

SEQUENTIAL HISTOLOGIC POSTMORTEM CHANGES IN
PORCINE KIDNEYS AND ADRENAL GLANDS

by

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INTRODUCTION

Autolysis is digestion of tissues by their own cellular hydrolytic enzymes¹. The rate of autolysis varies with environmental temperature, body size, nutritional status, pelage, and existing disease conditions. Autolytic changes proceed rapidly at 37 C, corresponding to the temperature optimum for mammalian enzymes². Therefore, the rate of cellular degradation is increased by warm weather, large carcass size, excessive adipose tissue, thick fur or wool, and antemortem hyperthermia caused by pyrexia, violent exercise, or heat exhaustion. Conversely, cooling of a carcass slows the autolytic process.

Body tissues are affected by autolysis at variable rates depending on sensitivity of their cells to anoxia, and cellular concentration of proteolytic enzymes. Renal proximal convoluted tubules^{3,4} and adrenal medulla^{5,6} are two tissues reported to be rapidly altered. It is important for diagnostic histopathologists to differentiate significant antemortem lesions from postmortem autolysis and processing artifacts.

Sequential postmortem changes in kidneys have been reported in cats⁷, dogs⁸, monkeys⁹, rats^{2,3,10-13}, mice¹⁴, and chickens⁴. Similar studies have not been reported in pigs. Autolytic changes in the central nervous system¹⁵⁻¹⁷, liver¹⁸⁻²⁰, gallbladder²⁰, and lung²¹ have been studied, while adrenal glands have not been subjected to specific controlled studies.

The objectives of this light microscopic study were to determine and pictorially depict sequential autolytic changes in kidneys and adrenal glands from porcine carcasses held at 4 C and at 24 C.

LITERATURE REVIEW

Cell death occurs when cells lose all ability to perform cellular functions. Within minutes of death, lysosomes liberate hydrolytic enzymes into the cytoplasm. These enzymes are activated by decreasing pH arising from decreased oxidative metabolism. Intracellular organelles and membranes are rapidly degraded in this process of selfdigestion. If this process occurs in a living animal, it is called necrosis. If it happens after somatic death, it is autolysis²².

In diagnostic histopathology, autolysis must be differentiated from coagulative necrosis. In autolysis and coagulative necrosis, cellular outlines and tissue architecture are maintained, but nuclei become pyknotic, karyorrhectic, or are lost by karyolysis. Necrotic changes are focally distributed amid viable tissue, while autolysis is generalized cell death. A zone of hyperemia and inflammation usually separates necrotic foci from viable tissue. No inflammatory or hyperemic response is elicited by autolysis⁶.

The renal cortex is composed of glomeruli, proximal convoluted tubules(PCT), distal convoluted tubules (DCT), and collecting tubules (CT) within medullary rays. Glomeruli are composed of tufts of capillaries in contact with mesangial matrix and glomerular epithelium. Tufts are enclosed in Bowman's capsules, composed of visceral and parietal epithelia

separated by a potential space, Bowman's space. The urinary pole of glomeruli leads into PCT that are tortuous tubules lined by pyramidal cells with basally located, round, vesicular nuclei with prominent nucleoli. The luminal surface of these epithelial cells is lined by a mucopolysaccharide brush border of microvilli. The bulk of the renal cortex is composed of these tubules. Distal convoluted tubules are lined by low cuboidal epithelial cells, resulting in larger luminal diameters than in PCT. These cells have centrally located nuclei and no brush borders on the luminal surface. Within the medullary rays, the CT are lined by low cuboidal epithelium with scalloped-edged luminal borders, pale cytoplasm, and dark, spherical, centrally located nuclei²³.

Adrenal glands are composed of an outer cortex and inner medulla. The cortex has three zones: zona arcuata (or zona glomerulosa), zona fasciculata and zona reticularis. The zona arcuata, located directly below the capsule, is composed of packets of columnar cells with uniformly staining cytoplasm and small, dark nuclei. The thickest zone, the zona fasciculata, consists of radially arranged cords of foamy, cuboidal cells lined along sinusoids. Nuclei are vesicular and may be centrally or eccentrically located within the cells. The foamy cytoplasm is due to dissolution of lipid droplets during routine processing. The zona reticularis is an irregular network of anastomosing cords of polyhedral cells with dark staining nuclei. Cells of the medulla are arranged in irregular bundles and clusters separated

by sinusoidal capillaries. Individual cell outlines are indistinct²³.

Kidneys and adrenal glands have a rich blood supply. Tubules in the renal cortex and the adrenal medulla are susceptible to rapid autolytic changes. Within a few hours, tubular nuclei disappear and brush borders of the proximal convoluted tubules are lost⁵. Lytic areas are found in the adrenal cortex within a few hours of death, and the medulla and inner cortex rapidly become liquefied^{5,6}.

In sequential light microscopic postmortem studies, feline autolytic kidneys had increased glomerular diameters and decreased size of nuclei compared to 0 hour controls. An increased amount of refluxed debris from the PCT into Bowman's space was found with increasing time, up to 24 hours. Canine autolytic kidneys similarly had swollen glomeruli due to cytoplasmic swelling and vacuolation, progressive nuclear chromatin clumping, and nuclear pyknosis and karyorrhexis. An increasing amount of refluxed debris in Bowman's space was seen with increasing time. In the rat, refluxed debris in Bowman's space was prominent after 4 hours. Lumens of PCT were closed in 0 hour controls due to cellular swelling. Debris was observed in the PCT lumens throughout the 24 hour experimental period, and increased with time. Brush borders were present after 24 hours. Cells of DCT retracted off basement membranes in 1 hour, and nuclei were pyknotic by 4 hours. Lumens of CT were open and lined by scalloped-edged epithelial cells. Chromatin clumping was prominent at 8 hours, and by 12 hours most nuclei were

fragmented. In the chicken, PCT underwent the most rapid autolytic changes and the brush borders were entirely lost within 9 hours. Cells retracted off basement membranes and individualized in the PCT earlier than in DCT or CT.

Other sequential postmortem autolysis studies on kidneys have dealt with changes seen by electron microscopy^{3,10,11} and histochemical alterations^{12,13}.

Due to the lack of literature pertaining to the adrenal gland, the only information found was rapid postmortem liquefaction of the medulla.^{5,6}

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I. SEQUENTIAL HISTOLOGIC CHANGES IN PORCINE KIDNEYS
AT 4 C AND 24 C.

INTRODUCTION

It is important for the histopathologist to differentiate postmortem autolysis from antemortem coagulative necrosis. Histologically, cells react similarly to both forms of self-digestion.¹

The renal cortex is composed of glomeruli, proximal convoluted tubules (PCT), distal convoluted tubules (DCT), and collecting tubules (CT) in the medullary rays. Glomeruli are composed of capillary tufts covered by visceral and parietal epithelium of Bowman's capsule. Between these two layers is Bowman's space, a potential space. Lumens of PCT are lined by brush borders. In general, they are larger in diameter than DCT, have smaller lumens, and tend to have cytoplasmic components that stain more intensely with routine hematoxylin and eosin. To identify these tubules, the periodic acid Schiff (PAS) reaction² can be used, providing autolysis has not extensively damaged the brush borders. The mucopolysaccharide brush borders are PAS+. Distal convoluted tubules lack brush borders, have low cuboidal epithelium and wider lumens. Scalloped-edged epithelium³ lines CT. Nuclei of this epithelium are stained darkly.

Sequential postmortem changes of kidneys have been studied in cats⁴, dogs⁵, rats⁶⁻¹¹, mice¹², monkeys¹³, and chickens¹⁴. Glomerular tufts swell and decrease Bowman's

space with increased time, in dogs and cats. Debris re-gurgitated into Bowman's space from PCT also increases in quantity with time, in dogs, cats, and rats. In the chicken, PCT underwent severe autolytic change faster than other cortical structures, while DCT in the rat were the first to undergo severe change. Brush borders in the chicken disappeared within 9 hours, but were maintained in rat PCT for up to 24 hours. Cellular swelling, luminal obliteration of the PCT, and noncellular debris within the lumens were more frequently seen with increasing time in the rat experiment.

This study was undertaken to determine the sequential histologic changes occurring in porcine kidneys at 4 C and 24 C.

MATERIALS AND METHODS

Experimental Design: Twenty-eight Yorkshire pigs of both sexes, 6-8 weeks of age and weighing 15 to 18 kg (average 17 kg), were used in this study. The 28 clinically normal pigs were randomly divided into 16 pairs, 12 test and 4 control. Twenty-six pigs were euthanatized by attaching an electrode to the left ear and tail and passing 110 volts for 10 seconds. Two test pigs were necropsied at each of the following time intervals postmortem at 4 C and 24 C, respectively: 3, 6, 12, 24, 48, 96 hours. Two control pigs were necropsied immediately following electrocution (0 hour controls). The other two control pigs were anesthetized with Ketamine^a and perfused intracardially with 10% buffered neutral formalin (BNF) and immediately necropsied (perfused).

Necropsy Examination: Each pig was subjected to a standardized necropsy and sections no thicker than 1 cm in width, of kidneys were taken and fixed in 10% BNF. Adrenal glands were bisected and fixed in 10% BNF. Kidney samples included longitudinal, midline and sagittal sections of the cortex, medulla, and pelvis. The sections were fixed in 10% BNF for at least a week before being trimmed and processed for histopathologic examination. Renal tissues were dehydrated through graded ethanols, cleared in xylene, and infiltrated with embedding paraffin in a routine automated processor cycle^b, cut at 6, mounted on glass slides, stained by hematoxylin

and eosin (H & E) by an automated slide stainer^C, and covered with glass coverslips. Periodic acid Schiff (PAS) counterstained with hematoxylin² were done by hand.

Histopathological Evaluation: Four structures of the renal cortex, including the glomeruli, proximal and distal convoluted tubules, and collecting tubules, were used to evaluate the extent of postmortem change in each kidney. Sections from perfused, 0 hour controls, 4 C and 24 C kidneys were scored for changes on a 1-plus to 3-plus system based on visual comparisons. Mild changes were scored 1-plus, moderate changes were 2-plus, and marked changes were 3-plus.

Glomerular changes were based on: the number of swollen glomerular tufts obliterating Bowman's spaces; quantity of debris regurgitated into Bowman's spaces from the proximal convoluted tubules; nuclear pyknosis; nuclear karyolysis.

Autolysis of the proximal convoluted tubules was evaluated using nine variables: tubular luminal obliteration by cellular swelling; the number of tubules containing luminal debris; disruption of the brush borders; segmental loss of the brush borders; cellular retraction off basement membranes; nuclear pyknosis; nuclear karyolysis; loss of tubular architecture; and bacterial invasion.

The severity of destruction of the distal convoluted tubules was based on five criteria: cellular retraction off the basement membranes; nuclear pyknosis; individualization of retracted cells within lumens; nuclear karyolysis; and loss of tubular architecture.

Collecting tubule alterations were evaluated using the 5 criteria used for the distal convoluted tubules.

Photomicrographs: Photomicrographs were taken with an automatic 35mm camera^d mounted on a Leitz Orthoplan microscope^e. Black-and-white film^f was used with a green number 58 Wratten filter^g. The camera magnification factor used was 3.2 x.

FOOTNOTES

^aKetaset (100 mg/ml), Bristol Laboratories, Syracuse, NY.

^bAutotechnicon, Technicon Corporation, Chauncey, NY.

^cHistotek, Ames Company, Div Miles Laboratories, Inc.,
Elkhart, Ind.

^dOrthomat, Leitz, Inc. Rockleigh, NJ.

^eDialux, Leitz, Inc. Rockleigh, NJ.

^fPanatomic-X, Eastman Kodak Company, Rochester, NY.

^gWratten, Eastman Kodak Company, Rochester, NY.

RESULTS

The sequential histologic changes are summarized in Table I and illustrated in Fig 1-14.

Perfused controls

Glomerular tufts were not swollen, so Bowman's spaces were visible in most glomeruli. Refluxed debris from the proximal convoluted tubules was minimal. No pyknotic nuclei or karyolysis was seen within the tufts (Fig 1 and 15). Proximal convoluted tubule epithelium showed little swelling and most of the lumens were patent. Brush borders were present and strongly periodic acid Schiff positive (PAS+) (Fig 5 and 21). Cells of the distal convoluted tubules (DCT) were in contact with the basement membrane and were not affected by nuclear pyknosis. The patent lumens were free of debris (Fig 9 and 26). Cells of the collecting tubules were in contact with the basement membrane, had no pyknotic nuclei, and the patent lumens were free of debris (Fig 13 and 29).

0 Hour controls

A small number of glomerular tufts were swollen, resulting in decreased size of Bowman's spaces. A few of the spaces had minute amounts of regurgitated noncellular debris present within them. No nuclear pyknosis or karyolysis was present

within the tufts (Fig 2). A moderate number of FCF had obliterated lumens due to cellular swelling. Brush borders were sharp and stained brightly with PAS. Marked nuclear pyknosis was seen in DCT cells, but the cells were still in contact with the basement membrane and no individualization was seen (Fig 10). Collecting tubules resembled those seen in perfused samples.

4 C EXPERIMENT

Glomeruli at 4 C.

Bowman's space of some glomeruli was reduced or obliterated in all specimens. This was most pronounced at 6 and 12 hours postmortem (Fig 3). By 24 hours, the glomerular tufts had shrunk and a prominent space was present. Debris was present in some Bowman's spaces of all tissue sections (Fig 4). No consistent pattern for the number of glomeruli affected developed throughout the experimental period. Pyknosis of glomerular nuclei was present from 24 hours (Fig 4).

Proximal convoluted tubules at 4 C.

All sections had some degree of cellular swelling and luminal obliteration (Fig 6-8). The number of obliterated lumens was minimal prior to 6 hours, but then became marked. Tubular lumens remained closed throughout the 96 hour test period. Debris accumulated in open lumens, and was present throughout the experimental period. Mild disruption of the borders was present at 48 and 96 hours (Fig 7). Cellular

retraction off the basement membranes was mild, and only observed from 24 hours. The number of pyknotic cells was minimal, and was only present from 48 hours (Fig 8).

