Escherichia coli. 2-Thiouracil did not inhibit the utilization of uracil and produced only partial inhibition (25%) of the utilization of orotic acid.

Dihydro-L-orotic acid, DL-ureidosuccinic acid, and L-aspartic acid were tested for ability to reverse 2-thiouracil inhibition. Only DL-ureidosuccinic acid clearly reversed this inhibition. An increase in 2-thiouracil concentration did not decrease the ability of DL-ureidosuccinic acid to reverse growth inhibition, indicating that 2-thiouracil does not inhibit L-ureidosuccinic acid utilization, but inhibits its formation or the formation of precursors. L-ureidosuccinic acid precursors are necessary for protein synthesis; thus aspartate transcarbamylase which catalyzes the formation of L-ureidosuccinic acid is considered to be the specific site of inhibition.

The skillful technical assistance of Mrs. Birgit Andersen is acknowledged.

1. Cardeilhac, P. T., Fed. Proc., 1967, v26, 540.
2. Lindsay, R. H., Nakagawa, H., Cohen, P. P., Endocrinology, 1965, v76, 728.
3. Maloof, F., Soodak, M., Pharmacol. Rev., 1963, v15, 43.
4. Wang, Shih-Chen, Lui, Hsiu-Ming, Yu, Ching-Hau, Fan, Chen-Fu, Shih Yen Sheng Wu Hsueh Pao., 1964, v9, 185. (Chem. Abstr., 1965, v62, 10760h).
5. Tupy, J., Biol. Plant., 1966, v8, 398.
6. Ralph, R. K., Matthews, R. E. F., Matus, A. I., Biochim. Biophys. Acta, 1965, v108, 53.
7. Amos, H., Vollmayer, E., Virology, 1955, v6, 337.
8. Tinacci, F., Boll. Soc. Ital. Biol. Sper., 1947, v23, 572.
9. Beckwith, J. R., Pardee, A. B., Austrian, R., Jacob, F., J. Mol. Biol., 1962, v5, 618.
10. Piérard, A., Science, 1966, v154, 1572.

Received January 27, 1967. P.S.E.B.M., 1967, v125.

Renal Compensation During Mild Water Diuresis and its Inhibition by Vagotomy.* (32183)

JOSEPH H. PERLMUTT
Department of Physiology, University of North Carolina School of Medicine, Chapel Hill

Vagal afferent nerve fibers are implicated in the reflex renal regulation of fluid volume, possibly by controlling the rate of secretion of antidiuretic hormone (ADH)(1). The inhibition of water diuresis(2) and increased blood antidiuretic activity(3) promoted by vagotomy support this concept. However, the increased water and salt excretion resulting from rapid expansion of extracellular fluid volume is not completely abolished(4,5) and mannitol diuresis is unaffected(6) by vagal section. If, indeed, ADH is released after vagotomy, this procedure would not be expected to inhibit a diuresis accompanied by significant natriuresis, since the hormone's activity on water reabsorption is inversely related to the magnitude of solute excretion (7). If fluid volume could be expanded in a manner which enhances only free-water excretion, then procedures which increase circulating ADH should conceivably inhibit the renal compensation. Functional exclusion of one kidney during mild water diuresis has been found to satisfy this condition. The characteristics of the renal response to this procedure and the effects of vagotomy thereon are presented here.

Methods. Mongrel dogs of both sexes (13.6-19.5 kg; mean body weight ± SE: 15.9 ± 0.5 kg), deprived of food for 18 hours, were anesthetized with pentobarbital sodium (30 mg/kg, with supplementation as needed). After a priming intravenous injection of creatinine and para-aminohippurate (PAH), warm 5% glucose, containing these substances, was infused continuously by pump at the rate of 2.5 ml/min. The ureters, exposed through a low mid-ventral incision, were cannulated with polyethylene tubing. A loose cupper clamp(8) was placed around the left renal artery, exposed retroperitoneally with minimal clearing of overlying tissue;

* Supported by USPHS research grant HE-02457 from Nat. Heart Inst.

