MICROSCOPIC POSTMORTEM CHANGES IN KIDNEYS AND ADRENAL GLANDS OF THE DOMESTIC FOWL

by

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INTRODUCTION

The importance of differentiating postmortem from antemortem changes is well-recognized and documented. In gross and microscopic examination of organs and tissues, basic information is required by pathologists to determine if the changes observed are significant.

One way of accurately interpreting microscopic lesions is to minimize the time interval between somatic death and tissue fixation. However, in clinical and field cases a certain amount of time usually elapses before necropsies are performed. Diagnostic samples may have undergone autolytic changes and become unsuitable for histopathological evaluation before reaching the laboratory. In these cases, little information may be derived and often, carcasses are discarded. There are cases, however, where necropsy is imperative for medico-legal reasons irrespective of the condition of the carcass.

It is necessary to know the extent and usefulness of various organs for critical histopathological evaluation to minimize futile necropsy studies which have little or no value. Moreover, favorable conditions should be devised in transporting diagnostic samples to reduce postmortem decomposition. Factors such as temperature, humidity and body condition should be considered.

Several investigators have studied and described postmortem changes in different organs of different species: man, guinea pig, rat, mice, rabbit, dog, cat, pig, cattle and ovine fetus. In birds, some investigations dealt with biochemical and physical

changes of the skeletal muscle in relation to the quality of meat and others with the usefulness of organs for histopathology.

In the light of existing knowledge, the aim of this study was to add to available information on postmortem changes in the kidney and adrenal glands of the domestic fowl (Gallus gallus domesticus). Specifically, the objectives were to:

1. Determine the type, rate and sequence of microscopic postmortem changes in the kidney and adrenal glands of intact chicken carcasses held at 18 or 29 C, 50% relative humidity; and
2. Evaluate and compare postmortem changes in the kidney and adrenal glands of intact chicken carcasses wet and not wet with detergent solution prior to storage at different temperatures.

I. REVIEW OF LITERATURE

Autolysis is the process of cellular dissolution by intracellular enzymes from lysosomes after somatic death.[1] It is differentiated from necrosis, the sum of morphological changes caused by enzymatic degradation of dead cells in a living body. The distinction between autolysis and necrosis is rather superficial since the same degradative reactions occur and both exhibit similar cellular changes. Some investigators consider necrosis an early stage prior to frank autolysis with differences only in rate and extent of changes.[2]

The basis of autolysis is hypoxia which explains the similarity of changes with the early lesions of ischemia.[3] If necropsy is delayed, difficulty arises in distinguishing histological and histochemical changes due to postmortem change or ischemia. Because of the similarity, patchy distribution of necrotic lesions, sharpness of outline of erythrocytes inside blood vessels outside necrotic foci, zone of hyperemia and inflammation around necrotic areas, and knowledge of the rate by which organs undergo autolysis, were criteria to be considered.[4]

All tissues undergo autolysis but the rate varies with different tissues, ambient temperature, humidity and condition of the animal.[3] Variation in the degree of autolytic change is partially due to variation in the content of proteolytic enzymes.[5] In tissues rich in proteolytic enzymes such as pancreas and gastric mucosa, autolysis is rapid while in connective tissues it is slow, and moderate in liver, kidney, muscle and brain.[6] In addition, during postmortem decomposition, organelles degenerate, the rate of change varies according to oxygen requirements.[3] Organs with rapid metabolic

activity undergo rapid autolysis.

Investigators have studied postmortem changes in several different organs of various species: man,[7-8] guinea pig,[9-10] rat,[2,11-14] mice,[15-20] rabbit,[7,21] dog,[22] cat,[23] pig,[24-26] cattle,[27] ovine fetus,[28-29] and chickens.[30-34] Studies ranged from morphological to histochemical and biochemical changes. Some studies were directed toward understanding the processes of cell degeneration and necrosis during life.

Most nuclear changes described during autolysis and necrosis were associated with nuclear chromatin but changes also occurred in other parts of the nucleus.[35] The classical nuclear changes are pyknosis, karyorrhexis and karyolysis but before such changes are detected by light microscopy, a certain amount of time elapses. The earliest nuclear change usually is condensation of the envelope with disappearance of chromatin from other places.[10,14,17] Nuclear shrinkage follows, proceeded by pyknosis where the nucleus shrinks and the chromatin condenses to a dense compact basophilic mass.

Karyorrhexis may be primary or secondary. It is secondary if the nucleus fragments after pyknosis.[12,14]

Karyolysis can be considered the end stage in which the nucleus loses the ability to stain with basic dyes leading to a non-nucleated cell.[6,12,36] Ultrastructurally, the nuclear envelope is intact but the contents are partially or completely lost.[35] Splitter and McGavin[10] reported nuclei or guinea pig hepatocytes disappeared almost entirely by karyolysis.

In addition to the basic nuclear changes, some observations cannot be strictly defined as one of the three. Aschoff[12] also

included nuclear swelling and vacuolation as a change in tissue undergoing necrosis. Nuclear fading is another change described in autolyzing chicken kidneys.[34]

Nuclear changes begin early but become marked only after severe cytoplasmic changes.[14] Cytoplasmic changes include acidophilia or eosinophilia, loss of basophilia if originally present, granulation, and mitochondrial fragmentation.[12] Increased eosinophilia is partially due to increased binding of greatly exposed negatively charged eosin to positively charged reactive sites along polypeptide chains of cytoplasmic proteins after denaturation.[36] Loss of basophilia reflects detachment and scattering of polysomes from rough endoplasmic reticulum.

Cellular changes vary from cell to cell within the same organ. Variations and combinations of nuclear changes are possible and any nuclear change may occur first.[12] Factors on sequence and rate of changes are unknown. Decreasing pH in autolysis, probably reflected by nuclear chromatin clumping,[36] activates lysosomes to release hydrolytic enzymes. Desoxyribonuclease catalyzes depolymerization of DNA into smaller units. Chromosomes containing DNA, histone and a residual protein, do not lose morphological integrity if histone only is removed. However, if DNA is removed, it is transformed into a mass of coiled protein threads and if attacked by a proteolytic enzyme forms a viscous gel invisible under light microscopy.[6]

Bacteria do not play an important role in the autolytic process itself. Postmortem bacterial invasion did not occur until 24 hours after autolysis had started in kidneys of canine carcasses held at 4 C.[22] The addition of bacteria, however, rapidly accelerated

structural changes.[9]

Kidneys and adrenal glands are two organs commonly thought to undergo rapid autolysis. Sequential studies have been undertaken in kidneys of man,[7] dog,[22] cat,[23] rabbit,[7] rats,[2,12-14] mice,[16] pigs,[26] and chickens.[30-32,34,37]

In chickens and rabbits, the proximal convoluted tubules underwent the earliest postmortem change.[7,34,37] Glomeruli were relatively resistant and so were the collecting tubules. In the rat and pig, the distal convoluted tubules underwent nuclear changes first.[2,14,26]

In the canine kidney, the sequential autolytic changes in glomeruli were cytoplasmic swelling and vacuolation of cells, progressive nuclear chromatin clumping, and finally pyknosis or karyorrhexis.[22]

In cats, autolytic kidneys had increased weight and glomerular diameter but diameter of cell nuclei decreased due to pyknosis. No differences were noted in basement membrane thickness and glomerular cell numbers between 0-hour controls and autolytic kidneys held at 4 C for 24 hours.[23]

In rabbits, kidneys placed in isotonic saline solution at room temperature (15 C) underwent progressive depletion of cytoplasmic and nuclear substances in proximal convoluted tubular epithelium.[7] At the end of 24 hours, cytolysis and karyolysis had progressed to a state of partial disruption of proximal convoluted tubules with cell detachment and intraluminal accumulation of granular and eosinophilic debris. After 74 hours, basement membranes were persistent.

In a recent review, Siller[38] reported conflicting results re-

garding postmortem changes in the kidney of the domestic fowl for which he had no plausible explanation. Matic[37] compared the nephrotic and autolytic lesions of chicken kidney and reported nephrotic lesions caused by zinc phosphide were characterized by karyorrhexis while postmortem autolysis was characterized by pyknosis.

Sequential studies on postmortem changes in adrenal glands are lacking. Only one study has been reported and this involved pigs.[26] Adrenal glands of intact pigs left at 4 or 24 C at varying periods after death had cells of the zona arcuata pyknotic, shrunken and individualized within 6 hours and moderate to marked changes in zona reticularis within 12 hours. Medullary cells were moderately shrunken with hyperchromatic nuclei at 4 C but marked changes occurred within 24 hours at 24 C. Bacteria were observed from 24 hours at 24 C only. Barber[26] claimed that adrenal glands of pigs were diagnostically useful at 48 and 12 hours after death in carcasses left at 4 and 24 C, respectively.

In the ovine fetus, no difference in the rate of autolysis between adrenal cortex and medulla was noted grossly.[28] Progressive softening started at 4 hours almost at the same time with renal cortex and spleen, and became brown by 8 hours. Shrinkage and sloughing of the cortical epithelium at 48 hours was the only significant microscopic change.[29]

The following brief review of the adrenal gland of the domestic fowl was based on the findings of Hodges[39] and Wells and Wight.[40]

The avian adrenal or suprarenal glands are small, paired, irregularly oval elongated structures located at the cranial end of the kidneys. They are yellowish and the size and weight depends

on breed, age, health, and various environmental factors. The glands are supplied mainly by the cranial renal arteries. They are encapsulated by dense fibro-elastic connective tissue. Histologically, the gland is composed of a cortex and medulla but there is no clear demarcation as in mammalian glands. It has been suggested that the avian cortex be renamed interrenal tissue and the medulla, chromaffin tissue. The adrenal parenchyma is divided into two zones: peripheral and central. The peripheral zone consists mainly of palely-staining cortical cells but with a subcapsular layer of basophilic medullary cells and has been likened to the zona glomerulosa of mammals. The central zone consists of cortical and medullary cells intermixed and has been referred to as the zona reticularis of mammals. Cortical cells occur as irregular cords in between groups of medullary cells. The ratio of cortical to medullary tissue varies depending upon several factors such as sex, age, health and environment.

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II. MICROSCOPIC POSTMORTEM CHANGES IN KIDNEYS OF THE DOMESTIC FOWL

INTRODUCTION

The priority of poultry pathologists to eliminate economically important and reportable diseases had led to some neglect in specific studies of the chicken kidney. However, with more infectious diseases affecting and complicating the kidney such as infectious bursal disease, infectious bronchitis, Newcastle disease and pasteurellosis,[1] interest in renal pathology is increasing.

Avian nephrosis due to toxins or disease manifestations may not be easily differentiated from postmortem autolysis grossly and microscopically. Many rely on experience but basic information is needed to form a sound basis for determining whether the changes observed are significant. To minimize confusion in interpreting microscopic lesions, immediate tissue fixation is recommended. Some samples, however, reach the laboratory poorly fixed or with ongoing autolytic changes.

Postmortem changes have been reported in kidneys of man,[2] dog,[3] cat,[4] rabbit,[2] mice,[5] rat,[6-9] pig[10] and chickens[11-15] under different physical conditions. In the light of existing knowledge, this study was conducted to add information regarding changes in kidneys of intact chicken carcasses and lead to a practical means of delaying postmortem changes.

MATERIALS AND METHODS

Birds - Eighty-four male White Leghorn chickens, 12 weeks of age, Babcock and Hi-sex strains, weighing 550 to 1250 g. were used in this study. They came from apparently healthy flocks in a commercial poultry farm.

Pretreatment and Experimental Design - The rectal temperature of all birds were taken prior to euthanasia using a scanning tele-thermometer[a] at a slow speed. The birds were euthanatized by placing them in groups of 5 to 8 in a CO_2 gas chamber for 25 to 30 seconds. Immediately after death, four birds were necropsied and served as controls (0 hour). The 80 test birds were randomly divided into four groups; half dry and half wet with detergent solution.[b]

Group I - 29 C - 18 birds - wet

II - 29 C - 18 birds - dry

III - 18 C - 22 birds - wet

IV - 18 C - 22 birds - dry

All birds were laid on their right side in a controlled environment chamber[c] with 50% relative humidity at 18 or 29 C.

Necropsy - Two birds from each wet and dry groups held at 29 C were necropsied at the following postmortem intervals (PI): 1, 3, 6, 9, 12, 18, 24, 36, and 48 hours. In test birds held at 18 C, PI was

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[a]YSI model 47TD, Yellow Springs Instrument Co., Inc., Ohio.

[b]Cen-O-Phen, Central Chemical Co., Inc., Kansas City, Kansas.

[c]ISCO chamber, Lincoln, Nebraska.

extended to 72 and 96 hours. Before necropsy, rectal and thoracic temperatures were taken and recorded. Data collected were statistically analyzed using one way analysis of variance.[16]

Histopathological Procedures - A transverse section of approximately 0.5 cm piece of tissue was taken from the middle of the caudal division of the left kidney and fixed in 10% buffered neutral formalin (BNF) for a minimum of 3 days, and trimmed to a thickness of about 3 mm for processing. The tissues were dehydrated in a series of ethanols, cleared in xylene, embedded in paraffin in a routine automated processor cycle,[d] and cut at 6 microns, mounted on glass slides, stained with Harris hematoxylin and eosin Y (H & E) using an automated slide stainer,[e] and covered with coverslips. Tissues were also stained with periodic acid Schiff (PAS) according to McManus[17] manually.

Two cortical lobules and two medullary areas were observed and evaluated by visual comparison using high dry (40x) objective and (10x) eyepieces and when in doubt, by oil immersion (100x). Changes were tabulated for the various components of the nephron and scored accordingly: 0 for absent; 1 for mild; 2 for moderate; and 3 for marked.

Photomicrographs were taken using a Leitz Orthomat camera mounted on a Leitz Ortholux microscope. Kodak Wratten green filter #58 was used with Kodak Panatomic-X black and white film.

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[d]Autotechnicon, Technicon Corporation, Chauncey, New York.

[e]Histotek, Ames Company, Div. Miles Laboratories, Inc., Elkhart, Indiana.

RESULTS

Temperature

The initial rectal remperature of the chickens ranged from 40.0 to 41.7 C, mean 40.9 C. Body temperature of the carcasses at each PI are given in the Appendix (Tables 1 and 2) and summarized in Table 1 and in Fig 1 and 2. Statistical data are given in the Appendix (Tables 3 and 4). No significant differences were noted between rectal and thoracic temperatures ($P < 0.05$).

1. 29 C Experiment - During the first hour after death, the temperature decreased from 40.9 to about 31.4 C in wet birds and 36.9 C in dry birds. It continued to decrease until 9 hr PI, then slightly increased until 12 hr PI. By 9 hr PI, dry birds had a temperature close to room temperature. In the wet group, body temperature was close to 29 C after 24 hr PI and about 9 hr PI in the dry group. Dry and wet birds had equilibrated close to room temperature by 24 hr PI. Decrease in temperature was statistically significant from 1 hr PI to about 18 hr PI ($P < 0.05$).

2. 18 C Experiment - Wet birds had a marked decrease in body temperature from 40.9 to 26.2 C one hour after death. The temperature of all birds decreased until 9 hr PI, then slightly fluctuated. The temperature of all birds remained almost constant throughout the experiment after 48 hr PI, slightly above room temperature. The temperature of dry birds did not decrease below room temperature. Decrease in temperature was significant until 48 hr PI ($P < 0.05$).

Microscopic changes: H & E stained sections

1. Control (0 hour) - Some Bowman's spaces in control kidneys were slightly dilated but free of debris. Glomeruli with a central compact mass of mesangial cells and tuft of glomerular capillaries were basophilic due to irregular ovoid mesangial nuclei with chromatin clumping (Fig 3).

