Kidney International, Vol. 6 (1974), p. 307–322

Vasopressin

RICHARD M. HAYS and SHERMAN D. LEVINE

Division of Nephrology, Albert Einstein College of Medicine, Bronx, New York

Antidiuretic hormone liberated the vertebrates from their aqueous environment, and permitted them to establish themselves on dry land. The combination of sensitive volume and osmoreceptors, a pituitary secretory apparatus which can vary its output from virtually zero to high levels in a short space of time, and receptor cells functioning in the countercurrent system of the renal medulla, has resulted in a water conservation system of great efficiency. The hormone rapidly alters the luminal membrane of receptor cells in the collecting tubule and collecting duct, increasing the permeability of these structures to water.[1] The permeability of the collecting duct to urea and sodium is also increased.

This review will be centered on the sequence of events that follows the attachment of vasopressin to its receptors in the distal nephron. This has become an area of intense activity since the discovery by Sutherland and his colleagues of the central role of cyclic AMP in the action of hormones [2]. The review will cover in brief much of the ground covered by the extensive review of Handler and Orloff [3], emphasizing recent additions to the literature. It is regrettable that the important advances in our understanding of the synthesis and release of antidiuretic hormone cannot be included; the reader is referred to recent symposia and reviews in this area [4–6], as well as in the comparative physiology of water regulation [7] and the countercurrent system [8].

[1] The distal convoluted tubule is usually also included in the segments of the nephron affected by vasopressin. Woodhall and Tisher [1] have questioned this in a recent study of the distal nephron of the rat, in which swelling of the epithelial cells was used as an index of vasopressin action. Swelling was not seen in the distal convoluted tubule, but was apparent along the collecting tubule and collecting duct.

Vasopressin levels in plasma

Release of vasopressin. Release of vasopressin from the pituitary may be triggered by a number of stimuli, the most important of which are plasma hyperosmolality and volume depletion. A role for the renin-angiotensin system in regulation of vasopressin secretion has been suggested [9, 10]. Dunn et al [11] have examined the relative potency of hyperosmolality and volume depletion in stimulating vasopressin release in the rat; they found that small changes in blood osmolality were much more effective than small changes in volume. When large reductions in volume were produced, however, arginine vasopressin (AVP) levels exceeded those produced by hyperosmolality; volume reduction also appeared to render the osmoregulatory system more sensitive to changes in plasma osmolality.

The extent to which circulating vasopressin is bound to plasma proteins is uncertain; Lauson [12], in a review of the studies of binding in human plasma, has concluded that the data are not wholly consistent, but seem to indicate that a minor fraction (perhaps less than one-third) may be bound.

The level of circulating vasopressin in various physiologic states has been a problem of great interest and will be considered in the next section.

Radioimmunoassay of AVP. Early estimates of the circulating level of AVP in man were based primarily on bioassay techniques [13–16]. Development of an accurate radioimmunoassay for AVP has been difficult; however, with refinement of techniques and acquisition of sensitive and specific antisera [17–20], several groups have reported values for plasma AVP under a variety of experimental conditions. The results of two recent studies with an antiserum developed by Glick and co-workers [19] are shown in Table 1 and Fig. 1. While these values must still be interpreted with caution, some tentative conclusions may be drawn. The levels of AVP in human serum,

Table 1. AVP levels as determined by radioimmunoassay

Authors	AVP levels (pg/ml)			
	Pituitary DI[a]	Water diuresis	Basal[b]	Dehydration
Husain et al [19]	undetect.	0.45	3.5 (1.3–7.0)[c]	3.9 (2.5–8.5)
Robertson et al [20]	0.8±0.3[d]	1.4±0.8	2.7±1.4	5.4±3.4

[a] DI = diabetes insipidus
[b] *ad lib* water intake
[c] range
[d] ±1 SD

translated to molar concentrations, are in the range of 10^{-12} to 10^{-11} M, and are somewhat lower than the earlier bioassay values. This discrepancy may be due to technical problems or variation in the physiologic state of the subjects; however, several authors have raised the question of an antidiuretic substance in addition to AVP in human plasma [15, 19, 21]. AVP release in man has a threshold of approximately 280 mOsm/kg. In normal subjects and subjects with nephrogenic diabetes insipidus or primary polydipsia, AVP levels were comparable at any given level of plasma osmolality. AVP levels in patients with pituitary diabetes insipidus were clearly below those of normals; they were still measurable in the studies of Robertson et al ([20], Fig. 1) but were undetectable in the studies of Husain et al ([19], Table 1). Of interest is the question of whether or not AVP levels go to zero with maximal hydration; in both studies shown in Table 1, measurable amounts were still present in normal subjects undergoing water diuresis. A striking increase in AVP levels was found following phlebotomy in sheep and rabbits [19], and following cigarette smoking in humans [18, 19]. In a study in a single normal subject, Arfonad-induced hypotension increased AVP levels sharply [20]. In four subjects with inappropriate ADH secretion, Beardwell [18] found very high AVP levels ranging from 24 to 102 pg/ml. Thus, the radioimmunoassay technique has yielded reasonable values for plasma AVP in a variety of normal and pathologic states.

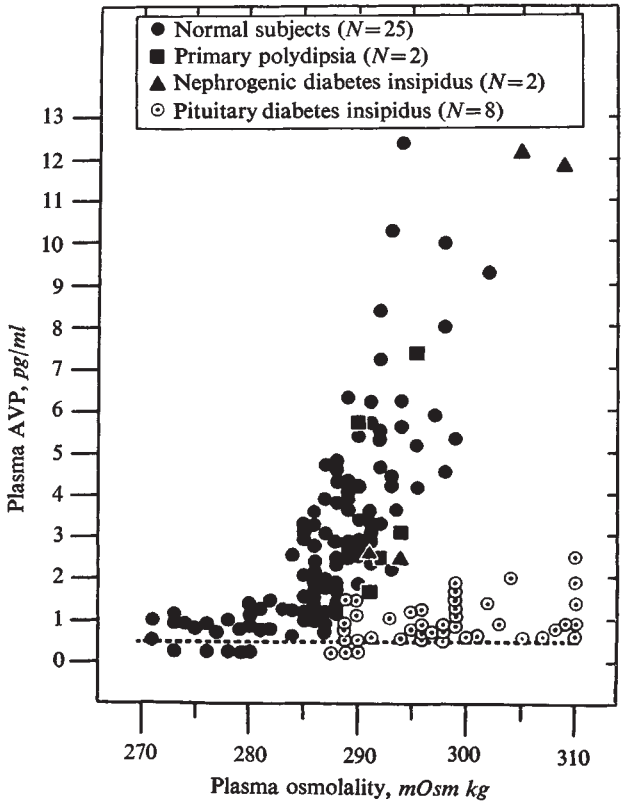

Fig. 1. *The relationship of plasma AVP to plasma osmolality in normal subjects and patients with polyuria of diverse etiologies.* Reprinted from [20].

Binding of neurohypophysial hormones to receptors. Vasopressin and other neurohypophysial hormones bind reversibly to receptors on the basolateral surface of their target cells. Binding to the receptor and activation of adenylate cyclase can be seen with hormone analogues lacking a disulfide bridge [22–24], and it would now appear that the specificity of binding of neurohypophysial hormones depends on the exact three-dimensional "fit" of a particular hormone into its receptor site. Walter and Urry [25] have constructed three-dimensional models of oxytocin and lysine vasopressin in which the molecule is arranged in a rather complex series of turns and foldings (Fig. 2), leaving certain residues free to react with the binding site and others to initiate the hormonal response.

Two recent studies have addressed themselves to the problem of vasopressin binding to "purified" membrane fractions rich in adenylate cyclase. Both employed plasma membranes from pig renal medulla. Bockaert and co-workers [26] carried out a detailed

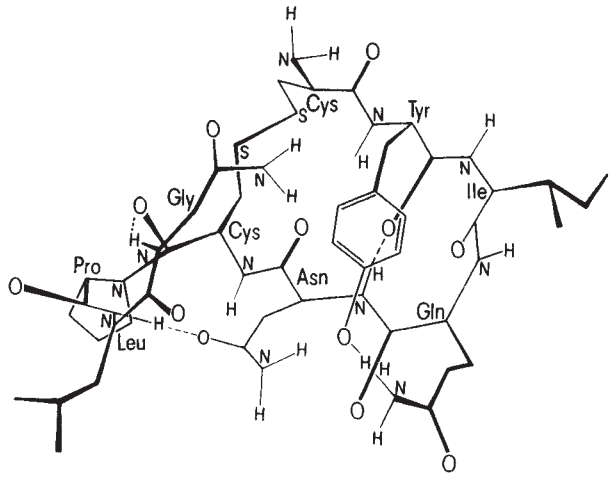

Fig. 2. *Hypothetical model of the biologically active conformation of oxytocin.* Reprinted from WALTER R: *Experientia* 28:959–960, 1972.

kinetic analysis of the binding of tritiated lysine vasopressin to medullary plasma membranes. They found that the saturation of the vasopressin receptors occurred in a dose range identical to that producing dose-dependent activation of adenyl cyclase (10^{-7} to 10^{-10} M). There was a striking similarity between the relative affinities of vasopressin, oxytocin and (*O*-Me) Tyr^2-oxytocin for the binding sites, and the ability of these compounds to activate the enzyme. Vasopressin binding was reversible; when the medium concentration of hormone was lowered by dilution from 10^{-8} to 5×10^{-10} M, virtually all of the hormone initially bound at 10^{-8} M left the receptor sites. The authors noted a delay of approximately 10 min in reaching maximal receptor binding of a given concentration (10^{-8} M) of vasopressin. Maximal activation of the enzyme was similarly delayed, and the authors concluded from the time course of binding and activation that enzyme activation was a function of the number of receptor sites occupied rather than the rate of turnover of the hormonal molecules on the receptor sites. The effect of calcium on the binding of tritiated lysine vasopressin to isolated membranes of pig renal medulla was reported by Campbell, Woodward and Borberg [27]. At concentrations of 1 to 10 mM, calcium inhibited vasopressin binding with complete inhibition at 10 mM. Lower concentrations of calcium (10^{-6} to 10^{-3} M) inhibited both basal and vasopressin-stimulated activity of the core enzyme with complete inhibition at 10^{-3} M calcium. Similar inhibition of adenylate cyclase had been reported by Marumo and Edelman [28]. These findings suggest two points in the vasopressin-adenylate cyclase sequence at which calcium may exert an inhibitory effect: *1*) the receptor site; *2*) the core enzyme.

