JCI The Journal of Clinical Investigation

The importance of aerobic metabolism in the renal concentrating process

Edward Weinstein, … , Andrzej Manitius, Franklin H. Epstein

J Clin Invest. 1969;48(10):1855-1861. https://doi.org/10.1172/JCI106151.

Research Article

The extent to which the concentrating function of the kidney depends on oxidative processes was investigated by infusing cyanide into one renal artery of dogs undergoing mild mannitol diuresis while receiving an infusion of vasopressin. This produced an abrupt fall in concentrating capacity ($T^c_{H_2O}$) that was reversed when the cyanide infusion was stopped. The change could not be accounted for by the accompanying solute diuresis, since it was not reproduced by increasing the rate of mannitol infusion. The reduction in $T^c_{H_2O}$ induced by cyanide did not result from increased delivery of dilute urine to the collecting ducts, since free water clearance (C_{H_2O}), studied in other dogs during water diuresis, was unchanged or decreased by cyanide. Cyanide produced renal vasodilatation, as did intraarterial acetylcholine, but in contrast to the striking reduction in concentrating capacity evoked by cyanide, $T^c_{H_2O}$ was not significantly changed by acetylcholine.

The data indicate that concentrating ability is closely tied to oxidative metabolism in the kidney, and it is suggested that the region where this is critically important is the red medulla and the thick ascending limb of Henle's loop.

The Importance of Aerobic Metabolism in the Renal Concentrating Process

EDWARD WEINSTEIN, ANDRZEJ MANITIUS, and FRANKLIN H. EPSTEIN

From the Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06510

ABSTRACT The extent to which the concentrating function of the kidney depends on oxidative processes was investigated by infusing cyanide into one renal artery of dogs undergoing mild mannitol diuresis while receiving an infusion of vasopressin. This produced an abrupt fall in concentrating capacity ($T^c_{H_2O}$) that was reversed when the cyanide infusion was stopped. The change could not be accounted for by the accompanying solute diuresis, since it was not reproduced by increasing the rate of mannitol infusion. The reduction in $T^c_{H_2O}$ induced by cyanide did not result from increased delivery of dilute urine to the collecting ducts, since free water clearance (C_{H_2O}), studied in other dogs during water diuresis, was unchanged or decreased by cyanide. Cyanide produced renal vasodilatation, as did intra-arterial acetylcholine, but in contrast to the striking reduction in concentrating capacity evoked by cyanide, $T^c_{H_2O}$ was not significantly changed by acetylcholine.

The data indicate that concentrating ability is closely tied to oxidative metabolism in the kidney, and it is suggested that the region where this is critically important is the red medulla and the thick ascending limb of Henle's loop.

INTRODUCTION

The ability of the kidneys to concentrate urine is impaired in several clinical disorders in which blood flow and oxygen supply to the kidney is reduced (1). Since concentrating capacity is largely a function of the renal medulla, it has been suggested that a portion of the energy supply of the medulla, necessary to pump sodium out of the ascending loop of Henle and thus contribute to the concentrating process, is dependent on oxidative metabolism (2). On the other hand, it is quite clear that the inner medulla of the kidney in most species is poorly equipped with mitochondria and oxidative enzymes (3) and derives much if not all of its energy from glycolysis (4, 5). The extent to which the concentrating function of the kidney depends upon oxidative processes is therefore uncertain.

An attempt was made to answer this question in the present experiments by inhibiting oxidative metabolism in the kidney of the dog with the infusion of cyanide into its renal artery. This resulted in a profound fall in concentrating capacity ($T^c_{H_2O}$) that could not be accounted for by the accompanying solute diuresis. The data indicate that concentrating ability is closely tied to oxidative metabolism in the kidney, and it is suggested that the region where this is critically important is the red medulla and the thick ascending limb of Henle's loop.

METHODS

Experiments were performed on male and female mongrel dogs (10–27 kg) which were fed a regular diet, supplemented by 0.5 lb. of meat daily. The animals were anesthetized with sodium pentobarbital (36 mg/kg) intravenously, and then studied under conditions of osmotic or water diuresis for measurement of $T^c_{H_2O}$ or C_{H_2O}, respectively.