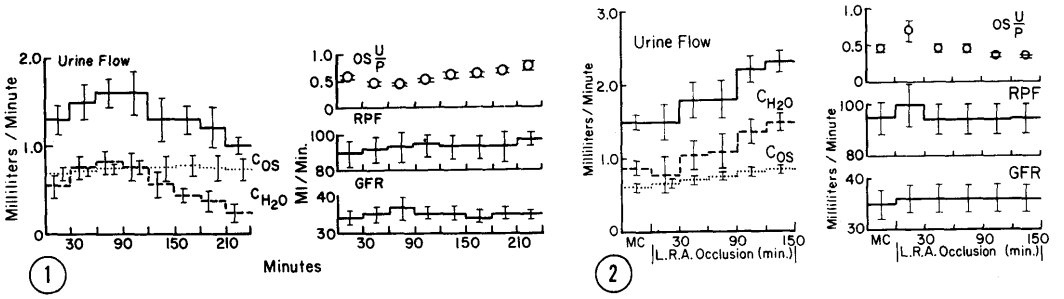

FIG. 1. Urine flow, free-water clearance (C_{H_2O}), osmolal clearance (C_{os}), glomerular filtration rate (GFR), effective renal plasma flow (RPF), and osmolal urine-to-plasma ratio (os U/P) for the right kidney during mild water diuresis. Each value is the mean ± S.E. of the mean for 5 dogs.

FIG. 2. Function of right kidney before (MC: mean of averaged control values) and during occlusion of left renal artery (L.R.A.). Two 15-min clearance periods were averaged to obtain the value for each 30-min interval. Abbreviations and compilation of data for 5 dogs in a state of mild water diuresis as in Fig. 1.

this type of clamp permitted subsequent occlusion of the artery without disturbing the wound. A femoral artery was cannulated in some animals for determining mean blood pressure with a mercury manometer, and an indwelling needle was inserted into an external jugular vein for obtaining blood samples at 30-minute intervals during subsequent clearance studies. In some animals, short segments of the cervical vagosympathetic trunks were exposed and subsequently sectioned as previously described(9).

Urine was collected separately from each kidney. When water diuresis was established, an animal was considered suitable if urine flow was reasonably stable during three 15-minute control periods, and the urine was hypoosmotic to plasma (during control phase, mean ± SE for osmolal U/P ratio was 0.48 ± 0.03).

Three types of experiments were performed in groups of 5 dogs each. *Group 1:* Spontaneous variations in renal function were determined over a 4-hour interval by using consecutive 30-minute clearance periods. Both kidneys and vagus nerves were intact. *Group 2:* At the end of the last control period, the left renal artery was occluded for 2½ hours, and the response of the right kidney was assessed by consecutive 15-minute clearance periods; the renal artery was then deoccluded, and the study was continued for several additional periods. About 2 minutes prior to clamping the renal artery, the original infusion fluid was changed to one containing one-half the concentration of creatinine and PAH in order to prevent a rise in their plasma concentration. *Group 3:* At the end of the last control period, bilateral vagotomy was performed. Fifteen minutes later, the left renal artery was occluded, and the experiment was conducted in the same manner as for the preceding group.

Urine and plasma samples were analyzed for creatinine(10), PAH(11), osmolality (Fiske osmometer), and Na and K (flame photometry). Exogenous creatinine and PAH clearances were used as estimates of glomerular filtration rate (GFR) and effective renal plasma flow (RPF), respectively. Osmolal clearance (C_{os}) and free-water clearance (C_{H_2O}) were calculated in the conventional manner(12).

