CARDIOVASCULAR DISEASE ASSOCIATED WITH INTERSTITIAL NEPHRITIS IN DOGS.

by

Lindsay J. Mackey, B.V.M.S., M.R.C.V.S.

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University of Glasgow.

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789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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Section III. A Series of Experiments Designed to Reproduce Leptospiral Nephritis in Dogs.

GENERAL INTRODUCTION.

It has been recognised for many years that among dogs renal disease is common and clinically very important. The various renal conditions which have been described in the dog include primary interstitial nephritis, pyelonephritis, glomerulonephritis, pyaemic nephritis, amyloidosis and nephrosis. Interstitial nephritis is of outstanding importance among these conditions, while the other nephropathies occur with comparative infrequency. The pattern of renal disease encountered in the dog differs from that in the human, in which pyelonephritis is relatively common and primary interstitial nephritis is unusual.

Figures illustrating the preponderance of interstitial nephritis in the dog have been given by two authors. Bloom (1939) examined 70 dogs which died of renal failure and found interstitial nephritis in 88.7%, while suppurative nephritis occurred in 7.1%, "necrotizing nephrosis" in 2.8% and amyloidosis in 1.4%. Wettimuny (1963) studied 178 dogs with renal disease. Of these, 74% showed interstitial nephritis, while glomerulonephritis (including amyloidosis) was present in 11%, pyelonephritis in 10% and pyaemic nephritis in 5%

Interstitial nephritis is associated with infection by the spirochaetal organism Leptospira canicola. Dogs become infected from the urine of animals excreting the organisms, the probable routes of infection being the oral and nasal mucosae. Leptospirae enter the bloodstream to become localised in the kidneys and the ultimate site of infection is thought to be the interstitial tissue of the renal cortex. It/

It has been suggested that the organisms reach this site in the blood-stream and later enter the lumina of proximal tubules, where they may be demonstrated in Levaditi-stained sections (McIntyre and Montgomery, 1952). In the renal cortex, the leptospirae provoke an intense reaction, characterised by the accumulation of dense foci of plasma cells and lymphocytes, with compression and necrosis of tubules. The nature of the cellular response suggests that the reaction has an immunological basis. An undetermined proportion of dogs which survive the acute disease develop chronic progressive renal fibrosis some months later and finally succumb to renal failure.

Klett (1899) first described an acute illness of dogs in which the principal symptoms were apathy, stomatitis and gastro-enteritis. He named the condition "Stuttgart disease." Spirochaetes were first seen in the organs of dogs dying of Stuttgart disease by Lukes and Derbek (1923) and their observation was soon confirmed, although controversy arose regarding the significance of the organisms. However, Lukes (1925) established the importance of the association by examining control dogs, in which no spirochaetes could be found. Wirth (1924) in Vienna first recognised the nephritic component of Stuttgart disease and a relationship was also noted between the acute illness and the subsequent development of chronic nephritis in surviving dogs. Lukes (1925) confirmed this and found small numbers of spirochaetes in Levaditi-stained sections of the kidneys of such animals.

Klarenbeek and Schuffner (1931) differentiated the causal organism of Stuttgart disease serologically from that of Weil's disease (Leptospira ictero haemorrhagiae) and named it Leptospira canicola. Icterus was the predominant symptom of Weil's disease, while renal failure was characteristic of most cases of L. canicola infection.

During the following twenty years, the frequency and importance of L. canicola infection of dogs was illustrated in several serological surveys carried out in Europe and Britain. The first British survey was undertaken by Stuart (1946) in Glasgow, who found positive titres in 40% of 100 unselected dogs passing through a small animal clinic. MacIntyre and Broom (1948) investigated the incidence of canine leptospirosis in England. Serum samples from 403 dogs aged 1 - 3 years were examined and agglutinins to L. canicola were found in 21%. The most recent survey was again carried out in a Glasgow clinic, by Cunningham, McIntyre and Ives (1957) and on this occasion positive titres to L. canicola were present in 28.9% of 197 unselected dogs. The importance of leptospiral nephritis was demonstrated in Edinburgh by McIntyre and Stuart (1949). They examined 416 dogs and 286 of these showed clinical evidence of renal disease, the vast majority apparently attributable to infection by L. canicola.

Clinical Features of Interstitial Nephritis.

Acute interstitial nephritis generally affects dogs during the first two years of life, but the disease can occur at any age (McIntyre, 1954;/

1954; Wettimuny, 1963). The incidence of chronic nephritis shows a marked increase with age (Joshua, 1949; McIntyre, 1954, Pearson, 1959; Wettimuny, 1963), though it has been found occasionally in dogs less than one year old. (McIntyre, 1954; Wettimuny, 1963).

There would appear to be no particular breed incidence of nephritis. McIntyre (1954) and Wettimuny (1963) encountered the disease in a very wide range of breeds without predominance in any.

A considerably greater frequency of interstitial nephritis in male dogs was noted by McIntyre (1954) and Wettimuny (1963). The sex ratio of affected animals observed by both of these writers was 3:1 (after adjusting the figures according to the total numbers of male and female dogs examined).

Following recognition of the nephritic basis of Stuttgart disease, the stomatitis and gastro-enteritis observed by Klatt (1899) came to be attributed to terminal toxaemia and uraemia resulting from renal failure. Bloom (1937) in the U.S.A. described briefly the symptoms of interstitial nephritis, characterised by anorexia, stomatitis, polydipsia, vomiting and diarrhoea, associated with uraemia. Lumbar pain was often a feature of acute cases, while loss of weight and anaemia generally occurred in the chronic phase. Albumin and hyaline casts were found in the urine; oliguria or anuria was often observed in acute nephritis while polyuria was seen in chronic cases.

Coffin and Stubbs (1944) reported symptoms similar to those described by Bloom. In addition, leucocytosis was regarded as a characteristic of acute nephritis and the writers considered that the acute disease progressed into chronic nephritis with death in uraemia.

McIntyre (1954) described interstitial nephritis and provided the first detailed correlation of symptomatology with laboratory and pathological features. The definition of these relationships has greatly facilitated understanding and diagnosis of the disease.

In a preliminary paper, McIntyre and Stuart (1949) divided leptospirosis canicola into invasive, primary renal and secondary renal stages on the basis of clinical signs, blood culture, serology, blood urea levels and examination of urine by dark ground microscopy for the presence of leptospirae. This work was subsequently extended and correlated with pathological studies (McIntyre and Montgomery, 1952; McIntyre, 1954). Meanwhile, Joshua (1949; 1950) described the clinical syndrome as seen in the London area. Primary cases were sub-divided into five groups. These were not clearly defined, however, nor substantiated by histopathological examination. Furthermore, in many of the dogs, agglutinins were present at very low levels, suggesting that the disease was not in the primary stages in such animals.

As a result of clinical, laboratory and pathological examinations, three/

three phases in the disease process emerged. (McIntyre and Montgomery, 1952; McIntyre, 1954). The initial invasive stage often passed unnoticed clinically, being characterised by bacteraemia with mild pyrexia. In the primary renal stage, all dogs had serological titres to L. canicola of > 1:10,000. This stage was graded as mild, severe and most severe, according to blood urea levels. Mild cases showed malaise, thirst and polyuria with blood urea < 50 mgms/100 ml. In severe cases, anorexia, apathy, thirst, emesis, polyuria (sometimes oliguria) halitosis and brown discoloration of the mouth were associated with a blood urea of 50 - 200 mgms/100 ml. In addition, the most severe cases, with blood urea > 200 mgms/100 ml., showed lumbar pain with stiffness and arching of the back. Buccal, lingual and glossal ulceration was found in most of these animals. In the primary renal stages many dogs showed leptospiruria, the frequency of which increased with rising of the serological titre. Protein and casts (both granular and hyaline) were always present in the urine. Leucocytosis was characteristic of this group, and a few of the most severe cases were anaemic.

In the secondary renal stage all dogs had titres to L. canicola of 1:30 - 1:1000 and this group was sub-divided according to the presence or absence of uraemia. Dogs without uraemia (with blood urea < 50 mgms/100 ml.) showed thirst, polyuria, occasional emesis and proteinuria. In the uraemic animals, typical signs were anorexia, frequent emesis (often with blood), thirst, weight loss, halitosis, buccal and lingual/

lingual ulceration with necrosis of the tip of the tongue in some cases. Anaemia was present in 16 out of 28 of these uraemic dogs. Leptospiruria was never found in the secondary stage. A "hardness" of the pulse wave was described and increased blood pressure was observed in some chronic cases. The development of the secondary stage from the acute illness was traced in 46 dogs during periods ranging from six months to four years.

Wettimuny (1963) confirmed McIntyre's classification of the phases of interstitial nephritis on the basis of pathological study. In addition to McIntyre's laboratory findings, an increase in erythrocyte sedimentation rate was noted in dogs with chronic nephritis and anaemia of macrocytic type occurred in 45% of chronic cases. Low urine urea levels were recorded, and attributed to loss of concentrating ability in chronically damaged kidneys.

Pathology of Interstitial Nephritis.

The earliest noteworthy pathological description of the disease was written by Henschen (1924), who recognised the interstitial nature of the renal inflammatory changes. Histopathological features were described in considerable detail and acute, sub-acute and chronic phases were distinguished. In acute interstitial nephritis, the interstitial tissue of the renal cortex was densely infiltrated by lymphocytes and plasma cells, while progressive fibrosis supervened in the later stages. Henschen's further observations of the microscopic features have been entirely corroborated in more recent studies.

In Britain, McFadyean(1929) first outlined the pathology of interstitial nephritis, describing acute and chronic stages of the disease. The principal observation in acute nephritis was pronounced lymphocytic infiltration of the interstitial tissue of the kidneys. In chronic nephritis, a very great increase of connective tissue in the medulla was associated with obstruction of many tubules and dilatation of those remaining.

Bloom (1937, 1939, 1941, 1954) studied the pathology of interstitial nephritis and found acute, sub-acute and chronic forms of the disease. The kidneys in acute nephritis were pale and swollen, containing many whitish nodules in the cortices. Microscopically, the outstanding feature was lymphocytic infiltration, either focal or diffuse, of the interstitial tissue. Tubules of the lower cortex showed epithelial degeneration and the straight tubules contained hyaline casts. The sub-acute phase was considered to develop from the acute, when the kidneys tended to become smaller, with a nodular appearance and clearly differentiated cellular foci in the cortices. The microscopic findings comprised progressive fibrosis with collapse and atrophy of tubules and "renewed" infiltrations of lymphocytes. In chronic nephritis, the kidneys were small and firm with granular surfaces. Extensive fibrosis and loss of tubules were observed at this stage and surviving tubules were often grossly dilated and contained hyaline casts. Sometimes the epithelium of the tubules showed hypertrophic change, developing a columnar appearance. Periglomerular fibrosis and dilatation of Bowman's capsules were also frequently present.

Platt (1952) studied the pathology of 8 acute and 25 chronic cases of interstitial nephritis. Although no sub-acute phase was recognised, the general description was in accordance with Bloom's observations. The infiltrating cells in acute nephritis were described in more detail. Plasma cells and lymphocytes formed the majority, while considerable numbers of histiocytes and occasional polymorphonuclear leucocytes were also present in the reaction. In chronic nephritis, hyalinization of glomerular tufts was a noteable feature and was considered to represent "the exaggeration of a normal senescent process."

The pathology of acute and chronic interstitial nephritis was described in detail by McIntyre and Montgomery (1952) and McIntyre (1954), whose findings correspond with those of Platt. Special features of the microscopic study included the finding of leptospirae in the convoluted tubules in many acute cases. Small numbers were present also in half of the chronic cases. Three dogs which were followed from the acute phase until death as a result of chronic nephritis were of particular interest. The animals were treated initially with penicillin, which terminated leptospiruria, following which there was a fall in the serological titre. However, on subsequent histopathological examination, leptospirae were found in the renal tubules, suggesting that small numbers of organisms may persist despite penicillin therapy.

Monlux (1953) classified the various canine renal diseases and briefly described the pathology of interstitial nephritis. Observations confirmed those of previous authors.

The most recent contribution was that of Wettimuny (1963), who surveyed and described the pathology of several forms of renal disease in the dog. The series included 36 acute and 96 chronic cases of interstitial nephritis. The characteristic acute infiltration of the interstitial tissue by mononuclear cells was described and the associated damage to tubules was attributed to compression with consequent degenerative change. In chronic nephritis, extensive fibrosis with dilatation and hyperplasia of the epithelium of surviving tubules were noted, as by previous authors. Additional changes were seen in the glomeruli, some of which showed atrophy of the capillary tuft, while others appeared to be hyperplastic. A number of glomerular tufts had undergone hyalinization and some were completely fibrosed.

Alimentary lesions.

Ulceration of the glossal, buccal and lingual surfaces and the gastric mucosa has been observed in dogs with advanced interstitial nephritis. The oral lesions form an important clinical feature of the disease and occasionally the tip of the tongue may slough off completely. Vomiting is generally associated with haemorrhagic erosions in the mucosa of the fundus of the stomach. These changes have been described by Bloom (1937), Platt (1951), McIntyre (1954) and Wettimuny (1963). These writers agree that the oral and gastric ulcers may occur in uraemic dogs in any phase of nephritis.

Macroscopic Cardiovascular Changes.

Certain changes in the heart and great vessels have been recorded in dogs suffering from interstitial nephritis by several authors.

Bloom (1939) described necrosis and inflammation of the endocardium in the left atrium and the intima of the origins of the aorta and pulmonary artery. The lesion was frequently found in all stages of nephritis and affected the three sites singly or together in different animals.

Platt (1951) also found necrosis in the left atrium, aorta and pulmonary artery. This occurred in all acute cases but in only one chronic case in Platt's series. A significant increase in the ratio of the left ventricular to right ventricular weight was found in dogs with chronic nephritis.

McIntyre (1954) observed "acute endocarditis" in the left atrium in 50% of dogs with acute nephritis, but never in chronic cases. Pronounced myocardial hypertrophy of the left ventricle was a characteristic of the chronic stage and this was attributed to hypertension.

Wettimuny (1963) found necrosis of the endocardium in the left atrium in many dogs with interstitial nephritis, in both acute and chronic stages of the disease. Similar necrotic change affected the intima of the origin of the pulmonary artery in a few chronic cases. Left ventricular hypertrophy was found in most dogs with chronic nephritis

Osteodystrophia fibrosa.

Several writers have described skeletal changes in dogs suffering from chronic nephritis. A proportion of such animals show decalcification of/

of the bones of the head and limbs, associated with hyperplasia of the parathyroid glands. The lesion is related to excessive retention of inorganic phosphate by chronically damaged kidneys. Stimulation of the parathyroid glands results and the secretion of parathormone is increased. The precise factor which stimulates the parathyroid is uncertain. The literature on this subject has been reviewed recently by Campbell (1963). Most evidence indicates that parathormone is secreted in response to reduction in serum calcium levels. In renal disease, this could result from intestinal excretion of calcium phosphate or from imbalance in the normal calcium/phosphorus ratio. Parathormone causes resorption of calcium from bones and in advanced cases, osteodystrophic softening becomes clinically obvious, with bones of the head showing most pronounced changes.

Platt (1951) studied the parathyroid glands and bones of nephritic dogs. A significant increase in weight of the glands was found in all cases of chronic nephritis. This change was greatest in dogs with appreciable "rubber jaw." The cranial bones were most markedly affected and 7 out of 22 dogs showed clinically obvious changes.

Dammrich (1958) studied 39 dogs with chronic nephritis. All showed hyperplasia of the parathyroid glands. Changes were found in the ribs, skull and sometimes in the scapula. Porosity, rarefaction and reduced specific gravity were observed in these bones.

Brodey, Medway and Marshak (1961) described the clinical and pathological/

pathological features of four dogs with chronic nephritis and osteodystrophy. Bones of the skull and jaw were affected and parathyroid hyperplasia was present in each case.

Wettimuny (1963) found osteodystrophia fibrosa in 17.5% of dogs with chronic nephritis. The condition was always associated with parathyroid hyperplasia. In these dogs, blood levels of inorganic phosphate were increased < 45 mg./100 ml. and serum calcium levels were low or normal. Again, bones of the head and limbs were found to be affected.

Discussion.

The foregoing general review of studies relating to interstitial nephritis shows that the clinical and pathological syndrome has been clearly defined and well recognised. However, many aspects of the pathogenesis of the disease remain obscure.

Although it is established that acute interstitial nephritis is associated with infection by L. canicola, the portal of infection is uncertain. After initial infection, it is not known where in the body the organisms multiply before the development of a severe reaction in the kidneys some time later and the ultimate site of infection within the kidney is uncertain. The nature of the characteristic cellular response in the interstitial tissue is not understood, though the predominance of plasma cells and lymphocytes indicates that the reaction has an immunological basis. It is not known whether damage to the renal tubules results solely from compression due to the accumulation of large numbers/

numbers of cells in the interstitial tissue or from direct or toxic injury by the organisms. The proportion of dogs which recover from the acute phase and the numbers which later develop progressive renal fibrosis have not been determined.

Chronic interstitial nephritis is considered to result from a preceding acute episode, but the mechanism of this sequence is uncertain. It may be suggested that persistant foci of cellular reaction cause destruction of small numbers of nephrons which become replaced by fibrous tissue. Continuation of such a process would produce extensive fibrosis with eventual renal failure. In this connection, the observation of leptospirae in the kidneys of dogs with chronic nephritis by Lukes (1925) and McIntyre (1954) is of interest.

Elevation of the arterial blood pressure may also play a significant part in the pathogenesis of chronic nephritis. In the human, an association between pyelonephritis and hypertension is well known (Freedman, 1963) and it has been shown that pyelonephritis may give rise to hypertension and vascular damage. This is discussed in Section 1.

In the dog, a positive relationship between nephritis and hypertension has never been demonstrated conclusively and therefore the role, if any, played by hypertension in the aetiology of the characteristic lesions is unknown. Damage to small blood vessels as a consequence of hypertension has long been recognised in the human (Castleman and Smithwick, 1943), but little study has been devoted to the vasculature of nephritic/

nephritic dogs. A number of reports and descriptions indicate that vascular lesions may be associated with chronic renal disease in the dog, but there is no general agreement as to the incidence, distribution and significance of such lesions. Should it be proved that interstitial nephritis gives rise to hypertension, the condition would have considerable interest in comparative studies. The disease might form a useful model, allowing further analysis of the mechanism of nephrogenic hypertension.

Although the pathology of interstitial nephritis has been described in the past in considerable detail, there has been no attempt to correlate quantitatively the degree of renal damage with the degree of renal failure or the blood pressure. The glomerular changes in chronic interstitial nephritis have not been thoroughly documented, nor is their pathogenesis understood. The nature of these lesions, which are described in detail later in the present work, suggest a hypertensive or immunological aetiology. The severity and consequences of glomerular involvement are uncertain.

Experimental reproduction of interstitial nephritis would provide a valuable opportunity to elucidate the disease process, allowing thorough clinical, laboratory and pathological study at all stages. Several authors have attempted this by infecting dogs with L. canicola but have failed to produce severe renal lesions. A practical benefit would also accrue from experimental reproduction, in that adequate testing/

testing of commercial leptospiral vaccines would become possible. These vaccines are used on a large scale in veterinary practice and at present, their protective efficiency is judged only on ability to stimulate a positive antibody titre to L. canicola. There is no direct evidence that vaccinated animals become fully resistant to natural infection.

The work described in this thesis was directed towards achieving greater understanding of the pathogenesis of interstitial nephritis. The study was particularly intended to establish the incidence of hypertension and its significance in this disease.

The thesis is divided into three sections. Section I is devoted to histopathological study of the organs of dogs suffering from severe nephritis. A survey has been carried out to establish the incidence, nature and distribution of vascular changes in such animals. The possibility of statistical relationships between vascular lesions and age, sex, degree of renal failure and blood pressure was investigated. As a control, a series of dogs with normal kidneys has been examined. Studies are described in which the numbers of damaged glomeruli in nephritic kidneys are estimated and the degree of damage noted. The effect of glomerular abnormality on the corresponding tubule has been studied by the use of serial sections. The results have been correlated with biochemical and urological findings.

In Section II, measurements of the blood pressure of nephritic and non-nephritic/

non-nephritic dogs are reported. To ascertain the precise relationship between renal disease and hypertension in this species, results of laboratory and post-mortem examinations are recorded.

Section III contains details of a series of experiments in which various methods were employed in an attempt to reproduce severe interstitial nephritis in dogs.

Literature concerning the specific investigations undertaken in this work is reviewed at the beginning of each section.

SECTION I.

CARDIOVASCULAR PATHOLOGY ASSOCIATED WITH INTERSTITIAL NEPHRITIS IN DOGS.

Introduction.

It has been recognised for many years that in man there is a close relationship between renal disease, hypertension and some forms of arterial disease. In most descriptions, two main types of vascular abnormality have been observed in association with hypertension. In the first, termed hyalinosis, sub-endothelial deposits of refractile hyaline material are found in small arteries and arterioles, often infiltrating the muscle of the media and causing reduction of the lumen. A more severe change, involving necrosis of the arterial media, occurs in arteries which also show deposition of fibrinous material within the necrotic media. Before studies of renal biopsies were undertaken, it was not clear from post-mortem examinations whether hypertension preceded the development of vascular damage and therefore the pathogenesis was obscure.

Weiss and Parker (1939) studied the pathology of 100 cases of pyelonephritis. A proportion of these had been hypertensive in life. It was found that in normotensive subjects, hyalinization of the renal arterioles was uncommon, while in the hypertensive group, vascular lesions showed greater severity and occurred more diffusely throughout the kidneys. These authors estimated that 15 - 20% of all cases of malignant/

malignant hypertension were caused by pyelonephritis and that hypertension only occurred if almost all of the renal arterioles were hyalinized. In their view (deriving from the experimental studies of Goldblatt, 1934) renal inflammation was considered to cause the arterial lesions, which in turn led to hypertension by producing renal ischaemia.

Fishberg (1925) examined the blood vessels of 72 patients with "essential" hypertension. The renal arterioles were invariably hyalinized, while vessels in other organs were affected less regularly. It was concluded that this form of hypertension could not be the result of generalized narrowing of the arterioles. The latter appeared to represent pathological exaggeration of a normal age change.

Bell and Clawson (1928) studied 420 cases of essential hypertension. The renal arterioles were hyalinized in 90%.

Moritz and Oldt (1937) confirmed that in hypertension, the kidney is the only organ in which hyaline change in the arterioles is nearly always present. Hyalinization of the renal vessels was found in more than 97% of hypertensive patients and in less than 10% of normotensives. From this it was considered that hyalinosis of the renal vessels was likely to be the cause of hypertension.

Castleman and Smithwick (1943) studied renal biopsies from 100 hypertensive patients and established that hypertension precedes the onset of vascular changes. In 7 cases, no vascular lesions were found; in/

in 21 the changes were very slight; in 25, lesions of grade 2 severity were found; 33 showed grade 3 changes and in 14 the lesions were of grade 4 (malignant) degree. Pyelonephritis was present in a few cases, especially in the group with most severe arterial lesions.

Bell (1950) described post-mortem findings in hypertensive patients. A relationship was established between hypertension (as reflected by the weight of the heart) and the degree of hyalin deposition in the renal arterioles.

Duguid and Anderson (1952) studied 72 cases of nephrosclerosis with hypertension and 70 cases of diabetis mellitus. Hyalinosis affecting renal and splenic vessels was found in 91 of these.

Smith (1955) examined the renal blood vessels from 528 consecutive autopsies. Of these patients, 266 had been normotensive and 226 hypertensive (the remainder were diabetic). Sclerosis of afferent and efferent arterioles was frequently found in the normotensive group. In the early stages of hypertension, there was no obvious difference in the degree of vascular damage but in later stages, changes in the afferent arterioles were much more severe. Smith concluded that hyalinosis is a normal age change which is exacerbated by hypertension.

The foregoing observations in the human illustrate that the association between renal disease, hypertension and arterial lesions is well established. It has been clearly shown that hypertension precedes the/

the development of severe vascular damage and that renal arterioles are affected to a greater degree than vessels in other organs. However, as recently discussed by Freedman (1963), the high incidence of essential hypertension and the difficulty of differentiating late-stage pyelonephritis from arteriolar nephrosclerosis raises doubt as to the precise importance of pyelonephritis in the aetiology of human hypertension. Nevertheless, that pyelonephritis may give rise to hypertension and vascular damage remains beyond dispute. This pattern of disease has not been demonstrated conclusively in the dog.

The nature of hypertensive vascular lesions will be discussed in greater detail in relation to the results of the present study.

The earliest reference to arterial changes in nephritic dogs was a report by Dayton (1914). He found that out of 21 unselected dogs obtained for teaching purposes, 10 had extensive chronic nephritis. One of these showed sclerosis of the intra-renal arteries.

MacNider (1916) described the pathology of the "naturally acquired chronic nephropathy of the dog." This was considered to be a primary chronic glomerulonephritis and in 4 out of 21 severe cases, sclerosis of the intra-renal arteries was observed. Three old dogs which showed "senile" changes were excluded from the series. In these, the kidneys were fibrosed and the major arteries contained atheromatous lesions. Severe arteriosclerosis affected the small arteries of the kidneys, heart and spleen.

McFadyean (1929) described acute and chronic nephritis in dogs. He did not find arteriosclerosis in five chronic cases and the blood vessels in the acute cases were not mentioned.

Innes (1930) discussed two dogs with chronic renal fibrosis. One of these showed such severe arterial sclerosis that this was considered to be the primary lesion and the cause of the renal fibrosis. The arteries around the cortico-medullary junction were most severely affected and showed hyaline sclerosis with calcium deposition in the intima and fat accumulation in the media. Hypertrophy of the media was also prominent in many vessels. This case was contrasted with another dog, whose fibrosed kidneys did not show vascular change. The renal lesion in the latter was presumed to result from nephritis.

Canine nephritis was described by Bloom in 4 publications (1937, 1939, 1941, 1954). In the three earlier descriptions, he stated that in all cases the arteries were entirely normal even although surrounded by dense inflammatory tissue. However, in 1954 arterial and arteriolar changes were discussed, in association with renal fibrosis. The incidence of vascular lesions was said to be low and when present, only a few irregularly distributed vessels were affected. Various forms of arterial change were described. The most common change was concentric or eccentric thickening of the arterial media. Necrotic endarteritis, fibrinoid alteration of the media and externa and fat and calcium deposits in the intima were also reported. These lesions were considered

to/

to result from the local inflammatory changes or to form part of the uraemic syndrome.

Another pathological study of interstitial nephritis was carried out by Platt (1952). In this work, the cardiovascular system was studied in some detail. Arterial abnormalities were found in 8 out of 22 dogs with chronic nephritis and these were interpreted as hypertensive changes. Fibrinoid necrosis, defined as the conversion of the vessel wall to brightly eosinophilic structureless material, was seen in glomerular tufts, small arteries and arterioles of the kidney. This lesion was observed less frequently in the small intestine and spleen. Hypertrophy of the media was also present in intra-renal arteries in chronic nephritis. The blood vessels of dogs with acute nephritis were never involved. Platt also found an increase in the ratio of the left ventricular to right ventricular weight in chronic cases. This evidence of left ventricular hypertrophy was also considered to indicate hypertension.

At this time a detailed study of interstitial nephritis was published by McIntyre and Montgomery (1952). The pathology of 13 dogs suffering from severe chronic nephritis was described. Vascular changes were found in the kidneys of all of the dogs and were presumed to be of hypertensive origin. The changes comprised hypertrophy of the media and thickening of the internal elastic lamina in interlobular arteries, while the afferent arterioles showed hypertrophy of the media with hyaline degeneration. In addition, the glomerular tufts sometimes contained

contained hyaline plaques. Fibrinoid necrosis was said to be absent and no vascular abnormalities were found in 21 acute cases. These results were reported again by McIntyre (1954), who described the clinical and pathological features of 20 dogs with chronic nephritis.

Thalmeier (1952) studied vascular changes in 12 dogs suffering from unspecified types of leptospirosis. In addition to widespread petechial haemorrhages (presumably in cases of L. icterohaemorrhagiae infection), the kidneys of other animals showed hyaline degeneration of the glomeruli in association with interstitial nephritis. Arterioles showed accumulation of fluid in the media, causing separation of muscle cells and often hyaline thickening of the intima and media was apparent. Arcuate and interlobular arteries sometimes contained foci of endarteritis and often the media of the interlobulars were hypertrophied to twice normal thickness. Frequently the adventitial connective tissue around larger vessels was increased. These vascular changes were observed only in the kidney.

Describing several forms of renal disease in the dog, Monlux (1953) reported renal vascular changes in cases of chronic interstitial nephritis. He found that the blood vessels in areas of renal fibrosis were often tortuous, with thickened walls and narrowed lumina.

Dahme (1957) carried out a pathological study of the cardiovascular system of nephritic dogs. The blood vessels of 41 dogs with chronic nephritis/

nephritis were examined and compared with those of 24 normal dogs. In the kidneys, certain changes were found to affect vessels of a particular size. The large arteries often showed muscle hypertrophy. Degenerative changes appeared first in the smaller arteries and arterioles in which oedema, fatty degeneration, hyalinosis and calcification were found. Hyalin accumulated diffusely in the walls of some vessels and was restricted to the intima of others, causing constriction or even closure of the lumen. In the renal arteries, the intima showed pronounced collagenous thickening. Dahme also observed similar changes quite frequently in the heart and occasionally in the brain. Other organs were examined less regularly but sclerosis of the thyroid arteries was reported. Hyalinization of the splenic vessels was present in nephritic and also in older normal dogs.

Wettimuny (1963) studied the various forms of canine renal disease. In the pathological descriptions he did not refer to vascular changes.

One additional study suggests that renal disease, hypertension and arterial lesions are related in the dog. Hamilton, Pund, Slaughter, Simpson, Colson, Coleman & Bateman (1940) found such a relationship in 5 dogs while carrying out a survey of blood pressure on 215 street dogs. The hypertensive dogs were examined post-mortem, and found to have fibrosed kidneys containing hyalinized arterioles.

This review of previous pathological studies of canine nephritis demonstrates/

demonstrates a general agreement among most writers that arterial abnormalities may occur in dogs with chronic nephritis. However, the published results show marked variations concerning the incidence, distribution, severity and significance of these lesions. These differences may be due to the fact that only small numbers of animals have been examined by most authors. The largest survey was that of Dahme, who studied 41 dogs with chronic nephritis. The development and possible aetiological importance of hypertension in relation to the canine arterial lesions has not been established.

The present thesis is based upon an investigation carried out at the University of Glasgow Veterinary Hospital to determine with certainty the relationship between renal disease, hypertension and vascular changes in dogs. The pathological study was intended to clarify the incidence of arterial abnormalities in nephritic dogs and the distribution of these throughout the body. Dogs suffering from acute and sub-acute nephritis were examined in addition to chronic cases and were of special interest because arterial changes have not been described in such animals, although they occur frequently in human acute nephritis. A detailed histological examination of the available material was carried out, using a variety of histochemical methods. When possible, blood pressure measurements were taken ante-mortem and correlated with pathological findings. This aspect of the work will be described in Section II of the thesis. Vascular lesions were also related to the phase of nephritis, biochemical urological and serological features and the age and sex of the dogs.

Materials and Methods.

A pathological study of 130 cases of interstitial nephritis was carried out. The animals in this series all suffered from severe nephritis with renal failure, which was the cause of death or the reason for euthanasia. In some dogs, intercurrent conditions were also present but in every case, nephritis was the predominent lesion.

The material for this survey came from the files of the Veterinary Hospital and from dogs which died or were destroyed following hospital treatment during the period of this investigation.

All available material from previous years was studied. This comprised 95 cases from which blocks of tissue were preserved for histological examination. The blocks differed in number and distribution in the individual cases.

On 35 recent cases a thorough post-mortem examination was carried out. Blocks of tissue for histological study were taken from kidneys, heart, lungs, livex, spleen, pancreas, adrenal, thyroid and parathyroid glands, brain, stomach, large and small intestines, tongue, testis and major arteries.

The blocks were fixed for 24 - 48 hours in 10% neutral formalin, then dehydrated, cleared and embedded in paraffin wax in the usual way.

Routine sections from every case were stained with haematoxylin and/

and eosin. To demonstrate with greater clarity any vascular changes, all kidney and other selected sections were stained by the picro-Mallory method.

To study the nature of vascular changes in detail, the following histochemical techniques were used on selected sections: Periodic acid Schiff, Phosphotungstic acid haematoxylin, Weigert's elastica, Van Gieson, Verhoeff van Gieson, Dunn's orange and blue.

The following blood biochemical estimations were carried out on most of the dogs.

1) Blood urea. (Varley, 1958)
2) Total protein. (Varley, 1958)
3) Albumin/globulin ratio. (Paper electrophoresis)
4) Serum bilirubin. (Jendrassik and Grof, 1938)
5) Alkaline phosphatase. (King and Wootton, 1956 and Bessey, Lowry and Brock, 1946).
6) Serum glutamic oxaloactic transaminase and serum glutamic pyruvic transaminase. (Sigma Tech. Bull No. 505)
7) Inorganic phosphate. (Fiske and Subbarow, 1925)
8) Chloride. (E.E.L. Chloride Meter Operating Instruction Book)
9) Sodium. (Varley, 1958)
10) Potassium. (Varley, 1958).

Examination of urine samples from many of the dogs was also carried out/

out. The urine protein content was determined by precipitation with salicylsulphonic acid and the turbidity was estimated visually against a set standard (King, 1947). The urine urea content was determined by the hypobromite method. Glucose content was measured by Benedict's method and the presence of ketones detected by Rothera's test.

A deposit was obtained by centrifugation of 10 ml. of urine at 1000 r.p.m. for 5 minutes and examined microscopically for the presence of cells and tubular casts. A drop of urine was examined by dark ground microscopy for the presence of leptospirae.

Blood samples were taken for serological examination. The agglutination-lysis test of Schuffner (Wolff, 1954) was employed to detect antibodies to leptospirae. Strains of both Leptospira canicola and Leptospira icterohaemorrhagiae were used as antigens in the test. Serum dilutions of of 1/10, 1/30, 1/100, 1/300, 1/1000, 1/3000, 1/10,000, and 1/30,000 were tested.

Routine haematological examinations were also carried out. These will not be described or reported as the haematological features of leptospiral nephritis have been thoroughly described by McIntyre (1954) and Wettimuny (1963).

Controls.

As a control, the blood vessels of 125 non-nephritic dogs were examined. These animals, though affected by a variety of disease conditions/

conditions, all had completely normal kidneys. They were selected from four age groups, so that the same number of nephritic and non-nephritic dogs were studied in each group. These groups were:

1) < 2 years.

2) 3 - 6 years.

3) 7 - 10 years.

4) 11 years and over.

The blocks of tissue available from the dogs varied in number and distribution. Sections of kidney and other selected sections were stained by the picro-Mallory method.

Pathological Classification of Three Phases of Interstitial Nephritis.

The 130 cases of severe interstitial nephritis were divided into three groups on the basis of the renal pathology. These were acute, sub-acute and chronic stages of nephritis. The number of cases in each group were as follows:

Phase of Nephritis	No. of Cases.
Acute	20
Sub-acute	18
Chronic	92

KIDNEYS.

Acute Nephritis.

In the acute phase, the kidneys were pale, swollen and nodular in appearance. The cortices contained numerous soft whitish nodular foci which bulged from the cut surface. Often these nodules were especially concentrated around the cortico-medullary junction. The capsules stripped easily from the kidneys and sometimes petechial haemorrhages were present over the cortical surfaces.

Microscopically, the kidneys showed an intense cellular reaction, mainly confined to the interstitial tissue of the cortices where much of the renal parenchyma was destroyed. The infiltrating cells were almost entirely mononuclear, with plasma cells predominating. Lymphocytes were also numerous and macrophages were present in lesser numbers. These/

These cells were generally arranged in nodular foci, in the centre of which a few polymorphonuclear leucocytes could often be found. Elsewhere, the mononuclear cells were scattered diffusely and less densely throughout the interstitial tissue of the cortices. Where the reaction was most pronounced, extensive destruction of tubules was apparent, with small groups of necrotic epithelial cells scattered among the mononuclear cells. Tubules adjacent to the reactive foci often showed evidence of compression, with the lumina obliterated and the epithelium degenerate. The glomeruli were normal in all cases except one. Hyaline and cellular casts were present in most of the surviving tubules. In the medulla, interstitial reaction was present around the boundary zone in the most severe cases.

Sub-Acute Nephritis.

In the previous descriptions of interstitial nephritis in dogs, McIntyre and Montgomery (1952), Platt (1952) and Wettimuny (1963) divided the disease into acute and chronic stages. Bloom (1954) included description of a sub-acute phase, in which progressive renal fibrosis was accompanied by "renewed" infiltrations of lymphocytes and plasma cells. In the present study, there emerged a distinct group of cases which could best be classified as sub-acute.

In sub-acute nephritis, the kidneys were pale, swollen and nodular. On section, fine radial bands of fibrous tissue could be distinguished between the cellular foci, causing contraction with pitting of the cortical surface. Sometimes the capsule was adherent at these points.

Histologically, the renal cortices contained many focal areas of reactive mononuclear cells of which plasma cells formed the majority. Interspersed between these aggregations were narrow radial bands of immature fibrous tissue, where remnants of destroyed tubules were often present. Periglomerular fibrosis involving Bowman's capsules was a fairly frequent feature. These kidneys contained small areas of perfectly normal renal tissue between the foci of cellular and fibrous reaction.

The most striking and distinguishing feature of the sub-acute phase was observed in the capillary tufts of the glomeruli. In most cases, a proportion of the glomeruli contained globular deposits of fibrinous material within the capillary loops. In a few of the glomeruli, several such deposits were present, becoming conjoined with collapse and fibrosis of the tuft. These changes formed the subject of a special study and will be described in more detail later.

Chronic Nephritis.

In chronic nephritis, the kidneys had a characteristic pale shrunken appearance, with irregular pitting of the cortical surfaces. The capsule was sometimes adherent and difficult to strip. The consistency of the renal tissue was always abnormally firm and a marked reduction in cortical depth was readily appreciable. Dense radial bands of fibrous tissue were clearly visible. In some cases, hydronephrosis of moderate degree was present and frequently small cystic dilatations/

dilatations could be seen in the cortices.

Histologically, the outstanding renal lesion was extensive fibrosis with replacement of functional nephrons. Large areas of a vascular fibrous tissue were often present in the cortices. Where the process was less advanced, glomeruli survived among immature fibrous tissue, remnants of tubules and small numbers of plasma cells and lymphocytes. Small scattered accumulations of mononuclear cells occurred throughout the cortices, without ever forming the dense nodules found in the acute and sub-acute stages. Periglomerular fibrosis was pronounced and the glomerular rufts frequently contained fibrinous deposits, with fusion, collapse and fibrosis in more severely affected glomeruli. Occasional glomeruli showed a different type of change, characterised by gross distension of the capsule and collapse and atrophy of the tuft. In a small proportion of cases, this lesion affected very many of the glomeruli, while fibrinous deposits were minimal or absent. Again, these capillary lesions will be described in detail later. Surviving tubules in the cortex and medulla often showed hypertrophic and hyperplastic changes, becoming dilated and forming double or treble layers of epithelium. Protein casts were present in most of the tubules.

Alimentary Lesions.

Many dogs in the terminal stage of nephritis, whether acute, subacute or chronic, showed ulceration of the oral and gastric mucosae. The tongue was frequently involved, with ulceration around the anterior and/

and lateral borders. Occasionally the tip of the tongue sloughed off completely. Often the buccal and lingual surfaces were also ulcerated and the mouth showed brown discolouration. In the stomach, the mucosa of the fundus was usually congested and often showed haemorrhagic erosions, varying from pinhead size to large ulcers of several centimetres diameter. The stomach generally contained brown mucoid fluid.

The Heart and Great Vessels.

Acute necrotizing endocarditis affecting the left atrium was found in many of the nephritic dogs. This was often associated with necrotizing enderteritis in the origins of the pulmonary artery and aorta. The lesion was seen as pale crumbling deposits, adherent to areas of necrosis in the endocardium, as in Figs. 1 & 2. Histologically, an intense acute inflammatory reaction was present, with superficial thrombus formation. The relative incidence of this lesion in each phase of nephritis is shown below.

Incidence of Necrotizing Endocarditis in Dogs

Incidence of Necrotizing Endocarditis in Dogs

with Interstitial Nephritis.

Phase of Nephritis	No. with data available	No. showing Necrotizing Endocarditis	% showing Necrotizing Endocarditis
Acute	15	10	66
Sub-Acute	11	7	64
Chronic	59	19	33

In four chronic cases, scar tissue was observed in the wall of the left atrium, suggesting the healed stage of a previous acute necrotizing lesion.

In six of the twenty dogs with acute nephritis, areas of necrosis and inflammation were present within the myocardium. These were cases 6337, 3387, 15790, 22329, 23689 and 23045. In only one case, 22329, was the lesion appreciable macroscopically. In this dog, the myocardium contained large areas which were almost white in colour, surrounded by a zone of congestion and haemorrhage. Histologically, the myocardium contained foci of intense polymorphonuclear leucocyte reaction with necrosis of muscle fibres. (Figs. 3 & 4). The severity varied in the individual cases. In case 22329, almost no part of the myocardium could be found which was entirely free of cellular infiltration. The lesion showed no special distribution and the inflammatory foci were scattered throughout the heart muscle.

In three chronic cases, nos. 7478, 15425 and 17312, the heart contained areas of myocardial fibrosis and in one, 8252, small foci of acute myocarditis were present.

Myocardial inflammation and necrosis do not appear to have been described previously in dogs with nephritis.

In chronic nephritis, hypertrophy of the myocardium of the left ventricle was readily appreciable in most cases.

Skeletal Changes.

Osteodystrophia fibrosa was noted clinically and post-mortem in 13 (14%) of the dogs with chronic nephritis.

RESULTS.

The Incidence of Vascular Lesions in Dogs with Interstitial Nephritis.

Details of the age, sex, breed, significant serological, biochemical and urological features and the distribution of vascular lesions in the individual dogs are shown in Appendix 1.

The incidence of intra-renal arterial changes in each phase of nephritis is shown in the following table.

Incidence of Intra-Renal Arterial Lesions in Dogs with Interstitial Nephritis.

Phase of Nephritis	No. of cases examined	No. showing arterial changes	% showing arterial changes.
Acute	20	10	50
Sub-Acute	18	8	55
Chronic	92	78	85
Total	130	96	74

The incidence of glomerular capillary lesions in each phase of nephritis was as follows:-

Incidence of Glomerular Capillary Damage in Dogs with Interstitial Nephritis.

Phase of Nephritis	No. showing glomerular lesions	% showing glomerular lesions.
Acute	1	5
Sub-Acute	11	61
Chronic	92	100

The Nature of the Vascular Changes.

The nomenclature applied by most authors to vascular changes associated with renal disease includes the terms fibrinoid necrosis, hyaline arteriosclerosis, arterial hyalinosis and hyaline degeneration. Fibrinoid necrosis is generally thought to be an acute change in which the smooth muscle of the vessel wall undergoes necrosis, associated with accumulation of fibrinous material in the media. The hyaline lesions are considered to represent a less acute change, in which the vessel wall becomes impregnated with and gradually converted to an amorphous hyaline material. On the basis of experimental results, Goldblatt (1938) concluded that uraemia is necessary in addition to hypertension to produce fibrinoid necrosis, while hyaline change develops with gradually increasing "benign" hypertension.

Muirhead, Turner and Grollman (1951B) and Montgomery and Muirhead (1953) considered that vascular changes in hypertension result from necrosis of smooth muscle cells. They found that the hyaline deposits had the same staining properties as smooth muscle and that the more intense reactions of hyalin were due to "unmasking" of normal constituents by a change in the physico-chemical state. Sub-endothelial accumulations of hyalin were thought to be derived from the media.

Duguid and Anderson (1952) proposed a very different pathogenesis for hypertensive vascular changes. They considered that material from the blood stream is deposited on the endothelium and then becomes covered/

covered by the endothelium so that the vessel is recanalised and the deposit incorporated into the vessel wall. On this basis, the largest lesions causing the greatest occlusion would be in the early phase. The authors illustrated their studies with photographs of hyaline deposits on the luminal surface of vessels with no apparent endothelial covering.

Smith (1955), largely on the evidence obtained from PAS-stained sections, concluded that hyalin results from degenerative change in the basement membrane and ground substance of an arteriole. Duguid and Anderson (1952) considered that since affected vessels do not become dilated, the lesion could not have a degenerative basis.

McKinney (1962) also studied the histo-chemistry of hyalinosis. He considered that hyalin is first deposited on the endothelium as fibrin and that it is then covered by the endothelium and gradually organized, eventually appearing as a collagen-like substance.

Lendrum, Fraser, Slidders and Henderson (1962) pointed out the limitations of existing methods of staining fibrin and described new histochemical techniques which gave almost automatic identification of fibrin in vascular lesions. The hypertensive diabetic kidney (Lendrum, 1963) was used as a model in which the complete range of changes could be studied. The vascular lesions were likened to those found in zones of infarction, in the lungs of patients with mitral stenosis,/

stenosis, in systemic hypertension and in systemic lupus erythematosus. The changes in all of these conditions were considered to result from the extrusion of plasmatic substances, including fibrin, through the vascular endothelium. In hypertension, the severity of the lesion would depend on the level of blood pressure, which would determine whether the fibrinous material traversed the internal elastic lamina, the media, the external elastica, to reach finally the advential tissue, as in the explosive lesions of malignant hypertension. Study of the aging of fibrin revealed that early deposits stained with medium molecular weight acid dyes. Later a transitional phase was apparent and the plasmatic substances came gradually to stain as collagen, with large molecular weight dyes. This change in staining affinity was attributed to alteration of the fine structure of the deposits. Lendrum discarded the term "fibrinoid" and adopted the name "plasmatic vasculosis," suggested by Goldblatt, to describe the fibrinous lesions in hypertension and collagen diseases in the human. To distinguish the older deposits which stained as collagen, the term "pseudo-collagen" was employed.

The nature of hyalin in arteriolar sclerosis was investigated using electron microscopy by McGee and Ashworth (1963), whose conclusions differ from those of Lendrum. The basis of the lesion appeared to be an excessive production of basement membrane material. In the initial phase, this was deposited mainly by endothelial cells and later also/

also by smooth muscle cells of the media. Eventually, the muscle cells were thickly encased in membrane material and became atrophic. Non-periodic filaments were present between the cells and a few collagen fibres were identified. The thickened basement membrane material coincided with PAS-positive hyalin seen by light microscopy.

As stated earlier, in chronic interstitial nephritis in dogs, many of the glomeruli undergo a change which terminates in conversion of the entire glomerulus to a mass of collagen-like material, resembling the substance termed "pseudo-collagen" by Lendrum (1963). Glomerular scar tissue has been studied in several diseases in the human. Farquhar, Vernier and Good (1957) examined by electron microscopy the glomeruli of patients with nephrosis, glomerulo-nephritis and lupus erythematosus. In the two latter conditions, an increase in thickness of the capillary basement membrane was observed. At a late stage, hyalinized glomeruli were composed of basement membrane-like material and a few atrophic cells. In contrast, Spiro (1959), also studying the electron microscopic appearance of diseased glomeruli, noted the production of collagen fibres in chronic glomerulo-nephritis.

Jones (1963) studied 3-dimensional reconstructions of glomeruli, made from photographs of thin serial sections. The scar tissue in chronic glomerulo-nephritis was found to have three components - excessive basement membrane material produced by epithelial and endothelial cells, sponge fibres possibly laid down by cells of the glomerular stalk and a hyaline substance of unknown origin.

The differing interpretations of vascular changes based on light and electron microscopic appearances demonstrate a need for further correlated study of the fine structure and histochemical properties of the lesions throughout their development.

In the present study, vascular changes of the fibrinoid and hyaline types were observed. However, there was no clear differentiation between these forms and many intermediate stages were encountered. This suggested that the lesions represent a spectrum of changes with similar aetiology and that the morphology observed is dependent on the severity of the causal factors. The separate terms "fibrinoid necrosis" and "hyaline arteriosclerosis" could not be applied accurately to the pattern of changes found in the renal vessels. The term "plasmatic vasculosis" has the advantages of covering the range of changes and also providing a precise indication of the probable pathogenesis and nature of the lesion. For these reasons it will be used in the description of the present findings.

In the series of nephritic kidneys, plasmatic vasculosis was found in all phases of the disease. Arcuate and interlobular arteries, afferent arterioles and glomerular capillaries were affected. The smallest lesions consisted of focal sub-endothelial deposits of amorphous brightly eosinophilic material which stained strongly for fibrin and in such cases the internal elastic lamina was intact. (Fig. 8). Sometimes larger deposits protruded into the lumen, apparently causing considerable reduction in patency. (Fig.9). In more advanced lesions, the internal elastic lamina was/

was ruptured and the plasmatic material extended into the media, with focal necrosis of smooth muscle cells. (Fig.10). In some vessels the entire circumference was affected, with total necrosis of the media, leaving a mass of fibrin, pyknotic nuclei and a few inflammatory cells. (Figs. 11 & 12). The most dramatic changes occurred in vessels in which the external elastic lamina also ruptured, with leakage of the plasmatic substances into the periarterial tissues, giving a splashed appearance. (Figs. 13 - 18). Sometimes there was a polymorphonuclear leucocyte reaction in and around the lesion, (Fig.19) though often no such reaction was present.

In sub-acute and chronic cases, plasmatic vasculosis often occurred in conjunction with hypertrophic and hyperplastic changes in the arterial wall. In such cases, the muscle of the media was considerably hypertrophied and surrounded by hyperplastic adventitial connective tissue, giving the vessel a characteristic thickened and whorled appearance. Plasmatic deposits were often present within the media of such vessels. (Figs. 20 & 21). In some cases, thickening of the media and adventitia occurred without plasmatic vasculosis.

The histochemical reactions of the plasmatic deposits showed a number of interesting features. In most cases of acute nephritis, the material stained strongly positive for fibrin, appearing blue with PTAH, (Fig. 22) red with picro-Mallory (Figs. 12 - 17) and strongly PAS-positive. In sub-acute and chronic cases, variable reactions occurred./

occurred. With PTAH, the deposits stained blue or purple in some instances and brown in others. (Figs. 22 & 27). With picro-Mallory, the deposits were either wholly red or pale blue or showed a mixed red and blue reaction. (Figs. 23 - 26). In the studies of Lendrum et al (1962) on the aging of fibrin, it was found that the plasmatic material first stains strongly as fibrin but later loses this property and comes gradually to stain as collagen. Hence the name "pseudo-collagen" was applied to describe the deposit at this stage. The variable staining reactions of the vascular lesions in sub-acute or chronic nephritis may also be due to differences in the age of the fibrin. This is strongly suggested by the alteration from red to blue staining with picro-Mallory.

In each phase of nephritis, the severity of plasmatic vasculosis and the number of vessels affected varied greatly. The incidence figures in the table include all grades of frequency and severity. In each group, approximately half of the cases showed severe or moderately severe lesions involving many renal vessels.

Intra-Renal Vascular Changes in Acute Nephritis.

In acute nephritis, plasmatic vasculosis was found in arcuate and interlobular arteries and afferent arterioles. The glomerular capillaries were not generally involved. In many cases, only a small proportion of vessels were affected and in kidneys in which the most severe changes were present, nearby vessels were often normal. The plasmatic deposits always stained strongly positive for fibrin. No hypertrophic changes were/

were found, suggesting that the vascular damage was of acute onset and short duration.

Intra-Renal Vascular Changes in Sub-Acute & Chronic Nephritis.

In sub-acute and chronic nephritis, plasmatic vasculosis was present at all levels of the vascular tree from the arcuate arteries to the glomerular capillaries and all grades of severity occurred.

In the glomeruli, globular deposits of fibrinous material were very frequently present in the tufts. These were seen as spherical amorphous eosinophilic droplets within the capillary loops. (Fig. 28). Often several deposits were present in a single glomerulus, with adhesion of the peripheral lesions to the capsule. Staining reactions were strongly positive for fibrin at this stage. As more of the tuft became involved, the deposits became conjoined and the capillary loops collasped. In such lesions, the staining properties were variable, with mixed red and blue reaction to picro-Mallory. (Fig. 29). Finally, the entire glomerulus became fibrosed and shrunken, staining as collagen. (Fig. 30). In chronic cases, such glomeruli were often very small, gradually merging with surrounding fibrous tissue. This suggested that some glomeruli might disappear altogether.

In the arteries and afferent arterioles, plasmatic deposits showed variable staining reactions and a "pseudo-collagen" appearance was very common.

Hypertrophic and hyperplastic changes were found more frequently in chronic than in sub-acute cases and generally involved interlobular arteries and afferent arterioles. The walls of affected vessels were often thickened to twice or three times normal size by hypertrophy of the media and hyperplasia of the adventitial tissue. The presence of plasmatic vasculosis was variable in these vessels and when present was seen as accumulations in the intima and media, usually of the pseudo-collagen type. In some chronic cases it appeared that the thickened arteries ran in a tortuous course, as many cross-sections could be seen within a very small area.

Another frequent finding in chronic nephritis was small areas of fibrosis of the media in lobar, arcuate and interlobular arteries. In some cases, duplication of the internal elastic lamina was present in larger arteries.

Vascular Changes in Other Organs.

The organs from which blocks were available for histological examination varied considerably in number in the individual cases.

Details of the incidence and distribution of vascular lesions in individual dogs is shown in Appendix I.

Plasmatic vasculosis was found very frequently in the arterioles of the splenic follicles. These vessels were the subject of a special investigation, which will be described later.

Arteries in the myocardium, gastric mucosa and muscle of the tongue showed plasmatic vasculosis in a small proportion of cases. (Figs. 31, 32 & 33). These findings are summarized below. In all of these cases, the renal vessels were also affected.

Incidence of Plasmatic Vasculosis in Extra-renal Sites.

Organ	No. examined	No. showing plasmatic vasculosis	% showing plasmatic vasculosis
Heart	87	13. 2 acute 1 sub-acute 10 chronic	15
Stomach	60	4. 1 acute 3 chronic	6.7
Tongue	39	4. 2 acute 1 sub-acute 1 chronic	10.2

Incidence of Plasmatic Vasculosis in Dogs with Normal Kidneys.

Histopathological examination of 125 non-nephritic dogs with the same age distribution as the nephritic animals revealed that plasmatic vasculosis is rare in the absence of renal disease. This conclusion applied to blood vessels in organs other than the spleen, which will be discussed later.

Details of the individual dogs and the incidence and distribution of vascular lesions is shown in Appendix 2.

Small focal plasmatic lesions were found in occasional arteries in the kidneys of three dogs and in the myocardium of one dog. The details of these cases are summarized in the following table.

Plasmatic Vasculosis in Dogs with Normal Kidneys.

Case	Age	Sex	Diagnosis	Plasmatic Vasculosis.
25228	6	M	Pericarditis	Kidney. 1 interlobular arte and 2 glomeruli contained small focal fibrinous deposi
17393	9	F	Tonsillar Carcinoma	Kidney. 1 interlobular arte showed a small focal lesion.
17274	14	M	Splenic Haemangioma	Kidney. Occasional inter-lobular arteries contained small focal lesions.
24254	3	F	Endocarditis	Myocardium. 1 small artery contained a fibrinous accum-ulation in the media.

This survey demonstrates that plasmatic vasculosis (in organs other than the spleen) is an uncommon sporadic finding in non-nephritic dogs, which may be related to individual differences in strength of the intima of small arteries.

Relationship between the Incidence of Plasmatic Vasculosis and Age.

