Otherwise, some of the free water formed will diffuse out of the distal tubule and the collecting duct. By way of contrast, reabsorption of solute-free water ($T^c_{H_2O}$) is maximal only when large amounts of circulating antidiuretic hormone are present. Abstraction of solutes from the ascending limb creates the osmotic force necessary to cause reabsorption of solute-free water from the collecting duct. $T^c_{H_2O}$ is defined as the amount of added water required to bring the osmotic pressure of hyperosmotic urine down to the same level as the osmotic pressure of plasma water. The effects of diuretics on $T^c_{H_2O}$ are parallel to those on C_{H_2O}. A drug that blocks reabsorption of sodium in the proximal tubule should increase the value of $T^c_{H_2O}$, and one that reduces transport of sodium in the ascending limb should lower $T^c_{H_2O}$.

Levitt and Goldstein (103), Porush *et al.* (136), and Farah and Miller (62) have reviewed most of the papers describing effects of mercurials on C_{H_2O}. Suffice it to say that many of the early reports of changes were invalid because the mercurial preparations employed contained theophylline. There were no detectable changes in C_{H_2O} when pure mercurials were used (117, 187). Levitt *et al.* (104) recently showed that organomercurials do not alter either C_{H_2O} or $T^c_{H_2O}$; nor do they influence the capacity of substances that act on the proximal tubule to increase C_{H_2O} and $T^c_{H_2O}$. These data indicate that mercurials act primarily on transport mechanisms located distal to the ascending limb. In contradistinction to these findings, Seldin *et al.* (157) pointed to the ascending limb as the site of action on the basis of clear evidence that mercurials in enormous doses (40 to 80 mg of Hg/kg intravenously followed by an infusion at the rate of 40 to 80 mg of Hg/kg/hr) reduce both $T^c_{H_2O}$ and C_{H_2O}. With doses such as these, it would be amazing if any transport function could remain intact. Blockade of sodium transport all along the tubule will also interfere with the formation of free water and the reabsorption of solute-free water. This difficulty of interpretation is precisely the sort of problem that plagues all studies in which water exchanges along the tubule are used to monitor the flux of electrolytes. The actions of drugs at more than one site will appear as a net effect at a single site. Moreover, some of the underlying assumptions have yet to be validated. For example, changes in renal blood flow and especially in distribution of flow between cortex and medulla will have profound effects on C_{H_2O} and $T^c_{H_2O}$. The assumption that diuretic drugs do not produce such changes is not warranted, for the evidence that many do so is overwhelming (3, 21, 30, 55, 175). Finally, the actual amount of free water formed

within the nephron cannot be measured exactly because some of it is reabsorbed even when antidiuretic hormone is minimal or absent. Diuretics may well influence the reabsorption of water by means of a direct action on tubular permeability or by increasing the osmotic strength of luminal fluid.

The vagaries of indirect methods accentuated the need for data obtained in such a way that they would not be subject to a number of alternative explanations. For this purpose, micropuncture studies seemed to afford the only hope and, in due course, they were carried out. Giebisch (67) established the existence of a transcellular potential difference of -72 mV, the inside of the cell being negative to the surrounding extracellular fluid pool, in the proximal tubule of *Necturus*. In large doses chlormerodrin depressed this potential. Since the potential is probably generated by active transport of electrolytes, the mercurial must have interfered with transport in the proximal tubule. The conditions of Giebisch's experiments, however, do not permit extension of this limited conclusion. Chlormerodrin was injected 24 to 48 hours before the potentials were measured and the concentration of mercury required was in excess of 200 μg/g of kidney, very likely a nephrotoxic amount. In addition, the potential difference was also found to be depressed in ischemic kidneys. Berliner *et al.* (9) and Dirks *et al.* (47) collected luminal fluid from the proximal tubule in the dog and then collected some more from the same puncture site after injection of chlormerodrin or other diuretics. The drug had no effect on the ability of the first two-thirds of the tubule to concentrate inulin (*i.e.*, to reabsorb water); and the reabsorption of water actually increased when urinary losses induced by the diuretic were not replaced. The authors could not rule out the possibility that the diuretics acted on the proximal tubule but stated that, if they did, local compensating adjustments (*e.g.*, an increase in the diameter of the tubule) precluded the chance of an appreciable contribution to the final diuresis. Evidence that the proximal tubule can make adjustments that regulate fractional reabsorption of sodium comes from the work of Bruner *et al.* (18) and Rector and his colleagues (137, 138, 140). They presented a considerable amount of data in support of their model. Its pertinent features are that the proximal tubule dilates when filtration rate is high or when reabsorption of water is depressed; reabsorption of fluid increases in proportion to the square of the tubular radius; and when bulk flow of isoosmotic tubular fluid into cells increases in response to an increase in tubular diameter, the rise in cytoplasmic concentration of sodium stimulates outward transport of sodium. Thus a diuretic acting on the proximal tubule might initiate a series of events that could ultimately oppose and even completely cancel its effect on net sodium transport. If this model is correct, failure to find a change in reabsorption of sodium in the proximal tubule after administration of a mercurial does not prove that the mercurial has no action in this part of the nephron. Thus micropuncture studies are inconclusive unless they yield positive results or simultaneous measurements of tubular diameter are also recorded. Considering some of the inherent problems of the micropuncture method—the inaccessibility of that all-important last third of the tubule in the dog; the large analytical errors; the wide scatter of data—it is easy to understand why application of the technique has not settled the question of site of action in the case of mercurials.