A number of problems related to membrane fractionation techniques make interpretation of these experiments difficult. For example, the concentration of vasopressin needed to activate adenylate cyclase in the broken cell preparation and the time required for activation are both greater than in the intact animal, where 10^{-11} to 10^{-12} M concentrations of AVP are rapid and effective. It is possible that the sensitivity of the receptor-adenylate cyclase system is reduced by the method of preparation. Further, until more is known about the location of the enzyme in the membrane fragments (for example, whether the enzyme occupies the inner or outer surface of membrane vesicles), it is difficult to relate the millimolar concentrations of calcium used in these experiments to the true levels of calcium that the enzyme is exposed to in the intact cell.

Nevertheless, it is useful to note the following conclusions from these studies even though they require further confirmation: *1*) The binding of vasopressin by membrane receptors appears to be reversible and permits the simplest possible model for water diuresis and antidiuresis. During antidiuresis, when plasma vasopressin levels are high, receptor occupancy is high; when vasopressin levels fall during water diuresis, vasopressin leaves the renal receptor sites. *2*) The ability of calcium to inhibit vasopressin-induced water reabsorption by the kidney [29] and the toad bladder may involve a direct inhibitory action of calcium on adenylate cyclase, and a second action in blocking the attachment of vasopressin to membrane receptors. *3*) Activation of adenylate cyclase may be a function of the total receptor sites occupied by vasopressin, rather than rate of turnover of hormone.

Activation and control of cyclic AMP

Activation of adenylate cyclase. Activation of adenylate cyclase by vasopressin accelerates the production of adenosine 3′,5′ monophosphate (cyclic AMP) from ATP. Cyclic AMP levels within the cell rise as shown in some representative tissues in Table 2, part *A*. The increase is modest (approximately threefold) in toad bladder epithelial cells, and more pronounced in rat inner and outer medulla. It is important to note that the cyclic AMP levels within the cell represent a balance between production by adenylate cyclase and destruction by the enzyme cyclic nucleotide phosphodiesterase. Thus, the concentration of cyclic AMP is far below that which would be achieved if phosphodiesterase were inhibited. In part *B* of Table 2, data are shown for toad bladder epithelial cells incubated in the presence of such an inhibitor of phosphodiesterase: 10 mM theophylline. A more pronounced response to 25 mU/ml vasopressin is apparent. Even higher levels

Table 2. Intracellular cyclic AMP levels

	Vasopressin	
	Absent	Present
A Intact cells		
Toad bladder epith. cells [52][a]	8.9	26.3[b]
Rat inner medulla [42]	3.5	36.8[c]
Rat outer medulla [42]	3.8	11.0[d]
B Intact cells + theophylline		
Toad bladder epith. cells [52]	15.0	81.5[e]

[a] Reference
[b] $\times 10^{-12}$ M/mg protein; 25 mU/ml vasopressin
[c] $\times 10^{-9}$ M/g wet tissue; 50 mU/ml vasopressin
[d] $\times 10^{-9}$ M/g wet tissue; 1 mU/ml vasopressin
[e] $\times 10^{-12}$ M/mg protein; 25 mU/ml vasopressin

of cyclic AMP (approximately 200×10^{-12} M/mg) are found in the theophylline-treated system if higher concentrations of vasopressin are used [30]. From these experiments it is clear that adenyl cyclase is capable of generating far higher levels of cyclic AMP than are seen in the normally functioning cell,[2] and that phosphodiesterase plays a major role in maintaining a low level of cyclic AMP.

Agents other than phosphodiesterase influence cyclic AMP levels: these include calcium, magnesium, prostaglandins, adrenergic agents and adrenal steroids. The actions of these agents will be briefly described; the reader is referred to the review by Handler and Orloff [3] for a more detailed treatment.

Calcium. As already noted, calcium inhibits the hydro-osmotic effect of vasopressin on receptor cells in the kidney and in the toad bladder. Bentley [32] observed that raising the calcium concentration in the solution bathing the serosal (blood) side of the toad bladder significantly reduced osmotic water flow in response to submaximal concentrations of vasopressin; an elevated concentration of magnesium had the same effect. An increased concentration of calcium or magnesium did not inhibit the increase in short-circuit current (active sodium transport), however [33], and it was concluded that two of the effects of vasopressin, on sodium and water movement, could be separated. That conclusion was confirmed by Peterson and Edelman [34], who went on to show that an elevated calcium concentration had no effect on water movement or short-circuit current stimulated by cyclic AMP. This finding placed the site of calcium inhibition of water movement at or before the generation of cyclic AMP. The site could be at the vasopressin receptor, or directly at adenyl cyclase; evidence exists for an inhibitory action of calcium at both sites [27, 28, 35]. These findings have made it necessary to consider at least two independent pathways for the action of vasopressin, the first directed towards osmotic water flow (and probably urea movement) across the cell, the second directed towards the entry of sodium across the luminal membrane. Both actions are mediated by cyclic AMP, but the fact that high concentrations of calcium can dissociate the water and sodium response to vasopressin suggests that there are separate adenyl cyclases, and the pools of cyclic AMP they generate remain separate from each other. There may also be two separate vasopressin receptors, one of which is blocked by calcium.

Prostaglandins. The prostaglandins, a group of naturally occurring long chain fatty acids, exhibit a variety of inhibitory and stimulatory effects on the actions of hormones on receptor cells [36]. In the isolated collecting tubule, prostaglandin E_1 (PGE_1) inhibits the antidiuretic action of vasopressin [37]; a comparable inhibitory effect is seen in the toad bladder [38–40]. Bergstrom [41] had originally proposed that the prostaglandins acted as modulators of adenylate cyclase, and the observation by Grantham and Orloff [37] that PGE_1 inhibited the effect of submaximal concentrations of vasopressin on water flow across the isolated rabbit collecting tubule, but not cyclic AMP-induced water flow, supported Bergstrom's proposal. On the basis of these observations, one would expect PGE_1 to decrease adenyl cyclase activity and cyclic AMP levels in vasopressin-treated cells. This has generally been the case: in homogenates of hamster medulla, for example, 10^{-7} M PGE_1 significantly inhibited adenyl cyclase activity in response to vasopressin [28]. Studies of slices of rat inner and outer renal medulla by Beck et al [42] showed that PGE_1 reduced the response of intracellular cyclic AMP to submaximal concentrations of vasopressin. Studies in the toad bladder failed in two cases [43, 44] to show an inhibitory effect of PGE_1 on adenyl cyclase, and, in a third case [45] showed an inhibitory effect at only one concentration of arginine oxytocin. Omachi et al [30] have recently reported that in intact toad bladder epithelial cells incubated both with and without 10 mM theophylline, PGE_1 produced a greater than 50% inhibition of vasopressin-stimulated cyclic AMP levels. Thus, in amphibian and mammalian receptor cells, there is evidence that PGE_1 exerts its modulating effect on vasopressin at the adenyl cyclase step.

[2] Eggena, Schwartz and Walter [31] have shown that certain analogues of vasopressin can produce a full hydro-osmotic response in the toad bladder, but have only about 30% of the stimulatory effect of arginine vasopressin on adenyl cyclase. Thus, the cyclic AMP produced by arginine vasopressin in excess of that achieved by less potent analogues is not translated into a hydro-osmotic response. This has been termed "receptor reserve."

The "adenylate cyclase step," however, is a broad description that could include the vasopressin receptors themselves, an intermediate step between receptor and enzyme, or an allosteric or substrate binding site in the core enzyme. In addition, there appear to be differences in the effect of PGE_1 on mammalian and nonmammalian receptor cells in the absence of vasopressin. In the isolated, perfused rabbit collecting tubule, PGE_1 (10^{-7} M) produced a slight *increase* in water flow in the absence of vasopressin, in contrast to its inhibitory effect in the vasopressin-treated tubule [37]. In slices of rat outer medulla, high concentrations of PGE_1 (10^{-6} M) produced a sharp rise in cyclic AMP; this effect was not seen in the inner medulla [42]. The fact that PGE_1 can induce a small osmotic flow in the absence of vasopressin, that it increases cyclic AMP production and that it inhibits the more potent effect of vasopressin on water flow, supports the view that in the mammalian tubule vasopressin and PGE_1 may compete for a site at or close to adenylate cyclase [37, 42].

In the toad bladder, PGE_1 has not been shown to increase water flow in the absence of vasopressin [43, 47]. PGE_1 at 2.5×10^{-6} M does increase cyclic AMP levels in the resting cell [30, 45]; this apparently contradictory finding may mean that a cyclic AMP pool unrelated to osmotic water flow but related to some other function, such as active sodium transport, is increased by PGE_1 [46]. (This may also be partly true in the mammalian renal tubule.) The possibility that this cyclic AMP pool in the toad bladder is unrelated to water flow is strengthened by observations in two laboratories that PGE_1 inhibits water flow induced by theophylline [38, 39]. Since theophylline promotes water flow by inhibiting phosphodiesterase, and PGE_1 has no effect on the access of theophylline to phosphodiesterase [45], it appears likely that in the toad bladder, PGE_1 inhibits, rather than stimulates, the adenylate cyclase involved in water flow. Thus, in the toad bladder, the evidence favors a direct, noncompetitive inhibitory action of PGE_1 on adenylate cyclase [31].

Irrespective of its exact mechanism of action, PGE_1 appears to be a modulator of the action of vasopressin. The endogenous production of small amounts of PGE_1 may prevent inappropriate responses of the tubule to small amounts of residual vasopressin [38]. An attempt to demonstrate a regulatory role for endogenous prostaglandins in the toad bladder has not yielded clear-cut results [48], and solution of this important problem may require better techniques for measuring trace amounts of prostaglandins in receptor tissues.

Adrenal steroids. Aldosterone and other adrenal steroids enhance the permeability response of the toad bladder to vasopressin and exogenous cyclic AMP [49–50], under certain experimental conditions. This "permissive effect" of adrenal steroids is seen in the response of both active sodium transport [50–51] and osmotic water flow [50] to vasopressin. Recent studies by Stoff et al [52, 53] have shown that this accelerated response is associated in the case of aldosterone with an increase in intracellular cyclic AMP, and that the elevated cyclic AMP levels, in turn, are the result of steroid-elicited decrease in cyclic AMP degradation by phosphodiesterase. These workers have suggested that aldosterone may initiate the synthesis of a protein inhibitor of phosphodiesterase. A comparable suppression of phosphodiesterase by dexamethasone has been observed in cultured hepatoma cells [54].

