Since concentration and secretion of I^{131} diodrast dose used in the standard test requires little kidney function, it is understandable why the renogram appears normal even when significant renal dysfunction exists. If the renal mechanism responsible for secreting diodrast is functioning at its maximum (for example, with Tm doses of diodrast or PAH), the I^{131} diodrast renogram would be expected to show a flat curve or essentially no tubular cell concentration or secretion of the I^{131} diodrast. Plasma level of the competing substance falls as the kidneys secrete it and/or it is distributed in extracellular fluids. Repeat I^{131} diodrast injections should show a gradual return of concentration and secretion by both kidneys. This sequence of events was demonstrated in normal dogs. If the functional capacity of one kidney is decreased the normal control kidney should show an earlier appearance of and a greater concentration and secretion of I^{131} diodrast as compared with the defective kidney. This was also demonstrated in the operative animals in this report.

Studies have been performed on human cases of unilateral and bilateral renal diseases. Currently we are employing a combination of I^{131} hippurate and PAH to eliminate the liver background problem encumbent with I^{131} diodrast(5).

Conclusions. (1) Routine I^{131} diodrast renograms frequently fail to demonstrate significant renal lesions. (2) Initial loading of tubular mechanism responsible for secretion of diodrast by competitive substance (for example, para-amino-hippurate) blocks concentration and secretion of I^{131} diodrast. (3) As tubules secrete the competing substance and plasma level gradually returns toward normal, the control kidney in cases of unilateral disease demonstrates, on successive injections of I^{131} diodrast, earlier appearance of uptake and secretion as well as greater quantitative uptake and secretion of I^{131} diodrast.

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Effects of Intra-Adrenal Infusion of Potassium on Urinary Potassium Excretion in the Dog.* (25980)

A. R. LAVENDER AND THEODORE N. PULLMAN

Dept. of Medicine, University of Chicago School of Medicine, Chicago, Ill.

It is well-known that intravenous infusion of potassium salts induces a prompt increase in urinary excretion rate of potassium. The kaliuresis is effected by a change in renal tubular activity since both glomerular filtration rate and serum potassium remain relatively constant(1). It is not known exactly how infusion of potassium salts provokes this change in renal tubular activity but it seems reasonable to suppose the existence of a site or sites sensitive to alterations in potassium concentration. Several lines of evidence suggest that at least one such site may be the adrenal cortex. There is a positive correlation between potassium intake and aldosterone excretion(2), and aldosterone acts directly on the mammalian renal tubule to increase potassium excretion(3). Furthermore, when potassium concentration is increased in perfusates of isolated beef adrenals, greater amounts of aldosterone are secreted(4). Similar results are obtained by addition of potassium to isolated rat adrenals(5). These observations suggest that potassium acts di-

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rectly on the adrenal cortex to increase aldosterone secretion, aldosterone then inducing kaliuresis. If this interpretation is correct, infusion of a potassium-containing solution into the adrenal blood supply of an intact animal should have a quantitatively greater effect on potassium excretion than infusion of an identical solution into a non-adrenal artery. To test this hypothesis, the adrenal gland of the dog has been infused *in situ* with potassium in high concentration, the results being compared with those obtained when potassium was infused into a non-adrenal artery.

Materials and methods. Healthy mongrel dogs weighing 12 to 20 kg were maintained on a constant diet for at least 2 weeks before an experiment was performed. Food and water were withheld for 18 hours prior to an experiment. All animals were anesthetized with intravenous pentobarbital sodium. A solution containing, per liter, 145 meq of sodium, 5 meq of potassium, 110 meq of chloride, and 40 meq of bicarbonate was administered intravenously in every experiment by a constant-speed infusion pump at the rate of 1.0 ml/min. Urine was collected in graduated cylinders from either ureteral catheters or an indwelling bladder catheter. The femoral artery was cannulated with a polyethylene tube for blood sampling. After placing the animal on its right side, a left flank, subcostal incision and a muscle-splitting procedure were used to expose the retroperitoneal space. A small-bore polyethylene cannula, connected to a second constant-speed infusion pump, was inserted into the lumbar branch of the lumbo-adrenal artery and advanced proximally until its tip was just distal to the origin of the small fan-like adrenal branches. The cannula was anchored with a silk ligature placed around its tip. Infusate was thus carried to the adrenal gland with its normal arterial blood supply. The control series consisted of 5 dogs who underwent a surgical procedure identical with the one used for the experimental animals except that in these sham-operated controls, a non-adrenal artery was cannulated for potassium infusion. Infusion rate in both the adrenal and non-adrenal groups was 0.86 ml/min. After a suitable period of time, during which urine flow became relatively constant, 3 or 4 urine collection periods of 20 minutes each were obtained. The composition of the arterial infusate during these control periods was identical with that of the intravenous infusate. The arterial infusate was then changed to one differing from the control solution by addition of 45 meq of potassium chloride to each liter of solution. Urine collection periods of 20 minutes each were continued for 2 to 3 hours. At the end of each experiment, 1 ml of india ink was injected into the lumbar artery cannula. An experiment was considered technically successful if 75% or more of the adrenal cortex was densely stained with ink. A total of 6 experiments satisfied this criterion. Sodium and potassium were determined in serum and urine by indirect flame photometry, using lithium as an internal standard. The statistical methods used are those described in standard textbooks of statistics.

