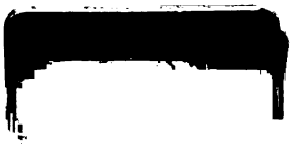
PLASMA DISAPPEARANCE OF PHENOLSULFONPHTHALEIN AS AN INDEX OF RENAL FUNCTION IN THE DOMESTIC CAT: NORMAL VALUES AND APPLICATION IN EXPERIMENTAL AND NATURALLY OCCURRING NEPHROPATHIES

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
JOHN JORDAN ANDREWS
1973



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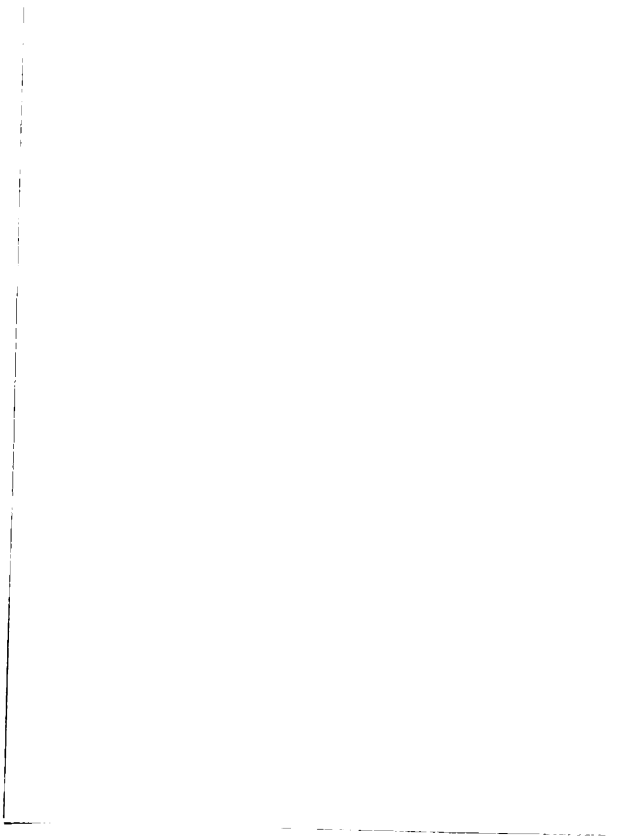

ABSTRACT

PLASMA DISAPPEARANCE OF PHENOLSULFONPHTHALEIN AS AN INDEX OF RENAL FUNCTION IN THE DOMESTIC CAT: NORMAL VALUES AND APPLICATION IN EXPERIMENTAL AND NATURALLY OCCURRING NEPHROPATHIES.

By

John Jordan Andrews

A study was undertaken to develop procedures utilizing phenolsulfonphthalein dye (PSP) for the detection of renal disease in cats. Twenty-four cats were divided into 6 groups to evaluate these procedures in normal animals and in cats with naturally occurring and experimentally induced nephropathies. Phenolsulfonphthalein biological plasma half-life (T 1/2), index (PSPI), plasma concentration at 30 minutes (PSP_{30}) and 40 minutes (PSP_{40}) after dye injection, clearance/kg. (C_{PSP}/kg.), and volume of distribution were determined in 12 nonanesthetized domestic cats with histologically normal kidneys. Values for T 1/2 ranged from 13.0 to 25.0 minutes (mean 18.2 $\pm$ 2.91), the PSPI values from 12.0 to 23.1 units (16.9 $\pm$ 2.69), PSP_{30} from 0.44 to 0.96 mg./100 ml. (0.65 $\pm$ 0.105), PSP_{40} from 0.28 to 0.66 mg./100 ml. (0.47 $\pm$ 0.082), the C_{PSP}/kg. from 6.42 to 12.36 ml./minute/kg. of body weight (9.23 $\pm$ 1.273) and the volume of distribution from 17 to 31 ml./100 Gm. of body weight (24.6 $\pm$ 3.62).

The biological half life of PSP (T 1/2) in 5 bilaterally nephrectomized nonanesthetized cats was markedly elevated, ranging from 41 to 60 minutes. One unilaterally nephrectomized cat had PSP T 1/2 values ranging from 25 to 49 minutes and elevated PSP_{30} values.

Four cats with mild, naturally occurring nephritis did not have elevated PSP T 1/2 or blood urea nitrogen values. One cat in this group did have slightly elevated PSP_{30} and PSP_{40} values. The nephritis observed in these cats was characterized by multifocal infiltration by mononuclear cells, tubular destruction and early fibrosis in the cortical areas.

Tubular nephrosis was produced in 4 cats by subcutaneous administration of a solution of uranyl nitrate at the rate of 5 mg./kg. of body weight. The PSP T 1/2 values from affected cats ranged from 23 to 60 minutes but were not considered a reliable index of renal damage. Glycosuria, ketonuria, proteinuria, and increased numbers of granular casts and small round epithelial cells in the urine sediment were observed at 24 hours after the administration of uranyl nitrate. Blood urea nitrogen values increased above normal values by the end of 48 hours. The PSP_{30} and PSP_{40} values increased significantly ($p < 0.001$) by 24 hours and appeared to be a more reliable indicator of early renal damage than did the T 1/2 values.

Two cats administered ethylene glycol orally at the rate

of 2 mg./kg. of body weight had markedly elevated PSP T 1/2 (53.5 to 150 minutes), PSP_{30} and PSP_{40} values by 24 hours following administration. Blood urea nitrogen values also rose above the normal range by this time. Serum calcium values of affected cats ranged from 2.3 to 6.8 mg./100 ml. and serum glutamic pyruvate transaminase (SGPT) levels were elevated (90 to 180 Sigma Frankel units). Obstruction of renal tubules and collecting ducts, pressure necrosis of the epithelium of those tubules and ducts, plus hepatic necrosis were observed histologically.

It was concluded that the measurement of plasma concentrations of PSP following intravenous administration is useful for the estimation of renal function in cats. It is of special value when urinalysis indicates acute or chronic nephritic processes and BUN values are still within the normal range.

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By

John Jordan Andrews

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Pathology

1973

To

My wife, daughter, and son

ACKNOWLEDGEMENTS

The author wishes to express his appreciation and thanks to his major professor, Dr. Robert L. Michel, for his guidance and encouragement during the course of the investigation and the writing of this thesis. The author also wishes to thank Drs. Robert L. Michel, Stuart D. Sleight, and William B. Buck for their assistance in acquiring financial aid for this project.

Also, the author wishes to thank Drs. Allan L. Trapp, Stuart D. Sleight, George E. Eyster and Robert G. Schirmer for serving on his guidance committee and for giving constructive criticisms on this manuscript.

A great deal of appreciation must be expressed to Dr. Vaughn A. Seaton and the staff of the Iowa State University Veterinary Diagnostic Laboratory for their assistance and encouragement that enabled the investigation to be completed.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Tests of renal function are valuable aids in veterinary research and practice. No single procedure has been sufficient to indicate to the investigator or clinician all aspects of the functional ability of the kidneys. Various renal units have distinct functions and impairments may be localized. For example, glomerulonephritis is commonly encountered in human medicine as a naturally occurring disease (Relman and Levinsky, 1963), while in veterinary medicine most nephritides are interstitial and tubular. Therefore, functional testing ideally should be directed at particular renal areas, and for an over-all estimation of renal function, several testing procedures should be employed.

Basically the clinical testing of renal function has been divided into 3 areas:

1. the examination of the urine,
2. the evaluation of serum levels of normal body substances primarily under renal control (i.e. blood urea or serum creatinine), and
3. the measurement of the kidneys' ability to rid the body of injected or ingested foreign materials.

For example, the determination of the specific gravity of the urine is useful to evaluate the ability of the distal

tubules to reabsorb water. Likewise, increased numbers of cells in the urine indicate particular areas of inflammation, and the presence of abnormal amounts of certain substances such as albumin suggests changes in the membrane integrity of the glomeruli and/or the renal tubules. Measurements of urea nitrogen and creatinine in the serum serve as crude estimates of glomerular filtration.

The injection of certain foreign materials such as para-amino hippurate and inulin has been useful to the researcher for evaluating renal blood flow and glomerular filtration rate, respectively. However, most of these methods are too cumbersome for clinical veterinary practice.

The measurement of the renal excretion of the indicator dye, phenol red or phenolsulfonphthalein (PSP), has been applied clinically in both human and veterinary medicine and has proven useful primarily as an index of renal tubular function. The methods of analysis have been relatively simple, and early detection of renal disease has been attributed to the application of procedures using PSP.

Techniques of testing have been reported for the canine, bovine, and ovine species but not for the feline species (Brobst et al., 1967; Mixner and Anderson, 1958; Osbaldiston, 1970). The purpose of this research was to adapt a method of determining PSP excretion for the domestic cat, to determine the normal range of values and to apply the method in cats with naturally occurring and experimentally induced nephropathies.

REVIEW OF LITERATURE

Characteristics of the feline kidney

The normal kidney of the domestic cat presents several characteristics not found in the kidneys of other domestic animals (Yadava and Calhoun, 1956; Lucke and Hunt, 1967; Bloom, 1954). The large amount of lipid material in the proximal convoluted tubules is particularly striking (Modell and Travell, 1934; Foote and Grafflin, 1938). The anestrus female has less of this intracellular lipid (Lobban, 1955) than other cats. No explanation of the function or significance of these lipids was offered.

Lucke and Hunt (1967) reported microscopic calcification in 7 of 33 otherwise normal feline kidneys. This calcification was located in the medulla and was preceeded by a "hyaline thickening of and an accumulation of cholesterol esters in the tubular basement membrane." They concluded that this represents a form of dystrophic calcification.

Prominent capsular veins are also a peculiar feature of the feline kidney (Yadava and Calhoun, 1956). Their functional significance, likewise, is not clear (Osbaldiston and Fuhrman, 1970). The capsule of the feline kidney is unusual in that smooth muscle fibers are absent (Yadava and Calhoun, 1956).

Pathology of the feline kidney

Various authors investigating naturally occurring feline renal disease reported that the most common lesion was a form of chronic scarring (primarily an interstitial nephritis) (Lucke, 1968; McClelland and McClelland, 1958; Hamilton, 1966; Bloom, 1954). The earliest changes reported in one survey (Lucke, 1968), and believed to be the precursor of this scarring, was a ballooning of the "epithelial cytoplasm of the proximal tubules by an excessive accumulation of fat, spatial derangement of the cells, and a slight focal hyaline change." Lucke's work thus suggests that tests of proximal tubular function would be most useful for early detection of this form of nephritis.

