STUDIES IN CANINE NEPHRITIS

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INTRODUCTION

Inflammatory lesions of the kidney are frequently observed in the dog at necropsy. Persson et al (1961) states that on a world wide basis diseases of the kidney constitute the largest disease group of dogs and that chronic interstitial nephritis is the predominant form. Considerable disagreement exists among various authors as to the etiology and pathogenesis of the interstitial inflammatory process.

Leptospirosis has been considered by many as the major cause of canine interstitial nephritis (Bloom, 1954; Jubb and Kennedy, 1965; Anderson, 1967 and Barron, 1966). Recent contributions by other investigators however have not been in agreement with this concept. Lowe et al (1967) reported that the kidneys of 11 dogs examined several months after recovery from acute infections with L. icterohaemorrhagiae or L. canicola did not exhibit functional or morphological evidence of renal damage. Persson et al (1961) stated that interstitial nephritis in dogs in Sweden existed with a frequency similar to that in other countries but that canine infections with leptospiral organisms were not known to occur.

The intent of this study was to investigate the possibility that infectious agents other than leptospiral organisms play an important role in the etiology and pathogenesis of canine interstitial nephritis.

Other bacteria and viruses comprise two additional classes of infectious agents that are known to be capable of inducing renal lesions. Bacteria have been incriminated as causative agents of nephritis in many species of animals and the majority of the experimental work in recent years has been directed toward assessing their significance in the etiology of progressive kidney lesions (Jackson, 1965; Beeson, 1955). A major concept that has evolved from these studies is that the hematogenous pathway is not likely to result in renal infection with bacteria unless a predisposing lesion is present in the urinary tract (Kass, 1966).

The potential of viruses as a class of infectious agents to localize and stimulate progressive lesions in the kidneys of man and animals has only recently been investigated to any extent (Jensen, 1967). Ginder (1964) demonstrated that a murine adenovirus can localize in the kidneys of mice and produce lesions which predispose that organ to secondary localization and infection with intravenously administered bacteria. The pathogenetic mechanism described by Ginder (1964) in mice conforms to the concept that a predisposing renal lesion is necessary for hematogenous bacterial infections to occur. This mechanism could be studied in any animal species in which a viral agent is known to regularly incite renal lesions. Infectious canine hepatitis (ICH) virus is an adenovirus that also localizes in the canine kidney and incites a focal inflammatory lesion similar to that produced in mice (Poppensiek and Baker, 1951; Stunzi and Poppensiek, 1953; Persson et al, 1961; Kapp, 1966; Hamilton et al, 1966 and Wright, 1967).

The purpose of the first phase of this study was to determine the intrarenal localization and pathogenicity of hematogenously administered

bacteria in normal dogs. Washed cultures of Escherichia coli and a Proteus species, both isolated from naturally occurring canine urinary tract infections, were utilized as the bacterial inoculum. The objective of the second phase of this experiment was to study the renal lesion of ICH and determine its potential to predispose the canine kidney to a superimposed hematogenous bacterial infection.

CHAPTER II

REVIEW OF THE LITERATURE

Canine Nephritis

Inflammatory lesions of the canine kidney can usually be grouped into diseases of the vascular system or diseases of the interstitial tissue (Monlux, 1953). Disparity exists among various authors as to the importance of interstitial nephritis in dogs as compared to glomerulonephritis. Interstitial nephritis is believed to be the predominant form.

Glomerulonephritis is defined as inflammation of the glomerular tuft; it can be acute or chronic and focal or diffuse and is usually accompanied by secondary changes in the tubules and interstitium (Jubb and Kennedy, 1970). Monlux (1953) and Lowe et al (1967) reported primary glomerular lesions in dogs but the frequency was low and the severity of the lesions was minimal. Obel et al, (1964) and Asheim, (1964) described a mixed membranous and proliferative glomerular lesion that occurred subsequent to pyometra in bitches. Lewis et al (1965) reported a chronic membranous glomerular lesion in dogs with systemic lupus erythematosus and Bloodworth (1965) portrayed a similar lesion in the glomeruli of diabetic dogs.

Chronic interstitial nephritis is a diffuse or multifocal progressive condition in which large portions of the renal parenchyma are

replaced by scar tissue and mononuclear inflammatory cells (Jubb and Kennedy, 1970). Its etiology and pathogenesis have not been determined. It has been the opinion of many that chronic interstitial nephritis results from progression of the acute and subacute lesions of leptospirosis (Bloom, 1954; Anderson, 1967; Wettimuny, 1967). Recent contributions have cast doubt on this relationship. Lowe (1967) conducted a long-term study of canine leptospirosis and concluded that a single leptospiral infection was not followed by the development of a chronic progressive interstitial lesion. Monlux (1953) attempted to follow the progression of leptospiral nephritis from the acute to the chronic stage, but was not able to demonstrate lesions that would bridge the gap between subacute leptospiral nephritis and chronic interstitial nephritis. Persson (1961) reported that infection with Leptospira canicola does not occur in dogs in Sweden but that the incidence of nephritis is as high there as in other countries. He believes that some factor or agent other than leptospirosis must be involved.

Pirie et al (1965) and Anderson and Fisher (1968) suggested that arterial disease was a prominent feature of the histopathology of interstitial nephritis. They speculated that the vascular damage was of hypertensive origin and was a significant factor in the progression of the renal lesion. Other conditions that have been associated etiologically with interstitial inflammatory changes include metritis, prostatitis, cystitis, filariasis, bronchopneumonia, wound infections, peritonitis, and various other chronic infections (Monlux, 1953; Bloom, 1954; Jubb and Kennedy, 1970).

Infectious canine hepatitis virus has been considered as a

possible etiological agent. Several authors have described the development of a focal interstitial nephritis subsequent to the acute phase of the disease (Hartley, 1958; Poppensiek and Baker, 1951; Stunzi and Poppensiek, 1953; Kapp, 1966; Wright, 1967). The possible role of this virus as a contributor to progressive renal disease will be expanded upon later in this review.

Bacterial isolates from the canine urinary tract have varied with different urological disorders. Wettimuny (1967) found a hemolytic Escherichia coli to be the most frequently encountered pathogen in his series of canine pyelonephritis cases. Goulden (1968) conducted a bacteriological study on urolithiasis cases and found urea splitting Staphylococcus species and Proteus species to be most common. Monlux (1953) believes that suppurative lesions in the kidney are usually associated with specific pyogenic infections which have reached the kidney by way of the blood stream or by ascending the urinary tract. Jubb and Kennedy (1970) refer to purulent lesions of hematogenous origin as embolic suppurative nephritis. Both authors utilize the term pyelonephritis to indicate a urogenous route of infection

Etiological Agents and Mechanisms of Infectious Nephritis - Bacteria

In recent years nephritides resulting from bacterial infections of the kidney of man and animals have been extensively studied (Jackson, 1965). The principal organisms studied have been E. coli, Enterobacter aerogenes and various strains of the genera Klebsiella, Proteus and Pseudomonas, which are all gram-negative organisms. Gram-positive organisms which have been investigated to some degree include staphylococci and enterococci.

The source of organisms and the route by which they invade the kidney have been subjects of extensive investigation. Beeson (1955) states that there is little doubt that the origin of the microorganisms which cause pyelonephritis in human patients is the flora of the intestinal tract. In his studies coliforms were responsible for about 80% of the early, uncomplicated cases. He concluded that ascending infection was the method by which pyelonephritis usually occurs, especially in those conditions associated with neurogenic bladder disturbances. Heptinstall (1966) proposed that if the ascending route is to be accepted one must assume that some degree of infection of the lower urinary tract occurs first. He pointed out the frequency with which the normal urethral flora becomes involved in cases of cystitis, especially in patients in whom catheters have been used. The abnormal transport of urine from the bladder to the kidney is referred to as vesicoureteral reflux. Although incompletely understood Beeson (1955) and Heptinstall (1966) both state that reflux can and does occur and that the process probably is important in carrying bacteria to the renal pelvis. Vesicoureteral reflux does occur in the dog but its role in the etiology of ascending renal infections in this species has not been studied (Lenaghan and Cussen, 1968).

Heptinstall (1966) believes there are certain situations in which the blood stream is undoubtedly the main route of renal infection such as infections arising from the bacteremia associated with boils or carbuncles. However, he believes that renal localization by gram-negative bacteria is unlikely by the hematogenous route. This hypothesis was supported by studies with experimental animals in which intravenous inoculations of large numbers of E. coli did not result in

pyelonephritis in the normal unobstructed urinary tract (Brumfitt and Heptinstall, 1959).

Heptinstall (1965) also compared the pathological changes resulting from both experimental ascending and blood-borne bacterial infections. He concluded that the sequence of changes was almost identical and the only significant difference was in the distribution of the lesions. In the blood-borne infection the lesions were widespread, involving all parts of the parenchyma, whereas in the ascending infection lesions were largely confined to the perihilar region. The route of infection was no longer distinguishable if lesions had progressed to the chronic state. Several investigators have shown that the renal medulla is more susceptible to bacterial infection than the cortex. Paplanus (1964) demonstrated that intravenous administration of a nonpathogenic strain of E. coli to rats resulted in the selective retention of organisms by the renal medulla. Shortly after inoculation bacteria were distributed equally in the cortex and medulla. Within 24 hours the cortex became sterile but the organisms multiplied and persisted in the medulla until 4 days after inoculation. Paplanus suggested that the difference in susceptibility to infection by the cortex and medulla was related to differences in their efficiency in clearing of the organisms but gave no indication of the events by which clearing occurred. Freedman and Beeson (1958) compared the relative susceptibility of the cortex and medulla of rat kidneys by direct inoculation of bacteria into the two different zones. As few as 10 E. coli cells injected into the medulla resulted in a wedge-shaped area of inflammation which extended into the adjacent cortex, whereas 10,000 organisms were required to establish infection in the cortex.

A variety of reasons for the apparently increased susceptibility of the renal medulla to infection have been offered. Beeson and Rowley (1959) noted that homogenized kidney tissue, unlike homogenates of other organs, interfered with the ability of normal serum to destroy coliform bacteria. They showed that this effect was the result of inactivation of the fourth component of complement by ammonia. Andriole and Epstein (1965) showed that water diuresis could protect the kidneys of rats from hematogenous infection with Staphylococcus aureus and Candida albicans. They concluded that hypertonicity of the renal medulla is an important determinant in increased susceptibility to infection. An isotonic or near isotonic environemnt was shown to be necessary for prompt leukocyte mobilization (Andriole, 1966). Water diuresis reduces medullary hypertonicity. Chernew and Braude (1962) and Knoll et al (1969) found that hypertonic urine not only decreased leukocyte mobilization at inflammatory sites but also impaired the phagocytic ability of the inflammatory cells.

The effect of urinary tract obstruction on the susceptibility of the kidney to infection has been studied extensively. Obstruction of urine flow enhances susceptibility to pyelonephritis by making available a stationary supply of media for bacterial growth (Guze and Beeson, 1956; Freedman et al, 1960). Fried and Wong (1969) postulated that temporary obstruction, induced by intraductal crystallization, might predispose the kidneys of rats to hematogenous bacterial infections.

The influence of localized injury on the susceptibility of the kidney to infection was investigated by Rocha (1963) and Beeson et al (1965). They showed that medullary scars produced by thermocautery and accompanied by intrarenal hydronephrosis predisposed the kidney to

pyelonephritis.

Guze (1969) recently discussed bacterial variants and their role in the production of disease. Under the influence of antimicrobial drugs or of antibody and complement, bacterial cells may lose constituents of their cell walls and assume unusual shapes. These forms are called protoplasts or spheroplasts and are susceptible to lysis in a non-isotonic environment. In appropriate solutions these variants may survive and later revert to pathogenic forms. Conditions favoring protoplast formation and survival seem to exist in the medulla of the kidney and in urine. The capacity of such organisms to survive in the kidney and later to revert to pathogenic forms and cause kidney infection has been demonstrated in experimental animals by Alderman and Freedman (1963) and by Kalmanson and Guze (1964). Bacterial transformation may play a role in the pathogenesis of chronic pyelonephritis.

Lesions resulting from a concurrent viral infection represent another mechanism which might enhance susceptibility of the kidney to pyelonephritis. This mechanism will be discussed in the next section of the review.

Etiological Agents and Mechanisms of Infectious Nephritis - Viruses

The possibility that viruses can cause or complicate renal lesions has received relatively little attention. Jensen (1967) reviewed much of the literature relating to viral infections which provoked manifestations of renal disease or diseases in which viruses were isolated from urine.

Some viruses, such as measles, canine distemper, infectious canine hepatitis, mumps, and viruses of the Herpes group, are known to produce

pathological changes in the kidney during the clinical stages of the disease (Coffin and Liu, 1957; Utz et al, 1964; Wright et al, 1967; Menser et al, 1967). Agents such as the Coxsackie and ECHO viruses, and lymphocytic choriomeningitis virus have been incriminated as etiological agents of immunologically-induced glomerular lesions (Sun et al, 1967; Baker et al, 1967; Burch and Hotchin, 1968).

Persistent infection and prolonged viruria result from many of the adenovirus and cytomegalovirus infections (Jensen, 1967; Fetterman et al, 1968). Some of these persistent infections are accompanied by focal interstitial nephritis (Poppensiek and Baker, 1951; Ginder, 1964; Shadduck et al, 1967; Kelly, 1967). Ginder (1964) studied a murine adenovirus that produced persistent renal infection in mice. The infection was accompanied by extensive mononuclear cell infiltrates in the cortex and medulla and a moderate amount of tubular necrosis. The virus-induced lesion predisposed mice to pyelonephritis when E. coli was injected intravenously or was instilled in the posterior urethra.

Infectious Canine Hepatitis

Infectious canine hepatitis (ICH) is a common viral infection of dogs. Serological surveys have shown that ICH virus exists throughout the world and naturally-acquired antibodies are present in more than 50 percent of the dog populations surveyed (Cabasso, 1962).

The virus was first recognized in 1930 as the causative agent of epizootic fox encephalitis (Green et al, 1930). It was later shown that the virus was pathogenic for the dog; the experimentally produced canine disease was described in 1934 (Green et al, 1934). Rubarth (1947) in Sweden, has been credited with recognizing that the experimental

disease produced by Green existed as a distinct, naturally-occurring entity in dogs.

Kapsenberg (1959) reported that the virus possessed properties characteristic of the adenovirus group. Kinjo and Yanagawa (1969) compared 14 strains of ICH virus and concluded that they represented a single serotype. A serological relationship exists between ICH virus and other adenoviruses. Carmichael and Baker (1962) noted that dogs inoculated with adenovirus type 4 and later with ICH virus developed a secondary response of complement-fixing antibodies to the type 4 antigen and that the viremic stage of ICH persisted for only one day in dogs with antibody to adenovirus type 4 as compared to 4 to 6 days in dogs given only ICH virus. Antibodies to adenoviruses of human origin occur naturally in dogs but their frequency is apparently low (Carmichael and Barnes, 1962).

Salenstedt (1963) studied the pathogenesis of the infection with fluorescent antibody procedures. After oral administration of the virus, the antigen localized in the tonsils and shortly was detected in the deep cervical lymph nodes. He postulated that the virus was then carried to the internal areas of the body by the cervical lymph channels. Viremia was detected 3 to 5 days after positive fluorescence was observed in the tonsils. Wright et al (1971) have recently demonstrated that puppies experimentally exposed to aerosolized ICH virus develop a necrotizing bronchiolitis. The role of ICH virus in naturally-occurring canine respiratory disease has not been clearly established.

