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GFR and the concentration of urine in the absence of vasopressin. Berliner-Davidson re-explored

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Robert W. Berliner made many important contributions to our understanding of the urinary concentrating mechanism. Among these, one must number his demonstration that urine can be rendered hyperosmotic to plasma even when vasopressin is absent, as well as his definition of the role of the glomerular filtration rate (GFR) in the concentrating process [1, 2]—points that had also been suggested by several other investigators [reviewed in 3, 4]. In postulating how urine might be concentrated without vasopressin purely by changes occurring within the kidney [1], Dr. Berliner expressed the essence of a message, which we still tend to overlook today, namely, that ". . . although it is commonly stated that the function of ADH is to cause the excretion of a hypertonic urine, its more important function might be better defined as preventing the excretion of a dilute urine."

The availability of the Brattleboro rat, which has an inherited deficiency for synthesizing vasopressin [5], made it possible to further explore the mechanisms by which urine can be concentrated in the apparent absence of the hormone. We here review these experiments, paying special attention to the role of GFR in the process.

Concentration of urine by Brattleboro homozygotes

Shortly after the discovery of the Brattleboro rat, we demonstrated its remarkable ability to concentrate urine when it was deprived of drinking water—to an osmolality of nearly 1,000 mOsm/kg H_2O after 24 hours of dehydration [6, 7].[1] This degree of urinary concentration was achieved at a time when the interstitial osmolality at the tip of the inner medulla was still significantly higher than the concurrent urinary osmolality (Fig. 1C, [6])—that is, when there was not osmotic equilibration between collecting duct fluid and the surrounding interstitium. This fact suggested that an increase in the water permeability of the collecting duct system (defined as the connecting tubule and the entire collecting duct) might not be a major part of the process, and we therefore invoked the Berliner–Davidson hypothesis [1] as a likely explanation. Inasmuch as that hypothesis begins with a decrease in GFR, it seemed important to learn what happens to GFR as Brattleboro homozygotes are deprived of drinking fluid. Some thirteen years passed before we were able to obtain that information by direct measurement [3], for we felt that the requisite experiment had to be performed in unanesthetized animals.

GFR during dehydration of Brattleboro homozygotes

A moment's reflection will show why the time course for changes in GFR and urine osmolality (Uosm) during dehydration is best determined in conscious rats. Aside from the interfering effects of anesthesia [11], which stimulates vasoactive systems [12], it would be difficult or impossible to maintain a viable preparation under continuous anesthesia for 24 hours or longer. The alternative, of examining different rats dehydrated for varying periods, would be hampered both by variability among animals and by the ultimate need for acute anesthesia and surgical preparation (with variable and unknown fluid losses) in order to measure clearances. (The reliability of the endogenous creatinine clearance as a measure of GFR in rats is doubtful, especially under conditions of rather severe volume contraction.) We therefore conducted these experiments in what we call the trained, conscious, chronically catheterized rat [3, 13], which permits serial measurements of renal clearances in the same, conscious animal.

The results are shown in Figure 1. There was a continuous loss of body weight, which amounted to approximately 20% at 24 hours of fluid deprivation (Fig. 1A). This volume contraction was accompanied by a steady rise in plasma osmolality to a high level, approximately 415 mOsm/kg H_2O. Urine osmolality rose to a mean of 700 mOsm/kg H_2O at 24 hours (Fig. 1B), and concurrently, GFR decreased strikingly by approximately 70%. The mirror images of the two curves in Figure 1B might suggest that the decline in GFR is the cause of the rise in Uosm. Yet, we found in subsequent experiments (to be described; [4, 14]) that the urine can be concentrated to a similar degree during the first three hours, without a change in GFR. Figure 1C shows that the urine was concentrated during dehydration without full osmotic equilibration between collecting duct fluid and the interstitium, and hence presumably in the absence of a maximal increase in water permeability of the collecting duct system. The apparently greater degree of equilibration as dehydration progresses might be ascribed to a greater papillary interstitial osmolality

[1] Although there have been reports of localized production of "vasopressin" in Brattleboro homozygotes [8, 9], it is probably safe to assume that circulating concentrations of the hormone are ineffective or zero, since its plasma and urinary concentrations remain below detectable limits even during dehydration [10].