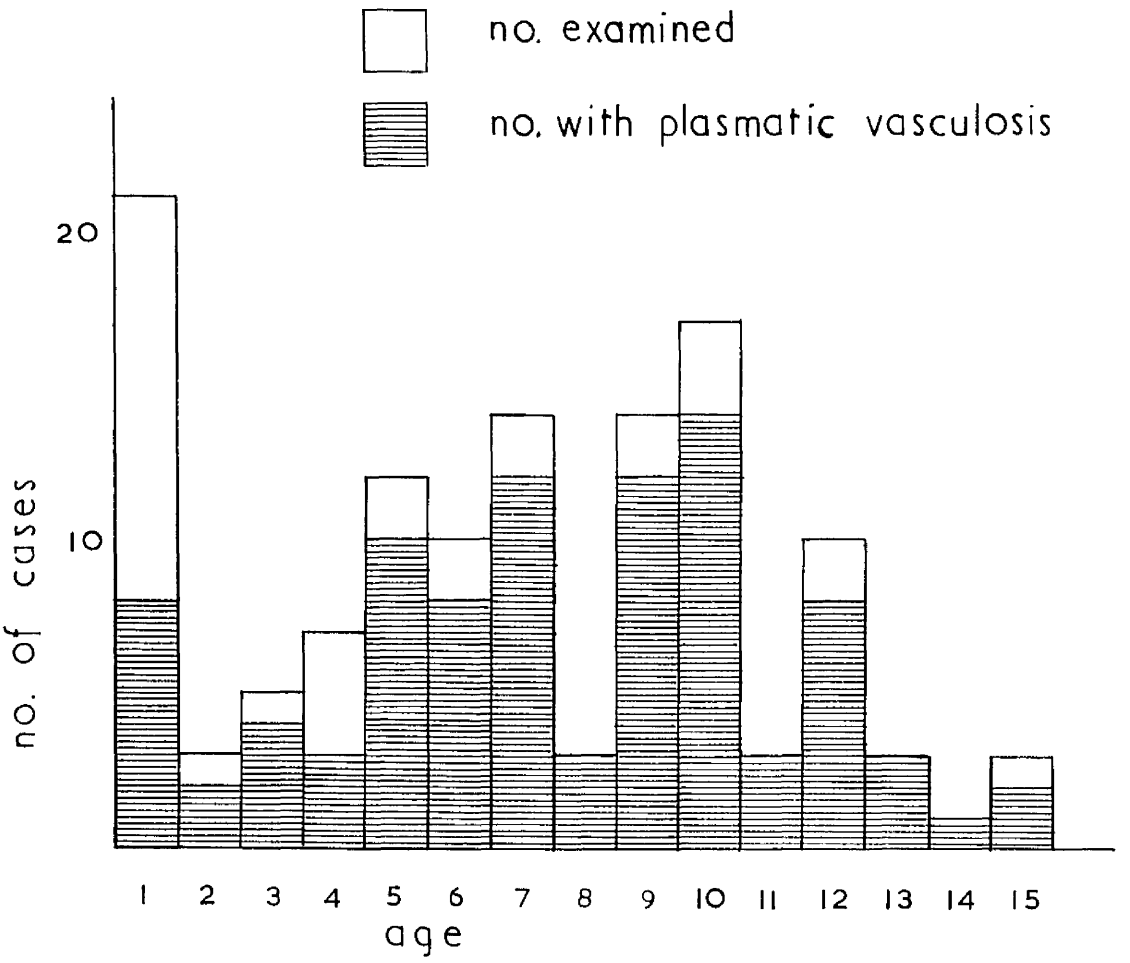
Smith (1955) found arteriosclerosis of the afferent arterioles in the kidneys of both normotensive and hypertensive human patients. The incidence and severity of the lesion increased with age and in association with hypertension. In the present study, the possibility of an association between the age of dog and frequency of vasculosis was investigated.

The number of nephritic dogs examined and the incidence of vascular lesions in relation to age is shown in the histogram overleaf.

This correlation shows that the incidence of vasculosis is not influenced by the age of the dog. In the first year of life, lesions were found in only 7 out of 20 dogs but thereafter the incidence rose sharply, to run in parallel with the incidence of nephritis. In the first year, most cases were of acute nephritis, which might complicate the interpretation of the lower number of affected dogs in this group. Acute nephritis may be less likely to give rise to vascular damage than factors associated with the more chronic phases of the disease.

There was no apparent relationship between age and the severity

of/



Correlation of the incidence of plasmatic vasculosis with age. After the first year, the incidence runs in parallel with age, indicating that the development of vasculosis is not influenced by the age of the dog.

of vasculosis. At all ages, a wide range was found in the number of vessels affected and the degree of vascular damage.

Examination of 125 non-nephritic controls also indicated that dogs have no predictable tendency to develop vascular changes, in organs other than the spleen, with increasing age. The four dogs in which small lesions were found were of widely differing ages.

Relationship between the Incidence of Vasculosis and Sex.

In normotensive humans, there is a higher incidence of vascular lesions in the male (Smith, 1955). In hypertensive subjects, this sex difference disappears and the female exhibits vascular changes of equal frequency and severity, suggesting a greater susceptibility to the effects of hypertension.

The possibility of any special predisposition to vascular damage was investigated in the present series of nephritic dogs. The proportions of male and female animals in which plasmatic vasculosis was found are tabulated below. The numbers include all animals whose sex had been recorded.

Incidence of Plasmatic Vasculosis in Nephritic Dogs in Relation to Sex.

	No. examined	No. with Vasculosis	% with Vasculosis
Male	97	70	72.2
Female	31	24	77.4
M/F ratio	3:1	3:1	

These figures show no sex difference in the incidence of vascular lesions. The percentages of male and female animals with vasculosis and the male/female ratios of examined and affected animals are very similar.

The series of 125 control dogs did not reveal any sex difference in susceptibility to vascular damage among non-nephritic animals. Of the four animals in which vasculosis was found, two were male and two female. The incidence of the lesion too low to demonstrate any predisposition.

It may be concluded from these findings that the sex of a dog does not influence the frequency of vascular lesions, whether or not nephritis is present.

Relationship between Incidence of Plasmatic Vasculosis and Blood Urea Levels.

The aetiology of vascular damage associated with human renal disease is generally attributed to hypertension. However, the evidence concerning the development of hypertension in nephritic dogs is inconclusive and no one has correlated blood pressure measurements with histopathological findings in such animals. Plasmatic vasculosis, found so frequently in nephritic dogs, cannot be assumed to be of hypertensive origin. Goldblatt (1938) from the results of experiments in which hypertension was induced in dogs by constriction of renal arteries, concluded that renal failure was necessary in addition to hypertension to produce severe fibrinoid necrosis./

necrosis. In the experimental dogs, moderate constriction of the renal arteries led to "benign" hypertension associated with arterial hyalinosis and hypertrophy. More severe constriction caused "malignant" hypertension with renal failure and widespread fibrinoid necrosis.

The incidence of vasculosis in the nephritic dogs of this series was compared with the blood urea levels (in cases where these records were available) to establish any positive relationship.

The result of this investigation is summarised in the following table.

The Incidence of Plasmatic Vasculosis in Relation to the Level of Uraemia.

Stage of Nephritis	Level of Uraemia (mg/100 ml)	No. with Vasculosis	No. without Vasculosis
Acute	50 - 100	1	0
	100 - 200	1	3
	200 - 300	1	1
	300 - 400	2	0
	> 400	5	2
Sub-Acute	50 - 100	3	2
	100 - 200	1	3
	200 - 300	1	0
	300 - 400	0	0
	> 400	3	2
Chronic	50 - 100	9	0
	100 - 200	13	1
	200 - 300	10	1
	300 - 400	4	1
	> 400	13	6

These results show that there is no significant difference in the incidence of plasmatic vasculosis between moderately uraemic and severely uraemic dogs with acute and sub-acute nephritis.

In chronic nephritis, a high incidence of vasculosis is reflected at all levels of uraemia. However, in a substantial number of dogs with blood urea > 400 mg/100 ml., vasculosis was absent. It may be suggested that in these animals the disease progressed to the terminal phase too rapidly to allow the development of vascular changes. Histologically, the kidneys in these cases showed an atypical lesion affecting many of the glomeruli, in which the capsules were grossly dilated and the tufts collapsed and atrophic. (Figs. 34, and 35). Clinical evidence, which will be discussed later, suggests that the course of the disease was unusually acute in such dogs.

It is clear that vasculosis and uraemia develop concurrently in dogs with interstitial nephritis. As the series did not include dogs with blood urea below 50 mg/100 ml. it is not known whether vasculosis may occur in the absence of uraemia. Therefore no conclusion can be drawn as to whether the incidence of vascular damage is influenced by uraemia.

The conclusions drawn from these figures may be questioned on the grounds that only one urea estimation was made on many dogs and the samples were taken at varying periods before death. However, in general the examination was made within a few days of death or euthanasia and the figures give an approximate guide to the terminal degree of uraemia.

Comparison of the Clinical Features of Nephritic Dogs with Vasculosis and with Normal Blood Vessels.

The clinical records of the nephritic dogs were studied to find any difference in the syndrome as it occurred in dogs with vascular damage and in those with normal blood vessels. Typical case histories from dogs in each group are outlined below.

Case 21540. Chronic Nephritis without Vascular Damage.

This was a fifteen-year-old female labrador, presented with a history of progressive loss of condition over the previous two months. During this time, the dog was excessively thirsty and vomited occasionally.

On admission to the hospital, the dog appeared dull and showed ulceration of the tongue and brown discolouration of the mouth. The pulse volume was poor. During the next six days, the dog's condition deteriorated, showing marked thirst, polyuria, anorexia and frequent vomiting. The level of blood urea was 710 mg/100 ml. and the inorganic phosphate 18.2 mg/100 ml. The serological titre to L. canicola was negative. Terminal chronic nephritis was diagnosed and euthanasia performed.

Pathologically, the kidneys showed advanced fibrosis. Small foci of necrotizing endarteritis affected the origin of the aorta. Small haemorrhagic erosions were present in the gastric mucosa. Histologically, diffuse renal fibrosis was observed. The intra-renal arteries were normal. Many glomeruli showed gross dilatation of the capsule, with collapse and atrophy of the tuft.

Case 19397. Chronic Nephritis with Severe Arterial and Glomerular Damage.

This was a seven year old male cairn, presented with a history of rapid loss of weight during the previous two weeks. According to the owner, the dog had shown excessive thirst for several years and generally drank two pints of water every day. Polyuria had been noted. The dog had vomited once daily for the past 3 - 4 months.

On admission to the hospital, the dog was very dull. Halitosis was marked and the mouth discoloured. The pulse volume was poor. During the following five days, the dog showed progressive apathy and remained anorexic, very thirsty, polyuric and vomited frequently. The blood urea was 540 mg/100 ml. and the inorganic phosphate 13.5 mg/100 ml. The serological titre to L. canicola was 1:10. Death occurred on the fifth day.

Pathologically, the kidneys showed advanced fibrosis and the gastric mucosa was ulcerated. Necrotizing endocarditis was absent. Histologically, typical features of chronic interstitial nephritis were observed. Plasmatic vasculosis affected many intra-renal arteries and plasmatic deposits occluded large numbers of glomeruli. The majority of the damaged glomeruli appeared as amorphous masses of pseudo-collagen material.

It is apparent from these typical case reports that the clinical features of both dogs were very similar while in the hospital. Routine examinations/

examinations did not reveal significant differences between dogs which proved to have severe vascular changes and those with normal blood vessels. However, if the owner's observations were reliable, it would seem that the course of the disease was much shorter in the dog which did not show vascular damage.

In the series of dogs with chronic nephritis, a small proportion were found in which the usual plasmatic form of glomerular damage was absent or affected only a very few glomeruli. Instead, large numbers of glomeruli showed gross distension of the capsule and collapse and atrophy of the tuft. (Figs. 34 and 35). The small arteries and arterioles in these cases were usually normal, or showed very mild vasculosis. As shown in the preceding investigation, the blood urea may be extremely high in such dogs. This variation on the typical syndrome may represent a more fulminating form of nephritis, in which rapidly progressive fibrosis occludes many tubules, resulting in distension of Bowman's capsules and collapse of the tufts and terminating in acute uraemia.

Further evidence for this hypothesis derives from the study of chronic nephritis in dogs one year old or younger. These were case nos. 19961, 16573, 10447, 23571 and 27108. The ages of 16573 and 10447 were 6 months and 5 months respectively and the others were approximately one year old. In these dogs, the disease progressed to the terminal phase within a few months and the renal histopathology is of considerable interest./

interest. In every case, with the exception of 19961, large numbers of glomeruli showed distension of the capsule and collapse of the tuft, while plasmatic changes were minimal or absent. The arteries were normal in all except 23571, which showed very mild vasculosis.

In conclusion, the combined evidence of the clinical and pathological studies and the correlation of vasculosis with blood urea levels indicates that the renal histopathology may reflect differences in the rate of progression of the disease and that the less typical glomerular changes described above occur with very rapidly developing renal fibrosis. The characteristic plasmatic vascular changes found in most cases may be a feature of more protracted chronic nephritis.

Discussion. The Significance of Vascular Damage in Dogs with Interstitial Nephritis.

Discussion. The Significance of Vascular Damage in Dogs with Interstitial Nephritis.

The survey of blood vessels in dogs suffering from severe interstitial nephritis has shown that vascular damage is extremely common in such animals, particularly in chronic nephritis. The considerable incidence of plasmatic vasculosis found in acute nephritis is of special interest, as there appears to have been no previous reference to arterial lesions at this stage of the disease.

Morphologically, the vascular changes were found to be the same as those encountered in hypertension in the human. It was clearly established that the lesions do not occur as a normal age change (in organs other than the spleen) and that there is no sex predisposition. In view of these findings, it is probable that plasmatic vasculosis in nephritic dogs is caused by hypertension and that the well-known relationship in the human between renal disease, hypertension and arterial damage also occurs in the dog.

Further evidence for the hypertensive origin of plasmatic vasculosis derives from the results of experimental studies carried out in dogs. In the classical experiments of Goldblatt (1934), hypertension was produced by partial occlusion of the renal arteries by means of silver clamps. In the dog, constriction of one renal artery was followed by a moderate or slight rise in blood pressure, which tended to return to the/

the control level within a few weeks. Moderate constriction of both renal arteries led to persistant hypertension without renal dysfunction and this was considered to resemble the hypertension of benign nephrosclerosis in the human. Severe constriction of both renal arteries (or unilateral constriction and contralateral nephrectomy) produced a very great increase in blood pressure and renal failure. This condition was thought to resemble malignant nephrosclerosis in man. Vascular damage was found in the hypertensive dogs in organs other than the kidneys, which were protected by the arterial clamps from the increased pressure (Goldblatt, 1938). Hyalinosis was found in dogs with moderate hypertension, while fibrinoid necrosis occurred in severely hypertensive animals. Goldblatt concluded that renal failure was necessary in addition to hypertension to produce fibrinoid necrosis. Perhaps the most significant observation in this work was that no vascular lesions developed in the protected kidneys, indicating the hypertensive origin of arterial damage in other organs.

The results of these experiments have stimulated a vast amount of research, which continues to the present time, directed towards elucidating the mechanism of nephrogenic hypertension in animals rendered hypertensive by Goldblatt techniques. It is not within the scope of the present studies to discuss the evidence on this subject.

One further series of experiments which is of direct relevance to the consideration of vascular changes in nephritic dogs was carried out by/

by Muirhead, Vanatta & Grollman (1949), Muirhead, Turner & Grollman (1951A; 1951B), Muirhead & Grollman (1951) and Muirhead, Stirman, Jones, Lesch, Burns & Fogelman (1953). Dogs in which hypertension was produced as a result of bilateral nephrectomy, were maintained by dialysis. Vascular damage, described as "necrotizing arteriolitis" was commonly observed in the heart and alimentary tract and less frequently in many other sites in dogs kept alive for 3 - 19.5 days (Muirhead et al, 1949). The lesions were considered to be identical to those occurring in human malignant hypertension. In dogs kept alive for prolonged periods (up to 70 days) vascular lesions were more often of a hyaline type, though acute necrosis was also found in these animals (Muirhead et al, 1951A) Again, the changes were likened to those of human malignant hypertension. In a succeeding publication (1951B), the histochemical properties of the vascular lesions were described. It was concluded that the changes resulted from necrosis of the smooth muscle of the media and that the appearance was identical to that of human hypertensive lesions. These observations were reinforced by Montgomery and Muirhead (1953) who compared necrotic arterioles in the kidneys of hypertensive humans with arterioles in the bowel of dogs with experimental hypertension. No differences were detected in the morphology or staining properties of the lesions. Muirhead and Grollman (1951) described necrotic arteries and arterioles in the organs of a patient who died of acute renal insufficiency. Again, the lesions were found to be the same as those in bilaterally nephrectomised dogs.

In conclusion, it is clear that the vascular changes observed in dogs with interstitial nephritis closely resemble lesions associated with hypertension in the human and those produced as a result of experimental hypertension in the dog. This gives a clear indication that vasculosis in nephritic dogs is likely to be caused by hypertension.

PLASMATIC VASCULOSIS IN THE SPLEEN.

Introduction.

Plasmatic vasculosis frequently affects the small arteries and arterioles of the spleen, both in the human and the dog. This lesion has been well known for some time and has generally been termed "hyaline arteriolosclerosis" or "arteriolar hyalinosis." The literature concerning human splenic vascular changes has been reviewed recently by Dustin (1962).

The vessels of the splenic follicles develop plasmatic vasculosis more often than any other blood vessels in the body and may be affected in both normotensive and hypertensive individuals of almost any age. However, in the human it is known that the incidence and severity of the lesion increase with age and become especially pronounced in association with hypertension. This relationship has been clearly demonstrated by Smith (1956), who studied the blood vessels in the spleen, pancreas and other viscera from 678 consecutive autopsies. He found that the spleen showed hyaline arteriolosclerosis of mild degree in 31% of normotensive patients under the age of 20. The incidence and severity increased sharply with each succeeding decade, with 85% of all cases over the age of 40 affected. The corresponding percentages for the pancreas were 7% and 64.5% respectively. Other organs became affected later in life and to a lesser degree. In patients with benign essential hypertension, the incidence and severity of the vascular lesions/

lesions were increased at all ages. It is also of interest that Smith found the distribution of arteriolosclerosis to be uniform in each spleen by examination of sections from several blocks of tissue in each case.

Similar observations were reported by McKinney (1962), who compared the arterioles of the trabeculae, follicules and pulp of spleens from human patients. Spleens were examined from normotensive and hypertensive patients in different age groups. The vessels of the follicles were affected more frequently than other arterioles. The incidence and severity of arteriolar lesions were found to increase with age and with hypertension and the difference between hypertensive and normotensive subjects was most marked in the 11 - 30 year age group.

There have been few studies of the splenic vessels of the dog. Krause (1923) examined the spleens of 78 old dogs and found hyalinosis of the intima of small arteries regularly in dogs above 5 years of age. Not all of the arteries were affected and the ellipsoidal vessels were never involved. In severe cases, the media of hyalinized arteries were also infiltrated. Platt (1952), in describing cardiovascular changes in nephritic dogs, referred briefly to fibrinoid necrosis of the splenic arterioles, which he dismissed as a normal change associated with age. Dahme (1957) found hyalinization of the splenic vessels in dogs with chronic nephritis and in older normal dogs. Oka (1963) studied the vessels of the splenic follicles in 171 dogs, aged between 80 days and

18/

18 years. He found "hyaline degeneration" of these vessels in 51.5% of the dogs. The incidence and severity was clearly related to age, with 7% of the dogs under one year old affected, increasing to 100% at eight years of age. A high incidence was found in association with certain disease conditions, including carcinoma, diabetis mellitus, pyometra, renal diseases and dirofilariasis. However, no direct relationship was established between the arteriolar lesions and these diseases since the incidence of the latter increased with age. There was no significant sex difference in frequency of the lesion.

The pathogenesis of vascular changes in the spleen is uncertain. It has been suggested by Moritz and Oldt (1937) that hyalinization is an aging phenomenon found most frequently in the abdominal organs supplied by relatively large short branches of the aorta and that small blood vessels in these organs might be exposed to a particularly forceful pressure. The more pronounced changes which occur in association with hypertension were considered by Smith (1956) to result solely from the increased blood pressure. He found that hypertensive patients who died as a direct result of hypertension showed more severe arteriolar changes in the spleen than those who died of unrelated causes. The latter patients were considered to be in earlier phases of hypertension, suggesting that raised blood pressure preceded the aggravation of the vascular lesions.

No relationship has been demonstrated in the dog between plasmatic vasculosis/

vasculosis in the spleen and renal disease or blood pressure. Because of their particular susceptibility, the splenic arterioles form a useful indicator in the study of the vascular effects of renal disease and hypertension. To establish whether renal disease influences the frequency and degree of plasmatic vasculosis in the spleen of the dog, a comparative histopathological study of spleens from nephritic and non-nephritic animals was undertaken.

Materials and Methods.

All available blocks of spleen from dogs dying of severe interstitial nephritis were examined and grouped according to age. The control series comprised 80 dogs which died of non-renal conditions and in which the kidneys were normal on macroscopic and microscopic examination. These animals were selected to form four groups of 20 according to age. The age groups were:

1) 0 - 2 years.

2) 3 - 6 years.

3) 7 - 10 years.

4) 11 years and over.

Sections from each spleen were stained with haematoxylin and eosin and by the picro-Mallory method. The results of the survey were based on the findings on Mallory-stained sections, which demonstrate vascular changes with greater clarity.

Plasmatic vasculosis was graded according to the number of follicular vessels affected and the severity of the lesion. The grades were defined as follows:

Grade 0 No vascular abnormalities.

Grade + Small focal sub-endothelial fibrinous deposits affecting less than half of the arterioles.

Grade ++ Small or moderately large deposits present in approximately half of the arterioles.

Grade +++ Severe vasculosis, often with total or almost total occlusion of the lumen, affecting the majority of the follicular arterioles.

Examples of Grade +, ++ and +++ lesions are shown in figs. 37,38 & 39.

Results.

The comparative study of arterioles in the spleens of nephritic and non-nephritic dogs gave a number of interesting and significant results.

The following table shows the total incidence of vasculosis observed in each series of dogs. A considerably greater frequency was found in the spleens of dogs with nephritis.

TABLE I./

TABLE I.

Incidence of Plasmatic Vasculosis in the Spleens of Nephritic and Non-Nephritic Dogs.

Status	No.examd.	No.positive	% positive
Nephritic	71	54	76
Non-Nephritic	80	33	41.3

The second table summarizes the incidence of vasculosis in the two series in relation to age. This investigation confirmed the known tendency for arteriolar damage to occur in the spleens of older dogs. However, the lesion appeared at an earlier age in the nephritic group. Vasculosis was not found in the spleens of dogs under three years of age in the control series, while 20% of the youngest dogs with nephritis were affected. The association of nephritis with increased frequency of vasculosis was reflected at all ages, though this difference was most pronounced in the 3 - 6 yr. groups, where an increase of 60% over the expected incidence was observed in the nephritic dogs.

TABLE II./

TABLE II.

Incidence of Plasmatic Vasculosis in the Spleens of Nephritic and Non-Nephritic Dogs in Relation to Age.

Age	% Nephritic Dogs affected	% Non-Nephritic Dogs affected
< 2 yrs.	23.5	0
3 - 6 yrs.	85	20
7 - 10 yrs.	95	55
> 10 yrs.	100	90

The third table outlines the severity of plasmatic vasculosis observed in the splenic arterioles. At all ages, the degree of damage tended to increase in the presence of nephritis. Grade +++ lesions were never found in control dogs, while a proportion of the nephritic animals showed changes of this severity in all but the youngest group.

TABLE III. /

TABLE III.

Comparison of the Degree of Plasmatic Vasculosis in the Spleens of Nephritic and Non-Nephritic Dogs in Different Age Groups.

Age	Status	No. exam'd.	Grade 0	Grade +	Grade ++	Grade +++
< 2 yrs.	Nephritic	17	13	4	"	"
	Non-Nephritic	20	20	"	"	"
3-6 yrs	Nephritic	20	3	10	5	2
	Non-Nephritic	20	16	4	"	"
7-10 yrs.	Nephritic	20	1	8	6	5
	Non-Nephritic	20	9	8	3	"
> 10 yrs.	Nephritic	13	"	3	8	3
	Non-Nephritic	20	2	13	5	"

The results of this comparative survey are sufficiently clear to require no statistical analysis. The study has re-emphasised the particular susceptibility of the arterioles of the spleen and a direct relationship between age and the frequency of vasculosis was apparent in both series of dogs. It is obvious that nephritis significantly affects the incidence, age of appearance and severity of plasmatic vasculosis in the spleen of the dog.

This survey forms a useful demonstration of the vascular effects of renal disease in the dog and provides further indirect evidence for the development of hypertension in association with nephritis in this species.

DAMAGE IN SUB-ACUTE AND CHRONIC INTERSTITIAL NEPHRITIS. ESTIMATION OF THE PROPORTION OF GLOMERULI SHOWING PLASMATIC DAMAGE IN SUB-ACUTE AND CHRONIC INTERSTITIAL NEPHRITIS.

In sub-acute and chronic interstitial nephritis, plasmatic deposits within the capillary loops of the glomeruli form a prominent feature of the renal histopathology. As described previously, the lesion appears first as a single globule of fibrin within the tuft. Gradually more fibrinous material accumulates, occluding the capillaries and showing mixed red and blue reaction with Mallory staining. Finally, the entire glomerulus is converted to an amorphous mass of pseudo-collagenous material.

In the present study, an attempt was made to estimate the proportion of glomeruli showing this form of damage in nephritic kidneys and to determine the relationship between glomerular lesions and the level of uraemia. The severity and significance of glomerular changes in interstitial nephritis are unknown.

Method.

Sections of kidney were studied from all cases of sub-acute and chronic nephritis from which blood urea levels had been recorded. The total number of glomeruli in one kidney section were counted. The numbers of glomeruli showing each of three grades of damage were then counted. The three grades were defined as follows:

Grade +: Isolated globular deposits of fibrinous material within the/

the glomerular tuft.

Grade ++: Several conjoined plasmatic deposits, showing mixed red and blue reaction with Mallory staining.

Grade +++: Conversion of the entire glomerulus to an amorphous pseudo-collagenous mass.

Examples of Grade +, ++ and +++ glomeruli are shown in figs. 28, 29 & 30.

The percentage of glomeruli damaged in each kidney was calculated and the percentage showing grade +++ damage. The results were correlated with the levels of blood urea.

Results.

The detailed results of this investigation are shown in the table overleaf.

In sub-acute nephritis, the numbers of damaged glomeruli varied considerably in the individual cases, with no relationship to the level of blood urea. (The table does not include cases 6104, 12732, 10622, 11879, 12424, 15553 and 21830, as they did not show plasmatic glomerular changes.)

The findings in cases of chronic nephritis are summarised below. In the majority of cases, between 20% and 50% of the glomeruli were affected/

affected and most of these showed grade +++ lesions. There was no relationship with the level of uraemia.

Proportion of Glomeruli Showing Plasmatic Lesions in Dogs with Chronic Nephritis.

Percentage of Glomeruli Damaged	No. of Dogs Affected.
< 10	12
11-20	3
21-30	13
31-40	12
41-50	9
51-60	5
> 60	3

A small number of the chronic cases showed very little glomerular damage of the plasmatic type. In cases in which 10% or less of the glomeruli were affected, a different pathological change was encountered. In these kidneys, the Bowman's capsules of large numbers of glomeruli were grossly distended, while the tuft was collapsed and atrophic, as in Figs. 34, and 35. . The intra-renal arteries and arterioles were either normal or showed plasmatic vasculosis of very mild degree. As discussed earlier, clinical evidence suggests that these changes occur in very rapidly progressive chronic nephritis. The brief course of the disease may not permit the development of hypertension and the typical plasmatic vascular changes.

Estimation of the Degree of Plasmatic Damage to Glomeruli in Dogs with Interstitial Nephritis, Related to the Level of Uraemia.

Case	Total No. of Glomeruli in one section	Total No. Showing Plasmatic Damage	% Damaged	No. Grade +
Sub-Acute Nephritis.				
14455	254	55	21.7	33
16864	218	24	11.0	0
11373	285	14	4.9	1
23422	276	1	0.4	1
21795	118	52	44.1	10
22682	186	82	44.1	21
17750	230	86	37.4	4
21506	135	15	8.1	6
Chronic Nephritis.				
16313	94	41	43.6	2
5712	70	22	31.4	0
13418	120	50	41.7	10
4444	90	31	34.4	6
12114	140	31	22.1	0
7089	164	49	29.9	10
1466	130	64	49.2	3
15425	160	12	7.5	0
13040	40	17	42.5	0
25270/2	94	25	26.6	9

No. Grade ++	No. Grade +++	% Grade +++	Blood Urea (mg/100 ml)
20	2	0.8	71
7	17	0.8	78
8	5	1.8	117
0	0	0	122
18	24	20.3	300
39	22	11.8	440
32	50	21.7	450
8	1	0.5	930
5	34	36.2	20
8	14	20.0	21
14	26	21.7	25
6	19	21.1	50
2	29	20.7	63.5
19	20	12.2	79
11	50	38.5	90
0	17	42.5	95
0	12	7.5	99
3	13	13.8	104

Case	Total No. of Glomeruli in one section	Total No. Showing Plasmatic Damage	% Damaged	No. Grade +
14101	72	30	41.7	3
6152	125	5	4.0	0
25529	100	20	24	0
7122	88	10	11.4	1
13835	164	57	34.8	7
14314	65	38	58.5	1
2717	30	7	23.3	0
7091	90	4	4.4	0
7478	150	86	57.3	21
13028	180	53	29.4	0
11277	120	70	58.3	4
9157	80	29	36.3	6
17129	110	52	47.2	4
21800	52	37	71.2	2
11170	30	8	26.7	0
17756	95	59	62.1	27
3186	176	82	46.6	7
20566	110	11	10.0	0
1543	60	20	33.3	1
26626	50	15	30.0	1
19196	146	53	36.3	2
16847	120	42	35.0	0
12507	100	28	28.0	2

No. Grade ++	No. Grade +++	% Grade +++	Blood Urea (mg/100 ml)
7	20	27.8	105
2	3	2.4	106
2	24	24.0	106
2	7	8.0	107
19	31	18.9	120
5	32	49.2	120
0	7	23.3	120
0	4	4.4	120
27	38	25.3	135
34	19	10.6	140
17	49	40.8	166.6
5	18	22.5	173.3
5	43	39.1	176
11	24	46.2	196
1	7	23.3	206
11	21	22.1	216
17	58	33.0	218
0	11	10.0	225
4	15	25.0	227
0	51	34.9	240
1	13	26.0	240
6	36	30.0	270
0	26	26.0	>200

Case	Total No. of Glomeruli in one section	Total No. Showing Plasmatic Damage	% Damaged	No. Grade +
23473	210	54	25.7	2
22249	140	53	37.8	1
13158	95	51	53.7	0
13161	40	16	40.0	1
12806	54	23	42.6	0
16636	120	4	3.3	0
4818	150	11	7.3	5
17327	130	29	22.3	3
17422	110	47	42.7	0
10835	105	11	10.5	0
17823	110	44	40.0	3
20132	110	9	8.2	0
18587	210	34	16.2	0
19258	150	54	36	0
20192	58	42	72.2	0
23571	104	27	26.0	2
10447	105	5	4.8	0
19397	170	103	60.6	0
27107	210	76	36.2	0
24819	230	3	1.3	0
19961	145	4	2.8	0
21540	158	10	6.3	0
25488	90	20	22.2	1
21460	45	6	13.3	0

No. Grade ++	No. Grade +++	% Grade +++	Blood Urea (mg/100 ml)
6	46	21.9	300
9	43	30.7	340
6	45	47.4	>300
5	10	25.0	>300
6	17	31.5	>300
0	4	3.3	400
2	4	2.7	400
2	24	18.5	400
16	31	28.9	430
1	10	9.5	440
8	33	30.0	450
0	9	8.2	450
12	22	10.5	450
5	49	32.6	450
19	23	39.7	450
2	23	22.2	>400
0	5	4.8	520
9	94	55.3	540
4	72	34.3	540
1	2	0.9	590
0	4	2.8	700
5	5	3.2	710
1	18	20.0	740
1	5	11.1	770

Discussion.

The variable and generally low percentage of glomeruli damaged in sub-acute nephritis might be expected, as the lesion first appears in this phase of nephritis.

In chronic nephritis, 20 - 50% of the glomeruli were damaged in most cases. The lack of correlation with blood urea levels may be due to the variable factors inherent in this study. Only one kidney section was examined from each case, which might not give accurate representation of the renal pathology. The urea levels were estimated at varying periods before death.

The small number of cases in which little evidence of plasmatic lesions was found were of interest. Glomeruli with greatly distended capsules and atrophic tufts seem likely to be non-functional. Arterial changes were minimal or absent in these kidneys, suggesting that hypertension was not an important factor. Clinical evidence presented earlier indicates that renal fibrosis progresses with unusual rapidity in such cases.

In the human, plasmatic deposits are found in the glomerular capillaries in hypertension (Lendrum, 1963). The morphological and histochemical features of the lesions observed in interstitial nephritis closely resemble the human glomerular changes and suggest that hypertension might play a significant part in the pathogenesis of the later phases of the disease.

THE EFFECTS OF GLOMERULAR DAMAGE OF THE PLASMATIC TYPE ON THE RELATED PROXIMAL TUBULES.

Estimation of the numbers of glomeruli showing plasmatic damage in sub-acute and chronic interstitial nephritis indicated that 20 - 50% of the glomeruli are frequently affected. Lesions range from single globular deposits of fibrinous material within the capillary tuft to complete conversion of the glomerulus to an amorphous mass with a pseudo-collagenous appearance. As such lesions may be caused by hypertension, it is important to assess the effect of glomerular damage on, the remainder of the nephron. This knowledge would give some indication of the importance of hypertension in the pathogenesis of progressive fibrosis and renal failure.

Method.

Serial sections were studied from six cases of chronic nephritis. These were nos. 3186, 1466, 7478, 15425, 17756 and 19258, selected because glomerular damage of the plasmatic type was a prominent feature of the renal histopathology.

Every fifth section was taken from each block of kidney tissue and a total of approximately 20 sections were examined from each case. Staining was by the picro-Mallory method.

Results. /

Results.

It was possible to study the proximal tubule arising from many glomeruli and the blood vessels related to a few glomeruli in each kidney.

Glomeruli and Related Proximal Tubules.

Proximal tubules arising from glomeruli showing Grade + lesions were always normal. As might be expected, small isolated deposits of fibrin in the capillary tuft had no detectable effect on the tubule.

The effects of Grade ++ glomerular damage appeared to be variable. In most instances, the related tubule was normal but sometimes the epithelial cells showed degenerative change. This was probably significant as the cells lining adjacent tubules were normal (Fig.40).

Despite examining several sections through large numbers of grade +++ glomeruli, it proved impossible to trace the related proximal tubules. Very often the glomerulus was embedded in an area of advanced fibrosis in which compressed remnants of tubules were present (Fig.30). However, sometimes the Grade +++ glomerulus was surrounded by normal proximal tubules (Fig.41) though no connection could be found with the tubules. These results suggested that damage of this degree to the capillary tuft may lead to degeneration and fibrous tissue replacement of the related tubule, probably as a result of ischaemia.

Lesions in Afferent Arterioles and Glomeruli.

This/

This study showed that there is no constant relationship between fibrinous vasculosis in afferent arterioles and lesions in the related glomeruli. Normal glomeruli were seen to be supplied by arterioles showing severe vasculosis (Fig. 42) and in other instances, both the glomerulus and the arteriole were affected (Figs. 43, 44 & 45). Again, damaged glomeruli were occasionally observed with normal arterioles.

Discussion.

Examination of serial sections from six cases of chronic nephritis has shown that the more advanced glomerular lesions may be associated with degeneration and disappearance of the related tubules. Lesions of lesser severity are unlikely to affect the tubule significantly.

If hypertension is indeed the cause of plasmatic damage in interstitial nephritis, then it would seem to play a considerable part in the destruction of nephrons and the progressive development of renal fibrosis. In many instances, severe damage to an isolated glomerulus might have little effect on the tubule because of the collateral blood supply. However, when large numbers of glomeruli are destroyed, as has been shown to happen in many cases of chronic nephritis, the collateral circulation may become inadequate.

Miller and Apfelbach (1927) produced infarction of the glomeruli in dogs by injecting fine particle charcoal into the renal arteries. This resulted in extensive fibrosis of the kidneys. Hypertension was not/

not recorded as the authors used an indirect method of measuring blood pressure which gave very variable readings. This evidence supports the view that occlusion of the capillary tufts in sub-acute and chronic nephritis may significantly contribute to the development of renal fibrosis.

The absence of a correlation between vasculosis in afferent arterioles and lesions in related glomeruli probably indicates that both structures are very susceptible to hypertension and may be damaged separately or together.

ARTERIAL SCLEROSIS.

An arterial change unrelated to plasmatic vasculosis was found in seven dogs in the series of 190 nephritic animals. This lesion has also been recognized in non-nephritic dogs and differs from plasmatic vasculosis in important respects.

The lesion is characterized by a great increase in thickness of the walls of small arteries, with severe constriction of the lumina and has been described in the small coronary arteries and arterioles of dogs. Lindsay, Chalkoff and Gilmore (1952) found "hyaline fibrous thickening" in the coronary arteries of four old dogs, one of which had areas of fibrosis in the myocardium of the left ventricle. Detweiler, Hubben and Paterson (1960; 1961), described pathological findings in 69 dogs which showed macroscopic cardiac abnormalities. In 54 of these, gross thickening of the walls and narrowing of the lumina affected the small coronary arteries. The major coronary vessels were always normal. The sclerotic lesion was usually associated with areas of degenerative or necrotic change in the myocardium.

Fisher and Pirie (1964), in a general outline of cardiovascular disease in domestic animals, described sclerosis of the intra-myocardial branches of the coronary arteries, which was said to occur quite frequently in old dogs. Thickening of the arterial wall resulted from the accumulation of eosinophilic material in the intima and media, with loss of/

of the internal elastic lamina and disappearance of smooth muscle cells. The lesion was associated with areas of fibrosis in the myocardium.

In the present study, seven nephritic dogs were found to show this form of arterial sclerosis. The lesion occurred in all phases of nephritis, in animals of 5 - 13 years of age. In each case, the small coronary arteries and arterioles were affected and in addition the hepatic arterioles were involved in three dogs and the arterioles of the testes in one. These details are shown in the table below. Plasmatic vasculosis was present in the kidneys of all of the affected dogs, with the exception of 13315.

ARTERIAL SCLEROSIS.

Case No.	Age	Stage of Nephritis	Affected arteries.
13315	7	Acute	Small coronary arteries and arterioles
22682	5	Sub-acute	Small coronary arteries and arterioles
21795	10	Sub-acute	Small coronary arteries, hepatic arteries, small arteries in the testes.
13835	-	Chronic	Small coronary arteries.
8951	11	Chronic	Small coronary and hepatic arteries.
8876	12	Chronic	Small coronary and hepatic arteries.
17312	13	Chronic	Small coronary arteries.

Arteries showing this form of sclerosis had greatly thickened walls and narrowed lumina, as described by previous authors. Large amounts of amorphous material were deposited within the wall and there was a loss of cellular detail. The elastic laminae disappeared and the vessel wall was composed of pale-staining ground substance in which widely separated nuclei persisted. The accumulated intercellular material was weakly eosinophilic, PAS-positive and showed negative reaction with P.T.A.H. and weakly positive reactions for collagen. These properties suggested the presence of mucopolysaccharide. The histological features of the lesions gave no apparent clue as to the origin of this material.

Lesions in the heart, liver and testis are illustrated in figs. 46 - 49.

This lesion was not found in any of the 125 dogs which formed the non-nephritic control series. However, the low incidence in nephritic dogs does not indicate a significant association with renal disease.

SUMMARY AND DISCUSSION OF SECTION I.

The studies described in this section were designed to establish the relationship between renal and vascular disease in the dog and to estimate the significance of vascular damage in the pathogenesis of chronic renal fibrosis.

In the histopathological study of 130 cases of interstitial nephritis, three phases of the disease were distinguished. Sub-acute nephritis has not been generally recognized. This stage was characterized by the early development of fibrosis in kidneys which still showed marked cellular infiltration. Plasmatic deposits in the glomeruli were observed in most of these cases, whereas the glomeruli were almost always normal in acute nephritis.

Damage to the intra-renal arteries and arterioles was observed in 50% of acute, 55% of sub-acute and 85% of chronic cases. This is a higher incidence than has been previously recorded and no reference could be found to vascular lesions in acute interstitial nephritis in dogs.

Extra-renal vascular changes were found in the heart, stomach and tongue in a few cases. Dahme (1957) described lesions in the small coronary arteries of dogs with chronic nephritis but there has been no record of such lesions in the stomach and tongue. Dahme (1957) also found vascular damage occasionally in the brain and Platt (1951) noted fibrinoid necrosis in the small intestine. No vascular lesions were observed in these sites in the present series.

The term "plasmatic vasculosis" has been adopted in the description of the vascular lesions, as the findings were in accordance with Lendrum's (1963) observations on the nature of vascular damage in human hypertension and other conditions. The lesions in the affected vessels appear as a spectrum of changes, showing graded severity with no clear division into two types which could be classified separately as hyalinosis and fibrinoid necrosis. Different interpretations of the nature of vascular lesions have been based on light and electron microscopic studies. As there is a high incidence in dogs with interstitial nephritis, this disease might provide a useful means of correlating the fine structure and histochemistry of vasculosis throughout its development.

Plasmatic vasculosis in the nephritic dogs was not influenced by the age or sex of the dog, nor was there any apparent relationship with the degree of uraemia. It was established that the lesions do not occur as a normal age change in non-nephritic dogs in organs other than the spleen.

A comparative histological survey of arterioles in the spleens of nephritic and non-nephritic dogs showed that nephritis is associated with a higher incidence, earlier appearance and greater severity of plasmatic vasculosis. This study formed a useful demonstration of the vascular effects of renal disease in the dog.

Estimation of the proportion of glomeruli showing plasmatic damage indicated/

indicated that 20 - 50% are affected in most cases of chronic nephritis. Failure to find any correlation with the level of uraemia may be attributable to the variable factors associated with this study. Examination of serial sections showed that the most severe glomerular lesions may cause degeneration of the related tubule. Thus, if hypertension is the cause of glomerular damage, it would appear to play a significant part in the development of chronic renal fibrosis.

The small number of dogs with chronic nephritis which showed little or no plasmatic changes in the arteries and glomeruli were of special interest. An atypical glomerular lesion formed an outstanding feature of the renal histopathology in this group. The capsules of large numbers of the glomeruli were grossly distended, while the tufts were collapsed and atrophic. Clinical evidence, and the findings of this lesion in almost all cases of one year of age or less, indicates that the chronic phase may have progressed with unusual rapidity in such dogs. The absence of plasmatic vasculosis suggests that hypertension was not a significant factor in these cases.

Arterial sclerosis of a type unrelated to plasmatic vasculosis was observed in the small coronary arteries and arterioles in seven of the nephritic dogs. It seems unlikely that nephritis influences the incidence of this lesion.

In conclusion, plasmatic vasculosis in nephritic dogs is essentially the same as vascular changes associated with hypertension in the human and/

and with experimental canine hypertension. The evidence of this investigation therefore strongly suggests that hypertension develops in many cases of canine nephritis, and is important in the pathogenesis of progressive renal fibrosis. The study described in Section II was designed to establish whether this is so.

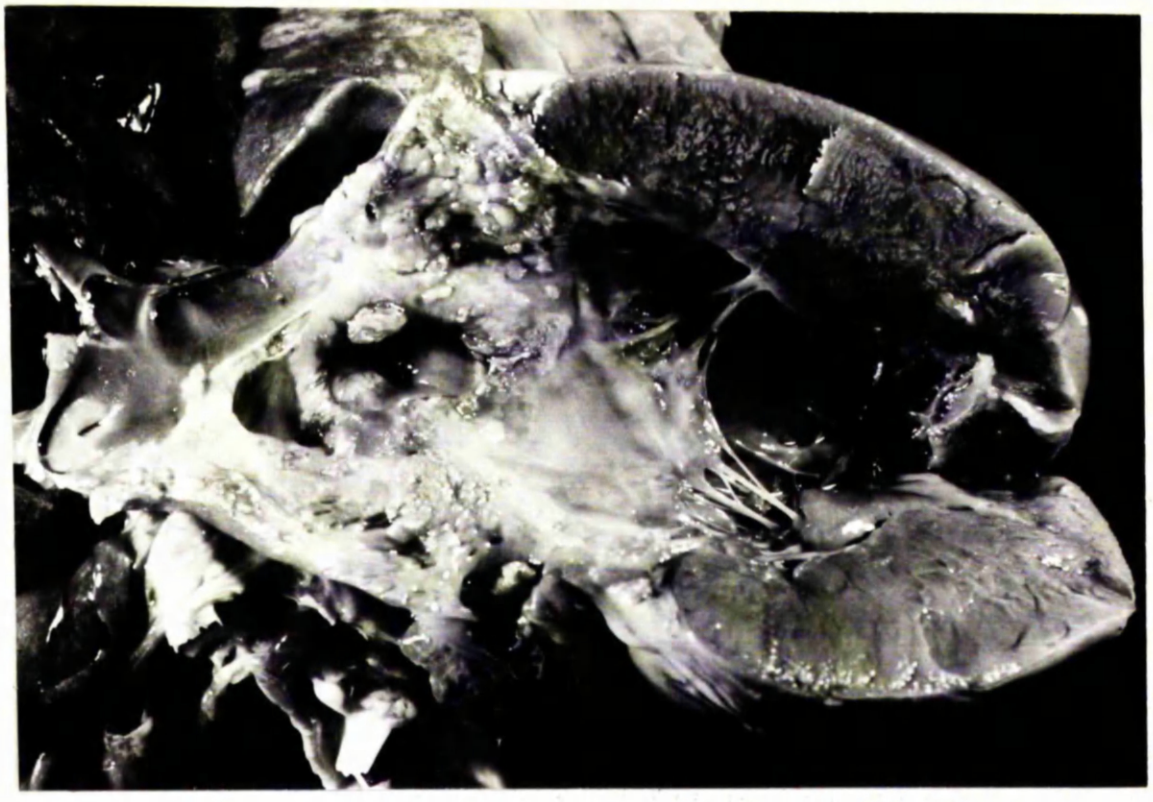


Fig. 1. Necrotizing endocarditis in the left atrium.

Fig. 2. Necrotizing endarteritis affecting the origin of the pulmonary artery.

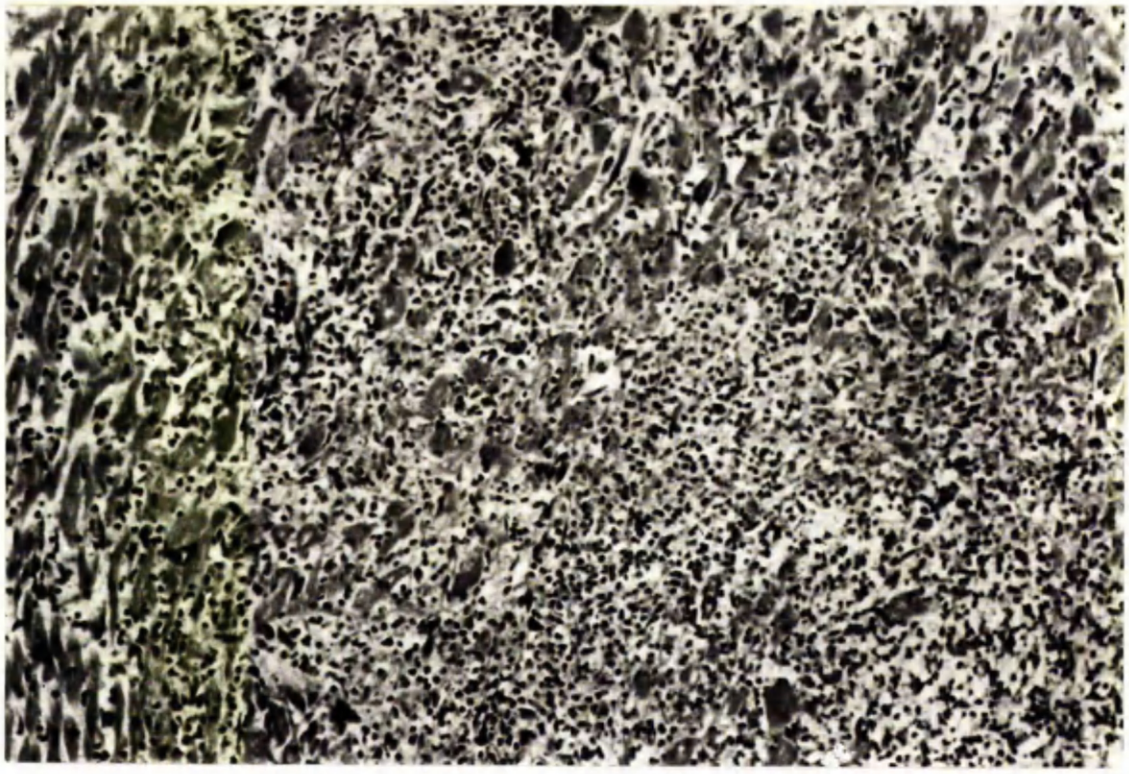


Fig.3. Case No. 22329. Acute myocarditis. Showing an intense polymorphonuclear leucocyte reaction and necrosis of myocardial fibres. H. & E. X 150.

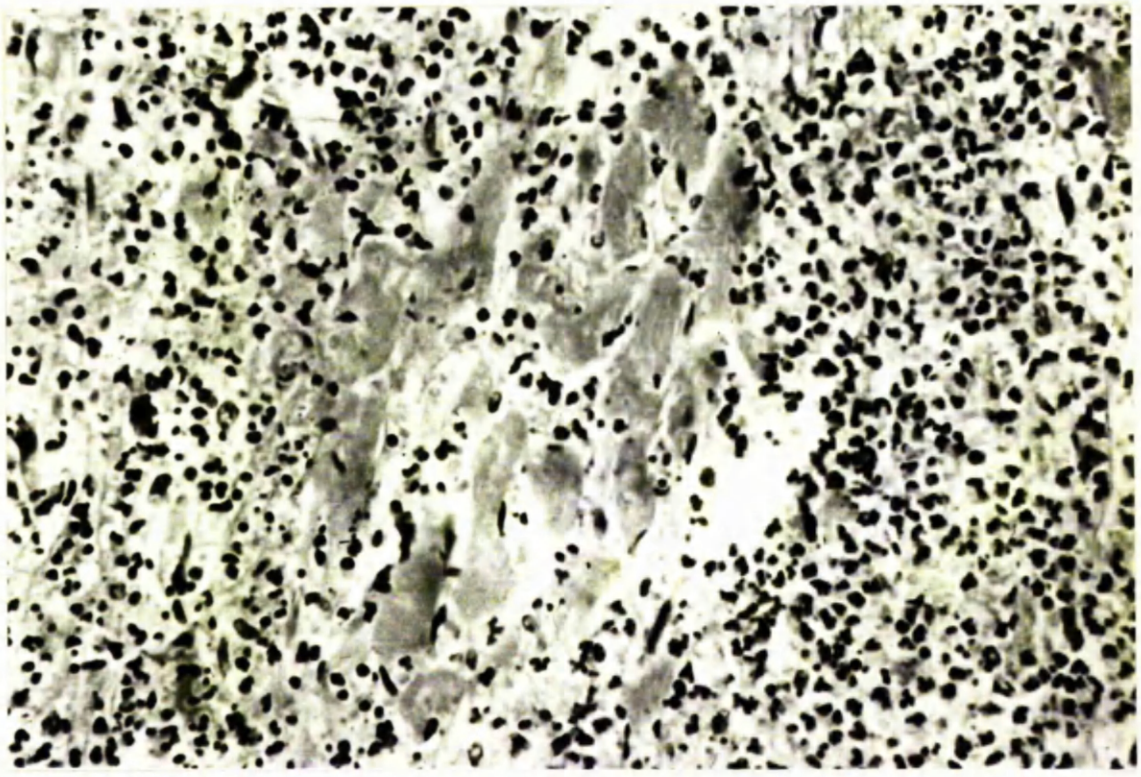


Fig. 4. Case No. 22329. A high-power view of an area of acute myocarditis. H. & E. X 300.

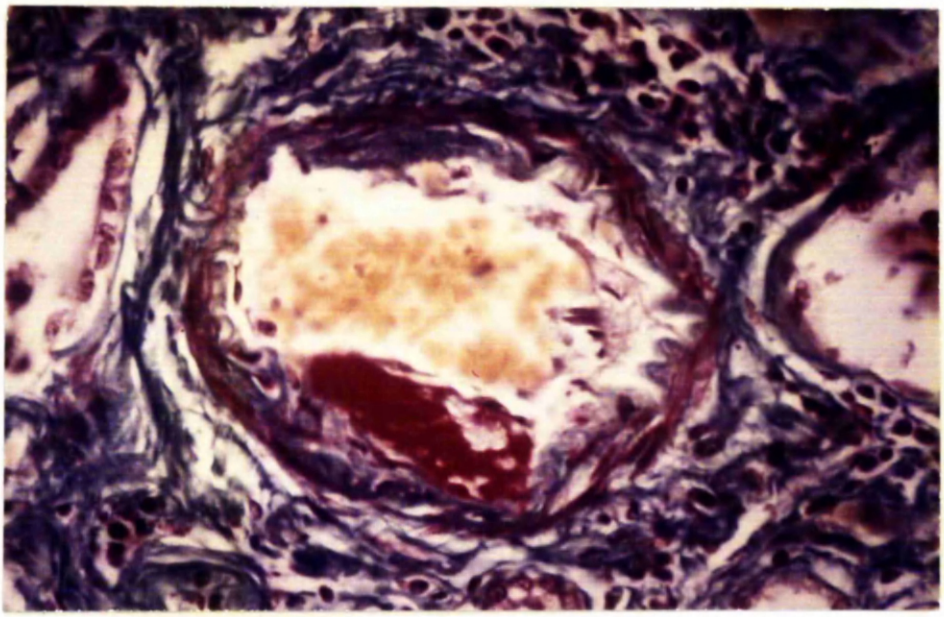


Fig.8. Case No. 17756. Chronic Interstitial Nephritis. Focal sub-endothelial deposit of plasmatic material in an interlobular artery. Mallory. x 500.

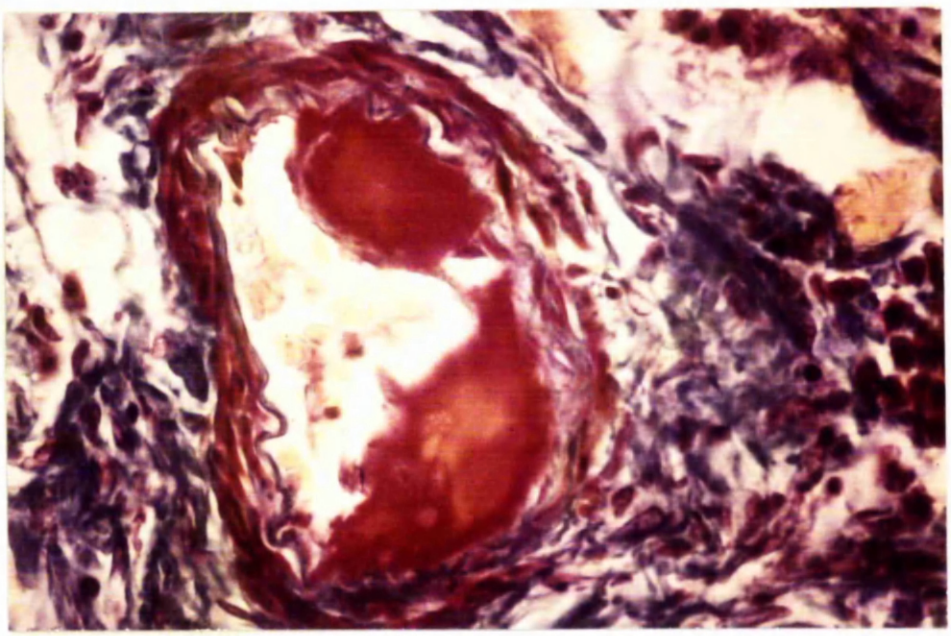


Fig.9. Case No. 17756. Chronic Interstitial Nephritis. Large sub-endothelial deposits of plasmatic material causing reduction of the lumen of an interlobular artery. Mallory. x 500.