In the first set of experiments (six dogs) a constant infusion of 0.5% inulin and 20 U of aqueous vasopressin in 1000 ml of normal saline was begun at 1.0 cc/min and continued throughout the experiment. After bilateral flank incisions through which both ureters were cannulated with polyethylene tubing, a 23 gauge needle connected to plastic tubing was inserted in the direction of flow into one renal artery (usually the left) near its origin at the aorta. Isotonic saline was infused at a constant rate of 0.5 cc/min through this needle via a Harvard infusion pump (Harvard Apparatus Co., Inc., Dover, Mass.). To minimize the influence of changes in solute excretion per se on $T^c_{H_2O}$, the experiments were conducted against a background of moderate mannitol diuresis produced by infusing a 5% solution of mannitol in 0.9% saline at 5.0 cc/min throughout the experiment. $T^c_{H_2O}$ had thus reached a plateau at the time that cyanide was given. Urine was collected from each

Dr. Weinstein was a Research Trainee, Metabolism Training Grant AM-5015, U. S. Public Health Service.

Received for publication 14 April 1969 and in revised form 6 June 1969.

ureter at intervals of 10–15 min and serum sampled at the midpoint of alternate collection periods. Once constant urine flows were achieved (usually within 1–2 hr after starting mannitol), the experiments were subdivided into four parts of at least three clearance periods each. *Part I:* Control clearances with 0.9% saline infused into the renal artery at 0.5 cc/min. *Part II:* A 12 mM solution of NaCN dissolved in normal saline was infused into the renal artery at 0.5 cc/min. This delivered 6 μmoles of NaCN/min to the infused kidney. In two animals the rate was subsequently doubled to deliver 12 μmoles of NaCN/min. Cyanide could not be infused more rapidly than that for any prolonged period without killing the dog. *Part III:* Normal saline was reinfused into the renal artery. *Part IV:* While continuing the normal saline infusion into the renal artery, the intravenous infusion of mannitol in saline was increased to 16.2 cc/min to increase osmolar clearance and produce a solute diuresis.

The second set of experiments (six dogs) was performed under conditions of water diuresis using hypotonic glucose. The protocol was similar to the first set of experiments with the following exceptions. No pitressin was used, and mannitol in saline was replaced by 2.5% glucose in water. This was infused intravenously, first as a loading dose designed to deliver 5–10% of the body weight in 60–90 min, then slowed to exceed urinary losses slightly. A minimum of 2 hr elapsed before urines became hypotonic and clearance measurements were begun. These experiments were subdivided into three parts, with infusions into the renal artery as described above. Parts I and III served as controls, and during part II NaCN was infused at 6 or 12 μmoles/min.

To study the effects of renal vasodilatation on $T^c_{H_2O}$ under conditions of osmotic diuresis, acetylcholine was infused into one renal artery in a third set of experiments (five dogs). Instead of placing a needle in one renal artery, catheters were placed by the retrograde femoral approach into one renal artery and the ipsilateral renal vein, so that *p*-aminohippurate (PAH) extraction across the kidney could be measured. The arterial catheter had inner and outer diameters of 0.054 and 0.094 cm, respectively, and the venous catheter 0.085 and 0.110 cm, respectively. In addition, an aortic catheter was inserted by the retrograde femoral route for measurement of mean aortic blood pressures, via a transducer recorder and oscilloscope. In addition to constant infusions of inulin, vasopressin, and mannitol, an intravenous priming dose of 0.06 cc/kg of 20% (PAH) was given, followed by a constant infusion of 0.4 cc/kg of 20% PAH in 250 cc saline at 1.0 cc/min. After the urine flow became relatively stable and control observations were made, a solution of acetylcholine containing 8 mg/100 ml of 0.9% saline was infused at 0.5 cc/min into the renal artery, to deliver 40 μg of acetylcholine/min to one kidney.

In a fourth group of experiments in which four dogs were prepared in a similar fashion, cyanide was infused at 6 μmoles/min into one renal artery to measure the effect of