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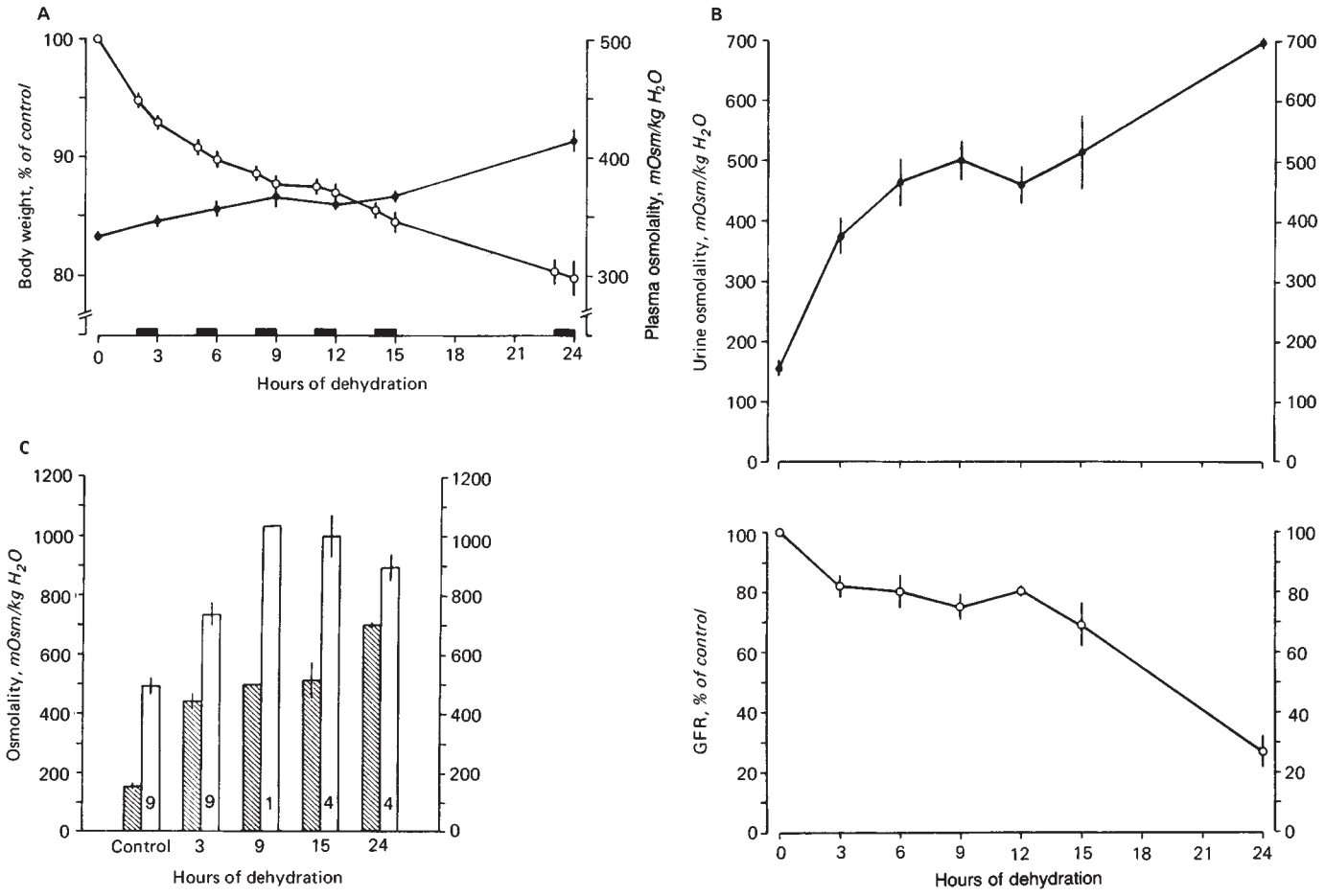

Fig. 1. *Serial changes during continuous withdrawal of drinking fluid for 24 hours in Brattleboro homozygous rats.* All values are means ± SEM in up to four females and six males. **A.** Changes in body weight (○–○) and plasma osmolality (●–●). Values at time 0 are means of three control periods before fluid deprivation was begun. Solid rectangles along the abscissa denote periods when the animals were in the restraining cage for measurement of renal clearances; during the rest of the time each rat was in its regular cage. **B.** Concurrent changes in urine osmolality and GFR. The decline in GFR during the first three hours of dehydration was not observed consistently in subsequent experiments [4, 14]. **C.** Interstitial osmolality at the tip of the inner medulla and concurrent urine osmolality at varying stages of dehydration. Although the degree of osmotic disequilibration tends to diminish with advancing dehydration, the difference between the two osmolalities is still statistically significant at 24 hours ($P < 0.01$). Number of rats studied at each time period is shown in the open columns. Published with permission from [3]. Symbols are: (▨) urine; (□) papilla.