The clinical and pathologic aspects of the acute disease have been reviewed by Thordal-Christensen (1957), Henderson (1959), Cabasso

(1962) and Cornwell and Wright (1969). The disease varies from a mild or inapparent infection to an acute syndrome in which the animal dies within 12 to 24 hours after the onset of symptoms. Clinical signs in severe cases are first manifested by a temperature elevation (as high as 106° F) which might persist as long as 6 days. Increased pulse and respiratory rates usually accompany the onset of fever. Loss of appetite, increased thirst, diarrhea and vomiting often occur concurrently with the temperature elevation or shortly thereafter. Tonsilitis and enlargement of superficial lymph nodes are seen in some cases. Hyperemia of mucous membranes is usually present early; later the membranes become blanched, and may contain petechial hemorrhages. Other features of circulatory disturbance such as cutaneous petechial hemorrhages and subcutaneous edema of the head, neck and trunk are sometimes present. Late in the course of the acute disease some animals exhibit signs of abdominal pain.

Hematologic and urinary abnormalities have been described by Coffin and Cabasso (1953), and more recently by Hamilton et al (1966). A progressive and pronounced leukopenia with reduction in all classes of leukocytes accompanies the onset of fever. An intense leukocytosis that is predominantly lymphocytic follows the febrile stage and is most marked the third or fourth day after fever has abated. Approximately 2 weeks after the fever subsides a transient neutrophilia occurs concurrently with a slight temperature elevation. Urinary bilirubin and albumin levels are frequently increased but the specific gravity is not altered significantly.

Prominent gross pathological findings are subcutaneous edema and enlarged hyperemic or hemorrhagic lymph nodes. A serosanguinous fluid

is often present in the abdominal cavity, and the gastrointestinal serosa has a dull, stippled, ground-glass appearance. Small strands of fibrin are apparent between the lobes of the liver and over the mesenteries. Hemorrhages are observed in many tissues but are most often present in the mesenteries, serosa of the stomach and intestines, and in the subcutis. The liver is moderately swollen and friable. Its surface presents a mottled appearance and the lobular pattern is accentuated. The state of hepatic congestion seems to vary from case to case and affects the overall color of the liver. Edematous swelling of the wall of the gall bladder is observed frequently and is considered by many authors to be pathognomonic for the disease.

The most characteristic histologic feature is the large basophilic or acidophilic intranuclear inclusion bodies which are present in hepatocytes, Kupffer cells and vascular endothelial cells throughout the body. Periacinar necrosis of the liver is described by most authors and microscopic hemorrhages and edema may be found throughout the body.

An immunologically induced transient, unilateral or bilateral corneal opacity occurs in approximately 20 percent of convalescent animals (Carmichael, 1964). ICH virus is believed to persist in the anterior uvea during convalescence. Local antibody production occurs in the iris and limbal region and reacts with the viral antigen to initiate a focal hypersensitivity response of the arthus-type. If sufficient damage to limbal vessels and corneal endothelium occurs, serum and fluid from the aqueous humor diffuse into corneal stroma causing edema.

Histologic evidence of renal infection in ICH has been reported by several authors (Green and Shillinger, 1934; Bloom, 1954; Wright,

1967). In the acute disease inclusion bodies were regularly observed in the vascular endothelium of the glomerular and larger renal vessels. A chronic renal infection, in the presence of circulating antiviral antibody, has been demonstrated by tissue culture procedures and animal inoculation experiments. The persistent excretion of virus particles in the urine has also been considered to be a primary method for dissemination of the virus. Poppensiek and Baker (1951) showed that viruria could persist for at least 161 days. Persson et al (1961) utilized tissue cultures of dog kidney cells to demonstrate viruria. He was able to isolate the virus from the urine of each of 14 dogs on the twenty-fifth day after infection. He also demonstrated viruria in 6 of 9 dogs 103 days after infection. Hamilton et al (1966) suggested that the virus was excreted in the urine intermittently because they were able to isolate the virus from only 7 of 25 urine samples obtained between the seventh and eleventh day post-inoculation. They also postulated that the virus was present in small amounts because of the length of time necessary to produce cytopathogenic effect after the urine was inoculated into canine kidney cell cultures.

Experimental studies on convalescent animals have proven that a persistent renal infection with ICH virus results in a focal, interstitial nephritis (Poppensiek and Baker, 1951; Stunzi and Poppensiek, 1953; Hamilton et al, 1966; Kapp, 1966; Wright, 1967). The focal lesions were in the cortex and medulla and consisted primarily of aggregates of mononuclear cells. Some of the medullary lesions exhibited tubular necrosis and infiltration by polymorphonuclear leukocytes.

CHAPTER III

MATERIALS AND METHODS

Introduction

The experiment was conducted in two phases: The purpose of phase I was to determine the intrarenal localization and renal pathogenicity of intravenously administered bacteria in dogs. Standardized bacterial inoculums were given to a group of dogs which were then sacrificed at predetermined intervals. Bacterial assays were conducted on renal cortex, renal medulla, urine and heart blood from each animal and histologic studies were performed on the kidneys.

The purpose of phase II was to characterize the renal lesions produced in dogs by infectious canine hepatitis virus and to evaluate their influence on the renal localization of a superimposed hematogenous bacterial exposure. Dogs were infected with ICH virus and 10 to 14 days after recovery from the acute disease, one-half were injected intravenously with the bacterial inoculums. At predetermined intervals the subjects were sacrificed and subjected to necropsy. Renal cortex, renal medulla, heart blood and urine were cultured quantitatively and the kidneys were assayed for pathologic changes.

Experimental Animals

Healthy unvaccinated puppies 6 to 14 weeks of age were purchased

for utilization in this study. They were comprised of 60 pure-bred beagles obtained from an Oklahoma State University experimental colony and a local breeder, and 19 puppies of mixed ancestry from isolated rural areas.

Dogs were housed in air-conditioned kennel facilities in individual stainless steel cages. Contact with animals and personnel not involved in the experiment was avoided. Each animal was permanently identified with a tattoo in the pinna of the left ear.

The diet consisted of a balanced commercial dog food[1,2] offered twice daily in amounts calculated to provide approximately 70 calories per pound of body weight per day.

Prior to any experimental procedure the animals were allowed a minimum of 5 days to adjust to their new environment. Two or more fecal samples from each puppy were examined for evidence of internal parasitism. An antihelminthic (Task[3] or BU-Chlorin[4]) was administered when indicated. During the adjustment period serum samples were collected and frozen for subsequent antibody titrations.

Body temperature, appetite, excretory habits and pertinent clinical observations were recorded daily throughout the experimental period. Blood for routine hemograms, blood urea nitrogen, blood creatinine and plasma protein was obtained from the left jugular vein 2 or 3 times weekly. Sodium ethylenediamine tetraacetic acid (EDTA) was used as the anticoagulant.

A major factor in the choice of animals utilized in the experiment was lack of exposure to the common infectious diseases. In order to avoid influences other than those produced by the experimental procedure, the dogs were not vaccinated for any of the canine diseases.

Bacteriologic Procedures

Two species of bacteria were utilized for inoculums. One was a strain of Escherichia coli (serotype 025: k+:H21)[5] isolated from a naturally occurring case of canine cystitis diagnosed at the Small Animal Clinic, College of Veterinary Medicine, Oklahoma State University. The other organism was isolated from the urine of a spontaneous case of cystitis which developed in one of the experimental animals (M-26) and was identified by cultural characteristics and biochemical reactions as a species of Proteus.

After isolation and identification, stock cultures of each organism were preserved for subsequent use in the following manner: Both isolates were heavily inoculated onto a nutrient agar medium, which contained 5% citrated bovine blood,[6] and incubated for 18 hours at 37°C. The bacterial cells were harvested from the plates by washing the growth from the surface of the agar with sterile horse serum. The suspensions of bacteria in horse serum were distributed in one milliliter (ml) ampules and lyophilized. The ampules were labeled and maintained at 72°F.

Bacterial inoculums were prepared from lyophilized cultures which had been resuspended and incubated in brain heart infusion (BHI)[7] broth for 12 hours at 37°C. The purity of the propagated cultures was confirmed through the use of bovine blood agar plates and biochemical media. The bacteria in the outgrowth broth culture were then further propagated in BHI broth for 12 to 18 hours at 37°C. Cultures were then centrifuged for 10 minutes at 2,000 revolutions per minute and the supernatant drawn off. Organisms remaining in the tubes were resuspended in 10 ml of sterile Zobell's solution and washed by continuous

agitation for 5 minutes. The tubes were recentrifuged and the supernatant removed. The procedure was repeated for a total of three washings. Suspensions were quantitated spectrophotometrically[8] by relating their optical density to the number of organisms per ml, as determined by the standard pour plate technique. The inoculum of E. coli used in the experiment contained 3.7×10^8 cells per ml and possessed an optical density of 0.35 at a wave length of 545 mμ. The inoculum of Proteus species used in the experiment contained 1.8×10^8 cells per ml and possessed an optical density of 0.55 at a wave length of 545 mμ. All of the above procedures were performed using aseptic technique.

The ability of each bacterial species to localize, proliferate and produce disease in the kidney, when administered by an intravenous route, was evaluated in experimental animals with a surgically induced urinary tract obstruction. The obstruction was effected in 5 dogs and 15 rats by unilateral ureteral ligation, which was done through a midline abdominal incision. Intravenous inoculation of the bacterial suspension was done 24 hours after the ureteral ligation. The animals were sacrificed from one to 26 days after inoculation. The bacteria were considered to be pathogenic if a suppurative pyelonephritis, from which the organisms could be re-isolated, was seen at the time of necropsy.

Virologic Procedures

The Cornell-1 strain of infectious canine hepatitis virus was obtained from a lyophilized suspension. It had been propagated in a dog kidney cell culture and contained approximately 10^5 tissue culture

infective doses (TCID) per 0.1 ml before lyophilization. The lyophilized virus suspension was reconstituted with 20 ml of sterile distilled water and immediately divided into 20 one ml ampules. The ampules were stored at -18°C.

Fifty-four dogs were inoculated subcutaneously with 1 ml injections of a 1×10^{-4} or 1×10^{-5} dilution of the stock virus in BHI broth. Urine samples, collected during convalescence and at the time the animals were killed, were frozen for subsequent virus assay.[9] The assay was done by inoculating 3 tubes of a dog kidney cell culture with 0.2 ml of urine. The inoculated cultures were examined microscopically 4 and 12 days postinoculation. Cytopathic effect in any of the 3 tubes which had been inoculated with urine was considered to be proof that the ICH virus was present.

Serology

Virus neutralization tests were performed on preinoculation serum samples and samples collected at the time of sacrifice.[9] The serum samples were diluted 1:10 and 1:100 before mixing with ICH virus. The serum-virus suspension was then inoculated into 3 tubes of dog kidney cell cultures and evaluated for cytopathic effect as described above. Microscopic slide agglutination test for leptospirosis was conducted on preinoculation serums and serums obtained at the time of terminal surgery.[10]

General Experimental Procedure

Phase I: Renal Distribution and Pathogenicity of Intravenously Inoculated Bacteria.

Seventeen puppies were used in these studies. Clinical and hematological data were monitored on all animals until terminal surgery.

Group I consisted of 9 animals, 8 of which were inoculated intravenously with the washed suspension of E. coli. One puppy was used as an uninoculated control. Six puppies received 4 x 10^8 organisms per pound of body weight. At intervals of 1, 4, 16, 24, 48, 96 and 192 hours and 30 days following the bacterial inoculation, the kidneys were removed for microbiologic and histologic evaluation.

Seven puppies comprising group II were inoculated intravenously with a washed suspension of a Proteus species. An eighth served as an uninoculated control. Ninety million organisms per pound of body weight were administered to 5 puppies and 2 received 12 x 10^8 organisms per pound of body weight. The intervals between inoculation and terminal surgery were 24, 48, 120, 264 and 312 hours and 30 days.

All animals were anesthetized with sodium pentobarbital and the abdominal area prepared for surgery. Access to the kidneys and urinary bladder was achieved through a midline abdominal incision. After clamping the renal vessels with hemostatic forceps, each kidney was removed and placed in a sterile Petri dish. Five ml of urine were aspirated into a syringe which contained sterile EDTA. Each sample was transferred to a sterile test tube for subsequent study.

A radial segment of one kidney approximately 1 cm thick was removed for histologic and fluorescent antibody procedures. The

remaining cortex and medulla of each kidney were then separated by sharp dissection at the corticomedullary junction and each placed into a sterile Petri dish.

The weight of the medulla and cortex was then determined and recorded. Each anatomical segment was homogenized with 100 ml of sterile water in a Waring blender for 3 minutes. A 5 ml aliquot of each crude homogenate was transferred to a Ten Broeck tissue grinder and further homogenized. Tenfold serial dilutions of the kidney homogenates and of urine and heart blood were cultured individually in desoxycholate and BHI agar pour plates. The pour plates were incubated aerobically for 48 hours at 37°C. The number of colonies per plate was quantitated on a Quebec colony counter and colony counts between 30 and 300 per plate were used to calculate the number of organisms per gram of renal tissue and per ml of urine and heart blood.

After collection of specimens for culture the animals were euthanatized with sodium pentobarbital and subjected to necropsy. Representative samples of all major parenchymatous organs were fixed in 10 percent formalin neutralized with sodium acetate.

Phase II: Renal Lesions of Infectious Canine Hepatitis and Localization of Superimposed Bacteria.

Fifty-four susceptible puppies were inoculated subcutaneously with ICH virus. The inoculum consisted of 1 ml of a 1×10^{-4} or 1×10^{-5} dilution of the stock virus suspension which contained 10^5 TCID per .1 ml. Postinoculation urine and serum samples were collected randomly and frozen for subsequent virus assay and virus-neutralization tests.[11] Clinical observations and periodic hematological data were recorded daily for each animal.

Of the 44 animals that survived the acute infection, 21 were given intravenous injections of bacteria 3 to 4 weeks after the administration of the virus. Eight puppies were given a single inoculation of 1.1×10^8 washed E. coli cells per pound of body weight and 11 received 1.2×10^8 washed Proteus species cells per pound of body weight. Two puppies were given multiple injections of washed E. coli cells at weekly intervals. M-22 received 1.1×10^8 cells, 1.1×10^7 cells and 2.2×10^7 cells per pound of body weight and M-32 received 1.1×10^8 cells and 3 injections of 2.2×10^7 cells per pound of body weight. Twenty-three animals surviving acute ICH did not receive bacteria.

At intervals of 4, 8, 12, 21, 30, 90, 144 and 160 days after the initial bacterial injections the animals were subjected to terminal surgery. Puppies not receiving bacteria were sacrificed at corresponding intervals. Blocks of kidney were removed for histologic and fluorescent antibody studies and samples of urine, heart blood and kidney were cultured quantitatively as described in phase I.

Necropsy was performed after collection of the surgical specimens and sections of major organs were fixed for histologic study.

Fluorescent-Antibody Procedures

Immediately after surgical removal of the kidneys, a specimen of one was frozen on a block of dry ice which had been covered with a piece of aluminum foil. Frozen sections were cut at 6 microns, mounted on cover slips, fixed in acetone for 10 minutes at room temperature and stored at -18° C for future use.

Antiserum to ICH virus for use in the fluorescent antibody technique, was obtained from one of the experimental puppies (M-13) 28 days

after inoculation with the virus. E. coli antiserum was produced in mature chickens by 6 consecutive weekly intravenous injections of 4×10^8 washed cells contained in 1 ml of Zobell's solution.