TABLE I

Representative Experiment Illustrating the Effect of Cyanide on $T^c_{H_2O}$

	V		U_{Osm}		U_{NaV}		GFR		C_{Osm}		$T^c_{H_2O}$	
Time	R	L	R	L	R	L	R	L	R	L	R	L
min	*ml/min*		*mOsm/kg H_2O*		*μEq/min*		*ml/min*		*ml/min*		*ml/min*	
	0.5% inulin in saline (20 U aqueous pitressin/1000 cc) begun at 1.0 cc/min. Both ureters catheterized. 5% mannitol in saline begun at 5.3 cc/min. Pentobarbital anesthesia 7.8 cc intravenously.											
102–112	0.9	1.4	871	707	143	230	39.3	39.1	2.60	3.28	1.50	1.88
119–143	Needle inserted into left renal artery—normal saline infusion begun at 0.5 cc/min.											
143–158	1.5	3.5	708	490	170	506	40.8	37.4	3.47	5.60	1.97	2.10
158–173	2.2	2.9	586	527	282	401	39.4	37.9	4.20	4.98	2.00	2.08
173–188	2.1	3.2	597	531	256	436	45.7	46.4	4.06	5.50	1.96	2.30
197–198	12 mM NaCN infusion begun into left renal artery at 0.5 cc/min (6 μmoles/min).											
208–218	1.3	9.1	725	346	86	1095	45.4	49.5	3.04	10.20	1.74	1.06
218–228	1.3	7.6	700	341	73	922	43.3	42.9	2.94	8.36	1.64	0.76
228–238	1.3	8.2	678	342	66	971	40.6	43.0	2.84	9.05	1.54	0.85
239–240	Normal saline restarted into left renal artery at 0.5 cc/min.											
255–265	1.45	3.80	689	474	85	484	40.3	40.9	3.21	5.79	1.76	1.99
275–285	1.50	3.60	692	501	85	464	42.9	39.0	3.31	5.74	1.81	2.14
285	5% mannitol in saline infusion increased to 16.2 cc/min.											
295–305	3.3	7.9	538	398	212	856	40.4	43.3	5.55	9.83	2.25	1.93
305–315	4.3	9.8	478	382	321	1046	39.2	45.1	6.38	11.6	2.08	1.83
315–325	4.2	10.2	463	373	302	1120	34.5	33.2	6.02	11.8	1.82	1.58

Abbreviations: GFR = glomerular filtration rate; R = right; L = left; $T^c_{H_2O}$ = concentrating capacity. Dog No. 305, weight 13.2 kg.

TABLE II

Effect of Cyanide and of Osmotic Diuresis upon $T^c_{H_2O}$

	Control		NaCN		Recovery		Mannitol-saline	
	C	E	C	E	C	E	C	E
Urine flow, *ml/min*	3.54 ±0.55 (6)	4.12 ±0.71 (6)	2.74 ±0.62 (6)	6.54* ±0.76 (6)	2.31 ±0.37 (5)	4.40 ±0.64 (5)	3.92 ±0.73 (4)	8.78* ±1.28 (4)
$U_{Na}V$, *μEq/min*	433.4 ±87.8 (6)	526.4 ±89.0 (6)	270.8 ±95.1 (6)	768.7* ±93.9 (6)	191.2 ±51.1 (5)	520.4 ±96.9 (5)	300.8 ±103.0 (4)	962.5* ±199.0 (4)
C_{In}, *ml/min*	40.29 ±2.92 (6)	40.15 ±4.21 (6)	38.92 ±1.97 (6)	35.08 ±4.09 (6)	37.73 ±3.82 (5)	38.21 ±5.99 (5)	34.50 ±4.78 (4)	42.14 ±7.86 (4)
C_{Osm}, *ml/min*	5.23 ±0.52 (6)	5.57 ±0.81 (6)	4.32 ±0.71 (6)	7.03* ±0.82 (6)	3.76 ±0.40 (5)	5.86 ±0.83 (5)	5.45 ±0.89 (4)	10.19* ±1.67 (4)
$T^c_{H_2O}$, *ml/min*	1.67 ±0.30 (6)	1.62 ±0.20 (6)	1.60 ±0.12 (6)	0.48* ±0.14 (6)	1.45 ±0.19 (5)	1.46 ±0.30 (5)	1.53 ±0.35 (4)	1.54 ±0.53 (4)
$T^c_{H_2O}$/GFR, ×100	4.10 ±0.54 (6)	4.02 ±0.33 (6)	4.20 ±0.45 (6)	1.33* ±0.47 (6)	3.87 ±0.18 (5)	3.63 ±0.45 (5)	4.25 ±0.56 (4)	3.08 ±0.77 (4)

Numbers represent mean ±SE. The number of experiments is shown in parenthesis. Three clearance periods were averaged to obtain a single value for each portion of every experiment. $P < 0.01$; C = control kidney; E = experimental kidney, infused with cyanide.
* Significantly different from control period.

this procedure on the extraction of O_2 and PAH by the kidney.

All plasma and urine samples were analyzed for osmolality with an osmometer (Advanced Instruments, Inc. Newton Highlands, Mass.). Sodium and potassium were determined with a flame photometer (model 143, Instrumentation Laboratory Inc., Watertown, Mass.) using lithium as an internal standard. Alkali-stable inulin was determined by the method of Walser, Davidson ,and Orloff (6) and PAH by the Bratton-Marshall reaction (7). Oxygen content of blood was measured with the Van Slyke apparatus.

