PATHOLOGY OF SPONTANEOUS CASES OF AVIAN TRANSMISSIBLE TUBULONEPHROSIS

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The present authors encountered a disease of chicks in which renal alterations were main gross pathological findings. An etiological agent which seemed to be a virus was isolated from the kidneys of these affected birds\(^1\). Furthermore, the same lesions as those in naturally affected birds were induced in 1-day-old and 28-day-old chicks by injection with this agent.

This disease was regarded as the same one that had been reported in North America and Australia and called avian nephrosis, avian nephritis, avian uremia, and so on\(^2-4,11,16,18\).

This paper describes the first incidence of the disease in Japan and the detailed pathological features of field cases, since the knowledge of the disease is limited.

The results of experimental studies on this disease will be reported in a paper to come.

MATERIALS AND METHODS

The materials used for the investigation consisted of 8 chicks which were reared on 3 poultry farms (named A, B, and C), as shown in Table 1. The aspects of occurrence of affected chicks could be examined in some detail only on poultry farm A, but not on any other farm. On poultry farm A, a total of 1,300 day-old chicks hatched at the same time were purchased. The birds were moved from the breeding house to the rearing one when they were at 28 days of age. After that, death occurred among them.

<table>
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<th>Poultry farm</th>
<th>Case No.</th>
<th>Autopsy No.</th>
<th>Breed**</th>
<th>Sex</th>
<th>Age (days)</th>
<th>Termination</th>
<th>Date of autopsy</th>
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<td>718</td>
<td>WC×WR</td>
<td>Female</td>
<td>37</td>
<td>Died</td>
<td>20/11 '68</td>
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<td>WL</td>
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* An agent which seemed to be a virus could be isolated from the kidney.

The cumulative number of deaths reached 101 (7.8%) up to 43 days of age. At 43 days of age and later, no incidence was observed. The affected chicks were found to be motionless and down-hearted.

After postmortem examination, specimens of almost all types of tissues were collected from all the birds, fixed in 10% formalin, and imbedded in paraffin. Sections were stained with hematoxylin and eosin.

RESULTS

A. Macroscopical findings

The most prominent change was observed in the kidneys and ureters (Figs. 1~4), as mentioned below. It was the most characteristic of this disease. The kidneys presented variable changes of severity ranging from low to high grade. In birds which showed changes of low grade, the kidneys exhibited slight cloudy swelling and discoloration (Fig. 1), and had pale whitish spotty foci distributed throughout the parenchyma. In birds which showed changes of medium to high grade, the kidneys swelled moderately or severely. Their parenchyma was cloudy and packed densely with pale-grayish or whitish minute foci spotty or meshy in shape (Figs. 2~4).

The ureters were often dilated in the whole length. They contained a small or large quantity of whitish fluid or muddy substance.

In addition to the changes of the uropoietic organs mentioned above, slight dehydration was noticed on the whole body in almost all the birds examined. Moreover, stagnation in the liver and an increase of mucus in the larynx and trachea were observed in some cases. In one case (case 2), there was an increase of pericardial fluid which contained some spotty whitish substances floating.

B. Microscopical findings.

Kidney (Figs. 5~12): There was stagnation in all the birds. In the proximal convoluted tubules, the epithelial cells often exhibited swelling, granular degeneration, and desquamation. Moreover, distinct hyaline-droplet degeneration was recognized in one case (case 3), and epithelial necrosis was found in two cases (cases 2 and 8). The lumina of the tubules were sometimes dilated, and a few heterophils infiltrated rarely in them. In the distal convoluted the collecting tubules, the lumen was frequently dilated and contained an accumulation of fluid substances stained poorly with eosin and sometimes hardly with hematoxylin. These fluid substances occasionally included heterophils and desquamated and degenerated epithelial cells. In some cases (cases 3, 5, and 6), needle-formed or minute globular crystalline substances were present within the lumina of those tubules in addition to the accumulated substances mentioned above. The epithelial cells of those tubules occasionally exhibited flattening, fatty degeneration, and desquamation. On the other hand, hyperplastic change was observed in the epithelium. In cases 3 and 7, furthermore, clumps of multinucleate giant cells were present around fluid or needle-formed substances accumulated within the tubules. In only one case (case 3) there were small necrotic foci in the parenchyma.