The proximal convoluted tubules (PCT) had readily distinguishable luminal brush borders with slightly visible lumina (Fig 3). Cells were slightly pyramidal with eosinophilic cytoplasm and rested on a basement membrane. Few cell nuclei were decreased in size and focal pyknosis was observed in some sections. A slight amount of intercellular space was present between the majority of cells.

Distal convoluted tubules (DCT) observed were confined around the central vein (Fig 4). They had cuboidal epithelium and could be differentiated from other segments of the nephron because the cells were flatter, cytoplasm was basophilic, and were without brush borders or apical mucopolysaccharide granules. The nuclei had a somewhat vesicular appearance.

The collecting tubular (CT) epithelial cells were cuboidal to low columnar and had apical mucopolysaccharide granules. The nuclei were round and vesicular and basally located (Fig 3).

Perilobular and medullary collecting ducts (PCD and MCD) were similar to cortical collecting tubules (Fig 3). Slight separation of medullary collecting ductular cells from the basement membrane was observed in two cortical sections.

The thick and thin segments of the medullary loop were located between MCD (Fig 5). Cells were cuboidal with round to oval nuclei

basally located and with coarse chromatin clumping.

Focal areas of lymphocytic infiltration were present in the cortical interstitium.

2. 29 C Experiment - Sequential microscopic changes of the wet and dry groups are presented in Tables 2 and 3, respectively.

a. Renal corpuscle - Eosinophilic debris in Bowman's space was detected in the wet group by 9 hr PI (Fig 11) and as early as 1 hr PI in one sample in the dry group. Few pyknotic glomerular nuclei were observed at 12 hr PI, pyknosis became marked by 36 hr PI (Fig 8). This change was observed at 9 hr PI in the dry group and became marked by 24 hr PI (Fig 13). Bowman's space increased in width with increasing time in both groups.

b. Proximal convoluted tubules - By 1 hr PI, nuclear fading, shrinkage and pyknosis were present in all birds (Fig 10). In the dry group, however, karyorrhexis was already present which only started 3 hours after death in the wet group. More tubular cells became affected with increasing PI. In wet birds, from 12 hr PI, PCT cell nuclei were either pyknotic or karyorrhectic with slight karyolysis. The three nuclear changes remained until 36 hr PI. In the dry group, nuclear pyknosis was only marked from 18 hr PI. Karyolysis was observed as early as 12 hr PI and continued until 36 hr PI. Karyorrhexis was present from 1 to 36 hr PI.

Initially, the cytoplasm was eosinophilic but became pale, vacuolated or mottled and disrupted once the nucleus became pyknotic. Some cells had homogeneous, markedly acidophilic cytoplasm during the later stages. This cytoplasmic change was observed in wet birds at 48 hr PI when nuclei were absent (Fig 9).

Cells were slightly to moderately separated from the basement membrane in all birds and by 3 hr PI, marked cell dissociation was evident (Fig 12). Brush borders were clearly visible initially but began to disappear with time.

c. Distal convoluted tubules - As early as one hour after death, slight chromatin margination accompanied by nuclear shrinkage was evident in all section (Fig 14). Most DCT cell nuclei in dry birds were pyknotic by 3 to 6 hr PI (Fig 15) but not until 18 hr PI in wet birds. From 6 hr PI, DCT were difficult to distinguish in the dry group and from 18 hr PI in the wet group due to loss of cytoplasmic basophilia. The nuclei began to disappear by 18 hr PI in one wet bird and by 36 hr PI in all birds.

Separation of DCT cells from the basement membrane was slight and inconsistent but was frequently observed in dry birds. Cell dissociation occurred at 3 hr PI in the dry group and 18 hr PI in the wet group.

d. Collecting tubules and perilobular collecting ducts - Nuclear changes occurred earlier in dry than wet birds. Moderate numbers of tubular cells were already undergoing karyorrhexis by 3 hr PI (Fig 16) and markedly increased by 24 hr PI. Pyknosis was infrequently seen. In wet birds, karyorrhectic nuclei moderately increased by 12 hr PI and became marked in one bird at 36 hr PI. A clear halo of cytoplasm was present around karyorrhectic nuclei (Fig 17). Degenerating nuclei seemed to lie more towards the lumen. Karyolysis was not observed in all sections.

e. Medullary collecting ducts - Separation of cells from the basement membrane was moderate to marked in all sections. Chromatin

margination was observed by 3 hr PI in the dry group (Fig 20) and by 6 hr PI in the wet group. Karyorrhexis was first observed at 6 hr PI in the dry group and became marked by 18 hr PI (Fig 21). In the wet group, moderate number of nuclei in a tubule were karyorrhectic by 18 hr PI and severe changes were noted by 24-36 hr PI (Fig 19). By 18 hr PI (Fig 21) cells of dry birds were disrupted; this change became diffuse and severe by 24 hr PI. Marked cell dissociation occurred only from 24 hr PI in wet birds.

f. Medullary loop - Most thin and thick segments of the medullary loop had marginated chromatin by 1 hr PI and slight nuclear shrinkage. From 18 hr PI, all nuclei in wet birds were pyknotic or karyorrhectic. Cells were dissociated from one another, sometimes separated from the basement membrane. In dry birds, mild pyknosis and karyorrhexis were noted at 6 hr PI, and more karyorrhectic nuclei were observed with increasing time.

g. Bacteria - Bacterial infiltration was observed by 24 hr PI and became marked by 48 hr PI. Forty-eight hours after death, all tubular cells were non-nucleated and individualized with homogeneous eosinophilic cytoplasm (Fig 9). Erythrocytes had pyknotic nuclei and unstained cytoplasm.

3. 18 C Experiment - Sequential microscopic changes are presented in Tables 4 and 5. Comparison between wet and dry birds and between 29 and 18 C experiments are summarized in Table 6.

Microscopic changes: PAS stained sections

1. Control (0 hour) - Proximal convoluted tubules had strongly PAS-positive luminal brush borders and basement membranes (Fig 6). The collecting tubules and ducts contained PAS-positive apical mucopolysaccharide granules (Fig 18).

2. 29 C Experiment - The basement membranes of glomeruli and tubules persisted until 36 hr PI in all birds. By 24 to 36 hr PI, the basement membrane was still recognizable but thin (Fig 7-8); PCT brush borders were still present but less intensely stained. Very few granules were present in the MCD. In the CT and PCD, granules were few. At 48 hr PI, basement membranes and granules no longer stained with PAS (Fig 9).

3. 18 C Experiment - Tubular basement membrane was still present at 96 hr PI although it appeared thinner than that of the control. Brush borders were less intensely stained and some PCT cells had lost the brush borders. Only a slight amount of granules was observed at this hour.

DISCUSSION

Microscopic changes observed were similar to those reported by other workers[12,15] although different methods of euthanasia were used. Intact chicken carcasses were used in this study to simulate natural diagnostic cases as near as possible when necropsy is delayed and carcasses are left in a warm or cool environment. Overexposure of birds to CO_2 did not cause significant changes in histocellular architecture that can alter postmortem examination.[18] Introduction of bacteria that could hasten rate of changes[19] as during intravenous injection was eliminated.

Temperature has the greatest effect on hastening or retarding postmortem change.[9] Munger and McGavin[15] held intact chicken carcasses at 20, 37, or 4 C at 50% relative humidity and reported changes occurred earlier at 37 C but varied widely. In forensic medicine, temperature is used as a guideline to estimate duration of death.[20]

Birds have feathers that protect them against cold. After death, feathers insulate the body and prevent heat dissipation. By wetting carcasses if necropsy is delayed, body temperature is lowered, thus, retarding postmortem autolysis. In this study, there was a significant difference between wet and dry groups ($P < 0.05$). This is further supported by earlier microscopic appearance of cellular changes in dry birds.

Body temperature of wet but not dry birds decreased below environmental temperature in both 29 and 18 C experiments. This may be due to evaporative cooling,[21] loss of heat from the carcass through evaporation was augmented by wetting the birds. At 9 hr PI at 29 C, feathers of wet birds started to dry and became marked by 18 hr PI.

Microscopically, the earliest segment of the nephron to undergo postmortem change was the proximal convoluted tubule followed by the distal convoluted tubule, collecting tubule and glomeruli confirming findings of earlier workers.[12,14,15] This is contrary to findings in porcine and rat kidneys where the distal convoluted tubules underwent nuclear changes first.[8-10]

Much emphasis was given to nuclear changes as done by previous pathologists and cytologists.[6] Nuclear changes are clearly evident by light microscopy and in most cases, staining artifacts obscured subjective analysis of cytoplasmic changes. Masson's trichome stain could be an excellent stain to recognize minute cytological changes that might be missed with routine stains.[2]

Different segments of the nephron underwent different rates and sequence of postmortem microscopic changes. Rate of postmortem change

varied between experiments: the higher the temperature, the faster the recognizable cellular changes.

The proximal convoluted tubular cells underwent a sequential nuclear change of shrinkage with chromatin margination, pyknosis, secondary karyorrhexis and karyolysis. It is interesting to note that not all cells in a PCT underwent degenerative changes at the same time. Some cells had pyknotic nuclei and were separated from the neighboring cells while others appeared unchanged. At 0 hour, pyknosis was already present, emphasizing the importance of immediate fixation after death.[10,15] This may be also interpreted as a "normal" finding in natural cases. Pyknosis was not evident in dry birds held at 29 C until 18-24 hr PI; instead, there was nuclear shrinkage with chromatin margination and primary karyorrhexis. Some nuclear changes observed commenced at the same time in kidneys of intact carcasses held at 18 C.[15]

Karyorrhexis was the most predominant nuclear change in collecting tubules and ducts. It appeared to be a primary change, the nucleus fragmenting without undergoing pyknosis.[6,8] This is similar to the finding in the light cells of the collecting tubules of rat kidneys incubated at 37 C at varying postmortem intervals.[6] In most collecting tubular and ductular cells, a clear halo of cytoplasm surrounded karyorrhectic nuclei. Like the PCT, not all cells in a cross section of a tubule or duct underwent postmortem change at the same time.

The distal convoluted tubules underwent postmortem changes later than PCT but the sequence of nuclear changes was similar. Medullary tubules representing thick and thin segments of the mammaliam loop had cells with nuclear chromatin marginated as early as 1 hr PI at

29 C and 18 C although the number of cell affected varied in each bird.

Separation of cells from the basement membrane was an inconstant artifact change, present in some control sections, although it became more frequent during the later PI. This change is a frequent finding in nephrosis.[1]

Bacteria were detected only at 24 hr PI in birds held at 29 C and at 72 hr PI and 96 hr PI in dry and wet birds, respectively, held at 18 C. At this stage, diffuse nuclear and cytoplasmic changes were evident. Those sections with marked bacterial invasion had all tubular cells non-nucleated and indistinguishable from each other. The erythrocytes were pyknotic with unstained cytoplasm. It is probable that bacteria hastened liberation of proteolytic enzymes[10] and/or bacteria released their own enzymes. The late postmortem bacterial invasion is consistent with findings in canine and feline kidneys and human cadavers.[3,4,22,23]

The persistence of PAS-positive structures such as basement membranes of glomeruli and tubules, luminal brush borders and acid-mucopolysaccharide granules of the collecting duct system until 36 hr PI at 29 C and 96 hr PI at 18 C suggests little availability of enzyme activity to split carbohydrates.[6] In H & E stained sections, however, brush borders were barely visible at 18 hr PI in dry birds held at 29 C. This is similar to findings in other species.[3-4,6-7,9-10] However, as in rat kidneys, basement membranes and brush borders became weakly defined or stained with PAS during the later PI. In an earlier study,[15] brush borders disappeared earlier than in the present study.

Renal glomerular nuclei were relatively resistant to autolysis. Bowman's space was narrow in control birds and initial PI probably

due to initial glomerular swelling.[7] It became wider with increasing PI. Eosinophilic debris, possibly edema fluid, as a result of fluid exchange[10] were observed at different times PI in each experiment. With increasing PI, tubular epithelial cells were present in the capsular space representing herniation of PCT as in rat and canine kidneys.[3,7] In cats, herniation of PCT in this space and possible pressure or fluid exchanges during autolysis resulted to an increase in renal corpuscular diameter.[4]

Earlier occurrence of autolytic changes in PCT of chicken kidneys implies greater susceptibility to anoxia and injury and suggests greater proteolytic activity than in PCT rats and pigs. Osvaldo et al.[6] described cytoplasmic bodies by electron microscopy but did not correlate them with autolytic enzyme activity in the PCT, DCT and CT. Some cytoplasmic bodies contained acid phosphatase like lysosomes but no cytoplasmic digestion in the immediate vicinity of these bodies was observed.

The changes observed are similar to those of avian nephrosis.[1] Usefulness of chicken kidneys for critical evaluation of lesions can best be attained if changes observed were compared with autolyzing diseased kidneys. Microscopic appearance of tissues varied at different PI, temperature, and body and environmental conditions. Even using the same technic under similar conditions produced different results. Wilhelm[14] and Gildmeister[12] used the same technic and found autolytic changes after 20 and 62 hours, respectively, in kidneys of exsanguinated chickens. These emphasized that several known and unknown factors govern the rate and sequence of postmortem changes.

As pointed in earlier published reports,[24-25] the kidney is one

of the organs undergoing early autolysis. Under similar conditions, postmortem changes occurred earlier in the kidney than the adrenal gland of intact chicken carcasses (Part III).

SUMMARY

Eighty-four male White Leghorn chickens were euthanatized by CO_2 gas to determine the type, rate and sequence of postmortem microscopic changes in kidneys of dry and wet intact carcasses. They were held at 29 or 18 C with 50% relative humidity at different postmortem intervals.

Microscopic postmortem changes in the different segments of the nephron underwent different rate and sequence of cellular changes. Cellular changes occurred earlier at 29 than at 18 C and in birds not wet with detergent solution prior to storage at different temperatures. Decrease in body temperature of wet birds over dry birds was significant ($P < 0.05$). Wetting carcasses delayed postmortem changes.

The proximal convoluted tubule (PCT) underwent the earliest postmortem changes followed by the distal convoluted tubule (DCT), collecting tubule (CT), medullary loop (ML), medullary collecting duct (MCD) and glomerulus. The PCT, DCT, and thin and thick segments of the ML underwent a sequential nuclear change of chromatin margination, progressive shrinkage, pyknosis, karyorrhexis, and karyolysis. Nuclei were pyknotic if cytoplasmic changes were severe. Primary karyorrhexis was the predominant feature of collecting tubules and ducts.

As early as one hour after death, some PCT cells of all kidney sections were already pyknotic emphasizing immediate tissue fixation was necessary for critical evaluation. By 9 and 18 hr PI, PCT of dry

and wet birds, respectively, held at 29 C had pyknotic and karyorrhectic nuclei with slight karyolysis and moderate to marked cytoplasmolysis that extended until 36 hr PI. At this time, DCT were headly distinguishable due to loss of basophilia. Karyorrhectic nuclei were already evident in collecting tubules and ducts. At 48 hr PI, massive bacterial invasion was present and all tubular cells were non-nucleated and individualized with homogeneous, acidophilic cytoplasm. Basement membranes no longer stained with PAS. Erythrocytes were pyknotic with unstained cytoplasm. Pyknotic glomeruli were first observed at 9 hr PI in dry birds and 12 hr PI in wet birds.

Histologic appearance of dry and wet birds at 9 and 18 hr PI at 29 C was similar to 12 hr PI in dry birds and 24 hr PI in wet birds held at 18 C with minor differences in some tubular changes. At 18 C, pyknotic glomeruli appeared by 6 hr PI in dry birds and 24 hr PI in wet birds. Most changes increased with increasing PI. Bacterial invasion was noted at 72 hr PI in dry birds and at 96 hr PI in wet birds.