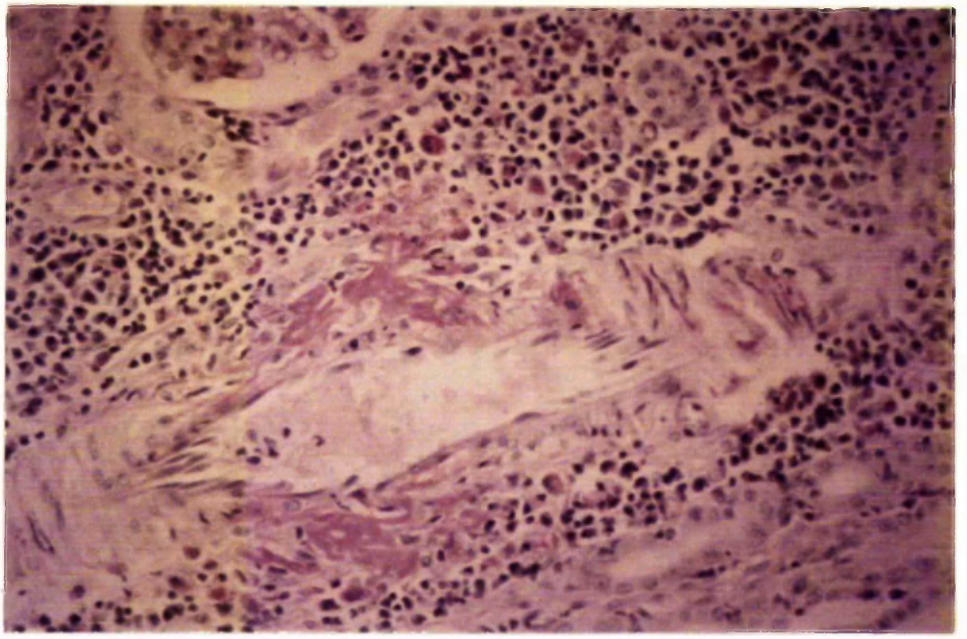


Fig.10. **Case No. 22882. Sub-acute Interstitial Nephritis. Severe plasmatic vasculosis in an interlobular artery, with rupture of the external elastica. H & E.** x 500.

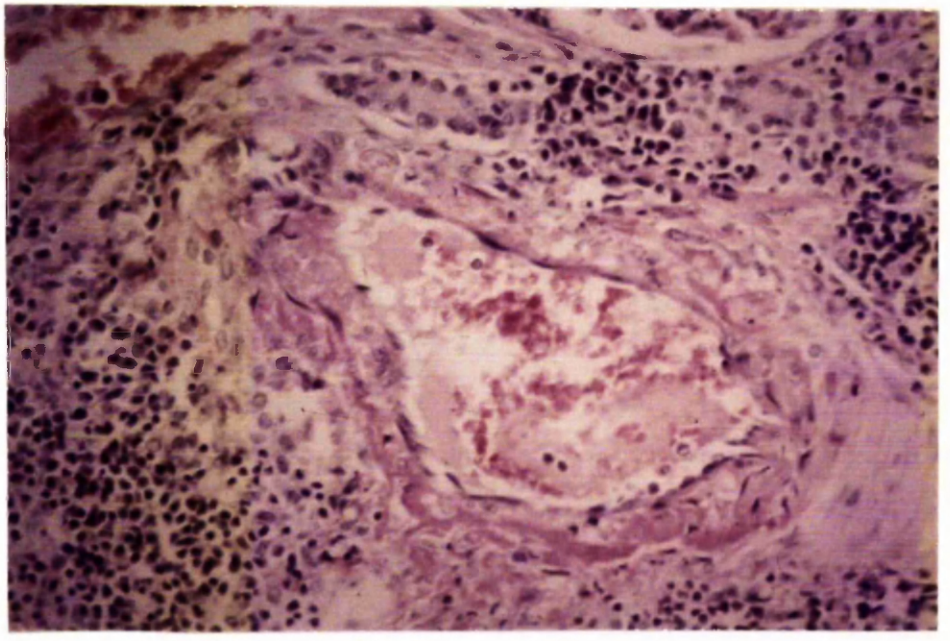


Fig.11. **Case No. 22882. Sub-acute Nephritis. Severe plasmatic vasculosis affecting the entire circumference of an interlobular artery. H. & E.** x 500.

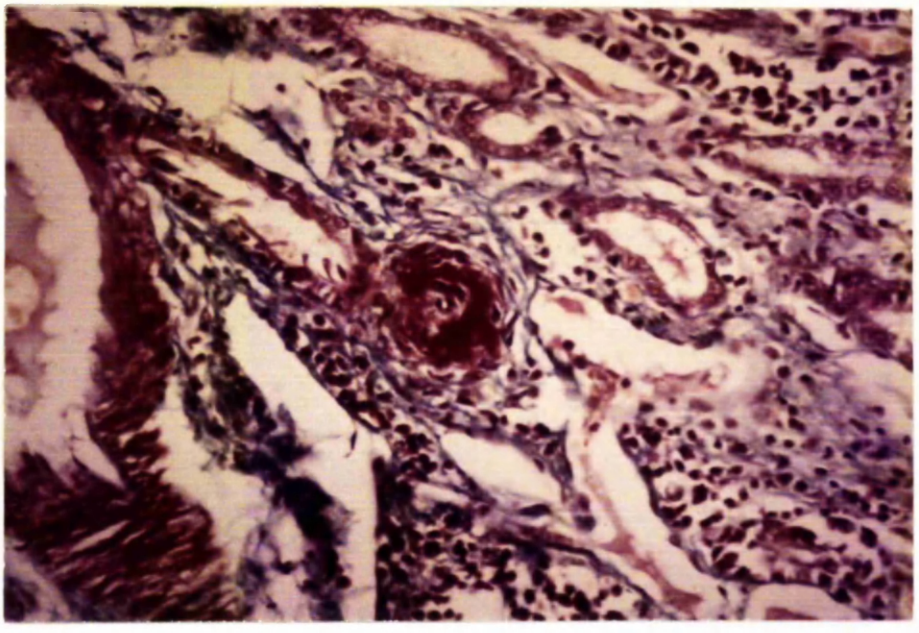


Fig.12. Case No. 21688. Acute Interstitial Nephritis. Plasmatic vasculosis in an afferent arteriole, causing occlusion of the lumen. Mallory. x 300.

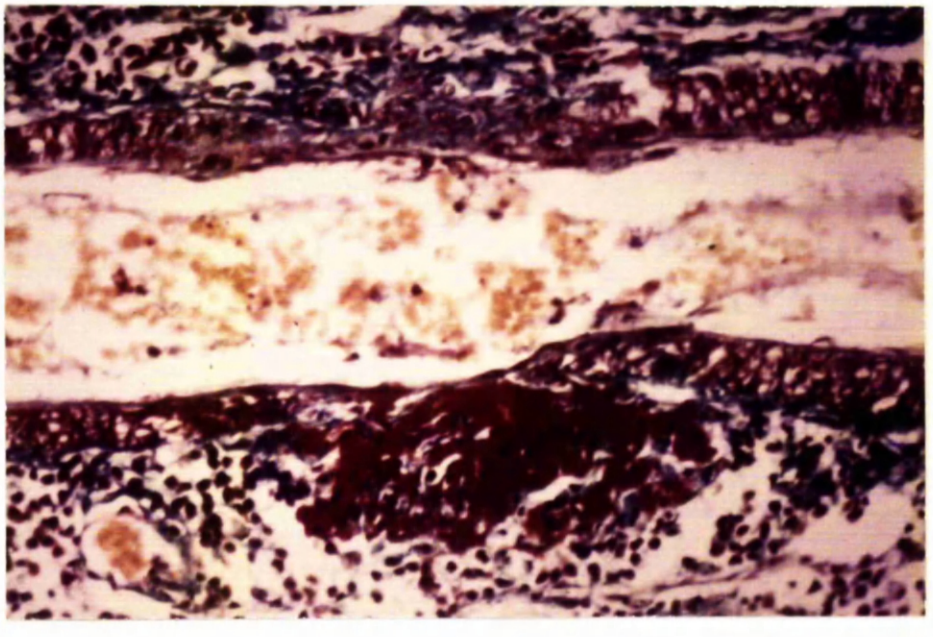


Fig.13. Case No. 21688. Acute Nephritis. Severe focal plasmatic vasculosis in an interlobular artery, with rupture of the external elastica and extension of plasmatic material into the adventitial tissue. Mallory. x 500.

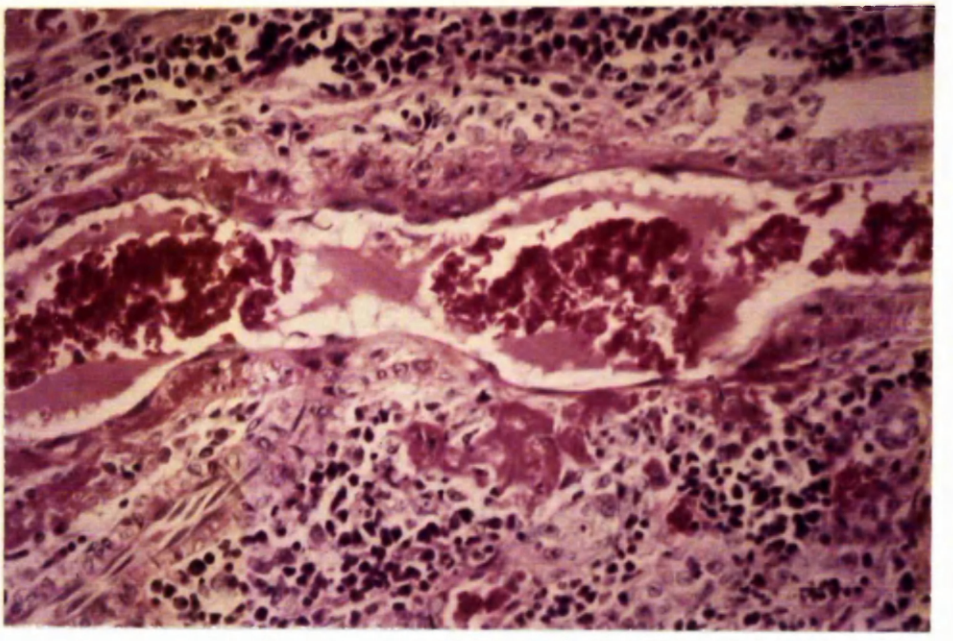


Fig.14. Case No. 21688. Acute Nephritis. Focal lesions along an interlobular artery, with rupture of the external elastica and dilatation of the vessel at the affected areas. H. & E. x 500.

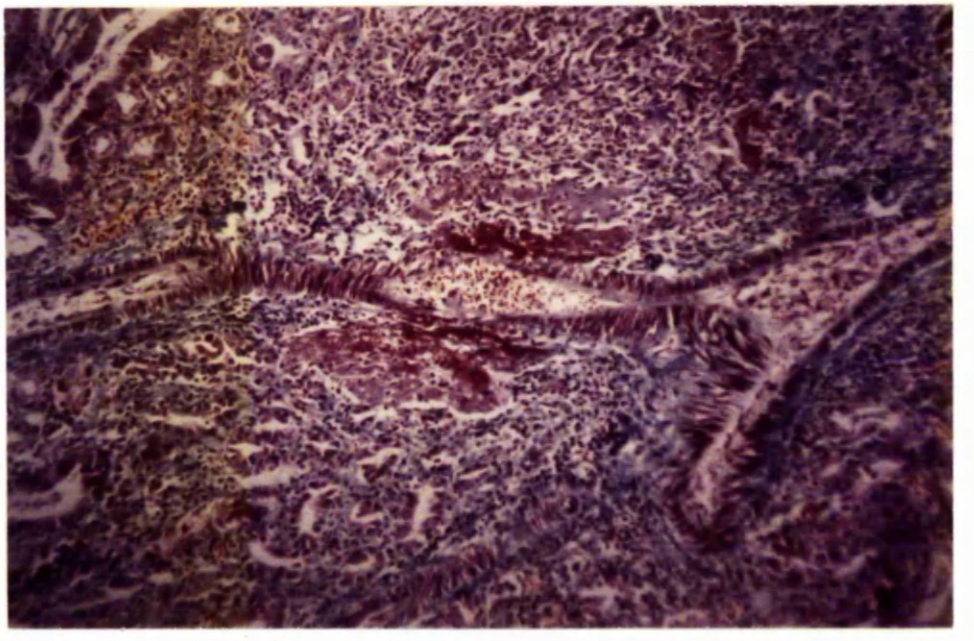


Fig.15. Case No. 21688. Acute Nephritis. Severe plasmatic vasculosis in an interlobular artery. Mallory. x 150.

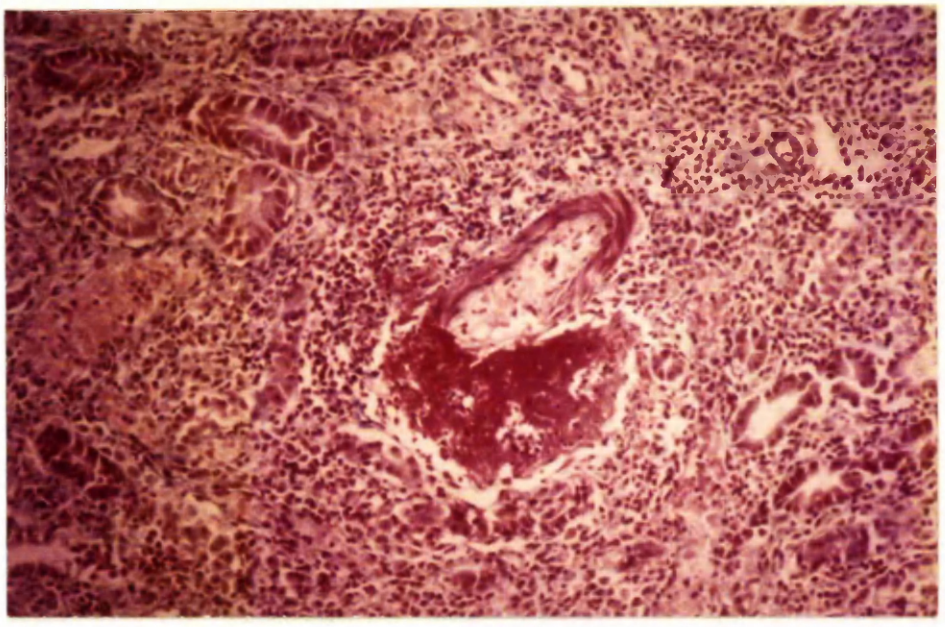


Fig.16. Case No. 21688. Acute Nephritis. Severe focal plasmatic vasculosis in an interlobular artery, showing necrosis of the vessel wall and extension of plasmatic material into the surrounding tissue. Mallory. x 300.

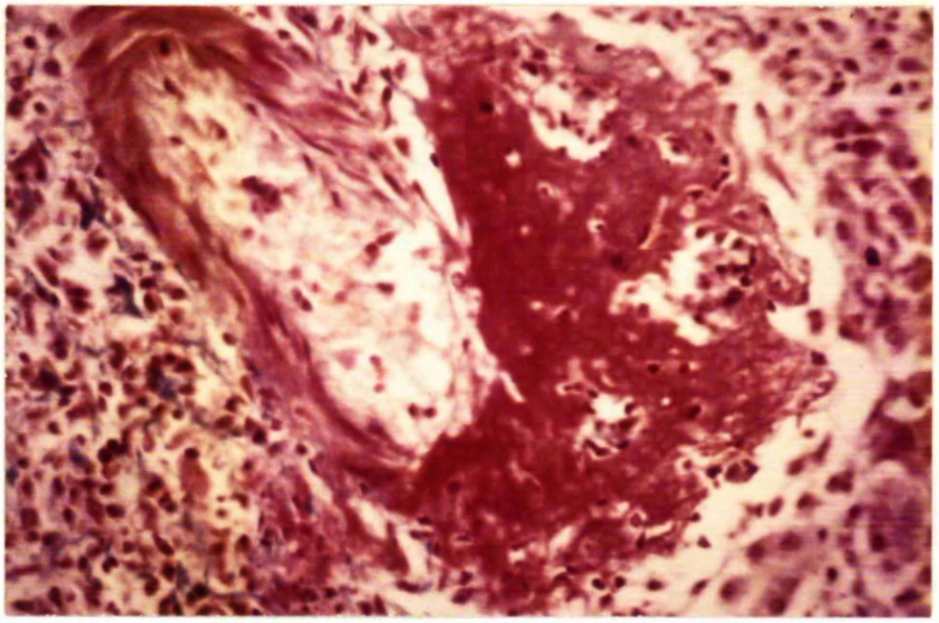


Fig.17. A high-power view of the same lesion. x 500.

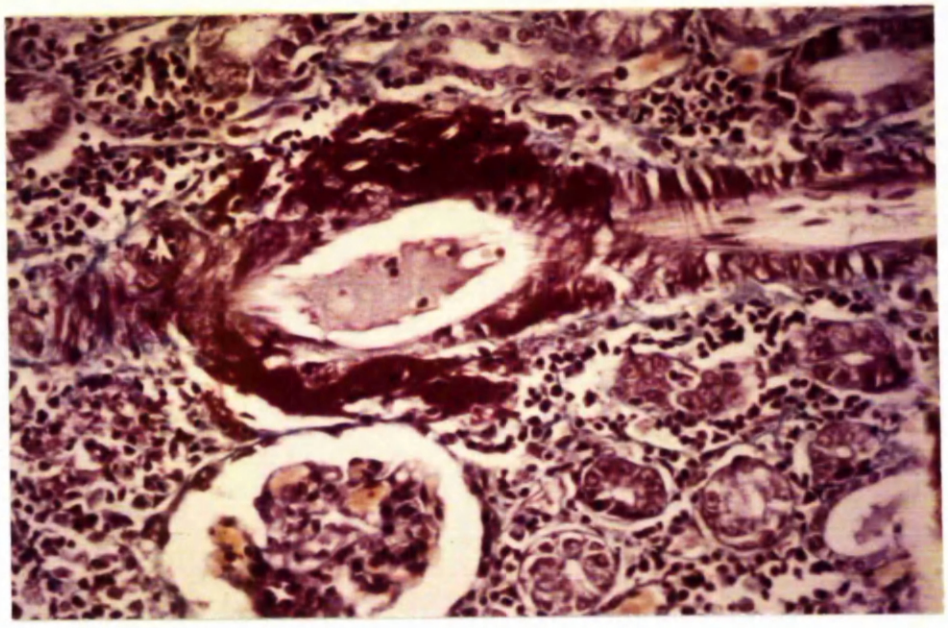


Fig.18. Case 18207. Acute Nephritis. Severe plasmatic vasculosis affecting the entire circumference of an interlobular artery. Mallory. x 300.

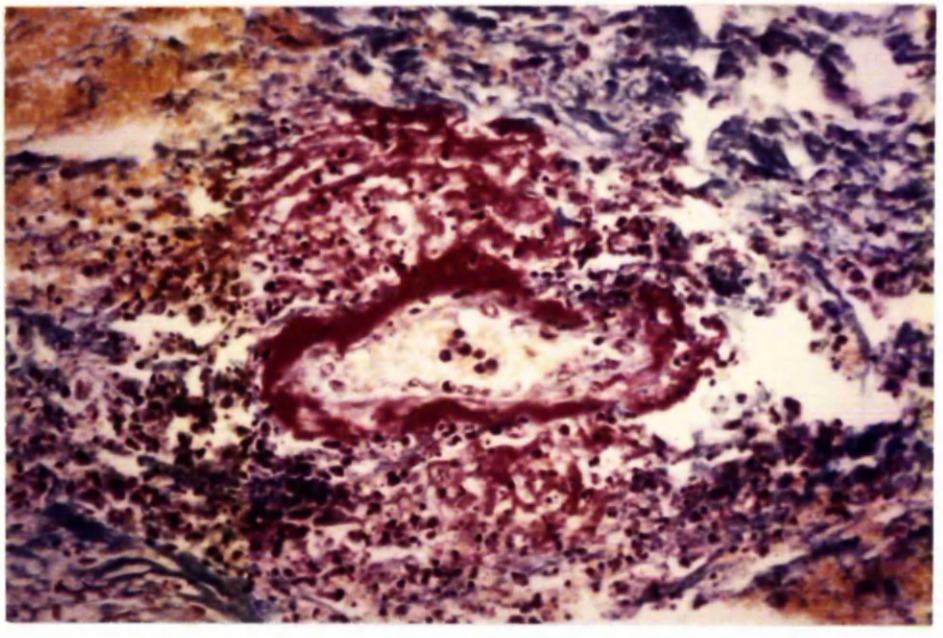


Fig.19. Case 21274. Chronic Nephritis. Severe plasmatic vasculosis in an interlobular artery, with plasmatic material in the adventitial tissue and an acute inflammatory reaction. Mallory. x 500.

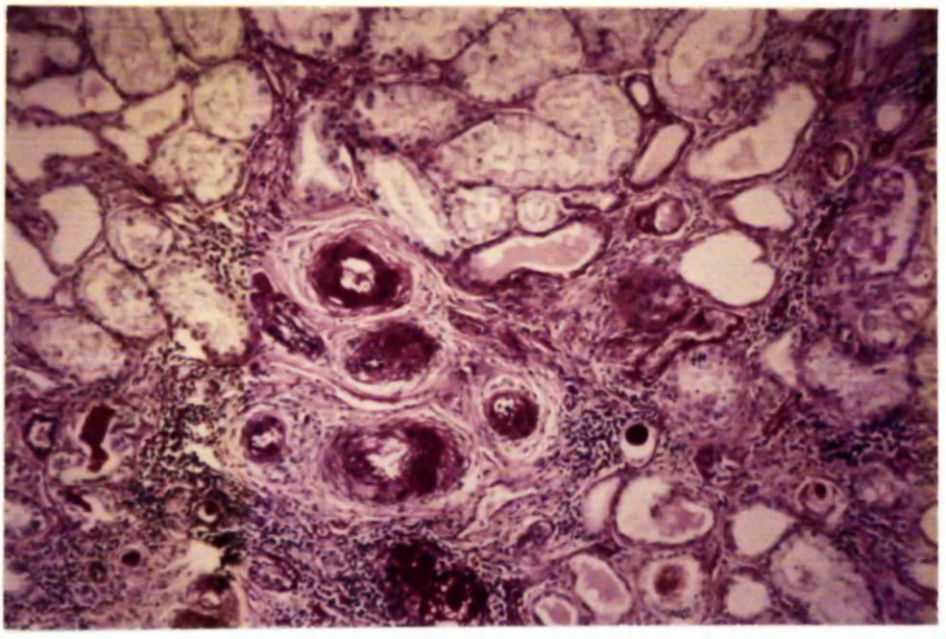


Fig.20. Case No. 17756. Chronic Nephritis. Interlobular arteries showing thickening of the wall due to muscle hypertrophy with plasmatic deposits and hyperplasia of the adventitial tissue. P.A.S. x 150.

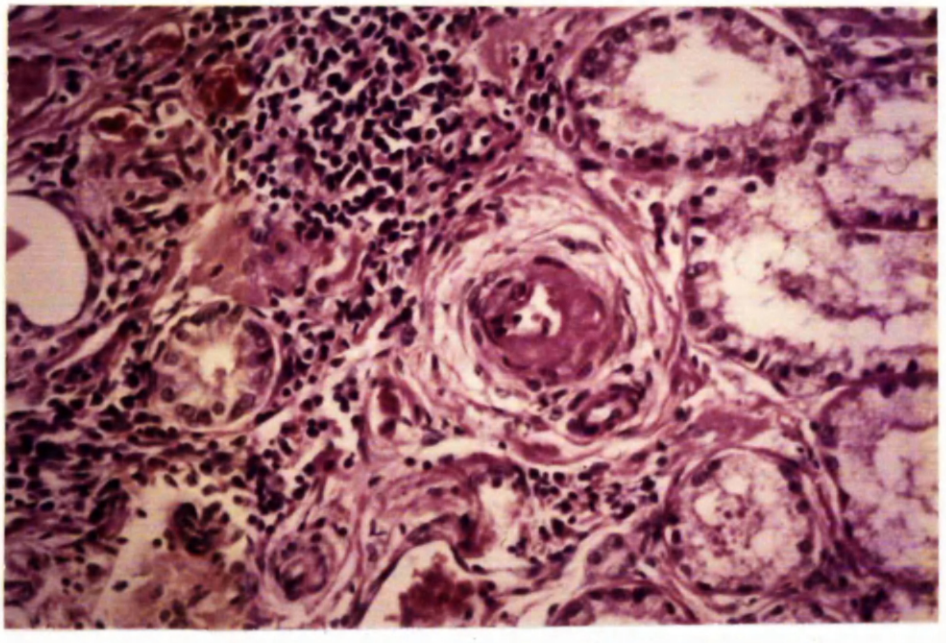


Fig.21. Case No. 17756. Chronic Nephritis. A thickened afferent arteriole, showing plasmatic vasculosis and hyperplasia of the adventitial tissue. P.A.S. x 500.

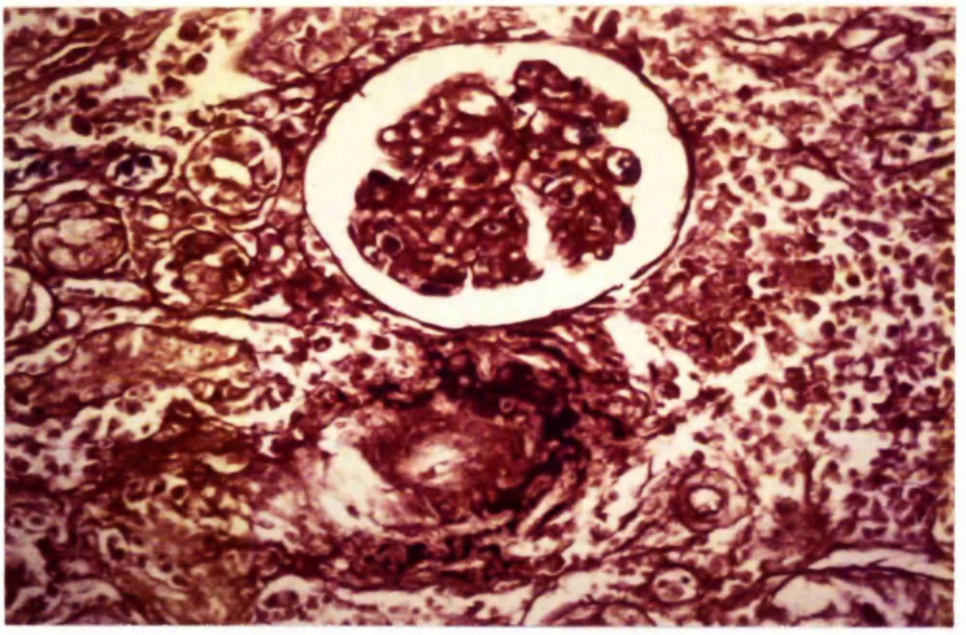


Fig. 22. Case No. 14840. Sub-acute Nephritis. Plasmatic vasculosis in an interlobular artery, showing positive reaction for fibrin. P.T.A.H. x 300.

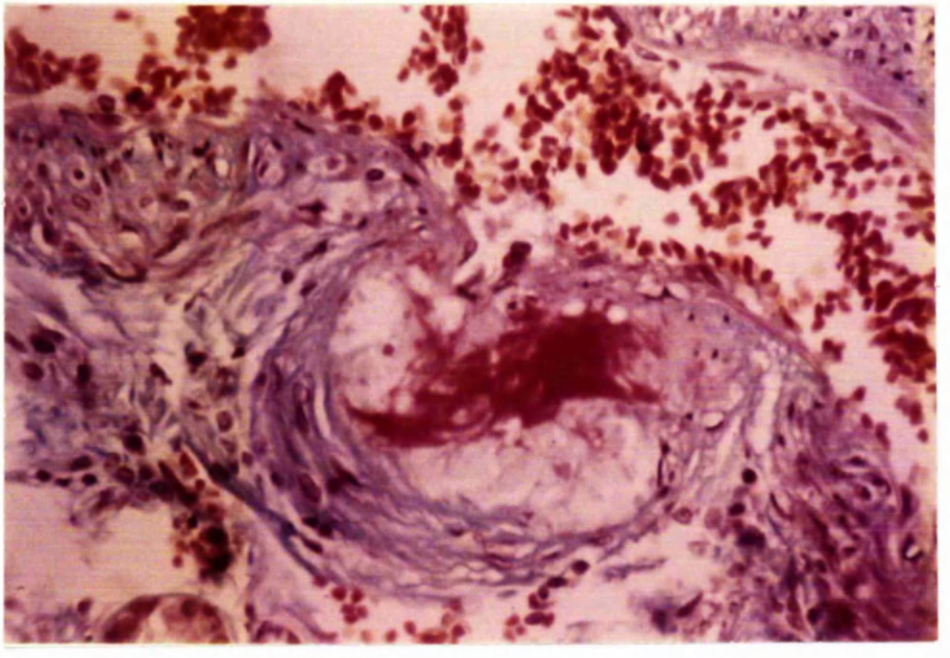


Fig. 23. Case No. 21274. Chronic Nephritis. A focus of plasmatic vasculosis in an arcuate artery, showing mixed red and blue reaction with picro-Mallory. x 500.

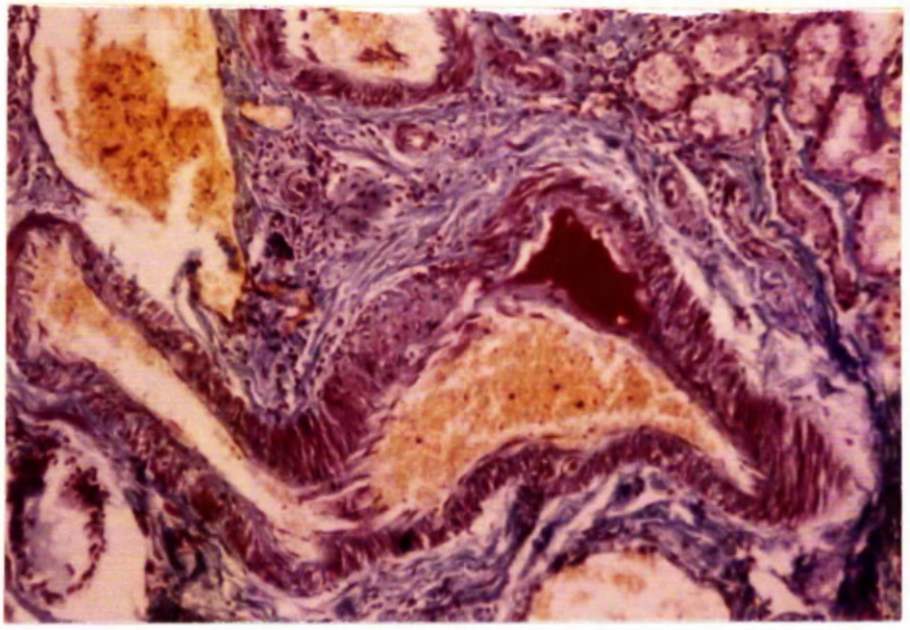


Fig.24. **Case No. 9157. Chronic Nephritis. A focal sub-endothelial deposit of fibrinous material in an arcuate artery. Mallory.** x 300.

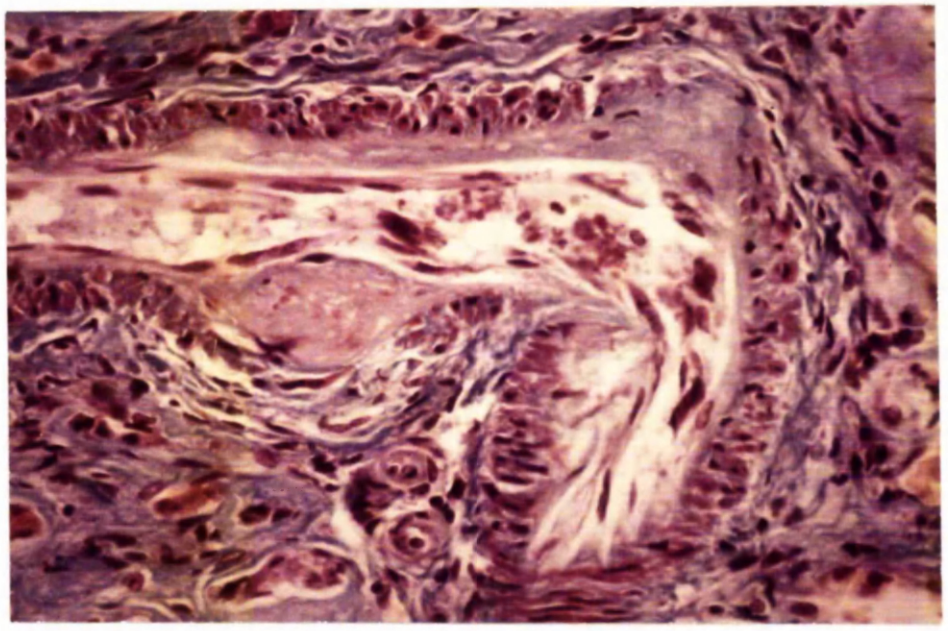


Fig.25. **Case 19258. Chronic Nephritis. Multiple deposits of pseudo-collagenous material in the wall of an arcuate artery. Mallory.** x 300.

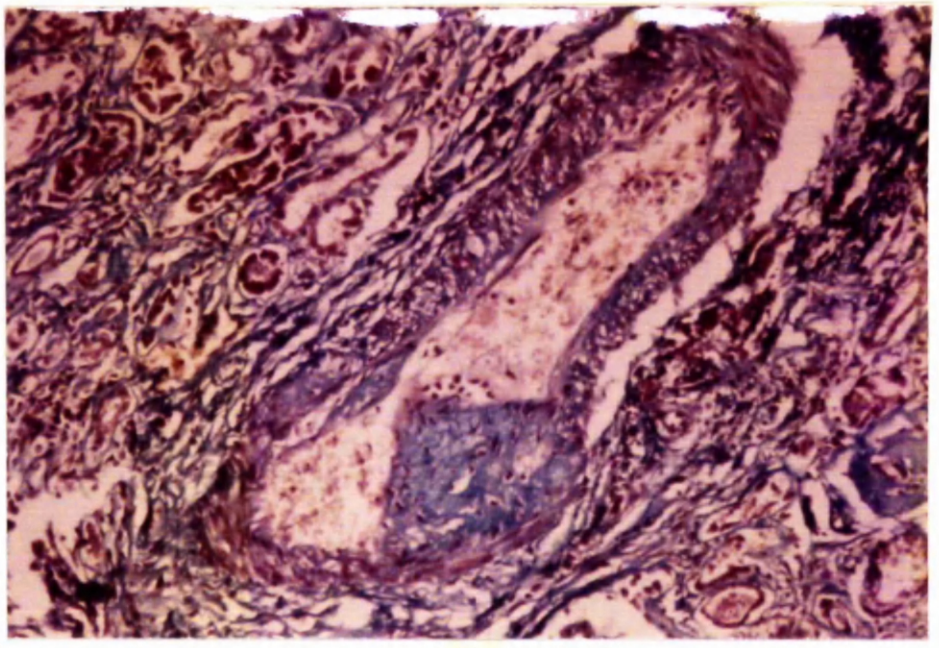


Fig.26. Case No. 7478. Chronic Nephritis. A focal accumulation of pseudo-collagenous material projecting into the lumen in an arcuate artery. Mallory. x 300.

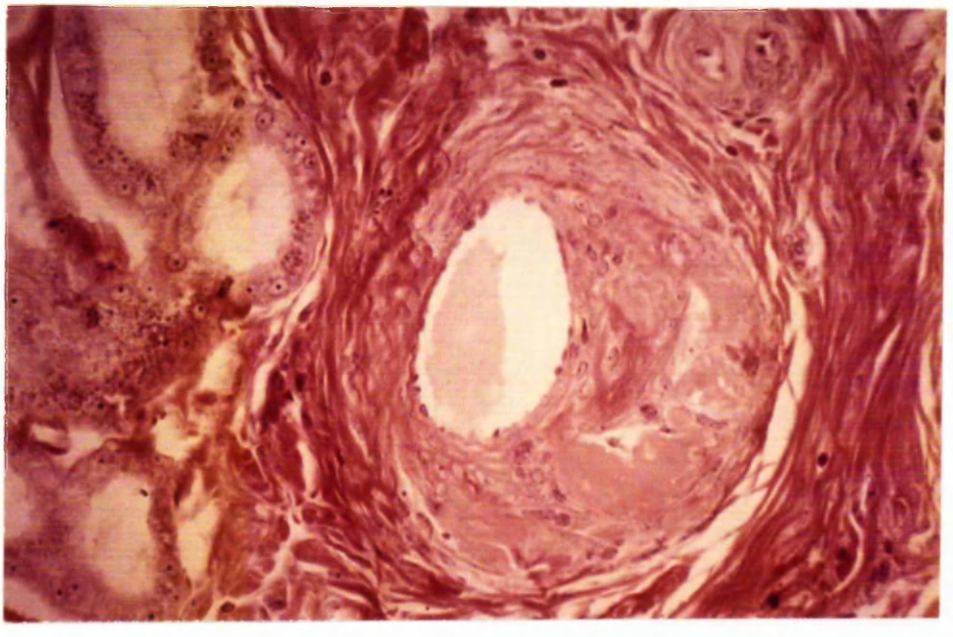


Fig.27. Case 12806. Chronic Nephritis. Thickening and partial occlusion of an interlobular artery by pseudo-collagenous material, showing a negative reaction for fibrin. P.T.A.H. x 500

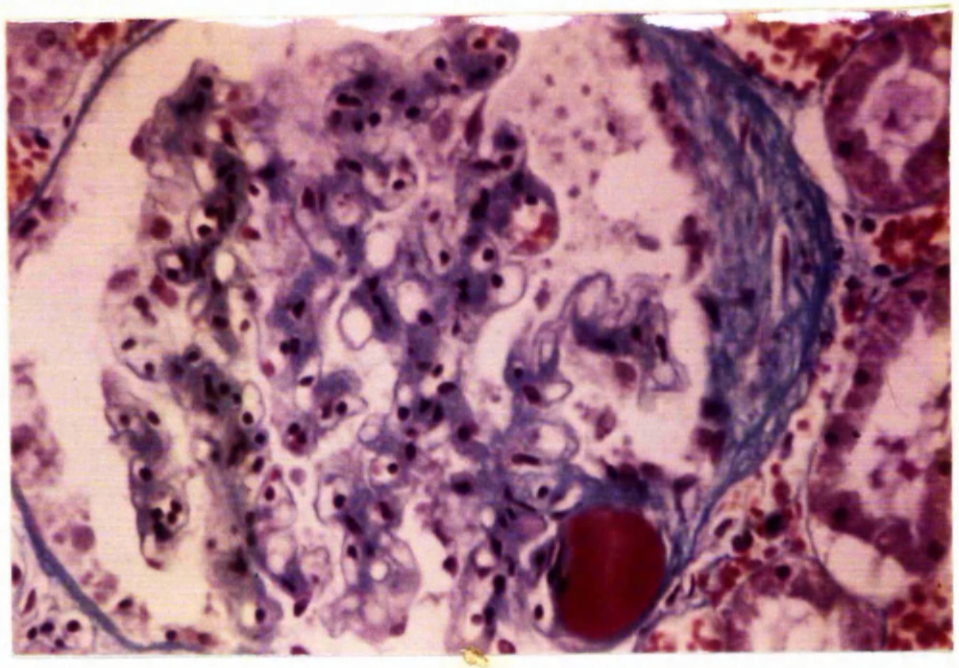


Fig.28. **Grade + glomerulus. A single globule of fibrinous material within a capillary loop. Mallory.** x 500.

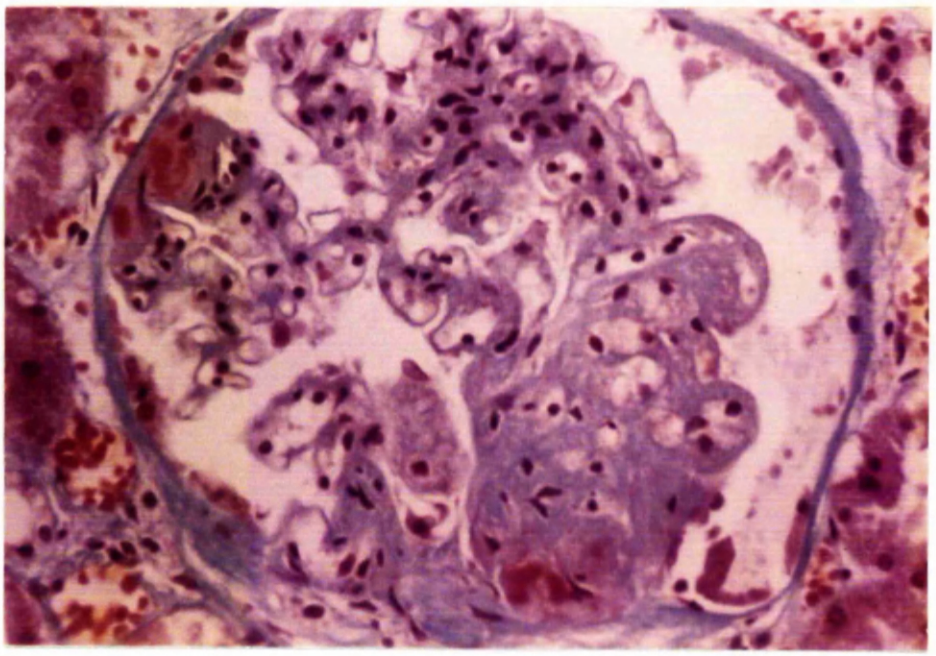


Fig.29. **Grade ++ glomerulus. Several conjoined deposits of plasmatic material, showing mixed reaction with Mallory staining.** x 500.

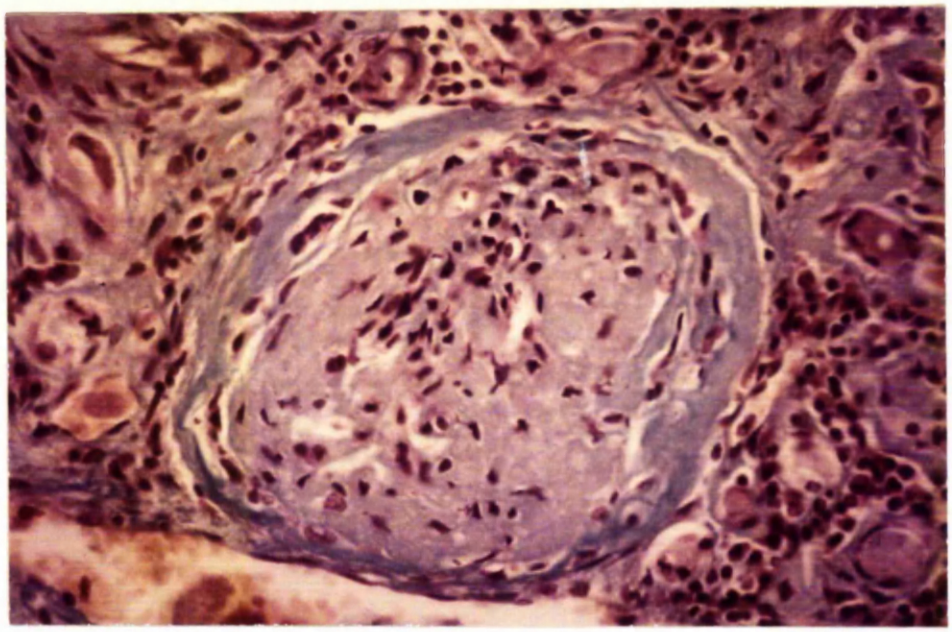


Fig.30. Grade +++ glomerulus. Complete conversion of the glomerulus to a mass of pseudo-collagenous material. Mallory. x 500.

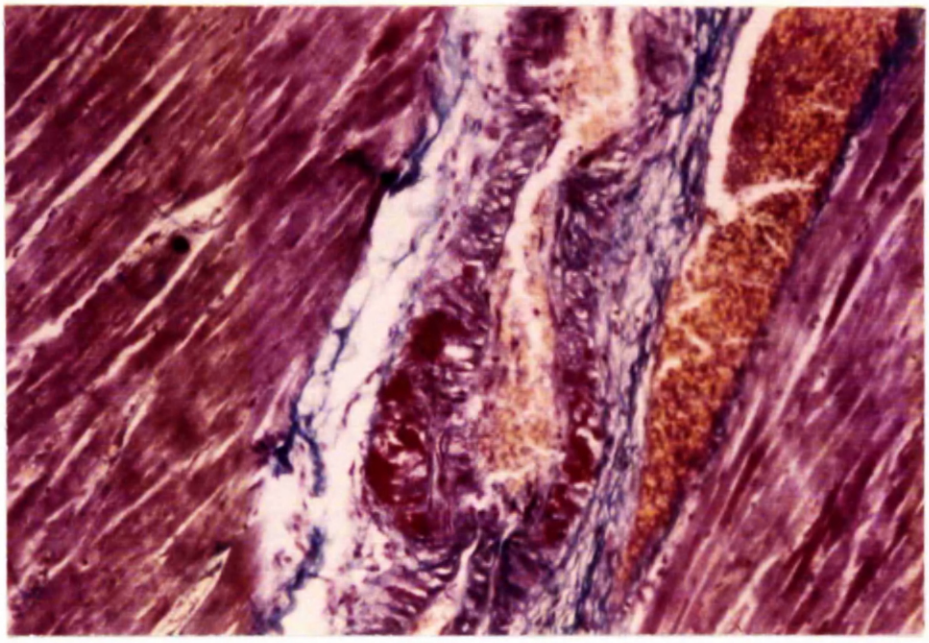


Fig.31. Case No. 22882. Chronic Nephritis. Plasmatic vasculosis in an intra-myocardial branch of a coronary artery. Mallory. x 500.

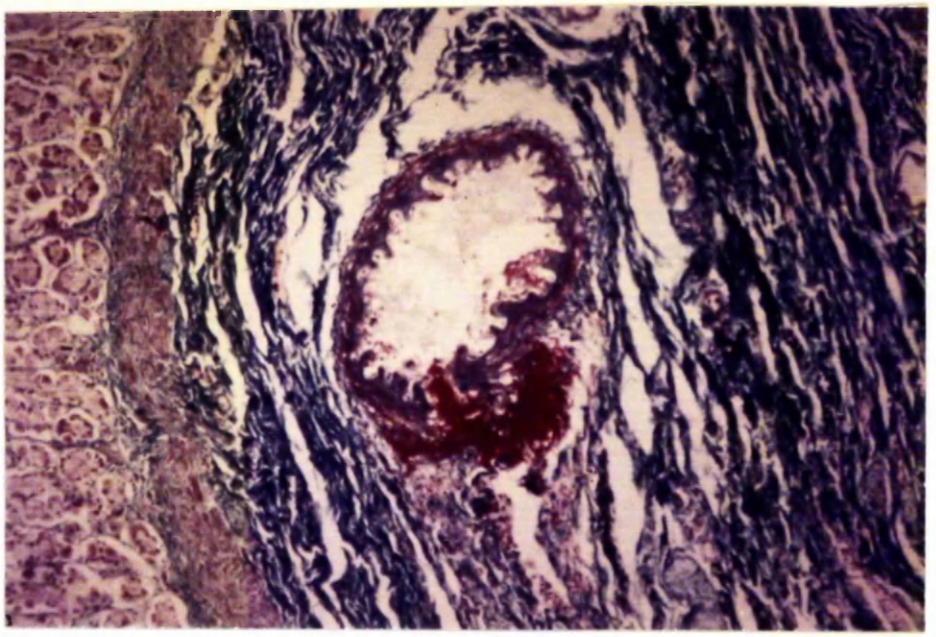


Fig.32. Case No. 4708. Chronic Nephritis. Severe focal plasmatic vasculosis in an artery in the sub-mucosa of the stomach. Mallory. x 150.

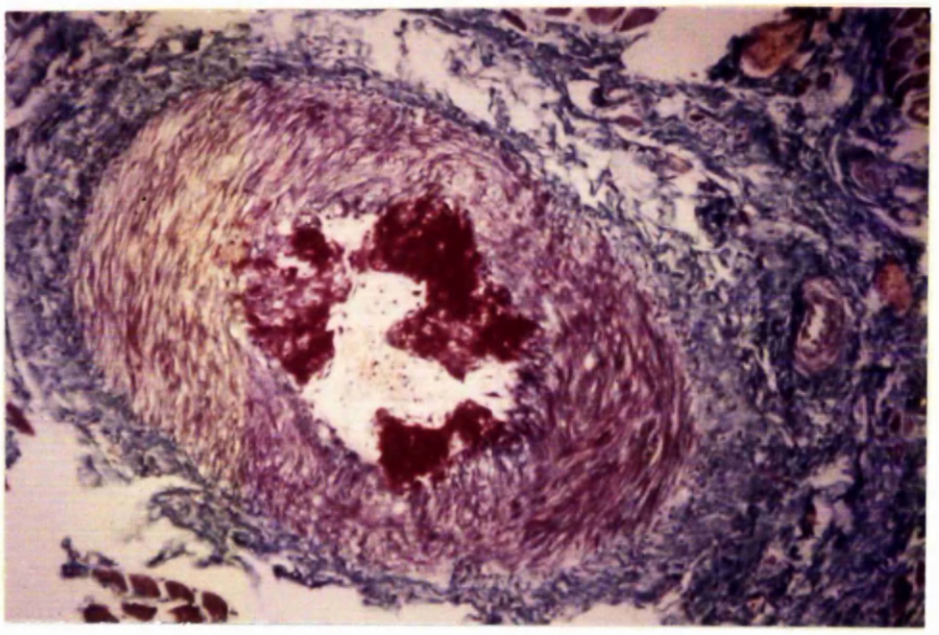


Fig.33. Case No. 23003. Acute Nephritis. Large sub-endothelial deposits of plasmatic material in an artery in the tongue. Mallory. x 300.

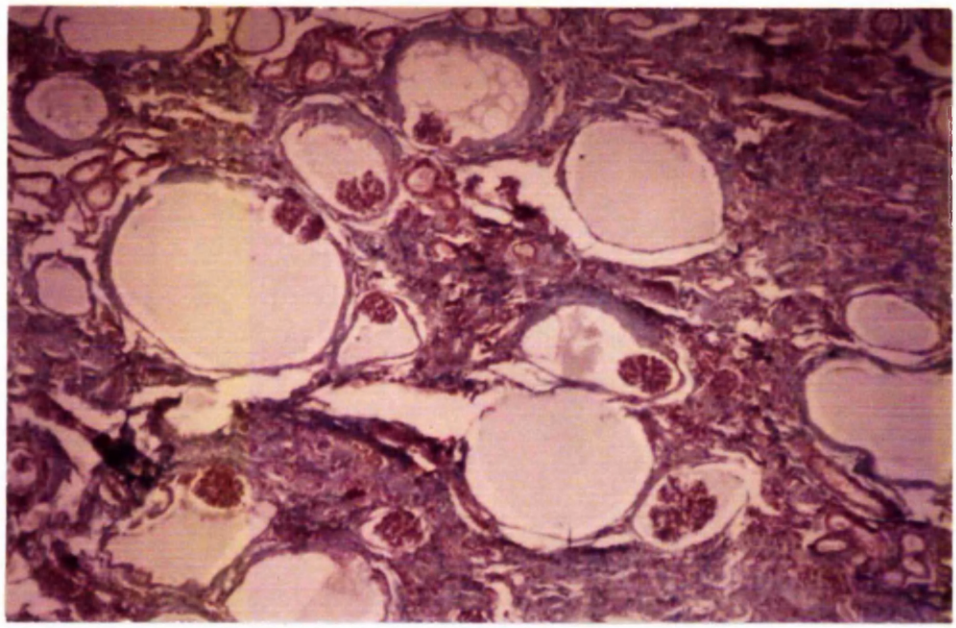


Fig. 34. Case No. 27108. Chronic Nephritis, showing distension of the glomerular capsules and collapse and atrophy of the tuft. Mallory. X 150.

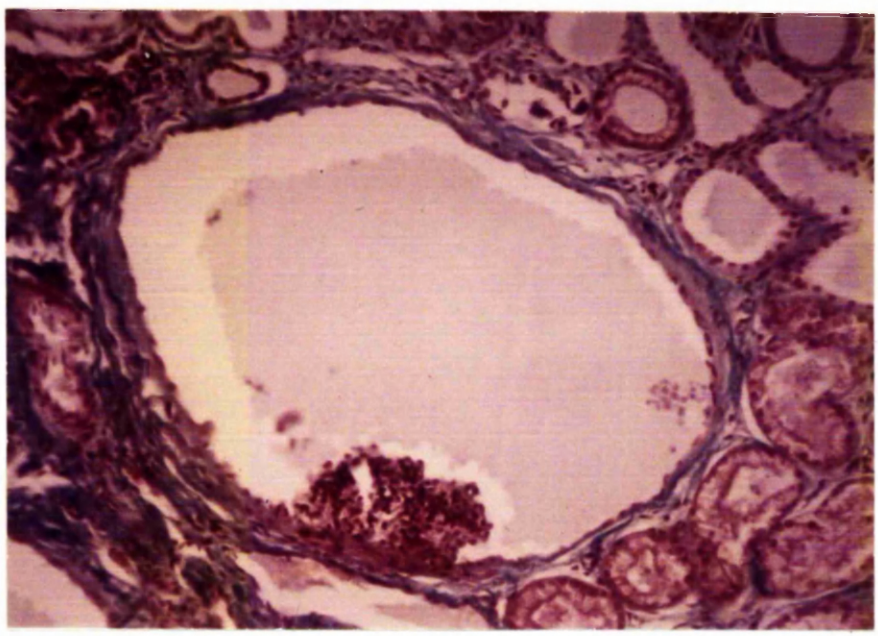


Fig. 35. Case No. 27108. A high power view of an affected glomerulus. Mallory. X 300.

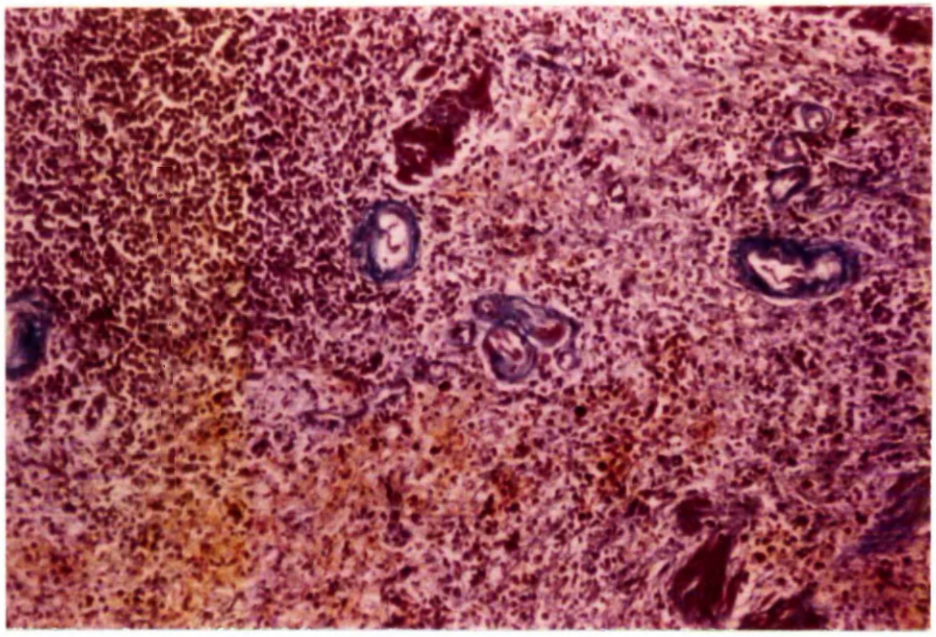


Fig. 37. Grade + Spleen. Small focal sub-endothelial deposits of plasmatic material affecting less than half of the follicular arteries. x150.

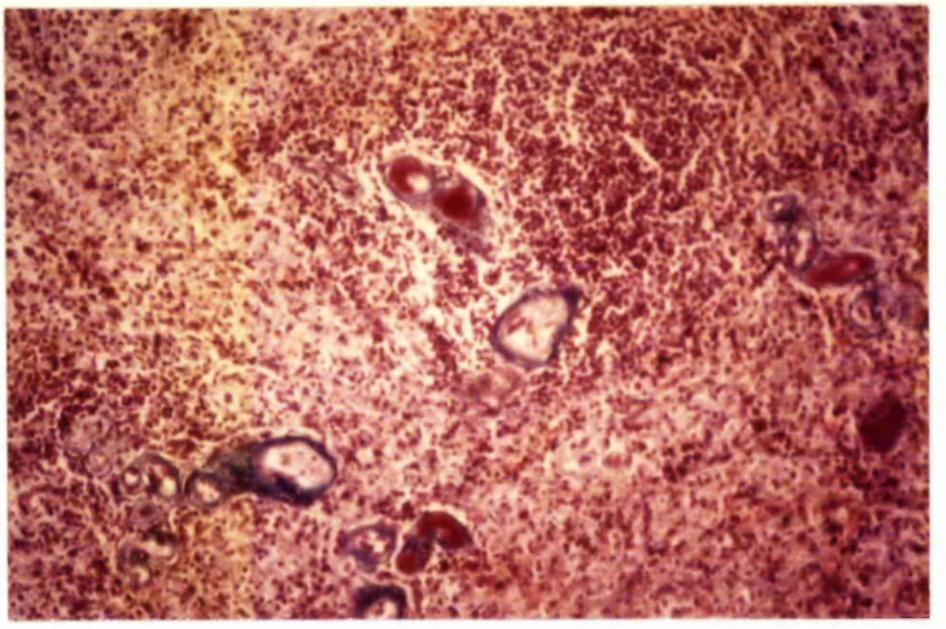


Fig.38. Grade ++ Spleen. Moderate and large plasmatic deposits in approximately half of the follicular arteries. X 150.