D. Conclusions

All lines of evidence considered, the most reasonable conclusion at this time is that mercurial diuretics block sodium reabsorption at all sites where the ion is actively transported. This concept in no way discounts either the possibility that inhibition of transport at any single locus may provide the bulk of the extra sodium excreted, or the evidence that inhibition of transport in the proximal tubule adds little or no sodium to the diuresis observed because compensatory adjustments intervene. The idea that only a single segment of the nephron is affected by a mercurial imposes requirements for which no evidence can be found. To accept it is to accept also that the sodium-transporting mechanisms of the types of renal cell differ biochemically, or that there are barriers that prevent entry of mercury into certain renal cells. Neither of these hypotheses is acceptable. On the contrary, the pattern of renal distribution of injected mercury and its reactions with protein-bound sulfhydryl groups (both discussed in a later section) support a "multiple site of action" hypothesis. The view that sodium transport mechanisms of all segments of the nephron have certain features in common is easier to accept. Parenthetically, sameness of one function in cells of different parts of the tubule does not imply sameness of all functions. Thus the reason cells of the terminal portion of the proximal tubule are more susceptible to the injurious effects of mercury may be that they actively transport mercury. The unisegmental appearance of the mammalian proximal tubule is no hindrance to this thought. Oliver (127) showed that glucose is absorbed throughout the proximal tubule but is not returned to the blood by the terminal portions. Edwards (52) concluded that the obvious presence of definite segments in the proximal convolution of the renal tubule in fish has been cytologically concealed but functionally retained in the kidney of other vertebrates.

III. PATHWAYS TO RENAL RECEPTORS

A. Glomerular filtration

Mercurials absorbed into the blood are rapidly and extensively bound to plasma protein. The results of experiments *in vitro* led Milnor (118) to predict that more than 90% of the organomercurial meralluride should be bound when diuretic doses are given. The data of Kessler *et al.* (95) upheld this prediction not only for meralluride but for 5 other diuretic and 7 nondiuretic mercurials. There is, in addition, strong evidence that a reactive sulfhydryl group of the albumin molecule is the group to which a mercurial binds (85, 86). On account of its large size (molecular weight about 69,000) only small quantities of albumin can be filtered across glomerular membranes of most species (159). Consequently, few investigators believe mercurial diuretics are filtered in significant amounts, and it has become almost axiomatic that their rapid urinary excretion must be abetted by a specialized renal transport system. Specialized transport notwithstanding, there are reasons for taking issue with the idea that filtration is unimportant. First, all of the mercury cannot be bound and whatever is free is subject to filtration. This being the case, we must ask what is an insignificant amount? Weiner *et al.* (184) have shown that only a minute fraction of the total

dose injected is necessary for sustaining a mercurial diuresis. Second, the time required for onset of action is much less when small quantities of a mercurial are injected into a renal artery than when large doses are injected intravenously (92). The most probable explanation for this observation is that a smaller fraction of a dose injected intraarterially reacts with plasma protein; more is filtered and gets to critical receptor sites more quickly.

Surprisingly, there is little information on glomerular filtration of mercurials. Berlin and Gibson (7) injected $HgCl_2$ into rabbits shortly after ligation of one ureter. Since renal accumulation of mercury was only slightly less than in the normal kidney, they concluded that uptake occurred directly from blood. However, the assumption that glomerular filtration ceases instantly when a ureter is ligated is not warranted. Salomon *et al.* (148, 149) found that glomerular filtration is measurable for many hours after complete ureteral obstruction. Cafruny *et al.* (24) measured excretion of chlormerodrin injected as a complex with a thiolated gelatin (average molecular weight about 100,000), as an albuminate (molecular weight about 69,000), or as the cysteine adduct (freely filterable). The rate of excretion varied inversely with molecular size for 30 to 40 minutes and then proceeded at the same rate for all three complexes. Interestingly, the chlormerodrin-thiogel complex did not produce a diuresis. These workers suggested that the shape of the excretion curves they obtained reflected differences in the amount of mercury cleared through glomerular membranes and that mercurial diuresis may depend on glomerular clearance and subsequent cellular uptake of filtered mercury. However, they could not exclude the possibility that excretion of chlormerodrin is accomplished normally by active secretory transport and that the chlormerodrin-thiogel combination was simply not secretable.

B. Tubular secretion

Lundquist (109) drew attention to the fact that many secreted substances are organic cyclic acids. Since most mercurial diuretics in therapeutic use are organic cyclic acids, mercury clearance might exceed glomerular filtration rate. Weston *et al.* (189) studied the renal clearance and extraction of mercury administered as thiomerin, an acidic mercurial. Renal clearance ranged from one-half to three-fourths of the simultaneously measured glomerular filtration rate. Extensive binding to plasma proteins and renal storage of mercury thwarted this attempt to demonstrate tubular secretion in man. In the dog Borghraef *et al.* (15) noted that the renal extraction of mercury (injected intravenously as chlormerodrin) was equal to filtration fraction. Since the filterable moiety of mercury ranged from 1 to 5% because of plasma binding, they postulated that essentially all of the mercury extracted must have entered tubular cells from peritubular fluid. Weiner *et al.* (182) were the first to show secretion of an acidic mercurial. Their results in the chicken were later verified by Campbell (32–35), who in addition, claimed that chlormerodrin, a neutral compound, was also secreted and that inhibitors of the acid secretory system blocked not only the transport of mercurials but also their diuretic action. Kessler *et al.* (94, 95) had already acquired evidence for secretion of chlormerodrin in the dog but had denied that the acid transport sys-

tem participated. On the basis of their findings in the aglomerular fish, *Lophius americanus*, Cafruny and Gussin (29) postulated that the acid transport system secretes mersalyl but not chlormerodrin. Cafruny *et al.* (24) confirmed the findings of Kessler *et al.* (94) in stop-flow studies but were unable to modify the secretory peak of the mercurial with large doses of probenecid, an inhibitor of organic acid transport. They suggested that chlormerodrin binds so readily to renal protein that it piles up in the cells and subsequently moves passively into urine when the concentration gradient is favorable. Thus, although it appears to be secreted, its transport is not the same as the usual carrier-mediated transport of organic acids. Weiner and Farah (183) pointed out that release of cellular chlormerodrin into urine during stop-flow (as suggested above) would conceal a true effect of probenecid on secretion of the mercurial. However, if release from cells accounts for the secretory peak, there is no reason to postulate that chlormerodrin is handled by the acid transport mechanism. Furthermore, probenecid increases the rate of excretion of chlormerodrin (24).