Distal convoluted tubules at 4 C.

Epithelial cells of the distal convoluted tubules started retracting off the basement membranes 3 hours following death (Fig 11). Most tubules were affected in all specimens. The number of pyknotic nuclei was marked in all specimens (Fig 11 and 12). Individualization of retracted cells occurred in nearly all DCT within 3 hours (Fig 11).

Collecting tubules at 4 C.

Within 3 hours of death, collecting tubules were slightly retracted off basement membranes. The number of affected tubules did not become extensive until 96 hours after death (Fig 14). Pyknosis was first observed at 24 hours. The quantity of affected cells remained small throughout the rest of the experimental period. Individualization of cells was observed only at 48 and 96 hours (Fig 14).

24 C EXPERIMENT

These sequential histologic changes are summarized in Table II, and illustrated in Fig 15-32.

Glomeruli at 24 C.

Glomeruli had narrowed or obliterated Bowman's spaces. Within 3 hours, this change had become markedly pronounced (Fig 16). The number of affected Bowman's spaces then decreased

through 24 hours. Glomerular spaces from cadavers held for 48 and 96 hours were not affected by this change. Debris was seen in Bowman's space from sections taken at all time intervals (Fig 17-20). The number of affected glomeruli was marked by 12 hours. The severity of this change did not diminish with time. A moderate number of pyknotic cells was first seen at 12 hours, and a marked quantity was affected in both 48 and 96 hour sections (Fig 18-20). Karyolysis within the glomerular tufts was seen 48 and 96 hours after death (Fig 20).

Proximal convoluted tubules at 24 C.

A moderate number of lumens were obliterated due to cellular swelling, in the 3 hour specimens. By 24 hours, the quantity of swollen cells had declined (Fig 22 and 23). Lumens were no longer obliterated by 96 hours. Debris in lumens was first observed 3 hours after death. The number of tubules affected became pronounced by 12 hours, and remained extensive through 96 hours (Fig 23-25). Disruption of the brush borders was seen occasionally in 12 and 24 hour sections and became markedly severe by 96 hours (Fig 23-25). Segmental loss of the borders was noted in a few tubules at 48 hours. An extensive number of tubules was affected by 96 hours after death, but the brush borders were not completely lost (Fig 25). Cellular retraction off the basement membranes was seen in a few tubules from 12 hours (Fig 23 and 24). No retraction was

observed in the 96 hour specimens. The number of cells affected by pyknosis was small at 12 hours, increased by 24 hours, and was extensive in 48 and 96 hour sections (Fig 24 and 25). Karyolysis and loss of tubular architecture were noticed occasionally in 48 hour samples, but changes were observed frequently in 96 hour sections (Fig 25). Large bacterial rods in moderate amounts were observed only in 96 hour tissues (Fig 25).

Distal convoluted tubules at 24 C.

These epithelial cells were extensively retracted off the basement membranes within 3 hours (Fig 27). Marked numbers of pyknotic cells were seen in all kidneys (Fig 27 and 28). Individualization of cells was moderate within 3 hours; became marked by 6 hours (Fig 27 and 28). These three changes in the DCT cells remained pronounced throughout the remaining postmortem intervals. Karyolysis and loss of tubular architecture were first noted at 48 hours. These changes progressed from mild to marked numbers of affected tubules by 96 hours.

Collecting tubules at 24 C.

Moderate numbers of CT epithelial cells started retracting off basement membranes by 3 hours. Within 12 hours, this change had affected an extensive number of tubules. Pyknosis was noted occasionally at 3 and 6 hours. By 12 hours, a moderate number of CT cells were pyknotic. Marked numbers were involved by 24 hours (Fig 30). Individualization of sloughed cells was seen within a few tubules at 3 and 6 hours.

By 12 and 24 hours, moderate numbers were involved (Fig 30). Most cells were individualized in 48 and 96 hour sections (Fig 31 and 32). Karyolysis and loss of tubular architecture were seen occasionally at 48 hours. By 96 hours, most tubules were affected by these changes (Fig 32).

DISCUSSION

Glomeruli initially swelled resulting in narrowed or obliterated Bowman's spaces. This was due to fluid uptake by the cells. This was seen in the 0 hour controls and was attributed to the time interval between immersion and actual penetration and fixation of the tissue with the buffered formalin fixative. These resembled findings in rat kidneys¹⁰. Refluxed debris from the proximal convoluted tubules was seen in all except the perfused control specimens. The quantity of affected glomeruli increased with time, resembling findings in canine kidneys⁵.

Proximal convoluted tubules had swollen cells and occluded lumens in all except the perfused control specimens. This was attributed to the delay between immersion, penetration and fixation of the samples in the 0 hour controls, resembling findings in the rat¹⁰. With the periodic acid Schiff (PAS) reaction, the brush borders were observed to be mildly disrupted, but still present after 48 hours at 4 C, and 12 hours at 24 C. These findings differed from earlier studies which found the borders to be extremely sensitive to autolytic destruction¹⁴. In the chicken, the brush border was only occasionally seen after 6 hours at 20 C. Persistence of the brush borders throughout the experimental period indicated there was little enzyme activity available to split the carbohydrates, resembling findings in rat kidneys¹⁰.

Earliest autolytic changes took place in the distal convoluted tubule cells. These changes were similar to the earliest changes seen in rat kidneys¹⁰. Refrigeration of the carcasses did not slow the rapid changes in these tubules, and this was probably due to the size of the cadavers being cooled, and the span of time needed to lower the abdominal cavity temperature.

Collecting tubules were second to show moderate autolytic change, resembling findings in rats and chickens^{10, 14}.

Erythrocytes appeared too fragile to be of any positive value as a postmortem indicator. One vessel would contain distinct, brightly stained cells, while an adjacent one contained totally lysed material, in the same tissue section.

Most renal structures, with the exception of the distal convoluted tubules, were still useful for diagnostic purposes after 96 hours at 4 C, but only up to 24 hours at 24 C.

SUMMARY

Glomeruli swelled and largely obliterated Bowman's space in all except perfused specimens. Refluxed debris from proximal convoluted tubules was present in Bowman's spaces, the number of glomeruli affected increasing with increased postmortem interval. Glomerular tufts became reduced in size and cellular pyknosis became conspicuous within 24 hours at 4 C, and 12 hours at 24 C.

Proximal tubules swelled extensively in all but the perfused specimens, but showed no other morphologic changes until 48 hours after death at 4 C, and 12 hours at 24 C. Distal convoluted tubules were severely affected within 3 hours after death. Collecting tubules were moderately changed within 12 hours at 4 C, and 3 hours at 24 C.

With the exception of the distal convoluted tubules, most renal structures were still useful for diagnostic purposes after 96 hours at 4 C, and up to 24 hours at 24 C.

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TABLE I

Histologic Changes of Porcine Kidneys Maintained at 4 C Compared to Perfused and 0 Hour Controls

Renal structure	Postmortem interval							
	Perfused control	0 hour control	3 hours at 4 C	6 hours at 4 C	12 hours at 4 C	24 hours at 4 C	48 hours at 4 C	96 hours at 4 C
<u>Glomeruli</u>								
Swollen glomerular tufts	0	+	+++	++	+	+	0	0
Debris in Bowman's space	0	+	+	+	+++	++	+++	+++
Nuclear pyknosis	0	0	0	0	++	++	+++	+++
Nuclear karyolysis	0	0	0	0	0	0	+	++
<u>Proximal convoluted tubules</u>								
Cellular swelling & lumen obliteration	0	++	++	++	++	+	+	0
Luminal debris	0	0	+	+	+++	+++	+++	+++
Brush border disruption	0	0	0	0	+	+	++	+++
Segmental loss of brush border	0	0	0	0	0	0	+	+++
Retraction off basement membrane	0	0	0	0	+	+	+	0
Nuclear pyknosis	0	0	0	0	+	++	+++	+++
Nuclear karyolysis	0	0	0	0	0	0	+	+++
Loss of tubular architecture	0	0	0	0	0	0	+	+++
Bacterial invasion	0	0	0	0	0	0	0	++
<u>Distal convoluted tubules</u>								
Retraction off basement membrane	0	0	+++	+++	+++	+++	+++	+++
Nuclear pyknosis	0	+++	+++	+++	+++	+++	+++	+++
Individualization	0	0	++	+++	+++	+++	+++	+++
Nuclear karyolysis	0	0	0	0	0	0	+	+++
Loss of tubular architecture	0	0	0	0	0	0	+	+++
<u>Collecting tubules</u>								
Retraction off basement membrane	0	0	++	++	+++	+++	+++	+++
Nuclear pyknosis	0	0	+	+	++	+++	+++	+++
Individualization	0	0	+	+	++	++	+++	+++
Nuclear karyolysis	0	0	0	0	0	0	+	+++
Loss of tubular architecture	0	0	0	0	0	0	+	+++

0=Change is absent
 +=Mild change
 ++=Moderate change
 +++=Marked change

TABLE II

Histologic Changes of Porcine Kidneys Maintained at 24 C Compared to Perfused and 0 Hour Controls

Renal structure	Postmortem interval							
	Perfused control	0 hour control	3 hours at 24 C	6 hours at 24 C	12 hours at 24 C	24 hours at 24 C	48 hours at 24 C	96 hours at 24 C
<u>Glomeruli</u>								
Swollen glomerular tufts	0	+	+++	++	+	+	0	0
Debris in Bowman's space	0	+	+	+	+++	++	+++	+++
Nuclear pyknosis	0	0	0	0	++	++	+++	+++
Nuclear karyolysis	0	0	0	0	0	0	+	++
<u>Proximal convoluted tubules</u>								
Cellular swelling & lumen obliteration	0	++	++	++	++	+	+	0
Luminal debris	0	0	+	+	+++	+++	+++	+++
Brush border disruption	0	0	0	0	+	+	++	+++
Segmental loss of brush border	0	0	0	0	0	0	+	+++
Retraction off basement membrane	0	0	0	0	+	+	+	0
Nuclear pyknosis	0	0	0	0	+	++	+++	+++
Nuclear karyolysis	0	0	0	0	0	0	+	+++
Loss of tubular architecture	0	0	0	0	0	0	+	+++
Bacterial invasion	0	0	0	0	0	0	0	++
<u>Distal convoluted tubules</u>								
Retraction off basement membrane	0	0	+++	+++	+++	+++	+++	+++
Nuclear pyknosis	0	+++	+++	+++	+++	+++	+++	+++
Individualization	0	0	++	+++	+++	+++	+++	+++
Nuclear karyolysis	0	0	0	0	0	0	+	+++
Loss of tubular architecture	0	0	0	0	0	0	+	+++
<u>Collecting tubules</u>								
Retraction off basement membrane	0	0	++	++	+++	+++	+++	+++
Nuclear pyknosis	0	0	+	+	++	+++	+++	+++
Individualization	0	0	+	+	++	++	+++	+++
Nuclear karyolysis	0	0	0	0	0	0	+	+++
Loss of tubular architecture	0	0	0	0	0	0	+	+++

0=Change is absent
 +=Mild change
 ++=Moderate change
 +++=Marked change

Fig 1. Perfused control: Glomeruli. Bowman's space (s) is prominent and has little debris. Cells within the glomerular tufts are round to ovoid. Capillaries are clear and free of erythrocytes, as a result of perfusion (410X reduced to 266X, PAS).

Fig 2. 0 hour: Glomeruli. Noncellular debris (d) is visible within Bowman's space. Cellular components of the glomerular tuft resemble perfused glomeruli, but the capillaries contain erythrocytes (410X reduced to 266 X, PAS).

Fig 3. 6 hours: Glomeruli at 4 C. These glomeruli have obliterated Bowman's spaces due to swelling of the glomerular tufts. Visceral and parietal layers of the capsule are in direct contact with one another (256X reduced to 166X, PAS).

Fig 4. 96 hours: Glomeruli at 4 C. The glomeruli have re-fluxed debris in Bowman's spaces. Nuclei within the glomerular tufts are pyknotic (410X reduced to 266X, PAS).

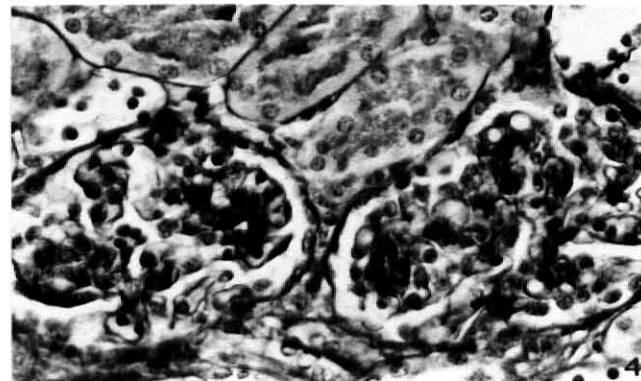
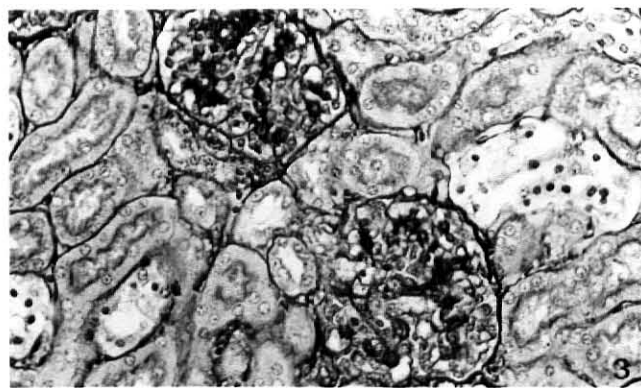
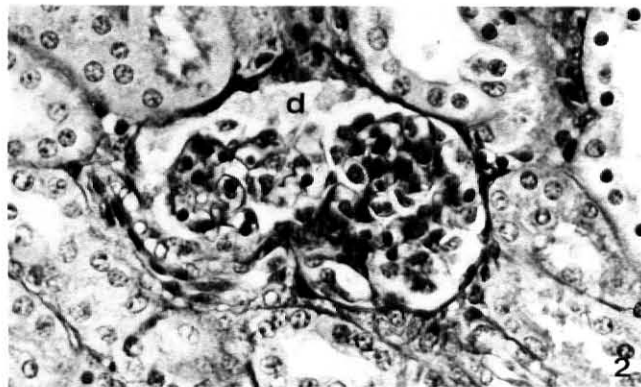
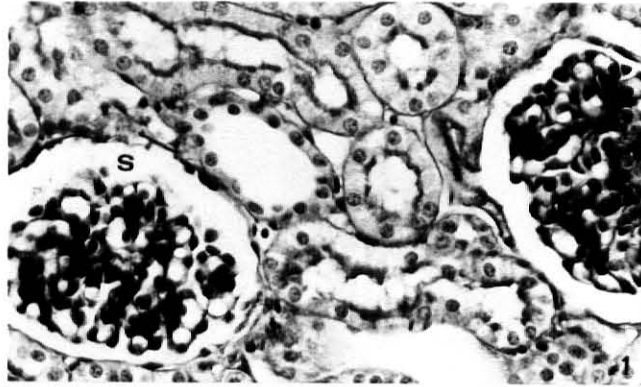


Fig 5. Perfused control: Proximal convoluted tubules. Tubular lumens are open, and contain some noncellular strands of debris. Periodic acid Schiff positive (PAS+) brush borders are seen lining the lumens. The tall pyramidal cells have centrally placed, vesicular nuclei with prominent clumped chromatin (256X reduced to 166X, PAS).

