A THESIS PRESENTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN THE

UNIVERSITY OF EDINBURGH.
STUDIES ON NEPHRITIS IN THE DOMESTIC FOWL.

by

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XII. APPENDIX
Pathologists working with poultry know how very frequently diseases of the urinary system are encountered. Generally speaking, such cases are often reported as "nephritis" when the kidneys show superficial deviations from the normal as swelling, alterations in colour, congestion and excessive prominence of urates within their substance. Frequently the diagnosis is unjustified; sometimes it is frankly incorrect. Because of the large numbers of autopsies carried out in routine laboratories, it is understandable that only in a proportion of autopsies can the macroscopical examination be supplemented by detailed histology. Consequently it is possible that in at least a minority of cases nephritis is diagnosed erroneously. Similarly, hasty examination with limited facilities may result in the more serious omission; when macroscopic lesions may not be discernible and a histological examination is not done. This error can occur easily because glomerulonephritis in its acute and possibly subacute, stages may not be associated with any macroscopic lesions (see below and Siller, 1959a).

With these reservations in mind, an approximate estimate of the incidence of nephritis can be made. A few reports are to be found in the literature, but unfortunately the figures contained therein often refer to the percentage incidence of
nephritis among total autopsies and this may have little relationship to the actual incidence of the disease in either the flock or the country as a whole. It must be remembered that the dead birds sent to the diagnostic laboratories form anything but random samples. If all birds which died were in fact submitted, the figures would be considerably more useful. Goss (1940) found a 15% incidence of nephritis among 7,408 autopsies. The figures of Blaxland (1958) are much lower at 5.3%, but Frischbier and Rindfleisch-Seyfarth (1948) record an incidence of macroscopic renal disease in 37% of their 581 poultry autopsies.

More significant, and undoubtedly more accurate, are the statistics published from the poultry progeny testing stations, where disease figures relate to the entire population and all birds are examined post-mortem. During the years 1952-54 (Poultry Production and Progeny Trials, 1952-54) nephritis was diagnosed in 20% of cases. It must be remembered again, however, that such figures cannot be applied directly to the poultry population as a whole. It is interesting to note that Blaxland (1958) illustrates this particular point when the Weybridge figures of egg peritonitis (4.2%) derived from material throughout the country are compared with those of the laying trials where the incidence is nearer 20%.
On a flock basis Greenwood and Blyth (1948) report nephritis to occur in 12.8% of pullets. Since the figures relate only to young birds it cannot have general application. Working with only a section of the same flock, the present author found that during the five years 1956 - 1960 nephritis was diagnosed in 233 (29%) of the 807 autopsies. Although this is again a figure calculated as a percentage of total autopsies, it is perhaps a little more valid, in that all dead birds were examined post mortem. Included in this 29%, however, are not only the macroscopically recognisable cases, but also those which were diagnosed histologically, sometimes as a mere chance finding, when the condition was unsuspected at autopsy.

Despite the indisputable importance of nephritis as probably the third most common disease of poultry in this country, few references to it appear in the literature. Many of the major text books on poultry diseases merely mention the condition in passing; Biester and Schwarte (1959) do not even list nephritis in the index or discuss the condition in the text.

Spector (1951) published a short monograph on this disease. He was supported in his study by the Agricultural Research Council who, as Spector writes in the preface, recognised "nephritis" as one of the most important causes of loss in poultry flocks.
The main purpose of the present investigation was to study the spontaneous nephritides in birds of the Poultry Research Centre. In view of the criticism already made, emphasis was placed on the histological study, this being the most neglected aspect of the disease. A classification of fowl nephritis can be suggested on the basis of this investigation.

It has long been recognised that gout, at least the visceral form of gout, in birds is associated with nephritis. This complex is therefore usually termed "nephritis and visceral gout". Thus it is obvious that any classification of avian nephritides would not be complete if gout were not included. For this purpose a detailed discussion of both forms of avian gout is included in this investigation.
II. MATERIAL and METHODS.

(A) Materials

The birds used in this investigation originated mainly from the in-bred flock of Brown Leghorns of the Poultry Research Centre. The main part of this study is a pathological investigation of the nephritides occurring in fowls and therefore the bulk of the material used came from routine autopsies. In addition, pathological material was obtained from other sources. Normal birds for anatomical study and experimental procedures were also used. For the sake of clarity the entire animals material used in this investigation is divided into appropriate groups according to their source and/or uses.

Group 1 (Routine autopsy material)

All animals of the Poultry Research Centre flock which died or were killed in extremis were examined post mortem by the author. In the five years (1956-1960) this amounted to 807 autopsies.

Group 2 (Additional pathological material)

(a) Proliferative Glomerulonephritis.

The tissue blocks of 21 birds which had been affected with "messy vent" and which had been collected by Dr. J.G. Campbell (of the BECC unit at the Poultry Research Centre) during the years 1954-1956, were used to supplement the study on proliferative Glomerulonephritis.
(b) Oestrogen-secreting tumours.

The kidney sections from five birds with oestrogen-secreting tumours were provided by Dr. J.G. Campbell, who diagnosed the neoplasms as thecal (1 case), granulosa (2 cases) and thecal and granulosa tumours (2 cases).

(c) Senecio poisoning.

Dr. J.G. Campbell also lent some kidney sections from fowls which had been fed on an experimental diet containing dried ragwort (Senecio jacobaea L.) or its component alkaloid seneciphylline.

(d) Membranous glomerulonephritis.

Tissue blocks for histology from nine turkey poults which had died from so-called Turkey-X-Disease (ground-nut poisoning) were provided by Mr. D.C. Ostler, M.R.C.V.S., Assistant Veterinary Investigation Officer, Reading.

Paraffin blocks of kidneys and other organs from ten field cases of ground-nut poisoning in fowls were received from Mr. L.A. Hemsley, M.R.C.V.S.

This histological material from turkeys and fowls forms the basis of the study on membranous glomerulonephritis.

(e) Articular Gout.

This condition is not encountered in the Poultry Research Centre flock. From the Ministry of Agriculture Veterinary Laboratory at Lasswade,
three cases of articular gout were obtained; one was received alive.

**Group 3 (Normal kidneys for Histology)**

Kidney sections were made from normal Brown Leghorn fowls (P.R.C.) at different ages. There were thus 72 males and 72 females, divided into 12 age groups ranging from 1 to 12 weeks of age (each of these age groups, therefore, contained 6 males and 6 females).

**Group 4 (Oestrogen administration)**

(a) Stilboestrol implants.

30 mg of stilboestrol, B.P. (Boots) was implanted subcutaneously into 6 males and 6 females, 7 weeks of age. These 12 chicks were killed 4 weeks later, when 11 weeks old.

(b) Lipamone feeding.

In the course of another experiment, Dr. A.W. Greenwood fed lipamone (dienoestrol diacetate) to fowls from hatching, at a level of 0.025% of dry mash. When any of these birds died they were examined by the author.

**Group 5 (Carbon tetrachloride poisoning)**

Six 10-week old fowls (3 males and 3 females) were given 6 oral doses of CCl₄ (1ml per day) in the course of one week. They were killed on the day after the last administration, at an age of 11 weeks.
(B) Methods

All birds were autopsied as soon after death as possible in order to minimise post-mortem autolysis. After noting any macroscopic changes, tissue blocks from the kidneys and other organs were routinely fixed in Susa. For specific purposes duplicate blocks were also fixed in 10% formol-saline and, for the demonstration of urate crystals, in absolute alcohol.

Sections from paraffin blocks were cut at 5µ, frozen sections of formalin fixed material were 10/µ-15/µ in thickness.

All kidney sections were routinely stained with haematoxyline and eosin and by the periodic-acid Schiff method (P.A.S.). Numerous other specific stains were used on the variously fixed kidney sections; the results of these special procedures are dealt with in the histological descriptions.
III. ANATOMICAL CONSIDERATIONS

The anatomy of the avian kidney has been extensively investigated. The main impetus on this subject was given by Spanner, who in 1924-25 published an important paper on the renal-portal system of birds. The gross anatomy is described in many books, theses and shorter publications (Bradley and Grahame, 1950; van den Broek, van Gerd and Hirsch, 1931-33; Goodchild, 1956; Gordeuk and Grundy, 1950; Spector, 1951; Sperber, 1949 and others).

Since the gross anatomy is of little direct relevance to the subsequent discussion, there is little necessity to go into its details.

The avian kidney is relatively much larger than the mammalian one, and the nephrons more numerous. In various species of birds, Li Koue Tsjang (1923) found between 90 and 450 glomeruli per cubic mm. of cortical substance, compared with 4-15 glomeruli in a comparable area of mammalian tissue. Kohda (1934) has shown that birds have more glomeruli relative to their body weight than do mammals, reptiles or amphibia. On the other hand, Kohda claims that mammalian glomeruli are larger on the average than those of birds.

The arrangement of the nephric tubules has been studied by Huber (1907) and Feldotto (1929). The central nephrons are comparable with those of mammals by virtue of the presence of both short and long loops,
Fig. 1
Normal peripheral glomerulus.
H & E. x 850

Fig. 2
Normal juxta-medullary glomerulus. This is considerably (about three times) larger than the peripheral glomerulus. The macula densa is well illustrated at the bottom left of the glomerulus. Note the large PCT surrounding the glomerulus.
PAS. x 850
while the peripheral ones are said to be similar to those of reptiles.

At first sight it would appear that the avian kidney has no sharp division between cortex and medulla; this is not so. In fact, the avian kidney is sub-divided into very many lobules, each of which is supplied by a centrally situated intra-lobular vein. In section the glomeruli are arranged in a horse shoe shape around this intra-lobular vein with the open ends of the horse shoe pointing inwards, away from the periphery. The central glomeruli are larger than the peripheral ones (Huber, 1907) as illustrated in figures 1 and 2.

(a) The normal Glomerulus.

The structures of the avian and mammalian glomeruli are substantially different. Smith (1951) states that the glomerular development in birds is poor: "The glomeruli are small and heavily invaded by inert tissue, the glomerular tuft sometimes being reduced to a few short capillaries. No aglomerular bird is known, but even the chicken is not far from this state". He further states that in marine birds which have no access to fresh water for very prolonged periods, glomerular function may be nearly zero: "The degeneration of the avian glomeruli", Smith believes, "is related to the uric acid habitus of birds".

Bowman's capsule is usually lined by flattened epithelium (Figs. 1 and 2). In birds as opposed to mammals, the glomerular tufts appear much denser and
Fig. 3
Normal rabbit’s glomerulus. Note the absence of a “central cell mass”. The tuft is much more highly vascularised than in the fowl.

H & E  x 850

Fig. 4
Normal glomerulus of Testudo graeca. There is no central mesangium and the capillary loops are much more highly developed than in the fowl. This glomerulus is rather similar to the mammalian one depicted above.

PAS  x 850
more cellular because of the prominent central mesangium about which the capillary loops are arranged (Figs. 1 and 2). Generally speaking, the lumen of these capillary loops is narrower in the mammal, where this central accumulation of cells is not present (Fig. 3). In birds, also, the glomerular capillary loops are fewer in number, although like the mammalian ones, they are lined by a distinct PAS-positive basement membrane. More diffusely arranged PAS-positive material extends without sharp demarcation from this basement membrane to the stroma of the tuft.

In an electron microscopical study of the avian glomerulus particular attention was paid by Pak Foy and Robertson (1957) to this mesangium or "central cell mass" as they termed it. They concluded that these "mesangial" cells were not endothelial but were connective tissue cells. The inter-cellular material surrounding these cells was shown by these workers to be continuous with the capillary basement membrane.

v. Möllendorf (1930) is of the opinion that the peripheral glomeruli of birds are similar to those of reptiles, while the central ones resemble those of mammals. It has been stated (Marshall, 1934) that the reptilian glomerulus is very similar to the avian and that its poor vascularisation presupposes a small glomerular filtration surface. Pak Foy (1959) studied the electron
TABLE I

SHOWING THE INCIDENCE OF COLUMNAR EPITHELIUM IN BOWMAN'S CAPSULE IN FOWLS OF DIFFERENT AGES AND SEX.

<table>
<thead>
<tr>
<th>AGE (WEEKS)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>MALES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO. OF SECTIONS COUNTED (ONE PER BIRD)</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>TOTAL NO. OF GLOMERULI (IN ALL SECTIONS)</td>
<td>1197</td>
<td>1105</td>
<td>960</td>
<td>1040</td>
<td>1402</td>
<td>1292</td>
</tr>
<tr>
<td>PERCENTAGE OF GLOMERULI WITH COLUMNAR CAPSULES</td>
<td>0.08</td>
<td>7.78</td>
<td>3.64</td>
<td>0.10</td>
<td>4.49</td>
<td>0.23</td>
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<tr>
<td>FEMALES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO. OF SECTIONS COUNTED (ONE PER BIRD)</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>TOTAL NO. OF GLOMERULI (IN ALL SECTIONS)</td>
<td>1044</td>
<td>1126</td>
<td>1029</td>
<td>832</td>
<td>1441</td>
<td>1264</td>
</tr>
<tr>
<td>PERCENTAGE OF GLOMERULI WITH COLUMNAR CAPSULES</td>
<td>0.1</td>
<td>0.09</td>
<td>0.87</td>
<td>0.12</td>
<td>0.14</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Fig. 5
Normal intermediary glomerulus of a fowl showing the normal squamous epithelium lining Bowman's capsule and merging into the columnar epithelium of the first part of the PCT.
PAS x 850

Fig. 6
Glomerulus of a normal 2-week old male fowl showing cuboidal epithelium lining Bowman's capsule. The beginning of the PCT is also shown.
PAS x 850
Fig. 7
Glomerulus of a normal 2-week old male fowl, showing the vascular pole and the cuboidal epithelium of Bowman's capsule.

PAS x 850

Fig. 8
Histogram taken from the results in table I, showing the percentage incidence of glomeruli with cuboidal metaplasia in males (yellow) and females (blue) of various ages.
microscopical appearance of the reptilian glomerulus, using the kidney of a lizard \( (Trachysaurus \text{rugosa}) \). Although a "central cell mass" was present, it was not as pronounced as that of the bird. The present author has had opportunity to examine a number of normal and diseased kidneys from various tortoises \( (Testudo \text{spp}) \) and has not observed this central cell mass with the light microscope \( (\text{fig. 4}) \). It therefore seems that the glomeruli of these reptiles may more closely approximate the mammalian type than the avian type.

\( (B) \) Columnar metaplasia of Bowman's capsule.

A number of kidney sections from normal fowls of different ages were examined and it was found that although the majority of avian glomeruli have a capsule with flattened squamous epithelium \( (\text{fig. 5}) \), there are some where this epithelium is cuboidal \( (\text{figs. 6 and 7}) \). This cuboidal "metaplasia" is found most frequently in males, particularly between 2 and 3 weeks of age and again at 5 weeks, although in all young birds of either sex (at least in those of the PAC flock of Brown Leghorns) some glomeruli of this type may be found; see table I and \( \text{fig. 8} \). In adults of both sexes, such capsules are rare.

This type of glomerular capsule has been observed in a number of mammals \( (\text{Helmholz, 1935}) \). He also saw it in a pyelonephritic human infant and in a 9-year-old boy. Risak \( (1928) \) reported a similar finding in an adult woman with pyelonephritis. More recently, it has been described in human kidneys of
both sexes (Finckh and Joshe, 1954). In the human kidney similar changes in Bowman's capsule were interpreted as adenomatoid transformation by Eisen (1946) and these were believed to be associated with adrenal neoplasia by Chappell and Phillips (1950) and with liver carcinoma by Nachman (1962). Crabtree (1941) has shown that cuboidal epithelium in Bowman's capsule occurs normally in male mice and actually constitutes a form of sex diathormism.

In birds as in mammals the visceral and parietal epithelium of the glomerulus develops by invagination of the tubular bud; at one stage of development, therefore, both these layers have a cuboidal appearance (Crabtree, 1941). Since the parietal layer is the first to assume the normal squamous appearance it is unlikely, according to Crabtree, that the occurrence of cuboidal capsular epithelium represents arrested development, as was originally suggested by Risak (1928).

Selye (1939) has observed large numbers of this type of capsule in female mice treated with testosterone. Crabtree (1941) found squamous epithelium in sexually mature mice of both sexes; with increasing age it becomes cuboidal. This metaplastic process is more rapid in males in which the final incidence may approach 100%.

Male chicks of less than 10 days old are heavily androgenised and between 30 and 40 days a further peak of androgenisation takes place (Breneman and Watson, 1951). These two peaks
of androgenisation would agree fairly well with the results obtained in the present study, which are summarised in table I and in the histogram (fig. 8). Campbell (1960) has also found by an indirect method that the androgen-oestrogen balance in male fowls under 6 weeks of age, appears to be on the androgen side. There seems little doubt, therefore, that this cuboidal metaplasia is a form of androgen response. The case of Chappell and Phillips (1950) is of interest here, since the adrenal neoplasm found in the patient with the cuboidal epithelium may well have been a virilising adrenal tumour.

(c) The tubules

Histologically the tubules are very similar to those of mammals. The large thick-walled proximal convoluted tubules (PCT) have tall columnar epithelium and a usually quite distinct luminal, PAS-positive brush-border (fig. 6). The distal convoluted tubules (DCT) are very much smaller, their walls are thinner and their comparatively low columnar epithelium is more basophilic than that of the PCT (figs 1 and 2). The epithelium of the loops of Henle is in some ways similar to that of the DCT but the cells are somewhat taller. Those of the very large, prominent collecting tubules, which are generally arranged in groups forming the medulla, are lightly stained and of greater diameter than the PCT. The ureter has a stratified epithelium which stains vividly with PAS; the epithelium of its branches has two layers.
Fig. 9
Cortical region of a kidney from a normal fowl, showing a secondary lymphoid focus.
H & E x 212

Fig. 10
Kidney of a 1-week old male chick, illustrating embryonic renal tissue.
H & E x 680
According to Goodchild (1956) the drainage area of each of these branches comprises a lobe. He maintains, rightly, that it is wrong to describe the avian kidney as consisting of three "lobes", as is so frequently done. These anterior, middle and posterior "lobes" are referred by him as divisions and they are made up of a number of lobes.

**Lymphoid nodules**

It is well known that areas of lymphoid tissue, so-called secondary lymphoid nodules are found in most organs of the fowl (Oakberg and Lucas, 1949). These are also present in the kidneys (fig.9) and should not be mistaken for pathological infiltrations of inflammatory cells.

**Embryonic rests**

In the kidneys of young birds there are numerous aggregations of embryonic tissue (fig.10) which stain more basophilically than mature renal tissue and which are usually situated at the periphery of the lobules. These embryonic cell masses are often unrecognisable as tubules or glomeruli, although they undoubtedly have the potential to develop into these. The embryonic renal tissue decreases in amount with increasing age and usually in birds over about 8 weeks of age it becomes difficult to find (Campbell, personal communication). The importance of these structures is shown by their neoplastic potentialities (Carr, 1956,1960).
Their physiological significance is not known, however.

(F) **Renal-portal system**

Consideration is now given to what is perhaps the best studied aspect of renal anatomy - the renal-portal system. The question of the existence or non-existence of such a renal-portal system in birds, and also in reptiles and amphibia, has been the source of considerable dispute. It is surprising that it is not only the physiological function of this system that was being disputed, for this is obviously difficult to prove, but also its purely anatomical existence. (The eminent anatomist Hyrtl (1863) actually denied such a system not only in birds but also in reptiles and other animals). This whole controversy is thoroughly reviewed by Sperber (1949) who points out that it was mainly the eminence and reputation of the participants in the argument, which influenced the opinions of other later workers.

Following the admirable work of Spanner (1924-25), however, there can be no doubt whatever of a renal-portal system being present in birds. It was particularly difficult to show that this system does, in fact, function. The early attempts to prove this by Spanner (1924-25) and Das (1931) were rejected by subsequent workers on the grounds that their experimental procedures had produced a non-physiological state which made the interpretation of their results unreliable. The same criticism is
applicable to the much more recent work of Gordeuk and Grundy (1950) and Cuypers (1959).

Sperber (1949) was the first to tackle the problem of functional activity of the renal-portal system in fowls by a different and more reliable method. He injected phenol red intra-muscularly into one leg and, collecting the output of each ureter separately, found the kidney ipsilateral to the injection site to secrete more phenol red than the kidney draining the opposite leg. This effect was interpreted as evidence that dye from the injected leg reached the ipsilateral kidney directly through the renal-portal system and was excreted, in part at least, before entering the general circulation. These results were confirmed by Rennick and Gandia (1954) using p-amino-hippurate. Levinsky and Davidson (1957) demonstrated a stimulated phosphate excretion by the kidney when the ipsilateral leg was infused with parathormone.

The criticism of unphysiological conditions applied far less, or not at all, in these experiments, but an observation made at this Centre confirms the existence of a functional renal-portal system in a fowl which had not been subjected to any experimental procedure whatsoever (Siller, 1959 b. see appendix). An osteogenic sarcoma had developed spontaneously at the proximal extremity of the right tibia of a Brown Leghorn hen. Although metastases of this neoplasm were present in both lungs, of all the other organs
in the body only the ipselateral kidney carried metastases. Since, in man at least, osteogenic sarcomata metastasise most frequently to the lungs (as much as 75% according to Willis, 1953) and since the spread of the tumour cells is usually by the haematogenous route, this involvement of the ipselateral kidney affords direct evidence of spread through the renal-portal system. This theory was later confirmed (Siller and Carr, 1961; see appendix) when metastases of experimental leg tumours were found to localise with extremely high frequency in the ipselateral kidney, usually in preference to other organs.

Although it has been established that there is not only an anatomical but also a functional renal-portal system in fowls, its true significance and its physio-pathological importance is still completely unknown. It is difficult to conceive the purpose served by "filtering" the venous blood from the legs through the kidneys. Smith (1951) believes that the existence of the renal-portal system is closely associated with the predominance of tubular excretion over glomerular filtration in birds. He further suggests that the renal-portal system is an inherent characteristic of the mesonephric kidney. Only mammals, which have developed a metanephric kidney, lose their renal-portal system during embryonic development. Thus Smith (1951) suggests that adult fowls do not have a metanephric but a mesonephric kidney. This is not correct.
Fig. 11

Diagram taken from Spanner (1924-25) to illustrate the circulation in the avian kidney. It shows the arterial supply (black), the afferent venous supply of the renal-portal system (dense stippling) and the efferent veins (light stippling).
Lillie (1952) describes the development of the metanephros in fowls; furthermore it is still present in very young chicks as a relatively large Wolffian body near the anterior pole of the definitive kidney.

Not all the afferent venous blood in this portal system is derived from the legs, since at the posterior end the efferent renal veins from either side are connected by a venous arch which also receives blood from the posterior part of the abdomen through the coccygeo-mesenteric vein, which anastomoses with the mesenteric vein (fig. 11). This mesenteric vein, therefore, communicates both with the hepatic-portal system (anteriorly) and the renal-portal system (posteriorly). The circulation in this vein is difficult to understand and no experiments have so far clarified the position. It would seem that there must be a point along the course of this mesenteric vein, proximal to which the blood flows in a cephalad direction, while distal to it, it is caudally directed.

Not all the afferent venous blood from the legs is filtered through the kidneys directly, some of it flows into the posterior vena cava. This "direct" flow is controlled by a valve (Kl.N. in Fig.11) situated at the junction of the femoral vein (V. iliaca, Spanner) and the posterior vena cava. The morphology of this valve was carefully studied in different birds by Spanner (1935). Rennick and Gandia (1954) have suggested that this valve is under nervous control and
Gilbert (1961) has actually demonstrated the nerve fibres within it.

The controlling mechanism of this valve, and therefore of the amount of blood permitted to go directly to the heart, or conversely via the kidneys, is not known.

Since the portal system supplied the tubules, however, it may well be of some direct importance in the development of the descending type of pyelonephritis (see section V), since inflammatory cells (heterophils) appear to accumulate in the peritubular region, or that which the afferent renal veins are believed to supply.

Smith (1951) suggests that in birds shunts may exist between the renal-portal system and the efferent renal vein, making it possible for blood from the former to by-pass the kidney. These organs may similarly be by-passed to some extent by way of the coccygeo-mesenteric veins. This would permit blood to flow from the renal-portal to the hepatic-portal system.
THE NEPHRITIDES OF THE FOWL

Many avian pathologists, when referring to nephritis, imply that condition which is described in Section V as pyelonephritis. A differentiation into glomerulonephritis, interstitial nephritis and pyelonephritis is rarely made. This is perhaps understandable since by far the most common form is pyelonephritis, which in most cases is readily recognised macroscopically and which is frequently associated with visceral gout. Pyelonephritis and "nephritis and visceral gout" were diagnosed in 78% of the nephritic cases in the present study.

That other forms of nephritis can occur in birds has been pointed out by Frischbier and Rindfleisch-Seyfarth (1948), but they mention only glomerulonephritis and interstitial nephritis. The 51 cases examined by Spector (1951) were all of the pyelonephritic type and he states quite categorically that in no case were the glomeruli or blood vessels the seat of primary disease. All three types of nephritis have, however, been described by various authors; reference to this may be found in Reis and Nobrega (undated).

In the material of the present study all three types of nephritis were observed and their macroscopic and histological appearance is described in detail below.
Because of the absence of adequate clinical symptoms and the fact that the aetiology of this condition is unknown, the purely histological classification of Allen (1951) has been adopted here. Allen mentions five types of acute glomerulonephritis; namely; proliferative, exudative, necrotising, haemorrhagic and membranous. Both membranous and proliferative types were observed in fowls of the present study; although Frischbier and Rindfleisch-Seyfarth (1948) described an exudative form of glomerulonephritis in birds, this was not observed.

A. Proliferative Glomerulonephritis

This form occurs more frequently than might be imagined. Its presence is often unsuspected at autopsy; macroscopically the kidneys may appear normal, yet under the microscope the glomeruli show the typical proliferative lesions.

A full description has already been published under the title of "The Pathology of Avian Glomerulonephritis" (Siller, 1959 a), which is enclosed in the appendix.

Material.

As already stated, 233 cases of nephritis were diagnosed during routine autopsies at the Poultry Research Centre, and 47 of these had proliferative glomerulonephritis. The following description is
based on the study of these, together with the histological material of another 21 cases kindly lent by Dr. J.G. Campbell.

(2) **Incidence**

During the years 1956-1960 the incidence of proliferative glomerulonephritis was in the region of 20% of all nephritis cases, or 5.8% of all autopsies. The majority of the 68 cases showed the histological characteristics of the chronic form; only 18 (26%) were diagnosed as acute glomerulonephritis. The limited data thus available suggest that females are more frequently affected than males. Among the 68 cases 62 birds were female. There were only 5 males and 1 capon. However, the female:male ratio in our routine autopsy material is about 5:1; in the older age groups particularly, more females than males are kept.

There is no definite age incidence; birds from less than 1 up to 3 years were affected. Most cases occur, however, in year-old pullets, at the height of egg production.

(3) **Clinical symptoms.**

Attention is drawn to chronically affected birds by a drop in egg production, accompanied by swelling and ulceration of the cloacal mucous membrane, which arises from irritation by the copious and continuous discharge of urine, as shown by a characteristic white soiling of the feathers surrounding the vent. The condition is
**TABLE II**

THE CAUSES OF DEATH AND INTERCURRENT CONDITIONS IN 26 BIRDS
IN WHICH GLOMERULONEPHRITIS WAS DIAGNOSED

<table>
<thead>
<tr>
<th>CAUSE OF DEATH</th>
<th>NO. OF BIRDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerulonephritis alone</td>
<td>6</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>4</td>
</tr>
<tr>
<td>Pericarditis</td>
<td>4</td>
</tr>
<tr>
<td>&quot;Egg peritonitis&quot;</td>
<td>3</td>
</tr>
<tr>
<td>Post-operative peritonitis</td>
<td>1</td>
</tr>
<tr>
<td>Rectal prolapse</td>
<td>2</td>
</tr>
<tr>
<td>Neoplasia (adenocarcinoma of the oviduct, histiocytic cell sarcoma)</td>
<td>2</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>1</td>
</tr>
<tr>
<td>&quot;Cage Layer Fatigue&quot; (Bell &amp; Siller, 1962)</td>
<td>1</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>1</td>
</tr>
<tr>
<td>Ventricular septal defect (Siller, 1958)</td>
<td>1</td>
</tr>
</tbody>
</table>
popularly known at the Poultry Research Centre as "messy vent".

Most acute cases, and undoubtedly also many of the more chronic cases of short duration, show no clinical abnormalities. They can be diagnosed only at autopsy and may require histological examination of the kidneys.

Blood films, especially in the later stages of the disease, show heterophilia and monocytosis: heterophil granulocytes rise from a normal of about 25-30\% to 35-40\% and monocytes from about 3-6\% to as much as 35-45\% in very severe cases.

Between 1955 and 1957 this form of proliferative glomerulonephritis was present at the P.R.C. in almost epidemic form. Since then, however, only sporadic cases have been seen. No satisfactory explanation can be given for this change in the epidemiological manifestations of this disease.

(4) Post-mortem findings.

During 1956 - 1960, 26 of the 47 birds affected with glomerulonephritis had died, while the remaining 21 had been killed. Of the 26 birds which had died, in only 6 (23\%) could the cause of death be directly attributed to renal disease; no complicating extra-renal lesions were found. Table II shows the variety of the other, and perhaps unrelated, conditions which were observed in the remaining 20 glomerulonephritic birds. It may be significant, however, that 14 of these 20 fowls (70\%)
had, besides the renal lesions, some infective conditions which precipitated death; these were: pneumonia (4), pericarditis (4), peritonitis (4), hepatitis (1) and endocarditis (1).

In view of the diversity of these intercurrent conditions and their infective agents, it is doubtful whether they bear any direct relationship to the development of the kidney lesions.

The macroscopic post-mortem examinations of birds which have died from glomerulonephritis, or those which were killed during the early, or even later stages of the disease, often fail to reveal any characteristic lesions. In some cases the kidneys may be somewhat enlarged, but there is no evidence of a granular surface or scarring.

There is evidence of neither cardiac enlargement, oedema nor accumulation of fluid in the serous cavities. The aorta and large systemic blood vessels show no macroscopic abnormalities. Occasionally, however, localised atheromatous plaques are present, which are also frequent in older birds without renal lesions (Siller, 1961). Atheromata were observed in 12 birds of the present series.

(5) Histological findings.

Even in cases where clinical manifestations are minimal and where the macroscopic examination shows no deviation from the normal, well marked and characteristic renal lesions can be recognised microscopically, and acute and chronic changes can be differentiated.
Very early glomerular changes in acute Glomerulonephritis. There is a tremendous enlargement and ischaemia of the glomerulus, swelling of the epithelium and proliferation of endothelial cells with obliteration of the capillary space.

**H & E**

x 580

---

**Fig. 13**

Acute Glomerulonephritis. Hyaline droplet degeneration of the swollen epithelial cells.

**H & E**

x 850
Fig. 14

High power photograph of a portion of a glomerular tuft, illustrating the hyaline droplet degeneration of the epithelial cells in acute glomerulonephritis. Note the thickened basement membranes.

PAS x 1900

Fig. 15

Glomerulus in acute glomerulonephritis. The capsular space is still obliterated and the tuft is swollen owing to massive endothelial proliferation.