The applicability of these findings to human renal physiology is complicated by other effects adrenal hormones have on the tubule, for example, in stimulating sodium transport by the ascending levels of the loop of Henle. However, as suggested by Handler et al [50], the abnormally low free water reabsorption by the kidneys of adrenalectomized animals, and its correction by adrenal steroids [55], may be due in part to the permissive effect of steroids on the permeability response of the tubule to vasopressin.

Adrenergic agents. Adrenergic agents modify water excretion in the mammalian kidney, with beta-adrenergic stimuli producing an antidiuretic effect [56, 57] and alpha-adrenergic agents a diuretic effect [58–60]. Beck and co-workers [61] showed that epinephrine increases both cortical and medullary cyclic AMP in the dog kidney, and that this effect is due to the beta- rather than the alpha-adrenergic action of this drug. Isoproteranol also increased medullary cyclic AMP. In the presence of vasopressin, alpha-adrenergic stimuli decreased the usual stimulus of vasopressin on cyclic AMP. These effects of alpha- and beta-adrenergic agents on medullary cyclic AMP levels were consistent with their effects on the intact kidney. Handler, Bensinger and Orloff [62] have proposed that alpha-adrenergic agents decrease cyclic AMP production by inhibiting adenylate cyclase. Beta agents may stimulate adenylate cyclase, but this was less clearly shown in their studies with the toad bladder.

Beyond Cyclic AMP. What steps lie beyond cyclic AMP, ending with the alteration of the luminal cell membrane? It has been thought that the major step is the activation of a protein kinase by cyclic AMP, with a resultant net transfer of a phosphate from ATP of a protein [63, 64]. This protein might conceivably be part of the membrane itself and play an important role in the permeability change in the membrane. Recent studies by Greengard and his colleagues [65, 66] have indicated that cyclic AMP has an effect

opposite to that predicted from earlier studies. Phosphorylation of a protein (protein D) in intact toad bladder epithelial cells, homogenates and membrane-rich subfractions was decreased rather than increased by vasopressin and cyclic AMP. This appeared to be due to the activation of a membrane-bound phosphatase by cyclic AMP, with the consequent net dephosphorylation of protein D. (The contribution of a kinase in this system could not be ruled out, but appeared to be less significant than that of the phosphatase.) The existence of such a phosphatase had been described by Dousa, Sands and Hechter [67] in studies of bovine kidney. This observation requires confirmation.

Summary. Fig. 3 summarizes in diagrammatic form what is currently believed to be the sequence of steps from the attachment of vasopressin to the alteration in the luminal cell membrane. The points at which calcium, prostaglandin E_1, adrenal steroids and alpha-adrenergic agents are thought to act are indicated.

Alteration of the cell membrane

The final step in the sequence initiated by vasopressin is a marked change in the permeability of the luminal cell membrane. Evidence that this portion of the cell membrane is indeed the barrier transformed by vasopressin has come from studies of frog skin [68], toad bladder [69, 70], rabbit cortical collecting tubule [71, 72] and rat collecting duct [1]. In general, these studies have shown that entry of water or urea across the luminal permeability barrier, as demonstrated by cell swelling or labeling of cell water by isotopic urea, is accelerated by vasopressin, an effect which can be most readily explained by an increase in the permeability of this barrier. It appears unlikely that vasopressin has any effect on the tight junction linking adjacent cells [72].

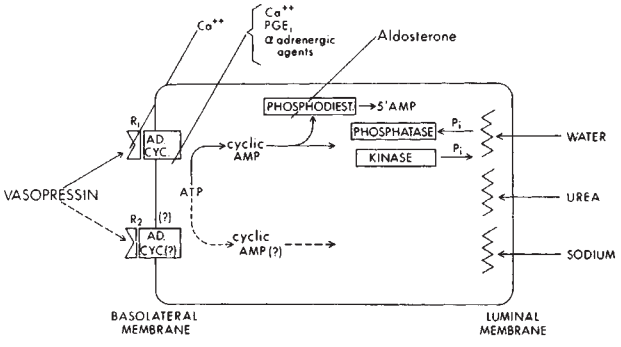

Fig. 3. *Schematic sequence of the steps in the action of vasopressin.* Two receptors (R_1 and R_2) are shown; R_2 and its associated adenylate cyclase are hypothetical, as is the second cyclic AMP pool indicated with dashed lines. A phosphatase, removing phosphate from a protein at or near the luminal membrane, is shown, along with a kinase, which could phosphorylate the membrane. The proposed inhibitory sites of calcium, PGE_1, alpha-adrenergic agents and aldosterone are shown.

The extent to which vasopressin increases the permeability of the luminal membrane is impressive, both in the mammalian collecting tubule and in anuran tissue such as toad bladder and toad skin. Osmotic water flow increases seven times, and urea movement one and one-half times across the rat collecting duct [73] following vasopressin; the increases in water flow and urea movement across the toad bladder are 50-fold and sixfold, respectively [69, 70]. The active transport of sodium is also increased by vasopressin, as well as the movement of a large number of solutes; indeed, it now appears that the effect of vasopressin on permeability is a very broad one involving not only hydrophilic but lipophilic solutes ([74], Levine, Einhorn, Franki and Hays, unpublished observations). In the following section, the nature of the effect of vasopressin on the cell membrane will be considered.

The pore enlargement hypothesis. In 1953, Koefoed-Johnsen and Ussing [75] published their important analysis of water flow across the frog skin. They had noted that while there was a greater than twofold increase in osmotic water flow across the skin following vasopressin, the diffusion rate of isotopically labeled water (the rate at which individual water molecules penetrated the skin) increased by only 10%. This apparent discrepancy between osmotic flow and net flow determined by diffusion could be explained if vasopressin enlarged pores (aqueous channels) in the membrane. Since the rate of osmotic flow through a porous structure would be determined by Poiseuille's law, it would be a function of the fourth power of the pore radius. The rate of diffusion of labeled water, on the other hand, would be a function of pore area, or r^2. Thus, with any increase in pore radius, osmotic flow would increase to a far greater extent than diffusion. This proposal accounted not only for the discrepancy between osmotic and diffusional net flow in the frog skin, but for the even greater apparent discrepancies in other epithelial tissues such as frog gastric mucosa, where the ratio of the osmotic to diffusional coefficients was 25 to 1 [76], and the vasopressin-treated toad bladder, where the ratio was greater than 100 to 1 [69]. Since rather large pore radii of the order of 15 to 40 Å would be required to account for these ratios, one would predict that even large solutes of the size of sucrose would penetrate tissues like the toad bladder more rapidly following vasopressin. Since this was not the case [77], it was necessary to propose that the luminal membrane might include two barriers in series, one a fine diffusion barrier capable of sieving out solutes, and the second a vasopressin-sensitive barrier, which becomes highly porous in the presence

of hormone and is responsible for the Poiseuille flow of water [78, 79]. This appeared to answer the question of how selectivity towards solutes could exist in the presence of large pores.

The pore enlargement hypothesis was supported by another observation: in the presence of net water flow there was an apparent acceleration of solute movement in the direction of the water flow [77, 78]. This "solvent drag" effect would only be seen if water and solutes interacted in aqueous channels, and thus it provided strong evidence for the presence of such channels in cell membranes.

Unstirred layers. An important part of the pore enlargement hypothesis was the small contribution that the process of diffusion appeared to make to total water flow. However, it has been recognized for some time that stagnant (unstirred) layers of water surrounding membranes can impede the rate of diffusion of molecules moving across the membrane [80–83]. Indeed, if the molecule is one that penetrates the membrane phase at a rapid rate (for example, a water molecule) the unstirred layers can provide a resistance to diffusion many times that of the cell membrane itself. If to the unstirred layers in series with the luminal cell membrane one adds the cell cytoplasm, the intercellular spaces and the layer of connective tissue that supports many epithelial structures, it becomes clear that the rate of movement of isotopic water across the luminal membrane may be exceedingly fast, and yet would be masked by the rate-limiting effect of the unstirred layers. This fact is shown diagramatically in Fig. 4.

In studies of the unstirred layer effect in the toad bladder [84, 85] it has been shown that the unstirred layers on either side of the membrane, plus the thick supporting layer, provide a major fraction of the resistance to the diffusion of isotopic water, but virtually no resistance to osmotic flow. The equations used in this estimate are included in references [84–86]. When one factors out the contribution of these extraneous layers, one finds that the diffusion rate of water across the epithelial cell layer increases sharply following vasopressin approximately 20-fold, rather than the 1.7-fold increase thought to take place in earlier experiments, in which the unstirred layer effect was not considered [69]. Thus, the discrepancy between osmotic flow and diffusion is reduced considerably by these studies; a further reduction would be anticipated when additional unstirred layers in the cytoplasm are taken into account (see below).

The conclusion that the rate of diffusion of water across the cell membrane is really very rapid was supported by the studies of Parisi and Piccinni [87], who measured the diffusion rate of tritiated water across the luminal border of the toad bladder epithelial cell over very short time periods. They concluded that uptake was rapid enough to account entirely for total water flow following vasopressin, and that barriers other than the luminal barrier had led to underestimation of the true rate of diffusion across the luminal membrane.

As pointed out by Handler and Orloff [3], the problem of unstirred layers in the renal tubule differs from that in flat epithelial structures such as the toad bladder. The luminal radius of a structure such as the cortical collecting tubule of the rabbit is less than 10 μ, providing little distance for an unstirred layer effect. There is only a thin supporting layer, and the external unstirred layer is thought to be small. Yet there is a large discrepancy between osmotic flow and diffusional net flow following vasopressin. The solution to this problem may lie in the retarding effect of the cell cytoplasm and other cellular structures. Schafer and Andreoli [88] have estimated in the vasopressin-treated isolated rabbit cortical collecting tubule that the diffusional resistance of structures beyond the luminal membrane is 15 to 25 times that of an equivalent thickness (6 μ) of water. Correcting for this "unstirred layer" effect increases the estimated diffusion rate of water across the luminal membrane enough to account for all of the water flow across this segment of the collecting tubule.