Fig 6. 24 hours: Proximal convoluted tubules at 4 C. Swollen cells partially occlude some of the lumens. Noncellular debris (d) is present within some lumens. Cells are in contact with the basement membranes. Vesicular nuclei are basally located within cells (410X reduced to 266X, PAS).

Fig 7. 24 hours: Proximal convoluted tubules at 4 C. Disruption of the brush borders (b) is evident, but most PAS+ brush borders are still intact (410X reduced to 266X, PAS).

Fig 8. 48 hours: Proximal convoluted tubules at 4 C. All lumens contain debris. PAS+ brush borders are disrupted, but some are still present. Cells are beginning to retract from the basement membranes, but most are still in contact. Some nuclei are hyperchromatic (410X reduced to 266X, PAS).

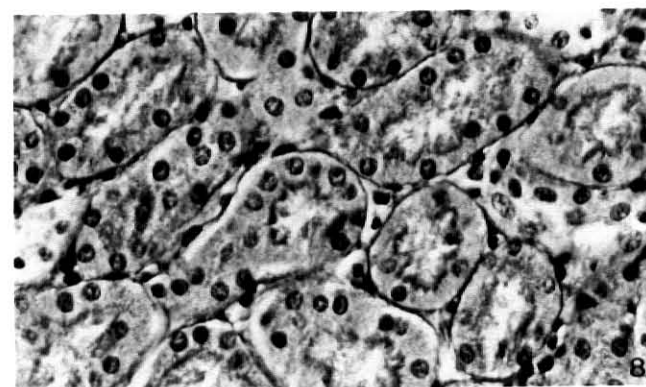
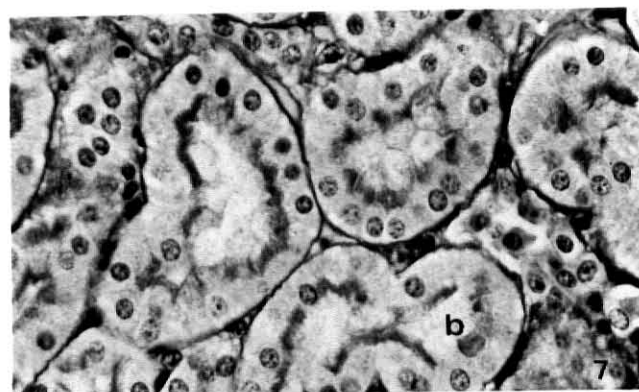
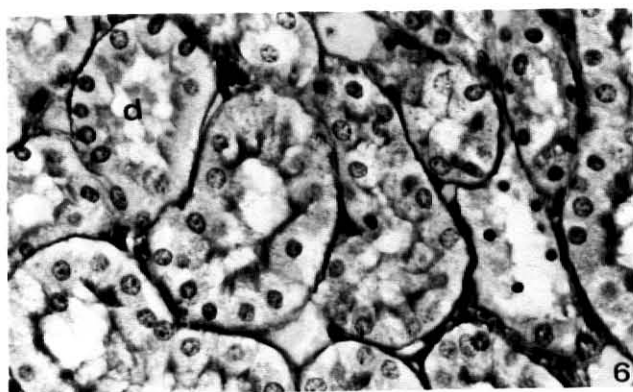
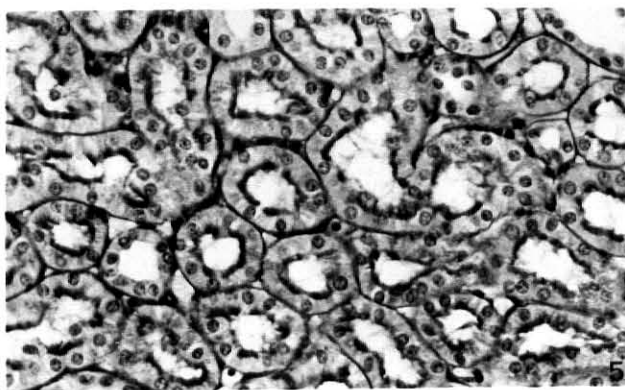


Fig 9. Perfused control: Distal convoluted tubules. The lumens (l) are free of debris. Low cuboidal epithelium lacks individual cell outlines, and brush borders on the luminal surface. Ovoid nuclei are centrally placed within the cells (256X reduced to 166X, PAS).

Fig 10. 0 hour control: Distal convoluted tubules. Some debris is visible within the lumens. Cytoplasm is vacuolizing, and nuclei are becoming pyknotic (256X reduced to 166X, PAS).

Fig 11. 3 hours: Distal convoluted tubules at 4 C. Cells have individualized and cytoplasmic strands are extending into the tubular lumens. Most nuclei are hyperchromatic to pyknotic (256X reduced to 166X, PAS).

Fig 12. 12 hours: Distal convoluted tubules at 4 C. Lumens are nearly obliterated by cellular debris. Indistinct cellular outlines, cytoplasmic strands and pyknotic nuclei characterize the tubular debris (256X reduced to 166X, PAS).

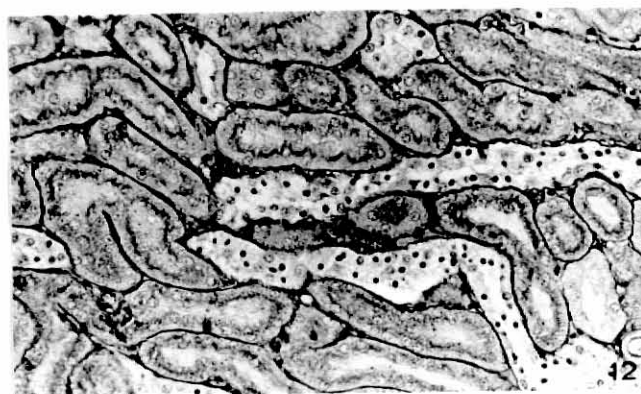
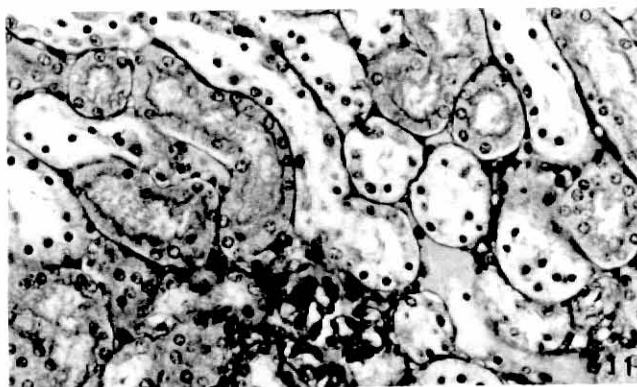
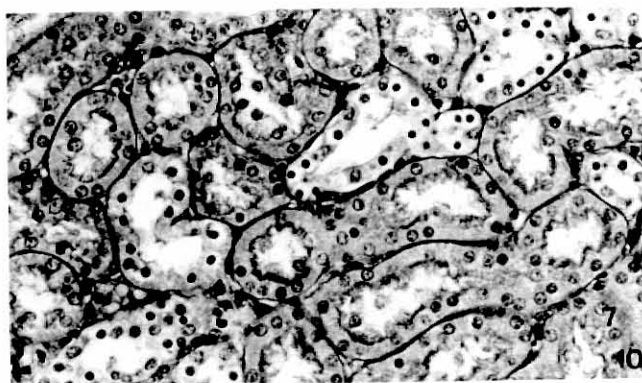
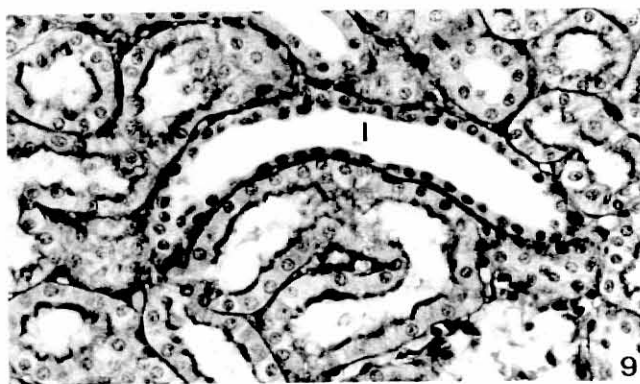


Fig 13. Perfused control: Collecting tubules. The lumen (1) is free of debris, and lined by low cuboidal epithelium. Cellular outlines are indistinct. Rounded, vesicular nuclei are centrally located within the cells (256X enlarged to 320X, PAS).

Fig 14. 96 hours: Collecting tubules at 4 C. The lumen (1) contains cellular debris. Epithelial cells are retracting from the basement membrane, and individualizing within the lumen. Some nuclear pyknosis is present (256X enlarged to 320X, PAS).

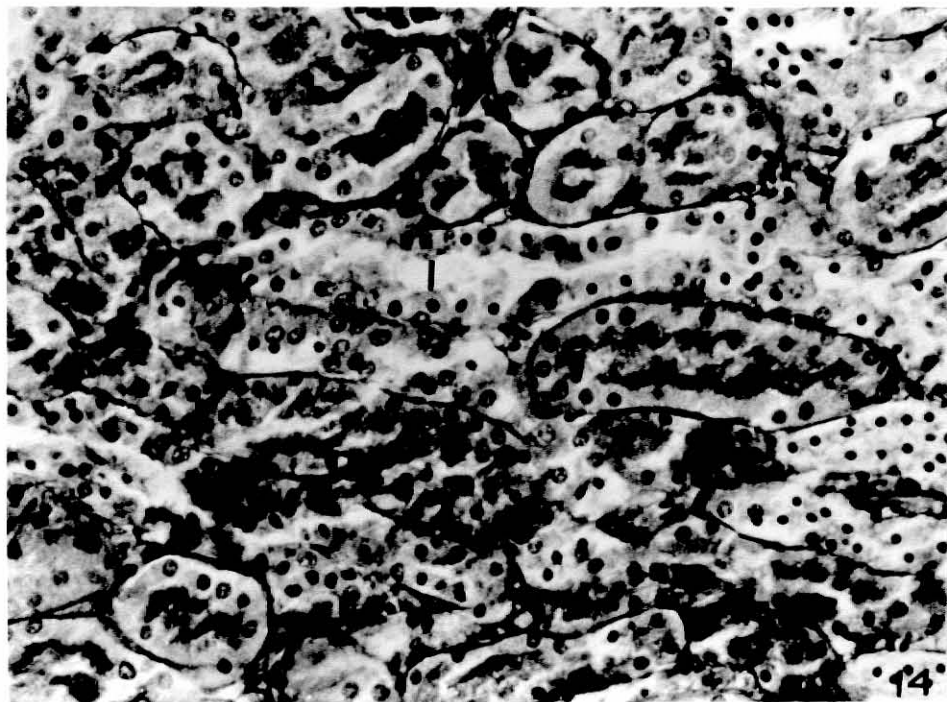
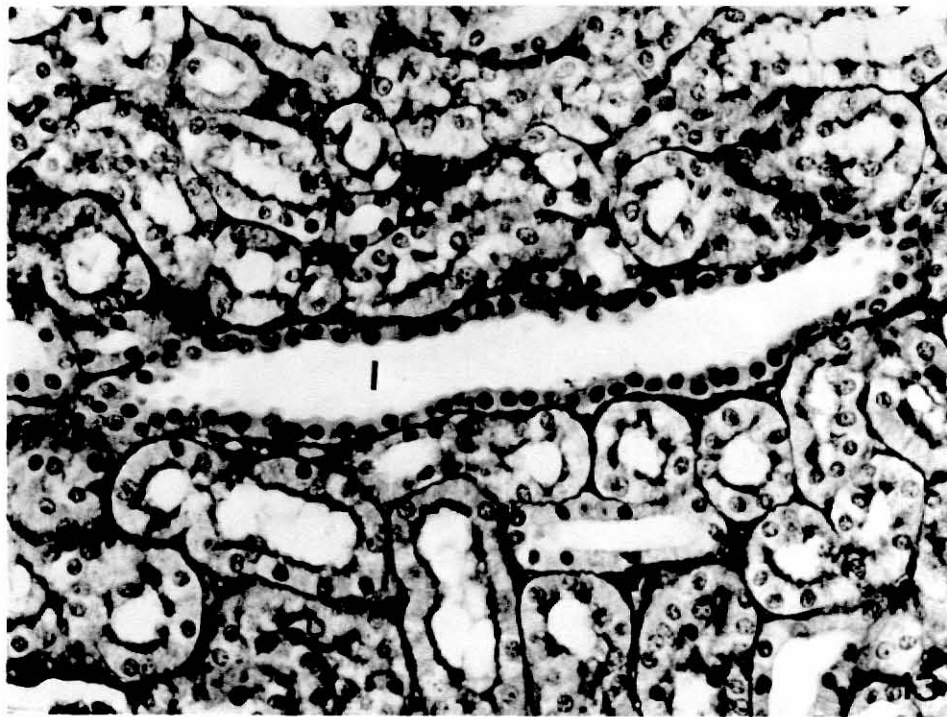


Fig 15. Perfused control: Glomeruli. Bowman's space (s) is prominent and contains little debris. Cells within the glomerular tufts are round to ovoid. Capillaries are clear and free of erythrocytes due to perfusion (410X reduced to 273X, PAS).