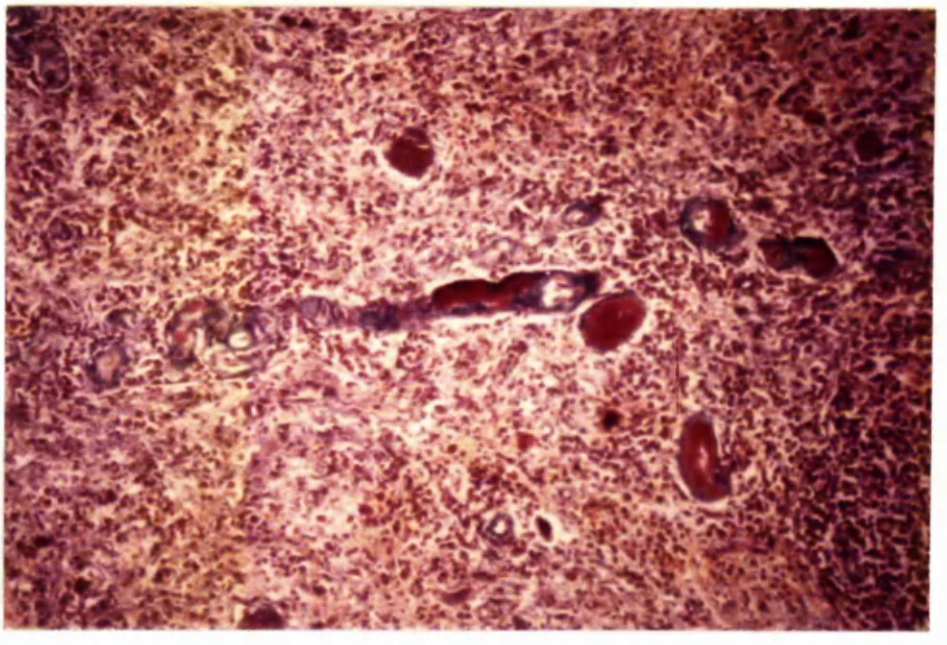


Fig.39. Grade +++ Spleen. Severe vasculosis affecting the majority of the arteries, some of which are occluded. X 150.

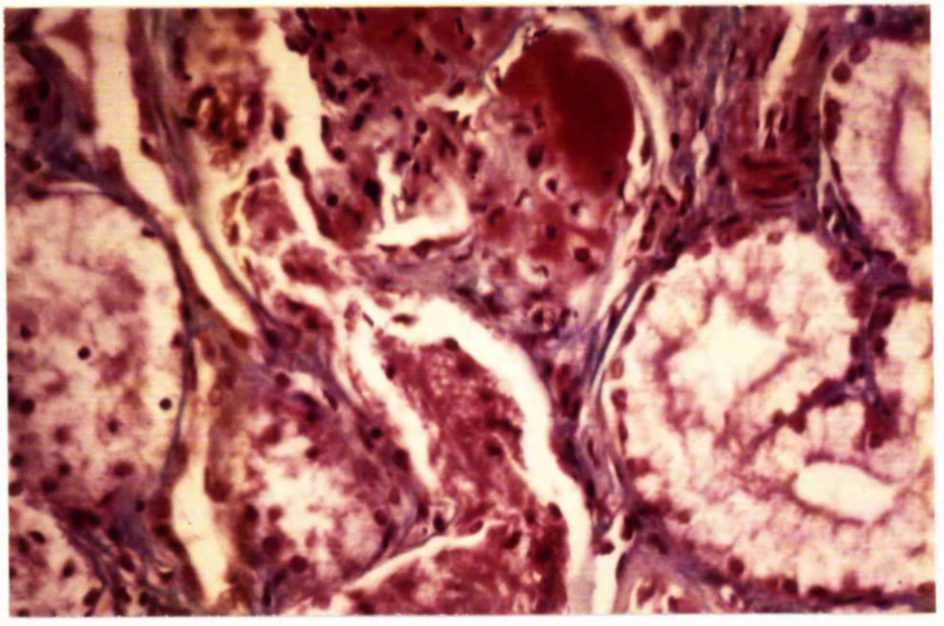


Fig.40. Glomerulus showing grade ++ damage with the related proximal tubule. The epithelium shows degenerative change, with separation from the basement membrane, pyknosis of nuclei and intensified staining. Adjacent tubules are normal. x 500.

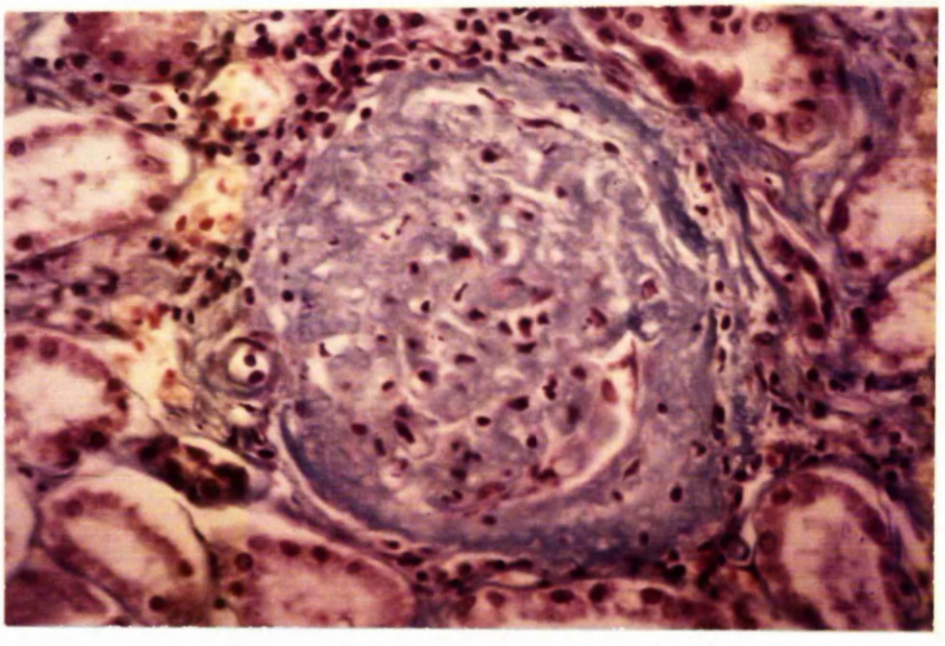


Fig.41. Grade +++ glomerulus surrounded by normal tubules. x 500.

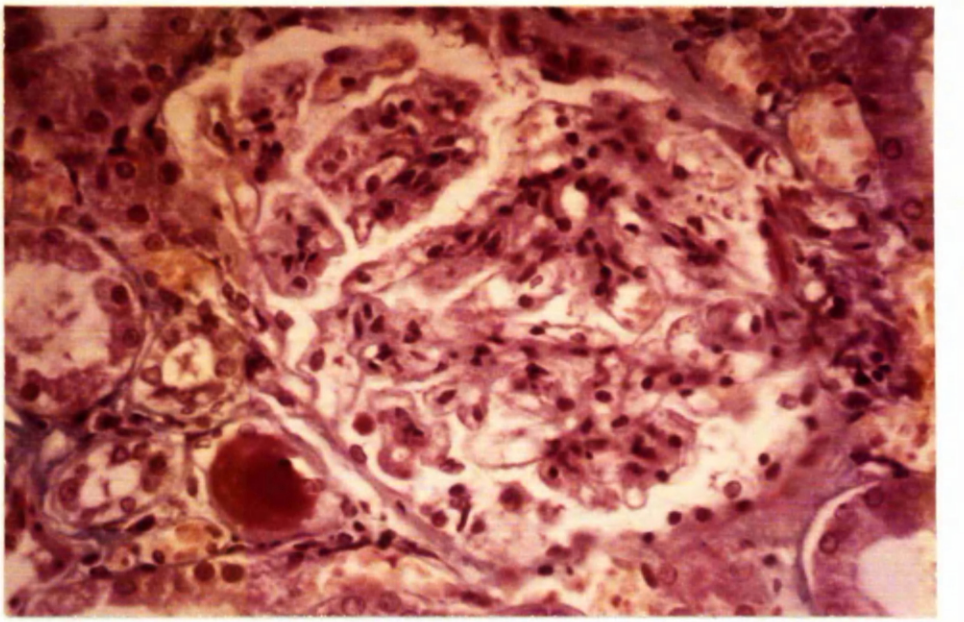


Fig.42. A normal glomerulus supplied by an arteriole showing severe vasculosis. x 500.

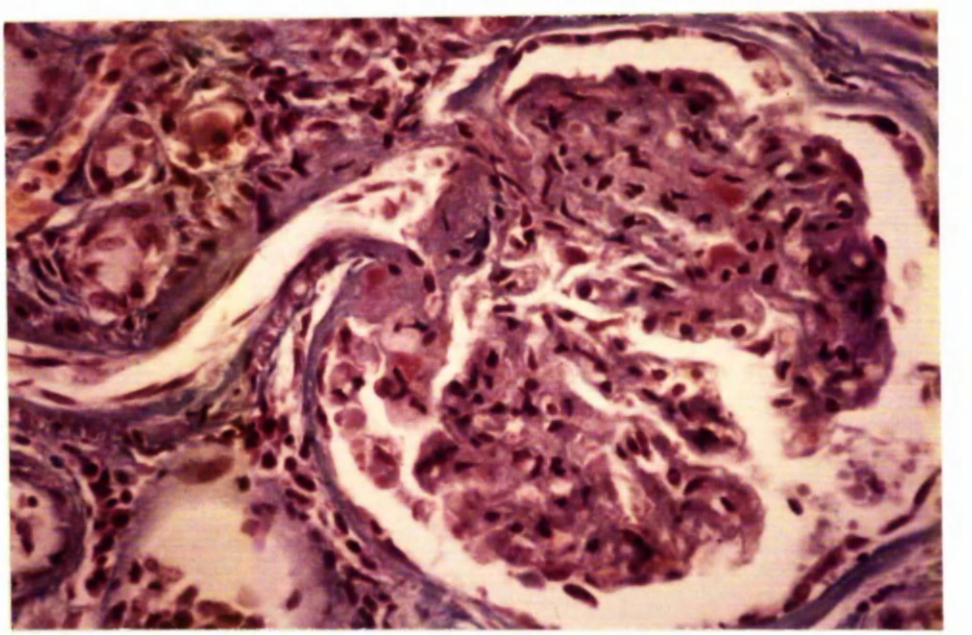


Fig.43. A grade ++ glomerulus supplied by an arteriole showing pseudo-collagenous deposits. x 500.

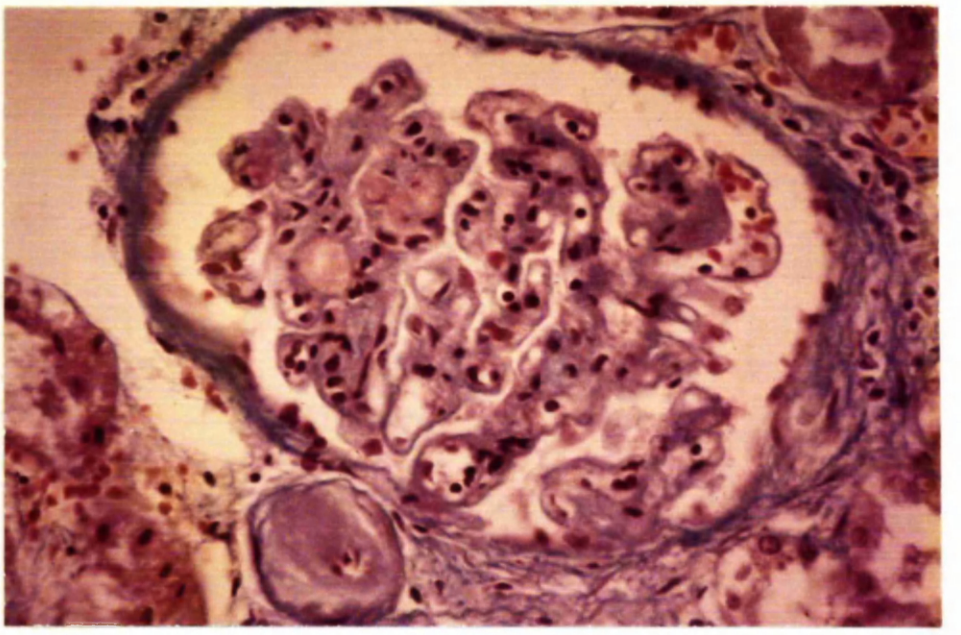


Fig.44. Glomerulus showing small pseudo-collagenous deposits in the tuft, supplied by a sclerotic arteriole. x 500.

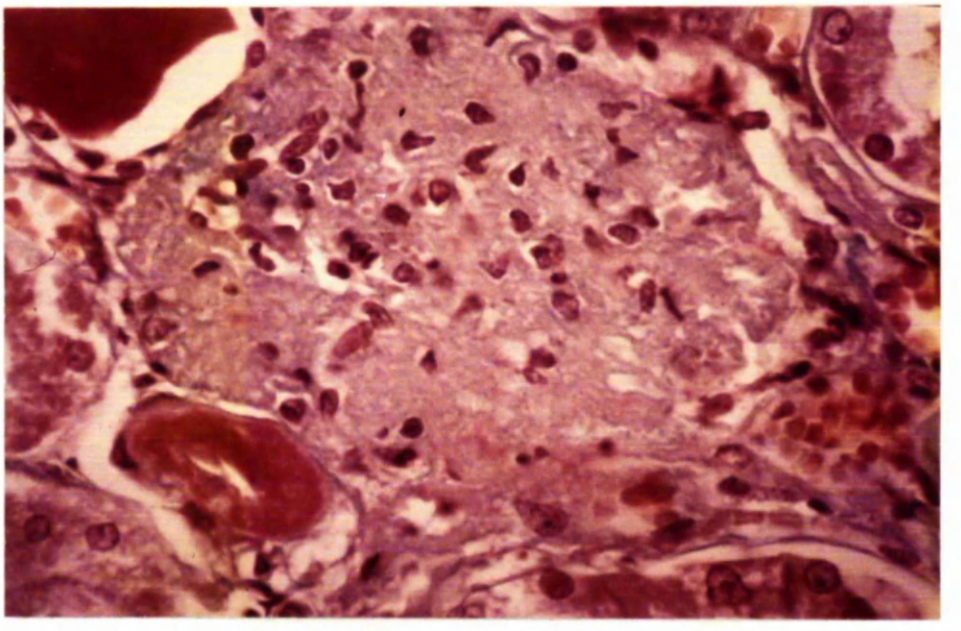


Fig.45. Grade +++ glomerulus with severe plasmatic vasculosis in the afferent arteriole. x 500.

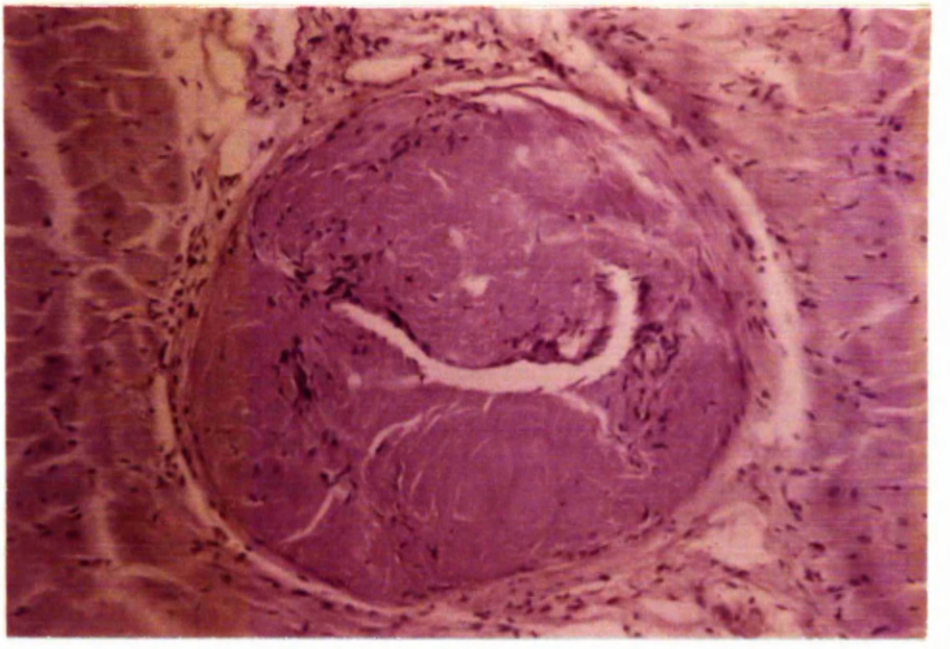


Fig.46. Case No. 17312. Chronic Nephritis. Arterial sclerosis in an intra-myocardial branch of a coronary artery, showing amorphous thickening and loss of smooth muscle cells. P.A.S. x 500.

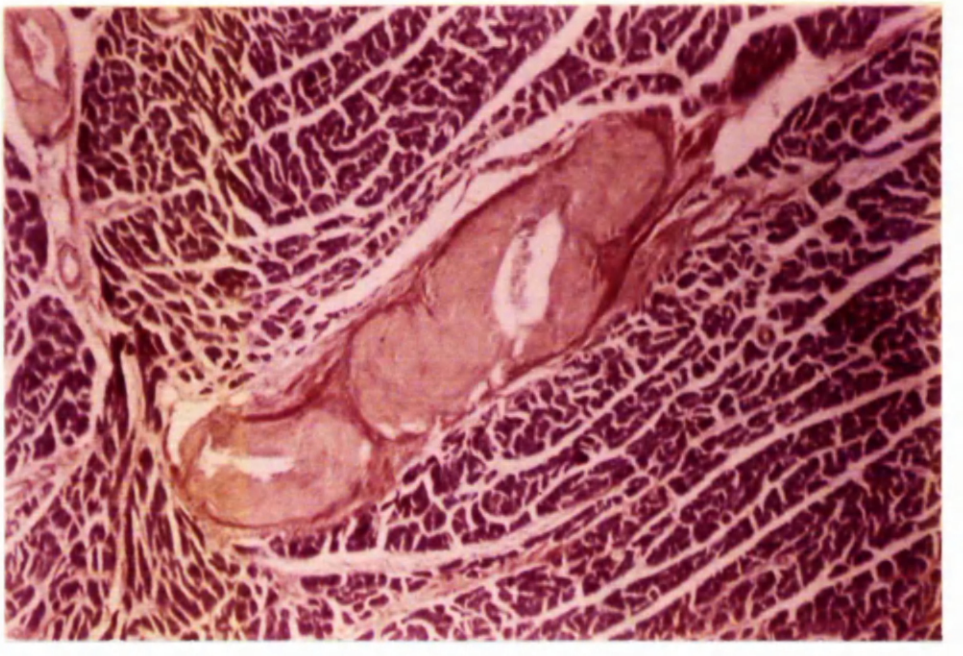


Fig.47. Case No. 17312. Arterial sclerosis in a branch of a coronary artery, showing negative reaction for fibrin. P.T.A.H. x 300.

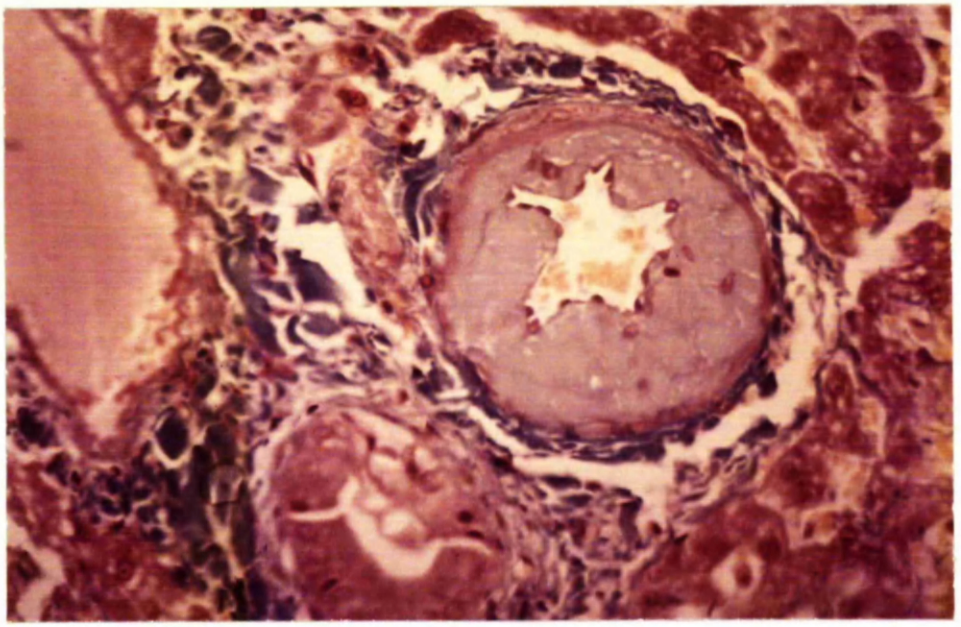


Fig.48. Case No. 8951. Chronic Nephritis. Arteriol sclerosis in a small branch of the hepatic artery. Mallory. x 500.

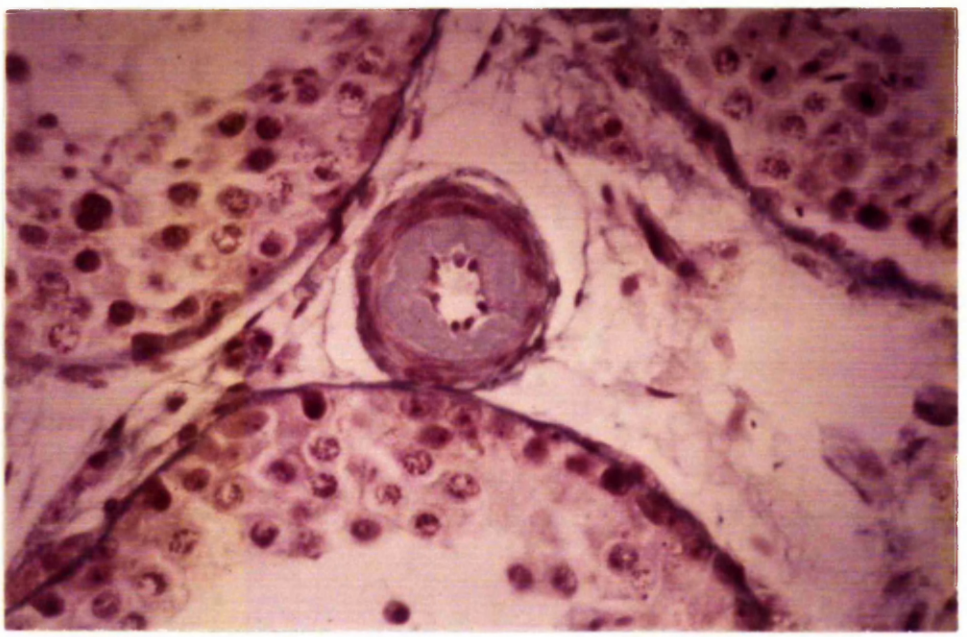


Fig.49. Case No. 21795. Sub-acute Nephritis. Arterial sclerosis in an artery in the testis. Mallory. x 500.

APPENDIX I.

Survey of Blood Vessels in Dogs with Interstitial Nephritis.

Details of the breed, age, sex, significant serological, biochemical and urological features and the distribution of plasmatic vasculosis in 130 dogs with interstitial nephritis.

Case No.	Age	Sex	Breed	Titre to L.canicola	Blood Urea (mg/100 ml)	Blood Inorganic phosphate (mg/100 ml)
Acute Nephritis.						
21260	1	M	Collie x	>1:30,000	630	10.4
21206	2	M	Cairn	>1:30,000	254	10.4
19920	5	F	Boxer	-	-	-
14593	6	M	Terrier x	-	3g%	18.0
21256	1½	M	Mongrel	>1:30,000	920	27.6
21688	1½	M	Terrier x	1:30,000	790	-
18207	6 mts.	M	Setter x	-	-	-
4086	1½	M	Alsatian	-	-	-
6337	7	F	Spaniel	-	-	-
3387	2	M	Collie	-	-	-
7562	-	M	-	-	-	-
17703	9	F	Spaniel	-	16	-
17521	9	M	Corgi	- ve	138	-
13315	7	F	Collie	-	-	-
22329	2	F	Mongrel	>1:30,000	184	25.3
23003	2	M	Collie x	>1:30,000	208	-
7090	2	M	Labrador x	>1:30,000	120	-
23689	1	M	Collie	1:30,000	320	20.2
23045	1½	M	Boxer	>1:30,000	415	14.5
24554	12	M	Labrador	>1:30,000	105	7.2
Sub-Acute Nephritis.						
21674	1½	M	Poodle	-	-	-

		Plasmatic Vasculosis.				
Urine Urea (g./100 ml)	Urine Protein (mg/100 ml)	Kidney	Spleen	Heart	Stomach	Tongue
2.4	80	-	0	-	0	0
"	"	"	"	"	"	"
"	"	+	0	-	"	0
"	"	+	+	-	0	0
2.5	200	-	"	"	"	"
1.8	550	+	"	"	"	"
"	"	+	0	-	0	0
"	"	+	0	+	0	0
1.5	120	-	0	-	0	-
"	"	"	"	"	0	0
"	"	+	0	0	+	0
1.9	300	+	0	0	0	0
4.0	1.7gm.	+	0	-	0	0
"	"	"	"	"	"	0
"	"	"	"	"	"	"
2.0	30	+	"	"	"	+
5.7	120	-	"	"	"	0
2.2	300	+	"	+	"	+
2.4	300	-	+	-	"	"
1.7	30	-	+	-	"	"
"	"	"	0	"	"	"

Case No.	Age	Sex	Breed	Titre to L.canicola	Blood Urea (mg/100 ml.)	Blood Inorganic phosphate (mg/100 ml)
21506	12	M	Labrador x	-	930	-
16864	7	M	Boxer	-	78	-
17750	11	M	St.Poodle	-	450	-
14840	15	M	Terrier	-	-	-
11373	9	M	Border Terrier	1: 300	116,6	-
6104	7 mts.	M	Collie x	>1:30,000	106	-
22882	8	F	Cock.Span.	- ve	440	24
14455	10	F	Collie	- ve	71	-
12732	12	F	Terrier	-	83	-
10622	6	M	Spaniel	-	185	-
11879	4	F	Mongrel	1: 3,000	98.3	-
12424	1	M	Alsatian x	1: 300	61	-
8198	4	M	-	-	-	-
21795	10	M	Alsatian	1: 30	300	-
15553	7	M	Shetland C.	-	600	-
23422	3	M	Terrier	1:10,000	122	5.3
21830	6	M	Boxer	1: 1,000	680	41.6
Chronic Nephritis.						
25488	6	M	-	-	740	25.9
21800	4	F	W.Highland	- ve	196	13.7
21540	15	F	Labrador	- ve	710	18.2
21460	10	M	Collie x	- ve	770	45.4

Urine Urea (g./100 ml)	Urine Protein (mg/100 ml)	Plasmatic Vasculosis. Kidney	Spleen	Heart	Stomach	Tongue
1.2	210	+	+	-	-	-
-	-	+	0	0	0	0
-	-	+	0	0	0	0
-	-	+	0	-	-	0
-	-	+	0	0	0	0
-	-	-	-	0	0	0
-	-	+	+	+	-	-
2.6	600	+	0	0	0	0
-	-	+	+	-	-	0
-	-	-	0	-	0	0
2.0	100	-	+	-	0	0
3.8	20	-	0	0	0	0
-	-	-	0	0	0	0
-	-	+	+	-	-	-
-	-	-	0	-	-	0
5.6	50	+	+	-	-	+
-	-	-	0	-	-	0
-	-	+	+	+	-	-
1.6	900	+	+	-	-	-
-	-	-	+	-	0	0
.07	>10	-	+	-	-	-

Case No.	Age	Sex	Breed	Titre to L.canicola	Blood Urea (mg/100 ml)	Blood Inorganic phosphate (mg/100 ml)
21274	5	M	"	"	"	"
20626	5	M	Bull Terrier	"	"	"
20466	"	M	"	1: 1,000	"	"
20192	7	M	Cairn x	- ve	450	"
19961	1½	M	Alsatian	"	700	"
19548	7	M	"	"	"	"
19397	7	M	Cairn	"	540	13.5
19258	10	F	Cairn	"	450	"
17756	10	M	Mongrel	"	216	12
7478	12	M	Boxer	"	135	7.6
8876	12	F	Spaniel	"	"	"
15425	12	M	Dalmatian	"	99	"
3186	9	M	Irish Wat.Span.	"	218	"
4514	4 mts.	M	"	"	"	"
4818	6	F	Collie x	"	400	"
4444	5	M	Boxer	1: 300	50	"
10447	5 mts.	M	Alsatian	- ve	520	"
17422	3	M	Alsatian	"	430	"
17823	6	M	Boxer	"	450	"
16847	6	M	Mongrel	"	270	"
16984	9	M	Spaniel x	"	>400	"

Urine Urea (g./100 ml)	Urine Protein (mg/100 ml)	Plasmatic Vasculosis. Kidney	Spleen	Heart	Stomach	Tongue
-	-	+	-	-	-	-
-	-	-	0	0	0	0
-	-	-	0	0	-	0
-	-	+	0	-	-	0
-	-	-	-	0	0	0
-	-	+	0	0	0	0
-	-	+	+	-	-	-
1.5	120	+	0	0	0	0
1.2	200	+	0	0	0	0
20.6	-	+	+	-	-	0
-	-	+	0	-	0	0
2.4	180	+	+	-	-	-
1.3	120	+	+	-	-	0
-	-	+	+	-	-	-
-	-	+	0	0	-	0
-	210-240	+	0	-	0	0
-	-	-	+	0	0	0
1.25	900	+	-	-	-	-
-	-	+	+	+	-	0
1.2	100	+	0	+	-	0
1.6	200	+	0	0	0	0

Case No.	Age	Sex	Breed	Titre to L.canicola	Blood Urea (mg/100 ml)	Blood Inorganic phosphate (mg/100 ml)
17327	10	M	Collie	-	400	-
17129	7	M	Alsatian	-	176	-
8252	10	M	-	-	-	-
5712	9	M	Spaniel	-	21	-
18587	4	F	Alsatian	-	450	-
22442	8	M	Labrador	-	-	-
22249	5	M	Corgi	-	340	17.4
22144	9	M	Cairn	-	-	-
20566	9	M	Lab x Span	1: 1,000	225	11
19196	10	M	Terrier x	- ve	240	-
23473	3	M	Collie	- ve	300	-
20132	5	M	Collie x	1: 100	450	-
23571	2	M	Boxer	- ve	>400	16.9
24019	3	M	Collie	-	590	24.6
1466	5	M	Mongrel	1: 1,000	90	-
1543	1	F	Alsatian	1: 3,000	227	-
2717	6	M	Collie	1: 300	120	-
6965	6	M	Boxer	- ve	-	-
9157	12	M	Spaniel	1: 1,000	173.3	-
11170	7	M	Bull Terrier	-	206	-
12114	7	M	Terrier x	1: 100	63.5	-
13418	-	M	Spaniel	1: 300	25	12.8

Urine Urea (g./100 ml)	Urine Protein (mg/100 ml)	Plasmatic Vasculosis. Kidney	Spleen	Heart	Stomach	Tongue
"	"	"	0	"	0	0
"	"	+	+	"	0	0
"	"	+	+	"	"	0
"	"	+	+	"	+	0
17	100	+	+	"	"	0
"	"	+	+	0	0	0
"	"	+	+	+	"	"
"	"	+	+	"	"	"
1.2	120	+	+	+	"	0
1.5	150	+	+	+	0	0
1.8	300	+	"	"	"	"
6.0	120	"	"	"	"	0
2.1	>300	+	+	"	"	"
"	"	+	+	+	"	"
"	200	+	+	"	0	0
"	300	+	+	"	0	0
"	"	+	0	"	0	0
1.6	160	+	+	"	"	0
1.0	100	+	+	0	0	0
1.6	120	+	0	"	0	0
2.0	110	+	0	"	0	0
0.9	300	+	+	"	0	0

Case No.	Age	Sex	Breed	Titre to L.canicola	Blood Urea (mg/100 ml)	Blood Inorganic phosphate (mg/100 ml)
13040	10	M	Terrier	-	95	-
16313	7	M	Boxer x Labrador	-	20	-
14910	9	F	-	-	-	-
12507	5	M	Collie	-	200	45
25270/2	-	F	Spaniel	-	104	4.7
6152	10	M	Collie x	1: 30	106	-
4708	6	M	Terrier x	1: 100	-	28
12806	14	M	Mongrel	1: 300	>300	-
41	11	F	Spaniel x	1: 100	220	-
11985	7	M	Collie	1: 1,000	-	-
16636	4	M	Labrador	- ve	400	-
17312	13	M	-	-	-	-
10578	10	M	Bull Terrier	1: 300	-	-
16573	6 mts.	F	Corgi	-	-	-
16733	5	M	Labrador x	-	-	-
16602	12	M	Bull terrier	1: 100	228	9.7
13835	10	F	Terrier	- ve	120	19.8
14101	8	M	Terrier x	-	105	27
13158	7	M	Labrador	- ve	>300	-
11277	15	M	Terrier	1: 80	166.6	-
10450	4	M	Irish Setter	-	-	-
11183	8	M	Cocker Sp.	-	-	-

Urine Urea (g./100 ml)	Urine Protein (mg/100 ml)	Plasmatic Vasculosis. Kidney	Spleen	Heart	Stomach	Tongue
-	-	+	-	-	0	0
-	-	+	+	+	+	0
-	-	+	+	+	0	0
-	-	+	0	-	-	-
-	-	+	+	-	-	-
-	80	-	0	0	0	0
-	280	+	+	+	+	+
1.75	10	+	+	0	0	0
1.4	240	+	0	0	0	0
1.6	100	+	0	0	0	0
2.0	100	+	0	0	0	0
-	-	+	+	-	0	0
2.1	100	+	+	0	0	0
-	-	-	0	0	0	0
-	-	+	0	-	0	0
-	-	+	0	0	0	0
-	-	+	0	-	0	0
-	-	+	0	0	0	0
-	-	+	+	-	-	-
2.1	110	+	0	0	0	0
-	-	-	0	-	0	0
2.5	120	+	0	0	0	0

Case No.	Age	Sex	Breed	Titre to L.canicola	Blood Urea (mg/100 ml)	Blood Inorganic phosphate (mg/100 ml)
10636	10	F	Alsatian	-	-	-
12804	10	F	-	-	-	-
13028	10	M	Corgi	-	140	14.2
13535	12	F	-	-	-	-
13161	5	M	Collie	1: 300	>300	-
13365	9	M	-	-	-	-
14314	5	M	Alsatian	-	120	-
8951	11	-	-	-	-	-
7409	4	F	Min.Poodle	- ve	220	-
7200	10	M	Cairn	-	-	-
7122	10	F	Collie x	-	107	-
7089	7	M	Alsatian	1: 30	79	-
7091	9	F	Spaniel	-	120	-
6186	13	F	-	-	-	-
10485	12	M	Collie x	-	-	-
10835	9	F	Spaniel	- ve	440	-
26822	9	M	Corgi	-	-	-
25529	5	M	Pomeranian	1: 30	106	6.0
26626	9	M	Alsatian	1: 30	240	10.5
27131	5	F	Kerry Blue	- ve	840	16.7
27107	13	M	Terrier	- ve	540	26.2
27108	1½	F	Bull Terrier	- ve	880	37.0

Urine Urea (g./100 ml)	Urine Protein (mg/100 ml)	Plasmatic Vasculosis. Kidney	Spleen	Heart	Stomach	Tongue
"	-	+	0	0	0	0
"	"	+	+	0	0	0
"	"	+	0	0	0	0
"	"	+	0	0	0	0
0.9	80	+	0	0	0	"
"	"	"	+	"	0	0
"	"	+	0	"	0	0
"	"	+	0	"	0	0
1.4	20	"	"	"	0	0
"	"	+	+	0	0	0
"	"	+	0	0	"	0
3.8	200	+	+	0	0	0
1.4	150	+	0	0	0	0
"	"	+	0	"	0	0
"	"	+	0	0	0	0
1.1	100	+	"	"	"	"
"	"	+	"	"	"	"
1.6	300	+	"	"	"	"
1.8	100	+	"	"	"	"
"	"	+	+	"	"	"
1.6	300	+	+	"	"	"
2.1	300	+	+	"	"	"

APPENDIX II.

Survey of Blood Vessels in Dogs.

Details of the breed, age, sex, disease status and incidence of plasmatic vasculosis in a control series of 125 dogs with normal kidneys.

APPENDIX II.

Survey of Blood Vessels in Dogs.

Control Series of 125 Dogs with Normal Kidneys.

No.	Age	Sex	Breed	Diagnosis	Plasmatic Vasculosis
Group 1. 24 Dogs < 2 years of age.					
24574	10 d.	M	-	Inconclusive	-
23041	1 m.	F	Labrador x	Distemper	-
21343	2 m.	M	Great Dane	Distemper	-
21194	2 m.	F	Alsatian x	Hepatitis	-
23723	2 m.	F	Collie x	Intussusception	-
21225	3 m.	M	Mongrel	Distemper	-
23333	3 m.	M	Scottie	Pneumonia	-
24927	3 m.	M	Labrador	Distemper	-
23897	3 m.	F	Collie	Inconclusive	-
23212	3 m.	M	Labrador	L. ictero-haemorrhagiae	-
23064	3 m.	M	Alsatian	Broncho-pneumonia	-
24520	4 m.	M	Alsatian	Inconclusive	-
23953	4 m.	M	W.High.Ter.	Brain haemorrhage	-
23187	5 m.	F	Cairn	Sod. chlorate poisoning	-
24049	6 m.	M	Poodle	Filaroides osleri	-
22213	7 m.	M	Border Ter.	Endocarditis	-
23503	7 m.	M	Mongrel	Fractured spine	-
23693	10 m.	F	Afghan hound	Lymphadenitis	-
21327	1 yr.	M	Collie	Toxoplasmosis	-

No.	Age	Sex	Breed	Diagnosis	Plasmatic Vasculosi
23986	1½ yr.	F	Irish Setter	Encephalitis	-
23905	1½ yr.	F	Dalmatian	Pleurisy & Pericarditis	-
23169	2	F	Cairn	Oesophageal F.B.	-
22834	2	M	Greyhound	Savaged	-
23537	2	M	Dobermann	Duodenal F.B.	-
Group 2. 33 Dogs of 3 - 6 years of age.					
25278	3	M	Labrador	Encephalitis	-
23500	3	M	Beddlington Terrier	Lymphosarcoma	-
11018	3	M	Spaniel	Spinal Fracture	-
22686	3	F	Poodle	Meningo-encephalitis	-
17883	3	F	-	Pneumonia	-
21348	3	F	Keeshond	Pancreatitis	-
22883	3	M	Poodle	Chronic enteritis	-
19927	3	M	Chow	Adeno-carcinoma	-
17260	3	F	Cairn	Oesophageal ulcers	-
16216	3	F	Labrador	Sinusitis	-
20810	3	M	Corgi	Tonsillar carcinoma	-
20143	3	F	Terrier	Cystitis	Spleen +
24254	3	M	Collie	Endocarditis	Small coro ary artery
24245	3	F	Pekinese	Thyroid carcinoma	-
25756	3	M	Alsatian	Cirrhosis	-

No.	Age	Sex	Breed	Diagnosis	Plasmatic Vasculosis
26412	3	M	Pekinese	Enteritis	-
24462	4	M	Alsatian	Gastro-enteritis	-
17611	4	M	Griffon	Tuberculosis	-
16570	4	M	Cairn	Tonsillar carcinoma	-
16277	4	M	Boxer	Meningeal-sclerosis	-
26090	4	M	Dachshund	Diaphragmatic hernia	-
22998	5	M	-	Tonsillar carcinoma	-
22231	5	F	Pekinese	Hepatic necrosis	-
18998	5	M	Corgi	Gastric F.B.	-
20569	5	M	Labrador	Enteritis	-
16844	5	M	Alsatian	Haemangio-sarcoma	Spleen +
19992	6	M	Alsatian	Cellulitis	-
22245	6	F	-	Endocardosis	-
19969	6	F	Spaniel	Cirrhosis	Spleen +
18891	6	F	-	Cellulitis	-
25228	6	F	Corgi	Pericarditis	Spleen + Kidne one interlobul artery +
26113	6	M	Labrador	Inconclusive	-
26028	6	F	Collie	Lymphosarcoma	-
<u>Group 3.</u>	<u>49 Dogs of 7 - 10 years of age.</u>				
23450	7	F	Boxer	Cervical disc protrusion	Spleen +
21132	7	F	Boxer	Lumbar disc protrusion	-

No.	Age	Sex	Breed	Diagnosis	Plasmatic Vasculosis
24619	7	M	Shetland collie	Tonsillar carcinoma	-
23312	7	F	Spaniel	Mammary carcinoma	-
23300	7	M	Alsatian	Heart block	-
22583	7	M	Alsatian	Osteosarcoma	Spleen +
22206	7	F	Shetland Collie	Encephalitis	-
19928	7	M	W. High.	Cystitis	-
23522	7	F	Boxer	Osteosarcoma	Spleen +
17147	7	F	Labrador	Rectal impaction	-
26067	7	M	Collie	Aortic thrombosis	-
24309	8	M	Poodle	Post-op death	-
23867	8	M	Dachshund	Intestinal F.B.	-
19807	8	F	Dachshund	Diabetes mellitus	Spleen +
23236	8	M	Alsatian	Oral carcinoma	-
21527	8	F	Cairn	Chronic enteritis	-
21605	8	M	-	Endocardosis	-
20332	8	M	-	Pituitary carcinoma	-
20459	8	F	Boxer	Ovarian carcinoma	-
19602	8	M	Cairn	Thyroid carcinoma	-
19741	8	M	Spaniel	Rectal carcinoma	-
19840	8	M	Mongrel	Nasal carcinoma	-
16638	8	F	Labrador	Bronchial carcinoma	-

No.	Age	Sex	Breed	Diagnosis	Plasmatic Vasculosis
24722	8	M	Cairn	Tonsillar carcinoma	-
24114	8	F	Corgi	Endocardosis	-
22539	9	F	Terrier	Pyometra	-
21533	9	M	Collie	Testicular tumour	-
22959	9	M	Terrier	Tonsillar carcinoma	-
19148	9	M	Alsatian	Cystitis	-
18910	9	F	Boxer	Vaginal carcinoma	-
19382	9	F	Terrier	Osteosarcoma	-
17393	9	F	Terrier	Tonsillar carcinoma	kidney: one interlobular artery +
16473	9	F	Spaniel	Adrenocortical adenoma	-
16541	9	M	Spaniel	Perianal adenomata	-
16752	9	M	Spaniel	Peritonitis	-
25704	9	F	Spaniel	Diabetes mellitus	-
25239	9	F	Boxer	Pituitary adenoma	Spleen +
24232	9	M	Cairn	Intestinal F.B.	-
25328	10	M	Alsatian	Osteosarcoma	Spleen +
25268	10	M	Terrier	Tonsillar carcinoma	Spleen +
19602	10	M	Cairn	Endocardosis	-
23665	10	F	Bull Mastiff	Mammary carcinoma	-
23011	10	M	Dachshund	Endocardosis	Spleen +
20853	10	F	Spaniel	Lymphohaemangioma	-
20010	10	F	Boxer	Osteosarcoma	-
16409	10	M	Scottie	Pancreatitis	-

No.	Age	Sex	Breed	Diagnosis	Plasmatic Vasculosis
16417	10	M	Mongrel	Bronchitis	-
16881	10	M	Fox Terrier	Haemangiosarcoma	-
26300	10	M	Spaniel	Lymphosarcoma	-
Group 4: 19 Dogs aged > 10 years.					
21806	11	M	Irish Setter	Myositis	Spleen +
15307	11	M	Spaniel	Pancreatic carcinoma	-
23410	11	M	Poodle	Enteritis	Spleen +
16846	11	M	Terrier	Endocardosis	Spleen +
20844	12	M	Collie	Inconclusive	Spleen +
5057	12	M	Terrier	Tonsillar carcinoma	Spleen +
24944	12	M	Terrier	Endocardosis	Spleen +
22931	12	M	-	Broncho-pneumonia	-
21544	12	M	Labrador	Broncho-pneumonia	Spleen +
20109	12	M	Spaniel	Haemangiosarcoma	-
17025	12	M	Labrador	Melanoma	-
26087	12	M	Labrador	Lymphosarcoma	Spleen +
22248	13	F	Mongrel	Mammary carcinoma	-
17835	13	M	Terrier	Haemangiosarcoma	-
17074	13	F	Terrier	Endocardosis	-
20329	14	M	-	Melanoma	Spleen +
17274	14	M	Schnauzer	Haemangioma	Kidney: few interlobular arteries +
24302	14	F	Shetland Collie	Tonsillar carcinoma	-
21690	15	M	Terrier	Tuberculosis	Spleen +

SECTION II.

A STUDY OF THE BLOOD PRESSURE OF NEPHRITIC AND NON-NEPHRITIC DOGS.

Introduction.

The vascular changes described in the preceding section closely resemble those associated with hypertension in the human and experimental canine hypertension and therefore suggest that hypertension may develop in a large proportion of nephritic dogs. To establish whether such animals do in fact have abnormally high blood pressure, recordings of arterial pressure were made from a series of dogs suffering from nephritis. The measurements were correlated with the stage and severity of the disease and with post mortem and histological findings when possible, to demonstrate any relationship between blood pressure, phase of nephritis and cardio vascular changes. The series was controlled by comparison with pressure recordings from normal dogs and dogs suffering from non-renal conditions.

Previous authors have employed a number of methods in the estimation of canine blood pressure and these will be discussed in more detail later.

The use of a sphygmomanometer in dogs was first described by Ferris and Hynes (1930), who measured the femoral arterial pressure of apparently normal animals. The observed range of systolic pressures was 110 - 130 m.m. Hg. and the diastolic pressure was approximately 80 m.m. Hg. Repeated observations on individual dogs gave gradually falling results.

Carotid arterial pressure was measured in experimental dogs by Gregg/

Gregg, Eckstein and Fineberg (1937). Exteriorized carotid loops were employed in these animals to permit repeated direct estimation. In well trained dogs the basal systolic pressure was approximately 124 and the diastolic 85.

Romagnoli (1953) compared the use of one direct and two indirect methods of measuring the femoral arterial pressure of dogs. The indirect methods gave very slightly lower systolic and higher diastolic pressures than the direct method. The mean pressures obtained were approximately 155/99 (direct) and 148/100 (indirect).

A study of the blood pressure of trained dogs over a prolonged period was made by Zapp (1949). The pressure of six adult male dogs was measured indirectly twice daily for 14 months. The systolic pressure range was 114 - 180 (mean 148) and the diastolic range 56 - 105 (mean 83).

Wilhelmj, Waldman and McGuire (1951) also carried out measurements on trained dogs. Repeated daily indirect estimations were made from thirteen dogs. The mean systolic pressure range was 95 - 136 and the mean diastolic range 43 - 66. The effect of fasting was studied and found to be associated with a fall in blood pressure and reduction in heart rate.

Surveys of blood pressure have been carried out on large numbers of dogs by several authors. In each series, a small proportion were found to have abnormally high pressure. These observations form an important/

important indication that hypertension may occur in dogs. The earliest of the large surveys was that of Hamilton, Pund, Slaughter, Simpson, Colson, Colman and Bateman (1940), who measured the femoral pressure of 215 street dogs by a direct method. The dogs were sedated with morphine. The observed systolic pressure range was 100 - 275 (mean 179.5) and the diastolic range 30 - 140 (mean 88.6). In five dogs with pressures greater than 200/100, hyalinized arterioles were found in the kidneys and in two of these extensive renal fibrosis was present.

Wakerlin (1943) described the use of indirect and direct methods of estimating the blood pressure of dogs. By the indirect method, the brachial arterial pressure was measured and the observed systolic range was 100 - 180 and the diastolic range 60 - 120. By the direct method employed, only the mean femoral pressure could be determined and this showed a range of 90 - 150. Wakerlin considered that hypertension probably does occur in dogs and he encountered one which had a mean femoral pressure of 150 - 180.

Three dogs with "spontaneous" hypertension were studied by Stamler, Katz and Rodbard (1949). Each of these dogs had pressures greater than 185/100 and one was known to develop hypertension from previously normal levels. Post-mortem observations were reported on two of the dogs in which areas of renal fibrosis and adrenal cortical hyperplasia were present. In life these animals did not show reduction in renal plasma flow or glomerular filtration rate and did not develop any impairment during/

during three years of observation. In the dogs, therefore, high blood pressure occurred in association with renal lesions which were not severe enough to cause renal failure.

An extensive survey of blood pressure was carried out by McCubbin and Corcoran (1953), who measured directly the femoral arterial pressure of 400 street dogs. Care was taken to ensure that the dogs were not excited or disturbed in any way, as only one recording was made from most of the animals. Only systolic pressures were reported and the observed range was 99 - 150, with the majority between 111 and 140. Regarding 150 as the upper limit of normality, nine dogs were found to be hypertensive and repeated measurements were made on these animals. Seven had pressures ranging from 150 - 157 and these fell in subsequent examinations. Two dogs showed persistantly high levels of 175 and 205. The renal plasma clearances of these dogs were low and at autopsy the kidneys were found to be extensively fibrosed.

Normal and hypertensive blood pressure levels were defined by Katz, Skom and Wakerlin (1957) on the basis of direct recordings from 1,000 trained dogs. The pressure of each dog was measured once or twice weekly for up to 8 months. Little or no change in pressure was found as the dogs became accustomed to the procedure. Normotension and hypertension were defined in terms of mean arterial pressure. The upper limit of normal was found to be 145 (135 - 155) ± 7.5 and the lower limit of hypertension 150 (140 - 160) ± 7.5. Of the 1,000 dogs examined, nine/

nine were found to be hypertensive. On post mortem examination, three of these showed chronic pyelo-nephritis, one had a gastric ulcer and in one adrenal adenomata were present. No abnormalities were apparent in the four remaining hypertensive dogs.

The hypertensive dogs described in these publications were all found in the course of surveys of blood pressure on large numbers of animals. The observation of persistantly high levels associated with renal fibrosis in a small proportion of the dogs was good evidence that a relationship might exist between chronic nephritis and hypertension in the dog, as in the human.

A significant contribution in this field was made by Doeglas (1953), who measured indirectly the pressure of a series of dogs suffering from a variety of conditions. Estimations of normal systolic and diastolic pressures were 140 - 150 and 80 - 100 respectively. The series included 26 uraemic and nephritic dogs (the latter without stated urea levels). The systolic and diastolic pressures of these dogs were 125 - 240 and 70 - 180 respectively (exclusing low pressures on two moribund dogs). In most of this group the pressures exceeded 150/100.

In the most recent study of canine blood pressure, Wilson and Clarke (1964) reported elevated pressure in association with uraemia, chronic interstitial nephritis and prostatic abscesses. However, no attempt was made to correlate the higher pressure with these conditions because of the/

the small numbers examined. These authors described an indirect method and reported the systolic pressure of 52 normal adult dogs. The range of pressure was 104 - 180, (mean 142) and more than 50% of the readings fell between 130 and 150. In addition, considerable day-to-day variation was observed in two dogs whose pressures were measured repeatedly (120 - 150 and 130 - 162 respectively). The pressure was found to increase steadily with age in dogs from 30 - 240 days old.

The published estimates of normal arterial pressure are summarized in the table overleaf. Although there is considerable variation in the results, it is apparent that in general the direct methods give higher systolic and lower diastolic measurements than the indirect methods. The normal systolic pressure has been considered to lie between 100 & 190, the normal diastolic pressure between 50 & 120 and the normal mean pressure 90 - 150. However, there is no proof that dogs with pressures at the top of these ranges were normal animals as no clinical or post-mortem observations accompany the results.

PUBLISHED ESTIMATES OF THE NORMAL ARTERIAL PRESSURE OF DOGS.

Author	Method	No.of Dogs	Systolic		Diastolic		Mean pressure
			Mean	Range	Mean	Range	
Ferris & Hynes (1930)	Indirect	-		110-130	80		
Gregg et al (1937)	Direct	-	124		85		
Hamilton et al (1940)	Direct	215	179.5	100-275 (abnormal)	88.6	30-140 (abnormal)	
Wakerlin (1943)	Indirect Direct	100	140	100-180	90	60-120	90-150
Zapp (1949)	Indirect	6	148	114-180	83	56-105	
Wilhelmj et al (1951)	Indirect	13		95-136		43-66	
Romagnoli (1953)	Indirect Direct	22 22	148 159	100-187 108-198	104 99	79-127 75-122	
McCubbin & Corcoran (1953)	Direct	400		99-150			
Doeglas (1953)	Indirect	50	140-150		80-100		
Katz et al (1957)	Direct	1000					135-155
Wilson & Clark (1964)	Indirect	52	142	104-180			

Four authors have studied specifically the blood pressure of dogs suffering from nephritis. The first of these was Robin (1948), who used an indirect method to measure the pressure of dogs suffering from various conditions, including many with chronic nephritis. No dog was found to have a systolic pressure greater than 120, even when evidence of left ventricular hypertrophy was present. Robin concluded that nephritic dogs do not develop hypertension as none showed rapid heart rate, retinal haemorrhages or cerebral symptoms (stated to be evidence of malignant hypertension in the human). Left ventricular hypertrophy was attributed to unknown factors associated with toxaemia. This work did not include details of the numbers of nephritic dogs and controls examined, individual observations or post-mortem and histological findings.

McIntyre and Montgomery (1952) measured indirectly the blood pressure of dogs suffering from chronic nephritis. It was found that in established cases the pressures were approximately 200/160. McIntyre (1954) listed the blood pressures of 16 dogs with chronic nephritis. The systolic pressure range was 110-280 and the diastolic range 40 - 180. In half of these animals the pressure exceeded 180/100. No post-mortem or histological examinations were carried out on the dogs, as they survived for several years after initial diagnosis.

Schulze (1958) measured the blood pressure of dogs with renal disease to find whether this could be used as an early diagnostic test in the absence of obvious clinical signs. The mean arterial pressure was determined by/

by a direct method using a mercury manometer. Previous examination of normal dogs had established a normal mean pressure range of 90 - 130 mm. Hg. Results were reported from 56 dogs with renal disease. Those which appeared clinically normal, with renal disease indicated only by urinary abnormalities, all had normal blood pressure. High pressure was found in dogs with uraemia, exceeding 150 mm. in severest cases. The latter were considered to be definitely hypertensive. Full post-mortem examinations were not described and no histological details were recorded. However, three of the uraemic dogs were examined post-mortem and the presence of chronic nephritis ascertained. As no correlation was apparent between blood pressure and the degree of uraemia and proteinuria, it was concluded that measurement of blood pressure was not a useful diagnostic method for the early detection of renal disease.

The results of McIntyre and Schulze conflict with observations published by Sporri and Leeman (1961), who described studies in fourteen dogs suffering from chronic interstitial nephritis, of which five were uraemic. They found that the non-uraemic dogs had normal systolic and diastolic pressures. The uraemic dogs sometimes showed an increase in diastolic pressure of 20 mm. above normal range, while the systolic pressures were above average but within normal range. The normal range was not defined, however, and no post-mortem examinations were reported. These authors considered that left ventricular hypertrophy found in most cases of chronic nephritis could not be attributed solely to a rise/

rise in blood pressure. They suggested that other factors, such as increased stroke volume caused by anaemia might also play a part.

One further study of canine blood pressure was published by Persson, Persson and Asheim (1961), who compared the pressures of normal dogs and dogs with congenital renal cortical hypoplasia. They found no significant difference between these groups. This observation is, of course, not directly relevant to the consideration of nephritic dogs, since in the latter a change takes place in the vascularization of a previously normal kidney, whereas in cortical hypoplasia the existing renal tissue does not suffer any superimposed disease process or deprivation of blood supply.