RESULTS

Effect of infusing cyanide into the renal artery upon concentrating ability (Tables I and II, Fig. 1). Cyanide is rapidly converted in the body to thiocyanate and it was, therefore, possible to observe a selective effect of intra-arterial cyanide in the infused kidney that could be compared with function on the opposite side and reversed when the infusion was stopped. Cyanide

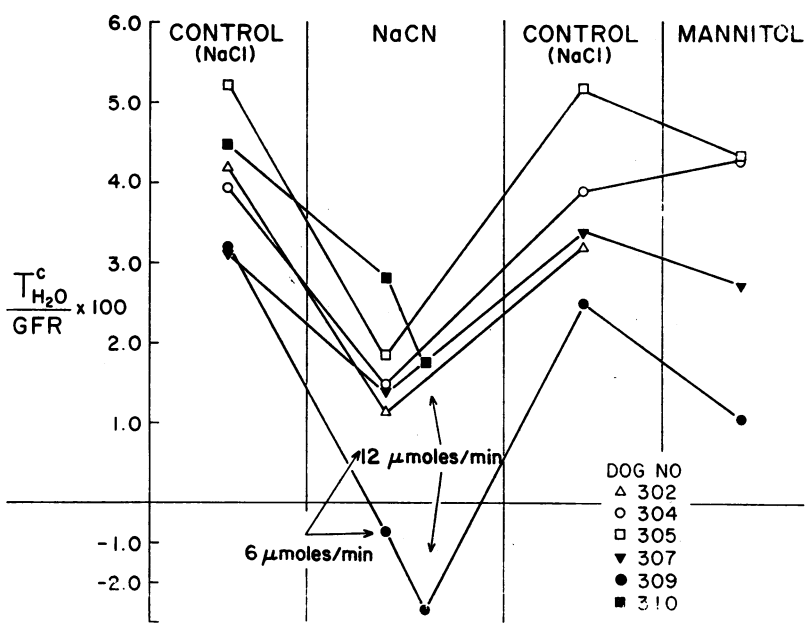

FIGURE 1 Effect of cyanide and of mannitol-saline diuresis upon concentrating capacity ($T^c_{H_2O}$). Cyanide infused into the renal artery decreased $T^c_{H_2O}$. The decrease in concentrating ability was not duplicated by osmotic diuresis induced with an infusion of mannitol in saline.

caused an immediate increase in urine flow on the infused side. Sodium excretion increased 2- to 3-fold, while falling slightly in the uninfused kidney. Osmolar clearance likewise increased to approximately twice the value of C_{osm} determined simultaneously on the opposite side. The average glomerular filtration rate (GFR) did not change significantly.

During cyanide infusion, $T^c_{H_2O}$ promptly fell to about one-third of its control level. Concentrating capacity in the opposite kidney was unchanged. When the infusion of cyanide was interrupted, $T^c_{H_2O}$ returned to normal. The impairment of concentrating ability produced by cyanide was not a simple consequence of solute diuresis, for when solute excretion was increased to the same level by a more rapid infusion of mannitol and saline, $T^c_{H_2O}$ did not fall.

Effect of cyanide on free water clearance (C_{H_2O}) during water diuresis (Tables III and IV). In the dog, urine leaving the distal tubule is not normally in osmotic equilibrium with blood, even when antidiuretic hormone is present (8). It was, therefore, conceivable that cyanide might have decreased $T^c_{H_2O}$ by increasing the formation of dilute urine by the distal tubule, and thereby increasing the delivery of dilute urine to the medullary collecting ducts. If such a process were operative, free water clearance should have been aug-