Clinical pathology of renal disease

Ante-mortem detection of renal disease by laboratory means had its origin historically with the "father of medicine," Hippocrates, and his methods of elemental urinalysis (Runes and Kiernan, 1964).

Rowntree and Geraghty (1910) reported that the injection of phenolsulfonphthalein (PSP, phenol red) was followed by reddish discoloration of basic urine. They measured the amount of this dye in the urine at specific time intervals following injection, and attempted to correlate PSP excretion with renal function and renal disease.

Hoe and O'Shea (1965) stated that methods for

determining renal function should be at least 90% accurate in predicting renal disease. They suggested using "a combination of the estimation of blood urea nitrogen (BUN), detection of casts and protein in the urine, and repeated measurement of the urinary specific gravity" to accurately assess renal function.

Clearance tests have also been used to estimate renal function. According to Gault (1966), the ideal substance for a clearance test to assess tubular function should remain in the plasma, be rapidly secreted by the renal tubules, be neither metabolized nor stored, and have no extrarenal excretion. He concluded "PSP (phenolsulfonphthalein) meets these requirements as well, or better than, other known substances."

Lapides and Bobbitt (1958) concluded that estimations of BUN, nonprotein nitrogen, and serum creatinine were entirely inadequate in man for the preoperative estimation of renal function and proposed the use of a 15 minute or fractional PSP urinary excretion test as fulfilling this purpose. Osbaldiston and Fuhrman (1970) stated "PSP urine excretion remains the most practical method for determining tubular function in animals. That is particularly true for the cat."

Pharmacodynamics of PSP excretion

Phenolsulfonphthalein is a weak organic acid. Its color changes from yellow to red and becomes accentuated as

the pH increases from 6.8 to 8.4 (Wesson, 1969). No clear evidence of undesirable side effects, either systemic or local, resulting from the administration of PSP has been reported (Gault et al., 1966). Likewise, no deaths have been attributed to the use of PSP.

Goldring et al. (1936) reported that PSP is primarily secreted by the renal tubules and consequently may be used to measure tubular function. The mechanism of secretion of PSP by the proximal renal tubules has been studied by Marshall (1931), Marshall and Crane (1924), Marshall and Vickers (1923), Sheenan (1931), and Chambers and Kempton (1933) and has been summarized by Wesson (1969) as follows: "1. No vertebrate class has yet been studied whose kidney, glomerular or aglomerular, does not actively secrete phenol red. 2. PSP belongs to a group of aromatic acids which are all transported by the same cellular carrier system. 3. PSP, PAH (para-amino hippurate), and diodrast are actively secreted in that their excretion rates are in excess of any possible amounts arising from filtration alone, are independent of pH, and are depressed by cellular poisons. 4. If diodrast is injected into the blood stream while PSP is being excreted, the phenolsulfonphthalein excretion rate is depressed and vice-versa. 5. Aromatic acid transport is in some manner dependent also on a supply of high-energy phosphate bonds."

The limiting factor of PSP tubular secretion is the rate of the delivery of the dye to the peritubular capillaries

rather than the tubular excretory capacity (Gault et al., 1967); and, thus, PSP excretion may also be a good indication of renal blood flow (RBF) or, more specifically, renal plasma flow (RPF) (Goldring et al., 1936; Osbaldiston, 1970). Factors affecting renal blood flow (fear, fever, decreased cardiac output), therefore, are reported to affect PSP excretion (Carper and Hougan, 1967; Osbaldiston, 1970). Only when renal plasma flow is within normal limits can one interpret prolonged clearance of PSP as an indication of renal tubular dysfunction (Carper and Hougan, 1967). These hemodynamic effects, however, appear to have less effect on PSP excretion values than on the blood levels of substances primarily filtered by the glomeruli such as urea nitrogen and creatinine (Osbaldiston, 1970).

"Because the tubule is dependent upon the glomerulus for its blood supply, it would appear also that the function of the tubule can rarely be better, comparatively, than the function of the glomerulus" (Lapides and Bobbitt, 1958). This suggests that PSP excretion would also be impaired in glomerular as well as in tubular disease.

After intravenous administration of PSP, the dye becomes largely bound to the plasma proteins, particularly the albumins (Blondheim, 1955). However, when serum albumin measures less than 1.5 Gm./100 ml. all the PSP is free. That dye which is not protein-bound is filtered by the glomerulus and the albumin-bound dye is released from the albumin in

the peritubular spaces. It is then actively absorbed by the tubular cells and transferred to the tubular lumen (Goldring et al., 1936). No dye is apparently stored in the renal tubules (Puck et al., 1952; Smith et al., 1938; Sheenan, 1931). In vitro, 80 to 85% of the PSP is bound to albumin (Gault, 1966). However, Gault (1966) found also that in human patients "after an injection of 1 mg./kg. of body weight (I.V.), the PSP behaves consistently as if it were only slightly bound to albumin." Ochwadt and Pitts (1956) demonstrated an increased clearance rate of PSP after replacement of plasma proteins with dextran. This would suggest that protein binding of the dye is functionally significant.

Phenolsulfonphthalein incubated for 24 hours with stool lost 50% of its color, apparently destroyed by bacteria (McLeod et al., 1968). Reabsorption of PSP from the intestinal tract also occurs readily. However, it has still not been satisfactorily explained why nearly all of an injected dose of PSP cannot be recovered. Cellular uptake and metabolism may be possible explanations (Gault, 1966).

Clinical use of PSP

Chapman and Halstead (1933), Varley (1956), and Shaw (1925) developed clinical methods of assessing renal function utilizing PSP. More recently, Gault (1966), Gault et al. (1966, 1967), Gault and Fox (1969), Vidt (1969), Henry (1965), Hoeffler et al. (1968), Axelrod (1966), and Young et al. (1969)

described clinical procedures using PSP in the diagnosis of renal diseases in human patients and discussed the theory and problems of these techniques.

The measurement of PSP excretion as a diagnostic procedure in veterinary medicine developed primarily from the work done in human medicine. Mixner and Anderson (1957, 1958) measured the plasma half-life (T 1/2) of PSP in normal dairy cattle. Osbaldiston and Moore (1971) and Anderson and Mixner (1959, 1960) reported the use of PSP as a measure of renal function in cattle. Brobst et al. (1967), Morgan (1967, 1968), and Carper and Hougan (1967) described and discussed PSP methods of evaluating renal function in the dog. Osbaldiston (1970) reviewed current methods of evaluation and theories regarding the use of PSP as a diagnostic aid in sheep, cattle, dogs, and cats.

Osbaldiston and Fuhrman (1970) reported PSP T 1/2 values in the cat and compared PSP clearance with clearance of para-amino hippurate, endogenous creatinine, and inulin. The complete details of his experimental determination of PSP values were not included in his paper.

The use of PSP to estimate renal function offers 2 basic approaches: the measurement of dye excreted into the urine and the measurement of the disappearance of dye from the plasma. Each method has distinct advantages and disadvantages (Gault, 1966).

The measurement of urinary excretion of PSP (designated

as PSPU) is theoretically an exact measurement of renal function (secretion plus filtration of the dye). However, to insure accuracy, this procedure required recovery of nearly all the urine produced within a time period (Gault, 1966). Osbaldiston and Fuhrman (1970) recommended the use of urecholine to facilitate urine collection in cats. Stimulation by urecholine of smooth muscles other than the urinary bladder has limited acceptance of this procedure. Prehydration of patients was also necessary to provide urine specimens of sufficient volume for accurate results (Brobst et al., 1967; Gault, 1966). A low urine volume resulted in high concentration of dye in the urine. Thus, any unrecovered urine (renal pelvis, ureters, residual bladder capacity) contained enough dye to alter the test results and caused an underestimation of renal function (Gault, 1966). Gault et al. (1967) also warned against "water-loading" patients with severe renal damage. Likewise, urinary obstructions significantly altered urine recovery and, thus, resulted in underestimation of renal function (Axelrod, 1966; Gault, 1966).

In cooperative patients with no urinary obstructions and in dogs where adequate urine samples were obtained, the measurement of PSPU values was of great value in estimating renal function (Gault, 1966; Lapides and Bobbitt, 1958; Hoeffler et al., 1968; Healy, 1968; Osbaldiston, 1970; Coles, 1967; Morgan, 1968). When the bladder washing procedure was

used, PSPU values were not affected by urine flow rates (Shannon, 1935; Kim and Hong, 1962) or by proteinuria and repeatability was good (Healy et al., 1964).

Cats and dogs normally excrete in the urine approximately 60% of the injected dose of PSP in the first 30 minutes following intravenous administration of 3 mg. and 6 mg. doses, respectively (Osbaldiston, 1970; Kim and Hong, 1962).

The determination of the plasma concentration of PSP and the biological half-life of the dye in the plasma (T 1/2) offered several advantages over PSPU measurement (Gault, 1966); the patient did not have to be "water-loaded" or prehydrated, urinary catheterization and bladder washing was not necessary, and patients with urinary obstructions could be tested accurately. The actual time required in the laboratory, however, was longer than for the PSPU determination; and it was necessary to obtain at least 4 plasma specimens to determine the T 1/2 value accurately (Gault, 1966).

Gault et al. (1966, 1967) determined both the PSPU (15 minute sample) and the PSP T 1/2 values simultaneously in human patients and concluded from a correlation coefficient of 0.96 that both tests (PSPU and PSP T 1/2) measured essentially the same function.

In human patients, the measurement of the plasma PSP concentration at 60 minutes after the administration of dye at the rate of 1 mg./kg. of body weight correlated well with other renal function tests and was judged to be a

reliable indicator of the degree of renal function (Gault and Fox, 1969). This test offered the advantage of requiring only 2 plasma samples.

Experimental renal disease

Uranyl nitrate was used to produce selective damage to the proximal renal tubules in the dog and the cat (Hepler and Simonds, 1945; Brobst et al., 1967; Simonds and Hepler, 1945). Modell and Travell (1934) investigated the role of the lipoid in the renal tubules of the cat in experimental uranyl nitrate nephrosis. Brobst et al. (1967) studied plasma PSP levels in dogs given uranyl nitrate intravenously and reported increased PSP T 1/2 times in these animals.

Naturally occurring ethylene glycol toxicosis and nephrosis has been reported in the domestic cat (Hadlow, 1957; Jonsson and Rubarth, 1967). Kersting and Nielson (1966) produced experimental poisoning in the dog by the use of ethylene glycol orally. Gessner et al. (1961) and Swanson and Thompson (1969) have investigated and discussed the renal handling of ethylene glycol and its metabolites. Twenty-five to 50% of an oral dose of ethylene glycol was excreted as such in the urine in 24 hours. Most of the remainder was oxidized to glyoxylic acid ($HCOCO_2H$) and, thence, to carbon dioxide. Smaller amounts were oxidized to oxalic acid and this combined with ionic calcium to form insoluble calcium oxalate crystals. The cat formed relatively large amounts of oxalic acid compared with other species and therefore, was more susceptible to the toxic effects of ethylene glycol.