The published results clearly show uncertainty as to the incidence and significance of hypertension in dogs. The wide ranges quoted as normal pressure levels are not supported by adequate clinical and post-mortem examinations to ensure the absence of renal disease. Significantly, several authors who examined large numbers of dogs have found high pressure in association with renal fibrosis. The four specific studies of blood pressure in nephritic dogs have not clarified the problem. Only McIntyre (1954) and Schulze (1958) provided clinical and biochemical estimation of the phase and severity of nephritis. The methods of measuring blood pressure employed by all of these authors were not the most accurate and a control level of normal pressure was stated only by Schulze. No post-mortem details were recorded and no histological examinations/

examinations were carried out. For these reasons, the development of hypertension in nephritic dogs has not been established conclusively and any cardio-vascular effects are unknown.

The present study was undertaken to determine the possible significance of hypertension in nephritic dogs, by correlation of blood pressure with clinical, biochemical, post-mortem and histological examinations. A second series of normal dogs and dogs suffering from non-renal conditions was examined for comparison.

Methods of Measuring the Blood Pressure of Dogs.

A number of methods have been used to measure the blood pressure of dogs and as the method employed significantly affects the accuracy of the estimation, these will be reviewed in some detail.

Indirect Methods.

Indirect methods measure the pressure required to collapse an artery and obstruct the flow of blood. The instrument used for this purpose is a sphygmomanometer. Essentially, this consists of an inflatable cuff which fits around a limb and is connected by rubber tubing to a mercury or aneroid manometer and to a pressure bulb. Detweiler (1959) has described the use of the sphygmomanometer in dogs. The cuff may be applied round the forelimb (radial artery) or hind limb (dorsalis pedis) while the animal lies in lateral recumbency. The cuff is inflated until the pulse wave (palpated distal to the cuff) disappears. The pressure may then be determined in one of four ways.

a) Palpation.

Using simple palpation, the systolic pressure is indicated by releasing the cuff pressure slowly until the pulse wave can just be detected once more. A reading is taken at this point. An approximate indication of diastolic pressure is given by watching the gauge or mercury column as the pressure is released still further. A point is reached at which maximum fluctuation of the needle or mercury level occurs. This is due to turbulence in the artery as the lumen re-opens almost to normal diameter and gives a guide to the diastolic pressure.

b) Auscultation.

The pressures may be measured by auscultating the artery distal to the inflated cuff. A stethoscope is placed over the artery and the pressure slowly released. Tapping sounds become audible as small jets of blood are permitted to pass through the artery. When these sounds first appear, the systolic pressure is recorded. As the pressure is further reduced, the sounds alter in intensity, first becoming softer, then louder and finally changing to soft and muffled before disappearing. The muffled sounds indicate turbulence just before smooth flow is restored in the fully opened vessel. Diastolic pressure is indicated at this point. The auscultatory method has been applied to dogs by Wakerlin (1943), Zapp (1949), Wilhelmj et al (1951) and Romagnoli (1953).

c) Electronic Pulse Meters.

To detect sounds below the sphygmomanometer cuff with greater ease and accuracy Grauwilder, Sporri and Wegman (1958) used a sensitive capacitance/

capacitance microphone in conjunction with an automatic pressure marker arranged to record both pressure signals and pulse waves. Detweiler (1960) also reported the use of this apparatus. Campbell, Lawson and Sanford (1964) tested three electronic pulse meters in dogs and cats. The instruments were of two types. In the first, pulsations were detected by a microphone transducer and pressure displayed as deflections on a meter, while in the second type, pulsations were detected by a phototransducer activated by a light bulb. These instruments were tested as a means of detecting and visually displaying the pulse and as a method of measuring blood pressure in dogs and cats. The phototransducer type was unsuitable because the transducer head was shaped for use on a human finger. The other instruments could be used to measure systolic pressure. The readings were compared with simultaneous recordings obtained from an intra-arterial canula attached to a Statham strain-gauge transducer, whereby both instruments were found to give systolic readings 10 - 30 mm. below true level. Identification of the end point was difficult owing to variations in pressure due to respiration and because meter deflections were reduced in size as the cuff was inflated. Diastolic pressure could not be measured as the point of maximum returning pulsation was impossible to determine with any accuracy. The instruments were used most satisfactorally in anaesthetised animals, as muscle tremor and voluntary movements introduced error in measurements on conscious animals.

d) Xylol Pulse Indicator.

Wilson & Clarke (1964) described the application of a sphygmomanometer and xylol pulse indicator in dogs. The apparatus was designed for use in infants and consisted of a standard sphygmomanometer connected to a glass capillary tube containing xylol to indicate the presence or absence of a pulse wave in the vessel from which the pressure was estimated. In this system, two inflatable cuffs were applied round the forelimb - a proximal occluding cuff and a distal monitoring cuff connected to the xylol indicator. The measurement was made in the usual way, except that the pulse wave was studied visually by movement of the xylol beads. As in all indirect methods, the diastolic pressure could not be estimated accurately.

Difficulties arise in the application of indirect methods to dogs due to differences in conformation. In small dogs, the forelimb is too small for even paediatric-sized cuffs. In all dogs, the cuff is not entirely suitable for use on the hind limb, due to the conical shape of the thigh which causes the cuff to slip down. Special cuffs have been designed to overcome this difficulty, but these have never come into general use. Allen (1941) used an adaptor moulded to fit the thigh from dental gutta percha. The adaptor held the cuff in place and slipping was prevented by suspending a weight from a cord attached to the top of the adaptor. Ferris and Hynes (1931) designed a canvas strip to fit the thigh and hold the cuff in position. Straps passed around the body supported the devise and prevented slipping. Rule (1944) /

(1944) used a bandage fixed to the end of the cuff to cover and support it by passing the bandage around the torso.

Campbell, Lawson and Sanford (1961) applied the cuff around the tail of dogs, to measure systolic pressure by means of an electronic pulse meter. The error due to tremor and voluntary movements was least at this site.

The indirect methods have several advantages. The essential apparatus is simple and portable and the procedure does not tend to excite the dog greatly and may be repeated readily any number of times. The disadvantages are also considerable. In general, the measurements are of a subjective nature and therefore liable to serious error. In particular, the diastolic pressure cannot be measured with accuracy by any indirect method. The pneumatic cuff is not well suited for use in dogs because of variation in size and conformation of the hind limb. The measurement may vary significantly according to the width of cuff used. Additional errors arise in small breeds with the auscultatory method. Because of shortness of the limb, the chest piece of the stethoscope may have to be slipped under the edge of the cuff. Hypersystolic sounds may then be audible as blood strikes the occluded artery. Sounds may be heard below the true diastolic level if the cuff becomes too tight as a result of introducing the chest piece (Detweiler, 1960). With electronic pulse meters the diastolic pressure cannot be measured and the instruments give satisfactory results only in anaesthetised animals.

Direct Methods.

The direct methods measure blood pressure by means of a needle or catheter inserted into an artery and connected to a manometer. Three direct methods have been used in dogs, of which two are applicable only to experimental animals.

a) Catheterization.

In acute experiments, a common method of measuring blood pressure is by cutting into an artery and inserting a catheter (Ruch and Fulton, 1960). By this means, the end pressure in the vessel is determined, as opposed to the lateral pressure impinging on the vessel wall. The lateral pressure is less than the end pressure by an amount equivalent to the kinetic energy of flow. This factor is of little significance in normal arteries, but becomes important in diseased vessels where the lumen is reduced, causing the velocity of flow to increase in the narrowed portion. Because the artery must be sacrificed after use, this method has no general application.

A technique for the percutaneous catheterization of the femoral artery has been described by Seldinger (1953) and Odman (1956). This method may be used to measure blood pressure without cutting or destroying the artery, though the usefulness of the procedure is limited in dogs by a marked tendency to haemorrhage when the catheter is withdrawn (Bain, 1965).

b) Exteriorized Carotid Loop.

Another experimental procedure has been used to allow repeated direct pressure recordings. Gregg et al (1937) measured the pressure in dogs by means of exteriorized carotid arterial loops.

c) Arterial Puncture.

In the usual direct method, the blood pressure is determined by means of a fine needle inserted into a femoral artery. The needle may be attached to a mercury or sensitive inductance manometer and thence to a physiological recorder. Using a mercury manometer, only the mean blood pressure can be determined because of the damping effect of the heavy mercury column. The most accurate method utilises a sensitive inductance manometer from which the output is fed into an electronic graphic recorder. A permanent record is obtained giving exact measurements and correlations with pulse rate and presence of sinus arrhythmia. A possible disadvantage associated with this method is a tendency to excite the dog to some extent. However, this can be overcome in the majority of animals with careful handling. Direct blood pressure recordings have been carried out on dogs by McCubbin and Corcoran (1953), Katz et al (1957) Ramagnoli (1953) and Wakerlin (1943).

The principal advantage of the direct measurement by femoral arterial puncture is the great accuracy of the method. The permanent record gives additional valuable information and the procedure can be carried out on dogs of almost any size. The measured pressure is the lateral pressure as it strikes the vessel wall since the orifice of the needle is slanted. For these reasons, this method was employed in the present study of blood pressure in nephritic and non-nephritic dogs.

Materials & Methods.

Measurement of Blood Pressure.

For the reasons discussed above, the blood pressure was measured by direct femoral arterial puncture. The apparatus consisted of 22 gauge, 1½ inch luer needles, connected by polythene tubing to a sensitive inductance manometer[1], from which the output was fed into a Siemens-Cardirex 6[2] for graphic recording. (Figs. 51-52). On each occasion, the machine was calibrated against a mercury manometer before use.

Considerable care was taken to avoid exciting the dogs during the procedure. The measurements were carried out in quiet surroundings and the dogs were handled gently. With the dog in lateral recumbency, the uppermost limb was raised and the femoral artery in the opposite limb palpated in the femoral triangle. At this site, the artery is almost subcutaneous in position. 2 mls. of local anaesthetic (xylocaine) were then injected subcutaneously over the artery. If the animal became distressed at all during the procedure, it did so upon injection of the anaesthetic. By allowing several minutes to elapse before proceeding further, most dogs became completely relaxed once more. However, many animals did not appear to notice the initial injection and remained relaxed throughout.

After injection of anaesthetic, the fine gauge needle was first passed under the skin over the artery. By palpating the artery and retaining/

1 Elema - Schonander, Sweden.

2 Siemens Ltd., Erlangen, Germany.

retaining it in suitable position, entry was generally effected without difficulty.

The pressure waves were recorded at a speed of 25 mm./sec. The mean pressure was also recorded and the record calibrated in each case.

Upon withdrawing the needle from the artery after recording, a pressure pack was applied over the vessel for 2 minutes to prevent haemorrhage.

From the records obtained, the systolic, diastolic and mean pressures were measured. In most cases, the presence of respiratory variation gave a record composed of repeated series of approximately four pressure waves of ascending size. The pressures were measured from waves in the middle of these series. The pulse rate was also measured from the record and the presence or absence of sinus arrhythmia noted.

In judging whether a dog was perfectly relaxed during the recording, three criteria were adopted. Firstly, the dog must appear to be calm and show no reaction to the femoral puncture procedure. Secondly, the pulse rate should not exceed 120/min. (Detweiler, 1959). Finally, sinus arrhythmia should be present. If a dog did not show all of these features, it was considered probable that some degree of excitement had been present and the recordings were not included in the survey.

Whenever possible, two or more recordings were taken from each dog. When/

When only one recording was obtainable, it was included in the series provided the three criteria were fulfilled. Repeated measurements were taken from dogs which exhibited evidence of excitement on the first occasion and the later recordings included if the dog became calm subsequently.

The Dogs in the Series.

The nephritic dogs in the series were all animals which were treated at the Veterinary Hospital. Some were treated once, recovered and went home and one series of pressure recordings were obtained from such cases. Others were treated and kept under observation for several months. These dogs were examined every few weeks during the period and pressure recordings taken on each occasion. Some dogs were admitted to the hospital in the terminal stages of nephritis. In such cases, one recording or series of recordings was obtained before death.

In all cases, the blood pressure was correlated with the clinical, biochemical, urological and serological features ofthe dogs. Details of these examinations have been described in Section I. In fatal cases, or in animals in which euthanasia was necessary because of hopeless prognosis, post-mortem and histological examinations were carried out.

The non-nephritic dogs in the series included animals which were treated in the hospital for conditions other than renal disease and a number of normal dogs. The absence of renal dysfunction was ascertained by biochemical and urological examinations. In some cases, post-mortem and histological examinations were also carried out.

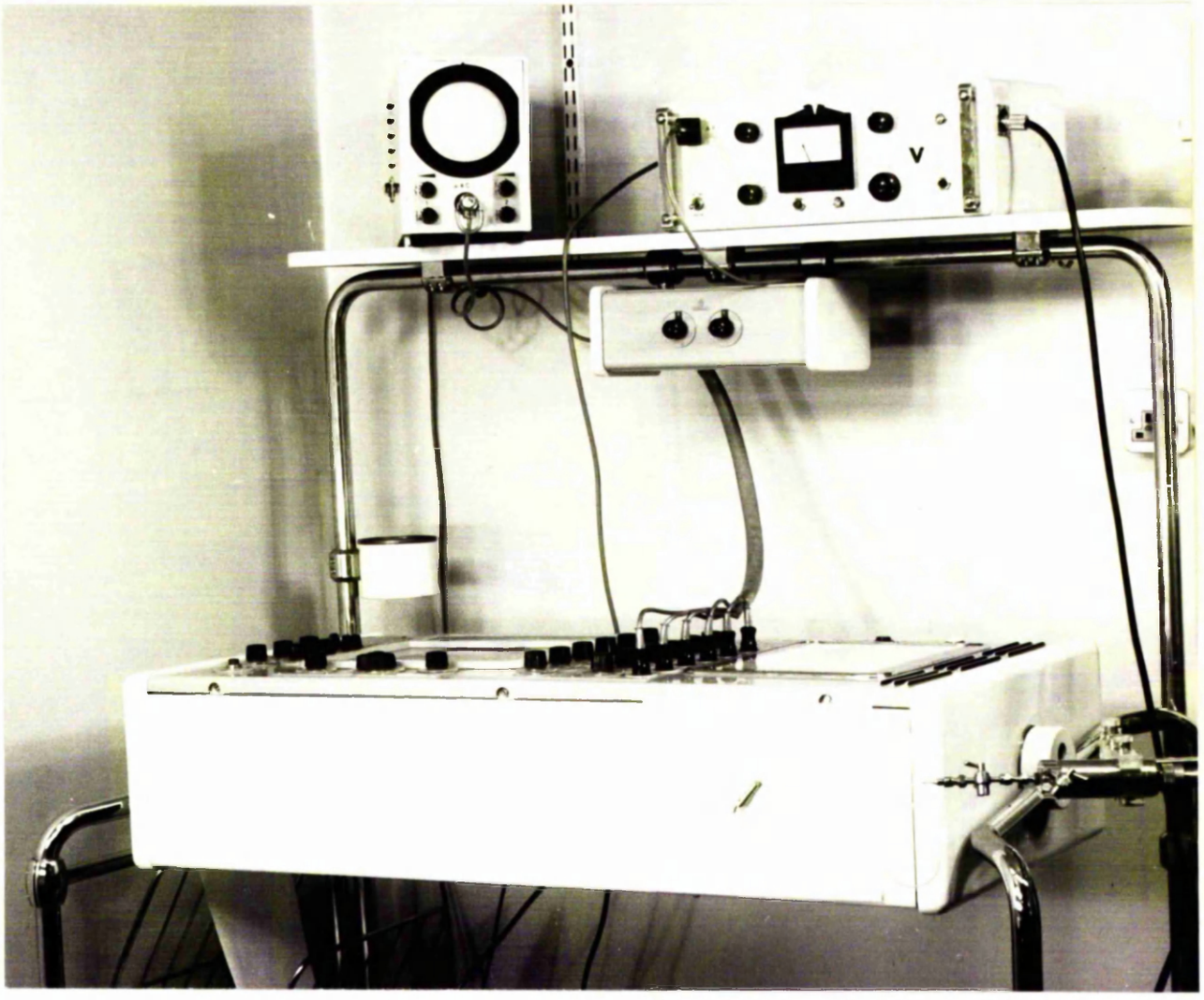


Fig. 51. Equipment used in the direct measurement of femoral arterial pressure of dogs. A 22-gauge luer needle is attached by polythene tubing to a sensitive inductance manometer, connected in turn to the Siemens-Cardirex 6 for graphic recording.

Fig. 52. Illustrating the direct measurement of femoral arterial pressure in a dog. The dog lies in lateral recumbency and the needle is inserted into the femoral artery.

Measurements of blood pressure were obtained from 22 dogs suffering from interstitial nephritis. These were 4 acute, 4 sub-acute and 14 chronic cases. The control series comprised 28 non-nephritic dogs.

RESULTS.

Blood pressure of Non-Nephritic Dogs.

The individual measurements of the femoral arterial pressure of 28 non-nephritic control dogs are shown in Table I overleaf, which also contains details of the age, sex, breed and disease status of these animals.

The levels of systolic, diastolic and mean pressure in this group are summarized in the following table.

Femoral Arterial Pressure of 28 Non-Nephritic Dogs.

	Systolic pressure (mm. Hg.)	Diastolic pressure (mm. Hg.)	Mean pressure (mm. Hg.)
Range	106-164	64-96	84-128
Mean	144	81	104
S.D.	±15	± 9	± 13
S.E.	± 2.8	± 1.7	± 2.5

In the present study, the highest levels observed in the non-nephritic dogs are of particular significance in defining the limits of normal arterial pressure.

To determine any relationship between blood pressure and age, the coefficient of correlation, "r," for systolic pressure and age was calculated and found to be $r = +0.17$. This indicates that there was no tendency for the blood pressure to alter with age in the present series.

Examples of blood pressure records from dogs in this series are shown in Figs. 55, 56, 57 and 58.

TABLE I.

The femoral arterial pressure of 28 non-nephritic dogs and details of the breed, age, sex and disease status of these animals.

TABLE I.

Case	Date	Breed	Sex	Age	Diagnosis
24163	30.1.64	Greyhound	M	5 yr.	Normal
	4.3.64				
23626	30.1.64	Spaniel	M	8 yr.	Normal
24305	30.1.64	Bull Ter.	F	13 yr.	C.N.S. lesion
24383	3.2.64	Collie x	M	6 yr.	Normal
24428	3.2.64	Boxer	M	4 yr.	Normal
24427	3.2.64	Alsatian	F	?	Normal
24445	3.2.64	Alsatian	M	?	Normal
24528	4.3.64	Greyhound	M	6 yr.	Lameness
24455	5.2.64	Alsatian	M	4 mths.	Gastro-enteritis
	1.4.64				
25316	1.6.64	Collie	M	8 yr.	Normal
25334	5.6.64	Collie x	M	6 yr.	Normal
25356	6.6.64	Terrier	M	7 yr.	Normal
25373	9.6.64	Collie	F	6 mths.	Normal
25384	9.6.64	Collie x	M	7 yr.	Cirrhosis
20014	9.6.64	Old Eng. Sheep Dog	M	8 yr.	Mitral incompetence
25422	12.6.64	Collie	M	6 yr.	Normal
25497	23.6.64	Collie x	M	5 yr.	Normal
	26.6.64				
25530	29.6.64	Lab x Beagle	M	6 yr.	Liver tumour

Systolic pressure	Diastolic pressure	Mean pressure	Pulse rate	Sinus Arrhythmia	Post-Mortem
148	84	104	105	+	-
148	88	115	100	+	N.A.D.
106	70	85	90	+	N.A.D.
132	72	90	120	+	-
152	70	-	90	+	-
136	84	104	120	+	-
170	88	116	120	+	-
136	68	86	90	+	-
148	82	100	100	+	-
125	74	104	110	+	-
115	70	88	120	+	-
136	88	118	110	+	N.A.D.
154	90	122	105	+	-
158	90	128	120	+	-
144	88	106	120	+	-
138	78	102	120	+	Cirrhosis
132	68	88	120	+	-
154	90	106	95	+	-
150	90	116	112	+	-
156	90	128	120	+	N.A.D.
152	84	112	90	+	-
130	84	104	120	+	Liver tumour

Case	Date	Breed	Sex	Age	Diagnosis
14080	16.9.64	Rhodesian Ridgeback	M	6 yr.	Normal
26079	1.10.64	Labrador	M	11 yr.	Inconclusive
26504	12.11.64	Boxer	M	5 yr.	Normal
27051	11.2.65 9.3.65	Labrador	M	5 yr.	Inconclusive Loss of weight
27086	12.2.65	Boxer	F	9 yr.	Thrombosis of iliac vein
27151	24.2.65	Terrier	M	9 yr.	Chronic Diarrhoea
27088	19.2.65	Labrador	M	4 yr.	Inconclusive ? Diabetis insipidus
24977	9.3.65	Labrador	M	10 yr.	Retropharyngeal abscess
27173	9.3.65	Collie x	F	10 yr.	Chronic Diarrhoea
27091	11.3.65	Collie x	M	?	Normal

Systolic pressure	Diastolic pressure	Mean pressure	Pulse rate	Sinus Arrhythmia	Post-Mortem
160	96	118	100	+	-
146	82	104	120	+	-
148	80	-	120	+	-
150	90	106	120	+	-
154	84	106	120	+	-
152	80	100	120	+	Iliac thrombosi
138	64	85	75	+	-
156	90	104	100	+	Normal kidneys
164	80	102	100	+	-
136	84	100	100	+	-
134	70	84	120	+	-

Blood Pressure of Dogs with Nephritis.

Individual measurements of femoral arterial pressure of the 22 nephritic dogs are listed in Table II. The significant biochemical, urological, serological and pathological features of these dogs are shown in Table III.

Blood Pressure in Acute Nephritis.

Four dogs suffering from acute leptospiral nephritis were examined and the clinical details of these cases will be outlined individually.

24756. This dog had been ill for one week when presented for examination. During this time it had shown dullness, anorexia, frequent vomiting and moderate thirst. The mouth was discoloured and halitosis pronounced. Renal damage was indicated by the presence of uraemia and proteinuria and the titre to L. canicola was > 1:30,000. Measurement of blood pressure revealed moderate hypertension. (163/110 and 166/102). Penicillin was administered for several days, when the condition improved and the dog was discharged.

Two months later, a second examination was carried out. In the interval, the dog had shown partial recovery, though occasional vomiting and increased thirst had persisted. At this time, biochemical and urological results indicated renal dysfunction of moderate severity. The titre had fallen to 1:10,000. Again hypertension was demonstrated. The blood pressure had risen further since initial examination and values up to/

to 186/120 were recorded on this occasion. (Fig. 59). The disease had evidently progressed to the sub-acute phase. Treatment was repeated and the dog was discharged.

24928. This dog had shown dullness, anorexia and occasional vomiting for two weeks when presented. During two weeks in hospital, infrequent vomiting and moderate thirst persisted. Leptospiruria and proteinuria were present, though there was no biochemical evidence of renal failure. The titre to L. canicola rose from 1:3,000 - 1:30,000 over this period. The blood pressure was normal. (117/77). The dog was treated and discharged.

26504. This dog was presented with a history of frequent vomiting, anorexia, dullness and excessive thirst for 1 - 2 weeks. Halitosis was marked and the mouth showed brown discolouration. These signs persisted during the following two weeks, with biochemical and urological evidence of renal failure. The titre rose from 1:3,000 - 1:10,000. The blood pressure was normal (148/80 & 158/90). The dog was treated and discharged.

24554. This dog had shown the typical signs of dullness, anorexia and vomiting for a week before examination. The unusual feature in this case was the dog's age - 12 years. Biochemical and urological evidence of renal failure was present, with some loss of ability to concentrate the urine. The titre to L. canicola was > 1:30,000. Marked hypertension was demonstrated in this dog, with pressures of 190/116 - 210/118. (Fig.60).

As the condition did not improve despite treatment, the dog was destroyed. On post-mortem examination, the kidneys showed the characteristic features of acute interstitial nephritis. There were no extra-renal lesions. Histologically, the interstitial tissue of the renal cortices was diffusely infiltrated by lymphocytes and plasma cells. The blood vessels were normal.

Blood Pressure in Sub-Acute Nephritis.

Four dogs with sub-acute nephritis were examined. The disease was estimated to be in the sub-acute phase when the duration was several weeks, the titre to L. canicola moderately high and proteinuria marked, with some loss of ability to concentrate the urine. The case histories of dogs in this group will be outlined individually.

25737. This dog was admitted to the hospital for examination because the owner had contracted leptospirosis canicola. The owner recalled that the dog had been unwell a few weeks previously. The dog appeared to be normal on clinical and biochemical examination. It proved impossible to obtain urine samples sufficient for urological testing, but small numbers of leptospirae were observed in the few drops collected. The titre to L.canicola was 1:30,000. The blood pressure was normal (146/90). In view of the duration of the condition, a tentative diagnosis of sub-acute nephritis was made.

The dog remained in the hospital for the following eight months, as the owner did not wish to keep it. No treatment was given and repeated clinical and laboratory examinations and measurements of blood pressure were carried out to estimate the course of the renal disease. Leptospiruria terminated within the first week and the dog remained clinically normal, with no biochemical indication of renal dysfunction. The titre fell to 1:1,000 after six months. The blood pressure showed interesting changes during this period. After six months, the dog became hypertensive (178/108)/

and the increase in pressure persisted during and after pregnancy, suggesting a sub-clinical progression of renal disease.

25848. This dog had shown loss of weight, thirst polyuria and occasional vomiting for 2 months when presented. There were no oral lesions and the dog was bright, though in poor condition. Uraemia was present and the titre to L. canicola was 1:1,000. During the following 3 months, partial recovery occurred but the dog remained thin, rather thirsty and polyuric. Hypertension was repeatedly demonstrated during this period, with a tendency to further increase in pressure latterly (Figs. 53 & 61). Euthanasia was performed after 3 months. Pathologically, the kidneys showed focal sub-acute interstitial nephritis. There were no vascular lesions.

25818. This dog had a history of loss of weight, increased thirst and occasional vomiting for several weeks. In the previous two weeks, the vomiting had become more frequent. Uraemia and marked proteinuria were present and the titre was 1:300. The blood pressure was normal. The dog responded to treatment and was discharged.

26694. This dog had a history of loss of condition, thirst and frequent vomiting during the previous few weeks. Uraemia and proteinuria were present and there was some loss of ability to concentrate the urine. The titre to L. canicola was 1:30,000. Moderate hypertension was found to be present (180/96). The dog was discharged after treatment.

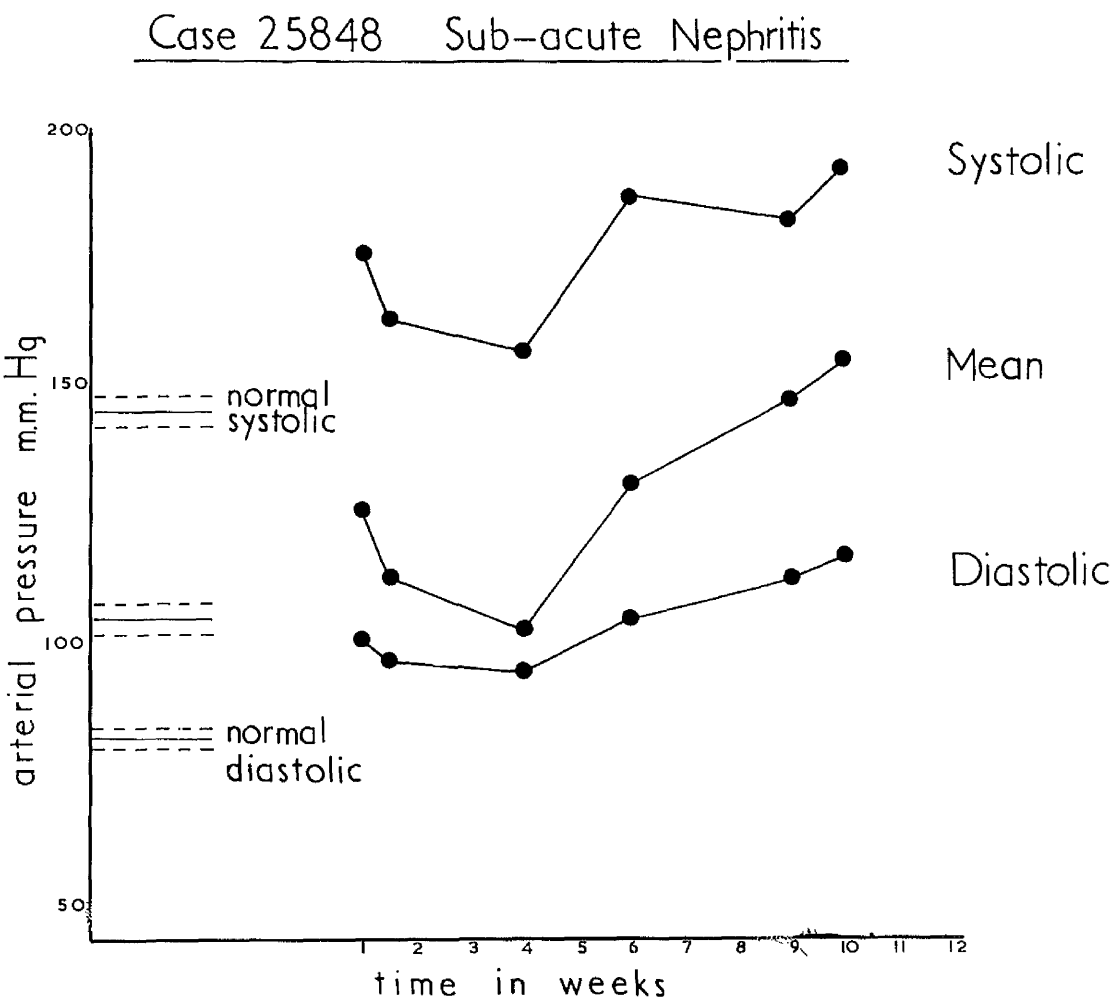


Fig. 53. The femoral arterial pressure of Case 25848, showing fluctuation and a tendency to rise over a ten-week period. The record includes the normal levels of systolic, mean and diastolic pressure determined from the control series.

Blood Pressure in Chronic Nephritis.

The case histories of the 14 dogs with chronic nephritis will not be described individually. All dogs in this group were presented with histories of loss of condition, excessive thirst, polyuria and vomiting for several weeks or months. Many of these animals were in the terminal stage of nephritis when examined, when they showed apathy, marked halitosis with discolouration and often ulceration in the mouth. At this stage, some degree of dehydration was often appreciable and such dogs vomited very frequently. None of the chronic cases were available for prolonged study.

It is of interest that the titre to L. canicola was negative in 10 of the 14 dogs in this group. There is therefore no indication that the renal lesion in these animals was of leptospiral origin, although the clinical and pathological features of the disease were indistinguishable.

Hypertension was demonstrated in 13 of the 14 dogs with chronic nephritis and post-mortem examinations were carried out on 7 of these. The measurements of blood pressure are summarized below.

Examples of blood pressure records from this group are shown in Figs. 62 - 66.

Femoral Arterial Pressure of 14 Dogs with Chronic Nephritis.

	Systolic pressure (mm. Hg.)	Diastolic pressure (mm. Hg.)	Mean pressure (mm. Hg.)
Range	124-212	80-132	96-142
Mean	175	107	125
S.D.	± 21	± 12	± 12
S.E.	± 5.6	± 3.2	± 3.2

The one normotensive dog in this series, no 27107, died within 12 hours of the examination. As the dog was dying at the time of the pressure measurement, the result may not have given an accurate estimate of the arterial pressure in the sub-terminal phase of nephritis. Therefore the above results were re-calculated, excluding this dog. The findings are shown in the following table.

Femoral Arterial Pressure of 13 Dogs with Chronic Nephritis.

	Systolic pressure (mm. Hg.)	Diastolic pressure (mm. Hg.)	Mean pressure (mm. Hg.)
Range	160-212	98-132	114-142
Mean	179	109	127
S.D.	± 16	± 10	± 9
S.E.	± 4.4	± 2.8	± 2.5

The results from the 14 dogs with chronic nephritis were compared with those from the non-nephritic dogs using Student's "t" test. The differences between the systolic, diastolic and mean pressures of the two groups were found to be highly significant (p=0.001). The results are shown in the table below.

Student's "t" test: Comparison of Arterial Pressure in 14 Dogs with Chronic Nephritis and 28 Non-Nephritic Dogs.

Status	Pressure	Mean (mm. Hg.)	S.E.	Level of probability.
Nephritic	Systolic	175	± 5.6	p=0.001
Non-Nephritic		144	± 2.8	
Nephritic	Diastolic	107	± 3.2	p=0.001
Non-Nephritic		81	± 1.7	
Nephritic	Mean	125	± 3.2	p=0.001
Non-Nephritic		104	± 2.5	

The possibility of a relationship between the blood pressure and level of blood urea in the dogs with chronic nephritis was investigated (Fig. 54). The coefficient of correlation for systolic pressure and blood urea was calculated and found to be $r = -0.35$. This indicates that there was/

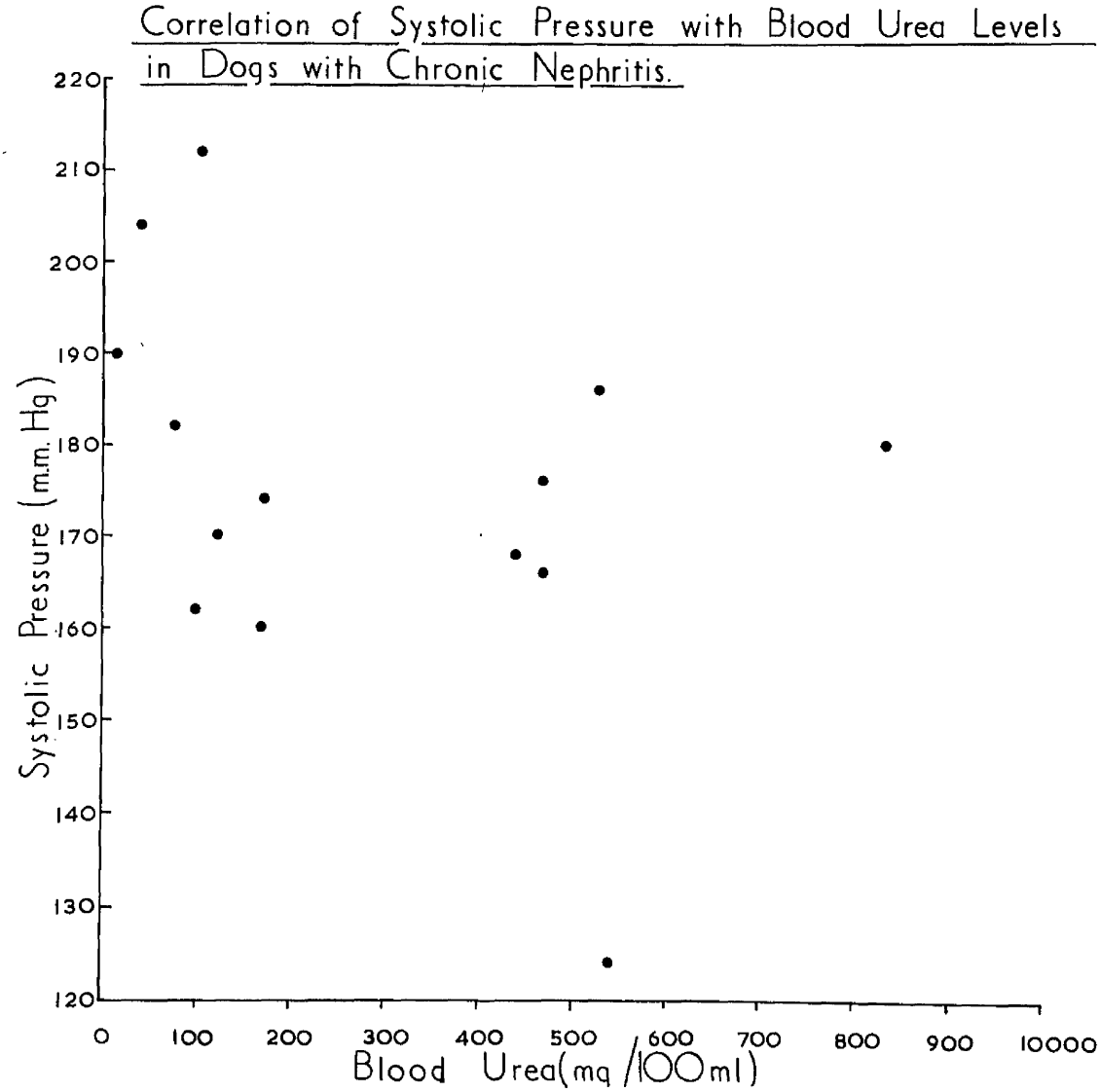


Fig. 54. Illustrating the absence of a relationship between systolic pressure and the level of blood urea in dogs with chronic nephritis. Coefficient of correlation "r" = - 0.35.

was no relationship between the degree of hypertension and the degree of uraemia. In two dogs with chronic nephritis, (24448 and 23744), hypertension was present in the absence of uraemia.

The moribund dogs in this series were of special interest. Apart from 27107, which died within a few hours of examination, the blood pressure of three moribund dogs was measured. These animals (nos. 4514, 27131 and 27308) died within 48 hours of examination. While the arterial puncture procedure was carried out, the dogs were profoundly dull and apparently unaware of being handled. As the possibility of excitement altering the blood pressure was totally eliminated in such animals, the demonstration of hypertension in moribund dogs is of particular significance.

All of the 7 dogs in this group which were examined post-mortem proved to have plasmatic vasculosis in the intra-renal arteries and the glomeruli. Individual pathological findings in these dogs are outlined below.

25270/2. Macroscopically, the kidneys showed the typical features of chronic nephritis, with advanced diffuse fibrosis and reduction of cortical depth. The heart showed left ventricular hypertrophy. Necrotizing endocarditis was absent and there were no oral or gastric ulcers. Histologically, in addition to the characteristic features of chronic interstitial nephritis, the kidneys showed moderately severe plasmatic vasculosis affecting arcuate and interlobular arteries, afferent arterioles (Fig. and/

and many glomeruli. Small focal lesions were present in a few small coronary arteries. Arteries in the spleen were also affected.

4514. The kidneys showed diffuse fibrosis. Left ventricular hypertrophy was present and necrotizing endocarditis affected the left atrium. Haemorrhagic erosions were present in the gastric mucosa. Plasmatic vasculosis of moderate severity was present in intra-renal arteries and glomeruli (Fig. 70) and in the arteries of the spleen.

10835. Diffuse renal fibrosis (Fig.68) and left ventricular hypertrophy were present. Moderately severe plasmatic vasculosis affected intra-renal arteries, glomeruli and arteries of the spleen.

27107. Typical severe chronic nephritis was present. The heart showed left ventricular hypertrophy and necrotizing endocarditis in the left atrium. The gastric mucosa was congested. Moderately severe plasmatic vasculosis affected intra-renal arteries and glomeruli (Fig. 71) and arteries in the spleen.

27131. Diffuse renal fibrosis was present. The heart showed left ventricular hypertrophy and necrotizing endocarditis in the left atrium. The mucosae of the lips, cheeks and tongue were ulcerated and the gastric mucosa was congested. Plasmatic vasculosis affected intra-renal arteries and glomeruli (Fig.72) and arteries of the spleen.

27308. Diffuse renal fibrosis was present. The heart showed left ventricular/

ventricular hypertrophy. The mucosae of the lips and cheeks were extensively ulcerated and the tongue was discoloured. Plasmatic vasculosis affected intra-renal arteries and glomeruli and arteries of the spleen.

In all of these cases, the arterial lesions were of moderate severity, with focal sub-endothelial plasmatic deposits affecting numerous interlobular arteries. None of the dogs showed the most extreme lesion, with muscle necrosis and bursting of plasmatic material into the adventitial tissue. Large numbers of glomeruli in each case showed plasmatic damage, the great majority of Grade +++ and severity.

TABLE II.

The femoral arterial pressure of 22 dogs suffering from interstitial nephritis and details of the breed, age and sex of these animals.

TABLE II.

Case	Date	Breed	Age	Sex	Stage of Nephritis
24756	18.3.64	Collie x	3 yr.	M	Acute
	19.3.64				
	21.5.64				
	22.5.64				
	26.5.64				
24928	15.4.64	Collie	1 yr.	M	Acute
26504	17.11.64	Boxer	5 yr.	M	Acute
	25.11.64				
24554	25.2.64	Labrador	12 yr.	M	Acute
	3.3.64				
	6.3.64				
25737	10.8.64	Terrier	9 mths.	F	Sub-Acute
	11.8.64				
	20.10.64				
	11.2.65)				
	)	Late pregnancy			
	12.2.65)				
	9.3.65	Post-partum			
25848	25.8.64	Bull Mastiff	2 yr.	M	Sub-Acute
	27.8.64				
	16.9.64				
	1.10.64				
	20.10.64				
	26.11.64				

Systolic pressure	Diastolic pressure	Mean pressure	Pulse rate	Sinus Arrhythmia
163	110	127	120	+
166	102	120	120	+
178	98	136	120	+
174	94	124	120	+
186	120	145	120	+
117	77	88	120	+
148	80	-	120	+
158	90	118	120	+
190	116	135	110	+
190	128	146	110	+
210	118	153	120	+
146	90	110	115	+
146	85	108	110	+
148	90	110	120	+
178	108	132	120	+
168	98	114	120	+
168	98	114	120	+
174	100	125	110	+
162	96	112	120	+
156	94	102	90	+
186	104	130	100	+
182	112	146	90	+
192	116	154	95	+

Case	Date	Breed	Age	Sex	Stage of Nephritis
25818	11.8.64 8.9.64	Terrier	1 yr.	M	Sub-Acute
26694	14.12.64	Terrier	1 yr.	M	Sub-Acute
24448	3.2.64	Collie	6 yr.	M	Chronic
23744	6.3.64	Labrador	10 yr.	M	Chronic
25270/2	21.5.64	Spaniel x	?	F	Chronic
25488	22.6.64	Alsatian	6 yr.	M	Chronic
4514	8.9.64	Collie	10 yr.	M	Chronic (moribund)
26000	16.9.64	Terrier	?	M	Chronic
26090	1.10.64	Terrier	5 yr.	M	Chronic
26161	26.10.64	Poodle	?	M	Chronic
26565	25.11.64	Spaniel	6 yr.	M	Chronic
10035	7.1.65	Spaniel	9 yr.	F	Chronic
27107	11.2.65	Terrier	13 yr.	M	Chronic (dying)
27131	15.2.65	Kerry Blue	5 yr.	F	Chronic (moribund)
25864	15.2.65	Boxer	13 yr.	F	Chronic
27303	12.3.65	Terrier	13 yr.	M	Chronic (moribund)

Systolic pressure	Diastolic pressure	Mean pressure	Pulse rate	Sinus Arrhythmia
136	62	84	120	+
164	84	110	110	+
180	96	128	110	+
190	100	124	120	+
182	116	138	120	+
212	132	116	110	+
186	110	130	85	+
176	100	124	110	+
162	120	114	110	+
204	100	130	100	+
184	118	136	80	+
174	110	136	110	+
166	100	120	120	+
124	80	96	130	+
180	116	136	110	+
160	106	124	120	+
168	98	114	120	+

TABLE III.

The significant biochemical, urological, sero-logical and pathological features of the 22 nephritic dogs.

TABLE III.

Case	Date	Blood Urea (mg/100 ml)	Inorganic phosphate (mg/100 ml)	Urine Urea g./100 ml)
24756	18.3.64	63	9.4	3.3
	20.5.64	68	7.0	2.2
24928	14.4.64	18	9	2.7
	21.4.64	21	8.5	3.6
	28.4.64	36	10.4	"
26504	11.11.64	104	"	"
	13.11.64	92	6.3	2.3
	16.11.64	119	"	2.4
	25.11.64	103	6.4	"
24554	20.2.64	150	"	2.1
	28.2.64	138	7.2	1.9
	12.3.64	105	"	1.7
25737	3.8.64	38	8.2	"
	11.2.65	44	5	"
25848	21.8.64	133	10.1	"
	31.8.64	76	5.7	"
	20.10.64	49	5.4	"
	26.11.64	38	3.9	"
25818	18.8.64	191	5.6	4.0
26694	10.12.64	147	15	1.8

Urine Protein (mg/100 ml)	Titre to L.canicola	Post-Mortem
20	>1:30,000	"
100	1:10,000	"
30	1: 3,000	"
20	1: 3,000	"
"	1:30,000	"
"	1: 3,000	"
30	"	"
60	"	"
"	1:10,000	"
70	>1:30,000	Acute interstitial nephritis with no vascular lesions.
100	"	
30	"	
"	1:30,000	"
"	1: 1,000	"
"	"	Mild sub-acute interstitial nephritis. No vascular lesions.
"	"	
"	"	
"	1: 1,000	
120	1: 300	"
100	1:30,000	"

Case	Date	Blood Urea (mg/100 ml)	Inorganic phosphate (mg/100 ml)	Urine Urea (g./100 ml)
24448	3.2.64	15	3.7	1.9
	20.2.64	16.5	7.8	1.4
	25.2.64	15	5.4	1.4
23744	3.3.64	76	5.0	3.1
	5.3.64	47	3.5	3.3
25270/2	26.5.64	104	4.9	-
25488	21.6.64	530	-	-
	22.6.64	740	26.9	2.2
4514	8.9.64	470	4.1	-
26000	15.9.64	98	4.6	4.6
26090	1.10.64	40	4.7	5.6
26161	26.10.64	123	-	8.1
26565	20.11.64	172	7.2	2.7
10835	6.1.65	470	-	1.2
	7.1.65	440	-	1.1
27107	11.2.65	540	26.2	1.6
27131	15.2.65	840	16.7	-
25864	10.2.65	170	18.2	-
	11.2.65	221	-	-
27308	12.3.65	440	15.5	1.5

Urine Protein (mg/100 ml)	Titre to L.canicola	Post-Mortem
30	- ve	-
30	-	-
30	-	-
20	1: 300	-
30	-	-
-	1: 10	Chronic nephritis. Plasmatic vasculosis in kidney & spleen.
-	-	-
200	- ve	Chronic nephritis. Plasmatic vasculosis in kidneys, heart & spleen
-	- ve	Chronic nephritis. Plasmatic vasculosis & kidneys & spleen.
300	- ve	-
30	- ve	-
300	1: 300	-
100	- ve	-
300	- ve	Chronic Nephritis. Plasmatic vasculosis in
100	-	kidney & spleen.
300	- ve	Chronic nephritis. Plasmatic vasculosis in kidney and spleen.
-	- ve	Chronic nephritis. Plasmatic vasculosis in kidney & spleen.
-	-	-
-	-	-
300	- ve	Chronic nephritis. Plasmatic vasculosis in kidney & spleen.

Discussion.

Measurements of blood pressure of 28 non-nephritic dogs indicated that there is considerable individual variation in normal pressure levels. However, the results suggest that the upper limits of normal are in the region of 160 systolic, 90 diastolic and 125 mean pressure. There was no relationship between blood pressure and age in this series.

A proportion of dogs with acute and sub-acute nephritis showed hypertension, which persisted and progressed over a period of months. The absence of vascular damage in two dogs examined post-mortem suggests that hypertension precedes the onset of vascular changes. Neither dog was in the terminal phase of nephritis.

In chronic nephritis, a high incidence of hypertension was demonstrated and there was a highly significant statistical difference between the pressures of dogs in this group and those of the non-nephritic dogs. The presence of plasmatic vasculosis in the seven dogs examined post-mortem establishes a positive relationship between chronic nephritis, hypertension and vascular damage in the dog.

Measurements from moribund dogs provided particularly clear evidence for the development of hypertension. It is certain that high pressure could not be attributed to excitement in such animals.

The low arterial pressure recorded from one dying dog with chronic nephritis which proved to have plasmatic vasculosis in the intra-renal vessels,/

vessels, suggests that hypertension may have been present in the sub-terminal phase of the disease.

There was no relationship between blood pressure and the degree of renal failure as judged by the level of uraemia. Hypertension was observed in one dog with sub-acute and two with chronic nephritis in the absence of uraemia. It would appear, therefore, that hypertension may develop in association with renal lesions which are not so severe as to cause renal failure. This relationship has been noted previously by Stamler et al (1949), who found hypertension in two dogs with sub-clinical renal disease.

In all chronic cases examined post-mortem, the arterial lesions were of moderate severity. The explosive lesion typical of malignant hypertension in the human was not encountered in these dogs. As this extreme form of vasculosis was found in several nephritic dogs in the survey described in Section I, it may be that on occasion the blood pressure rises to greater heights than found in the present study.

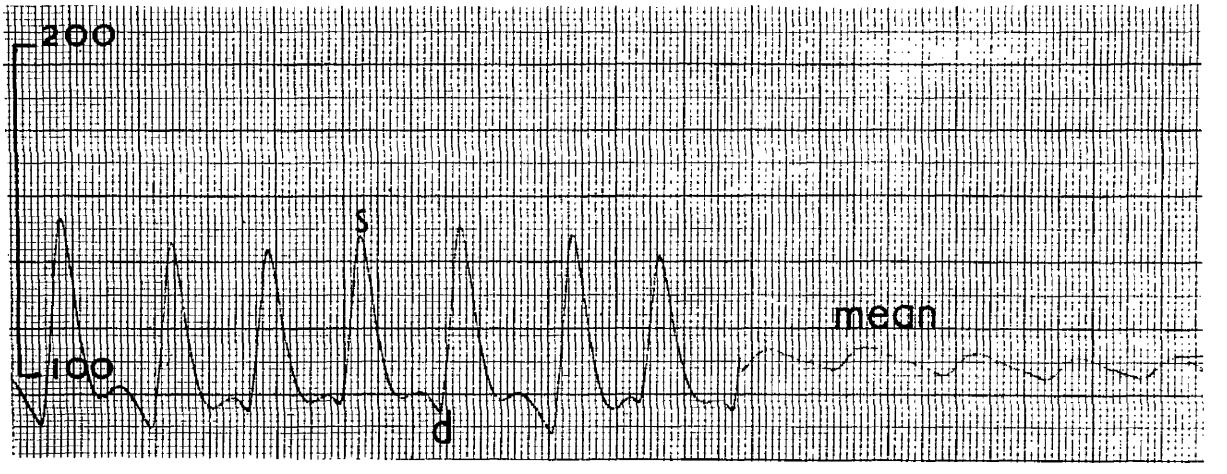
It is of interest that the high incidence of hypertension observed in chronic nephritis corresponds to the high incidence of arterial damage demonstrated in Section I. In relation to the conflicting evidence published by previous authors, the present findings reinforce the demonstration of hypertension by McIntyre (1954) and Schulze (1958). Robin (1948) and Spörri and Leeman (1961) did not find hypertension in association with canine nephritis. However, the absence of adequate clinical and post-mortem confirmation of the nephritic status of dogs in the latter studies/

studies raises doubt as to the significance of this conclusion.

This investigation provides the first demonstration of a positive relationship between nephritis, hypertension and arterial damage in the dog. It is almost certain, therefore, that plasmatic vasculosis is caused by hypertension in the dog as it is in man. The considerable significance of glomerular damage of the plasmatic type in the progressive development of renal fibrosis was indicated in Section I. The evidence of the present study suggests that hypertension causes plasmatic vasculosis and therefore plays a major part in the pathogenesis of chronic nephritis.

Case No.24163 - femoral arterial pressure

paper speed: 25 m.m./sec. heart rate: 100/min.



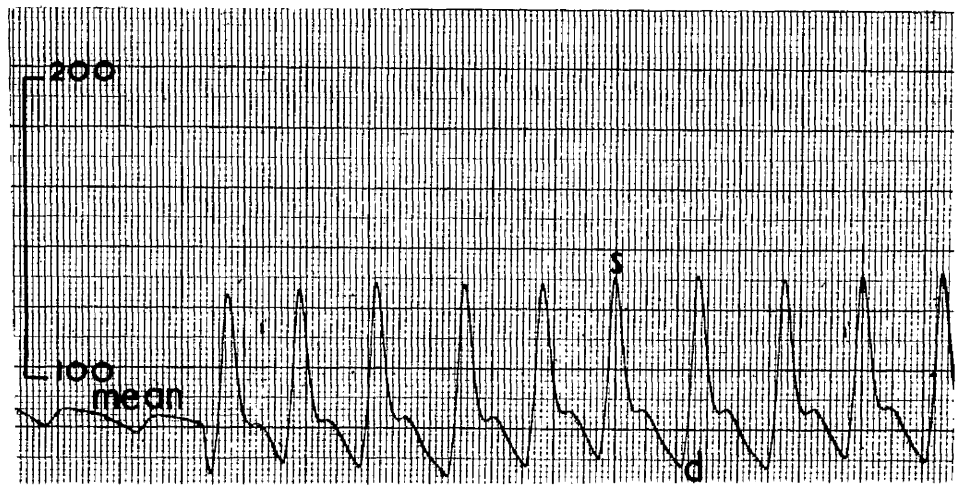
s – systolic

d – diastolic

Fig. 55. Normotension in a male greyhound, aged 5 years. 148/84 m.m./Hg.

Case No. 24305 – femoral arterial pressure

paper speed: 25 m.m./sec. heart rate: 120/min.



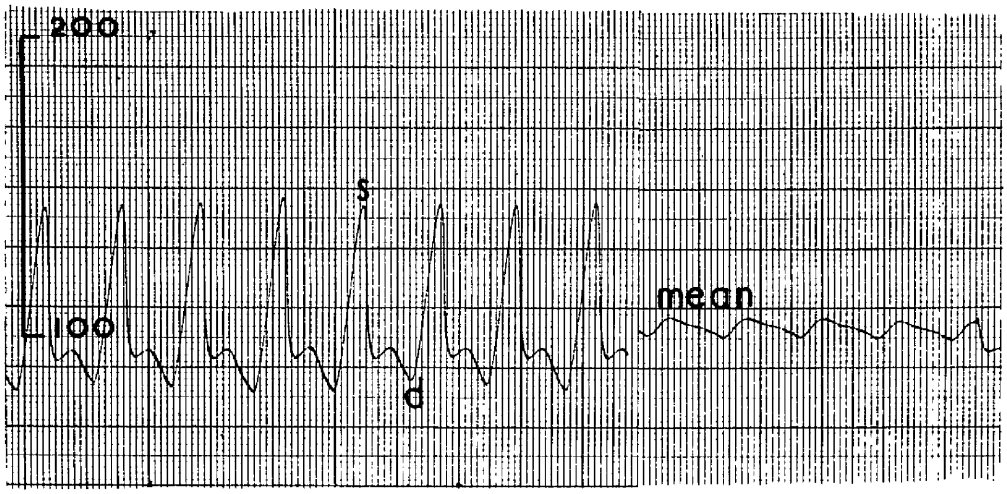
s – systolic

d – diastolic

Fig. 56. Normotension in a female bull terrier, aged 13 years. 132/72 m.m./Hg.

Case No. 26079 – femoral arterial pressure

paper speed: 25 m.m./sec. heart rate: 120/min.



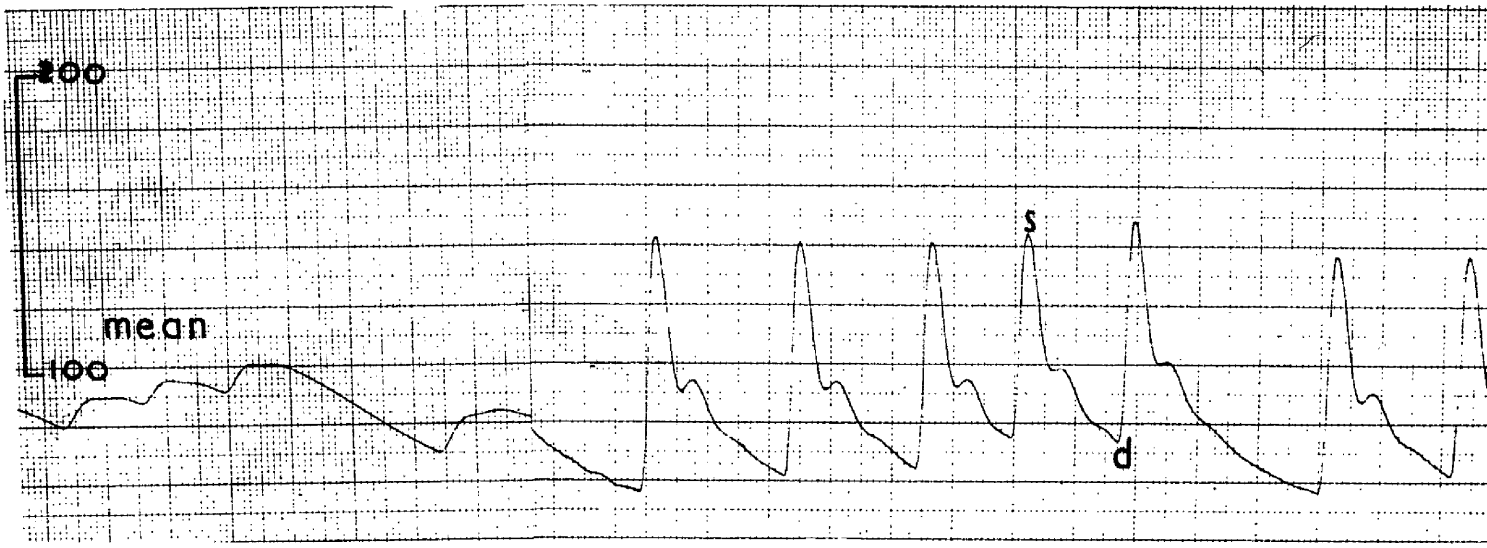
s – systolic

d – diastolic

Fig. 57. Normotension in a male labrador, aged 11 years. 146/82 m.m./Hg.