Basement membranes, brush borders and acid mucopolysaccharide granules were resistant to autolysis and persisted until 36 hr PI at 29 C and 96 hr PI at 18 C although they became less defined and weakly stained by PAS during the later periods after death.

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TABLE 1 - Mean body temperature of wet and dry intact chicken carcasses held at 29 or 18 C at varying postmortem intervals

Postmortem interval (hour)	29 C		18 C	
	Wet	Dry	Wet	Dry
1	31.4[a]	36.9[b]	26.2	34.6[b]
3	26.3	32.8[b]	19.0	28.5[b]
6	24.9	30.2[b]	16.6	22.2[b]
9	24.2	28.9[b]	16.2	19.6[b]
12	24.4	29.2[b]	17.2	20.2[b]
18	27.6	28.5[b]	17.8	19.6[b]
24	28.6	28.4	17.5	19.0[b]
36	28.7	29.0	16.9	18.3[b]
48	29.1	29.2	18.2	18.7[b]
72			18.2	18.6
96			18.6	19.0

[a]Mean of 2 rectal and 2 thoracic temperatures of 2 samples.

[b]Significantly different ($P < 0.05$).

TABLE 2 - Summary of microscopic postmortem changes in kidneys of wet intact chicken carcasses held at 29 C at varying postmortem intervals

Segment of the nephron	Postmortem interval (hour) 0	1	3	6	9	12	18	24	36	48
Glomerulus										
Bowman's space-increase	1	0-1	1	1	1	2	2	2	2	All tubular
debris	0	0	0	0	1	2	2	2	2	cells were
Small dark nuclei	0	0	0	0	0	1	1	2	3	individual-
										ized,
Proximal convoluted tubule										karyolysed,
Nucleus-fading	0	1	2	1	0	0	0	0	0	with homo-
-shrinkage	1	2	3	3	3	0	0	0	0	geneous
-pyknosis	0-1	1	2	2-3	3	3	3	3	3	eosinophilic
-karyorrhexis	0	0	1	1	1-2	2	2	1-2	1-2	cytoplasm.
-karyolysis	0	0	0	0	0	1	1	1	2	RBC were
Separation from basement										pyknotic
membrane	0	2	1	1-2	2	2	1	1-2	1	with
Cell dissociation	1	2	3	3	3	3	3	3	3	unstained
Visibility-lumen	1	1	1	1	2	1	0	0	0	cytoplasm.
-brush border	3	3	2	2	2	2	2	0-1	0	Marked
										bacterial
Distal collecting tubule										invasion was
Chromatin margination	0	1	1	1	2	2-3	2	2	0	present.
Nucleus-fading	0	0	1	1	2	0	0	0	0	
-shrinkage	0	1	1	1	1	2	0	0	0	
-pyknosis	0	0	1	1	2	2	2-3	3	3	
-karyorrhexis	0	0	0	0	0	1	1	1	2	
-karyolysis	0	0	0	0	0	0	0-1	0-1	1	
Separation from basement										
membrane	0	0	0	0	0	0	0-1	0	0-1	
Cell dissociation	0	0	0	0	0	0	0-1	0-1	3	
Collecting tubule &										
perilobular collecting duct										
Chromatin disappeared	0	0	1	1	1-2	2	2	3	3	
Nucleus-shrinkage	0	0	0-1	1	1	1	1	2	2-3	
-pyknosis	0	0	1	0-1	1	1	1	1-2	2	
-karyorrhexis	0	0	0	0-1	1	2	2	2	2-3	
-karyolysis	0	0	0	0	0	0	0	0	0	
Separation from basement										
membrane	0	0	0	0	0	0	1	0-1	1	
Cell dissociation	0	0	0	0	0	0	0	0	2	
Medullary collecting duct										
Chromatin disappeared	0	0	0	0-1	2	2	2	1-2	0	
Nucleus-shrinkage	0	0	0	0	0	0	1	2	0	
-pyknosis	0	0	0	0	0	0	0-1	1	0	
-karyorrhexis	0	0	0	0	0	0	2	2-3	3	
-karyolysis	0	0	0	0	0	0	0-1	0	0-1	
Separation from basement										
membrane	0-1	2-3	3	2	3	2	1-3	1-3	3	
Cell dissociation	0	0	0	0	0	0	0	0-3	3	
Medullary loop										
Chromatin disappeared	0	2	2	2	3	3	2	3	0	
Nucleus-fading	0	0	1	0	0	0-1	0	0	0	
-shrinkage	0	0-1	0-1	1	1-2	2	2-3	3	0	
-pyknosis	0	0	0	0	0-1	1	2-3	3	3	
-karyorrhexis	0	0	0	0	0	0	2	1-2	1-2	
-karyolysis	0	0	0	0	0	1	1	0	0	
Separation from basement										
membrane	0	0	0-1	0-1	2	0-1	1-2	0	0-1	
Cell dissociation	0	0	0	0	1	1	1-2	2-3	0-3	

0 = Absent
1 = Mild
2 = Moderate
3 = Marked

TABLE 3 - Summary of microscopic postmortem changes in kidneys of dry intact chicken carcasses held at 29°C at varying postmortem intervals

Segment of the nephron	Postmortem interval (hour) 0	1	3	6	9	12	18	24	36	48
Glomerulus										
Bowman's space-increase	1	0	2	1-2	2	1	2	3	0-2	All tubular
-debris	0	0-1	1	1	1	0-1	1	2	1	cells were
Small dark nuclei	0	0	0	0	1	1	2	3	3	individual-
										ized,
Proximal convoluted tubule										karyolysed,
Nucleus-fading	0	2	1	1	1	1	0	0	0	with
-shrinkage	1	1-2	2	3	3	2-3	3	0	0	homogeneous,
-pyknosis	0-1	1	1	1-2	1-2	1-2	3	2	1	eosinophilic
-karyorrhexis	0	1	2	2	3	3	2	3	3	cytoplasm.
-karyolysis	0	0	0	0	0	0-1	1	1	2	RBC were
Separation from basement										pyknotic
membrane	0	1-2	2	2	2	1	1	0	0	with
Cell dissociation	1	2	2-3	3	3	3	3	3	3	unstained
Visibility-lumen	1	0	1	0-1	0-1	1	1	0-1	1-2	cytoplasm.
-brush border	3	3	2	2	1	1	0	0	0	Marked
										bacterial
Distal collecting tubule										invasion
Chromatin margination	0	0-1	1	1-2	2	2-3	0	0	0	was present.
Nucleus-fading	0	0	0	0	0	0	0	0	0	
-shrinkage	0	0-1	3	0	2-3	2-3	0	0	0	
-pyknosis	0	0-1	3	2-3	3	2	3	2-3	2-3	
-karyorrhexis	0	0	1	2	2	2	2	2-3	2-3	
-karyolysis	0	0	0	0	0	0	0	0	1	
Separation from basement										
membrane	0	0	0-1	0-1	1	0-1	0	1	1	
Cell dissociation	0	0	1	1	2	1	2	3	3	
Collecting tubule &										
perilobular collecting duct										
Chromatin disappeared	0	0-1	0-1	1	1-2	1	2	0	0	
Nucleus-shrinkage	0	0-1	1	1	1	1	1	0	0	
-pyknosis	0	0	0	1	1	0	0-1	0	0	
-karyorrhexis	0	0-1	2	2	2	2	2	3	3	
-karyolysis	0	0	0	0	0	0	0	0	0	
Separation from basement										
membrane	0	0	0	0	0-1	1	1	1	1	
Cell dissociation	0	0	0	0	0	0	0-1	0-1	1	
Medullary collecting duct										
Chromatin disappeared	0	0	1	1	2	2	1	0	0	
Nucleus-shrinkage	0	0	0	1	2-3	2	0	0	0	
-pyknosis	0	0	0	0	0	0	0	0	0	
-karyorrhexis	0	0	0	1-2	2-3	2	3	3	3	
-karyolysis	0	0	0	0	0	0	0	0	0-1	
Separation from basement										
membrane	0-1	2	2	2	2	2-3	3	3	3	
Cell dissociation	0	0	0	0	0	0	2	3	2	
Medullary loop										
Chromatin disappeared	0	2	2-3	2	2	3	1	0	0	
Nucleus-fading	0	0	0	0	0	1	0	0	0	
-shrinkage	0	1	1	2	2-3	1-2	2	0	0	
-pyknosis	0	0	0	1	1	1	1-2	1	1	
-karyorrhexis	0	0	0	1-2	2-3	2-3	3	3	3	
-karyolysis	0	0	0	0	0	0	0	0	0	
Separation from basement										
membrane	0	1	2	2	3	2-3	3	3	1	
Cell dissociation	0	0	0	1	1	0-1	2	2	2-3	

0 = Absent
1 = Mild
2 = Moderate
3 = Marked

TABLE 4 - Summary of microscopic postmortem changes in kidneys of wet intact chicken carcasses held at 18 C at varying postmortem intervals

Segment of the nephron	Postmortem interval (hour)											
	0	1	3	6	9	12	18	24	36	48	72	96
Glomerulus												
Bowman's space-increase	1	0-1	1	1	1	1	2	2	3	3	3	3
-debris	0	0	0	0	0	0	1	2	2	3	3	3
Small dark nuclei	0	0	0	0	0	0	0	1	1	2	2	3
Proximal convoluted tubule												
Nucleus-fading	0	2	2	2	2	2	1	0	0	0	0	0
-shrinkage	1	2	3	3	3	3	3	0	0	0	0	0
-pyknosis	0-1	1	2	2-3	2-3	3	3	3	2-3	2	3	3
-karyorrhexis	0	0	0	1	1-2	1-2	1	2	2	2	2	1
-karyolysis	0	0	0	0	0-1	1	2	2	2	2	2	2
Separation from basement membrane	0	1	1	2	1	1	0-1	1	1-2	0-1	0-1	0
Cell dissociation	1	1	1	2	2-3	3	3	3	3	3	3	3
Visibility-lumen	1	1	1	1	1	1	0-1	0-1	0-1	0	0	0
-brush border	3	3	2	2	2	2	0	1	0	0-1	0	0-1
Distal collecting tubule												
Chromatin margination	0	0	0	0	1	1	2	2-3	2	2-3	1	0
Nucleus-fading	0	0	0	0	1	1	1	1	1	0	0	0
-shrinkage	0	0	0	0	1	0	1	1	1	2	3	0
-pyknosis	0	0	0	0	0	0	1	0-1	1	1	3	3
-karyorrhexis	0	0	0	0	0	0	0	1	1	1	3	2
-karyolysis	0	0	0	0	0	0	0	0	0	0	0	0
Separation from basement membrane	0	0	0	0	0	0	0	0	0	0	1	0
Cell dissociation	0	0	0	0	0	0	0	0	0	0	2	2
Collecting tubule & perilobular collecting duct												
Chromatin disappeared	0	0	0	0	0	0	1	1	1	1	1	0
Nucleus-shrinkage	0	0	0	0	0	0	1	1	1	1	1	0
-pyknosis	0	0	0	0	0	1	1	1	1	1	0-1	2
-karyorrhexis	0	0	0-1	0	0-1	0-1	0-1	2	1-2	2	2	3
-karyolysis	0	0	0	0	0	0	0	0	0	0	0	0
Separation from basement membrane	0	0	0	0	0-1	1	0	1	1	1	1	1
Cell dissociation	0	0	0	0	0	0	0	0	0	0	1	2
Medullary collecting duct												
Chromatin disappeared	0	0	0	0-1	1	1	2	2	2	1-2	1	0-1
Nucleus-shrinkage	0	0	0	0-1	1	1	0-1	0-1	1	1	0	0
-pyknosis	0	0	0	0	0	0	0	0	1	0	0	0
-karyorrhexis	0	0	0	0	0	0	0	0	1	1-2	3	3
-karyolysis	0	0	0	0	0	0	0	0	0	0	1	1
Separation from basement membrane	0-1	1-2	2	3	2	2	2	2	3	2	1-2	2-3
Cell dissociation	0	0	0	0	0	0	0	0	0-1	0	2	3
Medullary loop												
Chromatin disappeared	0	1	1-2	1	2	2-3	2	3	3	2	0	0
Nucleus-fading	0	0	0-1	0-1	0-1	1	0	0	0	0	0	0
-shrinkage	0	1	1-2	1	1	1	1	1	2	3	0	0
-pyknosis	0	0	0	0	0	0	0	0-1	1	2	3	2
-karyorrhexis	0	0	0	0	0	0	0	0	0	1-2	2	3
-karyolysis	0	0	0	0	0	0	0	0	0	0	0	0
Separation from basement membrane	0	0	0	0	0	0	0	0	0	0	0-1	0
Cell dissociation	0	0	0	0	0	0	0	0	0	1	2-3	2

0 = Absent
1 = Mild
2 = Moderate
3 = Marked

TABLE 5 - Summary of microscopic postmortem changes in kidneys of dry intact chicken carcasses held at 18 C at varying postmortem intervals

Segment of the nephron	Postmortem interval (hour)											
	0	1	3	6	9	12	18	24	36	48	72	96
Glomerulus												
Bowman's space-increase	1	1	1	1	1	1	1	2	1	2	2	1-2
-debris	0	0	1	1	1	1	1	1	1	2	3	1
Small dark nuclei	0	0	0	0-1	1	1	1	1	2	2	3	3
Proximal convoluted tubule												
Nucleus-fading	0	1	1	0	0	0	0	0	0	0	0	0
-shrinkage	1	2	2-3	3	0	0	0	0	0	0	0	0
-pyknosis	0-1	2	2	2-3	2-3	3	2-3	2	3	3	3	3
-karyorrhexis	0	0	1	1-2	2-3	2	2-3	3	3	2	3	2-3
-karyolysis	0	0	0	0-1	0	1	1	1	1	1-2	1	2-3
Separation of basement membrane	0	1-2	2	1	2	1-2	1	1-2	1-2	1	1-2	0-1
Cell dissociation	1	2	2	3	3	3	3	3	3	3	3	3
Visibility-lumen	1	1-2	0-1	1	1	1	1	1	1	1	1	2
-brush border	3	3	2-3	2	2	2	2	2	1	1	1	0
Distal convoluted tubule												
Chromatin margination	0	0	1	1	2	2	2	1	1-2	0-1	0	0
Nucleus-fading	0	0	0	1	1	2	1	0	2	0	0	0
-shrinkage	0	0	0-1	1	1	1	1	2	1	3	0	0
-pyknosis	0	0	0-1	1	1	2	3	2	2	3	3	3
-karyorrhexis	0	0	0	1	1	1-2	2	2-3	3	2-3	3	2-3
-karyolysis	0	0	0	0	0	0	0	0	0	0	0	0
Separation from basement membrane	0	0	0	0	0	0	1	1	1	1-2	1-2	0-1
Cell dissociation	0	0	0	0	0	0-1	2	2	1	2	2	2
Collecting tubule & periolbular collecting duct												
Chromatin disappeared	0	0-1	1	1	1	1	2	2	2	2	2	0
Nucleus-shrinkage	0	0	0	0-1	1	1	1	1	1	1-2	2	0
-pyknosis	0	0	0	1	1	1	1	0-1	0	1	0	0-1
-karyorrhexis	0	0	0-1	2	2	2	2	2	2	2	2-3	3
-karyolysis	0	0	0	0	0	0-1	0	0	0	0	0-1	2
Separation from basement membrane	0	0	0	0-1	0-1	1	0-1	1	0-1	1	2	2
Cell dissociation	0	0	0	0	0	0	0	0	0	0	0-1	3
Medullary collecting duct												
Chromatin disappeared	0	0	0	1	0-1	2	2-3	2	2	2	1	0-1
Nucleus-shrinkage	0	0	0	0	0	1	1	0	0	0	0-1	0
-pyknosis	0	0	0	0	0	0	0	0	0	0	0	0-1
-karyorrhexis	0	0	0	0	0	1	1-2	2	2-3	3	3	3
-karyolysis	0	0	0	0	0	0	0	0	0	0	0	0
Separation from basement membrane	0-1	2	3	2-3	3	2	2-3	2-3	2	2-3	3	1-2
Cell dissociation	0	0	0	0	0	0-1	0	0	0	2	2	3
Medullary loop												
Chromatin disappeared	0	1-2	1-2	2-3	2-3	3	1	2	2	0	0	0
Nucleus-fading	0	0	0-1	0-1	0	1	0	0	0	0	0	0
-shrinkage	0	1	1	2	2-3	2-3	3	2-3	3	3	0	0
-pyknosis	0	0	0-1	1	1	1-2	1-2	1	2	2	3	2
-karyorrhexis	0	0	0	0-1	1-2	1-2	3	3	3	3	3	3
-karyolysis	0	0	0	0	0	0	0	0	1	1	0-1	0-1
Separation from basement membrane	0	0-1	1	1	2	1-2	2-3	2	2	2-3	2	2
Cell dissociation	0	0	0	0-1	1	0-2	1	1-2	2	2	2-3	3