Thus, in several types of vasopressin-sensitive epithelia, there is evidence that the diffusion of individual water molecules across the luminal membrane is an exceedingly fast process and may account for the osmotic water flow observed. The studies cited

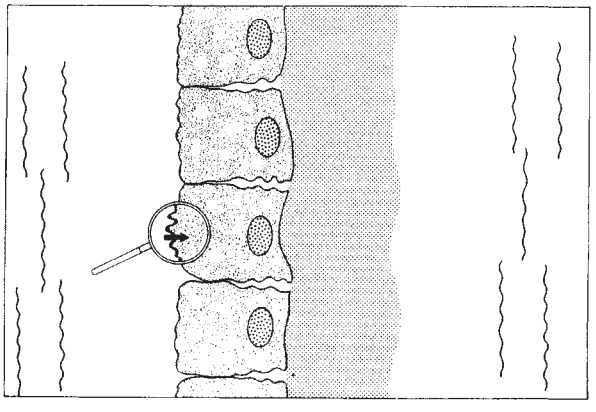

Fig. 4. *Extraneous barriers in an epithelial tissue.* The luminal membrane (under magnifying glass) is the membrane transformed by vasopressin. In series with the membrane are unstirred layers in the bulk solution, cell cytoplasm and organelles, basolateral cell membrane, intercellular channels and the supporting layer (stippled layer). Reprinted from [85].

do not permit us to distinguish between a "pure" diffusion model, in which water dissolves in the membrane more readily following vasopressin, and a model in which vasopressin simply increases the number of small aqueous channels, close to the size of a water molecule. The water in such channels may have an "ice-like" structure, judging from the high activation energy for water diffusion [89].

Independent pathways for water and solutes. The apparent interaction between water and solutes such as urea, as seen in the solvent drag effect, supported the idea that aqueous channels large enough to admit water and solutes were present in the cell membrane. A number of studies, however, raise the question of how extensive the solvent drag effect is, and where the major site of solute–solvent interaction is located in vasopressin-sensitive tissues. In the isolated rabbit cortical collecting tubule, there appears to be a complete dissociation between water and urea movement; water movement increases strikingly following vasopressin, but urea movement is unchanged [90]. In the toad bladder, urea movement was accelerated in the direction of net water flow when the experiments were carried out in conventional diffusion chambers, where bubbling provided the means of stirring the solutions [77]. However, in mechanically stirred chambers, the solvent drag effect on acetamide, an amide closely related to urea, was not apparent [85]. This finding suggests that the apparent solvent drag effect in relatively simple epithelia such as the toad bladder may be the result of the accumulation of isotopically labeled solute in unstirred layers near the luminal membrane, as water flow proceeds from lumen to serosa. Dilution of solute, on the other hand, would occur near the serosal membrane. This asymmetry of solute concentration would produce an asymmetry of flux rates in the two directions; mechanical stirring would tend to reduce solute asymmetry and hence reduce the apparent solvent drag effect. In more complex epithelia such as toad skin the opportunity for solute accumulation, and for water-solute interaction in intercellular channels is greatly increased. Ussing [91] has recently reported that sucrose, a large molecule whose diffusion pathway across the toad skin may be between cells, showed a pronounced solvent drag effect, while glycerol, a small molecule which probably penetrates the cell membrane, showed little or no solvent drag effect following vasopressin. He concluded that the long intercellular pathway afforded a maximum opportunity for solvent drag, while any solvent drag occurring across the cell membrane would be small because of the short path length involved. It seems reasonable to conclude that solute–solvent coupling may be most important in the intercellular channels, and that its existence in the cell membrane is open to question.

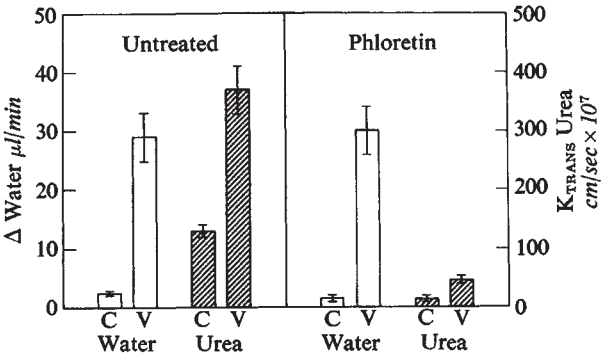

Fig. 5. *The effect of 10^{-4} M phloretin on the osmotic flow of water (open bars) and the movement of urea (hatched bars), in untreated bladders (left-hand panel) and phloretin-treated bladders (right-hand panel).* C, control period (vasopressin absent); V, following vasopressin. Reprinted from [93].

A more clear-cut demonstration of the independence of solute and water movement across the toad bladder was prompted by the observation of Macey and Farmer [92] that phloretin, the aglucone of phlorizin, blocked urea movement into the red cell, but had no effect on the entry of water. This proved to be the case in the toad bladder [93]; in the presence of 10^{-4} M phloretin in the luminal bathing medium, urea movement was markedly inhibited both before and after vasopressin, but water flow was unimpaired (Fig. 5). Identical results were obtained when 10^{-3} M chromate was placed in the luminal bathing medium [94]. Phloretin inhibited the movement of all amides tested, and certain nonamides (thiourea, formaldehyde) as well. Other solutes, such as ethanol and ethylene glycol, whose movement is accelerated by vasopressin, were not blocked by phloretin. Phloretin also blocks urea reabsorption by the dogfish kidney [95].

These findings indicate that many solutes, notably the amides, move across the luminal membrane of the bladder by a pathway which can be experimentally separated from the osmotic flow of water. Recent studies [96] have shown that this pathway has one of the properties of facilitated diffusion: it exhibits saturation at high substrate levels. In the presence of 125 and 150 mM acetamide on both sides of the bladder, for example, the movement of acetamide departs significantly from linearity (Fig. 6), indicating that at some point in the membrane there are a limited number of binding sites or recognition sites for acetamide which become saturated at high substrate concentrations. Other amides, such as methylurea, exhibit this type of self-saturation even more strikingly (LEVINE

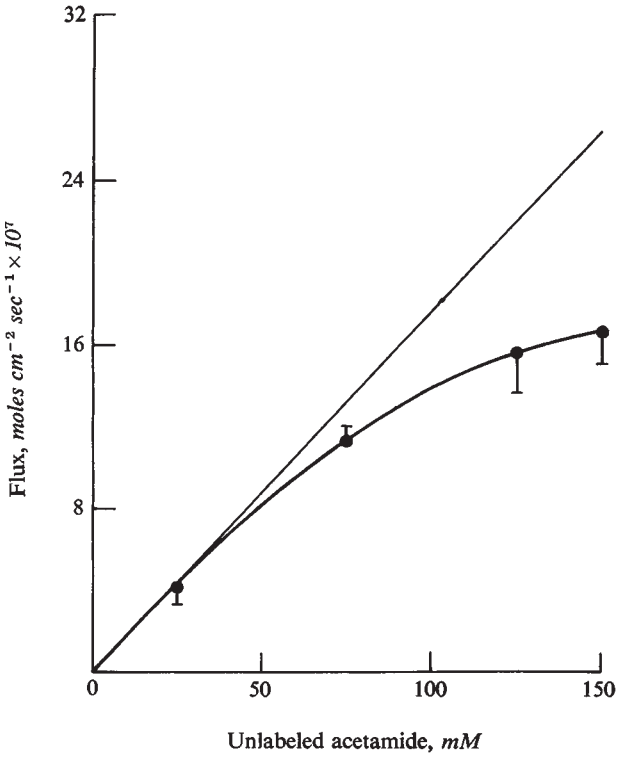

Fig. 6. *Velocity of acetamide movement across the toad bladder in the presence of increasing concentrations of acetamide in both bathing media.* Reprinted from [96].

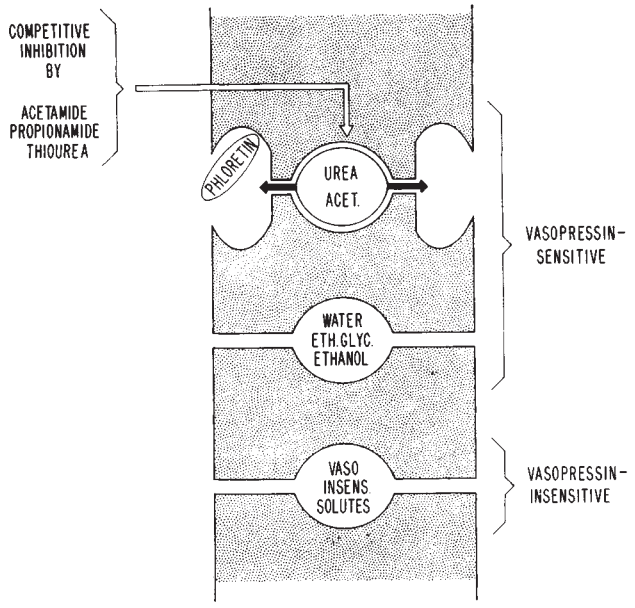

Fig. 7. *Schematic view of proposed pathways for water and solute movement across the toad bladder following vasopressin.* Amides move via a facilitated transport system in which saturation, mutual inhibition by amides and inhibition by phloretin take place. Other vasopressin-sensitive molecules, such as water, ethylene glycol and ethanol, move by one or more independent pathways, unaffected by phloretin. Vasopressin-insensitive solutes may move via additional pathways. Reprinted from [94].

and HAYS, unpublished observations). There is also competition between different amides for binding sites: thus, propionamide and methylurea both inhibit the movement of urea across the bladder. Eggena [97] has recently shown that thiourea inhibits urea movement across the bladder.

Summary. Water and solute movement across the cell membrane in response to vasopressin appears to have the following characteristics: *1*) Water movement across the luminal membrane may take place largely, if not completely, by the process of diffusion. This does not rule out the existence of small continuous aqueous channels, but such channels might be close to the size of a water molecule. *2*) Water movement can be experimentally dissociated from that of many solutes, notably the amides. *3*) Solvent drag may well take place in intercellular channels, but there is no compelling evidence that it takes place in membrane pores. *4*) The movement of amides and possibly other solutes appears to be by facilitated diffusion, and to exhibit saturation. Agents such as phloretin and chromate block this "amide pathway." Not all solutes move via this pathway; their mode of passage is as yet unknown. The proposed pathways are shown diagramatically in Fig. 7.

