TABLE II. Comparison of Hippuric Acid and p-Aminohippuric Acid Clearances in the Dog.

Exp		Hippurate clearance, cc/min	p-Aminohippurate clearance, cc/min	Hipp. Cl. / Creat. Cl.	PAH Cl. / Creat. Cl.
A	r	67.8	65.2	4.03	4.08
	l	66.3	59.0	4.57	5.46
B	r	37.5	49.5	3.35	3.06
	l	35.3	51.3	3.04	3.17
C	r	33.1	33.9	3.18	3.46
	l	41.2	35.0	3.85	3.50
D	r	82.1	109.3	4.11	5.44
	l	88.6	100.0	4.06	6.04
E	r	39.3	34.4	2.77	3.00
	l	35.0	34.6	2.50	3.23
F	r	43.0	40.5	3.00	3.30
	l	41.3	46.3	2.67	3.36
G	r	22.4	22.9	2.80	3.00
	l	34.6	36.1	2.58	3.06
H	r	40.6	22.0	3.87	3.70
	l	40.5	23.0	3.58	3.71
I	r	79.0	46.3	5.98	5.12
	l	54.3	47.3	4.38	4.34
J	r	59.8	49.6	3.08	3.20
	l	56.1	47.3	2.92	3.24
K	r	57.6	60.3	2.35	3.39
	l	62.7	58.1	2.59	3.82
L	r	68.7	55.8	3.97	5.17
	l	70.7	58.7	4.00	5.29
M	r	37.4	22.4	5.19	5.44
N*		162.0	165.0	5.90	5.70
O*		175.0	206.0	2.40	2.61
Mean		51.8	48.3	3.58	3.98
S.D.		17.7	21.2	.93	.96

* Bladder clearances. Omitted from statistical analysis.

sary to prevent contamination of the plasma with these substances. Temperature change is known to affect fluorescence, and we have found it necessary to make fluorescence measurements at constant temperature in this technique to assure reproducible results.

Statistical analysis of the clearance data by standard techniques shows that there is no significant difference in the values for clearance of hippuric acid *vs* p-aminohippuric acid at the 0.05 level. The results in Table II indicate the danger of using one kidney of an experimental animal as a "control" and the other as "experimental" in studies involving small numbers of animals. The data on clearance of p-aminohippuric acid by the dog are in essential agreement with those reported by Houck(8).

Summary. 1. A method for analysis of microgram quantities of hippuric acid in plasma with good precision and accuracy is presented. 2. There is no significant difference in the clearance of hippuric acid and p-aminohippuric acid by the dog.

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Effect of Vagotomy on Renal Function During Water Diuresis.* (29222)

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Henry and coworkers(1,2) demonstrated that increased stretch of the left atrium promoted diuresis which could be inhibited by blocking vagal transmission. As a possible explanation for these results, the hypothesis has been proposed that the secretion of anti-

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TABLE I. Renal Function in Anesthetized Dogs During Water Diuresis.

Time, min	Urine flow	GFR	RPF	C_{os}	U_{os}/P_{os}
	ml/min/100 g kidney				
0- 60	2.8 ± .3	69 ± 5	193 ± 18	1.38 ± .11	.54 ± .04
60-120	3.1 ± .3	70 ± 4	195 ± 16	1.45 ± .11	.55 ± .04
120-180	2.4 ± .3	70 ± 4	199 ± 14	1.50 ± .51	.68 ± .05

Each datum is the mean ± S.E. of mean for two 30-min or three 20-min clearance periods for 8 dogs.

diuretic hormone (ADH) is influenced by the activity of vagal afferent fibers(3). The results have been confirmed, but the mechanism has been questioned due to the failure of exogenous ADH to suppress the diuresis in anesthetized(4) or in conscious(5) dogs. In view of the conflicting opinions on this important concept of fluid volume control, a further test of the hypothesis was undertaken by investigating the effect of vagotomy on several parameters of renal function during water diuresis.

