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Standard renal function tests in normal beagles utilizing a computer analysis.

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STANDARD RENAL FUNCTION TESTS IN NORMAL BEAGLES UTILIZING A COMPUTER ANALYSIS

by

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I. INTRODUCTION

The kidney is an organ which has evolved to preserve a constant internal environment within its host. The environs, i.e., the air, may exhibit significant alterations in composition, but homeostasis of the internal environment is imperative. This does not infer that the body fluids are in a static state. Conversely, there is continual exchange occurring as a result of digestion, anabolism, catabolism and excretion. A means of control in either the entry or removal of substances from the body must be established to maintain homeostasis. Animals have some gross control over the entry of substances into their bodies. Organs of excretion have a more refined method of control. Principal function of the kidney is not the excretion of substances from the body but the regulation and control of excretion of these substances.

Three processes enable the kidney to accomplish its task. It is capable of filtration, secretion, and reabsorption. Micropuncture studies, stop-flow analysis, and clearance procedures have proved the existence of these processes. It has also been possible to denote whether many of these functions are active or passive. Many aspects of their exact mechanism of action and interrelationships are still unsolved today. The assessment of renal function is most satisfactorily accomplished by the evaluation of the various kidney functions separately and observing certain interrelationships. Subse-

quent to pathological alterations of the kidney not all parameters of function are affected equally affording us with additional methods of differential diagnosis.

A renal clearance procedure designed to measure several parameters of renal function simultaneously would be of value in veterinary medicine. This investigation was intended to observe as many aspects of renal function as possible. It is realized that the values obtained may not be "basal" observations of renal function but they were planned to be "standard" observations. The conditions of the experiment are closely and easily controlled to decrease the variability in repeated observations. This should facilitate the application of this procedure to the investigation of renal pathology in the dog.

The beagle has frequently been selected as the standard laboratory dog. They have the advantage of size and ease of handling, and they will most likely be employed more frequantly in the future. As many normal studies as possible should be done in the beagle in an attempt to standardize physiological parameters in the dog. There may be no functional differences in the beagle from other dogs but this must be studied before any statement could be made. Normal values are also becoming important in order to facilitate the calculation of mathematical models of renal function.

The choice of an experimental design is important in any experiment. The value of using littermate animals in an

attempt to decrease the animal to animal variation was observed. An attempt was made to determine where the largest variation in renal function parameters occurs. Normal values for osmolar clearance are not normally published since they are subject to alteration by many factors. Conditions altering osmolar clearance have been regulated in this investigation to determine a reproducible osmolar clearance. Osmolar clearance should afford some information regarding solute excretion by the kidney and might be valuable in evaluating canine nephritis during which the specific gravity of urine is altered.

Since the calculation of renal clearances is time consuming, the possibilities of computer analysis were investigated. The statistical analysis was largely accomplished by the computer which permitted the evaluation of a large number of correlations and variations. With the increased possibility of observing many correlations it was planned to compare them with correlations obtained by other investigators.

II. BACKGROUND

A. Renal Function

1. Blood supply

Adequate blood supply and blood pressure are maintained by means of a vascular system designed for glomerular filtration, maintenance of an osmotic gradient in the kidney, and nourishment of all cells. The renal artery of the dog usually bifurcates externally to the renal pelvis and then divides internally into interlobar arteries which branch to form arcuate arteries. Arcuate arteries in younger dogs apparently do not have an arched appearance as noted in the human. From the arcuate arteries the interlobular arteries go to the outer cortex of the kidney giving off branches, afferent glomerular arterioles, almost at right angles (12). This structural configuration is significant in explaining autoregulation of blood flow to the kidney by Kintner and Pappenheimer's theory of plasma skimming (33). Division of the afferent glomerular arteriole results in the formation of the glomerulus which is enveloped in Bowman's capsule, and combined they are entitled "the renal corpuscle" (51). Efferent arterioles arising from juxtamedullary glomeruli, i.e., vasa rectae dip into the renal medulla and accompany a loop of Henle making a similar hairpin turn. This anatomical feature is of consequence since it assists in preserving a concentration gradient of ions in the

renal medulla and also functions as a countercurrent exchange mechanism. Preservation of a concentration gradient is assisted by the sluggish rate of flow of blood in these vessels. Blood flow in the dog kidney medulla is only 0.7 - 1.0 ml/gm/min while cortex flow is 4 - 5 ml/gm/min (51). Efferent arterioles of cortical glomeruli form capillary networks which surround the distal and convoluted tubules of adjacent nephrons and, in addition, supply the loops of Henle in short cortical nephrons. Both the afferent and efferent arterioles contain smooth muscle enabling control of their diameters. The efferent arterioles are considered by most investigators to have a smaller diameter than afferent arterioles, and some believe that juxtamedullary efferent arterioles are larger than cortical ones since they must supply all the cortical vascular supply (12, 51). The venous drainage of the kidney is comprised of stellate veins in the capsule which drain into interlobular veins, then into arcuate veins followed by interlobar veins and subsequently into the major trunk of the renal vein.

RBF (renal blood flow) is not always constant, and it may be altered by several conditions. Examination of the effect of separate parameters is often times difficult owing to many complex interactions. Claude Bernard was the first one to elucidate the effect of sympathetic nervous fibers from the thoracolumbar trunk on the renal vasculature. They initiate

only a vasoconstrictor effect on renal vessels and therefore no vasodilator response (61). Conditions which will activate the sympathetic nervous system will consequently stimulate the renal nerves. This would include states such as fear, pain, and anxiety which, if severe enough, may induce renal ischemia. Alterations of emotional states may produce a significant reduction in RBF but the reduction is not consistent (50).

Epinepherine and norepinepherine, which are liberated into the systemic circulation during periods of sympathetic activity, elevate systemic blood pressure and can elevate or depress the RBF contingent upon the dosage. Infusion of norepinepherine, at rates below an average of 0.0006 mg/kg/min to areflex dogs, provoked increasing RBF which, apparently, was related to an elevation in systemic blood pressure. Support for this was elucidated when the RBF decreased if systemic blood pressure to the kidney was unaltered during norepinepherine infusion. Infusions at higher rates gave this same response (37). This represents a possible confliction to the theory of renal autoregulation of blood flow, which is supported by most investigators (51, 55, 56). Notation should be made of the fact that in this experiment when the blood pressure was elevated there was also a concomitant effect of epinepherine on the intrarenal vessels.

The kidney was shown to demonstrate a constant RBF while the perfusion pressure was increased from 88 mm Hg to 184 mm

Hg (55). This phenomenon is commonly called renal autoregulation. This ability of the kidney is dependent upon some internal alterations of the kidney which is explained by several theories. Unqualified proof for one theory is not available so several will be discussed here.

An increase in renal interstitial pressure, due to increased blood flow which would apply pressure to the peritubular capillaries, might restrict increases in blood flow. Increased pressure of the kidney was measured by inserting a needle into the kidney while perfusion pressure was increased. This procedure could not discriminate between pressure caused by hemorrhage or additional interstitial fluid. Subsequent micropuncture studies did not corroborate the increase in pressure seen by needle measurements (51).

The theory of cell separation and plasma skimming was advanced by Kintner and Pappenheimer (32, 33). This theory proposes that as a consequence of axial streaming of blood in the interlobular artery a larger portion of plasma is skimmed into the first afferent glomerular arterioles than the peripheral vessels. This creates a flow of blood with an increased red blood cell concentration and viscosity to perfuse the outer cortex resulting in increased resistance to flow. Elevation of perfusion pressure would increase axial streaming, viscosity of the peripheral blood, and resistance to blood flow. This theory has been supported by measurements of renal

hematocrits which increase from the medulla to the cortex and inhibition of autoregulation during severe anemia (33). These results have not been obtained by all investigators, and autoregulation was not completely eliminated in a kidney perfused with cell-free dextran solution (51, 69).

Another popular theory of autoregulation is entitled the myogenic theory which proposes that renal resistance is regulated by smooth muscle constriction of the afferent arteriole of the glomerulus. Confirmation of this theory is supported by evidence that autoregulation desists when cyanide, procaine and papaverine are injected into the renal artery. A common consequence of these drugs when injected in adequate concentrations is paralysis of smooth muscle.

Apparently, the kidney is capable of maintaining a constant RBF with some alteration of systemic blood pressure, but it must be remembered that RBF may fluctuate subsequent to sympathetic nerve stimulation, epinepherine and norepinepherine release, and other physiological conditions (47, 50). Doubling of the ERPF (effective renal plasma flow) has been accomplished by increasing the protein content of the diet. The diet was altered from one consisting of 9.4 gm cracker, 7 gm sugar, and 3 gm lard/kg of body weight to a diet furnishing 12 gm of protein/kg of body weight. The ingestion of 2.5 to 3.5% of a dog's body weight in water causes a significant increase in renal blood flow (57). A water load of 1,000 ml

administered per os to 13-17 kg dogs raised the ERPF by 30%. The ERPF could be increased an additional 10% when horsemeat was fed at the rate of 12 gm/kg of the dog's body weight just prior to ERPF measurements (31).

2. Glomerular filtration

Filtration of plasma by the renal glomerulus is necessary for the formation of urine. The glomerular capillary wall is adapted in some way to accomplish ultrafiltration of blood plasma. One hypothesis proposes that ultrafiltration is achieved by the existence of pores in the capillary wall. Electron microscopy has not revealed the existence of pores through the entire wall. Pore size has been estimated by utilizing Poiseuille's equation for fluid flow. These calculations indicate that the detection of these pores by electron microscopy would be difficult owing to their small size and infrequent occurrence. Some investigators advocate that filtration occurs through a gel with an absence of pores.

Three layers of the glomerular capillary have been described, but there is no proof as to which one is responsible for ultrafiltration. The inner lining of the capillary, "lamina fenestra", or "lamina attenuata", has perforations too large to allow ultrafiltration but adequate to prevent the diffusion of cellular elements. A gel without demonstrable porous structure comprises the middle layer or base-

ment membrane. The outer capillary layer consists of epithelial cells with foot-like processes called pedicels which abut on the capillary wall. Since the space between pedicels is 100 A^{o}, it has been proposed that they are the pores for filtration.

The confirmation of ultrafiltration is contingent upon the presence of certain conditions, which have been demonstrated mostly by micropuncture of the renal tubule. These include a glomerular filtrate, essentially protein-free, and containing crystalloid solutes in the same concentration as in plasma and an adequate force present in the form of hydrostatic pressure to permit ultrafiltration. Hydrostatic pressure in the glomerulus must be adequate to exceed the oncotic pressure of the blood and tissue pressure in Bowman's capsule. The effect of tubular fluid oncotic pressure may be neglected since the protein content of tubular fluid is very low.

GFR (glomerular filtration rate) is susceptible to variations of blood pressure within the glomerulus, alterations in plasma oncotic pressure, and modifications of tubular pressure. Changes in systemic blood pressure from 88 mm Hg to 184 mm Hg did not alter the GFR because of the kidney's ability of autoregulation (55). This implies the existence of a mechanism which maintains constant effective filtration pressure in the glomerulus. Conversely, GFR increases when the systemic blood pressure is elevated by the infusion of

norepinepherine. If the systemic blood pressure were unaltered, norepinepherine infusion decreased the GFR (37). Fright and pain, which can cause sympathetic stimulation and epinepherine release, can alter the GFR. Alteration in GFR is not as consistent as RBF alteration due to emotional stress (7, 50). During the measurement of normal renal clearance values in unanesthetized dogs, larger variations in RBF than GFR occurred (47).

Oral intake of large volumes of water has initiated increases in the GFR (31, 57). Alterations in the magnitude of GFR have been shown not to be the major factor influencing urine volume (58). One liter of water administered by stomach tube, 45 minutes prior to the determination of GFR in 13-17 kg dogs on a horsemeat diet, increased the GFR by 30% in contrast to dogs given water ad libidum (31). Sellwood and Verney (57) gave water in doses of 2.5 - 3.0% of the body weight and noted an increase in GFR which began within 15 minutes and was greatest prior to maximal water diuresis. Variations in arterial blood pressure did not explain these results. Changes in GFR were paralleled by RPF (renal plasma flow) adjustments which led these investigators to suggest that vascular resistance was decreased by relaxation of the afferent arteriole. They believed that an increase in pressure in the renal tubule initiated the relaxation of the afferent arteriole. Shannon (58) could not demonstrate any correla-

tion between urine flow from .5 - 4.0 ml/min and the GFR, but he acknowledged the existence of increases in GFR subsequent to the intake of large volumes of water.

Water administration is known to decrease the concentration of some plasma constituents. Within 2 hours after the administration of distilled water (5% of the body weight) at 1/2 hour intervals, the hemoglobin concentration decreased 10%, and with continued water administration it decreased to an average of 15%. The total serum proteins were depressed from an average of 6.8 - 5.9% during this experiment representing a decrease of 15%. The measurement of plasma volume by carbon monoxide and hemoglobin indicated an increase of 14 - 16% (23). More reliable plasma volume measurements can be obtained with T-1824 dye. In one observation by the author on a tranquilized dog given 80 ml of water/kg body weight, the plasma volume doubled for a brief period and returned to normal as water diuresis occurred. Plasma dilution was shown to be two times as great as dilution in the rest of the body when water was administered at the rate of 6.1% of the body weight (3).

Alterations in dietary protein intake are capable of increasing the GFR and also the RBF (29, 31, 45, 51). Protein ingestion caused maximal increases in the GFR within 3 - 4 hours and a reversion to fasting values within 24 hours (45). The consumption of 12 gm of protein, as horsemeat, per kg body

weight, just prior to GFR measurement elevated the GFR by 50% (31). Maximal increases of GFR, as much as 80% over 24-hour fasting values, were observed in dogs on a diet of 12 gm of bread and 50 ml of milk/kg of body weight when they were fed up to 70 gm/kg of body weight of protein just prior to clearance measurements (45). Glomerular filtration rates measured after a 16 - 18 hour fast in dogs with a protein intake of 180 gm/day after consumption of 9.6 gm of protein/day showed a 100% increase (29).

The regulation of GFR by renal tubular pressure or volume has been suggested. The time necessary for occlusion of the proximal tubule following cessation of glomerular filtration exhibited a direct linear correlation to the GFR. It was concluded that the proximal reabsorption of sodium was a tubular maximum type of process independent of but limiting the GFR (40).

Other factors have also been observed which vary the GFR. Position of the dog did not alter the GFR immediately, but a reduction in GFR occurred after 2 1/2 hours with animals in a Pavlov sling but not in those in a supine position (17). Exercise and hyperventilation did not appear to affect the GFR in dogs (7, 30).

3. Tubular secretion and reabsorption

The kidney tubule has the ability to reabsorb and secrete certain substances. The source of some of these substances is endogenous to the body while others are exogenous. The mechanisms responsible for these processes are still unknown. When these processes are passive, no energy is required for them to occur. The transport of chloride is believed to be an example of passive movement since its distribution is controlled by an electrical potential gradient in the proximal tubule (20). Water has also been shown to move passively from the renal tubule (38). During mannitol diuresis the movement of sodium against a concentration and electrical gradient has been shown by micropuncture studies. The amount of sodium transported across the proximal tubule is restricted by the amount of time allowed for reabsorption (51). Glucose reabsorption has been carefully analyzed, and it is evident that reabsorption is limited by the maximal capacity of the tubule to actively reabsorb glucose (60). This is an example of a transport maxima. Other substances which the tubule actively reabsorbs are phosphate, sulphate, malate, lactate, β-hydroxybutyrate, acetoacetate, vitamin C, and certain amino acids. Some examples of materials actively secreted are phenol red, creatinine PAH (p-aminohippurate), penicillin, chlorthiazide, Diodrast, Skiodan, and some strong organic bases.

Creatinine is a substance which has frequently been utilized to measure GFR since its clearance appeared to be identical to that of inulin and therefore was neither secreted nor reabsorbed (59). The creatinine to inulin ratio exceeded unity in an area coextensive with peak PAH to inulin concentration values during stop-flow analysis (48). Variation of the plasma concentration from 15 mg/100 ml to 80 - 450 mg/100 ml depressed but did not abolish creatinine secretion. The higher creatinine levels reduced both PAH free-flow and peak stop-flow clearance ratios possibly due to competitive inhibition of PAH secretion by creatinine. Further evidence for competitive inhibition was observed when the free-flow creatinine clearance ratio decreased from 1.15 to 1.05 after the plasma concentration of PAH was increased from 1.6 to 15 mg/100 ml and the plasma creatinine concentration was 15 mg/100 ml (67). Secretory activity of creatinine has only been exhibited in female dogs when 100 mg of testosterone were administered daily (48).

The secretion of PAH by the renal tubule is limited by a maximal rate (63). This secretion appears to be associated with oxidative processes. Mudge and Taggart (46) were able to increase tubular transport with acetate. The self-depression of the transport mechanism is noticed when the plasma PAH concentration is elevated and may be lessened by the infusion of acetate. The exact mechanism of the tubular

transport depression still remains to be discovered.

Stop-flow and micropuncture studies have provided most of the information for the localization of secretory and reabsorbtive mechanisms in the tubule. Active sodium reabsorption has been attributed to the proximal tubule, but evidence for active reabsorption in the distal tubule has not yet been presented (11, 73). Sodium reabsorption occurs in the distal tubule and is believed to be important for the dilution and concentration of urine (15, 38). No evidence for active urea reabsorption or secretion has been found. Urea is lost from the proximal tubule, but it is added to the descending limb of the loop of Henle. The urea which then enters the collecting duct diffuses into the renal medulla possibly to aid in the countercurrent mechanism (38). Creatinine and PAH are apparently secreted in approximately the same area of the proximal tubule (67).

4. Water and solute excretion

The kidney regulates the conservation and loss of electrolytes and water. A characteristic pattern of water diuresis ensues the oral intake of water by an animal. The principal reason for the increased urine flow is the suppression of ADH (antidiuretic hormone) liberation by the neurohypophysis. Ancillary factors influencing the urinary excretion pattern are GFR and solute load (1, 2, 4, 16, 29, 70). Sub-

sequent to water intake 10 minutes elapsed prior to an increase in urine flow which attained a maximum at 50 - 60 minutes. The absorption of water in the intestine was not governed by the rate of water flow through the pylorus since this was very rapid. Klisiecki et al. (35) proposed that water reabsorption in the small intestine was .028 ml/cm/min from a review of previous literature. The average length of the dog's small intestine was established as 24.7 cm/kg body weight from their and one other worker's investigations. The theoretical absorption time of 250 ml of water administered to a 10 kg dog applying the above values was 36 minutes. A close correlation exists with this time and the time of 35 minutes obtained experimentally. A maximal urine volume of 15.6 ml/kg/hr and a urine osmolar concentration of 78 Mm/l was attained after the administration of water (5 - 6% of body weight) (41). During the course of water diuresis there was a steady decline in electrolyte excretion. In nondiuretic dogs during an initial period of 30 minutes the electrolyte excretion decreased but then remained constant for the next 2 hours. Initial alterations in both conditions may have been due to excitement of the dogs (4).