TABLE III

Representative Experiment Illustrating the Effect of Cyanide on C_{H_2O}

	V		U_{Osm}		$U_{Na}V$		GFR		C_{osm}		C_{H_2O}	
Time	R	L	R	L	R	L	R	L	R	L	R	L
min	*ml/min*		*mOsm/kg H_2O*		*μEq/min*		*ml/min*		*ml/min*		*ml/min*	
0–39	Pentobarbital anesthesia 8.5 cc i.v. 0.5% inulin in saline begun at 1.0 cc/min. 2.5% glucose infusion at 11.0 cc/min. Both ureters catheterized.											
73–75	Glucose infusion decreased to 6.8 cc/min.											
50–80	0.10	0.53	972	655	14	75	18.0	37.2	0.33	1.19	−0.23	−0.66
86–105	Glucose infusion decreased to 2.8 cc/min. Needle inserted into left renal artery and intra-arterial infusion of normal saline begun at 0.5 cc/min.											
124–157	Glucose infusion increased to 5.6 cc/min.											
156–166	1.80	3.55	165	185	50	209	35.5	36.0	1.05	2.32	0.75	1.23
167–170	Glucose infusion increased to 7.5 cc/min.											
166–176	2.6	4.1	145	170	88	235	31.7	30.3	1.33	2.46	1.27	1.64
179	Glucose infusion increased to 8.1 cc/min.											
176–186	3.2	4.6	128	152	109	283	31.0	32.6	1.46	2.49	1.74	2.11
186–196	3.9	4.85	107	127	110	194	32.5	32.6	1.50	2.21	2.40	2.64
196–206	3.9	4.8	86	110	83	162	32.8	30.3	1.21	1.91	2.69	2.89
206–216	3.95	4.55	80	99	71	131	32.3	32.0	1.14	1.63	2.81	2.91
217–223	12 mM NaCN infusion begun into left renal artery at 0.5 cc/min (6 μmoles/min). Glucose infusion increased to 9.5 cc/min.											
218–228	3.10	6.95	77	177	47	511	26.9	31.5	0.86	4.46	2.24	2.49
228–238	3.00	5.70	86	176	46	406	32.3	29.5	0.93	3.63	2.07	2.07
238–248	2.85	6.05	89	195	46	487	30.1	30.8	0.92	4.27	1.93	1.78
249–250	Normal saline restarted into left renal artery at 0.5 cc/min.											
250–260	3.2	5.9	77	132	39	285	31.2	31.5	0.91	2.86	2.29	3.04
267	Glucose infusion decreased to 8.0 cc/min.											
260–270	3.50	4.65	68	93	42	126	30.1	28.6	0.88	1.61	2.62	3.04
270–280	3.80	4.50	65	77	44	90	31.3	29.2	0.93	1.30	2.87	3.20
280–290	3.90	4.30	60	59	49	68	30.6	25.6	0.89	0.96	3.01	3.37
292–293	12 mM NaCN restarted into left renal artery at 1.0 cc/min (12 μmoles/min).											
292–302	3.8	4.2	64	130	48	200	28.4	24.2	0.92	2.08	2.88	2.12
302–307	3.7	2.6	60	145	40	150	29.7	16.2	0.84	1.43	2.86	1.17
307–312	3.2	1.5	66	182	32	118	30.0	9.72	0.80	1.03	2.40	0.47

Dog no. 322, weight = 14.8 kg.

TABLE IV

Effect of Cyanide on Free Water Clearance (C_{H_2O})

	Control		NaCN		Recovery	
	C	E	C	E	C	E
Urine flow, *ml/min*	4.03 ±0.42 (6)	3.31 ±0.42 (6)	3.39 ±0.58 (6)	4.66 ±0.40 (6)	3.47 ±0.73 (6)	2.46 ±0.45 (6)
C_{H_2O}, *ml/min*	2.91 ±0.42 (6)	2.26 ±0.32 (6)	2.42 ±0.55 (6)	1.75 ±0.39 (6)	2.36 ±0.73 (6)	1.55 ±0.41 (6)
C_{Osm}, *ml/min*	1.12 ±0.08 (6)	1.05 ±0.18 (6)	0.97 ±0.09 (6)	2.91* ±0.46 (6)	1.10 ±0.17 (6)	0.91 ±0.16 (6)
GFR, *ml/min*	34.2 ±5.14 (6)	30.7 ±4.4 (6)	33.5 ±6.3 (6)	28.7 ±5.3 (6)	33.5 ±6.02 (6)	27.3 ±4.95 (6)
C_{H_2O}/V	0.71 ±0.03 (6)	0.69 ±0.03 (6)	0.68 ±0.06 (6)	0.38* ±0.11 (6)	0.61 ±0.10 (6)	0.60 ±0.08 (6)

Numbers represent mean and SE. The number of experiments is shown in parenthesis. Three clearance periods were averaged to obtain a single value for each portion of every experiment.

* $P < 0.05$; C = control kidney; E = experimental kidney, infused with cyanide.

mented by cyanide; instead it was unchanged or slightly diminished. This finding is especially significant in view of the fact that solute diuresis produced by saline infusions, for example, normally augments C_{H_2O} so that the fraction C_{H_2O}/V is constant or increases (9). In the present experiments, C_{H_2O}/V fell in every case, from an average of 0.69 to a mean of 0.38, when cyanide was infused.