Fig 16. 6 hours: Glomeruli at 24 C. The glomerular tufts are swollen and almost obliterate Bowman's space. Vessels within the tufts are visible, and the nuclei are round to ovoid (410X reduced to 273X, PAS).

Fig 17. 24 hours: Glomeruli at 24 C. Bowman's space is again prominent, containing debris (d) from the proximal convoluted tubules. Nuclei within the tufts are small and hyperchromatic (256X reduced to 170X, PAS).

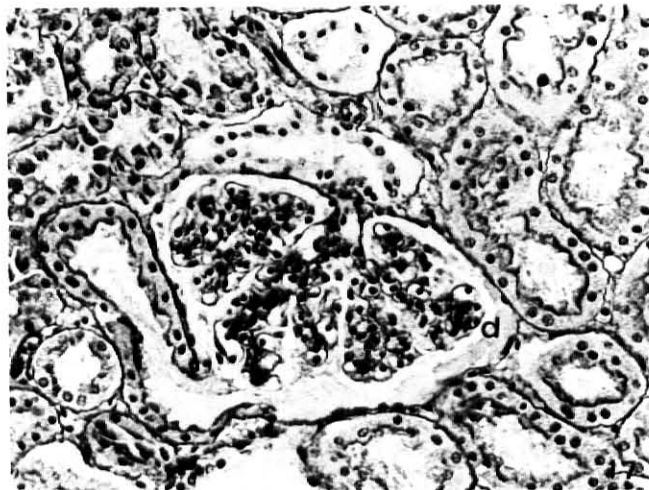
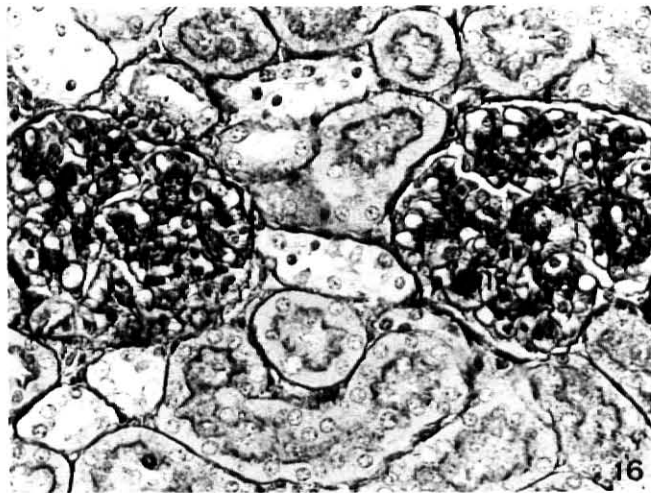
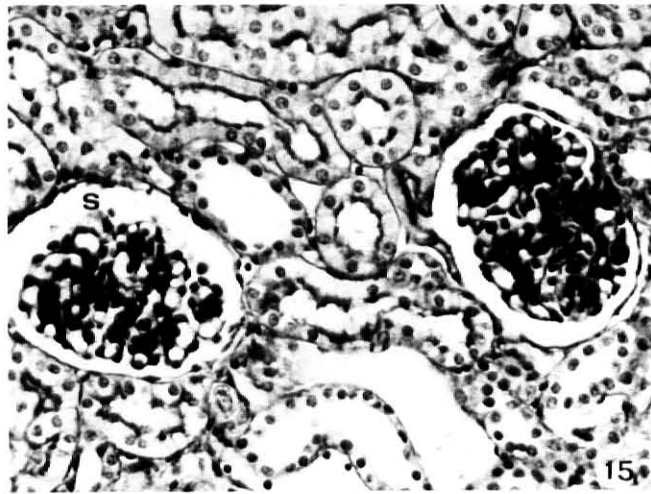
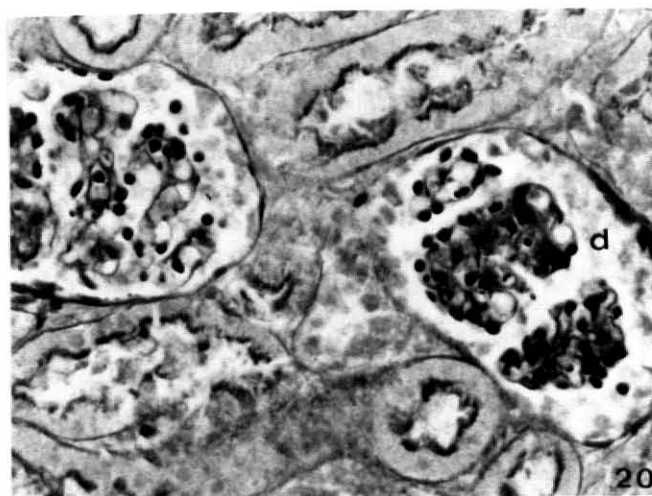
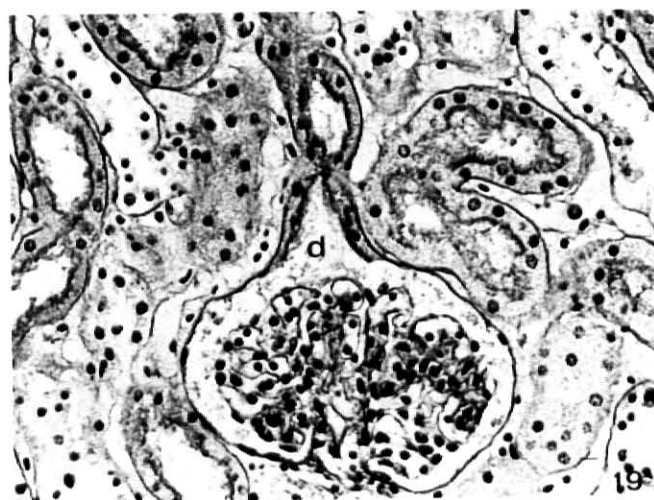
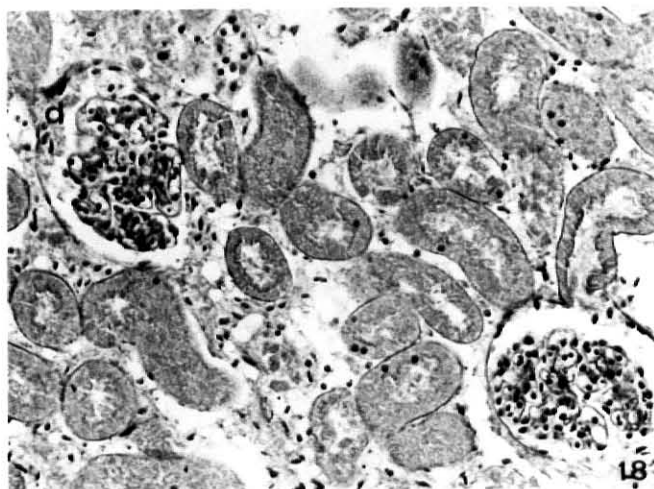


Fig 18. 48 hours: Glomeruli at 24 C. Shrunken glomerular tufts contain pyknotic nuclei. Refluxed debris (d) from the proximal convoluted tubules is present within conspicuous Bowman's space (256 X reduced to 170 X, PAS).

Fig 19. 48 hours: Glomeruli at 24 C. A glomerulus contains pyknotic nuclei and debris (d) within Bowman's space. The space is prominent due to shrinkage of the glomerular cellular components (256 X reduced to 170 X, PAS).

Fig 20. 96 hours: Glomeruli at 24 C. Two hypocellular glomerular tufts have lost nuclei and the remaining nuclei are pyknotic. The tufts have shrunk resulting in enlarged Bowman's space. Refluxed debris (d) from the proximal convoluted tubules is visible within Bowman's space (410 X reduced to 273 X, PAS).



- Fig 21. Perfused control: Proximal convoluted tubules. Tubular lumens are patent, and contain some non-cellular strands of debris. Periodic acid Schiff positive (PAS+) brush borders are observed lining the lumens. The tall cuboidal cells have centrally placed, open nuclei with prominent chromatin clumping (410 X reduced to 273 X, PAS).
- Fig 22. 6 hours: Proximal convoluted tubules at 24 C. Nearly all tubular lumens are obliterated by cellular swelling. Brush borders are indistinct, but still visible. Nuclei are vesicular and enlarged (410 X reduced to 273 X, PAS).
- Fig 23. 24 hours: Proximal convoluted tubules at 24 C. Patent lumens are again visible, and all contain some noncellular debris. Brush borders are present. The cytoplasm is finely granular, and is starting to retract off the basement membranes. Nuclei are hyperchromatic to pyknotic (256 X reduced to 170 X, PAS).

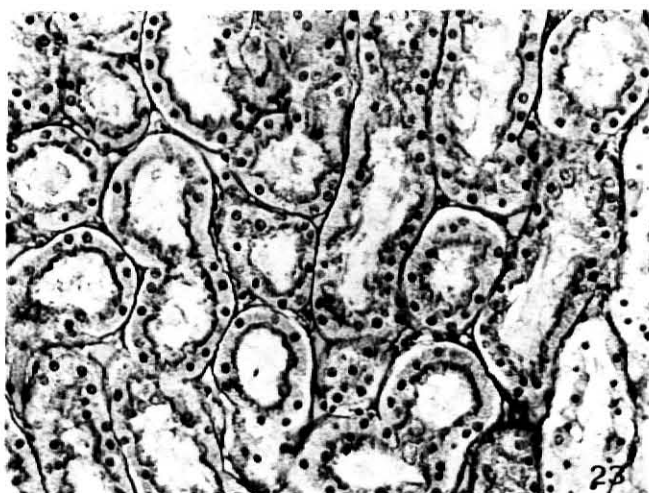
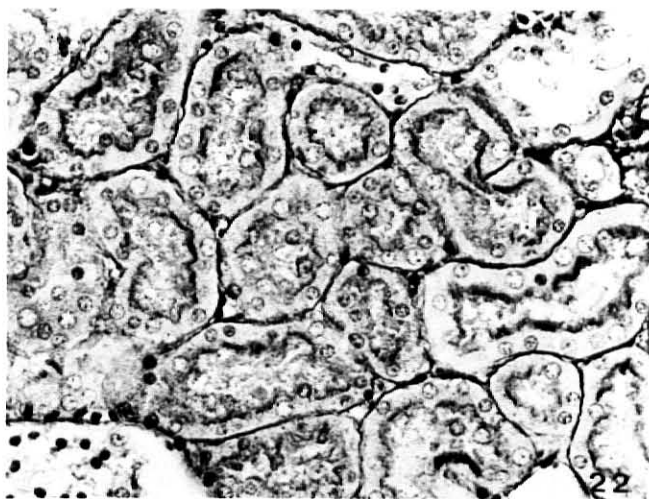
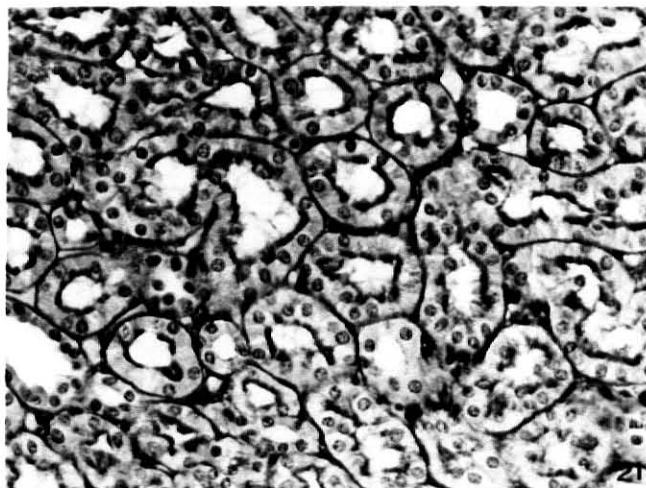


Fig 24. 48 hours: Proximal convoluted tubules at 24 C. Cells are retracting off the basement membranes in single units; no cellular individualization is visible. Noncellular debris is present in all lumens. Brush borders are still evident, but lack continuity due to partial loss. Nuclei are pyknotic (256 X enlarged to 320 X, PAS).

Fig 25. 96 hours: Proximal convoluted tubules at 24 C. Cells are in contact with basement membranes, and brush borders partially present. Nuclei have been lost through karyolysis. Bacterial colonies (b) were present throughout the entire cortex (256 X enlarged to 320 X, PAS).