Results. Group 1. Function of right kidney during mild water diuresis. Data for this group served as a basis for assessing the overall effects of exclusion of one kidney on the function of the contralateral kidney. Mean data for right kidney function during 4 hours, an interval encompassing the period of time in which subsequent experiments were performed, are presented in Fig. 1. Some fluctuations occurred in urine flow, free-water clearance, and osmolal U/P ratio, but differences between all means for the first half-hour and each subsequent interval are not statistically significant ($P>0.1$ to >0.9). Osmolal U/P ratio values of less than unity and positive values for C_{H_2O} persisted during the 4-hour interval. GFR, RPF, C_{os}, and K

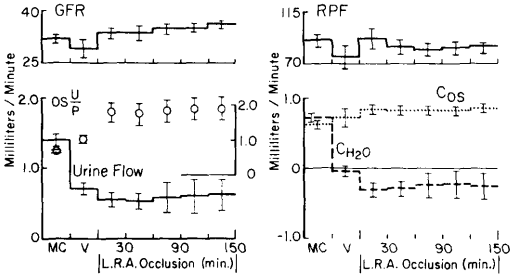

FIG. 3. Effect of vagotomy (V) on the response of the right kidney to occlusion of the left renal artery. Abbreviations and compilation of data for 5 dogs in a state of mild water diuresis as in Fig. 1 and 2.

excretion remained practically unchanged, while Na excretion increased insignificantly by 21 ± 14 μEq/min (P>0.3).

Group 2. Response of right kidney to occlusion of left renal artery. Mean data for the group before and during occlusion of the left renal artery for 2½ hours are plotted in Fig. 2. Urine flow and free-water clearance gradually increased and attained values during the last hour significantly higher than the mean control values (P<0.01). The net increase in urine flow was due primarily to the net increase in free-water clearance, and this was reflected in a falling tendency in osmolal U/P ratio. Compared to Group 1, the mean net increasees in urine flow and free-water clearance during the last hour are highly significant (for last period: P<0.001). C_{os}, Na and K excretion increased slightly (net increase for last period: 0.25 ml/min, 13 ± 6 and 13 ± 3 μEq/min, respectively), but compared to Group 1, these changes are not statistically significant. GRF and RPF during occlusion remained practically unchanged, and mean arterial blood pressure was not appreciably affected during occlusion.

After deocclusion of the left renal artery, there was a slight resumption of urine flow (about 0.3 ml/min) from the left kidney during an additional 45 minutes in 4 of the 5 animals. In these cases, urine flow from the right kidney declined to near control levels. In the animal in which there was no flow from the left kidney, the compensation continued. The subsidence of compensation was possibly due to the small dissipation of fluid volume rather than "wash-out" of a vasoactive substance, since renal hemodynamics and systemic blood pressure remained unchanged.

Group 3. Response of right kidney to vagotomy and occlusion of left renal artery. At the end of the last control period, bilateral vagotomy was performed, and the left renal artery was occluded for 2½ hours. Mean data for the group are presented in Fig. 3. During renal arterial occlusion in these vagotomized animals, diuresis was not enhanced, but rather, prolonged antidiuresis ensued; the decline in urine flow was due solely to the decline in free-water clearance. Differences between the mean control values and all subsequent values for urine flow and free-water clearance are statistically significant (P<0.02 to <0.001). Differences in net changes in these parameters between this group and Group 2 for all intervals during left renal arterial occlusion are highly significant (P<0.01 to <0.001). During all intervals after vagotomy, osmolal U/P ratio exceeded 1.0, the values being significantly higher than the mean control value of 0.47 (P<0.01 to <0.001). Net changes in C_{os} and Na and K excretion rates were essentially the same as those for Group 2 during the last 90 minutes of occlusion. Slight increases in Na and K excretion during two periods following vagotomy probably resulted from the evanescent rise in blood pressure. GFR and RPF did not change significantly.

After deocclusion of the left renal artery, only a few drops of urine were excreted by the left kidney during a 45-minute interval. Urine flow from the right kidney changed very little from the values during occlusion. This, of course, would be expected if vagotomy induces prolonged release of ADH(2,3).