MATERIALS AND METHODS

Experimental animals

Twenty-four mixed breed adult domestic cats of both sexes were initially divided into 5 experimental groups: 1) controls, 10 cats (4 cats later reclassified as naturally occurring nephritis); 2) bilateral nephrectomy, 5 cats; 3) unilateral nephrectomy, 1 cat; 4) uranyl nitrate toxicosis, 4 cats; and 5) ethylene glycol toxicosis, 4 cats. The pre-nephrectomy data for the cats in Groups 2 and 3 were also used as control data. A few other cats were used in a pilot study to develop the technique of jugular vein catheterization used in these experiments. All cats were housed in metal or concrete cages and fed a complete commercial ration [1,2]. Experimental animals were obtained through university laboratory animal resource departments[3].

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

[2]Purina Cat Chow®, Ralston Purina Company, St. Louis, Missouri.

[3]Center for Laboratory Animal Resources, Michigan State University and Laboratory Animal Resources, Iowa State University, Ames, Iowa

Catheterization of jugular vein

To facilitate accurate administration of the PSP dye and collection of the necessary blood samples, an indwelling polyethylene catheter[4] was threaded into the left jugular vein of each cat with the opening of the catheter positioned posterior to the thoracic inlet and anterior to the heart within the anterior vena cava. Once the catheter was properly located, the jugular vein was ligated anterior to the entry of the catheter. A ligature posterior to the entry of the tubing was tightened just enough to prevent leakage around the catheter without occluding the catheter lumen. A sterile, blunted 20 guage hypodermic needle[5] was inserted into the catheter and the tube was flushed with heparinized saline and stoppered. The tube and needle were then taped to the neck in a convenient position. After this procedure a minimum of 24 hours elapsed before testing procedures were initiated.

PSP solution for injection

Six Gm. of phenolsulfonphthalein powder[6] were added to 300 ml. of a 0.85% saline solution and sterilized for 20 minutes at 121 C, and 20 p.s.i. The addition of sodium hydroxide to produce a solution with a pH of approximately

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[4]Intramedic PE-90®, Clay Adams, Parsippany, New Jersey.

[5]Monoject 250®, Sherwood Medical Industries, Deland, Florida.

[6]Nutritional Biochemicals Corp., Cleveland, Ohio.

7.0 as suggested by Brobst et al. (1967) was not necessary in our experiments. The solution thus prepared contained 20 mg. of PSP/ml.

PSP testing procedure

Five milligrams of PSP/kg. of body weight (0.25 ml. solution/kg. of body weight) was injected intravenously via the jugular catheter at the initiation of each PSP testing period. This was followed by 3 to 5 ml. of 0.85% saline to insure removal of residual dye from the catheter. Pilot studies indicated that 5 mg./kg. of body weight was the most suitable dosage.

Collection of samples from the cats followed one of two basic patterns: 1) the collection of a serum sample, a whole blood sample mixed with the disodium salt of ethylenediamine tetraacetic acid (EDTA) as the anticoagulant, and a heparinized sample before the injection of the dye, and then the collection of 3 ml. heparinized samples at 2, 4, 6, 8, 10, 20, 30, and 40 minutes after PSP injection, or 2) the collection of preinjection samples as above and then the collection of heparinized samples at 20, 30 and 40 minutes after dye injection. Collection times were varied slightly when collection difficulties arose. Following the collection of each heparinized sample, 3 ml. of physiologic saline were flushed through the catheter to remove residual dye and to replace lost body fluids.

The heparinized blood samples were centrifuged and 1 ml.

of plasma was decanted into a glass tube. The tubes were sealed and labelled and the samples frozen if PSP concentrations were not determined the same day. Serum samples were likewise collected and frozen for future analysis. All hematologic values were determined on the collection day utilizing the whole blood sample mixed with the EDTA.

Clinical pathology procedures

Packed cell volumes were determined by standard methods using capillary pipettes and a high speed microcentrifuge.[7]

Blood urea nitrogen was determined by an automated diacetyl monoxime procedure[8], or by a photometric method using glycerol urease and Nessler's reagent (Lile et al., 1957). Total plasma protein was determined with a refractometer[9], serum albumin by a photometric method using the brom cresol green dye binding principle[10] and serum glutamic pyruvate transaminase (SGPT) by a photometric method[11]. Serum calcium was determined by atomic absorption spectrophotometry (Kerber, 1971).

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[7]Micro-Hematocrit Centrifuge (IEC), International Equipment Co., Needham Hts., Massachusetts.

[8]Technicon Autoanalyzer®, Technicon Corporation, Tarrytown, New York.

[9]T/S Meter®, American Optical, Buffalo, New York.

[10]Kit #630, Sigma Chemical Company, St. Louis, Missouri.

[11]Kit #505, Sigma Chemical Company, St. Louis, Missouri.

Urine collection was accomplished by manual expression of the urinary bladder after rapid intravenous administration of a 4% solution of thiamyl sodium[12] at the rate of approximately 0.25 ml./kg. of body weight to relax the sphincter muscle. The dosage of thiamyl sodium was varied slightly as necessary. Urine specimens were collected after the PSP testing was completed for that period.

Urine protein, glucose, pH, blood, and ketones were determined using commercially available reagent strips[13]. All positive urine protein determinations were confirmed using a sulfosalicylic acid test[14]. Urine specific gravity was determined with a refractometer[15]. Fifteen milliliters of urine were centrifuged in tapered tubes and the sediment examined microscopically using bright field illumination.

Photometric determination of plasma PSP

Three milliliters of spectrophotometric grade acetone[16] were added to each previously measured plasma sample (1 ml.), and the tubes were stoppered. The mixed samples were shaken vigorously for a minimum of 2 minutes. The acetone precipitates the plasma proteins and releases the PSP dye that is bound to these proteins (Carper and Hougan, 1967).

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[12]Surital®, Parke-Davis, Detroit, Michigan.

[13]Labstix®, Ames Company, Elkhart, Indiana.

[14]Bumintest®, Ames Company, Elkhart, Indiana.

[15]T/S Meter®, American Optical, Buffalo, New York.

[16]J. T. Baker Chemical Company, Phillipsburg, New Jersey.

After being shaken, the tubes were centrifuged for 10 minutes at approximately 2000 r.p.m. and the clear supernatant decanted to another stoppered tube to which 1 drop of a 50% solution of sodium hydroxide had been added. This mixture was mixed well and recentrifuged. The sodium hydroxide develops the deep red color of the dye (Carper and Hougan, 1967). The concentration of PSP in the supernatant fluid of this mixture was then determined by photometric means.

Two spectrophotometers[17] adapted for 12 X 75 mm. round glass cuvettes were used to determine plasma concentration of PSP dye. Each spectrophotometer was calibrated by standard methods to determine the most sensitive wavelength setting. A wavelength setting of 568 mμ was used on the first instrument (Michigan State University) and a wavelength setting of 563 mμ on the second spectrophotometer (Iowa State University). Standard curves were then prepared for both spectrophotometers utilizing solutions prepared from the injectable dye solution described earlier.

PSP T 1/2

The plasma concentration of PSP versus time was plotted on semi-logarithmic graph paper (Figure 1). The line of "best fit" was determined by visual estimation (Brobst et al., 1967). The time in minutes that was required for the plasma concentration to be reduced to 1/2 the original value was

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[17]Coleman Junior II Spectrophotometer, Coleman Instrument Corporation, Maywood, Illinois.

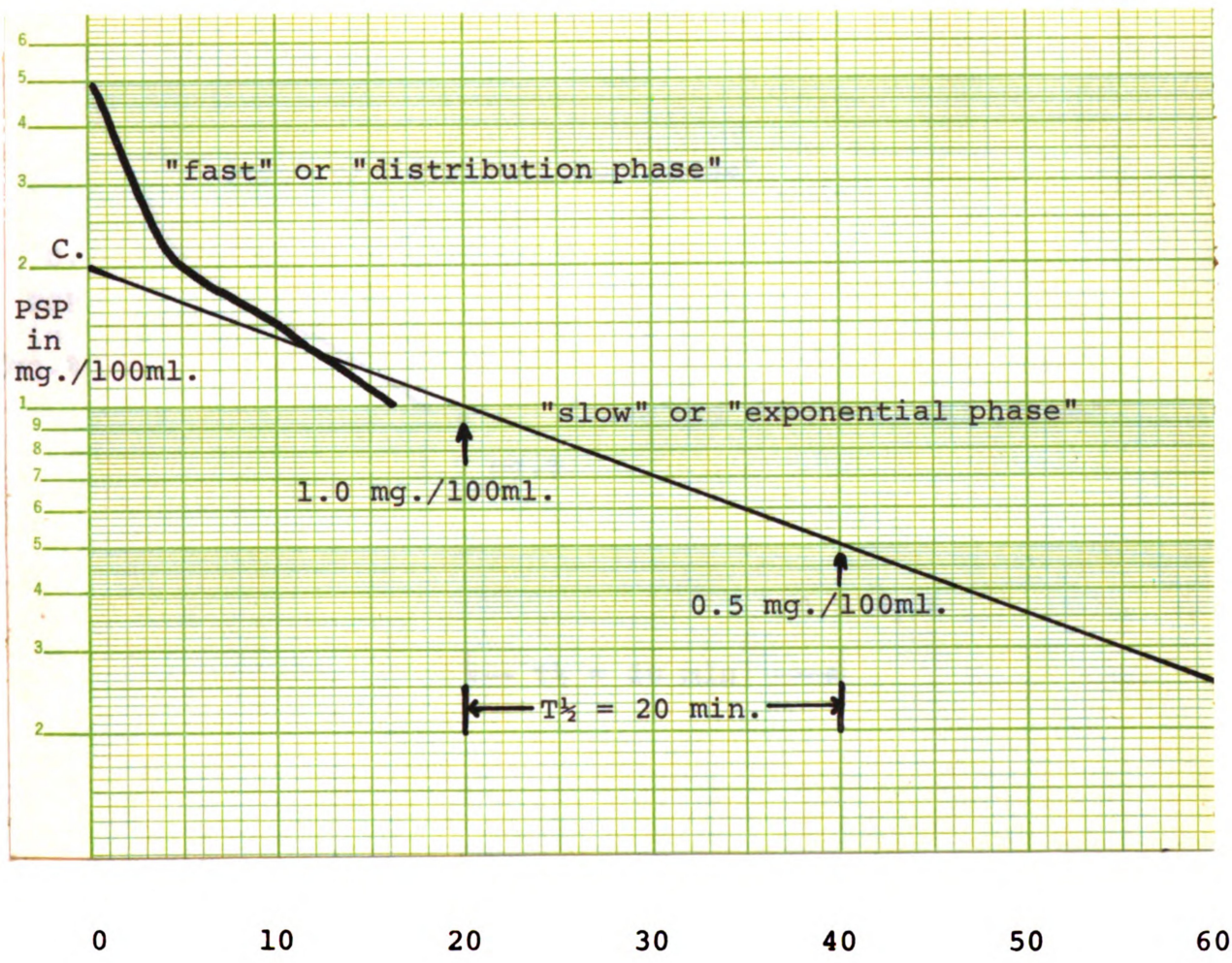

Figure 1. Typical PSP disappearance curve in the control cats. C. The theoretical plasma concentration at zero time used to determine volume of distribution of the dye. The two components of the disappearance curve are also illustrated.

recorded as the plasma half-life or T 1/2. The T 1/2 values were determined by using plasma concentrations of PSP in those samples collected between 19 and 41 minutes after the injection of the dye.