The immune sera were conjugated with fluorescein isothiocyanate[12] as follows: Three ml of undiluted serum at 4° C was adjusted to pH 8.8 by adding 0.5 N sodium bicarbonate buffer (15 percent of the serum volume). Fluorescein isothiocyanate crystals (1.5 mg/ml serum) were floated on the liquid surface and allowed to stand for approximately 10 minutes. The mixture was then stirred with a magnetic stirrer for 16 to 20 hours at 4° C. The conjugated serum was placed on a column of diethylaminoethyl cellulose (DEAE-cellulose). The cellulose and column were prepared as follows: The DEAE-cellulose was washed twice with 0.5 N NaOH; then with distilled water until the pH of the supernatant was 7.0-7.2. The material was then washed with a .025 N phosphate buffer, pH 7.2, until the supernatant was that of the buffer. All washings were done for 15 to 30 minutes with 10 times the original volume of DEAE-cellulose. After the DEAE-cellulose had equilibrated with the phosphate buffer at pH 7.2, the coarse particles were allowed to settle out and were packed into glass columns with a 12 mm inside diameter. The height of the cellulose was 3.5 for each ml of the sample fractionated. The antibody was eluted with 0.025 M phosphate buffer, pH 7.2. Collection of the conjugate began as soon as a yellow color appeared in the eluate and ended as soon as the color disappeared from the eluate. The conjugate was stored at 4° C.

An equal volume of a 20 percent homogenate of normal canine kidney in .01 M phosphate buffered saline was mixed with the conjugate to

remove some of the nonspecific staining components. Frozen sections were then flooded with the mixture and placed in a moist chamber at 37° C for 30 minutes. The stained sections were washed twice in 0.01 M phosphate buffered saline, pH 7.2, for 5 and 20 minutes, consecutively, rinsed once in distilled water and mounted on slides. The mounting medium was 0.01 M phosphate-buffered saline, pH 7.2, with 10 percent glycerol.

The stained sections were examined on a Zeiss microscope equipped with an Osram HBO 200-W maximal-pressure mercury-vapor arc. The excitation filter used was a UG 2 or 5 and the barrier filter system was the combination 0/41.

The effectiveness of the antibacterial conjugates was demonstrated by staining coverslip imprints of the corresponding bacterial colonies. The specificity of the ICH conjugate was demonstrated by the diminished fluorescence observed when tissues were treated with unconjugated anti-ICH serum prior to the application of conjugated antiserum. Frozen kidney sections from uninoculated puppies without demonstrable circulating ICH antibodies served as negative controls.

Histologic Procedures

All specimens collected for histological study were fixed in 10 percent neutralized formalin for at least 48 hours. They were dehydrated in alcohol, embedded in paraffin, cut at 6 microns and stained with hematoxylin and eosin. Selected kidney sections were also stained with Van Gieson's and Gomori's trichrome methods for the demonstration of connective tissue and the periodic acid-Schiff stain for the demonstration of basement membranes.

FOOTNOTES

1. Mark Morris Associates, Inc., Hill Packing Company, Topeka, Kansas.

2. Ibid.

3. Task (Dichlorvous), Shell Chemical Co., Division of Shell Oil Company, Agricultural Chemical Division, New York, New York, 10020.

4. BU-Chlorin (n-butyl chloride), Allied Laboratories, Inc., Indianapolis, Indiana.

5. Dr. W. B. Ewing, Ph.D., Chief, Enteric Bact., Department of Health, Education and Welfare, Public Health Service, Communicable Disease Center, Laboratory Branch, Atlanta, Georgia, 30333.
Dr. Paul J. Glantz, Ph.D., Pennsylvania State University, Veterinary Science Building, University Park, Pennsylvania, 16802.
Dr. Frank Siccardi, D.V.M., College of Veterinary Medicine, Department of Veterinary Bacteriology and Public Health, St. Paul, Minnesota, 55101.

6. Difco Laboratories, Inc., Detroit 1, Michigan.

7. Ibid.

8. Spectronic 20, Bausch and Lomb, Rochester, New York.

9. Dr. L. E. Carmichael, New York State Veterinary College, Cornell University, Ithaca, New York.

10. Dr. Charles Roberts, Alabama State Diagnostic Laboratory, Auburn, Alabama.

11. Dr. L. E. Carmichael, New York State Veterinary College, Cornell University, Ithaca, New York.

12. Baltimore Biological, Division of B-D Laboratories, Inc., Baltimore, Maryland.

13. Bio-Rad Laboratories, 32nd and Griffen, Richmond, California.

CHAPTER IV

RESULTS

Phase I

Shortly after receiving the bacterial inoculums the experimental subjects developed an acute shock syndrome characterized by elevation of body temperature, increased pulse and respiratory rate, pale mucous membranes, vomition, excessive salivation and watery, mucoid stools that occasionally contained blood. Body temperature fluctuations are listed in Table I. The dogs were depressed and anorectic for 2 to 3 days, and some developed lameness or stiffness in one or more limbs and the neck the day after inoculation. The stiffness or lameness gradually disappeared within 2 to 4 days. Hematologic data obtained subsequent to the bacterial injections were characterized by a marked but transient neutrophilic leukocytosis for 3 to 4 days. Total leukocyte and neutrophil counts during this period are presented in Tables II and III. All clinical and hematologic parameters monitored were normal within 5 days postinoculation.

Quantitative bacteriological counts obtained from cultural examinations of renal cortex, the renal medulla, urine and heart blood are presented in Tables IV and V. Bacterial counts in the renal cortex, the renal medulla and in heart blood were at their highest level one hour after administration of the organisms. The number of organisms

isolated from the kidney homogenates diminished gradually until 192 hours after inoculation at which time all samples were sterile. Bacteria were no longer demonstrable in the peripheral circulation after 48 hours. All urine specimens, with one exception (M-5), were sterile.

Gross pathologic alterations were not observed in the kidneys of any of these animals. Histologic changes in the kidneys attributable to the experimental procedure were limited to 5 dogs, (M-2, M-4, M-6, M-65 and M-66, and are presented in Tables VI and VII. Each of these was sacrificed within 24 hours after injection of the bacteria. The glomerular capillary lumens contained an increased number of neutrophils and an occasional neutrophil could be found in the cortical interstitium. The epithelial cells of the proximal convoluted tubules were swollen and often had pyknotic nuclei. Extrarenal alterations were observed in the same 4 animals. The sinusoids of the liver and adrenal glands were engorged with erythrocytes and neutrophils. Some hepatocytes exhibited cytoplasmic swelling and nuclear fragmentation. Large homogenous eosinophilic inclusion bodies were present in the cytoplasm of many of the degenerating hepatocytes. The vessels of the lungs were congested and the alveolar lumens frequently contained a faintly eosinophilic proteinaceous material. Distinct focal granulomas composed of lymphoreticular cells and eosinophils were observed in the outer renal cortex of 3 of the dogs. A degenerating parasite was located in the center of one of the granulomatous lesions.

Results of direct fluorescent antibody procedures performed on the frozen kidney sections from the animals in Phase I are presented in Table XVIII. Positive fluorescence was not observed in any of the

frozen sections when conjugated serum samples containing antibodies against E. coli or Proteus species were utilized.

Phase II

Clinical signs and hematological changes characteristic of acute infectious canine hepatitis developed after an incubation period of 4 to 6 days in most of the 54 dogs inoculated with ICH virus. The severity of the acute episode varied considerably. Eleven of the puppies (M-10, M-11, M-17, M-23, M-35, M-37, M-38, M-39, M-46, M-77 and M-82) succumbed to the acute infection 9 to 10 days after administration of the virus. Three of the puppies (M-73, M-74 and M-75) did not develop symptoms of the disease, and 7 (M-19, M-22, M-26, M-33, M-76, M-79 and M-80) exhibited minimal signs of infection manifested only by transient body temperature elevations. The clinical illness in the remaining 43 animals usually persisted for 4 to 6 days. Signs regularly observed consisted of fever (103.0° F. to 106.0° F.), Table VIII, anorexia, increased thirst and depression. Other signs manifested less consistently were vomition, excessive lacrimation, serous nasal discharge, increased pulse and respiration rates, stiffness of gait, arched back and a tense, tucked-up abdomen that was sensitive to palpation. The mucous membranes were frequently reddened early in the course of the disease but became blanched shortly before death. Icterus was observed in only one of the animals (M-20) and it developed on the sixth day of illness. Two of the dogs that survived (M-20 and M-24) developed marked subcutaneous edema of the head, neck and trunk after exhibiting a fever for 4 or 5 days. Prior to death some of the more severely affected animals were reluctant to recline on their side;

rather they preferred to rest on their sternum with limbs extended. Neurologic signs were not observed in any of the experimental subjects. Signs of the acute infection had disappeared by the twelfth day after inoculation of the virus. Unilateral or bilateral corneal opacity developed in 6 of the dogs (M-20, M-28, M-40, M-45, M-51 and M-52) shortly after the fever had subsided. The opacity persisted for 2 to 5 days and resolved without treatment.

Hematologic alterations during the acute phase of the disease are listed in Table IX. They were characterized by a marked reduction in circulating leukocytes and a slight decrease in hemoglobin concentration and packed cell volume. During the first and second days of fever the leukopenia was predominantly a lymphopenia but on the third and succeeding days it was predominantly a neutropenia. Eosinophils were not observed in peripheral blood smears during the febrile period. As the fever subsided a marked absolute lymphocytosis developed and persisted for 4 to 5 days.

Within 30 days after the acute signs had subsided 14 of the puppies developed an unexpected illness that lasted from 2 to 5 days. The episodes were characterized by elevations of body temperature ranging from 103° F. to 106° F., anorexia and mild depression. There was marked neutrophilic leukocytosis with a shift to the left. Blood cultures performed on samples obtained from 4 of the animals at the height of the fever were negative.

Twelve of the experimental animals (M-9, M-45, M-46, M-47, M-48, M-71, M-72, M-76, M-78, M-79, M-80 and M-81) developed canine distemper. Most of the dogs were sacrificed shortly after the tentative diagnosis of distemper was made. The most prominent findings consisted

of mild fever, conjunctivitis, anorexia, depression and lymphopenia. Histologic examination of the kidneys of these animals often revealed eosinophilic intranuclear or intracytoplasmic inclusion bodies in renal tubular epithelial cells and transitional epithelial cells of the pelvis. Other organs also contained histologic lesions supporting the diagnosis of distemper.

Necropsy performed on the animals that died during acute ICH revealed gross and histologic lesions characteristic of that disease. Prominent macroscopic alterations regularly observed consisted of a blanched oral mucosa, subcutaneous edema, edema of the wall of the gall bladder and a swollen, friable, mottled liver. Cut surfaces of the liver presented a speckled appearance that accentuated its lobular architecture. Perirenal edema, enlarged lymph nodes, edema and petechiation of the thymus and petechiation of the gastrointestinal serosa were commonly observed. Thin fibrinous strands were dispersed throughout the peritoneum, particularly over and between the lobes of the liver. Petechiae were sometimes present in the intestinal mucosa and the intestinal content was often dark and tarry.

Histologic alterations consisted of centrilobular liver necrosis and large basophilic intranuclear inclusion bodies in the hepatocytes and in vascular endothelial cells throughout the body. Inclusion bodies were frequently present in the endothelial cells of the glomerular capillaries, the vessels in the submucosa of the renal pelvis and the major vessels at the corticomedullary junction. Hemorrhage and edema were often present in the pelvic submucosa.

The dogs that received intravenous injections of bacteria during the early convalescent period developed the acute shock syndrome as

described for the animals in Phase I. Three (M-29, M-45, and M-50) died within 4 to 8 hours after receiving the bacteria. The results of bacteriological examination of the dogs in Phase II and a tabulation of renal lesions are presented in Tables X, XI, XII, XIII, XIV and XV. Bacteria were not isolated from the blood or from homogenized samples of renal cortex or medulla from any of the animals in Phase II. Eighty-six E. coli organisms per ml were isolated from the urine of M-32 and organisms of Paracolobactrum species and Proteus species were isolated from the urine of M-20 and M-26 respectively. Morphologic evidence of a mild chronic cystitis consisting of submucosal mononuclear cell aggregates was observed in each of these animals.

All dogs that survived the acute episode of infectious canine hepatitis developed a pyelitis and focal interstitial nephritis. Subsequent bacterial inoculations given intravenously to approximately 50 percent of the animals did not alter the nature or magnitude of the virus-induced renal or pelvic lesions.

The renal lesions attributed to ICH virus were grossly evident in approximately 50 percent of the dogs. The kidneys of 6 dogs (M-13, M-29, M-74, M-48, M-50 and M-78) contained randomly distributed subcapsular, gray, circular foci (Figure 1) that ranged from 4 to 8 mm in diameter which on cut surface extended through the cortex and medulla as thin, radially oriented, gray to white bands. The gross lesions in other animals had the same general appearance and distribution but were considerably smaller and less evident. The subcapsular lesions in some of the animals that had a longer convalescent interval before sacrifice were depressed below the cortical surface as small, shallow pits (Figure 2). Although the gross lesions were distrubuted at random

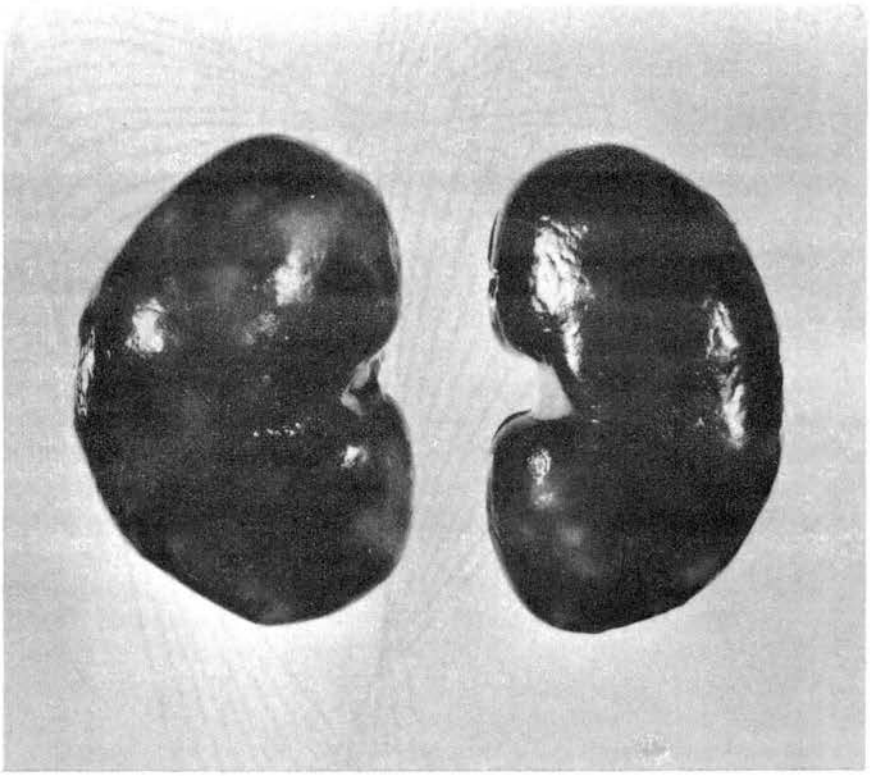

Figure 1. Kidney Illustrating Multifocal, Pale, Subcapsular Foci (M-78, 45 days post-viral inoculation).