Discussion. Mild water diuresis was maintained for several hours in anesthetized dogs. During such diuresis, functional exclusion of one kidney, by occluding its renal artery, induced significant increases in urine flow from the contralateral kidney primarily as a consequence of enhanced free-water clearance. Electrolyte excretion, GFR, and RPF were not significantly altered. The characteristics of the compensatory response to this procedure apparently depend upon the composition of the hydrating fluid; during iso-

tonic saline diuresis, the enhanced diuresis is accompanied by increased electrolyte excretion(13). A significant decline in renal plasma flow and a slight decline in urine flow for the left kidney during occlusion of the right renal artery have been reported(14); if no precautions were taken to prevent a rise in plasma PAH concentration during occlusion, the plasma PAH may have increased sufficiently to cause self-depression of its clearance, and/or the clamping technique may have been excessively traumatic.

Positive fluid balance (cumulative difference between infused fluid and urinary volumes) at the end of control periods was practically the same for the group with (3.3 $\pm$ 0.1% of body weight) and without (3.1 $\pm$ 0.8%) renal arterial occlusion; 90 minutes later, when significant compensation began, positive fluid balance increased by 0.5 $\pm$ 0.1% of body weight in the former group, and decreased by 0.3 $\pm$ 0.2% in the latter group ($P<0.02$). The compensation induced by renal arterial occlusion probably resulted from a gradual additional increase in fluid volume by an amount equivalent to the preexisting urine flow from the clamped kidney. An abrupt increase in blood volume is an unlikely explanation, since the onset of significant compensation was considerably delayed, and compensation resulted from clamping a ureter (3 dogs), in which case renal blood flow is not interrupted(15). The renal compensation promoted by this type of fluid volume expansion suggests decreasing activity of ADH, consonant with the report that administration of dextran to dogs with contracted extracellular fluid volume results in decreased blood antidiuretic titer(16). However, bioassay of blood cannot distinguish among the possibilities of decreased hormone release, dilution of the circulating hormone, or increased degradation. An indirect implication that decreased antidiuretic activity could have been present was provided by the following experiment. Continuous infusion of vasopressin (Pitressin, 8 mU/kg/hr) for 2½ hours during renal arterial occlusion prevented the compensation; urine flow declined, free-water clearance became negative, and osmolal urine-to-plasma ratio increased above 1.0. If there were a gradual decrease in ADH activity during renal arterial occlusion, plasma osmolality did not appear to be a contributing factor; plasma osmolality declined from a mean value of 296 $\pm$ 1 before infusion of the hydrating fluid to 276 $\pm$ 1 mOs/kg H_2O during the control periods ($P<0.001$) and did not decline further during occlusion. Therefore, a volume-related factor is implicated.

Under the present condition of hydration, vagotomy prevented the compensatory diuresis induced by renal arterial occlusion and promoted antidiuresis. Urine flow declined below control values, and the decline was due solely to the reduction in free-water clearance which attained negative values. Positive fluid balance in this group amounted to 3.0 $\pm$ 0.4% of body weight at the end of control periods and increased by 1.1 $\pm$ 0.1% 90 minutes later, a value significantly higher than that for the same interval for the group with intact vagus nerves subjected to the same procedure ($P<0.01$). Plasma osmolality decreased from a mean control value of 281 $\pm$ 1 to 275 $\pm$ 1 mOs/kg H_2O ($P<0.001$) during the same time interval, and reflects increased retention of hypoosmotic fluid. Thus, in the presence of fluid volume expansion and declining plasma osmolality, vagotomy prevented compensatory diuresis and induced antidiuresis, unaccompanied by declines in GFR, RPF or osmolal clearance. Several lines of evidence suggest that the renal response to vagotomy during water diuresis was possibly due to the absence of vagal impulses inhibitory to ADH release rather than to injury action potentials or painful stimuli: a. blockade of intact vagus nerves by prolonged application of xylocaine induced antidiuresis(2); b. electrical stimulation of the central end of a cut vagus nerve usually promoted a small increase in urine flow (unpublished observations); and c. the depression of urine flow induced by mechanical compression of a femoral nerve was accompanied by concomitant declines in GFR, RPF, and C_{os}(17) in contrast to insignificant changes in these parameters after vagotomy(2, Fig. 3). In view of a previous demonstration that the

sub-cardiac vagi are unessential for the antidiuretic response to cervical vagotomy during water diuresis(2), the present results support the hypothesis that vagal afferents originating in thoracic receptors serve as one of the mechanisms for the control of ADH release (1).