Alterations observed in the luminal membrane. Up to this point, the case for a vasopressin-induced change in the diffusional properties of the luminal membrane has rested largely on permeability studies. Several articles have appeared recently reporting direct observations on the change in the membrane following vasopressin. Grantham [98] has shown that the physical deformability of the urinary surface of rabbit collecting duct cells is increased following vasopressin. The measurement was made by determining the negative pressure required to suck a hemispherical bulge of the cells' urinary surface into the tip of a micropipette. There was a reduction of approximately 25% in the pressure recorded in the vasopressin-treated tubules. Masur, Holtzman and Walter [99] have reported that the plasma membranes of secretory granules lying below the luminal surface of the toad bladder become continuous with the surface membrane following vasopressin, permitting the granules to discharge their contents, which then may coat the cell surface. This type of exocytosis was apparent two hours after the addition of vasopressin, which raises a question of its relationship to the rapid physiologic action of the hormone; however, it may be necessary to wait until enough granules have attached to the surface to demonstrate this effect. Taylor et al [100] have called

attention to the possible role of microtubules and microfilaments in the action of vasopressin. Several agents which disrupt microtubules or microfilaments, including colchicine, vinblastine and cytochalasin B, inhibit the effect of vasopressin and cyclic AMP on osmotic water flow across the toad bladder. It is possible that microtubules and microfilaments, which were observed by the authors in the microvilli and subplasmalemmal region of the apical surface, exert their effects by releasing secretion granules, by their involvement in cytoplasmic streaming, or in some other fashion. A second possibility is that the agents employed in these studies have a direct action on the cell membrane, rather than on microtubules and microfilaments. Wunderlich, Muller and Speth [101], for example, have proposed a direct action of colchicine on the alveolar membrane of the protozoan *Tetrahymena pyriformis*, in studies using freeze-fracture techniques.

Oral agents in diabetes insipidus[3]

Although exogenous vasopressin is the most frequently utilized treatment for diabetes insipidus, the occurrence of occasional allergic, irritative or vasoconstrictive reactions, and the inconvenience of parenteral administration, makes alternative modes of treatment desirable. Over the past several years, the antidiuretic effects of a number of orally administered agents have become apparent.

Thiazides and other diuretics. Crawford and Kennedy [102] first showed that thiazides significantly reduced urine volume in diabetes insipidus, with an increase in urine volume toward, although not above, isosmotic levels. The antidiuretic effect appears to be the result of mild sodium depletion, with an increase in proximal salt and water reabsorption, and a consequent decrease in the delivery of fluid to the diluting segment of the nephron [103]. Other diuretics, as well as strict salt restriction, can effect or at least preserve a comparable antidiuresis [102, 104, 105]. Because its action does not directly involve vasopressin or the vasopressin-sensitive regions of the nephron, thiazides are the drugs of choice in nephrogenic diabetes insipidus. Their inability to produce a hypertonic urine, as well as their hyperglycemic, hyperuricemic and occasional hematologic side effects make them less than ideal for long-term administration in other forms of diabetes insipidus.

Chlorpropamide and other sulfonylureas. The antidiuretic effect of chlorpropamide was first observed by Arduino, Ferraz and Rodrigues [106] in a patient with idiopathic diabetes insipidus who took the drug mistakenly and noted a striking diminution in urine volume. The antidiuretic effect of chlorpropamide has been confirmed by many investigators in both adults and children with vasopressin-responsive diabetes insipidus [107–115]. It is ineffective in the nephrogenic form of the disease and has produced symptomatic hypoglycemia in a significant percentage of patients [116].

Chlorpropamide potentiates the antidiuretic effect of vasopressin. This was demonstrated in the toad bladder, where chlorpropamide alone had no effect on osmotic water flow but increased the sensitivity of the bladder to submaximal concentrations of vasopressin [117, 118]. This "sensitizing" effect of chlorpropamide was confirmed in studies of rats with hereditary hypothalamic diabetes insipidus [119] and dogs undergoing water diuresis [120]. In both cases, chlorpropamide alone had no antidiuretic effect, but it enhanced the effect of exogenous vasopressin. It was suggested [118] that chlorpropamide exerted its antidiuretic effect in man by sensitizing the renal tubule to the trace amounts of vasopressin that remained in circulation. The observation by Miller and Moses [121] that there was a significant correlation between the ability of patients to respond to chlorpropamide and their ability to respond to water deprivation by reducing free water clearance appeared to support this view.

However, the concept of "peripheral sensitization" did not appear adequate to explain several observations: *1*) chlorpropamide impairs water excretion in water-loaded normal subjects [122] and *2*) dilutional hyponatremia has been reported in patients with diabetes mellitus treated with chlorpropamide [123–126]. If chlorpropamide acted only by increasing peripheral sensitivity, this would imply that water loading does not completely inhibit the release of ADH. This may indeed be the case (see Table 1); however, Miller and Moses [127, 128] have proposed that in addition to its peripheral action, chlorpropamide stimulates the central release of vasopressin. They have found an increase in the urinary excretion of ADH, as determined by radioimmunoassay, in water-loaded patients given chlorpropamide. While these findings require confirmation, ideally with measurements of plasma vasopressin levels, they are of great interest.

With respect to other sulfonylureas, tolbutamide has slight antidiuretic activity, especially if given intravenously [129]; hyponatremia due to tolbutamide has been reported only infrequently [130]. Tolazamide,

[3] While this section deals with the action of the orally administered agents, it should be noted that the drug of choice is probably the ong-acting vasopressin analogue dDAVP [150, 151].

acetohexamide and glyburide, three other antihyperglycemic sulfonylurea compounds, enhance water excretion in water-loaded normal subjects, in contrast to the action of chlorpropamide [131], and glyburide has been reported to cause an increase in polyuria in patients with central diabetes insipidus [132]. Glyburide causes no change in urinary ADH excretion in hydrated normal subjects, and does not alter the excretion of radioactive ADH in the Brattleboro rat with total ADH deficiency [131]. In addition, glyburide does not alter the action of infused ADH in either man or the Brattleboro rat. Presumably, the diuretic action of glyburide may be related to an increase in glomerular filtration rate or a decrease in proximal salt and water reabsorption, increasing delivery to the diluting segments of the nephron. Because of the hazard of water intoxication in patients with cardiovascular disease treated with chlorpropamide for diabetes mellitus, the substitution of other sulfonylureas which are not antidiuretic has been recommended [131].

Cellular mechanism of action of chlorpropamide. As already noted, chlorpropamide in low concentrations (1 to 3×10^{-4} M) has no direct effect on osmotic water flow across the toad bladder, but enhances the effect of submaximal concentrations of vasopressin [117, 118]. Chlorpropamide does not enhance the effect of saturating levels of vasopressin [117, 118]. Chlorpropamide also intensifies the inhibitory action of PGE_1 on vasopressin-induced water flow in the toad bladder [133].[4] These observations, taken alone, suggest that chlorpropamide acts at the adenyl cyclase step, increasing the reactivity of the enzyme to both vasopressin and PGE_1. However, this single mode of action would not account for a number of effects of this agent: *1*) At high concentrations (1 to 7×10^{-3} M) chlorpropamide increases water permeability both in the absence of vasopressin [133, 134] and in the presence of saturating levels of vasopressin [135], a result consistent with a depressive effect of chlorpropamide on phosphodiesterase. *2*) The stimulatory effect of cyclic AMP on water flow when added to the serosal bathing medium is enhanced by mucosal chlorpropamide [135] and diminished by serosal chlorpropamide [117]. Urakabe et al have suggested that a local chlorpropamide-induced decrease in membrane permeability to cyclic AMP might be an additional mechanism of increasing the levels of intracellularly produced cyclic AMP, with a resultant increase in water permeability [134]. *3*) Recent measurements of cellular cyclic AMP levels by Omachi et al [30] have brought all of the foregoing explanations into question. Those authors have found that chlorpropamide added to bladders treated with vasopressin or theophylline does not increase cyclic AMP levels above those measured in the absence of chlorpropamide. On the contrary, in the vasopressin-treated bladders, chlorpropamide treatment caused a small reduction in cyclic AMP levels. It becomes difficult, therefore, to attribute the chlorpropamide-induced increase in water permeability in vasopressin-treated bladders to alterations in cyclic AMP levels, or to assign any single step in the vasopressin-cyclic AMP-membrane sequence as the point of action of chlorpropamide.

Analgesics. Although both aminopyrene [136] and acetaminophen [137] have an antidiuretic action in diabetes insipidus, the clinical usefulness of aminopyrine has been limited by its hematologic toxicity. These analgesics appear to have a dual effect when studied in the toad bladder: *1*) They increase the water permeability of the bladder in the absence of vasopressin, as well as in the presence of both low and saturating levels of vasopressin [133, 138, 139]. In addition, they increase the response of the bladder to exogenous cyclic AMP, suggesting an inhibitory action on phosphodiesterase. Supporting evidence for this hypothesis has been obtained by Lozada et al, although they were able to find inhibition only at high analgesic concentrations [133]. *2*) Like chlorpropamide, the phenylacetamide analgesics intensify the inhibitory action of PGE_1 on vasopressin-induced water flow [133], a finding which might be most easily explained by an action at adenyl cyclase as well.

Clofibrate. Recently, the hypolipidemic agent clofibrate has been found to have an antidiuretic action in patients with diabetes insipidus [140–142]. Clofibrate's mode of action has been clarified by studies of Moses et al [143], who demonstrated a decrease in urinary volume and free water clearance, as well as an increase in urinary ADH excretion, in patients with diabetes insipidus given clofibrate. These findings correlated with the ability of these patients to concentrate their urine and excrete ADH after dehydration. They suggested that clofibrate acts by enhancing the subnormal excretion of ADH in patients with diabetes insipidus, and that residual releasable ADH is necessary for clofibrate to induce an antidiuresis. Clofibrate had no antidiuretic effect in rats with hereditary hypothalamic diabetes insipidus, and did not increase the antidiuretic response of these rats to ADH; in fact, a small diuretic response was seen.

Clofibrate interfered with the ability of both patients and normal subjects to excrete a water load, and prevented the water load from inhibiting ADH excretion maximally in the normal subjects [143]. The

[4] Inhibition of PGE_1 has also been reported [152].

antidiuretic effect of clofibrate thus appears to be limited to increasing ADH release, without augmenting vasopressin's renal action, in contrast to chlorpropamide, which may affect release and renal action. This is consistent with the observation that although clofibrate and chlorpropamide are antidiuretic to similar degrees in normal water-loaded subjects, urinary ADH excretion is twice as great with clofibrate. The lack of apparent renal effect of clofibrate is also consistent with the findings of Shuchter and Hays (unpublished observations), who found no effect of clofibrate on water permeability of the toad urinary bladder either in the presence or the absence of vasopressin.

