anterior hypothalamus and lowered via the posterior "guide barrel" into the arcuate nucleus area. All four were diestrus within 2 days and remained so until sacrificed 1 month later.

Summary. The effect of estrogen-tipped steel tubes has previously been studied with permanent implant methods. An improved technique makes it possible to place and remove 27-gauge estrogen-tipped tubes through a short 22-gauge "guide barrel" permanently implanted in the calvarium. The "guide barrel" is implanted and the animal allowed to recover from the effects of surgery prior to placement of the estrogen tube. Three experiments are described demonstrating the value of this technique: (i) Castrated female rats with estrogen tubes placed in the preoptic–suprachiasmatic region of the anterior hypothalamus exhibited behavioral lordosis and mating in less time than that previously described for rats with permanent tube implants. (ii) Estrogen tubes placed in the estrogen-sensitive center of the posterior hypothalamus of intact, normally cycling female rats for 7 days suppressed the return of normal vaginal cycles for 16–37 days after the tubes were removed. In previous reports with permanently implanted tubes it was not possible to examine the delay in return to normal vaginal cycling following estrogen application to this hypothalamic center influencing gonadotropin release. (iii) Intact female rats with estrogen tubes placed in the anterior estrogen-sensitive center of the hypothalamus showed only temporary interference with normal vaginal cycling.

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Indirect Sodium-Retaining Action of Oxytocin on Dog Kidney (33001)

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The effect of oxytocin on renal salt and water excretion was found in past investigations to depend on the animal species studied, the dose applied, and the basal rate of urine flow. In the rat, injections of 10–100 mU of oxytocin per animal consistently increased renal sodium, chloride, and water excretion (1, 6–9). In the dog, oxytocin injected intravenously in dosage of about 10 mU/kg of body weight induced natriuresis only at low rates of urine flow (2–4,7). Least consistent results were obtained in human studies; some workers were unable to demonstrate any effect of oxytocin on renal water and electrolyte excretion (5,13), while others reported inconstant antidiuresis and antinatriuresis (10). It was usually admitted that in various species oxytocin possesses about 1/100 of antidiuretic potency of vasopressin (3,13).

Brooks and Pickford observed that when oxytocin was injected into the carotid artery

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of conscious dogs, much smaller doses were needed to produce saluresis than those necessary with intravenous injections (2).

Since in some studies oxytocin-induced natriuresis and diuresis occurred with no detectable changes in the glomerular filtration rate, it was concluded that the hormone inhibited renal tubular transport of sodium and water (1,2,4,9).

Though the results of previous studies were sometimes discordant, the conclusion most frequently arrived at was that oxytocin administration results in saluresis and diuresis. In the present study, we demonstrated that infusion of small doses of oxytocin to saline-loaded dogs under barbiturate anesthesia consistently decreased renal sodium and water excretion. The effect on the kidney appeared to be indirect, since an infusion of oxytocin into the left renal artery produced comparable effects of both kidneys, whereas reduction of oxytocin dosage simultaneously abolished sodium retention by the left and by the right kidney.

Methods. In the present experiments male and female mongrel dogs were used, weighing 8–18 kg. The animals were deprived of food 20 hours before study while drinking water was allowed *ad libitum*. They were anesthetized by intravenous injection of sodium *N*-methyl - β - bromallylisopropyl barbiturate (Eunarcon, Riedel A. G.), 50 mg/kg of body weight. Anesthesia was maintained with additional intravenous Eunarcon as necessary.

Urine was collected separately from the left and right kidney. The right ureter was catheterized after exposure through a suprapubic incision. The left kidney was exposed, the left ureter was catheterized, and a thin subcutaneous needle connected with the tubing for alternate infusions of saline and oxytocin was introduced into the left renal artery close to its origin at the aorta. Arterial blood samples were collected through an indwelling femoral artery catheter or needle.

A Y-shaped polyethylene cannula or a thin needle was introduced into the left common carotid artery for alternate infusions of saline and oxytocin. In five dogs, the external carotid artery was ligated just below the division of the common carotid.