If acidic mercurials are handled by the acid secretory system, it should be possible to demonstrate the phenomenon of competitive inhibition. Brun *et al.* (17a) and Berliner *et al.* (10) found that mersalyl reduced the capacity of the tubules to transport *p*-aminohippurate in man. Dicker (45) reported that mersalyl also inhibited the transport of diodrast in rats. However, inhibition is not readily demonstrable in the dog (10, 82) possibly because larger amounts of mercury may be required. Moreover, it is not known whether the inhibitory action in susceptible species is entirely competitive or is a reflection of metabolic disturbances, at least in part. The fact that the maximal capacity to reabsorb glucose is depressed by mercurophylline in man (188a) and by other mercurials in the dog (171) favors the latter view, for it is unlikely that mercurials may competitively inhibit transport of both glucose and organic acids.

Letteri and Wesson (102) recently reported that mercaptomerin prevents the depressant action of pentobarbital on the capacity of renal tubules of the dog to transport *p*-aminohippurate into urine. This is an exceedingly interesting but inexplicable finding. It is possible that in the dog the reabsorptive transport system for *p*-aminohippurate is more sensitive to the action of mercurials than is the secretory system.

C. Reabsorption

Only in recent years have there been any efforts to study reabsorptive transport of mercury. Gayer *et al.* (66) located an area where ^{203}Hg was reabsorbed slightly distal to the stop-flow locus of maximal transport of *p*-aminohippurate. They believed this area corresponded to the position of terminal segments of the proximal tubule. Cafruny *et al.* (24) injected chlormerodrin retrogradely through a ureteral catheter and found that chlormerodrin was reabsorbed as the cysteine adduct in the proximal tubule of the dog. Glucose reabsorption occurred in the same area. Probenecid blocked the reabsorptive transport of the mercurial but not that of glucose. Thus movement from urine to plasma appeared to be carrier-mediated. Subsequently, Cho and Cafruny (36) reported that *p*-aminohippurate

was also reabsorbed in the proximal tubule. Recent studies show that mersalyl is handled similarly (37) and that *p*-aminohippurate inhibits the reabsorption of mersalyl (38). It is probable that transport of organic acids across the proximal tubule is bidirectional and that reaction with cysteine enables a mercurial to be reabsorbed, perhaps by an amino-acid-transporting system.

D. Conclusions

Although there are several points to be settled, the following model is consistent with available data and clashes with no important concept: (a) That fraction of a mercurial that is not bound to plasma protein is filterable and enters urine both as a free organic mercurial and as a low-molecular-weight complex with circulating thiols (*e.g.*, cysteinate or glutathionate). (b) Acidic mercurials are transported to and fro across the proximal tubule by the acid-secretory system. (c) Any mercurial may enter renal cells from peritubular spaces. (d) Mercurials combine with amino acids and "ride free" on an amino-acid-reabsorptive system. (e) Diuresis may depend on filtration and reabsorption of an adequate amount of a mercurial. This scheme takes into account many of the major features of the renal actions of mercurial diuretics. It explains why they are apt to injure cells of the proximal tubule, why there is a lag period preceding the onset of diuretic action, and why, as Mudge and Weiner have shown (123), the conversion of Hg from the dichloride to the dicysteinate greatly increases its diuretic potency.

IV. RENAL DISTRIBUTION AND EXCRETION

Knowledge of the distribution and excretion of drugs often provides clues concerning their site and mechanism of action, and expectations were high when the appropriate studies were undertaken in the case of the mercurials. Unfortunately, the data acquired proved to be disappointing. In one sense, however, they have been most useful, for they have undoubtedly prevented the formulation of many a hypothesis based on statistical or numerical correlations that have no biological importance.

In one of the first reports, Borghraef and Pitts (16) made two important observations: There are marked species differences in renal uptake and rate of excretion of mercury, and most of the mercury accumulated in the kidney is found in the cortex, where levels greatly exceed those of plasma or any tissue. By autoradiographic or histological staining methods, many investigators (22, 106, 169, 181) then showed, in a variety of species, that most of the mercury present after administration of many different compounds was located in the cells or cellular membranes of the proximal tubules. Paradoxically, cellular distribution of chlormerodrin is not the same as distribution of other mercurials in the rat. Taugner *et al.* (166) found that after administration of ^{203}Hg-labelled chlormerodrin radioactivity was highest in distal tubules; and Laakso *et al.* (100) confirmed their work. In the dog it is found primarily in the proximal tubule (106). The data of Kessler *et al.* (95, 96), Borghraef *et al.* (15, 16), and Weiner *et al.* (185) form the basis for many of the relationships between mercurial diuresis and the distribution and excretion of mercury. Most notable are the

following points: Diuretic activity cannot be correlated with the concentration of mercury found in renal cortex. After equieffective doses, the concentration of Hg in the cortex after administration of chlormerodrin may be 10 times that after administration of mersalyl. Moreover, concentrations of certain nondiuretic mercurials (*e.g.*, *p*-chloromercuribenzoate) exceed those of mersalyl. Although mercurials that are not readily excreted in the urine possess little or no diuretic activity, there is no temporal relationship between rate of excretion and intensity of diuresis.