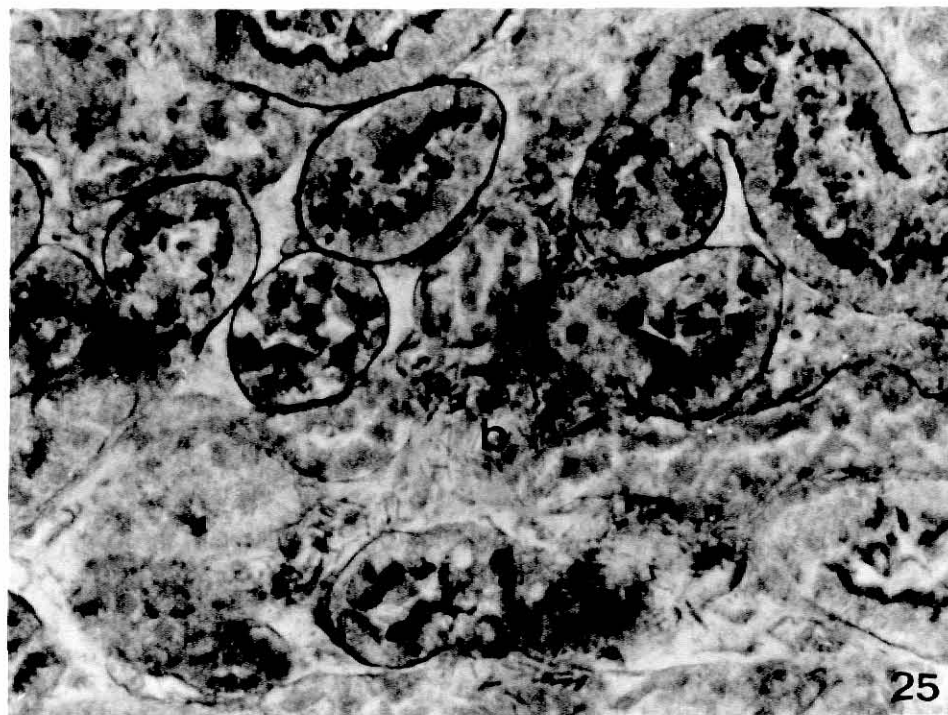
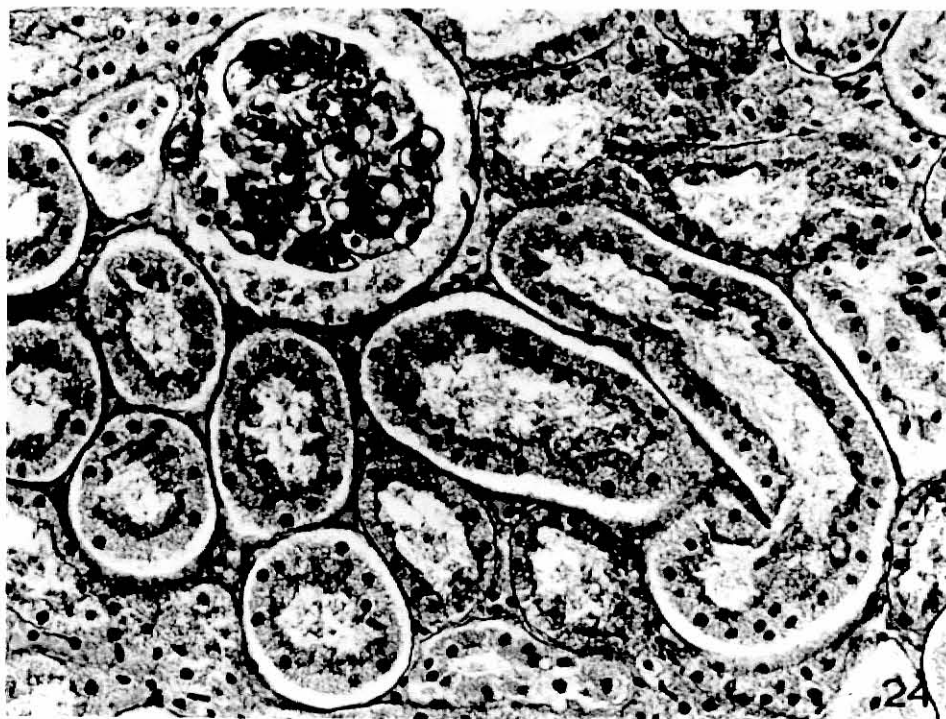


Fig 26. Perfused control: Distal convoluted tubules. Lumens (l) are free of debris. Low cuboidal epithelium lacks individual cellular outlines and periodic acid Schiff positive (PAS+) brush borders on the luminal surface. Ovoid nuclei are centrally located within the cells (410 X reduced to 273 X, PAS).

Fig 27. 3 hours: Distal convoluted tubules at 24 C. Most epithelial cells have retracted off the basement membranes, and individualized within the tubular lumens. Cytoplasm is scant around the pyknotic nuclei of the cellular debris. A few cells with vesicular nuclei are intact on the basement membranes (410 X reduced to 273 X, PAS).

Fig 28. 6 hours: Distal convoluted tubules at 24 C. Most of the cells have sloughed off the basement membranes and formed cellular debris within the lumen. Few cells remain in contact with the basement membranes (410 X reduced to 273 X, PAS).

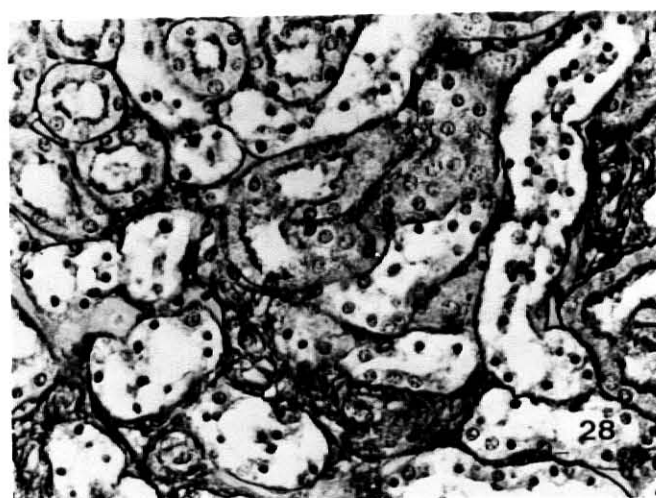
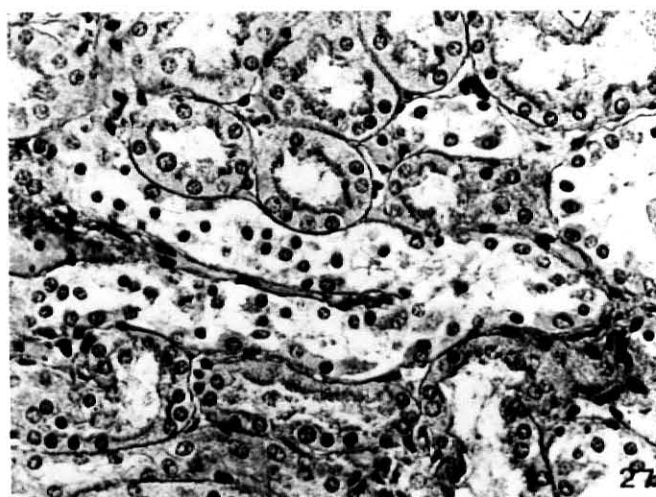
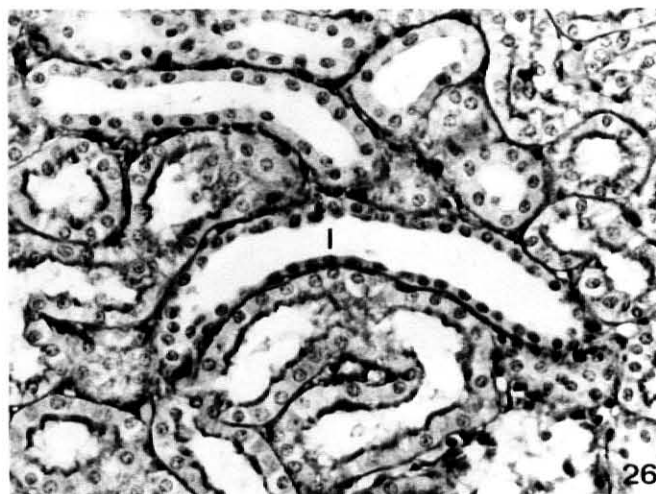
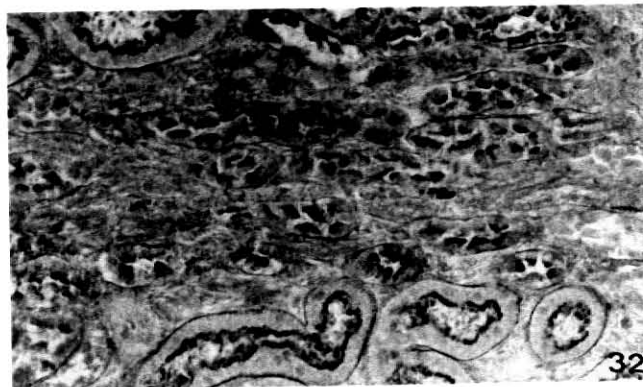
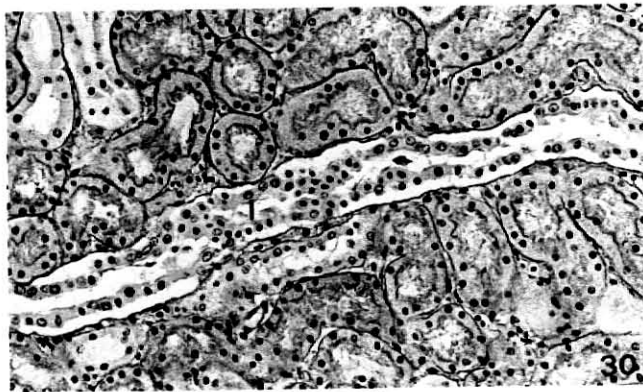
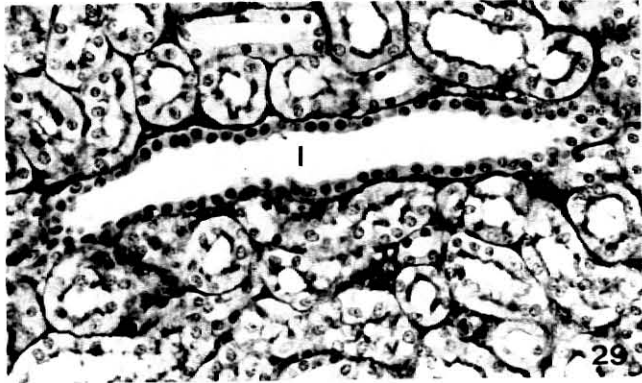


Fig 29. Perfused control: Collecting tubules. The lumen (1) is free of debris, and lined by low cuboidal epithelium. Cellular outlines are indistinct. Rounded vesicular nuclei are centrally located within the cells (256 X reduced to 166 X, PAS).

Fig 30. 24 hours: Collecting tubules at 24 C. Epithelial cells have retracted off the basement membrane and sloughed into the lumen (1). Some nuclei are pyknotic, but most are vesicular with clumped chromatin (256 X reduced to 166 X, PAS).

Fig 31. 48 hours: Collecting tubules at 24 C. Sloughed epithelium has individualized within the collecting tubule lumen. All nuclei are pyknotic (256 X reduced to 166 X, PAS).

Fig 32. 96 hours: Collecting tubules at 24 C. This medullary ray is composed principally of collecting tubules, although the architecture is disrupted, making identification difficult. Karyolysis of all nuclei is evident (256 X reduced to 166 X, PAS).



II. SEQUENTIAL HISTOLOGIC CHANGES IN PORCINE
ADRENAL GLANDS AT 4 C AND 24 C.

INTRODUCTION

It is important for histopathologists to differentiate postmortem autolysis from antemortem coagulative necrosis. Cells undergoing either type of self-digestion look similar histologically.¹

The adrenal gland is composed of a cortex and medulla.² The cortex has three distinct zones: zona arcuata, zona fasciculata, and zona reticularis. Beneath the capsule lies the thin zona arcuata composed of packets of columnar cells. The thickest zone, zona fasciculata, consists of radially arranged parallel cords of foamy cuboidal cells aligned on sinusoids. Irregular rete anastomosing cords of polyhedral cells amid vascular sinusoids characterize the zona reticularis. Medullary cells are arranged in irregular bundles separated by sinusoids. These cells react with chromium salts, and turn brown; this chromaffin reaction is due to oxygenation and polymerization of the catecholamines, epinephrine and norepinephrine.

A paucity of information exists concerning adrenal glands and autolytic changes. No sequential autolysis studies have been reported in the literature. Only two references mention autolysis, and both agree that the medulla undergoes rapid liquefaction.^{3,4}

This study was undertaken to determine the sequential histologic changes occurring in porcine adrenal glands at 4 C and 24 C.

MATERIALS AND METHODS

The experimental design, necropsy examination, and photomicrograph procedures are described in Paper I.

Histopathological Evaluation: The three cortical zones and the medulla were evaluated for the extent of postmortem change in each adrenal gland. Sections from perfused controls, 0 hour controls, 4 C and 24 C adrenal glands were scored for change on a 1-plus to 3-plus system based on visual comparisons. Mild changes were scored 1-plus, moderate changes were 2-plus, and marked changes were 3-plus.

Cells of the zona arcuata were evaluated for: nuclear hyperchromasia; nuclear pyknosis; nuclear karyolysis; and cellular shrinkage and individualization.

Autolysis of the zona fasciculata was evaluated using eight variables: prominence of sinusoids; prominence of endothelial nuclei; nuclear hyperchromasia; nuclear pyknosis; nuclear karyolysis; cellular shrinkage and individualization; bacterial invasion; and loss of zonal architecture.

The severity of autolysis in the zona reticularis was based on five criteria: prominence of sinusoids; prominence of endothelial nuclei; nuclear pyknosis; nuclear karyolysis; and cellular shrinkage and individualization.

Three variables were used to evaluate medullary autolytic changes: cellular shrinkage; nuclear hyperchromasia; and nuclear pyknosis.

RESULTS

The sequential histologic changes are summarized in Table I and illustrated in Fig 1-16.