PAS x 850
(a) **Acute proliferative Glomerulonephritis.**

During the acute stage the histology of the kidney is typical of acute proliferative diffuse glomerulonephritis, although all the glomeruli do not become involved simultaneously. The larger juxta-medullary glomeruli are the first to be affected. The most striking change is a marked enlargement due to an increase in the size of the tuft. This is brought about by a swelling of both the visceral and the parietal epithelium and a proliferation of the capillary endothelial cells and the mesangial cells in the centre of the tuft. The parietal epithelium of Bowman's capsule and the visceral epithelium of the tuft consequently lie, without adhesions, in close apposition and fill the capsular space, so that the glomerular capillaries are compressed and empty (fig. 12). Later the initial swelling of the visceral epithelium is succeeded by degenerative changes characterised by the appearance of PAS-positive hyaline droplets within the cells (figs. 13 and 14). The capillary loops resume patency, but their basement membranes become thickened and sometimes duplicated (figs. 15 and 16). Even with abatement of the early swelling of the epithelium, the tuft still remains somewhat enlarged, owing to the maintained proliferation of the endothelial cells. Subsequently more and more glomeruli become involved in this way until the majority show changes. The renal tubules remain normal.
Chronic glomerulonephritis. Note the cuboidal cells in the parietal epithelium, the periglomerular fibrosis and the proliferation of the mesangial cells.

H & E x 850

A group of severely affected peripheral glomeruli in the chronic stage, showing massive fibrosis and shrinkage of the tufts.

H & E x 170
Fig. 20
Enlarged and distorted glomeruli in chronic glomerulonephritis. There is a thickening of the blood vessels.
H & E
x 140

Fig. 21
Two glomeruli in chronic glomerulonephritis. Note the capsular fibrosis and the close chain of bead-like epithelial cells surrounding the tufts.
H & E
x 640
(b) **Chronic proliferative glomerulonephritis.**

The lesions characteristic of the chronic stage can generally be interpreted as a direct extension of the acute lesions. Blood vessels and, to a lesser extent, the tubules also become involved.

(i) **Glomerular changes.** All glomeruli are enlarged and the capsular spaces become more distinct because of shrinkage of the tufts (fig. 17). Bowman's capsule is frequently thickened, partly because the epithelium has become cuboidal (fig 18) and partly from the development of a pericapsular fibrosis, which may be massive (fig. 19). In the very distorted glomerular tufts the swelling and the hyaline droplet degeneration of the epithelium disappear and the thickened capillary basement membranes are no longer obvious. A striking feature at this stage is increased cellularity of the tuft, its centre being transformed into a mass of endothelial cells (figs. 16 and 20). The epithelium of the periphery of the tuft also undergoes considerable hyperplasia, resulting in the formation of a close chain of cells which envelop the tuft (fig. 21).

Though there is a mild increase in argyrophil fibres within the glomerular tuft, there is no true fibrosis, typical of human chronic glomerulonephritis. At the same time the tuft is shrunken, being markedly distorted and atrophic, showing a persistent ischaemia (fig. 22). Crescents are rare and, when present, consist of a more or less loose mass of proliferating
Fig. 24
Chronic glomerulonephritis. The blood vessels are thickened and the glomerular tufts are distorted.

H & E x 170

Fig. 25
Chronic glomerulonephritis. Atrophy of the glomerular tuft and thickening of the arterial walls. The vessel on the left shows some thickened collagen fibres.

H & E x 640
Despite Sperber's experimental results and his carefully summarised evidence for and against the existence of a functional renal-portal system there are some (e.g. Barth, 1949) who still question its existence in the adult fowl.

**SUMMARY**

A case of spontaneous osteogenic sarcoma is described in a seven-year-old female fowl. The primary tumour developed on the proximal extremity of the right tibia and there were metastases in the right kidney and both lungs. The radiological and histological examination of this neoplasm showed the characteristic picture of human osteogenic sarcomata. A high plasma alkaline phosphatase activity was observed in this fowl. Transmission experiments were negative.

The occurrence of the isolateral renal metastases is interpreted as additional evidence for the existence in adult fowls of a functional renal portal system.

The author thanks Dr. D. J. Bell of the Biochemical Section for the determination of the alkaline phosphatase activity; he also thanks Dr. J. G. Carr of the British Empire Cancer Campaign Unit for carrying out the transplantation experiments.

**REFERENCES**


**EXPLANATION OF PLATES**

**Fig. 1.**—Radiograph of the right leg, showing a large calcified tumour at the proximal end of the tibia. The femur and the femoro-tibial joint were not affected. Note the partially calcified tendons.

**Fig. 2.**—Section of the primary tumour shown in Fig. 1. Note the high degree of differentiation with the formation of osteoid and calcified bone. There are large masses of mature osteoblasts, but no osteoclasts. H. & E. ×95.

**Fig. 3.**—Illustrates the more anaplastic character of the osteoblasts at the periphery of the primary tumour. H. & E. ×385.

**Fig. 4.**—Calcified and non-calcified osteoid of the tibial neoplasm, showing various degrees of maturity in the osteoblasts; some bear very pronounced processes. H. & E. ×385.

**Fig. 5.**—Infiltrative growth of the primary tumour. Note the masses of anaplastic osteoblasts invading the neighbouring muscle tissue. H. & E. ×95.

**Fig. 6.**—Metastasis in the right kidney, showing atrophied tubules and glomerulus and also mitotic figures. H. & E. ×385.
W. G. SILLER: The tumour resembles a human osteoblastic osteogenic sarcoma on the basis of the predominance of more or less anaplastic osteoblasts and the presence of a variable but definite osteoid component. Calcification was seen not only in the primary tumour but also to some extent in the metastases. According to Dahlin (1957) multinucleated giant cells are rarely seen in osteogenic sarcomata; in the present case such cells were not observed.

It is unfortunately impossible to compare the histology of the present tumour with that of other osteosarcomata occurring in fowls. The only other cases which have been found in the literature were reported by Eber and Malke (1932) and Reis and Nobrega (undated), neither of whom give any histological details.

Authorities agree that the most common sites for osteogenic sarcomata in man are the distal extremity of the femur and the proximal end of the tibia (Christensen, 1925; Geschickter and Copeland, 1949; Dahlin, 1957; Amromin, 1959). While in the avian case of Eber and Malke the primary tumour was situated on the sternum, in the present case it was found to be at the proximal extremity of the right tibia. Reis and Nobrega make no mention of the location of their tumours.

Two-thirds of the human osteogenic sarcomata occur at an age of between 10 and 30 years; very young children and old people are seldom affected (Willis, 1953). If a true comparison were at all possible, the present case would be found to fall very much outside this range; at the time when the tumour was diagnosed the hen was over seven years old, an advanced age for a domestic fowl. It is possible that one reason why this counterpart of the most common primary malignant human bone tumour is so rarely observed in fowls, is that they are not kept commercially beyond a maximum age of about 3-4 years.

The plasma alkaline phosphatase activity in this present case was 360 units; the range for non-laying, non-moulting, normal hens lies between 25 and 120 units (Bell, 1960). High alkaline phosphatase activity is also characteristic of human osteogenic sarcomata (Willis, 1953). The alkaline phosphatase of the plasma is believed to originate largely if not entirely, in the osteoblasts; the enzymic activity of the plasma may therefore be considered to reflect the amount of osteoblastic activity in the bone. In birds suffering from "cage layer fatigue", which involves a bone dystrophy, high values for plasma alkaline phosphatase (300-600 units) have been invariably found (Bell, Siller and Campbell, 1959).

In view of the fact that some bone tumours in the fowl, such as the osteoma of Fujinami (1930), the osteo-chondro-sarcoma of Tytler (1913) and the osteoid sarcoma of Pikowski and Doljanski (1946), are transplantable, it is noteworthy that the transplantation experiments failed in the present case.

Metastases to the lung occur very frequently in man. Willis (1953) assesses the incidence of secondary growths in the lungs as high as 75 per cent. Spread is generally by the haematogenous route and lymphatic dissemination is rare. In the present case both lungs carried metastases. A very interesting phenomenon is the confinement of renal metastases to that organ situated isolaterally to the affected leg. Birds possess a renal portal circulation (Spanner, 1924-25), whereby venous blood from the leg is filtered through the capillary bed of the isolateral kidney. The fact that heavy metastases were found in the right kidney, and none in the left, supports the conclusive work of Sperber (1948) on the existence of a functional renal portal system. This would provide an immediate haematogenous pathway between the primary tumour and the capillary bed in the kidney.
kidney. Both lungs carried similar tumours of varying size. The other organs, including the left kidney, showed no macroscopic abnormality.

Transmission experiment

No tumours resulted from injections of saline tumour suspension in day-old chicks. Similar material grew on the chorio-allantoic membrane of 3 out of 6 inoculated 10-day chick embryos, but further passage failed.

Histo-pathology

Material from the primary growth on the tibia was fixed in 10 per cent formal saline and decalcified. Blocks of other tissues and organs were fixed in Susa, embedded in paraffin and sectioned without previous decalcification.

The tibial tumour showed a structure typical of osteogenic sarcomata, with large areas of osteoid and calcified bone (Fig. 2) and, particularly at the periphery, areas of more or less undifferentiated spindle-shaped osteoblasts (Fig. 3). The osteoid and calcified bone was arranged in irregular trabeculae which were bordered by chains or sometimes large groups of pleomorphic, somewhat spindle-shaped osteoblasts, which frequently bore numerous processes (Fig. 4). In section the free "edge" of the tumour showed sheets of closely knit undifferentiated cells, exhibiting marked infiltrative growth into the surrounding muscle tissue (Fig. 5). Although multinucleated giant cells may have been present they were by no means obvious, even in the calcified portions of the neoplasm. In the undifferentiated peripheral portion mitotic figures, although present, were not numerous; the nucleoli of the osteoblasts in these areas were, however, large and very prominent (Fig. 3). The tumour was well vascularised and there was no evidence of necrosis.

The histological appearance of the metastases was essentially similar to that of the primary tumour. In the lungs, as well as in the kidney, these secondary growths appeared to be fairly well circumscribed and more or less spherical; they were not encapsulated and, in fact, manifested apparently rapid infiltrative growth into the tissues of the affected organs.

In the centre of these metastases, well differentiated and partially calcified osteoid was present, particularly in the lungs. This was irregularly interspersed by groups of readily recognisable osteoblasts. The peripheral infiltrating zone had a much more anaplastic character, the tumour cells exhibiting great pleomorphism and numerous mitotic figures (Fig. 6).

The apparently rapid infiltrative growth of the metastatic tumour cells in the kidney is illustrated in Fig. 6, where atrophic, but in no way degenerate, renal tubules and glomeruli were seen embedded in tumour tissue. In the lungs the appearance was essentially similar in that the alveoli tended to become filled with tumour cells, and in the peripheral portion at any rate, they retained a suggestion of lobulated structure. Areas of necrosis were not observed in the metastatic growths.

DISCUSSION

There can be little doubt that this tumour is an osteogenic sarcoma. Its histological and radiological characteristics are virtually identical with those of human osteosarcomata. According to the classification of Coventry and Dahlir
AN OSTEOGENIC SARCOMA IN THE FOWL

W. G. SILLER

From the Agricultural Research Council, Poultry Research Centre, Edinburgh, 9

Received for publication October 27, 1959

SPONTANEOUS primary bone tumours are so rare in the fowl that the occurrence of an osteogenic sarcoma is of sufficient intrinsic interest to warrant report, particularly since Feldman and Olson (1952) state that the rarity of these neoplasms has precluded adequate study.

Osteomata have been observed in the fowl by Fujinami (1930) and Heim (1931). Kaupp (1933) described a tumour which he termed a sarco-chondro-osteoma. Avian osteoclastomata have been reported by Baker (1928) and Campbell (1947). Tytler (1913) described in detail a transplantable osteo-chondro-sarcoma in fowls. Eber and Malke (1932) mentioned the occurrence of an osteosarcoma among their large collection of avian neoplasms. Reis and Nobrega (undated) state that in birds they have seen five osteogenic sarcomata, three of which presented the appearance of osteo-chondro-fibromata. Neither Eber and Malke nor Reis and Nobrega described these tumours in any detail.

This communication describes the finding of a malignant osteogenic sarcoma, which had arisen spontaneously at the proximal extremity of the right tibia, and had metastasised to the right kidney and to both lungs.

Case history

A Brown Leghorn female (A.3466) was found to be lame when over seven years old. She was one of a group of normal hens kept at the Poultry Research Centre under ordinary semi-intensive conditions as controls to a long-term environmental experiment, briefly reported on by Greenwood (1958) and Wilson (1958). A hard swelling had developed in the region of the right femoro-tibial articulation. Movement of this joint was somewhat restricted but the degree of lameness was mild. On palpation the swelling was found to be hard, apparently painless and firmly adhering to the bone. A radiograph (Fig. 1) showed a tumour involving the proximal extremity of the tibia; it affected neither the femur nor, apparently, the joint cavity. The tumour was considerably calcified and the "explosive" pattern on the X-ray was very suggestive of that seen in human osteosarcoma. The plasma alkaline phosphatase activity of this bird was 360 units (mg. of 4-nitrophenol liberated by 100 ml. of plasma at pH 10, during 60 minutes at 37° C.).

Post mortem findings

A large tumour, about 3 cm. in diameter and of very firm consistency was situated near the proximal extremity of the tibia. The femoro-tibial joint itself and the distal extremity of the femur were unaffected. No other bones of the body were found to be involved. Numerous very firm nodular metastases of up to 5 mm. in diameter were scattered throughout the substance of the right


HEIM, F.—(1931) Z. Krebsforsch., 33, 76.


Made and printed in Great Britain for H. K. Lewis & Co. Ltd., by Adlard & Son, Ltd., Bartholomew Press, Dorking.
THE SPREAD OF TUMOUR METASTASES VIA THE RENAL-PORTAL SYSTEM IN THE FOWL

BY

W. G. SILLER and J. G. CARR


BLACKWELL SCIENTIFIC PUBLICATIONS
OXFORD
The Pathology of Avian Glomerulonephritis

BY

W. G. SILLER

Agricultural Research Council Poultry Research Centre,
West Mains Road, Edinburgh
THE PATHOLOGY OF AVIAN GLOMERULONEPHRITIS

W. G. SILLER

Agricultural Research Council Poultry Research Centre,
West Mains Road, Edinburgh

(Plates XII-XVI)

"Nephritis" is the third most frequent cause of death in poultry, Goss (1940) recording this disease in 15 per cent. of 7408 autopsies. The records of the Poultry Production and Progeny Trials (1952-54) show that 20 per cent. of all deaths were due to urinary diseases. Greenwood and Blyth (1948) in their survey of pullet mortality at the Poultry Research Centre, Edinburgh (P.R.C.) for 12 years give an incidence of 12.8 per cent. My own results, which cover only a certain division of the same flock, and were obtained from birds of all age groups, suggest an even higher figure. Yet little has been written on the pathology of this condition, and what there is deals mainly with the more common pyelonephritis or the kidney lesions in visceral gout. Spector (1951) deals exclusively with these forms and states that among the 51 cases of nephritis examined histologically there were no cases in which either the glomeruli or the blood-vessels were the seat of the primary disease. This is all the more surprising since his material included 15 cases of "messy vent" obtained from the P.R.C.

The only reference to spontaneous glomerulonephritis of the fowl was made by Frischbier and Rindfleisch-Seyfarth (1948) who claimed that acute diffuse glomerulonephritis occurs in septic conditions. Their statement, with that of Spector (1951), would lead one to conclude that primary lesions of glomeruli are rare in fowls. However, glomerulonephritis has been repeatedly observed in the necropsy material of the P.R.C., where this condition is present in an endemic form.

MATERIAL AND METHODS

At the P.R.C. all birds that die or are killed because of obvious clinical disease are examined post mortem. All birds from a particular division of the flock representing all inbred lines and crosses were examined as a routine by me, and in 486 such autopsies done during two years nephritis was found in 145 cases (29.8 per cent.) among which 31 (21.4 per cent.) had glomerulonephritis. The histological and macroscopic findings as well as the clinical data on these cases form the basis of the present study. The histological material of a further 21 cases, collected during the years 1954 and 1955 and kindly lent to the author by Dr J. G. Campbell of the B.E.C.C. Unit at the P.R.C., is also included.

A simple technique has been developed for the removal of kidney biopsy material in the fowl. It allows the development of the renal lesions to be
followed histologically in the living animal, but owing to shortage of material it could be employed only on a small number of birds. The bird is anaesthetised with nembutal, and then, with appropriate precautions against haemorrhage, a portion of the posterior division of either the left or the right kidney is removed through a trephine hole in the sacrum.

Material for histology from necropsy and biopsy was fixed in Susa, embedded in paraffin and sections cut at 5 μ.

**Observations**

In the fowl, glomerulonephritis appears to affect females more frequently than males, and predominantly females at the height of egg production. There is no definite age incidence, since birds from less than one year to up to three years of age are affected, but it seems probable that most cases develop at about one year. The symptom complex which draws attention to affected birds consists in a drop in egg production, accompanied by swelling and ulceration of the cloacal mucous membrane, probably brought about by the irritation of a continuous and copious urinary discharge. This is shown by a characteristic white soiling of the feathers surrounding the vent. For this reason the condition is popularly known at the P.R.C. as "messy vent".

Blood films, especially in the later stages, show heterophilia and monocytosis: heterophil granulocytes rise from a normal of about 25-30 per cent. to 35-40 per cent. and monocytes from about 3-6 per cent. to as much as 35-45 per cent. in very severe cases. The malady runs a protracted course and the eventual mortality is comparatively high. It has not been possible so far to correlate the condition with any specific primary form of infection, although egg peritonitis or cloacal prolapse, or both, are frequently also present.

Epidemics begin with isolated cases but the condition may eventually involve 30-40 per cent. of the birds in a pen. Clinical symptoms characteristically appear in winter and subside during the summer months, but the condition frequently reappears, year after year, in the same and also other pens. All the inbred lines of the P.R.C. flock seem to be equally susceptible.

Because of the technical difficulties of collection no data are available so far on biochemical studies of urine from affected birds. Biochemical blood-analysis shows no significant deviation from the normal; the results are summarised in the table. Blood-pressure determinations arecumbersome and have not been attempted on affected birds.

**Macroscopic findings**

Nineteen of the 31 birds in the present series were killed at various stages of the disease. The remaining 12 died naturally, and in only 2 of these could the cause of death be attributed to renal failure, intercurrent disease being responsible in the rest. Three birds died of egg peritonitis, 2 of rectal prolapse with subsequent haemorrhage, 1 each of
endocarditis, internal haemorrhage, hepatitis, pulmonary oedema and post-operative peritonitis. The macroscopic post-mortem examinations of birds that have died from glomerulonephritis, or those that were killed during the early or even the latter stages of the disease, fail to reveal any characteristic lesions. In some cases the kidneys may be somewhat enlarged, but there is no evidence of a granular surface or scarring. Roughly triangular areas, reminiscent of haemorrhagic infarcts, and extending some way into the substance of the organ were observed in 8 of the 31 cases. Histology shows them, however, to be areas of extramedullary haematopoiesis, and as such areas are occasionally seen in kidneys not showing glomerular lesions they cannot be considered specific for this condition.

There is no evidence of cardiac enlargement, oedema or the accumulation of fluid in serous cavities. The aorta and large systemic blood-vessels show no macroscopic abnormalities, other than localised atheromatous plaques, which are frequent in older birds without renal lesions. Atheromata were observed in 4 birds of this series.

| Table |

**Hæmatological and biochemical data on five cases of chronic glomerulonephritis**

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
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</thead>
<tbody>
<tr>
<td>Ranges found in healthy birds (fed)</td>
<td>Laying</td>
<td>Non-laying</td>
<td></td>
<td></td>
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<td>Packed cell volume, per cent.</td>
<td>30-5</td>
<td>25-5</td>
<td>31-5</td>
<td>34-0</td>
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<tr>
<td>Buffy coat, per cent.</td>
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<td>1-0</td>
<td>1-5</td>
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<td>Red-cell volume, per cent.</td>
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<td>23-3</td>
<td>28-5</td>
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<td>Hæmoglobin, g./100 ml. of blood</td>
<td>...</td>
<td>...</td>
<td>9-8</td>
<td>12-0</td>
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<td>Mean corpuscular haemoglobin concentration, per cent.</td>
<td>...</td>
<td>...</td>
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<td>40</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate (Westergren, mm. in 1 hr)</td>
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<td>2</td>
<td>1-5</td>
<td>3-0</td>
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<tr>
<td>Plasma protein, g./100 ml.</td>
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<td>3-7</td>
<td>5-2</td>
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<td>Non-protein nitrogen*, mg./100 ml.</td>
<td>56</td>
<td>33</td>
<td>48</td>
<td>44</td>
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<td>Whole blood</td>
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<td>23</td>
<td>22</td>
<td>21</td>
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<tr>
<td>Plasma</td>
<td>20</td>
<td>23</td>
<td>22</td>
<td>21</td>
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<tr>
<td>Erythrocytes</td>
<td>137</td>
<td>66</td>
<td>113</td>
<td>87</td>
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<tr>
<td>Blood urea-nitrogen, mg./100 ml.</td>
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<td>2-2</td>
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</tr>
<tr>
<td>Plasma glucose, mg./100 ml.</td>
<td>219</td>
<td>185</td>
<td>255</td>
<td>260</td>
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* Compared with mammalian blood, the amount of urea-N in avian blood is negligible, urea being formed solely in kidney tissue (Clementi, 1945).
† Bell (1957). Other normal values, Bell et al. (1959).
The ovaries are generally quiescent. In 22.8 per cent. of cases yolk substance was found free in the abdominal cavity. In 4 birds there was evidence of a fresh or long-standing cloacal prolapse, sometimes showing severe trauma. Swelling and ulceration of the cloaca was encountered in 29 per cent. of cases, sometimes without evidence of prolapse.

Staphylococci have been isolated from the kidneys and other organs of affected birds, but this is by no means a constant finding. Whilst mammalian glomerulonephritis is generally secondary to some preceding condition, no primary cause has so far been established in the fowl.

**Histological findings**

Even in cases where clinical manifestations had been minimal and where the macroscopic examination showed no deviations from the normal, well marked and characteristic renal lesions can be recognised microscopically, and acute and chronic changes can be differentiated.

**Acute glomerulonephritis**

During the acute stage the histological appearance of the kidney is typical of acute diffuse glomerulonephritis, although all the glomeruli do not become involved simultaneously. The larger juxtamedullary glomeruli are the first to be affected. The most striking change is a marked glomerular enlargement due to an increase in the size of the tuft. This is brought about by a swelling of the epithelium and a marked proliferation of the endothelial cells which, even in the normal animal, form a distinct cellular core about which the capillary loops are arranged (fig. 1). The parietal epithelium of Bowman’s capsule and the visceral epithelium of the tuft consequently lie, without adhesions, in close apposition and fill the capsular space, so that the glomerular capillaries are compressed and empty (fig. 2). Later the initial swelling of the visceral epithelium is succeeded by degenerative changes characterised by the appearance of P.A.S.-positive hyaline droplets within the cells (fig. 3). The capillary loops resume patency, but their basement membranes become thickened and sometimes duplicated (fig. 4). Even with abatement of the early swelling of the epithelium the tuft still remains somewhat enlarged, owing to the continuing proliferation of endothelial cells (fig. 5). Subsequently more and more glomeruli become involved until the majority show changes. The renal tubules remain normal.

**Chronic glomerulonephritis**

The characteristic lesions of the chronic stage can generally be interpreted as a direct extension of the acute lesions. However, blood vessels and to a lesser extent the tubules become involved.

*Glomerular changes.* All glomeruli are enlarged, but with shrinkage of the tufts the capsular spaces become more distinct (figs. 6 and 7).
**FIG. 1.**—Cortical glomeruli of a normal fowl. Note endothelial-cell core in tuft. Hematoxylin and eosin. ×640.

**FIG. 2.**—Very early glomerular changes in acute glomerulonephritis. Tremendous enlargement and ischaemia of glomerulus, swelling of epithelium, proliferation of endothelial cells and obliteration of capsular space. H. and E. ×650.
Fig. 3.—Portion of glomerular tuft in acute glomerulonephritis showing hyaline droplett degeneration of epithelial cells and beginning thickening of capillary basement membrane. H. and E. × 1300.

Fig. 4.—Glomerular tuft during acute stage showing thickening and duplication of capillary basement membranes. Note disappearance of glomerular ischemia. Periodic acid-Schiff. × 1200.

Fig. 5.—Glomerulus in acute glomerulonephritis. The capsular space is still obliterated and the tuft is swollen owing to massive endothelial proliferation. P.A.S. × 670.

Fig. 6.—Chronic stage. Note cuboidal cells in parietal epithelium, pericapsular fibrosis and central core of proliferated endothelial cells. H. and E. × 710.
PLATE XIV

Avian glomerulonephritis

Fig. 7.—Enlarged ischaemic glomeruli with distorted, shrunken tufts and well-developed capsular spaces in chronic glomerulonephritis. H. and E. ×160.

Fig. 8.—A group of severely affected peripheral glomeruli, showing massive capsular fibrosis and shrinkage of the tufts. H. and E. ×170.

Fig. 9.—Two glomeruli in chronic glomerulonephritis. Note capsular fibrosis and close chain of epithelial cells surrounding the distorted tufts. H. and E. ×650.
Fig. 10.—Glomerular crescent of proliferating parietal epithelium in chronic phase. H. and E. \( \times 650 \).

Fig. 11.—Chronic glomerulonephritis. Atrophy of glomerular tuft and thickening of arterial wall. The vessel on the left shows some thickened collagen fibres. H. and E. \( \times 650 \).
Fig. 12.—Asymmetrical thickening of the wall of a small artery with hyaline degeneration of the media. Note perivascular mononuclear-cell infiltration seen during the chronic stage. H. and E. × 540.

Fig. 13.—Nucleated red-cell casts in distal convoluted tubules in glomerulonephritis. H. and E. × 640.

Fig. 14.—Cloacal muscularis in chronic glomerulonephritis showing advanced hyalination of muscle fibres and thickening of interfibrous connective tissue. H. and E. × 800.

Fig. 15.—Cloacal mucous membrane with cyst-like accumulation of "urates". Note also mononuclear-cell infiltration. H. and E. × 100.
Bowman's capsule is frequently thickened, owing in part to the epithelium becoming cuboidal (fig. 6), and in part to the development of pericapsular fibrosis, often considerable (figs. 6, 8). In the glomerular tufts, which become very distorted (fig. 7), the swelling and the hyaline droplet degeneration of the epithelium disappear and the thickened capillary basement-membranes are no longer obvious. A striking feature at this stage is increased cellularity, the centre of the tuft being transformed into a dense mass of endothelial cells (fig. 8). The epithelium of the periphery of the tuft also undergoes considerable hyperplasia, resulting in the formation of a close chain of cells enveloping the tuft (fig. 9).

Though there is a mild increase in argyrophil fibres within the glomerular tuft, no true glomerular fibrosis, such as is typical of human chronic glomerulonephritis, is ever observed in the fowl. Yet there is shrinkage of the tuft, resulting in marked distortion and atrophy, with persistence of the ischæmia (fig. 11). Adhesions between parietal and visceral epithelium are frequent. Crescents are rarer, but when present consist of a more or less loose mass of proliferating epithelial cells, probably originating from the parietal layer of Bowman's capsule (fig. 10). Completely fibrotic crescents were not observed in the fowl.

Vascular changes. In advanced cases of chronic glomerulonephritis the renal arterioles and small arteries show characteristic alterations. There would appear to be no doubt about the secondary nature of the vascular lesions, since they do not occur during the acute phase and become pronounced only in the later stages of the chronic phase. The walls of the vessels are thickened, but in general their lumina are not greatly reduced (fig. 11). Intimal proliferations are not well developed. The thickening of the wall is due to fibrosis of the media and adventitia. Even without the use of specific stains for connective tissue, thickened strands of collagen can readily be recognised within the media (fig. 11). Both the P.A.S. and trichrome stains vividly illustrate this point; in such sections the blood-vessels are strikingly differentiated and in the media and adventitia there is a marked increase in P.A.S.-positive fibres. The absence of true hyalinisation of the vessels, especially in afferent glomerular arterioles, is particularly important. Hyaline droplet degeneration is sometimes seen in the media of a few arterioles (fig. 12). Necrosis or fibrinoid degeneration of the vessel is never observed.

In many smaller arteries the internal elastic lamina seems to have disappeared, because it is no longer demonstrable with the recognised elastic-tissue stains. There is no suggestion of a duplication of the internal elastic layer.

Tubular lesions. The renal tubules are surprisingly free from degenerative changes even in the chronic stage. "Urate" and red-blood-cell casts are sometimes seen in the proximal and distal convoluted tubules (fig. 13). Localised areas of both mild tubular atrophy
and cyst formation are occasionally seen; these lesions follow the interstitial fibrosis which may be present, though focally distributed, in advanced chronic cases.

Changes in the interstitial tissue. An infiltration of lymphocytes and macrophages may be seen in the interstitial tissue during the latter stages of the acute phase; they increase during the chronic stage. These infiltrations by mononuclear cells may surround glomeruli or blood-vessels (fig. 12), or they may form extensive foci in the interstitial tissue. These foci must not be confused with discrete lymphocyte accumulations (secondary lymphoid nodules), which are normally present in the fowl in the kidneys as well as other organs (Oakberg and Lucas, 1949).

The interstitial fibrosis which develops during the chronic stage of glomerulonephritis is readily demonstrable by silver impregnation, trichrome and P.A.S. staining methods. It is localised and tends to be most pronounced in the medulla. Mild perivascular and periglomerular fibrosis is also common.

Histological lesions in other organs

The liver of affected birds is frequently involved and presents the picture of diffuse perisinusoidal degeneration. The cytoplasm of cells bordering the venous sinuses is swollen, thus compressing their lumina. The cytoplasm of the affected liver cells stains only lightly; the nuclei are unaltered in the majority of cases. Under low magnification the liver has a patchy appearance with these pale areas scattered more or less evenly throughout the organ. Fatty change is not uncommon and may sometimes be severe; generally the cell nuclei remain intact.

Cloaca. The high incidence of cloacal swelling, ulcerations and prolapse has already been mentioned. Histological examination of such cloaces often reveals not only an acute inflammatory reaction but also extensive mononuclear-cell infiltrations into the superficial and deep layers of the mucosa. The muscularis shows hyalinisation of fibres, and fibrosis (fig. 14). In long-standing cases, where continual severe urate discharge was recorded intra vitam, masses of "urate s" may be found enclosed in a cyst-like manner within the crypts of the cloacal villi (fig. 15).

Ureter. Mononuclear-cell infiltrations occur in the ureteral submucosa and may be so extensive that the convolutions of the mucosa are almost lost.

Biopsies have been made in 7 affected birds. Nothing of importance has been added to the picture of the disease. However, in 2 birds which biopsy showed to have acute lesions, complete resolution was found to have taken place when later biopsies and post-mortem examinations were done.
AVIAN GLOMERULONEPHRITIS

DISCUSSION

The changes observed in the glomeruli during the acute phase of the illness closely resemble the glomerular lesions characteristic of mammalian acute glomerulonephritis, particularly in respect of the increased epithelial and endothelial cellularity, the thickened basement-membranes of the capillary loops, and the glomerular ischaemia, though there is no intraglomerular accumulation of acute inflammatory cells. In the fowl, however, the chronic glomerular changes do not appear to develop beyond a stage comparable to the human subacute lesion, since complete glomerular fibrosis and hyalinisation has not been found.