The volume of glomerular filtrate is greatly decreased during its passage through the tubule necessitating large tubular reabsorption of water. The reabsorption of water appears to be a passive process since it seems to move along

concentration gradients. The proximal tubular fluid was always isosmotic with plasma during water diuresis and antidiuresis (13, 14). The inulin F/P (glomerular filtrate to plasma) ratio at the end of the proximal convoluted tubule increased to a level indicating 60% of the fluid had been removed from the tubule (38). By extrapolation 80% of the glomerular filtrate was predicted to be removed in the entire proximal tubule. Isotonicity of the filtrate was maintained despite the removal of fluid indicating a proportional loss of solute and water. Processes occurring may be inferred by comparing tubular fluid from the end of the proximal convoluted tubule and the beginning of the distal convoluted tubule. The osmolar F/P ratio was less than unity at the origin of the distal convolution, but the inulin F/P ratio had increased. This indicated the removal of both solute and water, but a greater loss of solute. The osmolar F/P ratio at the tip of the loop of Henle in the hamster was greater than unity inferring that water loss occurs in the descending limb and solute reabsorption takes place in the ascending limb of the loop of Henle. In nondiuretic rats the inulin F/P ratio doubled and the osmolar F/P ratio increased but not as much as inulin F/P ratio in the distal tubule. An increase of 40 times in the inulin F/P ratio was observed in the collecting, but the osmolar F/P ratio increased only 6.4 times which was evidence for solute reabsorption in the collecting duct (38).

The countercurrent multiplier and exchange mechanism are thought to enable the formation of a hypertonic urine. A concentration gradient of solute presumably sodium in the renal medulla is created by active sodium transportation out of the loop of Henle, which is relatively impermeable to water. The renal medullary solute concentration is maintained by the countercurrent exchange mechanism operating in the hairpin loops of the blood vessels and renal tubules. The collecting duct which passes through this area has an increased permeability to water in the presence of ADH. Water diffusion occurs because of the large concentration gradient between collecting duct fluid and the renal medullary interstitium. Support for this theory has been obtained by stop-flow experiments in the dog. Two peaks of urine osmolarity were recorded and appeared to be from the area of the collecting duct and the descending limb of the loop of Henle. An increase in creatinine concentration was observed with the distal but not the proximal area of the tubule. The osmolarity between the two osmolar peaks was below that of free-flow urine. The osmolarity peak only in the area of the collecting duct persisted after the administration of ADH (15).

Urea was also capable of enhancing water reabsorption in the renal medulla. The presence of urea in the collecting duct decreased the amount of nonpermeating solute in the tubule. Since urea passed freely through this membrane, more

free water was available for reabsorption (28).

B. Clearance Determinations

1. Creatinine

The clearance procedure is a method employed to evaluate various functions of the kidney by measuring the amount of a substance excreted in relation to its plasma concentration. The product of the urinary concentration of a substance and the rate of urine formation per minute determines the amount of the substance excreted per minute. The division of this quantity by the plasma concentration calculates the amount of plasma which has a specific substance completely removed from it during its passage through the kidney. The process by which the kidney excretes a substance must be known before its clearance has any physiological significance (61). Creatinine is a substance which, until recently, was thought only to be filtered by the glomerulus and not secreted or reabsorbed (59). Recent experiments have proved active creatinine secretion in small quantities in the male dog (48, 67). O'Connell et al. (48, p. 989) said,

> In general these results do not discredit the use of creatinine as a reasonably good approximation of the filtration rate under free-flow conditions, particularly since most studies of renal function in the dog have been carried out on females.

There is always a delay from the time that blood perfuses the kidney until a portion of it appears in the bladder as

urine. An appearance time of 100 seconds was obtained by Morales et al. (44) in the dog. This appearance time is very significant if there are excessive fluctuations in the concentration of the test substance in the plasma. Rapid alterations in the blood concentration of test substances did not affect clearance measurements if arterial blood samples were analyzed and the appearance time considered. During changing plasma levels of the substance venous samples were inaccurate. Constant intravenous infusion eliminated the large variations in plasma concentration, and the renal clearances calculated with arterial and venous samples were almost identical (8). Equilibration of plasma and urine concentrations were calculated to require approximately 25 minutes after instantaneous changes in plasma concentration of a solution. These calculations considered 150 seconds as the minimal appearance time (44). To maintain an adequate and constant plasma creatinine level Sellwood and Verney (57) injected 80 mg creatinine/kg body weight into the malleolar vein and infused intravenously creatinine at about .4 mg/kg/min.

The concentration of creatinine is determined from protein-free filtrates of plasma. The plasma creatinine concentration of blood and plasma did not equilibrate after 2 hours indicating the lack of movement of creatinine into the red blood cell. It is unlikely that any significant changes

in plasma concentration would occur prior to the precipitation of a plasma sample (57). The concentration of sodium tungstate utilized for protein precipitation may be altered 100% without affecting the measurement of creatinine by the method of Peters (49).

2. PAH and Tm_{PAH}

The clearance of PAH when the plasma concentration is from 1 - 6 mg/100 ml is called the ERPF. When the plasma concentration of PAH exceeds 10 mg/100 ml, the secretion of PAH becomes constant and independent of the plasma concentration and the Tm_{PAH} (tubular maxima secretion of PAH) can be determined (51). To maintain adequate plasma concentrations of PAH to measure ERPF, Sellwood and Verney (57) infused .25 mg PAH/kg body weight/min preceded by a prime injection of 15 mg PAH/kg body weight intravenously.

To determine the actual RPF, it is necessary to adjust the ERPF to account for the incomplete removal of PAH from the plasma as it passes through the kidney. The PAH extraction ratio may only be determined by measuring the renal arterial and renal venous plasma PAH concentrations. Smith (62) in a review of the literature found mean extraction ratios of 74, 84, and 87%. Difficulties in the measurement of the extraction ratio are encountered because of the diffusion of PAH from the red blood cells into the plasma after a large portion

of the PAH has been extracted by the kidney (10, 22). Gömöri (22) obtained an extraction ratio of 75% even when the blood was centrifuged immediately upon collection. The extraction ratio was noted to increase when anemia was corrected by blood transfusion suggesting variations due to the red blood cell volume (52). The extraction ratio has been observed to be greatly reduced in infants (10).

The standard formula of Smith (63) which was $Tm_{PAH} = U_{PAH} V - C_{in}P_{PAH} b$ was utilized for the Tm_{PAH} determination. The abbreviations represent the following: U_{PAH} = urine PAH concentration; V = volume of urine formed per minute; C_{in} = clearance of inulin but could be creatinine clearance as well; P_{PAH} = plasma PAH concentration, and b = fraction of PAH in plasma which is freely diffusible. Taggart (68) has found that 92% of the PAH is freely diffusible with plasma concentrations up to 60 mg/100 ml of plasma. Tm_{PAH} is not always constant due to the self inhibition of PAH transport by the tubule which has been observed experimentally (5, 54). Acetate was capable of elevating the Tm_{PAH} and partially reversing the self depression (46, 54). These investigations indicated that self depression was a consequence of alterations in the transport mechanism, but other evidence implicates back diffusion of free water (71). An elevated load PAH/Tm_{PAH} was present when depression occurred, but depression was not always evident with an elevated Tm_{PAH} (54). Asheim et al.

(5) found a negative correlation between the PAH load/Tm_{PAH} ratio when the formula for P_{PAH} was P_{PAH} - 10/5. They believed that depression of Tm_{PAH} begins even at the lowest concentrations necessary to determine Tm_{PAH}. The load PAH/Tm_{PAH} is generally recommended to be maintained between 1.5 and 4.0 when attempting to measure the Tm_{PAH}.

Concomitant increases in GFR and Tm_{PAH} were observed in varying degrees in two independent experiments (24, 46). McDonald et al. (42) have recorded frequent incidences of decreased GFR and increased RPF during the maintenance of high plasma PAH concentrations. The injection of 50 ml of 20% PAH intravenously elevated the pulse rate and systolic and diastolic pressure, but they reverted to preinjection levels within 20 minutes. Other investigators observed no alteration in GFR during elevated PAH plasma levels but noticed increased urinary flow, increased sodium elimination, and reduced urine osmolarity (19).

3. Osmolar and free water

The regulation of water excretion may be monitored by observing the urine and plasma osmolarities and the urine volume. The osmolar clearance which represents the quantity of urine isosmotically associated with solute may be calculated by multiplying the urine osmolarity times the volume of urine collected per minute and dividing by the plasma osmolar-

ity. The water in the urine which is not isosmotically associated is called free water and may be calculated by subtracting the osmolar clearance from the volume of urine formed per minute. If the urine is hypertonic to plasma, the free water clearance is negative; and it represents the amount of free water reabsorbed.

The administration of a water load initiates an increased urine flow and the formation of hypotonic urine. A direct correlation between the rate of excretion of solute and the urinary flow was observed (9, 34). In response to an increased salt load the minimal urine osmolarity was decreased. This, therefore, resulted in increases in both the osmolar clearance and the free water clearance (34). The free water clearance has been observed to increase directly with the osmolar clearance if the GFR is normal (70). The free water and osmolar clearances were reduced when the salt intake was reduced from normal (9). No correlation was noticed between the minimal urine osmolarity and the plasma osmolarity (34).

Since alterations in the solute load regulate the process of water excretion, the GFR which governs the amount and flow rate of solute through the tubule should also affect water excretion. At the height of water diuresis with maximal urinary flow, the urine volume and free water clearance are directly related to the GFR (34). Berliner and Davidson (6) were the first to observe that by decreasing the GFR a hyper-

tonic urine could be produced in the absence of ADH. This was reproduced by other investigators (2, 16, 70) but was not observed by Leaf et al. (39). The urine osmolarity could be decreased while the GFR was reduced if the solute load was increased (70). These results could not be verified by another investigator (74). Decreasing the renal blood flow has also decreased the elevated urine osmolarity subsequent to a decreased GFR (1). The mechanism for Berliner and Davidson's observation still remains confusing and unknown. The decreased urine flow, decreased solute delivery to the distal tubule, decreased sodium delivery to the distal tubule, increase in the free water back diffusion in the collecting duct or variations in RBF may be responsible for this phenomenon. Many aspects of water diuresis remain unknown, and research in this area has increased.

4. Correlation of renal functions

Various correlations between measured clearances have been utilized to learn more about renal function. The most commonly calculated interrelationship is the FF (filtration fraction) which is the per cent of the GFR to the ERPF (62). It is maintained relatively constant during fluctuations in blood pressure but was shown to be elevated in water diuresis due to a larger increase in GFR than the ERPF (56, 57). The Tm_{PAH} may be considered as a measurement of the relative mass

of the kidneys in dogs. The Tm_{PAH} might have a closer relationship to relative kidney weight than the dog's body weight or surface area. The relationship of GFR and ERPF to Tm_{PAH} was proposed to be more constant than values on a weight basis. The GFR/Tm_{PAH} is also an index of the balance between glomerular and tubular function, sometimes called the "glomerulotubular balance." The relative blood flow per functional nephron is inferred from the RPF/Tm_{PAH} ratio. Only under controlled experimental conditions are the relationships of osmolar and free water clearance to other renal functions considered. In one experiment during maximal diuresis the free water clearance/GFR was calculated (26).

C. Normal Studies of Renal Function

Many normal renal function studies have been performed but probably no two under the same circumstances. A comparison of the values obtained and statistical evaluation are presented in Table 1. When the values were calculated by another author for body surface area by the Meeh-Rubner equation:

$$SA = \frac{11.2 \times W^{2/3}}{100}$$

it was possible to recalculate a value on a kg body weight basis. It was not possible though to determine the standard deviation or other statistical analysis. Each author's experiment will be discussed separately in an effort to disclose

Table 1. Normal renal clearance values

Clearance	Author	Mean per kg	Range	S.D.	Mean per m^2	Range	S.D.	CI 95%	FF	Range	S.D.
GFR[a]	Houck	4.29	2.15-8.32	1.01	84.4	43-133	19.1	--	.317	.225-.247	.052
	Asheim	3.77	1.74-5.86	.84	76.0	42-102	15.0	--	.31	.19-.44	.051
	Russo	3.75[b]	--	--	94	59-125	18[b]	±36	.40	--	--
	Stamler	3.90	--	--	104	86-112	15.3	--	.353	--	--
	Kubicek	--	--	--	88.4	63-119	12.1	--	.306	--	.032
	White	--	--	--	86.2	--	14.9	--		--	--
ERPF[a]	Houck	13.51	8.05-22.43	3.26	266	139-430	66	--	--	--	--
	Asheim	12.88	6.30-21.18	3.21	263	160-419	62	--	--	--	--
	Russo	8.92[b]	--	--	238	--	60.4[b]	±133	--	--	--
	Kubicek	--	--	--	286[c]	203-425	50.5	--	--	--	--
	White	--	--	--	242.9	--	54.6	--	--	--	--
	Stamler	11.06	--	--	295	221-390	57.6	--	--	--	--
Tm_{PAH}[c]	Handley	.69	--	.07	--	--	--	--	--	--	--
	Asheim	1.21	--	.34	25.43	--	7.04	--	--	--	--
	Russo	.62[b]	--	--	16.5	--	2.2[b]	±4.7	--	--	--
	White	--	--	--	18.72	--	3.58	--	--	--	--

[a]Abbreviations, see Appendix E.

[b]Values calculated from author's data.

[c]RBF value extraction ratio .90.

any variation in their techniques and analysis.

Russo et al. (53) determined renal clearances in 31 female dogs over a period of 6 years. PAH, which was given orally, and creatinine were utilized as test materials, but no other facts concerning the experimental procedure were described. A large difference in renal clearances among dogs was noticed from year to year. A standard deviation of 9 ml/min/m^2 body surface area of the GFR was obtained for a single determination on any given dog for any one occasion. The study included 2,045 determinations of creatinine clearance. No trend in the GFR due to the dog's age was indicated over a period of 3-7 years. The analysis of renal plasma flow was done 290 times on 12 dogs. The variation of any renal blood flow calculation for any 1 dog on any one occasion was 32 ml/min/m^2 body surface area. Values for Tm_{PAH} were obtained from 20 determinations from 7 dogs. An analysis of variance did not show a significant difference among dogs. The standard deviation of a single determination on any given dog for any one occasion was 1.8 mg/min/m^2 body surface area. These observations were from experiments designed for purposes other than the measurement of normal renal function.

Houck (27) compiled the GFR, ERPF and FF obtained from 75 normal trained female dogs by various investigators in his laboratory. The clearances were determined in unanesthetized dogs in the postabsorptive state, and most of the time they

were well hydrated. Diuresis was produced either by the administration of 50 ml water/kg of body weight by stomach tube 30 minutes prior to clearance measurements or by the intravenous infusion of a 10% mannitol solution. Creatinine and PAH were administered by constant infusion. The correlation coefficient of GFR to ERPF was 0.79. It was concluded from their observations that GFR and ERPF were essentially as well correlated to body weight as to surface area. The statistical analysis was computed on the average clearance value for each dog.

Asheim et al. (5) determined the GFR, ERPF, and Tm_{PAH} with inulin and PAH on 21 female and 11 male dogs. Sixteen of the dogs utilized were cocker spaniels from five different litters. The animals were fasted 20 hours prior to the experiment and either 500 ml of water was administered 1/2 hour before the experiment, or it was given ad libidum. The diuresis was never below 0.10 ml/min. The dogs were anesthetized and inulin and PAH were infused. The plasma PAH level was maintained close to 2 mg/100 ml for ERPF measurements and usually exceeded 15 mg/100 ml for Tm_{PAH} determinations. The plasma inulin concentration was maintained between 15-30 mg/100 ml. The average clearance values for each dog were utilized for statistical analysis. There was no tendency toward a variation of function with age. No significant difference due to sex was noted even though the mean GFR for

females was 71 ± 3.1 and males was 81 ± 4.9 while the ERPF for males was 286 ± 24.3 and females was 251 ± 10.5. The ratio of PAH load/Tm_{PAH} was shown to have a linear regression which was tested to be significantly different from 0. The values for PAH load were designated by $\frac{\text{(plasma PAH concentration - 10)}}{5}$ thereby giving a precision of 5 mg/100 ml to plasma PAH concentration.

Kubicek et al. (36) determined GFR and RPF in 32 trained conscious mongrel dogs with creatinine and PAH. The plasma concentrations of creatinine and PAH were maintained close to 2 and 12/mg/100 ml, respectively. The dogs were fasted for 18 hours and 500 ml. of water were given by stomach tube 30 minutes before starting the clearance procedure. They calculated the RPF by considering .90 as the extraction ratio of PAH. The values of FF are based upon the GFR/RPF ratio.

White et al. (72) determined the PAH clearance, inulin clearance and Tm_{PAH} in trained female dogs. The PAH and creatinine were administered subcutaneously to provide the necessary plasma values. To obtain a diuresis, water (3.5% of the body weight) was administered. Data from six of his dogs were used to calculate the mean and standard deviation of the various clearances. The values used were the average clearances obtained in at least two collection periods on one day. The values are calculated on a body surface area basis, but the formula used for this calculation is not given.

The calculation of Tm_{PAH} was done by Handley et al. (25) on four trained unanesthetized dogs. The values used for statistical evaluation were an average of three collection periods. All the dogs weighed from 14 - 17 kg.

Six male dogs were utilized by Stamler et al. (65) to determine the GFR and ERPF with creatinine and PAH. Priming injections of PAH and creatinine were followed by constant infusion to maintain unchanging plasma values. Hydration was accomplished with 40 ml of water/kg body weight via stomach tube 50 - 90 minutes before collecting urine for the first clearance period. Intravenous infusion of 5% glucose at a rate of 6 ml/min was instituted to produce an osmotic diuresis. The number of clearance periods during each observation was usually three and the observations on dogs varied from one to three. His normal values are based upon 11 observations and 34 clearance periods. The values were calculated on a body surface area basis using the Meeh-Rubner equation.

The only normal values with free water clearance was a ratio of free water clearance/GFR times 100 with a value of 9.0 in the dog (26).