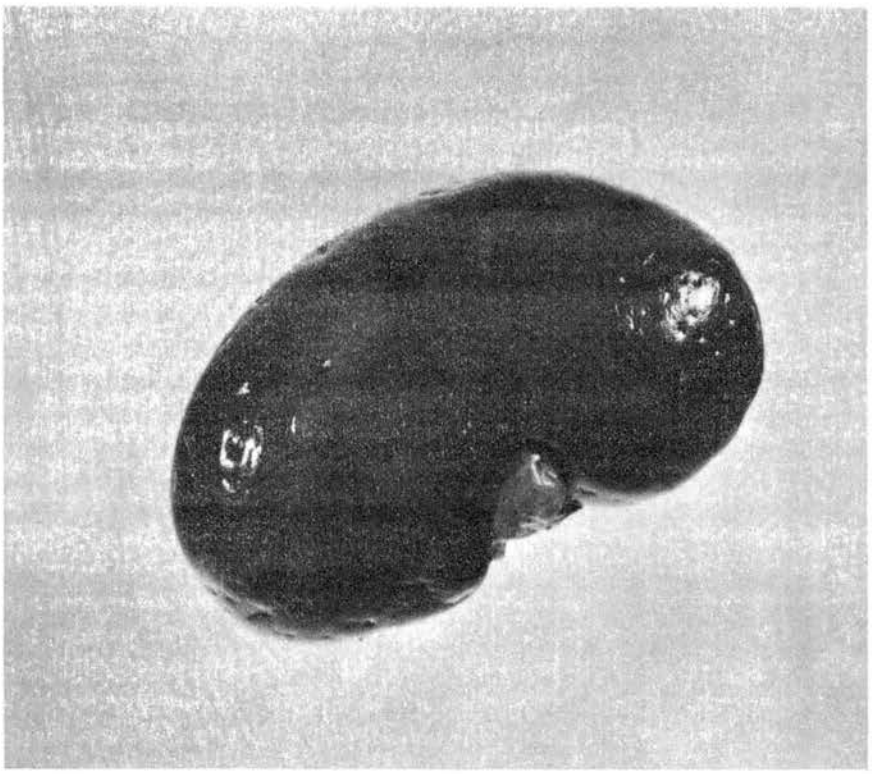

Figure 2. Kidney Illustrating Small, Multifocal Scars (M-52, 117 days post-viral inoculation).

over the capsular surface they were usually more numerous at the poles of each kidney.

Histologic lesions of diverse nature were present within the kidney proper. In general they were characterized by multiple, focal inflammatory lesions distributed randomly in the cortex and medulla. The extent of inflammation in terms of the number and size of individual lesions varied considerably. They were not as numerous in the cortex as in the medulla but cortical lesions were frequently larger and, in some instances, appeared to coalesce into a diffuse inflammatory reaction. As the interval between virus administration and sacrifice was prolonged, the number of inflammatory cells in the individual foci diminished and collagen deposition became more apparent.

Perivascular aggregates of mononuclear cells were observed in the cortex around arcuate, inter- and intralobular arteries and afferent arterioles of 35 of the 40 convalescent animals (Figure 3 and Figure 4). A periglomerular infiltrate of mononuclear cells was present in 32 of the dogs and occasionally the inflammatory cells distorted the glomerular architecture. Lymphoreticular cells were interspersed between branches of some of the vasa recta bundles in the medulla of all animals studied (Figure 5). The inflammatory process frequently extended from the inner cortex to the inner medulla where the bundle branches diverge. Occasionally an individual vessel was found with a distended lumen that contained intact or degenerating erythrocytes and brown, granular pigment.

Sections of kidney from all but 2 of the 50 dogs studied exhibited mild to severe, focal, peritubular, mononuclear cell aggregates in the cortex. A comparable lesion was present in both zones of the medulla

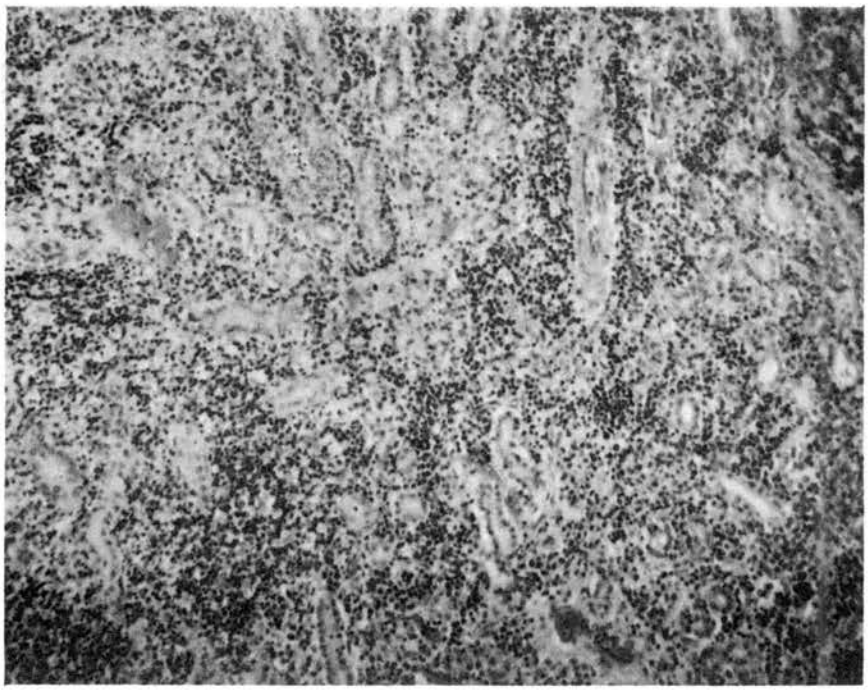

Figure 3. Extensive Mononuclear Cell Infiltrates in the Renal Cortex (M-29, 26 days post-viral inoculation).

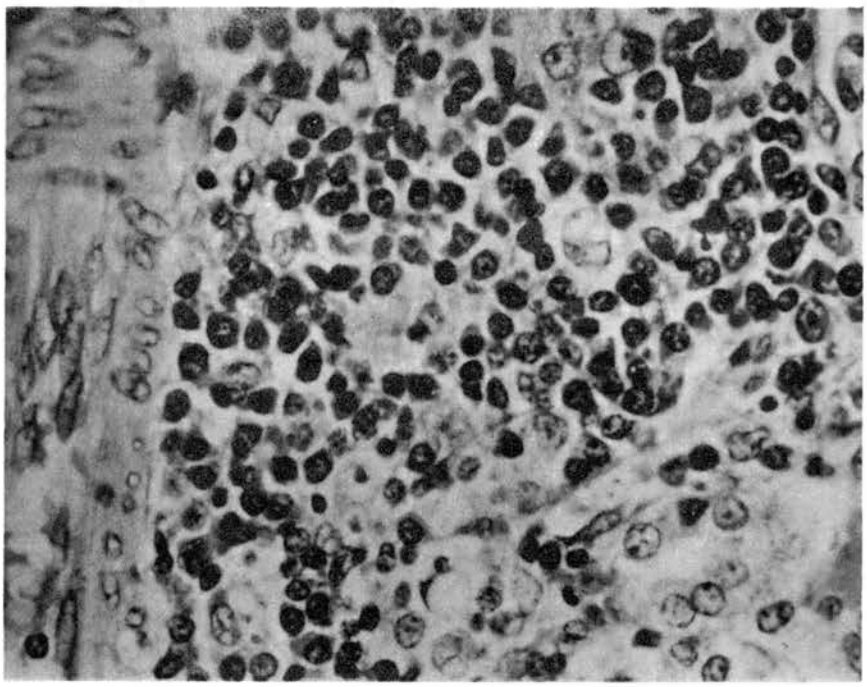

Figure 4. Renal Cortex Showing Mixed Mononuclear Cell Infiltrate and Many Plasmacytoid Cells. (M-29, 26 days post-viral inoculation; high magnification).

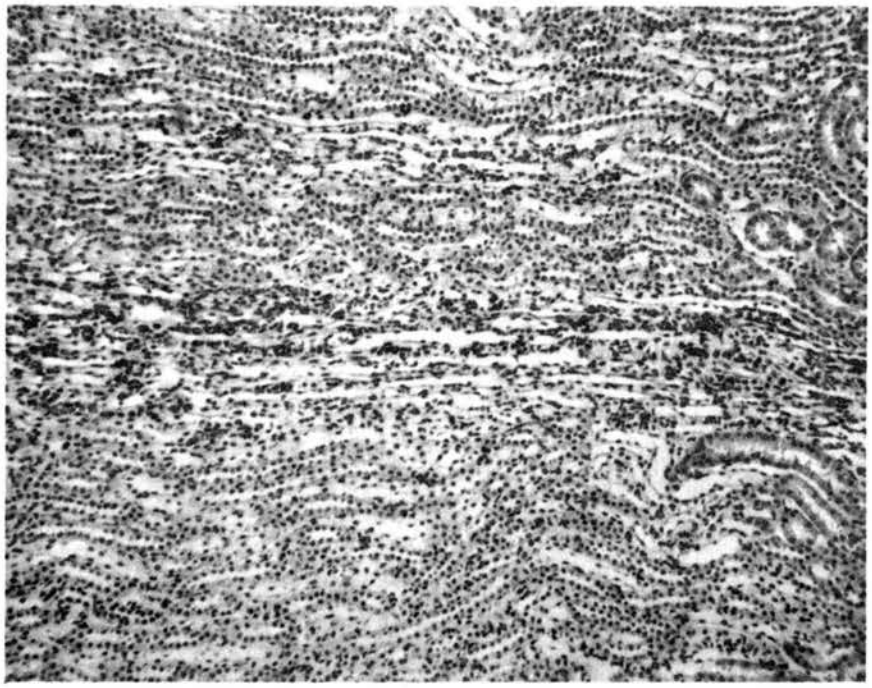

Figure 5. Lymphoreticular Cell Proliferation within Vasa Recta Bundle (M-59, 118 days post-viral inoculation; low magnification).

of all animals sacrificed (Figure 6 and Figure 7). Some degree of tubular epithelial cell damage usually accompanied the inflammatory foci. The epithelial changes varied considerably and included hypertrophy, increased cytoplasmic basophilia with flattening of epithelial cells, increased mitotic activity and necrosis. Tubular lumens above the more severe lesions were sometimes dilated. Basophilic intranuclear inclusion bodies were present in epithelial cells of collecting tubules or papillary ducts in 23 of the 40 convalescent cases (Figure 8). One of the 23 (M-59), was sacrificed 118 days after administration of the virus. Sometimes inclusion bodies were found in epithelial cells that were otherwise unaltered and were not accompanied by a peritubular inflammatory process. More frequently however, the cells were enlarged and sometimes desquamated. In these instances a lymphoreticular infiltrate was usually present around the affected tubule.

Fibroplasia in the medullary inflammatory foci occurred in 19 of 40 of the experimental subjects (Figure 9 and Figure 10) and was first observed in a dog (M-56) sacrificed 35 days after administration of the virus. Collagen deposition was observed in the cortex of 14 of the dogs (Figure 11). Secondary glomerular atrophy and tubular dilatation invariably accompanied the fibrosis. In specimens that had been sectioned in a plane parallel to the longitudinal axis of the tubules, some of the lesions occurred as a continuous inflammatory and fibroplastic process that extended from the outer cortex through the corticomedullary junction and coalesced with similar lesions in the outer or inner medulla. Collagen deposition in the cortex was always accompanied by collagen deposition in the outer or inner medulla. In many instances

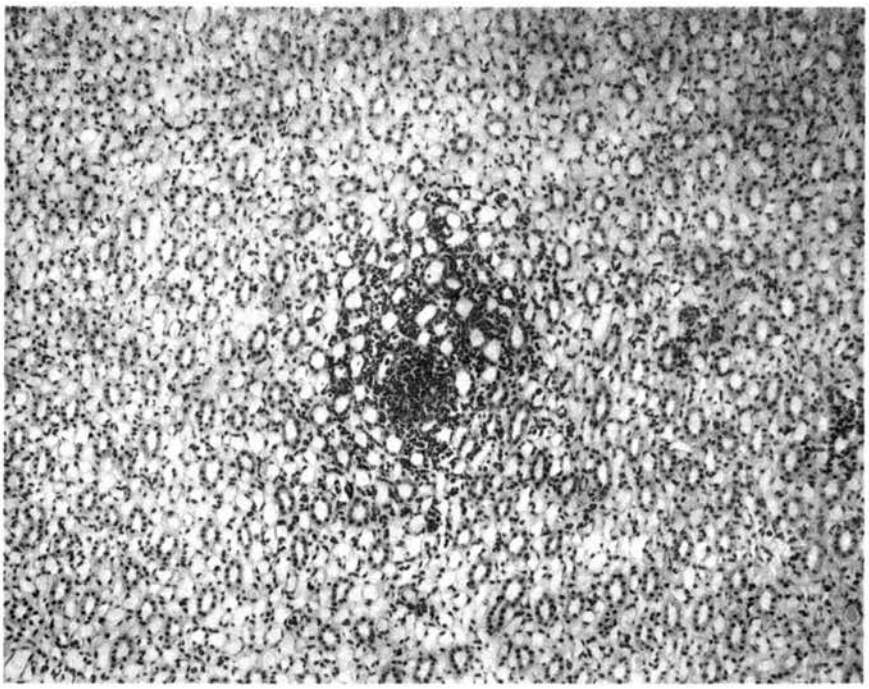

Figure 6. Focal Peritubular Aggregate of Mononuclear Cells in the Medulla (M-8, 34 days post-viral inoculation; low magnification).

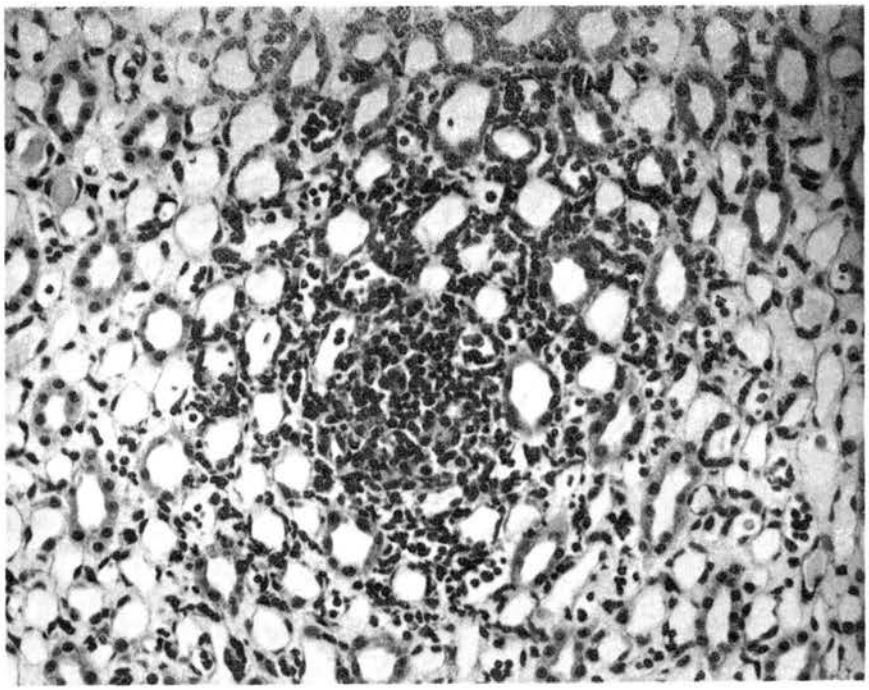

Figure 7. Focal Peritubular Aggregate of Mononuclear Cells in the Medulla (M-8, 34 days post-viral inoculation; high magnification).

