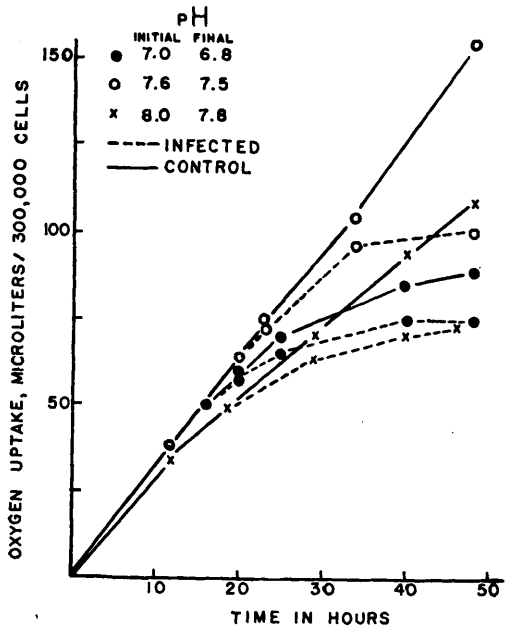

FIG. 1. Oxygen uptake of infected and control HeLa cell cultures at initial pH of 7.0, 7.6 and 8.0.

virus titrations should be carried out in adequately buffered media and that reduction of medium pH below 7.0 during incubation should be corrected. It is also apparent that in screening potential chemotherapeutic or chemoprophylactic agents the effect of pH should be considered. A test agent could act upon cells to increase glycolysis. The resultant increased acid production would thus lower virus yield to simulate specific interference with virus production.

Summary. The pH of tissue culture medium was altered purposefully to measure quantitatively the effects upon virus yield and host cell metabolism. By use of strain HeLa cells and poliovirus Type 1 (strain Mahoney) for growth in media of pH from 6.3 to 8.0 it was established that virus yield was affected by the pH of the culture medium. Decreased virus yield at decreased or increased pH levels was related to a decrease in metabolic rate of host cell cultures.

1. Enders, J. F., *Ann. Rev. Microbiol.*, 1954, v8, 473.

2. Syverton, J. T., and Scherer, W. F., *Ann. N. Y. Acad. Sci.*, 1954, v58, 1056.

3. Gifford, G. E., Robertson, H. E., and Syverton, J. T., *J. Cell and Comp. Phys.*, 1956.

4. Gifford, G. E., Robertson, H. E., and Syverton, J. T., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 515.

5. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.

6. Fogh, J., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 494.

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Chronic Catheterization of the Renal Artery: Technic for Studying Direct Effects of Substances on Kidney Function.* (22744)

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During the course of studies on the pathogenesis of sodium retention in experimental congestive heart failure(1) it was shown that sodium retention occurred in dogs with normal basal glomerular filtration rates, suggesting the presence of increased tubular reabsorption of sodium. In order to obtain more direct information concerning the increased tubular reabsorption of sodium, a method has been developed for chronic catheterization of one renal artery, with collection of urine from each kidney individually. Such a technic affords the opportunity of studying the direct effect of salt solutions, hormones and drugs injected directly into the renal artery of the unanesthetized dog, using the opposite kidney as a control. Furthermore such experiments are relatively uncomplicated by extrarenal factors, and allow the separation of renal from

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extrarenal effects in the unanesthetized dog. The presence of the catheter in the renal artery has not altered the glomerular filtration rate of that kidney, the renal plasma flow, the daily urine volumes, daily sodium excretions, or the response of the kidney to intravenous loads of sodium chloride.

Methods. Technic of urine collection from each kidney individually is a modification of the method described by Desautel.§ The dog, under sodium pentobarbital anesthesia, is placed in a supine position. Through a midline suprapubic incision the urinary bladder is exposed, and drained by needle puncture. The neck of the bladder is mobilized and the urethra cut between two heavy ligatures. The bladder is then hemisected in the sagittal plane, starting on the anterior surface, continuing up over the fundus and posteriorly down between the ureteric orifices. The hemisected bladder is then reconstructed into two separate bladder pouches. Each half of the bladder is sutured around a lucite funnel (Fig. 1A). Starting at the urethral end of the bladder the serosal surfaces of each half of the bladder are brought together by mattress sutures, inverting the bladder mucosa; a similar inverting suture is started at the fundal end of the bladder and continued down toward the midportion of the cut surface. The cup of the funnel is inserted into the bladder pouch thus formed and the sutures are continued up to the threaded stem of the funnel. A purse-string suture in the serosa is then drawn tightly around the funnel stem. The stem of each funnel is brought through a stab wound in the abdominal wall 2 to 3 cm lateral to the midline. Omentum is then sutured between the two pouches to reduce the likelihood of fistula formation, and the abdominal incision is closed. A lucite-nut with smoothed edges is screwed on to the stem of the funnel and tightened down to the abdominal wall to hold the bladder snugly against the anterior abdominal wall. A length of rubber tubing is attached to each funnel and is in turn connected to the plastic bags for urine collection (Fig. 1B). Rubber tubing is also attached to the distal end of

§ To be published.