Antineoplastic drugs. In the past several years, hyponatremia and water intoxication have been reported as untoward side effects of the drugs cyclophosphamide and vincristine. Studies by DeFronzo et al [144] have demonstrated a consistent impairment of water excretion in 13 of 17 patients 4 to 12 hr after intravenous administration of cyclophosphamide in doses of 50 mg/kg or higher, as demonstrated by decreased urine flow, weight gain, hyponatremia and inappropriately concentrated urine. The changes in urinary osmolality showed a close temporal relationship to the appearance of alkylating metabolites of cyclophosphamide in the urine and serum. No significant changes in GFR or urinary sodium excretion were observed. Although the mechanism of this antidiuresis has not been established, the water-loading that these patients frequently undergo to prevent cystitis and uric acid lithiasis makes testing for hyponatremia especially important.

Vincristine neurotoxicity has been implicated in several reports of apparent inappropriate ADH secretion. Most of these occurred in children [145–147] and at least one adult has been reported as well [148]. The patients have responded well to fluid restriction. Robertson, Bhoopalam and Zelkowitz [149] have confirmed by radioimmunoassay the presence of elevated ADH concentrations in this condition.

Acknowledgments

Drs. Joseph Handler and Pedro Cuatrecasas critically reviewed portions of this manuscript. Dr. Hays is a Career Scientist, Health Research Council of the City of New York. Dr. Levine is the Molly Berns Senior Investigator of the New York Heart Association.

Reprint requests to Dr. Richard M. Hays, Department of Medicine, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461, U.S.A.

References

1. Woodhall PB, Tisher CC: Response of the distal tubule and cortical collecting duct to vasopressin in the rat. *J. Clin Invest* 52:3095–3108, 1973
2. Sutherland EW, Rall TW: The relation of adenosine-3′,5′-phosphate and phosphorylase to the actions of catecholamines and other hormones. *Pharmacol Rev* 12:265–299, 1960
3. Handler JS, Orloff J: The mechanism of action of antidiuretic hormone, in *Handbook of Physiology*, Section 8, Renal Physiology, edited by Orloff J, Berliner RW, Washington, Am Physiol Soc, 1973, pp. 791–814
4. Thorn NA: Antidiuretic hormone synthesis, release and action under normal and pathological circumstances, in *Advances in Metabolic Disorders*, edited by Levine R, Luft R, New York, Academic Press, 1970, pp. 39–73
5. Physiology Society Symposium: Control of antidiuretic hormone secretion. *Fed Proc* 30:1376–1394, 1971
6. *Topics in Neuroendocrinology*: vol. 38 of *Progress in Brain Research*, edited by Kappers SA, Schade JP, Amsterdam, Elsevier, 1972
7. Schmidt-Nielsen B, Mackay WC: Comparative physiology of electrolyte and water regulation with emphasis on sodium, potassium, chloride, urea and osmotic pressure, in *Clinical Disorders of Fluid and Electrolyte Metabolism* (2nd ed), edited by Maxwell MH, Kleeman CR, New York, McGraw-Hill, 1972, pp. 45–93
8. Wirz H, Dirix R: Urinary concentration and dilution, in *Handbook of Physiology*, Section 8, Renal Physiology, edited by Orloff J, Berliner RW, Washington, Am Physiol Soc 1973, pp. 415–430
9. Andersson B, Westbye O: Synergistic action of sodium and angiotensin on brain mechanisms controlling fluid balance. *Life Sci, [I]* 9:601–604, 1970
10. Malvin RL: Possible role of the renin-angiotensin system in the regulation of antidiuretic hormone secretion. *Fed Proc* 30:1383–1386, 1971
11. Dunn FL, Brennan TJ, Nelson AE, Robertson GL: The role of blood osmolality and volume in regulating vasopressin secretion in the rat. *J Clin Invest* 52:3212–3219, 1974
12. Lauson HD: Metabolism of the neurohypophysial hormones, in *Handbook of Physiology*, Section 7, Endocrinology, Washington, Am Physiol Soc, 1974, in press
13. Yoshida S, Motohashi H, Ibayahi H, Okinata S: Method for the assay of antidiuretic hormone in plasma with a note on the antidiuretic titer of human plasma. *J Lab Clin Med* 62:279–285, 1963
14. Czaczkes JW, Kleeman CR, Koenig M: Physiologic studies of antidiuretic hormone by its direct measurement in human plasma. *J Clin Invest* 43:1625–1640, 1964
15. Ahmed ABJ, George BC, Gonzalez-Auvert C, Dingman JF: Increased plasma arginine vasopressin in clinical adrenocortical insufficiency and its inhibition by glucosteroids. *J Clin Invest* 46:111–123, 1967
16. Segar WE, Moore WW: The regulation of antidiuretic hormone release in man: I. Effects of change in position and ambient temperature on blood ADH levels. *J Clin Invest* 47:2143–2151, 1968
17. Robertson GL, Klein LA, Roth J, Gordon P: Immunoassay of plasma vasopressin in man. *Proc Natl Acad Sci USA* 66:1298–1305, 1970

18. BEARDWELL CG: Radioimmunoassay of arginine vasopressin in human plasma. *J Clin Endocrinol* 33:254–260, 1971
19. HUSAIN MK, GERNANDO N, SHAPIRO M, KAGAN A, GLICK S: Radioimmunoassay of arginine vasopressin in human plasma. *J. Clin Endocrinol* 37:616–625, 1973
20. ROBERTSON GL, MAHR EA, ATHAR S, SINHA T: Development and clinical application of a new method for the radioimmunoassay of arginine vasopressin in human plasma. *J Clin Invest* 52:2340–2352, 1973
21. PAVEL S: Tentative identification of arginine vasotocin in human cerebrospinal fluid. *J Clin Endocrinol* 31:369–371, 1970
22. RUDINGER J, JOST K: A biologically active analogue of oxytocin not containing a disulfide group. *Experientia* 20:570–571, 1964
23. SCHWARTZ IL, RASMUSSEN H, RUDINGER J: Activity of neurohypophysial analogues lacking a disulfide bridge. *Proc Natl Acad Sci USA* 52:1044–1045, 1964
24. PLISKA V, RUDINGER J, DOUSA T, CORT JH: Oxytocin activity and the integrity of the disulfide bridge. *Am J Physiol* 215:916–920, 1968
25. URRY DW, WALTER R: Proposed conformation of oxytocin in solution. *Proc Natl Acad Sci USA* 68:956–958, 1971
26. BOCKAERT J, ROY C, RAJERISON R, JARD S: Specific binding of ^{3}H lysine-vasopressin to pig kidney plasma membranes. *J Biol Chem* 248:5922–5931, 1973
27. CAMPBELL BJ, WOODWARD G, BORBERG V: Calcium-mediated interactions between the antidiuretic hormone and renal plasma membranes. *J Biol Chem* 247:6167–6175, 1972
28. MARUMO F, EDELMAN IS: Effects of Ca^{++} and prostaglandin E_1 on vasopressin activation of renal adenyl cyclase. *J Clin Invest* 50:1613–1620, 1971
29. EPSTEIN FH: Calcium and the kidney. *Am J Med* 45:700–714, 1968
30. OMACHI RS, ROBBIE DE, HANDLER JS, ORLOFF J: Effects of ADH and other agents on cyclic AMP accumulation in toad bladder epithelium. *Am J Physiol*, 226:1152, 1974
31. EGGENA P, SCHWARTZ IL, WALTER R: Threshold and receptor reserve in the action of neurohypophyseal peptides: A study of synergists and antagonists of the hydroosmotic response of the toad urinary bladder. *J Gen Physiol* 56: 250–271, 1970
32. BENTLEY PJ: The effects of ionic changes on water transfer across the isolated urinary bladder of the toad *Bufo marinus*. *J Endocrinol* 18:327–333, 1959
33. BENTLEY PJ: The effects of vasopressin on the short-circuit current across the wall of the isolated bladder of the toad *Bufo marinus*. *J Endocrinol* 21:161–170, 1960
34. PETERSON MJ, EDELMAN IS: Calcium inhibition of the action of vasopressin on the urinary bladder of the toad. *J Clin Invest* 43:583–594, 1964
35. HYNIE S, SHARP GWG: Adenyl cyclase in the toad bladder. *Biochim Biophys Acta* 230:40–51, 1971
36. RAMWELL PW, SHAW JE: Biological significance of the prostaglandins. *Recent Prog Horm Res* 26:139–187, 1970
37. GRANTHAM JJ, ORLOFF J: Effect of prostaglandin E_1 on the permeability response of the isolated collecting tubule to vasopressin, adenosine 3′,5′-monophosphate and theophylline. *J Clin Invest* 47:1154–1161, 1968
38. ORLOFF J, HANDLER JS, BERGSTROM S: Effect of prostaglandin (PGE_1) on the permeability response of toad bladder to vasopressin, theophylline and adenosine 3′,5′-monophosphate. *Nature* 205:397–398, 1965
39. LIPSON LC, SHARP GWG: Effect of prostaglandin E_1 on sodium transport and osmotic water flow in the toad bladder. *Am J Physiol* 220:1046–1052, 1971
40. LOZADA ES, GOUAUX J, FRANKI N, APPEL GB, HAYS RM: Studies of the mode of action of the sulfonylureas and phenylacetamides in enhancing the effect of vasopressin. *J Clin Endocrinol Metab* 34:704–712, 1972
41. BERGSTROM S: Prostaglandins: Members of a new hormonal system. *Science* 157:382–391, 1967
42. BECK NP, KANEKO T, ZOR U, FIELD JB, DAVIS B: Effect of vasopressin and prostaglandin E_1 on the adenyl cyclase-cyclic 3′,5′-adenosine monophosphate system of the renal medulla of the rat. *J Clin Invest* 50:2461–2465, 1971
43. BAR HP, HECHTER O, SCHWARTZ IL, WALTER R: Neurohypophyseal hormone-sensitive adenyl cyclase of toad urinary bladder. *Proc Natl Acad Sci USA* 67:7–12, 1970
44. WONG PDW, BEDWANI JR, CUTHBERT AW: Hormone action and the levels of cyclic AMP and prostaglandins in toad bladder. *Nature [New Biol]* 238:27–31, 1972
45. LIPSON L, HYNIE S, SHARP G: Effect of prostaglandin E_1 on osmotic water flow and sodium transport in the toad bladder. *Ann NY Acad Sci* 180:261–277, 1971
46. BOURGUET J, MAETZ J: Evidence of independent action of neurohypophyseal peptides on osmotic water flow and active sodium transport in the same target organ: Studies on *Rana esculanta* skin and bladder. *Biochim Biophys Acta* 52:552–565, 1961
47. OZER A, SHARP GWG: Effect of prostaglandins and their inhibitors on osmotic water flow in the toad bladder. *Am J Physiol* 222:674–680, 1972
48. FLORES AGA, SHARP GWG: Endogenous prostaglandins and osmotic water flow in the toad bladder. *Am J Physiol* 233:1392–1397, 1972
49. EGGENA P, SCHWARTZ IL, WALTER R: Action of aldosterone and hypertoxicity on toad bladder permeability to water, in *Regulation of Body Fluid Volumes by the Kidney*, edited by CAT JH, LICHARDUS B, Basel, Karger, 1970, pp. 182–192
50. HANDLER JS, PRESTON AS, ORLOFF J: Effect of adrenal steroid hormones on the response of the toad's urinary bladder to vasopressin. *J Clin Invest* 48:823–833, 1969
51. FANESTIL DD, PORTER GA, EDELMAN IS: Aldosterone stimulation of sodium transport. *Biochim Biophys Acta* 135:74–88, 1967
52. STOFF JS, HANDLER JS, ORLOFF J: The effect of aldosterone on the accumulation of adenosine 3′,5′-cyclic monophosphate in toad bladder epithelial cells in response to vasopressin and theophylline. *Proc Natl Acad Sci USA* 69: 805–808, 1972
53. STOFF JS, HANDLER JS, PRESTON AS, ORLOFF J: The effect of aldosterone on cyclic nucleotide phosphodiesterase activity in the toad urinary bladder. *Life Sci* 13:545–552, 1973
54. MANGANIELLO V, VAUGHAN M: An effect of dexamethasone on adenosine 3′,5′-monophosphate content and adenosine 3′,5′-monophosphate phosphodiesterase activity of cultured hepatoma cells. *J Clin Invest* 51:2763–2767, 1973
55. SIGLER MH, FORREST JN JR, ELKINTON JR: Renal concentrating ability in adrenalectomized rat. *Clin Sci* 28: 29–37, 1965
56. BLOTTING R, FARMER JB, LOCKETT MF: The effect of subcutaneous adrenaline and isoprenaline on the excretion of electrolytes by rats. *Arch Intern Physiol Biochem* 69: 203–212, 1961