D. Computers in Medicine

The computer is becoming more an integral part of medicine each year. It can be adapted for many diverse problems. Computers are capable of diagnosing disease conditions by a

process similar to pattern recognition. This relies on the presumption that the pattern of symptoms determines what the disease is. The mathematical solution and explanation of physiological events can be accomplished by the design of a computer model of a biological system. This analysis has been applied to the flow of ions from one site to another and is called compartment analysis (75). Many computing centers now have a variety of prepared program packages, e.g., plotting routines, analysis of variance, regression and correlation programs. Analog computers are capable of solving differential equations and have been used to evaluate the electroencephalogram and electrocardiogram (18).

The computer has certain capabilities necessary for its function. It can read input material and write out information. This requires the development of a language or a program which the computer is capable of understanding. The programmer must perform his task in presenting the computer with the instructions in an efficient and understandable form by the computer. Any program must completely define the problem, and the method of solution of this problem must be presented to the computer in logical arithmetical steps. Computers are capable of memorizing data for short or long periods of time. They can follow and respond to instructions. Another attribute of the computer is its rapid, accurate, and efficient method of calculation (43).

No specific references to programs for the calculation of renal clearances are available. Computer programs for renal clearance measurements may be written in Fortran language and with slight modifications can be adjusted to different experimental designs.

III. MATERIALS AND METHODS

A. Experimental Animals

Six female beagles were selected as the experimental animals. They were littermates (American Kennel Club (A.K.C.) litter BE-356954) which were whelped on April 26, 1963. The sire was Sta-Lor Duke (A.K.C. registration number HA-383604) and the dam was Sta-Lor Princess (A.K.C. registration number HA-383605). Prior to their acquisition a modified live canine distemper and infectious canine hepatitis vaccine[1] was administered twice. The dogs were 13 and 18 weeks of age when the vaccine was given. They were vaccinated with modified live rabies vaccine[2] when they were 22 weeks old.

An antibody titer of 1:100 against canine distemper was present in all the dogs when they were 11 months old. A titer of 1:20 was also present at this time against infectious canine hepatitis. Both of these titer levels are generally considered to indicate satisfactory immunity. The titers were determined by serum neutralization.[3]

The dogs' diet during the period of renal clearance measurements was uniform but was not the same ration they were

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[1]Cabvac, American Cyanamid Company, Princeton, New Jersey.

[2]Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

[3]Fromm Laboratories, Inc., Grafton, Wisconsin.

fed prior to their acquisition. The diet consisted of a dry expanded ration from weaning until 5 months of age. The ingredients included: yellow corn, soybean oil meal, meat meal, wheat dried cheese meal, dried tomato pomace, brewers dried yeast, animal fat, and wheat germ meal. The calculated analysis was: crude protein 25.52%, crude fat 8.37%, crude fiber 3.69%, moisture 8.65%, and ash 6.95%. Each dog received 1 pound of p/d[1] dog food once daily, beginning at 5 months of age and continuing throughout the experiment. This food has the following ingredients: horse meat, horse meat by-products, whole egg, corn grits, soy grits, and corn oil. It consists of protein 8.5%, fat 5.0%, fiber 1.0%, nitrogen free extract 15.8%, ash 1.9%, and moisture 69%. The total caloric intake per day was approximately 660 Calories. The food intake was not altered during the experiment or varied among dogs, therefore the caloric intake ranged from 60 - 85 Calories/kg body weight. Occasional supplements to the diet, dried biscuits,[2] were given for positive reinforcement. All food was withheld for at least a period of 15 hours before a clearance determination. No p/d ration was offered to the dogs within a 24-hour period prior to any experiment.

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[1]p/d, Prescription Diet, Hill Packing Company, Topeka, Kansas.

[2]Gaines Biscuits, General Foods Corporation, White Plains, New York.

The dogs were procured[1] when they were 5 months of age and subjected to a physical examination. The only abnormality revealed in this examination was a mild seborrheic otitis externa present in all dogs. Otodectes cynotis was the etiological parasite. Treatment and eradication were accomplished with an ointment[2] containing neomycin 5%, sulfacetamide 10%, sodium caprylate 10%, piperonyl butoxide 1%, and tetracaine hydrochloride as active ingredients. Hematological examinations revealed normal values which are summarized below.

	713	714	715	716	717	718
WBC/cmm	11,600	11,900	8,300	8,650	9,450	--
Sed. rate, mm in 30 min	0	0	0	0	0	0
Packed cell volume, %	48.5	48.0	46.5	46.5	49.0	45.0
Blood urea nitrogen, mg %	30	24	28	24	20	20
Hemoglobin, g/100 ml	16.00	15.25	15.50	14.75	16.50	13.80

It was necessary to train the dogs to remain quiet on a restraint board. The animals were placed on this board for increasing periods of time up to 1 hour. The training varied with each individual dog to compensate for their different temperaments. Positive reinforcement with dried biscuits

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[1]Beagles for Research Inc., Jeffersonville, New York.

[2]Mitox, Norden Laboratories, Lincoln, Nebraska.

was utilized at the termination of each procedure. Voice commands were employed during the training session as negative reinforcement. The restraint board was 40 inches long by 36 inches wide. A slight V shaped trough was provided by having the center of the board 2 inches lower than the sides. A 3-inch thick piece of foam rubber functioned as a cushion on top of the board. The animal was restrained in lateral recumbency during the procedure. The dog was placed under a 12-inch strip of plastic fastened on both sides of the restraint board. This strip was then tightened and four padded tie ropes were secured to the dog's legs and the restraint board.

B. Clearance Procedure

The renal clearance procedure was designed to evaluate several kidney functions and to insure accuracy in their measurement. The dogs were provided with their daily food intake at least 24 hours prior to the experiment. To insure that the dogs were not dehydrated 500 ml of water were administered *per os* via a stomach tube, approximately 16 hours before the experiment. Water was then given *ad libidum*. The experimental period commenced by giving 80 ml/kg of water via stomach tube. The vulvar area was swabbed with an antiseptic

solution.[1] A sterile urethral retention catheter[2] modified with a syringe adapter was introduced into the urethra and bladder with the aid of a metal stylet and an infant nasal speculum. The operator wore sterile surgical gloves to prevent bacterial contamination of the sterile instruments. The dog was then placed in lateral recumbency on the restraint board, and the plastic strip and leg ties were fastened securely but not tightly. An intravenous catheter[3] was introduced into the saphenous vein. This catheter included a sterile 17-gauge needle and an 8-inch polyethylene catheter with a syringe adapter. The catheter did not come in direct contact with the operator during insertion and thereby assures sterility. The needle was introduced into the vein, and then the catheter was inserted through the needle and into the vein. The needle was withdrawn from the vein and the needle and catheter taped to the leg. A 5 ml pretreatment blood sample was obtained at this time. The catheter was filled with a sodium heparin solution (100 units/ml) to remain until the ensuing blood sample was withdrawn. Another intravenous catheter was introduced into the cephalic vein and taped in

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[1]Nolvosan-S, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

[2]Bardex two-wing Malecot-3 #12 Fr., C. R. Bard Inc., Murray Hill, New Jersey.

[3]Intracath 1617, C. R. Bard, Inc., Murray Hill, New Jersey.

place. A constant infusion pump,[1] utilizing a 50 ml syringe and rubber infusion tubing delivered the infusion solution to the cephalic catheter at the rate of .387 ml/min. A priming solution was also introduced into the cephalic catheter at this time. The priming and infusion solutions will be discussed, subsequently in more detail. The urine was collected in a graduate cylinder and measured at 10-minute intervals to monitor the urine flow. When the rate of urine flow attained at least 2 ml/min and 30 minutes had elapsed since the infusion commenced, the clearance periods would be initiated. The bladder was evacuated by instilling 10 ml of water and 5 ml of air into it, mixing and then removing all bladder contents with a syringe. This was performed at the beginning and end of each urine collection period. The urine was collected for a 10-minute interval and the solution, which was withdrawn from the bladder by rinsing at the completion of the urine period, was added. Two blood samples were withdrawn from the saphenous catheter, one at the commencement and the other at the completion of the urine collection period. Three consecutive urine samples and four blood samples were obtained in this manner. The infusion solution was changed to one containing a 10-fold increase in PAH concentration upon completion of the first three urine samples. The second priming solution

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[1]Infusion/withdrawal pump Model 600-900, Harvard Apparatus Company, Inc., Dover, Massachusetts.

was administered at this time, containing only PAH. Subsequent to a 25-minute interval, during which equilibration of the plasma PAH occurs, three urine collection periods were repeated as previously done. This concluded the experimental period. The intravenous and urethral catheters were removed and the animal released from the restraint board. A positive reinforcement in the substance of dry biscuits, and the daily p/d ration was given.

Two prime and infusion solutions were prepared for each experiment. The initial prime and infusion solutions were calculated to maintain adequate plasma concentrations of creatinine and PAH to assess GFR and ERPF. The first prime solution contained 40 mg of creatinine[1] and 4 mg of sodium aminohippurate/kg dissolved in 5 ml of isotonic saline.[2] The PAH utilized was a 20% solution of sodium aminohippurate.[3] The infusion solution consisted of 400 mg of creatinine plus 50 mg of PAH/kg dissolved in 100 ml of isotonic saline. This solution was infused intravenously at the rate of .387 ml/min. To measure the maximal tubular secretion of PAH, it was necessary to elevate the plasma concentration of PAH sufficiently

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[1]Creatinine, Eastman Kodak, Rochester, New York.

[2]Sodium chloride isotonic U.S.P., Abbott Laboratories, North Chicago, Illinois.

[3]Sodium aminohippurate N.F., Merck Sharp and Dohme, West Point, Pennsylvania.

so that not all of the PAH can be removed from the plasma during one passage through the kidney. This condition was fulfilled by administering intravenously 100 mg of isotonic saline containing 400 mg of creatinine and 400 mg of PAH/kg.

The blood samples obtained were heparinized and a microhematocrit tube was filled for each sample. The blood samples were centrifuged immediately and 2 - 3 ml of serum were removed. Precipitation of plasma proteins was accomplished immediately following centrifugation. Samples to be utilized for creatinine determinations were precipitated with sodium tungstate and those for PAH determinations with trichloroacetic acid. Precipitation by sodium tungstate was accomplished by the addition of 0.5 ml of plasma to 3 ml of 1/9 normal sulfuric acid, then adding 1.5 ml of 3.33% sodium tungstate and centrifuging. Protein free filtrate for PAH determinations was obtained by the addition of 0.5 ml of plasma to 3.5 ml of a 3.5% solution of trichloroacetic acid. The samples were all centrifuged and the supernatant removed and refrigerated for subsequent chemical determinations within 48 hours.

Various dilutions of urine and plasma must be performed to enable the measurement of chemical concentrations within a narrow range of optical transmittancy. The urine samples were diluted 1:100 for determinations of creatinine. When renal plasma flow was determined with the lower plasma PAH levels,

the urine was diluted 1:200. The urine was diluted to 1:2,500 when the tubular maximal secretion of PAH was determined. Due to the precipitation techniques, the plasma was diluted 1:10 for creatinine and 1:8 for plasma during the lower PAH plasma levels. When the plasma level of PAH was increased, it was necessary to dilute the plasma to 1:88.

The concentration of creatinine and PAH was determined by measuring colorimetric changes with a spectrophotometer[1] subsequent to the addition of certain chemicals (see Appendix A and B). Two ml of alkaline picrate (5 parts saturated picric acid solution and 1 part of 10% sodium hydroxide) were added to 1 ml of protein free filtrate or urine, and the optical density of this solution was measured at a wavelength of 525 m to determine the creatinine concentration (49). The optical density of standard solutions containing .01, .02, .04, and .05 mg of creatinine were recorded in duplicate for every set of samples using the same reagents which were prepared fresh daily. PAH concentration was determined by the method of Smith (52). To 2 ml of protein free filtrate or urine were added 0.4 ml of 1N hydrochloric acid, 0.2 ml of sodium nitrite (100 mg %), 0.2 ml ammonium sulfamate (500 mg %) and 0.2 ml of ethylene-diamine (100 mg %). All reagents must be added separately and with a 5-minute pause between

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[1]Beckman Model B, Beckman Instrument Company, Fullerton, California.

each addition except after the addition of hydrochloric acid. Spectrophotometric recordings of the samples and standards, which contained .005, .01, .02, and .03 mg of PAH, were then done 15 minutes later at a wavelength of 540 m . This method depends on diazotizing the para-amino group of PAH with nitrous acid destruction of excess nitrous acid with sulfamate and coupling with N (1-napthyl) ethylene-diamine.

The osmolarity of the urine and plasma were determined by freezing point depression utilizing an osmometer.[1] The osmometer was calibrated by the use of standards to give values in millosmols.

C. Computer Analysis

It was necessary to have a tabulation sheet for all values so that they could be transferred to computer punch cards. Included on this sheet were: concentration of creatinine and PAH standards, optical densities of urine, plasma and standard samples obtained in creatinine and PAH determinations, urine volume, urine and plasma osmolarities, packed cell volume, dog number, experiment number, and duration of each collection period.

The computer program was devised to convert the input

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[1]Fiske osmometer Model G-62, Fiske Associates, Bethel, Connecticut.

data into values for GFR, ERPF, Tm_{PAH}, U/P (urine to plasma) ratio of creatinine, plasma and urine concentrations of creatinine and PAH, FF, osmolar clearance, and free water clearance. These values are then printed out in a format for use in obtaining punch cards. The entire program can be seen in Appendix C. To convert the optical density recordings to actual concentrations, a regression was calculated from the standards. This regression is computed by the least squares method based on the formula that concentration equals a (y intercept) plus the optical density times b (slope). The computer obtains values for both a and b for creatinine and PAH for each experiment.

The methods utilized to compute the clearance values are those routinely employed but will be reviewed here. Some variations must be taken into account due to the addition of 10 ml of bladder rinse. The GFR was calculated by dividing the quantity of creatinine excreted per minute by the plasma concentration of creatinine. The plasma concentration was obtained by averaging the concentrations of the plasma samples withdrawn at the beginning and end of each urine collection period. The quantity of creatinine in the urine was computed by multiplying the creatinine concentration of the urine sample, which includes 10 ml of wash solution, times the volume of this sample on a per minute basis. When this quantity of creatinine was divided by the actual urine flow per

minute, the concentration of creatinine in the urine can be obtained. When this concentration in the urine was divided by the plasma concentration, it gave the U/P ratio.

The ERPF was evaluated in the initial three collection periods during which time the plasma PAH concentration was low. It was calculated in the identical manner as the GFR, but the concentrations of PAH instead of creatinine were used. The ERBF was determined by dividing the renal plasma flow by the per cent of plasma volume. The blood packed cell volumes of the samples at the beginning and end of a urine collection period were averaged to obtain the per cent of plasma volume. The filtration fraction is that per cent of plasma entering the kidney that was filtered in the glomerulus to become the glomerular filtrate. This was calculated by dividing the GFR by the ERPF.

The measurement of the maximal ability of the tubules to secrete PAH required the simultaneous measurement of GFR and PAH in the urine and the plasma. The portion of PAH filtered by the glomerulus was subtracted from the total amount of PAH present in the urine leaving the amount transported from the tubule to the urine. To calculate the amount of PAH filtered, the concentration of freely diffusable PAH in the plasma was multiplied times the GFR. The quantity of freely diffusable PAH is dependent upon the protein binding and in this investigation 92% of the PAH was considered to be freely diffusable.

Water and electrolyte balance may be assessed by certain calculations using plasma and urine osmolarities. The osmolar clearance was determined by dividing the number of milliosmols excreted per minute in the urine by the plasma osmolarity. To obtain a value for the osmolarity of the actual urine, the total number of milliosmols in the sample of urine and wash are divided by the actual urine volume. Free water clearance was derived by subtracting the osmolar clearance from the volume of urine collected per minute. The computer also calculated the averages of the three periods for: GFR, ERPF, ERBF (effective renal blood flow), Tm_{PAH}, osmolar clearance, free water clearance, urine volume per minute, urine osmolarity, filtration fraction, and the U/P ratio of creatinine.

The determinations of renal clearances and other calculated values were evaluated statistically by an analysis of variance. The program used was "Estimation of Variance Components for the Completely Nested Model." This program which is applicable when unequal replicates are present calculates means and an analysis of variance table for each variable including degrees of freedom, sums of squares, components of variance and the percentages that the components contributed to the total variance.

The "Regression and Correlation"[1] program was then

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[1]Iowa State University Computation Center, Ames, Iowa.

utilized to compute the correlation between all factors, and specific regressions were requested to be calculated. This program calculated the means, sums of squares, cross products, correlations and a regression analysis.

The "Graph Plotter"[1] program was adapted to plot the water diuresis curves. The urine flow versus time was plotted for each individual experiment. A program was then written to average these values for each dog and then a graph was plotted (see Appendix D).

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[1] Ibid.

IV. RESULTS AND DISCUSSION

A. Conditions of the Experiment

1. Plasma concentrations

The maintenance of constant creatinine and PAH-blood concentrations is necessary to obtain accurate and reproducible clearance values. The plasma creatinine concentration during the RPF clearance period (the first 3 urine collections) and the Tm_{PAH} clearance period (the second 3 urine collections) was perpetuated by the constant infusion of creatinine at the rate of 1.55 mg/min/kg of body weight. The creatinine concentration of the plasma varied from 8.7 - 31.0 mg/100 ml during the RPF clearance period and from 17.5 - 29.9 mg/100 ml in the Tm_{PAH} period. The greatest change in the creatinine concentration on any 1 day's experiment during the RPF clearance period was 7.2 mg/100 ml and only 3.5 mg/100 ml in the Tm_{PAH} clearance period. The mean plasma concentration of creatinine for the RPF clearance period was 18.46 mg/100 ml with a standard deviation of 3.1 mg/100 ml. The analysis of variance (66) indicated that 88.7% of the variance component was present due to observations on different days within the same dog, 11.3% due to the observations on 1 day on 1 dog and no component among dogs. The F test indicated a significant difference ($P < .01$) in the plasma concentration of creatinine among days. The mean creatinine concentration while measuring

Tm_{PAH} was 23.7 mg/100 ml with a standard deviation of 3.2 mg/100 ml. The largest component of the variance 86.8% was noticed among days within dogs, while 13.2% was evident among observations within days within dogs. The variance among days within dogs was significant ($P < .01$) with these results being almost identical to those observed during the RPF period.