Thus it is apparent that there are two types of mercurial attachment to cellular constituents. We may call these A (active) and I (inactive). Reactions of type A inhibit the transport of sodium; reactions of type I produce no measurable pharmacological effects. Since numerically the ratio A/I is not fixed but must vary with dosage and especially from mercurial to mercurial, the total amount of mercury present in renal cortex is not necessarily related to the intensity of diuresis. In fact there is a considerable amount of renal binding before the onset of diuresis (96). Nor does the distribution among the segments of the nephron supply information on site of action. Transport mechanisms account for unequal distribution between segments of the nephron, and for the rapid excretion of the diuretic compounds in use.

The major excretory product of common organic mercurials is the cysteine complex, R-Hg-cysteine (185, 186), but mercuric chloride and certain organic compounds that possess very labile carbon-mercury bonds are excreted as dicysteinates, cysteine-Hg-cysteine (185). Weiner *et al.* (185) converted a nondiuretic compound into an active one by injecting a mixture of the compound with cysteine. Its rate of excretion, normally less than 1% in 3 hours, increased to 7%. Mercurial-monothiol complexes being freely filterable, cysteine probably enhanced filtration of the mercurial and its uptake into renal cells. Since most nondiuretic compounds cannot be converted by the simple expedient of injecting along with a molar excess of a thiol, it is necessary to assume that they form only type I attachments.

Subcellular localization of radiomercury administered as labeled chlormerodrin has been examined by Greif *et al.* (76). In the kidneys of rats and dogs, the highest concentration of mercury was found in the "soluble" fraction, the supernatant fluid from which "nuclear" and "granular" solids had been separated by centrifugation. Mercury was also present in the other two fractions. While these results lead to no suggestions or conclusions concerning the identity of enzyme systems subject to mercurial blockade, they suggest that there is a large number of possible candidates. There is obviously a need for additional studies of subcellular distribution of mercurials that do not bind to renal proteins as extensively and nonspecifically as chlormerodrin.

V. MECHANISM OF ACTION

A. Inactivation of succinic dehydrogenase

A report by Gremels (78) in 1929, that mercurial and xanthine diuretics increased oxygen consumption of the isolated dog kidney, evoked the possibility

that mercurials might affect the activity of one or more enzymes involved in the energetics of cellular metabolism. Most investigators tacitly subscribed to this idea and channeled it into the following hypothesis: Active transport of sodium requires an expenditure of energy. Since the availability of the energy depends on the activity of enzyme systems, mercurials must interfere with or inactivate enzymes. There is nothing profound or exacting in this hypothesis. In fact it would probably have had no value if it were necessary to examine piecemeal the vast number of enzymes with which mercurials could interact. For a decade the idea sat. Then it was refined.

Hildes (64) found that glutathione reversed the antibacterial action of mercuric chloride and that the sulfhydryl group of glutathione was responsible for its protective action. Salle and Ginoza (147) showed that cysteine also had this effect. Peters *et al.* (133) and Waters and Stock (180) reported on the effectiveness of dimercaprol (BAL) as an antidote in poisoning with many heavy metals. Barron and Singer (4a, 158a) inactivated a large number of sulfhydryl-containing enzymes with *p*-chloromercuribenzoate and succeeded in reactivating them by adding compounds that possess free sulfhydryl groups. Thus the stage was set for Long and Farah (107–108) to show that mono- and dithiols reduced the cardiac toxicity of mercurials. The dithiol, BAL, also prevented or stopped mercurial diuresis but monothiols did not do so (49, 61, 80). These reports all furnished additional support for the notion that mercurials inhibit enzymes involved in the maintenance of the ionic reabsorptive processes of renal cells. Furthermore, they implicated only those enzymes that possess free sulfhydryl groups.

Many such enzymes catalyze reactions of both anerobic and oxidative metabolism (4a, 158a). Handley and Lavik (81) began the search by studying the effects of a mercurial on the activity of succinic dehydrogenase, a sulfhydryl-containing enzyme. Substantial inhibition of the enzyme was observed in homogenates of rat renal cortex 1 hour after the administration of 4 to 8 mg of Hg/kg, and also after addition of the diuretic (5×10^{-6} to 5×10^{-5} M) to Warburg flasks containing renal tissue. In cardiac and hepatic succinic dehydrogenase there was comparable inhibition *in vitro* but assayable enzyme was unchanged in the injected animals. Fawaz and Fawaz (63) repeated this work but could not confirm it.

With the development of histochemical staining procedures for qualitative determination of succinic dehydrogenase, a large number of groups began to study the actions of mercurials. Mustakallio and Telkka (124, 125, 168) found that inhibition of the enzyme was almost complete in the ascending limb of Henle's loop. They used 20 to 30 mg of Hg/kg. Wachstein and Meisel (177, 178) noted that inhibition was prominent only in terminal portions of the proximal tubule when the dose of mercury injected was 10 mg/kg. Rennels and Ruskin (142) did not detect any changes on administration of diuretic doses, but large (nondiuretic) doses produced effects after 24 to 48 hours. Using a semiquantitative procedure, Bahn and Longley (2) concluded that no major change in renal succinic dehydrogenase activity occurs during active diuresis. Bickers *et al.* (13)

found no changes in succinic dehydrogenase activity unless definite mercurial nephrotoxic lesions were present. Rodin and Crowson (146) recently presented a convincing account which explains why these many histochemical reports have been controversial. They showed that histologic lesions precede enzymatic changes by at least 1 hour. Mercury initiates cellular destruction. Disintegration of mitochondria then results in loss of enzymatic activity. Since most workers have employed large, necrotizing doses, the evidence for inactivation of succinic dehydrogenase does not withstand inspection.