Methods. Healthy mongrel dogs (12.7-20.7 kg) were anesthetized with pentobarbital sodium (30 mg/kg, i.v., with supplementation as required). After a priming intravenous injection of creatinine and para-aminohippurate (PAH), warm 5% glucose in water, containing appropriate amounts of these substances, was infused continuously by pump at the rate of 2.5 ml/min throughout the experiment. The ureters were exposed through a mid-ventral incision and were cannulated with polyethylene tubing. Short segments of the cervical vagus nerves (vagosympathetic trunks) were isolated approximately 5-7 cm below the lower border of the larynx and double loose ligatures were placed around each nerve. A femoral artery was cannulated for determining mean blood pressure with a mercury manometer, and an indwelling needle was placed in an external jugular vein for obtaining blood samples during subsequent clearance studies. An animal was considered suitable for an experiment if the urine was hypotonic, and urine flow was reasonably stable during three 15-min control periods. At the end of the last control period, bilateral vagotomy was performed; manipulation of the nerves preparatory to sectioning, either with or without prior application of local anesthesia, was not accompanied by bodily movements or blood pressure changes.

Conventional renal clearances were performed before and after vagotomy. Exogenous creatinine and PAH clearances were used as estimates of glomerular filtration rate (GFR) and effective renal plasma flow (RPF), respectively. Methods used previously(6) were employed for analyses of creatinine, PAH, osmolality, Na, and K in plasma and urine. Osmolal clearance (C_{os}) and free-water clearance (C_{H_2O}) were calculated as follows: $C_{os} = U_{os}/P_{os} \times V$, where U_{os} and P_{os} are the urine and plasma osmolalities in mOs/kg H_2O, respectively, and V is the urine flow in ml/min; $C_{H_2O} = V\text{-}C_{os}$.

Results. Stability of renal function during water diuresis. Renal function was determined in 8 dogs subjected to the above hydration and surgical procedures except that the vagus nerves were intact during the study. Table I summarizes the data obtained over a 3-hr interval. It is evident that the indices of renal function investigated were reasonably stable, which demonstrates the suitability of this type of preparation for the investigation. Differences between the means of all flow rates for any 2 periods are not statistically significant. Osmolal urine-to-plasma ratio (U_{os}/P_{os}) was below unity during the 3-hour interval, indicating that the urine remained hypotonic to plasma.

Renal function after vagotomy during water diuresis. Changes in renal function after vagotomy were qualitatively similar in each of 9 dogs. Fig. 1 presents mean values for the group for urine flow, GFR, RPF, osmolal clearance and osmolal U/P ratio. Mean urine flow declined while mean osmolal U/P ratio increased to values in excess of 2.0 after vagotomy; by 45 minutes after the procedure the values remained reasonably con-

stant. Differences between mean control values for these 2 determinations and means for all periods subsequent to the first period after vagotomy are statistically significant ($p<0.01$ to <0.001); differences between means for the first period after vagotomy and those for subsequent periods are also significant ($P<0.05$ to <0.01). The antidiuresis occurred in the presence of declining total solute concentration of the plasma (Fig. 1, P_{os}). Slight fluctuations in GFR and RPF after vagotomy amounted to less than 5% of control values and are not statistically significant. The slight rise in osmolal clearance after vagotomy is statistically insignificant. Under the same experimental conditions, renal responses of similar magnitude were obtained by continuous infusion of 50 mU/hr of ADH (Pitressin) into dogs with intact vagus nerves.

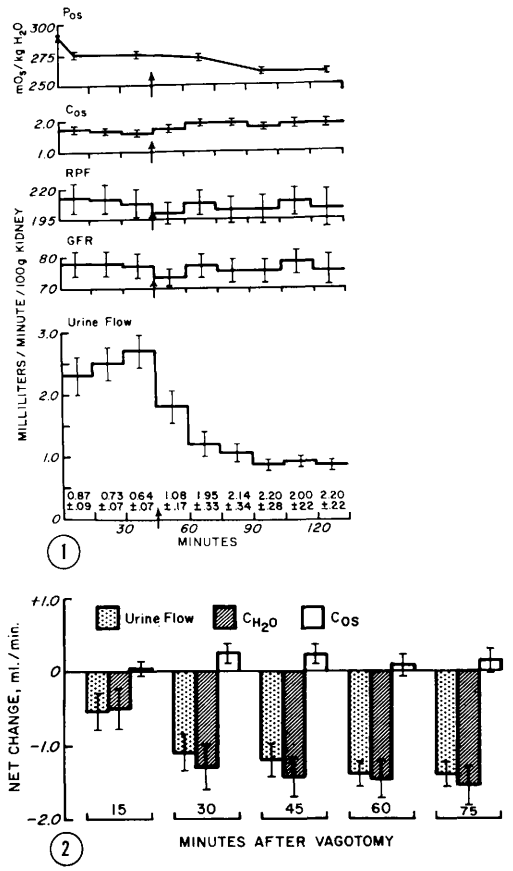

FIG. 1. Urine flow, glomerular filtration rate (GFR), effective renal plasma flow (RPF), osmolal clearance (C_{os}), and plasma osmolality (P_{os}) before and after bilateral cervical vagotomy (arrow) during water diuresis. Numbers below urine flow curve are the osmolal U/P ratios. X on P_{os} ordinate is mean pre-hydration value for P_{os}. Each value is mean ± S.E. of the mean for 9 dogs.