Perfused controls

Cells of the zona arcuata were columnar with large vesicular nuclei. Cells were clumped together, and cell outlines were indistinct. No shrinkage or individualization of cells was seen (Fig 1 & 17). Sinusoids of the zona fasciculata were prominent, and free of erythrocytes. Endothelial cells were visible, but not pyknotic. Cells were arranged in parallel, radiating cords along the sinusoids. Nuclei were large and vesicular. Cells of the zona reticularis had cytoplasm which stained more prominently than cells in other zones (Fig 9 & 29). Sinusoids free of erythrocytes were visible. No shrinkage of cells off the reticular framework was observed. Medullary cells were round, and distributed in clusters. nuclei were moderately stained, while the cytoplasm was pale (Fig 13 & 31).

0 Hour controls

The major difference in these tissues from perfused controls was seen in the zona fasciculata. Cord cells had swollen, and obliterated sinusoidal lumens (Fig 5 & 23). The parallel cord arrangement was maintained. All other zones resembled perfused tissue sections.

4 C EXPERIMENT

Zona arcuata at 4 C.

Nuclear hyperchromasia in cells of this zone was evident in few cells at 3 hours, became prominent at 6 hours, and affected moderate number of cells at 24 and 48 hours (Fig 2 & 3). Nuclei first became noticeably pyknotic at 6 hours. Variable numbers of nuclei were affected in each successive time interval; by 96 hours, all nuclei were pyknotic (Fig 4). Cellular shrinkage and individualization was first observed at 3 hours, and varied throughout the experimental period.

Zona fasciculata at 4 C.

Sinusoids between the parallel cords of cells were detectable at all times; by 12 hours they were moderately prominent, but were obliterated by swollen cord cells at 24 hours (Fig 6). Endothelial nuclei were hyperchromatic at 12 and 96 hours (Fig 8). Decreased cell size and individualization within the cords varied in severity with subsequent time intervals, but was most prominent by 48 hours (Fig 7). Few nuclei were hyperchromatic at 6 hours; by 24 hours, most were affected. A few nuclei were pyknotic at 24 and 96 hours.

Zona reticularis at 4 C.

Sinusoids of this zone were prominent at 12 and 96 hours (Fig 12). Decreased cytoplasmic stainability was first noted

at 6 hours, and was pronounced at 24 and 96 hours (Fig 10 & 12). Cellular shrinkage and individualization proceeded at variable rates from 6 to 48 hours (Fig 10 & 11), being most pronounced at 12 hours. Pyknosis was not observed before 24 hours (Fig 10).

Medulla at 4 C.

Shrinkage of these cells was noted as early as 3 hours postmortem. Only at 48 hours were cells severely shrunken (Fig 15). Hyperchromasia was not observed before 12 hours, and varied in intensity as time increased (Fig 14-16).

24 C EXPERIMENT

The sequential histologic changes are summarized in Table II and illustrated in Fig 17-34.

Zona arcuata at 24 C.

Nuclei of this zone were markedly hyperchromatic at 3 and 6 hours (Fig 18 & 19). From 12 hours, nearly all nuclei were pyknotic (Fig 20). Karyolysis was first noted at 24 hours; was marked at 48 hours (Fig 21 & 22). Cellular shrinkage and individualization was marked from 3 hours (Fig 18-20).

Zona fasciculata at 24 C.

Sinusoids and endothelial cells were prominent from 3 to 24 hours (Fig 24-26), but barely noticeable at 48 hours. Nuclei were markedly hyperchromatic at 6 and 12 hours (Fig 25), pyknotic by 24 hours (Fig 26), while few nuclei remained by 48 hours (Fig 28). Karyolysis was first noted at 12 hours;

the number of affected nuclei increased with time.

Cellular shrinkage and individualization was evident at 3 hours; most cells were affected from 6 hours (Fig 24-28). Moderate numbers of large bacterial rods were present at 24 and 48 hours (Fig 27 & 28). Disruption of the linear parallel cord architecture of cells was noted within 6 hours, and was moderate by 12 hours (Fig 25). One 24 hour specimen was mildly affected, while the other was markedly so; by 48 hours, the cords were severely disrupted (Fig 28).

Zona reticularis at 24 C.

Sinusoids were mildly prominent by 3 hours; became markedly noticeable at 6 hours postmortem. Their prominence decreased at 12 hours, but became marked again later on. Endothelial cells varied in prominence throughout the experimental period. They were most easily seen at 24 hours (Fig 30).

Pyknotic nuclei were most noticeable at 12 and 24 hours (Fig 30). By 48 hours, most nuclei were gone. Marked shrinkage and individualization were observed only at 48 hours. Cytoplasmic stainability decreased from 6 to 48 hours.

Medulla at 24 C.

Slight cellular shrinkage was noted at 12 hours; from 24 hours, most cells were affected. The percentage of pyknotic nuclei varied throughout the experiment; by 48 hours, nearly all nuclei were affected (Fig 32).

96 Hour adrenal glands at 24 C.

All nuclei were lysed. The shrunken, pale-staining cellular remnants of the various zones could not be distinguished. Destruction of the zonal architecture was complete, and bacterial invasion was marked. The cellular remains were no longer identifiable as adrenal tissue (Fig 33 and 34).

DISCUSSION

Contrary to printed literature,^{3,4} the medullas of these porcine adrenal glands did not undergo rapid liquefaction. At 4 C, cellular shrinkage and nuclear hyperchromasia were only moderate. These changes were marked by 48 hours at 24 C, but most cells of this zone were still identifiable. By 96 hours at 24 C, all nuclei had been lost and medulla could not be differentiated from cortex.

Bacterial invasion at 24 C by 24 hours hastened cell lysis and disruption of the cortical architecture. Cells adjacent to the bacteria were lysed and cellular arrangement was more disorganized than cells of the same zone not in contact with the bacteria. This could be due to liberation of proteolytic enzymes by the bacteria or by adrenal cells in response to the bacteria.

These porcine adrenal glands were found to be useful for diagnostic purposes after 48 hours at 4 C, but only up to 12 hours at 24 C.

SUMMARY

Cells of the zona arcuata became shrunken, pyknotic, and individualized within 6 hours after death at both 4 C and 24 C. Large bacterial rods were found in the zona fasciculata at 24 and 48 hours at 24 C. No bacteria were observed in refrigerated specimens throughout the experimental period. Moderate disruption of the parallel cord architecture of this zone appeared by 12 hours, and increased in severity through 96 hours at 24 C. Moderate to marked changes were noted in the zona reticularis by 12 hours at both temperatures. Medullary cells were moderately shrunken and hyperchromatic in refrigerated adrenal gland specimens through 96 hours, but at 24 C, these changes were marked within 24 hours postmortem.

Porcine adrenal glands were considered to be diagnostically useful after 48 hours if cooled to 4 C. At 24 C, however, adrenals remained useful for only 12 hours.

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TABLE I

Histologic Changes of Porcine Adrenal Glands Maintained at 4 C Compared to Perfused and 0 Hour Controls

Adrenal zone	Perfused control	Postmortem interval						
		0 hour at 4 C	3 hours at 4 C	6 hours at 4 C	12 hours at 4 C	24 hours at 4 C	48 hours at 4 C	96 hours at 4 C
<u>Zona arcuata</u>								
Nuclear hyperchromasia	0	0	+	+++	+++	++	++	0
Nuclear pyknosis	0	0	0	++	++	+	++	+++
Nuclear karyolysis	0	0	0	0	0	0	0	0
Cellular shrinkage & individualization	0	0	+	+++	+	0	0	++
<u>Zona fasciculata</u>								
Prominent sinusoids	++	0	+	+	++	0	+	+
Prominent endothelial nuclei	+	0	0	0	++	+	0	++
Nuclear hyperchromasia	0	0	0	+	+	+++	+	++
Nuclear pyknosis	0	0	0	0	0	+	0	+
Nuclear karyolysis	0	0	0	0	0	0	0	0
Cellular shrinkage & individualization	0	0	+	+	++	0	+++	++
Bacterial invasion	0	0	0	0	0	0	0	0
Loss of zonal architecture	0	0	0	0	0	0	0	0
<u>Zona reticularis</u>								
Prominent sinusoids	0	0	+	++	+++	+	+	+++
Prominent endothelial nuclei	0	0	0	0	0	0	0	0
Nuclear pyknosis	0	0	0	0	0	++	+	+
Nuclear karyolysis	0	0	0	0	0	0	0	0
Cellular shrinkage & individualization	0	0	0	++	+++	+	+	++
<u>Medulla</u>								
Cellular shrinkage	0	0	+	+	++	+	+++	+
Nuclear hyperchromasia	0	0	0	0	+	++	+	++
Nuclear pyknosis	0	0	0	0	0	0	0	0

0=Change is absent
 +=Mild change
 ++=Moderate change
 +++=Marked change

TABLE II

Histologic Changes of Porcine Adrenal Glands Maintained at 24 C Compared to Perfused and 0 Hour Controls

Adrenal zone	Perfused control	Postmortem interval							96 hours at 24 C
		0 hour at 24 C	3 hours at 24 C	6 hours at 24 C	12 hours at 24 C	24 hours at 24 C	48 hours at 24 C		
<u>Zona arcuata</u>									
Nuclear hyperchromasia	0	0	+++	+++	0	0	0	0	
Nuclear pyknosis	0	0	0	++	++	+++	+++	+++	
Nuclear karyolysis	0	0	0	0	0	++	+++	+++	
Cellular shrinkage & individualization	0	0	+++	+++	+++	+++	+++	+++	
<u>Zona fasciculata</u>									
Prominent sinusoids	++	0	+++	+++	+++	+++	+++	+	Due to com-
Prominent endothelial nuclei	+	0	+++	++	+++	+++	+++	+	plete karyo-
Nuclear hyperchromasia	0	0	0	+++	+++	0	0	0	lysis and
Nuclear pyknosis	0	0	0	++	++	+++	+++	+	total
Nuclear karyolysis	0	0	0	0	0	++	+++	+++	destruction
Cellular shrinkage & individualization	0	0	++	+++	+++	+++	+++	++	of glandular
Bacterial invasion	0	0	0	0	0	+	+	+++	architecture
Loss of zonal architecture	0	0	0	0	+				individual
<u>Zona reticularis</u>									
Prominent sinusoids	0	0	+	+++	++	+++	+++	+++	zones are
Prominent endothelial nuclei	0	0	0	0	+	++	+	+	not dis-
Nuclear pyknosis	0	0	0	0	0	0	+++	0	cernible.
Nuclear karyolysis	0	0	0	0	0	0	++	+++	Bacterial
Cellular shrinkage & individualization	0	0	0	0	0	0	0	+++	invasion is
<u>Medulla</u>									
Cellular shrinkage	0	0	0	0	++	0	+++	+++	extensive.
Nuclear hyperchromasia	0	0	++	+++	+++	+++	+++	+++	
Nuclear pyknosis	0	0	++	+	+	++	++	+++	

0=Change is absent
 +=Mild change
 ++=Moderate change
 +++=Marked change

Fig 1. 0 hour: Zona arcuata. Located beneath a prominent capsule (c), this narrow zone is composed of round to ovoid cells with incomplete cell margins. The cytoplasm is finely granular. Round, vesicular nuclei are prominent, resulting in a large nuclear to cytoplasmic ratio. (410 X reduced to 266 X, PAS).

Fig 2. 48 hours: Zona arcuata at 4 C. The capsule (c) has been partially pulled loose during sectioning. The cells have become shrunken away from their basement membranes, individualization is conspicuous, and the nuclei are hyperchromatic. (256 X reduced to 166 X. PAS).

Fig 3. 48 hours: Zona arcuata at 4 C. Cellular shrinkage and retraction off the reticular framework is visible. Cellular individualization and pyknosis are present (410 X reduced to 266 X, PAS).

Fig 4. 96 hours: Zona arcuata at 4 C. Little cytoplasm is visible in these shrunken cells and pyknosis of most nuclei is prominent (410 X reduced to 266 X, PAS).

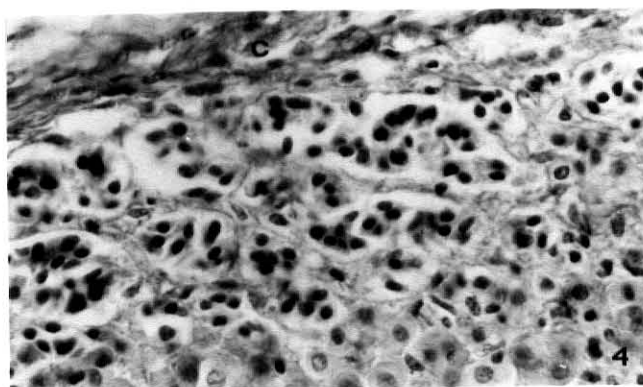
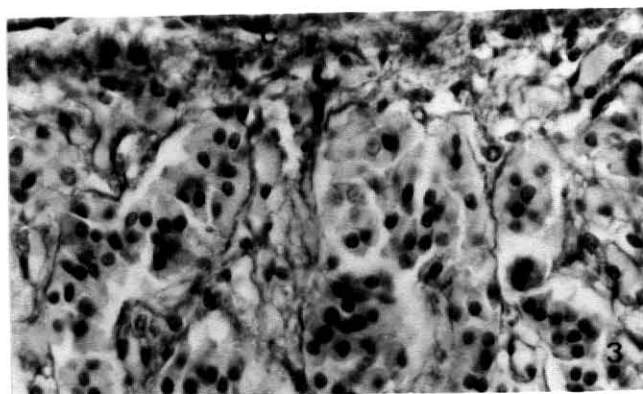
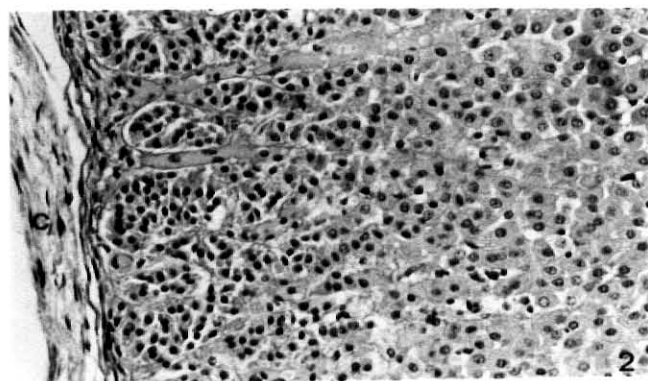
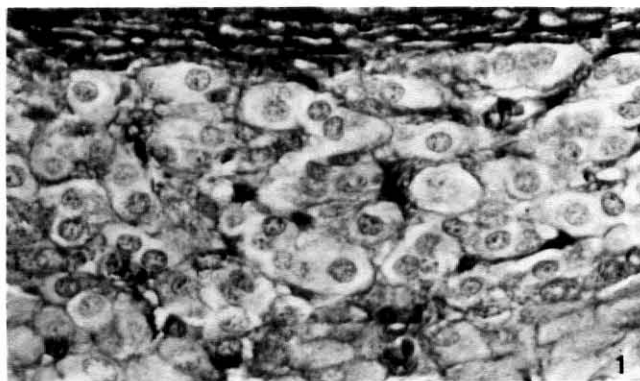


Fig 5. 0 hour: Zona fascicularis. Parallel cords of cells radiating toward the medulla are one to two cells in width oriented longitudinally on a meshwork of sinusoids lined by endothelium (s). The large, polyhedral cells have distinct membrane borders, foamy cytoplasm, and prominent, vesicular, round nuclei located both centrally and eccentrically (410 X reduced to 266 X, PAS).