B. Reaction with protein-bound sulfhydryl groups

The failure of early attempts to correlate the diuretic action of mercurials with inactivation of succinic dehydrogenase did not dampen enthusiasm for sulfhydryl-containing enzymes as the receptor molecules with which mercurials reacted. The extremely strong affinity between mercury and thiols could not be overlooked, and the opportunity to obtain experimental verification of the proposed reaction in renal cells finally arrived. Barrnett and Seligman (4) published a histochemical procedure for staining protein-bound sulfhydryl groups (PBSH). With slight modification, Cafruny *et al.* (26) were able to make quantitative microspectrophotometric measurements of PBSH in the cytoplasm of renal cells. In the rat, diuretic doses of mersalyl reduced sulfhydryl concentration in cells of the terminal, straight portion of the proximal tubule; in the brush borders of these cells; in both limbs of the loop of Henle; and in the collecting ducts. BAL, injected before or after administration of the mercurial, restored sulfhydryl concentration, but the monothiol, cysteine, was ineffective. Mersalyl produced no change in PBSH of cells of the proximal or distal convoluted tubules. Since the diuretic action of mercurials is not impressive in the rat, the work was repeated in the dog. In all essentials, results were the same and a reduction of PBSH correlated well with the occurrence of diuresis (27, 28). The authors concluded that the diuretic effect of mercurials was related to suppression of the activity of sulfhydryl-containing enzymes, and that all sites of reduction of PBSH were sites of action; by exclusion they believed mercurials did not affect reabsorption of sodium in convoluted tubules.

The first conclusion has stood the test of time, but it was not so persuasive when it was first expressed, and the observation that several mercurials, including *p*-chloromercuribenzoate, a potent inhibitor of sulfhydryl enzymes, were nondiuretic (95) almost destroyed it. The case for involvement of PBSH in the production of mercurial diuresis is most compelling when one enumerates the separate pieces of evidence: (a) All mercurials tested to date react with PBSH of renal cells (60). (b) The nondiuretic mercurial, *p*-chloromercuribenzoate, reduces PBSH (60) and also competitively inhibits mercurial diuresis (115, 116). (c) Acidosis potentiates mercurial diuresis and also enhances the reduction of PBSH produced by an organic mercurial whereas alkalosis has opposite effects (56). (d) Many substances that react with sulfhydryl groups possess diuretic properties. The list includes bismuth (114), ethacrynic acid (99, 156), and N-ethylmaleimide (23, 172). (e) Cessation of mercurial diuresis brought about by in-

jections of the dithiol, BAL, is associated with reactivation of PBSH (27, 28) but large doses of the monothiols, cysteine or glutathione, neither retard diuresis nor reactivate PBSH (60). The affinity of mercuric ions for SH groups is so great that only after all its SH groups are saturated will a protein begin to interact with mercury (51).

Although no one of these points is sacred, collectively they comprise an argument that is difficult to refute, especially since there is no opposing evidence. The chief argument against involvement of PBSH is that a relationship between PBSH and active transport of sodium has not been established. That is to say, it is first necessary to show that sulfhydryl enzymes catalyze reactions deemed to be part of a sodium-transporting system. In essence, this is a call for proof, not evidence against a hypothesis. On the same grounds, it would be just as reasonable to deny that acetylcholine is a transmitter substance.

One additional point needs to be emphasized. The hypothesis that PBSH is somehow tied in with the diuretic response to mercurials does not impose the condition that other reactive groups of proteins do not participate. The mercuric ion hypothesis (discussed below) depends on SH groups as anchors for only one valence of mercuric ion, the second valence being free to react with other groups. There is no inconsistency between the two hypotheses.

As evidence for involvement of PBSH began to accrue, the development of counter-evidence against sites of PBSH reduction being the precise or only sites of action of mercurials kept pace. In retrospect, the conclusion was absurd, and it is not in accord with the following points: (a) Autoradiographic studies of ^{203}Hg distribution reveal the presence of mercury in convoluted tubules (6, 106, 166). (b) After the injection in dogs of any one of several mercurials most of the mercury present is found in proximal convoluted tubules (22). (c) There appears to be no active transport of sodium in the descending limb of Henle's loop, a site where mercury reduces PBSH. (d) Recent stop-flow experiments indicate that the distal tubule is a site of action (154). (e) There is a considerable amount of nonspecific binding to PBSH (*i.e.*, attachments of the I type).

The absence of PBSH changes in the convoluted part of the proximal tubule when mercury is obviously present in this region is not a cause for alarm. Rather, the divergence of these observations proclaims the limitations of histochemical techniques. In the convoluted portions, mercury accumulates in the apical and basal membranes of cells (22). To achieve maximal accuracy measurements of PBSH were made in cytoplasm as far from the limiting membranes as possible. Estimates of PBSH in the very broad brush borders of cells of the terminal segment were obtained only with difficulty, but were not even possible to get in the case of the narrow brush borders of the convoluted segment. Since a reduction of PBSH adjacent to or within the membranes of convoluted tubules would not be detectable, it was decidedly improper to exclude any part of the renal tubule as a site of action of mercurial diuretics. No less in error was the assumption that a reduction of PBSH in a given cell type marks that cell as a locus of action.