(due mainly to accumulation of urea; [6, 15]), as well as to a lesser tubular flow rate. As discussed below, however, the possibility that there was some increase in water permeability has not been fully excluded.

In order to ascertain whether the decrease in GFR shown in Figure 1B is the explanation for the rise in urine osmolality, we in effect repeated the Berliner–Davidson experiments in Brattleboro homozygotes [4]. The results are shown in Figure 2.

Again, these experiments are conducted in trained, chronically catheterized, conscious rats in order to avoid the confounding influences of anesthesia and acute surgical preparation [11, 12]. Stable, partial constriction of the aorta above both renal arteries for three hours was effected by means of a specially constructed, inflatable cuff [4, 13]. Figure 2A shows that, while the mean GFR decreased slightly and significantly during aortic constriction, in some animals it barely changed at all, and in several it actually increased. Yet, in every instance, urinary osmolality increased rather markedly. From these findings, we drew the conclusion that a decrease in GFR is not essential to concentrating the urine in the absence of vasopressin, even to levels hyperosmotic to plasma. That conclusion, which was borne out in subsequent experiments [14] to be described below (Fig. 3D), is also supported by Figure 2B, which shows a lack of correlation between changes in GFR and those in urinary osmolality under the conditions of these experiments.

Although most people tend to associate the Berliner–Davidson mechanism with a decrease in GFR, it was Dr. Berliner himself who stressed that the essence of the hypothesis is the decreased delivery of tubular fluid to the diluting segment of the nephron [1]. Such decreased delivery might come about, even when GFR has not changed, through an increase in the filtration fraction and hence an increase of the oncotic pressure within peritubular capillaries, which would enhance proximal reab-

