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THE PATHOGENESIS OF NEPHRITIS IN CHICKENS INDUCED BY INFECTIOUS BRONCHITIS VIRUS

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INTRODUCTION

The kidneys of vertebrates are an excellent source of tissue culture cells with a broad spectrum of susceptibility to viruses. However, no virus has been shown to be the direct cause of renal disease in man (Jensen, 1967; Smith and Aquino, 1971; Morrison and Wright, 1977). Many different viruses have been isolated from human urine but there are no specific "nephrotropic" viruses comparable to the hepatitis viruses (Utz, 1974). On the contrary, most human renal disease, especially chronic nephritis, is still of unknown aetiology (Pincherle, 1977). Although the role of immune complexes in the pathogenesis of renal disease is well established, the nature of the antigens involved remains obscure (Sissons, 1975).

Nephritis in the fowl is a serious cause of economic loss, and its pathology has been thoroughly investigated (Spector, 1951). The association of avian nephritis with infectious bronchitis virus (IBV) was recognized relatively recently, with the isolation of the virus from the kidneys after natural outbreaks of the disease (Winterfield and Hitchner, 1962; Cumming, 1962, 1963; Julian and Willis, 1969). The histopathology of IBV-induced acute avian nephritis is well documented (Pohl, 1974; Siller and Cumming, 1974; Purcell, Tham and Surman, 1976). More recently, Alexander, Gough and Pattison (1978) showed that the virus also induces a chronic nephritis. However, there has been no demonstration of viral antigens in the kidneys, nor attempts to explain the pathogenesis of the disease. In this study we provide evidence that both acute and chronic nephritis in the fowl are probably due to a focal progressive destruction of the tubular epithelium by IBV.

MATERIALS AND METHODS

Virus. The Australian "T" strain (Winterfield, Hitchner and Appleton, 1964) and the Massachusetts 41 strain (M41) were kindly supplied by Dr D. J. Alexander, Central Veterinary Laboratory, Weybridge, Surrey. These viruses were grown in 9-day-old specific pathogen-free (SPF) embryonated eggs inoculated by the allantoic

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route. Infected eggs were incubated at 37 °C for 72 h before harvesting. Allantoic fluid was clarified by centrifuging at 5000 *g* for 15 min to produce stock virus suspensions, which were stored at −70 °C. The virus was concentrated before freezing by centrifuging at 35 000 *g* for 45 min, and the pellet was re-suspended and stored in 0·01 M Tris/HCl buffer, pH 6·5.

Birds. The chicks used were hatched from SPF eggs. The Rhode Island Red (RIR) eggs were purchased from Houghton Poultry Research Station, Huntingdon, and the White Leghorn eggs from Wickham Laboratories Ltd, Hants.

Haemagglutination inhibition (HI Test). The serum antibody titre to IBV was measured by the HI test as described by Alexander and Chettle (1977), with phospholipase-C-treated M41 virus as antigen. The concentrated virus was incubated at 37 °C for 30 min with an equal volume of phospholipase C type 1 (Sigma Ltd) containing one unit of enzyme per ml of phosphate buffered saline (PBSA). The enzyme-treated "T" strain of IBV does not agglutinate red blood cells and was therefore not used in the assay.

Virus isolations. Faecal samples were homogenized in PBSA and after centrifugation at 6000 *g* for 30 min the supernates were used as inocula. Virus isolations from the kidney were made by allantoic inoculation of 9-day-old embryonated eggs with (i) tissue samples homogenized in PBSA, and (ii) supernatants from kidney explant cultures.

Immunofluorescence. High titre convalescent serum from an IBV-infected fowl was first tested for absence of autoantibody to kidney brush-border, and was then conjugated with fluorescein isothiocynate (FITC). Immunoglobulins were precipitated from serum with saturated sodium sulphate solution. The immunoglobulin concentration was determined by spectrophotometric measurement, and the FITC-protein ratio was 50:1. After filtration through a Sephadex G-25 column, the conjugate was absorbed twice with fowl kidney homogenate. The indirect method was used mainly for detection of IgG deposits in glomeruli and also of auto-antibodies to kidney brush-border. FITC-conjugated rabbit anti-chicken globulin was obtained from Nordic Immunological Laboratories, Maidenhead, U.K. Fowl tissues were frozen in liquid nitrogen and sections cut in a cryostat. Observations were made with a Leitz Orthoplan microscope equipped for incident light fluorescence.

Histology. For light microscope studies, tissues were fixed in 10 per cent neutral buffered formaldehyde. They were processed for paraffin wax embedding and sections were stained with haematoxylin and eosin (HE) and periodic acid-Schiff (PAS).