In many cases, the papillary ducts were highly dilated and filled with a large quantity of fluid substance stained poorly with eosin and occasionally containing desquamated epithelial cells or cellular debris. In cases 5 and 7, an accumulation of needle-formed crystals was recognized within the lumen. The epithelial cells of those ducts frequently presented flattening and desquamation. Aggregation of multinucleate giant cells was rarely observed around the substances accumulated within the lumen (case 7). Moreover, fluid substances stained poorly with eosin occasionally exuded in
the interstitial connective tissue around the affected ducts.

Interstitial alterations of the kidney were observed chiefly in the areas correspond-
ing to the medulla, and frequently in the regions adjoining to those of the parenchymal
changes mentioned above. They were mild edema, a slight proliferation of juvenile
fibrocytic cells, and an infiltration of heterophils. Besides, a slight increase of plasma
cells and lymphoid cells or large mononuclear cells was recognized in some circum-
scribed areas.

Alteration was scarcely observed in the renal corpuscles. Bowman’s spaces rarely
showed slight dilation.

- Ureter (Fig. 13): In both ureters, the lumen was dilated in almost the whole
length and frequently contained fluid substances stained poorly or somewhat deeply
with eosin. The epithelial cells of the ureters were hyperplastic to various degrees in
some portions, and were desquamative and deficient in others. In the submucous coat
of the ureters, an increase of lymphocytic cells was often observed, a proliferation of
histiocytic or fibrocytic cells was occasionally recognized.

Other organs: In addition to the histological changes of the uroepithelial organs
mentioned above, the following alterations were noticed. General congestion, especially
congestive edema of the lungs, and acute catarhal pneumonia were frequently present
as important alterations ranking next to the renal damage. Besides, acute catarhal
laryngitis and tracheitis were recognized in 4 cases. Furthermore, a slight increase of
lymphocytes and a slight activation of reticulo-endothelial system cells were occasionally
observed in the general lymphoid tissue. As compound lesions, encephalonecrobiosis
(case 4), fresh degenerative alterations of the cross-striated muscle tissue in the femoral
area (case 5), and an initial lesion of visceral lymphomatosis (case 8) were confirmed.

DISCUSSION

A. Pathological changes characteristic of this disease

The present authors described in detail the pathomorphological alterations of 8
spontaneous cases of this disease. As stated at the beginning of this article, this disease
seems to be quite identical with what has been reported under the name of avian
nephrosis, uremia, infectious nephritis-nephrosis, avian nephritis, and so on. In the previous reports, the gross pathology of the kidney and the ureter was noticed as the most characteristic changes of the disease.

As to the histopathological changes of this disease, the description is very scanty.
Especially, as to those of the spontaneous case, it was only Cosgrove who described.
He mentioned that the lesions of the disease were confined to the renal tubules and
consisted of cloudy swelling indicating a degenerative process.

On the other hand, experimental studies were carried out not infrequently. Cumming pointed out the occurrence of cloudy swelling in the renal tubules, but did not find nephritis in his cases. Newton and Simmons described changes of the kidney varying from acute to chronic. These changes were observed in the glomeruli and tubules, especially the proximal convoluted portions. These tubules exhibited distention of the lumen and pyknosis or some other type of degeneration of the epithelial cells. Cellular debris, heterophils, amorphous casts, urates, and uric acid crystals were seen in the tubules. Furthermore, plasma cells and aggregations of young lymphoid cells frequently appeared, suggesting the occurrence of active changes. Payor and Woo regarded the lesion of the kidney as non-inflam-

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The present authors' findings were very similar to those described by the researchers cited above. The present authors, however, noticed the degenerative changes of the renal tubular epithelium, which were regarded as the initial lesion of this disease. Consequently, they considered that such degenerative changes had given rise to an accumulation of abnormal excrements or substances within the proximal and distal convoluted and the collecting tubules, the papillary ducts, and the ureters. There were an infiltration of inflammatory cells, an increase of lymphocytes, and a formation of multinucleate giant cells in the kidney and ureter. The present authors regarded these changes as responsive alterations to the accumulation of abnormal excretions or substances within the renal tubules. In addition to the lesions of the uropoietic organs mentioned above, a high degree of general congestion was noticed.