PSPI

The PSP Index, designated PSPI, was calculated by dividing 301 by the T 1/2 value (Gault, 1966). This index presents T 1/2 as a linear rather than an exponential function.

PSP_{30} and PSP_{40}

The plasma concentrations of PSP at 30 and 40 minutes after dye injection were designated as PSP_{30} and PSP_{40}, respectively.

PSP volume of distribution and clearance

The volume of dye distribution and the clearance of PSP were determined in control cats by a method reported by Osbaldiston and Fuhrman (1970).

The volume of dye distribution was estimated by plotting the "slow phase" or "exponential phase" of the distribution curve to the "zero time" and calculating the theoretical plasma concentration of PSP as if the dye had been completely distributed the instant it was injected (Figure 1). Using this plasma concentration value, the volume of distribution was estimated by the following formula:

$$\text{Volume of distribution} = \frac{K \cdot Z \cdot 100}{C}$$

where K = dose of dye/kg. of body weight
Z = kg. of body weight
and C = theoretical plasma concentration of dye at zero time (mg./100 ml.)

The clearance of PSP was determined by multiplying the volume of distribution by the disappearance rate. The disappearance rate was determined according to the following formula:

Disappearance rate = 1-X where

$$X = \sqrt[n]{0.5}$$

and n = PSP T 1/2 time

Clearance of PSP was designated C_{PSP} and expressed as ml./minute. The clearance values were also expressed per kg. of body weight (C_{PSP}/kg. of body weight).

The volume of distribution as ml./100 Gm. of body weight was calculated by dividing the volume of distribution by the body weight as measured in centigrams.

Histopathology procedures

Specimens collected from each cat at necropsy included kidneys, liver, small intestine, large intestine, adrenal gland, stomach, lungs, heart, spleen, urinary bladder, and brain. Tissues were fixed in either 10% buffered formalin or Zenker's fixative according to standard procedures (Armed Forces Manual of Histologic and Special Staining Techniques, 1960).

Hematoxylin and eosin stain was applied to sections of all tissues and periodic acid-Schiff stain was applied to sections of all kidneys. Oil red O stain was also applied as necessary to fulfill specific needs.

Experimental groups

The PSP T 1/2, PSPI, PSP_{30}, PSP_{40}, volume of distribution, C_{PSP}, C_{PSP}/kg. and packed cell volume were determined in 10 cats. Four of these cats were later reclassified as examples of naturally occurring nephritis on the basis of histologic examination of the kidneys. Five cats were bilaterally nephrectomized, and plasma PSP disappearance curves determined and compared with the pre-nephrectomy curves. One cat was unilaterally nephrectomized and the disappearance curves were determined for 4 days following the surgery. The prenephrectomy data from bilaterally and unilaterally nephrectomized cats are listed with the control data (Tables 2 and 3). These and the following groups are summarized (Table 1).

A sterile solution containing 5 mg./ml. of uranyl nitrate[18] was administered subcutaneously at a rate of 5 mg./kg. of body weight to 4 cats following determination of PSP, BUN, serum calcium, and uninalysis values. The determinations were then repeated at 24 hour intervals until euthanasia of each cat.

Five milliliters/kilogram of body weight of ethylene glycol[19] were administered *per os* to 2 cats (015, 016) following the determination of PSP, BUN, serum calcium and SGPT values.

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[18]J. T. Baker Chemical Company, Phillipsburg, New Jersey.

[19]J. T. Baker Chemical Company, Phillipsburg, New Jersey.

Death occurred in these cats before tests could be repeated. Two milliliters/kilogram of body weight of ethylene glycol were administered to 2 cats (013, 014) following PSP and other determinations (see above), and repeat determinations were performed at 24 hour intervals until these cats were euthanatized when obviously near death.

TABLE 1. Summary of experimental procedure and groups according to cat identification number

Controls	Bilateral nephrectomy	Unilateral nephrectomy	Reclassified as naturally occurring nephritis	Uranyl nitrate toxicosis	Ethylene glycol toxicosis
001*			001		
002*			002		
004					
005					
006					
007					
008*			008		
009**		009			
010					
011					
012*			012		
					013
					014
					015
					016
021**	021				
022**	022				
023**	023				
024**	024				
025**	025				
				026	
				027	
				028	
				029	

*Data was not used as control data.
**Prenephrectomy data was used as control data.

RESULTS

Controls

The PSP T 1/2, PSPI, PSP_{30}, and PSP_{40} values are listed (Table 2). The PSP volume of distribution, volume of distribution expressed as ml./100 Gm. of body weight, C_{PSP}, and C_{PSP}/kg. of body weight are also listed (Table 3).

There was little correlation between C_{PSP}/kg. of body weight and the average packed cell volume ($r = 0.13$). Packed cell volumes ranged from 22 to 36% for all cats in all groups.

The only gross and microscopic changes observed in the control cats were subacute catarrhal sinusitis (cats 007 and 010), mild intestinal parasitic infestation with ascarids and tapeworms (species not identified) (cats 005 and 009), and thrombus formation around the intravenous catheter tips in those cats that were catheterized for longer than 5 days (cats 004, 005, and 010). No renal lesions were present.

Bilateral nephrectomy

Five cats were bilaterally nephrectomized after control data had been collected. Marked increases in PSP T 1/2, PSP_{30}, and PSP_{40} and decreases in PSPI occurred in all cats following nephrectomy (Table 4).

Unilateral nephrectomy

Elevations of PSP T 1/2 and PSP_{30} were recorded following unilateral nephrectomy of 1 cat (009). Blood urea nitrogen levels remained within normal limits during this experiment (Table 5).

Naturally occurring nephritis

Microscopic renal lesions were found in 4 cats (001, 002, 008, and 012) previously designated as controls (Table 1). Three had normal PSP and BUN values (Table 6). One cat (012) had slight elevations of PSP_{30} and PSP_{40}.

Multifocal areas of lymphocytic and reticulo-endothelial cell infiltration involving approximately 5% of the nephrons were observed in the renal cortices of both kidneys of Cat 001. Several foci were as large as 180 by 3500 μ and involved glomeruli and periglomerular tubules. The basement membranes of many Bowman's capsules were thickened and a mild increase in interstitial connective tissue was observed in portions of the renal medulla. The unaffected proximal tubules appeared to be laden with lipid.

Scattered throughout the cortices of both kidneys of cat 002 were multiple foci of mononuclear inflammatory cells, which in some cases surrounded centers of neutrophils. The foci were estimated to be from 90 to 600 μ in diameter and involved only a small percentage of nephrons. The proximal tubules of these kidneys also appeared to be laden with lipid.

There were a few scattered foci of lymphocytes and reticulo-endothelial phagocytes in the renal cortices of

cat 008. Two large (4.6 mm. by 1.6 mm. and 1.9 mm. by 0.2 mm.) pyramids of inflammatory reaction were observed in a longitudinal section of the left kidney. These areas extended from the region of, or near, the renal surface, centrally toward the cortico-medullary junction. Lipid was prominent in the proximal tubular epithelium.

The right kidney of cat 012 was histologically normal. The left kidney, however, contained inflammatory areas involving the medullary rays. These varied from small foci of lymphocytes and plasma cells to larger areas (100 X 800 μ) containing the same cellular infiltration. A few areas also contained neutrophils.

Uranyl nitrate toxicosis

Four cats (026, 027, 028, and 029) exhibited anorexia, depression, and mild ataxia within 3 days after subcutaneous administration of uranyl nitrate at a rate of 5 mg./kg. of body weight. Hemorrhagic ileitis was observed in 3 cats (026, 028, and 029). This was associated with extreme hyperemia of 3 to 6 inches of the lower portion of the ileum, not directly adjacent to the cecum (Figure 2). A small amount of blood-tinged mucus was in the intestinal lumen in these areas, and the mesenteric lymph nodes were edematous.

Two cats (026 and 028) had mild to severe perirenal edema (Figure 3). This edema was most extensive in Cat 028 and extended from the area directly surrounding the kidneys, caudad along the ureters in the periureteral tissues for a distance of approximately 5 cm. A focal hemorrhagic cystitis

in the mucosa and muscularis of the fundic region of the urinary bladder and multiple renal cysts (approximately 0.1 cm. in diameter) were also observed in this cat. Cat 026 also had hemorrhages in the thymus (Figure 4).

Gross changes in the kidneys of these 4 cats administered uranyl nitrate included paleness, swelling of the parenchyma and hemorrhage at the cortico-medullary junction (Figure 5).

The essential microscopic renal change was degeneration of the proximal tubular epithelium, particularly that portion that forms the medullary rays (Figures 6 and 7). The cellular reaction ranged from cloudy swelling of these tubular cells to complete loss of the epithelium. Tubular epithelial loss was particularly severe in Cats 028 and 029. Focal mineralization in the medulla and mild cellular infiltrations were also observed in several of these kidneys.

Within 24 hours after the administration of the uranyl nitrate, significant increases in PSP_{30} and PSP_{40} occurred ($p < 0.001$). By 48 hours the BUN concentration had risen markedly (Tables 7 and 8). Changes in T 1/2 and PSPI were erratic and not reliable indicators of renal damage.

Serum calcium values decreased markedly in 1 cat (028) 72 hours following the administration of the uranyl nitrate (Table 7). A slight decrease in serum calcium occurred in 1 cat (029) given uranyl nitrate.