Ultrastructural studies. Pieces of renal cortex and medulla were placed in cacodylate-buffered glutaraldehyde (2·5 per cent at pH 7·2) and cut into 1 mm^3 blocks. The tissues were pre-fixed in glutaraldehyde at 4 °C overnight, post-fixed for 1 h in 1 per cent osmium tetroxide, and then dehydrated and embedded in Epon. Ultrathin sections were cut with a Reichert OmU3 microtome and stained with uranyl acetate followed by lead citrate. All specimens were examined with a Philips EM300 electron microscope.

Negative staining for electron microscopy. Faecal suspensions were clarified by low speed centrifugation and then centrifuged at 40 000 *g* for 1 h. The pellets were re-suspended in distilled water and stained with equal volumes of 3 per cent potassium phosphotungstate, pH 7·0.

Infection of birds. Embryos at the 16th day of incubation were inoculated by the allantoic route with 0·1 ml of diluted stock virus containing 10^5 EID_{50} (embryo infectious dose 50). The eggs were candled daily until hatching. In addition, 1-day-old and 15-day-old chicks were infected by both conjunctival and nasal instillation of diluted stock virus (10^6 EID_{50}). The control birds were given appropriate dilutions of uninfected allantoic fluid. Infected chicks were housed separately and examined daily. All dead birds were removed for post-mortem examination. Moribund birds were killed and sera were collected for HI testing. Organs (kidneys, liver, bursa of Fabricius, gut, trachea, and lungs) were collected from birds that died and from

those killed when moribund, for histological, immunofluorescent and virus-isolation studies. Faecal material was collected for virus isolation, and also for the electron microscopic examination of negatively stained material.

RESULTS

Production of Acute Nephritis in RIR and WL Chicks with 2 Strains of Virus

It was found that susceptibility to nephritis depended on the strain of virus. Sixty newly hatched White Leghorn (WL) and 60 Rhode Island Red (RIR) chicks were infected with two strains of IBV—M41 and T—as shown in Table 1. The 4 groups of chicks were housed separately until the end of the experiment 4 weeks after infection.

TABLE 1

SUSCEPTIBILITY OF 2 BREEDS OF FOWL TO 2 STRAINS OF INFECTIOUS BRONCHITIS VIRUS

Strain of IBV virus	*Rhode Island Red*		*White Leghorn*	
	Mortality	*Mean HI titre**	*Mortality*	*Mean HI titre**
T	16/30 (53·3 per cent)	1/16 (1/8–1/32)	15/30 (50·0 per cent)	1/16 (1/8–1/32)
M41	5/29 (17·2 per cent)	1/28 (1/32–1/256)	1/30 (3·3 per cent)	1/64 (1/32–1/256)

*HI titre of surviving birds at the fourth week. Ten birds from each group were bled and tested separately. The range of titre is shown in brackets.

Difference in mortality between T and M41, $P > 0{\cdot}009$ by Fisher's exact probability test.

Clinical observations. Tracheal râles and gasping were detected 2 to 7 days after infection in 80 per cent of the WL chicks infected with strain M41. Only 30 per cent of the RIR chicks infected with the same virus showed respiratory signs. In contrast, the T-infected chicks (RIR and WL) showed only very mild respiratory signs, with 15 per cent of the chicks developing mild râles. However, despite the absence of respiratory signs, severely ill chicks were observed from the third day onwards. These chicks stood hunched and appeared depressed, with ruffled feathers. Some had faeces and urine matted to the cloaca. The disease in the T infected chicks was rapid and some died within 24 h while a small number, although ill, recovered. The difference in mortality between the 2 strains of virus was statistically significant (Table 1).

Necropsy findings. Pathological changes were seen only in the kidneys. In all the T, infected chicks examined the kidneys were 2 to 4 times the normal size, pale in colour, and often had ureters distended with whitish fluid. Of the 5 M41-infected RIR chicks that died, 3 had enlarged kidneys. In the surviving birds killed 4 weeks after infection, no abnormalities were observed.

Four pools of faecal material, 2 from M41-infected and 2 from T-infected moribund birds, were examined by negative staining and electron microscopy. Abundant coronavirus particles were seen in both groups of birds. Material from normal chicks was negative.

Serological examination. All the chicks had high IBV-antibody titres. The HI-antibody titres of the M41-infected chicks were much higher than those of the T-infected group, but this probably reflected the use of the M41 virus as antigen in the HI test. In immunofluorescence tests with homologous antigens, the titres in both groups were similar. It is concluded that the T strain readily induced acute nephritis in the fowl.

Pathogenesis of Acute Nephritis in RIR Chicks Infected at Different Stages

Three groups of RIR chicks of different ages were infected with the T strain, as shown in Table 2, and studied by virological, immunofluorescent, histological and electron-microscope techniques.