Fatty change, vacuolation and the formation of hyaline droplets in the tubular epithelium are common in acute glomerulonephritis in mammals (Allen, 1951), yet none of these changes is encountered in the fowl. Mammalian tubules are supplied only with post-glomerular arterial blood and the tubular lesions of acute glomerulonephritis are secondary to glomerular ischaemia. The avian kidney has a dual blood-supply, the tubules receiving not only post-glomerular arterial blood but, in addition, venous blood from the afferent renal portal vein (Spanner, 1924-25). It is reasonable to attribute the survival of the tubules in birds with acute glomerulonephritis to this double blood-supply, since ischaemia of the glomerulus will have less severe consequences on the kidney as a whole than in mammals.

Interstitial fibrosis in the chronic stage is not nearly so extensive as in mammals and this may well be due to a species difference in the reactivity of fibrous tissue. It has been pointed out by Campbell (1955-57) that true liver cirrhosis is rarer in fowls than in mammals subjected to similar hepatotoxic influences. The tubular changes, such as atrophy, which are so evident in mammalian chronic glomerulonephritis and which, in part at any rate, are due to the extensive intertubular fibrosis, are therefore much less apparent in the fowl. It seems safe to assume that this is also the reason for the insignificant fibrosis of the glomerular tuft, where there is only a moderate increase in argyrophil fibres, and also for the absence of fibrosis in the glomerular crescents, which is common in long-standing cases of subacute glomerulonephritis of man (Allen, 1951). The tubular changes of both the acute and the chronic phase are, therefore, much milder in the fowl than in mammals with glomerular lesions of equal severity.

In the fowl, acute glomerular lesions, characterised by cellular proliferation, epithelial swelling and degeneration, and thickening of the basement membrane, appear to be completely reversible. This is shown by the biopsies of the present investigation. Similar lesions produced experimentally can also resolve (Paver, Robertson and Wilson, 1953; and personal observation).

The results of the biochemical blood-analyses listed in the table may be misleading in that they show no significant abnormalities,
although chronic glomerulonephritis was diagnosed. These 5 birds were killed and though they showed definite clinical symptoms and marked histological lesions of the disease, the condition was obviously not sufficiently advanced to have caused any recognisable alterations in the blood constituents examined. It is possible, furthermore, that the kidneys of such birds may have their excretory capacities unimpaired although, in fact, they are functionally "aglomerular". This assumption is supported by the primitive histological appearance of the normal avian glomerulus and by the existence of the renal portal system. It seems possible that the tubules may possess functions attributed to the glomeruli in mammals. It is unfortunate that it has not yet been possible to do blood-analyses on birds in the final stages of the disease.

Selye (1942) described in fowls experimental glomerular lesions apparently identical to those of the spontaneous condition now under discussion. He claimed that his experimental syndrome was very similar to human nephrosclerosis and, since it was caused by repeated subcutaneous injections of deoxycorticosterone acetate, he suggested that this steroid might play a part in the etiology of essential hypertension of man. He also concluded that the extrarenal lesions, such as edema, ascites, pericardial effusion and cardiac enlargement, result from changes in the electrolyte metabolism which may not be due to the direct action of DOCA but be secondary to the renal lesions. The data under review do not support this view, for none of Selye's extrarenal lesions was observed during either the acute or chronic stages of the spontaneous disease in the fowl, though the glomerular and renal vascular changes appear to be identical.

The changes in the renal vessels, although not so severe as in chronic glomerulonephritis or nephrosclerosis of man, suggest that in this spontaneous disease of fowls there is also an increase in blood-pressure. Since there is none of the glomerular fibrosis and hyalinisation of the afferent arterioles seen in human malignant hypertension it is safe to assume, even though no measurements have been made, that any hypertension which may accompany this disease will be relatively insignificant. This is presumably the reason for the inconclusive blood-pressure readings obtained by Selye in his experimental birds. In another paper Selye and Stone (1943) state that chicks on a high NaCl intake develop lesions identical to those obtained after DOCA. They claim that the condition so produced is similar to pullet disease of the fowl (Jungherr and Levine, 1941). Unpublished work in this laboratory suggests that pullet disease is an acute pyelonephritis and bears no pathological relationship to primary glomerulonephritis, although secondary glomerular changes are, of course, frequent. Furthermore, pullet disease is not accompanied by generalised edema, which Selye and Stone (1943) describe as typical for their experimental conditions. Paver, Robertson and Wilson (1953) who studied the toxicity of salt on young chickens and describe generalised oedema
and acute and subacute glomerulonephritic lesions in the experimental birds, do not suggest any correspondence to pullet disease.

The aetiology of the spontaneous disease is so far unknown, although this problem is under investigation.

**Summary**

In 486 post-mortem examinations on fowls there were observed 145 cases of nephritic disease including 31 of glomerulonephritis. Histologically the acute glomerular lesions of fowl glomerulonephritis are similar to those of mammalian acute diffuse proliferative glomerulonephritis, whilst the chronic lesions are comparable to those of the human subacute form. Tubular changes and clinical symptoms are, however, minimal. These major differences from the human disease can probably be accounted for by the lesser tendency to fibrosis seen in fowl tissues generally, and by the presence of the renal portal blood-supply which permits tubular function to occur in the presence of glomerular damage. This condition does not appear to have been described previously in the fowl. The aetiology is unknown.

I thank Dr D. J. Bell of the Biochemistry Section of the P.R.C. for the analytical data used in the table.

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The Spread of Tumour Metastases via the Renal-portal System in the Fowl

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SUMMARY. Evidence is presented to show that the renal-portal system of the adult fowl is functional. Experimentally-induced virus tumours (Rous I, MH endotheioma and 'D' of Duran-Reynals) of the leg musculature metastasize first to the ipsilateral kidney. 57 birds had renal metastasis; in 26 the ipsilateral kidney only was affected; in 22 cases other non-renal organs were also affected, while both kidneys were involved in only 9 birds, in all of which liver, lung and spleen also had secondary growths.

WHEN CONSIDERING the distribution of tumour metastases in the fowl, conclusions are often drawn on the basis of tumour behaviour in mammals, without due appreciation of the biological difference. In fact, mammals are one of the few classes of chordates which do not have a renal-portal system, and the present work indicates that this system can be of major importance in controlling the distribution of blood-borne metastases from the legs.

Siller (1959) has shown in a recent report of a spontaneous osteogenic sarcoma in a fowl that haematogenous renal metastases were present only in the organ ipsilateral to the primary tibial tumour. This was interpreted as evidence of the existence of a functional renal-portal system in adult fowls.

While anatomical proof is readily available, it is no simple matter to demonstrate conclusively that this renal-portal system is, in fact, functional. Sperber (1949) produces evidence and briefly outlines the history of the controversy which has lasted from the early part of the nineteenth century to the present day. Criticism has often been levelled against some of the investigators (Spanner, 1925; Das, 1931), who present evidence in favour of such a system, that their observations were made on animals in which the experimental procedure had produced non-physiological states. The same objection applies to the more recent work of Gordeuk and Grundy (1950) and of Cuypers (1959). The most convincing experiments, in which the criticism mentioned above does not apply to the same extent, are those of Sperber (1949), Rennick and Gandia (1954) and Levinsky and Davidson (1957). Sperber injected phenol red into the musculature of one leg and found that the ipsilateral kidney excreted a greater amount of the dye than the contralateral one. Similar results were obtained by Rennick and Gandia, who injected p-amino-hippurate into the leg vein, while Levinsky and Davidson showed that parathormone infused into one leg vein stimulated phosphate excretion by the kidney on the same side.

Observations on the renal metastases of primary leg tumours support the view that notwithstanding the complicated pelvic pattern at least some of the venous drainage from the leg goes first through the ipsilateral kidney. In the spontaneous osteogenic sarcoma described by Siller (1959) no experimental interference had taken place, and in the present series this was confined to one initial injection of tumour-producing virus into the leg musculature of healthy young chicks. The criticism of the 'unphysiological abnormal state' due to the experimental procedure does not therefore apply.

* British Empire Cancer Campaign Unit.
MATERIAL AND METHODS

The observations reported here were made on material from fowl tumour virus assays, which were carried out according to the method of Carr and Harris (1951). Decimal dilutions of active material were injected into the left legs of young brown Leghorn chicks between 7 and 21 days old. While some birds were killed as soon as tumour growth was detected, others were maintained for a further period. The infective agents used were those of the Rous I sarcoma and the 'D' tumour of Duran-Reynals (1946), neither of which induce any cancers of renal origin (Carr, 1959). Some results from similar work with MH₂ reticuloendothelioma (Murray and Begg, 1930) are also included. Since this virus can cause kidney carcinomas (Carr, 1960), only those cases were considered in which histological examination verified that all the renal tumour growths were purely sarcomatous metastases.

RESULTS

Metastatic tumour spread occurred frequently in the test animals which were maintained for the longer period. The ipsilateral kidney was usually affected first, while the contralateral one was rarely involved and then only when other organs also carried metastases. The liver was usually the first organ to develop metastases subsequent to the ipsilateral kidney; the spleen and lungs were less frequently affected.

The results, which are summarized in Table I, are quite clear cut. Renal metastases developed in 57 birds; in 26 cases these were confined to the ipsilateral kidney; in 22 this kidney and other non-renal organs were involved, while in only 9 birds were both kidneys as well
as liver, spleen and lung affected also. At the time of autopsy, the earliest macroscopically recognizable metastases in the kidneys were frequently in the form of pale circumscribed nodular areas situated on the surface of the organ at the junction of the middle and caudal division of the kidney where the ischiatic vein enters that organ (see Fig. 1). In advanced cases more numerous nodules developed (Fig. 2) which showed a tendency towards confluence, finally affecting the whole organ (Fig. 3).

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Distribution of renal metastases</th>
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<tbody>
<tr>
<td></td>
<td>Left kidney alone</td>
<td>Left kidney and other non-renal organs</td>
<td>Both kidneys and other organs</td>
<td></td>
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<tr>
<td>Rous</td>
<td>12</td>
<td>9</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>'D'</td>
<td>11</td>
<td>11</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>MH₁₂</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
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<td></td>
<td>26</td>
<td>22</td>
<td>9</td>
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Histological examination of the affected kidneys shows masses of rapidly growing tumour cells infiltrating and replacing the otherwise normal renal tissue; it does not throw any light on the route by which the metastatic tumour cells gained access to the kidney.

DISCUSSION

The evidence quoted in the table demonstrates that tumour cells are carried by venous drainage from the leg musculature to the ipsilateral kidney where they frequently form secondary growths. These findings confirm the experimental results of Sperber (1949), Rennick and Gandia (1954) and Levinsky and Davidson (1957), which provided strong evidence for the existence of a functional renal-portal system in the adult fowl.

One finding, however, deserves further consideration. The prominent femoral vein enters the abdominal cavity on the ventral surface of the cranial division of the kidney. It runs forwards towards the posterior vena cava, where a valve is situated which allows a variable amount of blood to return directly to the right atrium. The remainder is shunted caudad in the afferent renal vein, which again runs along the surface of the kidney for some distance. While this route is generally believed to account for the bulk of the venous blood entering the kidney, the small and relatively insignificant ischiatic vein also plays an important part in draining venous blood from the leg through the kidney. This is made clear by the frequency of the small early metastases at the point of entry of the ischiatic vein. While the function of the renal valve is undoubtedly important in controlling the amount of venous blood entering the kidney, there is thus another important source of afferent venous blood, outside the direct control of the valve.

Although the renal afferent veins of both sides are connected by the coccygeo-mesenteric vein and there is a venous arch, there appears to be no significant transport of tumour cells to the contralateral kidney by this route, since that organ was affected only when generalized metastasis had taken place.
Fig. 2. Four discrete metastases have developed in the left kidney. Note the large muscle tumour of the ipsilateral leg.

Fig. 3. Confluent metastatic involvement of the ipsilateral kidney with a tumour in the left leg.
Foulds (1934) studied the distribution of metastases of several virus tumours of fowls. He inoculated 928 birds into the breast muscle. Metastases occurred in 288 cases. Except with MH₂ endoendothelioma in which there were 29 cases with renal 'metastases', none was found in this organ with any other tumour type. It should be noted that MH₂ gives kidney carcinoma (Carr, 1960), which are due to virus infection, not cell metastases. This shows that renal metastases are extremely rare unless the primary growth is in the leg.

The fact that in this investigation 9 metastases occurred in the contralateral kidney, whereas Foulds found none, may be explained by the fact that very young birds (under 3 weeks old) were used, and such birds give a much higher incidence of frequency and spread than older birds such as were usually employed by Foulds.

In this connexion it should also be mentioned that when both kidneys carried metastases the ipsilateral organ tended to be much more severely affected.

It might be suggested that these renal metastases were not blood-borne, but carried in lymphatics. It was first established by Rous in 1911 and repeatedly confirmed (Fujinami and Inamoto, 1914; Foulds, 1934) that these metastases are spread by the blood stream, and that they are not the result of re-infection by virus.

Any tumour cells entering the small lymph vessels in the region of the primary tumour would be carried directly in the ischiatic lymph vessel which enters the abdominal cavity along with the femoral artery and vein and then passes either through the middle division of the kidney or dorsal to it, to join the thoracic duct (Baum, 1930). Even if the lymph vessel from the leg goes through the kidney, the metastatic tumour cells would have no opportunity to become established in this organ. The first metastases, if they were lymphatic in origin, would, since the fowl has no lymph nodes, be in the lungs, i.e., the most proximal capillary bed.

Furthermore, the solitary tumour nodules seen in the ipsilateral kidney of some early cases were caudal to the entry of the ischiatic lymph duct, in the region of the ischiatic vein (see Fig. 1). There is no evidence, therefore, to suggest that these renal metastases are lymph-borne.

ACKNOWLEDGMENTS

One of us, J. G. Carr, is on a full-time grant of the British Empire Cancer Campaign.

Received for publication September 14th, 1960.

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The Histopathology of an Entero-hepatic Syndrome of Turkey Poults

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SUMMARY.—The clinical and macroscopic features of 3 outbreaks of a disease of turkey poults with very high mortality are described. The characteristic and very striking histological changes in duodenum, liver, and kidney are described in considerable detail.

The livers of such turkey poults are diffusely affected with very severe necrobiotic changes accompanied by marked regeneration. The kidneys show the typical lesions of membranous glomerulonephritis and hyaline droplet nephrosis, while marked catarhal enteritis is the characteristic finding in the duodenum.

These histological lesions lend support to the possibility of a toxic aetiology which has been suggested before on several occasions, but the toxic agent remains unidentified.

NUMBER of outbreaks of a disease affecting turkey poults, with high mortality, were reported in the counties of Berkshire, Buckinghamshire, Northamptonshire and Oxfordshire, during June, July, and August, 1960. The losses usually occurred when the poults were 2 to 4 weeks old, but in some cases deaths began to occur within 2 to 4 days of hatching while in other outbreaks birds up to 8 weeks old were affected.

Poults often were found dead and usually in good bodily condition. In a few instances the birds were seen to be ill; they were depressed, had an unsteady gait, and later the muscles of the limbs appeared to stiffen and the birds would fall on their sides before death. Some birds showed torticollis.

In the 11 outbreaks investigated the birds were being fed on proprietary food "Brand X" turkey crumbs containing an anti-blackhead drug and a coccidiat. In cases where some of the birds survived, deaths stopped shortly after the food was changed to another brand or to a simple cereal diet.

The following account deals with 3 out of the 11 outbreaks of disease which were typical of all.

Clinical Findings

Flock 1
Fifty Broad Breasted Bronze turkey poults (day-old) were purchased and put into brooders which had given satisfactory rearing results with earlier batches. The first deaths began when the poults were 7 days old and continued steadily until, after a period of 21 days, all the birds had died. The owner did not notice any symptoms of illness while the birds were on their legs; the poults fell on their sides and died within a few minutes. Therapeutic courses of Aureomycin and furazolidone did not appear to affect the incidence or the course of the disease.

Post-mortem findings. The bodily condition was good. The feathers around the vent were not soiled. The carcases were congested and there was general slight oedema. The livers were severely congested, enlarged, turgid and firm, and appeared as if they had been preserved for some time in formalin. The gall bladders were well filled. The kidneys were very swollen and congested. The myocardium was congested and the pericardial sac distended by clear straw-coloured fluid. There was food in the crops, and in some birds, there were particles of litter in the gizzards; otherwise the alimentary tracts were empty. Catarhal enteritis, most severe in the duodenum, was present. No pathogenic organisms could be isolated when the heart blood and the liver were cultured on 5 per cent. blood agar and desoxycholate citrate agar. Material from this outbreak was examined histologically.

It is of interest that, when all the original poults had died, 50 1-month-old turkey poults were purchased from the same source and fed on the remainder of the food for 14 days, when the diet was changed to turkey grower pellets. Morbidity and mortality were negligible in this group.

Flock 2
Seventy 1-day-old Broad Breasted White turkey poults and 100 Broad Breasted Bronze poults from separate hatcheries together made up a batch for rearing. The Bronze poults started to die immediately and Salmonella saint paul was consistently isolated from the internal organs. Furazolidone was administered in the food at a therapeutic level of 0.04 per cent. to the whole group for 10 days. Twenty-seven Bronze birds died during 7 days of treatment but no deaths occurred in the White birds which had been in contact. At the end of 7 days the White birds started to die but no Salmonellae were isolated from the carcases. There were only 40 survivors out of the original 170 when the poults were 3 weeks old.

During the same period deaths commenced in a second group of 70 2-month-old White poults housed well away from other stock and managed by a separate attendant. There had been no previous deaths in this group from 1 day old. There were no significant bacterial findings and 30 out of 70 birds died.

While deaths were occurring in the first batches a third group of 100-day-old Bronze poults was purchased. These started to die at 4 to 5 days of age; deaths occurred at a rate of approximately 12 per day. Within 14 days 78 out of 100 had died. The symptoms and post-mortem findings shown by these 3 batches were similar to those seen in Flock 1 and in some poults loose whitish droppings were observed. Material from this outbreak was also examined histologically.
Flock 3

One hundred and sixty 1-day-old Broad Breasted Bronze turkey pouls started to die within 24 hours of arrival on the farm. *Salmonella typhimurium* phage type IA, was consistently isolated and during 4 days approximately 100 pouls died. The remaining birds were immediately changed to another food, “Brand B” turkey starter crumbs, because it was readily available and contained the therapeutic level of furazolidone. Very few deaths occurred subsequently; approximately 60 pouls were reared successfully. Two hundred and seventy 14-day-old turkey pouls were in a brooder only a few feet from the birds infected with *Salmonella typhi-murium*. Furazolidone at the prophylactic level of 0.01 per cent, was mixed with “Brand X” turkey starter crumbs and deaths commenced in this group 3 days later. The birds were then divided equally between 4 outside Motley brooders but deaths continued. Bacteriological examinations were negative and it was thought that the isolation of *Salmonella* might have been inhibited by the presence of furazolidone. To clarify this the livers and terminal intestines of 17 birds which had died were cultured using selenite and desoxycholate citrate agar. As sparse colonies were obtained from 2 birds only, salmonellosis was not considered to be the responsible agent of disease. Estimations of sodium and chloride of the food gave results within the normal range. Ketones were not detected in blood serum samples from 5 typically affected birds. Mortality persisted in this group until 250 out of 270 had died during 18 days.

The farm was visited when over half of the 270 birds had died; there was a high standard of husbandry with very experienced labour. The remaining pouls were dull, mildly interested in food, cheeped continuously, developed an unsteady gait as they became weak, and finally fell on their sides and died. Two typical birds, which seemed reasonably alert, were chosen for observation at the laboratory but deaths continued. Bacteriological examinations were negative and it was thought that the isolation of *Salmonella* might have been inhibited by the presence of furazolidone. To clarify this the livers and terminal intestines of 17 birds which had died were cultured using selenite and desoxycholate citrate agar. As sparse colonies were obtained from 4 birds only, salmonellosis was not considered to be the responsible agent of disease. Estimations of sodium and chloride of the food gave results within the normal range. Ketones were not detected in blood serum samples from 5 typically affected birds. Mortality persisted in this group until 250 out of 270 had died during 18 days.

The survivors were then obtained for observation. On reaching the laboratory they were subdued, huddled to heat sources, and the feathers appeared ruffled and unduly loose in the skin. Drinking water containing 3 per cent. glucose, and a proprietary chickmeal, were readily consumed. One bird died during the first 24 hours, but there were no further casualties and the birds grew normally over the next 2 months.

Post-mortem findings in this outbreak were almost identical with those described above, but there was no oedema in the birds which died during the later stages. Material for histological examination was taken at various stages of the disease.

Another batch of 100 1-day-old Broad Breasted White pouls were fed on “Brand B” turkey starter crumbs containing furazolidone as a prophylactic measure and the rearing percentage was completely satisfactory.

Sixty well grown 5-week-old Broad Breasted Bronze pouls were purchased to replace the casualties and fed on the remainder of the consignment of “Brand X” turkey crumbs for 10 to 14 days. Two of these birds died of moniliasis. The farmer noticed an unusually strong smell from the droppings. Turkey grower pellets were fed after 14 days and a satisfactory number of birds were reared. Older birds appeared to be more resistant. In Flock 1, though there was a 100 per cent. mortality in birds between the ages of 7 and 28 days, birds imported from the same hatchery when 25 days old and fed on the remainder of the food for 10 days did not show clinical disease. In Flock 3, 78 out of 100 birds died between the ages of 1 to 3 weeks, whereas only 30 out of 70 8-week-old birds succumbed.

It may be relevant to mention that during the last 2 years somewhat similar macroscopic post-mortem features have been noted in older turkeys, particularly 7 to 10 days after a change of food, from growing to fattening pellets.

**Histological Observations**

Prompt fixation in Susa of only fresh material ensured that post-mortem change was minimal in all tissues examined. Paraflin sections were routinely stained with haematoxylin and eosin. Other special techniques such as the periodic acid Schiff reaction and the Gordon-Sweet Silver impregnation method were employed also. The most striking and constant abnormalities were observed in the liver, kidney and duodenum.

**Liver**

Marked retrogressive and regenerative changes were present in the liver parenchyma. The cells were swollen, had a more or less homogeneous eosinophilic appearance, and were sometimes vacuolated. The very large, vesicular nuclei with margined chromatin had prominent spherical nucleoli (Fig. 4). Unlike the circumscribed focal necrosis, characteristic of a variety of avian liver conditions, the present disease was manifested by diffuse necrosis of practically all the perisinusoidal regions. This affected especially the “ends” of the liver cords where there was evidence of true necrosis. This indicated that the peripheral areas of the parenchymal sheets were most severely involved. These areas, which were distributed diffusely throughout the liver, were characterised by the presence of cell debris, karyorrhexis and karyolysis. Haemorrhage was not observed but venous congestion was marked.

In early cases the degenerative and necrobiotic changes dominated the picture (Fig. 3) but were superseded in the more advanced stage by regenerative processes. In such advanced cases the groups of regenerating liver cells were distributed throughout the organ (Fig. 1). Although the regeneration appeared to originate at the periportal region, no evidence of a true nodular regeneration could be seen. The groups of regenerating cells were characteristically arranged as distinct tubules (Fig. 2) and in some instances almost the entire section was composed of such tubules (Fig. 1). The regenerating cells were markedly basophilic, often contained large vacuoles, and were sometimes multinucleated but contained few mitotic figures (Figs. 2 and 3) despite the obviously active proliferation. Islets of necrobiotic parenchyma were scattered between the groups of regenerating liver cells (Fig. 4). While the sinusoidal reticulo-endothelial cells were...
W. G. SILLER & D. C. OSTLER.—THE HISTOPATHOLOGY OF AN ENTERO-HEPATIC SYNDROME OF TURKEY POULTS

Fig. 1.—"Tubules" of regenerating liver cells distributed evenly throughout the organ. Note the marked proliferation of sinusoidal reticulo-endothelial cells. H. & E. x 100.

Fig. 2.—Groups of regenerating liver cells arranged in the characteristic tubular form. Note the mitotic figure in the centre. H. & E. x 400.

Fig. 3.—Illustrates the edge of an area of necrobiosis (above) the regenerating liver cells appear as well differentiated tubules, but their hepatic origin cannot be questioned. A mitotic figure is seen bottom left. H. & E. x 350.

Fig. 4.—In the centre of the picture there is an islet of degenerated liver tissue surrounded by hyperplastic reticulo-endothelium and tubules of regenerated liver tissue. Note the prominent nucleoli and the margination of chromatin in the enlarged nuclei of the necrobiotic cells. H. & E. x 500.
Fig. 5.—Two glomeruli showing the typical membranous changes. Note the thickened, dense basement membranes and the nodular thickening (top right). There are no proliferative changes but the capillary loops are ischaemic. P.A.S. × 560.

Fig. 6.—A glomerulus showing very marked membranous thickening of the looser more diffuse type. Ischaemia is pronounced but the glomerulus is not enlarged nor are there any proliferative changes, but P.A.S.-positive granules may be seen in Bowman's capsule. P.A.S. × 680.

Fig. 7.—A proximal convoluted tubule with the typical changes of hyalin droplet nephrosis. The cytoplasm of the epithelial cells is tightly packed with P.A.S.-positive droplets. Note the still intact nuclei of this and the neighbouring tubules. P.A.S. × 400.

Fig. 8.—Normal liver showing the reticulum fibres surrounding the liver cell cords. Gordon & Sweet × 400.

Fig. 9.—Necrobiotic liver cells. The reticulum network is sparse and incomplete. Note the swelling of the parenchymal cells and the enlargement of the nuclei with their prominent nucleoli. Gordon & Sweet × 400.
markedly hyperplastic (Fig. 1), there was only a moderate localised increase in collagen and reticulum fibres. The bile ducts were only moderately hyperplastic and some of the larger ones were hypertrophic, showing multi-layered epithelium.

Although a few heterophils and mononuclear inflammatory cells such as plasma cells and lymphocytes were sparsely scattered through the affected livers, there was no evidence of a true inflammatory reaction or focal cellular infiltration. The blood vessels were congested but showed no other abnormality.

**Kidneys**

Apart from a mild intertubular oedema present in some cases, only the glomeruli and the proximal convoluted tubules showed any significant changes. The glomeruli showed a very pronounced thickening of the capillary basement membrane (Fig. 5). Practically every glomerulus in the section was affected to some degree. In a single section the severity of the lesions ranged from moderate thickening of the basement membrane of 1 capillary loop in 1 glomerulus to a pronounced thickening of practically all the membranes in another. These membranes were usually dense and stained deeply with P.A.S. (Fig. 5), but in some instances they were of a looser texture (Fig. 6) and in some others nodular laminated thickenings were present along the course of the capillary walls (Fig. 5). Ischaemia was noticeable particularly in the more seriously affected glomeruli (Fig. 6). P.A.S.-positive spherical granules of varying size were, from time to time, observed within the Bowman's capsules (Fig. 6). While these membranous lesions were very prominent, the glomeruli were of normal size and there was no evidence of any proliferative changes typical of avian glomerulonephritis (Siller, 1959a).

In sections stained with P.A.S., tubular lesions, when present, were striking but sparsely distributed. Those kidneys affected with membranous glomerulonephritis also showed hyaline droplet nephrosis. The proximal convoluted tubules were usually affected, although the collecting tubules sometimes showed similar lesions. On the other hand, the distal convoluted tubules were invariably normal. The epithelium of the nephroptic tubules was swollen. In those severely affected the cytoplasm was completely filled by P.A.S.-positive hyaline droplets of varying size (Fig. 7). This dense accumulation of granules obscured the nuclei. Although most were normal (Fig. 7), a few nuclei in the affected epithelium showed such degenerative changes as moderate pyknosis and margination of the nuclear chromatin. In more mildly affected tubules small droplets only were present. It would appear that these first develop at the apical part of the epithelial cell, immediately below the brush border lining the lumen. The blood vessels of the kidney were normal.

**Duodenum**

There was evidence of a very heavy catarrhal enteritis. Large portions of mucosal epithelium, otherwise normal, were desquamated and lay free in the lumen of the gut. In a number of sections practically all the mucosa was desquamated leaving only the Brunner glands of the submucosa intact. In some cases there was a very characteristic coagulative necrosis of groups of villi similar to what one would expect to see in an infarct, although no thrombosis was discovered. There was only moderate secondary inflammation in the mucosa and submucosa; no para- sites could be found in the sections.

**Heart**

Mild granular myocardial degeneration was distributed fairly even throughout the heart. SomeWhat more marked was the subepicardial oedema, which was prominent in the coronary region and extended some short distance into the adjacent myocardial tissue. A rather loose type of fibrous tissue containing sparsely distributed myocardial fibres is normally present in avian hearts at the base of the aorta, Siller (1958) has suggested that this represents the remains of the endocardial cushion. In the oedematous hearts of these turkey pouls this fibrous tissue showed a considerably looser structure and large vacuoles, presumably due to oedema.

**Other organs**

Pancreas and brain showed no deviations from the normal.

**Discussion**

Campbell (1955-57) has pointed out that the avian liver does not readily react to irritant substances by the development of true cirrhotic changes. In fowls, at any rate, the connective tissue response appears to be much more moderate than in mammals. In mammals such a severe liver degeneration as was observed in the present cases would undoubtedly have initiated considerable fibrosis. The moderate increase in the connective tissue together with the bile duct hyperplasia must, however, be interpreted as evidence of a cirrhotic process. This is supported by the report of Wannop (1960), who describes mild cirrhosis in similarly affected turkeys.

The avian liver, like that of the mammal, has a great regenerative ability. Saphir (1959) stated that in man the cords of regenerating liver cells are a "perfect imitation of those of the normal liver." In birds regenerating liver cells tend to assume the above-mentioned tubular appearance which suggests a reversion to the embryonic type. Campbell (1960b) described a similar tubular regeneration in the liver of fowls experimentally dosed with seneciphylline. He compares these structures with embryonic liver tissue. In some mammals neoplastic liver tissue, such as occurs in hepatocellular carcinomata, also tends to assume the embryonic tubular appearance (Berman, 1951). The turkey livers showed no evidence of tumour formation, but the similarities between neoplastic and actively regenerating tissues are often very marked and the borderline may not be easy to draw.

The lumina of these tubules of regenerating parenchyma represent an enlarged or dilated bile capillary. The normal avian liver has a proportion of the cell cords which can appear almost circular in section with the apices of the cells pointing towards the centre where the bile capillaries is situated. In normal birds these circular cords have no obvious "lumen," such as is seen in the regenerating "tubules." There is no doubt that these "tubules" are not hyperplastic bile ducts but are, in fact, regenerating parenchyma
Although at first sight the differentiation may not be easy, it cannot be certain, however, whether these regenerating cells were not derived from bile duct epithelium. Such a possibility is contested by Cameron (1952) and by Willis (1958) and it seems generally accepted that regenerating cells originate from still viable hepatic tissue (Maximow & Bloom, 1952).

Some of the regenerating cells showed evidence of mild retrogressive changes, which was not comparable in severity, however, with the degenerative process seen in the liver parenchyma. Both degenerative and regenerative processes were obviously highly active at the time of death and it is therefore reasonable to assume that the regenerating liver cells were also subjected to the effects of the toxic agent. Yet the amount of degeneration which had taken place in the regenerating cells was minimal. This confirms the observations of Cameron (1952) and Campbell (1960a), that regenerating cells are much more resistant to poisons.