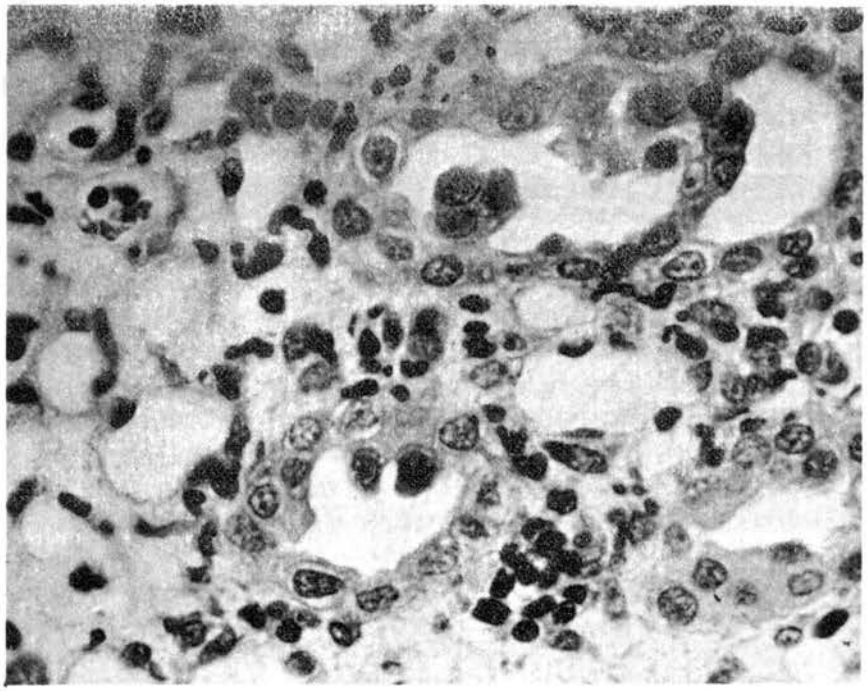

Figure 8. Intranuclear Inclusion Bodies Typical of ICH (M-18, 51 days post-viral inoculation; high magnification).

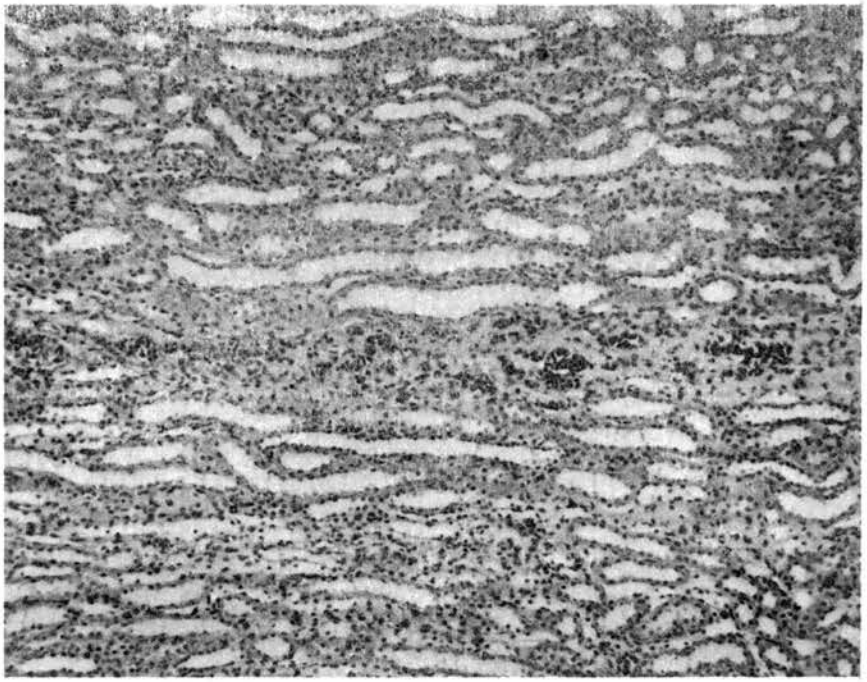

Figure 9. Early Fibrosis and Lymphoreticular Cell Proliferation in the Medulla (M-17, 112 days post-viral inoculation; low magnification).

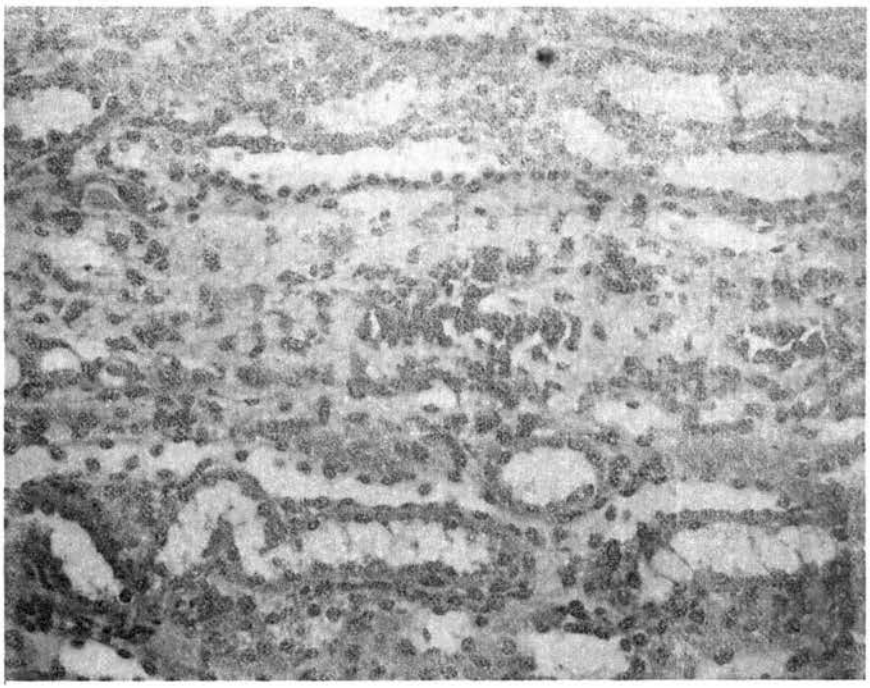

Figure 10. Early Fibrosis and Lymphoreticular Cell Proliferation in the Medulla (M-17, 112 days post-viral inoculation; high magnification).

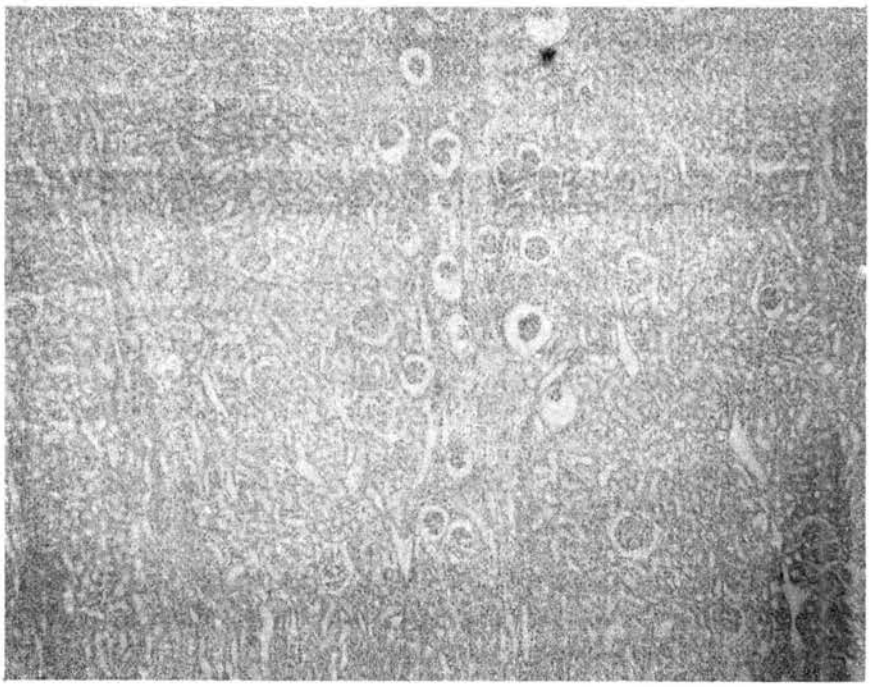

Figure 11. Radially Oriented Segment of Cortical Fibrosis, with Glomerular and Tubular Atrophy (M-17, 112 days post-viral inoculation; low magnification.

both collagen deposition and interstitial lymphoreticular cell proliferation appeared to be more extensive where the course of the nephrons forms a sharp angle to enter the inner medulla. Connective tissue proliferation was present in the inner medulla of 12 of the cases but it was never as extensive as in the outer medulla or cortex.

The inner medullary zone of the kidneys of 16 of the 40 dogs had diffuse areas of inflammation that were characterized by the presence of neutrophils usually located in capillaries but sometimes in the interstitial tissue (Figure 12 and Figure 13). The size of the inflammatory lesions varied from a few clusters of neutrophils to large, irregularly shaped areas that involved 1/3 of the medulla.

Twelve of the 40 animals that survived the acute infection had small focal deposits of necrotic cellular debris in the interstitial tissue of the papilla (Figure 14). These lesions were frequently associated with dilated capillaries whose lumens contained masses of sequestered degenerating leukocytes, erythrocytes and intravascular, brown, granular pigment. The cellular remnants in the capillary lumens were sometimes mineralized.

A mild to severe pyelitis was present in each of the 40 convalescent animals. It was characterized by trans-epithelial migration of lymphoreticular cells, hypertrophy and necrosis of individual epithelial cells and submucosal aggregates of lymphoreticular cells (Figure 15). In 11 of the dogs the lymphoreticular aggregates assumed the form of lymphoid follicles located adjacent to the pelvic epithelium. In 10 of the dogs the sub-pelvic lesion extended around the apex of the pelvic lumen and into the outer medulla. In these cases the inflammatory process continued as a linear zone adjacent to the

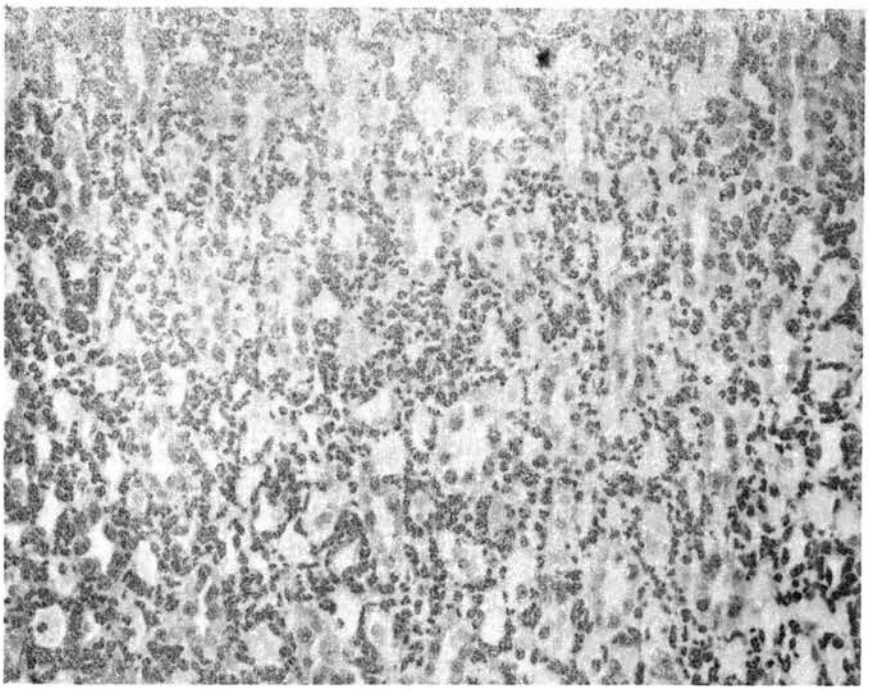

Figure 12. Interstitial and Intracapillary Aggregates of Neutrophils in the Medulla (M-13, 31 days post-viral inoculation; low magnification).

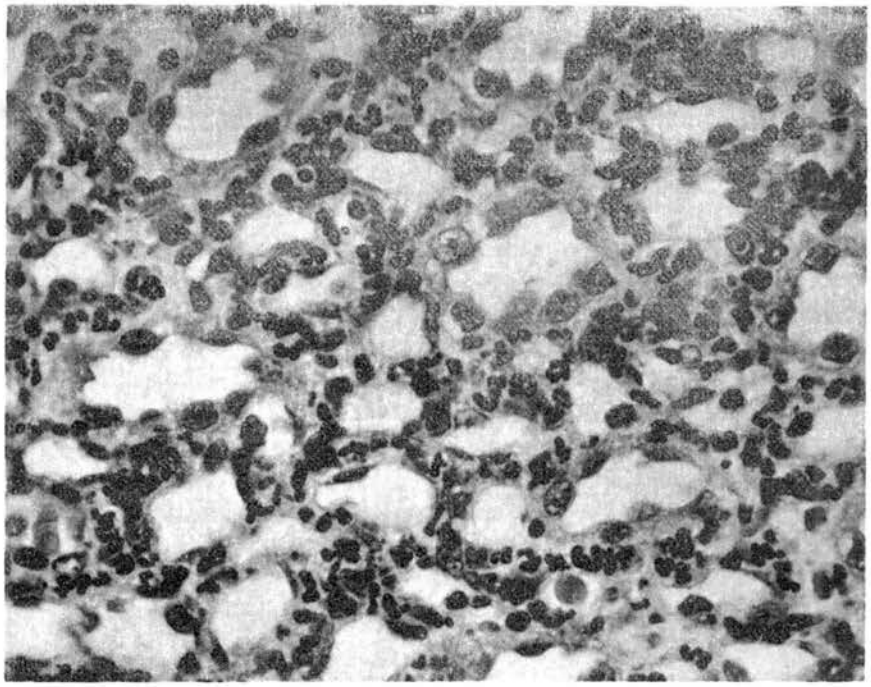

Figure 13. Interstitial and Intracapillary Aggregates of Neutrophils in the Medulla (M-13, 31 days post-viral inoculation; high magnification).

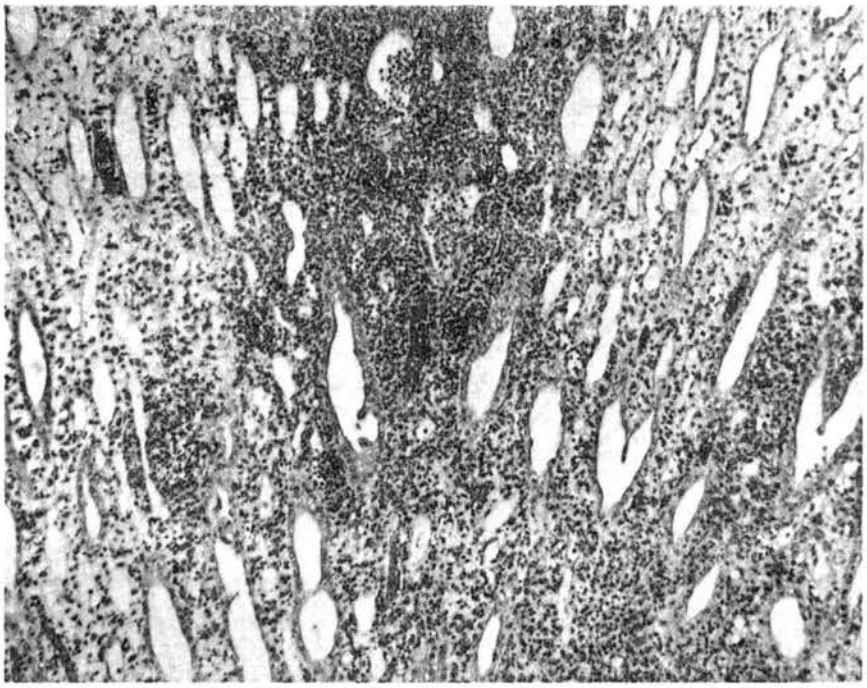

Figure 14. Focal Papillitis. Extensive Cellular Infiltration of the Papillary Interstitial Tissue, Engorgement of Vasa Recta and Degenerating Cellular Debris in Tubular Lumens (M-51, 138 days post-viral inoculation; low magnification).