Glycosuria, ketonuria, proteinuria and increased numbers of urinary casts were evident in all 4 cats 24 hours after the administration of the uranyl nitrate (Tables 9 & 10). Increased

numbers of small and large round epithelial cells in the urine sediment indicated the advancing severity of the renal lesions (Table 10). Hematuria occurred in 3 cats near the end of the experiment (48 to 72 hours).

Ethylene glycol toxicosis

Two cats (015 and 016) given 5 ml./kg of body weight of ethylene glycol *per os* died within 12 hours. No data were collected from these cats. Severe hemorrhagic gastritis was seen at necropsy of both cats.

One cat (013) administered 2 ml./kg. of body weight of ethylene glycol *per os* was severely ataxic and depressed within 12 hours. This cat was comatose at 40 hours and was euthanatized. The other cat (014) given the same dosage lived four days and was euthanatized *in extremis*. Clinical signs included mild ataxia (24 hours), severe ataxia with rigid flexion of the claws and head (48 hours), severe weakness with no rigid flexion (72 hours), and prostration (90 hours).

The only gross lesions were gastric hyperemia (Figure 8), the presence of blood-tinged mucus in the intestinal tract and a finely granular appearance of the cut surfaces of the kidneys. Microscopic renal lesions included the presence of birefringent crystals in the lumens of the proximal and distal convoluted tubules and the collecting ducts. Those tubules near the kidney capsule were particularly affected by crystal deposition (Figure 9). Other observations included necrosis of the tubular epithelium adjacent to these crystals,

debris and hyaline material in the lumens of many tubules and collecting ducts, a few granular and hyaline casts in the medullary collecting ducts, swollen glomerular tufts, and distended Bowman's capsules with granular debris along the basement membrane.

The adrenal gland of one cat (013) had multifocal medullary necrosis. The livers of both cats also had centrolobular cloudy swelling and coagulative necrosis of the hepatocytes. Crystals believed to be calcium oxalate were observed in the liver of Cat 014 (Figure 10).

Phenolsulfonphthalein T 1/2, PSP_{30}, and PSP_{40} and SGPT values were extremely elevated 24 hours after ethylene glycol administration (Table 11). Serum calcium and the PSPI values were markedly decreased at this same time. Blood urea nitrogen levels were moderately elevated at 24 hours and continued to rise until euthanasia.

The serum albumin and plasma protein levels were monitored in most of the cats in all groups, and these values remained within normal limits.

One cat (027) began vomiting immediately after intravenous injection of the PSP dye solution on 2 consecutive days. Slower administration of the dye on subsequent days apparently prevented further emesis.

Figure 2. Terminal ileum and anterior colon. Hyperemic to hemorrhagic ileitis. Note the inflammation is not continuous into the colon. Uranyl nitrate toxicosis. Cat #029.

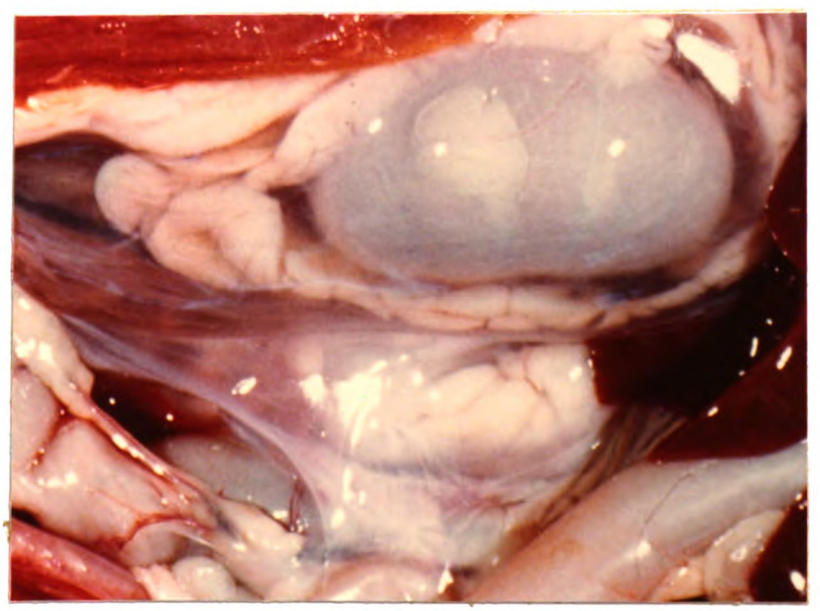

Figure 3. Kidneys and perirenal area. Perirenal edema. Uranyl nitrate toxicosis. Cat #028.

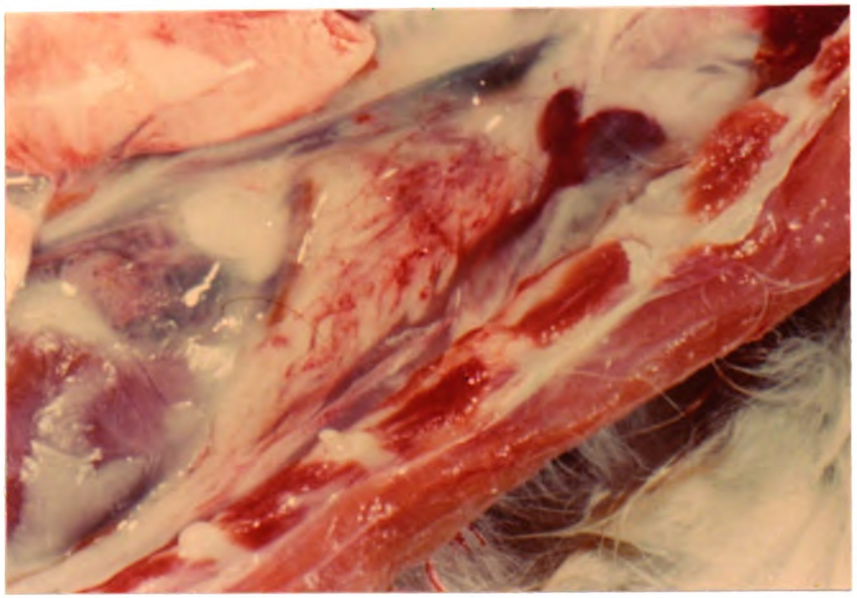

Figure 4. Thymus. Hemorrhages involving the thymus. Uranyl nitrate toxicosis. Cat #026.

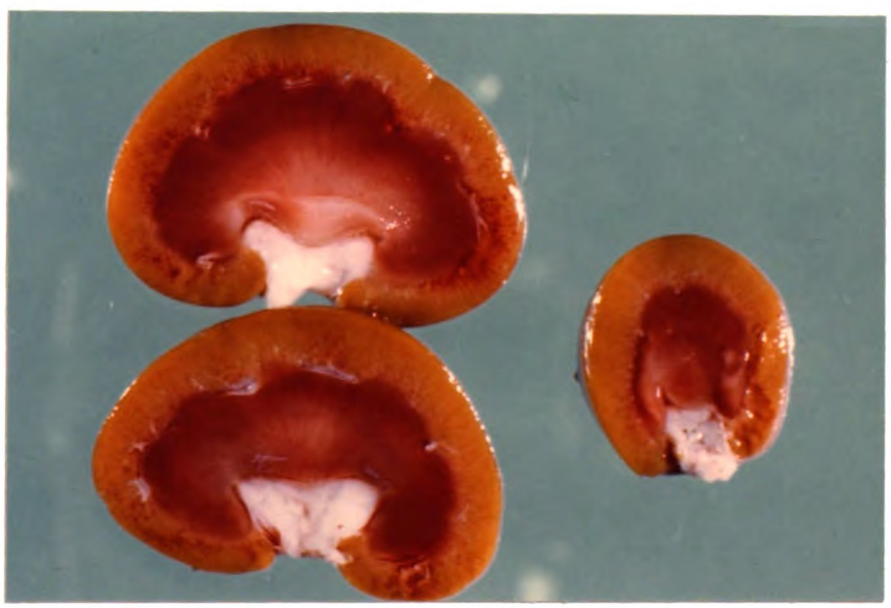

Figure 5. Longitudinal and cross sections of the kidneys. Congestion and hemorrhage at the cortico-medullary junction. Uranyl nitrate toxicosis. Cat #029.

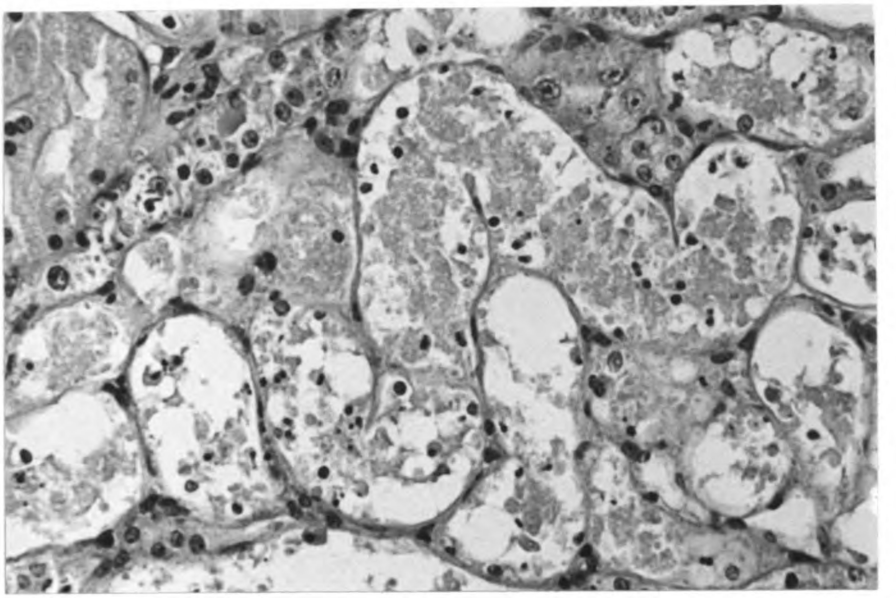

Figure 6. Renal cortico-medullary junction. Extensive loss of tubular epithelium. Uranyl nitrate toxicosis. Cat #028. Hematoxylin and eosin. X 288.