Both the retrogressive and regenerative processes seen in these livers suggest a toxic basis. The picture is in a number of respects similar to that of ragwort poisoning in fowls (Campbell, 1955-57 and 1960b). It does seem, however, that in these turkey poult's both processes are much more acute; despite the massive regeneration up to 90 per cent. of the cases died, whereas in experimental Senecio poisoning in fowls survival is frequent.

If the arrangement of the reticulum network of the liver is altered during the retrogressive phase (as was the case here, see Figs. 8 and 9), the orientation of the regenerating liver cells, in correct relation to the sinusoids and central veins, is hindered. This may cause interference with the normal portal circulation to give a "portal hypertension with all its complications" (Saphir, 1959). Ascites is a frequent symptom in these turkey poult's; the destruction of the normal liver tissue and its disorientated regeneration may be contributory. Ascites was also observed by Campbell in fowls poisoned with ragwort.

In fowls, glomerulonephritis is characterised in the acute stage by a thickening of the capillary basement membranes and by both epithelial and endothelial proliferations within the glomeruli (Siller, 1959a). In the turkey poult's, on the other hand, the glomeruli showed neither enlargement nor proliferative changes. The membranous lesions were very much more severe than in fowls and are comparable with the changes observed in human membranous glomerulonephritis. This condition is characteristic for eclampsia and lipoid nephrosis (Allen, 1951). It should be mentioned that the nodular thickenings of the turkey glomerular capillary basement membranes bear a superficial resemblance to the Kimmelstiel bodies seen in cases of human diabetes. However, the latter are usually laminated, while in the poult's the nodules have a more or less homogeneous although somewhat pitted appearance. There was, of course, no histological evidence of diabetes; the pancreas was normal and diabetes is unknown in graminivorous birds although it may be noted that the normal blood glucose level is more than twice that in mammals (cf. Erlenbach, 1938; Bell, 1957).

It has been found that, in fowls, diffuse proliferative glomerulonephritis may be a sequel to some forms of liver cirrhosis (Siller, unpublished data), but such strictly membranous lesions without proliferative changes appear not so far to have been recorded in birds. Liver damage causes a decreased inactivation of oestrogen by conjugation, and therefore accumulation of free oestrogen (Campbell, 1957). Proliferative glomerular lesions occur in fowls exposed to excessive oestrogen, regardless of whether it is of endogeneous or exogeneous origin (Siller, quoted by Campbell, 1960a). In such cases, however, the glomerular changes are not membranous but proliferative. It is unknown to what extent oestrogen participated in the development of the membranous glomerulonephritis of the present cases.

Hyaline droplet nephrosis in man is associated with various renal diseases such as membranous glomerulonephritis, lipoid nephrosis, and nephrosclerosis, and it occurs in tubular degeneration following poisoning with certain sulphonamides (Allen, 1951).

It is noteworthy that most of the investigators, including the present authors, stress the macroscopic enlargement of the kidneys in this condition. Histologically the interstitial oedema was slight and not nearly so severe as in acute pyelonephritis of the fowl, in which the kidneys are greatly swollen (Siller, 1959b). On the other hand, in human lipoid nephrosis the kidneys are greatly enlarged (large white kidney).

The aetiology of this "new disease of turkey poult's" is unknown. Stevens (1960) suggests that the liver lesions of this complex are similar to those of virus hepatitis in turkeys reported by Snoeyenbos et al. (1959) and Mongeau et al. (1959), who describe fatty change, focal parenchymal necrosis, foci of granulocytic and lymphocytic infilrations and haemorrhage in the liver. Not one of these changes was observed in the present cases. Haemorrhage and foci of cellular infiltration were completely absent from the liver and there was no focal necrosis although diffuse necrobiosis was severe. Regeneration of liver tissue and renal lesions were both very pronounced but they appear not to be characteristic for turkey virus hepatitis in which, however, pancreatic lesions occur. (The very prominent nuclei of the degenerating liver cells should not be confused with virus inclusion bodies.) Wannop (1960) also denies the identity of these 2 conditions.

An alimentary toxemia in chickens presumed to be due to a toxic factor in nutritional fat was described by Sanger et al. (1958). The main macroscopic findings were hydropericardium and mottled scarred livers. The histological features consisted of large areas of necrosis, petechiation, and cirrhosis of the liver, but regeneration was not observed. In the kidneys there was interstitial oedema, swelling of the glomerular tufts, erythrocytes within the nephron and tubular degeneration. The tips of the duodenal villi were necrotic and there was a sub-epicardial oedema with myocardial degeneration. There are some points of similarity, therefore, between this and the condition in turkeys under discussion, but judging from the, unfortunately rather poor, illustration in Sanger's paper, it is particularly
the liver and renal glomerular changes which will readily differentiate them.

It may be noted that 3 outbreaks of disease in broilers aged approximately 3 weeks, each one on separate premises and in different breeds, had showed the following macroscopic post-mortem features. The carcases were in good condition but congested. The livers were enlarged and congested with scattered haemorrhages. The kidneys were very swollen with blanched areas, the congested blood vessels standing out in relief. The myocardium was severely congested and hydro-pericardium was present to varying degrees. There was catarrhal enteritis with congestion of the blood vessels. The intestinal contents were mucilaginous. Bacteriological and parasitological examinations produced nothing significant. Although the mortality figures were not recorded, they were not unduly alarming and in 2 flocks mortality subsided over 2 weeks. Later on, the growth rate was obviously uneven. Food consumption did not fall throughout the period when the birds were affected. No material from these broilers was available for histology.

Most investigators suggest that nutritional toxic factors may be responsible for a new disease (Stevens et al., 1960; Smith, 1960; Wannop, 1960; Wiley, 1960). Blount (1960b) stressed the anorexia as due to unpalatability. Mann (1960) implicates the increased addition of the cereal milo in the poultry foods during the past year, but Blount (1960a) points out that some outbreaks of “X”-disease were definitely not associated with rations containing milo. Blount (1960b) lists the exhaustive but so far unrewarded search which has been, and still is being, made for a chemical toxin which may be responsible for this economically important disease.

In Flock 3, the presence of a toxic factor in the food was strongly suggested by the result of changing the food in order to provide rapid treatment with furazolidone. Many of the authors quoted above have noted the beneficial effect of a change of diet. A fertile etiology is strongly supported by the post-mortem and histological findings. The catarrhal enteritis, along with the severe retrogressive liver changes resembling those produced by known hepatic toxins such as ragwort alkaloids, the hyaline droplet nephrosis, and myocardial degeneration support the concept of a toxic cause.

A meeting of research workers and representatives of the National Food Compounders was held at the Agricultural Research Council Headquarters on August 19th, 1960, to discuss this condition. Their conclusions, which appeared in The Veterinary Record (1960, 72: 710) were: "It seems unlikely that any nutritional deficiency or excess per se could give rise to the rapid onset of symptoms and high mortality experienced. Therefore, one is driven to the conclusion that if the disease originates via the intestinal tract then it is probably by some form of pre-formed toxin."

The renal and hepatic lesions would undoubtedly account for the generalised oedema, a symptom closely associated in human medicine with lipid nephrosis. It is noteworthy that this connexion that a diuretic treatment has had some success in outbreaks of this turkey disease (Swarbrick, 1960).

While the histological changes described above were constant in the cases examined, Wannop (1960) records some differences in the macroscopic post-mortem findings of his cases. He did not observe generalised oedema, but he saw haemorrhages in the liver and pancreas. It may be that the autopsy findings may not be identical in all the outbreaks. Clinical symptoms, too, appear to be variable, which suggested to Wannop that there may be 3 forms of a single disease entity.

The isolation of Salmonellae in flocks 2 and 3 would appear at first sight, to confuse the picture, but it must be remembered that turkeys frequently are carriers of these organisms. Furthermore, in flock 2 no Salmonellae were isolated from the White birds which had died and in which the histological changes were characteristic for this condition. In this flock, the Bronze birds in which Salm. saint paul had been isolated originally continued to die even after they were treated with furazolidone. These, and the fact that the histological changes were not typical for salmonellosis, indicate that the cause of death in these outbreaks was not due to the concomitant infection with Salmonella.

In view of the predominant enteric and hepatoxic changes it is suggested that this condition be termed "an entero-hepatic syndrome" of turkeys.

Acknowledgments.—The authors are indebted to Mr. D. W. Menzies, M.R.C.V.S., and Mr. Loosmore, B.V.Sc., M.R.C.V.S., for their help in connexion with the clinical section of this paper.

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Avian Nephritis and Visceral Gout

W. G. Siller, Dr. Med. Vet., M.R.C.V.S.

The term "gout" should be employed with reservation in connection with avian pathology. Avian articular gout resembles in many respects the condition in man; it may even have a similar etiology. In fowls, however, only a very small percentage of cases can be diagnosed as "articular gout"; the remainder show deposition of urates only on various internal organs and serous membranes when they are said to have "visceral gout." Since the visceral form is frequently associated with inflammatory changes in the kidneys, the syndrome is termed "nephritis and visceral gout," or "renal gout."

MATERIAL AND METHODS

The present study is based on material obtained from the closed and inbred flock of Brown Leghorns kept at the Poultry Research Centre at Edinburgh. All birds that die or are killed because of obvious clinical diseases are examined postmortem.

Among the 631 autopsies of the last 3½ years, there were 35 cases (5.5 per cent) of visceral gout. No case of articular gout was observed. This article therefore describes only the visceral form.

Of these 35 cases, 22 were examined histologically and collectively summarized. Routinely, tissues were fixed in Susa and embedded in paraffin, while frozen sections of formal-fixed material were used to demonstrate fat. Latterly, duplicate tissue blocks were fixed in absolute alcohol to demonstrate urates.
by Gomori's methenamine silver method. Stained and unstained alcohol-fixed sections were also examined under polarized light.

In cooperation with Dr. W. M. McIndoe, a small pilot experiment was set up following Kionka and later workers, who noted gouty lesions in fowls that had received food rich in protein. Six chicks, 4 females and 2 males (6 weeks old), were maintained for 14 weeks on a diet containing approximately 30 per cent total protein that was derived from the ordinary balanced ration (18 per cent protein) fed at this Centre, supplemented by a 2:1 mixture of casein and gelatin. This was followed by a further period of 11 weeks on the ordinary ration supplemented to the 60 per cent protein level by 2:1 casein-gelatin mixture and a vitamin-mineral mixture. Blood samples for plasma uric acid determinations were taken at intervals.

As controls, 3 birds (1 male and 2 females) were kept on the normal diet throughout the experimental period. The uricase-ultraviolet spectrophotometric method of Fechtmeir and Wrenn was slightly modified and used by Dr. McIndoe for the determination of plasma uric acid.

**SEX AND AGE INCIDENCE**

In the fowl, visceral gout occurs in both sexes. It was not possible to establish a definite sex incidence in our flock because many more females were presented for examination than males (of the 35 cases under discussion, only 3 were males).

The age at which birds show symptoms of visceral gout varies; the present series ranged from 1 week to 6 years of age, with a majority between 1 and 2 years. Hartwig reported visceral gout occurring in newly hatched chicks. Ward and Gallagher suggested a hereditary predisposition to avian gout but the present study produced no evidence of a hereditary basis for visceral gout; none of the 8 inbred lines at this Centre showed a higher susceptibility to this condition.

**SEASONAL INCIDENCE**

Craig and Kearney reported a higher incidence of visceral gout during the winter months, and this was confirmed to some extent in the present series. Twenty-three birds were affected during the period from October to March, while 12 cases, showing a peak in the true summer months of May to August, occurred during the rest of the year. It is suggestive that the high winter incidence coincides roughly with molting. There is a tendency for the plasma uric acid to rise during molt (Bell, McIndoe, and Gross, and McIndoe, unpublished data).
TABLE I. Seasonal Incidence of Visceral Gout Over the Three Years, 1956–1958

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CLINICAL SYMPTOMS

A clinical diagnosis of visceral gout is not readily established. Any symptoms that may be manifest are so general that they could equally well be part of another syndrome. There are no pathognomonic clinical features: some birds in good bodily condition and without a clinical history of the disease may die suddenly, and others may have a prolonged history of ill health—generally in poor bodily condition, out of lay, listless, and with dry, small, and often cyanosed combs. Vent feathers are frequently soiled with solid “urates” but this, too, is a common observation in birds not affected with visceral gout. Nervous symptoms as observed by Gmelin and Coles were not noted in the present series. Of the birds in this investigation, 32 died naturally and only 3 were killed in the latter stages of the disease.

MACROSCOPIC AUTOPSY FINDINGS

Advanced visceral gout is readily diagnosed postmortem. There is the well-known and characteristic diffuse deposition of white material, generally accepted as urates, on the serous surfaces of various abdominal and thoracic organs, particularly the heart, liver, lungs, gut, and air sacs (Fig. 1). The amount of these deposits may vary from tiny flecks, hardly discernible with the naked eye, to massive encrustation of the organs.

There is no need to discuss separately the appearance of these various organs since all present the same basic picture, which varies only in degree. Urate deposition is best seen on the heart, where in early stages it takes the form of delicate white streaks on the epicardium and the fat of the coronary region. With increased deposition, urates appear on the visceral layer of the pericardium and also as white, semifluid material in the pericardial space. In very advanced cases the heart itself is no longer visible below the dense white mass of urate, and the two membranes covering this organ become firmly adherent, with virtual occlusion of the pericardial space. Similar urate deposits develop on the surface of Glisson’s capsule, which may, in severe cases, become fused with the sternum due to a fibrin-like exudation.

The deposits on the visceral peritoneum covering the gut and those on the costal and pulmonary pleura are essentially similar, except that they do not
appear to attain the same proportions as do those on the heart and liver. In some cases (5 of the 35 examined) the only macroscopic deposition of urate apart from that in the kidneys, was found in the left ventricle, where it formed a clearly defined white line under the endocardium just below the aortic valve (Fig. 2). In 1 of these 5 cases the subendocardial deposit appeared as a very delicate white line running along the posterior border of an unlipped subaortic septal defect, of Type I as described by Siller. Subendocardial deposits, more widely distributed in both left and right ventricles, often occur with other visceral deposits. The liver is frequently swollen, friable, and congested and may on occasion show numerous scattered miliary necrotic foci.
Fig. 2. Small linear subendocardial "urate" deposit below the aortic valve.

Fig. 3. The two kidneys from one bird with visceral gout. The organ at the top shows only moderate enlargement and urates in the form of thin white streaks. The lower kidney is grossly enlarged and shows numerous nodular urate deposits.
The macroscopic changes in the kidneys in visceral gout deserve special consideration. It should be stressed that it is not uncommon for the two kidneys to be affected differently, as regards both type and severity of lesion (Figs. 3 and 4). Partial aplasia and hypoplasia of the kidneys is a common finding, especially in the “red” line of this flock, where it occurs with a frequency of about 25 per cent (Pun, unpublished data). This abnormality, illustrated in Fig. 5, complicated 4 of the cases under review.

In only 3 of the 35 birds with visceral gout could the kidneys be classified as macroscopically normal. In all other instances these organs showed some degree of pathologic change, which justified the diagnosis of nephritis. In about half the cases the kidneys were pink and enlarged, sometimes considerably, presenting the characteristic appearance of acute pyelonephritis (Fig. 3). In the remainder the changes were typical of those in chronic pyelonephritis (Fig. 5), which consist of shrinkage with the development of an irregular surface, often showing evidence of scar and cyst formation.

Urateles of varied appearance are discernible in the kidneys of every bird affected with visceral gout. One type, consisting of thin, white, radiating streaks confined to a lobular distribution, can be seen on the surface as well as in the substance of the organ (Figs. 3 and 4). Similar lesions almost invariably accompany acute pyelonephritis, even when there is no evidence of visceral gout. This change represents merely a stasis within the nephrons. The other type of

Fig. 4. Transverse section of the two kidneys in Fig. 3. Note the confluent nodular deposits in the right and the more linear arrangement in the left kidney.
renal urate deposit appears as yellowish-white nodules scattered over the surface as well as throughout the tissue. These nodules have a marked tendency to confluence, particularly in severe cases, where they show as extensive white areas (Figs. 3 and 4).

The ureters are frequently distended with semifluid, white urine (Fig. 5), which can extend along the branches of the ureter a considerable distance into the substance of the kidneys. On sectioning the organ these tenaceous ureter contents ooze out over the cut surface. Urolithiasis has not been observed in visceral gout.

The interphalangeal toe joints are not enlarged and do not appear to be involved, since they show neither the intra-articular nor periarticular urate deposits so characteristic of articular gout. In the latter condition visceral deposits occur rarely, if ever. In a comparatively high percentage of cases of visceral gout semifluid urates are found within the synovial capsule of the femorotibial joints, but there is no apparent deposition of urates in the articular cartilage and no evidence of tophi in these joints.

In rare instances urates are deposited like fine snowflakes on the surface of

Fig. 5. The two kidneys of one bird with visceral gout, showing partial aplasia and ureteral distention in the lower organ. Note the irregular surface, cysts (left pole), and the nodular as well as streaklike urate deposits in the organ at the top.
the thigh musculature without any evidence of surrounding granulomatous tissue.

Other intercurrent diseases were observed in 10 birds of this series: 4 cases of aortic atheromatosis, 2 of egg peritonitis, 2 of enteritis, 1 of metastasizing adenocarcinoma of the oviduct, and 1 of pneumonia.

HISTOLOGY

The most striking histologic feature of the urate deposits in the serous membranes is the absence both of tissue damage and of inflammatory reaction, acute or chronic, with the exception of fibrin exudation. Tophi were never observed. The needle-shaped crystals appear singly or in radiating bundles in the otherwise normal membrane. There is a tendency, however, for urates to extend beyond the limit of the covering membrane into the surface of the organ. This is particularly noticeable in liver and intestines. Apparently independently of these peripheral lesions, urates, in the characteristic radiating arrangement, may be found lying in a PAS-positive matrix within the substance of some organs, especially in necrotic areas of the liver (Fig. 6). In the lungs urates

Fig. 6. Needle-shaped urate crystals in a necrotic focus of the liver. (Gomori-Leishman, × 200)
are sometimes surrounded by inflammatory cell debris or are apparently free within the otherwise normal tissues in close association with the alveoli (Fig. 7). Similar radiating deposits may be found in the myocardium, again without secondary inflammatory reaction. Very extensive fibrin exudate, also containing urates, may be seen on the surface of affected membranes. This is well illustrated in the case of Glisson’s capsule.

The heavy urate deposits on the heart differ substantially from those of other serous membranes. In advanced cases, needle-shaped crystalline urates, strikingly refringent and staining brown to black with Gomori’s method, line the parietal surface of the epicardium and the visceral surface of the pericardium along practically the entire length (Fig. 8). It is interesting that here the crystals lie more or less parallel to one another with their long axes perpendicular to the membranes. At intervals the course of this crystalline layer is interrupted by the formation of dense structureless homogeneous material (intensely black with Gomori’s stain). Reticular structures that also stain extend into the pericardial space from the border of these deposits. Lying apparently free within the pericardial space itself are irregularly shaped but sometimes almost spherical bodies varying in size and giving a positive urate
stain; they contain numerous needle-shaped, anisotropic crystals (Fig. 8) and may attain considerable dimensions, in places bridging the pericardial and epicardial urate deposits.

Mild subendocardial inflammatory reaction, characterized by the presence of mainly heterophil leukocytes, occurs in some areas (Fig. 9), and is noteworthy in view of the absence of inflammation in other serous membranes. Even so, this reaction is not very marked and involves only acute inflammatory cells without participation of giant cells or fibrosis. Hyperemia of the subendocardial myocardium may be marked.

The histologic appearance of the kidney in visceral gout is complex and somewhat confusing. It is, however, of the greatest importance to the understanding of this condition. The lesions can be considered under two headings: (1) primary changes, and (2) secondary changes.

Although, generally speaking, both kinds of changes are present in any one bird, there were 3 atypical cases in this present series. These showed none of the primary lesions to be described below, although the nonrenal urate deposits were indistinguishable from those of other cases with visceral gout.
Primary Changes

These consist of lesions that are considered typical for pyelonephritis, in agreement with Spector.61 They may be acute, chronic, or healed. Pyelonephritis of the fowl differs in many respects from that of man or other mammals, on both anatomic and physiologic grounds. The avian kidney possesses a peculiar renal portal blood supply60 and a renal pelvis is absent. It is not appropriate here to give a detailed account of pyelonephritis in the fowl; it will suffice to point out those salient features that have a bearing on visceral gout.

The histologic features of acute pyelonephritis are interstitial edema and
heterophil infiltration. Subsequent active invasion of the intact tubular epithelium by acute inflammatory cells is followed by an intratubular accumulation of such cells, often very marked (Fig. 10). Both proximal and distal portions of the nephron can participate in this process and their epithelium becomes flattened and later shows various degenerative changes and sometimes even necrosis. A most characteristic change seen in the acute stage is a selective dilatation of the distal convoluted tubules; this is particularly noticeable where these are related to their isonephric glomeruli, in the region of the macula densa (Fig. 11). The number of nephrons thus affected varies considerably from case to case. In the severest form, the bulk of the tubules are involved.

The chronic pyelonephritic changes are characterized by interstitial, periglomerular, and perivascular fibrosis, scar and cyst formation with tubular and glomerular atrophy, and the development of colloid casts. Interstitial inflammatory cells are predominantly of the more chronic type, involving mainly lymphocytes and plasma cells (Fig. 12).
Fig. 11. Acute pyelonephritis in visceral gout. Note the characteristic dilatation of the proximal convoluted tubule in the region of the macula densa. At the top left there is a necrotic tubule with pyknotic epithelial cells. Urates infiltrate the necrotic epithelium to form intratubular tophi. (Hematoxylin and eosin, × 800)

Fig. 12. Chronic pyelonephritis in visceral gout. Interstitial fibrosis and infiltration with chronic inflammatory cells. Note the severe tubular hypertrophy and the amorphous urates in a tubule at top right. (Hematoxylin and eosin, × 200)
The chronic form may progress to the healed type where the characteristic lesion is often a very considerable hypertrophy of the tubules due to marked increase in the number of their cells (Fig. 12). Scars and cysts are prominent and colloid casts are often abundant. Glomerulonephritic changes, somewhat similar to the chronic type described by Siller, frequently accompany chronic pyelonephritis.

Any or all of these changes are simultaneously present to a varying degree in almost every case of visceral gout.

Secondary Changes

These consist in the deposition of urates in the kidney. Amorphous urates are a common finding in cases of pyelonephritis, even when uncomplicated by visceral gout. The deposits are finely granular, staining bluish with hematoxylin and eosin, and are confined to the lumen of the tubule. Although similar amorphous deposits may be seen in cases of visceral gout, they are overshadowed by other and more significant ones, which are principally of three types. First, there are the delicate needle-shaped crystals that infiltrate the epithelium of necrotic tubules from the still-patent lumen, thus giving a striated appearance to the more or less homogeneous and eosinophilic epithelium (Fig. 11).

Fig. 13. Small intratubular deposit of radiating urate crystals. Note the pyknotic nuclei and the absence of inflammatory reaction. (Hematoxylin and eosin, × 800)
The necrotic tubules have pyknotic nuclei and are often surrounded by a few acute inflammatory cells, which also invade the epithelium from outside (Fig. 13).

These early lesions may develop into the second type, the advanced renal uratic deposits, substantially similar to a tophus. They consist of an indeterminate PAS-positive center, sometimes containing cell debris (Fig. 14), into which the urate crystals are deposited in a radiating, rosette-like arrangement (Fig. 15). The number of crystals, as well as their microstructure, is very variable. The aforementioned central portion is sometimes surrounded by giant cells (Fig. 16) and some fibrosis; at other times giant cells are apparently absent from the otherwise similar lesion (Fig. 14).

Third, necrotic tubules, showing a peripheral chain of pyknotic nuclei but no giant cells and a tendency to confluence, are frequently observed (Fig. 17). These contain granular PAS-positive material in which, also, urates may occasionally be found.

Although the renal urate deposits are generally of any or all these types just described, an occasional case may be seen where delicate needle-shaped urate crystals are scattered indiscriminately throughout the kidney parenchyma. They infiltrate the tubular epithelium from within the lumen, appear in the inter-

Fig. 14. Renal tophus. The crystals have been removed in processing. Note the comparatively poor development of giant cells and fibrosis. (Hematoxylin and eosin, × 800)
Fig. 15. Section of the right kidney depicted in Fig. 4. Note the radiating bundles of urate crystals in the numerous and sometimes confluent renal tophi. (Gomori-Leishman, × 200)

Fig. 16. Several intratubular tophi with well-developed giant cells and a PAS-positive central portion. (PAS, × 200)
Fig. 17. Necrotic renal tubules showing granular content and pyknotic nuclei. Such tubules show a tendency to confluence. (Hematoxylin and eosin, × 800)

Fig. 18. Renal change, atypical in visceral gout. There is no evidence of inflammatory reaction. Some of the proximal convoluted tubules are lined by dense, homogeneous, PAS-positive material; compare the staining reaction of the brush borders. Note the spherical bodies in the distal convoluted tubule at top right. (PAS, × 800)
stitial tissue, within Bowman's space, in the capillary loops of the glomeruli, and even inside the blood vessels.

Another atypical lesion was seen in only 1 of the 35 cases: The kidney showed no inflammatory changes whatsoever; glomeruli, blood vessels, and interstitial tissue all appeared to be normal. On the other hand, a large number of tubular lumina were lined by an apparently homogeneous substance staining deeply with periodic acid–Schiff reagent (Fig. 18). Some of the lumina were tightly packed by spheroid structures that stained in the same way. Such spheroids, which stain black with Gomori's stain, and the homogeneous lining are frequently seen within the tubular lumen in cases of visceral gout (Fig. 19).

**EXPERIMENTAL RESULTS**

In the feeding trial the first blood samples were taken 2 months after the commencement of the experiment. A marked difference was observed between the plasma uric acid of the controls and of the experimental groups. The former showed an average level of 1.3 mg. per 100 cc., while that of the experimental group was 7.3 mg. per 100 cc. Two subsequent samples at monthly intervals showed that the average plasma values of both control and experimental groups had risen to about 5 mg. per 100 cc. However, 3 weeks after raising the protein level to 60 per cent, a further increase to 9 mg. per 100 cc. was observed.

![Fig. 19](image). Necrobiotic tubule in visceral gout. The lumen contains numerous spherical crystals. Note the homogeneous appearance and "fibrous" projections of the periluminar portion of the epithelium. (Hematoxylin and eosin, × 2000)
in the experimental group, while no change occurred in the controls. This difference was maintained when the final blood samples were taken a month later.

The opportunity presented itself to measure the plasma uric acid in 3 cases of spontaneous visceral gout. These showed 18.5, 19.3, and 22.3 mg. per 100 cc. respectively. The highest urate level so far found in healthy birds is 9.6 mg. per 100 cc. One case of genuine articular gout without visceral deposits, obtained from outside this flock, had a plasma uric acid value of 19.2 mg. per 100 cc.

Gross chemical analysis of the pericardial deposit in 1 case of visceral gout showed it to contain less than 5 per cent lipid. Thirty per cent of the fat-free residue was protein and the remaining 70 per cent was largely or wholly urate. X-ray crystallographic studies on this and other samples indicated that the urates may have been entirely monosodium urate. Although the periarticular deposit of 1 case of articular gout contained monosodium urate, the lipid content was 15 per cent and the protein 30 per cent of the dried material.*

DISCUSSION

There appears little doubt that visceral gout and articular gout in the fowl are distinct syndromes. Their lesions differ considerably, not only in somatic distribution but also in the type of tissue reaction evoked. This fact, one of primary importance, is not sufficiently stressed in some textbooks on avian diseases, where visceral and articular gout are generally discussed together, as different manifestations of a single entity, under the heading of either nutritional or metabolic diseases. Blount and Stonebrink on the other hand, consider them quite separately. As mentioned above, an articular involvement sometimes occurs in visceral gout, but there is no similarity between this and the joint lesions of articular gout. It is rarely made clear that these two conditions seldom, if ever, occur concomitantly. Martinaglia, cited by Coles, observed that joints were not implicated in his cases of visceral gout, and Coles, in agreement, points out that in South Africa there was no record of articular gout, while visceral gout occurred frequently. Although avian articular gout shows considerable morphologic similarities with human gout, the latter appears to be unrelated to visceral gout.

Kidney lesions are commonly associated with gout in man. Characteristic are urate deposits, which, according to Ebstein, are specific only when necrotic foci with crystalline urates and an inflammatory reaction are present. Urate crystals alone are said to occur in other conditions as well. In advanced cases it is uncertain whether the lesions, which are surrounded by tissue dam-

*I am indebted to Dr. W. M. McIndoe for these chemical data.
age, inflammatory reaction, and fibrosis, originated within the tubules or in the interstitial tissue. Hench et al.\textsuperscript{24} and Brown and Mallory\textsuperscript{5} found such lesions within the tubules; Minkowski\textsuperscript{11} went even further, postulating that the first deposition occurred in the convoluted tubules and that the involvement of the collecting tubules followed. Garrod\textsuperscript{17} believed the lesions to be of interstitial origin; both tubules and interstitial tissue were involved in Talbott's cases.\textsuperscript{63}

Interstitial renal urate deposits do occur in visceral gout, but they appear to be rare. As a rule, the characteristic lesions develop within the tubules and the urates are laid down in necrotic tubular epithelium. As the lesions enlarge they certainly spread beyond the limits of the tubular basement membrane and there is evidence of actual confluence of neighboring necrotic tubules. A marked but localized inflammatory reaction may follow interstitial urate deposits. In rare instances needle-shaped urate crystals can be seen lying apparently free in an irregular arrangement throughout the kidney substance. Such deposits are not accompanied by a significant inflammatory reaction and the crystals are sometimes found to extend even across structures such as Bowman's capsule. This seems to be due to a sudden deposition of urate.

The secondary renal lesions described above appear essentially similar to the specific gouty changes in the human kidney. In the fowl, however, visceral gout is almost invariably accompanied by some degree of pyelonephritis. Chronic renal changes frequently complicate human gout. Pyelonephritis has been reported on several occasions.\textsuperscript{5, 28, 59} Its significance is not settled but Spitz et al.\textsuperscript{62} believe that there is a direct relationship between the typical kidney lesions of gout and of pyelonephritis. The latter may follow mechanical obstruction of the tubules by urates.\textsuperscript{3} In man, pyelonephritic lesions are not interpreted as primary, although they seem to be so in visceral gout of the fowl. Although Craig and Kearney\textsuperscript{12} mention the possibility that irritation of the avian kidney during uric acid excretion may cause nephritis, this seems most unlikely, despite the suggestion of Talbott\textsuperscript{63} that, in man, excessive and prolonged uric acid excretion may cause secondary renal damage.