0 = Absent
1 = Mild
2 = Moderate
3 = Marked

TABLE 6 - Comparison of microscopic postmortem changes in kidneys of wet and dry intact chicken carcasses held at 29 and 18 C

Criteria	29 C		18 C	
	Wet(hr PI)	Dry(hr PI)	Wet(hr PI)	Dry(hr PI)
Glomerulus				
Increase in Bowman's space evident	12	3	18	48
Debris in Bowman's space evident	9	1	18	3
Small dark nuclei evident	12	9	24	6
Proximal convoluted tubule				
Nuclear fading evident	1	1	1	1
Nuclear shrinkage evident	1	1	1	1
Pyknosis evident	1	1	1	1
Pyknosis marked	9	18	12	9
Karyorrhexis evident	3	1	6	3
Karyolysis evident	12	12	9	6
Cell dissociation evident	1	1	6	1
Cell dissociation marked	3	3	9	6
Brush border absent	36	18	36(?)	96
Basement membrane lost[a]	48	48	-	-
Distal convoluted tubule				
Chromatin margination evident	1	1	9	3
Nuclear fading evident	3	-	9	6
Nuclear shrinkage evident	1	1	9	3
Pyknosis evident	3	1	18	6
Pyknosis marked	18	3	72	48
Karyorrhexis evident	12	3	24	6
Karyolysis evident	18	36	-	24
Cell dissociation evident	18	3	72	12
Collecting tubule and peri-lobular collecting duct				
Chromatin disappearance evident	3	1	18	1
Nuclear shrinkage evident	3	1	18	6
Pyknosis evident	3	6	12	6
Karyorrhexis evident	6	1	9	3
Karyolysis evident	-	-	-	72
Cell dissociation evident	36	18	72	72
Medullary collecting duct				
Chromatin disappearance evident	6	3	6	6
Nuclear shrinkage evident	18	6	6	12
Pyknosis evident	18	-	36(?)	96
Karyorrhexis evident	18	6	36	12
Karyolysis evident	18	36	72	-
Cell dissociation evident	24	18	36(?)	48
Medullary loop				
Chromatin margination evident	1	1	1	1
Nuclear fading evident	12	12	3	3
Nuclear shrinkage evident	1	1	1	1
Pyknosis evident	9	6	24	3
Pyknosis marked	18	-	72	72
Karyorrhexis evident	18	6	48	6
Karyolysis evident	12	-	-	36
Cell dissociation evident	9	6	48	6
Bacterial invasion evident	24	24	96	72

[a]PAS-stained sections.

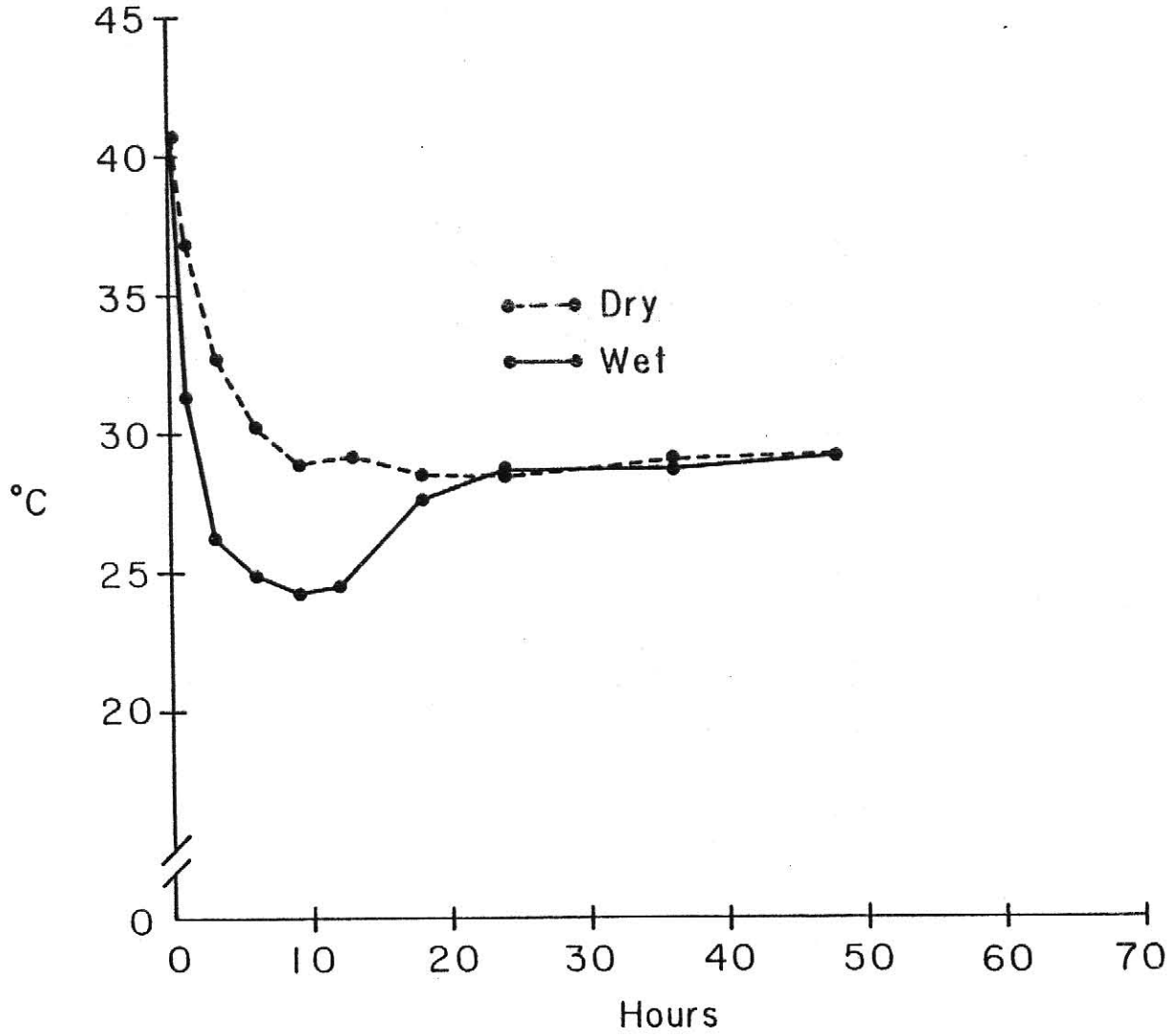


Fig 1 - Mean body temperature of birds held at 29 C at different postmortem intervals

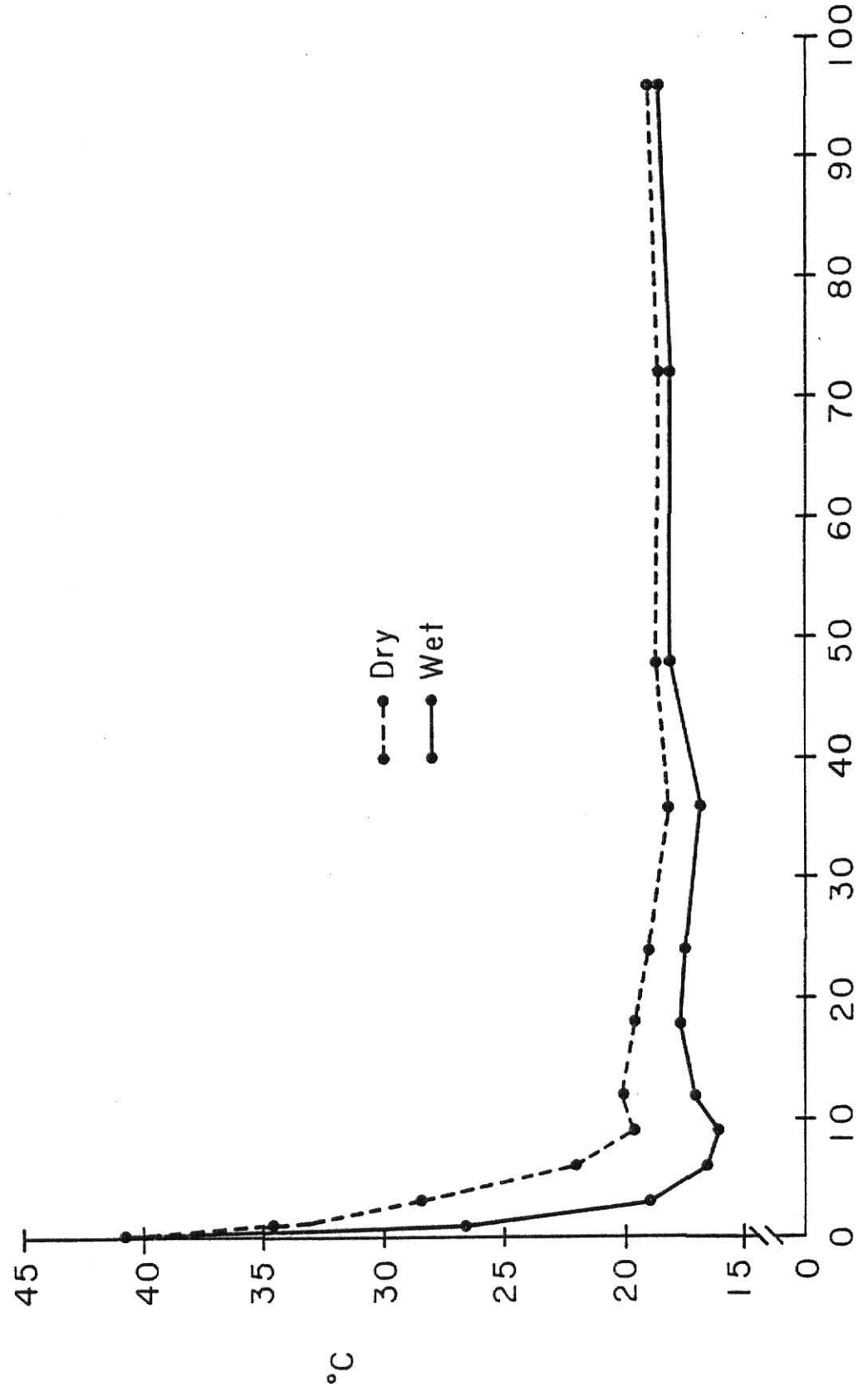
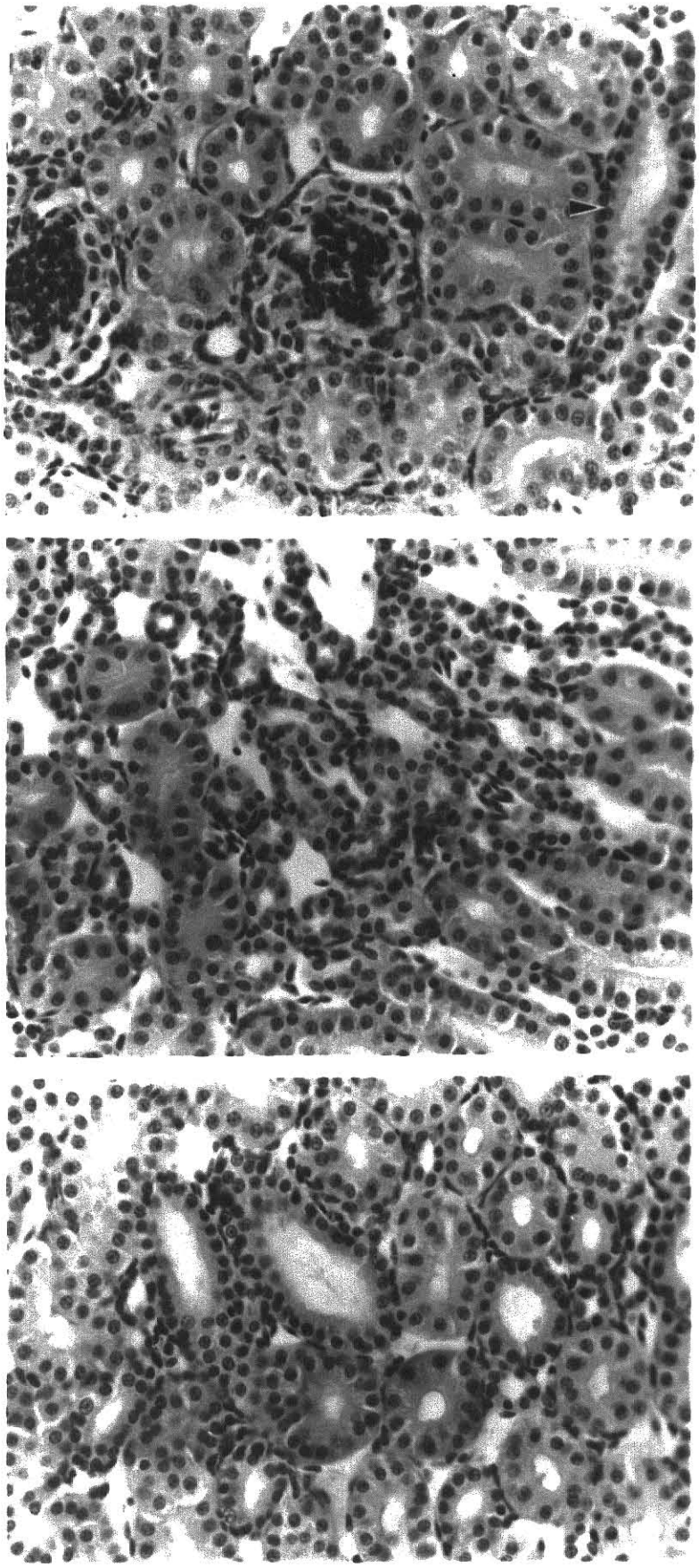


Fig 2 - Mean body temperature of birds held at 18 C at different postmortem intervals

Fig 3 - Kidney, Control
Glomeruli have a central compact mass of basophilic cells. Proximal convoluted tubules have prominent luminal brush borders and cells are slightly separated from one another. Collecting tubule (arrow) has cuboidal to low columnar epithelial cells with basally-located nuclei. H & E stain; x640.

Fig 4 - Kidney, Control
Distal convoluted tubules are mostly confined around the central vein. The epithelium is cuboidal and flatter, cytoplasm is basophilic and the nucleus has a somewhat vesicular appearance. H & E stain; x640.

Fig 5 - Kidney, Control
The medulla consists of collecting ducts lined by low columnar epithelial cells with apical mucopolysaccharide granules and thin and thick segments of the medullary loop of the mammalian nephron. H & E stain; x640.



