also a progressive depletion. Considering the amounts of the hormone stored in the organs examined here and the depletions observed, it seems possible that the spleen furnished the major portion of the circulating noradrenaline. The situation, if true, may, however, be peculiar to the dog. The spleen has been implicated in shock by Hardaway(26).

Summary. Catecholamine levels were determined in tissues obtained from dogs subjected to prolonged oligemic hypotension. The lung and liver were found to have retained their normal complement of catecholamines while the cerebral hemisphere, spleen and heart were depleted. In the brain the depletion appeared to be a terminal phenomenon, possibly associated with the respiratory difficulties experienced by the animal at that time. In the spleen the depletion seemed to take place progressively throughout the experimental period. An attempt is made to reconcile these data with known metabolic and functional parameters of the organs studied.

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Renal Responses to Acetylcholine.* (30328)

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We reported recently that malachite green, a cationic triphenylmethane dye, acts directly on the kidney to increase excretion of calcium, phosphate, sodium, potassium, chloride and water(1). Evidence that triphenylmethanes inhibit cholinesterase(2) suggested

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that malachite green might act to increase renal tissue concentration of acetylcholine (Ach) and that Ach might exert renal actions analogous to those of the dye.

No data are available concerning the effects of Ach on renal excretion of calcium and phosphate, although others(3,4) have reported that infusion of Ach into the renal arterial blood supply increases sodium, potassium, chloride and water excretion. Moreover, even though Ach hydrolyzes rapidly in body fluids, the possibility that products of Ach hydrolysis are responsible for the drug's renal effects has not been investigated. A third as yet unresolved problem is the renal response to cholinesterase inhibition. If Ach is present in renal tissue, cholinesterase inhibition should evoke a diuresis, but infusion of the anticholinesterase, physostigmine, failed to do so (3). Since an anticholinesterase which is effective in one tissue may be ineffective in another, study of additional inhibitors appeared to be desirable.

We have examined the renal effects of Ach by infusing the drug directly into one renal artery of the dog. The renal responses to choline, acetate, several anticholinesterases and atropine also have been examined. The data extend previous observations on the renal actions of Ach and demonstrate that the effects are a specific response to the drug.

Methods. Fasting, hydropenic mongrel dogs, 18 to 20 kg body weight, were anesthetized with intravenous pentobarbital sodium. A constant-speed pump (1.4 ml/min) infused intravenously inulin and p-aminohippurate (PAH) in isosmotic saline. A second constant-speed pump (1.23 ml/min) infused isosmotic saline into the right renal artery *via* an 18 gauge cannula inserted by a previously described technique(5). Ureteral catheters conducted urine from each kidney into graduated cylinders. Blood samples were taken from a femoral arterial cannula at the midpoint of odd-numbered collection periods and arterial blood pressure was monitored with a mercury manometer. Chemical methods are described elsewhere(1) and statistical methods used were those described in standard textbooks.

Acetylcholine infusion. After 4 urine collection periods of 10 or 20 minutes each, Ach chloride, 10 μg per ml in isosmotic saline, was infused into the renal artery and urine collected for 2 to 4 additional periods of 10 or 20 minutes each. The infusate then was changed to isosmotic saline and urine collected for 2 more periods of 10 or 20 minutes each. Ten experiments were performed in 10 dogs.

Atropine infusion. In 4 of the 10 experiments above, following recovery from Ach effects, atropine sulfate, 37 μg per ml in saline, was infused for 1 to 3 collection periods of 10 or 20 minutes each. Acetylcholine then was infused again for 1 period, 10 μg per ml, and for 1 period, 100 μg per ml.

Anticholinesterase infusions. In 4 experiments, physostigmine sulfate, 0.4 to 17 μg per ml in saline, was infused for 1 to 3 collection periods of 10 or 20 minutes each. In one experiment, diisopropylfluorophosphate (DFP), 10 to 1000 μg per ml in saline, was infused for several periods. In one experiment, neostigmine methyl sulfate, 18 to 72 μg per ml in saline, was infused for several periods.