TABLE 2

ACUTE NEPHRITIS AND VIRUS ISOLATION STUDIES IN RIR CHICKS INFECTED WITH THE "T" STRAIN

Age infected	*Total mortality at day 23*	*Incidence of nephritis in dead and moribund birds*	*Virus isolation positive-examined*		
			Faecal material	*Kidney homogenate*	*Kidney explants*
16-day-old chick embryo 27/54*	23/27=85·2 per cent (1—23)†	1/23= 4·4 per cent	6/7	3/4	3/3
1-day-old chicks	13/29=44·8 per cent (5—13)	10/13=76·9 per cent	5/6	4/6	4/4
15-day-old chicks	7/28=25·0 per cent (7—14)	1/7 =14·3 per cent	3/3	1/1	Not done

* Number of chicks hatched. Control chicks have a greater than 95 per cent hatching rate.

† Period of deaths in days.

For each age group of infected birds, 15 control birds were similarly housed. There was no mortality and virus was not isolated.

Clinical observations. Of the chicks hatched from infected embryos, 80 per cent were much smaller, less alert, and less active than the control chicks. Between day 2 and day 9 after hatching 25 per cent of chicks developed mild râles. Half of these also developed diarrhoea from day 6. The infected chicks had a much reduced weight gain and a reduced feed consumption compared to uninfected chicks. From about 4 weeks, the affected chicks began to recover and gained weight. The clinical picture for chicks infected when 1 day old was as described in the previous section. Of the chicks infected at 15 days, 20 per cent had ruffled feathers and were stunted but did not show any respiratory signs.

About 80 per cent of the moribund and dead birds from the group infected at day 1 showed kidney lesions. In contrast, the other groups had a much lower incidence of kidney lesions. However, serological examination showed that all infected birds had significant levels of HI antibody to IBV.

Virus isolations. IBV was isolated from faecal material of both dead and moribund chicks. The infectivity titres varied between 10^4 and 10^6 EID_{50} per g of material. Large numbers of coronavirus-like particles were seen and were confirmed to be IBV by immune electron microscopy.

IBV was also isolated from kidney samples collected from moribund birds with acute nephritis after the samples had been grown as explant cultures.

The cells grew to confluence in about 7 days, appeared normal, and no cytopathic effect was seen in unstained cultures. However, in stained preparations small syncytia were occasionally seen. Virus was consistently recovered from the supernate of explant cultures.

Histology. The main features in acute nephritis were similar to those reported by Siller and Cumming (1974). The kidneys showed varying degrees of tubular damage and interstitial infiltration with mononuclear cells in the medullary tracts and in the cortex. In the cytoplasm of many cells of the tubules basophilic inclusions were seen. These probably represent the viral inclusion bodies seen by electron microscopy and have not been reported in previous studies. Air-dried cryostat sections of frozen kidney fixed in cold acetone and stained by May-Grünwald Giemsa and viewed in polarizing light showed widespread accumulation of urate crystals in the tubules of moribund birds with enlarged kidneys. When the same sections were stained with haematoxylin and eosin, vacuoles in tubular cells and the lumen were obvious. Presumably, these are sites where the crystals had been dissolved. In control kidneys, only very few and fine crystals were seen.

Immunofluorescent findings. Virus-specific fluorescence was demonstrated in tracheal smears from moribund and dead chicks, and also in infected birds with no clinical signs. Specific cytoplasmic fluorescence was seen in the tubules of enlarged kidneys from all dead or moribund chicks as scattered foci in tubular epithelial cells in both cortex and medullary areas (Fig. 1). The staining was