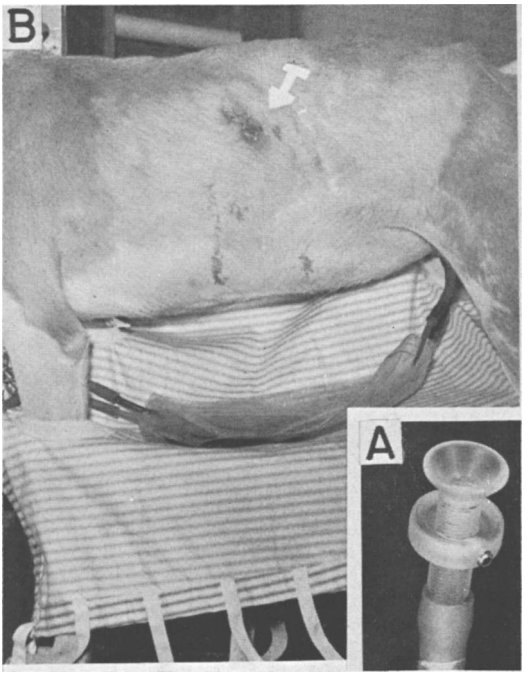

FIG. 1. A. Lucite funnel for urine collection. B. Illustration showing plastic bags attached to lucite funnels, and renal artery catheter secured to skin suture on flank of dog.

the urine bags and closed with Hoffman clamps, which allow fixation to the inside of the canvas jacket which is worn by the dog. The sterilized urine bags are changed daily. After a 7 to 10-day recovery period creatinine, para-amino-hippurate and sodium clearances are measured to assess any possible changes in these functions resulting from the subsequent catheterization of the renal artery. *Technic for implantation of catheter in renal artery.* The dog, anesthetized with sodium pentobarbital, is placed in the right lateral decubitus position. A left flank incision is made below the costal margin and the left kidney exposed. Blunt dissection in the retro-peritoneal space allows mobilization of the aorta for 2 inches above and below the left renal artery. The operation is abandoned if more than a single renal artery supplies the left kidney. Light rubber tubing is passed around the aorta, above and below the root of the renal artery, for counter traction and control of blood loss during subsequent manipulation. A 10-inch length of smooth, polyvinyl catheter is used. This catheter has an inside

diameter of .015 inch and an outside diameter of .028 inch with a total volume of approximately .05 ml. A stainless steel wire is threaded through the catheter to the tip to facilitate handling of the catheter. A purse-string suture is placed on the postero-lateral aspect of the aorta near the origin of the renal artery. The wall of the aorta is then perforated through this purse-string suture with a sharp needle. While countertraction is applied to the rubber tubes around the aorta, a blunt thin-walled curved needle, with a removal plug in the hub, is passed into the lumen of the aorta and across into the orifice of the renal artery and advanced approximately 1 cm. The plug is then withdrawn and the catheter passed through the needle and gently advanced beyond the end of the needle. The needle is cautiously withdrawn while the catheter is held firmly in place. The stainless steel wire is then removed. The purse-string suture around the catheter is then tied for hemostasis. The catheter is flushed with heparin and the external tip clamped. Branching of the renal artery usually occurs within the first inch of its origin from the aorta. To insure the perfusion of the entire kidney with injected substances, the catheter must be withdrawn until its tip lies proximal to the first bifurcation. Palpation of the catheter in the renal artery is not always reliable. Indigo carmine, injected through the catheter, produces a short-lived dark blue discoloration of the portion of the kidney through which the dye passes and sharply delineates the tissues not perfused with the dye. The catheter is then withdrawn 2 or 3 mm after each injection of indigo carmine until a single injection perfuses the entire kidney, indicating that the catheter tip now lies in the main stem of the renal artery and proximal to any bifurcation. The discoloration of the entire kidney also indicates that adequate mixing occurs in the renal artery. The catheter is then secured to the aortic wall. A small loop is left in the retroperitoneal space with further fixation to muscle and subcutaneous tissue. A second loop is left in the subcutaneous tissue and the external tip brought through the skin. The catheter is flushed with concentrated heparin (10,000 units per ml) and closed with a stainless steel obturator inserted into the tip. A fine tie is placed on the catheter over the metal obturator and this is secured to a loop sutured in the adjacent skin. A small protective covering is applied over the end of the catheter before the jacket is drawn on to the animal. The catheter is flushed each day with heparin.