The association between visceral gout and nephritis is well authenticated.\textsuperscript{12, 22, 32, 46, 47, 61} Gmelin,\textsuperscript{19} however, states definitely that in his single case there was no "renal insufficiency," and mentions no gross or histologic abnormalities in the kidneys. Likewise, Nieberle and Cohrs\textsuperscript{43} make no mention of nephritis in connection with visceral gout, and Seifried\textsuperscript{56} states that in the early stages the kidneys show no lesions and that the inflammatory changes of the later stages are secondary and are confined to the areas of urate deposition. With the exception of 3 cases, the present series emphasizes the importance of primary renal inflammatory changes. This clearly confirms the statement
of Blount:4 "Visceral gout is that form of acute nephritis which at death is characterized by the deposition of naked eye accumulations of uric acid compounds. . . ." Birds, being uricotelic, excrete the bulk of their waste nitrogen as uric acid which, as shown by Mayrs,15 Gibbs,18 and Marshall,30 is mainly excreted by the tubules. At normal plasma levels, 87 to 93 per cent of the total urate excreted is eliminated by this pathway.53 This being so, one of the consequences of severe and widespread renal tubular damage, inflammatory or degenerative, is the development of hyperuricemia. Jungherr and Matterson30 found that in subacute avian monocytosis, where the outstanding lesion is nephritis, the blood uric acid level rises. Reinhart46 also states that the blood urate is raised in both renal insufficiency and acute nephritis.

The question arises whether it is at all logical to apply the term "gout" to a condition that does not appear to be due to faulty uric acid metabolism. Visceral gout must in most cases be regarded as following damage to the kidneys. More definitely, it appears usually to arise as a sequel to pyelonephritis, although not every case of nephritis is followed by visceral deposition of urates; this may be determined by the severity of the kidney damage. If visceral "gout" is to be so termed it will have to be considered as secondary, or renal, gout. Secondary gout is a term used in human pathology to indicate, according to Gutman,21 a rare form of nonhereditary gout due to an increased uric acid production following the accelerated degradation of nucleic acid that occurs in some blood dyscrasias. Gutman further points out that on rare occasions secondary gout is associated with the hyperuricemia of chronic nephritis; Sokoloff39 draws attention to Magnus-Levy's35 statement that concretions are found in the joints of many uremics who have had no history of primary gout. But even in the primary gout of man the importance of the kidney lesions must not be lost sight of, although, according to Gutman,21 they only accelerate the already existing condition. This is all the more significant when one remembers that tubular excretion has been reported in man.54

There is no evidence of any relationship between gout and leukemia in birds. In visceral gout, enteritis16 and rupture of ovarian follicles, with spilling of yolk material into the peritoneal cavity,16 are common. Egg peritonitis complicated 2 cases of visceral gout in the present series. Uninfected yolk from ruptured ovules is usually absorbed from the peritoneal cavity and could cause a significant rise in endogenous nitrogen convertible to uric acid. Whether a hyperuricemia results in such cases is not known.

Our understanding of the etiology of avian gout remains by no means complete. Despite this, Bechade1 introduced her paper on nutritional or visceral gout, delivered at the Ninth World Poultry Congress in Paris, with the following sentence: "This report does not intend to bring new elements to the knowl-
edge of nutritional gout, the etiology and pathogeny of which are already known."

Craig and Kearney list the following four factors which may account for this condition: (1) Increased endogenous uric acid production, (2) increased exogenous production, (3) decrease in solubility of blood urates, and (4) defective uric acid elimination by the kidney. Stonebrink blames a disturbed watersalt balance.

High-protein intake is frequently incriminated as one of several possible causes of spontaneous gout in fowls. Coles, however, states categorically that "the percentage of available protein in the diet is definitely not to be associated in any way with the disease." This statement, although probably accurate in respect to the etiology, is too dogmatic, since it is quite feasible that a high-protein intake will tend to aggravate an already existing gouty condition.

A connection between a high-protein intake and the development of avian gout appears to be supported by the experimental production of the disease by feeding protein-rich diets. Schlothauer and Bollman induced articular gout in turkeys by increasing the dietary protein to 40 per cent by the addition of horse meat or by supplementing the food with 5 per cent urea. Deposits in both articular and visceral gout were found by Kionka in fowls fed 150 Gm. of horse meat daily, and he believes avian and human gout to be identical in respect of cause and mode of development. The addition of vegetable protein to a level of 36 per cent of the diet produced typical articular lesions in turkey poults.

On the other hand, Hansen has fed fowls for 1½ to 2 months exclusively on meat, liver, and pancreas (all cooked) without producing gouty deposition. He believes that a high-protein diet is of etiologic importance only if accompanied by excessive over-all feeding. Prolonged feeding of unbalanced rations is a cause postulated by Hartwig.

The results of our own very limited feeding trial so far agree with those of Hansen. Our birds were kept on a 30 per cent protein diet for 3 months and subsequently, without a break, on 60 per cent protein for 2 months. Although the plasma uric acid levels during both periods rose in comparison with control birds kept on 18 per cent protein, they did not exceed physiologic levels. It must be pointed out, however, that the total food consumption of the birds was not estimated and may well have decreased considerably, as did indeed happen when a gelatin-casein mixture comprised the sole diet. The trial is still in progress and no evidence of articular gout has so far appeared. A single bird killed for autopsy showed no gouty lesions and no evidence of nephritis.

There are several possible explanations for the apparent discrepancy between our results and those of the workers cited above. In the first place, when
our experiment started the birds were 6 weeks old, and during the course of
the trial their protein requirements altered considerably, particularly when
they came into lay in the latter period. Second, it is possible that over the
comparatively short period of the experiment the kidneys had adapted them-
selves to excrete excessive amounts of uric acid. This is supported by the state-
ment of Folin, Berglund, and Derick that a high-protein diet increases the re-
sponsiveness of the kidney and thus lowers the circulating uric acid. Third.
the protein supplement fed in this experiment consisted of a 2:1 mixture of
casein and gelatin and was therefore fairly well balanced in amino acids. If
the amino-acid balance is incorrect, even in comparatively low-protein diets,
only a certain percentage will be utilized in the body, leaving the remainder to
be converted to uric acid and excreted.

A high wastage of amino acids may be one factor that might explain why
high-protein diets (more likely, unbalanced protein) are so often said to cause
gout in birds under field conditions. This cannot be the whole answer, however,
since at this Centre there is a high incidence of both nephritis and visceral
gout although the diet is balanced in respect of amino acids and has a total
protein content of 18 per cent.

The earliest record of the experimental production of visceral gout goes
back to 1766, when Galvani ligated both ureters in a fowl. This procedure
was repeated later by various workers, who observed urate deposits
on the kidneys, liver, lungs, myocardium, serous membranes, and joints, which
increased in severity the longer such birds survived. A rise of plasma uric acid
to 284 mg. per 100 cc. was obtained by Levine, Wolfson, and Lenel after
ureteral ligation. Similar results were recorded by Folin, Berglund, and
Derick.

Ebstein claimed that lesions typical of both visceral and articular gout
were produced following the subcutaneous administration of potassium dichro-
mate, and Jungherr and Levine obtained a gross pathologic picture indistingui-
shable from renal gout by a similar procedure. Other poisons such as mer-
curic chloride, oxalic acid, acetone, and phenol were reported by von Kossa to
cause similar lesions. Von Kossa also noted the development of renal urate
deposits after the injection of aqueous solutions of various sugars. Injections
of aloin, alloxan, and the administration of yohimbine (van der Plank, cited
by Coles) are all said to cause visceral urate deposition. Reinhart suggested
that the accidental intake of poisons may be responsible for the spontaneous
development of visceral gout.

Witter observed that the clinical use of sodium bicarbonate in the usual
concentration of 0.6 per cent in fowl's drinking water is sometimes accom-
panied by renal change and visceral gout. He produced lesions identical to
those of spontaneous gout by adding 1.2 per cent sodium bicarbonate to the
drinking water. He noted necrotic lesions in the kidney, and the blood uric acid
level rose 6 to 8 times above that of controls. Jungherr confirmed Witter’s
findings. This is all the more interesting since sodium bicarbonate is frequently
suggested as a therapeutic agent in this condition. Hansen was able to
prevent the development of gouty symptoms and elevated blood urate in his
aloin-injected birds by the addition of sodium bicarbonate to the drinking
water.

That visceral gout can develop after the administration of poisonous chemi-
cals again draws attention to the importance of primary renal damage, par-
ticularly when such well-known tubular poisons as mercuric chloride and
potassium dichromate are involved. Innes demonstrated renal cortical necro-
sis in sheep following the administration of alloxan. Some authors who favor
a high-protein diet as the etiologic agent, suggest that this also causes primary
renal damage. Furthermore, a high-protein diet is believed by some to cause
nephritis in the fowl.

Jarmai reported visceral gout in a goose following the feeding of moldy
maize. He believed, also, that in this case the renal damage was primary, he
went far as to say that all cases of renal damage are followed by visceral
gout in birds. On the other hand, Ronk and Carrick found no toxic effects
among chicks fed on maize infected with a variety of molds.

The cause of spontaneous pyelonephritis is obscure. The disease bears the
hallmark of an infection, but so far no specific agent has been isolated. Numer-
ous inconclusive etiologic factors have been suggested in the literature. Avian
monocytosis is said by Waller and by Watanabe to be caused by a filterable
agent.

No definite information is available on the mechanism of the visceral depo-
sition of urates. Reinhart considers that they become precipitated on the
serous membranes owing to the sluggish circulation in these areas. Seifried
thinks that “an alteration in the colloidal state” is responsible. Mayall’s unique explanation is as follows: “The secretion of the urate . . . is brought
about in a reactionless way and thanks to the presence of a stasis transudate
it passes out from the well filled capillaries and veins of the subserosa. The
blood plasma rich with uric acid comes on the surface and there the uric acid
loses its solvent (acid phosphate of soda) and is deposited.” Gmelin suggests
that the heavy uratic deposition on the liver is due to the fact that this organ is
the site of uric acid formation in the fowl. This argument is not sound for,
apart from the fact that uric acid is also produced in the kidney, the heaviest
extrarenal deposits usually occur on the heart. No xanthinoxidase has been
demonstrated in the avian heart. Roberts and Brugsch and Citron have
shown that cartilage can absorb urates from saturated solutions. Folin et al.
suggest that connective tissue and dead animal tissue may have a similar affinity for urates. They also point out that the sites of gouty depositions have poor circulation.

It seems probable that the bulk of the visceral urate deposits, at least the extrarenal ones, are metastatic and not dystrophic, as defined by Sokoloff in the instance of human gout. In the membranes there is no evidence of preceding necrosis, while in the parenchymas of the liver, lung, and kidneys, earlier necroses and the presence of PAS-positive matrices are usually demonstrable. Lipids have been demonstrated by Sokoloff in the center of some human tophi; such was not observed in the cases of the present series.

Deposits on serous membranes do not induce a secondary inflammatory reaction, with the exception of deposits on the epicardium, in which event a localized low-grade cellular reaction can occur. The often considerable fibrin exudation from liver surface and pericardium must, however, be considered as evidence of inflammation. The hyperemia of the subendocardial myocardium is presumably of similar significance. This observation is confirmed by the findings of Nieberle and Cohrs, who even record the development of recent granulation tissue arising from the epicardium and infiltrating the mass of urates. Gmelin failed to confirm Joest's interpretation of the uratic lesion as a chronic inflammation and, examining but 1 case, denies the presence of an inflammatory reaction. The very mild tissue reaction suggests that the crystalline deposits are of short duration and that they are probably formed a short time before death.

Of considerable interest is the chemical composition of the "urate deposits" in visceral gout, a subject given little attention in the literature. McCrudden states that the gouty concretions in man consist essentially of monosodium urate. In fowls the identification is most commonly based on the murexide test, which does not preclude the presence of other salts besides urates, nor does it identify the cation(s) involved. Brandl, cited by Seifried, believes the deposits are composed of monosodium urate and calcium urate. Sokoloff states that it is generally believed that ammonium urate is involved. Unfortunately, he gives no reference to the origin of this statement and it has not been possible to inspect the evidence. It is likely that at least a high proportion of urinary urates are in the form of the ammonium salt, but the visceral deposition of this substance seems very unlikely; ammonia and ammonium ion are extremely toxic and are not measurable in avian blood. Revolta, Delprato, and Farsinarie record 1 case of so-called visceral gout in which the deposits on the serosae and viscera were said to be calcium phosphate; similar depositions of calcium have also been described by Pallaske, but without the characteristic pathologic picture of visceral gout.
It appears from our limited studies that the visceral deposits contain lipid and protein, in addition to substantial amounts (more than 50 per cent) of monosodium urate. It is quite possible, however, that small amounts of other cations, particularly calcium, may also be present.

It is noteworthy that although the plasma-urate level is considerably elevated in visceral gout, a similar high level was observed in 1 case of articular gout. Schlothauer and Bollman noted a high plasma-urate level in articular gout of turkeys. Why visceral deposits do not develop in articular gout is therefore difficult to understand.

SUMMARY

A pathologic study was carried out on 35 cases of visceral gout in Brown Leghorns, with the following conclusions:

1. Visceral and articular gout appear to be separate entities.
2. Visceral gout is usually secondary to renal damage, most commonly that caused by pyelonephritis.
3. The cause of nephritis is still obscure, but high-protein diet or poisonous chemicals do not appear to have been the primary etiologic factors in the cases of the present series.

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SOME CASES OF GOUT IN REPTILES

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(PLATES CXXI AND CXXII)

Urate deposits similar to those of human gout have not been described in other mammals except for a single case of peri-articular deposits of sodium urate in a dog (Bruckmüller, 1869). In the fowl, however, two distinct forms of gout are recognised (Siller, 1959): in the less common articular form, the urate deposits appear similar to those in man, whilst the visceral form is characterised by deposits occurring mainly on the serous surfaces of internal organs.

Although reptilian gout must be familiar to pathologists who handle these animals, published reports of this condition are few and superficial. A brief report of nine cases of gout in reptiles is presented because of its comparative interest.

MATERIALS

The cases were collected over a period of nine years and include one monitor lizard Varanus exanthematicus (case 1), one alligator of unknown species (case 9) and seven tortoises—one Testudo sulcata (case 3), one Kinixys belliana (case 6), one Testudo radiata (case 7) and four which were either Testudo graeca or Testudo hermani (cases 2, 4, 5 and 8).

PATHOLOGICAL CHANGES

Macroscopic findings

Joints. The monitor and all seven tortoises showed deposits of white or pale yellow material of varying consistency within the synovial cavities of one or more joints. In case 7 this material was chemically analysed and found to contain urate.

The severity of the condition varied from case to case in respect of the amount of urate contained within joints and the number of joints affected. Intervertebral articular articulations (fig. 1) as well as limb joints were involved (fig. 2). In the majority of cases the urates were confined to within the joint capsule; in case 6 there were also peri-articular deposits.

Liver. Cases 5 and 6 showed traces of visceral deposits in addition to the articular involvement. In case 5 there was a fine white tracery on the liver capsule, in case 6 a yellowish crusty deposit in the same situation.

Kidneys. In six cases, macroscopic urate was found in the kidneys. The deposits were usually multiple, small foci being scattered throughout the kidney substance, but in case 6 a large fusiform calculus was present in the hilar region of the fibrotic right kidney, and several smaller calculi were present in the left kidney.

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Pericardium. In the alligator, case 9, only the heart was received for examination. Here extensive chalky white deposits were present on the epicardium (fig. 3).

Histological findings

Kidney. The most frequent and easily recognised feature in these cases is the presence of tophi in the tubules. These have a characteristic appearance, the tubular epithelium being replaced by a roughly circular band of striations radiating from the lumen, which is either empty or contains granular or amorphous material.

Tophi were seen in five of the eight cases where renal tissue was available for histological examination. Though the great majority are sited in the tubules, the possibility of interstitial tophus formation could not be entirely ruled out. In general, the appearance of the tophi is very similar to that described in the fowl by Siller (1959) (figs. 4-6). The degree of cellular reaction is minimal and consists only of a ring of multinucleate giant-cells (figs. 4 and 5). In some cases these are very few in number or are absent altogether (fig. 6). The central zone, but not the outer radiating portion, of the tophus is frequently periodic acid-Schiff-positive. Fatty change is present in the tubular epithelium, but there is no fat in the tophi.

Other retrogressive tubular changes, varying from epithelial vacuolation to necrosis, are present in all eight cases. In case 5 some of the renal tubules are distended by dense masses of degenerating inflammatory cells, similar to those occurring in avian pyelonephritis (Spector, 1951). Some degree of interstitial edema is present in several cases, and in case 7 this is accompanied by a widespread heavy cellular interstitial infiltration of polymorphonuclear cells (fig. 4).

No histological changes relevant to gout are present in the other organs. Not even in the liver and epicardium where urates had been observed macroscopically can histological abnormalities be seen. However, many livers contain macrophages laden with pigment (probably melanin), a finding which is common in reptiles and amphibia.

Discussion

Pagenstecher (1863-64) observed articular gout in an *Alligator sclerops* with sodium urate deposits in both hip joints and diffusely distributed throughout the entire musculature. Sodium urate deposits in alligator muscle had previously been reported by Liebig (1849), but without reference to any gouty arthritis. Fox (1925) noted urates on serous membranes of a squamate. Osman Hill (1954-55), surveying metabolic disorders in animals kept at the London Zoological Gardens, described the frequent occurrence of urate deposits on viscera and in the kidneys and joints of birds and reptiles, and assumed the cause to be disordered purine metabolism. Hamerton (1932, 1933, 1939) described three cases in Teguexin lizards (*Tupinambis*) and one in a Sharp-nosed crocodile (*Crocodilus americanus*) and noted that the condition is common to birds and reptiles. The kidneys were enlarged with many urate deposits in distended tubules and ureters; nearly all cases had deposits on the viscera, especially the pericardium and liver capsule and not infrequently around the limb joints. Two cases were noted as having gouty deposits in soft tissues.

Since gout is unknown in mammals other than man, and yet is seen in the fowl and in reptiles, remarks on the renal excretion in the latter are appended.

Amphibia are ammonotelic during their immature, wholly aquatic, phase of life and assume urootelism when adult. Birds, on the other hand, excrete the bulk of their waste nitrogen as uric acid, i.e. they are uricotelic. All three forms of nitrogen excretion are represented among the reptiles, depending on whether they have a wholly aquatic, semi-aquatic or entirely terrestrial existence (Baldwin,
Fig. 2.—Case 7. Radiated Tortoise. Joints of the fore limb displayed showing deposits of urate material on the serous surface of the joint capsules.

Fig. 1.—Case 7. Radiated Tortoise (Testudo radiata). Urate deposits at the intervertebral articulations.

Fig. 3.—Case 9. Alligator (species unknown). Heart, showing white streaky deposits on the epicardial surface.

Gout in reptiles
GOUT IN REPTILES

Fig. 4.—Case 7. Radiated Tortoise. Kidney showing several tophi, and oedema and cellular infiltration of the interstitial tissue. Haematoxylin and eosin. × 60.

Fig. 5.—As fig. 4. Higher-power view of portion of tophus showing radiating lines and peripheral giant-cells. H. and E. × 350.

Fig. 6.—Case 5. Moorish Tortoise (Testudo greca or hermani). Tophus showing P.A.S.-positive centre. Periodic acid-Schiff. × 300.
1962). In alligators the bulk (67-87 per cent.) of urinary nitrogen is ammonia; in the turtle *Chrysemys*, most (24-48 per cent.) is urea and in the terrestrial horned lizard *Phrynosoma*, nearly all (98 per cent.) is uric acid (Scheer, 1948). Moyle (1949) found that the urinary nitrogen of aquatic turtles was largely urea and ammonia, whilst uric acid predominated in terrestrial tortoises.

In view of their uricotelism and the fact that in reptiles, as in the fowl, the uric acid is secreted by the tubules (Marshall, 1932), the occurrence of gouty deposits in terrestrial reptiles such as tortoises and lizards is not surprising. Though at first sight the occurrence of gout in an alligator and in Hamerton's crocodile may seem remarkable, it must be remembered that there is still a much higher percentage of uric acid in the urine of these animals than there is in that of mammals; Scheer's (1948) figure for alligator urine is 20 per cent.

In the present cases the articular form has predominated over the visceral form of gout, although the latter is by far the most common in the fowl. Hamerton, however, had had much experience and his remarks tend to suggest that visceral deposits occur in association with articular deposits in the majority of reptilian cases.

The aetiology of human gout frequently involves the excessive production of uric acid. In the fowl visceral gout has been shown to follow primary renal damage in some, if not all, cases (Siller, 1959). Schlumberger (1959) suggests that in parakeets even synovial gout may be secondary to renal damage. There was no conclusive evidence of primary renal damage in our series of reptilian cases, but there appeared to be some correlation between the amount of urate material deposited in the joints and the degree of pathological changes in the kidney. The joint deposits were heaviest in the cases which showed prominent and numerous tophi in the kidneys.

One factor may have some bearing on the occurrence of "gout" in tortoises. The animals are ungainly and movement is restricted by the heavy carapace. Drinking from a dish or pool with vertical sides is thus difficult. In the opinion of one of us the condition of the tortoises in the Edinburgh Zoological Gardens was noticeably improved when water was made more readily accessible. Dehydration may be significant in the aetiology of gout in captive reptiles. A similar suggestion has been made by Ratcliffe (1959) in respect of reptiles and birds.

**Summary**

Nine cases of gout in reptiles are described, eight showing deposits of urate-like material in the joint capsules and three showing deposits on the viscera. A brief description of the renal pathological appearances is given and the condition is compared to that which commonly occurs in the fowl.

The alligator heart was submitted for examination by Messrs Whittle & Thurmand, Rochdale, Lancashire, and one Moorish Tortoise by the clinical department of the Royal (Dick) School of Veterinary Studies.

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epithelial cells, probably originating from the parietal layer of Bowman's capsule (fig. 23). Completely fibrotic crescents were not observed in the fowl.

(ii) Vascular changes. In cases of advanced chronic glomerulonephritis the renal arterioles and small arteries show characteristic lesions. There would appear to be no doubt about the secondary nature of the vascular changes, since they do not occur during the acute phase and become pronounced only in the later stages of the chronic phase. The walls of the vessels are thickened, but in general their lumina are not greatly reduced (figs. 20 and 24). The thickening of the wall is due to hyperplasia and fibrosis of the media and adventitia. Even without the use of specific stains for connective tissue, thickened strands of collagen can be recognised within the media (fig. 25). Both the PAS and Trichrome stains vividly illustrate this point; in such sections the blood vessels are strikingly differentiated and in the media and adventitia there is a marked increase in PAS positive fibres. The absence of true hyalinisation of the vessels, particularly of afferent glomerular arterioles, is particularly important. Hyaline droplet degeneration is sometimes seen in the media of some of the small arteries (fig. 26). Necrosis or fibrinoid degeneration of the vessels is never observed.
Fig. 26
Asymmetrical thickening of the wall of a small artery with hyaline degeneration of the media. Note the perivascular mononuclear-cell infiltration seen during the chronic stage.
H & E x 640

Fig. 27
Nucleated red-cell casts in the DCT in chronic glomerulonephritis. The tubular epithelium appears normal.
H & E x 850
(iii) **Tubular lesions.** The renal tubules are surprisingly free from degenerative changes even in the chronic stage. "Urate" and red-blood-cell casts are sometimes seen in the proximal and distal convoluted tubules (fig. 27). In advanced chronic cases, localised areas of both mild tubular atrophy and cyst formation are occasionally seen; these lesions are subsequent to such focally distributed interstitial fibrosis as may be present.

(iv) **Changes in the interstitial tissue.** During the later stages of the acute phase, an infiltration of lymphocytes and macrophages may be seen in the interstitial tissue; they become more prominent in the chronic stage. These infiltrations by mononuclear inflammatory cells may surround glomeruli and blood vessels (figs. 18, 25 and 26), or they may form extensive foci in the interstitial tissue. These foci must not be confused with discrete lymphocyte accumulations (secondary lymphoid nodules), which in the fowl are normally present in the kidney and in other organs (fig. 9).

The interstitial fibrosis which develops during the chronic stage of glomerulonephritis is readily demonstrable by silver impregnation, trichrome and PAS staining. It is localised and tends to be most pronounced in the medulla. Mild perivascular and periglomerular fibrosis are also common.
Fig. 28
Diffuse perisinusoidal degeneration of the liver in glomerulonephritis.
H & E x 640

Fig. 29
Cloacal muscularis in chronic glomerulonephritis showing advanced hyalinisation of the muscle fibres and thickening of the interstitial connective tissue.
H & E x 212
Fig. 30
Cloacal mucous membrane with cystlike accumulation of urates. Note also the mononuclear-cell infiltration.
H & E x 212

Fig. 31
Massive lymphocytic infiltration into the submucosa of the ureter in glomerulonephritis.
H & E x 85
(v) **Histology of other organs.** The liver of affected birds is frequently involved and presents a picture of diffuse perisinusoidal degeneration (fig. 28). The cytoplasm of the cells bordering the venous sinuses is swollen, thus compressing their lumina. The cytoplasm of the affected cells stains only lightly; the nuclei are unaltered in the majority of cases. Under low magnification the liver has a patchy appearance with pale areas scattered more or less evenly throughout the organ. Fatty change is not uncommon and may sometimes be severe.

The high incidence of cloacal swelling, ulceration and prolapse has already been mentioned. Histological examination of such cloacae often reveals not only an acute inflammatory reaction, but also extensive mononuclear-cell infiltrations into the superficial and deep layers of the mucosa. The muscularis shows hyalinisation of fibres (fig. 29) and fibrosis. In long-standing cases, where continual severe urate discharge was recorded intra vitam, masses of "urates" may be found enclosed in a cyst-like manner within the crypts of the cloacal villi (fig. 30). Mononuclear-cell infiltrations occur in the ureteral submucosa and may be so extensive that the convolutions of the mucosa are almost lost (fig. 31).

(6) **Kidney biopsies.**

Kidney biopsies were performed in 7 affected birds, but they added nothing of importance to the picture of the disease. However, in 2 birds
which biopsy showed to have acute lesions, complete restitutio ad integrum was found to have taken place when later biopsies and post-mortem examinations were done.

(7) **Aetiology.**

Frischbier and Rindfreisch-Seyfarth (1948) and Reinhart (1950) claim that a diet with an excessive content of sodium chloride can cause nephritis in fowls. While this may be true in some cases, and it has been confirmed experimentally by Paver, Robertson and Wilson (1953), it cannot be accepted as the usual cause of glomerulonephritis.

Staphylococci were occasionally isolated from kidneys and other organs of affected birds of the present series, but this finding is too inconstant to be significant. Inoculations of staphylococcal cultures and organ homogenates into healthy birds have failed to produce the glomerulonephritic changes.

(8) **Proliferative glomerular lesions in hyperoestrogenisation.**

(a) **Physiological hyperoestrogenisation.**

Apart from the higher incidence in male chicks, as compared with females, of cuboidal metaplasia of the epithelium which lines Bowman's capsule (see pg. 12), there is no obvious histological sex diamorphism in the kidneys of fowls. Kidney sections from healthy chicks of different ages were studied and it was found that in 10-12 week old birds of both sexes, the glomeruli show changes which would generally be regarded
Fig. 32
Physiological hyperoestrogenisation in an 11-week old male fowl: 2 glomeruli showing massive epithelial proliferation and thickening of the basement membranes.
PAS x 850

Fig. 33
Physiological hyperoestrogenisation in an 11-week old female. All the glomeruli are enlarged due to epithelial proliferation.
PAS x 212
abnormal. This "abnormality" is diffuse, affecting all the glomeruli to a greater or less extent and it is completely reversible in that these changes disappear when the chicks have passed this "critical" age.

Figure 32 illustrates two such glomeruli in an 11-week old male chick, while figure 33 is taken from the kidney section of a similarly aged female and shows a diffuse involvement of the glomeruli. It is apparent that the glomeruli are greatly enlarged, the swollen tufts filling the capsular space, and that there is a considerable hyperplasia, not only of the central mesangial and endothelial cells, but also the cells of the visceral epithelial layer (Fig. 35).

These epithelial cells have increased not only in size, but also in number and they lie in close contact with those of the parietal epithelium of Bowman's capsule. This may cause difficulty in determining whether the proliferations originate in the visceral or the parietal layer, or in both.

The glomeruli are more or less ischaemic. This is due not only to the swelling of the tuft as a whole and the epithelial cells in particular, but also to the thickening of their basement membranes; the latter may only be focal and not very obvious.

While the glomeruli of both sexes are affected in this manner, those of the males are more severely involved. Furthermore, there appears to be a gradual increase in the severity of the glo-
Fig. 34
Physiological hyperoestrogenisation in an 11-week old male. This glomerulus shows marked proliferation of mainly the capillary endothelium leading to ischaemia.

PAS x 850

Fig. 35
Glomerulus in a hen with a thecal tumour showing the acute phase of the oestrogen effect.

H & E x 850
merular involvement from about 9 weeks of age onwards, reaching a maximum at 11 weeks.

It will be suggested in the discussion, that these glomerular peculiarities of 11-week old chicks are probably due directly to a physiological endogenous hyperoestrogenisation.

(b) Oestrogen-secreting tumours.

Dr. J.G. Campbell has kindly provided histological sections of kidneys from fowls bearing oestrogen-secreting tumours. There were 5 cases in all; in 2 the neoplasma were diagnosed (by Dr. Campbell) as granulosa tumours, in 1 as thecal tumour and in the remaining 2 as showing both thecal and granulosa moieties.

Renal glomerular changes were observed histologically in all these cases. In 3 cases they were of the acute type while in 2 birds they showed more chronic characteristics. The changes were diffuse, in that, especially in advanced cases, all the glomeruli in the section were affected. In early cases, where not all the glomeruli were involved, it was obvious that the larger juxta-medullary ones were the first to become affected.

The acute lesions are not dissimilar from those of the acute form of proliferative glomerulonephritis, the tufts being swollen, ischaemic and filling the entire capsular space (fig. 35). The degenerative hyaline droplets of the swollen visceral epithelium are not so marked. The basement membranes
Fig. 36
Severely distorted glomerulus in a bird with a thecal tumour.
H & E  x 850

Fig. 37
Scar formation in a kidney from a bird with a thecal tumour. Note the hyaline casts.
H & E  x 212
may be thickened. Tubules and blood vessels are still normal at this stage.

In the chronic form the glomeruli may be shrunken, distorted (fig. 36) and surrounded by extensive fibrosis. Areas of genuine scar tissue involving a number of such glomeruli may be encountered (fig. 37). Vascular changes in the form of thickening of the arterial walls become very prominent. The tubules, however, still only show very slight retrogressive changes.

(c) Experimental oestrogen administration.

In view of the possible influence of oestrogens on the renal glomeruli, it was thought important to examine kidney sections from fowls which had received experimental oestrogen administrations. These were of two kinds:

(1) Oestrogen implants. 30 mg stilboestrol were implanted subcutaneously into 6 males and 6 females, all 7 weeks old. These birds were killed when 11 weeks of age and their kidneys were examined histologically.

The glomeruli of both sexes showed proliferative lesions considerably more severe than found in normal fowls of that "critical" age (fig. 38). The distribution of these altered glomeruli was much more diffuse in females than in males. Chronic changes were not observed, but all the characteristic features of the acute type of lesions were represented.
Fig. 38
Chronic proliferative glomerular lesion with distortion and lobulation of the tuft in an 11-week old male bird with 30 mg stilboestrol implantation.
H & E x 850

Fig. 39
A group of distorted glomeruli showing chronic proliferative changes in a bird fed an oestrogenic diet.
H & E x 212
(ii) Oral oestrogen administration. Dr. A.W.