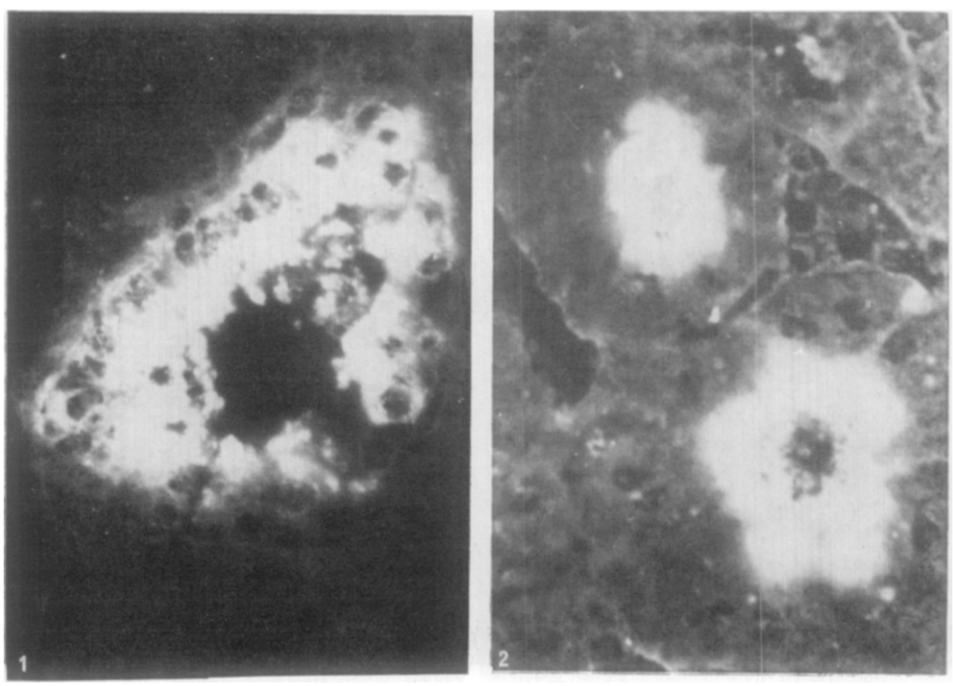
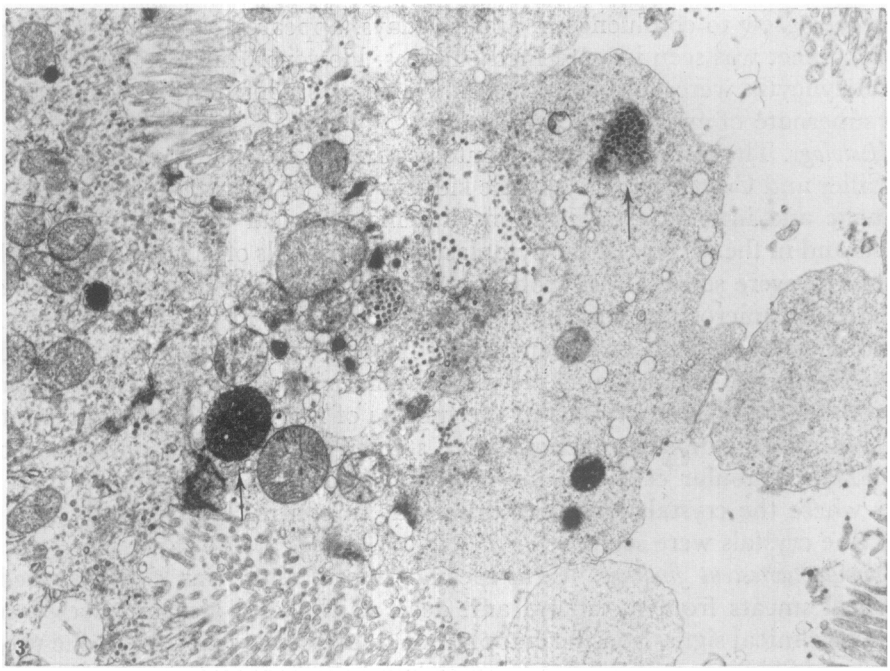


Fig. 1. A proximal tubule showing cytoplasmic fluorescence and infected casts in the lumen. ×375.
Fig. 2. A cryostat section of normal chick kidney showing tubular auto-antibody fluorescence, mainly near the brush border and apical cytoplasm. ×500.



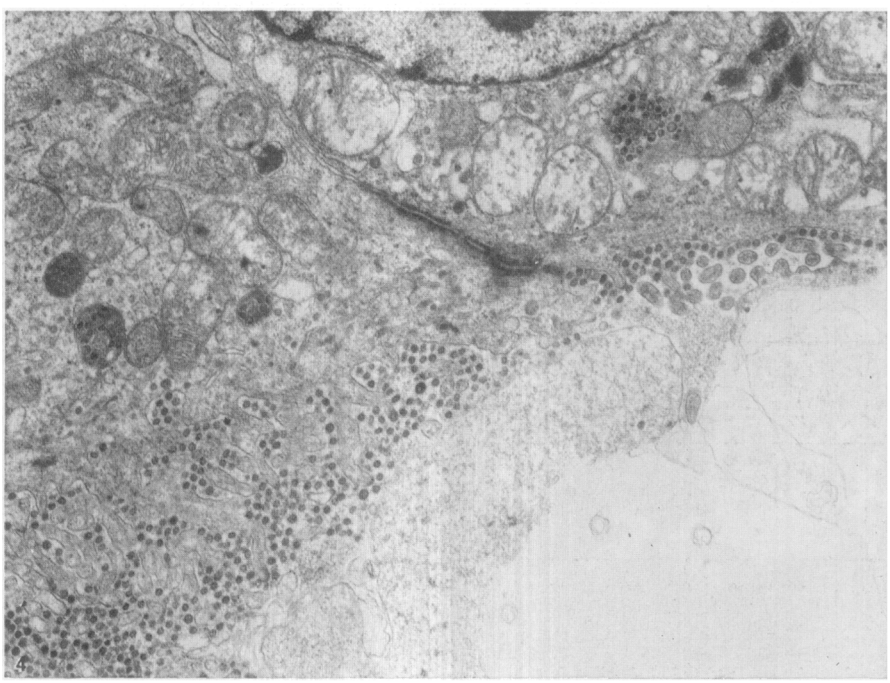


Fig. 3. A tubular cast in the lumen of an infected proximal tubule showing virus in vesicles and viral inclusion bodies. Arrows show dark, osmiophilic, round, single membrane-bound inclusion bodies with dense, smooth, round particles. ×8000.