Renal vasodilatation produced by cyanide. Comparison with the effects of intraarterial acetylcholine (Tables V and VI). Intraarterial infusions of 6 μmoles of cyanide/min increased renal plasma flow in four dogs from 136 ±16 to 220 ± 51 ml/min without altering systemic arterial blood pressure significantly. Renal extraction of PAH was depressed to 35% from a mean control value of 60%. Renal oxygen uptake, measured in three dogs, was decreased by an average of 43% (range 25–63%).

It seemed possible that renal vasodilatation and the consequent increase in renal blood flow produced by cyanide might be responsible for the changes in concentrating capacity we observed. Accordingly, similar experiments were carried out in which acetylcholine was infused into the renal artery (Table VI). The increase in renal plasma flow produced by acetylcholine (from 168 ±22 to 277 ±25 ml/min) was comparable to that induced by cyanide, and, as with cyanide, systemic blood pressure did not change appreciably. There was a slight increase in urine flow, osmolar clearance, and sodium excretion. GFR was not significantly altered. In contrast to the striking reduction in concentrating ability produced by cyanide, $T^c_{H_2O}$ was not significantly changed by acetylcholine.

DISCUSSION

The importance of oxidative processes in the metabolism of the renal medulla is a matter of some controversy, though it is generally conceded that the major fuel of the medulla is glucose, and the predominant metabolic process is glycolysis (4, 5). Mitochondria and mitochondrial enzymes of oxidation are found in much greater abundance in the cortex than in the medulla (3, 10). Nevertheless, some mitochondrial material is present in cells lining the thin loops of Henle (10), and some oxygen is presumably utilized by the medulla in vivo, to account for the lowered partial pressure of oxygen at the tip of the renal papilla (11) and in the urine (12). Although slices of medulla convert glucose almost quantitatively to lactic acid (4, 5), a small uptake of oxygen can be demonstrated in vitro (2). Interestingly enough, in the intact kidney of the hamster, only 25% of the glucose disappearing from vasa recta blood in its journey through the medulla can be accounted for by the appearance of lactate (13). An

TABLE V

Effect of Cyanide on Renal Hemodynamics

	Control	Cyanide
GFR, *ml/min*	21.4 ± 5.3	17.7 ± 5.1
C_{PAH}, *ml/min*	84.4 ±17.3	76.0 ±21.2
E_{PAH}	0.60 ± 0.06	0.35 ± 0.05
RPF, *ml/min*	136.3 ±15.7	220.7 ±51.4

Values represent mean and SE of four experiments. Clearances shown are those of the single kidney receiving an intraarterial infusion of cyanide. The abbreviations used are PAH, *p*-aminohippurate and RPF, renal plasma flow.

TABLE VI

Effect of Acetylcholine on $T^c_{H_2O}$ and Renal Hemodynamics

	Control		Acetylcholine	
	C	E	C	E
Urine flow, *ml/min*	4.46 ± 0.51	4.31 ± 0.28	4.01 ± 0.68	6.04 ± 1.28
GFR, *ml/min*	38.0 ± 2.56	33.3 ± 2.29	40.1 ± 4.06	38.8 ± 4.72
C_{PAH}, *ml/min*	118.5 ±12.6	104.9 ± 9.25	118.4 ±14.7	144.4 ±14.6
E_{PAH}		0.66 ± 0.05		0.52 ± 0.03
RPF, *ml/min*		168.6 ±21.8		277.4 ±25.0
C_{Osm} *ml/min*	6.35 ± 0.45	5.85 ± 0.46	5.89 ± 0.63	7.42 ± 1.43
$T^c_{H_2O}$, *ml/min*	1.89 ± 0.29	1.54 ± 0.31	1.87 ± 0.29	1.38 ± 0.39

Values represent mean and SE of five experiments. Clearances are shown as in previous tables. Calculation of renal plasma flow and extraction ratios could only be made in the kidney infused with acetylcholine, since renal venous blood was not sampled in the other kidney. C=control period; E=experimental period, during the acetylcholine infusion.

important difference between studies of kidney slices in vitro and of whole kidneys *in situ* is that under the latter circumstances, the tubules are perfused by glomerular filtrate and transport sodium actively while in slices the tubular lumen is collapsed and tubular reabsorption of sodium is minimal. It seems possible, therefore, that oxygen utilization by medullary tissue might be more prominent in the intact kidney than in kidney slices. Windhager was able to abolish the intraluminal electrical potential in the ascending limb of Henle's loop in the intact kidney of hamsters by perfusing the tubule with potassium cyanide to inhibit oxidative metabolism, as well as by perfusing with iodoacetate, an inhibitor of glycolysis (14). Cyanide might thus be expected to inhibit the formation of a concentrated urine by interfering with the active transport of sodium out of the loop of Henle, upon which process the osmotic gradient between cortex and medulla depends.