Greenwood had fed, for different reasons, chicks from hatching on a dry mash diet containing 0.025% of "Lipamone" (Diencestrodiol diacetate). All birds which subsequently died were examined post-mortem by the author; the kidneys again showed marked glomerular lesions. Unlike those of oestrogen-implanted birds, however, the lesions were decidedly chronic in character, showing distortion and shrinkage of the glomerular tufts, fibrosis of the capsule and the "chain-like" appearance of the visceral epithelial cells (fig. 39). The intra-renal arteries were also greatly thickened and very prominent.

(d) Secondary hyperoestrogenisation following liver damage.

Campbell (1956) has shown that damage to the liver can lead to a hyperoestrogenic state in fowls, because such livers are unable to conjugate and excrete oestrogen, which therefore accumulates in the tissues.

(i) Senecio poisoning. Kidney sections from Dr. Campbell's ragwort experiments were examined by the author. All cases associated with severe long-standing liver damage invariably showed diffuse chronic lesions in the renal glomeruli. These were of the typical kind with distortion of the tufts, fibrosis of the capsule and the characteristic epithelial changes.
Fig. 40
Severe diffuse liver cirrhosis. Almost all of the liver parenchyma is replaced by fibrous tissue. Islets of hepatic tissue, probably regenerative, remain.
H & E x 212

Fig. 41
Cortical region of a kidney from the bird with severe liver cirrhosis (fig. 40). There is a tremendous and diffuse thickening of the tubular basement membranes and a selective dilatation of the DCT.
H & E x 212
(ii) Carbon tetrachloride poisoning. All 6 birds (3 males and 3 females) which had received a course of oral carbon tetrachloride administration, showed severe retrogressive tubular changes in the kidneys and also glomerular lesions of the acute proliferative type. The glomerular lesions were less severe than in birds under direct oestrogenisation (following the administration of oestrogenic substances, of the development of oestrogen-secreting tumours), or in those with Senecio poisoning.

(iii) Liver cirrhosis. True liver cirrhosis, of a severity comparable with that of man, is rare in fowls. Nevertheless it was possible to collect at least 3 such cases, where the fibrous proliferations in the liver were sufficiently extensive to warrant a clear-cut diagnosis of hepatic cirrhosis. In each of these cases the kidneys again showed changes similar to those of proliferative glomerulonephritis, although the severity and distribution of the glomerular lesions was only moderate.

One unusual and very severe case of liver cirrhosis deserves special mention, since the renal lesions were strikingly different from the others. The liver itself was practically completely replaced by fibrous tissue, only small islets of normal, possibly regenerating liver cells being present (fig. 40).

The glomeruli showed only moderate proliferative changes. The basement membranes of the tuft capillaries were only focally thickened, while the
fig. 42
Kidney in liver cirrhosis. There are only moderate glomerular lesions and the capsule is thickened. The thickening of the tubular basement membrane and the dilatation of the DCT is shown.
H & E x 850

Fig. 43
Medulla of the kidney in liver cirrhosis. There is a marked interstitial fibrosis. Although present in some tubules, the thickening of the basement membranes is not so prominent as in the cortex.
H & E x 212
tubular basement membranes were diffusely and tremendously affected (figs. 41 and 42). The renal medulla showed marked fibrosis (fig. 43).

(9) Discussion,

The changes observed in the glomeruli during the acute phase of the illness closely resemble the glomerular lesions characteristic of mammalian glomerulonephritis, particularly in respect of the increased epithelial and endothelial cellularity, the thickened basement membranes of the capillary loops and the glomerular ischaemia. In the fowl, however, the chronic glomerular changes do not appear to develop beyond a stage comparable with the subacute lesions in man, since complete tuft fibrosis and hyalinisation have not been observed.

Fatty change, vacuolation and the formation of hyaline droplets in the tubular epithelium are common in acute glomerulonephritis of man (Allen, 1951), yet none of these changes is encountered in the proliferative form of glomerulonephritis of fowls. Mammalian tubules are supplied only with post-glomerular arterial blood and the tubular lesions of acute glomerulonephritis are secondary to glomerular ischaemia. The avian kidney has a dual blood-supply, the tubules receiving blood from the afferent renal-portal vein (see page 16) as well as post-glomerular arterial blood. It is reasonable to attribute the survival of the tubules in birds affected
with acute glomerulonephritis to this double blood-supply, since ischaemia of the glomerulus will have less severe consequences on the kidney as a whole than in mammals.

Interstitial fibrosis in the chronic stage is not nearly so extensive as in mammals, and this may well be due to a species difference in the reactivity of fibrous tissue. It has been pointed out by Campbell (1956) that true liver cirrhosis is rarer in fowls than in mammals subjected to similar hepatotoxic influences. The tubular changes, such as atrophy, which are so evident in mammalian chronic glomerulonephritis and which, in part at any rate, are due to the extensive intertubular fibrosis, are therefore much less apparent in the fowl. It seems safe to assume that this is also the reason for the insignificant fibrosis of the glomerular tuft, where there is only a moderate increase in argyrophil fibres, and also for the absence of fibrosis in the glomerular crescents, which is common in long-standing cases of subacute glomerulonephritis of man (Allen, 1951).

The tubular changes of both the acute and the chronic phase are, therefore, much milder in the fowl than in mammals with glomerular lesions of equal severity.

In the fowl, acute glomerular lesions, characterised by cellular proliferation, epithelial swelling and degeneration and thickening of the basement membrane, appear to be completely reversible. This is shown by the biopsies of the present investigation.
Similar lesions produced experimentally by salt administration, can also resolve (Paver, Robertson and Wilson, 1953).

Selye (1942) described, in fowls, experimental glomerular lesions apparently identical with those of the spontaneous condition now under discussion. It was found that this experimental syndrome was very similar to human nephrosclerosis and, since it was caused by repeated subcutaneous injections of desoxycorticosterone acetate, he suggests that this steroid may play a part in the aetiology of essential hypertension of man. The conclusion was reached that the extra-renal lesions, such as oedema, ascites, pericardial effusion and cardiac enlargement, result from changes in the electrolyte metabolism, which may not be due to the direct action of DOCA but be secondary to the renal lesions. The data under review do not support this view, for none of Selye's extra-renal lesions was observed during either the acute or chronic stages of the spontaneous disease in the fowl, though the glomerular and renal vascular changes appear to be identical.

The changes in the renal vessels, although not so severe as in chronic glomerulonephritis or nephrosclerosis of man, suggest that there is also an increase in blood-pressure in this spontaneous disease of fowls. Since there is none of the glomerular fibrosis and hyalinisation of the afferent arterioles seen in human malignant hypertension, it is safe to
assume, even though no measurements have been made, that any hypertension which may accompany this disease will be relatively insignificant. This is presumably the reason for the inconclusive blood-pressure readings obtained by Selye in his experimental birds. In another paper Selye and Stone (1943) state that chicks on a high sodium chloride intake develop lesions identical with those obtained after DOCA administration.

It has already been stated that the aetiology of proliferative glomerulonephritis of fowls is unknown. According to Allen (1951) in man, diffuse glomerulonephritis may be looked upon as a complication of, or a sequel to, focal infections in any of a number of organs. He believes it to develop on an immunological basis. Such a mechanism has not been established in the fowl with any degree of certainty, although in some cases (see table II) various complicating conditions, many of them infective, were present; a definite relationship between these and glomerulonephritis could not be established.

The importance of oestrogens in the development of glomerulonephritis is illustrated by the description of the lesions observed in hyperoestrogenised fowls. Of particular interest is the fact that, normally, 11-week old fowls show proliferative glomerular changes. Campbell (1960) has already demonstrated, by an indirect method using the bromsulphthalein liver clearance test, that both male and female fowls of this age are heavily
oestrogenised. The exact coincidence of this "trough" of oestrogenisation (see Campbell's fig. 1), with the appearance of proliferative glomerular changes strongly suggests a direct connexion between the two, particularly since a number of other changes are known to occur at this "critical" age (Campbell, personal communication).

Direct evidence to prove the effect of oestrogen on glomerular morphology was obtained by the administration of oestrogenic substances and by the examination of kidney sections from birds with oestrogen-secreting tumours. Oestrogen implantation provoked only acute changes while feeding of oestrogenic substances had a more chronic effect on the glomeruli. This is undoubtedly only a question of concentration and duration of the oestrogen influence. Similarly some oestrogen-secreting tumours produce acute, and others chronic, lesions. This can be interpreted as a variation in duration and amount of oestrogen secretion.

Campbell (1956) has shown, again by his indirect method, that liver damage leads to hyperoestrogenisation. His ragwort-poisoned birds showed well developed chronic glomerular lesions, while the rather more acute and less severe liver damage due to carbon tetrachloride administration produced only acute changes in the glomeruli. In these cases, however, the possibility of a direct nephrotoxic effect of CCl₄ cannot altogether be neglected.
The few cases of liver cirrhosis do not afford very convincing support to a hepato-renal relationship in glomerulonephritis. This is particularly so, since in the only really advanced case of hepatic cirrhosis, the severest renal changes were extra-glomerular. The fact must not be overlooked that the hepatic cirrhosis producing noxa may have had a direct effect also on the kidney, inducing changes in this organ, which may be comparable in some respects to a "cirrhotic" process.

In all, however, the weight of the evidence does suggest that directly or indirectly, excessive circulating oestrogen affects the renal glomeruli and produces in them changes indistinguishable from proliferative glomerulonephritis.
B. Membranous Glomerulonephritis.

(1) Introduction.

While focal thickening of the basement membranes of the glomerular capillaries is part of the histo-pathological complex of proliferative glomerulonephritis, the most significant changes are the proliferative ones. True membranous glomerulonephritis, unaccompanied by epithelial, mesangial or endothelial cell-proliferation was seen only in cases of ground-nut poisoning. A part of this study has already been published as "The histopathology of an entero-hepatic syndrome in turkey poults", (Siller and Ostler, 1961 - see appendix). The clinical and macroscopic features of this disease were studied by Ostler and are not considered here.

Highly fatal epidemics among turkey poults reported from various parts of the country were ultimately attributed to ground-nuts in the ration. The toxic factor has not yet been positively identified, but it is now generally held that fungal infection of the nut plays an important part.

(2) Histological lesions in ground-nut poisoning.

(a) Turkeys.

(1) Liver: The most striking and easily recognisable lesions occur in the liver and are practically pathognomonic for this disease. Both retrogressive and regenerative changes are invariably co-existent.
Fig. 44
Retrogressive and regenerative changes in the liver of a turkey with Brazilian ground-nut poisoning. Note the various stages of necro-biosis (right) and the regenerating liver cells in tubular arrangement (left).
H & E x 680

Fig. 45
Membranous glomerulonephritis. Two glomeruli showing mostly diffuse but also nodular thickening of the basement membranes. There are no proliferative changes.
PAS x 850
The retrogressive changes, which appear to predominate in the early cases, are of necrobiotic character and all stages from swelling of the parenchymal cells to complete necrosis are observed. In the early stages the cells are merely vacuolated, later they take on a homogeneous eosinophilic appearance.

The regenerative phase is characterised by the development of larger liver cells than normal, the nuclei of which contain very prominent nucleoli. These regenerating cells usually form tubules. Both retrogressive and regenerative changes are seen in figure 44.

(ii) Duodenum: There is evidence of severe catarrhal enteritis. Large portions of mucosal epithelium, otherwise normal, are desquamated and lie free in the lumen of the gut. In a number of sections the mucosa is almost totally desquamated, leaving only the Brunner glands of the submucosa intact. In some instances there is a characteristic coagulative necrosis of groups of villi.

(iii) Kidney: Apart from mild inter-tubular oedema present in some cases, only the glomeruli and the PCT show any significant changes. In the glomeruli there is a very pronounced and widespread thickening of the capillary basement membranes (fig. 45). Practically every glomerulus in the section is affected to some degree. In one and the same section the severity of the lesion can range from moderate thickening
Membranous glomerulonephritis. Diffuse thickening of the capillary basement membranes which are of rather loose texture. Note the hyaline droplet in Bowman's capsule (top left).

PAS \hspace{1cm} x 1275

Ground-nut poisoning in a turkey. A PAS is tightly packed with hyaline droplets. Such epithelial nuclei as are visible are seen to be normal.

PAS \hspace{1cm} x 1275
of the basement membrane of one capillary loop in one glomerulus to a pronounced thickening of all the membranes in another. These membranes are usually dense and stain deeply with PAS (fig. 45), in some instances they are of looser texture (fig. 46), and in some others nodular, non-laminated thickenings are present along the course of the capillary walls (fig 45). PAS-positive spherical granules of varying size are, from time to time, observed in the parietal epithelium of Bowman's capsule (fig. 46). Although these membranous lesions are prominent, the glomeruli are of normal size and show no evidence of any proliferative changes.

When present in sections stained with PAS, lesions of the tubules are striking but sparsely distributed. Those kidneys affected with membranous glomerulonephritis also show hyaline droplet nephrosis. The PCT are usually involved and, sometimes, the collecting tubules as well. On the other hand, the DCT are invariably normal. The epithelium of the nephrotic tubules is swollen. In those severely affected, the cytoplasm is packed with PAS-positive hyaline droplets of varying sizes (fig. 47). This dense accumulation of granules tends to obscure the nuclei. Although most of them are normal (fig. 47) a few in the affected epithelium show degenerative changes such as moderate pyknosis and margination of the nuclear chromatin. In less severely affected tubules, small droplets only are present. It would
appear that these first develop at the apical part of the epithelial cell, immediately below the brush border which lines the lumen. The blood vessels of the kidney are normal.

(b) **Fowls.**

(i) **Liver:** The liver lesions in the 10 fowls affected with this disease were virtually identical with those of turkeys. However, it is more usual to find either the regressive or the regenerative lesions predominating in any one section.

(ii) **Kidneys:** The glomerular changes are identical with those of the turkeys. In some instances, however, the membranous thickening is even more advanced. Hyaline droplet nephrosis was not observed in any of the kidney sections from ground-nut poisoned fowls.

(3) **Discussion**

The more common proliferative type of glomerulonephritis in fowls is characterised in the acute stage by both epi- and endothelial hyperplasia in the glomeruli and it is accompanied by only moderate and focal thickening of the capillary basement membranes. In ground-nut poisoning of both turkeys and fowls, on the other hand, the important renal lesion appears to be the very marked and extensive thickening of these membranes. According to Allen's(1951) classification these latter lesions are typical for membranous glomerulonephritis.
The fact that hepatic changes in ground-nut poisoned turkeys are very similar to those of Senecio intoxication in fowls (Campbell, 1956) was first shown by Siller and Ostler (1961). Since then, other investigators have confirmed the similarity of liver lesions in ground-nut and Senecio intoxication in ducks and fowls (Asplin and Carnaghan, 1961), in cattle (Loosemore and Markson, 1961) and in pigs (Loosemore and Harding, 1961).

Whereas the liver changes in both ground-nut and Senecio intoxication are very similar, changes in the kidneys are not, although there is a glomerulonephritis in both cases. It was argued above (pg. 41) that the proliferative glomerular lesions in Senecio poisoning are due to a secondary hyperoestrogenisation following liver damage. In the ground-nut poisoned birds, the glomerular and tubular lesions do not resemble those seen in hyperoestrogenisation. While it is true that ground-nut poisoning runs a much more rapid course than does Senecio intoxication, and while it may be argued that a hyperoestrogenic state had not had time to develop, it must be remembered that, even in the very early stages, such membranous lesions do not occur in proliferative glomerulonephritis.

Hyaline droplet nephrosis was seen in many kidney sections from turkeys with ground nut intoxication. In animals, this lesion occurs both in
glomerular and interstitial nephritis (Nieberle, 1952). In man, this type of nephrosis is associated with various renal diseases such as membranous glomerulonephritis, lipoid nephrosis and nephrosclerosis. It also occurs in tubular degeneration following chemical poisons including certain sulphonamides (Allen, 1951).

It is not understood why hyaline droplet nephrosis did not occur in fowls with ground-nut poisoning. Asplin and Carnaghan (1961) described this condition in fowls and ducks, and also failed to find renal lesions similar to those of turkeys (Siller and Ostler, 1961). Asplin and Carnaghan suggest that the renal lesions of turkeys were due to the combined effect of sulphaquinoxalin and ground-nuts. It is true that the turkeys had received sulphonamide supplementation and this may perhaps account for the nephrosis, but both turkeys and fowls (which had not received sulphonamides) showed membranous glomerular changes. It is therefore incorrect to attribute the renal lesions to the action of sulphonamides. It is still difficult to understand why Asplin and Carnaghan did not observe membranous thickening in their ground-nut poisoned fowls.
V. PYELONEPHRITIS.

(A) Introduction.

Pyelonephritis is by far the most common form of nephritis in the fowl. The 51 cases of renal disease described by Spector (1951) were all of this type, although judging from his description he saw mainly that form which is described below as chronic. Blount (1947) does no more than mention nephritis in his otherwise excellent book on poultry diseases. No indication is given of the pathological type involved but he must be referring to pyelonephritis, since he associates it with visceral gout. Reinhart (1950) is no more lucid on the histological description, merely stating that hyperaemia, exudative and proliferative changes are present. Reis and Nobrega (undated) quote various authors as having observed pyelonephritis in birds, but do not describe the lesions of this condition.

(B) Incidence.

As already stated, during 1956-1960, 233 cases of nephritis were found among 807 autopsies. Of these 233 cases, 126 (55%) were diagnosed as pyelonephritis. It must be remembered that although this type of nephritis may occur without any extra-renal urate deposits, frequently it is associated with visceral gout as it was in a further 54 birds (23%) where the diagnosis was "nephritis and visceral gout". These 54 cases will be dealt with separately in section VIII (gout).
but they must be taken into consideration from the point of view of the overall incidence of pyelonephritis, which was therefore present in 78% of the PRC birds showing renal inflammatory changes.

(C) Clinical Signs.

Peracute, acute and chronic forms can be differentiated.

(1) Peracute Pyelonephritis

Birds in good bodily condition suddenly show a drop in egg production, their head appendages are often severely cyanosed and their crops may be distended with fluid. Such birds are inappetent and febrile. They die within 1-2 days, or death may be the first symptom to be noted. In the peracute form, it is not uncommon for a number of birds in the same pen to become affected more or less simultaneously and die within a short time of one another. Usually, laying pullets are affected. There is a severe absolute leucocytosis, characterised by a very marked heterophilia and monocytosis.

Such "outbreaks" were observed in the PRC flock in 1958 and 1960. Mortality was never very high, amounting to only 10-20% of the birds in one pen. Only a few pens were affected. This peracute form is usually uncomplicated by other diseases.

(2) Acute Pyelonephritis.

Affected birds may show no characteristic symptoms, but may die suddenly. On the other hand, they are often listless and febrile for a few
days. The vent feathers may or may not be soiled by continuous and copious urine discharge. Bodily condition is usually good when they die. Since this form can occur either by itself, or in association with other disease conditions, the clinical symptoms are not at all clear-cut and diagnostic.

In acute pyelonephritis the blood picture is usually abnormal, showing heterophilia and monocytes.

(3) **Chronic Pyelonephritis.**

This form, too, is frequently complicated by other diseases and it is difficult to give a generally valid description of the clinical symptoms. However, the chronic protracted course usually causes affected birds to be in poor bodily condition; they are frequently emaciated. In the later stages, such birds may show signs of somnolence; they sit hunched up with eyes closed and feather ruffled. Egg-production ceases; their small combs are dry and scaly. The pericloacal region may be soiled with dried urates.

(D) **Autopsy findings.**

The majority of birds (108) died while only 20 were killed in extremis.

(1) **Peracute form.**

The autopsy findings in this form are almost sufficiently characteristic to permit a correct diagnosis being made on them alone. The birds are fewered, in good condition, and usually ovulating. A number of ovules are large and mature, although they may be somewhat congested. The oviduct is large and
functional. The kidneys are huge. They bulge into the abdominal cavity from their bony confines in the pelvis. They are bright pink in colour and have streaky urates deposited throughout their substance.

The liver is invariably enlarged, toxic and friable. It has a greasy, smudgy appearance. The adrenal glands are usually enlarged. Extra-renal urate deposits do not usually occur.

(2) Acute form.

While both kidneys are usually affected in the peracute form, only one organ may be involved in the acute and chronic forms, or the involvement of the two kidneys may be unequal. In uncomplicated cases the kidneys are somewhat enlarged and paler than normal, but the swelling is not nearly so pronounced as in the peracute form. Urates are usually present in the substance of these organs; they form streaks which are due to urine stasis within the tubules. Nodular urate deposits are not usually seen in this form.

In every one of the 50 birds with acute pyelonephritis the liver was enlarged and toxic. The other abdominal organs are either normal or bear evidence of other complicating diseases.

Urate deposits on the visceral organs occur in a number of these acute cases of pyelonephritis; these will be described in detail under visceral gout in section VIII.
Fig. 48

Macroscopic view of the eviscerated abdominal cavity of a bird with chronic pyelonephritis showing very large cystic kidneys. A somewhat more normal appearance may be seen in the anterior division of the right kidney (top right).

Fig. 49

Macroscopic view of the two kidneys from a bird with chronic pyelonephritis. One kidney is hyperplastic; the ureter is obstructed and distended.
(3) **Chronic form.**

A considerable variety of macroscopic kidney lesions can be seen in this form.

The swelling of the acute stage has entirely regressed. The kidneys are more or less normal in size and are in many cases atrophied. They are in general abnormally dark coloured, with an irregular surface. Cysts frequently develop. These vary in size between very small and extremely large, when they may bulge above the surface. Such cysts can be uni- or multilocular. They usually contain a clear fluid which can contain flecks of urate. Such cysts may attain tremendous size and distribution (fig. 48), and simulate the appearance of a congenital cystic kidney.

Obstruction of the ureter is frequent, leading to the development of hydro-ureter (fig. 49). Such distended ureters contain not only a milky white urine, but sometimes also inspissated pus.

**(E) Histology.**

The histological differences between the peracute, acute and chronic types of pyelonephritis are usually marked, but it must be remembered that transitional stages occur between these forms. It is particularly common to find acute exacerbative lesions in kidneys with the chronic type.

On the strength of the histological examination, it is possible to classify all the 128 birds with pyelonephritis into one or other of the following
Fig. 50
Peracute pyelonephritis. The marked interstitial oedema has caused a wide separation of the tubules, which normally lie in close apposition one to the other.

H & E  x 850

Fig. 51
Acute Pyelonephritis. There is the typical distension of a DCT in the juxta-glomerular position and necrobiosis of a PCT.

H & E  x 850
categories:

- Peracute - 9 birds (7%)
- Acute - 50 birds (39%)
- Chronic - 69 birds (54%)  

(a) peracute pyelonephritis.

The greatly enlarged kidneys of peracute pyelonephritis show very extensive interstitial oedema of both the cortical and the medullary regions. The tubules have become widely separated and the inter-tubular stroma has a very loose appearance (fig. 50). Acute inflammatory cells, the so-called heterophils, and spindle-shaped fibroblasts are seen in the oedematous interstitium. Cells of the mononuclear series are generally absent. The inter-tubular capillaries are often congested.

A variety of changes is seen in the tubules, some of which may be completely normal, while others may be dilated and their lumina appear empty. This distension is characteristic of both the peracute and the acute form and it affects mainly the DCT (fig. 51). This is particularly well illustrated in the region of the macula densa, where the DCT is closely related to the isonephric glomerulus.

In some tubules the otherwise normal epithelium may contain heterophil leucocytes (fig. 52) which presumably have migrated from the interstitial tissue. The lumina, also, of many such tubules may contain these inflammatory cells. However, this cellular response is not usually as severe as in the
Fig. 52

Acute pyelonephritis. Interstitial infiltration with heterophil granulocytes which are actively invading the tubular epithelium and accumulating within the lumen of the tubules.

H & E  x 850

Fig. 53

Very early acute pyelonephritis. Small foci of interstitial heterophil infiltration are present. Some of the DCT contain inflammatory cells in their lumina. In this bird no involvement of the medulla was found.

H & E  x 212
Fig. 54
Very early acute pyelonephritis. This is a section of the same kidney as in fig. 53. It shows active invasion of the tubular epithelium by inflammatory cells.
H & E x 850

Fig. 55
Acute pyelonephritis. The glomerulus shows moderate hyperplasia of the visceral epithelium. A PCT is heavily invaded by acute inflammatory cells from the interstitial tissue which also shows the more chronic mononuclear-cell reaction.
H & E x 850
acute form of pyelonephritis, where it forms the characteristic lesion.

In the peracute form the glomeruli are generally normal, although in some, Bowman's capsule may be dilated and even contain small amounts of granular material. The blood vessels are unaffected.

(b) acute pyelonephritis.

The renal lesions in acute pyelonephritis are extremely multifarious. They vary from tiny focal infiltrations, of e.g. heterophils, to severe involvement of most of the renal tissue with massive cellular infiltration and destruction. The first lesions appear to occur in the interstitial tissue where, in the very early cases, they show as small accumulations of heterophils (fig. 53). Oedema is usually not marked. These early lesions may be confined to only a very small area and probably affect no more than a few nephrons. Some of the tubules in such an area are dilated; this is most readily seen in the DCT (fig. 53). The active invasion of the tubular epithelium is demonstrated by the presence of heterophils within the otherwise normal tubular lining (fig. 54). It would appear as though it is mainly the DCT which are involved. In the very early case illustrated in figure 53 the lesions were confined to a very small area of the cortex. No medullary infiltrations were seen and the collecting tubules did not contain any inflammatory cells.

As the disease advances the renal lesions
**Fig. 56**

Acute pyelonephritis. General view showing a dilatation of the DCT, the interstitial inflammatory reaction, the active invasion of the tubular epithelium and the accumulation of inflammatory cells in the lumina of several DCT.

H & E 

x 212

**Fig. 57**

Acute pyelonephritis. This photomicrograph shows the characteristic dilatation of the DCT.

H & E 

x 212
Fig. 58
Acute pyelonephritis. Complete obliteration of the lumina of all the dilated tubules in the field with inflammatory cells. There is little interstitial reaction.
H & E  x 850

Fig. 59
Acute pyelonephritis. Degeneration of a PCT. There is a more chronic inflammatory reaction in the interstitium.
H & E  x 850
Fig. 60
Acute pyelonephritis. Tubular degeneration with subepithelial accumulation of cell debris.
H & E x 850

Fig. 61
An early "rosette" in pyelonephritis, showing the necrosis of the tubule and the invasion of peripheral inflammatory cells.
H & E x 850
Massive accumulation of inflammatory cells in the renal medulla in acute pyelonephritis.

Fig. 62
PAS  x 50

A cortical abscess in acute pyelonephritis.

Fig. 63
H & E  x 212
Fig. 64
Glomerulus in acute pyelonephritis. Heterophil granulocytes are present not only in the tuft but also in the capsular space.

H & E x 850

Fig. 65
Partial glomerular necrosis in acute pyelonephritis. Note the dilated juxta-glomerular DCT (bottom left).

H & E x 850
the later stages of acute pyelonephritis.

While these changes occur in the cortical region, lesions in the medulla also occur. There, interstitial oedema can be observed, and perhaps inflammatory cell infiltration, which may not be so prominent as in the cortical region. The collecting tubules are often tightly packed and distended with heterophils (fig. 62); this is particularly noticeable in advanced severe cases, and may be followed by abscess formation. Such lesions become surrounded by giant cells; the appearance of the normal medullary architecture may be ultimately lost.

Similarly, though usually much smaller, abscesses can sometimes be seen also in the cortical area (fig. 63); again, tissue destruction is so marked that it is no longer possible to recognise any structures. Such diffuse necrotising involvement of the cortex is, however, rare.

Although in general the glomeruli show comparatively little involvement in the acute form, isolated glomeruli which are infiltrated with heterophils may, on occasion, be encountered. The inflammatory cells are then present, not only in the tuft, but also in Bowman's space (fig. 64). Necrotising glomerular lesions may follow, but these are also very infrequent (fig. 65).

During the initial stages there are few vascular lesions and these consist mainly of a hyperaemia or perhaps even a passive congestion. This
Fig. 66
Chronic pyelonephritis. Note the interstitial inflammatory reaction of a chronic type and the hyperplasia of the very distorted tubules.
H & E  x 212

Fig. 67
Chronic pyelonephritis. The medulla shows a local fibrosis.
Picro-Mallory  x 170
Fig. 68
Chronic pyelonephritis showing a typical scar leading to the periphery of the kidney.

H & E x 212

Fig. 69
Chronic pyelonephritis. Note the distension of many tubules and the epithelial hyperplasia. Many of the tubules are severely distorted.

H & E x 212
is very noticeable in the cortical region, where the inter-tubular sinusoids become engorged with red blood corpuscles. In the final stages of both the acute and chronic forms, mild fibrosis of the arterial walls may be noticed.

(c) **chronic pyelonephritis.**

Tubular, interstitial, glomerular and vascular changes are observed in the chronic form of pyelonephritis. In addition to those lesions which are considered characteristic for the chronic form there are also those typical for the acute phase, which may become superimposed on a chronic nephritis.

(i) **Interstitial changes.** The general character of the interstitial cellular infiltration has changed from the acute to the chronic type, in that the bulk of the very numerous cells are of the lymphocytic and plasma cell series (fig. 66). This heavy interstitial reaction leads to a marked separation of the tubules, as does the fibrosis which has become prominent in some areas of the kidney, particularly the medulla (fig. 67). The capillaries no longer show the engorgement seen during the acute phase, nor is the heterophilia as severe. Areas of peripheral scar tissue are seen quite frequently during the chronic phase (fig. 68).

(ii) **Tubular changes.** The most striking changes observed in the chronic pyelonephritic kidney is tubular hypertrophy (figs. 66 and 69). The overall diameter of many tubules is increased and their outline is severely distorted. The epi-
Fig. 70
Complete homogenisation of the brush border in pyelonephritis.

H & E  x 2120

Fig. 71
Tubular necrosis in pyelonephritis. Practically only cell debris is left of the tubular epithelium.

H & E  x 850
Fig. 72
Inflammatory cells invading the necrotic tubule from the periphery.
H & E \( \times 850 \)

Fig. 73
Chronic pyelonephritis. There is a marked hyperplasia of the tubular epithelium. A large "abscess" is illustrated which is surrounded by typical giant cells, and fibrosis.
H & E \( \times 212 \)
Fig. 74
An early "rosette" with giant cells in chronic pyelonephritis. Note the tubular hyperplasia.
H & E x 850

Fig. 75
A necrotic tubule containing urate crystals, in chronic pyelonephritis. Note the development of giant cells at the periphery.
H & E x 850
thelium is frequently flattened and sometimes contains many more nuclei per unit area than normal; the tubules presenting a beaded appearance (fig. 66). Not only the DCT, which show the characteristic dilatation in the acute stage, but also the PCT may be affected. The lumen of such a dilated and distorted tubule may either appear empty or contain granular, eosinophilic material.

The most varied manifestations of tubular necrosis may be observed in one and the same bird. Figure 51 illustrates an early lesion during the acute phase. The cytoplasm of the tubular epithelium has become almost homogeneous, the nuclei are pyknotic and pushed against the basement membrane, and the lumen is still patent. In other tubules (fig. 70) the luminal region has become homogeneous, and hyaline in appearance with a progression to complete necrosis of the tubular epithelium (fig. 71). While at first the lumen of the tubule is still recognisable it is later completely lost and inflammatory cells begin to invade this region from the periphery (fig. 72). Giant cells often surround such necrotic tubules which may attain a tremendous size (fig. 73).