FIG. 2. Net changes in urine flow, free-water clearance (C_{H_2O}), and osmolal clearance (C_{os}) for 5 periods after vagotomy during water diuresis. Each value is mean ± S.E. of mean for the 9 dogs of Fig. 1.

Fig. 2 shows mean net changes (post-vagotomy minus control values) in urine flow, free-water clearance and osmolal clearance for 5 periods after vagotomy. It is evident that the net decline in urine flow was due solely to the decline in free-water clearance, *i.e.*, increased renal tubular reabsorption of water.

Vagotomy had no conclusive effect on Na excretion. In 5 of 9 animals after vagotomy, as well as in 5 of 8 animals with intact vagus nerves, Na excretion rate increased by approximately 70 μEq/min during the same time intervals; no appreciable change occurred in the remaining animals of either group. K excretion for both groups remained practically unchanged from control levels.

Additional findings. Average mean arterial blood pressure remained practically constant throughout the experiments, except for a transient rise immediately after vagotomy.

Renal responses to vagotomy similar to those above were obtained from dogs with a denervated kidney. Antidiuresis also occurred during an established water diuresis under the following conditions: (a) continuous pump respiration before and after vagotomy, (b) prolonged application of xylocaine ointment to the intact vagus nerves after control periods, and (c) bilateral cervical vagotomy after previous bilateral sub-cardiac vagotomy.

Discussion. Under conditions of the present study, water diuresis could be maintained for several hours in anesthetized dogs (Table I). During such diuresis, bilateral cervical vagotomy invariably promoted a prolonged decline in urine flow and an increase in urine osmolality above that of plasma. The decline in urine flow was due solely to increased renal tubular reabsorption of solute-free water. No evidence for renal vasoconstriction or increased solute reabsorption was

apparent as judged by clearances of creatinine, PAH, and total solute. Thus, the renal response to vagotomy was typical for increased ADH activity(7). Decreased osmolality of the plasma was present throughout the experiments; thus, stimulation of "osmoreceptors" was not a factor. It is unlikely that changes in activity of renal nerves, in activity of sub-cardiac efferent vagal fibers, or in respiration were responsible for the renal response to cervical vagotomy; antidiuresis occurred after cervical vagal section in animals with a denervated kidney, with vagi severed below the heart, or during continuous pump respiration.

Reports on bioassay of blood or plasma from non-hydrated dogs indicate increased antidiuretic activity after vagotomy(8,9). Present results agree with these findings and demonstrate their functional significance. Both types of investigation support the hypothesis of vagal control of ADH secretion.

Summary. 1. Prolonged inhibition of water diuresis occurred after bilateral cervical vagotomy in anesthetized dogs. 2. The urine became hypertonic to plasma, and the decline in urine flow was due solely to increased tubular reabsorption of solute-free water. 3. Glomerular filtration rate, renal plasma flow, and solute excretion did not change significantly.

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Effects of Adenosine Triphosphate on Ethionine-Induced Resorption.* (29223)

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Ethionine administration to the pregnant rat has been shown to produce a marked increase in fetal resorption(1). In an attempt to determine the role of ethionine in the initiation of the death and subsequent resorption of the fetus, studies from our laboratory have shown that ethionine is incorporated into the amino acid pool and into the proteins of the rat fetus and placenta and is associated with a fluctuation in methionine content of these tissues(2). However, these same studies indicate that the fetus may persist, apparently normally, with ethionine present in its proteins.

In recent studies on the mechanism of ethionine inhibition of protein synthesis, it has been shown that ethionine administration leads to a rapid fall in concentration of ATP in the liver, that this effect is counteracted by injection of ATP, and that administration of ATP to ethionine-treated rats counteracts the inhibitory effect of ethionine upon leucine incorporation into liver ribosomes(3).

The present study was undertaken to determine whether ATP administration would in a similar manner reverse the ethionine-induced resorption of the rat litter.

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