B. Etiology

An etiological agent which seemed to be virus was isolated from 4 of the 8 birds subjected to this study¹⁰. In an experiment, suspensions of kidneys of these birds were injected into the allantoic cavity of embryonating chicken eggs. Then, one drop of the affected allantoic fluid was inoculated intraocularly and intraperitoneally in 1-day-old and 28-day-old chicks. Consequently, the inoculated chicks showed the same renal alterations as the spontaneous cases. The results of this experiment will be reported in a paper to come. From such experimental results, the present authors are convinced that the etiological agent of this disease is a virus.

Isolation of a virus was successfully performed by some of the other researchers³,⁴,¹⁷. Accordingly, it seems to be almost free from doubt that the etiological agent of this disease is a virus. The virus reported by the previous researchers belongs to an infectious bronchitis virus type⁴ and is an aberrant strain of infectious bronchitis virus⁴. It is related to the known infectious bronchitis virus type¹⁷.

On the other hand, nutritional factors have also been considered to be related to the cause of such kidney lesions as mentioned above. For instance, it has been reported that these lesions were brought about when the birds had been reared with a meat-meal diet¹³, a high protein-high energy diet¹²,¹³, and a high-calcium diet¹⁴. No nutritional factors, however, were taken into consideration in the present authors' studies for the time being. McClymont¹⁰, and Pryor and Woo¹² have had doubt about the opinion that nutritional factors should be taken into consideration in the etiological study of uremia.

C. Differential diagnosis

Gumboro disease has come into question in the first place, since Cosgrove² reported a disease inducing swelling of the bursa of Fabricius and renal changes. At present, it is generally presumed that Gumboro disease may have been mixed with his disease. After that, the infectious bursal agent was isolated by Winterfield and Hitchner¹⁷ from spontaneous cases. According to Helmboldt and Garner⁵ it is probably a misnomer that Gumboro disease is called by the term nephritis-nephrosis. On the other hand, Landgraf et al.⁹ described essentially the same renal changes in Gumboro disease as observed by Cosgrove². These changes were swelling of the kidney and reddish-gray discoloration of prominent tubules. Furthermore, Helmboldt and Garner⁵ recognized histopathological changes of the kidney in 5% of the birds experimentally infected with the etiological agent of Gumboro disease.

In the present authors' cases, those changes which could be observed in Gumboro disease were found in no tissues of the whole body. Gumming⁴ described that swelling of the bursa of Fabricius was not present. The present authors themselves examined pathologically in detail a large number of birds affected naturally with Gum-
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borno disease. As a result, no lesions were observed in the kidney of any bird. Kaneko et al.\(^{8}\) reported the same findings in Gumboro disease as those mentioned by the present authors. Accordingly, the present authors are convinced that differential diagnosis between Gumboro disease and this disease can be done even on the basis of the morphological changes of the kidney and the bursa of Fabricius.

In the next place, the difference between this disease and visceral gout comes into question on account of the close resemblance of renal alterations. Visceral gout of chickens has been investigated by one of the present authors himself\(^{6,18}\). This problem will be discussed in detail in experimental studies on this disease to come. The following changes in this disease, however, may attract attention only from a histopathological point of view. Main alterations were recognized in the tubular system of the kidney. No embolism of urate crystals was observed within any blood vessel in the whole body tissue, including the interstitium of the kidney. It was very rare to see urate deposition on the serosa in various areas. Cummin\(^{4}\) described essentially the same findings as those observed in avian visceral gout in birds induced by injection with a virus isolated from infectious avian nephrosis (uremia) in Australia. Because the etiology of avian gout has not yet been established, the difference between this disease and that nephrosis should be investigated from various points of view in the future. In the present authors' cases, the parenchymal changes of the tubules of the kidney occurred first, as mentioned above. Subsequently, such lesions as resembling those of avian visceral gout may have been brought about to the whole body tissue. Schloththauer and Bollman\(^{14}\) regarded renal insufficiency as one of the etiological factors of gout in turkeys.