Needle-shaped urate crystals can often be seen in the necrotic tubules (figs 74 and 75), even in cases where no evidence is present of visceral gout. Such intra-renal urate deposits are described in greater detail in section VIII under "gout".
Fig. 76

Chronic pyelonephritis. Large cysts have developed in the renal medulla.

H & E x 212

Fig. 77.

A fibrosed glomerulus in chronic pyelonephritis. Note the beading of the visceral epithelial cells of the tuft.

H & E x 850
During the chronic form of pyelonephritis extreme tubular distension, presumably subsequent to obstruction of the lower nephron, is accompanied by formation of cysts (fig. 76). These are often recognisable macroscopically as they may attain considerable size which, however, varies tremendously. In very rare cases almost the entire kidney may become replaced by cysts (fig. 48).

Cellular casts are seen mainly during the acute stage, but hyaline casts predominate in the chronic phase, where, on occasion, they may be extremely numerous.

Acute tubular lesions may, and often do, become superimposed on these chronic changes and presumably represent acute exacerbations of the condition.

(iii) Glomerular lesions: The glomerular changes which occur in chronic pyelonephritis are very similar to those of chronic glomerulonephritis in that they show beading of the epithelial cells of the tuft and, frequently, a thickening and fibrosis of Bowman's capsule (fig. 77). The tufts are often severely distorted. While these changes, when present, are readily recognisable, they do not have the same diffuse distribution as in glomerulonephritis. Furthermore, glomeruli with distended capsules containing eosinophilic granular material are frequently encountered. Atrophied, shrunken glomeruli usually occur only in the peripheral scarred areas.
### TABLE III

THE CAUSES OF DEATH AND INTERCURRENT CONDITIONS IN 128 BIRDS
IN WHICH PYELONEPHRITIS WAS DIAGNOSED

<table>
<thead>
<tr>
<th>CAUSE OF DEATH</th>
<th>NO. OF BIRDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyelonephritis alone</td>
<td>62</td>
</tr>
<tr>
<td>&quot;Egg peritonitis&quot;</td>
<td>23</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>18</td>
</tr>
<tr>
<td>Renal hypoplasia</td>
<td>9</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>6</td>
</tr>
<tr>
<td>Prolapse</td>
<td>5</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>6</td>
</tr>
<tr>
<td>Enteritis</td>
<td>4</td>
</tr>
<tr>
<td>Pericarditis</td>
<td>3</td>
</tr>
<tr>
<td>Tumours (histiocytic 1, Adenocarcinomata 2)</td>
<td>3</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>1</td>
</tr>
<tr>
<td>Liver conditions</td>
<td>1</td>
</tr>
</tbody>
</table>

More than one of the complicating conditions were present in some of the birds.

Visceral gout is not included in this table; it is discussed in section (VIII) on gout.
(iv) Vascular lesions: The walls of the intra-
renal arteries are thickened, mainly through fibrosis
of the media. No changes are observed in the intima
of these vessels.

(2) Other Organs.

A variety of histological changes may
be seen in the non-renal organs, depending largely on
which complication of pyelonephritis is present. It
is only the liver which always shows lesions when
peracute or acute pyelonephritis is present.

The liver changes consist of cloudy swelling
and perisinusoidal degeneration. The acute liver
changes regress and, in the chronic phase, this organ
is usually atrophied but otherwise normal. Urate de-
posits which occur in various organs are described
in the section on gout.

(F) Secondary Conditions.

Uncomplicated pyelonephritis of the peracute,
acute or chronic types was the cause of death in 62
(48.4%) of the 128 birds. In the remainder, the
renal disease was associated with one or more con-
ditions which are listed in table III. It should
be noted that a condition, frequently occurring in
this category, is "egg peritonitis" (23 cases, 18%).
It may also be of interest that hypoplasia of one
or both kidneys was associated with pyelonephritis
in 9 birds (7%).

Every one of the 59 birds affected with
either the peracute or acute form of pyelonephritis
showed an enlarged and toxic liver. Since the latter is considered to be part of the pathological picture of these forms of renal disease, it is not specially listed in table III.

(3) Discussion.

The aetiology and pathogenesis of human pyelonephritis has caused considerable controversy for many years. The consensus of opinion appears to be that the condition arises in the renal pelvis (Saphir, 1958). It is not known, however, just how the infecting organisms reach the kidney pelvis, whether by the lymphatic, the haematogenous or the ascending routes. Some authors, e.g. Smith (1951) and Willis (1950), believe that pyelonephritis is always secondary to obstruction of the urinary tract. Longcope (1937) presented evidence that renal infection is usually blood-borne. Mallory, Crane and Edwards (1940) discount the ascending route, even in cases of ureteral obstruction, and stress that the organisms reach the kidney by the haematogenous route.

Allen (1951) divides the pyelonephritides into two more or less distinct types: (a) haematogenous and (b) ascending or obstructive pyelonephritis. It is assumed that, in the absence of urinary tract obstruction, the disease is of haematogenous origin, but Weiss and Parker (1941) indicate that a physiological obstruction may have the same effect as an organic one. Nevertheless, mere obstruction does not preclude the possibility of haematogenous entry of
the organism into the kidney (Allen, 1951). In mammals it has been shown that the experimental obstruction of the ureter produces haematogenous localisation in the ipsilateral kidney of intravenously injected bacterial suspensions (Lepper, 1921; Mallory et al., 1940; Gorrill, 1956; Guze and Beeson, 1956; Brumfitt and Neptinstall, 1958; Freedman, Kaminskas and Beeson, 1960; and others). Saphir (1958) writes that a differentiation between metastatic pyaemic abscesses and pyelonephritic abscesses is often very difficult because in both instances the infective organism may be carried by the lymphatic or the haematogenous route.

In domestic animals Nieberle (1952) also differentiates between the ascending or "urinogenous" pyelonephritis which is secondary to obstruction of the distal urinary passage and the so-called "pyelonephritis bacteritica" which is particularly common in cattle and is of haematogenous origin. In 1925 Jones and Little have shown that Corynebacterium renale is the specific cause of bovine pyelonephritis. This was confirmed by Morse (1950), although Weitz (1947) recovered C. renale from the vaginae of apparently healthy cows. Lovell and Harvey (1950) have shown that the selective action of this organism and its urease activity are linked and Lovell (1959) is of the opinion that the infection is blood-borne.

In the fowl it is difficult to decide, on purely histological grounds, whether the disease is ascending or descending. Very early lesions are
confined to the cortex, without evidence of any inflammatory changes in the medullary areas. Ureteral obstruction is sometimes found in chronic cases, but it cannot be considered as a general primary cause. The collecting tubules may contain masses of inflammatory cells, but in fowls, the severe necrosis seen in the pelvis of the human or the bovine kidney with pyelonephritis, does not occur. While an ascending spread may be accepted in some cases, in at least a proportion the disease definitely commences in the cortical region. The bacterial colonies and large, true abscesses which occur in pyogenic nephritis of man and other mammals are never encountered. The interstitial peri-tubular accumulation of acute inflammatory cells in the cortex would obviously appear to be a response to a noxa acting in that area. Heterophils then actively invade the tubular epithelium and so gain access to the lumen.

Only in rare cases of the acute form was the primary lesion situated in the glomeruli, although in mammalian descending nephritis the glomeruli are usually initially involved. The difference between the avian and mammalian disease may be explained by the presence in birds of a renal-portal system, which supplied afferent venous blood directly to the peri-tubular region.

It may be argued that this disease is not a pyelonephritis but an interstitial nephritis because it does not commence in the lower nephron, is frequently unassociated with obstruction of the urinary
tract and because the interstitial tissue is the first site of involvement. The other characteristics, mainly the presence of masses of inflammatory cells within the tubules in the acute stage, and the fibrosis, scarification, glomerular lesions, the presence of numerous hyaline casts and the severe involvement of the medullary region in the advanced cases, justify the classification of this condition as pyelonephritis, even though it is not identical with the human or the common bovine disease of that name.

In both the peracute and the acute forms the histological renal lesions are of an acute inflammatory nature. In the chronic type, degenerative and reparative changes predominate, while chronic inflammatory lesions are also present. These chronic changes can be interpreted as sequela to the damage caused to the renal structures during the acute phase. Fibrosis and scarification are responsible for the cyst formation and the shrinkage of certain glomeruli. The "rosettes" of the necrotic tubules are expressions of retrogressive and attempted reparative processes of the tubular epithelium. It is suggested that the tubular hypertrophy observed during the chronic stage may represent an attempted compensation.

The aetiology of pyelonephritis in the fowl is unknown. The clinical as well as the pathological symptoms bear the hallmarks of an infection, yet the causal agent has not been found.
Routine bacteriological examination yields a variety of organisms, possibly non-specific, and frequently not in pure culture. Escherichia coli and Staphylococci predominate, but both these organisms are commonly found in birds and their aetiological significance in this disease is difficult to assess. The experimental production of pyelonephritis in healthy fowls of various ages by the injection (usually intra-peritoneal) of organ homogenates or bacteria isolated from affected fowls has failed invariably. It has not been possible, so far, to attempt ligation of the ureter before injection of these bacteria or organ homogenates.

The relationship between pyelonephritis and its complication, visceral gout, is discussed below. The significance of other complicating conditions is not known; in most instances they are undoubtedly non-specific, but the fact that "egg peritonitis" does occur in 35% of the complicated cases and renal hypoplasia in 14%, may suggest some direct relationship.

Lesbouyries (1941) suggests that, in the fowl, nephritis is almost invariably secondary to nutritional errors, infectious or parasitic diseases or to poisoning. Bacteria and toxic substances excreted by the kidney are said to damage the renal epithelium and cause a descending nephritis. Lesbouyries further states that acute congestive nephritis may
follow a sudden cold spell in the weather. It can be produced experimentally by exposure to cold (Kaupp, quoted by Lesbouyries). Reinhart (1950) believes that an increase in the protein content of the diet may produce nephritis in the fowl. Blount (1947) suggests that various factors such as dietetic errors, moulds, bacteria, toxins (especially staphylococcal and streptococcal), poisons and the like may be involved, where a number of birds are affected with nephritis. There is little evidence to implicate this array of suspects. The aetiology of all three forms of pyelonephritis still remains obscure.
VI. INTERSTITIAL NEPHRITIS

Nieberle (1952) points out that, while interstitial nephritis is far less important than glomerulonephritis in man, the converse holds good for animals where interstitial nephritis is the most common form of renal inflammation. The well known and almost ubiquitous interstitial nephritis in the dog is but one example; it is also encountered in cattle and pigs.

Nieberle (1952) also states that, in the fowl, interstitial nephritis is common and is found to accompany various infectious diseases such as pullorum disease. Reis and Nobrega (undated) observed interstitial nephritis in 28 of their 17,755 autopsies on various species of birds. This is an incidence of only about 0.2%. Kaupp (quoted by Reis and Nobrega), Huray (quoted by Reis and Nobrega), Bayon (1936) and Lee (1938) are all reported to have observed interstitial nephritis in fowls.

In man, Saphir (1958) points out that acute interstitial nephritis is rare in the absence of other forms of renal disease; it is an important constituent of acute pyelonephritis. It is therefore not easy to differentiate histologically between human chronic interstitial nephritis and chronic pyelonephritis.

It is difficult to be certain that interstitial nephritis, as a distinct entity separate
from pyelonephritis, occurred in the fowls of the present series. In 4 birds the histological renal lesions consisted solely in a peri-tubular and periglomerular interstitial infiltration by mononuclear inflammatory cells (mainly lymphocytes and plasma cells); there was no infiltration into or through the tubular epithelium. The lumina of the tubules, both of the cortex and the medulla, were in each case free from inflammatory cells. The glomeruli showed no abnormalities. On the strength of these distinctions from pyelonephritis the lesions were tentatively diagnosed as interstitial nephritis.

No special clinical data or macroscopic renal changes were observed in these birds. The renal lesions were diagnosed during routine histological examination.

The PAR flock is free from the major infectious diseases of poultry; there was thus no opportunity for studying renal changes secondary to these diseases, where according to Nieberle (1952) interstitial nephritis is common. Nevertheless, the 4 cases showing purely interstitial inflammatory lesions of a somewhat chronic character would suggest that such a form of nephritis can occur in fowls. This category is therefore included in the classification of the nephritides.
This condition has been given many synonyms e.g. avian monocytosis, blue comb disease, housing disease, unknown disease, new disease, X disease, XX disease, cholera-like disease, contagious indigestion, battery nephritis, Bright's disease, Tom Barron's disease, acute toxemia of colibacillosis, hepato-nephrosis, mud fever of turkeys, avian infectious diarrhoea, uraemia, new wheat disease.

Many diseases whose causes are unidentified receive a variety of synonyms over the years. Pullet disease is no exception. Many of the names describe certain prominent, if not pathognomonic, characteristics of the condition, but exception must be taken to the use of the term "uraemia". This is used regularly in Australia (Hungerford, 1951) and would suggest that in affected birds there was a high blood urea level, whereas, of course, there is a hyperuricaemia. It is interesting that Bollman and Schlotthauer (1936) made a similar mistake in their paper on "uremia in turkeys" - an astonishing misnomer.

Under the name X-disease, Beaudette (1929) described a disorder of pullets in heavy lay which was characterised by cyanosis of the head appendages and sudden death, although flock mortality was low. Jungherr (1959) reviewed the literature on this
subject. The disease was reported from many American states and also from Britain by Gordon and Blaxland (1945). Jungherr and Levine (1940) studied its pathology and amongst other typical features observed renal changes and monocytosis. These renal changes varied from insignificant lesions to gross enlargement, finally giving the typical picture of visceral gout. The characteristic histological changes were cloudy swelling, pyknosis and desquamation of the epithelium of the ureter. Later, the tubules were distended, the epithelium was flattened and even necrotic, forming typical tophi similar to those described above under pyelonephritis. Intra-tubular accumulations of heterophils were also described.

In this country, pullet disease is reported in the diagnostic centres when the kidneys are nephritic and the anamnesis suggests that a large number of birds are affected. The writer has had the opportunity of examining histologically the kidneys of such birds in which pullet disease was diagnosed. Changes identical with those of peracute pyelonephritis were seen. Oedema and heterophil infiltrations were most prominent. Neither tophi nor tubular necrosis appeared to be characteristic for the "British" form of pullet disease. The striking macroscopic feature of peracute pyelonephritis lies in the tremendous enlargement of the kidneys. This is also characteristic of pullet disease as it occurs in Britain and of the so-called "uraemia" in Australia (Hungerford, 1951). Only a percentage
of the cases described by Jungherr and Levine (1940) showed renal enlargement.

The question arises whether the form seen in this country is in fact identical with that of the USA. Dr. Jungherr has kindly lent the writer slides of the "American" form of pullet disease; in those specimens the lesions were not nearly so acute or diffuse as in peracute pyelonephritis. Neither oedema nor the accumulation of intra-tubular heterophils were at all prominent. If the British and the American forms are in fact the same disease, it would seem that the former is of a more acute character in both its clinical and histological manifestations.

The liver lesions of avian monocytosis consist of local areas of necrobiocis, while in peracute pyelonephritis the livers are enlarged, toxic, showing diffuse perisinusoidal degeneration and necrosis. It is not known which of these two types of liver change is present in the field cases of pullet disease in this country.

The monocytosis described by Jungherr and Levine (1940), as characteristic for pullet disease, is in fact found in many other acute diseases of fowls. Apart from being present in both the peracute and acute forms of pyelonephritis it occurs also in glomerulonephritis and is, for instance, a constant feature of spontaneous and experimental staphylococcal arthritis (Siller, unpublished data). It is suggested that monocytosis in the fowl is a non-specific response to a variety of noxae.
The aetiology of pullet disease is not known. Waller (1942) and Watanabe (1952) have isolated a virus. Jungherr and Matterson (1944) suggest, however, that secondary non-specific factors must be implicated to account for the often explosive outbreaks. The attempted experimental transmission of peracute pyelonephritis has always been unsuccessful but this may have been due to technical factors and does not disprove the possibility of a virus aetiology.

Other aetiological factors which have been suggested are wheat poisoning (Quigley, 1943, 1944) due to an abnormal bacterial and fungal flora in the wheat (Petty and Quigley, 1947).

Jungherr (1959), in the discussion of the aetiology, does not differentiate between avian monocytosis and visceral gout, presumably on the assumption that they are identical. This is probably based on the findings that the so-called "salt effect" produced in birds by feeding various inorganic acids (Correll, 1941) is preventable by potassium administration (Scott, Jungherr and Matterson, 1944), which is also said to have a curative effect in spontaneous avian monocytosis.

DOCA (Selye, 1942) and sodium chloride (Selye and Stone, 1943) produced glomerular lesions in fowls similar to those of proliferative glomerulonephritis described above, yet Selye and Stone (1943) claim the experimental renal lesions to be similar to those of pullet disease. These workers describe
generalised oedema as typical of their experimental condition. Faver, Robertson and Wilson (1953), who studied the toxicity of salt in young fowls, also observed both generalised oedema and acute and sub-acute glomerulonephritis; they do not suggest any similarity to pullet disease.

It would appear, therefore, that pullet disease or avian monocytosis is a very acute form of pyelonephritis of as yet undefined cause. There seems to be some evidence that in the USA avian monocytosis is a less acute disease than in Britain and Australia, where the histological renal lesions are identical with peracute pyelonephritis. It is doubtful whether pullet disease should be considered as a specific disease entity, distinct from pyelonephritis, particularly since Jungherr and Levine (1941) and Blaxland (1957) describe tubular changes and renal tophi typical for both the acute and the chronic forms of pyelonephritis.
VIII. GOUT.

The term "gout" should be employed with reservations in connexion with avian pathology. Avian articular gout resembles in many respects the condition in man; it may even have a similar aetiology. In fowls, however, only a very small percentage of cases can be diagnosed as articular gout; the remainder show deposition of urates only on the various internal organs and serous membranes, when they are said to have "visceral gout". Since the visceral form is usually associated with inflammatory changes in the kidneys, the syndrome is termed "nephritis and Visceral gout" or "renal gout".

The results of the present study on visceral gout have already been published (Siller, 1959c - see appendix) and they formed the basis of a paper presented at the Conference of the Comparative Pathology of Arthritis and Rheumatism, held at the NIH, Bethesda, Maryland on the 5th and 6th of February, 1959.
(A) Visceral Gout.

(1) Incidence.

Among the 807 autopsies of the years 1956-1960 there were 54 cases (6.7%) of visceral gout. Of these 54 cases, 37 were examined histologically and they are collectively summarised.

In the fowl, visceral gout occurs in both sexes; no definite sex incidence was established. The age at which birds show symptoms of visceral gout varies; the present series ranged from 1 week to 6 years of age, with a majority between 1 and 2 years. Hartwigk (1931) reported visceral gout occurring in newly hatched chicks.

Ward and Gallagher (1928) suggested a hereditary predisposition to visceral gout. Hicks (1958) believes that not only visceral gout but also nephritis in fowls has a hereditary basis. The present study produced no evidence of a hereditary susceptibility to visceral gout; none of the 8 inbred lines at the PAG showed a higher incidence of this condition.

Craig and Kearney (1931) reported a higher incidence of visceral gout during the winter months, and this was confirmed to some extent in the present series. It is suggestive that the high winter incidence coincides roughly with moulting. There is a tendency for the plasma uric acid to rise during the moult (Bell, McIndoe and Gross 1959 and McIndoe, unpublished data).
(2) **Clinical symptoms.**

A clinical diagnosis of visceral gout is not readily established. Any symptoms that may be manifest are so general that they could equally well be part of another syndrome. There are no pathognomonic clinical features: some birds in good bodily condition and without clinical history of disease may die suddenly, and others may have a protracted history of ill health - generally in poor bodily condition, out of lay, listless and with dry, small and often cyanosed combs. Vent feathers are frequently soiled with solid "urates" but this, too, is a common observation in birds not affected with visceral gout. Nervous symptoms as observed by Gmelin (1924) and Coles (1934) were not noted in the present series.

(3) **Macroscopic autopsy findings.**

Advanced visceral gout is readily diagnosed post-mortem. There is the well-known and characteristic diffuse deposition of white material, generally accepted as urates, on the serous surfaces of various abdominal and thoracic organs, particularly the heart, liver, lungs, intestine and air sacs (fig 78). The amount of these deposits may vary from tiny flecks, hardly discernible with the naked eye, to massive encrustation of the organs.

There is no need to discuss separately the appearance of these various organs since all present the same basic picture, which varies only in degree. Urate deposition is best seen on the heart, where in
Fig. 78
Visceral gout in an adult hen. Note the heavy deposition on the heart. Urates are also present on Glisson's capsule and the visceral peritoneum of the gut (top right).

Fig. 79
A focal, almost linear sub-endocardial urate deposition below the middle aortic cusp; this was the only evidence of visceral gout in this bird with pyelonephritis.
early stages it takes the form of delicate white streaks on the epicardium and the fat of the coronary region. With increased deposition, urates appear on the visceral layer of the pericardium and also as white, semifluid material in the pericardial space. In very advanced cases the heart itself is no longer visible below the dense white mass of urate, and the two membranes covering this organ are firmly adherent, with virtual occlusion of the pericardial space. Similar urate deposits develop on the surface of Glisson's capsule, which may, in severe cases, become fused with the sternum due to a fibrin-like exudation.

The deposits on the visceral peritoneum covering the intestine and those on the costal and pulmonary pleura are essentially similar, except that they do not appear to attain the same proportions as do those on the heart and liver. In some cases (7 of the 54) the only macroscopic deposition of urate apart from the kidneys, was found in the left ventricle, where it formed a clearly defined white line under the endocardium just below the aortic valve (fig. 79). In 1 of these 7 cases the subendocardial deposit appeared as a very delicate white line running along the posterior border of an unlined subaortic ventricular septal defect, of type I as described by Siller (1958). Subendocardial deposits, more widely distributed in both left and right ventricles, often occur with other visceral
deposits. The liver is frequently swollen, friable and congested and may on occasion show numerous scattered miliary necrotic foci.

The macroscopic changes in the kidneys in visceral gout deserve special consideration. It should be stressed that it is not uncommon for the two kidneys to be affected differently, as regards both type and severity of lesions (figs 80 and 81). Partial aplasia and hypoplasia of the kidneys is a common finding, especially in the "R" line of this flock, where it occurs with a frequency of about 25% (Pen, 1961). This abnormality, illustrated in figure 49, accompanied 6 of the cases under review.

In only 3 of the 54 birds with visceral gout could the kidneys be classified as macroscopically normal. In all other instances these organs showed some degree of pathological change, which justified the diagnosis of nephritis. In about half the cases the kidneys were pink and enlarged, sometimes considerably, presenting the characteristic appearance of acute pyelonephritis (fig. 80). In the remainder the changes were typical of those in chronic pyelonephritis, which consist of shrinkage with the development of an irregular surface, often showing evidence of scar and cyst formation.

Uratcs varying in appearance are discernible in the kidneys of every bird affected with visceral gout. One type, consisting of thin, white, radiating straks confined to a lobular distribution, can be seen
The two kidneys from one bird with visceral gout. The organ at the top shows only moderate enlargement and urates in the form of thin white streaks. The lower kidney is grossly enlarged and shows numerous nodular urate deposits.

Transverse section of the kidneys shown in figure 80. Note the confluent nodular deposits in the left and the more linear arrangement in the right kidney.
on the surface as well as in the substance of the organ (figs. 80 and 81). Similar lesions almost invariably accompany acute pyelonephritis, even when there is no evidence of visceral gout. This change merely represents a stasis within the nephron. The other type of renal urate deposit appears as a yellowish-white nodular speckling over the surface as well as throughout the tissue. These nodules have a marked tendency to confluence, particularly in severe cases, where they show as extensive white areas (figs. 80 and 81).

The ureters are sometimes distended with semifluid, white urine (fig. 49), which can extend along the branches of the ureter a considerable distance into the substance of the kidneys. On sectioning the organ these tenaceous ureter contents ooze out over the cut surface. Urolithiasis has not been observed in visceral gout.

The interphalangeal toe joints are not enlarged and do not appear to be involved, since they show neither the intrarticlar nor periarticular urate deposits so characteristic of articular gout. In the latter condition visceral deposits occur rarely, if ever. In a comparatively high percentage of cases of visceral gout semifluid urates are found within the synovial capsule of the femero-tibial joints, but there is no apparent deposition of the urates in the articular cartilage and no evidence of tophi in these joints.

In rare instances urates are deposited
Fig. 82
Needle-shaped urate crystals are present in a necrotic focus of the liver in a bird with visceral gout.
Gomori's methenamine silver x 212

Fig. 83
A tophus with giant cell development is present in the liver of this bird with visceral gout.
H & E x 680
like fine snowflakes on the surface of the thigh musculature without any evidence of surrounding granulomatous tissue.

(4) **Histology.**

The most striking histological feature of the urate deposits in the serous membranes is the absence of both tissue damage and inflammatory reaction, acute or chronic, with the exception of fibrin exudation. Tophi were never observed. The needle-shaped crystals appear singly or in radiating bundles in the otherwise normal membrane. There is a tendency, however, for urates to extend beyond the limit of the covering membrane into the surface of the organ. This is particularly noticeable in the liver and intestine. Apparently independently of these peripheral lesions, urates, in the characteristic radiating arrangement, may be found lying in a PAS-positive matrix within the substance of some organs, especially in necrotic areas in the liver (fig. 82). One single case was encountered where the urate deposits in the liver parenchyma were accompanied by true tophus formation with giant cells (fig. 83). In the lungs urates are sometimes surrounded by inflammatory cell debris or are apparently free within the otherwise normal tissues in close association with the alveoli (fig. 84). Similar radiating deposits may be found in the myocardium, again without secondary inflammatory reaction. Very extensive fibrin exudate, also containing urates,
Fig. 84
Radiating urate crystals in the lung of a bird with visceral gout. Note the absence of any inflammatory reaction.
Gomor's methenamine siler x 850

Fig. 85
Epicardial urate deposits in visceral gout. A continuous line of urates may be seen along the entire length of the parietal surface of the epicardium. Note the parallel arrangement of these crystals. A mass of crystalline urates is situated in the pericardial space.
Gomori's methenamine siler x 212
my be seen on the surface of affected membranes. This is well illustrated in the case of Glisson's capsule.

The heavy urate deposits on the heart differ substantially from those of other serous membranes. In advanced cases, needle-shaped crystalline urates, strikingly refringent and staining brown to black with Gomori's methenamine silver method, line the parietal surface of the epicardium and the visceral surface of the pericardium along practically the entire length (fig. 85). It is interesting that here the crystals lie more or less parallel to one another with their long axes perpendicular to the membranes. At intervals the course of this crystalline layer is interrupted by the formation of dense structureless homogeneous material (intensely black with Gomori's stain). Reticular structures that also stain, extend into the pericardial space from the border of these deposits. Lying apparently free within the pericardial space itself are irregularly shaped but sometimes almost spherical bodies varying in size and giving a positive urate staining reaction; they contain numerous needle-shaped, anisotropic crystal (fig. 85) and may attain considerable dimensions, in places bridging the pericardial and epicardial urate deposits.

Mild sub-epicardial inflammatory reaction, characterised by the presence of mainly heterophil leucocytes, occurs in some areas (fig. 86), and is
Fig. 86

Epicardial inflammation. Note the sub-epicardial hyperaemia and the acute inflammatory cells. The urate crystals have been removed from the epicardial deposits in processing; their negative image is still apparent.

H & E  x 212

Fig. 87

Homogenisation of the periluminar part of the tubule, accompanied by other necrobiotic changes. Note the pyknosis of some of the cells.

H & E  x 850
noteworthy in view of the absence of inflammation in other serous membranes. Even so, this reaction is not very marked and involves only acute inflammatory cells without participation of giant cells or fibrosis. Hyperaemia of the sub-epicardial myocardium may be marked (fig. 86).

The histological appearance of the kidney in visceral gout is complex and somewhat confusing. It is, however, of the greatest importance to the understanding of this condition. The lesions can be considered under two headings: (a) primary changes and (b) secondary changes.

Although generally speaking, both kinds of changes are present in any one bird, there were 3 atypical cases in this present series, which showed none of the primary lesions, although the non-renal urate deposits were indistinguishable from those of other cases of visceral gout.

The primary changes are identical with the lesions described in section V as pyelonephritis. They can be acute or chronic.

The secondary changes in the kidney consist of urate deposition. Amorphous urates are a common finding in cases of pyelonephritis, even when unaccompanied by visceral gout. The deposits are finely granular, staining bluish with haematoxylin and eosin, and are confined to the lumen of the tubule. Although similar amorphous deposits may be seen in cases of visceral gout, they are overshadowed by other more significant ones, which are
Fig. 88
Necrotic tubule showing homogenisation of the central part of the epithelium. Note the pyknotic cells at the periphery and the invasion of chronic inflammatory cells, which undoubtedly would have developed into giant cells.

H & E  

x 850

Fig. 89
Visceral gout. Early degenerative lesion in the epithelium of a PCT. Urate deposition appears to have developed between the epithelium and the basement membrane.

H & E  

x 2120
Fig. 90
Necrotic tubule with complete occlusion of the lumen. Some of the epithelial cells appear to be proliferating.
H & E x 1700

Fig. 91
A renal tophus in visceral gout. The crystals have been removed in processing. Note the comparatively poor development of giant cells and fibrosis.
H & E x 850
Fig. 92
Section of a pyelonephritic kidney in visceral gout. Note the radiating bundles of urate crystals in the numerous and sometimes confluent renal tophi.
Gomori's methenamine silver  x 212

Fig. 93
Multiple tophi in a kidney with chronic pyelonephritis. Note the very prominent giant cells, the PAS-positive centres and in some tophi the radiating lines where urate crystals were dissolved out.
PAS  x 212
principally of three types. First, there are the delicate needle-shaped crystals that infiltrate the epithelium of necrotic tubules from the still-patent lumen, thus giving a striated appearance to the more or less homogeneous and eosinophilic epithelium (fig. 51). The necrotic tubules have pyknotic nuclei and are often surrounded by a few acute inflammatory cells, which also invade the epithelium from outside (fig. 75), but the tubular epithelium becomes necrotic prior to urate deposition (figs 87 and 88). An early degenerative tubular lesion with urate deposition is shown in fig. 89. The lumen may become completely occluded (fig. 90).

These early lesions may develop into the second type, the advanced renal uratic deposits, substantially similar to a tophus. They consist of an indeterminate PAS-positive centre, sometimes containing cell debris (fig. 91), into which the urates are deposited in a radiating rosette-like arrangement (fig. 92). The number of crystals, as well as their microstructure, is very variable. The aforementioned central portion is sometimes surrounded by giant cells (fig. 93) and some fibrosis; at other times giant cells are apparently absent from the otherwise similar lesion (fig. 91). In some cases the giant cell reaction is already advanced, while urate deposition is minimal and the lumen appears still to be patent (fig. 94).

Third, necrotic tubules, showing a peripheral chain of pyknotic nuclei but no giant cells and a
**Fig. 94**

Chronic pyelonephritis. Necrosis of tubular epithelium; the lumen appears still patent but giant cell formation is advanced despite minimal urate deposition.

H & E x 850

**Fig. 95**

A large, necrotic tubule in visceral gout, showing urates and pyknotic cells at the periphery.

H & E x 850
Fig. 96

Visceral gout. There is an interstitial deposition of urate crystals. Note the tubular necrosis.