Fig. 4. Part of cytoplasm of 2 cells near the lumen of a proximal tubule. Adsorbed to the lumen wall and microvilli of the brush border were many intact and mature virus particles. Viral inclusion bodies of various sizes were also visible. ×13 800.

mainly in the proximal tubules, although other segments also showed some staining. Moribund chicks with no gross kidney abnormality showed sparse staining, often in isolated tubules. It was necessary to apply the direct fluorescent staining method because of the presence of auto-antibodies in the convalescent sera (Fig. 2).

There was no virus-specific fluorescence in the glomeruli. Thus, it is considered that the virus can infect the whole nephron except the glomerulus. There was also no staining in the intestine or the bursa of Fabricius. When FITC conjugated antiglobulins were used, immunoglobulin deposits were occasionally seen in the capillary loops of glomeruli of moribund chicks.

Ultrastructural findings. Evidence of virus replication was found in the cells of the proximal convoluted tubules. The infected cells contained many cytoplasmic vesicles filled with mature virions (Figs 3, 4). Desquamated cells in the lumen showed strong evidence of virus replication (Fig. 3). The cells with greater cytoplasmic disorganization contained many characteristic viral inclusion bodies. The latter were usually bound with a single membrane, had an electron-dense matrix, and contained many electron-dense particles about 120 nm in diameter with a smooth outline. They varied in size from 0·5 to 2 μm in diameter (Figs 3 to 5). Although many lymphocytes were seen throughout the interstitial spaces none showed evidence of viral replication.

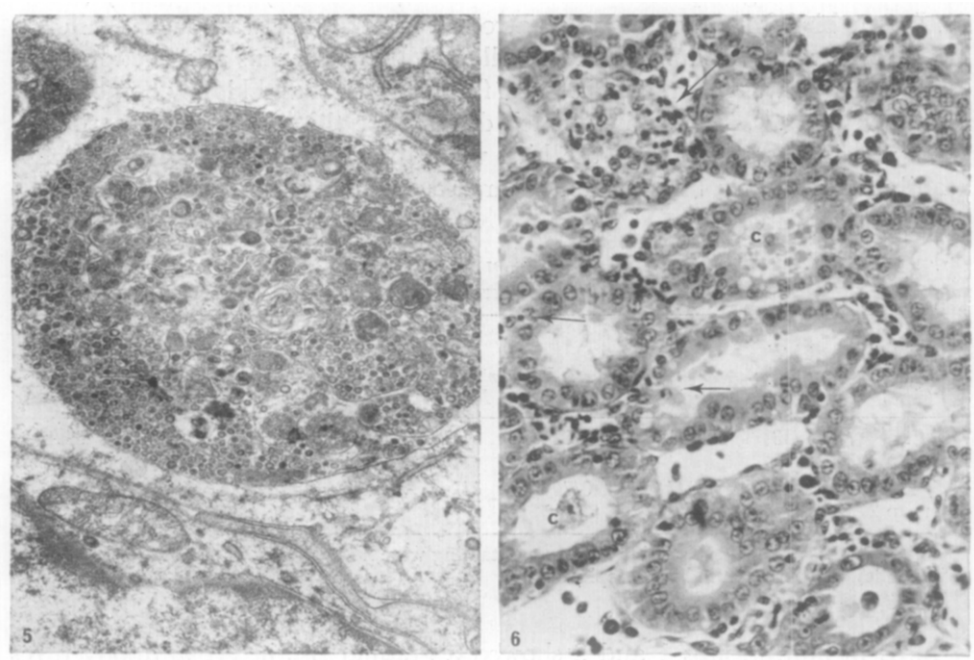


Fig. 5. A large inclusion body in the cytoplasm of an infected tubular cell from a chronically infected fowl at 29 weeks after the initial infection. It contained abundant, dense, round particles about 100 nm in diameter, and necrotic cellular material. ×11 970.

Fig. 6. Medullary region of an enlarged kidney from a chronically infected fowl at 29 weeks after initial infection. Note cellular casts (c), inclusion bodies (arrow head) and a tubule with fused cells (arrow). HE ×280.