After completing the operative procedure, 0.45% saline in 5% glucose solution or 0.9% saline was infused rapidly through a femoral vein catheter until urine flow was at least 1 ml/min per kidney. Futher infusion was carried out in one of the two ways. In some experiments, the rate of infusion was adjusted to produce an approximately stable diuresis and then, after obtaining three control periods, raised 1–2 ml/min. In most experiments, after a stable urine flow had been obtained, the volume excreted by both kidneys in the latest 10-min period, plus the volume of the blood sample, was replaced each time from a 100-ml burette, to maintain body fluid volume as constant as possible.

Left renal or common carotid artery infusion of 0.9% saline at a rate about 0.5 ml/min was started directly after the needle or cannula had been placed in the vessel. Subsequently, when urine flow was at least 1 ml per min per kidney and stable, priming doses of creatinine and sodium *p*-aminohippurate (PAH) were injected, followed by a maintenance infusion containing clearance substances in concentrations appropriate for clearance measurement and also lysin-vasopressin (Sandoz, A. G.) in a concentration which provided the infusion rate of 2 mU/kg per hour. To insure the stability of vasopressin, this solution was acidified to pH 5.2–5.6 with acetic acid.

After 30–40-min equilibration period, urine collection was started. Arterial blood samples were withdrawn in the midpoint of each 10-min clearance period. The exchange of 0.9% saline for oxytocin solution in saline, both given at a rate about 0.44 ml/min, was effected through adjusting distant clamps, without any direct manipulation at the renal or carotid artery. Three control (saline) periods were always collected; the urine from the 10-min period which followed the exchange of saline for oxytocin was discarded and then three or four experimental (oxytocin) periods were obtained. Clearance substances plus vasopressin, as well as oxytocin solutions were infused using motor-driven syringes. The order of the two parts of the experiment, that studying the effect of oxytocin infusion into the common carotid artery (A) and the one

in which the hormone was infused into the left renal artery (B) was varied; experiments of AB and BA type were almost equal in number. At least 40 min elapsed from the end of oxytocin infusion of the first part and beginning control periods of the second part of the experiment.

Two commercial preparations of synthetic oxytocin used in this study were those of Sandoz, A.G., Basel (Syntocinon) and of G. Richter, Budapest. The rate of oxytocin infusion was 5 mU/kg per hour in experimental series I, and 0.5–1.5 mU/kg per hour in series II.

Sodium excretion ($U_{Na}V$) and potassium excretion (U_kV) were calculated in the usual way and expressed in μeq/min. The glomerular filtration rate (GFR) was measured as the clearance of exogenous creatinine, and effective renal plasma flow (ERPF) as the clearance of *p*-aminohippurate. The osmotic clearance (C_{osm}) was calculated as $U_{osm}V/P_{osm}$ and free water clearance as $V - C_{osm}$.

Plasma and urinary sodium and potassium were determined by flame photometry and plasma and urinary osmolality using a Fiske osmometer. Creatinine was determined by the method of Popper *et al.* (11) and PAH by the method of Smith *et al.* (12).

Results. I. Oxytocin infusion (rate 5 mU/kg per hour). The effect of oxytocin on sodium excretion in this series of experiments is presented in Table I. In the period of 30–40 min after beginning oxytocin infusion into the left renal artery, $U_{Na}V$ of the left kidney decreased 49 μeq/min or 38%. Simultaneously, there was a 36 μeq/min or 25% decrease in sodium excretion by the right kidney. Despite a small number of observations (eight and seven, respectively) the differences in $U_{Na}V$ from the initial control periods were, for both the left and right kidney, statistically significant ($p<0.05$). Following oxytocin infusion into the common carotid artery mean sodium excretion fell 27 μeq/min or 17%, but this difference was not significant ($p<0.2$).

The fall in $U_{Na}V$ did not visibly depend on the control rate of sodium excretion, since it occurred with a wide $U_{Na}V$ range of 36–336 μeq/min per kidney. Plasma sodium concentration showed no important changes during the experiments. The changes in urine flow, as summarized in Table II, paralleled those in $U_{Na}V$.