The plasma PAH level was maintained while the RPF was measured by the infusion of .155 mg/min/kg of body weight and during the Tm_{PAH} period by the infusion of 1.55 mg/min/kg of body weight. A standard deviation of .247 mg/100 ml and a mean PAH concentration of 1.294 mg/100 ml were measured in the RPF clearance period. The range of concentrations was from 0.4 - 2.2 mg/100 ml of plasma with the greatest change during 1 day of 0.4 mg/100 ml. The variance due to observations on different days within the same dog was significant (P .01) and accounted for 82.3% of the variance component. The remaining 17.7% of the variance was attributed to the 3 different observations on 1 day on 1 dog. During the Tm_{PAH} clearance period the plasma concentration had a mean value of 23.4 $\pm$ 3.4 mg/100 ml and a range of 11.1 - 28.6 mg/100 ml. The largest variation in plasma PAH concentration occurring in any experiment during 1 day was 7.2 mg/100 ml. The determination of Tm_{PAH} was not considered as valid unless the plasma concentration of PAH exceeded 15.5 mg/100 ml. A significant difference (P .01) of PAH concentration was proven among

days within the dogs and this component of the variance comprised 68.1% of the variance. The variance component of the plasma PAH concentration among observations within days within dogs was lower than that in the PAH clearance period and accounted for only 7.6% of the variance while the difference among dogs contributed 24.2%. Only the difference among days within dogs was significant ($P < .01$).

The plasma PAH and creatinine concentrations seem to exhibit essentially the same pattern. The greatest variation was evident due to observations on different days within the same dog. The cause of this may be a consequence of variations in actual quantities infused, due to measurement inaccuracy or technical difficulties with the infusion pump. The extent of plasma dilution and the difference in time of measurement following the prime injection likewise are factors. Variations in the RPF, GFR, and Tm_{PAH} can also cause variations in the plasma concentrations of creatinine and PAH. The PAH and creatinine plasma concentrations varied only slightly during the Tm_{PAH} and RPF clearance periods on any 1 day and would preclude any errors of the clearance measurement due to "dead space" of the kidney and permit the utilization of venous instead of arterial blood samples (8). The Tm_{PAH} was measured in a range where self inhibition is not considered to occur, but Asheim (5) demonstrated its existence at all plasma levels necessary to determine the Tm_{PAH}.

2. Comparison of PAH clearance and Tm_{PAH} periods

It was decided that the normal values for GFR, osmolar clearance, and free water clearance would only be obtained from the measurements in the RPF clearance period. The profound increase in the PAH-infusion concentration might alter these measurements either by affecting the circulatory hemodynamics or by a direct affect on the tubular mechanism of solute excretion and reabsorption. The Tm_{PAH} period also was concomittant with the falling portion of the diuresis curve. The observation of the renal clearance values in the 2 periods indicated that some of them were different. The GFR values obtained in the RPF clearance period were 19.6% higher than those during the Tm_{PAH} period. These clearances were proven to be significantly different ($P < .01$) with the use of a paired analysis (64). The phenomenon of decreasing GFR can be analyzed further if a few experiments are considered. On several occasions the normal procedure for clearance measurements were altered. In one instance in a previous study, the Tm_{PAH} was measured during the entire experiment with a plasma level of 18 - 20 mg/100 ml. The GFR decreased 12.8% from the early observations to those later in the experiment. In another experiment, when the RPF was determined with a plasma PAH concentration of 1.5 - 1.9 mg/100 ml throughout the experiment, a decrease of 23.2% in the GFR was noted. In dogs 714

and 715, on one occasion, a second water load of 200 and 250 ml, respectively, were administered by stomach tube 30 minutes prior to the Tm_{PAH} clearance period. The GFR of dog 714 decreased only 2.8% between the RPF clearance period and the Tm_{PAH} period while dog 715 decreased 7.5%. In dogs 714 and 715 which were given a second water load the GFR was 10.5 and 18.2% higher than the GFR normally recorded in the Tm_{PAH} clearance period. This represents a very small number of observations which cannot be expected to provide statistical or final proof as the basis of the decreased GFR. Several factors such as increased plasma concentration of PAH, lapse of time, effect of the water load and difference in the state of excitement might be implicated. Since the decrease in GFR occurred when the plasma PAH level was high during the entire experiment and also when it was at a low level throughout, it appears that PAH was not the responsible factor. This decrease was almost completely eliminated by the administration of water and therefore it is doubtful whether time or emotional state contributed a major portion to the decrease in GFR. It is believed that the water load is the factor which increases the GFR and as this water is excreted, the GFR begins to decrease.

The osmolar clearance could not be shown to be significantly different at the 1% level in the 2 clearance periods with a paired analysis. A decrease of 1.2% was noted from the

osmolar clearance in the RPF period to the Tm_{PAH} period. A significant difference ($P < .01$) was evident between the free water clearance in the RPF and the Tm_{PAH} periods. The difference was 28.6% with the higher value occurring in the RPF period. Since the free water clearance value was different in the Tm_{PAH} period, only its value in the RPF period was used. To be consistent only the osmolar clearances observed in the RPF period were utilized in calculating normal values.

3. Water administration

The oral administration of water produced a rapid dilution of the blood and a prompt diuresis. The packed cell volume decreased within 15 minutes of the administration of water, and within 30 minutes it had stabilized at a value 10 - 15% less than its normal level. A very rapid rate of water absorption occurred in the alimentary tract. The packed cell volume remained relatively constant during the RPF clearance period and a progression to higher values occurred in the Tm_{PAH} period but did not attain pre-water load values. The decrease in plasma osmolarity mimicked the decrease in packed cell volume but was not as extensive. Some elevation of the plasma osmolarity occurred from the RPF period to the Tm_{PAH} period but did not attain pre-water load values. An accurate estimation of the increase in blood volume cannot be obtained by observing the packed cell volume and the plasma

osmolarity.

The urine excretion curves of each dog were plotted for each experiment and for the average of the experiments by the computer plotting program. A sample of one of these graphs may be seen in Appendix D and all the dogs' average diuresis curves are seen in Figures 1 and 2. The exact time of the commencement of urine flow was not always determined since urine collection did not begin immediately after the administration of water. In the instances where observed, the urine volume began its increase within 20 minutes after the dogs received the water. The time of the peak diuresis and the maximal urine flow varied among dogs and are summarized as follows:

Dog	Minutes before peak urine flow	Maximum urine volume ml/min
718	96	4.09
716	67	4.46
713	97	4.56
715	86	5.00
717	106	5.26
714	85	5.40

The diuresis was still present at the conclusion of the experiments (2 1/2 - 3 hours after water load) with urine flows of at least 2.0 ml/min.

The pattern of solute excretion during the experiment was not consistent in the RPF period, but it usually decreased

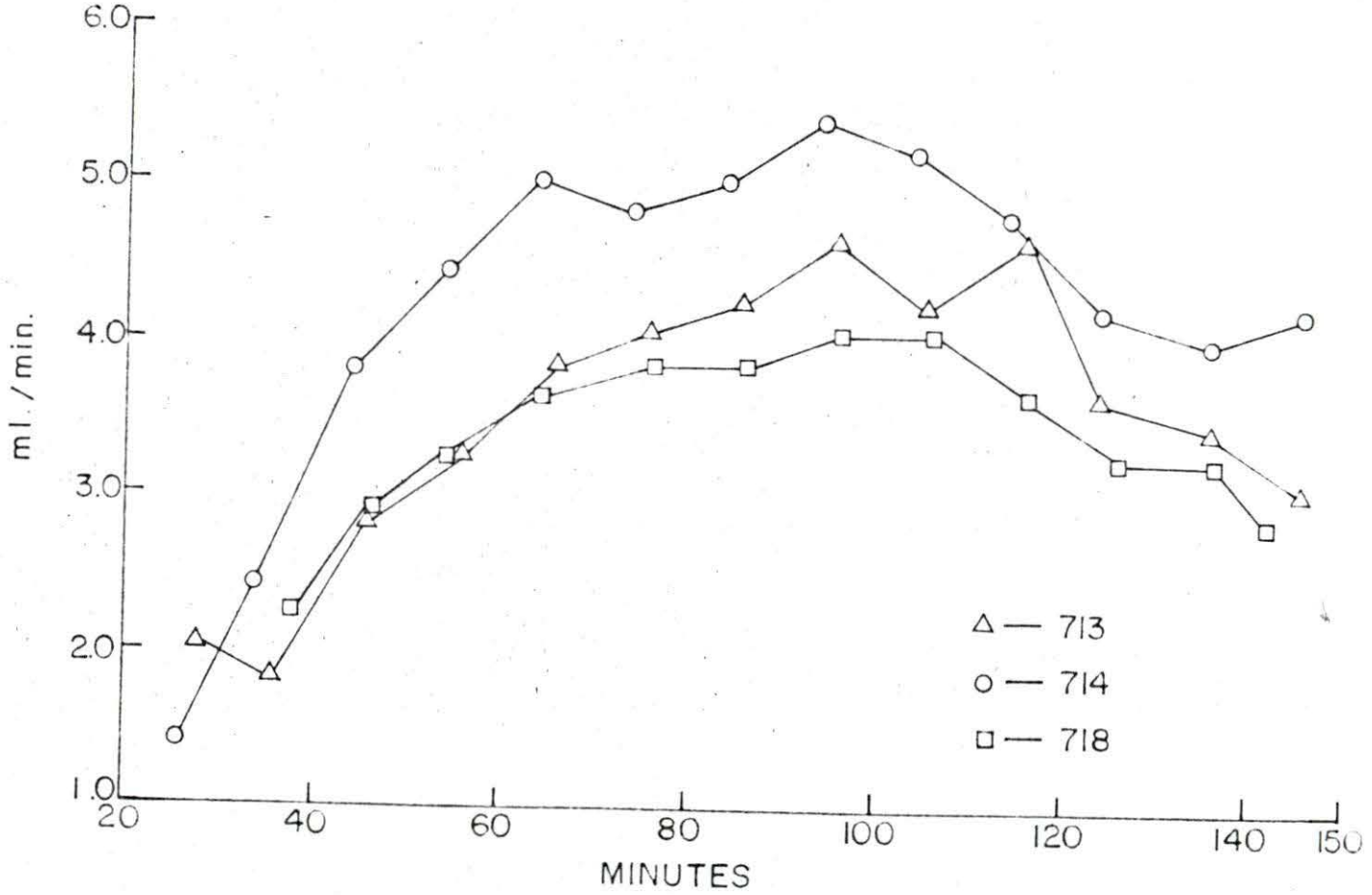

Figure 1. Diuresis curves of dogs 713, 714 and 718

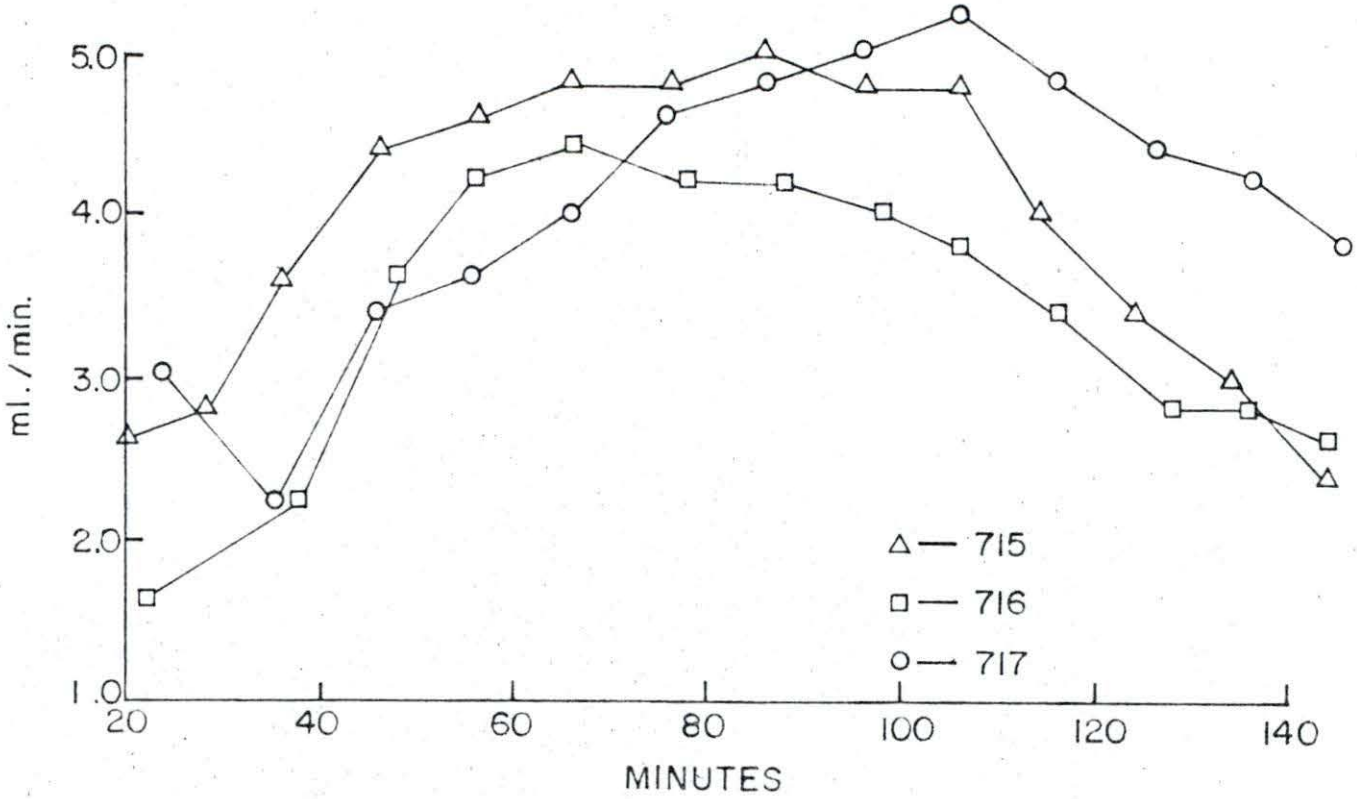

Figure 2. Diuresis curves of dogs 715, 716 and 717

during the Tm_{PAH} period. The factor which appears to have the most influence on the solute excretion is the urine flow.

4. Food intake

The effect of protein ingestion prior to renal clearance measurements was observed on 3 occasions. The dogs were fed 38.5 gm of protein 2 - 6 1/2 hours prior to the clearance measurements. The protein source was p/d ration which was fed to the dogs the morning of the experiment. The results from these experiments were not incorporated in the calculation of the normal clearance values. All the serum samples were lipemic, but there was no indication of any interference with the measurements of the renal clearances. The GFR was increased over its normal value by 10.2% to 43.4 ml/min, and it was not significantly different at the 1% level ($.10 > P > .05$) than the normal values with a paired analysis (64). The ERPF was significantly changed ($P < .01$) and increased by 14.5% to a mean value of 111.9 ml/min. The urine osmolarity, osmolar clearance, and urine volume were elevated 44.9%, 76.7, and 23.8%, respectively. A statistical significance ($P < .01$) was shown by the osmolar clearance and urine osmolarity increases which were from 1.03 - 1.82 ml/min and from 67 - 97 milliosmols respectively. The postprandial solute excretion was elevated from 283 - 502 milliosmoles/min. This increased solute excretion accounts for the augmentation

of the osmolar clearance. In contrast to this the free water clearance was only 6.5% greater after eating and could not be shown with this small sampling to be different from the normal value.

The increases of the GFR and ERPF agree closely with those obtained by Kerr (31) when horsemeat was fed just prior to clearance measurements. No clearance determinations in this study were attempted prior to the water load because of the large error incurred by the small urine flow. It is believed that the GFR has been elevated over basal conditions by the administration of a water load. This statement is based on the decrease in GFR observed during the Tm_{PAH} clearance period, larger normal clearance values obtained in this study than in others when a water load was not administered, and the experiments of Sellwood and Verney (57). Larger increases in renal clearances would most likely have resulted subsequent to the protein intake if the clearance values had not already been elevated by the administration of water. These alterations in the renal clearance indicate the importance of a fasting condition for at least 6 hours prior to an experiment. A constant protein load could be given to dogs just prior to the clearance measurement in an attempt to obtain maximal clearance values which might have minimum fluctuations. The difficulty with this procedure would be in procuring a diet in which the solute content was constant since

this would affect the osmolar clearance.

A correlation apparently existed between the increase in urine volume and the increased solute excretion. The increased solute excretion did not increase the free water clearance to any degree. More solute was presented to the tubule due to the increased GFR and almost constant plasma osmolarity. More water in relation to solute was removed from the tubule after feeding than in the fasting animal. No conclusions as to the mechanism by which the kidney accomplishes this can be obtained from this type of experiment.