Free virus particles were seen lining the microvilli of the brush-border of infected proximal tubules (Fig. 4). Although cytoplasmic vesicles containing virus particles were only rarely seen in distal tubules, particles with projections were often seen in the lumen. The glomeruli of all moribund birds examined appeared normal.

Pathogenesis of Chronic Nephritis Induced in RIR Fowls

Birds which survived the acute IBV infection were observed for 30 weeks. There were 2 groups. Group 1 consisted of 13 birds infected at Day 1, and Group 2 of eight chicks infected at Day 15. The birds were examined daily, and bled at regular intervals up to 30 weeks, when the experiments were terminated.

Clinical observations. Most of the birds appeared normal throughout the period of observation (Table 3). However, 77 days after infection one bird from Group 1 was unable to stand but no abnormality was seen at necropsy. Another bird from the same group died at the 18th week and necropsy showed greatly enlarged pale kidneys but no other gross abnormality. In Group 2, also at the 18th week after infection, one bird could not stand and had diarrhoea but no gross abnormality was seen at necropsy.

Necropsy findings. At 30 weeks after infection a total of 4 birds from group 1 and 2 birds from group 2 were found to have gross kidney abnormality (Table 3). In one bird from each group an atrophic right kidney was found, while the left kidneys were pale and slightly swollen. All other organs appeared normal. Virus isolation was attempted from the faecal material and kidney homogenate of all the birds. Of the 3 birds which died before the end of the experiment, virus was isolated only from the bird with abnormal kidneys. IBV was also readily isolated from birds exhibiting marked kidney lesions at the end of the experiment (Table 3).

TABLE 3

CHRONIC NEPHRITIS INDUCED BY IBV

Infected at age (days)	*Total mortality at week* 30	*Gross kidney lesions*	*Birds alive at week* 30				
			*Isolation**		*Specific** *fluorescence*	*HI*	*Kidney auto-antibodies*
			Faeces	*Kidney*			
1 (Group 1)	2/13	4†/11	3/11	2/11	4/11	Mean=1/128 (1/32—1/512)‡	5/11
15 (Group 2)	1/8	2†/7	0/7	1/7	2/7	Mean=1/128 (1/64—1/256)‡	2/7

*All positive isolations and specific fluorescent staining were from birds with gross kidney lesions.
†Includes one bird with kidney atrophy.
‡Range of titres.

Birds in both groups 1 and 2 showed a gradual rise in HI antibody titres until the 16th week after infection, and the titre remained high thereafter (Table 4). In group 1 birds, at the 20th week after infection, 4 showed very high HI antibody titres (1:128 to 1:512), while 4 others showed lower HI titres 1:32). These sera were then titrated by the indirect fluorescent antibody (IFA) method with IBV-infected primary chick kidney cultures as substrate. Although

the IFA titres corresponded well with the HI titres, the differences were smaller (1:80 to 1:320), indicating that all these birds had high titres of anti-IBV antibody.

TABLE 4

MEAN SERUM ANTIBODY TITRES OF RIR CHICKENS AT UP TO 30 WEEKS AFTER INFECTION WITH INFECTIOUS BRONCHITIS VIRUS

Week	*HI titre (Geometric mean)*	
	Group 1	*Group* 2
4	1/16 (1/8 —1/32)*	1/16 (1/16—1/32)
8	1/32 (1/16—1/64)	1/64 (1/16—1/128)
12	1/64 (1/32—1/64)	1/64 (1/32—1/128)
16	1/128(1/32—1/256)	1/128(1/32—1/256)
20†	1/128(1/32—1/512)	1/128(1/16—1/256)
30†	1/256(1/32—1/512)	1/128(1/64—1/512)

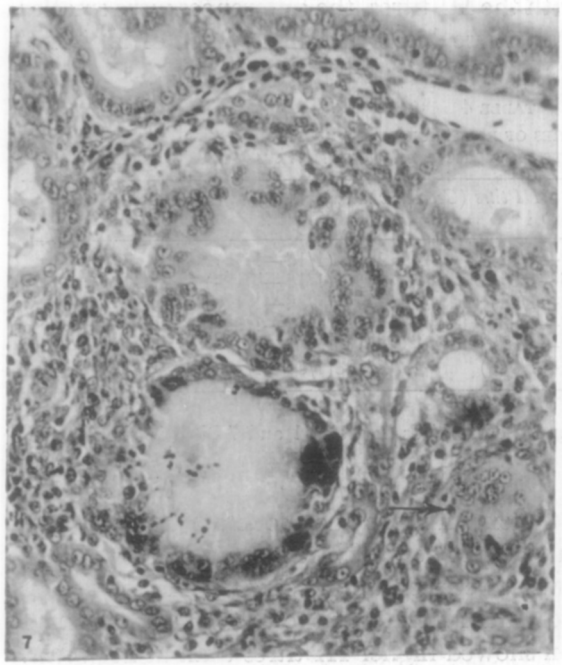
*Range of titres.