TABLE I. Representative Daily Urine Volume (ml) of the 2 Kidneys before and after Chronic Catheterization.

Preoperative		Postoperative	
Left	Right	Left	Right
390	380	360	305
380	365	175	140
142	145	255	225
290	287	370	320

Results. Successful bladder division and catheter implantation has been performed in 5 normal dogs and 2 dogs with right-sided congestive failure. Such animals have now been followed for periods as long as 5 months without functional or pathological evidence of renal damage.

The ratios of the daily urine volumes of the 2 kidneys have not been altered by the insertion of the catheter into the left renal artery (Table I). Furthermore, the presence of the catheter has not modified the creatinine or par-aminohippurate clearances. In a representative dog (Table II) control creatinine clearances of the left kidney varied from 30 to 38 ml/min; after catheterization values ranged from 29 to 31 ml/min differences which are of the same order of magnitude observed in the right or control kidney. The para-aminohippurate clearances of the left kidney varied from 82 to 89 ml/min before,

TABLE II. Representative Creatinine and Para-aminohippurate Clearances (ml/min.) before and after Chronic Catheterization.

C_{CR}				C_{PAH}			
Pre-operative		Post-operative		Pre-operative		Post-operative	
Left	Right	Left	Right	Left	Right	Left	Right
36	32	29	28	82	79	83	85
38	37	30	31	89	87	90	94
31	28	31	33	85	80	94	95
30	28	31	32	85	81		

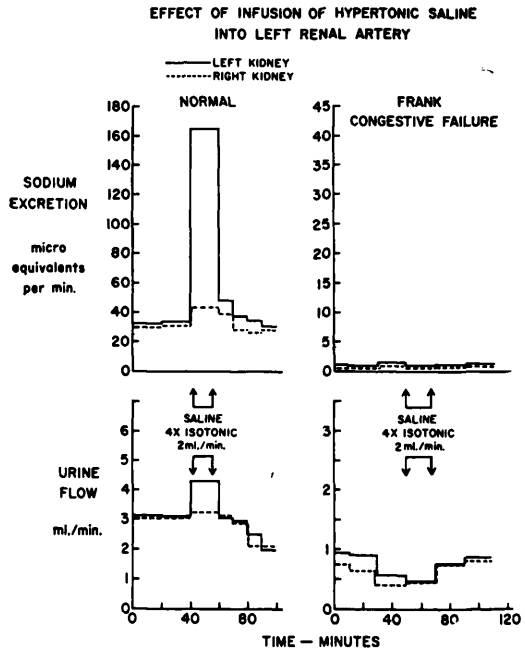

FIG. 2. Effect of infusion of hypertonic saline into left renal artery of normal dog, and dog with congestive heart failure.

and 83 to 94 after, catheterization. Repeated clearance measurements over a period of 5 months have shown no significant changes. The animals were sacrificed at varying intervals after catheterization; neither the control nor catheterized kidneys showed any histological abnormalities. The bladder mucosa was hyperemic and hypertrophied.

With the successful development of the technic for chronic catheterization of the renal artery preliminary studies have been performed on renal tubular reabsorption of sodium in normal dogs and in dogs with chronic congestive heart failure. For example, as indicated in Fig. 2, the infusion of hypertonic saline (580 meq/L) into one renal artery of a normal dog at the rate of 2 ml/min produces a 5 to 10 fold increase in sodium excretion on the injected side with a smaller increment in water excretion. Little or no change is observed in the control kidney. A similar infusion into the renal artery of a dog in congestive failure, with marked sodium retention but a normal basal glomerular filtration rate, does not produce any increase in sodium or water excretion—further evidence for increased tubular reabsorption of sodium and water in congestive heart failure.

Summary. A method has been developed for chronic catheterization of one renal artery, with collection of urine from each kidney separately. The presence of the catheter has not altered renal function over a period of months. Preliminary studies with infusion of hypertonic saline into the renal artery of the dog in congestive heart failure indicate a marked increase in tubular reabsorption of sodium.

1. Barger, A. C., Yates, F. E., and Rudolph, A. M., *Am. J. Physiol.*, 1955, v183, 595.

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Adenosine and Ash as Unidentified Chick Growth Factors in Fish Meal.* (22745)

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Growth stimulation of many microorganisms by adenine and other purines has long been recognized(1). It has also frequently been postulated that in animal nutrition it may be possible that the rate of purine synthesis is inadequate for optimal growth, and at various times adenine and its derivatives have been stated to possess vitamin-like properties. For example, vit. B_4 has been postulated to be adenine and vit. L_2 has been stated to be adenylthio-methyl pentose. Huff and Bosshardt(2) have reported that the growth retardation brought about by the inclusion of succinylsulfathiazole in the diet of mice is

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