Results. Serum potassium. The mean serum potassium concentration for all the periods prior to potassium infusion was 3.81 meq/l in the control series and 3.92 meq/l in the adrenal infusion series. (Table I). Serum potassium increased slightly in 4 of the 5 control experiments and in 4 of the 6 adrenal infusion experiments. Mean increase during potassium infusion was 0.35 meq/l in control experiments and 0.17 meq/l in adrenal infusion experiments. The difference between the means for the 2 series could not be proved to be statistically significant (Table II).

Potassium excretion. Potassium excretion increased moderately in 4 of the 5 control experiments and in 5 of the 6 adrenal infusion experiments during infusion of potassium. Mean increase was 23 μeq/min in control series, and 16.3 μeq/min in adrenal infusion series. Although potassium excretion increased in both groups, the difference between the 2 groups could not be proved to be statistically significant.

K/Na ratio. There was no consistent change in urinary K/Na concentration ratio in either group during potassium infusion. The mean changes of 0.44 in control experiments and −0.24 in adrenal infusion experi-

TABLE I. Renal Effects of Systemic and Intra-adrenal Infusion of Potassium in Dog.

Dog No.	Serum K (meq/l) Control	Exp.	Δ	Urine K (μeq/min.) Control	Exp.	Δ	Urine K/Na Control	Exp.	Δ	Urine Na (μeq/min.) Control	Exp.	Δ
					Systemic infusion exp.							
1	3.73	4.40	+.67	55.6	82.7	+27.1	.50	.98	+ .48	110.4	84.6	−25.8
2	3.69	3.55	−.14	33.4	68.3	+34.9	1.66	.73	− .93	20.1	92.9	+72.8
3	3.67	4.29	+.62	22.4	49.3	+26.9	2.73	7.03	+4.30	8.2	7.0	− 1.2
4	4.07	4.35	+.28	44.1	36.9	− 7.2	.88	.94	+ .06	50.3	39.1	−11.2
5	3.87	4.22	+.35	22.6	55.9	+33.3	3.70	2.00	−1.70	6.1	27.9	+21.8
Mean	3.81	4.16	+.35	35.6	58.6	+23.0	1.89	2.34	+ .45	39.0	50.3	+11.3
					Adrenal infusion exp.							
6	3.96	4.07	+.11	55.8	52.9	− 2.9	.38	.47	+ .09	145.5	103.9	−41.6
7	4.37	4.31	−.06	59.1	83.8	+24.7	1.47	.79	− .68	40.1	107.4	+67.3
8	3.93	4.08	+.15	54.8	91.9	+37.1	4.00	1.79	−2.21	13.6	50.7	+37.1
9	3.80	3.99	+.19	58.5	73.0	+14.5	.95	1.63	+ .68	61.9	44.8	−17.1
10	3.83	3.79	−.04	40.5	52.6	+12.1	2.18	1.82	− .36	18.6	28.3	+ 9.7
11	3.61	4.29	+.68	14.1	26.2	+12.1	4.17	5.24	+1.07	3.3	5.0	+ 1.7
Mean	3.92	4.09	+.17	47.1	63.4	+16.3	2.19	1.96	+ .23	47.2	56.7	+ 9.5

ments could not be proved to be statistically significant.

Sodium excretion. There was no consistent change in sodium excretion in either group during infusion of potassium. The mean change was an increase of 11.5 μeq/min in control series and 9.5 μeq/min in adrenal infusion series. The change could not be proved to be statistically significant in either series.