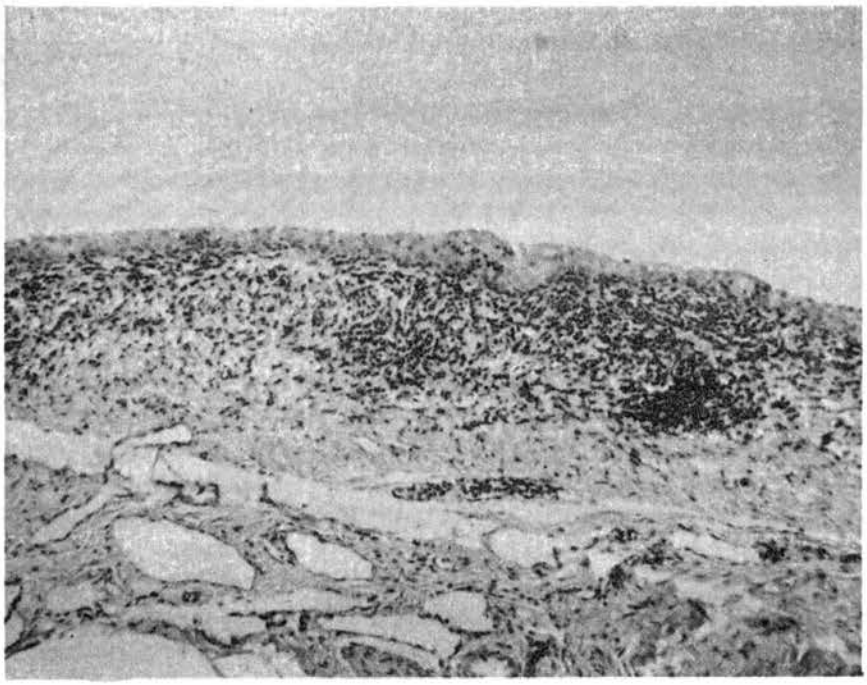

Figure 15. Aggregation of Lymphoreticular Cells in the Pelvic Submucosa (M-19, 112 days post-viral inoculation; low magnification).

visceral epithelium of the pelvis (Figure 16).

Focal solitary granulomas were found in the outer cortex of 5 of the experimental animals and were believed to be the result of migrating parasite larvae.

A single, well-circumscribed abscess was present in the renal cortex of one of the dogs that had received multiple injections of bacteria. A Staphylococcus species was isolated from the abscess but organisms were not isolated from the urine.

The results of attempts to isolate ICH virus from urine samples collected at sacrifice are listed in Table XVI. Virus was not isolated from any of the specimens examined.

Results of virus neutralization tests performed on serum samples obtained before and after exposure to ICH virus and at the time of sacrifice are listed in Table XVII. Virus neutralizing antibody was present in all of the postinoculation serum samples.

The results of direct fluorescent antibody procedures conducted on frozen kidney sections from the animals in Phase II are listed in Table XVIII. When the anti-ICH serum was applied to the frozen sections positive fluorescence was observed only in the renal glomeruli of animals that died during the acute episode of the disease (Figure 17). Specific fluorescence was not observed when antibacterial conjugates were utilized.

Microscopic agglutination tests for the presence of leptospiral antibodies were not detected in any of the animals. The results are listed in Table XIX.

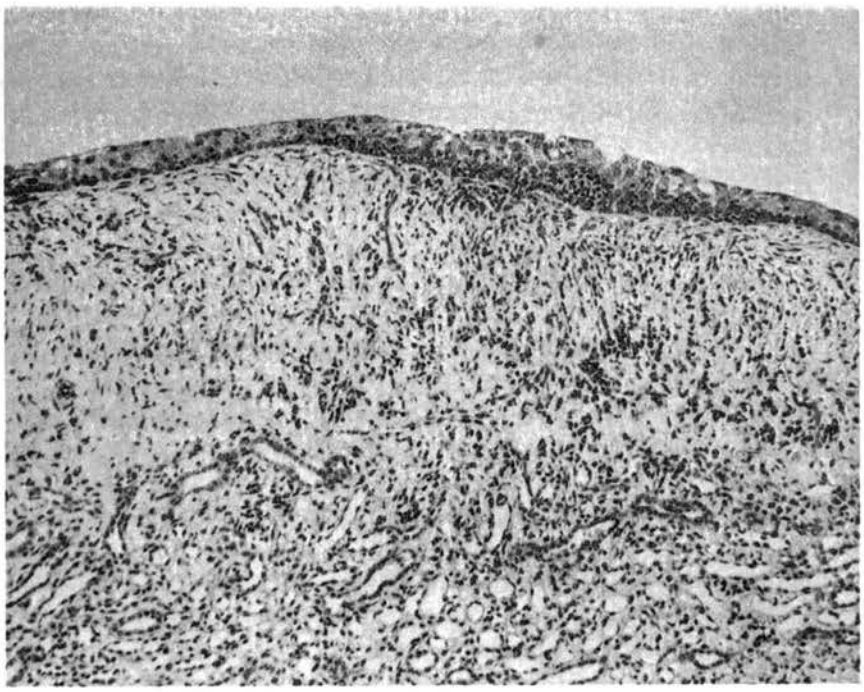

Figure 16. Linear Zone of Chronic Inflammation Adjacent to the Visceral, Pelvic Epithelium. Note Intraepithelial Inflammatory Cells (M-33, 52 days post-viral inoculation; low magnification).

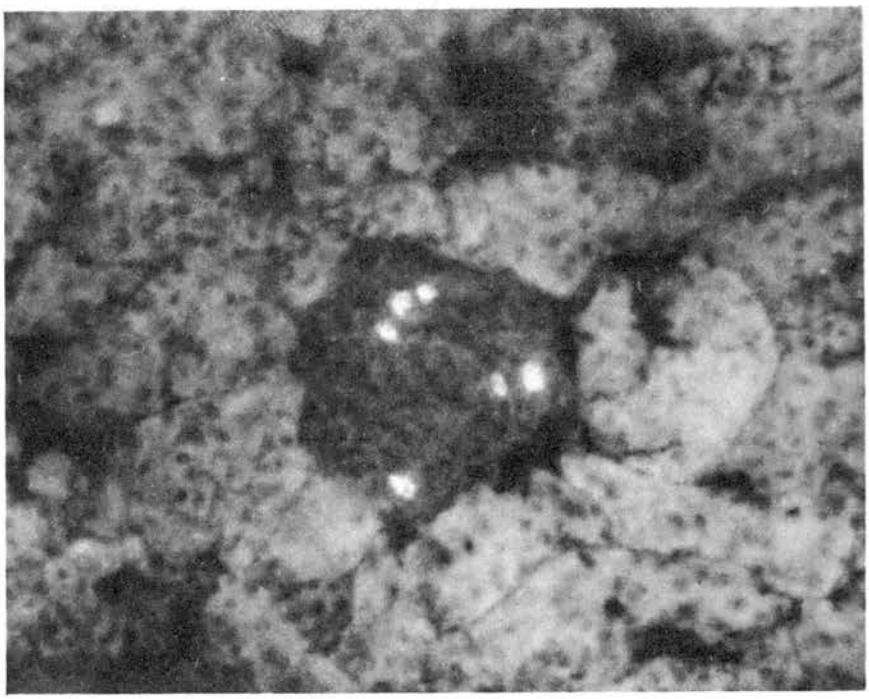

Figure 17. Specific Fluorescence in the Glomerulus of a Dog that Succumbed to Acute ICH (M-11, 7 days post-viral inoculation; high magnification).

CHAPTER V

DISCUSSION AND CONCLUSIONS

Phase I

Phase I was designed to determine the intrarenal pathogenicity of intravenously administered bacteria in normal dogs. Cultural assay revealed that only a few organisms were culturable from the cortices 96 hours postinoculation and after 192 hours all parts of the kidney were sterile. Histologic lesions indicative of bacterial localization and resultant inflammation were absent. It is therefore concluded that hematogenous exposure of the healthy kidney is unlikely to result in a persistent infection or to be accompanied by lesions. It has been repeatedly observed in many species of animals that intravenously inoculated organisms do not localize in normal, unobstructed kidneys (Guze and Beeson, 1956; Rocha, 1963; Corone et al, 1959).

Paplanus (1964), investigating the clearance of organisms from the kidneys of rats subjected to intravenously administered bacteria, found that organisms were cleared from the cortex within 24 hours whereas they multiplied for a short period of time in the medulla and were still present through the fourth day. Results in the present experiment show that organisms were removed from both the cortex and medulla at comparable rates; there was no differential in the survival time of organisms between cortex and medulla. Whether the differences are properties of

the host, the organisms utilized or experimental procedures is not clear. However, the pathogenicity of bacterial inoculums used in these experiments was established by demonstrating the capacity of the intravenously administered organisms to induce pyelonephritis in dogs and rats following ureteral ligation. The success of this procedure in establishing a renal infection also suggests that the age of the culture was satisfactory.

With a single exception (M-5) all urine specimens were bacteriologically sterile. Guze et al (1961) reported that bacteria residing in the kidney do not gain access to the urine unless tissue destruction exists in that kidney. Destructive lesions were not observed in the kidneys of the experimental subjects. The presence of a few bacteria in the urine of M-5 might be the result of contamination, a small undetected lesion in the urinary bladder, or they might represent a spontaneous asymptomatic bacteruria (Vejlsgaard, 1965). The precise fate of the bacteria was not investigated. It was established that they were not eliminated through the urine. They might have been carried away by the lymphatic or venous circulation or lysed by specific antibodies, or by fixed or circulating phagocytic cells.

Phase II

Infection of the dogs with ICH virus resulted in a renal lesion regardless of the severity of the systemic clinical response elicited. Ginder (1966) experimentally produced descending bacterial pyelonephritis in mice convalescing from a murine adenovirus infection. Intravenous exposure of the dog to bacteria during the convalescent phase of ICH did not result in localization or persistence of bacteria in the

kidney, nor was morphological evidence of bacterial infection consistently observed. On the basis of Ginder's observations it had been anticipated that ICH virus would predispose the canine kidney to infection with hematogenously administered bacteria. A number of factors might be responsible for the failure of the dog kidney to respond similarly. Results of studies reported in Phase I indicate a difference exists in the medullary localization of bacteria between the dog and rat. Differences may also exist between the dog and the mouse and these species variations may be critical to localization of bacteria in the dog kidney. A second potential factor for our failure relates to the interval between exposure to ICH virus and inoculation of bacteria. If the murine adenovirus predisposed to bacterial nephritis by virtue of tubular obstruction, the time interval applied to the subjects of this experiment may have been too short. Bacteria were injected approximately 28 days after exposure to ICH virus. The focal renal lesions at that time consisted of interstitial cellular infiltrates and tubular damage, but no recognizable fibrosis. Tubular dilatation and presumably obstruction were not observed until 35 days following exposure to the virus. Bacterial inoculums administered later, subsequent to the development of intrarenal obstruction, might have resulted in bacterial localization in the kidney. The negative results do not refute the validity of the hypothesis that viral-induced lesions would predispose to bacterial localization; rather they do not support it. Additional efforts varying the experimental procedures appear warranted.

Histologic changes observed in the kidneys of dogs in phase II were attributed to the viral infection itself and are similar to the focal convalescent lesions described by other investigators (Poppensiek

and Baker, 1951; Stunzi and Poppensiek, 1953; Hamilton et al, 1966; Kapp, 1966; Wright, 1967). The following sequence of development of lesions was postulated from a study of the sections. Inclusion bodies were observed in the nuclei of some tubular epithelial cells that had no other discernable alterations. They were also observed in the nuclei of cells whose cytoplasm was enlarged to the point of infringement on the tubular lumen. The hypertrophic cells were often accompanied by hyperplasia of contiguous tubular epithelial cells and by mononuclear cell aggregates in either a peritubular or intracapillary location. In other instances the swollen, inclusion body-bearing cells had sloughed into the tubular lumens; the remaining epithelial cells were hypertrophic and the peritubular interstitium was densely infiltrated by mononuclear cells. Progressively affected tubules contained necrotic cellular debris, hypertrophic epithelial cells and an even more extensive peritubular lymphoreticular cell reaction. Later in the sequence lesions contained fibroplasia in conjunction with the mononuclear cell infiltrates. Cellular reactions as described above occurred in many foci in which inclusion bodies were not observed. Cabasso (1962) demonstrated that viral antigen is present within the inclusion bodies and Wright (1967) showed that viral antigen exists in many cells that do not contain inclusions. It is assumed that the focal cellular reaction in the presence or absence of inclusion bodies is provoked by the presence of viral antigen. Since a single kidney from a dog infected several weeks or months previously might contain recently acquired tubular lesions as well as mature cellular infiltrates and fibroplastic lesions, it is believed that cellular invasion by the virus occurs not only during the viremic phase, but also long after

the viremic phase has subsided.

Attempts to isolate ICH virus from the urine of experimental subjects were unsuccessful. Viruria has been reported repeatedly and is particularly prevalent in early convalescence (Poppensiek and Baker, 1951; Persson, et al, 1961; Hamilton, et al, 1966). Hamilton et al (1966) suggested that viruria occurred intermittently and that the virus was present in low concentration. It is believed that virus was present in at least some of the urine specimens but that technical inadequacies, such as failure to use a preservative before freezing, and failure to concentrate viral particles in urine prior to inoculation of the tissue cultures rather than absence of virus were responsible for failure to isolate virus.

Aggregates of neutrophils were observed in the medullary interstitium of 16 of the 40 convalescent animals examined. Bacteria were not recovered from the kidney of any of the animals in the present study. Wright (1967) also found that a characteristic feature of the larger medullary lesions was infiltration by numerous polymorphonuclear leukocytes. His study did not include bacteriologic examinations of the kidney nor did he speculate as to why the neutrophils were present. It is possible that anaerobic or other forms of bacteria not detectable by cultural procedures used might have been responsible for the lesions. Mobilization of neutrophils may have occurred as a result of the virus itself or as a response to the tubular necrosis. It has also been established that neutrophils can accumulate in the vasa recta as a result of a sluggish circulation (Heptinstall, 1966). If circulatory impairment associated with the renal lesions of ICH did occur, it could be responsible for the aggregation of neutrophils in the medulla.

Focal papillitis was present in 12 of the 40 convalescent animals. Eleven of the 12 that had papillary lesions were among the 16 in which neutrophils were found in the inner medulla. Papillary lesions were often associated with vasa recta that were engorged and that contained sequestered cellular debris which was sometimes partially or completely mineralized. The vascular lesions may have diminished medullary circulation creating papillary ischemia and the focal papillary lesion.

Similarities exist between the renal perivascular mononuclear cell aggregates observed in the present experiment and the ocular lesions described by Carmichael (1964) in convalescent animals. Carmichael (1964) demonstrated that local antibody production occurred in the iris and limbal region, and postulated that the local antibody reacted with cell-associated viral antigen initiating a focal hypersensitivity response. Cell associated viral antigen also exists in the renal lesion. Although local antibody production has not been demonstrated in the dog kidney, the existence of dense infiltrates of plasma cells in the kidney indicates that local antibody synthesis occurs. If ICH antibody synthesis occurs in the canine kidney, it could react locally with viral antigen and produce focal hypersensitivity reactions similar to those in the eyes. This could be the basis of the perivascular mononuclear cell response that occurs in the kidney and the subpelvic tissue.