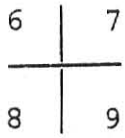


Fig 6 - Kidney, Control
Basement membranes of glomeruli and proximal convoluted tubules and luminal brush borders are strongly PAS-positive. PAS stain; x640.

Fig 7 - Kidney, wet bird, 24 hr PI at 29 C
Basement membranes are still recognizable but thin. Proximal convoluted tubular brush borders are recognizable but less intensely stained. PAS stain; x640.

Fig 8 - Kidney, wet bird, 36 hr PI at 29 C
Basement membranes and brush borders are still present. There is marked diffuse glomerular pyknosis. PAS stain; x640.

Fig 9 - Kidney, wet bird, 48 hr PI at 29 C
Basement membranes and brush borders are no longer stained by PAS. Marked bacterial invasion is present. PAS stain; x640.

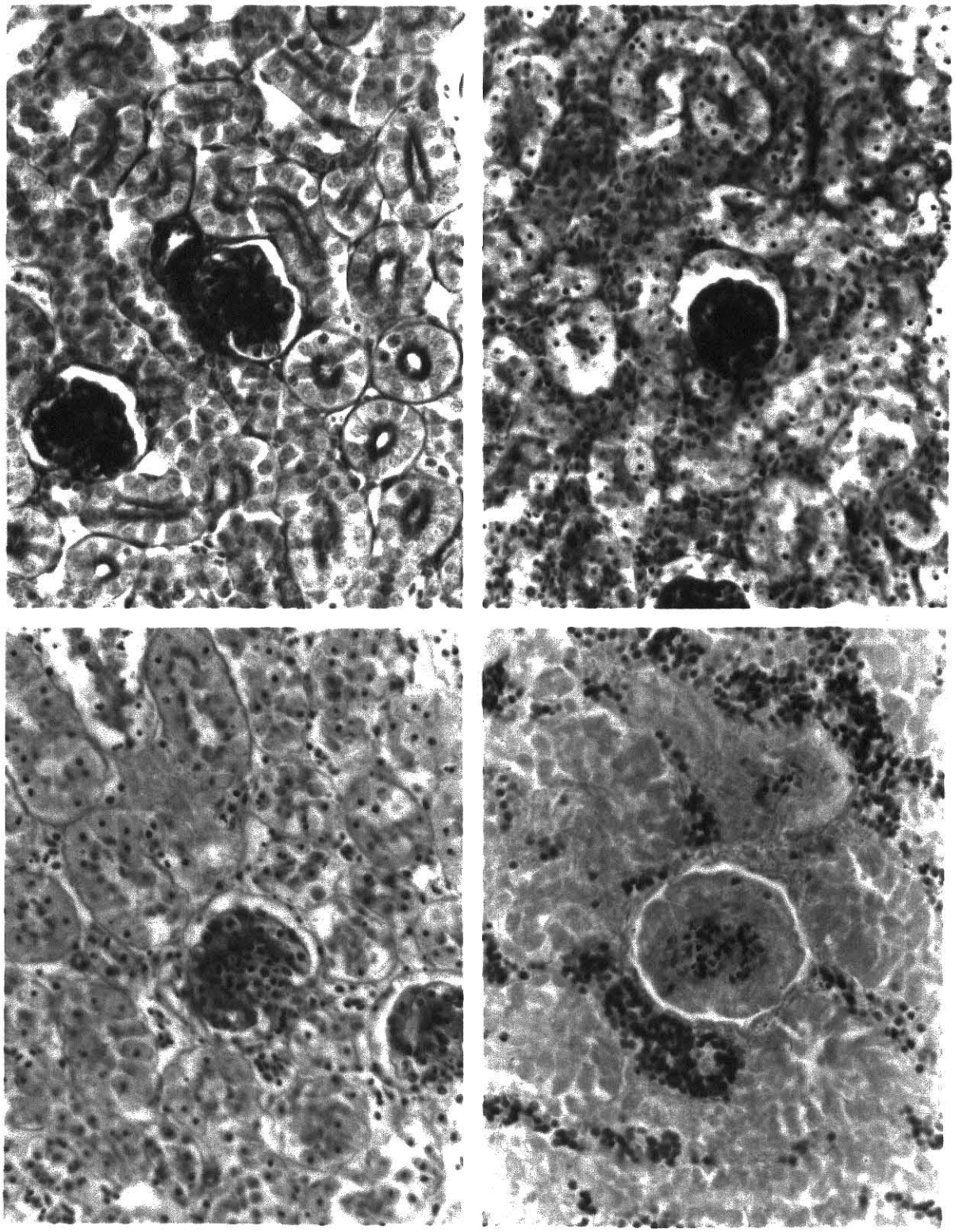
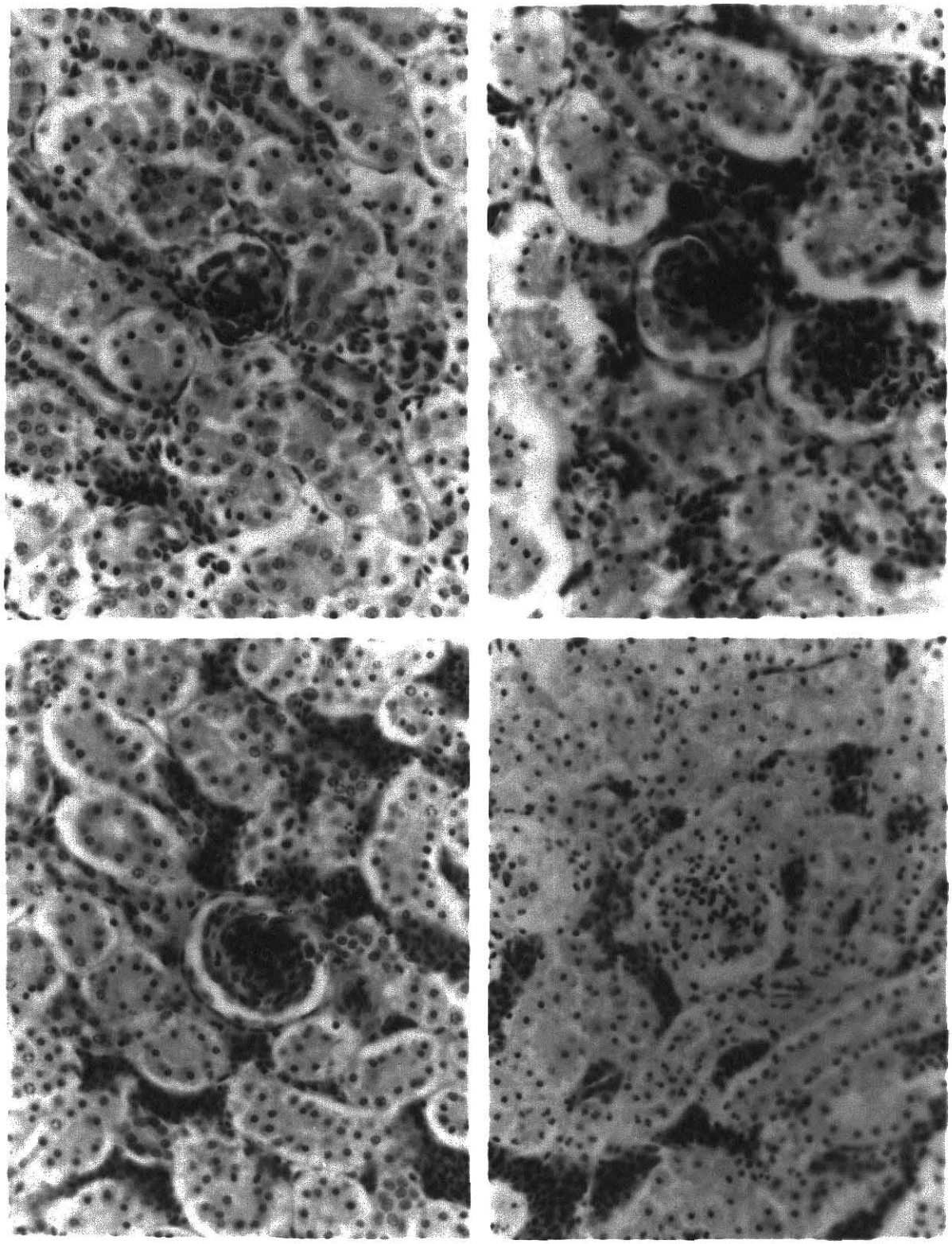


Fig 10 - Kidney, wet bird, 1 hr PI at 29 C.
Some epithelial cells in a proximal convoluted tubule are pyknotic, dissociated from one another and separated from the basement membrane. Nuclear fading and shrinkage are also present. H & E stain; x640.

Fig 11 - Kidney, wet bird, 9 hr PI at 29 C.
Eosinophilic debris in Bowman's space becoming evident. Proximal convoluted tubular cells are well-separated from the basement membrane and are mostly pyknotic or karyorrhectic. H & E stain; x640.

Fig 12 - Kidney, dry bird 3 hr PI at 29 C.
Eosinophilic debris in the form of epithelial cells is present in Bowman's space. Proximal convoluted tubules are pyknotic or karyorrhectic and cell dissociation is marked. H & E stain; x640.

Fig 13 - Kidney, dry bird, 24 hr PI at 29 C.
Glomerular nuclei are diffusely pyknotic. Nuclei of perilobular collecting ductular cells are mostly karyorrhectic. H & E stain; x640.



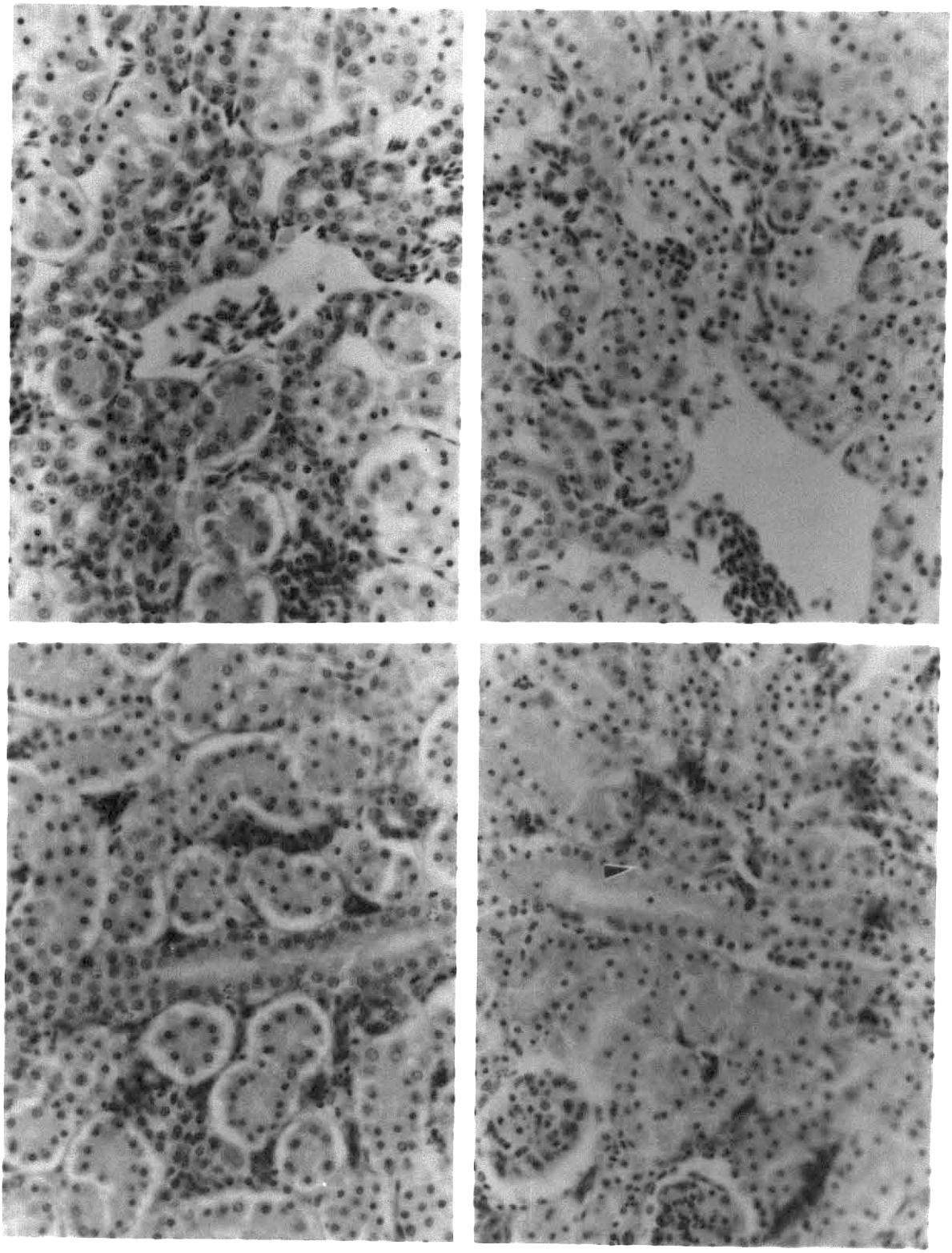
14	15
16	17

Fig 14 - Kidney, wet bird 1 hr PI at 29 C
Slight chromatin margination accompanied by nuclear shrinkage is present in distal convoluted tubular cells. H & E stain; x640.

Fig 15 - Kidney, dry bird, 3 hr PI at 29 C
Most convoluted tubules are undergoing pyknosis but not until 18 hr PI in wet birds held at 29 C. H & E stain; x640.

Fig 16 - Kidney, dry bird, 3 hr PI at 29 C
Some cells in a perilobular collecting duct are already undergoing karyorrhexis. H & E stain; x640.

Fig 17 - Kidney, dry bird, 18 hr PI at 29 C
Most cells in a perilobular collecting duct are karyorrhectic. A clear halo of cytoplasm is usually present around karyorrhectic nuclei (arrow). H & E stain; x640.