Case No. 27151 - femoral arterial pressure

heart rate: 75/min. paper speed: 25 m.m./sec.

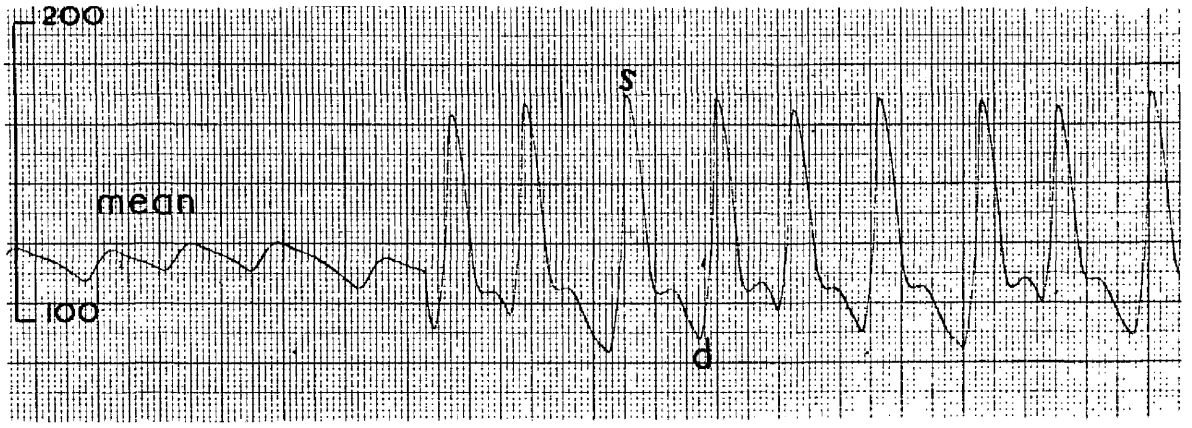


s - systolic
d - diastolic

Fig. 53. Normotension in male terrier, aged 9 years. 138/64 m.m./Hg.

Case No. 24756 – femoral arterial pressure

paper speed : 25 m.m./sec. heart rate : 105/min.



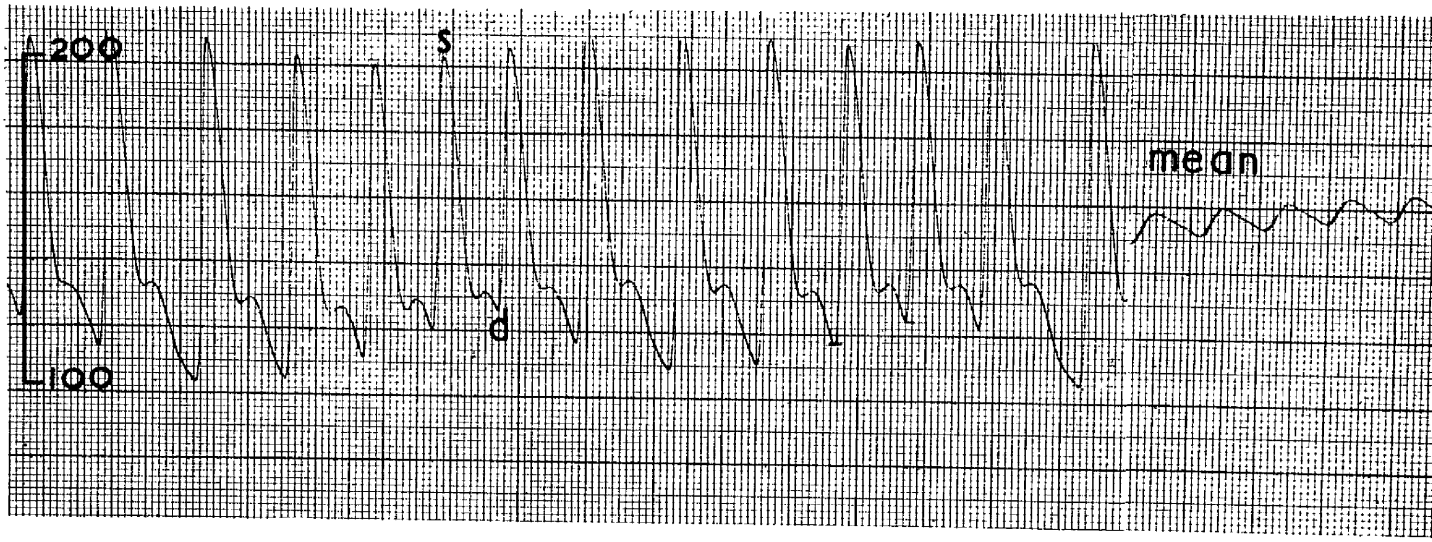
s – systolic

d – diastolic

Fig. 59. Hypertension in a dog suffering from acute interstitial nephritis. 174/94 m.m./Hg.

Case No. 24554 – femoral arterial pressure

paper speed: 25 m.m/sec. heart rate 120/min.



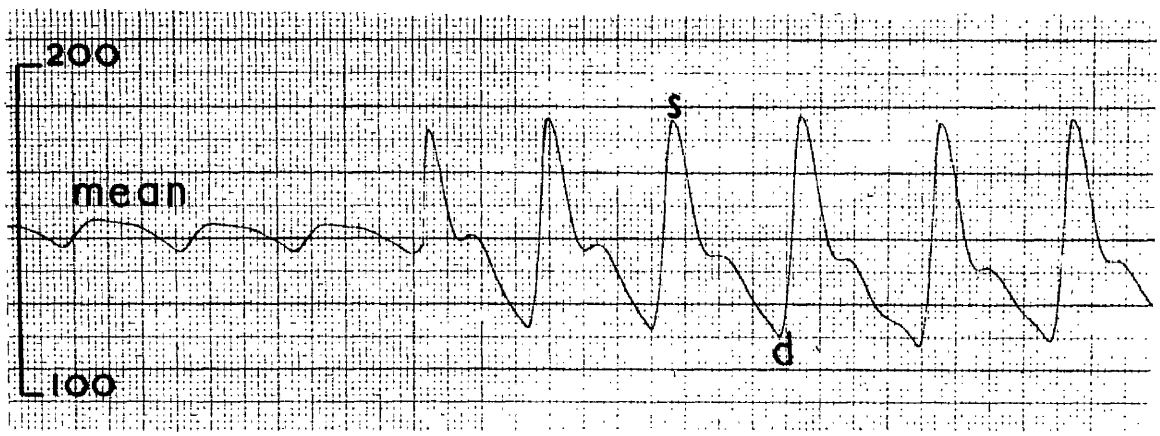
s – systolic

d – diastolic

Fig. 60. Hypertension in a dog suffering from acute interstitial nephritis. 210/118 m.m./Hg.

Case No. 25848 - femoral arterial pressure

paper speed: 25 m.m./sec. heart rate: 95/min.



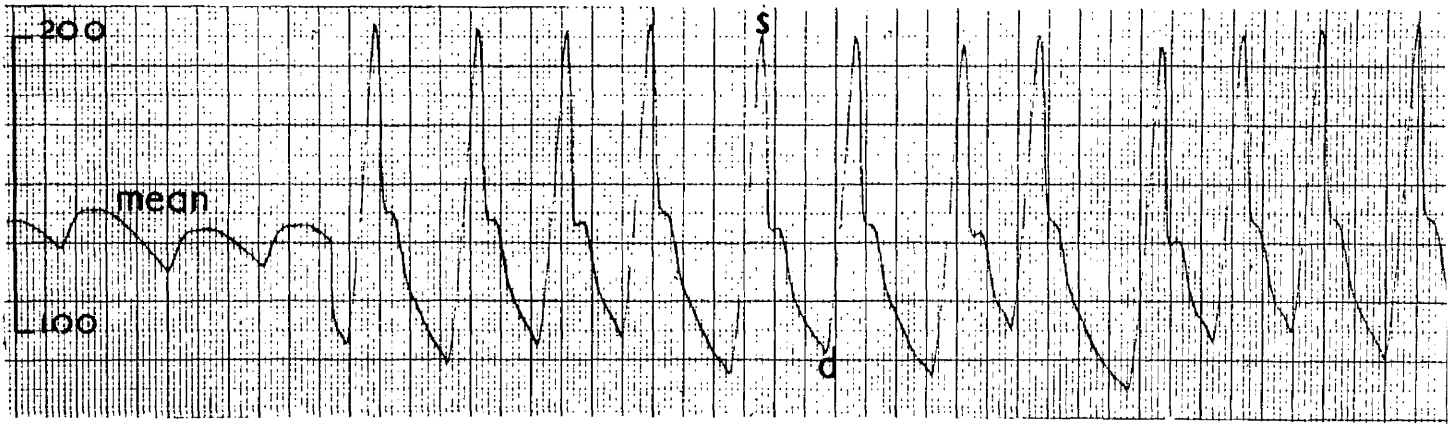
s - systolic

d - diastolic

Fig. 61. Hypertension in a dog suffering from sub-acute interstitial nephritis.

Case No. 26090 - femoral arterial pressure

paper speed : 25 m.m/sec. heart rate 100/min



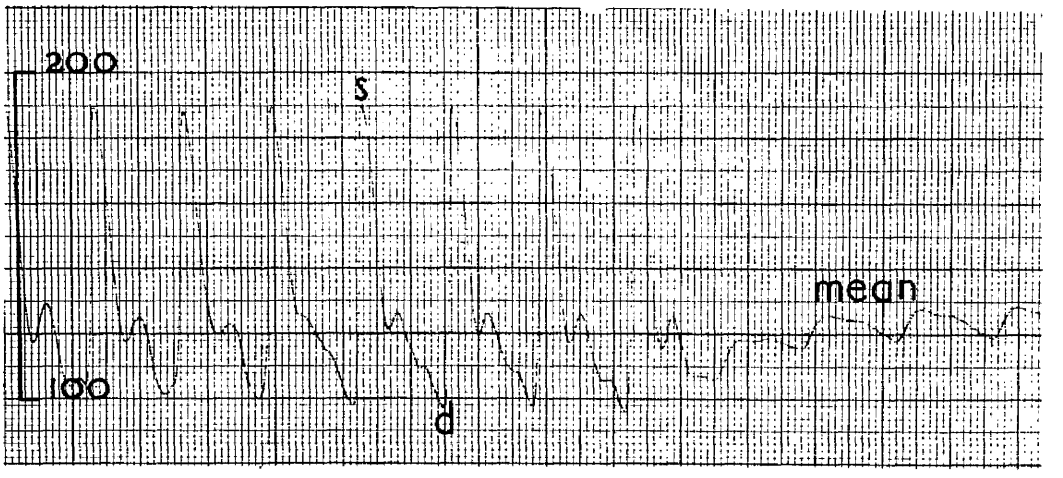
s – systolic

d – diastolic

Fig. 62. Hypertension in a dog with chronic nephritis. 204/100 m.m./Hg.

Case No. 24448 - femoral arterial pressure

paper speed: 25 m.m./sec. heart rate: 120/min



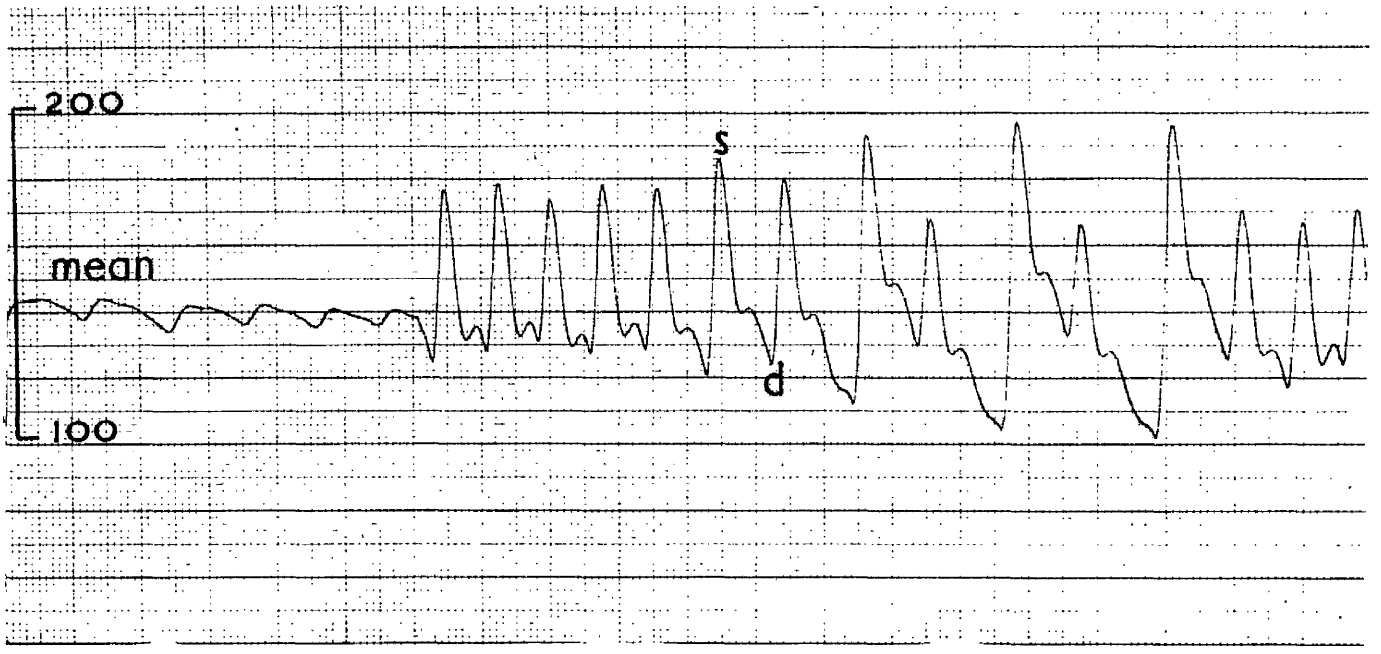
s – systolic

d – diastolic

Fig. 63. Hypertension in a dog with chronic nephritis. 190/100 m.m./Hg.

Case No. 26161 – femoral arterial pressure

paper speed: 25 m.m./sec. heart rate 80/min



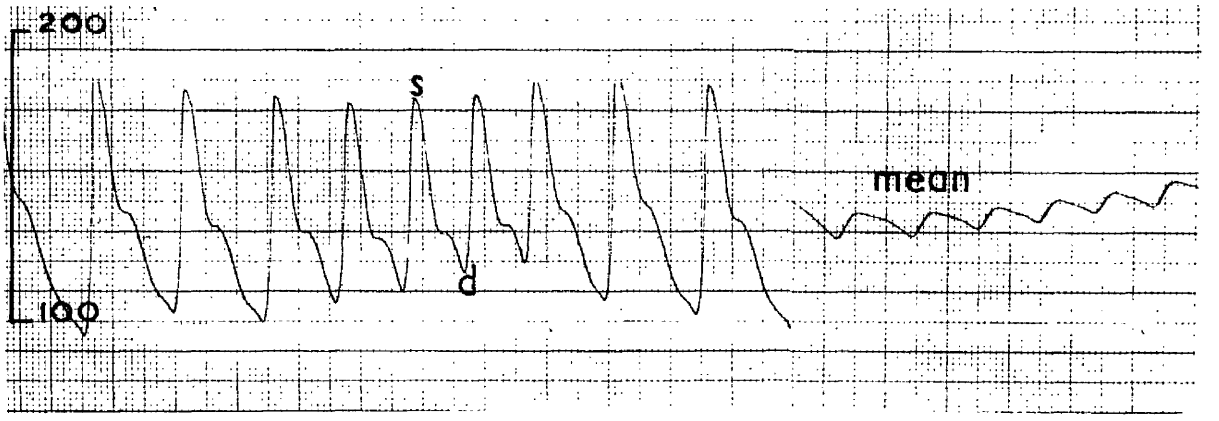
s – systolic

d – diastolic

Fig. 64. Hypertension in a dog suffering from chronic nephritis. This dog also showed extra systoles, occurring at regular intervals. 104/118 m.m./Hg.

Case No.26565 - femoral arterial pressure

paper speed : 25 m.m./sec. heart rate: 110/min



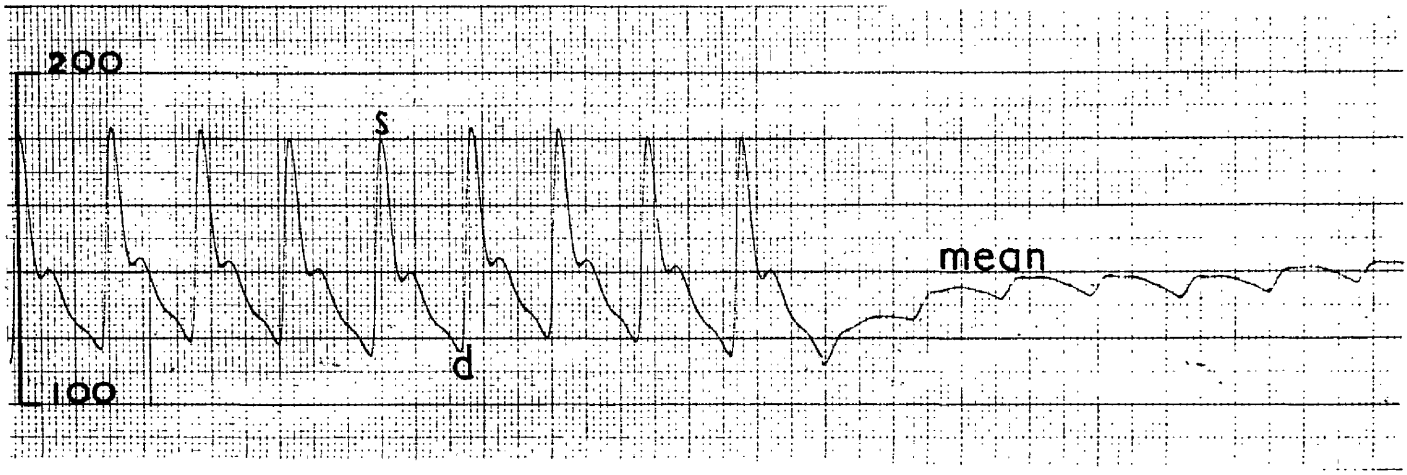
s – systolic

d – diastolic

Fig. 65. Hypertension in a dog with chronic nephritis. 174/110 m.m./Hg.

Case No. 27131 - femoral arterial pressure

paper speed : 25 m.m./sec. neart rate : 110/min.



s – systolic

d – diastolic

Fig. 66. Hypertension in a dog with chronic nephritis. 180/116 m.m./Hg.

Fig. 67. Case No. 25488. Advanced diffuse chronic nephritis, showing irregular granular surface, reduction of cortical depth and cystic dilatation of tubules.

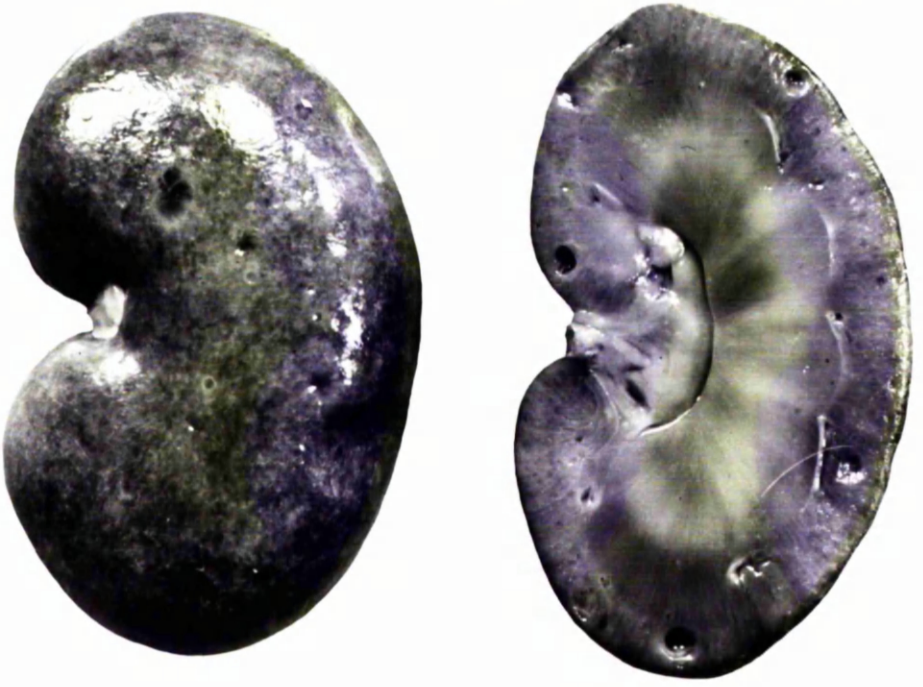


Fig. 68. Case no. 10835. Diffuse renal fibrosis with cystic dilatation of tubules.

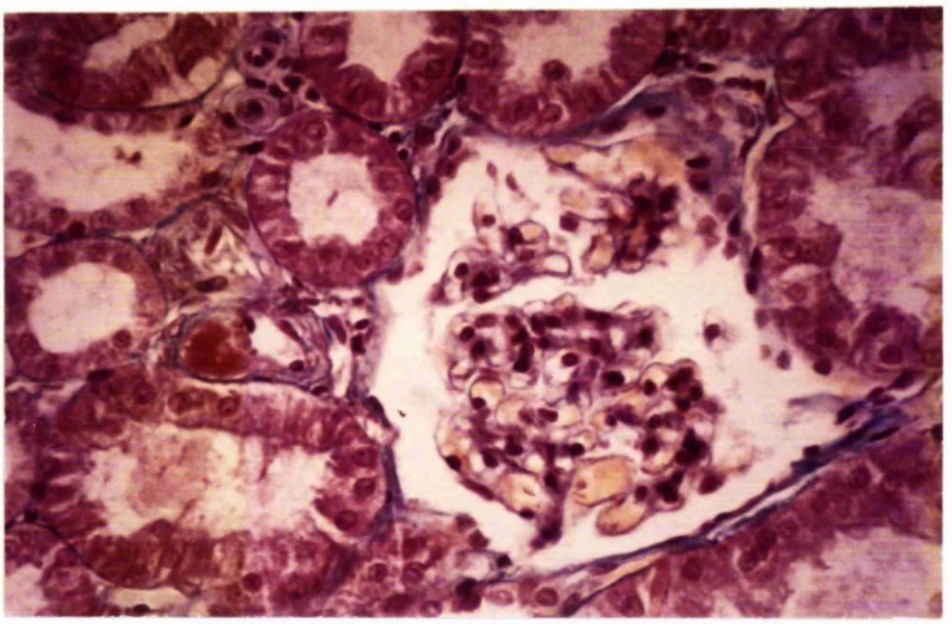


Fig. 69. Case No. 25270/2. Plasmatic vasculosis in an afferent arteriole. Picro-Mallory. X 500.

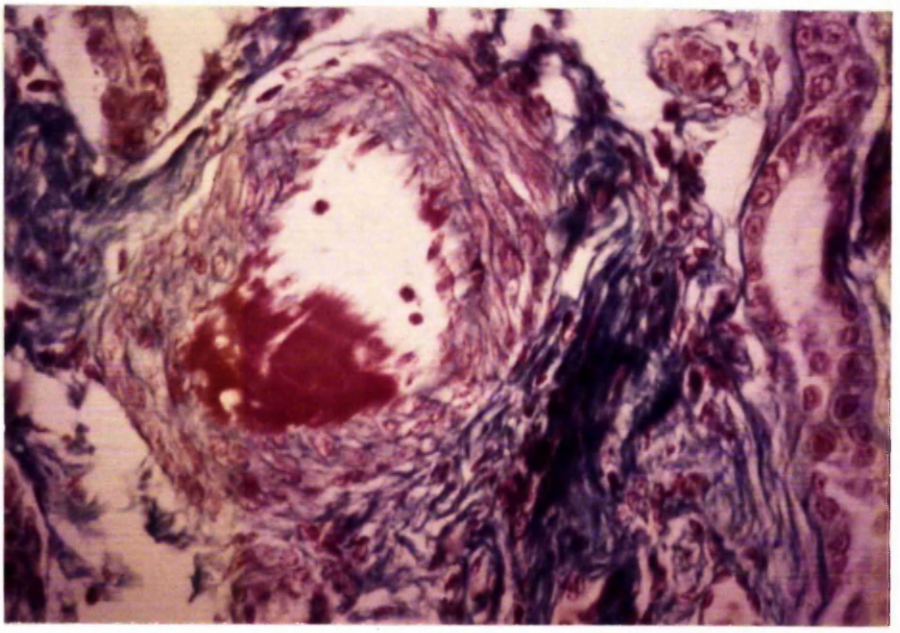


Fig. 70. Case No. 4514. Plasmatic vasculosis in an interlobular artery. Picro-Mallory. X 300.

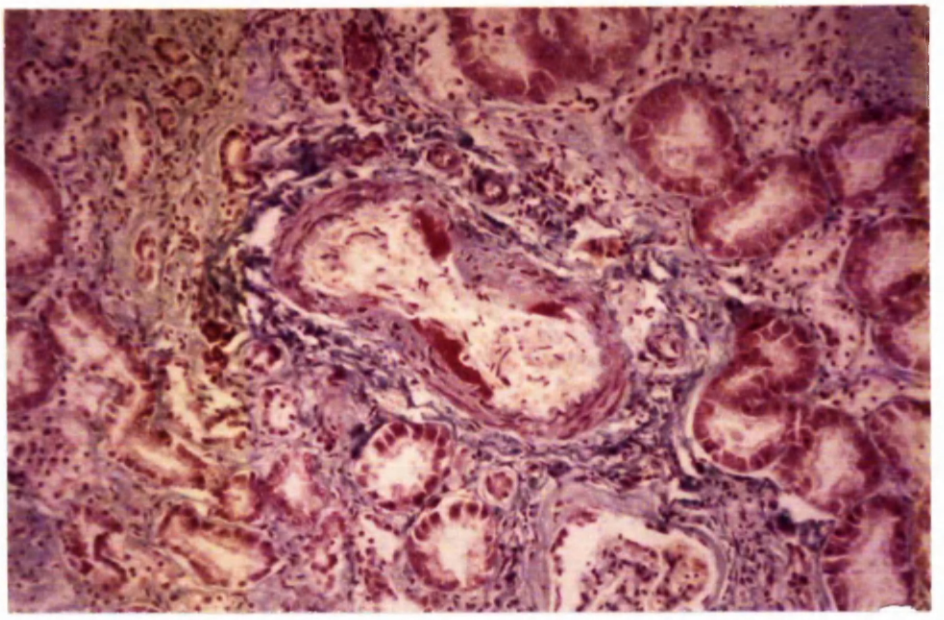


Fig. 71. Case No. 27107. Plasmatic vasculosis in an interlobular artery, showing mixed red and blue reaction with picro-Mallory. X 150.

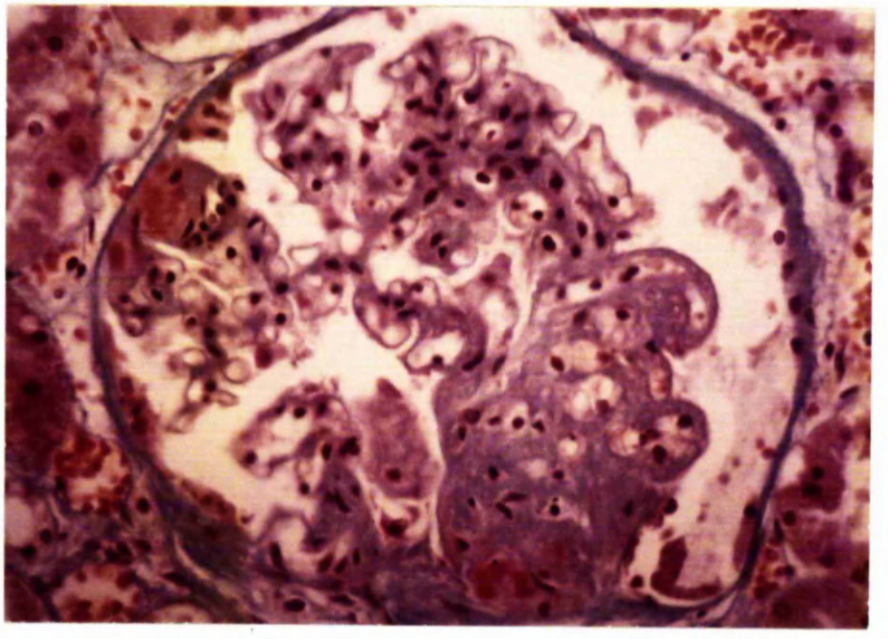


Fig. 72. Case No. 27131. Plasmatic deposits in the capillary tuft of a glomerulus, showing mixed red and blue reaction with picro-Mallory. X 500.

SECTION III.

A SERIES OF EXPERIMENTS DESIGNED TO REPRODUCE LEPTOSPIRAL NEPHRITIS IN DOGS.

Introduction.

The foregoing pathological investigation has shown that 74% of all dogs dying as a result of interstitial nephritis show cardiovascular changes which are likely to be of hypertensive origin and measurements of blood pressure have demonstrated that hypertension may be present in nephritic dogs, particularly in the chronic phase. To study in detail the pathogenesis of interstitial nephritis and the development of hypertension, it is necessary to reproduce the disease experimentally.

Many aspects of the pathogenesis of interstitial nephritis are not understood. It is not known where in the body the leptospirae multiply between initial infection and the later development of severe reaction within the kidneys, nor is the nature of the acute mononuclear cell response understood. The relative importance of persistant foci of infection and of hypertension in the mechanism of the progression to the chronic phase cannot be ascertained from study of field cases. The investigations described in Section I indicated that vascular damage is of considerable significance in this sequence. To establish the precise role of hypertension in producing the characteristic vascular lesions, it would be necessary to correlate their appearance with measurements of blood pressure.

Reproduction of the syndrome would have considerable interest in the comparative study of hypertension. Hitherto, experiments in dogs have involved ligation of renal arteries and other artificial manipulations. A reproducible canine renal disease associated with the development of hypertension would form a useful model, allowing further analysis of the mechanism of nephrogenic hypertension.

It is also important that interstitial nephritis be reproduced to investigate the protective efficiency of commercial leptospiral vaccines. These vaccines are used on a large scale in veterinary practice and their effectiveness is judged solely in terms of ability to stimulate a positive serological titre to L. canicola. There is no direct evidence to indicate that vaccinated dogs are fully resistant to natural infection.

For many years it has been known that experimental infection of dogs with Leptospira canicola can be readily established. However, infected dogs do not become clinically ill and renal damage is slight, with no retention of non-protein nitrogen in the blood.

Wirth (1937) demonstrated that leptospiruria could persist in dogs for 7 months following infection with a spirochaete isolated from a dog suffering from Stuttgart disease. Presumably this organism was L.canicola although it was not typed antigenically.

Klarenbeek and Winsser (1938) infected young dogs and cats with strains/

strains of L. canicola and L. icterohaemorrhagiae. With both serotypes, the pups developed jaundice fairly regularly and showed high serological titres and leptospiruria. In the cats, a serological response occurred without clinical signs.

Monlux (1948) infected dogs with untyped strains of leptospirae. The dogs developed leucocytosis and an increased erythrocyte sedimentation rate but did not show evidence of severe renal disease, either clinically or pathologically.

McIntyre (1954) in a series of experiments, infected dogs with L. canicola. The organism was originally isolated from a dog and maintained in culture in Schuffner's medium. Infected dogs developed transient bacteraemia, pyrexia, leucocytosis, positive serological titres to L. canicola and leptospiruria. However, they remained clinically healthy and pathologically showed only small foci of mononuclear cell reaction in the renal cortices. In this series, infection was established by intra-venous, intra-peritoneal and intra-nasal inoculation. One dog became infected while sharing a cage with another which was excreting the organisms. In another experiment double infection was carried out at 5 day intervals. None of these variations resulted in the development of severe renal lesions. McIntyre concluded that other unknown factors must be involved in the aetiology of severe interstitial nephritis and that attenuation of virulence in organisms after prolonged culture might be an important reason for the experimental results.

Jaundice was produced by Jull and Heath (1961) in dogs infected with L. canicola. While testing a combined L. canicola and L. icterohaemorrhagiae vaccine, two out of three unvaccinated control dogs infected with L. canicola developed jaundice. One of these became acutely ill and died on the 5th day, showing widespread haemorrhages, while the other dog recovered.

Wettimuny (1963) infected dogs with a strain of L. canicola isolated from a dog and maintained in hamsters, hoping that loss of virulence would be prevented by growing the organisms in vivo. The dogs were infected with various numbers of organisms and destroyed at different time intervals. Again, only small renal lesions developed and the dogs remained healthy. Wettimuny concluded that the virulence of his organisms might have become attenuated and that any other factors associated with the aetiology of severe nephritis remained unknown.

The results of previous investigations show that experimental infection of dogs can be established, with haematological and serological responses and localization of the organisms in the kidneys with subsequent leptospiruria. Various routes of infection have been used with success and transmission between dogs has been demonstrated. However, no one has reproduced the severe illness with renal failure such as is seen so frequently in the field.

A number of possible reasons for the lack of success in previous experiments/

experiments may be suggested. Attenuation of virulence in organisms after prolonged culture in vitro, or in hamsters must be considered. Normal healthy dogs may have a fundamental resistance to the disease since any stress factors which might act in conjunction with the organism under natural conditions of infection are unknown. The results of serological surveys carried out in Glasgow indicate that a large proportion of the dog population is exposed to infection although a much smaller number succumb to the disease. Stuart (1946) found positive titres to L. canicola in 40% of dogs and Cunningham et al (1957) found positive titres in 28.9% of dogs in their series. The endemic nature of the disease in the city may have given rise to a population in which many animals have well-developed tissue immunity. It may be of considerable significance that none of the 60 city pups purchased for the present experimental work had serologically detectable antibodies at the age of 8 weeks, contrasting with the known incidence of positive titres in the whole population. Any other immune mechanism operating in this disease is unknown.

In view of the results of other investigations, it was concluded that straightforward inoculation of city dogs with cultured leptospirae would be unlikely to cause severe interstitial nephritis. The series of experiments was designed to overcome in various ways the difficulties resulting from possible loss of virulence in cultured organisms and the apparent resistance of healthy dogs to the potential effects of the pathogen.

During the time in which the experimental work was carried out, attempts were made to isolate L. canicola from dogs suffering from acute interstitial nephritis in the hospital, to obtain a fresh strain of the organism with full virulence for dogs. Isolation was eventually achieved and the work carried out with the fresh strain will be described later.

Before the isolation of L. canicola from a field case of nephritis, attempts were made to enhance the virulence of cultured organisms by passage in series through dogs.

Several methods designed to increase the susceptibility of healthy dogs to L. canicola were investigated.

Dogs were infected by a number of different routes, to determine whether the mode of infection influences the penetration of the organisms and severity of the subsequent renal lesions.

To avoid any possible immunity in city-bred dogs, a litter of country-bred pups was also infected.

The various attempts to reproduce severe interstitial nephritis are described below. Most of the experiments took the form of pilot trials designed to test the effectiveness of various procedures before using unnecessary control animals.

Materials and Methods. /

Materials and Methods.

The dogs used in most of the experiments were purchased from a city pet shop at the age of 6 - 8 weeks. The majority were females as these were less expensive. For one experiment, farm collie pups were purchased. The dogs were kept in warm dry pens out of any contact with other animals. The age at time of infection varied from 9 weeks to 16 weeks. All of the dogs had negative serological titres to L. canicola and L. icterohaemorrhagiae before infection and were in good health.

The strain of L. canicola used in most of the experiments was supplied by the Department of Bacteriology, Royal Infirmary, Glasgow. This was originally isolated from a dog and maintained in culture in Stuart's modification of Schuffner's medium, (Stuart, 1946). A second strain was later isolated from a field case of acute nephritis.

Details of the methods of infection are given with each experiment. In the series, intra-venous, intra-peritoneal, intra-renal, intra-nasal and oral routes were employed.

The dogs were clinically examined each day and rectal temperatures recorded in several experiments.

Blood samples were taken from the dogs for cultural, serological, haematological and biochemical examinations. For L. canicola culture, samples of 2 mls. were withdrawn aseptically from the cephalic vein into sterile bijou bottles containing anti-coagulant. For serological examination, 2 mls. blood were drawn off into bijou bottles. Samples of/

of 2 mls. were collected for haematological examination into bijou bottles treated with sodium ethylene diamine tetra-acetate, (E.D.T.A.). For biochemical examination, samples of 10 mls. were collected in heparinized tubes. Details of the methods employed have been given in Section I.

In some instances, urine samples were examined for the presence of leptospirae by dark ground microscopy.

At the end of experiments, the dogs were destroyed by intra venous injection of sodium pentobarbitone. Post-mortem and histological examinations were carried out on every dog.

In the descriptions of the experiments, individual special procedures employed will be noted.

Experiment I.

In healthy dogs, injected leptospirae may be rapidly destroyed in the bacteraemic phase and fail to colonise the kidneys in sufficient numbers to cause excessive damage. To test this hypothesis, leptospirae were injected directly into the renal arteries of two dogs and into the renal tissue of one dog.

Method.

The three dogs (21294/3, 4 and 5) were three months old at the time of infection. The culture of L. canicola contained 500 x 10^6 organisms /ml.

Under cyclopropane anaesthesia, the abdominal cavities were opened and kidneys and renal arteries exposed. A tuberculin syringe with a bent needle was used to effect intra-arterial injection. 1 ml. of culture was injected into both renal arteries of 21294/3 and 4. In dog 21294/5, 1 ml. of culture was injected directly into each kidney from the posterior pole. The dogs made uneventful recoveries from the operation.

Results.

The dogs were kept under observation for two months following infection, during which time they remained clinically healthy.

On the day after infection, urine samples were examined by dark ground microscopy for leptospirae and numerous non-motile crumpled organisms were present in each sample. Urine examinations were carried out daily/

daily for a further week and then once weekly until the end of the experiment. No leptospirae were detected.

Two days after infection, the dogs developed mild pyrexia, with temperatures between 102.5° and 103.5°F., which persisted for three days. On the third day, blood samples for L. canicola culture were taken, with positive results in each case.

Biochemical examinations of blood and urine samples were carried out two weeks after infection. There were no abnormalities.

Blood samples were taken for serological examination on post-infection days 10,17,27,40 and 60. The titres of each dog to L. canicola are listed in the following table.

Titre to L. canicola.

Post-Infection Days.

Dog	10	17	27	40	60
21294/3	1: 3,000	1: 3,000	1: 3,000		
21294/4	1: 1,000	1:10,000	1: 1,000	>1:30,000	1: 1,000
21295/5	1: 3,000	1: 1,000	1: 1,000	1: 1,000	1: 100

The serological response to infection was very marked in each of the dogs. However, in field cases, a titre of > 1:30,000 is generally associated with very severe acute nephritis, whereas the experimental dogs/

dogs remained clinically normal.

Dog 21294/3 was destroyed after 1 month, and the kidneys were examined as at this time acute reaction is thought to be well developed in the natural disease. The other dogs were destroyed after two months, since their titres were falling and no clinical abnormality had occurred.

On post-mortem examination, 21294/3 showed numerous small renal lesions, seen as white foci of pin-head size scattered throughout the cortices.

Histologically, these foci were discrete areas of interstitial nephritis, with mononuclear leucocyte reaction in which plasma cells predominated. (Fig. 73).

Post-mortem examination of the dogs destroyed after 2 months revealed little. 21294/4 showed no macroscopic lesions, while the kidneys of 21294/5 contained a white spot on the cortex at the posterior pole, almost certainly scar tissue resulting from intra-renal injection at this site. Histologically, the kidneys of both of these dogs contained a few very small scattered foci of interstitial mononuclear leucocyte reaction, mainly in the junta-medullary region of the cortices. (Fig.74).

Discussion.

Although experimental infection was readily established by intra-renal arterial and intra-renal injection, with good serological response, only/

only very small renal lesions developed, resembling those described in the experiments of McIntyre (1954) and Wettimuny (1963). Lesions of similar type and cellular pattern are found on a much larger scale in field cases.

The establishment of bacteraemia after intra-renal infection is of interest. The scattered distribution of renal lesions in 21294/5 seems to indicate that the kidney was re-colonised after blood-born dissemination of the organisms from the injection site in the posterior pole.

In this experiment, there is no doubt that large numbers of organisms were introduced into the kidneys. Therefore, it seems that the actual number of leptospirae which reach the kidney may not be of prime importance in determining the severity of the subsequent reaction.

Experiment II.

The results of Experiment I illustrated the ability of healthy dogs to resist the potential effects of L. canicola. Several methods of overcoming this resistance were devised. The first of these involved the infection of dogs previously treated with cortisone.

Cortisone was administered to depress reticulo-endothelial activity to a level which would permit extensive invasion of the kidney by the organisms, with subsequent intense cellular reaction.

Working with syphilis (another spirochaetal disease), Turner and Hollander (1953) found that after pre-treating rabbits with cortisone, more severe lesions developed than in control animals. When treatment was stopped, a rebound reaction occurred. Lewis (1953) showed that cortisone-treated rabbits succumbed to fatal septicaemia following skin injection of group A streptococci, with no cellular reaction to the organisms, while control animals showed only local inflammation of the skin. Inhibition of antibody production was demonstrated by Fischel (1953) in vaccinated rabbits treated with cortisone.

On the basis of these observations, the experiment was intended to utilise the inhibition of cellular reaction and antibody production, allowing the leptospirae to invade the renal tissue extensively. Termination of cortisone treatment might induce a severe rebound reaction, resulting in the type of lesion found in clinical cases of nephritis.

Method.

Two dogs were used in this experiment, numbers 21294/20 and 21, aged 9 weeks. The corticosteroid preparation employed was Betamethasone ("Betsolan," Glaxo.)

The dogs each received 1 mgm. Betsolan sub-cutaneously daily on days 1 - 4 of the experiment. The dose was calculated as approximately 10 times the therapeutic level. On day 3, the dogs were infected intra-peritoneally, each receiving 4 mls. of L. canicola culture.

Results.

During the following 6 days, both dogs became abnormally thirsty, but remained otherwise healthy. On day 9, 21294/20 suddenly became extremely ill, showing profound dullness, deep jaundice, haematuria and ataxia. Blood samples for biochemical examination were taken on this day and on day 10, when the dog was moribund and destroyed. The results of these examinations are given in the table.

Blood Biochemistry of 21294/20.

	Day 9		Day 10	
Blood urea	79	mgms/100 mls.	260	mgms/100 mls.
Total bilirubin	16.8	" "	21.6	" "
Direct bilirubin	8.6	" "	7.8	" "
A/G ratio	0.6	" "	0.5	" "
S.G.O.T.	59	S.F.	79	S.F.
S.G.P.T.	29	S.F.	28	S.F.
Alk. Phos.	37	K.A.	49	K.A.
Protein			4.9	gms/100 mls.
Inorganic Phosphate			20	m.eq/litre

These features indicated that jaundice was both haemolytic and hepatic in origin, since approximately half of the bilirubin was direct. Liver damage was also shown by the raised alkaline phosphatase and serum transaminase levels. Renal failure was shown especially in the results of day 10, by the high blood urea and inorganic phosphate levels.

On post-mortem examination, the body was deeply jaundiced with generalized petechiation of serous membranes, especially the pleura. A haemorrhage was present in the wall of the left ventricle. The kidneys were deeply congested. Mild fatty change of the liver was appreciable. Histologically, haemorrhages were present throughout the kidneys with blood lying freely in Bowman's capsules outwith the glomerular tufts and also inside most of the tubules. (Fig. 75). The liver showed separation of parenchymal cells and early centrilobular fatty change. (Fig.76). The meninges were markedly congested and over the cerebellum a mild polymorphonuclear leucocyte reaction was apparent in places around blood vessels. Haemorrhages were scattered throughout the molecular layer of the cerebellum. (Fig.77).

Immediately after death, kidney samples and cerebrospinal fluid were taken for culture of L. canicola and a urine sample was examined for leptospirae. No leptospirae were found in the urine and none were cultured.

The other dog, 21294/21, remained in good health and was destroyed 8 weeks after the start of the experiment. There were no macroscopic or microscopic lesions.

Discussion.

Cortisone pre-treatment did not result in the development of typical acute interstitial nephritis in the infected dogs. The severe acute icteric and haemorrhagic syndrome which affected one dog did not resemble the disease generally associated with L. canicola, but closely simulated the disease caused by L. icterohaemorrhagiae. Also, the lesions - and especially the renal haemorrhages - were very similar to those observed in hamsters infected with L. canicola. (Fig. 78). It seems possible that the cortisone administration may have allowed a far more acute form of leptospirosis to develop. However, as the second dog remained healthy and showed no lesions at all, interpretation of the results cannot be certain.

Experiment III.

The acute illness of one dog in the previous experiment indicated that further investigation of the combined effects of infection with inhibition of immunological reaction could be of value. In this experiment, dogs were dosed with "Methotrexate," (4 Amino N^{10} - methyl pteroylglutamic acid. Lederle), a folic acid antagonist and powerful antimitotic agent, which causes repression of cellular reactions and antibody production. Since the results of Experiment II were difficult to interpret, it would be of interest to find whether a similar acute icteric syndrome might result from treatment of infected dogs with a different anti-mitotic drug.

Ferguson, Thiersch and Philips (1950) investigated the toxicity of Methotrexate in dogs. Severe intoxication or death occurred in 5 - 6 days following intra-muscular injection of 1.0 mgm/kgm/day, in 10 - 11 days with doses of 0.2 mgm/kgm/day and in 14 - 37 days with 0.05 mgm/kgm/day. In the present experiment, a dose of 0.1 mgm/kgm/day was selected as a suitable sub-toxic level for short-term administration.

Method.

Four dogs were used in this experiment, 21294/28 1 31 aged 9 weeks. The organisms were cultured from the Royal Infirmary strain. Methotrexate was administered orally in doses of 0.1 mgm/kgm/day.

The dogs were dosed with methotrexate on days 1 -4 of the experiment.

On/

On day 3, infection was carried out by intra-peritoneal injection of 3 mls. L. canicola culture.

Results.

The dogs showed no abnormality until day 8, when 21294/28 and 31 developed mild jaundice. On day 9, 21294/28 had recovered, while 21294/31 was extremely dull, deeply jaundiced and ataxic. On day 10, this dog had recovered partially and although still very jaundiced, was able to walk almost normally and was much brighter. The condition remained unchanged until day 14, when the dog became moribund and was destroyed.

On day 13, dog no. 21294/30 became very dull with marked pallor and mild jaundice. This dog died on day 14.

Blood samples for biochemical examination were taken on day 13 from 21294/30 and 31. The results are tabulated below.

Blood Biochemistry on Day 13.

	21294/30	21294/31
Total bilirubin	2.4 mgms/100 ml.	6.0 mgms/100 ml.
Direct bilirubin	1.1 " "	"
Indirect "	1.2 " "	"
Urea	28 " "	42 mgms/100 ml.
Alk.Phos.	40 K.A.	25 K.A.
S.G.O.T.	78 S.F.	22 S.F.
S.G.P.T.	190 S.F.	18 S.F.
A/G ratio	0.3	0.6
Protein	"	3.8 mgm/100 ml.
Inorg. Phos.	8.6 mg/100 ml.	"

These results indicate severe liver damage, particularly in 21294/30 which had high alkaline phosphatase and serum transaminase levels. The division of bilirubin into approximately half direct and half indirect suggests that jaundice was both hepatic and haemolytic in origin.

On post-mortem examination, dog no. 21294/30 showed pronounced jaundice. The liver was pale orange in colour and the capsule petechiated. The kidneys and myocardium were pale and the pleura petechiated. Histologically, the liver showed severe acute focal hepatitis (Fig.79) and the kidneys showed nephrosis with no cellular reaction.

The post-mortem features of dog no. 21294/31 were generally similar to those of 21294/30. The liver was bronze in colour and the kidneys contained numerous white spots of pin-head size scattered throughout the cortices. Paleness of kidneys and myocardium and petechiation of the pleura were also present in this dog. Histologically, the liver showed a moderate degree of generalised fatty change. Mild interstitial nephritis was present in the kidneys, consisting of foci of plasma cell and lymphocyte reaction with some destruction of tubules.

Dogs 21294/28 and 29 remained healthy, with no further sign of jaundice in 28. Both were destroyed one month after infection. Neither dog showed macroscopic post-mortem changes. Histologically, the kidneys of 28 contained a few small interstitial accummulations of lymphocytes and plasma cells. No lesions were found in dog 29.

Discussion.

In this experiment an acute syndrome occurred in three dogs out of four following methotrexate treatment and infection. In two dogs the condition was fatal and the clinical biochemical and pathological features in these dogs were very similar to those observed in the jaundiced dog in Experiment II. As there was no indication of methotrexate poisoning (diarrhoea) it is unlikely that the illness was due to effects of the drug alone.

It would appear that both cortisone and methotrexate, by inhibiting immune mechanisms, may have allowed a more acute form of leptospirosis to develop following infection with L. canicola. The syndrome was very similar to that generally associated with L. icterohaemorrhagiae infection.

Experiment IIII.

In the two preceding experiments, attempts to render dogs more susceptible to L. canicola infection by treatment with cortisone and methotrexate resulted in the production of an acute hepatic syndrome. A further trial with methotrexate was planned in the hope that nephritis might develop in a proportion of infected dogs. As the aetiology of the liver failure in previous experiments was uncertain, it was also necessary to determine the relative effects of the drug and the organisms. For these reasons, a controlled experiment was carried out.

Methotrexate was used in the same dosage as before (0.1 mgms/kgm/day)as there had been no sign of toxicity and not all treated dogs developed lesions in the preceding experiment.

Infection was carried out by intra-nasal instillation of L. canicola culture. Since infection under natural conditions may occur by the intra-nasal route, it was of interest to find whether this method would favour the establishment of infection and development of severe renal lesions. McIntyre (1954) demonstrated that small renal inflammatory lesions resulted from experimental intra-nasal infection.

Method.

Eighteen dogs aged 9 weeks were used in the experiment. These were divided into three groups of six and each group was housed separately.

L. canicola was obtained in culture from the Royal Infirmary. The culture contained 180 x 10^6 organisms /ml.

Group I (Nos. 22661 - 22666) received methotrexate treatment and infection.

Group II (Nos. 22667 - 22672) received infection only.

Group III (Nos. 22673 - 22678) received methotrexate only.

Groups I and III were dosed daily on days 1 - 4 of the experiment, receiving 0.1 mg. methotrexate solution orally on each occasion. On days 3 and 4, groups I and II were infected.

The infection procedure involved the intra-nasal instillation of 0.5 ml. culture into each nostril. The dogs were tranquillised by intravenous injection of diethyl-thiambutene ("Themalon," Burroughs Wellcome & Co.) at the rate of 2 mgms./lb body weight. Then, with the dogs in dorsal recumbency with necks extended, the culture was slowly dripped into each nostril from a syringe. No sneezing occurred. Finally, tranquillisation was terminated by sub-cutaneous injection of 1 ml. nalorphine hydrobromide solution ("Lethidrone," B.W. & Co.).

This procedure was carried out on day 3 and repeated on day 4 to ensure that each dog received a large infective dose.

Results.

The dogs were kept under observation for 2 months. During this time, none showed any evidence of renal or hepatic disease. Temperatures were recorded daily on days 1 - 14. Transient pyrexia was observed in the /

the infected dogs (Groups I and II), with temperatures between 102° and 103° on days 7 and 8. Blood samples for serological examination were taken from all dogs on day 18, 28 and 38. The results are tabulated below.

Dog. no.	Titre to L. canicola.		
	Day 18	Day 28	Day 38
22661	1: 1,000	1: 100	1: 30
22662	1: 3,000	1: 1,000	1: 100
22663	1: 30	1: 30	1: 10
22664	1: 30	1: 30	1: 10
22665	- ve	- ve	- ve
22666	1: 3,000	1: 1,000	1: 100
22667	1: 10	1: 10	- ve
22668	1: 30	1: 10	- ve
22669	1: 100	1: 100	1: 1000
22670	1: 30	1: 30	1: 10
22671	- ve	1: 10	1: 10
22672	1: 300	1: 100	1: 30
22673	- ve	- ve	- ve
22674	- ve	- ve	- ve
22675	- ve	- ve	- ve
22676	- ve	- ve	- ve
22677	- ve	- ve	- ve
22678	- ve	- ve	- ve

The rather higher titres in the dogs of Group I are of interest. It seems likely that methotrexate, by delaying cellular reaction to the organisms, allowed the infection to become better established, subsequently stimulating greater antibody production when the drug effects terminated.

As the dogs remained perfectly healthy and the serological titres fell, it was evident that infection was not maintained. The dogs in groups I and II were destroyed after two months.

On post-mortem examination, macroscopic changes were found in only one dog, no. 22662. For this reason, it was considered unnecessary to destroy the methotrexate-treated control dogs in group III.

The kidneys of dog 22662 contained many white pin-head sized foci scattered throughout the cortices. Histologically, these lesions were areas of interstitial nephritis, consisting of cellular reaction with some destruction of tubules. The infiltrating cells were mainly plasma cells and lymphocytes but multinucleate giant cells were also present in considerable numbers. (Figs. 80 & 81). Giant cells have not been found previously either in natural or experimental cases.

None of the kidneys or other organs from the remaining 11 dogs showed microscopic lesions.

Discussion.

This second attempt to produce severe interstitial nephritis by stressing dogs with methotrexate before infection was unsuccessful. However,/

However, the greater serological response in the methotrexate-treated dogs probably indicates that infection became more firmly established in those animals.

The acute hepatic syndrome encountered in previous experiments did not occur on this occasion. Possibly this was related to individual differences in susceptibility or to the fact that intra-nasal infection may have caused bacteraemia to develop more slowly and to be overcome more readily.

In dog no. 22662, foci of interstitial nephritis resulted from intra-nasal infection. The results of the experiment show that dogs do not succumb to infection any more readily by this route, although it may be a natural portal of infection in the field.

The appearance of giant cells in the kidney of 22662 suggests that methotrexate caused a modification of the usual cellular reaction, probably attributable to abnormal mitosis.

Experiment 5.

Attempts to increase the susceptibility of dogs to L. canicola by depression of cellular responses in the initial stages of infection did not result in the development of severe interstitial nephritis. Although infection was readily established in such dogs, inflammatory reaction in the kidneys was never extensive. It would appear, therefore, that the leptospirae did not achieve widespread colonisation of the renal tissue. In the previous experiments healthy kidneys were found to be resistant to heavy infective doses of L. canicola. Consequently, an attempt was made to stress the kidneys alone in the hope of permitting the organisms to achieve widespread invasion.

To stress the kidneys, mercuric chloride was selected as a suitable nephrotoxic agent. This chemical causes injury specifically to cells of the proximal tubules. Since proximal tubule damage is a pronounced feature in naturally occurring interstitial nephritis, it was hoped that injury to these cells would provide a suitably non-resistant environment where the leptospirae might readily invade.

Rocha et al (1959) produced proximal tubule damage in rats using mercuric chloride in doses of 1 mgm./rat. To avoid risk of causing renal failure as a result of the chemical action alone, the dosage used in this experiment was rather smaller on a body weight basis. The dogs were given 1 mgm. mercuric chloride/kgm. body weight, administered sub-cutaneously in 1 mgm./cc. solution.