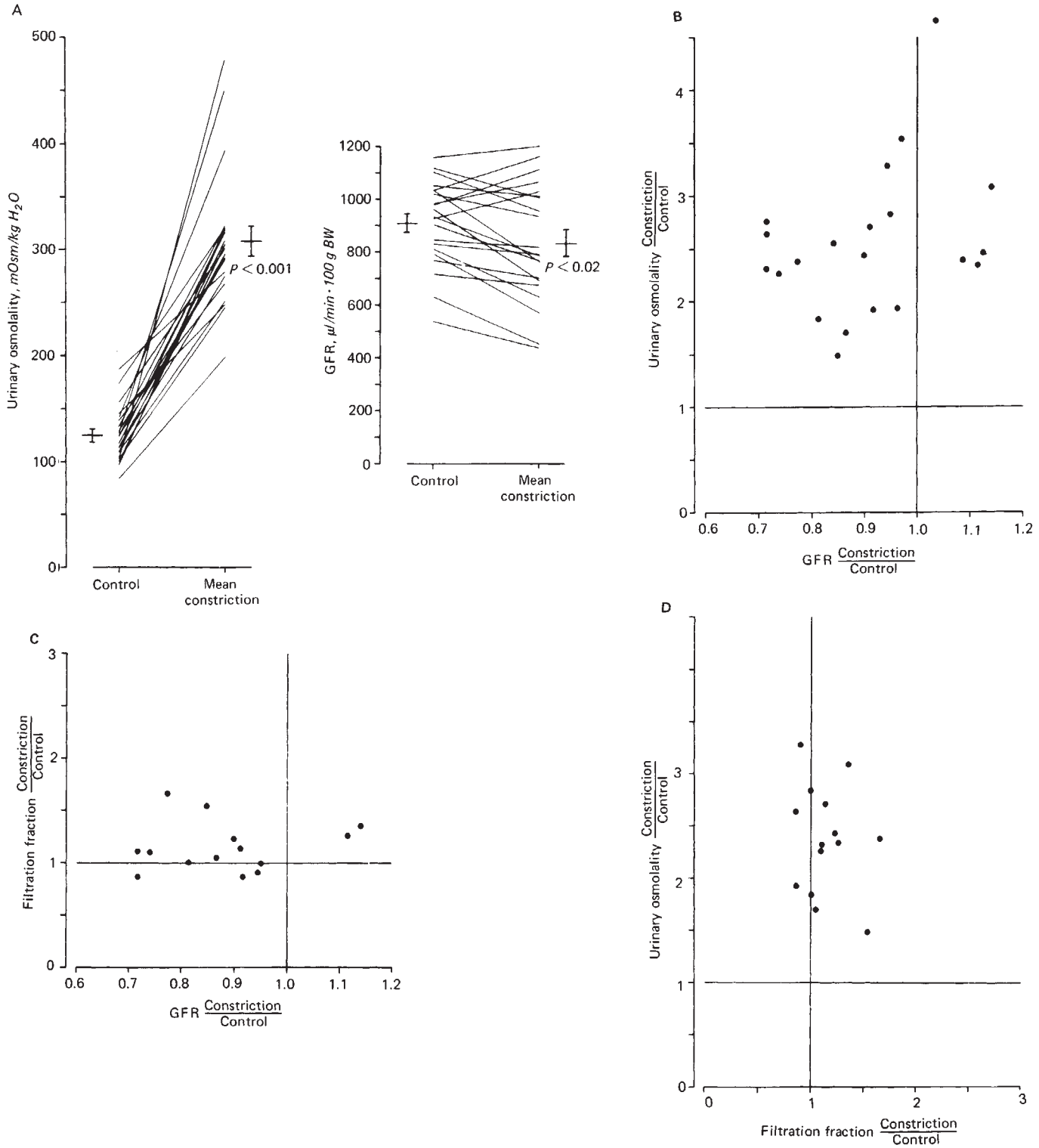

Fig. 2. *Changes in urine osmolality during constriction of the aorta above the renal arteries in 13 female and 8 male Brattleboro homozygotes.* In each instance, the values for control represent the mean of three determinations prior to aortic constriction, and those for constriction the mean of five determinations during three hours of stable, partial aortic constriction. **A.** Concurrent measurements of Uosm and GFR. Each line connects values in individual animals. Note that while Uosm rose in all animals, the response in GFR varied, decreasing in some rats, staying the same in some, and increasing in others. The horizontal lines and brackets represent the means ± SEM for the entire group. **B.** Relative increase (from control) of Uosm as a function of relative change in GFR. Each point represents simultaneous determinations in each animal. There is no clear-cut correlation between a change in GFR and that in Uosm. **C.** Relative change in filtration fraction as a function of relative change in GFR. Again, there is no correlation. **D.** Lack of correlation between increases in filtration fraction and the degree of urinary concentration. Reproduced, with permission, from [4].