D. The name of the disease

Various names have been given to this disease, as mentioned above. The present authors attached great importance to the histopathological alterations observed in this study. Furthermore, an agent which seems to be a virus was isolated from the present cases. This agent could induce the same lesions as those in naturally affected birds when injected into chicks. From these results, the present authors think that the term avian transmissible tubulonephrosis is suitable for the designation of this disease at the present time.

SUMMARY

Histopathological investigation was carried out on 8 chicks affected naturally with a disease identical with what had been reported under the name avian nephrosis, nephritis, or uremia in North America and Australia. As a result, alterations of the uriniferous tubules were pointed out and regarded as initial lesions from scrupulous studies. Furthermore, an agent which seemed to be a virus was isolated from these chicks. On the basis of these results it was proposed that avian transmissible tubulonephrosis be the most suitable name for this disease.

REFERENCES.

6) Inoue, M., Itakura, C., Yokoyama, T., Takemura, N., Ono, T., Ueda, A. and


鶏に自然発生した伝達性尿細管腎症の病理について

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著者らは、1962年以来、北アメリカおよびオーストラリアにおいて、avian nephrosis, nephritisあるいはuremiaの名のもとに報告されて来た病と、同類であるとみなされる症例に遭遇したので、これらについて病理組織学的に検索した。

検索材料は、鶏飼養家において自然発生した8症例よりなる。

病変は、全症例と共に臨して SHR と腎尿管の変化が、最も特徴的であった。すなわち、肉眼的には、腎は渕溝腫脹を示し、実質内に、点状または細目状をなした灰白色果が形成されていた。腎尿管は拡張し、管腔内に白色混濁物質を認めていった。

組織変変は、尿細管、集合管に主座し、乳頭管、輸尿管に及んで認められた。すなわち、尿細管上皮の変性、剥離、混濁死を発端とし、集合管、乳頭管をも含めて管腔は拡張し、蛋白性または尿酸様物質、顆粒細胞などを、多少の著者に認められた。反応性変化として、軽度ではあったが、偽好酸球浸潤、間質に単核性細胞の増数、幼若線維性細胞の増殖が認められ、多核巨細胞に周囲された病変性組織もみられに認められた。

これらの検索材料8例のうち、4例の腎から、ウイルスと思われる病原体を分離し得た。そして、その病原体を初生腎および28日未熟に接種することにより、自然発生例と同様の病変を再現することができた。

以上の結果を基として、文献研究を加えて、若干の考察を行なった。なかでも、本病はGumboro病との関係の問題として残ることを述べた。また、上記の組織病理発生学的所見を重視して、本病を伝達性(恐らくウイルス性)尿細管腎症と呼称するのが適当であることを提唱した。

EXPLANATION of PLATES

Figs.1～4 show macroscopical changes of kidneys. Figs.5～13 are photomicrographs, all of which were taken from sections of kidneys, except one (Fig.13 for the ureter), and stained with hematoxylin and cosin.

PLATE I

Fig. 1. Slight swelling and discoloration of kidneys. The parenchyma is slightly cloudy. The left ureter is dilated. Case 4 (autopsy No.725). 41 days old.

Fig. 2. Kidneys are swollen fairly markedly. The parenchyma is cloudy, containing a number of pale whitish-gray minute foci spotty or meshy in shape. Both ureters are dilated and filled with whitish muddy substance. Case 6 (autopsy No.728). 41 days old.

Fig. 3. Kidneys show swelling and marked discoloration. The parenchyma contains a number of whitish foci spotty or meshy in shape. Both ureters, especially the left one, are
dilated and filled with whitish muddy substance. Case 7 (autopsy No. 942). 50 days old.