Six dogs were used in this experiment, Nos. 24478/1 - 6, aged 8 weeks.

On day 1 of the experiment, the dogs were injected with mercuric chloride as described. Eight hours later, they were infected by intraperitoneal injection of 1 ml. L. canicola culture. The dogs were apparently healthy for the next 4 days.

On day 5, all of the dogs were partially anorexic and slightly dull. There was no change on day 6. On the morning of day 7, two dogs, 24478/4 and 24478/6, were found dead.

Post-mortem examination of these dogs revealed pronounced jaundice, with orange-coloured livers, petechiation of the pleura and very pale kidneys. Histologically, the renal cortices of both dogs contained a few small foci of lymphocyte and plasma cell reaction. In 24478/4, the cells of the proximal tubules showed pronounced fatty change. (Fig.82). The livers also showed marked fatty change.

The remaining four dogs recovered from the three days of malaise without showing specific symptoms. Blood samples for serological examination were taken on days 14, 28 and 42. These results are tabulated overleaf.

Dog	Titre to L. canicola. Day 14	Day 28	Day 42
24478/1	1: 3,000	1: 1,000	1: 300
24478/2	1: 3,000	1: 1,000	1: 100
24478/3	1: 300	1: 100	1: 30
24478/5	1: 1,000	1: 100	1: 100

After this, the dogs were destroyed as they showed no evidence of nephritis.

On post-mortem examination, the kidneys of all the dogs contained numerous small white foci scattered throughout the cortices. Histologically, these were areas of mild interstitial nephritis, characterised by plasma cell and lymphocyte reaction. (Fig. 83).

Discussion.

In this experiment, six dogs treated withmercuric chloride before infection all developed numerous small foci of interstitial nephritis. In previous experiments only a proportion of infected dogs showed these renal changes. Therefore it is possible that damage to the proximal tubules caused by mercuric chloride rendered the kidneys more susceptible to invasion by L. canicola. However, as two dogs became extremely ill and died and none of the dogs succumbed to severe nephritis, this method of enhancing susceptibility appeared to be impractical.

Once again, the acute icteric condition which occurred in two of the/

the dogs, resembled the disease generally associated with L. icterohaemorrhagiae infection.

In this series of experiments designed to increase the susceptibility of healthy dogs to leptospiral nephritis a large proportion of the dogs developed small foci of interstitial nephritis. A number of the dogs died after a brief severe illness of which jaundice and hepatic failure were prominent features. This syndrome may have represented a hyper-acute form of leptospirosis. In view of the fatalities affecting several dogs, it would have been impossible to increase the dosage of the various drugs employed, although some dogs remained perfectly healthy. As none of the dogs developped severe nephritis, no further attempts of this type were made.

Experiment 6.

In previous experiments, attempts to increase the susceptibility of dogs to L. canicola were not successful in producing severe nephritis. The organisms used in the experiments (and those of other workers) were cultured for long periods after original isolation from dogs, with possible loss of virulence for that species. In the next two experiments, leptospirae were passaged in series through dogs in an attempt to enhance their pathogenicity. Low et al (1956), working with L. icterohaemorrhagiae, found that cultured organisms became more virulent as they were passaged through dogs. In their experiments, each dog was infected from a previous dog. Arean (1962) increased the virulence of L. icterohaemorrhagiae for guinea-pigs by passage after the organisms had been cultured for several years.

In experiments 6 and 7, L. canicola was passaged by infecting dogs with blood taken in the bacteraemic phase from the preceding member of the series.

Method.

Nine dogs aged 8 weeks were used in this experiment. (Nos. 21294/10 - 18). Infection was effected by intra-peritoneal injection. Following infection by this route, bacteraemia is established by the 4th day (McIntyre. 1954) and therefore blood samples were taken on post-infection day 4 from each dog.

The blood samples were taken from the jugular veins. 10 mls. of blood were removed on each occasion and this volume was replaced with dextran solution.

Infection of the next dog was then effected by intra-peritoneal injection of 8 mls. blood. The remaining 2 mls. were used for the culture of L. canicola, so that the presence of bacteraemia could be verified in retrospect.

The first dog of the series, 21294/10 was infected by intra-peritoneal injection of 1000 x 10^6 leptospirae in culture. On day 4, a blood sample was taken to infect dog 21294/11.

This procedure of taking blood samples on the 4th day to infect the next dog in the series was repeated until nine dogs had been infected.

Results.

Blood cultures were positive only in the first 4 dogs, indicating that bacternemia was not present in the fifth dog and therefore succeeding dogs were not infected.

During the experiment, the dogs did not show a pyrexic response to infection. The main clinical observation was the development of jaundice in the first and third dogs of the series. The third dog showed jaundice of moderate degree.on the 8th day after infection. The first dog developed mild jaundice 18 days after infection. Neither dog showed malaise, anorexia,/

anorexia, fever or other symptoms at this time. In both cases, jaundice persisted for 3 days, and was followed by recovery. Blood samples for biochemical examination were taken from these dogs. The results are tabulated below.

Blood Biochemistry of the Jaundiced Dogs.

	21294/10		21294/12.	
Bilirubin	2	mgm./100 ml.	2.15	mgm./100 ml.
Protein	4.4	gm./100 ml.	5.1	gm./100 ml.
A/G ratio	0.5		0.5	
Alkaline Phosphatase	27	K.A.	3	K.A.
Urea	27	mgm./100 ml.	35	mgm./100 ml.
S.G.O.T.	40	S.F.	25	S.F.
S.G.P.T.	23	S.F.	22	S.F.

These findings indicate liver damage, shown by raised bilirubin levels, low serum protein and A/G ratios and elevation of alkaline phosphatase in 21294/10.

No further abnormalities were observed in any of the dogs and they were destroyed 10 weeks after the start of the experiment. No post-mortem or microscopic lesions were found in any organ.

The organisms isolated from the 4th dog of this series were maintained/

maintained in culture to be used in a second passage series in experiment 7.

Discussion.

In this experiment, L. canicola was passaged through four dogs. In the fifth dog, either the bacteraemic phase was missed or else the organism failed to become established. McIntyre (1954) demonstrated that bacteraemia may occur from the third day after intra-peritoneal infection. Therefore this transient phase may have passed before the blood sample was taken on the fourth day.

Passage through four dogs did not enhance the virulence of the leptospirae sufficiently to cause renal disease. However, the presence of jaundice in two dogs with biochemical evidence of liver damage was of considerable interest. Liver disease also resulted from infections of pups in Experiments 2, 3 and 5. As young animals have been used in these experiments, it may be that in young pups the liver is more susceptible to L. canicola than the kidneys. The great majority of field cases of leptospiral nephritis occur in dogs over 6 months of age.

Experiment 7.

In the preceding experiment, no obvious increase in virulence resulted from four serial passages of L. canicola through dogs. As the organism was "lost" after the fourth dog, a second series was planned to continue the passage and to determine whether an obvious increase in virulence would develop.

Method.

In this second series, six pups aged 8 weeks were used, (nos. 21294/22 - 27). This time blood samples were taken from the dogs on both days 3 and 4 after infection, to minimise any risk of missing the bacteraemic phase. Again, the samples were taken from the jugular veins. 6 mls. of blood were taken from each dog on both days. 4 mls. of this were injected intra-peritoneally into the next member of the series and 2 mls. were used to culture L. canicola. The blood volume was replaced with dextran solution.

The first dog of this series was infected from culture maintained after isolation of L. canicola from the fourth dog in Experiment 6. At this time, the organism had been cultured for 8 weeks. 4 mls. of culture were injected intra-peritoneally. On days 3 and 4, blood samples were taken for passage and culture as described.

The second dog, infected twice, was bled 3 and 4 days after the second infection. This procedure, whereby each dog received two injections/

injections of blood from the preceding dog and in turn had blood samples taken on the following third and fourth day, was repeated with the six dogs in succession.

Results.

The results of blood culture were again positive only in the first four dogs. The individual results from each dog on days 3 and 4 are tabulated below.

Results of Blood Culture.

	Day 3	Day 4
21294/22	+	+
21294/23	+	+
21294/24	+	-
21294/25	+	-
21294/26	-	-
21294/27	-	-

During the experiment, 21294/22 showed no clinical abnormality.

Eight days after infection, 21294/23 became severely ill, showing extreme dullness, complete anorexia and advanced ataxia. Locomotion was limited to a few shaky steps, after which the dog fell over on its back. Euthanasia was performed. No macroscopic abnormalities were found/

found on post-mortem examination. Histologically, meningitis was present, with especially marked changes over the cerebrum where the meninges showed oedematous thickening and mononuclear cell reaction. (Fig.84). Cultural examination of cerebro-spinal fluid for the presence of L. canicola was carried out, with negative results.

Dog no. 21294/24 developed jaundice 17 days after infection. Jaundice was of moderate degree and the dog was rather dull and partially anorexic. These signs persisted for four days, then resolved gradually. Biochemical examination of a blood sample from this dog gave the following results:

Blood Biochemistry of 21294/24.

Bilirubin	=	4 mgm/100 ml.
Protein	=	4.5 gms/100 ml.
A/G ratio	=	0.4
Alkaline phosphatase	=	59 K.A.
S.G.O.T.	=	12 S.F.
S.G.P.T.	=	18 S.F.
Urea	=	25 mgm/100 ml.

Liver damage was indicated by the high bilirubin and alkaline phosphatase levels and low protein and A/G ratio.

None of the other dogs in the series showed any abnormality and they were destroyed 8 weeks after the start of the experiment. No post-mortem or histological lesions were found.

Discussion.

Passage of L. canicola through a second series of dogs did not lead to the development of nephritis in the infected dogs. The severe miningitis in 21294/23 may have been caused by L. canicola, as occasionally happens in the human. Again, liver disease occurred in one of the pups, with similar clinical and biochemical observations to those noted in jaundiced dogs in previous experiments. This further case indicates once more that the livers of very young dogs may be more susceptible to L. canicola than the kidneys.

It cannot be entirely certain whether the illnesses in two dogs of the series resulted from an increase in virulence of the organism. Passage was maintained through only four dogs despite precautions to ensure that the bacteraemic phase was not missed. Apparently the fifth dog received too few organisms or had sufficient natural immunity to overcome the infection very rapidly.

As none of the infected dogs developed renal lesions and passage could not be continued beyond four dogs in either series, no further attempts of this type were made, although the organisms isolated from the fourth dog in this series were cultured and used in later experiments.

Experiment 8.

The difficulties encountered in the experimental reproduction of leptospiral nephritis have been well illustrated in the work of other investigators and in the preceding experiments. Infection as it occurs naturally cannot be repeated accurately, since the natural route is uncertain and the condition of dogs at the time of infection is quite unknown. McIntyre (1954), in an attempt to simulate field conditions, infected dogs intra-nasally and produced small renal lesions. This procedure was also followed in the 4th of these experiments with similar results. The oral route is the other probable natural portal of infection. No one has infected dogs in this way previously. In this experiment, dogs were infected orally to establish definitely whether the mode of infection has any bearing on the severity of the disease.

On this occasion, older dogs were used because liver disease had been encountered with considerable frequency in young infected pups in other experiments.

Method.

Five dogs aged 6 months were used (Nos. 22673 - 22677). These dogs had been used as controls in Experiment 4, when they were dosed with methotrexate. They had been housed out of any contact with other dogs and had negative serological titres to leptospirae during Experiment 4 and at the start of this experiment.

Oral infection was carried out by feeding the liver and kidneys of infected hamsters to the dogs. As organisms grown in vitro were used in preceding experiments without producing severe renal lesions, leptospirae were cultured in hamsters in this instance to find whether such organisms would show greater pathogenicity.

Twelve hamsters aged 9 weeks were each injected with L. canicola culture on the first day of the experiment. An intra-peritoneal dose of 0.5 ml. culture was given to each hamster at 4 p.m. The following morning, 5 hamsters were dead and the other 7 moribund. The dead animals were discarded and the others destroyed by placing them in jars containing trichlorethylene-soaked cotton wool. The livers and kidneys were then removed, chopped and mixed. Examination of a smear of this mixture by dark ground microscopy showed the presence of large numbers of motile leptospirae.

The infected tissues were then fed to the dogs in approximately equal amounts. This was done by opening the dogs' mouths and pouring the mixture to the back of the throat.

Results.

During the following 6 weeks, the dogs showed no clinical abnormality and appeared to be perfectly healthy. On the 14th and 28th days after infection, blood samples were taken for serological examination. The results were as follows:

Titre to L. canicola.

Dog	Day 14	Day 28
22673	1: 10	1: 10
22674	1: 300	1: 100
22675	1: 300	1: 100
22676	1: 300	1: 300
22677	1: 100	1: 10

The dogs were destroyed six weeks after infection. On post-mortem examination, visible lesions were present only in the kidneys of 22676. The surface of the cortices were uneven with many tiny white pits of pin-head size. These minute foci were also present more deeply in the cortices. Histologically, these were areas of interstitial nephritis with plasma cell and lymphocyte reaction. There were no microscopic lesions in the kidneys of the other dogs.

Discussion.

Dogs infected orally with L. canicola developed specific serum agglutinins, indicating that infection was successfully established by this route.

Focal interstitial nephritis developed in only one animal in the group and it is of interest that no lesions were found in the other dogs although the serological titres were positive. Apparently the organisms persisted/

persisted in the dogs without demonstrable renal reaction.

In this series of experiments, dogs have been infected by intra-renal, intra-peritoneal, intra-venous, intra-nasal and oral routes. With each method, a serological response occurred and a proportion of the dogs developed small renal foci of interstitial reaction. It seems certain, therefore, that the route of infection has little importance in determining the subsequent course of the disease. Provided that the organisms reach the blood stream by any means, the results of infection are similar.

No apparent difference in pathogenicity resulted from growing the leptospirae in hamsters instead of artificial medium.

The older dogs used in the experiment did not show evidence of liver disease.

Experiment 9.

Several pathological features of leptospiral nephritis suggest that an immunological mechanism might play a part in the aetiology of some of the characteristic lesions.

In the acute phase of nephritis, lymphocytes and plasma cells accumulate in the kidneys to form an extremely dense infiltrate, in which polymorphonuclear leucocytes are almost entirely absent. This cellular pattern indicates that an intense antigen-antibody reaction ensues, which may in itself be responsible for much of the renal damage. There is thought to be a time interval of 2 - 3 weeks between initial infection and the development of an extensive cellular response in the kidneys. During this time, hypersensitivity or hyperimmunity to leptospiral antigen could build up. Serum agglutinins rise very sharply during this phase.

In sub-acute and chronic nephritis, a large proportion of the glomeruli become altered, with deposition of plasmatic material within the capillary loops. In Section II, it was found that this change is associated with hypertension. However, the nature of the lesion suggests that it could have an immunological basis, as Lendrum (1963) has shown that plasmatic vasculosis has a similar appearance in hypertension and certain collagen diseases in the human.

In chronic nephritis, a frequent complication is endocardosis of the/

the atrio-ventricular valves of the heart. This condition, in which fibro-elastic proliferation with excessive ground substance deposition occurs in the valves, has some resemblence to chronic rheumatoid valvular disease in humans. The lesion could result from a local antigen-antibody reaction.

An experiment was designed in which both hypersensitivity and hyperimmunity to L. canicola were induced in dogs by exposing them repeatedly to the organism. In this way, hypersensitivity was induced in the early part of the experiment, with subsequent hyperimmunisation. The dogs were examined for any renal or cardiac effects.

Method.

Five dogs, Nos. 23248 - 23252, aged four months were used. A cardiovascular examination was carried out on each dog before the start of the experiment. This included electro-cardiographic and phonocardiographic recordings in addition to auscultation of the heart, palpation of femoral pulse and observation of mucosal colour and venous distension. There were no abnormalities in any of the dogs.

Repeated exposure to L. canicola was carried out by infecting the dogs every week for 12 weeks. On each occasion, 1 ml. of L. canicola culture was injected intra-venously.

Every week before re-infection, the dogs were thoroughly examined and recordings taken as described. Blood samples were taken for serological/

serological examination each week for 3 weeks, then fortnightly for the rest of the experiment.

Results.

After the second infection, three of the dogs showed transient anaphylaxis (Nos. 23248, 49 and 51). These dogs collapsed about five minutes after the injection, with very fast weak pulses and shallow respiration. They recovered rapidly after a few minutes.

After the third infection, 23251 and 52 showed anaphylaxis of milder degree. After fourth infection, only 23252 became mildly anaphylactic. Thereafter, the infection had no immediate effect on any of the dogs.

Weekly infection and examination were repeated for 12 weeks. The dogs were kept for a further 8 weeks, during which they were examined each week and fortnightly blood sampling was continued.

During the experiment, the dogs remained clinically normal and no cardio-vascular abnormalities were detected. The serological results are tabulated overleaf.

Serological Titres to L. canicola.

Week of Expt.	23248	23249	23250	23251	23252
1	- ve	- ve	- ve	- ve	- ve
2	1: 100	1: 300	1: 3,000	1: 10	1: 30
3	1: 3,000	>1:30,000	>1:30,000	1: 300	1: 3,000
5	1: 300	1: 3,000	1: 3,000	1: 1,000	1: 3,000
7	1:10,000	1:30,000	1:10,000	1:10,000	1:30,000
9	1: 3,000	1: 300	1: 1,000	1: 300	1: 1,000
11	1:10,000	1: 1,000	1:10,000	1: 1,000	1:10,000
13 (last infection)	1:30,000	1: 300	1: 300	1: 300	1: 1,000
15	1: 100	1: 100	1: 30	1: 100	1: 100
17	1: 10	1: 100	1: 10	1: 10	1: 30
19	- ve	1: 30	- ve	- ve	- ve
21	- ve	1: 10	- ve	- ve	- ve

The dogs were destroyed 5 months after initial infection. On post-mortem examination, the kidneys of each dog contained numerous white foci of 1 - 2 mm. diameter scattered throughout the cortices. There were no cardiac or other lesions. Histologically, the kidneys showed mild interstitial nephritis consisting of foci of lymphocyte and/

and plasma cell reaction with localised destruction of tubules. (Figs. 85 & 86). There were no abnormalities elsewhere.

Discussion.

In this experiment, dogs repeatedly exposed to L. canicola showed transient anaphylactic reactions but did not develop severe renal lesions or any cardio vascular abnormalities.

The most interesting feature of this experiment was the pattern of the serological response to repeated infection. After rising rapidly to maximum levels at about the third week, the titres then fluctuated for several weeks although infection was continued. The titres of 23249 - 52 all showed a marked fall between the 11th and 13th weeks, that is before infection was discontinued. This demonstrates a sharp decrease in antibody production despite continued antigenic stimulation. When infection was stopped, the titres of all of the dogs decreased with remarkable rapidity, becoming negative in four of the dogs six weeks after the last infection.

Dixon et al (1952) found the half-life of homologous gamma-globulin in dogs to be 8 ± 1.1. days. Therefore a cessation of antibody production is followed by a very rapid fall in circulating levels. The serological results of this experiment indicate that production stopped before the dogs were infected for the last time, thus demonstrating immune paralysis. not necessarily

It is of interest that foci of cellular reaction persisted in the kidneys in the absence of demonstrable serum agglutinins.

Experiment 10.

In this experiment, the isolation of a fresh strain of L. canicola from a dog suffering from acute nephritis and the subsequent infection of dogs with this strain will be described. Earlier unsuccessful attempts to isolate the organism will be mentioned briefly.

During the time in which the experimental work was carried out, urine samples were examined for the presence of leptospirae from every dog admitted to the hospital suffering from nephritis. It was essential to infect dogs with newly isolated organisms to establish whether previous experimental infections failed to cause severe nephritis because of attenuated virulence in cultured organisms.

Attempts 1 and 2.

On two occasions, dogs were admitted to the hospital in the terminal stages of acute nephritis. Being moribund, these dogs were destroyed and the kidneys removed aseptically. One kidney was then used to culture L. canicola and the other used immediately to infect young hamsters and pups. In the latter procedure, renal cortical tissue was homogenized with sterile sand and saline, then injected intra-peritoneally into the hamsters and pups. In both of these attempts, the hamsters and pups did not develop any clinical, serological or post-mortem evidence of leptospirosis and kidney culture was unsuccessful.

These attempts may have failed because of the very high levels of antibody/

antibody in the kidneys of dogs dying in the acute stage. Rudge (1958) stated that the efficiency of the animal inoculation test for demonstration of viable leptospirae in kidney tissue may be seriously reduced if the specimen is obtained from an animal with a very high titre. The organisms may be inactivated by exposure to antibody during preparation of the tissue suspension used as an inoculum.

Attempt 3.

On another occasion, a dog suffering from acute nephritis without complete renal failure was found to have leptospiruria. Urine was taken from this dog through a sterile catheter into sterile bottles. Some of the urine was used to culture L. canicola and the remainder injected intra-peritoneally into hamsters and pups. Again, the infected animals did not show evidence of leptospirosis and urine culture was unsuccessful. It was suggested by Dr. S.W. Michna that this attempt may have failed because the glassware used to hold the urine samples was washed in detergent before sterilization and any trace of detergent would be rapidly toxic to leptospirae. This possibility was tested on the following occasion.

Attempt 4.

The fourth attempt to isolate L. canicola was successful. A dog suffering from non-fatal acute nephritis was found to be excreting leptospirae. Urine was collected through a sterile catheter into two sets of bottles, one set of which had been detergent-washed before sterilization while/

while the other was boiled in buffered saline, pH 7.2. Half an hour then elapsed before media could be inoculated for culture. At the end of this time, the leptospirae in the detergent-washed bottles were non-motile while those in the other bottles were still actively motile. Leptospirae were successfully cultured from the motile samples, both directly and by isolation from an infected guinea pig.

Urine was also injected directly into 4 pups intra-peritoneally. The pups were 6 - 8 weeks old (Nos. 24478/7 - 10).

Results.

Seven days after infection, 24478/7 died, showing liver necrosis and widespread petechial haemorrhages. At this time the other pups showed malaise and anorexia for 3 days and one developed keratitis. Contagious canine hepatitis was suspected, but no evidence of this was found on serological examination of blood samples from the pups, or later by the use of liver as antigen in the complement-fixation test. Histologically, no inclusion bodies were found in the livers.

Blood samples for serological examination were taken 14 and 28 days after infection. The results were as follows:

	Titre to L. canicola.	
Dog	Day 14	Day 28
24478/8	- ve	1: 30
24478/9	- ve	1:1000
24478/10	- ve	1: 10

The pups showed no further abnormalities after the brief illness described. They were destroyed 6 weeks after infection. One kidney from each was used to culture L. canicola and the liver used as antigen for the contagious hepatitis complement fixation test.

L. canicola was cultured from the kidneys of 24478/8 and 9. Tests for virus hepatitis were negative.

On post-mortem examination, the kidneys of 24478/9 contained several small white cortical foci. Histologically, these were areas of interstitial nephritis with lymphocyte and plasma cell reaction. There were no macroscopic or histological lesions in 24478/8 and 10.

Discussion.

The tests in which leptospirae were collected into detergent-washed and non-detergent washed bottles indicate that the organisms are extremely susceptible to the most minute residue in the glassware.

The acute illness which occurred in the pups one week after infection, killing one pup, resembled virus hepatitis. In the absence of inclusion bodies and serological evidence of Rubarth's virus, it seems probable that this was a further example of acute liver disease in young pups caused by L. canicola.

Serum agglutinins were not present in the pups two weeks after infection, although they were detected after four weeks. This was an unusually delayed response.

The culture of L. canicola from the kidneys of 24478/8 is of interest, as no renal lesions were detected in this dog.

The culture from the kidneys of 24478/9 was used in the next experiment.

Experiment 11.

L. canicola, freshly isolated from a nephritic dog, was passaged through dogs in Experiment 10, then re-isolated and cultured. This culture was used 2 weeks later to infect 4 more pups. The results of the previous experiment were unsatisfactory in that the dogs did not develop nephritis and a liver disease of uncertain origin occurred. A further trial was required to establish whether dogs would be resistant to infection with a fully virulent strain of L. canicola.

Method.

Five dogs were used in this experiment (Nos. 24478/11 - 15). They were 10 weeks old at the time of infection. Four of the dogs were infected (24478/11-14) and the fifth (24478/15) was kept in the same pen to find whether transfer of infection would occur.

The organisms used were from the first sub-culture grown from the kidney of 24478/9 in Experiment 10. The dogs were infected by intra-peritoneal injection of 1 cc. of this culture.

The temperatures of the dogs were recorded before infection and once daily for the next 10 days.

Results.

A pyrexic response developed in the dogs three days after infection and this persisted for three days. The temperatures over the pyrexic period are tabulated overleaf.

Temperatures of the Infected Dogs.

Post-infection		24478/11	24478/12	24478/13	24478/14.
Day	2	102°	102.1°	101.5°	102°
	3	102.4°	104°	102°	103°
	4	102.4°	103.4°	102°	101.8°
	5	102.8°	103.2°	101.6°	101.5°
	6	101.4°	102°	101.6°	101.7°
	7	101.4°	101.8°	101.5°	101.5°

The dogs showed slight malaise on the 3rd - 5th days after infection. This was most pronounced in 24478/12, which was also partially anorexic at this time.

The dogs recovered and showed no further abnormality during the next 2 months, with the exception of 24478/11, which failed to grow as well as the others and was very thin.

The serological titres to L. canicola during the experiment are tabulated below.

Serological Titres to L. canicola.

Post-infection week.	24478/11	24478/12	24478/13	24478/14	24478/15.
2	1: 3,000	1:10,000	- ve	- ve	
3	1: 3,000	1:30,000	- ve	- ve	
4	1: 3,000	>1:30,000	- ve	- ve	1: 100
5	1:10,000	>1:30,000	- ve	1: 10	1: 1,000
6	>1:30,000	>1:30,000	1: 10	- ve	1: 300
8	1:30,000	>1:30,000	- ve	- ve	1: 100
10	1: 1,000	>1:30,000	- ve	- ve	1: 10
12		1: 1,000	- ve	- ve	- ve

The development of a positive titre in 24478/15 is of interest, indicating that cross-infection took place.

On the 72nd day, dog no. 24478/11 died suddenly. At post-mortem examination, the liver was found to be pale and enlarged and the kidneys contained numerous white foci of 2 - 3 mm. diameter scattered throughout the cortices, especially around the cortico-medullary junction. The thymus was oedematous and petechiated. Histologically, the liver showed a moderate degree of fatty change. (Fig.87). Interstitial nephritis of moderate severity affected the kidneys, in which fairly numerous areas of plasma cell and lymphocyte accumulation were present. (Fig.88). In addition, there were several radial bands of chronic reaction with well developed fibrosis. (Fig.89). Serological and histological examinations for virus hepatitis proved negative.

During the next month, the remaining dogs showed no clinical abnormality and were destroyed three months after infection.

On post-mortem examination, very well developed macroscopic renal lesions were present in dog 24478/12. The kidneys were grossly swollen and very pale. The capsules were partially adherent and the cortical surfaces rough and nodular. On section, the tissue was abnormally firm and very numerous white nodular foci were present throughout the cortices. These foci were up to 3 mm. in diameter. Narrow radial bands of fibrous tissue were also apparent in the cortices. The macroscopic appearance of

a /

a kidney from the dog is shown in Fig. 90. The difference in size between this kidney and that of 24478/15 is shown in Fig.91. (All of the dogs in this group were of the same age and size.)

Because of the highly significant renal changes in this dog, the heart blood was taken for biochemical estimation of serum urea. The result was as follows:

Serum urea = 150 mg/100 ml.

The kidneys of dogs 24478/13, 14 and 15 were macroscopically normal.

Histologically, the kidneys of 24478/12 showed severe changes characteristic of sub-acute interstitial nephritis. The cellular reaction was nodular in pattern, with large dense areas of plasma cell and lymphocyte accumulation scattered throughout the cortices. Smaller foci were also present in the medulla. In the centre of some of the larger reactive areas, polymorphonuclear leucocytes were present in considerable numbers. Numerous radial bands of fibrosis were also present in the cortices and in these areas the glomeruli showed varying degrees of fibrosis of the capillary tuft. The arteries and arterioles were normal. The lesions are illustrated in Figs. 92, 93 and 94.

L. canicola was successfully cultured from a kidney of this dog and the re-isolated organism was used in the next experiment.

No microscopic abnormalities were found in the kidneys of 24478/13 and 14. Mild interstitial nephritis was present in the kidneys of 24478/15, seen/

seen as small foci of plasma cell and lymphocyte reaction situated in the juxta-medullary region of the cortices and in the outer medulla. (Fig. 95).

Discussion.

The development of severe sub-acute interstitial nephritis in one dog in this experiment is of very considerable significance. This is the first time that the disease has been reproduced in a severe form with retention of non-protein nitrogen in the blood. The fact that only one dog in the group of four showed these features may be due to individual variations in susceptibility among the city-bred pups.

It is of interest that the sub-acute lesions were present three months after infection and that the serological titre had fallen at this time to levels which are generally associated with the sub-acute phase of the disease in field cases.

The results of this experiment suggest that the virulence of the organism is of major importance in determining the outcome of an infection. Out of the group of five dogs, one died of liver failure and showed moderately severe renal lesions and one developed severe nephritis with renal failure.

Unfortunately, as dog 24478/12 showed no clinical symptoms, the blood biochemistry was not followed throughout the experiment.

The transient serological response and small renal lesions in the cross-infected dog, 24478/15, suggest that infection by "natural" means has little influence on the severity of the disease process.

Experiment 12.

The recently isolated strain of L. canicola was twice passaged through dogs, in experiments 10 and 11. On the second occasion, one infected dog developed severe sub-acute interstitial nephritis with uraemia and another died of liver failure, with moderately severe sub-acute nephritis. This result suggested that the field strain of L. canicola was considerably more virulent than organisms maintained in prolonged culture and that passage through dogs may have enhanced the virulence still further. The organism was therefore re-isolated, cultured and used to infect another series of dogs to ascertain the significance of these observations.

For this experiment, a litter of farm-bred collie pups was obtained. It was hoped that such animals, bred and reared out of any contact with city dogs, would have no maternally transferred immunity to L. canicola. As discussed earlier, the endemic nature of the infection among city dogs suggests that many pups may have some resistance to the potential effects of L. canicola.

Method.

Four farm collie pups were used in this experiment (Nos. 26238, 26239, 26240 and 26241). They were three months old at the time of infection and had negative serological titres to L. canicola and L. icterohaemorrhagiae.

The organisms were from the first sub-culture grown from a kidney of dog 24478/12 in Experiment 11. The dogs were infected by intra-peritoneal injection of 1 cc. of this culture.

The temperatures of the dogs were recorded before infection and once daily for the following ten days. Blood samples were taken from each dog for serological and biochemical examination once every week throughout the experiment. Urine samples were examined towards the end of the experiment.

Results.

The dogs showed a pyrexic response from the 2nd - 6th days after infection. The temperatures over this period are shown in Table I.

TABLE I.

Temperatures of the Infected Dogs.

Post-infection Day	26238	26239	26240	26241
1	101.7°	101.5°	101.8°	101.6°
2	102°	102.2°	102.4°	101.8°
3	103°	103.1°	103.4°	102.4°
4	103.5°	103.6°	103.8°	103.5°
5	103.1°	102.8°	103.5°	103.5°
6	102.6°	102.5°	102.4°	102.8°
7	101.8°	101.7°	101.9°	101.8°

The dogs showed mild malaise from the 3rd - 5th days, though none became anorexic.

No further clinical abnormality was detected in the dogs during the next two months. From the 8th - 10th weeks, dog 26238 became appreciably anaemic.

The serological titres to L. canicola during the experiment are shown in Table II.

TABLE II.

Serological Titres to L. canicola.

Post-infection Week.	26238	26239	26240	26241
1	1:3000	>1:30,000	1: 1,000	>1:30,000
2	1:300	>1:30,000	1: 3,000	1:10,000
3	1:3000	1:10,000	1:1, 000	>1:30,000
4	1:300	1: 1,000	1: 1,000	1: 1,000
5	1:300	1: 3,000	1: 300	1: 3,000
6	1:300	1: 3,000	1: 300	1: 3,000
7	1:300	1: 3,000	1: 1,000	1: 1,000
8	1:100	1: 3,000	1: 300	1: 3,000
9	1:300	1: 3,000	1: 3,000	1: 1,000
10	1:100	1: 3,000	1: 3,000	1: 300
11		1: 1,000	1:10,000	1: 300
12		1: 300	1: 3,000	1: 1,000
13		1: 300	1: 300	1: 300
14		1: 100	1: 300	1: 300

All of the dogs developed high titres, which attained levels of > 1:30,000 in 26239 and 26241. In the latter part of the experiment, the titres fell to low levels.

Although there were no significant clinical findings during the three months following infection, the weekly blood biochemical examinations revealed interesting abnormalities. As the observations varied in the individual animals, the biochemical results are tabulated in full (see Table III). In addition to the recorded findings, estimations of bilirubin, sodium potassium and chloride were also carried out but these are omitted from the tables as no abnormal levels were found.

TABLE III.

Blood Biochemistry : Dog 26238.

Post-infection Week	Urea (mg/100 ml)	Inorganic phosphate (mg/100 ml)	Protein (gm/100 ml)	Albumin/ Globulin ratio	Alkaline phosphatase (SLB. units)	S.G.O.T. (S.F. units)	S.G.P.T. (S.F. units)
2	43	7.6	5.5	1.3	4.9	53	40
3	32.3	8.5	-	-	-	-	-
4	32	7.3	5.8	1.1	4.2	38	76
5	29	6.8	3.9	0.6	3.8	20	22
6	31	6.3	5.6	0.7	3.7	107	42
7	40	7.7	4.9	0.7	5.4	56	44
8	40	7.0	5.7	1.0	2.7	58	48
9	37	9.0	5.3	1.1	2.3	37	45
10	54	7.9	4.7	1.3	1.3	104	76

Blood Biochemistry : Dog 26239.

Post-infection Week.	Urea (mg/100 ml)	Inorganic phosphate (mg/100 ml)	Protein (gm/100 ml)	Albumin/ Globulin ratio	Alkaline phosphatase (BLB. units)	S.G.O.T. (S.F.units)	S.G.P.T. (S.F.units)
2	43	6.4	5.8	0.8	3.2	53	22
3	46.5	6.8	-	-	-	-	-
4	42	-	6.0	0.9	-	-	-
5	30	7.5	6.1	0.6	3.5	10	10
6	28	3.6	6.8	0.7	8.7	30	13
7	40	7.1	5.7	0.7	3.7	38	14
8	28	6.0	6.1	0.8	3.7	23	11
9	35	9.7	6.9	1.2	4.2	16	16
10	33	8.1	8.1	1.1	3.8	33	26
11	40	8.7	5.5	1.0	3.6	44	24
12	28	7.1	5.1	1.5	3.1	10	0
13	34	7	6.2	1.0	3.2	32	70
14	26	6.1	6.4	0.9	5.8	43	15

Blood Biochemistry : Dog 26240.

Post-infection Week	Urea (mg/100 ml)	Inorganic phosphate (mg/100 ml)	Protein (gm/100 ml)	Albumin/ Globulin ratio	Alkaline phosphatase (BLB units)	S.G.O.T. (S.F. units)	S.G.P.T. (S.F. units)
2	54	8.3	5.8	1.5	3.6	76	15
3	34.5	9.0	-	-	-	-	-
4	30	7.0	6.2	1.4	4.6	63	27
5	31	6.0	6.8	0.7	3.2	32	8
6	33	7.2	6.1	0.8	3.9	50	27
7							
8	37	5.8	6.6	1.0	3.2	74	28
9	41	8.6	6.9	1.2	3.3	65	32
10	52	3.2	8.3	1.3	4.6	154	180
11	56	5.8	5.2	0.9	1.7	67	57
12	32	7.4	5.6	1.1	4.5	42	>115
13	40	7.4	5.6	1.1	1.4	50	15
14	34	9.1	6.3	1.0	2.6	28	19

Blood Biochemistry : Dog 26241

Post-infection Week	Urea (mg/100 ml)	Inorganic phosphate (mg/100 ml)	Protein (gm/100 ml)	Albumin/ Globulin ratio	Alkaline phosphatase (BLB units)	S.G.O.T. (S.F. units)	S.G.P.T. (S.F. units)
2	56	8.1	5.4	1.5	7.0	56	33
3	35.6	8.6	-	-	-	-	-
4	32	7.5	6.0	1.3	4.3	34	30
5	37	6.7	6.0	0.8	4.5	35	32
6	41	6.5	5.6	0.9	4.2	40	20
7	50	7.5	4.9	0.7	5.2	26	20
8	43	5.5	4.9	0.8	5	25	19
9	43	10.6	6.3	1.0	4.8	21	10
10	37	5.9	8.3	1.0	3.9	39	28
11	44	8.5	5.1	0.8	4.3	55	43
12	35	6.4	5.4	1.5	1.7	16	11
13	38	7.5	5.5	1.3	1.9	26	11
14	48	8.5	5.4	1.0	4.4	43	22

These results indicate significant liver damage in all of the dogs and mild renal dysfunction in two.

Dog 26238 showed an increase in serum transaminase levels throughout the experiment, with the exception of the 5th week. The protein level was consistently low, especially in the 5th, 7th and 10th weeks. Evidence of impaired renal function was shown by a slight rise in blood urea in the 10th week.

Dog 26239 had raised S.G.O.T. levels in the 2nd, 11th and 14th weeks, suggesting liver damage.

Dog 26240 showed almost persistantly raised serum transaminase levels, which were extremely high in the 10th and 12th weeks. Blood urea was increased in the 10th and 11th weeks.

Dog 26241 showed a moderate increase in transaminase levels in the 2nd, 11th and 14th weeks.

As dog 26238 became noticeably anaemic, blood samples were taken for haematological examination in the 9th and 10th weeks. The results are shown below.

Haematology : Dog 26238.

	9th week.	10th week.
B.S.R. (mm. in 1 hr.)	-	-
P.C.V. (ml/100 ml.)	26	24.5
Hb (g/100 ml.)	10.1	9.6
R.B.C. (m/c mm.)	4.3	4.0
W.B.C. (th/c mm.)	16.800	14.800
Neutrophils %	79.5	72.5
Eosinophils %	1.0	0
Lymphocytes %	19.5	17.5

Severe anaemia is shown in these results. The packed cell volumes, haemoglobin levels and red cell counts were all very low.

This dog died suddenly during the 11th week. On post-mortem examination, the only abnormality was slight paleness of the liver. Histologically, the liver showed separation of parenchymal cells with mild degenerative change. The kidneys contained fairly numerous small foci of interstitial nephritis, mainly situated around the cortico-medullary junction. It would appear that the dog died of combined liver failure and anaemia.

Samples of urine were examined from the three remaining dogs in the 12th week. No leptospirae were detected in any sample. Urological abnormalities were absent, except in dog 26241. The urine of this dog contained 100 mg. protein/100 ml.

As the dogs showed no evidence of progressive renal damage and the serological titres fell to low levels, they were destroyed 14 weeks after infection.

On post-mortem examination no abnormalities were apparent in the liver or kidneys of any of the dogs. 26241 showed pneumonia of proliferative type, affecting small areas of the apical and cardiac lobes. There were no lesions in other organs.

Histologically, the kidney of 26239 contained numerous small foci of mild interstitial nephritis scattered throughout the cortex. (Fig.96.). The/

The liver was normal. Focal lesions of interstitial nephritis were also found in the kidney of 26240, affecting all levels of the cortex. In this dog, the liver was moderately congested and centrilobular cells showed mild fatty change. The kidney of 26241 contained many small foci of interstitial nephritis in the cortex. The liver was moderately congested and numerous small accumulations of lymphocytes were scattered among the cords of parenchymal cells. (Fig.97). Contagious hepatitis inclusion bodies were not found in the liver of any of the dogs.

Samples of liver from each dog were tested for the presence of contagious hepatitis virus. The complement-fixation test was negative in each case.

One kidney from each dog was removed aseptically for L. canicola culture. The organisms were successfully isolated from all three kidneys.

Discussion.

In this experiment, infection of country-bred dogs with freshly isolated leptospirae was followed by pronounced pyrexia, sharp serological response and biochemical indication of liver damage and mild renal dysfunction. However, renal lesions were restricted to small foci of interstitial nephritis similar to those produced in most of the preceding experiments. This result suggests that the dogs used on this occasion were no more susceptible to infection than city pups. The renal changes in this experiment and the more severe lesions encountered in Experiment 11/

11 indicate that considerable individual differences in susceptibility to L. canicola occur irrespective of the habitat of the dogs.

It seems unlikely that the virulence of the organisms could have become appreciably attenuated since the previous experiment. However, the effect of prolonged exposure to antibody in the kidney before re-isolation is unknown.

Biochemical evidence of liver damage was an interesting aspect of the experiment, providing yet another example of liver disease in pups infected with L. canicola. Microscopic changes were found in two dogs which had shown the most pronounced biochemical abnormalities. The presence of groups of lymphocytes in the liver of 26241 suggests a local reaction to L. canicola.

In the absence of extensive renal lesions, the significance of the slight rise in blood urea levels in two of the dogs and the proteinuria found in a third is uncertain. Possibly a more severe inflammatory reaction had resolved slightly by the time the dogs were destroyed.

In conclusion, the failure to produce severe nephritis in country-bred dogs infected with a field strain of L. canicola, compared with the noteably varied response in the previous experiment, demonstrates that wide differences in susceptibility must exist in individual dogs, regardless of habitat. It seems that maternally-transferred immunity is unlikely to be important in dogs over 2 - 3 months of age. Unknown factors must operate to render a proportion of infected dogs particularly susceptible/

susceptible to L. canicola. Serological surveys in Glasgow showed that infection is very common in the dog population, but not all dogs with positive titres suffer from clinical nephritis. Any other immune mechanisms operating in this disease are unknown.

Discussion on Section III.

The series of experiments which have been described in this section were carried out in an attempt to reproduce severe interstitial nephritis in dogs. Various methods designed to increase the susceptibility of healthy animals to L. canicola were investigated and differences in virulence of cultured, passaged and freshly isolated organisms were studied.

In general, the majority of dogs throughout the experiments responded to infection with transient pyrexia, development of high serological titres and mild interstitial nephritis. The renal lesions were usually small and focal and consisted of the characteristic infiltration of the cortices by plasma cells and lymphocytes, as found on a larger scale in field cases. These changes also closely resembled lesions produced in the experiments of McIntyre (1954) and Wettimuny (1963).

Severe sub-acute nephritis with retention of urea developed in one dog during the series. This was of considerable importance, as it is the first recorded occasion on which progressive renal disease has been shown to follow experimental infection. This encouraging result proves that the disease can be reproduced, though the reasons why one particular animal became severely affected remain uncertain. The dog was one of a group of city pups infected with a field strain of L. canicola which had been passaged once through dogs since initial isolation.

In contrast, a group of farm collie pups did not develop severe nephritis/

nephritis when infected with the reisolated field strain. This result suggested that individual dogs may show wide differences in susceptibility to L. canicola, regardless of habitat. Maternally-transferred immunity may not be an important factor in determining the degree of resistance in dogs over 2 - 3 months of age and any other immune mechanisms operating in this disease are unknown.

A particularly interesting feature of the experiments was the development of liver disease in many of the infected dogs. During the series of experiments, 60 dogs were infected and 12 of these showed evidence of liver disease. In stressed dogs and in two healthy dogs infected with the field strain, the liver failure was fatal. As examinations for contagious hepatitis proved negative and the syndrome resembled Weil's disease, these findings suggest that the liver may be more susceptible to L.canicola than the kidneys in very young dogs. Jaundice was observed in pups infected with L. canicola by Klarenbeek and Winsser (1938) and by Jull and Heath (1963). Mayer, Stewart-Anderson and Eddie (1939), describing the field syndrome in dogs infected with L. canicola as it occurred in California, observed jaundice in 48 of 88 dogs examined post-mortem. The illness in these animals apparently showed close resemblance to Weil's disease. The present results indicate that L. canicola infection should be considered in the differential diagnosis of liver disease in young dogs.

Experiments carried out with a freshly isolated field strain demonstrated/

demonstrated that such organisms are more virulent for dogs than those maintained in prolonged culture. In general, the pyrexic response was more marked, serological titres rose to higher levels and renal lesions were more extensive following infection with freshly isolated organisms. In the final experiment, failure to produce severe nephritis in farm collies may have been due to individual differences in susceptibility or to loss of virulence of the organism as a result of exposure to antibody in the kidney for a long period before re-isolation. It seems probable that different field strains would show different degrees of virulence and it would be of value to isolate several strains and test their effects in dogs.

Any change in virulence of cultured organisms following passage through dogs was difficult to assess. Dogs in both series developed liver disease and one died of meningitis, though none showed severe nephritis.

Experiments in which dogs were stressed before infection by administration of cortisone, methotrexate or mercuric chloride were of interest because of the development of a hepatic syndrome closely resembling Weil's disease. These forms of stress did not increase the renal lesions, though in the mercuric chloride trial, inflammatory foci were more numerous than usual.

During the series of experiments, infection was achieved by intravenous,/

intra-venous, intra-peritoneal, intra-renal arterial, intra-nasal and oral routes. In every case, the renal lesions were small and scattered. A similar result was observed in a dog which became infected by natural transfer through sharing a pen with infected dogs (expt.11). The route of infection therefore seems un-important and provided the organisms reach the blood-stream, infection will become established. The effects of injecting leptospirae into the renal arteries indicated that the number of organisms reaching the kidney is not of prime importance in determining the severity of the subsequent renal reaction. Other factors associated with the virulence of the organism or the susceptibility of the dog must be involved.

Repeated weekly infection of dogs in expt. 9 elicited an interesting serological response. Immune paralysis was demonstrated by a fall in titres before infection was stopped and an abnormally rapid return to negative thereafter.

Attempts to isolate a fresh field strain from dogs suffering from severe acute nephritis illustrated the care which must be taken in preparation of glassware for containing infected urine samples. The failure to isolate from kidney tissue was probably due to inactivation of the organisms as a result of exposure to high concentration of antibody during preparation of the tissue suspension used as an inoculum.

In conclusion, the experimental work has shown that severe interstitial/

interstitial nephritis is reproducible in dogs by infection with L. canicola and that this organism may be a significant cause of liver disease in pups. The difficulties encountered in producing extensive renal lesions in most of the dogs indicated wide individual differences in susceptibility to L. canicola. The virulence of the organism may vary between particular strains and this is probably of considerable importance. However, any predisposing or synergistic factors which operate under natural conditions to produce the severe disease in a proportion of infected dogs remain unknown.

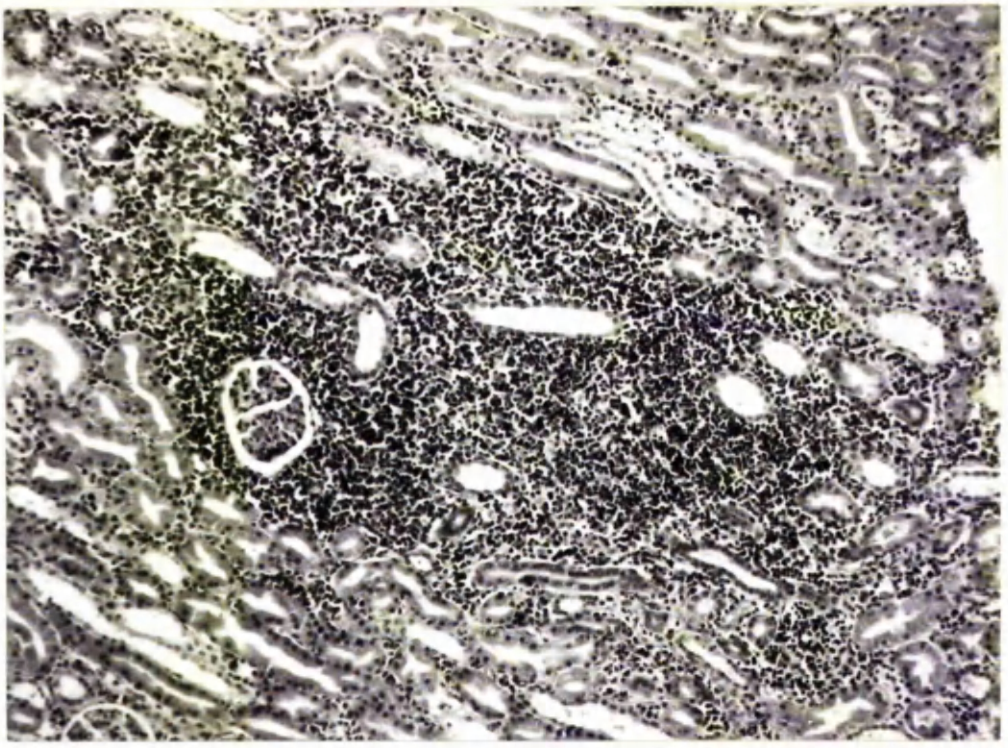


Fig. 73. Expt.1. Dog 21294/3. A focus of interstitial nephritis, showing plasma cell and lymphocyte reaction and localised destruction of tubules. H. & E. X 150.

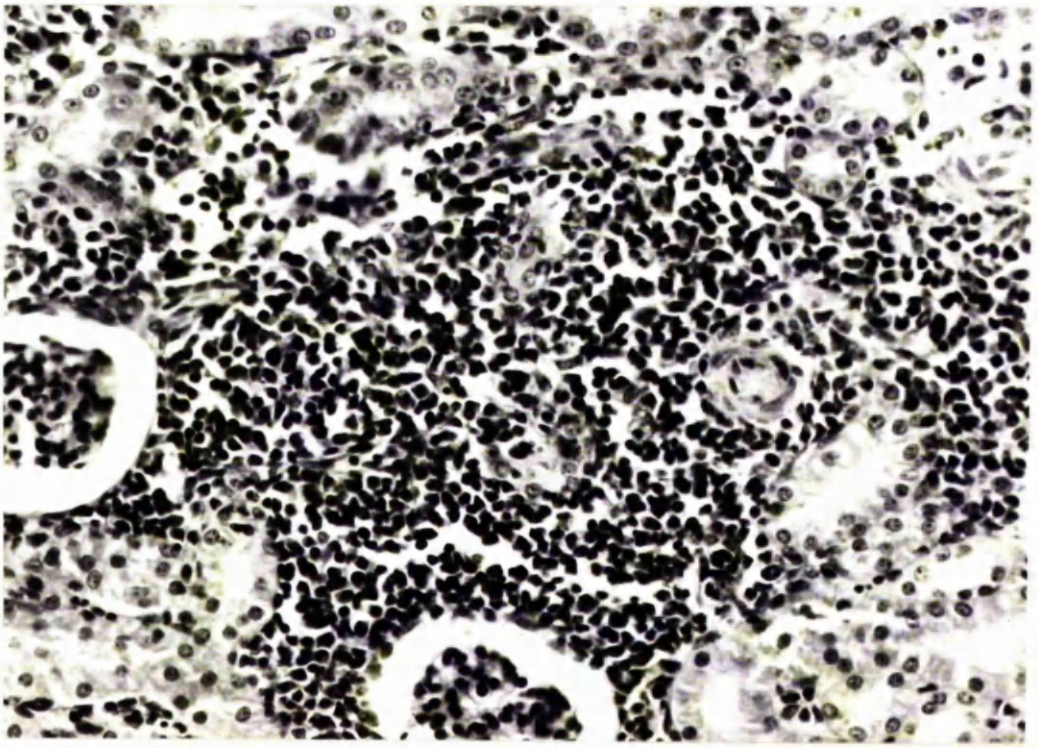


Fig. 74. Expt.1. Dog 21294/4. A small focus of interstitial nephritis. H. & E. X 600.

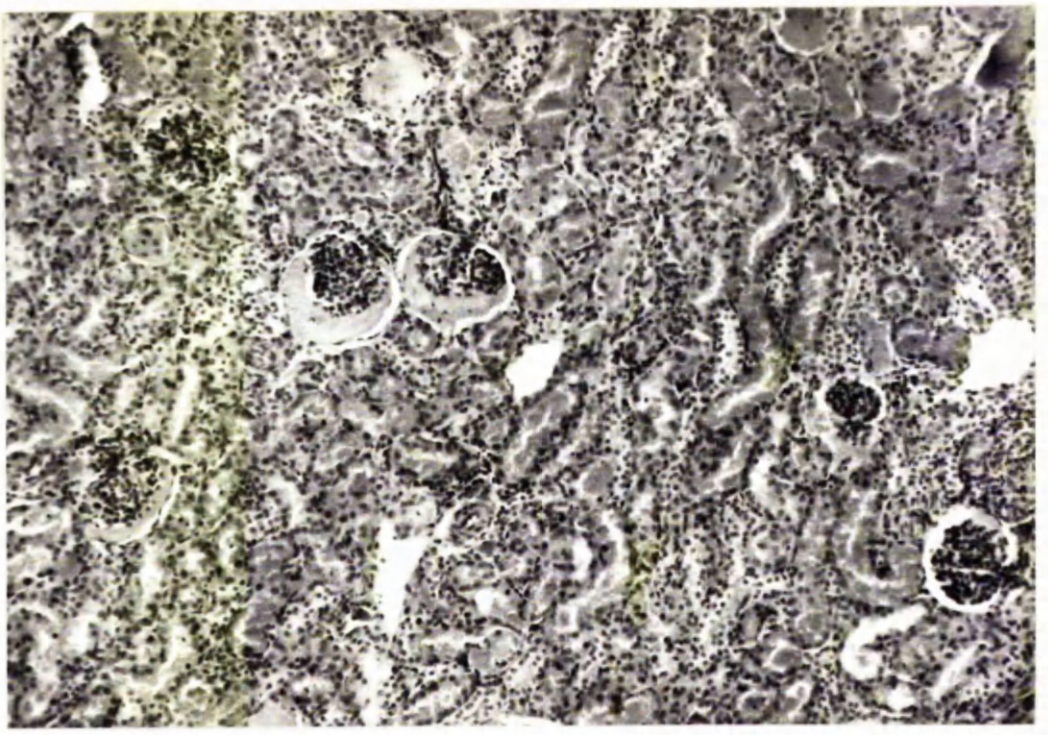


Fig. 75. Expt.2. Dog 21294/20. A section of kidney showing haemorrhage into the glomeruli and tubules. H. & E. X 150.

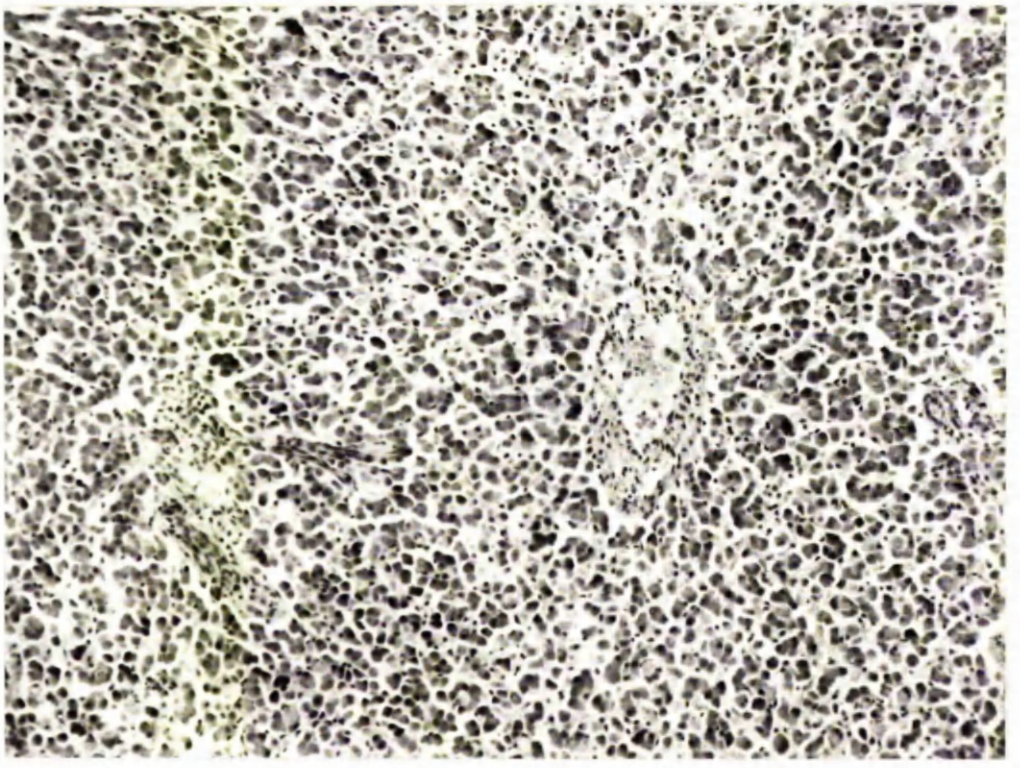


Fig. 76. Expt.2. Dog 21294/20. A section of liver showing separation of parenchymal cells and degenerative change. H. & E. X 150.