57. Lehr D, Mallow J, Krugowski M: Copious drinking and simultaneous inhibition of urine flow elicited by β-adrenergic stimulation and contrary effect of adrenergic stimulation. *J Pharmacol Exp Ther* 158:150–163, 1967
58. Baldwin DS, Gombos EA, Chasis H: Changes in sodium and water excretion induced by epinephrine and norepinephrine in normotensive and hypertensive subjects. *J Lab Clin Med* 61:832–857, 1963
59. Dexter D, Stoner HB: The role of the adrenal medulla in water diuresis in rats. *J Physiol (Lond)* 118:486–499, 1952
60. Liberman B, Klein LA, Kleeman CR: Effect of adrenergic blocking agents on the vasopressin inhibiting action of norpinephrine. *Proc Soc Exp Biol Med* 133:131–134, 1970
61. Beck NP, Reed SW, Murdaugh HV, Davis B: Effect of catecholamines and their interaction with other hormones on cyclic 3′,5′-adenosine monophosphate of the kidney. *J Clin Invest* 51:939–944, 1972
62. Handler JS, Bensinger R, Orloff J: Effect of adrenergic agents on toad bladder response to ADH, 3′,5′-AMP, and theophylline. *Am J Physiol* 215:1024–1031, 1968
63. Kuo JF, Greengard P: Cyclic nucleotide dependent protein kinases: IV. Widespread occurrence of adenosine 3′,5′-monophosphate dependent protein kinase in various tissues and phyla of the animal kingdom. *Proc Natl Acad Sci USA* 64:1349–1355, 1969
64. Jard S, Bastide F: A cyclic AMP-dependent protein kinase from frog bladder epithelial cells. *Biochem Biophys Res Commun* 39:559–566, 1970
65. DeLorenzo RJ, Walton KG, Curran P, Greengard P: Regulation of phosphorylation of a specific protein in toad bladder membrane by antidiuretic hormone and cyclic AMP, and its possible relationship to membrane permeability changes. *Proc Natl Acad Sci USA* 70:880–884, 1973
66. DeLorenzo RJ, Greengard P: Activation by adenosine 3′,5′-monophosphate of a membrane-bound phosphoprotein phosphatase from toad bladder. *Proc Natl Acad Sci USA* 70:1831–1835, 1973
67. Dousa TP, Sands H, Hechter O: Cyclic AMP-dependent reversible phosphorylation of renal medullary plasma membrane proteins. *Endocrinology* 91:757–763, 1972
68. MacRobbie EAC, Ussing HH: Osmotic behavior of the epithelial cells of frog skin. *Acta Physiol Scand* 53:348–365, 1961
69. Hays RM, Leaf A: Studies on the movement of water through the isolated toad bladder and its modification by vasopressin. *J Gen Physiol* 45:905–919, 1962
70. Maffly RH, Hays RM, Lamdin E, Leaf A: The effect of neurohypophysial hormones on the permeability of the toad bladder to urea. *J Clin Invest* 39:630–641, 1960
71. Ganote CE, Grantham JJ, Moses HL, Burg MB, Orloff J: Ultrastructural studies of vasopressin effect on isolated perfused renal collecting tubules of the rabbit. *J Cell Biol* 36:355–367, 1968
72. Grantham JJ, Ganote CF, Burg MB, Orloff J: Paths of transtubular water flow in isolated renal collecting tubules. *J Cell Biol* 41:562–576, 1969
73. Morgan T, Berliner RW: Permeability of the loop of Henle, vasa recta, and collecting duct to water, urea, and sodium. *Am J Physiol* 215:108–115, 1968
74. Pietras RJ, Wright EM: Non-electrolyte probes of membrane structure in ADH-treated toad urinary bladder. *Nature* 247:222–224, 1974
75. Koefoed-Johnsen V, Ussing HH: The contribution of diffusion and flow to the passage of D_2O through living membranes. *Acta Physiol Scand* 28:60–76, 1953
76. Durbin RP, Frank H, Solomon AK: Water flow through frog gastric mucosa. *J Gen Physiol* 39:535, 1956
77. Leaf A, Hays RM: Permeability of the isolated toad bladder to solutes and its modification by vasopressin. *J Gen Physiol* 45:921–932, 1962
78. Andersen B, Ussing HH: Solvent drag on non-electrolytes during osmotic flow through isolated toad skin and its response to antidiuretic hormone. *Acta Physiol Scand* 39:228–239, 1957
79. Lichtenstein NS, Leaf A: Effect of amphotericin B on the permeability of the toad bladder. *J Clin Invest* 44:1328–1342, 1965
80. Teorell T: Discussion in *Trans Faraday Soc* 33:1020–1021, 1937
81. Ginzburg BZ, Katchalsky A: The frictional coefficients of the flows of non-electrolytes through artificial membranes. *J Gen Physiol* 47:403–418, 1963
82. Dainty J: Water relations of plant cells. *Adv Botan Res* 1:279–326, 1963
83. Cass A, Finkelstein A: Water permeability of thin lipid membranes. *J Gen Physiol* 48:543–557, 1965
84. Hays RM, Franki N: The role of water diffusion in the action of vasopressin. *J Membr Biol* 2:263–276, 1970
85. Hays RM: The movement of water across vasopressin-sensitive epithelia, in *Current Topics in Membrane and Transport*, vol. 3, edited by Bronner F, Kleinzeller A, New York, Academic Press, 1970, p. 339
86. Hays RM: A new proposal for the action of vasopressin, based on studies of a complex synthetic membrane. *J Gen Physiol* 51:385–398, 1968
87. Parisi M, Piccinni ZF: The penetration of water into the epithelium of toad urinary bladder and its modification by oxytocin. *J Membr Biol* 12:227–246, 1973
88. Schafer JA, Andreoli T: Cellular constraints to diffusion. The effect of antidiuretic hormone on water flows in isolated mammalian collecting tubules. *J Clin Invest* 51:1264–1278, 1972
89. Hays RM, Franki N, Soberman R: The activation energy for water diffusion across the toad bladder: Evidence against the pore enlargement hypothesis. *J Clin Invest* 50:1016–1018, 1971
90. Burg M, Orloff J: Perfusion of isolated renal tubules, in *Handbook of Physiology*, Section 8, Renal Physiology, edited by Orloff J, Berliner RW, Washington, *Am Physiol Soc*, 1973, pp. 145–159
91. Ussing HH: Effect of ADH in transport paths in toad skin, in *Alfred Benzon Symposium V: Transport Mechanisms in Epithelia*, edited by Ussing HH, Thorn NA, Copenhagen, Munksgaard, 1973, pp. 11–19
92. Macey RI, Farmer REL: Inhibition of water and solute permeability in human red cells. *Biochim Biophys Acta* 211:104–108, 1970
93. Levine S, Franki N, Hays RM: Effect of phloretin on water and solute movement in the toad bladder. *J Clin Invest* 52:1435–1442, 1973
94. Shuchter SH, Franki N, Hays RM: The effect of tanning agents on the permeability of the toad bladder to water and solutes. *J Membr Biol* 14:177–191, 1973
95. Levine SD, Kaplan MA, Franki N, Myers JD, Hays RM: The effect of phloretin on renal urea and sodium transport in *Squalus acanthias*. *Bull Mt Des I Biol Lab* 13:70–71, 1973