Choline and acetate infusions. Choline chloride, 7.7 μg per ml in saline, was infused in 3 experiments and sodium acetate, 7.7 μg per ml in saline, was infused in 2 of the 3 experiments. In all 3 experiments, 10 μg per ml of Ach chloride was infused. The molarity of each solution was 5.5×10^{-5}.

Results. Renal arterial infusion of acetylcholine (Table I). Acetylcholine promptly increased excretion of calcium, phosphate, water, sodium, potassium and chloride from the infused kidney. Both GFR and ERPF increased and filtration fraction fell. When saline was substituted for Ach, all changes returned rapidly towards pre-infusion values.

A. *Hemodynamic effects.* In the majority of the infused kidneys, GFR and ERPF increased during Ach infusion (Table II). In the majority of the non-infused kidneys, GFR and ERPF decreased during Ach infusion. Arterial blood pressure and pulse rate remained constant in all experiments.

B. *Excretory effects.* Excretion of water, sodium, potassium, calcium, chloride and phosphate increased in all the infused kidneys except for potassium and phosphate in Dog 1. Excretion of ions and water decreased in many of the non-infused kidneys. Ion and

TABLE I. Renal Responses to Infusion of Acetylcholine Chloride into Right Renal Artery of the Dog.*

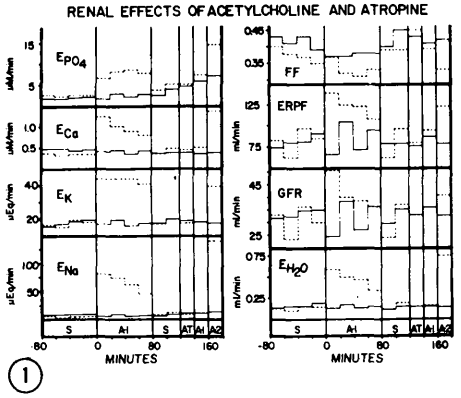
Time, min	Hemodynamics GFR, ml/min E	GFR, ml/min C	ERPF, ml/min E	ERPF, ml/min C	Urine excretory rates H_2O, ml/min E	H_2O, ml/min C	Na, μEq/min E	Na, μEq/min C	K, μEq/min E	K, μEq/min C	Ca, μM/min E	Ca, μM/min C	Cl, μEq/min E	Cl, μEq/min C	PO_4, μM/min E	PO_4, μM/min C	Plasma concentrations Na, mEq/l	K, mEq/l	Ca, mM/l	Cl, mEq/l	PO_4, mM/l
-90-0	Begin renal arterial infusion of .85% saline at 1.23 ml/min and i.v. infusion of saline, inulin and PAH at 1.4 ml/min.																				
0-20	18	33	52	83	.10	.12	9	12	7	13	.6	.6	7	5	.7	3.5	149	4.0	2.8	116	1.7
20-40	26	27	69	68	.10	.10	6	10	11	11	.6	.6	3	4	.7	2.8					
40-60	26	33	68	82	.10	.13	15	18	13	15	.6	.7	9	6	.7	3.3	152	3.8	2.6	117	1.6
60-80	27	38	74	102	.10	.16	9	26	14	20	.6	.8	11	9	.9	4.4					
80	Renal arterial infusate changed to contain 10 μg/ml (5.5×10^{-5} M) acetylcholine chloride in .85% saline.																				
80-100	37	31	108	84	.49	.12	95	18	27	17	1.8	.8	82	8	3.4	4.5	151	3.8	2.7	121	1.7
100-120	30	29	95	75	.49	.11	100	15	24	17	1.7	.7	88	9	4.9	4.7					
120-140	30	31	90	81	.64	.10	128	11	29	18	2.1	.7	118	6	6.4	5.7	156	3.7	2.5	120	1.9
140-160	30	32	97	82	.57	.10	128	11	31	21	2.0	.6	118	6	8.4	6.7					
160	Renal arterial infusate changed to .85% saline.																				
160-180	19	28	48	71	.16	.11	26	10	17	18	.6	.5	23	5	4.0	7.1	148	3.7	2.5	121	2.0
180-200	29	32	75	79	.16	.11	24	13	22	21	.7	.6	23	6	5.5	8.0					

* Continuous infusions of inulin (15 mg/min) and p-aminohippurate (2.5 mg/min) were given throughout the experiment following appropriate priming doses of each. The acetylcholine containing and control infusates were isosmotic. E = experimental; C = control kidney. 20 kg dog. (Dog No. 9 in Table II.)