Fig 6. 24 hours: Zona fascicularis at 4 C. Endothelial cells of the sinusoids (s) are quite prominent. Cells of this zone have shrunk slightly, but no individualization is obvious. Nuclei are hyperchromatic and contain clumped chromatin (410 X reduced to 266 X, PAS).

Fig 7. 48 hours: Zona fascicularis at 4 C. Individualization of shrunken cells is apparent. Endothelial cells and sinusoids are not as obvious in this section (410 X reduced to 266 X, PAS).

Fig 8. 96 hours: Zona fascicularis at 4 C. Pyknotic endothelial cells are prominent. Some individualization of the shrunken cord cells is visible (410 X reduced to 266 X, PAS).

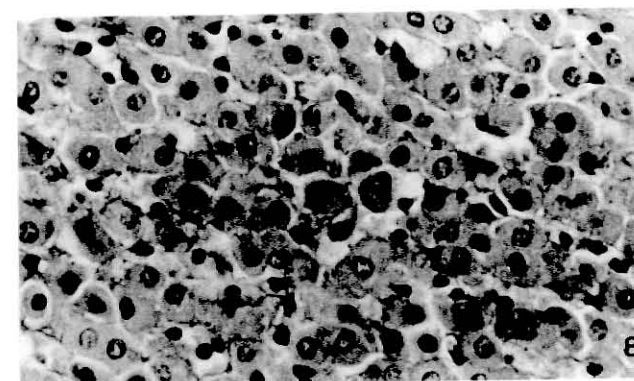
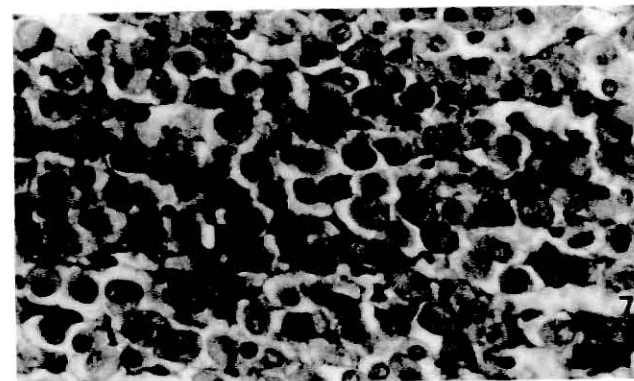
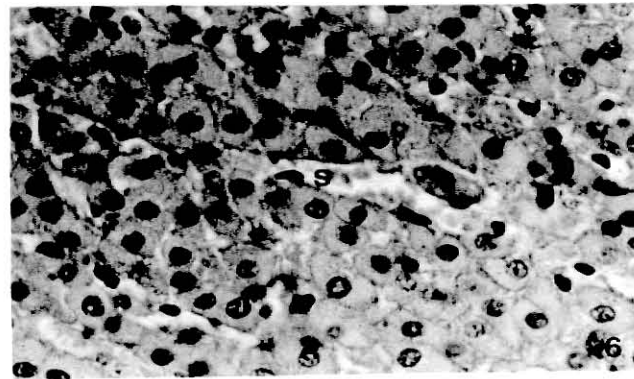
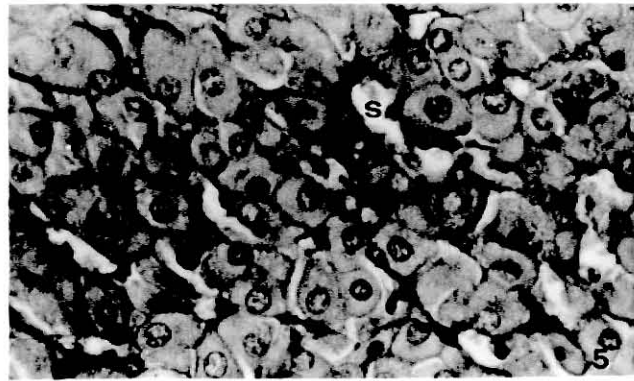
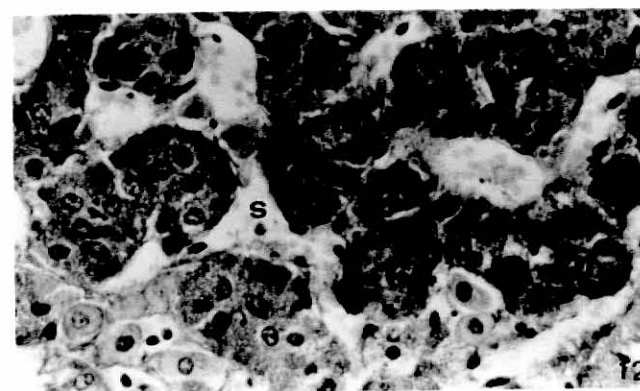
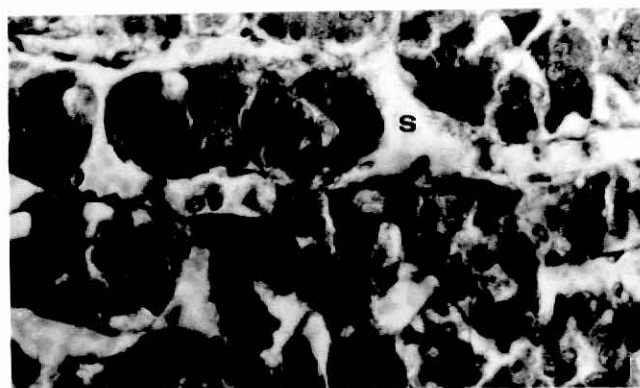
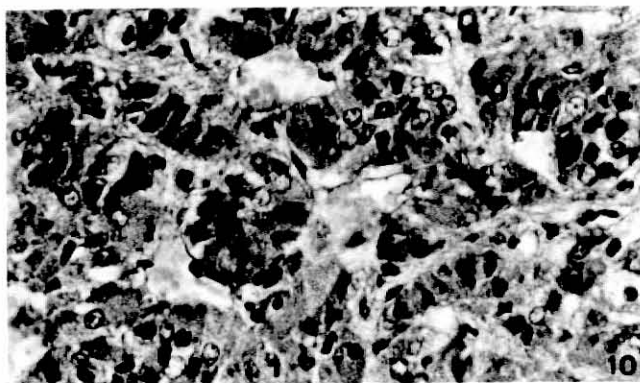
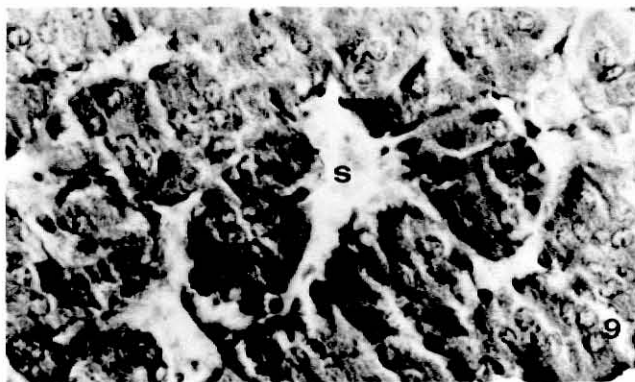


Fig 9. 0 hour: Zona reticularis. Cells arranged in a rete network lack distinct cellular borders. The cytoplasm of the cells stains darkly and has a granular texture. Nuclei are round and vesicular, and chromatin clumping is prominent. Vascular sinusoids (s) are visible (410 X reduced to 266 X, PAS).

Fig 10. 24 hours: Zona reticularis at 4 C. Some cellular shrinkage and retraction from the basement membranes is visible. The cytoplasm is staining deeply and has maintained its granular texture. Most nuclei have shrunk and are hyperchromatic. Sinusoids and endothelial cells are visible, but not prominent (410 X reduced to 266 X, PAS).

Fig 11. 48 hours: Zona reticularis at 4 C. Clusters of shrunken, dark staining cells lack individual cell outlines. Nuclei are vesicular and contain clumped chromatin. Sinusoids (s) are prominent, but endothelial cells are not (410 X reduced to 266 X, PAS).

Fig 12. 96 hours: Zona reticularis at 4 C. Individual cell outlines are visible, because cells have retracted away from each other. The cytoplasm is granular and nuclei contain clumped chromatin. Sinusoids (s) and pyknotic endothelial cells are conspicuous (410 X reduced to 266 X, PAS).



- Fig 13. 0 hour: Medulla. Medullary cells form nests separated by vascular channels. The cells lack distinct cytoplasmic outlines, have stippled cytoplasm, and large, round, vesicular nuclei. Nucleoli are prominent in some cells (410 X reduced to 266 X, PAS).
- Fig 14. 24 hours: Medulla at 4 C. Cellular configuration resembles control (0 hour) tissue. Clumped chromatin and nucleoli are prominent but vascular sinusoids are not readily apparent (410 X reduced to 266 X, PAS).
- Fig 15. 48 hours: Medulla at 4 C. Cellular shrinkage and retraction from the reticular framework is conspicuous. Clumps of shrunken cells have feathered-edged cellular outlines (410 X reduced to 266 X, PAS).
- Fig 16. 96 hours: Medulla at 4 C. Cellular shrinkage is not as prominent as in 48 hour tissues. Cellular outlines are indistinct, and the cytoplasm is stippled with granules. Most nuclei are vesicular with clumped chromatin, but a number are becoming pyknotic. Vascular sinusoids and endothelial cells are becoming more conspicuous (410 X reduced to 266 X, PAS).

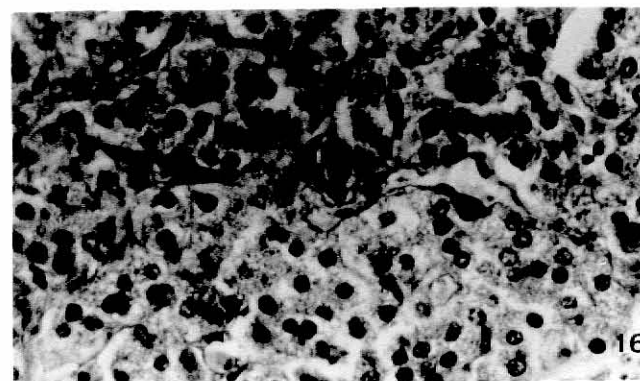
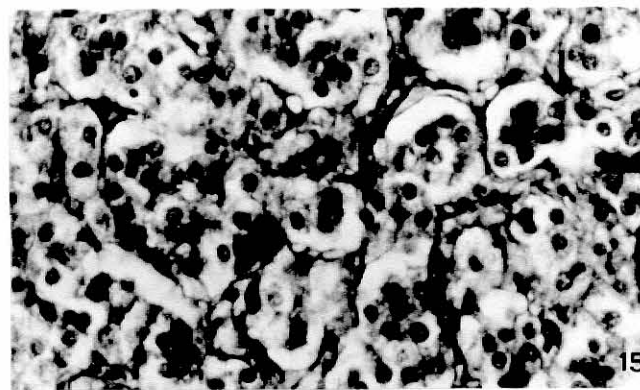
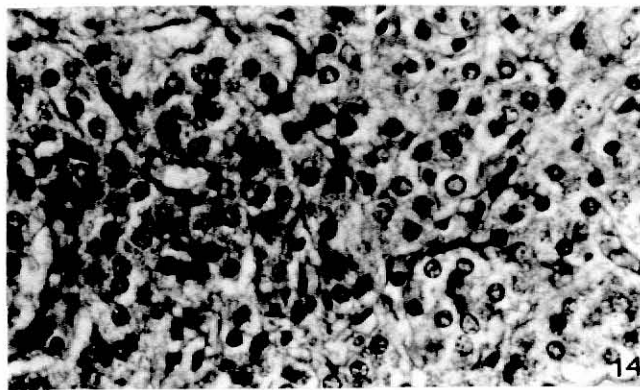
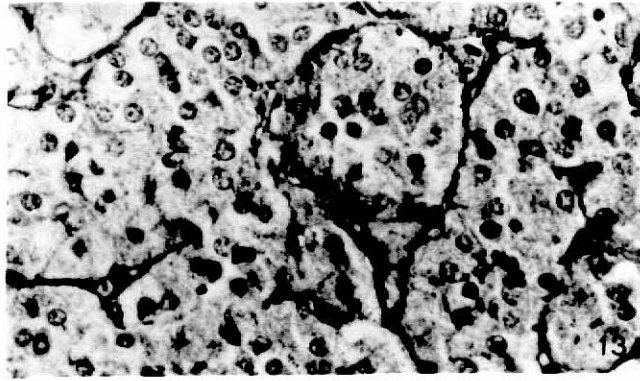


Fig 17. 0 hour: Zona arcuata. Located beneath the capsule (c), this narrow zone is composed of round to ovoid cells with incomplete cellular outlines and finely granular cytoplasm. Round, vesiculated nuclei are prominent, resulting in a large nuclear to cytoplasmic ratio (410 X reduced to 273 X, PAS).