B. Normal Values

The clearance values in this study are considered as normal values for the conditions of this experiment and are summarized in Table 2. The values for each determination are in Appendix F. The intervals of the clearance determinations and the dogs' weight are recorded in Figures 3 and 4. These values are not basal values; but since renal function in the dog is very labile, certain conditions must be maintained. A large urine flow is desirable for the accurate measurement of renal clearances. Osmotic diuretics are capable of increasing urine flow, but it was considered that a diuresis produced by water was more physiological and might not alter the normal renal function as much. It is realized that the oral intake of 80 ml of water/kg of body weight by a hydrated

Table 2. Mean clearance values of 6 female beagles

	GFR ml/min Creatinine clearance			ERPF ml/min PAH clearance			ERBF ml/min		
	Actual value	Per kg body wt.	Per m^2 BSA	Actual value	Per kg body wt.	Per m^2 BSA	Actual value	Per kg body wt.	Per m^2 BSA
Number of dogs	6	6	6	6	6	6	6	6	6
Mean	39.4	4.35	94.6	97.8	10.85	235.3	154.1	17.1	371.37
Range	37.2-40.6	4.0-4.7	90.0-100.8	91.9-111.6	10.0-12.2	213.2-270.9	145.6-165.9	14.9-20.7	337.7-432.0
Standard deviation	1.23	0.26	3.95	7.55	0.98	21.27	8.36	2.12	35.9
Coefficient of variation	3.1	6.0	4.2	7.7	9.0	9.0	5.4	12.4	9.7
Standard error	0.50	0.11	1.61	3.08	.40	8.68	3.41	0.87	14.6

Table 2. (continued)

	T_m PAH mg/min			Osmolar clearance ml/min			Free water clearance ml/min				
	Actual value	Per kg body wt.	Per m^2 BSA	Actual value	Per kg body wt.	Per m^2 BSA	Actual value	Per kg body wt.	Per m^2 BSA	FF per cent	U/P creatinine
Number of dogs	6	6	6	6	6	6	6	6	6	6	6
Mean	5.41	0.60	13.02	1.03	0.115	2.48	3.19	0.353	7.67	40.6	9.67
Range	4.92-6.00	0.54-0.66	11.7-14.6	.94-1.22	0.09-0.13	2.14-2.79	2.69-3.72	0.27-0.41	6.07-9.03	35.5-43.0	8.3-1.05
Standard deviation	0.43	0.05	1.11	.11	.016	.27	.40	.05	1.05	2.89	1.13
Coefficient of variation	7.9	8.3	8.5	10.7	13.9	10.9	12.5	14.2	13.7	7.1	11.7
Standard error	0.17	0.02	0.45	.04	.007	.11	.164	.021	.429	1.18	.46

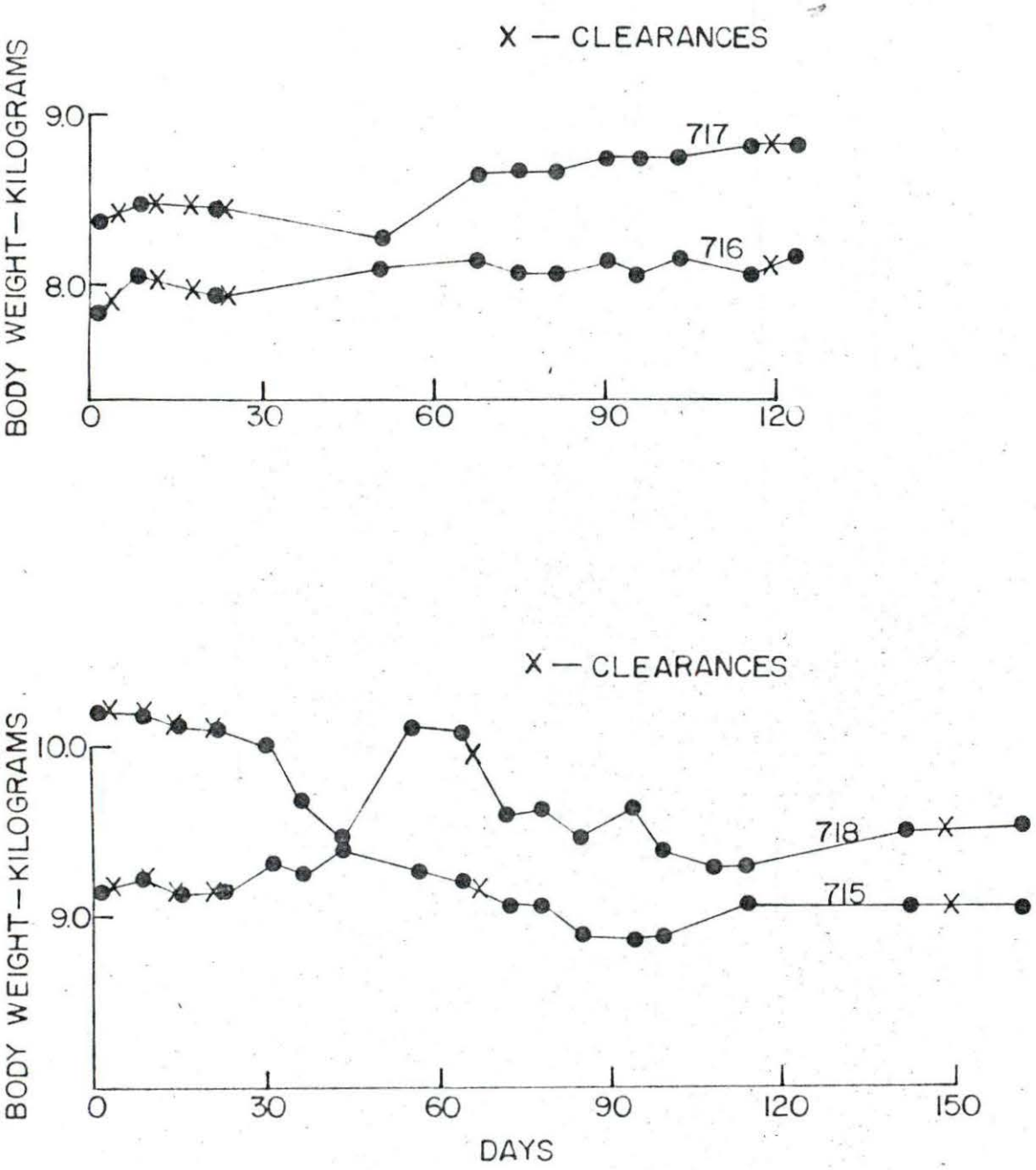

Figure 3. Time of clearance determinations and body weight in dogs 715, 716, 717 and 718

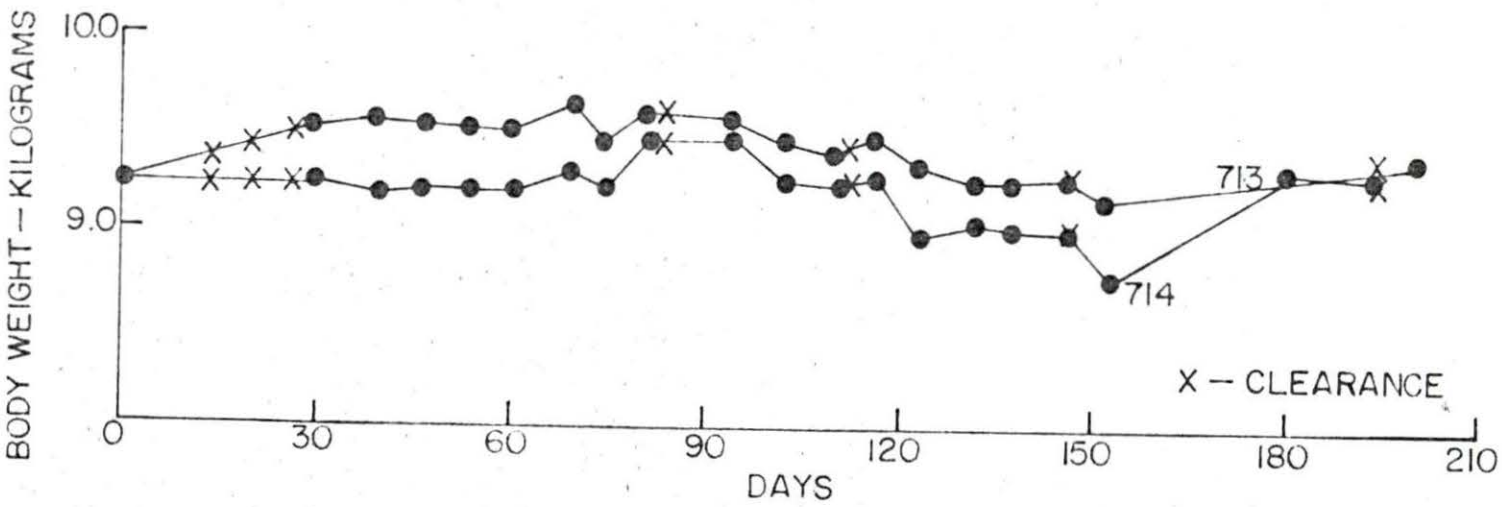

Figure 4. Time of clearance determinations and body weight in dogs 713 and 714

dog is not completely physiological.

1. GFR

The mean GFR measured during the RPF clearance period was 39.40 ml/min with a standard deviation of 1.23 ml/min in 6 dogs. The average GFR per kg of body weight was 4.35 ± .26 ml/min. The analysis of variance charts for both these values may be seen in Table 3. A significant difference among days ($P < .01$) was present in the actual clearance values and those calculated on a kg of body weight basis. In the analysis of variance in which the actual clearance value was tested, no significant difference at the 1% level could be detected among dogs but there was a difference ($P < .01$) when the value was based on body weight. When the GFR was adjusted for body surface area, the mean value was 94.6 ml/min/m^2 of body surface area with a standard deviation of 3.95 ml/min which gives a very low coefficient of variation (4.2%). The GFR shows less variation among dogs when it is based on the body surface area instead of body weight.

The mean value of GFR per kg of body weight was almost identical to the value obtained by Houck (27). The value calculated on a body surface area basis was greater than Houck's value which was determined from 75 trained female mongrel dogs. His method of diuresis was similar to the procedure used in this study. Asheim's (5) normal values

Table 3. Analysis of variance of the GFR

Source	Degrees freedom	Sum of squares	Mean square	Component	Percentage	F
Analysis of variance of GFR actual values						
Dogs	5	125.700	25.140	.524	5.54	1.59
Days	30	474.620	15.820	3.540	37.36	2.92**
Observations	70	378.740	5.410	5.41	57.10	
Total	105	979.060	9.324	9.475	100.00	
Analysis of variance of GFR/kg of body weight						
Dogs	5	4.971	.994	.046	30.88	5.74**
Days	30	5.197	.173	.035	23.57	2.52**
Observations	70	4.809	.068	.068	45.55	
Total	105	14.979		.150	100.00	

**Significant at the 1% level.

which were lower than the mean obtained in this study may have been due to the fact that the dogs were anesthetized and the diuresis was not as large. Russo (53) calculated the GFR to be 94 ml/min/m^2 of body surface area which is almost identical to the GFR obtained in this study.

The standard deviation of the GFR per kg of body weight in this experiment was similar to Houck's (27) and Asheim's (5) who utilized 75 female mongrels and 32 male and female cocker spaniels and mongrel dogs, respectively. When analysed on a square meter of body surface basis, the deviation

in this experiment was much less than those previously calculated. The fact that littermates were used may have made it possible to obtain similar standard deviations as procured with much larger numbers of dogs. Some dogs indicated a greater variation within all their determinations than others. The standard deviation within each dog's GFR was between 2.45 and 3.34 ml/min and dogs 715 and 716 had the largest while 714 and 718 had the least.

2. ERPF and ERBF

The mean ERPF of 6 beagles measured by the PAH clearance was 97.8 ml/min with a standard deviation of 7.55 ml/min. When the ERPF was adjusted for body weight, it resulted in a value of 10.85 $\pm$ .98 ml/min/kg of body weight. Adjustment of the ERPF to body surface area disclosed a mean of 235.3 ml/min/m^2 with a standard deviation of 21.27 ml/min. The ERPF determined from the 6 beagles was lower than that proposed by other investigators except Russo (53) and White (72). The examination of experimental procedures did not disclose any consistent variation of technique which might account for this difference. Houck and Stamler (27, 65) used osmotic diuretics and Asheim (5) utilized anesthetized animals. Kubicek's (36) mean ERPF of 257 ml/min/m^2 of body surface area was higher and the experimental procedure was similar to that used in this study. In none of the normal experiments

was a value of ERBF estimated. No other experiment appeared to administer as large a per cent of the body weight as water and this could result in a lower packed cell volume. It is possible that in all the normal studies ERBF may be identical and the variation of ERBF is dependent upon fluctuations in the packed cell volume. A mean value of 154.1 ml/min with a standard deviation of 8.36 ml/min was calculated for the ERBF. The coefficient of variation was almost identical to that obtained for the ERPF indicating that the ERBF can be utilized without fear of large fluctuations in its value. The ERBF was 17.1 $\pm$ 2.12 ml/min/kg of body weight and 371.37 $\pm$ 35.87 ml/min/m^2 of body surface area.

The analysis of variance on the ERPF and ERBF actual values and on a weight basis indicated a significant portion ($P < .01$) of the variance among dogs and among days in all instances except the difference among dogs could not be shown to be significant at the 1% level, when the actual value of the ERBF was observed. There are large fluctuations of ERPF and ERBF in one dog between different measurements. Much more instability of ERPF and ERBF than GFR was observed, agreeing with the observations of other investigators. It was possible to obtain much lower coefficients of variation in this study than any previously obtained. The greatest variation was observed in dogs 715 and 716 while 713 and 718 had the slightest. It must be noted here that 716 and 717 had only 11 and

14 observations while the others had from 16-20 observations.

In the discussion of the normal values of GFR, osmolar clearance, and ERPF the variation within each dog was mentioned. There was reason to believe that the emotional state of the animal might influence the variability of the clearances. In an attempt to assess the effect of emotional state, the laboratory technician, who was present during all clearance measurements but did not know about the actual variation of clearances in any one animal, described each dog's emotional state during clearance determinations. She rated dogs 713, 716, and 717 as generally calm and 714, 715, and 718 as occasionally nervous. These do not appear to correspond very well since 718 had the least variation and 716 had the greatest variation of all values. Dog 715 did have a greater variation in the GFR and ERPF and was thought to be slightly nervous. The fact that emotional states can cause variation in the clearances can not be discredited since the accurate determination of anxiety in the dog is extremely difficult.

The relationship of the ERPF and ERBF to the body weight and the body surface area did not reduce the variability among dogs. The coefficient of variation was smallest when the actual clearance value was used in contrast to those adjusted for weight or body surface area.

3. Tm_{PAH}

The tubular maxima secretion of PAH should denote the functional amount of renal tubular tissue. Occasional lower values of Tm_{PAH} were observed and examined for the possible existence of self-depression. The correlation coefficient between the plasma PAH concentration and the Tm_{PAH} was positive (.0796) but could not be shown to be different from zero at the 1% level of significance. The load of PAH reaching the tubules may be calculated by determining the amount of PAH that flows through the kidney per minute and subtracting the amount filtered by the glomerulus. Asheim (5) found a linear relationship between the Tm_{PAH} and the load/Tm_{PAH} ratio. In this study the load/Tm_{PAH} ratio varied only from 1.65-5.49 and included only 34 observations but a statistically significant portion ($P < .01$) of the Tm_{PAH} variance was explained by the load/Tm_{PAH} ratio. There were some indications of a linear relationship between the ratio of the blood perfusing the tubule (ERPF-GFR) and the GFR. Four observations prevented the statistical proof that this regression explained a significant proportion of the variance of the Tm_{PAH}. These four values all had an ERPF which was noticeably different from the mean ERPF value for that dog. The higher the ratio of blood perfusing the tubule to the GFR the greater the Tm_{PAH} was. It is to be noted that in this

ratio no account is made for the plasma PAH concentration. This may just be a phenomenon which occurs at the plasma level range of PAH present in this study. Further investigations would be necessary at more varied plasma PAH levels to study this possible mechanism of Tm_{PAH} self-depression.

The Tm_{PAH} had a mean value of 5.41 $\pm$ .43 mg/min with a coefficient of variation 7.9%. The coefficient of variation was 8.3 and 8.5%, respectively, with a mean of .60 $\pm$ .05 mg/min/kg of body weight and 13.02 $\pm$ 1.11 mg/min/m^2 of body surface area. This Tm_{PAH} value was lower than any value reported in the literature. The variance among dogs was less than in any other study reported. The analysis of variance did not show at the 1% level any significant portion of the variance of Tm_{PAH} actual value and Tm_{PAH}/kg of body weight due to different dogs. In both instances the variation of experiments on the same dog on different days was significant ($P < .01$) and comprised the major component of the variance.

4. Water and solute excretion

No other normal values for osmolar clearance are available, presumably because of their great dependence on the experimental conditions. The values obtained for these clearances in this study could only be used as a standard as long as the experimental conditions of this study are strictly followed.

The coefficient of variation of the osmolar clearance was approximately 10% when the actual clearance and the clearance based on body surface area were used. The variation was almost 15% when the osmolar clearance was adjusted to body weight. No significant difference of the osmolar clearance at the 1% level was found to exist among dogs. A significant portion ($P < .01$) of the variance was found to exist among the observations done on different days within the same dog. The largest variation in any one dog was in dog 716 and the least was in dog 718. Dog 717's variance was also slightly higher than dogs 713, 714, and 715 which had intermediate values.

Since the osmolar clearance is calculated from the urine volume, and the urine and plasma osmolarities, it was decided to determine their individual effects on the osmolar clearance. Their effects were observed within the PAH clearance observations and the Tm_{PAH} periods. Significant positive correlation coefficients of .7063 and .8664 were calculated for the PAH and Tm_{PAH} clearance periods, respectively, between the osmolar clearance and urine volume. In both instances the regression of osmolar clearance on urine volume accounted for a very large portion of the variation in samples, There was a positive correlation of .7498 between the osmolar clearance and the urine volume with an analysis of all clearance periods. The effect of fitting urine volume to the osmolar

clearance is statistically significant ($P < .01$) even after fitting both urine osmolarity and plasma osmolarity. The osmolar clearance utilizing only the urine volume was .436 + .153 times the urine volume. Studies with greater variations in urine volumes would have to be conducted to see if the osmolar clearance could be predicted from urine volume alone.

In these experiments urine osmolarity had a significant negative correlation ($P < .01$) to osmolar clearance with coefficients of .2824 and .4162 in the PAH clearance and Tm_{PAH} periods. The regression analysis indicated that a significant portion of the variations of the osmolar clearance could be explained by predicting an effect of urine osmolarity on the osmolar clearance but it did not explain as much of the variation as accomplished by urine volume. The urine osmolarity was found to explain a significant portion of the variation of the osmolar clearance after the urine volume had been adjusted for with an analysis of all the clearance periods. In the combined analysis of the ERPF and Tm_{PAH} clearance periods the correlation of urine osmolarity and osmolar clearance could not be shown to be different from zero at the 1% level, and the urine osmolarity's effect on the variance of the osmolar clearance was not significant. The osmolar clearance utilizing the urine volume and urine osmolarity was equal to -.267 + .227 times the urine volume + .005 times the urine osmolarity.

The plasma osmolarity did not show any correlation with the osmolar clearance during the entire or any portion of the experiment. It still did not explain a significant portion of the variance after the osmolar clearance was adjusted for urine volume and urine osmolarity. It must be noted here that the plasma osmolarity did not vary very much during the measurement of the osmolar clearance and no information on the osmolar clearance is available during the initial rapid change in plasma osmolarity or during the time when it returned to normal values. This analysis reveals that alterations in urine volume do have significant effects on osmolar clearance in this experiment and must be considered when trying to obtain standard values. Even with this effect of urine volume on osmolar clearance coefficients of correlation of only 10.7%, 13.9% and 10.9% were found when the osmolar clearance actual value per kg of body weight and per m^2 of body surface area are calculated. The mean osmolar clearance of 1.03 $\pm$ .11 ml/min was seen in the 6 dogs with a value of .115 $\pm$ .016 ml/min/kg of body weight and 2.48 $\pm$ .27 ml/min/m^2 of body surface area. These variations are relatively no greater than those observed in measuring the RBF, RPF, and Tm_{PAH}.

The free water clearance was a more variable determination and its value was heavily dependent upon urine flow. A mean of 3.19 $\pm$ .40 ml/min and a correlation of 14.2% were measured. The values per kg of body weight and per m^2 of

body surface area were .353 ± .05 and 7.67 ± 1.05 ml/min, respectively. The correlations of free water clearance with any factor which indicates a concentration of the urine or changes coincidentally with increased urine concentration normally had a large value. The free water clearance was very highly correlated to the urine volume and most of the variance of the free water clearance may be accounted for by the regression on the urine volume. The urine volume of course is utilized in calculating the free water clearance and therefore should have a large influence on the free water clearance. Other indications of urine flow such as increased urine concentration, urine osmolarity and the U/P creatinine ratio show correlations with the free water clearance. No correlation at the 1% level could be exhibited between the plasma osmolarity and the free water clearance in the ERPF and Tm_{PAH} periods separately but there was a significant correlation ($P < .01$) when the entire experiment was considered.