TABLE II. Mean Changes in Hemodynamic and Excretory Functions Induced in Both Kidneys by Infusion of Acetylcholine into Renal Artery of One Kidney.*

Dog No.	Hemodynamics GFR, ml/min E	GFR, ml/min C	ERPF, ml/min E	ERPF, ml/min C	Urine excretory rates H_2O, ml/min E	H_2O, ml/min C	Na, μEq/min E	Na, μEq/min C	K, μEq/min E	K, μEq/min C	Ca, μM/min E	Ca, μM/min C	PO_4, μM/min E	PO_4, μM/min C	Cl, μEq/min E	Cl, μEq/min C
1	-4	-5	- 5	-13	.22	-.11	50	-19	0	-15	.3	0	0	-2.8	41	-11
2	-1	-6	18	-16	.98	-.12	163	-19	13	- 6	.9	- .5	2.9	.3	172	-14
3	0	-1	15	- 2	.49	-.01	92	- 1	7	- 1	.2	.1	.3	- .5	89	1
4	3	-3	19	-14	1.60	.04	257	4	19	- 1	3.0	.2	4.4	- .8	238	6
5	3	-3	12	- 7	2.58	-.34	344	-37	16	1	2.9	- .1	3.5	- .8	315	-28
6	3	1	37	4	2.35	.13	420	13	47	7	4.2	.4	6.9	1.0	412	-16
7	3	-3	12	-17	1.93	-.25	347	-61	26	- 5	3.5	- .7	4.0	-2.4	322	-23
8	3	-4	22	- 5	1.17	-.01	220	- 2	6	3	3.0	0	6.4	-1.5	207	1
9	5	-3	27	- 6	.46	-.03	108	- 4	17	4	1.3	0	5.7	2.1	100	1
10	7	0	36	8	.30	-.01	55	- 1	31	0	.6	- .1	5.6	.6	43	0
Mean	2.2	-2.7	19.3	-6.8	1.21	-.07	205.6	-12.7	21.2	-1.3	1.99	-.07	3.97	-.48	193.9	-8.3
S.E. (±)	.99	.68	3.91	2.67	2.75	.04	42.0	6.9	4.58	1.96	.47	.1	.75	.48	40.4	3.7
P	.05-.1	.01-.001	<.001	.05-.1	.01-.001	.1-.2	<.001	.1-.2	.01-.001	.5-.6	.01-.001	.5-.6	<.001	.3-.4	.001	.05-.1

* Changes in infused kidney for each experiment appear under E and represent the difference between mean values obtained during and prior to acetylcholine infusion. Similarly calculated changes for non-infused kidney appear under C. The first period during acetylcholine infusion was omitted in these calculations to minimize dead-space collection errors.



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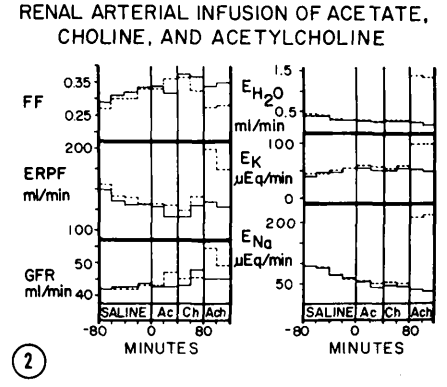

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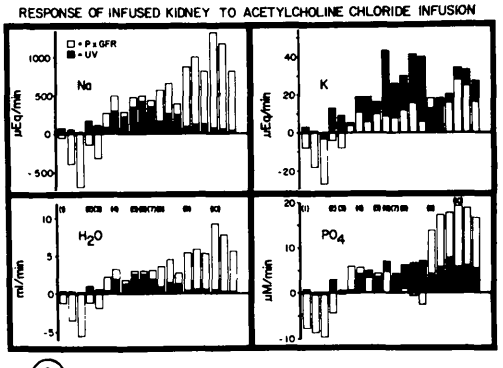