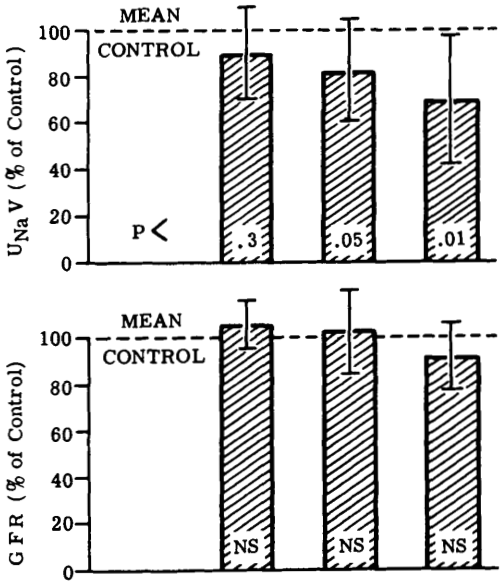

FIG. 1. Changes in sodium excretion ($U_{Na}V$) and glomerular filtration rate (GFR) following oxytocin infusion at a rate of 5 mU/kg per hour (mean values and SD). The data from carotid artery infusions of oxytocin are not included.

The relationship between changes in sodium excretion and in glomerular filtration rate for the two groups of observations which showed significant decreases in $U_{Na}V$ (effects of left renal artery infusion of oxytocin on the left and on the right kidney) is shown in Fig. 1. It indicates that significant changes in sodium excretion in the second and third oxytocin period occurred with no significant changes in glomerular filtration rate.

Effective renal plasma flow (ERPF) tended to fall with the decrease in sodium excretion; while mean $U_{Na}V$ per one kidney for nine observations fell 24 μeq/min or 15% (six decreases and three increases), mean ERPF per one kidney fell 17 ml/min or 18% (seven decreases and two increases).

The behavior of osmotic and free water clearances was examined in 11 instances. It was found that while mean $U_{Na}V$ decreased 39 μeq/min or 35% (eight decreases and three increases) mean C_{H_2O} fell slightly from

TABLE I. Effect of Oxytocin Infusion (rate 5 mU/kg per hour) on Sodium Excretion ($U_{Na}V$) in Dogs under Barbiturate Anesthesia.

No.	Kidney: (L)eft (R)ight	$U_{Na}V$ (μeq/min) Control (mean of 3 periods)	Min from start of infusion 10–20	20–30	30–40	Change in $U_{Na}V$ (μeq/min)	(%)	p value
Left renal artery								
1	L	44	32	22	22			
2	L	74	53	59	65			
3	L	250	199	101	96			
4	L	78	83	52	19			
5	L	212	167	195	209			
6	L	73	62	69	57			
7	L	174	242	170	75			
8	L	126	110	137	103			
Mean (1–8)		130	119	101	81	—49	—38	<0.05
Left renal artery								
9	R	184	119	93	134			
10	R	133	144	26	58			
11	R	79	82	39	21			
12	R	231	166	184	180			
13	R	101	94	89	84			
14	R	86	67	77	49			
15	R	184	221	212	223			
Mean (9–15)		143	128	103	107	—36	—25	<0.05
Common carotid artery								
16	L	326	258	238	233			
17	R	336	270	246	214			
18	L	36	22	24	27			
19	R	36	14	19	25			
20	R	264	287	240	142			
21	L	103	97	109	117			
22	R	173	147	159	174			
23	L	59	70	88	80			
24	R	181	230	250	240			
25	R	70	58	51	56			
Mean (16–25)		158	145	142	131	—27	—17	<0.2

TABLE II. Effect of Oxytocin Infusion (rate 5 mU/kg per hour) on Urine Flow of Dogs under Barbiturate Anesthesia.

Site of infusion	Kidney: (L)eft (R)ight	No. of observations	Urine flow[a] (ml/min) Mean control	Last oxytocin period	Mean change in urine flow (ml/min)	(%)	p value
Left renal artery	L	8	1.59 ± 0.71	1.04 ± 0.77	—0.55	—35	<0.02
Left renal artery	R	7	1.42 ± 0.21	0.95 ± 0.41	—0.47	—33	<0.05
Common carotid artery	L, R	10	1.95 ± 0.65	1.62 ± 0.78	—0.33	—17	<0.2

[a] Mean values ± SD.