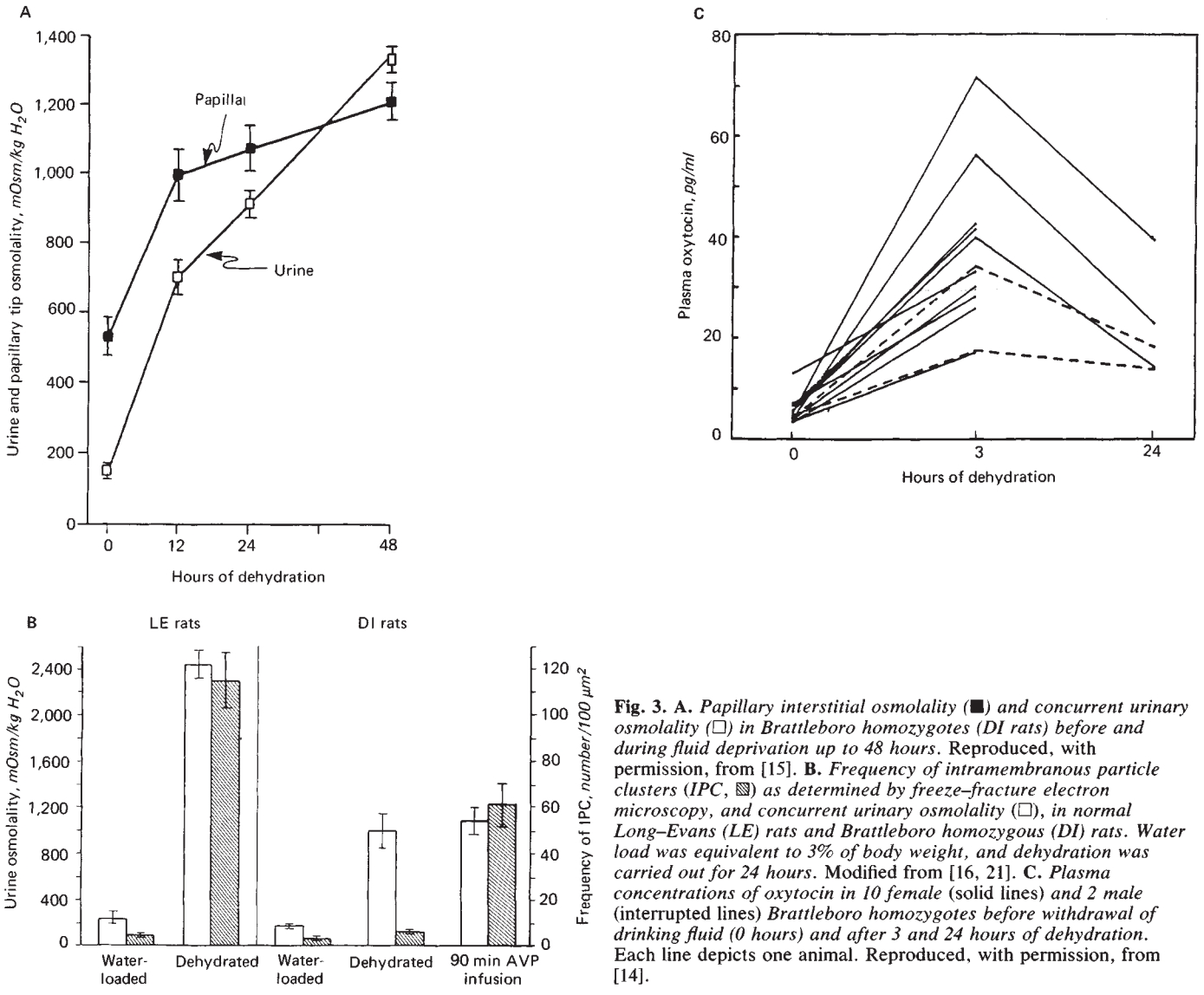

Fig. 3. A. *Papillary interstitial osmolality (■) and concurrent urinary osmolality (□) in Brattleboro homozygotes (DI rats) before and during fluid deprivation up to 48 hours.* Reproduced, with permission, from [15]. **B.** *Frequency of intramembranous particle clusters (IPC, ▨) as determined by freeze-fracture electron microscopy, and concurrent urinary osmolality (□), in normal Long-Evans (LE) rats and Brattleboro homozygous (DI) rats. Water load was equivalent to 3% of body weight, and dehydration was carried out for 24 hours.* Modified from [16, 21]. **C.** *Plasma concentrations of oxytocin in 10 female* (solid lines) *and 2 male* (interrupted lines) *Brattleboro homozygotes before withdrawal of drinking fluid (0 hours) and after 3 and 24 hours of dehydration.* Each line depicts one animal. Reproduced, with permission, from [14].

sorption. Filtration fraction did, in fact, increase in the majority of our experiments (Fig. 2C), but the lack of correlation evident in Figure 2D makes it doubtful that that mechanism was responsible for the rise in urinary osmolality.

Thus, we have not identified the mechanism by which decreased delivery of fluid to the diluting segment might have come about in those of our experiments in which GFR did not decline. Possibilities include a lower renal perfusion pressure and hence a lessened peritubular hydrostatic pressure, or increased reabsorption of fluid from the proximal straight tubule or the descending limb of Henle because of a rising medullary interstitial osmolality. Nor do we mean to say—by pointing out that urine can be concentrated in the absence of vasopressin with minimal or no decrease in GFR—that a decline in GFR is unimportant in the Berliner–Davidson mechanism. It may well constitute a major means by which delivery to the diluting segment is decreased in those experiments in which GFR declined (Fig. 1B).