It was considered that if the water and solute clearances were observed during the period of maximum urine flow it might reveal certain correlations not evident throughout the entire experiment and it might have less variation than those in which urine volume is fluctuating. In 36 observations the osmolar clearance was 1.06 ± .20 ml/min and the free water clearance was 3.45 ± .59 ml/min. These values indicate that there is a greater deviation than if all 3 urine collections

in the PAH clearance period are considered. The mean values are slightly higher than those obtained in all 3 periods but not by a large amount. A correlation of greater than zero could not be proven at the 1% level between the plasma osmolarity and free water clearance and between plasma osmolarity and osmolar clearance. The relation between urine osmolarity with the free water and osmolar clearances could not be shown to be significant at the 1% level with this smaller sampling. The urine volume explained a significant portion ($P < .01$) of the variations of the osmolar and free water clearance. By observing the peak urine flow a correlation of greater than zero ($P < .01$) was found between the GFR and the osmolar clearance and between the ERPF and the free water clearance. The regression of the osmolar clearance on the GFR accounts for a significant portion ($P < .01$) of the variation and the GFR equals 31.6 + 8.12 times the osmolar clearance. The partition of the mean squares using regression of the free water clearance on the ERPF accounted for a significant portion ($P < .01$) of the mean square.

5. Correlations

The FF was calculated by dividing the creatinine clearance by the PAH clearance. The mean value of the FF was 40.6 $\pm$ 2.89% with a coefficient of variation of 7.1%. The only other investigator to find a FF this high during normal

clearance determinations was Russo (53). The coefficient of variation of the FF that occurred in this study was less than that of the ERPF but greater than that of the GFR. Commonly it is believed that the GFR and the ERPF vary in proportion to each other and therefore the FF should remain constant. There was a significant correlation ($P < .01$) between the GFR and ERPF. The regression of the ERPF on the GFR explained a significant amount ($P < .01$) of the variation of the ERPF. The ERPF was calculated to be 14.59 + 2.12 times the GFR. The correlation of the GFR to FF could not be shown to be greater than zero at the 1% level but the correlation of ERPF to the FF was -.7824 and was significantly (P .01) greater than zero.

The determination of the per cent of blood filtered at the glomerulus is obtained by dividing the GFR by the ERBF instead of the ERPF. The normal value was 26.0 $\pm$ 3.3% in an analysis of each individual simultaneous determination of the GFR and ERBF.

In an attempt to find a more accurate method of relating renal function among dogs than on body weight or body surface area, the clearances were compared on the dogs Tm_{PAH} value. This was accomplished by dividing the clearance under consideration by the mean Tm_{PAH} for that dog. The GFR/Tm_{PAH} ratio was 7.30 $\pm$ .46 with a coefficient of variation of 6.35%. This did not reduce the variation among dogs. The only

instance where relating a clearance to the Tm_{PAH} reduced variation was with the ERPF. A mean ratio of 18.11 with a standard deviation .781 and a correlation coefficient of 4.31% were determined. The ERBF/Tm_{PAH} ratio did not reduce the variance and had a mean of 28.62 $\pm$ 2.72 with a coefficient of variation of 9.51%. The free water clearance/GFR ratio was 12.5 $\pm$ 1.6 with a coefficient of variation of 13%.

The final urine formed has the varying properties of volume and osmolarity. It is of interest to observe if any renal functions have a correlation with these parameters. In interpreting correlations it must be remembered that if two values are correlated it does not prove that there is a cause and effect relationship. Their relationship may be entirely independent and linked to another factor.

In an examination of the entire experiment the GFR had a positive correlation of .6220 with the urine volume. The urine volume explained a very large portion of the variation in the GFR and the GFR was 23.47 + 3.29 times the urine volume. It was observed that following water ingestion the GFR and urine volume were increased in the Tm_{PAH} period. The formation of a large volume of urine is known to occur due to the suppression of ADH release. Apparently ADH release has been suppressed during this experiment as evidenced by the smooth urine excretion curves and its influence may be discounted. The increase and decrease in urine volume must then

be independent of the action of ADH. It is possible that the dilution of the blood or other consequence of water ingestion may affect urine volume directly or the GFR might be the regulating factor of urine volume during maximal water diuresis. The correlation of the GFR to plasma osmolarity was .6204 and negative. The portion of variance of the GFR due to the regression of the plasma osmolarity was significant ($P < .01$) and the GFR was calculated to be 153.82 - .43 times the plasma osmolarity. There was also a significant negative correlation ($P < .01$) of .2346 between the plasma osmolarity and the urine volume. The regression of urine volume on plasma osmolarity was significant ($P < .01$) and was 156.81 - .555 times the plasma osmolarity. This relationship of plasma osmolarity to urine flow was not as evident as the relationship between plasma osmolarity and the GFR. There was a negative correlation between ERPF and the plasma osmolarity of .3359, and the regression was significant ($P < .01$). A correlation of greater than zero at the 1% level could not be shown between the ERBF and the plasma osmolarity. A positive correlation of .3986 of the ERPF to the urine volume was observed with a significant portion ($P < .01$) of the urine variance explained by the ERPF. The correlation of the ERBF to the urine volume was also shown to be greater than zero and was .2768. The ERPF could be expected to increase with a decrease in plasma osmolarity if the ERBF remained constant

since a larger portion of the blood would be non-cellular. A decrease in the plasma osmolarity would also indicate a decrease in the plasma dilution of protein and a decreased osmotic pressure of the blood. This might be responsible for alterations in the glomerular filtration rate. Also since the plasma flow to the kidney increased with decreased osmolarity, it would be expected that more glomerular filtrate would be formed.

The urine osmolarity also bears some relationship to other kidney functions. The deviations in urine volume and plasma osmolarity may be observed concomittantly with the urine osmolarity if the osmolar clearance is determined. The ERPF could not be shown to have a correlation of greater than zero at the 1% level with urine osmolarity and the same was true of GFR during the PAH clearance period. The correlation of the GFR with urine osmolarity was significant when the whole experiment was considered and also during the Tm_{PAH} clearance period. It may be coincidental that the GFR decreases as the urine osmolarity increases with the decreased urine volume.

A positive correlation of .3596 and .3507 was observed, respectively, with the ERPF and the GFR with the osmolar clearance. Both of these could be shown to have significant linear regressions ($P < .01$). The increased GFR would present the tubule with a larger volume and consequently cause more

osmotically active substances to be excreted. When the GFR and ERPF was increased, there was a decrease in the plasma osmolarity which would also increase the osmolar clearance. Another factor which could increase the osmolar clearance was an increase in the urine volume which was also associated with an increase in GFR and ERPF. To try and separate the effect of urine volume and GFR a regression with both factors and then with each factor separately was analyzed. The GFR still had a significant effect ($P < .01$) in explaining the variation of the osmolar clearance after the effect of urine volume was considered. This suggests that the increased filtrate might in some way alter solute transfer in the tubule.

V. SUMMARY AND CONCLUSIONS

A standard procedure has been developed to accurately measure as many renal clearances as possible at one time. Creatinine and PAH are infused continuously and a water load of 80 ml/kg of body weight was administered per os. Chemical procedures were modified slightly to adapt to the volumes and concentrations encountered in this procedure. A computer program was developed to enable the calculation of all clearances from the urine volumes, optical densities obtained from the chemical analysis of plasma and urine samples, and the plasma and urine osmolarities. Further computer analysis also enabled extensive statistical analysis and was used in obtaining diuresis curves.

The main purpose of this paper has been to provide normal renal clearance values for female beagles. The dogs utilized were approximately 1 year of age, but no difference in renal function during the early years of life might be expected. The mean GFR was 39.4 ml/min, 4.35 ml/min/kg of body weight, and 94.6 ml/min/m^2 of body surface area as measured by the clearance of creatinine. The PAH clearance designed to measure the ERPF was 97.8 ml/min, 10.85 ml/min/kg of body weight, and 235.3 ml/min/m^2 of body surface area. The ERBF was calculated to be 154.1 ml/min, 17.1 ml/min/kg of body weight and 371.4 ml/min/m^2 of body surface area. Values for the Tm_{PAH}

were 5.41 mg/min, .60 mg/min/kg of body weight, and 13.0 mg/min/m^2 of body surface area. The FF determined was 40.6%. The values of the osmolar and free water clearance could only be considered standard when the experimental procedure as outlined in this study is unaltered. The osmolar clearance was 1.03 ml/min, .115 ml/min/kg of body weight and 2.48 ml/min/m^2 of body surface area. Values for the free water clearance were 3.19 ml/min, .35 ml/min/kg of body weight and 7.67 ml/min/m^2 of body surface area. The free water clearance was influenced by the volume of urine excreted and its coefficient of variation was larger than any other clearance measured. The GFR/Tm_{PAH} ratio was 7.3, the ERPF/Tm_{PAH} ratio was determined to be 18.1, and the ERBF/Tm_{PAH} ratio was 28.6. A calculation of the free water clearance/GFR gave a ratio of 12.5.

The clearances were shown to be influenced by certain conditions in the experiment. A decrease in the GFR during the experiment was apparently prevented by the administration of a second water load immediately following the measurement of the PAH clearances. It was originally thought that water administration at this time would cause excessive anxiety in the dog which might alter the clearances. This did not occur in several instances where it was used, and it almost completely prevented the clearance values from decreasing in the Tm_{PAH} clearance periods. In future experiments the use of

this second water load would be recommended. The ingestion of food within 6 hours of the experiment was shown to increase the GFR, ERPF, urine osmolarity, and the osmolar clearance. This stresses the importance of fasting prior to the measurement of renal clearances.

The variability in this study was less than any published which is apparently due to the homogenicity of the animals utilized. The use of littermate animals would be beneficial when a small number of dogs are needed in a study of renal function. The least variability among the mean clearances for each dog was found when the actual clearance value for each dog was considered. The variability generally increased slightly with the clearance adjusted for body weight and then more when it was adjusted for body surface area. The only incidence in which the variability of any clearance was reduced was when the ERPF was related to the Tm_{PAH}. This indicates in this experiment that renal clearances were more closely controlled by genetic factors than by weight or body surface area.

The clearance values done on different days on the same dog were the greatest source of variability. This would indicate that frequent observations on different days would be most valuable in obtaining the most efficient experimental design. This does not mean that a large number of animals should not be observed in any experiment as long as observa-

tions are made on each dog on several different days.

The correlations between the various clearances produced some interesting relationships, but no proof for the regulation of one clearance by another can be inferred. The relationships that were noted merit further observations and study to disclose any causal relationships. The plasma osmolarity was shown to vary inversely with the GFR and the ERPF. The GFR and the ERPF varied directly with the urine volume. The GFR and ERPF also varied directly with the osmolar clearance.

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VIII. APPENDIX A. CREATININE DETERMINATION

Reagents:

Sulfuric acid: 1/9 N

Sodium tungstate: 3.333 gm/100 ml

Picric acid: 11.75 gm/1000 ml

Sodium hydroxide: 10 gm/100 ml

Alkaline picrate solution: To be made up immediately before use. One volume 10% sodium hydroxide added to 5 volumes of picric acid solution.

Stock standard creatinine: Creatinine in water, 50 mg/100 ml

Procedure:

(1) Precipitation of plasma protein

Add 0.5 ml of plasma to 3.0 ml of sulfuric acid

Add 1.5 ml sodium tungstate, mix, and remove the supernatant after centrifugation

(2) Preparation of standards

From the stock standard solutions of .01, .02, .04, and .05 mg/ml are made up in water

(3) Determination

One ml of each protein free filtrate, each urine diluted 1 - 100 and each standard is placed in test tubes. Duplicate samples of the standards are done.

Two ml of alkaline picrate solution are added to all samples.

Photocolorimetric recordings are obtained after 20 minutes at a wavelength of 525 mμ.

IX. APPENDIX B. PAH DETERMINATION

Reagents:

Trichloroacetic acid: 3.486 gm/100 ml

Hydrochloric acid: 1 N

Sodium nitrite: 100 mg/100 ml

Ammonium sulfamate: 500 mgm/100 ml

N-(1-naphthyl) ethelenediamine: 100 mg/100 ml

Stock standard of PAH: 25 mg/100 ml

Procedure:

(1) Precipitation of plasma protein

Add 0.5 ml of plasma to 3.5 ml of trichloroacetic acid

The mixture is shaken well and the supernatant removed after centrifugation

The protein-free filtrates from the Tm_{PAH} clearance period are diluted 1-11

(2) Urine dilution

Urine samples during the PAH clearance period are diluted 1-200

Urine samples from the Tm_{PAH} clearance periods are diluted 1-2500

(3) Preparation of standard

Dilutions of the stock standard to .005, .01, .02, and .04 mg/ml are made

(4) Determination

Two ml samples of each urine dilution, each protein free filtrate and each standard are added to test tubes. Duplicate samples of the standard are prepared.

Hydrochloric acid, 0.4 ml, is added to each sample

Sodium nitrite, 0.2 ml, is added to all samples

After a 3-5 minute pause 0.2 ml of ammonium sulfamate is added to all samples

After another 3-5 minute pause 0.2 ml of ethylenediamine is added

The optical density of the samples is determined in the spectrophotometer at a wavelength of 540 mμ after 15 minutes