TABLE III. Effect of Oxytocin Infusion (rate 0.5–1.5 mU/kg per hour) on Sodium Excretion ($U_{Na}V$) in Dogs under Barbiturate Anesthesia.[a]

Site of infusion	Kidney: (L)eft (R)ight	No. of observations	Mean $U_{Na}V$ (μeq/min) Control (mean of 3 periods)	Mean $U_{Na}V$ (μeq/min) Oxytocin (40–50 min of infusion)	Change in $U_{Na}V$ (μeq/min)	Change in $U_{Na}V$ (%)
Left renal artery	L	12	154	137	−17	−11.1
Left renal artery	R	10	221	233	+12	+ 5.4
Common carotid artery	L, R	22	265	240	−25	− 9.4
	L, R[b]	(6)	(300)	(286)	(−14)	(−4.7)
Totally		44	225	210	−15	− 6.7

[a] The differences in $U_{Na}V$ in individual groups and totally for 44 observations were statistically insignificant.

[b] Data concerning dogs in which the external carotid artery had been ligated are given in parentheses.

0.13 to 0.03 ml/min (five decreases and six increases). There was no correlation of changes in sodium excretion with free water clearance.

Simultaneous measurement of sodium and potassium excretion (U_KV) was carried out in nine instances and showed that while mean $U_{Na}V$ fell 70 μeq/min or 40% (a decrease in each case), mean U_KV decreased 11 μeq/min or 26% (six decreases, two unaltered, one increase).

II. Oxytocin infusion (rate 0.5–1.5 mU/kg per hour). The protocol of experiments in which oxytocin was infused at a rate 0.5–1.5 mU/kg per hour did not differ from that in the previous series except that four instead of three oxytocin periods were collected, so that the last period fell into 40–50 min from the beginning of hormone infusion.

Effects of small oxytocin doses on sodium excretion are summarized in Table III. Although the number of observations (44) was much greater than in the previous series of experiments (25), the differences in sodium excretion between control and oxytocin periods were relatively small and proved statistically insignificant.

Discussion. An intraarterial infusion of synthetic oxytocin at a rate 5 mU/kg per hour (i.e., about 0.08 μg/kg per hour) was found in these studies to inhibit sodium and water diuresis in moderately saline-loaded dogs under barbiturate anesthesia.

Although the average fall in $U_{Na}V$ was relatively largest in the left kidney after oxytocin infusion into the left renal artery, the difference in the response of the two kidneys was not great; decreases for the left and for the right kidney from ipsilateral control periods were statistically significant at the same level of 0.05. Thus, the results would be incompatible with direct action of oxytocin on the kidney unless the dose of 5 mU/kg per hour applied in experimental series I was large enough to produce maximal antinatriuresis, irrespective of the site of hormone infusion into the circulation. This possibility is ruled out by the results of experimental series II in which the dose of oxytocin was reduced to 0.5–1.5 mU/kg per hour. With this rate of infusion, the change in sodium excretion after oxytocin, also that observed in the left kidney following left renal artery infusion, was inconsistent and insignificant. Since blood flow per single kidney approximates 1/10 of the cardiac output, oxytocin infusion into the left renal artery at a rate 0.5–1.5 mU/kg per hour would produce a concentration of the hormone in the left renal artery blood equal or higher than oxytocin concentration in right renal artery blood during left renal artery infusion of oxytocin at a rate 5 mU/kg per hour. However, "fast" infusion resulted in a significant sodium retention by the right kidney while "slow" infusion was without any significant effect on sodium ex-

cretion by the left kidney. It appears, therefore, that the concentration of oxytocin in blood perfusing the kidney is not the factor determining sodium retention after intravascular oxytocin infusion, i.e., that the action of oxytocin on the kidney is indirect.

In their studies of oxytocin action on the kidney of conscious dogs, Brooks and Pickford found that at low urine flow 0.31 mU/kg of oxytocin injected into the carotid artery increased renal sodium and chloride excretion; several times larger doses were needed to obtain this effect with intravenous administration of the hormone (2). They concluded that the saluretic action of oxytocin was in some way mediated by the central nervous system.

In our experiments, the antinatriuretic action of oxytocin was not demonstrable with common carotid artery infusion of oxytocin, not even in dogs in which the external carotid artery had been ligated below the division of the common carotid artery, a procedure which must have further increased the concentration of oxytocin in the blood supplying the brain. It might be speculated that the saluretic component of oxytocin action, found by Brooks and Pickford to be particularly prominent with the hormone injected into the carotid artery, opposed the sodium-retaining component demonstrated in the present study; the net result was no significant sodium retention.