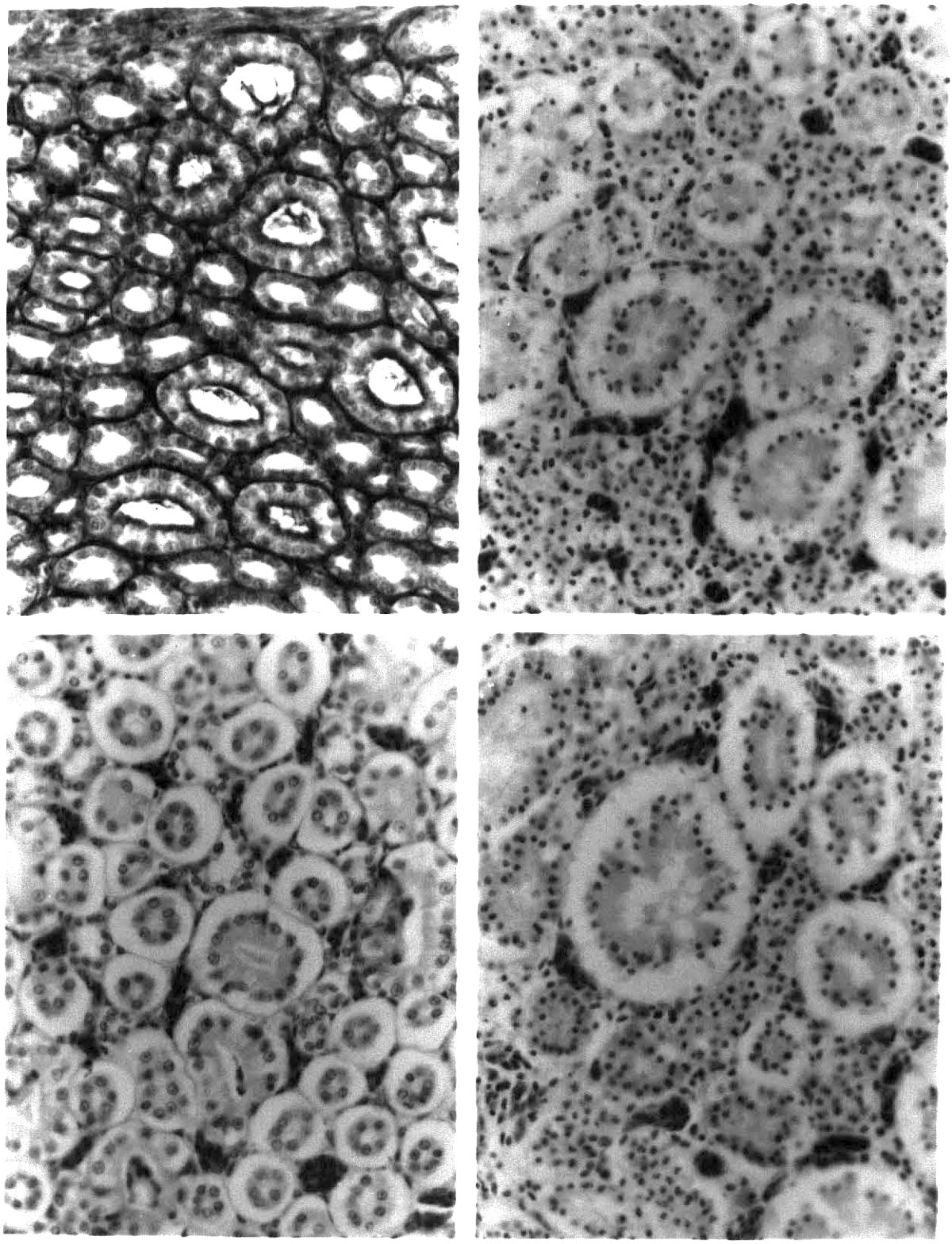
18	19
20	21

Fig 18 - Kidney, Control
Control kidneys have strongly PAS-positive acid mucopolysaccharide granules and basement membranes in medullary collecting ducts. Cells are closely apposed to the basement membrane. PAS stain; x640.

Fig 19 - Kidney, wet bird, 36 hr PI at 29 C
Medullary collecting ductular cells are markedly separated from the basement membrane. The nuclei are undergoing karyorrhexis and cytoplasmolysis is evident. Cells of the medullary loop are pyknotic or karyorrhectic. H & E stain; x640.

Fig 20 - Kidney, dry bird, 3 hr PI at 29 C
Marked separation of cells from the basement membrane is present in medullary collecting ducts and thin and thick segments of the medullary loop. Nuclear chromatin margination of medullary cells is evident. H & E stain; x640.

Fig 21 - Kidney, dry bird, 18 hr PI at 29 C
Medullary collecting ducts are mostly karyorrhectic and markedly separated from the basement membrane. Cell disruption is present. H & E stain; x640.



III. MICROSCOPIC POSTMORTEM CHANGES IN ADRENAL GLANDS OF THE DOMESTIC FOWL

INTRODUCTION

The rate by which organs undergo autolysis is one criterion for estimating the degree of postmortem decomposition in a carcass and to differentiate postmortem from antemortem changes.[1] The adrenal gland specifically the medulla, is an organ that undergoes rapid autolysis. In cattle, this is thought to be due to its intimate contact with the rumen.[2]

The adrenal gland of the domestic fowl consists of cortical and medullary cells but unlike mammalian glands, has no definite demarcation: loops of cortical cells are intermingled with islands of medullary cells.[3] The avian adrenal gland is conveniently divided into peripheral and central zones. Size and weight of the gland varies depending upon factors such as sex, age, breed, health and environment.[4]

Sequential studies on postmortem changes in adrenal glands are lacking. Only one study has been reported and this involved pigs.[5] Barber reported that adrenal medulla of pigs did not undergo postmortem change as rapidly as previous reports indicated.[5] In the ovine fetus, no difference in the rate of autolysis between the cortex and medulla was noted grossly.[6]

The study presented here was conducted to determine the type, rate and sequence of microscopic postmortem changes in adrenal glands of dry and wet intact chicken carcasses held at 18 or 29 C.

MATERIALS AND METHODS

General procedures were outlined in Part II.

Experimental Design and Necropsy -

Group I - 29 C - 16 birds - wet

II - 29 C - 16 birds - dry

III - 18 C - 22 birds - wet

IV - 18 C - 22 birds - dry

V - Control - 4 birds

Eighty male White Leghorn chickens were used. Immediately after death, four birds were necropsied and served as controls (0 hour). Two birds from each wet and dry groups were necropsied at different postmortem intervals (PI). At 29 C, PI was until 36 hours. In test birds held at 18 C, PI was until 96 hours.

Histopathological Procedures - The whole adrenal glands were immersed and fixed in 10% buffered neutral formalin (BNF) for a minimum of 3 days. A transverse cut was made in the middle of the glands and half of each was placed in a tissue holder for processing. The tissues were dehydrated in a series of ethanols, cleared in xylene, embedded in paraffin, and cut at 6 microns. They were mounted on glass slides, stained with Harris hematoxylin and eosin Y (H & E) and covered with coverslips.

Sections of the left and right adrenal glands were observed and evaluated. Nuclear and cytoplasmic changes in cortical and medullary cells were tabulated and scored accordingly: 0 for absent; 1 for mild; 2 for moderate; and 3 for marked.

RESULTS

Temperature

Temperatures were reported in Part II.

Microscopic changes

1. Control (0 hour) - Cortical cells were mostly columnar arranged radially in loops or cords without lumens although round, oval and pyramidal cells were also present. Cells laid on a basal membrane closely apposed to blood capillaries. The cytoplasm was eosinophilic, granular and vacuolated and the nucelus was round to oval almost centrally located, hyperchromatic with slight shrinkage (Fig 1). Few isolated pyknotic cells were present. Subcapsular cortical cells were more lightly-staining than the cells in the central zone.

Groups of polygonal medullary cells were present between cortical cords (Fig 1). Medullary cells had a very basophilic cytoplasm due to numerous small basophilic granules, some appeared as brownish granules. The nucleus was round and centrally-placed and had a diffuse fine chromatin pattern. Slight amount of space was present between cells. Few cells were pyknotic and had a very basophilic cytoplasm.

2. 29 C Experiment - Sequential postmortem changes are summarized in Tables 1 and 2 and illustrated in Fig 2 to 8. Comparison of important changes between wet and dry groups is presented in Table 5.

In wet birds, at 3 hr PI, pyknotic medullary cells had either intensely basophilic or vacuolated cytoplasm (Fig 2). Brownish granules were no longer observed. Cortical changes started to appear

by 9 hr PI when chromatin marginated and the nucleus decreased in size (Fig 3). At this stage, few cells had pyknotic nuclei. Cytoplasmic vacuoles appeared finer and cells became detached from the basal membrane. Moderate number of medullary cells had shrunk and some were pyknotic and dissociated from one another.

At 12 hr PI, moderate chromatin margination and detachment from the basal membrane were observed in cortical cells. Moderate number of medullary cells were pyknotic. Diffuse medullary pyknosis was observed at 18 hr PI (Fig 4) and by 24 hr PI, cellular changes were severe (Fig 5).

In dry birds, changes at 9 hr PI in wet birds were observed at 3 hr PI. Postmortem changes increased with increasing time. Marked diffuse pyknosis of medullary cells was noted at 18 hr PI and at about 24 hr PI, severe nuclear shrinkage was noted in cortical cells (Fig 5).

Bacterial invasion was observed from 24 hr PI in dry birds (Fig 7) and 36 hr PI in wet birds. By 36 hr PI, dry birds had all cells karyolytic and individualized (Fig 8); cortical cells could not be differentiated from medullary cells. Cytoplasm of cells was eosinophilic and finely vacuolated. Erythrocytes were pyknotic with cytoplasmolysis. Wet birds at 36 hr PI had shrunken and pyknotic cortical and medullary cells hardly distinguishable from each other (Fig 6). Moderate bacterial infiltration was present and the cytoplasm of red blood cells was unstained. Karyorrhexis of cortical and medullary cells was not observed at any test period.

3. 18 C Experiment - Sequential microscopic postmortem changes are summarized in Tables 3 and 4 and illustrated in Fig 9 to 12. Comparisons between the wet and dry groups in the 18 and 29 C experiments are presented in Table 5.

At 3 hr PI, few cortical cells were pyknotic or had marginated nuclear

chromatin and the nuclei of medullary cells began to shrink (Fig 9). Pyknotic cells with vacuolated cytoplasm were present in all sections. Cortical changes became noticeable by 6 hr PI in dry birds and by 9 hr PI in wet birds when nuclei decreased in size. Moderate to marked pyknosis of cortical cells were not noted in all birds. Diffuse pyknosis of medullary cells occurred at 48 hr PI in both dry (Fig 11) and wet birds.

Karyorrhexis and karyolysis were observed first in cortical cells by 72 hr PI and by 48 hr PI in medullary cells of dry birds. Slight karyorrhexis was detected only by 72 hr PI in medullary cells but not in cortical cells of wet birds.

At 36 hr PI, wet and dry birds had almost similar microscopic appearance (Fig 10). Bacterial invasion was noted only in one 96-hour dry bird (Fig 12). In this bird, nuclei of all cells were lysed. There was loss of tissue and cellular architecture. Red blood cells were pyknotic and the cytoplasm was unstained.

DISCUSSION

No significant tissue changes such as severe congestion or hemorrhage attributable to the euthanasia method were observed. Overexposure to CO_2 proved to be a good method of euthanasia aside from being inexpensive, non-flammable, practical, and minimally hazardous to man.[7]

In general, slight cellular changes were observed in adrenal sections initially and only during the later postmortem periods did changes become more noticeable and marked. Medullary cells underwent postmortem changes earlier than cortical cells. This was

contrary to findings in pigs where cortical cells underwent nuclear changes before medullary cells.[5]

Medullary pyknosis alone could not be used as an indicator of postmortem autolysis. Histologic appearance of adrenal glands has a physiological basis[8] and medullary cells with pyknotic nuclei and filled with cytoplasmic granules are considered inactive and present in normal glands. Other factors influencing histologic appearance include age, sex, breed, health and environment[4] but some of these were eliminated by using chickens possessing similar characteristics and raised under similar environmental conditions. Postmortem change in medullary cells has started if moderate number of pyknotic cells have vacuolated cytoplasm due to loss of granules.

Medullary cells underwent prominent sequential changes of progressive nuclear shrinkage with chromatin clumping, pyknosis with cytoplasmic loss of granules, cell shrinkage and cell dissociation. Karyolysis was the end stage of nuclear changes.

Karyorrhexis was not a prominent feature except at 48 and 72 hr PI in dry and wet birds, respectively, held at 18 C. Karyorrhexis was primary, nuclear fragmentation occurring prior to pyknosis.[9-10] A similar pattern of observation was noted in chicken livers of intact carcasses held at 20 or 37 C.[11]

Various stages of pyknosis are present in cortical cells of normal glands.[3] These stages, however, were not markedly observed. Cortical cells were hyperchromatic compared to medullary cells and they appeared more resistant to autolysis. At 18 C, slight pyknosis was observed only throughout the experimental period although chromatin margination, nuclear fading and shrinkage were slight to moderate. Cells remained

hyperchromatic except when bacterial invasion occurred. *Karyorrhexis* was not a feature as in medullary cells. Cytoplasm remained eosinophilic, granular and vacuolated but vacuoles appeared finer and less prominent during the later postmortem periods.

Slight difficulty was encountered in evaluating cytoplasmic changes due to unavoidable staining artifacts. More and Crowson[12] suggested special stain in kidneys to recognize minute variations in cytological structure. This study, however, was more concerned with practicality and applicability so conditions set up included regular H & E staining of adrenal gland sections of intact chicken carcasses.

It is well-known that a positive correlation exists between temperature and postmortem autolysis - the higher the temperature, the earlier the postmortem changes. Conditions of storage produced only minor qualitative changes but marked quantitative effect in guinea pig[12] and chicken[13] kidneys. In chicken adrenal glands, slight quantitative and qualitative differences were noted between wet and dry birds held at 18 C. Primary karyorrhexis was seen at 18 C but not at 29 C. Diffuse pyknosis of medullary cells occurred at 18 hr PI in all birds held at 29 C.

Bacterial invasion hastened lysis of nucleus and cytoplasm of cells[5,14] but appeared 12 hours later in adrenal glands than in kidneys of chickens held at 29 C (Part II). Bacteria were observed only in one bird held at 18 C at 96 hr PI. This may be related to differences in blood supply and anatomical structure of avian kidneys and adrenal glands.

One interesting observation was the extreme resistance of chicken erythrocytes to autolysis: changes were noted only when bacteria started

to appear in tissues. The cytoplasm became lysed followed by nuclear pyknosis.

The findings of the present study suggest that adrenal glands do not autolyse rapidly as published reports indicated although medullary cells underwent postmortem changes earlier than cortical cells. Under similar conditions, kidney cells underwent postmortem changes earlier than adrenal cells which is the reverse of that in guinea pigs.[14] Adrenal glands may still be useful for examination before 18 hr PI at 29 C and before 48 hr PI at 18 C.

SUMMARY

Eighty male White Leghorn chickens were euthanatized by CO_2 to determine the type, rate and sequence of microscopic postmortem changes in adrenal glands of dry and wet intact carcasses. They were held at 29 or 18 C with 50% relative humidity at different postmortem intervals (PI).

Sequence of microscopic postmortem changes were similar in all birds except at 18 C when karyorrhexis of cortical and medullary cells was observed. Cellular changes occurred earlier at 29 C than at 18 C and in dry but not birds wet with detergent solution prior to storage at different temperatures although slight quantitative and qualitative differences were noted between wet and dry birds.

Medullary cells underwent postmortem changes earlier than cortical cells. Nuclei of medullary cells decreased in size with chromatin clumping leading to pyknosis followed by cytoplasmic vacuolation, cellular shrinkage, and finally, karyolysis and cell dissociation.

Cortical cells had nuclear chromatin marginated and nuclei reduced

in size initially with some nuclear fading, followed by pyknosis and karyolysis. Karyorrhexis was not a prominent feature of cortical and medullary cells although it occasionally occurred prior to pyknosis. Cytoplasm of cortical cells remained eosinophilic, granular and vacuolated but vacuoles became finer during the later postmortem periods. Postmortem changes increased with time.

Pyknotic medullary cells with vacuolated cytoplasm were observed as early as 3 hr PI regardless of the temperature. Diffuse pyknosis of medullary cells were noted at 18 hr PI in birds at 29 C and at 48 hr PI in birds held at 18 C. Marked cortical pyknosis was noted only at 36 hr PI in wet birds held at 29 C when bacterial invasion started. Dry birds held until 36 hr PI at 29 C had diffuse cellular dissociation, karyolysis and cytoplasmic acidophilia and marked bacterial invasion. Erythrocytes were pyknotic with cytoplasmolysis. Bacterial invasion was noted only at 96 hr PI in one bird held at 18 C. It was concluded that adrenal glands may still be useful for histopathological examination before 18 hr PI at 29 C and before 48 hr PI at 18 C.