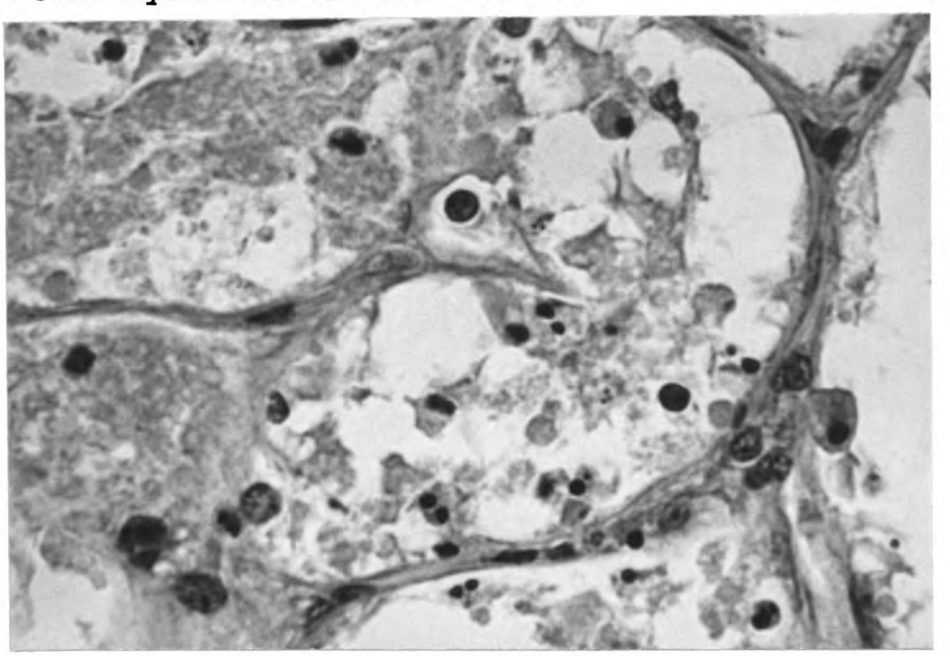

Figure 7. Higher magnification of Figure 6. Note loss of tubular epithelium and pyknosis and loss of nuclei of tubular epithelium remaining. Cat #028. Hematoxylin and eosin. X 576.

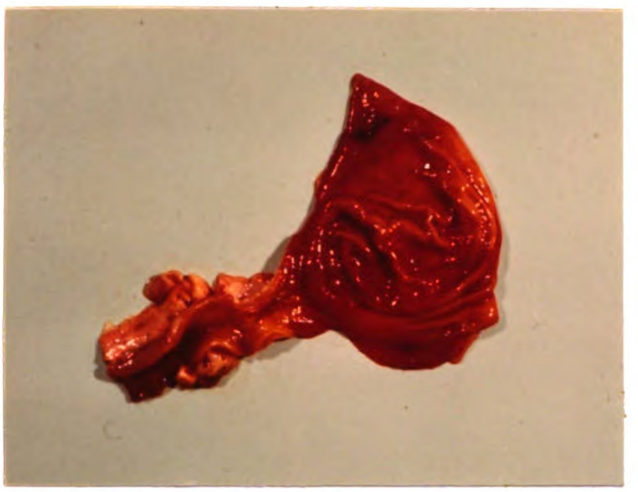

Figure 8. Gastric mucosa. Severe hyperemia of the gastric mucosa. Ethylene glycol toxicosis. Cat #013.

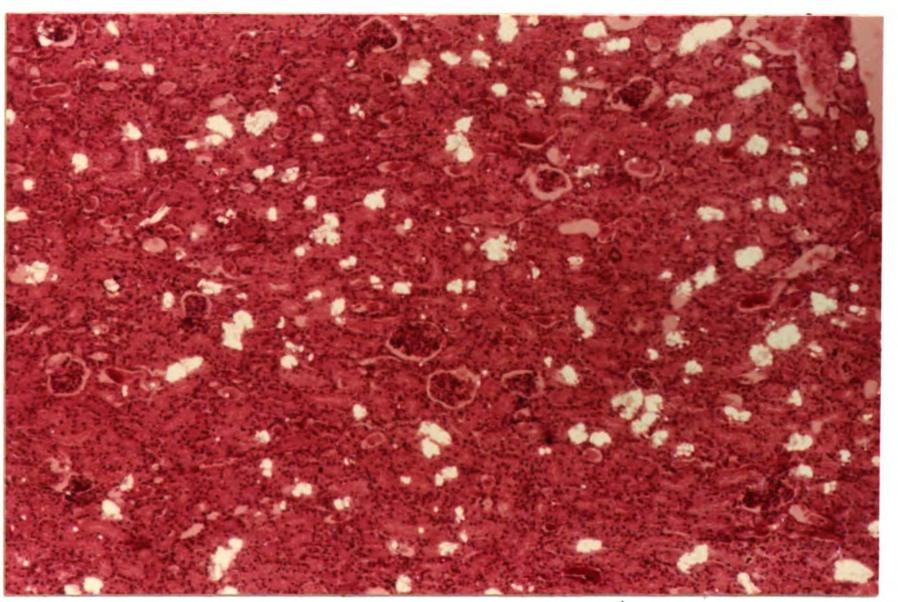

Figure 9. Renal cortex. Photographed using partially polarized light. Note the presence of large numbers of birefringent crystals. Ethylene glycol toxicosis. Cat #014. Hematoxylin and eosin. X 48.

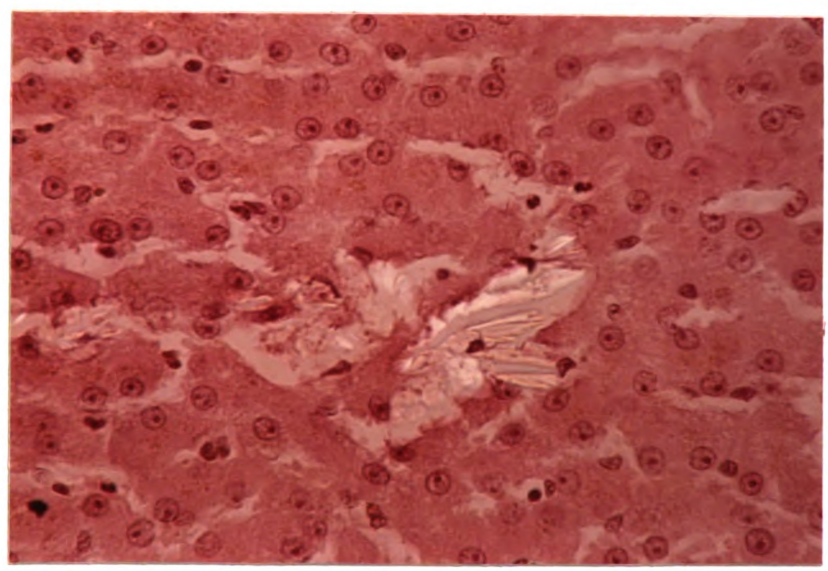

Figure 10. Liver. Photographed using a partially polarized light source. Note the presence of birefringent crystals within sinusoidal spaces. Ethylene glycol toxicosis. Cat #014. Hematoxylin and eosin. X 480.

DISCUSSION

Normal PSP T 1/2 and clearance

The average PSP T 1/2 for control cats (18.2 minutes) was similar to that reported by Brobst *et al.* (1967) for the dog (19.6 minutes) and lower than that reported for the dairy cow (24.4 minutes) (Mixner and Anderson, 1958). The mean T 1/2 of anesthetized cats in experiments by Osbaldiston & Fuhrman (1970), however, was much lower (10.3 minutes).

The reason for the higher T 1/2 values in nonanesthetized cats is not apparent. Osbaldiston infused cats with saline. However, Gault (1966) stated that urine flow rates do not alter plasma disappearance of PSP. Determination of the slow phase using plasma concentrations before 20 minutes may result in falsely low T 1/2 times. Osbaldiston did not reveal the time of collection of plasma samples used in his work. Also, Osbaldiston used micromethods which enabled determination of PSP plasma concentrations produced by doses of the dye as small as 3 mg. In the experiments reported here a total dose of approximately 10 to 15 mg. of PSP per cat was used to produce accurately measurable plasma concentrations. Although it was not evident, suppression of the excretory mechanisms may have occurred by exceeding the enzymatic carrier system capacity.

This would result in higher T 1/2 values.

Gault (1966) attempted to estimate the "PSP space" or distribution volume by back extrapolation of the decreasing plasma concentration of the dye. He concluded that "the PSP space may be a rough estimate of the extracellular fluid volume". Smith et al. (1938), however, stated that backward extrapolation is not an accurate method of determining the distribution volume, especially in those patients with renal disease. Distribution volumes reported here and determined by this method were calculated only in cats with histologically normal kidneys.

In cattle, distribution volumes of PSP between 19.0 and 31.9% of body weight (19 - 31 ml./100 Gm. of body weight) were reported by Mixner and Anderson (1958). In control cats distribution volumes ranged from 17 to 32 ml./100 Gm. of body weight. This indicates a similar PSP distribution compartment in the two species.

Phenolsulfonphthalein clearance/kg. (C_{PSP}/kg.) determined in nonanesthetized cats was significantly higher ($p < 0.001$) than the value reported in 7 anesthetized (thiamyl sodium) cats by Osbaldiston and Fuhrman (1970). Barbiturate anesthesia has been reported to reduce phosphorylation and oxygen availability which theoretically would suppress the PSP tubular transport system (Wesson, 1969). Also reduction of the effective renal blood flow by anesthesia would result in lower renal clearance of PSP. However, it is paradoxical that both T 1/2 and C_{PSP}/kg. values were higher in nonanesthetized than in anesthetized cats.

Normal PSP plasma disappearance

The disappearance curve of plasma PSP in cats with normal renal function can be divided into two components: the so-called "fast component" which declined rapidly following injection of the dye for approximately 10 to 20 minutes and the "slow component" which declined at an exponential rate from approximately 20 minutes to approximately 50 minutes. After 50 minutes the concentration of PSP was below accurately measurable amounts (0.1 mg./100 ml.) using the analytic methods reported here (Gault, 1966). This curve conforms to the PSP disappearance curves in human, canine, and bovine subjects (Gault, 1966; Brobst et al., 1967; Carper and Hougan, 1967; Mixner and Anderson, 1958). Although the fast component was reported to be complete in normal human subjects, dogs, and cattle by 15 minutes, in many control cats the curve did not become exponential until 15 to 20 minutes.

Carper and Hougan (1967) stated the fast component represented glomerular filtration of unbound dye in the dog. The persistence of this component in bilaterally nephrectomized cats indicated this is not true in the cat. Others (Gault, 1966; Osbaldiston, 1970) interpreted the fast component to represent a "distribution phase" in which PSP is distributed to various body compartments. This phase in cats is apparently the combined effects of the distribution of dye, glomerular filtration of the free dye, extrarenal excretion and early renal tubular secretion.