The observation that significant decrease in sodium excretion in the second and third oxytocin periods occurred with no significant changes in glomerular filtration rate (see Fig. 1) suggests very strongly that the mechanism of sodium retention following oxytocin infusion at this dosage was an increase in renal tubular reabsorption of sodium. This conclusion contradicts the previous data indicating saluresis and inhibition of tubular sodium transport by oxytocin (1,2,4,9). In our laboratory also intravenous injections of oxytocin in the dose of 10 mU/kg of body weight were found to produce natriuresis and diuresis in both conscious and anesthetized dogs (unpublished observations). Possibly, increased sodium excretion occurs with single injections of relatively large doses of oxytocin, the usual method of the hormone administration in past investigations, while constant infusion of small doses is associated with sodium retention.

The physiological significance of oxytocin in the renal handling of sodium is unknown; however, the present observation that a consistent decrease in sodium excretion can be elicited with an infusion at a rate as low as about 0.08 μg/kg per hour suggests that the hormone could have some part in the control of sodium excretion by the kidney.

Summary. The action of synthetic oxytocin on renal sodium excretion was studied in dogs under barbiturate anesthesia given a moderate saline load. Left renal artery infusion of oxytocin at a rate 5 mU/kg per hour produced comparable and significant decreases in sodium excretion by the left and by the right kidney. Since reduction of oxytocin dose to 0.5–1.5 mU/kg per hour simultaneously abolished sodium retention by the infused and the contralateral kidney, it was concluded that oxytocin's action on the kidney was indirect. The observation that postoxytocin decrease in sodium excretion occurred with no significant changes in glomerular filtration rate suggested very strongly that the cause of sodium retention was an increase in renal tubular sodium reabsorption. These findings contradict most of the previous data which indicated saluresis and inhibition of tubular sodium transport by oxytocin, and suggest that oxytocin may have differential effects, depending on dosage and mode of administration. The mechanism of antinatriuretic action of oxytocin is unknown.

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Transcholesterin Titers and Their Biological Significance in Experimental Atherogenesis in Rats* (33002)

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A previous report(1) described the development of a distinctive reaction line between components of human serum and cholesterol when subjected to immunoelectrophoresis in an agar gel. The specificity of the reaction was indicated by the fact that the line was neither elicited by cholesterol esters, close homologues, a variety of other steroids nor by the vehicle, Teepol "L". It was suggested that the reactive globulins, later found to be alpha-1 and alpha-2 globulins,[2] represent a natural cholesterol transport similar in principle to homeostatic mechanisms of transcortin, transferrins, haptoglobins, and natural antibodies; the term "transcholesterin" was proposed(1). Both the whole serum and lipoprotein-free serum demonstrated the reaction line in the regions of alpha globulins.[2] Furthermore, it was found that whole sera could be titrated in the transcholesterin assay with serial dilution of the sera in the same manner as antibodies are quantitated.

The studies of Neff *et al.*(2) on the development of atherosclerosis including coronary involvement in rats on an atherogenic diet and on various high-fat diets were repeated to provide serum samples for testing the reactivity of transcholesterin from such animals at different periods in the dietary and pathogenetic regimens.

The present paper reports the alterations in titers of transcholesterin and the total serum cholesterol values of rats on experimental diets in comparison with those on stock chow.

Materials and Methods. Sera of rats on experimental diets. Whole sera from young adult male Sprague-Dawley strain rats with an average weight of 200 gm on the four dietary regimens—high-fat(3) (peanut oil), high-fat(3) (butter), atherogenic(3,4), and stock chow, were assayed for transcholesterin titers without previous knowledge of their dietary regimens or their cholesterol levels. The numbers representing the serum samples were decoded after the procurement of the experimental values.

Total cholesterol determination. A modified Liebermann-Burchard procedure(5) utilizing the Bausch and Lomb Spectronic 505 spectrophotometer to read the density of the color at wavelength 640 mμ was adapted for cholesterol quantitation. The reproducibility of the analytical procedure was good with a standard deviation of ± 5%. The error was sometimes greater than ± 5% if the serum samples were thick and creamy (such as those obtained in rats during the later stages of

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