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FIG. 1. Renal arterial infusion of acetylcholine and atropine. Dashed lines refer to the right (experimental) kidney and solid lines to the left (control) kidney. Vertical line at zero time indicates substitution of acetylcholine-containing solution for saline. Vertical line at 120 min indicates substitution of atropine-containing solution for saline. Vertical lines at 140 and 160 min indicate substitution of acetylcholine-containing solutions. S = saline; A-1 = acetylcholine chloride, 12.3 μg/min; A-2 = acetylcholine chloride, 123 μg/min; AT = atropine sulfate, 46 μg/min.

FIG. 2. Renal arterial infusion of acetate, choline and acetylcholine. Dashed lines refer to right (experimental) kidney and solid lines to left (control) kidney. Ac = sodium acetate, 9.47 μg/min; Ch = choline chloride, 9.47 μg/min; Ach = acetylcholine, 12.3 μg/min.

FIG. 3. Comparison of simultaneously determined changes in filtered load and excretion rate in the experimental kidney during acetylcholine infusion. See text for details.

water excretion increased in 3 experiments in which GFR decreased or was unchanged (Dogs 1, 2, 3).

C. *Plasma composition.* Acetylcholine did not alter significantly plasma concentrations of sodium, calcium, chloride or phosphate. The mean change for all experiments was +0.7 mEq/l for sodium, 0 mM/l for calcium and phosphate and +0.8 mEq/l for chloride. A slight mean decrease of 0.1 mEq/l for potassium was statistically significant ($P<0.001$) but of doubtful biological significance.

Atropine sulfate. Atropine sulfate blocked the renal response to a previously effective Ach dose in each of 4 experiments, but a 10-fold increase of Ach dose again evoked a diuresis. A representative experiment is shown in Fig. 1. Acetylcholine infusion increased excretion of sodium, potassium, calcium, phosphate and water by the infused kidney with little or no change in the non-infused kidney. Atropine sulfate affected none of these functions but did abolish the response of GFR, sodium, potassium and water to Ach and diminished the response of ERPF, calcium and phosphate. When the Ach dose was increased 10-fold, all functions again increased in the infused kidney.

Choline and acetate. When choline chloride or sodium acetate, in doses equimolar to the effective Ach dose, were infused, no changes occurred in the infused kidney (Fig. 2).

Cholinesterase inhibitors. Three cholinesterase inhibitors, neostigmine methyl sulfate, physostigmine sulfate and DFP, were infused into the renal artery in graded doses. Although the largest dose of each drug induced bradycardia and hypotension, no unilateral changes in hemodynamic or excretory functions were observed. In each experiment in which these agents were ineffective, Ach produced its characteristic effects.

Discussion. These experiments demonstrate that Ach acts directly on the kidney to increase calcium and phosphate excretion, and

they confirm earlier reports(3,4) that the drug increases GFR, ERPF and excretion of sodium, potassium, chloride and water. The renal responses are a specific Ach effect as shown both by the failure of choline and acetate to alter renal function and by the blocking action of atropine sulfate.

Renal hemodynamics. Renal arteriolar vasodilation, evidenced by a rise in GFR and ERPF, probably involved both afferent and efferent glomerular arterioles since ERPF increased more than GFR. The decreased GFR and ERPF observed in the non-infused kidney of most experiments may have been secondary to vasodilation in the infused kidney. Assuming that cardiac output was constant, the maintenance of arterial blood pressure despite lowered resistance and increased blood flow in the infused kidney would require compensatory increased resistance and decreased flow elsewhere in the arterial circuit. The control kidney might be expected to participate in these changes.