†For weeks 20 and 30, figures represent mean of 11 birds in group 1 and 7 birds in group 2. All other figures represent means of 6 birds.

Although all birds at the end of the experiments had moderate to high titres of HI antibody to IBV, only about 30 per cent of birds showed gross kidney abnormality. There was no correlation between the presence of kidney lesions and the titre of HI antibody in individual birds, but the 4 birds with marked nephritis at 30 weeks after infection showed higher HI titres than at 20 weeks.

Histological observations. The enlarged kidneys showed massive interstitial infiltration of lymphocytes, with some polymorphs. In both the cortex and medulla, many tubules, including proximal and distal convoluted and collecting tubules, showed degenerative changes, with swollen and vacuolated epithelial cells (Fig. 6). In intact tubules, individual cells were "rounded" and separated from neighbouring epithelial cells. These cells often contained basophilic inclusions (Fig. 6). Foci of more severe tubular necrosis were also present. Some isolated tubules were seen with tubular cells completely fused (Fig. 6) and others lying within the cellular infiltrate were completely degenerated (Fig. 7). The medullary collecting ducts were often distended and impacted with polymorphs and other necrotic cells. Portions of amorphous material surrounded by a giant cell reaction, presumably the remains of urate-impacted tubules, were also found (Fig. 7). The dilated tubules were most prominent in the medullary area and often contained eosinophilic and PAS-positive hyaline casts. The atrophied kidney showed extensive scar tissue, with fibrosis and isolated foci of cellular infiltration, mainly lymphocytes (Fig. 8). Kidneys which did not show gross abnormality contained scattered small foci of lymphocytic infiltration. The glomeruli of all birds appeared normal, although very occasionally PAS-positive material was seen within Bowman's capsule.

Immunofluorescence findings. Specific viral fluorescence was detected in the renal tubules of all the birds with macroscopic evidence of nephritis. In atrophied kidneys, fewer foci of fluorescent tubular epithelial cells were seen. The distribution of viral fluorescence was similar to that in acute nephritis, but fewer tubules were affected. Immunoglobulin staining was not detected in the glomeruli.



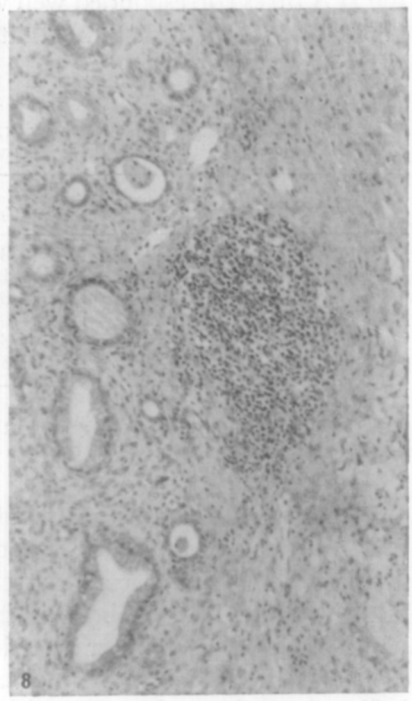


Fig. 7. Medullary region of same section as Fig. 6 showing urate-impacted tubules with surrounding giant cell reaction. Arrow shows a completely degenerated tubule. HE ×250.
Fig. 8. Atrophied kidney of a chronically infected fowl showing extensive scar tissue with fibrosis and a focus of cellular infiltration. HE ×100.

Ultrastructural findings. Evidence of virus replication was also found in the kidneys with chronic nephritis. Although both virus particles and viral inclusion bodies were seen in the chronic enlarged kidney, they were less prominent than in acute nephritis. The ultrastructure of the glomeruli of most birds appeared normal.

Auto-antibodies. Antibodies to normal kidney tubular brush-border (Fig. 2) were detected in sera from chronically infected RIR fowl 6 weeks after infection. They were present in about 50 per cent of the chronically infected birds from group 1 and 30 per cent of birds from group 2 at the 20th week after infection, and persisted till the 30th week. Of the 6 chickens which developed chronic nephritis, only one showed kidney auto-antibody. It is noteworthy that most of the birds which had high auto-antibody to kidney also had high antibody titres to IBV.

DISCUSSION

The results support previous evidence that some IBV strains produce nephritis more readily than others. Although the T strain produced a similar mortality for both breeds of fowl, chronic nephritis was not induced in the WL (data not shown). This agrees with an earlier report by Alexander *et al.* (1978) that chronic nephritis was predominantly induced in RIR chickens.