While some part of the action of cyanide in reducing renal concentrating capacity may have been localized to thin loops of Henle in the papilla (white medulla), it seems reasonable that a major effect was on the thick ascending limbs of the loop, located in the red, or outer, medulla. Cells of the thick ascending limb are well supplied with mitochondrial and mitochondrial oxidative enzymes (15). Slices of the outer medulla of the dog utilize oxygen actively, and are similar in this respect to cortex, rather than to inner medulla (16). It is in the outer medulla that the largest increment in tissue concentration of sodium is observed along the gradient from cortex to papillary tip (17), and it is probably here that the major portion of sodium reabsorption responsible for $T^c_{H_2O}$ is carried out. Inhibition of oxidative metabolism driving the active transport of sodium in this part of the kidney should therefore reduce $T^c_{H_2O}$, as we observed. A diminution in diluting ability might also be predicted. In the present experiments, small decreases in free water clearance were seen after the infusion of cyanide. These must be interpreted in light of the associated solute diuresis caused by cyanide. Increased delivery of proximal urine to distal portions of the nephron usually results in an increase in C_{H_2O} proportional to the increase in urine volume (9). The fall in C_{H_2O}/V seen after cyanide indicates that the diluting capacity of the thick ascending limb and distal convoluted tubule had been substantially impaired by the infusion.

Though cyanide is an inhibitor of many enzymes, especially those containing heavy metals, its predominant effect in the concentrations used here is probably on cytochrome oxidase, which is exquisitely sensitive to inhibition by cyanide (18). Cyanide and other inhibitors of electron transport like azide, amytal, ubiquinone, and hydroxylamine have been shown previously to inhibit sodium reabsorption and oxygen consumption by the kidney (19–22). A major site of action is in the proximal tubule (21), although Herms and Malvin deduced from stop-flow studies that cyanide retarded sodium transport in the distal portion of the nephron as well (22). Herms and Malvin also reported that urinary osmolality was decreased when cyanide was infused into the renal artery of dogs, but it was not clear from their experiment whether this might not have been a result of osmotic diuresis, an explanation excluded by the present data.

The effect of cyanide to reduce renal vascular resistance and increase renal blood flow might conceivably have changed concentrating ability by increasing the rate at which sodium was removed from the medulla by the circulation, thereby interfering with the development of a corticomedullary concentration gradient for sodium. This explanation seems unlikely because acetylcholine, which has a similar vasodilating action when infused into the renal artery (23), did not reduce $T^c_{H_2O}$.

The concentrating function of the kidney is evidently dependent on both glycolytic and oxidative processes. Inhibition of anaerobic glycolysis in vivo is known to reduce urinary osmolality without greatly changing the excretion of sodium (22). The present experiments indicate that renal concentrating ability can be profoundly disturbed if oxidative metabolism of the kidney is inhibited. The results are relevant to certain clinical situations in which blood flow and oxygen supply to the medulla may be selectively reduced and concentrating ability appears to be disproportionately affected. Examples include sickle-cell anemia (24) and hydronephrosis (25). Despite the well-known capacity of the papilla for glycolysis, oxidative metabolism must proceed unimpeded if the concentrating function of the renal medulla is to operate efficiently. Selective ischemia of the medulla probably impairs the formation of a concentrated urine by interfering with the production of energy derived from oxidative processes for use in the active transport of sodium by cells of the thin and thick portions of the ascending limb of Henle's loop.

ACKNOWLEDGMENTS

This work was aided by Grants HE-00834 and AM-5015 from the National Institutes of Health, U. S. Public Health Service. Dr. Epstein is a recipient of a Research Career Award 4-K6-AM-21,578.