In spite of their limitations, the PBSH studies have played one of the leading roles in the search for the mechanism of action of mercurials. By pointing to

sulfhydryl receptors, they have influenced and still guide the course of investigation. Yet, because they represent an attack along a broad front, they can afford only a panoramic view. A more circumscribed approach is essential.

C. Inactivation of adenosine triphosphatase (ATPase)

Clues to the identity of a specific enzyme system with which mercurials could interact came not only from studies of PBSH but also from observations on sodium and potassium transport in many tissues. Of paramount importance was the demonstration that cardiac glycosides inhibited electrolyte transport in erythrocytes (72, 91, 153, 162). This provided the impetus for conducting investigations of the effect of glycosides on sodium reabsorption in the kidney and, in due course, the fact that these drugs blocked reabsorption was established (87, 165, 191). Since this effect of glycosides is associated with concomitant inhibition of a sulfhydryl-containing enzyme that splits ATP when there are appropriate amounts of sodium and potassium in the medium containing the enzyme [$Na^+ + K^+$ ATPase, hereafter referred to merely as ATPase] (73, 143), there was a distinct possibility that mercurials also could react with and inactivate ATPase.

Goth *et al.* (74) had considered this possibility even before the relationships outlined above had been recorded. Although they showed that mersalyl or $HgCl_2$ inhibited ATPase, the amounts required were nephrotoxic. Cohen *et al.* (42) and DeGroot *et al.* (43) reported that mersalyl, in diuretic doses, interfered not only with dephosphorylation of ATP but also with its formation in rat kidney. While the work of Taylor (167) and Rendi (141) with preparations of renal ATPase left no doubt that mercurials could inhibit the enzyme *in vitro*, the relevance of these findings to the mechanism of action of mercurials was still open to question.

Jones *et al.* (90) responded to the need for correlative biochemical and pharmacological data by studying the effects of diuretic and nondiuretic mercurials in the rat. Compounds of both classes inhibited ATPase *in vitro* but only diuretic compounds were effective when given by injection. They postulated that, by reacting with the Na^+ and K^+ binding sites of the ATPase, mercurials prevent hydrolysis of ATP formed by means of the phosphoglycerate kinase reaction of cytoplasmic glycolysis. Consequently, the rate of glycolysis is reduced. The capacity to reabsorb sodium also diminishes because cytoplasmic glycolysis furnishes the energy for transport. The work of Jones *et al.* (90) supplies the strongest evidence for involvement of ATPase in the mechanism of action of mercurials, but it also raises many issues. When they failed to show that nondiuretic mercurials possessed activity *in vivo*, they assumed that such compounds did not concentrate at "the critical site of the ATPase system." This conclusion is not warranted, for the nondiuretic compounds they studied do inhibit ATPase *in vitro*. Moreover, one of them (*p*-chloromercuribenzoate) blocks the diuretic action of other mercurials *in vivo* (116). Another point of contention is their inference, based on the lack of correlation observed between pH and mercurial inhibition of ATPase, that the mercuric ion hypothesis is incorrect. A more appropriate conclusion is that failure to obtain a correlation argues for rejection

of ATPase as the active receptor for mercurial diuretics. Finally, it is not certain that the nondiuretic mercurials used are inactive in the rat over a wide range of dosage.

These objections were upheld when Nechay *et al.* (126) discovered that renal microsomal fractions of dogs given chlormerodrin or *p*-chloromercuribenzoate contained equal quantities of mercury. As in the rat both diuretic and nondiuretic mercurials inhibited ATPase *in vitro*. However, there were important differences between the species. In dogs the inhibitory activity of mercaptomerin increased as the pH of the incubating medium was lowered and, most important of all, none of the mercurials affected the activity of ATPase *in vivo*. Nechay *et al.* (126) cautioned that this latter finding does not permit a verdict in the case against ATPase for two reasons: redistribution of mercury may occur while the microsomes are being isolated, and in their experiments diuretic mercurials prevented the diuretic action of ouabain. However, since large amounts of mercury were found in the microsomal fraction, it is unlikely that redistribution could account for the negative results obtained. Nor is it possible to conclude that diuretic mercurials and ouabain must occupy the same renal receptors because each suppresses the diuretic activity of the other. The quantitative response to a diuretic is often inversely related to rate of sodium excretion at the time the diuretic is injected. Thus, for example, if ouabain is given during mercurial diuresis, its effects on sodium excretion could be less pronounced than if it is given alone because renal losses of sodium are certain to cause activation of sodium-retaining mechanisms.

Clearly the case for ATPase is incomplete at this time. The strongest part of it is the simple fact that mercurials can react with the enzyme *in vitro*. This is insufficient to establish cause and effect in the intact animal, and the inability of injected mercurials to alter ATPase activity of renal microsomal preparations in the dog is inauspicious. The data of Kessler and his associates (93, 97) show how difficult it is to attribute the activity of mercurials to an effect on renal metabolic processes. They showed that injection of chlormerodrin into a single renal artery decreases ATP synthesis by the same amount in both kidneys but diuresis occurs only on the injected side. Perhaps more attention should be given to the possibility that mercurials react with a sodium carrier—that any other metabolic disturbances they induce have no impact on sodium reabsorption.