H & E  x 850

Fig. 97

Renal changes in atypical visceral gout. There is no evidence of an inflammatory reaction. Some of the PCT are lined with dense, PAS-positive material. Compare the staining reaction of the intact brush borders. Note the spherical bodies in the DCT.

PAS  x 850
Fig. 98

A necrobiotic tubule in visceral gout. The lumen contains numerous spherical urates. Note the homogeneous appearance and "fibrous" projections of the periluminar portion of the epithelium.

H & E x 2120
tendency to confluence, are frequently observed (fig. 95). These contain granular PAS-positive material in which, also, urates may occasionally be found.

Although the renal deposits are generally of any or all these types just described, an occasional case may be seen where delicate needle-shaped crystals of urate are scattered indiscriminately throughout the kidney parenchyma (fig. 96). They infiltrate the tubular epithelium from within the lumen, appear in the interstitial tissue, within Bowman's space, in the capillary loops of the glomeruli and even inside the blood vessels.

Another atypical lesion was seen in only 1 of the 54 cases: The kidney showed no inflammatory changes; glomeruli, blood vessels and interstitial tissue all appeared normal. On the other hand, a large number of tubular lumina were lined by an apparently homogeneous substance staining deeply with periodic acid-Schiff reagent (fig. 97). Some of the lumina were tightly packed by spheroid structures, that stained in the same way. Such spheroids, which stain black with Gomori's stain, and the homogeneous lining are frequently seen within the tubular lumen in cases of visceral gout (fig. 98).

(5) Experimental Procedures.

In co-operation with Dr. W.M. McIndoe, a small pilot experiment was set up following Kiinaka (1900) and later workers, who noted gouty lesions in
Six chicks, 4 females and 2 males (6 weeks old) were maintained for 14 weeks on a diet containing approximately 30% total protein that was derived from the ordinary balanced ration (18% protein) fed at the PRC (Bolton, 1959), supplemented by a 2:1 mixture of casein and gelatin. This was followed by a further period of 11 weeks on the ordinary ration supplemented to 60% of protein by 2:1 casein-gelatin mixture and a vitamin-mineral mixture. Blood samples for plasma uric acid determination were taken at intervals.

As controls 3 birds (2 females and 1 male) were kept on the normal diet throughout the experimental period. The uricase-ultraviolet spectrophotometric method of Rechtmeir and Wrenn (1955) was slightly modified and used by Dr. McIndoe for the determination of plasma-uric acid.

In the feeding trial the first blood samples were taken 2 months after the commencement of the experiment. A marked difference was observed between the plasma uric acid levels of the control and the experimental groups. The former showed an average level of 1.3 mg per 100 ml, while that of the experimental group was 7.3 mg per 100 ml. Two subsequent samples at monthly intervals showed that the average plasma values of both controls and experimental birds had risen to about 5 mg per 100 ml.
However, 3 weeks after raising the protein content of the diet to 60%, a further increase to 9 mg per 100 ml was observed in the experimental group, while no change occurred in the controls. This difference was maintained when the final blood samples were taken a month later.

The opportunity presented itself to measure the plasma uric acid in 3 cases of spontaneous visceral gout. These showed 18.5, 19.3 and 22.3 mg per 100 ml respectively. The highest urate level so far found in healthy birds is 9.6 mg per 100 ml. One case of genuine articular gout without visceral deposits was obtained alive its plasma uric acid level was 19.2 mg per 100 ml.

Gross chemical analysis of the pericardial deposit in 1 case of visceral gout showed it to contain less than 5% lipid. 30% of the fat-free residue was protein and the remaining 70% was largely or wholly urate. X-ray crystallographic studies on this and other samples indicated that the urates may have been entirely monosodium urate. Although the periarticular deposits of 1 case of articular gout contained monosodium urate, the lipid content was 15% and the protein 30% of the dried material.

(The author is indebted to Dr. W.M. McIndoe for carrying out all these chemical determinations).
Fig. 99

Articular gout in a hen. Note the swelling of the toe joints.
Kodachrome.

Fig. 100

Articular gout. Toe joint exposed to show the intra- and mainly peri- articular urate deposits.
Kodachrome.
(B) Articular gout.

It is not the intention here to give a detailed description of articular gout of fowls; excellent descriptions have already been published (Schlotthauer and Bollman, 1934a; Schlumberger, 1959). The writer is of the opinion that (a) articular gout and visceral gout are two distinct disease entities and (b) that articular gout is not secondary to renal damage and therefore does not strictly speaking come into the scope of this discourse on nephritides of the fowl.

The reason for inclusion of this short chapter on articular gout is to stress the distinction between the visceral and articular conditions.

A first-hand description of articular gout must of necessity be brief as it is based on but three cases, since this was all the material available. In fowls, this condition appears to be rare and in fact human gout too is seen much less commonly than in the past.

(1) Gross pathology.

The most prominent macroscopic lesions occur on the toes. At least some of the joints are swollen (fig.99). This swelling may be soft or firm and extend some distance up the leg. Incision of such swollen joints shows heavy periarticular deposition of urates lying in the muscles, tendons and
**Fig. 101**

Brilliant white, semi-solid intra-articular urate deposits are present besides the peri-articular deposits shown in fig. 100

Kodachrome.

**Fig. 102**

Articular gout in a fowl. A wedge-shaped incision of the musculature exposes the tibia in the depth and shows almost complete replacement of the muscles by urates.

Kodachrome.
ligaments surrounding the joint. Deposits of solid urates are also present on the articular surfaces within the joint cavity (fig. 100). Semi-solid brilliant white, intra-articular accumulations of urate may also occur together with peri-articular deposits (fig. 101). In one case the musculature of the lower leg appeared to be practically replaced by urates (fig. 102).

In the same bird subcutaneous tophi were found in the wattles (fig. 103).

There are no changes in the internal organs which are characteristic of articular gout. The kidneys show no inflammatory changes but urate deposits and tophi may be present. Visceral urate deposits have not been observed in articular gout.

(2) Histology.

For a detailed description of the histology of articular gout in birds, the reader is referred to the excellent work by Schlumberger (1959) on gout in parakeets. In the present series the kidneys were found to be free from inflammatory lesions other than tophaceous ones.

In fowls the formation of articular tophi is similar to that in the parakeet (Schlumberger, 1959). The muscular deposition shown in figure 102 is worthy of further histological description. Sections stained with haematoxylin and eosin show massive replacement of the musculature by a more or
Fig. 103
The wattle in this bird with articular gout shows a nodular, subcutaneous tophus formation (arrow).

Fig. 104
Articular gout. A large part of the leg muscle is replaced by homogeneous material, which is in part subdivided by fibrous tissue trabeculae. A strip of intact muscle is shown at the top. Micro-Mallory x 68
Fig. 105

Extensive tophus formation at the edge of the diffuse muscle lesion in a case of articular gout.

H & E  x 170

Fig. 106

Part of the field shown in figure 105. This shows the giant cells around the tophus.

H & E  x 850
Fig. 107
Gomori's methenamine silver stain shows the massive deposition of urates (black) within the altered musculature in a bird with articular gout.
Gomori's methenamine silver x 85

Fig. 108
This section was made just distal to one of the inter-phalangeal articulations in a 10-day old chick with very severe visceral gout. The synovial capsule (left and right) contains masses of urates.
Gomori's methenamine silver x 68
less homogeneous material which is roughly subdivided by trabeculae of fibrous tissue (fig. 104). The architecture of the muscle fibres is completely lost in such areas which may be bordered at the edge by normal muscle tissue (fig. 104). Tophi are present throughout this homogeneous material (fig. 106), but they are best developed at the periphery, where they are accompanied by considerable fibrosis (fig. 105). Gomori's (1946) methenamine silver method shows the altered musculature to contain huge amounts of urate (fig. 107).

Finally it should be mentioned that one additional chick (10 days old), received from the Veterinary Laboratory at Lasswade, appeared to be affected with both articular and visceral gout. This seemed of great interest because usually the two conditions do not occur together.

Macroscopic examination showed that typical visceral urate deposits were severe on the heart, liver, pleura and peritoneum. Semi-fluid urates were present in the toe joints, the knee joints and some of the inter-vertebral articulations. The tendon sheaths of the feet also contained urates, which reached a considerable way up the leg. True peri-articular gout with tophus formation was, however, not observed.

Histologically the joint capsules of the interphalangeal joints contained masses of amorphous urates (fig. 108) and urate crystals
Fig. 109

Section through a toe of the same bird as in figure 108. The tendon sheaths show a parallel deposition of urate crystals. Note the absence of an inflammatory reaction and tophus formation.

Gomori's methenamine silver x 112
were perpendicularly arranged also on the synovial surfaces of the affected tendons and tendon sheaths (fig. 109). This arrangement of the urate crystals is identical with the type of deposition which occurs on the epi- and pericardium in cases of visceral gout, which was also well marked in this case.

The absence of typical peri-articular deposits proves that this is not a case of articular but of very severe visceral gout. The kidney lesions were typical of acute pyelonephritis.
(C) Discussion.

There appears little doubt that visceral and articular gout in the fowl are distinct syndromes. Their lesions differ considerably, not only in somatic distribution but also in the type of tissue reaction evoked. This fact, one of primary importance, is not sufficiently stressed in some textbooks dealing with avian diseases (e.g. Hutyra, Marek, Männinger, 1946; Reinhart, 1950; Nieberle, 1952 and Reis and Nobrega, undated), where visceral and articular gout are generally discussed together, as different manifestations of a single entity, under the heading of either nutritional or metabolic diseases. Blount (1947), on the other hand, considers them quite separately. As mentioned above, an articular involvement sometimes occurs in visceral gout, but there is no similarity between this and the joint lesions of articular gout. This is well illustrated in the 10-day old chick with visceral gout where the articular urate deposition was heavy and involved the tendon sheaths, but where the type of deposition differed substantially from that in articular gout but was similar to that of visceral urate deposits. It is not surprising that joint capsules and tendon sheaths should be involved in visceral gout, since these are also serous membranes and they become implicated along with those of the viscera.

It is rarely made clear that these two con-
ditions seldom, if ever, occur concomitantly. Martinaglia, cited by Coles (1934) observed that the joints were not implicated in his cases of visceral gout and Coles, in agreement, points out that in South Africa there was no record of articular gout, while visceral gout occurred frequently. Although avian articular gout shows considerable morphological similarities with human gout, the latter appears to be unrelated to visceral gout.

Kidney lesions are commonly associated with gout in man (Folin, Berglund and Derick, 1924). Characteristic are urate deposits, which, according to Ebstein (1882), are specific only when necrotic foci with crystalline urates and an inflammatory reaction are present. Urate crystals alone are said to occur in other conditions as well. In advanced cases it is uncertain whether the lesions, which are surrounded by tissue damage, inflammatory reaction and fibrosis, originate within the tubules or in the interstitial tissue. Hench et alia (1948) and Brown and Mallory (1950) found such lesions within the tubules; Minkowski (1903) went even further, postulating that the first deposition occurred in the convoluted tubules and that the involvement of the collecting tubules followed. Garrod (1863) believed the lesions to be interstitial in origin; both tubules and interstitial tissue were involved in Talbott's (1943) cases.

Interstitial urate deposits do occur in visceral gout, but they appear to be rare. As a rule, the
characteristic lesions appear to develop within the tubules and the urates are laid down in the necrotic tubular epithelium. As the lesions enlarge they certainly spread beyond the limits of the tubular basement membrane and there is evidence of actual confluence of neighbouring necrotic tubules. A marked localised inflammatory reaction may follow interstitial urate deposits. In rare instances needle-shaped urate crystals can be seen lying apparently free in an irregular arrangement throughout the kidney substance. Such deposits are not accompanied by significant inflammatory reactions and the crystals are sometimes found to extend even across structures such as Bowman's capsule. This seems to be due to a sudden deposition of urate.

The secondary renal lesions described above appear essentially similar to the specific gouty changes in the human kidney. In the fowl, however, visceral gout is almost invariably accompanied by some degree of pyelonephritis. Chronic renal changes frequently complicate human gout. Pyelonephritis has been reported on several occasions (Brown and Mallory, 1950; Mayne, 1955 and Sokoloff, 1957). Its significance is not settled but Smith et alia (1949) believe that there is a direct relationship between the typical kidney lesions of gout and of pyelonephritis. The latter may follow mechanical obstruction of the tubules by urates (Bell, 1946). In man, pyelonephritic lesions are not interpreted as primary, although they seem to be so in visceral gout of the fowl. Although Craig
and Kearney (1931) mention the possibility that irritation of the avian kidney during uric acid excretion may cause nephritis, this seems most unlikely, despite the suggestion of Talbott (1943) that, in man, excessive and prolonged uric acid excretion may cause secondary renal damage. 

In fowls the association between visceral gout and nephritis is well authenticated (Hansen, 1923; Kitt, cited by Buzna, 1924; Craig and Kearney, 1931; Reinhart, 1950; Spector, 1951; Jungherr, 1959, Reis and Nobrega, undated). However, Gmelin (1924) states definitely that in his single case there was no "renal insufficiency", and mentions no gross or histological abnormalities in the kidneys. Likewise, Niberle (1952) makes no mention of nephritis in connexion with visceral gout and Seifried (1950) states that in the early stages the kidneys show no lesions and that the inflammatory changes of the later stages are secondary and are confined to the areas of urate deposition. With the exception of 3 cases, the present series emphasises the importance of primary renal inflammatory changes. This clearly confirms the statement of Blount (1947): "Visceral gout is that form of acute nephritis which at death is characterised by the deposition of naked eye accumulations of uric acid compounds ....". Birds, being uricotelic excrete the bulk of their waste nitrogen as uric acid which, as shown by Mayrs (1924), Gibbs (1929) and Marshall (1932), is mainly excreted by the tubules. At normal plasma levels, 87 to 93% of the total urate excreted
is eliminated by this pathway (Shannon, 1938). This being so, one of the consequences of severe and wide-
spread renal tubular damage, inflammatory or degenera-
tive, is the development of hyperuricaemia. Jungherr
and Matterson (1944) found that in subacute avian monon-
cytosis, where the outstanding lesion is nephritis,
the blood uric acid level rises. Reinhart (1950) also
states that the blood urate is raised in both renal
insufficiency and acute nephritis.

Birds are not the only representatives of the
animal kingdom which are affected with gout. It is
ture that man is the only mammal susceptible to this
disease, even though Bruckmüller (1869) has reported
a dog with peri-articular deposits of sodium urate
and Braun (1919) claims to have seen uratic arthritis
in a rabbit. In reptiles, however, gout, both visceral
and articular, is common (e.g. Fox, 1925; Hamerton,
1932, 1933, 1939; Hill, 1954-55; Appleby and Siller,
1960 - see appendix). Of particular comparative
interest in relation to the heavy uratic deposition
in the musculature of one fowl (fig. 102) is the ob-
servation of Pagenstecher (1863-64). He saw articular
gout in an Alligator sclerops with sodium urate de-
posits in both hip joints and diffusely distributed
throughout the entire musculature. Sodium urate de-
posits in alligator muscle had previously been re-
ported by Liebig (1849), but without reference to any
gouty arthritis.
The fact that both birds and reptiles are so susceptible to gout is explained by the peculiarities of physiology in these phyla. Amphibia are ammnonotelic during their immature, wholly aquatic, phase of life and assume ureotelism when adult. Birds, on the other hand, excrete the bulk of their waste nitrogen as uric acid, i.e. they are uricotelic. All three forms of nitrogen excretion are represented among the reptiles, depending on whether they have a wholly aquatic, semi-aquatic or entirely terrestrial existence (Baldwin, 1952). In alligators, the bulk (67-87%) of urinary nitrogen is ammonia; in the turtle Chrysomys, most (24-48%) is urea and in the terrestrial horned lizard Phrynosoma, nearly all (98%) is uric acid (Scheer, 1948). Moyle (1949) found that the urinary nitrogen of aquatic turtles was largely urea and ammonia, whilst uric acid predominated in terrestrial tortoises.

The question arises whether it is at all logical to apply the term "gout" to a condition that does not appear to be due to faulty uric acid metabolism. Visceral gout must in most cases be regarded as following damage to the kidneys. More definitely, it appears usually to arise as a sequel to pyelonephritis, although not every case of nephritis is followed by visceral depositions of urates; this may be determined by the severity of the kidney damage and the rapidity of the disease. If visceral "gout" is to be so termed it will have to be considered as secondary, or renal, gout. Secondary gout is a term used in human pathology to
indicate, according to Gutman (1953), a rare form of non-hereditary gout due to an increased uric acid production following accelerated degradation of nucleic acid which occurs in some blood dyscrasias. Gutman further points out that on rare occasions secondary gout is associated with the hyperuricaemia in chronic nephritis; Sokoloff (1957) draws attention to Magnus-Levy's (1909-10) statement that concretions are found in the joints of many uraemics who have had no history of primary gout. But even in the primary gout of man the importance of the kidney lesions must not be lost sight of, although, according to Gutman (1953), they only accelerate the already existing condition. This is all the more significant when one remembers that tubular excretion has been reported in man (Schintker and Richter, 1936), normally of course, uric acid is excreted by the glomeruli and reabsorbed to about 90% by the tubules. Tubular damage would therefore be expected to produce a reduction of uric acid reabsorption and therefore a hypouricaemia.

There is no evidence of any relationship between gout and leukaemia in birds. In visceral gout, enteritis (Reinhart, 1950) and rupture of the ovarian follicles, with spilling of yolk material into the peritoneal cavity (Coles, 1934), are common. Egg peritonitis complicated 6 cases of visceral gout in the present series. Uninfected yolk from ruptured ovules is usually absorbed from the peritoneal cavity and could cause a significant rise in endogenous nitrogen convertible to uric acid.
whether a hyperuricaemia results in such cases is not known.

Our understanding of the aetiology of avian gout remains by no means complete. Despite this, Bechade (1951) introduced her paper on nutritional or visceral gout, delivered at the Ninth World Poultry Congress in Paris, with the following sentence: "This report does not intend to bring new elements to the knowledge of nutritional gout, the aetiology and pathogenesis of which are already known."

Craig and Kearney (1931) list the following four factors which may account for this condition: (1) increased endogenous uric acid production, (2) increased exogenous production, (3) decrease in solubility of blood urates, and (4) defective uric acid elimination by the kidney.

High protein intake is frequently incriminated as one of several possible causes of spontaneous gout in fowls (Coles, 1934; Wirth and Diernhofer, 1943; Hutryra, Marek and Manninger, 1946; Reinhart, 1950; Bechade, 1951; Lesbouyries, 1955 and others). However, Coles (1934) states categorically that "the percentage of available protein in the diet is not associated in any way with the disease". This statement, although probably accurate in respect of the aetiology, is too dogmatic, since it is quite feasible that a high-protein intake will tend to aggravate an already gouty condition.

A connexion between a high-protein intake and the development of avian gout appears to be supported
by the experimental production of the disease by feeding protein-rich diets. Schlotthauer and Sollman (1934b) induced articular gout in turkeys by increasing the dietary protein to 40% by the addition of horse meat or by supplementing the food with 5% urea. Articular and visceral deposits were found by Kionka (1900) in fowls fed 150 g of horse meat daily, and he believes avian and human gout to be identical in respect of cause and mode of development. The addition of vegetable protein to a level of 36% of the diet produced typical articular lesions in turkey poult’s (Lloyd, Reed and Fritz, 1949).

On the other hand, Hansen (1923) had fed fowls for 18-24 months exclusively on meat, liver and pancreas (all cooked) without producing gouty deposition. He believes that a high-protein diet is of etiological importance only if accompanied by excessive over-all feeding. Prolonged feeding of unbalanced rations is a cause postulated by Hartwigk (1931).

The results of our own limited feeding trial so far agree with those of Hansen. Our birds were kept on 30% protein for 3 months and subsequently without a break, on 60% protein for 2 months. Although the plasma uric acid levels during both periods rose in comparison with control birds kept on 18% of protein, they did not exceed physiological levels. It must be pointed out, however, that the total food consumption of these birds was not estimated and may well have decreased considerably, as did indeed happen when the
diet consisted entirely of the gelatin-casein mixture. The birds were killed for autopsy and showed no gouty lesions (visceral or articular) and no evidence of nephritis.

There are several possible explanations for the apparent discrepancy between our results and those of the workers cited above. In the first place, when our experiment started the birds were 6 weeks old, and during the course of the trial their protein requirements altered considerably, particularly when they came into lay in the latter period. Second, it is possible that over the comparatively short period of the experiment the kidneys had adapted themselves to excrete excessive amounts of uric acid. This is supported by the statement of Folin, Berglund and Derick (1924), that a high-protein diet increases the responsiveness of the kidney and thus lowers the circulating uric acid. Third, the protein supplement fed in this experiment consisted of a 2:1 mixture of casein and gelatin and was, therefore, fairly well balanced in amino acids. If the amino acid balance is incorrect, even in comparatively low-protein diets, only a certain percentage will be utilised by the body, leaving the remainder to be converted to uric acid and excreted.

A high wastage of amino acids may be one factor that might explain why high-protein diets (more likely, unbalanced protein) are so often said to cause gout in birds under field conditions. This cannot be the whole answer, however, since at this Centre there is a high incidence of both nephritis and visceral gout.
although the diet is balanced in respect of amino acids and the total protein content is only 18%.

The earliest record of the experimental production of gout goes back to 1766, when Galvani ligated both ureters in a fowl. This procedure was repeated later by various workers (Zaleski, 1865; Chrzowszczewsky, 1866; Pavlinoff, 1875; von Schröder, 1880; Colasanti, 1881) who observed urate deposits on the kidneys, liver, lungs, myocardium, serous membranes and joints, which increased in severity the longer such birds survived. A rise in blood uric acid to 264 mg per 100 ml was obtained by Levine, Wolfson and Lenel (1947) after ureteral ligation. Similar results were recorded by Folin, Berglund and Derick (1924).

Ebstein (1882) claimed that lesions typical of both visceral and articular gout were produced following the subcutaneous administration of potassium dichromate, and Jungherr and Levine (1940) obtained a gross pathological picture indistinguishable from renal gout by a similar procedure. Other poisons such as mercuric chloride, oxalic acid, acetone and phenol were reported by von Kossa (1898-99) to cause similar lesions. Von Kossa also noted the development of renal urate deposits after the injection of aqueous solutions of various sugars. Injections of aloin (Hansen, 1923); alloxan (Saviano, 1948) and the administration of yohimbine (van der Flank, cited by Coles, 1934) are all said to cause visceral urate deposits. Bollman and Schlotthauer (1936) produced
visceral gout lesions by the intramuscular injection into the leg of uranium acetate. Reinhart (1950) suggested that the accidental intake of poisons may be responsible for the spontaneous development of visceral gout.

Witter (1936) observed that the clinical use of sodium bicarbonate in the usual concentration of 0.6% in fowl's drinking water is sometimes accompanied by renal changes and visceral gout. He produced lesions identical with those of spontaneous gout by adding 1.2% sodium bicarbonate to the drinking water. He noted necrotic lesions in the kidney, and the blood uric acid level rose 6 to 8 times above that of controls. Jungherr (1936) confirmed Witter's findings. This is all the more interesting since sodium bicarbonate is frequently suggested as a therapeutic agent in this condition (Buzna, 1924; Reinhart, 1950; Reis and Nobrega, undated). Hansen (1923) was able to prevent the development of gouty symptoms and elevated blood urate in his alcin-injected birds by the addition of sodium bicarbonate to the drinking water.

That visceral gout can develop after the administration of poisonous chemicals again draws attention to the importance of primary renal damage, particularly when such well known tubular poisons as mercuric chloride and potassium dichromate are involved. Innes (1950) demonstrated renal cortical necrosis in sheep following the administration of alloxan. Some authors who favour a high-protein diet
as the aetiological agent, suggest that this also causes primary renal damage (Reinhart, 1950). Furthermore, a high-protein diet is believed by some to cause nephritis in fowls.

Jarmai (1925) reported visceral gout in a goose following the feeding of moldy maize. He believed, also, that in this case the renal damage was primary, he went so far as to say that all cases of renal damage in birds are followed by visceral gout. On the other hand Ronk and Carrick (1930-31) found no toxic effects among chicks fed on maize infected with a variety of molds.

The cause of spontaneous pyelonephritis is obscure. The disease bears the hallmark of an infection, but so far no specific agent has been isolated. Numerous inconclusive aetiological factors have been suggested in the literature. Stonebrink (1947) believes that an upset in the water-salt balance is responsible.

No definite information is available on the mechanism of the visceral deposition of urates.

Reinhart (1950) considers that they become precipitated on the serous membranes owing to the sluggish circulation in these areas. Seifried (1950) thinks that "an alteration in the colloidal state" is responsible. Mayall's (1929) unique explanation is as follows: "The secretion of the urate ... is brought about in a reactionless way and thanks to the presence of a stasis transudate it passes out
from the well filled capillaries and veins of the subserosa. The blood plasma rich with uric acid comes on the surface and there the uric acid loses its solvent (acid phosphate of soda) and is deposited". Gmelin (1924) suggests that the heavy uratic deposition on the liver is due to the fact that this organ is the site of uric acid formation in the fowl. This argument is not sound for, apart from the fact that uric acid is also produced in the kidneys, the heaviest extra-renal deposits usually occur on the heart. No xanthine oxidase has been demonstrated in the avian heart (Morgan, 1926). Roberts (1892) and Brugsch and Citron (1908) have shown that cartilage can absorb urates from saturated solutions. Folin, Berglund and Derick (1924) suggest that connective tissue and dead animal tissue may have a similar affinity for urates. They also point out that the sites of gouty deposition have poor circulation.

It seem probable that the bulk of the visceral urate deposits, at least the extra-renal ones, are metastatic and not dystrophic, as defined by Sokoloff (1957) in the instance of human gout. In the membranes there is no evidence of preceding necrosis, while in the parenchymata of the liver and kidneys, earlier necroses and the presence of PAS-positive matrices are usually demonstrable. Lipids have been demonstrated by Sokoloff (1957) in the centre of some human tophi; such was not the case in the lesions of the present series.
Deposits on serous membranes do not induce a secondary inflammatory reaction, with the exception of deposits on the epicardium, in which event a localised low-grade cellular reaction can occur. The often considerable fibrin exudation from the liver surface and pericardium must, however, be considered as evidence of inflammation. The hyperaemia of the subepicardial myocardium is presumably of similar significance. This observation is confirmed by the findings of Nieberle (1952) who even records the development of recent granulation tissue arising from the epicardium and infiltrating the mass of urates. Gmelin (1924) failed to confirm Joest's interpretation of the uratic lesions as chronic inflammation and, examining but one case, denies the presence of an inflammatory reaction. The very mild tissue reaction suggests that the crystalline deposits have little duration and that they are probably formed a short time before death.

Of considerable interest is the chemical composition of the "urate deposits" in visceral gout, a subject given little attention in the literature. McCrudden (1905) states that the gouty concretions in man consist essentially of monosodium urate. In fowls the identification is most commonly based on the murexide test, which does not preclude the presence of other salts besides urates, nor does it identify the cation (s) involved. Brandl (cited by Seifried (1950) believes the deposits to be composed of monosodium urate and calcium urate. Sokoloff (1957)
states that it is generally believed that ammonium urate is involved. Unfortunately, he gives no reference to the origin of this statement and it has not been possible to inspect the evidence. It is likely that at least a high proportion of urinary urates are in the form of the ammonium salt, but the visceral deposition of this substance seems very unlikely; ammonia and ammonium ion are extremely toxic and are not measurable in avian blood (Conway and Cooke, 1939). Revolta, Dalprato and Farsinarie (cited by Kitt, 1927), record one case of so-called visceral gout in which the deposits on the serosae and viscera were said to be calcium phosphate; similar depositions of calcium have been described by Pallaske (1933), but without the characteristic pathological picture of visceral gout.

It appears from our limited studies that the visceral deposits contain lipid and protein, in addition to substantial amounts (more than 50%) of monosodium urate. It is quite possible, however, that small amounts of other cations, particularly calcium, may also be present.

It is noteworthy that although the plasma urate level is considerably elevated in visceral gout, a similar high level was observed in 1 case of articular gout. Schlotthauer and Bollman (1934b) noted a high plasma urate level in articular gout in turkeys. Why visceral deposits do not develop in articular gout is therefore difficult to understand.
The purpose of this study was to ascertain whether more than one type of nephritis occurs in the fowl and to study in detail the histology of these nephritides.

Pyelonephritis is known to affect fowls frequently but many poultry pathologists neglect the significance of glomerulonephritis, which was seen in about 20% of the 233 nephritic birds of the present series. With such a frequency, therefore, the disease must not be ignored, even though the aetiology is still obscure. Considerable evidence was produced to show that at least some cases of proliferative glomerulonephritis may be secondary to a hyperoestrogenic state. The histological diagnosis of glomerulonephritis must be interpreted with care, since in young birds proliferative glomerular changes appear to be physiological and reversible.

Membranous glomerulonephritis was only found to occur in turkeys and fowls which had been poisoned with Brazilian ground-nuts.

Interstitial nephritis was seen in only 4 cases but since it is supposed to occur as a complication of various infectious diseases, it is included in the classification. Similarly exudative glomerulonephritis was not observed in the present series, but it is mentioned in the literature as occurring in fowls and is therefore also included.
Pyelonephritis can occur in three forms: the peracute, the acute and the chronic. The peracute form may be similar to or identical with pullet disease, as seen in this country.

Acute and chronic pyelonephritis are those types which are often associated with visceral gout. The causes of all three types of pyelonephritis are still unknown; although a number of possibilities have been suggested by various workers, they are by no means applicable in every case.

Two distinct forms of gout are seen in birds - the articular and the visceral. Their characteristic lesions do not occur simultaneously in one and the same individual. While the cause of articular gout is obscure in fowls as it is in man, the visceral form usually follows severe renal tubular damage and should be interpreted only as a symptom.

On the basis of the present study, therefore, the following tentative classification of the nephritides in the fowl can be drawn up. It must be pointed out however, that future investigations may eventually lead to certain alterations in this classification.
Classification of the Nephritides in the Fowl

A. Glomerulonephritis
   (1) Proliferative glomerulonephritis.
   (2) Membranous glomerulonephritis
   (3) Exudative glomerulonephritis

B. Interstitial nephritis

C. Pyelonephritis
   (1) Peracute - (Pullet disease)
   (2) Acute
   (3) chronic

D. Gout
   (1) Visceral gout (usually associated with acute or chronic pyelonephritis)
   (2) Articular gout (this is unrelated to nephritis, in fowls)
X. ACKNOWLEDGEMENTS

The writer acknowledges with thanks the official supervision extended by Dr. A.W. Greenwood, C.B.E., F.R.S.E. and Professor C.H. Waddington, F.R.S.

He further acknowledges many helpful conversations with his colleagues on the staff of the Agricultural Research Council’s Poultry Research Centre and also the skilled assistance of Mr. Bernard Dugdale, who printed and mounted the numerous illustrations. Thanks are also due to Dr. W.M. McIndoe for carrying out the chemical determinations and to Drs. C.A. Beevers and H.W.E. Ehrlich of the Chemistry Department, Edinburgh University for providing facilities and advice in carrying out the x-ray crystallographic studies.

Thanks are especially due to Dr. J.G. Campbell, F.R.S.E., Dr. J.E. Wilson, F.R.S.E., Mr. R.H. Duff, M.R.C.V.S., Mr. D.C. Ostler, M.R.C.V.S., and many others who have provided certain specimens which are duly acknowledged in the text of this thesis.
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