During the first month after the acute clinical signs had subsided 14 of the animals developed fever and marked leukocytosis with a neutrophilic shift to the left. Spontaneous bacteremia was suspected to be the cause in the present experiment but blood cultures performed on 4 of these animals at the height of the fever were negative. A similar

phenomenon has been observed by Hamilton et al (1966) but he did not speculate as to the cause.

ICH antigen was demonstrated by direct fluorescent antibody procedures in the renal glomeruli of animals that succumbed to the acute infection. Specific fluorescence was not observed in any of the convalescent cases in spite of the presence of inclusion bodies. Application of an indirect procedure as described by Wright (1967), might have yielded positive results in some of the recovered animals. Specific fluorescence was not observed with any of the bacterial conjugates. This probably reflects the low concentration of organisms in the renal tissue at the time of sacrifice. Bacterial antigen might have been observed if additional sections had been examined.

The isolation of a few bacteria (Proteus species, Escherichia coli and Paracolobacterum species) from the urine of 3 of the dogs is without explanation but is not regarded as a product of the experimental procedure.

The occurrence of a staphylococcal abscess in the cortex of one of the puppies that had received multiple injections of E. coli was not expected. Rocha (1963) however, noted that spontaneous staphylococcal infection occurs frequently in dogs after the experimental induction of medullary scars by electrocautery.

CHAPTER VI

SUMMARY

The primary objective of the experiment was to study a potential etiologic mechanism of interstitial nephritis in dogs. The experiment was divided into 2 phases.

Phase I was designed to determine the intrarenal pathogenicity of intravenously administered Escherichia coli and Proteus species in the normal canine kidney. The intravenously injected bacteria did not become established in the kidney; the number of organisms diminished progressively to near zero from both the cortex and medulla within 96 hours.

Phase II was designed to characterize the renal lesion produced by ICH virus and to determine the potential of the viral-induced lesion to predispose the canine kidney to a superimposed, hematogenous infection with bacteria. Infection of dogs with ICH virus consistently resulted in a focal interstitial nephritis and pyelitis. Intranuclear inclusion bodies were present in glomerular endothelial cells during the acute phase of the disease and in epithelial cells of the collecting tubules during convalescence. A sequence of development of the lesions was postulated from a study of the histologic sections. The renal lesion consisted primarily of focal aggregates of mononuclear cells distributed randomly throughout the cortex and medulla. Focal papillitis and focal accumulations of neutrophils in the medulla were

also observed in some of the experimental subjects. Cultural assays performed on the kidneys of convalescent dogs that had received intravenous inoculation of organisms were invariably negative.

With the procedures utilized in this study, the renal lesion produced by ICH virus did not predispose the canine kidney to a superimposed infection with bacteria. The negative results do not refute the validity of the hypothesis that viral-induced lesions would predispose to bacterial localization; rather they do not support it. Additional efforts varying the experimental procedures appear warranted.

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APPENDIX

TABLE I

PHASE I: DAILY BODY TEMPERATURE VALUES BEFORE AND AFTER ADMINISTRATION OF BACTERIA

Pre- and Post-Inoculation Interval	-1	0	1	2	3	4	5	6	7	8	9
DOGS											
M3	101.5	101.8	...	...	...	...	...	...	...	...	...
M6	101.8	102.6	...	...	...	...	...	...	...	...	...
M2	101.8	102.4	103.0	...	...	...	...	...	...	...	...
M4	101.8	101.6	102.8	...	...	...	...	...	...	...	....
M65	102.0	101.8	103.6	...	...	...	...	...	...	...	...
M1	101.5	102.0	105.0	105.2	...	...	...	...	...	...	...
M66	101.8	101.8	103.2	102.6	...	...	...	...	...	...	...
M7	102.0	102.5	103.0	102.6	102.3	103.0	...	...	...	...	...
M43	100.2	100.4	103.4	102.6	100.9	100.7	100.2	...	...	...	...
M5	102.2	102.6	102.8	103.4	103.4	103.4	102.8	102.0	101.8	101.6	...
M44	101.2	100.8	104.0	103.0	102.2	101.6	101.6	101.6	101.0	100.5	101.6
M53	101.7	101.2	103.0	102.4	100.2	102.2	99.8	100.0	100.2	100.0	100.2
M42	101.2	100.8	102.3	102.0	101.4	101.0	100.0	101.0	100.4	101.4	101.2
M15	101.3	101.2	102.9	103.0	103.0	102.0	102.0	101.6	101.5	101.3	101.5
M54	100.8	101.0	102.4	102.4	102.0	101.5	101.6	1012	101.8	101.2	102.0
*M14	101.2	101.8	101.4	101.0	100.8	101.8	101.8	101.8	101.6	101.4	101.3
*M49	101.2	101.0	101.8	101.0	100.8	101.0	101.0	101.0	100.8	101.4	101.0

*Control

TABLE II

PHASE I: TOTAL BLOOD LEUKOCYTE COUNTS OBTAINED BEFORE AND AFTER ADMINISTRATION OF BACTERIA

Days Pre- and Post-Inoculation	-1	0	1	2	3	4	5	6	7	8	9
M3	8,200	...	...	...	...	...	...	...	...	...	...
M6	6,150	...	...	...	...	...	...	...	...	...	...
M2	15,250	...	13,650	...	...	...	...	...	...	...	...
M4	...	...	22,300	...	...	...	...	...	...	...	...
M65	10,100	9,600	15,800	...	...	...	...	...	...	...	...
M1	20,900	...	...	32,250	...	...	...	...	...	...	...
M66	13,400	...	24,600	27,400	...	...	...	...	...	...	...
M7	11,500	...	...	32,750	...	...	...	...	...	...	...
M43	9,800	10,500	41,000	...	11,300	...	...	15,900	...	...	...
M5	14,950	...	...	21,650	...	...	...	14,900	...	...	...
M44	9,250	9,550	32,100	...	...	...	...	13,950	...	9,900	...
M53	14,200	15,300	...	...	9,700	...	...	14,850	...	...	17,300
M42	9,250	9,800	28,250	...	11,850	...	...	...	...	12,200	...
M15	14,550	14,550	...	35,150	...	...	...	13,250	...	...	12,600
M54	8,850	...	...	...	8,850	...	...	...	...	...	13,300
*M14	9,950	13,750	11,300	...	11,950	...	10,550	...	7,350	...	6,300
*M49	10,350	8,400	11,380	...	7,800	...	11,000	...	11,800	...	11,000

*Control

TABLE III

PHASE I: TOTAL NEUTROPHILIC LEUKOCYTE COUNTS OBTAINED BEFORE AND AFTER ADMINISTRATION OF BACTERIA

Days Pre- and Post-Inoculation	-1	0	1	2	3	4	5	6	7	8	9
M3	6,150	...	...	...	...	...	...	...	...	...	...
M6	3,567	...	...	...	...	...	...	...	...	...	...
M2	...	...	12,012	...	...	...	...	...	...	...	...
M4	...	...	18,286	...	...	...	...	...	...	...	...
M65	5,353	4,320	13,746	...	...	...	...	...	...	...	...
M1	...	...	...	26,122	...	...	...	...	...	...	...
M66	5,896	...	21,402	23,838	...	...	...	...	...	...	...
M7	...	...	...	29,475	...	...	...	...	...	...	...
M43	4,508	4,200	37,720	...	3,729	...	...	9,036	...	...	...
M5	...	...	...	17,969	...	...	...	6,897	...	...	...
M44	3,237	3,724	28,890	...	...	...	...	6,975	...	3,960	...
M53	7,384	11,628	...	...	6,111	...	...	8,910	...	...	11,072
M42	4,995	4,802	26,272	...	7,939	...	...	...	...	5,734	...
M15	6,838	7,857	...	30,580	...	...	...	7,817	...	...	5,544
M54	5,044	...	...	...	4,956	...	...	...	...	...	8,246
*M14	4,140	4,368	6,554	...	6,692	...	5,940	...	6,018	...	3,087
*M49	5,074	5,225	5,576	...	4,134	...	4,853	...	3,160	...	4,180

*Control

TABLE IV

PHASE I: BACTERIAL COUNTS FROM RENAL CORTEX, RENAL MEDULLA, URINE AND HEART BLOOD FOLLOWING INTRAVENOUS ADMINISTRATION OF ESCHERICHIA COLI

Animal Identification	Post Inoculation Interval	Organisms Inoculated/lb Body Weight	Organisms Isolated per Gram of Cortex		Organisms Isolated per Gram of Medulla		Organisms Isolated per ml of	
			Left	Right	Left	Right	Blood	Urine
M3	1 hour	500×10^6	788.1	1244.2	1853.2	1252.9	17	0
M6	4 hours	336×10^6	747.6	719.7	368.4	661.0	3	0
M2	16 hours	336×10^6	217.5	273.8	201.5	134.9	4	0
M4	24 hours	$336 \cdot 10^6$	482.1	339.9	227.4	144.6	1	0
M1	48 hours	500×10^6	415.6	432.5	218.0	605.1	0	0
M7	96 hours	336×10^6	6.0	30.4	0	0	0	0
M5	192 hours	336×10^6	0	0	0	0	0	0
M15	720 hours	$112 \cdot 10^6$	0	0	0	0	0	0
*M14	0 hours	0	0	0	0	0	0	0

*Control

TABLE V

PHASE I: BACTERIAL COUNTS FROM RENAL CORTEX, RENAL MEDULLA, URINE AND HEART BLOOD FOLLOWING INTRAVENOUS ADMINISTRATION OF PROTEUS SPECIES

Animal Identification	Post Inoculation Interval	Organisms Inoculated/lb Body Weight	Organisms Isolated per Gram of Cortex		Organisms Isolated per Gram of Medulla		Organisms Isolated per ml of	
			Left	Right	Left	Right	Blood	Urine
M65	24 hours	$90x10^6$	167.3	...	83.3	...	1	0
M66	48 hours	$90x10^6$	0	...	0	...	0	1
M43	120 hours	$90x10^6$	0	...	0	...	0	0
M44	264 hours	$90x10^6$	0	...	0	...	0	0
M53	264 hours	$120x10^6$	0	...	0	...	0	0
M42	312 hours	$90x10^6$	0	...	0	...	0	0
M54	720 hours	$120x10^6$	0	...	0	...	0	0
*M49	0 hours	0	0	...	0	...	0	0

*Control

TABLE VI

PHASE I: HISTOLOGICAL FINDINGS IN KIDNEYS OF DOGS RECEIVING ESCHERICHIA COLI

Animal Identification	Neutrophils in Glomeruli	Tubular Epithelial Degeneration	Parasitic Granuloma	Interstitial Nephritis	Pyelitis
M1	-	-	+	-	-
M2	+	-	+	-	-
M3	...	...	...	...	...
M4	+	+	-	-	-
M6	+	+	-	-	-
M7	-	-	-	-	-
M15	-	-	-	-	-
*M14	-	-	-	-	-

*Control

TABLE VII

PHASE I: HISTOLOGICAL FINDINGS IN KIDNEYS FROM DOGS RECEIVING PROTEUS SPECIES

Animal Identification	Neutrophils in Glomeruli	Tubular Epithelial Degeneration	Parasitic Granuloma	Interstitial Nephritis	Pyelitis
M42	-	-	-	-	-
M43	-	-	-	-	-
M44	-	-	-	-	-
M54	-	-	-	-	-
M65	+	-	-	-	-
M66	+	-	-	-	-
*M49	-	-	+	-	-

*Control

TABLE VIII

PHASE II: DAILY BODY TEMPERATURE VALUES DURING THE CLINICAL COURSE OF ACUTE ICH

Day After Onset of Clinical Illness	-1	0	1	2	3	4	5	6	7
Dog Identification									
M8	102.1	103.9	104.6	104.8	104.2	105.5	103.5	101.2	100.5
M9	101.8	104.2	105.1	106.5	105.4	104.4	101.5	101.5	101.2
M12	102.2	103.8	104.6	104.4	104.0	104.2	103.2	101.4	101.8
M13	102.0	103.2	104.2	104.8	104.4	105.2	102.8	101.2	100.6
M17	101.2	103.2	104.2	103.8	103.4	105.2	103.8	101.8	100.0
M18	102.4	104.6	104.8	103.8	103.8	104.6	104.8	102.2	101.8
M19	102.2	103.0	103.4	103.4	102.2	101.8	103.2	101.0	102.2
M20	101.8	103.0	104.0	104.2	104.2	104.2	105.0	103.0	102.2
M22	101.8	102.0	101.8	101.8	101.8	101.8	101.8	101.8	102.0
M24	101.8	103.6	104.0	104.0	103.2	101.0	101.4	101.4	101.6
M25	102.0	103.8	103.6	104.0	104.0	104.0	104.2	101.0	101.6
M26	101.8	102.8	101.6	101.8	102.0	102.0	102.0	102.0	102.0
M28	101.8	103.8	104.0	104.2	104.2	101.6	101.8	102.0	101.8
M29	102.0	104.4	104.8	104.1	104.2	104.0	100.8	101.4	101.8
M30	101.8	103.8	104.0	104.4	104.8	104.2	101.6	102.0	102.0
M31	102.2	102.8	104.8	105.2	104.4	104.2	101.8	101.6	100.4
M32	101.8	104.0	104.2	101.6	101.8	102.0	101.6	102.0	101.8
M33	101.6	101.8	101.8	101.8	101.8	101.6	102.0	102.0	102.2
M40	102.1	103.0	104.6	103.6	102.1	101.1	100.2	100.2	100.2

TABLE VIII (continued)

Day After Onset of Clinical Illness	-1	0	1	2	3	4	5	6	7
Dog Identification									
M45	101.2	104.0	104.2	103.6	104.4	105.0	104.2	102.8	100.7
M46	101.8	103.9	104.5	104.4	104.2	104.4	104.4	101.8	100.5
M47	102.8	104.0	105.2	104.2	104.2	105.1	105.2	102.4	102.0
M48	102.0	102.6	103.7	104.0	105.6	104.8	109.3	102.4	104.4
M50	101.6	104.0	104.0	104.2	104.4	104.4	103.4	100.8	100.8
M51	101.6	103.0	104.2	103.5	103.3	100.0	101.0	101.2	101.1
M52	102.7	104.2	103.2	102.4	101.2	101.0	100.6	101.8	101.6
M55	101.6	103.0	103.2	102.3	100.6	100.4	99.8	99.8	101.6
M56	101.6	103.0	102.9	103.2	104.0	103.6	101.0	100.6	100.6
M57	101.8	101.8	101.5	103.2	102.4	100.0	100.8	101.4	101.6
M58	102.0	102.5	104.0	104.4	103.4	100.4	100.6	101.9	101.6
M59	101.4	102.2	103.8	103.4	104.2	103.2	101.4	100.0	100.0
M61	101.4	102.0	104.2	104.4	104.2	104.4	103.6	99.6	100.6
M71	101.4	102.5	104.5	104.1	103.3	103.9	104.2	101.2	99.3
M72	101.8	103.7	104.3	103.5	105.4	104.7	105.0	101.0	99.6
M76	102.2	102.9	103.9	102.8	101.4	101.4	100.6	100.9	100.9
M79	101.6	103.9	103.0	102.8	103.5	104.2	104.3	102.0	102.0
M80	101.8	104.8	106.0	105.7	104.8	103.2	102.0	102.0	102.5
M81	102.3	103.4	104.6	103.7	104.7	104.5	103.7	101.0	101.0