D. Effects on renal transport of electrolytes

Any concept of the diuretic action of mercurials must take into account their highly specific effects on electrolyte excretion. Although renal losses of many substances may be accelerated (135), the only consistent change is an increase in excretion of NaCl. Because mercurials regularly increased urinary concentration of chloride to a greater extent than concentration of sodium (7, 162), it was generally accepted that they interfered primarily with active reabsorption of chloride. This view had to be amended when the electrophysiological studies of Giebisch (68, 69) established beyond reasonable doubt that chloride passively follows sodium, the actively transported partner, across the renal tubule. While it

is possible that a fraction of total chloride reabsorption may be accomplished by means of a chloride "pump" (139), and that mercurials may decrease its capacity, active transport of sodium predominates. Significant also is the fact that certain drugs (*e.g.*, acetazolamide) increase excretion of sodium independently of chloride but there is no way to modify excretion of chloride without effecting a conjoint change in sodium excretion. Since it is unlikely that mercurials alter tubular permeability to chloride (89), most workers agree they inhibit active transport of sodium; and Berliner's (8) solution to the extra chloride output—exchange of a portion of luminal sodium for other cellular cations—is widely accepted.

The chance that mercurials might alter permeability of renal cellular membranes has not been overlooked. Most pertinent are the studies of White *et al.* (190), who occluded ureters of dogs undergoing mannitol diuresis and subsequently injected ^{22}Na into a renal artery. Mercuhydrin increased the specific activity of proximal tubular urine (collected in stop-flow manner) relative to specific activity of plasma. They attributed this effect to a change in passive leak of sodium across the cells from blood to tubular urine, and suggested that the mercurial acted, at least in part, by increasing the permeability of cellular membranes to sodium (190). However, glomerular filtration does not cease entirely during ureteral occlusion, and it is possible that mercuhydrin merely blocked reabsorption of filtered ^{22}Na. Many workers (17, 98, 110, 144) have shown that mercurials can influence movements of electrolytes into and out of renal slices, but there is no way to relate such effects to diuretic activity.

E. Structure-activity analysis

No aspect of the pharmacology of mercurial diuretics has created as much stir as the portion that dealt with structure-activity analysis; and none has furnished as much valuable data. The preamble to all work in this field was the necessary assumption that the mercury atom of an organic molecule is the evocator of activity. This assumption is incontestable, but its converse—the organic fragment has no activity—does not necessarily follow. Moreover, even an inactive organic fragment could control the mode of attachment of mercury to renal receptors and, thereby, the entire diuretic response.

Until 1930 most workers believed that the diuretic potency of organic mercurials was superior to potency of inorganic compounds. Since the evidence for this was not impressive, Sollman and Schreiber (160, 161) began the study which ultimately led to formulation of the "mercuric ion hypothesis." Colloidal, inorganic, or organic mercury was injected into nonedematous patients. Both magnitude and duration of diuresis were measured and related to urinary excretion of mercury. When the compounds were ranked according to the amount of urine produced (in excess of control values) per milligram of mercury excreted, the responses to inorganic compounds far surpassed all others. The effect of mercuric chloride, for example, was about 450 times as great as the effect of mersalyl. On the assumption that a urinary excretion-response relationship is a more logical measure of potency than dose-response, Sollman and Schreiber (161) concluded that the diuretic action of all mercurial compounds may be due to the

liberation of mercuric ions. It is well to remember that this somewhat parochial view of the way to define the potency of mercurials was expressed before adequate data on renal distribution and transport were available. The conclusion may be correct but the original premise is false. The next step in the evolution of the mercuric ion hypothesis was the demonstration of Lehman *et al.* (101) that BAL *in vitro* caused rupture of the carbon-mercury bond of several mercurials. This report prompted Weston *et al.* (189) to suggest that a renal enzyme with two adjacent sulfhydryl groups could also break off and capture a mercuric ion. Thus a rational idea was taking form.

Before it could be tested, however, Kessler *et al.* (95) published results of a structure-activity study which contained many points in favor of an "intact molecule hypothesis." Their major findings were: (a) There was no relation between pattern of distribution or renal concentration and diuretic activity of 13 mercurials. (b) Three mercurial compounds widely used as inhibitors of sulfhydryl enzymes were devoid of diuretic properties. (c) A chain of not less than 3 carbon atoms with mercury attached to the terminal carbon and a hydrophilic group not less than 3 carbons distant from the mercury (R—C—C—C—Hg) was associated with diuretic activity. Kessler *et al.* (95) then proposed that the intact molecule attaches through one valence of mercury and through the hydrophilic group to a double renal receptor (X—R—C—C—C—Hg—X); mercuric chloride is active because it reacts with cysteine in the body and the complex formed (HOOC—C—C—S—Hg) satisfactorily mimics the proposed structural requirements. Kessler and his associates emphasized that their proposal should be considered merely as a working hypothesis; other structures might be compatible with diuretic activity. In their reviews of the chemical literature, Friedman (65) and Sprague (163) did not uncover any irreconcilable exception to this proposed structure-activity relationship.

While the intact molecule hypothesis seemed to be secure, in reality the experimental evidence on which it was founded was scanty. If the rival hypothesis had been abandoned at this point, it would clearly have been a case of default. Fortunately, this did not happen. Mudge and Weiner (123) reissued the mercuric ion hypothesis in 1958. Levy *et al.* (105) later added supporting data and Weiner *et al.* (185) completed the presentation in 1962. Stripped of embellishments, the hypothesis has two focal points. The first is that rupture of the carbon-mercury bond of diuretic mercurials *in vitro* occurs more rapidly in an acidic medium. Biological correlates of this observation—the well known potentiation of the diuretic activity of mercurials during metabolic acidosis (54, 79) and the fact that mercurials with stable carbon-mercury bonds are devoid of diuretic activity (185)—attest to its significance. The second point is that acidosis enhances the activity of mercurials with labile carbon-mercury bonds to a much greater extent than activity of mercuric cysteine (carbon-sulfur bond). Weiner *et al.* (185) examined 32 organic mercurials. All active diuretics were acid-labile; no acid-stable compound was a diuretic. They concluded that the diuretic response is attributable to the intrarenal release of mercuric ions.