Fig. 4. Kidneys show marked swelling and discoloration. The parenchyma is cloudy and contains whitish foci presenting a minute network pattern. This picture was taken from a chick experimentally affected in the following manner. A suspension of kidney from a naturally infected chick (case 6 in Fig. 2) was injected into the chorioallantoic cavity of an embryoing hen's egg. Then one drop of the infected chorioallantoic fluid was inoculated intraocularly into a 1-day-old chick, which died (autopsy No. 757) 11 days after inoculation and from which the picture was taken. The experimental results of this investigation will be reported in a paper to come.

PLATE II

Fig. 5. Almost all the tubules (mainly the proximal and distal convoluted portions) are dilated highly. Their lumen contains amorphous fluid substances stained poorly with eosin, and a few desquamated degenerative epithelial cells. The renal epithelial cells show degenerative and necrobiotic alterations. Case 2 (autopsy No. 719). 37 days old. ×263.

Fig. 6. Four focal necrotic foci are seen in the parenchyma of the kidney. In each of them a few heterophils are present. Degenerated nuclei are in the peripheral zone of each focus. An arrow indicates an accumulation of urate-like substance with multinucleate giant cells. Some of the distal convoluted and collecting tubules present dilatation of the lumen and infiltration of a few heterophils. Degenerative changes occurred to renal epithelial cells. Case 3 (autopsy No. 721). 37 days old. ×131.

Fig. 7. Distal tubules are dilated chiefly. Some portions of these tubules exhibited infiltration of heterophils, accumulation of a small amount of fluid substances stained poorly with eosin, and desquamation, flattening, and degenerative alterations of renal epithelial cells. Some epithelial cells show fatty degeneration. Proximal tubules exhibit degenerative and desquamative changes of the epithelium and sometimes infiltration of a few heterophils. Interstitial connective tissue also presents infiltration of a few heterophils. Case 1 (autopsy No. 718). 37 days old. ×131.

Fig. 8. Degenerative and desquamative changes are shown in the epithelium of some distal tubules. These tubules contain small round crystals (arrow) stained rather deeply with eosin. Accumulations of heterophils are present in the parenchyma and interstitial connective tissue in the central part of the picture. Case 6 (autopsy No. 728). 41 days old. ×263.

Fig. 9. Coagulation necrosis of parenchymatous cells is shown. Infiltration of a few heterophils is present in the interstitial connective tissue. Case 8 (autopsy No. 90054). 51 days old. ×131.

PLATE III

Fig. 10. Part of the medulla of the kidney is shown. Many collecting tubules or ducts are highly dilated. Their lumina contain accumulations of desquamated renal epithelial cells and fluid substances stained poorly with eosin. Their epithelia are affected with degeneration, desquamation, loss, and flattening. A slight proliferation of juvenile fibrocytic cells is present in the interstitial connective tissue. Case 1 (autopsy No. 718). 37 days old. ×131.

Fig. 11. Collecting and papillary ducts are highly dilated. The former contain accumulated heterophils in some portions. The latter have a lumen filled with fluid substance, including a small amount of cell debris, stained poorly with eosin. In the papillary ducts, the wall has fallen down, and the fluid substance has exuded into the peripheral interstitial connective tissue. Diffuse proliferation of juvenile fibrocytic cells is seen in the interstitial connective tissue. Case 8 (autopsy No. 90054). 51 days old. ×32.

Fig. 12. Part of the medulla of the kidney is shown. Collecting and papillary ducts are dilated. In them, the lumen contained desquamated epithelial cells, heterophils,
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fluid substance stained poorly with cosin, and needle-shaped crystalline substances. The epithelial lining was lost in some ducts and multinucleate giant cells appeared in others. Juvenile fibrocytic cells were proliferated in the interstitial connective tissue. Case 7 (autopsy No. 942). 50 days old. ×52.

Fig. 13. Ureter. The dilated lumen contains a large amount of fluid substance, including heterophils, stained poorly with cosin. The epithelial cells are hyperplastic. The submucous coat shows an increase of lymphocytes and a proliferation of fibrocytic cells. Case 3 (autopsy No. 721). 37 days old. ×151.