

MATERIALS AND METHODS

The experiments were performed on 18 mongrel dogs of both sexes which weighed on 10 to 19 kg. The animals were divided into three groups: Group 1) Renal hypothermia (6 dogs); Group 2) Renal normothermia (6 dogs); and Group 3) Renal compensation (6 dogs). The experiments were started at approximately the same time of the day, about 08:00 - 09:00 a.m. The animals were anesthetized with 30 mg per kg body weight of sodium pentobarbital intravenously and maintained at a surgical plane of anesthesia by administering supplemental doses of the anesthetic as needed. The trachea was cannulated immediately to ensure a patent airway throughout the experiment. The right femoral vein was cannulated with polyethylene tubing (P.E.240) for infusion of solutions. The left femoral vein was also cannulated for infusion of normal saline solution in order to replace surgical losses. The left femoral artery was cannulated with polyethylene tubing (P.E.240) for collection of blood samples and recording of blood pressure with a Statham arterial pressure transducer (Model P23AC) connecting to a Grass polygraph (Model 79D). The kidneys were exposed by an abdominal mid-line incision and both ureters were catheterized with polyethylene tubing (P.E.205) for quantitative collection of urine samples separately. The catheters were inserted as close to the renal pelvis as possible. The left renal artery and vein were gently identified and cleared of fat and surrounding tissues. Normal saline solution, 30 ml per kg body weight was infused in order to hydrate the animals.

The urine flow rate was induced by infusion of 25 per cent mannitol 50 ml, then a priming doses of 1.0 per cent inulin and 0.4 per cent para-aminohippuric acid (PAH) in 25 ml normal saline solution were administered rapidly to raise the plasma concentrations. This was followed by the continuous infusion of a solution containing 0.1 per cent inulin, 0.05 per cent PAH and 5.0 per cent mannitol in 0.6 per cent sodium chloride solution, with the rate of 0.5 - 1.0 ml per minute, to maintain relatively constant levels of plasma inulin and PAH. The animals were allowed to stabilize for 90 minutes before clearance measurements were measured. The urine samples were separately collected for two consecutive 15-minute periods and approximately 8 ml heparinized arterial blood samples were withdrawn at the midpoint of the urine collection periods. Urine volume was measured with measuring cylinder and all blood samples were immediately centrifuged to separate the plasma from cellular elements. Microhematocrits of all blood samples were estimated by Sherwood micro-hematocrit tubes. After the control period, the left renal artery and vein were immediately occluded by a rubber-covered clamp for 60 minutes in renal hypothermic and renal normothermic groups and throughout the experimental period in renal compensation group.

In the renal hypothermia group, iced-saline was applied directly to the left kidney which was placed in the plastic container. The intrarenal temperature was monitored by means of a needle thermocouple (Tele-Thermometer, Yellow Springs, Ohio. range 0 - 50 °C). The thermocouple needle was inserted deeply into the parenchyma of the

kidney throughout the experiment. At the end of 60 minutes the clamp and iced-saline were removed immediately and the first 15-minute period was allowed for flushing stagnant urine out of the kidney before collection of urine samples. Blood and urine samples were collected every 15-minute interval for 90 minutes.

In the renal normothermia group, the animals were performed in the same manner as those in renal hypothermia group with an exception that no iced-saline was applied to the left kidney.

In the renal compensation group, the left renal artery and vein were occluded and the function of the right kidney was determined every 30-minute interval for 120 minutes during the occlusion.

All plasma and urine samples of the renal hypothermia and renal normothermia groups were analyzed for inulin and PAH. In the renal compensation group the plasma and urine samples were analyzed for inulin, PAH, urea nitrogen, osmolality and potassium. The total solid content (refractometer method) and pH (Beckman pH-meter) of urine samples were also determined in this group of animals. Osmolality was estimated by freezing point depression technique using an osmometer (Advanced Osmometer, Model 64-31). Potassium concentration was determined by a Baird-Atomic flame photometer (Model KY). Urea nitrogen content was determined by a Technicon Auto-analyzer. Inulin was analyzed by a modification of the resorcinol-iron method of Kulka (1955). PAH was analyzed by the benzaldehyde method of Brun (1951).

From the measurements of plasma and urinary inulin, PAH, potassium and osmolality, clearances of these substances were computed by using standard clearance formula as follows:

$$C = \frac{U \times V}{P}$$

where C = clearance (ml/min)

U = concentration of a substance in urine (mg/100 ml or $\mu\text{g/ml}$ or mOsm/L)

V = urine flow rate (ml/min)

P = concentration of a substance in plasma (mg/100 ml or $\mu\text{g/ml}$ or mOsm/L).

When the above equation is applied to renal handling of inulin and PAH, they are universally known as glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively. From RPF and hematocrit (Hct.), renal blood flow (RBF) was calculated as follows:

$$\text{RBF} = \text{RPF} \left[\frac{1}{1 - \text{Hct.}} \right]$$

Free water clearance ($C_{\text{H}_2\text{O}}$) was estimated as follows:

$$C_{\text{H}_2\text{O}} = V - C_{\text{osm}}$$

where V = urine flow rate (ml/min)

C_{osm} = osmolal clearance (ml/min)

The following calculations were performed at various plasma concentrations of urea nitrogen and potassium:

Filtration rate = $GFR \times (\text{concentration in plasma})$

Excretory rate = $(\text{urine flow rate}) \times (\text{concentration in urine})$

Reabsorption rate = $(\text{Filtration rate}) - (\text{Excretory rate})$

Secretary rate = $(\text{Excretory rate}) - (\text{Filtration rate})$.

Variations in renal function were expected and subsequent changes were related to the control values. All numerical data are given as means \pm standard error (S.E.). The statistical significance between mean values was assessed using standard statistical method and Student's "t" test.



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