TABLE IX

PHASE II: TOTAL BLOOD LEUKOCYTE COUNTS DURING THE CLINICAL COURSE OF ACUTE INFECTIOUS CANINE HEPATITIS

Day After Onset of Clinical Illness	-1	0	1	2	3	4	5	6	7
Dog Identification									
M8	13,500	10,100	10,300	7,400	4,350	3,700	2,950	...	13,550
M9	12,050	8,250	4,500	3,200	4,900	9,050	...	...	14,650
M12	12,250	...	...	...	...	7,000	...	...	16,050
M13	10,600	...	16,900	...	...	5,450	4,300	...	25,900
M17	10,250	5,250	8,450	...	4,200	4,750	6,050	...	...
M18	10,650	...	2,550	5,100	3,850	6,050	8,650	...	...
M19	8,050	...	9,208	9,400	8,500	10,200	9,800	...	10,950
M20	7,600	8,450	5,050	...	8,850	...	8,350	...	8,600
M22	12,250	14,850	...	14,600	...	15,350	14,650	...	...
M24	14,750	11,300	...	5,100	...	...	8,050	12,650	...
M25	12,050	...	9,100	...	5,350	...	14,100	22,500	...
M26	15,750	...	15,300	...	...	...	...	15,150	...
M28	12,500	...	8,900	...	5,900	...	16,850	...	...
M29	15,450	...	8,700	...	...	...	...	11,900	...
M30	12.000	14,850	...	2,000	...	...	11,350	...	23,500
M31	8,450	...	5,300	6,600	3,850	3,700	...	...	13,650
M32	10,850	...	2,650	...	28,050	...	...	...	24,200
M33	12,700	...	8,350	...	29,200	...	...	...	18,500
M40	15,700	...	6,050	...	5,550	...	...	...	14,650

TABLE IX (continued)

Day After Onset of Clinical Illness	-1	0	1	2	3	4	5	6	7
Dog Identification									
M45	14,550	8,450	4,400	6,450	...	...	...	...	20,100
M46	19,450	12,950	5,950	5,900	...	5,350	...	...	23,600
M47	19,350	...	9,500	5,900	5,800	5,800	...	6,700	...
M48	8,400	6,900	5,050	5,100	5,300	...	...	...	15,600
M50	10,400	...	...	5,350	...	...	...	...	...
M51	7,650	...	...	4,250	...	...	...	...	...
M52	10,300	...	...	6,450	...	...	...	...	...
M55	9,700	...	5,400	...	3,450	...	...	...	15,800
M56	8,800	...	3,100	...	...	...	...	...	14,300
M57	10,150	...	7,650	...	...	6,650	...	...	...
M58	7,200	...	...	1,700	...	...	...	...	...
M59	16,150	...	...	2,300	...	...	...	...	...
M61	10,500	...	...	5,350	...	...	...	...	22.000
M71	8,850	...	...	4,800	3,300	...	...	...	13,850
M72	8,600	...	...	3,300	2,950	...	...	...	21,250
M76	10,600	...	9,650	...	...	...	...	...	15,550
M79	13,900	...	11,800	...	...	...	...	...	12,150
M80	14,750	13,300	...	6,500	...	...	...	17,900	...
M81	16,600	...	8,750	...	...	...	...	...	...

TABLE X

PHASE II: BACTERIAL COUNTS FROM DOGS INOCULATED WITH INFECTIOUS CANINE HEPATITIS VIRUS ONLY

Dog Identification	Days Post-Viral Inoculation	Organisms Isolated per Gram of Cortex	Organisms Isolated per Gram of Medulla	Organisms Isolated per ml Blood	Organisms Isolated per ml Urine
M47	21	0	0	0	0
M20	30	0	0	0	3
M61	30	0	0	0	0
M13	31	0	0	0	0
M48	32	0	0	0	0
M60	35	0	0	0	0
M78	45	0	0	0	0
M31	48	0	0	0	0
M26	49	0	0	0	4
M28	49	0	0	0	0
M80	49	0	0	0	0
M25	51	0	0	0	0
M33	52	0	0	0	0
M79	52	0	0	0	0
M78	54	0	0	0	0
M76	70	0	0	0	0
M71	81	0	0	0	0
M81	93	0	0	0	0
M19	112	0	0	0	0
M59	118	0	0	0	0
M72	256	0	0	0	0

TABLE XI

PHASE II: BACTERIAL COUNTS FROM DOGS INOCULATED WITH ESCHERICHIA COLI SUBSEQUENT TO INOCULATION WITH INFECTIOUS CANINE HEPATITIS VIRUS

Dog Identification	Days Post-Viral Inoculation	Days Post-Bacterial Inoculation	Organisms Isolated per Gram		Organisms Isolated per ml	
			Cortex	Medulla	Blood	Urine
M8	34	12	0	0	0	0
M12	32	12	0	0	0	0
M30	47	21	0	0	0	0
M18	51	30	0	0	0	0
*M22	50	30	0	0	0	0
M24	51	30	0	0	0	0
**M32	50	30	0	0	0	86
M17	112	90	0	0	0	0
M9	166	144	0	0	0	0
M29	26	0	...	...	...	...

*M22 was given three bacterial inoculations at approximately weekly intervals. Euthanasia was thirty days after the first bacterial inoculation.

**M32 was given four bacterial inoculations at approximately weekly intervals. Euthanasia was thirty days after the first bacterial inoculation.

TABLE XII

PHASE II: BACTERIAL COUNTS FROM DOGS INOCULATED WITH PROTEUS SPECIES SUBSEQUENT TO INOCULATION WITH INFECTIOUS CANINE HEPATITIS VIRUS

Dog Identification	Days Post-Viral Inoculation	Days Post-Bacterial Inoculation	Organisms Isolated per Gram		Organisms Isolated per ml	
			Cortex	Medulla	Blood	Urine
M45	33	0	...	...	...	...
M50	26	0	...	...	...	...
M46	37	3	0	0	0	0
M55	32	5	0	0	0	0
M56	35	8	0	0	0	0
M40	38	12	0	0	0	0
M57	47	21	0	0	0	0
M58	56	30	0	0	0	0
M52	117	90	0	0	0	0
M51	138	111	0	0	0	0

TABLE XIII

PHASE II: RENAL CORTICAL LESIONS IN DOGS SUBSEQUENT TO INFECTION WITH INFECTIOUS CANINE HEPATITIS VIRUS

Dog ID	Days Post-Viral Inoculation	Perivascular Mononuclear Cells	Periglomerular Mononuclear Cells	Collagen Deposition	Glomerular Atrophy	Peritubular Mononuclear Cells	Parasite Granuloma
M47	21	+	+	-	-	+	+
M29	26	+	+	-	-	+	-
M50	26	+	+	-	-	+	-
M61	30	+	+	-	-	+	-
M13	31	+	+	-	-	+	+
M48	32	+	+	-	-	+	-
M12	32	-	-	-	-	+	+
M55	32	+	+	-	-	+	-
M45	33	+	-	-	-	+	-
M8	34	+	+	-	-	+	-
M56	35	+	+	-	-	+	-
M60	35	+	+	-	-	+	-
M46	37	+	-	-	-	+	-
M40	38	+	+	-	+	+	+
M78	45	+	+	-	+	+	-
M30	47	+	+	+	+	+	-
M57	47	+	+	-	-	+	-
M31	48	+	+	+	+	+	-
M26	49	+	+	-	+	+	-
M28	49	+	+	+	+	+	-

TABLE XIII (continued)

Dog ID	Days Post-Viral Inoculation	Perivascular Mononuclear Cells	Periglomerular Mononuclear Cells	Collagen Deposition	Glomerular Atrophy	Peritubular Mononuclear Cells	Parasite Granuloma
M80	49	+	+	+	+	+	-
M22	50	-	-	-	-	+	-
M32	50	+	+	+	+	+	-
M18	51	+	+	-	-	+	+
M24	51	+	-	-	+	+	-
M25	51	-	-	-	-	+	-
M20	52	+	+	-	-	+	-
M33	52	+	+	-	-	+	-
M79	54	+	+	+	+	+	-
M58	56	+	+	+	-	+	-
M76	70	-	-	-	-	-	-
M71	81	+	+	+	+	+	-
M81	93	+	+	+	+	+	-
M17	112	+	+	+	+	+	-
M19	112	+	+	+	+	+	-
M52	117	+	+	+	+	+	-
M59	118	+	+	+	+	+	-
M51	138	+	+	+	+	+	-
M9	166	-	-	-	-	-	-
M72	256	+	+	-	-	+	-

TABLE XIV

PHASE II: LESIONS PRESENT IN THE OUTER MEDULLA OF DOGS SUBSEQUENT TO INFECTION WITH INFECTIOUS CANINE HEPATITIS VIRUS

Dog Identification	Days Post-Viral Inoculation	Vasa Rectae Mononuclear Cell Aggregates	Peritubular Mononuclear Cells and Tubular Distortion	Collagen Deposition
M47	21	+	+	-
M29	26	+	+	-
M50	26	+	+	-
M61	30	+	+	-
M13	31	+	+	-
M48	32	+	+	-
M12	32	+	+	-
M55	32	+	+	-
M45	33	+	+	-
M8	34	+	+	-
M56	35	+	+	+
M60	35	+	+	-
M46	37	+	+	-
M40	38	+	+	+
M78	45	+	+	+
M30	47	+	+	+
M57	47	+	+	-
M31	48	+	+	+
M26	49	+	+	+
M28	49	+	+	+

TABLE XIV (continued)

Dog Identification	Days Post-Viral Inoculation	Vasa Rectae Mononuclear Cell Aggregates	Peritubular Mononuclear Cells and Tubular Distortion	Collagen Deposition
M80	49	+	+	+
M22	50	+	-	-
M32	50	+	+	+
M18	51	+	+	-
M24	51	+	+	+
M25	51	+	-	-
M20	52	+	+	-
M33	52	+	+	-
M79	54	+	+	+
M58	56	+	+	+
M76	70	-	+	-
M71	81	+	+	+
M81	93	+	+	+
M17	112	+	+	+
M19	112	+	+	+
M52	117	+	+	+
M59	118	+	+	+
M51	138	+	+	+
M9	166	-	+	-
M72	256	+	-	-

TABLE XV

PHASE II: INNER MEDULLARY LESIONS IN DOGS SUBSEQUENT TO INFECTION WITH INFECTIOUS CANINE HEPATITIS

Dog Identification	Days Post Inoculation	Inclusion Bodies	Fibrosis	Neutrophil Aggregates	Focal Papillary Lesion
M47	21	+	-	-	-
M29	26	+	-	+	-
M50	26	+	-	-	-
M61	30	-	-	+	+
M13	31	-	-	+	+
M48	32	+	-	+	+
M12	32	+	-	+	+
M55	32	+	-	-	-
M45	33	+	-	-	-
M8	34	+	-	+	-
M56	35	+	-	-	-
M60	35	+	-	+	+
M46	37	-	-	-	-
M40	38	-	-	+	-
M78	45	-	-	-	-
M30	47	-	-	-	-
M57	47	+	-	-	-
M31	48	+	-	-	-
M26	49	-	+	+	+
M28	49	+	+	+	+

TABLE XV (continued)

Dog Identification	Days Post Inoculation	Inclusion Bodies	Fibrosis	Neutrophil Aggregates	Focal Papillary Lesion
M80	49	-	+	-	-
M22	50	+	-	-	+
M32	50	+	-	+	+
M18	51	+	-	+	+
M24	51	-	-	-	-
M25	51	-	-	-	-
M20	52	+	-	-	-
M33	52	+	-	-	-
M79	54	+	+	-	-
M58	56	+	+	-	-
M76	70	-	-	-	-
M71	81	+	+	-	-
M81	93	-	-	-	-
M17	112	-	+	+	-
M19	112	-	+	+	-
M52	117	-	+	-	-
M59	118	+	+	+	+
M51	138	-	+	+	+
M9	166	-	-	-	-
M72	256	-	+	-	-

TABLE XVI

PHASE II: ICH VIRUS ISOLATION FROM URINE SAMPLES

Dog Identification	Test 1	Test 2
M47	-	-
M29	...	...
M50	-	-
M61	-	-
M13	-	-
M48	-	-
M12	-	-
M55	-	-
M45	-	-
M8	-	-
M56	-	-
M60	-	-
M46	-	-
M40	-	-
M78	-	-
M30	-	-
M57	-	-
M31	-	-
M26	-	-
M28	-	-
M28	-	-
M80	-	-
M22	-	-
M32	-	-
M18	-	-
M24	-	-
M25	-	-
M20	-	-
M33	-	-
M79	-	-
M58	-	-
M76	-	-
M71	-	-
M81	...	...
M17	-	-
M19	-	-
M52	-	-
M59	-	-
M51	-	-
M9	-	-
M72	-	-
M49 (Control)	-	-

TABLE XVII

PHASE II: RESULTS OF VIRUS NEUTRALIZATION TEST ON SERUM SAMPLES

Dog Identification	Pre-inoculation	Post-inoculation
M47	-	+
M29	-	...
M50	...	+
M13	-	+
M48	-	+
M12	-	+
M55	...	+
M45	-	+
M8	-	+
M56	...	+
M60	...	+
M46	-	+
M40	-	+
M78	...	+
M30	-	+
M57	-	+
M31	-	+
M26	...	+
M28	-	+
M80	...	...
M22	...	+
M32	-	+
M18	-	+
M24	...	+
M25	...	+
M20	-	+
M33	...	+
M79	...	+
M58	...	+
M76	...	...
M71	-	...
M81	...	...
M17	-	+
M19	-	+
M52	...	+
M59	-	...
M51	...	+
M9	-	+
M72	-	+
M49	-	-

TABLE XVIII

RESULTS OF DIRECT FLUORESCENT ANTIBODY TEST ON KIDNEY

Dog Identification	Anti-ICH Serum	Anti-*E. coli* Serum	Anti-Proteus Serum
M10	+	-	-
M11	+	-	-
M16	+	-	-
M23	+	-	-
M35	*	*	*
M36	*	*	*
M37	*	*	*
M38	*	*	*
M39	*	*	*
M77	*	*	*
M82	*	*	*
M47	*	*	*
M29	*	*	*
M50	-	-	-
M61	-	-	-
M13	-	-	-
M48	*	*	*
M12	-	-	-
M55	-	-	-
M45	*	*	*
M8	-	-	-
M56	-	-	-
M60	-	-	-
M46	-	-	-
M40	-	-	-

TABLE XVIII (continued)

Dog Identification	Anti-ICH Serum	Anti-*E. coli* Serum	Anti-Proteus Serum
M78	*	*	*
M30	-	-	-
M57	-	-	-
M31	-	-	-
M26	*	*	*
M28	*	*	*
M80	*	*	*
M22	-	-	-
M32	-	-	-
M18	-	-	-
M24	-	-	-
M25	-	-	-
M20	-	-	-
M33	-	-	-
M79	*	*	*
M58	-	-	-
M76	*	*	*
M71	-	-	-
M81	*	*	*
M17	-	-	-
M19	-	-	-
M52	-	-	-
M59	-	-	-
M51	-	-	-
M9	-	-	-
M72	-	-	-
M49	-	-	-

TABLE XIX

PHASE II: RESULTS OF MICROSCOPIC AGGLUTINATION TEST FOR LEPTOSPIROSIS

Dog Identification	Pre-inoculation	Post-inoculation
M47	-	-
M29	-	...
M50	...	-
M13	-	-
M48	-	-
M12	-	-
M55	...	-
M45	-	-
M8	-	-
M56	...	-
M60	...	-
M46	...	-
M40	-	-
M78	-	-
M30	-	-
M57	-	-
M31	-	-
M26	-	-
M28	-	-
M80	-	...
M22	...	-
M32	-	-
M18	-	-
M24	...	-
M25	...	-
M20	-	-
M33	-	-
M79	...	-
M58	...	-
M76	...	...
M71	-	...
M81	-	...
M17	-	-
M19	-	-
M52	...	-
M59	-	-
M51	...	-
M9	-	-
M72	-	...
M49 (Control)	-	-

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