We also provide evidence for the replication of the virus in the tubules as a direct cause of the disease. In agreement with other authors we found no evidence of glomerular disease. It appears that the virus is first established in the trachea, where specific fluorescence is readily demonstrated as early as 24 h after infection. Thereafter, specific immunofluorescence was demonstrated only in the kidneys of birds which showed enlarged kidneys. Although, during viraemia, the virus may infect many visceral organs, it presumably persists only in the kidneys. The virus infection could spread along the tubules, as it was found frequently that whole lengths of tubule showed specific immunofluorescence alongside other normal tubules. In very young chicks (less than 2 weeks of age) severe necrotic changes were found in the tubules, with little mononuclear cell infiltration.

From the histology, the specific immunofluorescence findings and the electron microscopic evidence of virus replication in the tubular cells, it may be concluded that the pathological changes result from the cytolytic action of the virus. After the acute stage, about 50 per cent of the birds survive and develop high titres of specific antibody. The virus could be isolated from the cloacal contents of some chicks. This faecal shedding of virus from apparently normal birds continues for more than 30 weeks (Table 3). Efforts to isolate virus from extracts of the trachea, liver, spleen, bursa of Fabricius and lungs failed, as also was reported by Alexander and Gough (1977). The site of virus replication in these silent excreters of virus could not be demonstrated by immunofluorescence. It is probable that after the initial infection the virus is not completely cleared and is being shed from a few infected cells not detectable by immunofluorescence.

It has been speculated that the virus may replicate in the intestinal tract or in the caecal lymph nodes (Alexander and Gough, 1977). Indeed, in a more recent report, Alexander *et al.* (1978) showed that virus can be isolated from the caecal tonsil as frequently as from the faeces. However, contamination of caecal lymph nodes by faeces cannot be excluded. We failed to detect IBV replication in the intestine and caecal lymph nodes by immunofluorescent methods and so it is unlikely that the caecal lymph nodes or the intestinal tract are sites of replication. We suggest that the shedding of virus from infected renal tubules could account for virus found in faecal material.

The results show that the chronic nephritis is due to a persistent progressive IBV infection. The persistence of high titres of IBV antibody and the mononuclear infiltration indicate adequate immune response. It is known that persistent viral infection can occur in immunologically competent hosts and that some virus infection may persist in the presence of neutralizing antibodies (Notkins, 1974). Although there are many factors which may contribute to such persistent infections (Porter, 1975), most of these have not been investigated for IBV infection. However, there are 2 features of coronavirus replication which may contribute to the persistence of the infection. Firstly, coronaviruses do not appear to induce new viral antigens on the cell surface (Oshiro, Schieble and Lennette, 1971). Secondly, most coronaviruses, including IBV, readily induce cell fusion. These properties may enable IBV to replicate and spread to neighbouring cells without alerting the host's immunological defences.

The eventual lysis of infected cells, and the accompanying release of viral antigens, would then attract inflammatory cells to the site of the infection and also boost antibody production. Alternatively, virus may persist by suppressing the host immune response, although evidence of virus replication in lymphocytes was not seen by us.

Another interesting feature of chronic nephritis is the development of auto-antibodies against normal tubular brush-borders. This has not been reported before as a consequence of virus infection and it is not clear whether renal auto-antibody contributes to the pathogenesis of nephritis.

Lastly, although the chronic nephritis caused by IBV in chickens could be a good model for studies on persistent virus infections, the relevance to human chronic nephritis remains to be established.

SUMMARY

Nephritis in chickens caused by infectious bronchitis virus (IBV) was studied by virological, histological and electron microscopical methods. The T strain of the virus caused only mild respiratory signs in both Rhode Island Red (RIR) and White Leghorn (WL) breeds; the 50 per cent mortality induced was due to acute nephritis. All the infected birds developed high titres of antibody to IBV for up to 30 weeks. In spite of the persistence of antibody, about 35 per cent of the RIR developed chronic progressive nephritis. The histology showed varying degrees of pathological changes in the tubules, with relatively unaffected glomeruli. Foci of mononuclear cell infiltration were prominent in the cortex and medulla, particularly in chronic nephritis. Cytoplasmic IBV immunofluorescence was found in all segments of the tubules, but not in the glomeruli. There was no evidence of virus replication in the caecal tonsil and bursa of Fabricius. Evidence of extensive coronavirus replication was found in the cells of the tubules. A large number of viral inclusion bodies as seen containing dark smooth particles 120 nm in diameter within a single membrane. Virus was readily recovered from the kidneys as well as faeces of birds with acute and chronic nephritis. It is concluded that direct virus-induced cell lysis is the primary cause of IBV nephritis. In addition, about 50 per cent of the chronically infected birds also developed brush-border auto-antibody.

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