Other factors

Our group has further explored the mechanisms that contribute to the concentration of urine as Brattleboro homozygotes are deprived of water [14–16]. These subsequent experiments addressed the question whether urine becomes more concentrated under these circumstances entirely without an increase in the water permeability of the collecting duct system, or whether an increase of permeability is induced by substances other than vasopressin. We used several experimental approaches to this question, and the results are shown in Figure 3.

Urinary versus papillary interstitial osmolality

The rationale of the first approach is one that we have used previously in rats [6] and mice [17]: If papillary interstitial osmolality is significantly higher than the concurrent urinary osmolality, then it is likely, though not proven [18], that a low water permeability of the collecting duct system has prevented osmotic equilibration between tubular fluid and the surrounding

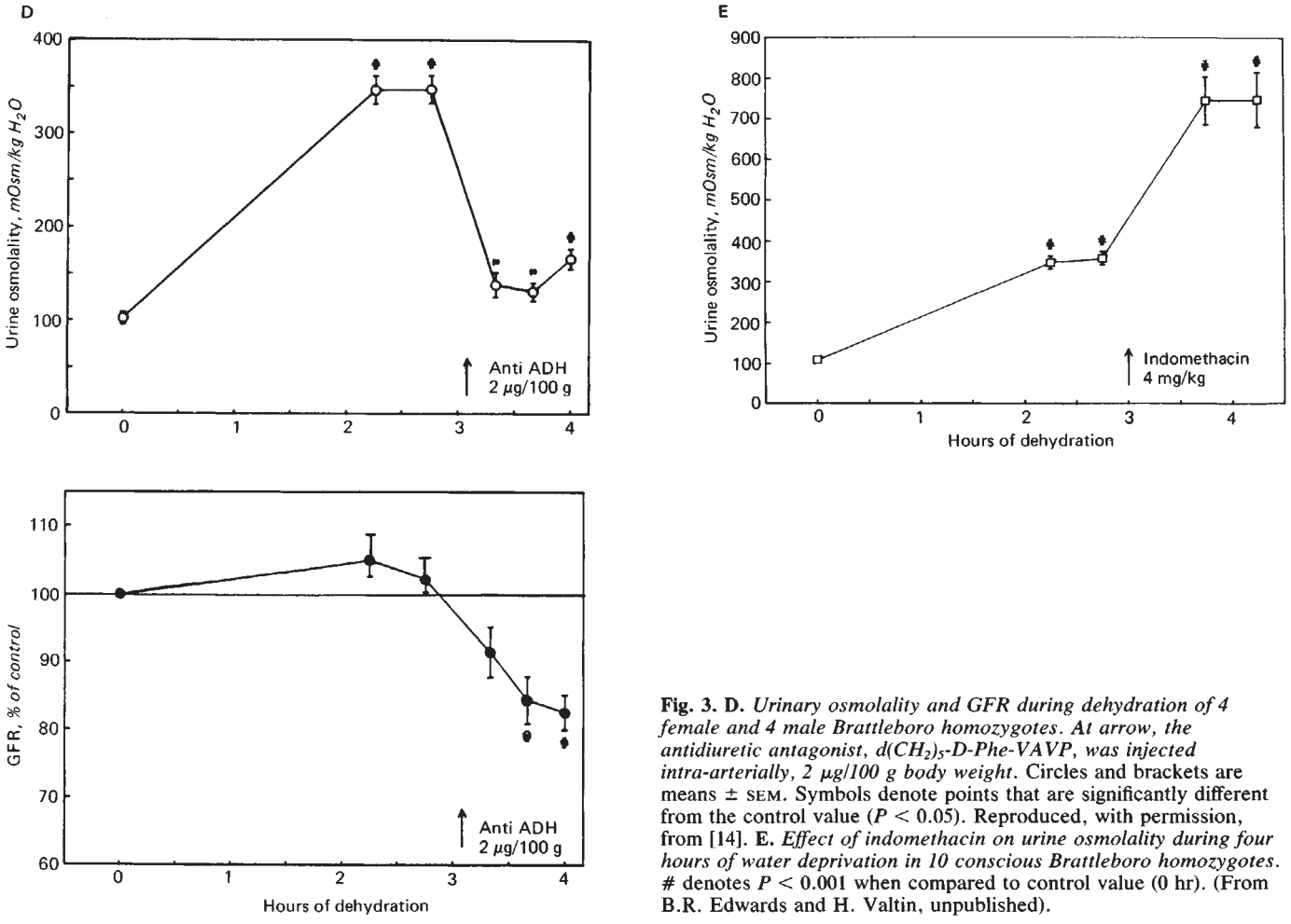

Fig. 3. D. *Urinary osmolality and GFR during dehydration of 4 female and 4 male Brattleboro homozygotes. At arrow, the antidiuretic antagonist, d(CH₂)₅-D-Phe-VAVP, was injected intra-arterially, 2 μg/100 g body weight.* Circles and brackets are means ± SEM. Symbols denote points that are significantly different from the control value ($P < 0.05$). Reproduced, with permission, from [14]. **E.** *Effect of indomethacin on urine osmolality during four hours of water deprivation in 10 conscious Brattleboro homozygotes.* # denotes $P < 0.001$ when compared to control value (0 hr). (From B.R. Edwards and H. Valtin, unpublished).

interstitium. Figure 3A shows that while initially there was a large osmotic disequilibrium between papilla and urine, the disparity decreased steadily up to 24 hours of dehydration, and after 48 hours urinary osmolality was actually recorded to be slightly higher than papillary osmolality. (The latter result is likely to reflect osmotic equilibrium, since the measured papillary osmolality represents an integral over a finite portion of the corticopapillary gradient and therefore is a slight underestimate of the osmolality at the very tip of the papilla.) The curves suggest, furthermore, that during the initial 12 hours of dehydration, increases in papillary osmolality and in the degree of osmotic equilibration both contributed to the greater concentrating ability, whereas from 12 to 48 hours it was mainly a matter of greater osmotic equilibration. The latter may result mainly from decreased flow rate of tubular fluid, and hence greater contact time with the reabsorbing epithelium. As shown by further experiments (below), however, there may also be an increase in water permeability.