The slow or exponential phase of PSP disappearance represents primarily the tubular secretion of the dye when plasma concentrations are below 1.5 mg./100 ml. (Brobst et al., 1968). A dosage of 5 mg. PSP/kg. of body weight administered intravenously resulted in plasma levels in the control cats between 0.1 mg./100 ml. and 1.5 mg./100 ml. during the slow phase.

The slow phase in anuric animals was reported to be linear rather than exponential (Osbaldiston and Fuhrman, 1970). However, the slow phase (extrarenal component) in bilaterally nephrectomized cats retained its exponential function.

Extrarenal PSP disappearance

It is necessary to consider the extrarenal disappearance of PSP when using plasma concentration values in diagnostic procedures. The PSP index or PSPI is a linear function of T 1/2 and is a more useful term when comparing renal to extrarenal disappearance (Gault, 1966).

"The extrarenal component of the PSPI which ordinarily amounts to 3 or 4 units (in humans) probably is due to the passage of PSP from interstitial fluid into cells. Hepatic uptake is quantitatively small and the degree of metabolism of PSP insignificant during the test period" (Gault, 1966). An average PSPI value of 16.9 ($\pm$ 2.69) was recorded in control cats in the present study. The PSPI values in bilaterally nephrectomized cats averaged 6.2 units ($\pm$ 0.94) and ranged from 28.5 to 43.0% of the pre-nephrectomy values.

These percentage values are much higher than the 18% reported in anuric human patients (Gault, 1966). Because the percentage of extrarenal PSP disappearance is so high in the cat, unless the component is constant or nearly so, PSP plasma disappearance (T 1/2) cannot be a reliable indicator of only renal function. The measurement of PSP in the urine then is theoretically a better indicator of renal function than the measurement of plasma concentrations of the dye. The urinary method, however, has not proven clinically useful in the cat due to the difficulty of collecting adequate urine samples. Comparison studies between simultaneously determined PSP T 1/2 and PSP urinary excretion values should be done to resolve the question of whether these 2 indices measure the same function in the cat.

Effect of packed cell volume on PSP clearance

There was no apparent correlation between packed cell volume and PSP clearance. Ewald (1967) suggested that clearance is dependent on effective renal blood flow and would therefore be unaffected by minor variations in packed cell volumes.

The packed cell volumes of the control cats were generally below normal (Schalm, 1965; Velasco et al., 1971). However, the samples were collected via a jugular cannula with its opening immediately anterior to the right auricle of the heart in the anterior vena cava. Centrally collected blood specimens have a slightly lower packed cell volume than peripheral specimens. Also, surgical blood loss may

have reduced the packed cell volumes.

Clinical application of PSP

Though limited studies were done, it appeared that the PSP T 1/2, PSP_{30} and PSP_{40} are increased when 50% of the renal mass is removed (unilateral nephrectomy). However, PSP T 1/2, PSP_{30}, PSP_{40} and PSPI were of little value in detecting the presence of mild naturally occurring nephritis.

Uranyl nitrate injected subcutaneously in cats at the rate of 5 mg./kg. of body weight produced toxic tubular nephrosis. The primary degeneration involved the epithelium of the proximal tubules of the medullary rays.

Although the PSP T 1/2 times were elevated in the cats with uranyl nitrate nephrosis, the PSP_{30} and PSP_{40} provided more sensitive indices of the increasing severity of the renal damage and were more useful in early detection of damage. Urinalysis was particularly useful in predicting early damage. The BUN levels rose markedly by 48 hours and thus were another indicator of renal impairment.

In clinical detection of uranyl nitrate toxicosis, urinalysis was considered to be of more value as a diagnostic and prognostic test than either PSP or BUN values. Phenolsulfonphthalein could be used alone, however, to detect this type of renal disease but not to predict the nature of the renal damage (nephrosis).

Values for PSP were markedly affected in two cats with ethylene glycol toxicosis. The T 1/2 and PSP concentration

values in these affected cats were above even those values recorded for bilaterally nephrectomized cats. Not only were the renal mechanisms of PSP disappearance inhibited, but much of the extrarenal excretion was also slowed by ethylene glycol toxicity. The plugging of the renal tubules with oxalate crystals is apparently the major mechanism inhibiting the renal excretion of PSP. Decreased hepatic excretion of PSP was also probable. Histologic hepatic changes, elevated SGPT values and oxalate crystal deposition in the liver were present in cats receiving ethylene glycol. Calcium is necessary for the transfer of PSP across cellular membranes (Richards and Barnwell, 1927). A depression of PSP excretory mechanisms due to hypocalcemia apparently also contributed to altered PSP values.

Conclusions

The use of PSP does not offer advantages over urinalysis in detection of most cases of acute nephritis. The difficulty of the PSP procedures when compared to the accuracy of the information gained, does not warrant using only PSP procedures when acute nephritis is suspected. However, in chronic nephritis where 50% or more of the renal mass is nonfunctional and urinalysis and BUN values do not indicate the damage, PSP_{30}, PSP_{40}, and PSP T 1/2 may be of diagnostic usefulness.

In clinical studies of renal disease, determination of PSP_{30} and PSP_{40} would be worth the effort in an attempt to detect renal dysfunction associated with obstructive

urolithiasis, chronic renal disease, or antifreeze (ethylene glycol) toxicosis. The PSP_{30} and PSP_{40} values may also be more reliable in the cat than in the dog because of the general uniformity of the feline anatomy among different breeds as compared with the dog.

Urinalysis probably is the one greatest aid in diagnosing renal disease. Phenolsulfonphthalein techniques detect decreases in renal function before BUN values increase. However, the laboratory determination of BUN is considerably less difficult. The determination of PSP values in the cat, therefore, is of value in specific circumstances and is not recommended for routine clinical evaluation of renal function.

SUMMARY

A study was undertaken to develop procedures utilizing phenolsulfonphthalein dye (PSP) for the detection of renal disease in cats. Twenty-four cats were divided into 6 groups to evaluate these procedures in normal animals and in cats with naturally occurring and experimentally induced nephropathies. Phenolsulfonphthalein biological plasma half-life (T 1/2), index (PSPI) plasma concentration at 30 minutes (PSP_{30}) and 40 minutes (PSP_{40}) after dye injection, clearance/kg. (C_{PSP}/kg.), and volume of distribution were determined in 12 nonanesthetized domestic cats with histologically normal kidneys. Values for T 1/2 ranged from 13.0 to 25.0 minutes (mean 18.2 $\pm$ 2.91), the PSPI values from 12.0 to 23.1 units (16.9 $\pm$ 2.69), PSP_{30} from 0.44 to 0.96 mg./100 ml. (0.65 $\pm$ 0.105), PSP_{40} from 0.28 to 0.66 mg./100 ml. (0.47 $\pm$ 0.082), the C_{PSP}/kg. from 6.42 to 12.36 ml./minute/kg. of body weight (9.23 $\pm$ 1.273) and the volume of distribution from 17 to 31 ml./100 Gm. of body weight (24.6 $\pm$ 3.62).

The biological half life of PSP (T 1/2) in 5 bilaterally nephrectomized nonanesthetized cats was markedly elevated, ranging from 41 to 60 minutes. One unilaterally nephrectomized cat had PSP T 1/2 values ranging from 25 to 49 minutes and elevated PSP_{30} values.

Four cats with mild, naturally occurring nephritis did not have elevated PSP T 1/2 or blood urea nitrogen values. One cat in this group did have slightly elevated PSP_{30} and PSP_{40} values. The nephritis observed in these cats was characterized by multifocal infiltration by mononuclear cells, tubular destruction and early fibrosis in the cortical areas.

Tubular nephrosis was produced in 4 cats by subcutaneous administration of a solution of uranyl nitrate at the rate of 5 mg./kg. of body weight. The PSP T 1/2 values from affected cats ranged from 23 to 60 minutes but were not considered a reliable index of renal damage. Glycosuria, ketonuria, proteinuria, and increased numbers of granular casts and small round epithelial cells in the urine sediment were observed at 24 hours after the administration of uranyl nitrate. Blood urea nitrogen values increased above normal values by the end of 48 hours. The PSP_{30} and PSP_{40} values increased significantly ($p < 0.001$) by 24 hours and appeared to be a more reliable indicator of early renal damage than did the T 1/2 values.

Two cats administered ethylene glycol orally at the rate of 2 mg./kg. of body weight had markedly elevated PSP T 1/2 (53.5 to 150 minutes), PSP_{30} and PSP_{40} values by 24 hours following administration. Blood urea nitrogen values also rose above the normal range by this time. Serum calcium values of affected cats ranged from 2.3 to 6.8 mg./100 ml.

and serum glutamic pyruvate transaminase (SGPT) levels were elevated (90 to 180 Sigma Frankel units). Obstruction of renal tubules and collecting ducts, pressure necrosis of the epithelium of those tubules and ducts, plus hepatic necrosis were observed histologically.

It was concluded that the measurement of plasma concentrations of PSP following intravenous administration is useful for the estimation of renal function in cats. It is of special value when urinalysis indicates acute or chronic nephritic processes and BUN values are still within the normal range.

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VITA

The author was born July 25, 1944, in University Park (Oskaloosa), Iowa and attended public grade school in North English, Iowa. In 1962 he graduated from United Community High School, Boone, Iowa and the fall of that same year entered Iowa State University in a pre-veterinary program. In 1964 the author was accepted in the Veterinary College at Iowa State University and graduated in May, 1968.

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The author is married and has two children.

APPENDIX

TABLE 2. Summary of PSP values in control cats and prior to nephrectomy in nephrectomized group.

Cat No.	T 1/2 (minutes)	PSPI (units)	PSP_{30} (mg./100ml.)	PSP_{40} (mg./100ml.)
004	22	13.6	0.80	0.60
004	15	20.0	0.71	0.49
004	20	15.0	0.73	0.54
004	19	15.8	0.65	0.51
005	14	21.5	0.58	0.40
005	20	15.0	0.67	0.43
005	19.5	15.4	0.65	0.44
005	18	16.7	0.69	0.46
005	18	16.7	0.67	0.46
005	18.5	16.2	0.72	0.48
005	18	16.7	0.72	0.50
005	18	16.7	0.67	0.43
005	20	15.0	0.96	0.66
006	22	13.6	0.63	0.50
007	15.5	19.4	0.65	0.58
007	23.5	12.8	0.56	0.42
007	18	16.7	0.78	0.59
009	13	23.1	0.56	0.39

TABLE 2. continued on next page.