One of the virtues of the mercuric ion hypothesis is that it ties together many

heretofore inexplicable observations. Potentiation of the action of organic mercurials by administration of NH_4Cl or NH_4NO_3 (54, 84), by chronic administration of acetazolamide (113), or during hypokalemic alkalosis (181) is associated with low urinary pH; a reduction in response brought about by administration of $NaHCO_3$ (54, 105) or during acute administration of acetazolamide (113) is associated with elevated urinary pH. All may be explained in terms of alterations in renal cellular or urinary acidity. Axelrod and Pitts (1) believed that refractoriness to mercurials in alkalosis was related to low levels of plasma chloride and an associated reduction in concentration of the ion in tubular urine. In some of the conditions mentioned above there is no fixed relationship between plasma concentration of chloride and the diuretic response. Because changes in acid-base balance do influence the response to mercuric cysteine, albeit to a lesser extent than to mercurials with carbon-mercury bonds, Weiner and Farah (183) conceded that filtered chloride load may also control the magnitude of mercurial diuresis. While this is a reasonable assumption, it is not an essential one. An alternative is the possibility that acidosis not only speeds rupture of carbon-mercury bonds but also increases the diuretic response to mercuric ion. If this is true, acid-base balance must necessarily condition responses to all mercurial diuretics, including mercuric cysteine. Quantitatively, mercurials with carbon-mercury bonds would show greater sensitivity because mercury ions would be liberated. In addition, this explanation might account for the observation that acidosis increases renal cortical binding of mercury (measured histochemically) injected as chlormerodrin or $HgCl_2$, but not as *p*-chloromercuribenzoate, which has a stable carbon-mercury bond (22). These data indicate that acidosis influences renal binding of mercuric ion. However, they do conflict with a report that there is no increase of total mercury in the kidney (184). Furthermore, Farah and his associates (56, 60) found that acidosis accentuates the reduction of PBSH induced by organic mercurials but not by $HgCl_2$. Since mercury bound to macromolecules in renal cortex represents only a fraction of the total cellular content and changes in PBSH are not detectable in convoluted tubules, no definitive conclusions can be drawn.

The recent report of Cafruny *et al.* (24) appears to challenge mainstays of the mercuric ion hypothesis. The stable mercurial, *p*-chloromercuribenzoate, produced a small, unilateral diuresis when it was given by retrograde injection through a renal catheter. $HgCl_2$ by this route was approximately twice as potent as the stable mercurial, but the response to chlormerodrin was even larger. On the surface, it seems that chlormerodrin has greater intrinsic activity than mercuric ion. If this were so, it would be a blow to the mercuric ion hypothesis. In actuality, this conclusion is not warranted, for it is clear that potency of mercurials can be assessed properly only when the number of attachements to critical receptors is known. Distributional factors may not be disregarded. Cafruny *et al.* (24) agree with the mercuric ion hypothesis insofar as mercuric ion is held to be the most active form of mercury. They consider intact molecules containing mercury to be much less active and, as has been shown (115, 116), sometimes competitively antagonistic. In this view, *p*-chloromercuribenzoate is a weak

agonist that reacts with a receptor, initiates a short-lived response (not apparent in conventional experiments), and then acts as a competitive antagonist.

In support of the mercuric ion hypothesis, Clarkson *et al.* (41) reported that homogenates of renal tissue of rats injected with chlormerodrin contained free ion and that observed levels of ions correlated well with an increase in urine flow. However, the actual change in urine flow did not begin for at least 24 hours and the effect persisted for almost a week (41). This "late" diuresis may be associated with tubular injury (19). Clarkson and Greenwood (40) noted later that mercuric ion was also released *in vivo* after the injection of *p*-chloromercuribenzoate in the rat. In this instance, excretion of sodium chloride decreased. Significance of these studies is not readily apparent primarily because mercurial diuresis in the rat is unpredictable and has many complex features. It is often associated with a marked elevation of glomerular filtration rate (45). Clarkson's (39) incipient studies in the dog should be most revealing.

Unpublished data of Cafruny *et al.* (25) show that acidosis does in fact release mercuric ion. Mersalyl^{14}C mixed with equal amounts of mersalyl^{203}Hg was injected into the renal artery of alkalotic or acidotic dogs. The average value for the ratio, [mercury as ^{203}Hg/mercury as ^{14}C], was 1.36 in renal cortex removed from six diuresing, acidotic animals but only 1.04 in cortex of six nondiuresing, alkalotic animals. The difference was significant.

F. Conclusions

Mercurial diuretics act primarily on active transport of sodium. The mechanism of action probably involves a firm attachment of mercury to a sulfhydryl group of a renal enzyme that helps to generate energy for sodium transport, or to a sodium carrier. In either case, the transporting system fails. To date, no known enzyme or specific carrier substance has been identified as the receptor for mercurials.

Structure-activity analysis makes it necessary to reject the diuretic structure proposed by Kessler *et al.* (95), for there are too many exceptions to it (185). Although there is no remaining barrier to acceptance of mercuric ion as the most active form of mercury, the intact molecule hypothesis should not be discarded because there is still reason to believe that a single attachment to a receptor through one valence of mercury may also produce a diuresis.

A better understanding of mechanism of action depends on the acquisition of additional information on: (a) the carrier system that transports sodium; (b) the mechanism whereby acidosis potentiates the diuretic action of mercuric cysteine; (c) renal transport and distribution of mercurials, especially diuretic compounds that do not pile up in large amounts (*e.g.*, mersalyl); (d) the identity of the specific renal receptor to which mercurials bind.

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