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TABLE 1 - Summary of microscopic postmortem changes in adrenal glands of wet intact chicken carcasses held at 29 C at varying postmortem intervals

Adrenal cells	Postmortem interval (hour)								
	0	1	3	6	9	12	18	24	36[a]
Cortical cells									
Nucleus-chrom. margination	0	0	0	0	1	2	3	3	3
-fading	0	0	0	0	0	0	0	1	0
-shrinkage	1	1	1	2	1	2	2	2	3
-pyknosis	0-1	0	1	1	0-1	1	1	1	3
-karyorrhexis	0	0	0	0	0	0	0	0	0
-karyolysis	0	0	0	0	0	0	0	0	0
-hyperchromasia	3	2-3	2	2	2	2	2	2	2
Cytoplasm-eosinophilia	3	3	3	3	3	3	3	3	3
-granularity	3	3	3	3	3	3	2-3	2	1
-vacuolation	3	3	3	3	2	2	2	1	2
Detachment from basal membrane	0	0	0	0	1	1-2	2	1-2	2
Cellular shrinkage	0	0	0	0	0	0-1	1	1	2-3
Cell dissociation	0	0	0	0	0	0-1	0-1	1-2	2
Medullary cells									
Nucleus-fading	0	0	0	0	0	0	0	0	0
-shrinkage	1	0	1	2	2	2-3	3	3	3
-pyknosis	0-1	0	1	2	1-2	2	3	3	3
-karyorrhexis	0	0	0	0	0	0	0	0	0
-karyolysis	0	0	0	0	0	0	0	0	1
Cytoplasm-granularity	3	3	2-3	2-3	2	2	1-2	2	1-2
-vacuolation	0	0	0-2	1-2	2	2-3	2-3	2-3	2
Cellular shrinkage	0	0	0	1	1	2	2	2	2
Cell dissociation	1	1	1-2	1-2	2	3	3	3	3

0 = Absent
1 = Mild
2 = Moderate
3 = Marked

[a]One sample had all cells pyknotic; cortical cells were hard to differentiate from medullary cells. RBC cytoplasm was unstained.

TABLE 2 - Summary of microscopic postmortem changes in adrenal glands of dry intact chicken carcasses held at 29 C at varying postmortem intervals

Adrenal cells	Postmortem interval (hour)								
	0	1	3	6	9	12	18	24	36
Cortical cells									
Nucleus-chrom. margination	0	0	1	2	3	2-3	3	2	All cells were individualized and karyolysed with eosinophilic cytoplasm. RBC were pyknotic with unstained cytoplasm. Bacterial invasion was marked.
-fading	0	0	0	1	0-1	1	1-2	1	
-shrinkage	1	1	2	2	2	2	2	3	
-pyknosis	0-1	1	1	2	1	0-1	1	2	
-karyorrhexis	0	0	0	0	0	0	0	0	
-karyolysis	0	0	0	0	0	0	0	0	
-hyperchromasia	3	2	2	2	2	2	1-2	2	
Cytoplasm-eosinophilia	3	3	3	3	3	2-3	2	3	
-granularity	3	3	3	3	3	2	2	2	
-vacuolation	3	3	2	1-2	1	1	0-1	1	
Detachment from basal membrane	0	0	2	1	1-2	2	2	2	
Cellular shrinkage	0	0	0-1	1	1	1-2	2	1	
Cell dissociation	0	0	0-1	1	1	1-2	2	0-1	
Medullary cells									
Nucleus-fading	0	0	0	0	0	0	0	0	
-shrinkage	1	1	2	2	2-3	2-3	3	3	
-pyknosis	0-1	1	1-2	1	2	2	3	3	
-karyorrhexis	0	0	0	0	0	0	0	0	
-karyolysis	0	0	0	0	0-1	0	0-1	1	
Cytoplasm-granularity	3	3	2	2	2	2	2	2	
-vacuolation	0	0	2	2-3	2	1	2	2	
Cellular shrinkage	0	0	1	1-2	2	2	3	2-3	
Cell dissociation	1	1	2	2-3	3	3	3	3	

0 = Absent
1 = Mild
2 = Moderate
3 = Marked

TABLE 3 - Summary of microscopic postmortem changes in adrenal glands of wet intact chicken carcasses held at 18 C at varying postmortem intervals

Adrenal cells	Postmortem interval (hour)											
	0	1	3	6	9	12	18	24	36	48	72	96
Cortical cells												
Nucleus-chrom. margination	0	0	0-1	1	1	2	2	3	3	3	3	3
-fading	0	0	0	0	0	0	1-2	1	1-2	1-2	2	1-2
-shrinkage	1	1-2	1	2	2	2	2	2	1-2	2	2	2
-pyknosis	0-1	1	0-1	2	1	1	1	1	0	1	1	1
-karyorrhexis	0	0	0	0	0	0	0	0	0	0	0	0
-karyolysis	0	0	0	0	0	0	0	0	0	0	0	0
-hyperchromasia	3	2-3	3	3	2-3	3	2	2	2	2-3	3	2
Cytoplasm-eosinophilia	3	3	3	3	3	3	3	3	3	3	2-3	2-3
-granularity	3	3	3	3	3	3	3	3	3	2	1	2
-vacuolation	3	2-3	3	3	3	2	2	1	1	1	1	2
Detachment from basal membrane	0	0-1	0	0	0	0	0	0-1	0	1	2	1-2
Cellular shrinkage	0	0-1	0	0	0	0	0	0	0	1	1	1
Cell dissociation	0	0	0	0-1	0	0	0	0-1	0	1	1-2	1
Medullary cells												
Nucleus-fading	0	0	0	0	0	0	0-1	1	0	0	0	0
-shrinkage	1	0-1	1	1	1	2	3	3	3	3	3	3
-pyknosis	0-1	0	1	1	1	2	2	2	2	3	3	2
-karyorrhexis	0	0	0	0	0	0	0	0	0	0	1	0-1
-karyolysis	0	0	0	0	0	0	0	0	0	0	0	0
Cytoplasm-granularity	3	3	3	3	2	2	2	2	2	2	2	2
-vacuolation	0	0	1	1	1	1	1	2-3	1	2-3	2-3	2
Cellular shrinkage	0	0	0-1	0	1	1	1	1	1-2	2	2	2
Cell dissociation	1	1	1	1	1-2	1	2	2	2-3	2	3	3

0 = Absent
1 = Mild
2 = Moderate
3 = Marked

TABLE 4 - Summary of microscopic postmortem changes in adrenal glands of dry intact chicken carcasses held at 18 C at varying postmortem intervals

Adrenal cells	Postmortem interval (hour)											
	0	1	3	6	9	12	18	24	36	48	72	96[a]
Cortical cells												
Nucleus-chrom. margination	0	0	1	1	1	2	2	2	2-3	3	3	3
-fading	0	0	1	1	1	1-2	2	2	2	2	2	2
-shrinkage	1	1-2	1	2	2	1-2	1	1	1-2	1	1-2	1-2
-pyknosis	0-1	1	1	1	1	0-1	1	0-1	1	1	1	1-2
-karyorrhexis	0	0	0	0	0	0	0	0	0	0	0-1	2
-karyolysis	0	0	0	0	0	0	0	0	0	0	0-1	1
-hyperchromasia	3	2	2	2	2	2	1-2	1-2	2	2	1	2
Cytoplasm-eosinophilia	3	3	2-3	3	3	3	3	3	2-3	2-3	2-3	2
-granularity	3	3	3	2-3	3	3	3	2-3	2	2	2	2
-vacuolation	3	2-3	2	1-2	2	2-3	2-3	1	1	1	1-2	2
Detachment from basal membrane	0	0	0-1	0	0-1	1	1	1-2	2	2	1-2	1
Cellular shrinkage	0	0	0-1	0	0-1	0-1	0	1	1	1	1	1
Cell dissociation	0	0	0	0	0	0	0-1	0	1	1	1	2
Medullary cells												
Nucleus-fading	0	0	0	1	1	0-1	1	0	0-1	0	0	1
-shrinkage	1	1	1	2	2	2	2-3	2-3	2-3	3	3	3
-pyknosis	0-1	1	1	2	1	2	2	2	2	3	3	2
-karyorrhexis	0	0	0	0	0	0	0	0	0	0-1	1	1
-karyolysis	0	0	0	0	0	0	0-1	1	1	1	1	2
Cytoplasm-granularity	3	3	2-3	2	2	2	2	2	2	2	2	1-2
-vacuolation	0	0	1-2	2	2	2	2	2	2	2	2-3	2
Cellular shrinkage	0	0-1	1	2	2	2	2	2	1-2	2	1-2	0-1
Cell dissociation	1	2	2	2	2	3	2	3	3	2-3	2	3

0 = Absent
1 = Mild
2 = Moderate
3 = Marked

[a]One sample had all cells individualized and karyolysed with eosinophilic cytoplasm. RBC were pyknotic with unstained cytoplasm. Bacterial invasion was marked.

TABLE 5 - Comparison of microscopic postmortem changes in adrenal glands of wet and dry intact chicken carcasses held at 29 or 18 C

Criteria	29 C		18 C	
	Wet(hr PI)	Dry(hr PI)	Wet(hr PI)	Dry(hr PI)
Cortical cells				
Chromatin margination evident	9	3	3	3
Nuclear shrinkage evident	6	3	9	6
Pyknosis marked	36	-	-	-
Karyorrhexis evident	-	-	-	72
Karyolysis evident	36[a]	36	-	72
Granularity decreased	24	12	48	36
Basal membrane detachment evident	9	3	48	9
Cellular shrinkage evident	12	6	48	24
Cell dissociation evident	12	6	48	36
Medullary cells				
Nuclear shrinkage evident	6	3	12	6
Pyknosis increased	6	9	12	6
Pyknosis marked	18	18	48	48
Karyorrhexis evident	-	-	72	48
Karyolysis evident	36	18-24	-	18
Vacuolation evident	3	3	3	3
Cellular shrinkage evident	6	3	9	3
Cell dissociation marked	12	9	72	24
Bacterial invasion evident	36	24	-	96[a]

[a]One sample only.

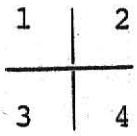
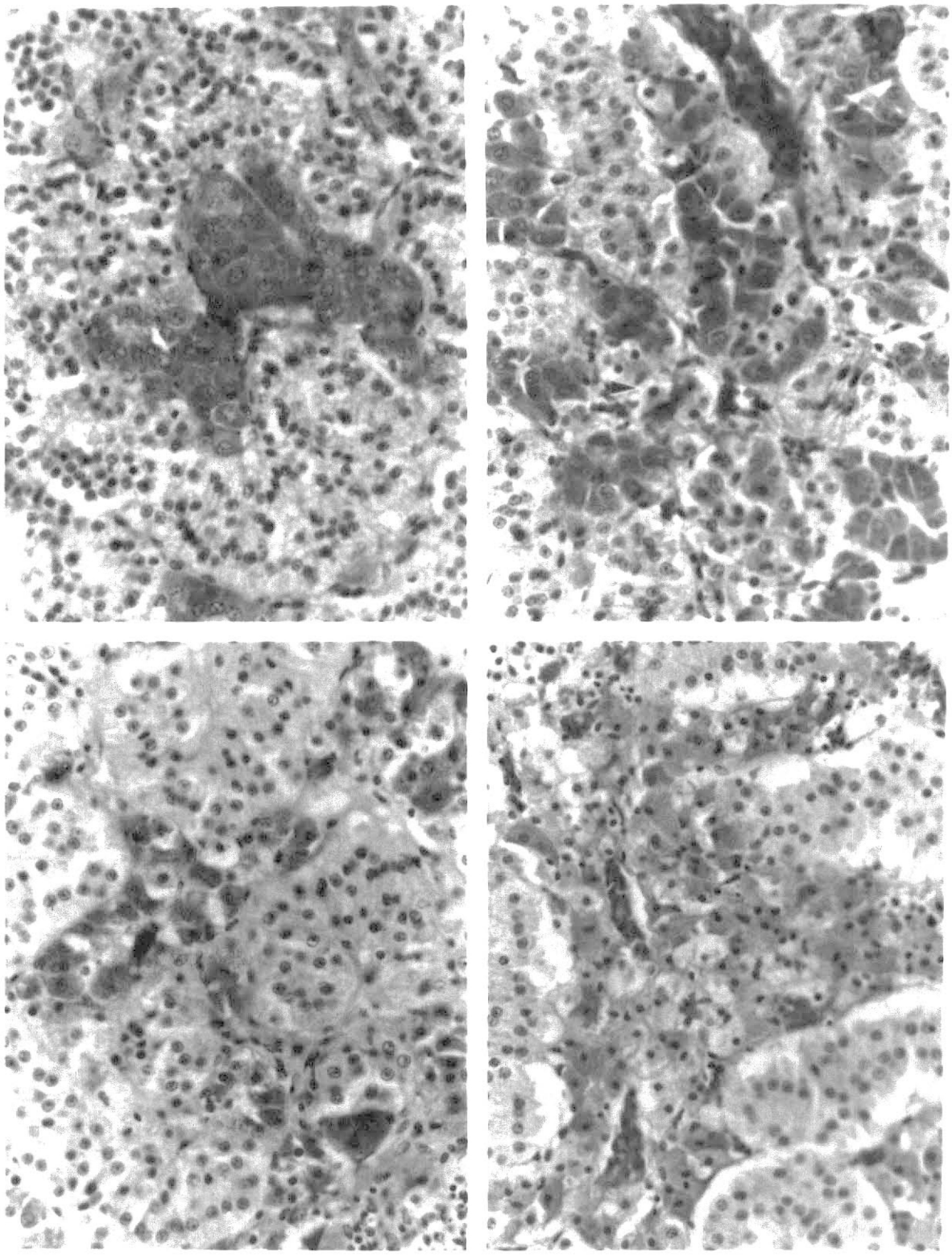


Fig 1 - Adrenal gland, Control
Groups of intensely basophilic medullary cells are surrounded by granular and vacuolated cortical cells arranged in loops without lumens. Nuclei of medullary cells are round with fine chromatin pattern. Cortical cell nuclei are smaller and hyperchromatic. H & E stain; x640.

Fig 2 - Adrenal gland, wet bird, 3 hr PI at 29 C
Medullary cells have separated from each other. Pyknotic medullary cells have either intensely basophilic or vacuolated cytoplasm (arrow). H & E stain; x640.

Fig 3 - Adrenal gland, wet bird, 9 hr PI at 29 C
Nuclear chromatin of cortical cells marginated and the nucleus decreased in size. H & E stain; x640.

Fig 4 - Adrenal gland, wet bird, 18 hr PI at 29 C
Most medullary cells are pyknotic with vacuolated cytoplasm. Cortical cells are moderately detached from the basal membrane and there is nuclear chromatin margination. H & E stain; x640.