X. APPENDIX C. CLEARANCE DETERMINATION PROGRAM AND RESULTS

A. Program

```
C       CALCULATION OF RENAL CLEARANCES
        DIMENSION X(15),Y(15),B(15),V(15),U(15),XCR(15),YCR(15),BCR(15),
       1UCR(15),UO(15),BO(15),IDOG(15),IEXP(15),ICD(15),TIME(15),HCT(15)
        ISW=ISW
C       NO OF DOGS AND EXPS IS INO
        READ INPUT TAPE 1,600,INO
  600 FORMAT(I3)
        DO 9 N=1,INO
        READ INPUT TAPE 1,100,K
  100 FORMAT(I2)
        DO 3 I=1,K
        READ INPUT TAPE 1,101,X(I),Y(I),B(I),V(I),U(I),XCR(I),YCR(I),
       1BCR(I),UCR(I),UO(I),BO(I),IDOG(I),IEXP(I),ICD(I),TIME(I),ISW
       2,HCT(I)
  101 FORMAT(F3.1,F5.1,F3.0,F5.3,F3.0,F3.0,F5.1,F3.0,F3.0,2F6.2,I3,I2,
       1I2,F2.0,I2,F3.2)
    3 CONTINUE
        XCRM=(XCR(1)+XCR(2)+XCR(3)+XCR(4))/4.0
        YCRM=(YCR(1)+YCR(2)+YCR(3)+YCR(4))/4.0
        XDCR=XCR(1)*XCR(1)+XCR(2)*XCR(2)+XCR(3)*XCR(3)+XCR(4)*XCR(4)
        XSCR=XCR(1)+XCR(2)+XCR(3)+XCR(4)
        XSSCR=XSCR*XSCR
        DCR=XDCR-(XSSCR/4.0)
        YSCR=YCR(1)+YCR(2)+YCR(3)+YCR(4)
        SXYCR=XCR(1)*YCR(1)+XCR(2)*YCR(2)+XCR(3)*YCR(3)+XCR(4)*YCR(4)
        RCR=SXYCR-((XSCR*YSCR)/4.0)
        BBCR=RCR/DCR
        ACR=YCRM-(XCRM*BBCR)
        CRFAC=DCR/RCR
```

```
PCR1=(((BCR(2)+BCR(3))/2.0)-ACR)*(10.0*CRFAC) /1000.0
PCR2=(((BCR(3)+BCR(4))/2.0)-ACR)*(10.0*CRFAC) /1000.0
PCR3=(((BCR(4)+BCR(5))/2.0)-ACR)*(10.0*CRFAC) /1000.0
PCR4=(((BCR(5)+BCR(6))/2.0)-ACR)*(10.0*CRFAC) /1000.0
PCR5=(((BCR(6)+BCR(7))/2.0)-ACR)*(10.0*CRFAC) /1000.0
PCR6=(((BCR(7)+BCR(8))/2.0)-ACR)*(10.0*CRFAC) /1000.0
PCR7=(((BCR(8)+BCR(9))/2.0)-ACR)*(10.0*CRFAC) /1000.0
PCR8=(((BCR(9)+BCR(10))/2.0)-ACR)*(10.0*CRFAC)/1000.0
UV1=V(1)-(10.0/TIME(1))
UV2=V(2)-(10.0/TIME(2))
UV3=V(3)-(10.0/TIME(3))
UV4=V(4)-(10.0/TIME(4))
UV5=V(5)-(10.0/TIME(5))
UV6=V(6)-(10.0/TIME(6))
UV7=V(7)-(10.0/TIME(7))
UV8=V(8)-(10.0/TIME(8))
VCCR1=(UCR(1)-ACR)*100.0*CRFAC/1000.0
VCCR2=(UCR(2)-ACR)*100.0*CRFAC/1000.0
VCCR3=(UCR(3)-ACR)*100.0*CRFAC/1000.0
VCCR4=(UCR(4)-ACR)*100.0*CRFAC/1000.0
VCCR5=(UCR(5)-ACR)*100.0*CRFAC/1000.0
VCCR6=(UCR(6)-ACR)*100.0*CRFAC/1000.0
VCCR7=(UCR(7)-ACR)*100.0*CRFAC/1000.0
VCCR8=(UCR(8)-ACR)*100.0*CRFAC/1000.0
UCCR1=(VCCR1*V(1))/UV1
UCCR2=(VCCR2*V(2))/UV2
UCCR3=(VCCR3*V(3))/UV3
UCCR4=(VCCR4*V(4))/UV4
UCCR5=(VCCR5*V(5))/UV5
UCCR6=(VCCR6*V(6))/UV6
UCCR7=(VCCR7*V(7))/UV7
UCCR8=(VCCR8*V(8))/UV8
```

```
GFR1=(VCCR1*V(1))/PCR1
GFR2=(VCCR2*V(2))/PCR2
GFR3=(VCCR3*V(3))/PCR3
GFR4=(VCCR4*V(4))/PCR4
GFR5=(VCCR5*V(5))/PCR5
GFR6=(VCCR6*V(6))/PCR6
GFR7=(VCCR7*V(7))/PCR7
GFR8=(VCCR8*V(8))/PCR8
UPCR1=UCCR1/PCR1
UPCR2=UCCR2/PCR2
UPCR3=UCCR3/PCR3
UPCR4=UCCR4/PCR4
UPCR5=UCCR5/PCR5
UPCR6=UCCR6/PCR6
UPCR7=UCCR7/PCR7
UPCR8=UCCR8/PCR8
XMEAN=(X(1)+X(2)+X(3)+X(4))/4.0
YMEAN=(Y(1)+Y(2)+Y(3)+Y(4))/4.0
XD=   X(1)*X(1)+X(2)*X(2)+X(3)*X(3)+X(4)*X(4)
XS=  X(1)+X(2)+X(3)+X(4)
XSS=XS*XS
D=XD-(XSS/4.0)
YS=Y(1)+Y(2)+Y(3)+Y(4)
SXY=X(1)*Y(1)+X(2)*Y(2)+X(3)*Y(3)+X(4)*Y(4)
R=SXY-((XS*YS)/4.0)
BB=R/D
A=YMEAN-(XMEAN*BB)
CFAC=D/R
P1=(((B(2)+B(3))/2.0)-A)*(8.0*CFAC)/1000.0
P2=(((B(3)+B(4))/2.0)-A)*(8.0*CFAC)/1000.0
P3=(((B(4)+B(5))/2.0)-A)*(8.0*CFAC)/1000.0
P4=(((B(5)+B(6))/2.0)-A)*(8.0*CFAC)/1000.0
P5=(((B(6)+B(7))/2.0)-A)*(88.0*CFAC)/1000.0
P6=(((B(7)+B(8))/2.0)-A)*(88.0*CFAC)/1000.0
P7=(((B(8)+B(9))/2.0)-A)*(88.0*CFAC)/1000.0
P8=(((B(9)+B(10))/2.0)-A)*(88.0*CFAC)/1000.0
```

```
VC1=(U(1)-A)*200.0*CFAC/1000.0
VC2=(U(2)-A)*200.0*CFAC/1000.0
VC3=(U(3)-A)*200.0*CFAC/1000.0
VC4=(U(4)-A)*200.0*CFAC/1000.0
VC5=(U(5)-A)*2500.*CFAC/1000.0
VC6=(U(6)-A)*2500.*CFAC/1000.0
VC7=(U(7)-A)*2500.*CFAC/1000.0
VC8=(U(8)-A)*2500.*CFAC/1000.0
UC1=(VC1*V(1))/UV1
UC2=(VC2*V(2))/UV2
UC3=(VC3*V(3))/UV3
UC4=(VC4*V(4))/UV4
UC5=(VC5*V(5))/UV5
UC6=(VC6*V(6))/UV6
UC7=(VC7*V(7))/UV7
UC8=(VC8*V(8))/UV8
RPF1=(VC1*V(1))/P1
RPF2=(VC2*V(2))/P2
RPF3=(VC3*V(3))/P3
RPF4=(VC4*V(4))/P4
RPF5=(VC5*V(5))/P5
RPF6=(VC6*V(6))/P6
RPF7=(VC7*V(7))/P7
RPF8=(VC8*V(8))/P8
RBF1=RPF1*2.0/(2.0-(HCT(2)+HCT(3)))
RBF2=RPF2*2.0/(2.0-(HCT(3)+HCT(4)))
RBF3=RPF3*2.0/(2.0-(HCT(4)+HCT(5)))
TMPH1=(VC5*V(5))-(P 5 *.92*GFR 5 )
TMPH2=(VC6*V(6))-(P 6 *.92*GFR 6 )
TMPH3=(VC7*V(7))-(P 7 *.92*GFR 7 )
TMPH4=(VC8*V(8))-(P 8 *.92*GFR 8 )
```

```
RBF1=RPF1*2.0/(2.0-(HCT(2)+HCT(3)))
RBF2=RPF2*2.0/(2.0-(HCT(3)+HCT(4)))
RBF3=RPF3*2.0/(2.0-(HCT(4)+HCT(5)))
PFF1=GFR1/RPF1*100.0
PFF2=GFR2/RPF2*100.0
PFF3=GFR3/RPF3*100.0
PFF4=GFR4/RPF4*100.0
PFF5=GFR5/RPF5*100.0
PFF6=GFR6/RPF6*100.0
PFF7=GFR7/RPF7*100.0
PFF8=GFR8/RPF8*100.0
FF1=GFR1/RBF1*100.0
FF2=GFR2/RBF2*100.0
FF3=GFR3/RBF3*100.0
PO1=(BO(2)+BO(3))/2.0
PO2=(BO(3)+BO(4))/2.0
PO3=(BO(4)+BO(5))/2.0
PO4=(BO(5)+BO(6))/2.0
PO5=(BO(6)+BO(7))/2.0
PO6=(BO(7)+BO(8))/2.0
PO7=(BO(8)+BO(9))/2.0
PO8=(BO(9)+BO(10))/2.0
CUO1=V(1)/UV1*UO(1)
CUO2=V(2)/UV2*UO(2)
CUO3=V(3)/UV3*UO(3)
CUO4=V(4)/UV4*UO(4)
CUO5=V(5)/UV5*UO(5)
CUO6=V(6)/UV6*UO(6)
CUO7=V(7)/UV7*UO(7)
CUO8=V(8)/UV8*UO(8)
```

```
      COSM1=UO(1)*V(1)/PO1
      COSM2=UO(2)*V(2)/PO2
      COSM3=UO(3)*V(3)/PO3
      COSM4=UO(4)*V(4)/PO4
      COSM5=UO(5)*V(5)/PO5
      COSM6=UO(6)*V(6)/PO6
      COSM7=UO(7)*V(7)/PO7
      COSM8=UO(8)*V(8)/PO8
      FWC1=(V(1)-(10.0/TIME(1)))-COSM1
      FWC2=(V(2)-(10.0/TIME(2)))-COSM2
      FWC3=(V(3)-(10.0/TIME(3)))-COSM3
      FWC4=(V(4)-(10.0/TIME(4)))-COSM4
      FWC5=(V(5)-(10.0/TIME(5)))-COSM5
      FWC6=(V(6)-(10.0/TIME(6)))-COSM6
      FWC7=(V(7)-(10.0/TIME(7)))-COSM7
      FWC8=(V(8)-(10.0/TIME(8)))-COSM8
   26 WRITE OUTPUT TAPE 2,899,IDOG(1),IEXP(1)
  899 FORMAT(36H1    DR. EWALD---DOG EXPERIMENTS 1964,5X,I3,5X,I2)
      WRITE OUTPUT TAPE 2,914
  914 FORMAT(119
     1          HOVAR.  DOG EXP   1              2              3
     2   4              5              6              7              8    )
      WRITE OUT PUT TAPE 2,900,
     1 GFR1,GFR2,GFR3,GFR4,GFR5,GFR6,GFR7,GFR8
  900 FORMAT(4HOGFR,3X,          8(2X,F12.3))
      WRITE OUTPUT TAPE 2,901,RPF1,RPF2,RPF3,RPF4,RPF5,RPF6,RPF7,RPF8
  901 FORMAT(4HORPF,3X,8(2X,F12.3))
      WRITE(2,820)RBF1,RBF2,RBF3
  820 FORMAT(4HORBF,3X,   3(2X,F12.3))
      WRITE(2,821)HCT(1),HCT(2),HCT(3),HCT(4),HCT(5),HCT(6),HCT(7),
     1HCT(8)
```

```
821 FORMAT(4HOHCT,3X,8(2X,F12.3))
    WRITE OUTPUT TAPE 2,902,TMPH1,TMPH2,TMPH3,TMPH4
902 FORMAT(6HOTMPAH,1X,4(2X,F12.3))
    WRITE OUTPUT TAPE 2,903,UPCR1,UPCR2,UPCR3,UPCR4,UPCR5,UPCR6,UPCR7,
   1UPCR8
903 FORMAT(5HOUPCR,2X,8(2X,F12.3))
    WRITE OUTPUT TAPE 2,904,PCR1,PCR2,PCR3,PCR4,PCR5,PCR6,PCR7,PCR8
904 FORMAT(4HOPCR,3X,8(2X,F12.3))
    WRITE OUTPUT TAPE 2,905,UCCR1,UCCR2,UCCR3,UCCR4,UCCR5,UCCR6,UCCR7,
   1UCCR8
905 FORMAT(5HOUCCR,2X,8(2X,F12.3))
    WRITE OUTPUT TAPE 2,906,P1,P2,P3,P4,P5,P6,P7,P8
906 FORMAT(2HOP, 5X,8(2X,F12.3))
    WRITE OUTPUT TAPE 2,907,UC1,UC2,UC3,UC4,UC5,UC6,UC7,UC8
907 FORMAT(3HOUC,4X,8(2X,F12.3))
    WRITE(2,908)PFF1,PFF2,PFF3,PFF4,PFF5,PFF6,PFF7,PFF8
908 FORMAT(4HOPFF,3X,8(2X,F12.3))
    WRITE(2,855)FF1,FF2,FF3
855 FORMAT(4HOFF ,3X,   3(2X,F12.3))
    WRITE OUTPUT TAPE 2,909,PO1,PO2,PO3,PO4,PO5,PO6,PO7,PO8
909 FORMAT(3HOPO,4X,8(2X,F12.3))
    WRITE OUTPUT TAPE 2,910,CUO1,CUO2,CUO3,CUO4,CUO5,CUO6,CUO7,CUO8
910 FORMAT(4HOCUO,3X,8(2X,F12.3))
    WRITE OUTPUT TAPE 2,911,COSM1,COSM2,COSM3,COSM4,COSM5,COSM6,COSM7,
   1COSM8
911 FORMAT(5HOCOSM,2X,8(2X,F12.3))
    WRITE OUTPUT TAPE 2,912,FWC1,FWC2,FWC3,FWC4,FWC5,FWC6,FWC7,FWC8
912 FORMAT(4HOFWC,3X,8(2X,F12.3))
    WRITE(2,822)UV1,UV2,UV3,UV4,UV5,UV6,UV7,UV8
822 FORMAT(4HOUV ,3X,8(2X,F12.3))
    AGFR1=(GFR1+GFR2+GFR3)/3.0
    AUV1=(UV1+UV2+UV3)/3.0
    ARBF=(RBF1+RBF2+RBF3)/3.0
    ARPF=(RPF1+RPF2+RPF3)/3.0
    ATMPH=(TMPH1+TMPH2+TMPH3)/3.0
```

```
      AUPCR=(UPCR1+UPCR2+UPCR3)/3.0
      APFF=(PFF1+PFF2+PFF3)/3.0
      AFF=(FF1+FF2+FF3)/3.0
      ACUO=(CUO1+CUO2+CUO3)/3.0
      ACOSM=(COSM1+COSM2+COSM3)/3.0
      AFWC=(FWC1+FWC2+FWC3)/3.0
      AUV2=(UV5+UV6+UV7)/3.0
      AGFR2=(GFR5+GFR6+GFR7)/3.0
      WRITE(2,700)AGFR1,AGFR2
  700 FORMAT(13HOAVE. GFR 1-3,16X,F9.2,
     15X,
     2                                    11HAVE.GFR 5-7,16X,F9.2  )
      WRITE(2,701)ARPF
  701 FORMAT(13HOAVE. RPF 1-3,16X,F9.2)
      WRITE(2,702)ARBF
  702 FORMAT(13HOAVE. RBF 1-3,16X,F9.2)
      WRITE(2,703)ATMPH
  703 FORMAT(14HOAVE. TMPH 1-3,15X,F9.2)
      WRITE(2,704)ACOSM
  704 FORMAT(14HOAVE. COSM 1-3,15X,F9.2)
      WRITE(2,705)AFWC
  705 FORMAT(13HOAVE. FWC 1-3,16X,F9.2)
      WRITE(2,706)AUV1,AUV2
  706 FORMAT(12HOAVE. UV 1-3,17X,F9.2,
     15X,
     2                                  11HAVE. UV 5-7,16X,F9.2)
      WRITE(2,707)ACUO
  707 FORMAT(13HOAVE. CUO 1-3,16X,F9.2)
      WRITE(2,708)APFF
  708 FORMAT(13HOAVE. PFF 1-3,16X,F9.2)
      WRITE(2,709)AFF
```

```
709 FORMAT(12HOAVE. EF 1-3,17X,F9.2)
    WRITE(2,710)AUPCR
710 FORMAT(14HOAVE. UPCR 1-3,15X,F9.2)
    ICDD=ICDD+ICD(1)
    TIMEE=TIMEE+TIME(1)
 27 CONTINUE
    DO  699 KKK=1,15
    X(KKK)=0.0
    Y(KKK)=0.0
    B(KKK)=0.0
    V(KKK)=0.0
    U(KKK)=0.0
    XCR(KKK)=0.0
    YCR(KKK)=0.0
    BCR(KKK)=0.0
    UCR(KKK)=0.0
    UO(KKK)=0.0
    BO(KKK)=0.0
    IDOG(KKK)=0
    IEXP(KKK)=0
    ICD(KKK)=0
    TIME(KKK)=0.0
    HCT(KKK)=0.0
699 CONTINUE
  9 CONTINUE
    END
```

B. Results

DOG EXPERIMENT 713 8 DATE 9-8-64 WT 9.3

VAR. DOG EXP	1	2	3	5	6	7
GFR	39.674	38.560	38.335	34.276	34.019	32.694
RPF	88.642	77.920	81.120			
RBF	138.504	121.751	126.749			
HCT	0.420	0.360	0.360	0.360	0.360	0.360
TMPAH	4.421	4.834	3.929			
UPCR	12.798	12.050	10.088	8.569	9.583	10.138
PCR	.173	0.181	0.193	0.218	0.226	0.230
UCCR	2.220	2.182	1.948	1.867	2.161	2.335
P	0.015	0.016	.016	0.242	0.236	0.228
UC	0.440	0.397	0.347	3.013	3.441	3.347
PFF	44.757	49.486	47.258	68.819	65.685	69.132
FF	28.644	31.671	30.245			
PO	271.375	267.750	267.375	268.125	273.125	276.000
CUO	70.097	70.875	67.579	78.750	80.746	81.880
COSM	0.901	0.847	0.960	1.175	1.050	0.957
FWC	2.299	2.353	2.840	2.825	2.500	2.268
UV	3.100	3.200	3.800	4.000	3.550	3.225

AVE. GFR 1-3	38.86	AVE.GFR 5-7	33.66
AVE. RPF 1-3	82.56		
AVE. RBF 1-3	129.00		
AVE. TMPH 1-3	4.39		
AVE. COSM 1-3	0.87		
AVE. FWC 1-3	2.50		
AVE. UV 1-3	3.37	AVE. UV 5-7	3.59
AVE. CUO 1-3	69.52		
AVE. PFF 1-3	47.17		
AVE. FF 1-3	30.19		
AVE. UPCR 1-3	11.65		

XI. APPENDIX D. URINE EXCRETION CURVE PROGRAM AND RESULTS

A. Program

```
C       URINE EXCRETION CURVE
C       BETWEEN DOGS HAVE AN 88 CARD        AT END 99 CARD
C       DR. EWALD URINE EXCRETION CURVE
        DIMENSION IDOG(15),IEXP(15),TIME(15),VOL(15)
    8   READ(1,1)ICNTL
    1   FORMAT(I2)
        WRITE(2,36)
   36   FORMAT(1H1)
        CALL PUT (0,0,0,0,0,0,0,0,-1)
        IF(ICNTL-88)12,69,800
   12   DO9 I=1,ICNTL
        READ(1,2)IDOG(I),IEXP(I),TIME(I),VOL(I)
    2   FORMAT(I3,I2,F5.1,F4.2)
    4   CALL PUT (0.0,TIME(I),200.0,0.0,VOL(I),10.0,36,26,0)
        IF(TIME(I)-20.0)21,21,22
   22   IF(TIME(I)-30.0)23,23,24
   24   IF(TIME(I)-40.0)25,25,26
   26   IF(TIME(I)-50.0)27,27,28
   28   IF(TIME(I)-60.0)29,29,30
   30   IF(TIME(I)-70.0)31,31,32
   32   IF(TIME(I)-80.0)33,33,34
   34   IF(TIME(I)-90.0)35,35,56
   56   IF(TIME(I)-100.0)37,37,38
   38   IF(TIME(I)-110.0)39,39,40
   40   IF(TIME(I)-120.0)41,41,42
   42   IF(TIME(I)-130.0)43,43,44
   44   IF(TIME(I)-140.0)45,45,46
   46   IF(TIME(I)-150.0)47,47,9
```

```
 21 VHL 1=VHL 1+VOL(I)
    THL 1=THL 1+TIME(I)
    TD1=TD1+1.0
    GO TO 9
 23 VHL 2=VHL 2+VOL(I)
    THL 2=THL 2+TIME(I)
    TD2=TD2+1.0
    GO TO 9
 25 VHL 3=VHL 3+VOL(I)
    THL 3=THL 3+TIME(I)
    TD3=TD3+1.0
    GO TO 9
 27 VHL 4=VHL 4+VOL(I)
    THL 4=THL 4+TIME(I)
    TD4=TD4+1.0
    GO TO 9
 29 VHL 5=VHL 5+VOL(I)
    THL 5=THL 5+TIME(I)
    TD5=TD5+1.0
    GO TO 9
 31 VHL 6=VHL 6+VOL(I)
    THL 6=THL 6+TIME(I)
    TD6=TD6+1.0
    GO TO 9
 33 VHL 7=VHL 7+VOL(I)
    THL 7=THL 7+TIME(I)
    TD7=TD7+1.0
    GO TO 9
 35 VHL 8=VHL 8+VOL(I)
    THL 8=THL 8+TIME(I)
    TD8=TD8+1.0
    GO TO 9
 37 VHL 9=VHL 9+VOL(I)
    THL 9=THL 9+TIME(I)
    TD9=TD9+1.0
    GO TO 9
```