TABLE 2. (continued)

Cat No.	T 1/2 (minutes)	PSPI (units)	PSP_{30} (mg./100ml.)	PSP_{40} (mg./100ml.)
010	17	17.7	0.60	0.49
010	16	18.8	0.65	0.55
010	16.5	18.2	0.58	0.42
010	25	12.0	0.53	0.35
011	18.5	16.2	0.61	0.42
021	14.5	20.7	0.44	0.28
022	15.5	19.8	0.47	0.30
023	18	16.7	0.56	0.42
024	22	13.6	0.73	0.45
025	17	17.7	0.70	0.47
mean value	18.2	16.9	0.65	0.47
standard deviation	± 2.91	± 2.69	± 0.105	± 0.082
range	13.0 to 25.0	12.0 to 23.1	0.44 to 0.96	0.28 to 0.66

TABLE 3. Summary of PSP volume of distribution and clearance values in control cats and prior to nephrectomy in nephrectomized group.

Cat No.	volume of distribution (ml.)	vol. dist. per 100 Gm. of body wt. (ml./100 Gm.)	C_{PSP} (ml./min.)	C_{PSP}/kg. (ml./min./kg.)
004	1038	27	31.14	8.06
004	644	17	28.98	7.50
004	1104	29	37.54	9.72
004	920	24	33.12	8.57
005	488	21	23.91	10.05
005	650	27	22.10	9.29
005	559	23	20.97	8.81
005	541	23	21.10	8.87
005	553	23	21.57	9.06
005	517	22	19.65	8.26
005	524	22	20.44	8.59
005	449	19	15.27	6.42
006	1157	31	34.71	9.26
007	864	20	38.02	8.81
007	1136	26	32.94	7.63
007	1136	26	43.17	10.00
009	764	23	38.96	11.84

TABLE 3. continued on next page.

TABLE 3. (continued)

Cat No.	volume of distribution (ml.)	vol. dist. per 100 Gm. of body wt. (ml./100 Gm.)	C_{PSP} (ml./min.)	C_{PSP}/kg. (ml./min./kg)
010	1094	27	43.76	10.70
010	942	23	39.56	9.67
010	1076	26	44.12	10.79
010	1303	32	36.48	8.92
011	758	26	28.80	9.76
021	639	26	30.03	12.36
022	770	27	33.88	10.07
023	762	28	28.96	9.10
024	491	29	14.73	8.76
025	417	21	16.68	8.34
mean value	782	24.6	29.65	9.23
standard deviation	± --	± 3.62	± 9.012	± 1.273
range	417 to 1303	17 to 32	16.68 to 44.12	6.42 to 12.36

TABLE 4. Comparison of pre-and post-nephrectomy PSP values in bilaterally nephrectomized cats.

Cat No.	PSP T 1/2 (minutes) Pre-Neph.	PSP T 1/2 (minutes) Post-Neph.	PSPI (units) Pre-Neph.	PSPI (units) Post-Neph.	PSP_{30} (mg./100ml.) Pre-Neph.	PSP_{30} (mg./100ml.) Post-Neph.	PSP_{40} (mg./100ml.) Pre-Neph.	PSP_{40} (mg./100ml.) Post-Neph.
021	14.5	43.0	20.7	7.0	0.44	1.02	0.28	0.90
022	15.0	52.0	20.0	5.7	0.47	1.18	0.30	1.07
023	18.0	41.0	16.7	7.3	0.56	1.27	0.42	1.10
024	22.0	60.0	13.6	5.0	0.73	1.14	0.45	1.01
025	14.5	49.0	20.7	6.1	0.70	1.29	0.47	1.19
mean	16.8	49.0	18.3	6.2	0.58	1.18	0.38	1.05
standard deviation	± 3.81	± 7.58	± 0.94		± 0.109		± 0.108	
ratio (%) of post./pre.	291.7%		34.4%		203.4%		276.3%	

TABLE 5. Comparison of pre- and post-nephrectomy PSP and BUN values in a unilaterally nephrectomized cat (cat 009).

time after surgery (hours)	T 1/2 (minutes)	PSP_{30} (mg./100ml.)	PSPI (units)	blood urea nitrogen (mg./100ml.)
0*	22	0.90	13.6	-
0*	13	0.56	23.1	-
0*	17.5	0.81	17.2	20
24	45	1.14	6.6	25
48	27.5	1.05	10.9	24
72	49	1.15	6.1	19
96	25	1.05	12.0	20

*Pre-surgical determinations.

TABLE 6. PSP and BUN values in 4 cats with naturally occurring nephritis.

Cat No.	PSP T 1/2 (minutes)	PSPI (units)	PSP_{30} (mg./100ml.)	PSP_{40} (mg./100ml.)	blood urea nitrogen (mg./100ml.)
001	14	21.5	0.59	0.35	18
002	16	18.8	0.55	0.37	15
008	16.5	18.2	0.80	0.52	20
012	21.5	14.0	1.01	0.73	17

TABLE 7. Summary of the PSP, BUN, and serum calcium values of 4 cats receiving uranyl nitrate (5 mg./kg. of body weight).

Cat No.	time after admin. (hours)	PSP T 1/2 (minutes)	PSPI (units)	PSP_{30} (mg./100ml.)	PSP_{40} (mg./100ml.)	blood urea nitrogen (mg./100ml.)	serum calcium (mg./100ml.)
026	0	21	14.3	0.80	0.60	20	9.3
	24	54	5.5	1.27	1.16	30	9.3
	48	26	11.5	1.33	1.11	110	9.3
	72	39	7.7	1.55	1.38	136	-
027	0	17	17.7	0.76	0.54	17	9.3
	24	28	10.7	1.16	0.81	20	8.8
	48	21.5	14.0	1.53	1.05	36	8.8
	72	27.5	10.9	1.46	1.19	65	-

TABLE 7. continued on next page.

TABLE 7. (continued)

Cat No.	time after admin. (hours)	PSP T 1/2 (minutes)	PSPI (units)	PSP_{30} (mg./100ml.)	PSP_{40} (mg./100ml.)	blood urea nitrogen (mg./100ml.)	serum calcium (mg./100ml.)
028	0	21	14.3	0.87	0.68	27	-
	24	29	10.3	1.03	0.95	22	7.5
	48	32	9.4	1.18	1.01	70	6.8
	72	33.5	8.9	1.48	1.26	125	5.8
	96	36	8.3	1.66	1.38	135	-
029	0	25.5	11.8	0.97	0.77	22	-
	24	23	13.0	1.11	0.81	26	8.5
	48	60	5.0	1.55	1.33	63	8.0
	72	37	8.1	1.61	1.33	97	8.0
	96	53.5	5.6	1.43	1.34	132	-

TABLE 8. Average PSP and BUN values arranged in time interval groups for 4 cats receiving uranyl nitrate (5 mg./kg. of body weight).

time after administration (hours)	PSP T 1/2 (minutes)	PSPI (units)	PSP_{30} (mg./100ml.)	PSP_{40} (mg./100ml.)	BUN (mg./100ml.)
24	33.5	9.9	1.14	0.93	26
S.D.*	(±13.92)	(±3.15)	(±0.100)	(±0.165)	(± 5.9)
48	34.8	10.0	1.40	1.12	63
S.D.	(±17.34)	(±3.81)	(±0.176)	(±0.143)	(±34.9)
72	34.2	8.9	1.53	1.29	97
S.D.	(± 5.0)	(±1.42)	(±0.069)	(±0.083)	(±38.6)
96	44.7	7.0	1.55	1.36	132

*S.D. = standard deviation

TABLE 9. Summary of urinalysis findings in 4 cats receiving uranyl nitrate (5 mg./kg. of body weight).

Cat No.	time after administration (hour)	protein	specific gravity	glucose	pH	blood	ketones
026	0	trace	1.060	-	6.5	-	-
	24	++	1.023	+++	6.0	-	+++
	48	+++	1.024	+++	6.0	-	+++
	72	+++	1.021	+++	6.0	++	++
027	0	trace	1.060	-	7.5	-	-
	24	++	1.039	+++	7.5	-	+++
	48	++	1.021	+++	6.5	-	+++
	72	++	QNS*	+++	QNS	-	+
028	0	trace	1.040	-	7.0	-	-
	24	+	1.030	+++	6.0	-	+++
	48	+++	1.015	+++	6.0	++	-
	72	+++	1.013	+++	6.0	+++	-
	96	++	1.014	+++	6.0	+++	-
029	0	trace	1.040	-	6.5	-	-
	24	+	1.033	+	6.0	-	+
	48	++	1.019	+++	6.0	-	++
	72	+++	1.017	+++	6.0	-	+
	96	++	1.017	+++	6.0	-	-

*Quantity not sufficient.

TABLE 10. Summary of microscopic examination of urine sediment for 4 cats receiving uranyl nitrate (5 mg./kg. of body weight).

Cat No.	time after admin. (hour)	red blood cells	squamous epith. cells	small round epith. cells	large round epith. cells	white blood cells	protein casts
026	0	-	-	-	-	-	-
	24	-	+	++	-	-	++ gran.*
	48	-	+	TNTC**	-	-	++
	72	TNTC (obscured other sediment)					
027	0	-	++	+	-	±	± hyaline
	24	-	+	++	+	+	+ gran.
	48	-	+	++	++	+	+
	72	quantity of urine recovered not sufficient for test					
028	0	-	-	-	-	-	-
	24	-	+	++++	++	-	+ gran.
	48	TNTC	+	TNTC	++	++	+++
	72	TNTC (obscured other sediment)					
	96	TNTC (obscured other sediment)					
029	0	-	-	-	-	-	-
	24	-	-	+	+	-	+ gran.
	48	++	+	+++	++	++	++
	72	+	-	TNTC	+++	++	+++
	96	+	-	TNTC	+++	++	+++

*gran. = granular casts
**TNTC = too numerous to count
+ = few
++ = moderate number
+++ = many
++++ = very many

TABLE 11. Summary of PSP, BUN, serum calcium, and SGPT values in cats receiving ethylene glycol orally (2 ml./kg. of body weight).

Cat No.	time after admin. (hours)	PSP T 1/2 (minutes)	PSPI (units)	PSP_{30} (mg./ 100ml.)	PSP_{40} (mg./ 100ml.)	BUN (mg./ 100ml.)	serum calcium (mg./ 100ml.)	SGPT (S.F.* units)
013	0	26.5	11.3	1.00	0.73	9	9.3	30
	24	53.5	5.6	1.95	1.67	60	6.3	120
	just prior to death	--	--	--	--	100	2.3	180
014	0	20.5	14.6	0.75	0.58	17	9.3	27
	24	72	4.1	1.42	1.21	44	5.0	160
	48	150	2.0	1.89	1.75	100	5.0	140
	72	98.5	3.0	1.59	1.49	120	5.3	90
	just prior to death	--	--	--	--	125	6.8	110

*Sigma Frankel units