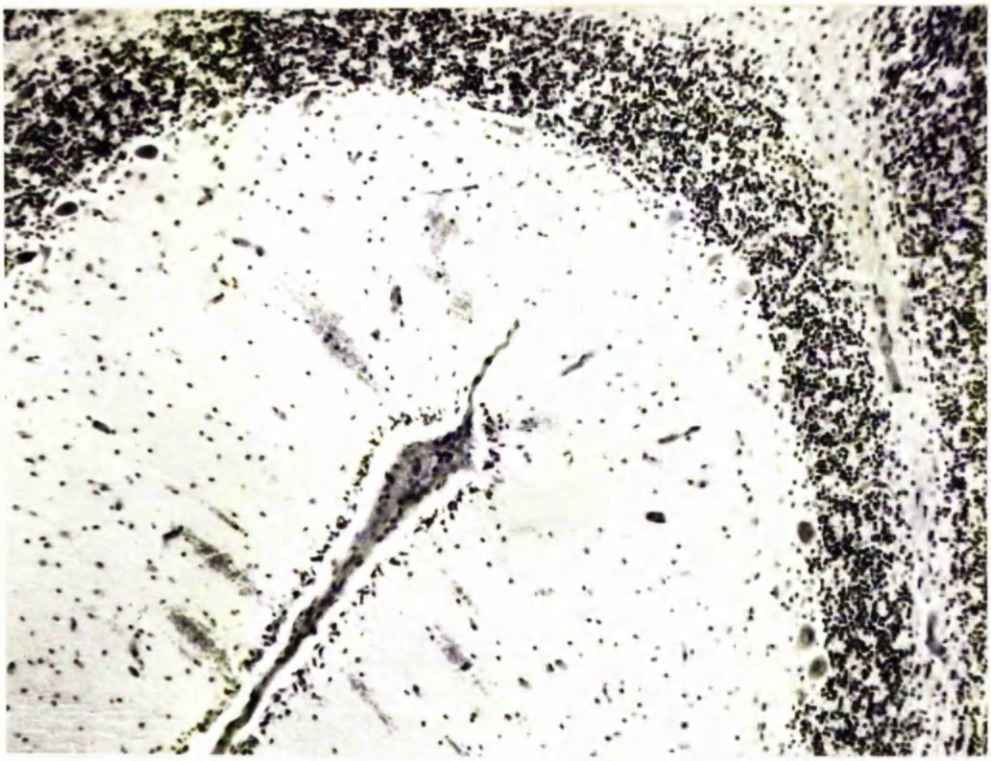


Fig. 77. Expt.2. Dog 21294/20. A section of cerebellum, showing several small haemorrhages in the molecular layer. H. & E. X 150.

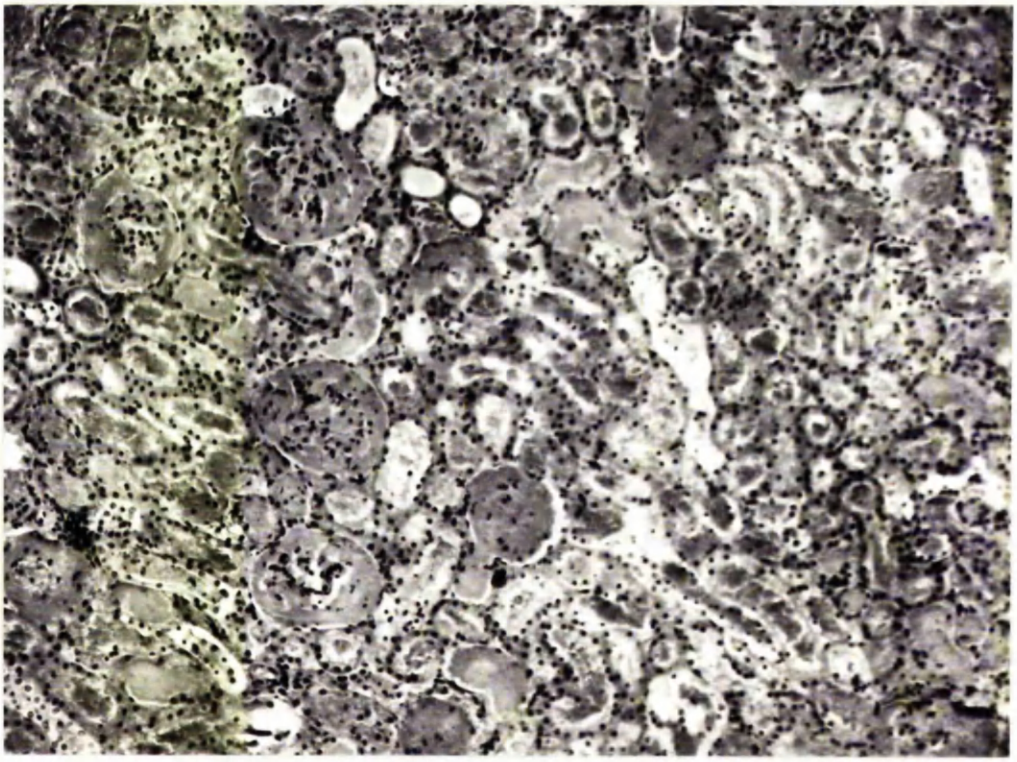


Fig. 78. A section of kidney from a hamster infected with L. canicola, showing haemorrhage into glomeruli and tubules. H. & E. X 150.

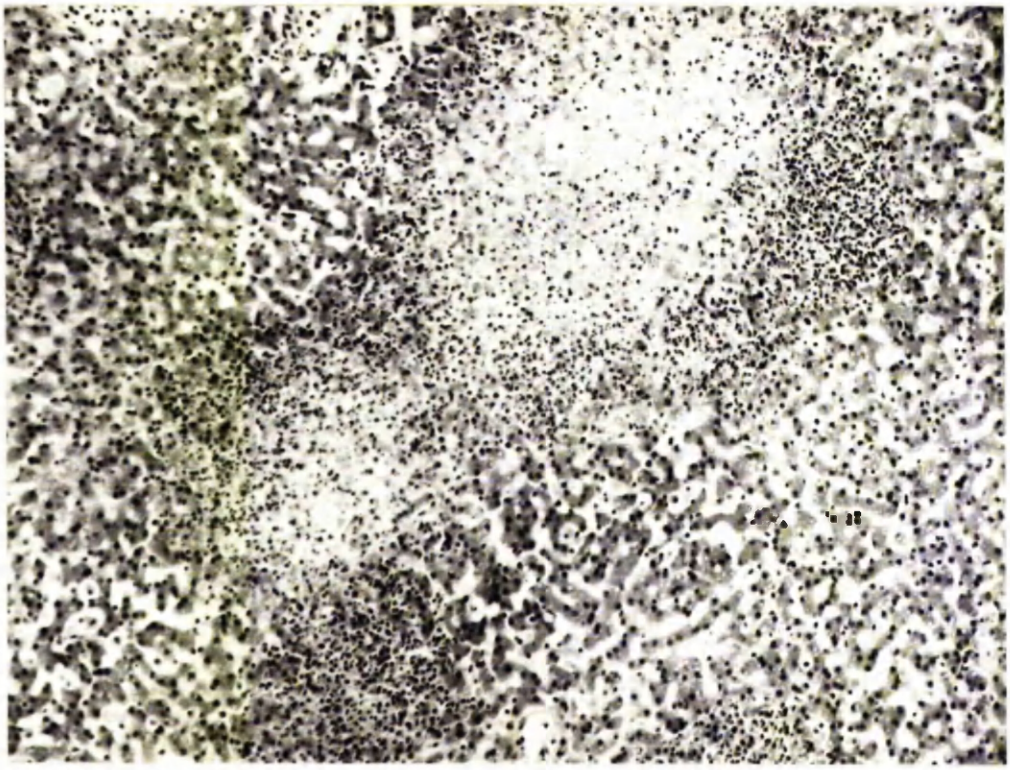


Fig. 79. Expt. 3. Dog 21294/30. Acute focal hepatitis. H. & E. X 150.

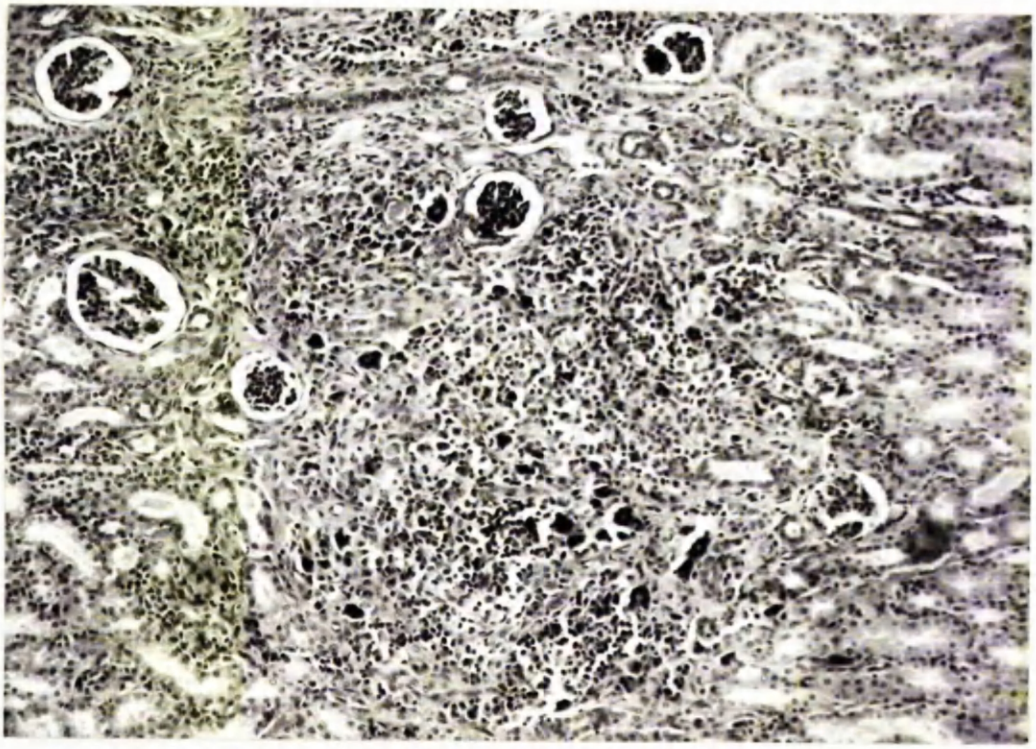


Fig.80. Expt.4. Dog 22662. A focus of interstitial nephritis with giant cells among the plasma cells and lymphocytes. H.& E. X 150.

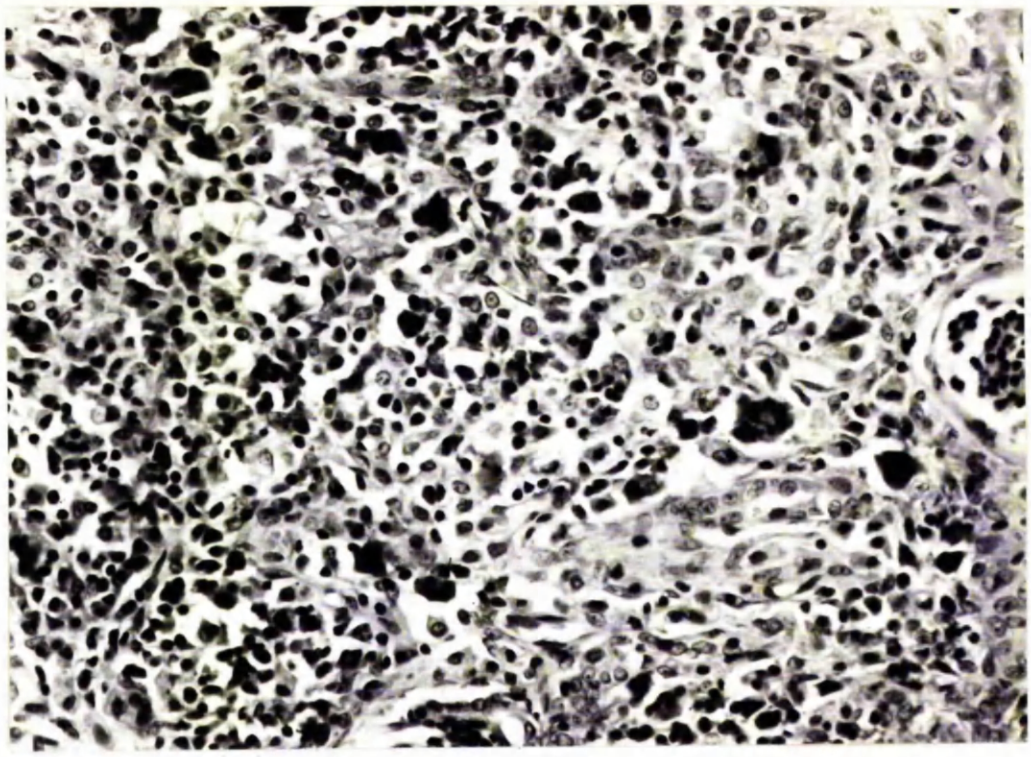


Fig. 81. Expt.4. Dog 22662. Showing giant cells in a focus of interstitial nephritis. H. & E. X 600.

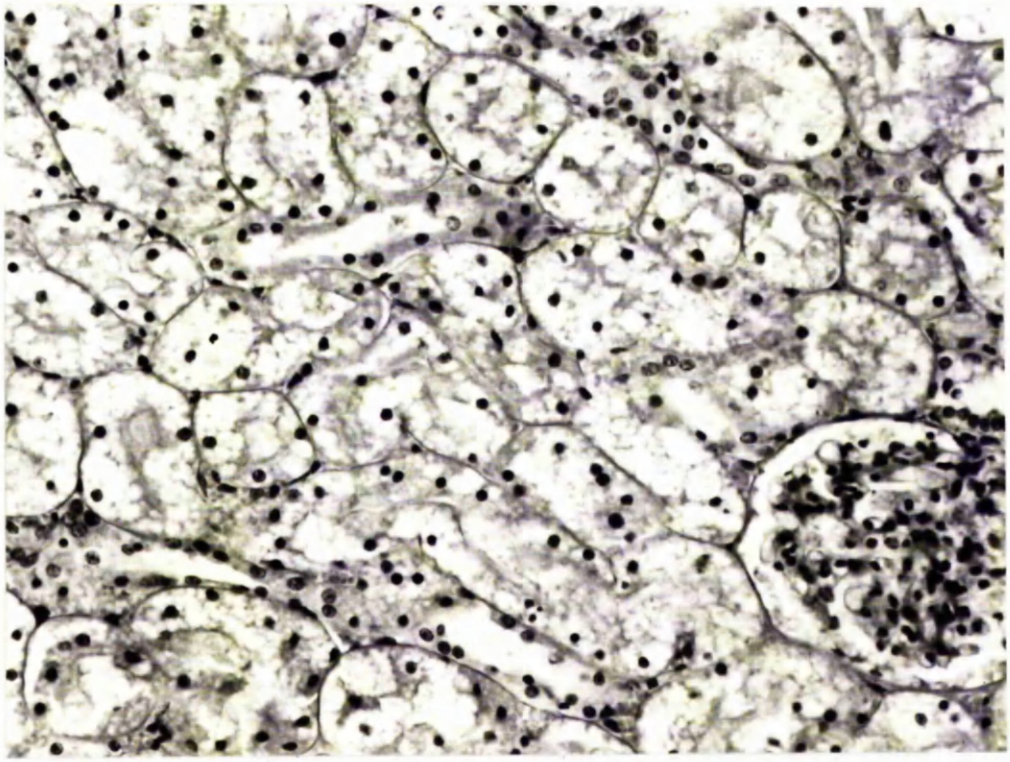


Fig. 82. Expt.5. Dog 24478/4. Showing pronounced fatty change in epithelial cells of the proximal tubules. H.& E. X 300.

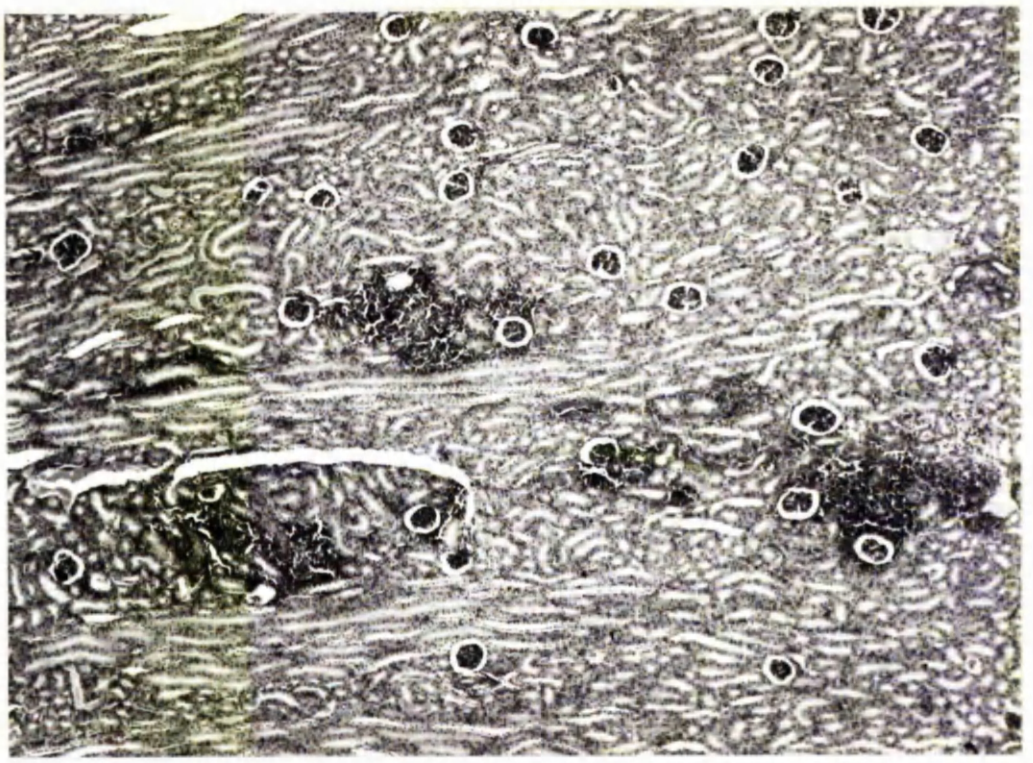


Fig. 83. Expt.5. Dog 24478/3. Small foci of interstitial nephritis. H. & E. X 50.

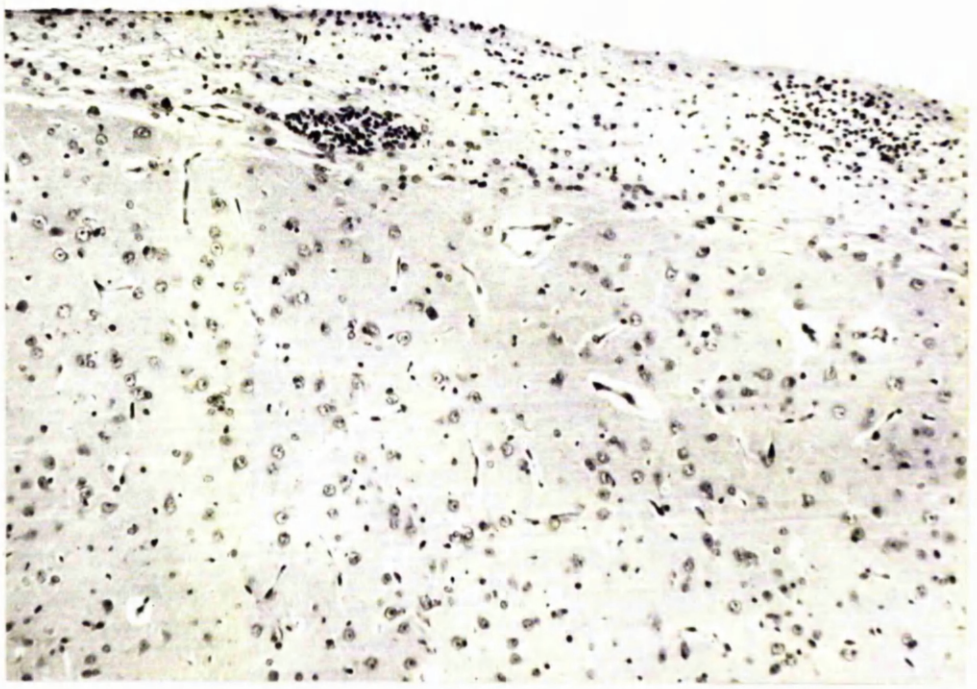


Fig. 84. Expt.7. Dog 21294/23. Meningitis, characterised by oedematous thickening of the meninges and plasma cell and lymphocyte reaction. H. & E. X 150.

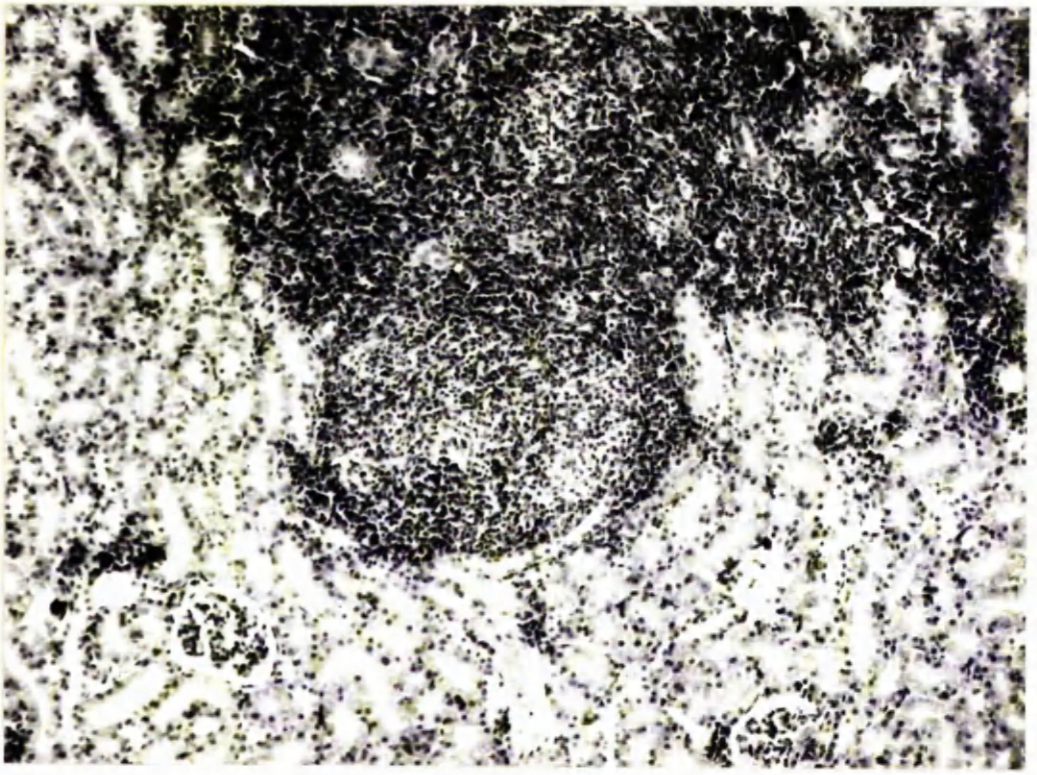


Fig. 85. Expt. 9. Dog 23248. A focus of interstitial nephritis, showing mononuclear cell reaction and localised destruction of tubules. H. & E. X 150.

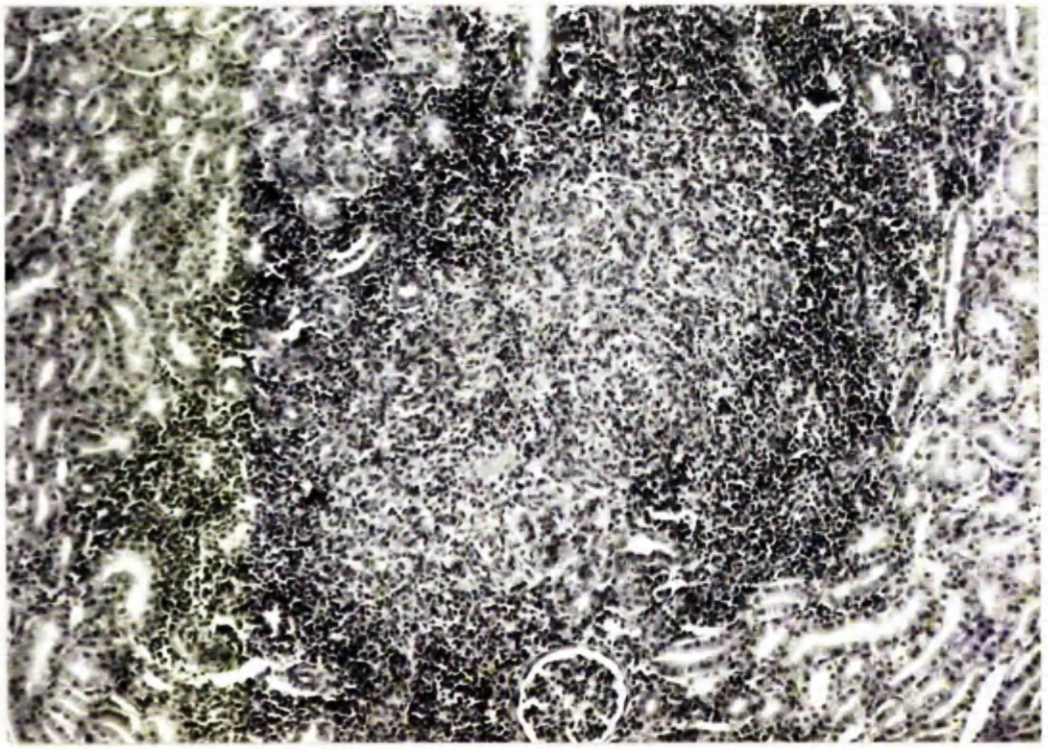


Fig. 86. Expt.9. Dog 23251. A focus of interstitial nephritis. H.& E. X 150.

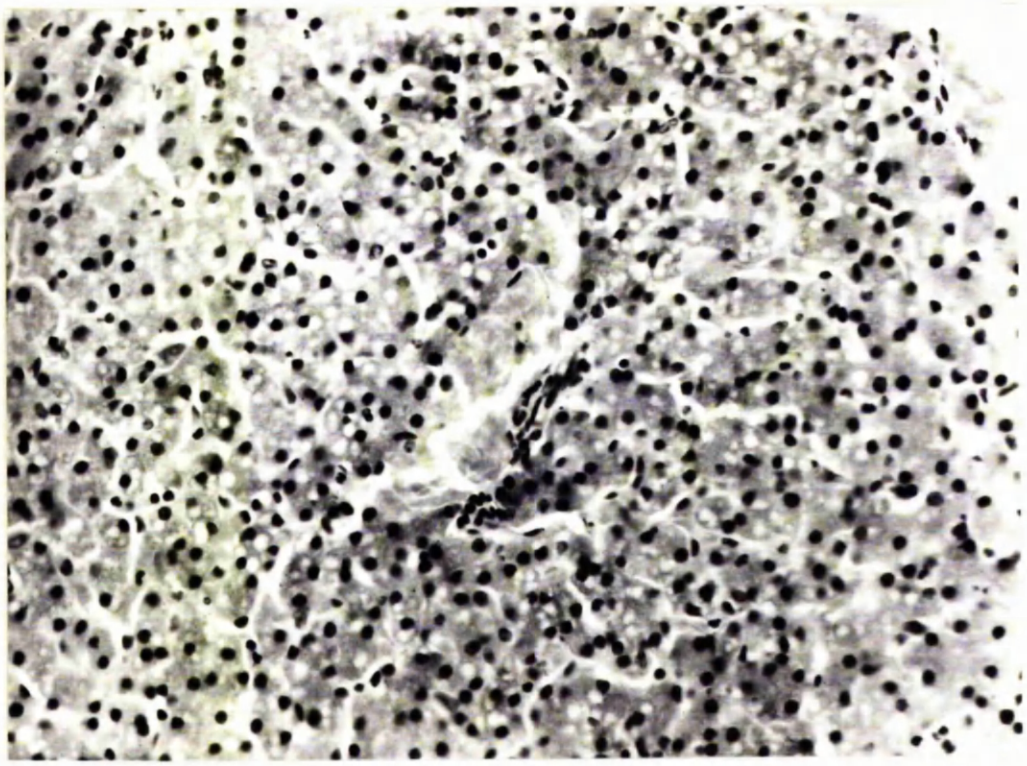


Fig. 87. Expt.11. Dog 24478/11. A selection of liver showing moderate fatty change. H. & E. X 300.

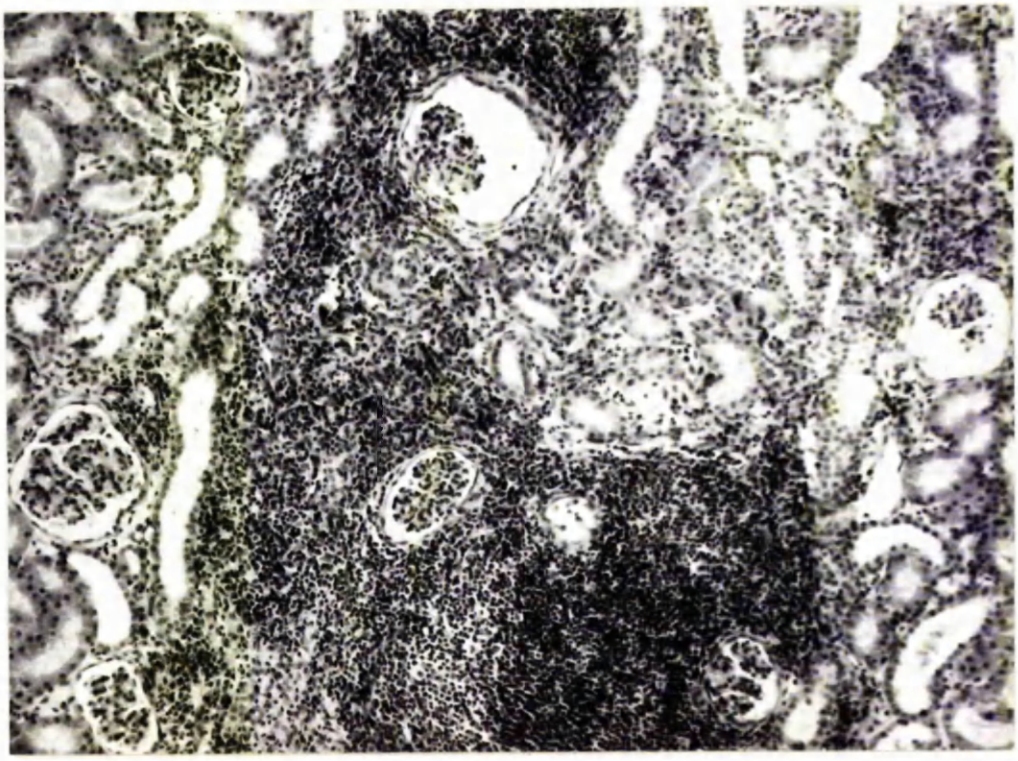


Fig. 88. Expt.11. Dog 24478/11. An area of interstitial nephritis. H. & E. X 150.

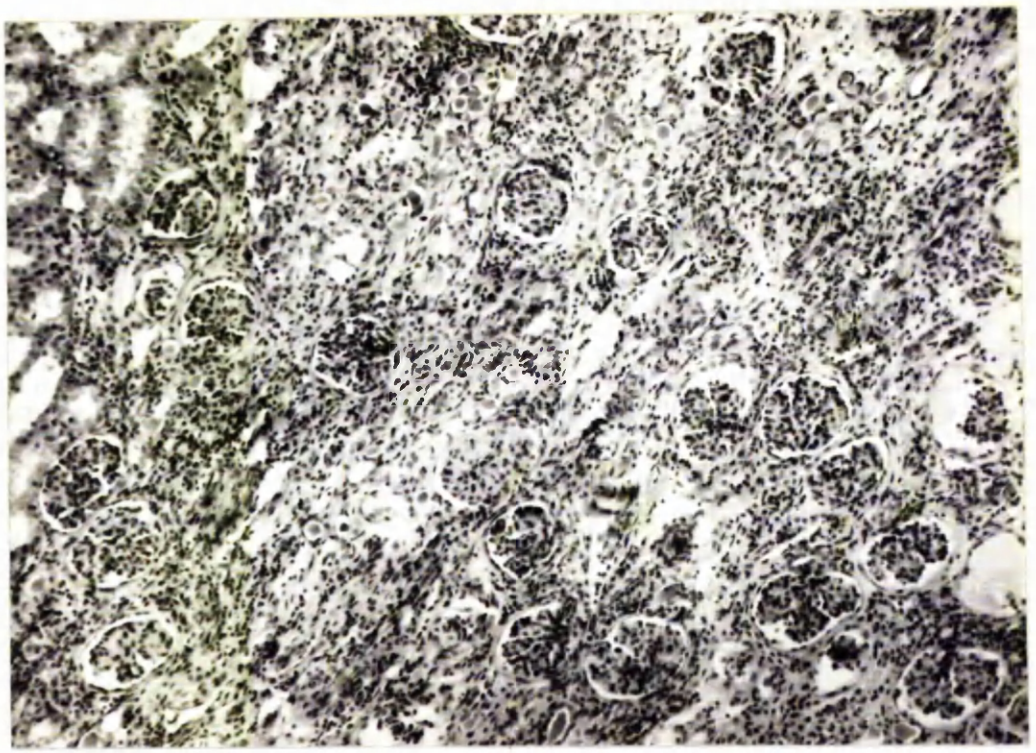


Fig. 89. Expt.11. Dog 24478/11. An area of chronic nephritis, showing interstitial fibrosis, hyalinisation of some glomeruli and scattered mononuclear leucocytes. H. & E. x 150.

Fig. 90. Expt. 11. Dog 24478/12. A kidney, showing the pale, swollen, nodular appearance of the cortex.

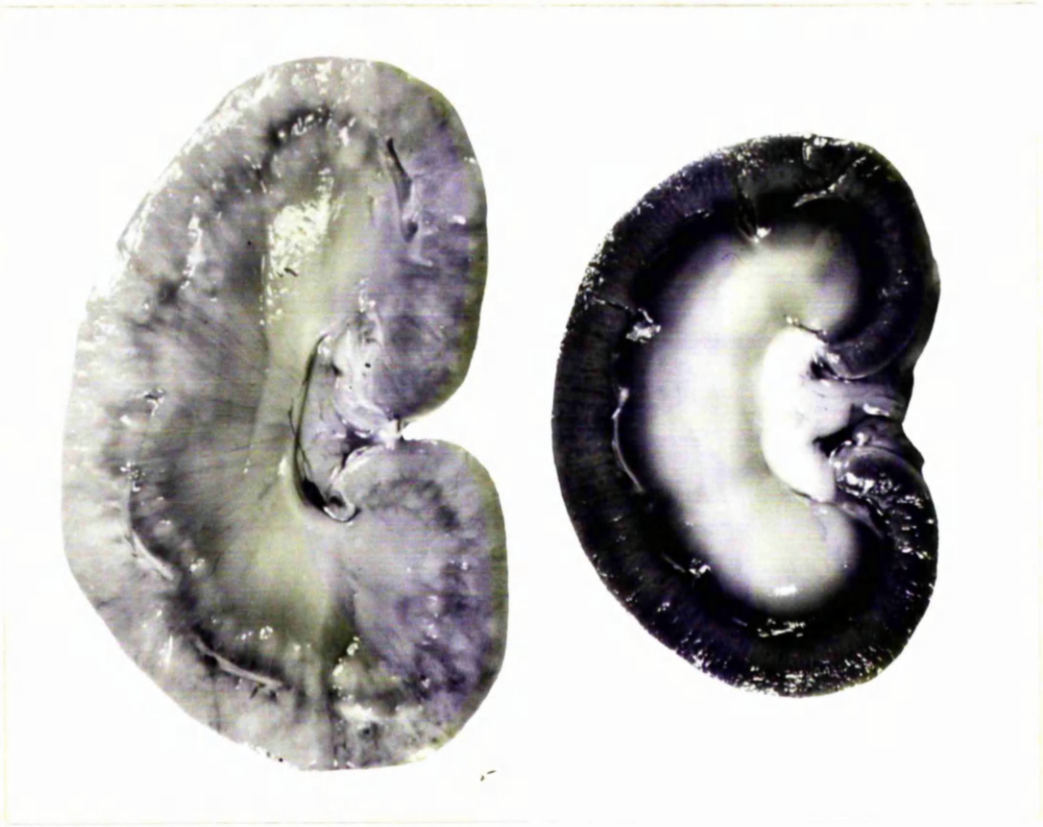


Fig. 91. Expt.11. Dogs 24478/12 and 24478/15. Showing the increase in size of the kidney with severe sub-acute interstitial nephritis.

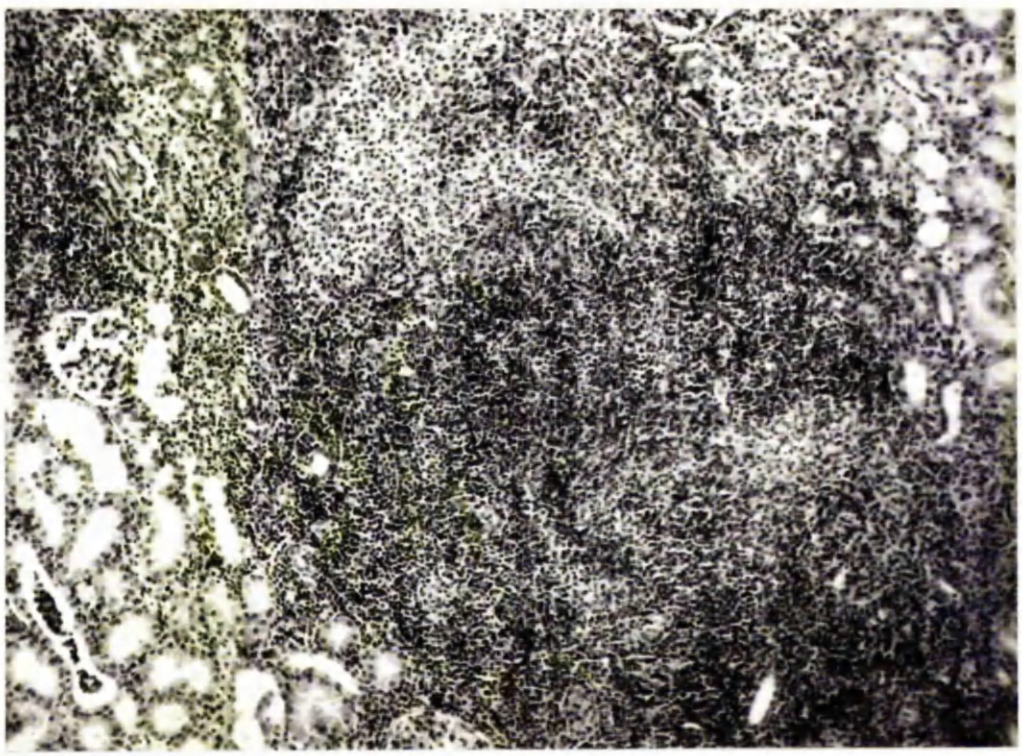


Fig. 92. Expt.11. Dog 24478/12. A large focus of interstitial nephritis. In the reaction, plasma cells and lymphocytes predominate, with a few polymorphonuclear leucocytes in the centre of the nodules. H.& E.

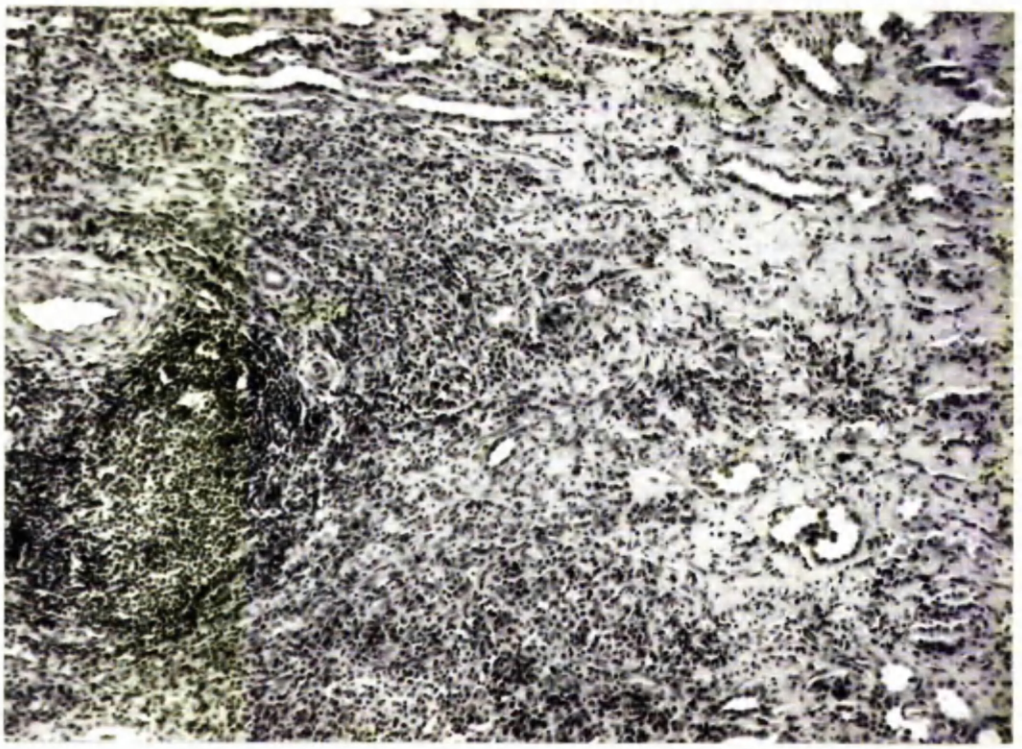


Fig. 93. Expt. 11. Dog 24478/12. Illustrating the sub-acute nature of the reaction. Interstitial fibrosis is well-marked in places, while there remains an extensive cellular infiltration. H. & E. X 1

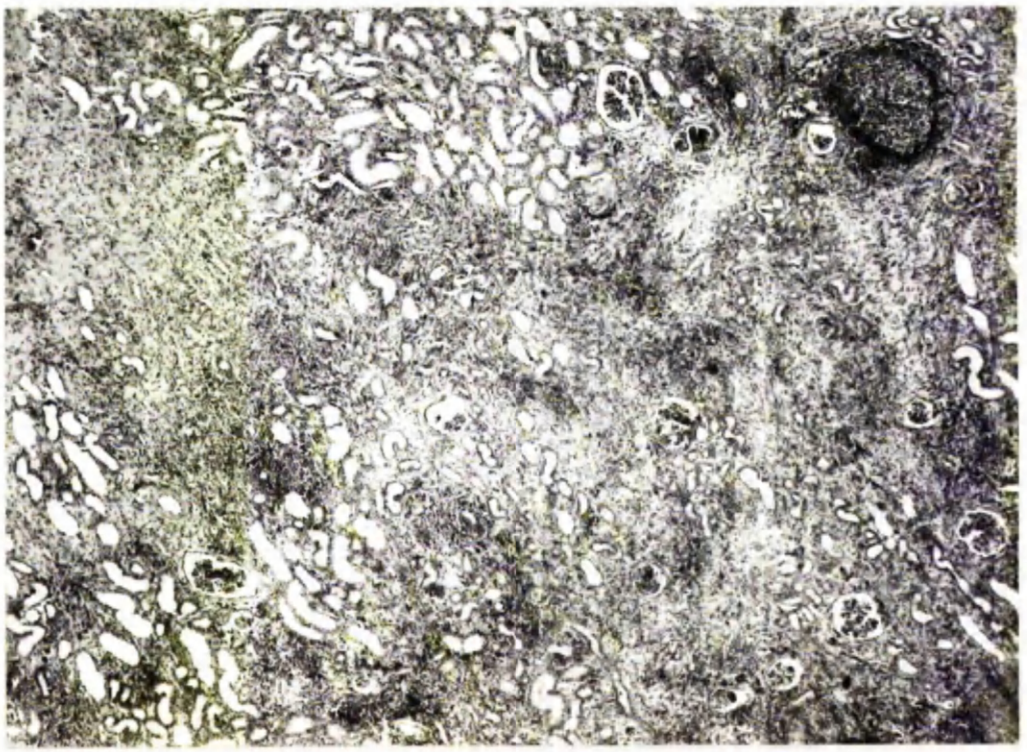


Fig. 94. Expt.11. Dog 24478/12. An area of well developed renal fibrosis with scattered accumulations of lymphocytes and plasma cells. H. & E. X 50.

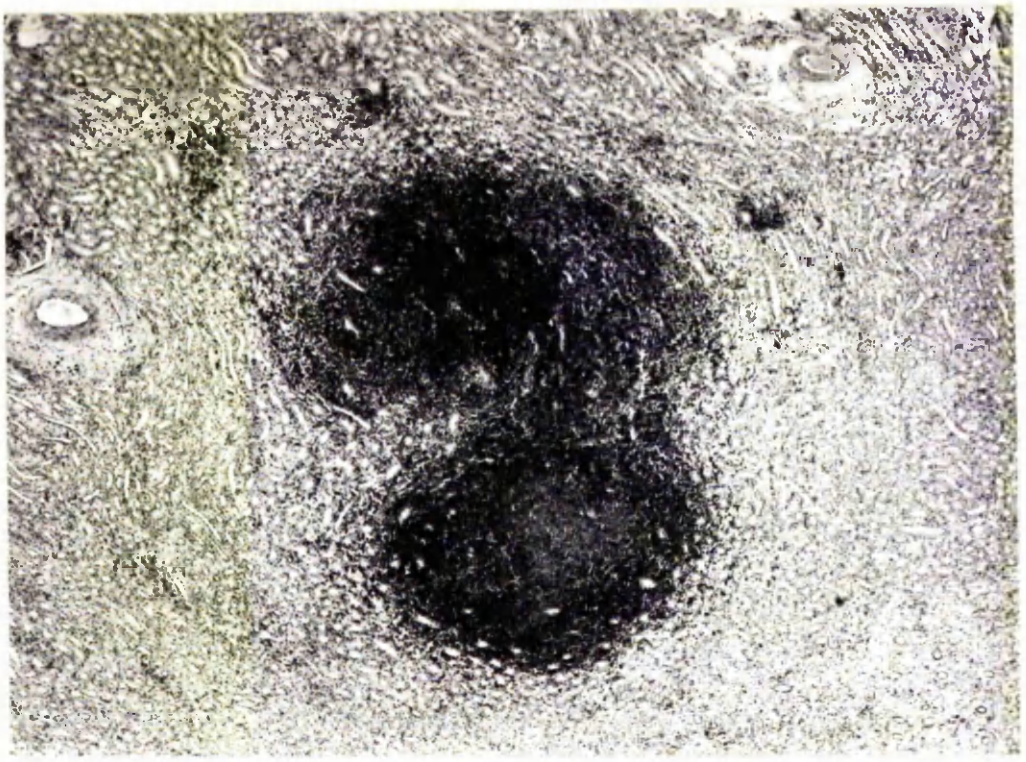


Fig. 95. Expt. 11. Dog 24478/15. A section of kidney showing a nodule of plasma cells and lymphocytes in the outer medulla. H. & E. X 50.

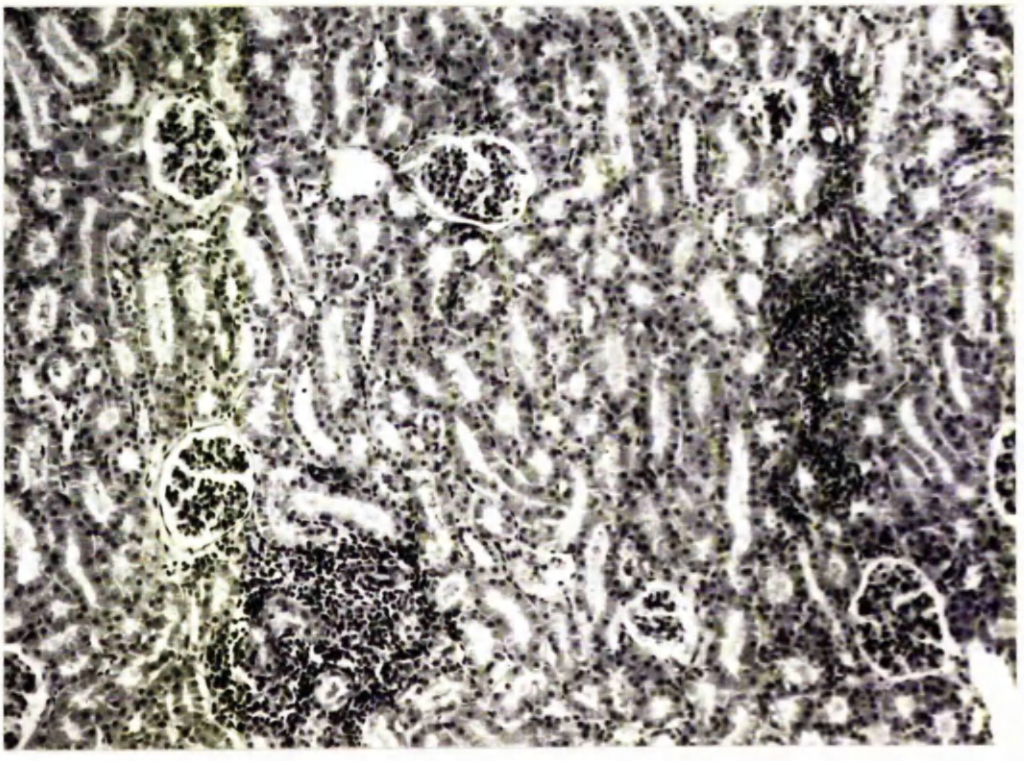


Fig. 96. Expt.12. Dog 26239. Very small foci of interstitial nephritis. H. & E. X 150.

Fig. 97. Expt.12. Dog 26241. A section of liver showing small accumulations of lymphocytes among the cords of parenchymal cells. H. & E. X 150.

FINAL CONCLUSIONS.

The studies described in this thesis have shown that vascular lesions of the type associated with hypertension in the human and experimental hypertension in the dog occur frequently in dogs with severe interstitial nephritis. Such lesions appear to be of considerable importance in the pathogenesis of progressive renal fibrosis.

Measurements of blood pressure by a direct method demonstrated that hypertension develops in many dogs with nephritis, particularly in the chronic phase of the disease. Correlations with clinical and pathological features established a positive relationship between nephritis, hypertension and vascular damage in the dog. From this evidence it is concluded that hypertension is the probable cause of vascular lesions and therefore significantly contributes to the progression of chronic nephritis.

A series of experiments was carried out in an attempt to reproduce severe interstitial nephritis in dogs. It was hoped to establish a reliable method whereby detailed study of the pathogenesis of the disease and thorough testing of commercial leptospiral vaccines would be possible. The disease, if reproducible, might form a useful model, allowing further analysis of the mechanism of nephrogenic hypertension. During the series, one dog developed severe sub-acute nephritis with uraemia, indicating that progressive nephritis can be produced by infection with L. canicola. The results suggested that the virulence of different strains of the organism may determine the outcome of infection. Further investigation of this possibility would be of value.

Acknowledgements.

With great pleasure I record my thanks to my supervisors, Professor W.L. Weipers and Dr. W.F.H. Jarrett, for their guidance and advice and for providing many facilities.

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CARDIOVASCULAR DISEASE ASSOCIATED WITH INTERSTITIAL NEPHRITIS IN DOGS.

by

Lindsay J. Mackey, B.V.M.S., M.R.C.V.S.

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Interstitial nephritis, associated with infection by the spirochaetal organism Leptospira canicola, is common and clinically important in dogs. Many aspects of the pathogenesis of the disease are not understood and the well-known relationship in the human between renal disease, hypertension and certain arterial lesions has not been established in the dog.

The present thesis is based upon an investigation carried out at the University of Glasgow Veterinary Hospital to determine the incidence of hypertension in dogs with interstitial nephritis and its significance in the pathogenesis of the disease. The thesis contains three sections and at the start of each, literature related to the specific studies is reviewed.

In Section I, an account is given of pathological studies carried out on the organs of 130 dogs with severe interstitial nephritis. Acute, sub-acute and chronic phases of the disease are described. In the heart, acute necrotizing endocarditis was found in 66% of acute, 64% of sub-acute and 33% of chronic cases. The left atrium was affected in all cases and sometimes the origin of the pulmonary artery and the aorta showed necrotizing endarteritis. In 30% of acute cases, the myocardium contained areas of acute inflammation and necrosis. In most chronic cases, hypertrophy of the myocardium of the left ventricle was readily appreciable.

Arterial lesions were found within the kidneys in 50% of acute, 55% of sub-acute and 85% of chronic cases. Arteries in the spleen were also frequently involved and those in the myocardium, sub-mucosa of the stomach and muscle of the tongue were affected less regularly. There was no relationship between the incidence of vascular damage and the age, sex or level of/

of uraemia of these dogs. In a control series of 125 dogs with normal kidneys, small infrequent arterial lesions were found in only four animals. The arterial changes in the nephritic dogs showed a gradation, first appearing as small focal sub-endothelial deposits of fibrinous material, then showing rupture of the internal elastic lamina and necrosis of the media and finally, involving rupture of the external elastica with extrusion of plasmatic material into the adventitial tissue. In chronic cases, small arteries and arterioles in the kidneys often showed hypertrophy of the media and hyperplasia of the adventitia. These changes were of the same type as those associated with hypertension in the human and experimental canine hypertension.

A comparative histological survey of spleens from nephritic and non-nephritic dogs in four age groups clearly demonstrated that lesions in the follicular arterioles show a higher incidence, greater severity and appear at earlier ages in association with nephritis. This illustration of the vascular effects of renal disease provided further indirect evidence for the development of hypertension in nephritic dogs.

In sub-acute and chronic interstitial nephritis, plasmatic damage in the capillary tufts of the glomeruli forms a prominent feature of the renal histopathology. This is characterised by the deposition within the capillary loops of fibrinous material which accumulates, coalesces and results finally in the conversion of the entire glomerulus to an amorphous mass of pseudo-collagen substance. As the lesion may be caused by hypertension, its role in the progression of the latter phases of nephritis was estimated. By counting the number of glomeruli showing each of three grades of capillary damage, it was found that in most chronic cases, 20 - 50% of the glomeruli were affected. Study of serial sections revealed that the more advanced changes were associated with degeneration and disappearance of the related proximal tubules, indicating that glomerular damage is important in the pathogenesis of chronic nephritis.

Section II contains details of a study of the blood pressure of nephritic and non-nephritic dogs. A direct method was employed to give maximum accuracy/

accuracy and to provide a permanent pressure record. The femoral arterial pressure of 28 non-nephritic dogs was measured and the upper limit of normotension was found to be approximately 150/90 mm. Hg. Hypertension was demonstrated in 2 out of 4 dogs with acute nephritis, in 3 out of 4 sub-acute and 13 out of 14 chronic cases. There was a highly significant statistical difference between the pressures of the chronic group and those of the non-nephritic dogs. Pathological examination of 7 of the dogs with chronic nephritis established a positive relationship between renal disease, hypertension and vascular damage in the dog. This investigation indicated that hypertension is the probable cause of vascular damage and therefore plays a significant part in development of chronic renal fibrosis.

In Section III, a series of 12 experiments is described. These were carried out in an attempt to reproduce severe interstitial nephritis in dogs, so that the pathogenesis could be studied in detail and adequate testing of commercial leptospiral vaccines would be possible. The disease, if reproducible, might form a useful model, allowing further analysis of the mechanism of nephrogenic hypertension. In the experiments, a number of methods designed to increase the susceptibility of healthy dogs to L. canicola and to enhance the virulence of cultured organisms were investigated and dogs were infected with a freshly isolated field strain. One dog developed severe sub-acute nephritis with uraemia - the first recorded occasion on which progressive renal disease has followed experimental infection with L. canicola. During the series, 20% of infected pups developed liver disease similar to that generally associated with L. icterohaemorrhagiae infection. The results suggested that differences of virulence between strains of L. canicola is important in determining the outcome of infection.