Thus, when fluid volume was expanded in a manner which primarily promoted enhanced free-water excretion, vagotomy abolished the renal response. Presumably, extrathoracic receptors were not significantly activated. It has been repeatedly demonstrated that vagotomy and/or carotid sinus denervation do not completely abolish the diuretic and natriuretic response to rapid expansion of extracellular fluid volume(5,18,19); in this situation other receptors appear to be involved(20). It is possible that expansion of total body water to a greater degree than achieved in the present investigation may result in activation of intra- and extrathoracic receptors; conceivably, interruption of vagal pathways alone would then incompletely prevent renal compensation.

Summary. Occlusion of a renal artery in anesthetized dogs during mild water diuresis promoted enhancement of the diuresis from the contralateral kidney. The compensation was primarily due to increased free-water clearance and was not accompanied by any significant changes in glomerular filtration rate, effective renal plasma flow, or electrolyte excretion. After vagotomy, a compensatory renal response did not occur, and free-water clearance declined to negative values.

The technical assistance of Mr. Alan Gordon is acknowledged.

1. Gauer, O. H., Henry, J. P., Physiol. Rev., 1963, v43, 423.

2. Perlmutt, J. H., Proc. Soc. Exp. Biol. & Med., 1964, v116, 270.

3. Share, L., Levy, M. N., Am. J. Physiol., 1962, v203, 425.

4. Atkins, E. L., Pearce, J. W., Can. J. Physiol. and Pharmacol., 1959, v37, 91.

5. Gilmore, J. P., Weisfeldt, M. L., Circulation Res., 1965, v17, 144.

6. Perlmutt, J. H., Federation Proc., 1963, v22, 385.

7. Orloff, J., Wagner, H. N., Jr., Davidson, D. G., J. Clin. Invest., 1958, v37, 458.

8. Heymans, C., De Schaepdryver, A. F., De Vleeschhouwer, G. R., Circulation Res., 1960, v8, 347.

9. Perlmutt, J. H., Proc. Soc. Exp. Biol. & Med., 1966, v121, 177.

10. Kennedy, T. J., Jr., Hilton, J. G., Berliner, R. W., Am. J. Physiol., 1952, v171, 164.

11. Smith, H. W., Finkelstein, N., Alimonisa, L., Crawford, B., Graber, M., J. Clin. Invest., 1945, v24, 288.

12. Wesson, L. G., Jr., Anslow, W. P., Jr., Am. J. Physiol., 1952, v170, 255.

13. Peters, G., ibid., 1963, v205, 1042.

14. Friedman, S. M., Radcliffe, R. W., Turpin, J. E. H., Friedman, C. L., Can. J. Biochem. & Physiol., 1956, v34, 158.

15. Thurau, K., Am. J. Med., 1964, v36, 698.

16. Share, L., Am. J. Physiol., 1962, v202, 791.

17. Perlmutt, J. H., ibid., 1963, v204, 197.

18. Mills, I. H., de Wardener, H. E., Hayter, C. J., Clapham, W. F., Clin. Sci., 1961, v21, 259.

19. Pearce, J. W., Can. J. Biochem. & Physiol., 1959, v37, 81.

20. Pearce, J. W., Sonnenberg, H., Can. J. Physiol. and Pharmacol., 1965, v43, 211.

Received January 30, 1967. P.S.E.B.M, 1967, v125,