96. LEVINE S, FRANKI N, HAYS RM: A saturable, vasopressin sensitive carrier for urea and acetamide in the toad bladder epithelial cell. *J Clin Invest* 52:2083–2086, 1973
97. EGGENA P: Inhibition of vasopressin-stimulated urea transport across the toad bladder by thiourea. *J Clin Invest* 52:2963–2970, 1973
98. GRANTHAM JJ: Vasopressin: Effect on deformability of urinary surface of collecting duct cells. *Science* 168:1093–1095, 1970
99. MASUR SK, HOLTZMAN E, WALTER R: Hormone-stimulated exocytosis in the toad urinary bladder: Some possible implications for turnover of surface membranes. *J Cell Biol* 52:211–219, 1972
100. TAYLOR A, MAMELOK M, REAVEN E, MAFFLY R: Vasopressin: Possible role of microtubules and microfilaments in its action. *Science* 181:347–349, 1973
101. WUNDERLICH F, MULLER R, SPETH V: Direct evidence for a colchicine-induced impairment in the mobility of membrane components. *Science* 182:1136–1138, 1973
102. CRAWFORD JD, KENNEDY GC: Chlorothiazide in diabetes insipidus. *Nature* 183:891–892, 1959
103. EARLEY LE, ORLOFF J: The mechanism of antidiuresis associated with the administration of hydrochlorothiazide to patients with vasopressin-resistant diabetes insipidus. *J Clin Invest* 41:1988–1997, 1962
104. KLEEMAN CR, CUTLER R: The neurohypophysis. *Ann Rev Physiol* 25:385–432, 1963
105. CUTLER R, KLEEMAN CR, MAXWELL MH, DOWLING JT: Physiologic studies in nephrogenic diabetes insipidus. *J Clin Endocrinol* 22:827–838, 1962
106. ARDUINO F, FERRAZ FPJ, RODRIGUES J: Antidiuretic action of chlorpropamide in idiopathic diabetes insipidus. *J Clin Endocrinol Metab* 26:1325–1328, 1966
107. EHRLICH RM, KOOH SW: Oral chlorpropamide in diabetes insipidus. *Lancet* 1:890, 1969
108. MENDERS AE, TOUBER JL, DEVRIES LA: Chlorpropramide treatment in diabetes insipidus. *Lancet* 2:544–546, 1967
109. KUNSTADTER RH, CABANA EC, OH W: Treatment of vasopressin-sensitive diabetes insipidus with chlorpropamide. *Am J Dis Child* 117:436–441, 1969
110. FROYSHOV I, HAUGEN HN: Chlorpropamide treatment in diabetes insipidus. *Acta Med Scand* 183:397–400, 1968
111. REFORZO-MEMBRIVES J, MOLEDO LI, LANARO AE, MEGIAS A: Antidiuretic effect of 1-propyl-3-*p*-chlorobenzenesulfonylurea (chlorpropamide). *J Clin Endocrinol Metab* 28:332–336, 1968
112. EHRLICH RM, KOOH SW. The use of chlorpropamide in diabetes insipidus in children. *Pediatrics* 45:236–245, 1970
113. VALLET HL, PRASAD M, GOLDBLOOM RB: Chlorpropamide treatment of diabetes insipidus in children. *Pediatrics* 45:246–253, 1970
114. HOCKEN AG, LONGSON D: Reduction of free water clearance by chlorpropamide. *Br Med J* 1:355–356, 1968
115. DRIEDGER AA, LINTON AL: Familial ADH-responsive diabetes insipidus: Response to thiazides and chlorpropamide. *Can Med Assoc J* 109:594–597, 1973
116. WEBSTER B, BAIN J: Antidiuretic effect and complications of chlorpropamide therapy in diabetes insipidus. *J Clin Endocrinol Metab* 30:215–227, 1970
117. MENDOZA S: Effect of chlorpropamide on the permeability of the urinary bladder of the toad and the response to vasopressin, adenosine 3′,5′-monophosphate and theophylline. *Endocinology* 84:411–416, 1969
118. INGELFINGER JR, HAYS RM: Evidence that chlorpropamide and vasopressin share a common site of action. *J Clin Endocrinol Metab* 29:738–740, 1969
119. BERNDT WO, MILLER M, KETTYLE WM, VOLTIN H: Potentiation of the antidiuretic action of vasopressin by chlorpropamide. *Endocrinology* 86:1028–1032, 1970
120. ZWEIG SM, ETTINGER B, EARLEY LM: Mechanism of antidiuretic action of chlorpropamide in the mammalian kidney. *Am J Physiol* 221:911–915, 1971
121. MILLER M, MOSES AM: Potentiation of vasopressin action by chlorpropamide in vivo. *Endocrinology* 86:1024–1027, 1970
122. MILLER M, MOSES AM: Mechanism of chlorpropamide action in diabetes insipidus. *J Clin Endocrinol Metab* 30:488–496, 1970
123. GARCIA M, MILLER M, MOSES AM: Chlorpropamide-induced water retention in patients with diabetes mellitus. *Ann Intern Med* 75:549–554, 1971
124. FINE D, SHEDROVILSKY H: Hyponatremia due to chlorpropamide: A syndrome resembling inappropriate secretion of antidiuretic hormone. *Ann Intern Med* 72:83–87, 1970
125. HAGEN GA, FRAWLEY TF: Hyponatremia due to sulfonylurea compounds. *J Clin Endocrinol Metab* 31:570–575, 1970
126. WEISSMAN PN, SHENKMAN L, GREGERMAN RI: Chlorpropamide hyponatremia: Drug-induced inappropriate antidiuretic-hormone activity. *N Engl J Med* 284:65–71, 1971
127. MILLER M, MOSES AM: Radioimmunoassay of urinary antidiuretic hormone in man: Response to water load and dehydration in normal subjects. *J Clin Endocrinol Metab* 34:537–545, 1972
128. MOSES AM, NEUMANN P, MILLER M: Mechanism of chlorpropamide-induced antidiuresis in man: Evidence for release of ADH and enhancement of peripheral action. *Metabolism* 22:59–65, 1973
129. LUETHI A, STUDER H: Antidiuretic action of chlorpropamide and tolbutamide. *Minn Med* 52:33–36, 1969
130. HAGEN GA, FRAWLEY TF: Hyponatremia due to sulfonylurea compounds. *J Clin Endocrinol Metab* 31:570–575, 1970
131. MOSES AM, HOWANITZ J, MILLER M: Diuretic action of three sulfonylurea drugs. *Ann Intern Med* 78:541–544, 1973
132. RADO JP, BORBELY L: Glybenclamide enhancement of polyuria in patients with pituitary diabetes insipidus. *Endokrinologie* 59:397–402, 1972
133. LOZADA ES, GOUAUX J, FRANKI N, APPEL GB, HAYS RM: Studies of the mode of action of the sulfonylureas and phenylacetamides in enhancing the effect of vasopressin. *J Clin Endocrinol* 34:704–712, 1972
134. URAKABE S, SHIRAI D, ANDO A, TAKAMITSU Y, ORITA Y, ABE H: Effect of the sulfonylureas on the permeability to water and electrical properties of the urinary bladder of the toad. *Jap Circ J* 34:595–601, 1970
135. URAKABE S, SHIRAI D: Effect of vasopressin, cyclic 3′,5′-AMP, and chlorpropamide on water permeability of toad urinary bladder. *Med J Osaka Univ* 21:151–159, 1971
136. DEGOWIN EL: The urinary concentration in diabetes insipidus: A comparison of the effects of several drugs. *Am J Med Sci* 190:747–756, 1935
137. NUSYNOWITZ ML, FORSHAM PH: The antidiuretic action of acetaminophen. *Am J Med Sci* 252:429–435, 1966
138. NUSYNOWITZ ML, WAGRINKA LC, BOWER BF, FORSHAM PH: Effect on vasopressin action of analgesic drugs *in vitro*. *Am J Med Sci* 252:424–428, 1966

139. Shirai D, Urakabe S, Ando A, Takamitsu Y, Orita Y, Abe H: Effect of urea derivatives and analgetics on the permeability to water and electrical properties of the urinary bladder of the toad. *Jap Circ J* 34:603–608, 1970

140. Uhlich E, Loeschke K, Eigler J, Holbach R: Clofibrat bei diabetes insipidus. *Klin Wochenschr* 49:436–437, 1971

141. DeGennes LJ, Bertrand C, Bigorie B, Truffert J: Études preliminaires de l'action antidiuretique du clofibrate (ou atromid S) dans le diabete insipide pitressosensible. *Ann Endocrinol* 31:300–308, 1970

142. DeGennes JL, DesBois JC, Marie J: Étude therapeutique du clofibrate au cours des diabetes insipides pitressosensibles de l'enfant. *Ann Pediatr (Paris)* 17:754–759, 1970

143. Moses AM, Howantiz J, Van Gemert M, Miller M: Clofibrate-induced intidiuresis. *J Clin Invest* 53:535–542, 1973

144. DeFronzo RA, Braine H, Colvin M, Davis PJ: Water intoxication in man after cyclophosphamide therapy. *Ann Intern Med* 78:861–869, 1973

145. Fine RN, Clark RR, Shore NA: Hyponatremia and vincristine therapy—syndrome possibly resulting from inappropriate antidiuretic hormone secretion. *Am J Dis Child* 112:256–259, 1966

146. Haggard ME, Fernback DJ, Holcomb TM, Sutow WW, Vietti TJ, Windmiller J: Vincristine in acute leukemia of childhood. *Cancer* 22:438–444, 1968

147. Slater LM, Warner RA, Serpick AA: Vincristine neurotoxicity with hyponatremia. *Cancer* 23:122–125, 1969

148. Cutting HO: Inappropriate secretion of antidiuretic hormone secondary to vincristine therapy. *Am J Med* 51: 269–271, 1971

149. Robertson GL, Bhoopalam N, Zelkowitz LJ: Vincristine neurotoxicity and abnormal secretion of antidiuretic hormone. *Arch Intern Med* 132:717–719, 1973.

150. Vavra I, MacHova A, Holecek V. Cort JH, Zaoral M, Sorm F: Effect of a synthetic analogue of vasopressin in animals and in patients with diabetes insipidus. *Lancet* 1:948–952, 1968

151. Edwards CRW, Kitau MJ, Chard T, Besser GM: Vasopressin analogue dDAVP in diabetes insipidus: Clinical and laboratory studies. *Br Med J* 3:375–378, 1973

152. Ozer A, Sharp GWG: Modulation of adenyl cyclase action in toad bladder by chlorpropamide: Antagonism to prostaglandin E_1 *Eur J Pharmacol* 22: 227–232, 1973