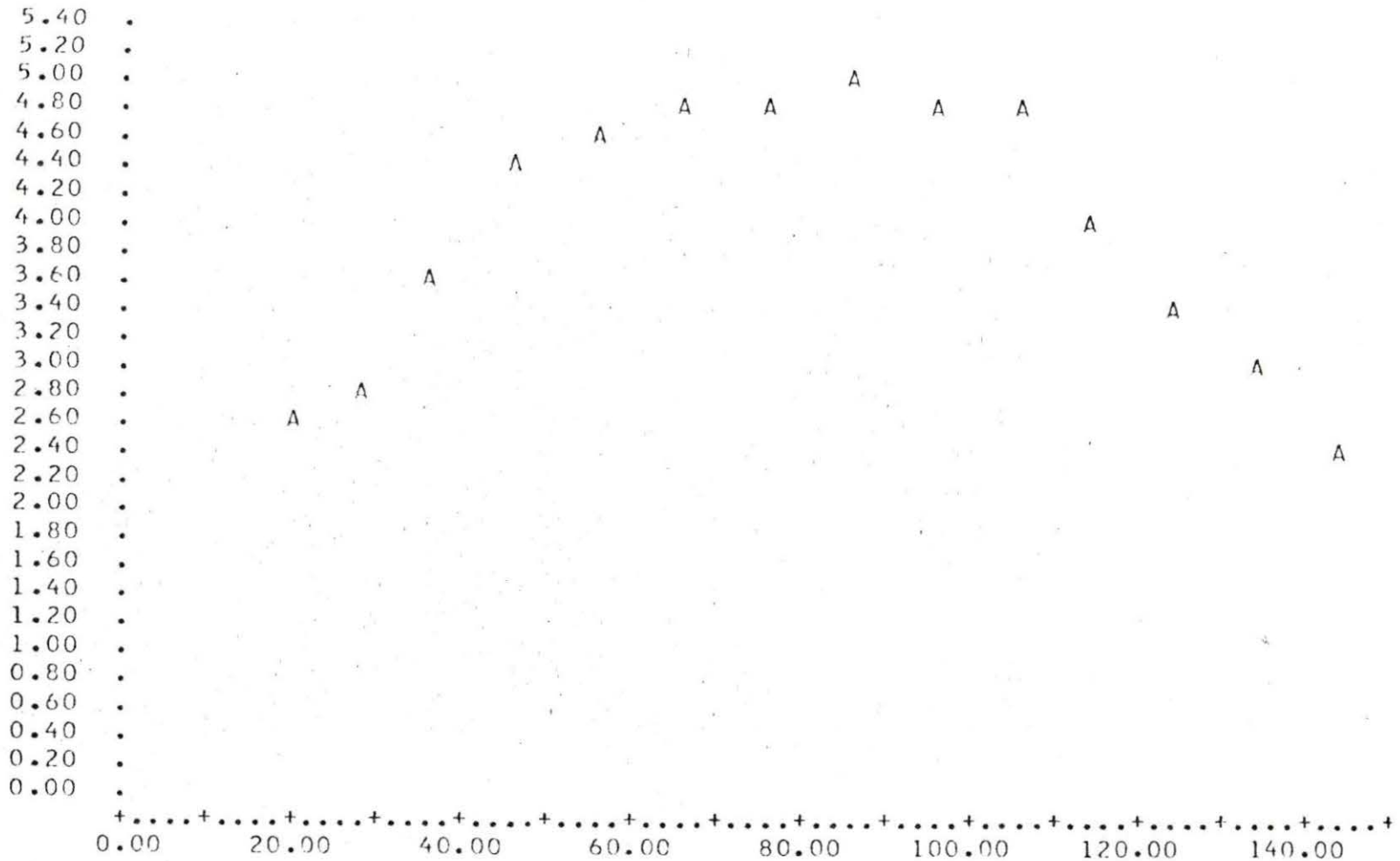
```
 39 VHL 10=VHL 10+VOL(I)
    THL 10=THL 10+TIME(I)
    TD10=TD10+1.0
    GO TO 9
 41 VHL 11=VHL 11+VOL(I)
    THL 11=THL 11+TIME(I)
    TD11=TD11+1.0
    GO TO 9
 43 VHL 12=VHL 12+VOL(I)
    THL 12=THL 12+TIME(I)
    TD12=TD12+1.0
    GO TO 9
 45 VHL 13=VHL 13+VOL(I)
    THL 13=THL 13+TIME(I)
    TD13=TD13+1.0
    GO TO 9
 47 VHL 14=VHL 14+VOL(I)
    THL 14=THL 14+TIME(I)
    TD14=TD14+1.0
    GO TO 9
  9 CONTINUE
100 CALL PUT (0.0,0,200.0,0.0,0,10.0,0,0,+1)
    WRITE(2,200)
200 FORMAT(1HO,50X,22H URINE EXCRETION CURVE)
    WRITE(2,300)IDOG(1),IEXP(1)
300 FORMAT(1HO,43X,11H DOG NUMBER,3X,I3,3X,11H EXPERIMENT,3X,I2)
    IDGHL=IDOG(1)
    DO 79 I=1,ICNTL
    IDOG(I)=0
    IEXP(I)=0
    TIME(I)=0.0
    VOL(I)=0.0
 79 CONTINUE
    GO TO 8
```

```
69 AV  T1=THL 1/TD1
   AV  V1=VHL 1/TD1
   CALL PUT (0.0,AV  T1,200.0,0.0,AV  V1,10.0,1,26,0)
   AV  T2=THL 2/TD2
   AV  V2=VHL 2/TD2
   CALL PUT (0.0,AV  T2,200.0,0.0,AV  V2,10.0,1,26,0)
   AV  T3=THL 3/TD3
   AV  V3=VHL 3/TD3
   CALL PUT (0.0,AV  T3,200.0,0.0,AV  V3,10.0,1,26,0)
   AV  T4=THL 4/TD4
   AV  V4=VHL 4/TD4
   CALL PUT (0.0,AV  T4,200.0,0.0,AV  V4,10.0,1,26,0)
   AV  T5=THL 5/TD5
   AV  V5=VHL 5/TD5
   CALL PUT (0.0,AV  T5,200.0,0.0,AV  V5,10.0,1,26,0)
   AV  T6=THL 6/TD6
   AV  V6=VHL 6/TD6
   CALL PUT (0.0,AV  T6,200.0,0.0,AV  V6,10.0,1,26,0)
   AV  T7=THL 7/TD7
   AV  V7=VHL 7/TD7
   CALL PUT (0.0,AV  T7,200.0,0.0,AV  V7,10.0,1,26,0)
   AV  T8=THL 8/TD8
   AV  V8=VHL 8/TD8
   CALL PUT (0.0,AV  T8,200.0,0.0,AV  V8,10.0,1,26,0)
   AV  T9=THL 9/TD9
   AV  V9=VHL 9/TD9
   CALL PUT (0.0,AV  T9,200.0,0.0,AV  V9,10.0,1,26,0)
   AV  T10=THL 10/TD10
   AV  V10=VHL 10/TD10
   CALL PUT (0.0,AV  T10,200.0,0.0,AV  V10,10.0,1,26,0)
   AV  T11=THL 11/TD11
   AV  V11=VHL 11/TD11
   CALL PUT (0.0,AV  T11,200.0,0.0,AV  V11,10.0,1,26,0)
```

```
      AV  T12=THL 12/TD12
      AV  V12=VHL 12/TD12
      CALL PUT (0.0,AV  T12,200.0,0.0,AV  V12,10.0,1,26,0)
      AV  T13=THL 13/TD13
      AV  V13=VHL 13/TD13
      CALL PUT (0.0,AV  T13,200.0,0.0,AV  V13,10.0,1,26,0)
      AV  T14=THL 14/TD14
      AV  V14=VHL 14/TD14
      CALL PUT (0.0,AV  T14,200.0,0.0,AV  V14,10.0,1,26,0)
      CALL PUT (0.0,0,200.0,0.0,0,10.0,0,0,+1)
      WRITE (2,600)
  600 FORMAT(2H0,50X,22H URINE EXCRETION CURVE)
      WRITE (2,650)IDGHL
  650 FORMAT(1H0,47X,11H DOG NUMBER,2X,I3)
      WRITE (2,700)AV T1,AV T2, AV T3, AV T4,AV T5, AV T6,AV T7,AV T8,
     6AV T9,AV T10, AV T11,AV T12,AV T13,AV T14
  700 FORMAT(1H1,2X,9H0AVE TIME,2X,14(2X,F6.2))
      WRITE(2,750)AV  V1,AV  V2,AV  V3,AV  V4,AV  V5, AV  V6,AV  V7,
     6AV  V8,AV  V9,AV  V10,AV  V11,AV  V12,AV   V12,AV  V13,AV  V14
  750 FORMAT(9H0AVE VOL.,2X,14(2X,F6.2))
      TD 1=0
      TD 2=0
      TD 3=0
      TD 4=0
      TD 5=0
      TD 6=0
      TD 7=0
      TD 8=0
      TD 9=0
      TD 10=0
      TD 11=0
      TD 12=0
      TD 13=0
      TD 14=0
      IDGHL=0
```

```
      VHL 1=0.0
      VHL 2=0.0
      VHL 3=0.0
      VHL 4=0.0
      VHL 5=0.0
      VHL 6=0.0
      VHL 7=0.0
      VHL 8=0.0
      VHL 9=0.0
      VHL 10=0.0
      VHL 11=0.0
      VHL 12=0.0
      VHL 13=0.0
      VHL 14=0.0
      THL 1=0.0
      THL 2=0.0
      THL 3=0.0
      THL 4=0.0
      THL 5=0.0
      THL 6=0.0
      THL 7=0.0
      THL 8=0.0
      THL 9=0.0
      THL 10=0.0
      THL 11=0.0
      THL 12=0.0
      THL 13=0.0
      THL 14=0.0
      GO TO 8
  800 END
```

B. Results



URINE EXCRETION CURVE

DOG NUMBER 715

XII. APPENDIX E. ABBREVIATIONS

GFR	glomerular filtration rate
ERPF	effective renal plasma flow
RPF	renal plasma flow
ERBF	effective renal blood flow
RBF	renal blood flow
FF	filtration fraction
FWC	free water clearance
PAH	para-aminohippurate
Tm_{PAH}	tubular maxima of para-aminohippurate
BSA	body surface area
U/P	urine to plasma ratio
F/P	glomerular filtrate to plasma ratio
ADH	antidiuretic hormone
X	concentration of PAH standard
Y	optical density of PAH in the standard
B	optical density of PAH in the plasma
V	urine volume plus wash solution
U	optical density of PAH in the urine
XCR	concentration of creatinine standard
YCR	optical density of creatinine in the standard
BCR	optical density of creatinine in the plasma
UCR	optical density of creatinine in the urine
UO	osmolarity of urine and wash solution
BO	plasma osmolarity

UV	urine volume
COSM	osmolar clearance
PFF	filtration fraction

XIII. APPENDIX F. INDIVIDUAL CLEARANCE OBSERVATIONS IN 6 BEAGLES

Table 4. Individual clearance observations in 6 beagles

Dog	Date	GFR	RPF	Tm_{PAH}	PFF	COSM	FWC	UV
713	3/10	38.1	87.0	--	43.8	1.22	3.43	4.65
		38.0	90.1	4.83	42.2	1.14	3.53	4.68
		37.9	95.5	5.17	39.7	1.11	3.64	4.75
713	3/17	36.7	86.2	--	42.6	1.01	2.72	3.72
		36.0	86.4	--	41.7	0.88	2.82	3.70
		33.5	82.4	--	40.6	0.82	2.98	3.80
713	3/24	40.2	100.6	5.73	39.9	0.79	2.86	3.65
		36.6	94.7	6.58	38.7	0.82	2.98	3.80
		34.4	85.4	7.11	40.3	0.73	2.95	3.68
713	5/19	41.9	97.7	4.23	42.9	0.92	2.88	3.80
		41.4	91.4	4.68	45.3	0.90	3.10	4.00
		43.3	102.6	--	42.2	1.00	3.55	4.55
713	6/17	38.2	83.6	5.05	45.7	0.84	2.61	3.45
		38.2	86.8	4.82	43.9	0.82	2.48	3.30
		46.1	107.9	5.05	42.8	1.10	3.25	4.35
713	7/22	39.7	103.0	5.29	38.5	1.07	3.03	4.10
		41.4	105.7	5.82	39.1	1.05	3.10	4.15
		37.1	94.3	4.25	39.3	1.03	3.07	4.10
713	9/8	39.7	88.6	4.42	44.8	0.80	2.30	3.10
		38.6	77.9	4.83	49.5	0.85	2.35	3.20
		38.3	81.1	3.93	47.3	0.96	2.84	3.80
714	3.10	38.5	92.8	5.62	41.5	1.18	3.32	4.50
		40.9	90.4	5.40	45.2	1.23	3.77	5.00
		39.9	92.7	5.44	43.0	1.23	3.97	5.20
714	3/17	39.8	91.4	5.65	43.6	1.20	2.94	4.15
		43.3	113.2	5.68	38.2	1.36	4.09	5.45
		--	--	5.36	--	--	--	--
714	3/24	38.3	96.7	7.64	39.7	1.25	3.42	4.68
		40.2	111.6	5.57	36.0	1.25	3.75	5.00
		40.9	109.2	6.60	37.4	1.23	3.69	4.92

Table 4. (continued)

Dog	Date	GFR	RPF	Tm_{PAH}	PFF	COSM	FWC	UV
714	5/19	40.5	99.5	3.71	40.7	1.40	3.35	4.75
		40.9	98.7	3.57	41.4	1.43	3.54	4.98
		41.2	98.7	3.32	41.7	1.36	3.68	5.05
714	6/17	42.4	106.2	4.54	39.9	1.50	4.10	5.60
		40.3	86.4	3.92	46.6	1.32	4.18	5.50
		41.0	81.2	4.31	50.5	1.24	4.26	5.50
714	7/22	36.1	79.3	6.30	45.6	1.01	3.29	4.30
		35.6	78.9	6.85	45.1	0.98	3.19	4.17
		36.6	78.7	6.91	46.5	0.87	2.88	3.75
714	9/8	40.8	90.8	3.51	45.0	1.05	2.97	4.02
		43.6	95.3	3.40	45.8	1.15	3.55	4.70
		45.5	95.9	4.35	47.4	1.08	3.82	4.90
715	4/7	49.5	125.9	5.94	39.3	1.16	3.54	4.70
		39.0	108.2	5.73	36.0	1.07	3.34	4.41
		37.8	109.0	5.69	34.7	1.14	3.97	5.11
715	4/14	38.0	98.5	5.26	38.6	1.06	3.24	4.30
		42.6	113.0	5.28	37.7	1.22	4.28	5.50
		41.3	109.4	6.10	37.8	1.14	4.38	5.52
715	4/21	34.9	98.3	6.41	35.5	0.85	3.27	4.12
		38.8	106.4	6.37	36.4	1.06	3.54	4.60
		37.9	110.0	6.31	34.5	1.04	3.76	4.80
715	4/28	40.6	129.0	5.22	31.5	1.32	4.04	5.35
		39.5	124.7	5.64	31.6	1.33	4.17	5.50
		40.2	126.4	6.44	31.8	1.16	4.01	5.18
715	6/10	--	--	6.79	--	--	--	--
		37.3	107.4	6.42	34.8	0.78	3.67	4.45
		42.1	118.2	6.78	35.6	0.95	4.45	5.40
715	9/1	39.1	100.8	5.76	38.7	0.92	2.98	3.90
		37.5	109.6	5.90	34.2	0.90	3.20	4.10
		35.1	102.8	5.90	34.1	0.92	3.38	4.30
716	2.10	40.4	105.4	3.36	38.3	0.83	1.07	1.90
		38.6	102.0	3.94	37.9	0.74	1.66	2.40
		39.1	196.1	--	39.0	0.76	1.84	2.60

Table 4. (continued)

Dog	Date	GFR	RPF	Tm_{PAH}	PFF	COSM	FWC	UV
716	2/17	30.4	75.7	4.82	40.2	0.89	2.80	3.70
		38.5	92.4	3.93	41.6	1.00	3.57	4.58
		33.4	83.7	4.61	39.9	0.87	3.38	4.25
716	2/24	37.9	86.4	5.13	43.9	0.95	3.15	4.10
		35.6	82.9	4.91	42.9	0.81	2.89	3.70
		39.2	98.2	5.43	40.0	0.95	3.35	4.30
716	3/2	35.3	87.3	4.39	40.4	0.84	2.66	3.50
		35.9	83.5	4.52	43.0	0.78	2.67	3.45
		33.0	79.8	5.69	41.4	0.65	2.50	3.15
716	6/3	38.6	102.6	6.17	37.6	1.68	3.82	5.50
		43.6	113.8	6.17	38.3	1.50	4.50	6.00
		38.0	119.8	5.74	31.7	1.06	4.04	5.10
717	2/12	35.8	95.6	5.25	37.9	1.00	3.20	4.20
		38.3	98.6	5.40	38.8	0.91	3.24	4.15
		36.4	95.2	5.28	38.2	0.92	3.35	4.28
717	2/17	38.0	88.5	5.89	42.9	1.14	3.41	4.55
		48.2	85.0	6.03	56.7	1.36	4.09	5.45
		41.3	92.2	5.89	44.8	1.24	3.96	5.20
717	2/24	39.2	94.7	5.35	41.4	1.45	3.04	4.50
		39.1	90.6	5.54	43.2	1.32	3.32	4.65
		39.8	95.4	5.05	41.8	1.25	3.45	4.70
717	3/2	38.5	79.9	3.91	48.2	0.86	2.29	3.15
		39.5	83.6	4.51	47.2	0.77	2.53	3.30
		41.4	78.2	4.52	52.9	0.81	2.94	3.75
717	6/3	38.2	105.9	6.54	35.9	1.06	2.19	3.25
		40.6	105.5	6.41	38.5	1.01	3.34	4.35
		41.0	111.7	6.96	36.7	0.98	3.77	4.75
718	4/7	41.1	100.4	6.37	40.9	0.83	2.27	3.10
		41.7	103.8	6.19	40.2	0.93	2.57	3.50
		41.8	108.4	6.15	38.5	0.93	2.97	3.90
718	4/14	41.1	105.4	5.80	39.0	0.84	2.36	3.20
		41.4	113.0	6.15	36.6	0.89	2.89	3.78
		41.2	107.2	5.68	38.5	0.89	2.71	3.60

Table 4. (continued)

Dog	Date	GFR	RPF	Tm_{PAH}	PFF	COSM	FWC	UV
718	4/21	42.4	98.4	6.23	43.1	0.89	2.73	3.62
		45.0	105.5	5.74	42.6	1.00	2.95	3.95
		46.2	114.7	5.97	40.3	1.05	3.40	4.45
718	4/28	40.4	105.4	5.82	38.3	0.84	2.61	3.45
		41.0	104.2	5.93	39.4	0.85	2.70	3.55
		41.5	102.4	5.62	40.6	0.88	2.67	3.55
718	6/10	37.1	97.9	5.39	37.9	1.20	2.50	3.70
		38.4	97.4	5.49	39.4	1.10	2.88	3.98
		38.6	94.0	5.57	41.0	1.00	2.75	3.75
718	9/1	36.1	89.2	6.05	40.5	0.92	2.22	3.15
		40.0	96.2	4.44	41.5	1.05	2.85	3.90
		36.1	86.8	5.59	41.6	0.94	2.36	3.30