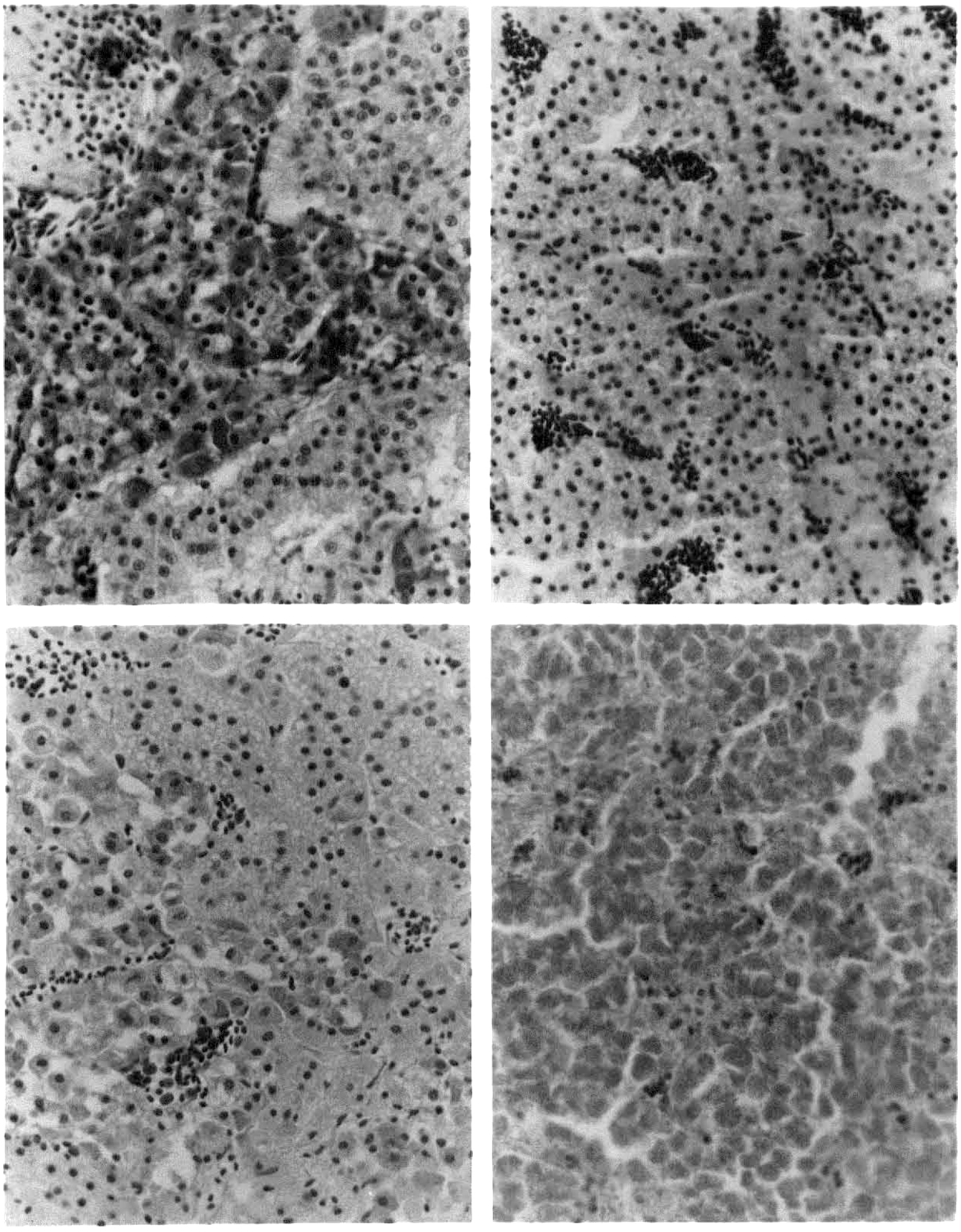
5	6
7	8

Fig 5 - Adrenal gland, wet bird, 24 hr PI at 29 C
Medullary cells are diffusely pyknotic with vacuolated cytoplasm. Note the close resemblance between 18 and 24 hr PI. H & E stain; x640.

Fig 6 - Adrenal gland, wet bird, 36 hr PI at 29 C
Cortical and medullary cells are shrunken, pyknotic and hard to differentiate from one another. Moderate bacterial invasion is present (arrow) with cytoplasmolysis of erythrocytes. H & E stain; x640.

Fig 7 - Adrenal gland, dry bird, 24 hr PI at 29 C
Medullary cells have shrunken and are separated from one another. Some cells are pyknotic with vacuolated cytoplasm. Cortical cells decreased in size with nuclear chromatin margination and some pyknosis. Bacterial invasion starts. H & E stain; x640.

Fig 8 - Adrenal gland, dry bird, 36 hr PI at 29 C
All cells are individualized and karyolysed with eosinophilic cytoplasm. Erythrocytes are pyknotic with unstained cytoplasm. Bacterial invasion is marked. H & E stain; x640.



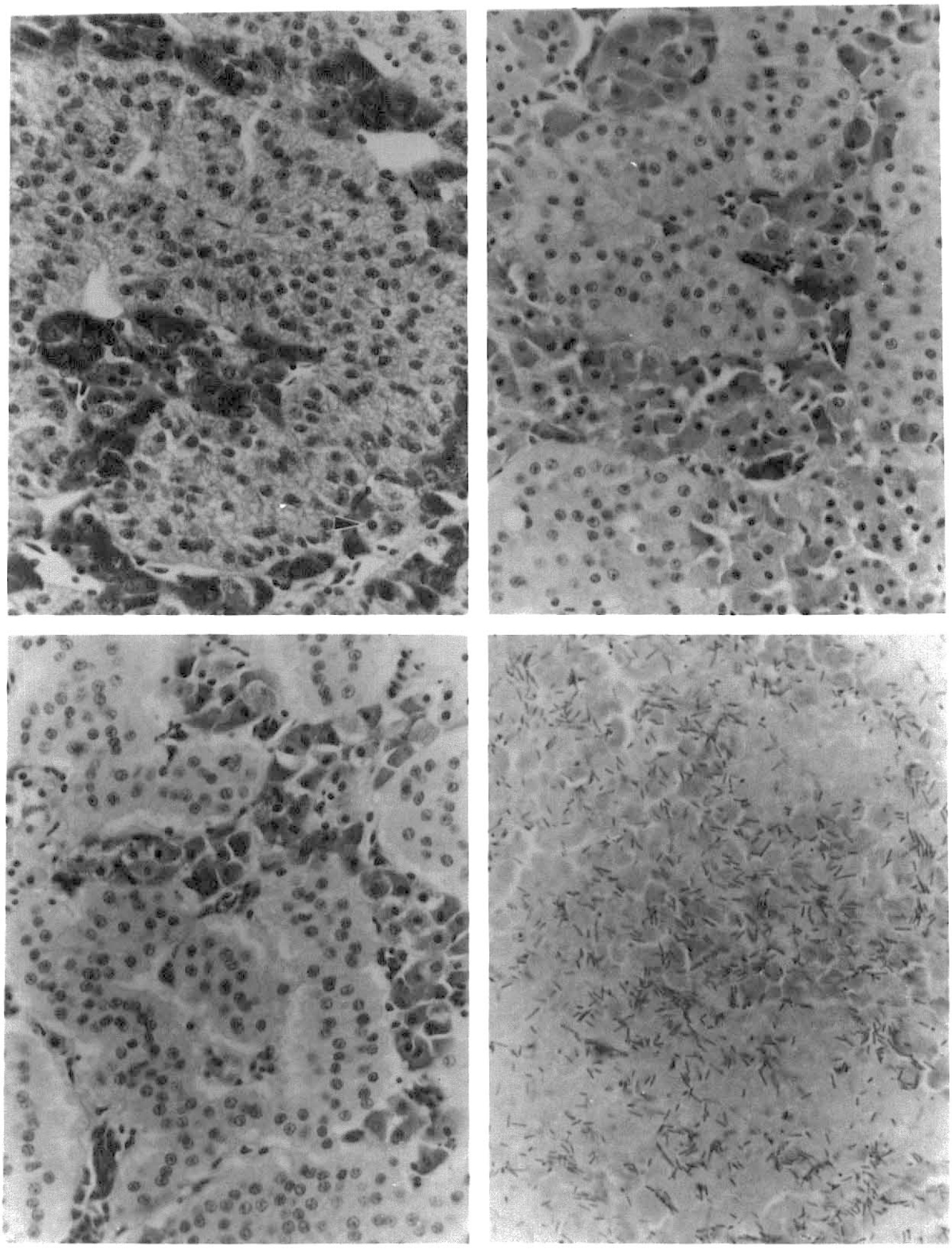
9	10
11	12

Fig 9 - Adrenal gland, wet bird, 3 hr PI at 18 C
Slight changes are noted. Cortical cells have hyperchromatic nuclei with slight chromatin margination. Some medullary cells are pyknotic (arrow) with slightly vacuolated cytoplasm. H & E stain; x 640.

Fig 10 - Adrenal gland, wet bird, 36 hr PI at 18 C
Medullary cells are less intensely stained, moderately separated from each other with shrunken or pyknotic nuclei. Most cortical cells have fading nuclei or marginated chromatin. H & E stain; x640.

Fig 11 - Adrenal gland, dry bird, 48 hr PI at 18 C
Diffusely pyknotic medullary cells are distinctly separated from each other with vacuolated cytoplasm. H & E stain; x640.

Fig 12 - Adrenal gland, dry bird, 96 hr PI at 18 C
Nuclei of all cells are lysed. Marked bacterial invasion is present. H & E stain; x640.



IV. APPENDIX

TABLE 1 - Rectal and thoracic temperatures of wet and dry intact chicken carcasses held at 29 C at varying postmortem intervals

Postmortem Interval (hour)	Bird #	Wet group (C)		Dry group (C)	
		Thoracic	Rectal	Thoracic	Rectal
1	1	30.6	31.7	37.2	36.1
	2	31.7	31.7	37.2	37.2
3	1	27.2	26.7	33.9	33.3
	2	26.1	25.3	32.2	31.7
6	1	25.6	25.0	30.6	30.6
	2	25.0	23.9	30.0	29.4
9	1	24.4	24.2	28.9	28.9
	2	24.4	23.9	28.9	28.9
12	1	24.7	24.2	29.4	29.7
	2	24.4	24.4	28.9	28.9
18	1	27.8	27.8	28.6	28.9
	2	27.5	27.2	28.3	28.3
24	1	28.3	28.3	28.3	28.3
	2	28.9	28.9	28.3	28.6
36	1	28.6	28.9	29.2	29.4
	2	28.6	28.6	28.6	28.9
48	1	28.9	28.9	29.4	28.9
	2	29.2	29.4	29.4	29.2

TABLE 2 - Rectal and thoracic temperatures of wet and dry intact chicken carcasses held at 18 C at varying postmortem intervals

Postmortem Interval (hour)	Bird #	Wet group (C)		Dry group (C)	
		Thoracic	Rectal	Thoracic	Rectal
1	1	26.7	26.7	34.4	35.6
	2	25.3	26.1	34.2	34.4
3	1	18.9	18.3	27.8	28.6
	2	19.4	19.4	28.9	28.9
6	1	16.7	16.7	21.1	21.4
	2	16.4	16.7	23.1	23.3
9	1	16.1	16.7	19.4	19.4
	2	16.1	16.1	19.4	20.0
12	1	17.5	17.0	20.6	20.0
	2	17.0	17.5	20.0	20.0
18	1	17.8	17.8	19.4	19.4
	2	17.8	17.8	19.4	20.0
24	1	17.2	17.2	18.9	18.9
	2	17.8	17.8	18.9	19.0
36	1	17.0	17.0	18.5	18.5
	2	16.7	17.0	18.0	18.0
48	1	18.3	18.1	18.6	18.6
	2	18.3	18.1	18.9	18.6
72	1	18.3	17.8	19.2	18.5
	2	18.3	18.6	18.3	18.3
96	1	18.6	18.6	19.2	19.2
	2	18.5	18.6	18.6	18.9

TABLE 3 - Analysis of variance of temperatures of wet and dry intact chicken carcasses held at 29 C at varying postmortem intervals

Source	DF	Sum of squares	Mean square	F value	PR>F	R-square	C.V.
Model	1	172.9800	172.9800	26.52	0.0001	0.2747	8.8694
Error	70	456.6594	6.5237		STD DEV		Temp Mean
Corrected total	71	629.6394			2.5542		28.7972
Source	DF	ANOVA SS	F value		PR>F		
Group	1	172.9800	26.52		0.0001		

TABLE 4 - Analysis of variance of temperatures of wet and dry intact chicken carcasses held at 18 C at varying postmortem intervals

Source	DF	Sum of squares	Mean square	F value	PR>F	R-square	C.V.
Model	1	229.1364	229.1364	14.11	0.0003	0.14098	20.1160
Error	86	1396.1268	16.2340		STD DEV		Temp Mean
Corrected total	87	1625.2632			4.0291		20.0295
Source	DF	ANOVA SS	F value		PR>F		
Group	1	229.1364	14.11		.0003		

MICROSCOPIC POSTMORTEM CHANGES IN KIDNEYS AND ADRENAL GLANDS OF THE DOMESTIC FOWL

by

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Microscopic postmortem changes in kidneys and adrenal glands of dry and wet intact male White Leghorn chickens euthanatized by CO_2 gas and held at 29 or 18 C, 50% relative humidity at different postmortem intervals (PI) were determined, compared and evaluated.

Cellular changes occurred earlier in kidneys than in adrenal glands, in birds held at 29 than at 18 C and in dry but not birds wet with detergent solution prior to storage at different temperatures. Decrease in body temperature of wet over dry birds was significant ($P < 0.05$). Wetting carcasses delayed postmortem changes in kidneys but slight quantitative and qualitative differences were noted in adrenal glands between wet and dry birds.

In the kidney, the proximal convoluted tubule (PCT) underwent the earliest postmortem changes followed by the distal convoluted tubule (DCT), collecting tubule (CT), medullary loop (ML), medullary collecting duct (MCD) and glomerulus. Adrenal medullary cells underwent postmortem changes earlier than cortical cells.

The PCT, DCT and thin and thick segments of the ML underwent a sequential nuclear change of chromatin margination, progressive shrinkage, pyknosis, karyorrhexis and karyolysis. Karyorrhexis was the predominant feature of collecting tubules and ducts.

As early as one hour after death, some PCT cells of all kidney sections were already pyknotic emphasizing immediate tissue fixation was necessary for critical evaluation. By 9 and 18 hr PI, PCT of dry and wet birds, respectively, held at 29 C had pyknotic and karyorrhectic nuclei with slight karyolysis and moderate to marked cytoplasmolysis that extended until 36 hr PI. At this time, DCT were hardly distinguishable due to loss of basophilia. Karyorrhectic nuclei were

already evident in collecting tubules and ducts. At 48 hr PI, massive bacterial invasion was present and all tubular cells were non-nucleated and individualized with homogeneous, acidophilic cytoplasm. Basement membranes no longer stained with PAS. Erythrocytes were pyknotic with unstained cytopplasm. Pyknotic glomeruli were first observed at 9 hr PI in dry birds and 12 hr PI in wet birds.

Histologic appearance of dry and wet birds at 9 and 18 hr PI at 29 C was similar to 12 hr PI in dry birds and 24 hr PI in wet birds held at 18 C with minor differences in some tubular changes. At 18 C, pyknotic glomeruli appeared by 6 hr PI in dry birds and 24 hr PI in wet birds. Most changes increased with increasing PI. Bacterial invasion was noted at 72 hr PI in dry birds and at 96 hr PI in wet birds.

Basement membranes, brush borders and acid mucopolysaccharide granules were resistant to autolysis and persisted until 36 hr PI at 29 C and 96 hr PI at 18 C although they became less defined and weakly stained by PAS during the later periods after death.

Adrenal medullary cells underwent sequential changes of progressive nuclear shrinkage with chromatin clumping leading to pyknosis with cytoplasmic vacuolation, cellular shrinkage, and finally, karyolysis and cell dissociation. Cortical cells had nuclear chromatin marginated and the nuclei reduced in size initially with some nuclear fading followed by pyknosis and karyolysis. Karyorrhexis was not a prominent feature of cortical and medullary cells although it occasionally occurred prior to pyknosis. Cytoplasm of cortical cells remained eosinophilic, granular and vacuolated. Postmortem changes increased with time.

Pyknotic medullary cells with vacuolated cytoplasm were observed as early as 3 hr PI regardless of temperature. Diffuse pyknosis of medullary cells were noted at 18 hr PI in birds held at 29 C and at 48 hr PI in birds held at 18 C. Marked cortical pyknosis was noted only at 36 hr PI in wet birds held at 29 C when bacterial invasion started. Dry birds held until 36 hr PI at 29 C had diffuse cellular dissociation, karyolysis and cytoplasmic acidophilia with marked bacterial invasion. Erythrocytes were pyknotic with cytoplasmolysis. Bacterial invasion was noted only at 96 hr PI in one bird held at 18 C. Adrenal glands may still be useful for histopathological examination before 18 hr PI at 29 C and before 48 hr at 18 C.