Intramembranous particle clusters

Following the exciting discovery by two groups [19, 20] that exposure of amphibian urinary bladders to oxytocin and vasopressin resulted in the appearance of intramembranous particle (IMP) clusters on freeze–fracture electron microscopy, we used this approach to estimate vasopressin–induced water permeability in the mammalian collecting duct [21, 22]. In a variety of preparations, there appears to be a direct correlation between the degree of vasopressin–induced water permeability and the number of IMP clusters per unit area [reviewed in 22]. Consistent with these results are the findings shown in the left half of Figure 3B, namely, that when normal Long–Evans (LE) rats are given an oral water load equivalent to 3% of their body weight, the resulting water diuresis is accompanied by very few IMP clusters; by contrast, when such rats are dehydrated for 24 hours, urine osmolality and number of IMP clusters rise in parallel. These two situations are known to be accompanied by low and high water permeability, respectively, of the collecting duct system [23]. When Brattleboro homozygotes (DI rats) are similarly water loaded (or, for that matter, when they are drinking ad libitum), the number of IMP clusters is very low, as is the Uosm; and when DI rats are treated with vasopressin, both Uosm and the number of clusters rise (Fig. 3B, right panel). But the parallelism between Uosm and frequency of clusters breaks down when DI rats concentrate their urine through 24 hours of dehydration (Fig. 3B).

The last results suggest that the urine was concentrated in dehydrated Brattleboro homozygotes without an increase in water permeability. Nevertheless, the slight rise in frequency of

clusters, from 2.9 ± 1.0 per 100 μm^2 of membrane surface in water-loaded DI rats, to 5.9 ± 0.7 in dehydrated DI rats (Fig. 3B), although not statistically significant, leaves open the possibility that a small increase in permeability might have occurred. There is suggestive evidence, shown in Figure 3C and D, that such an increase might be mediated through oxytocin.

Possible intermediation of oxytocin

Because the effects of dehydration on Uosm can be observed during the first three hours of fluid withdrawal (Fig. 1B), we limited subsequent experiments to this period. Figure 3D shows that, as in our previous experiments (Fig. 2A), conscious Brattleboro homozygotes elaborated hypertonic urine after three hours of dehydration even though GFR did not decline. In fact, there was a slight increase of GFR, which we think was mediated by oxytocin [24].