REFERENCES

1. Epstein, F. H. 1966. Disorders of concentrating ability. *Yale J. Biol. Med.* **39**: 186.
2. Bernanke, D., and F. H. Epstein. 1965. Metabolism of the renal medulla. *Amer. J. Physiol.* **208**: 541.
3. Kean, E. L., P. H. Adams, H. C. Davies, R. W. Winters, and R. E. Davies. 1962. Oxygen consumption and respiratory pigments of mitochondria of the inner medulla of the dog kidney. *Biochim. Biophys. Acta.* **64**: 503.
4. Kean, E. L., P. H. Adams, R. W. Winters, and R. E. Davies. 1961. Energy metabolism of the renal medulla. *Biochim. Biophys. Acta.* **54**: 474.
5. Lee, J. B., V. K. Vance, and G. F. Cahill, Jr. 1962. Metabolism of C^{14} labeled substrates by rabbit kidney cortex and medulla. *Amer. J. Physiol.* **203**: 27.
6. Walser, M., D. G. Davidson, and J. Orloff. 1955. The renal clearance of alkali-stable inulin. *J. Clin. Invest.* **34**: 1520.
7. Goldring, W., and H. Chasis. 1944. Hypertension and Hypertensive Disease. Commonwealth Fund, New York. 203.
8. Clapp, J. R., and R. R. Robinson. 1966. Osmolality of distal tubular fluid in the dog. *J. Clin. Invest.* **45**: 1847.
9. Eknoyan, G., W. N. Suki, F. C. Rector, Jr., and D. W. Seldin. 1967. Functional characteristics of the diluting segment of the dog nephron and the effect of extracellular volume expansion on its reabsorptive capacity. *J. Clin. Invest.* **46**: 1178.
10. Rhodin, J. 1958. Electron microscopy of the kidney. *Amer. J. Med.*, **24**: 661.
11. Aukland, K., and J. Krog. 1960. Renal oxygen tension. *Nature (London).* **188**: 671.
12. Washington, J. A., II, and J. M. Holland. 1966. Urine oxygen tension: effects of osmotic and saline diuresis and of ethacrynic acid. *Amer. J. Physiol.* **210**: 243.
13. Ullrich, K. J., G. Pehling, A. Ruiz-Guinazu, and M. Espinar-Lafuente. 1961. Veränderung des Blutes bei der Passage durch das vasculäre Gegenstromsystem im Nieren-mark. *Pflügers Arch. Gesamte Physiol. Menschen Tiere.* **274**: 64.
14. Windhager, E. E. 1964. Electrophysiological study of renal papilla of golden hamsters. *Amer. J. Physiol.* **206**: 694.
15. Wachstein, M. 1955. Histochemical staining reactions of the normally functioning and abnormal kidney. *J. Histochem. Cytochem.* **3**: 246.
16. Ullrich, K. J., and G. Pehling. 1958. Aktiver Natriumtransport und Sauerstoffverbrauch in der äusseren Markzone der Niere. *Pflügers Arch. Gesamte Physiol. Menschen Tiere.* **267**: 207.
17. Ullrich, K. J., and K. H. Jarausch. 1956. Untersuchungen zum Problem der Harnkonzentrierung und harnverdünnung. *Pflügers Arch. Gesamte Physiol. Menschen Tiere.* **262**: 537.
18. Dixon, M., and E. C. Webb. 1964. Enzymes. Longmans, Green & Co. Ltd. London. 2nd edition. 337.
19. Strickler, J. C., and R. H. Kessler. 1963. Effects of certain inhibitors on renal excretion of salt and water. *Amer. J. Physiol.* **205**: 117.
20. Fujimoto, M., F. D. Nash, and R. H. Kessler. 1964. Effects of cyanide, Q_o, and dinitrophenol on renal sodium reabsorption and oxygen consumption. *Amer. J. Physiol.* **206**: 1327.
21. Chertok, R. J., W. H. Hulet, and B. Epstein. 1966. Effects of cyanide, amytal, and DNP on renal sodium absorption. *Amer. J. Physiol.* **211**: 1379.
22. Herms, W., and R. L. Malvin. 1963. Effect of metabolic inhibitors on urine osmolality and electrolyte excretion. *Amer. J. Physiol.* **204**: 1065.
23. Earley, L. E., and R. M. Friedler. 1966. The effects of combined renal vasodilatation and pressor agents on renal hemodynamics and the tubular reabsorption of sodium. *J. Clin. Invest.* **45**: 542.
24. Perillie, P. E., and F. H. Epstein. 1963. Sickling phenomenon produced by hypertonic solutions: a possible explanation for the hyposthenuria of sicklemia. *J. Clin. Invest.* **42**: 570.
25. Berlyne, G. M., and A. Macken. 1962. On the mechanism of renal inability to produce a concentrated urine in chronic hydronephrosis. *Clin. Sci. (London).* **22**: 315.