Discussion. There can be little doubt that infusion of a solution containing 50 meq/l of potassium at a rate of 0.86 ml/min into an adrenal artery raises potassium concentration in the blood being supplied to that adrenal gland. This concentration is probably on the order of magnitude of 10 meq/l, as can be deduced from the following considerations. Rate of adrenal blood flow in the dog has been reported to be .297 ± .18 ml/kg/min (6). If there were a maximal flow of .5 ml/kg/min, a body weight of 20 kg, a hematocrit of 40% and a plasma potassium concentration of 3.8 meq/l, infusion of potassium in a concentration of 50 meq/l at the rate of .86 ml/min would be expected to elevate potassium concentration in adrenal artery plasma to 9.6 meq/l. This is a conservative estimate of the actual elevation of serum potassium concentration, higher concentrations probably being reached in the smaller animals. If the kaliuretic response to potassium infusion is mediated by the adrenal cortex, elevations in plasma potassium concentration of this magnitude should be a more than adequate stimulus for this response, provided they were not toxic to the gland. A toxic effect is unlikely as shown by the observation that potassium concentrations as high as 29.6 meq/l did not impair aldosterone production in isolated adrenal preparations(5). Since response to intra-adrenal infusion of potassium did not differ significantly from response to non-adrenal infusions, it can be concluded that the augmentation of potassium excretion is dependent upon some other mechanism.

TABLE II. Statistical Comparison of Renal Effects of Systemic *vs* Intra-adrenal Infusion of Potassium in Dog.

Parameter	Paired comparison of systemic *vs* adrenal series Diff.	"t"	P
Serum K (meq/l)	.18	1.008	.3–.4
Urine K (μeq/min.)	6.73	.710	.4–.5
Urine K/Na	.68	.596	.5–.6
Urine Na (μeq/min.)	2.00	.086	>.9

These studies do not exclude the possibility that intra-adrenal infusion of potassium stimulates production of aldosterone, an effect which has been observed *in vitro*. On the other hand, in experiments on isolated calf adrenals(4) and isolated rat adrenals(5), the rise in aldosterone secretion evoked by high potassium concentrations was not quantitatively great. For this reason, Giroud and coworkers(5) suggested that alterations of serum sodium and potassium concentrations may modify aldosterone output by an indirect

action through some other system. Regardless of the effect of serum potassium concentration on rate of secretion of aldosterone these experiments demonstrate that the characteristic kaliuresis accompanying potassium infusion is not dependent upon a potassium-sensitive receptor mechanism in the adrenal cortex.

Summary. 1) A technic has been described whereby the dog adrenal may be infused *in situ* without disturbing its arterial blood supply. 2) Utilizing this technic, infusion into left adrenal arteries of a solution containing high concentrations of potassium did not affect serum potassium concentration or urinary potassium excretion any differently than infusion of similar solution into a non-adrenal artery. It is concluded that the kaliuretic response to potassium infusions is not mediated by a direct action of potassium on the adrenal cortex.

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Electron and Fluorescence Microscopy of Mouse Hepatitis Virus.* (25981)

THEODORE J. STARR,† MORRIS POLLARD, DONALD DUNCAN, MARVIN R. DUNAWAY
Depts. of Preventive Medicine and Public Health, and Anatomy, University of Texas Medical Branch, Galveston

Interest in comparative viral hepatitis stems from lack of methods for propagation and detection of the virus(es) of human hepatitis (1). Since identification of this agent depends on the hazardous exploitation of man, other host-specific viruses associated with hepatitis infections of dog(2), duck(3-5), and mouse (6-9) have been studied as possible prototypes. This communication describes procedures for visualization of the *Eperythrozoon*-free strain of mouse hepatitis virus (MHV 1)(10) by electron and by fluorescence microscopy.

Materials and methods. Newborn Swiss mice were infected *via* the peritoneal route with MHV 1(11). After 5 days, mice were sacrificed and infected livers were examined. Tissue imprints were prepared on coverslips, fixed without air drying in acid-alcohol, and stained with acridine orange fluorochrome at pH 3.8 and with fluorescein-tagged antibody by methods described for psittacosis infection of human cell lines(12). Antiserums were prepared in adult Swiss mice by repeated intraperitoneal inoculation of normal and of infective mouse-liver homogenates(13). Globulin fractions were conjugated with fluorescein isothiocyanate for use in the direct fluorescein-staining procedures(14). Paraffin sections were cleared in xylol, then stained with acridine orange and with fluorescein-tagged antibody. A Reichert Zetopan microscope and illumination system were used with appropriate ultraviolet light source and filters. Other paraffin sections and tissue imprints were stained by routine procedures with hematoxylin-eosin. Preparations of infected liver were fixed in Palade's(15) veronal acetate buffered osmium tetroxide, embedded in methacrylate, and sectioned for examination with an electron microscope (RCA EMU-3C).

Results. Tissue imprints and paraffin sec-

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† Present address: Kaiser Foundation Research Inst., Lab. of Comparative Biol., Richmond, Calif.