Excretory changes. Acetylcholine could have increased water and solute excretion by increasing GFR, decreasing net tubular reabsorption, or both. In several experiments, the increase in excretory rate exceeded the increase in filtered load, indicating a change in tubular activity. Fig. 3 summarizes simultaneous changes in filtered load and excretory rate in the infused kidney and was constructed in the following way. Mean excretion rate and filtered load during saline infusion periods were subtracted from the corresponding value for each Ach infusion period, omitting the first Ach period to minimize dead-space collection errors. Each bar represents the change from mean control in one experimental period, clear bars indicating filtered load and solid bars indicating excretion rate. Both functions were plotted from the zero baseline, values above the line indicating an increase and values below the line, a decrease, from control values. Each bar consists of 2 superimposed functions so that when the solid area exceeds the clear area, excretion rate increased more than filtered load, and when the clear area exceeds the solid area, filtered load increased more than excretion rate. The bars are arranged from left to right in the order of experiments listed in Table II and numbers in parentheses indicate the dog number. In the 20 experimental periods, excretion rate increased more than filtered load in 5 periods for sodium, 18 periods for potassium, 5 periods for water, and 7 periods for phosphate. Furthermore, excretory rate increments approached closely filtered load increments in an additional 5 periods for sodium, one period for potassium, 3 periods for water and 3 periods for phosphate. Since an increase in excretion attributable to an increase in filtered load cannot exceed the change in filtered load, and since increments in excretion rarely approach increments in load, these results suggest that the excretory changes were not secondary solely to increased filtered load. Also since both phosphate and sodium reabsorption are predominantly proximal tubular functions, it might be anticipated that changes in excretion of the 2 ions would be comparable if a rise in GFR were the only means by which excretion rates were increased. However, this was not the case.

The increased water excretion may be secondary to increased solute excretory load or may represent a direct tubular action of the drug on water reabsorption.

The increased calcium and phosphate excretion suggests that Ach acts at a proximal tubular site and stop-flow studies support this view(3). The effect on calcium excretion is of particular interest since some evidence indicates that Ach may act elsewhere by altering membrane permeability to calcium ions (6).

The rise in potassium excretion may be secondary to the increased phosphate excretory load, increased distal tubular sodium-potassium exchange or a specific action of Ach on distal tubular potassium secretion.

The influence of changes in renal blood flow on tubular functions is poorly understood but Pinter *et al*(4) have postulated that excretory changes may be secondary to increased renal blood flow. However, the proposition that Ach exerts a direct tubular action is consistent with its actions on secretory cells elsewhere in the body, such as salivary glands and pancreas, and with its actions on secretory organs of other species, such as the salt gland of marine birds(7).

Atropine sulfate. Atropine's blocking ac-

tion could be reversed by large Ach doses, indicating that atropine did not act as a nonspecific tubular toxin. The lack of response to atropine alone argues against, but does not exclude, the presence of endogenous renal tubular Ach. Urine flow was low in these hydropenic animals and it would be difficult to demonstrate the expected antidiuretic action of atropine. Since pentobarbital is an inhibitor of acetylation(8), Ach synthesis may have been impaired also, thereby masking any response to atropine.

Cholinesterase inhibitors. The anticholinesterases, neostigmine, physostigmine and DFP, were all inactive, even when infused to the point of systemic toxicity. Systemic toxicity excludes possible renal destruction of the drugs and their inactivity may represent either absence of endogenous Ach or failure to reach cellular cholinesterase. It is also possible that renal cholinesterase is resistant to the drugs used and the presence of eserine-resistant esterases in mammalian renal tubules (9) supports this alternative explanation.

The observations reported here strengthen the view, developed by several workers in recent years(10-12), that cholinergic mechanisms may be regulators of cellular cation transport systems. The wide distribution of cholinesterases in tissues of several animal species(13), the abundant supply of cholinergic nerve fibers to exocrine organs, and the evidence that Ach mediates nerve impulse transmission by altering membrane permeability(14) make such an hypothesis an attractive one.

Summary. Infusion of Ach into one renal artery of the dog induced unilateral increase in calcium and phosphate excretion and increased GFR, ERPF and excretion of sodium, potassium, chloride and water. Products of Ach hydrolysis, choline and acetate, were inactive. Atropine blocked the renal responses to Ach but exerted no renal actions in the absence of exogenous Ach. Several anticholinesterases failed to alter renal hemodynamic or excretory functions. The excretory changes produced by Ach were not explicable on the basis of changes in GFR alone and indicate that Ach exerted a direct renal tubular action. The data constitute further evidence that cholinergic mechanisms may be regulators of cellular cation transport systems.

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