For some time, we had thought of the possibility—but rejected it [7]—that since DI rats can secrete oxytocin [5], and since oxytocin secretion is known to be stimulated during dehydration [14], oxytocin might increase the water permeability of the collecting duct system, just as it increases that of the amphibian urinary bladder [19]. The most recent findings [14], some of which are shown in Figure 3C and D, make that possibility plausible.

Figure 3C shows changes in the plasma concentration of oxytocin as Brattleboro homozygotes were dehydrated. On the average, there was a 6.5-fold rise in plasma oxytocin after three hours of dehydration, and after 24 hours the level was significantly reduced (although still higher than before dehydration), presumably because releasable stores of the hormone were depleted. In order to test whether the rise in plasma oxytocin might be involved in raising the Uosm after three hours of dehydration, an antagonist to the antidiuretic action of vasopressin [25] was given (Fig. 3D). (Although this antagonist was designed to inhibit the effect of vasopressin by occupying receptors to the hormone, it has been shown [14] that it also antagonizes the antidiuretic and other effects of oxytocin administered to hydrated Brattleboro rats.) The antagonist reversed the rise in Uosm induced by three hours of dehydration (Fig. 3D). Our interpretation of this result is that oxytocin contributes to the concentrating ability during dehydration—at least initially—presumably by occupying vasopressin receptors. What, then, of the failure of IMP clusters to rise significantly during dehydration (Fig. 3B, right panel)? It is possible that a significant rise was not seen because clusters were counted after 24 hours of dehydration, rather than after three hours, when the plasma concentration of oxytocin was highest (Fig. 3C).

The analysis has been taken one step further. On the assumption that oxytocin, like vasopressin, can stimulate the renal production of prostaglandins [26], we tested the effect of indomethacin, an inhibitor of prostaglandin production, injected after three hours of dehydration (Fig. 3E). In 10 conscious Brattleboro homozygotes, Uosm rose from 108 ± 8 mOsm/kg H_2O in the control state, to 352 ± 14 mOsm/kg H_2O after three hours of dehydration, and then rose further to 745 ± 57 mOsm/kg H_2O after indomethacin. These results suggest yet an additional element that might modulate the ability to concentrate urine during dehydration.

Concluding comments

On the basis of experiments in Brattleboro homozygous rats, we propose that the process of concentrating urine during dehydration in the absence of vasopressin be divided into two stages. In the early stage (up to approximately 12 hours of dehydration), the major mechanisms are a rise in papillary interstitial osmolality and an increase in the degree of osmotic equilibration between collecting duct fluid and interstitium (Figs. 1C and 3A). A decrease in GFR is not essential to the concentrating process during this initial phase. On the contrary, a rise in the plasma concentration of oxytocin during this period may actually increase GFR which, as described by Levinsky, Davidson, and Berliner [2], might be partly responsible for the increase of the corticopapillary interstitial gradient (Fig. 3A). The rise in plasma oxytocin also might increase the water permeability of the collecting duct system (Fig. 3D), a change which may be partially offset by simultaneous, oxytocin–stimulated production of prostaglandin(s) (Fig. 3E).

We believe that the classic Berliner–Davidson mechanism [1] becomes more instrumental in the second stage (beyond approximately 12 hours of dehydration), when volume contraction is more severe (Fig. 1A). During this phase, a decrease in GFR with its consequent reduced delivery of fluid to the tubular diluting segment and decreased tubular flow through the collecting duct system, combine to further concentrate the urine (Fig. 1B).

We stress that this postulated schema applies specifically to the Brattleboro homozygous rat during water deprivation. The mechanisms may vary when other experimental approaches are used to eliminate vasopressin, such as acute water loading and volume expansion, or surgical removal of the neurohypophysis, in which elaboration of oxytocin, as well as of vasopressin, is blocked. What seems clear is that a multiplicity of changes, both demonstrated and as yet to be tested (in plasma oxytocin and possibly other peptide hormones; in the corticopapillary interstitial gradient; in water permeability of the collecting duct system; in prostaglandins; in GFR; in flow rate of tubular fluid; and in medullary blood flow) are involved in concentrating the urine in the absence of vasopressin.

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