Fig 18. 3 hours: Zona arcuata at 24 C. Beneath the capsule (c), cells of this zone have shrunk away from their reticular framework and the nuclei are deeply basophilic (410 X reduced to 273 X, PAS).

Fig 19. 6 hours: Zona arcuata at 24 C. Individualization of shrunken cell clusters has occurred. Nuclei are hyperchromatic to pyknotic (410 X reduced to 273 X, PAS).

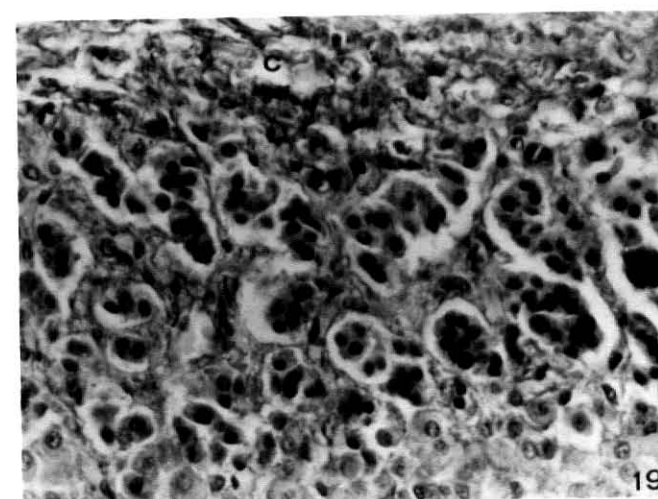
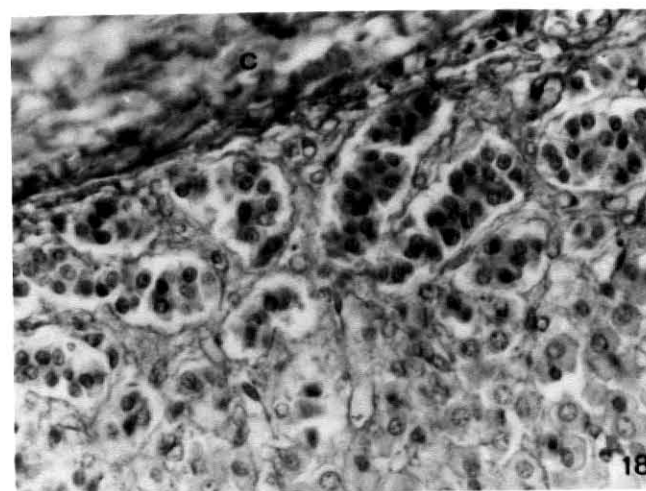
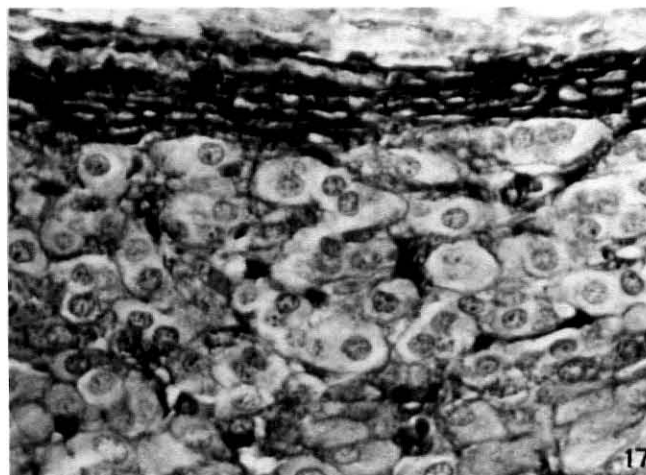


Fig 20. 12 hours: Zona arcuata at 24 C. Cells in this thin zone beneath the capsule (c) have little stainable cytoplasm and are in the process of individualization (410 X reduced to 273 X, PAS).

Fig 21. 48 hours: Zona arcuata at 24 C. Beneath the capsule (c), few nuclei remain. Cellular individualization is conspicuous (256 X reduced to 170 X, PAS).

Fig 22. 48 hours: Zona arcuata at 24 C. Nuclei have been lost by karyolysis. Cells are markedly shrunken into small clusters (410 X reduced to 273 X, PAS).

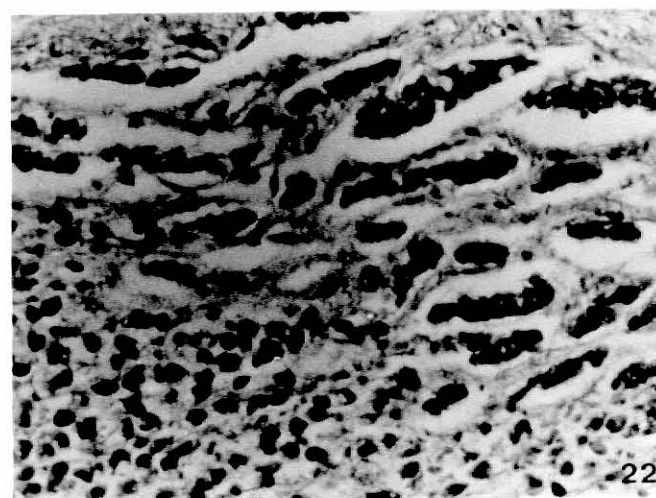
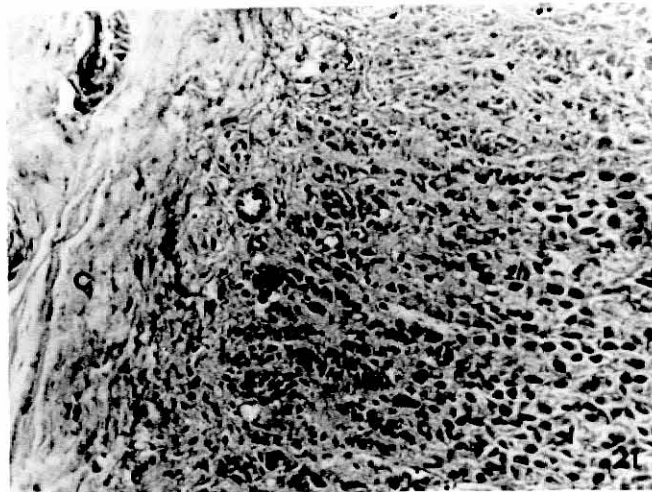
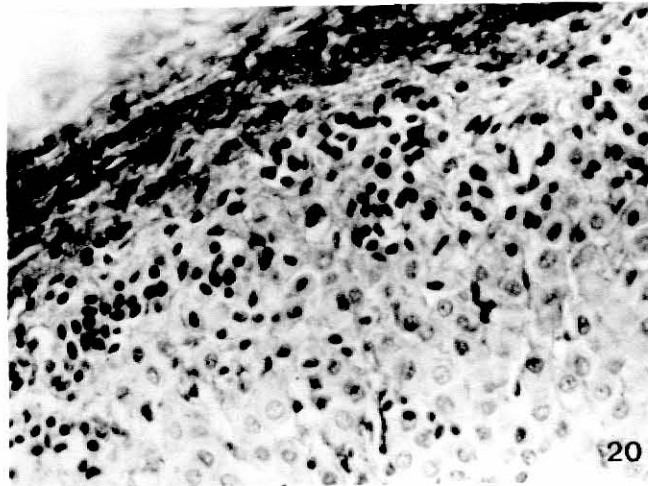


Fig 23. 0 hour: Zona fasciculata. Parallel cords of cells radiating towards the medulla are one to two cells in width, oriented longitudinally on a meshwork of sinusoidal capillaries (s). The large, polyhedral cells have distinct membrane borders, foamy cytoplasm and prominent, vesicular, round nuclei. Nuclei are located both centrally and eccentrically (410 X reduced to 273 X, PAS).

Fig 24. 6 hours: Zona fasciculata at 24 C. Cells of this zone have started to shrink and individualize, but the parallel cord architecture is intact. Chromatin clumping is visible within nuclei. Endothelial cells, and sinusoids (s) are easily seen (410 X reduced to 273 X, PAS).

Fig 25. 12 hours: Zona fasciculata at 24 C. Sinusoids (s) are conspicuous between cords of shrunken, individualized cells of this zone. Endothelial cells are plump, oval and basophilic (410 X reduced to 273 X, PAS).

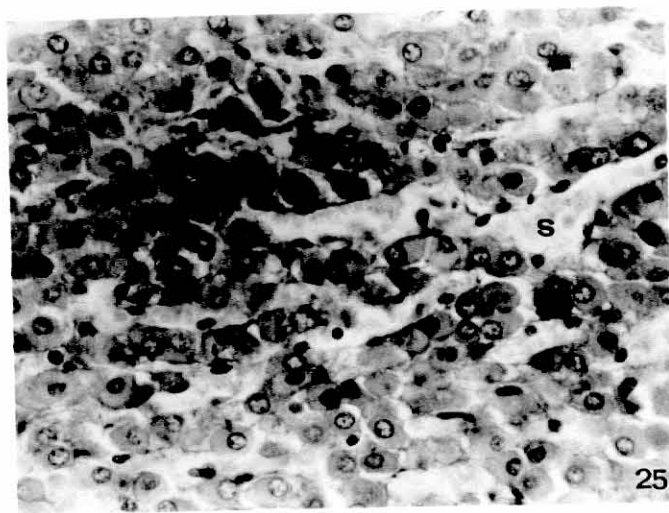
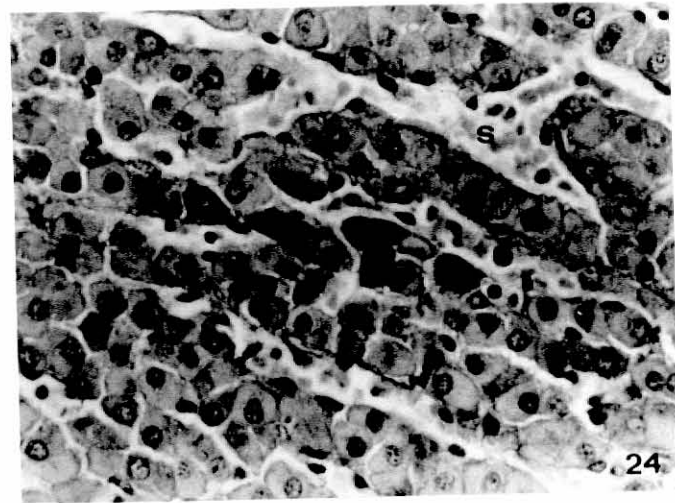
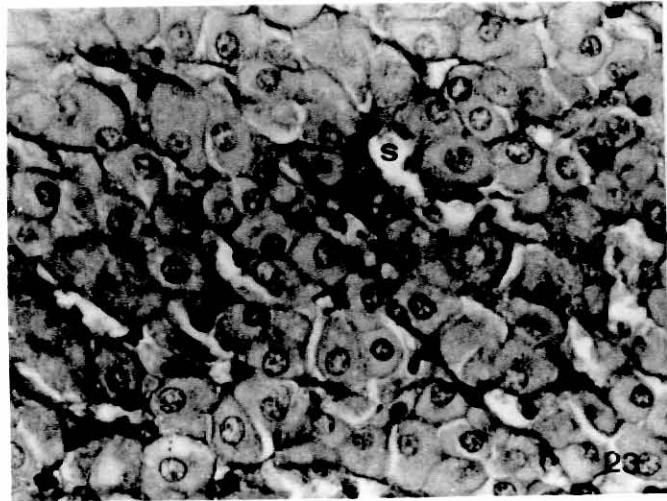


Fig 26. 24 hours: Zona fasciculata at 24 C. Parallel cord architecture is still present, but the cells have shrunk and individualized. Nuclei are pyknotic and karyolytic. Pyknotic endothelial cells within prominent sinusoids (s) are evident between the cords of cells. Some endothelial cells have sloughed into vessel lumens (410 X reduced to 273 X, PAS).

Fig 27. 24 hours: Zona fasciculata at 24 C. Disruption of the parallel architecture, karyolysis, pyknosis, and cellular debris are prominent (410 X reduced to 273 X, PAS).

Fig 28. 48 hours: Zona fasciculata at 24 C. Karyolysis, loss of parallel cord architecture, and cellular shrinkage and individualization are visible. Bacterial colonies (b) are dispersed among severely autolyzed cells of this zone (410 X reduced to 273 X, PAS).

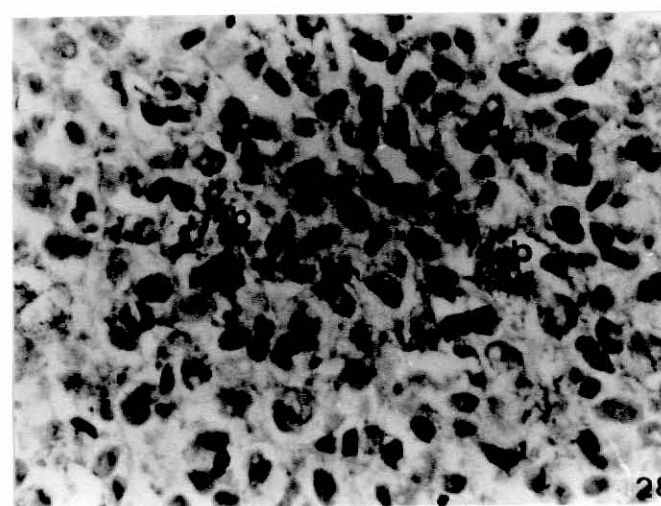
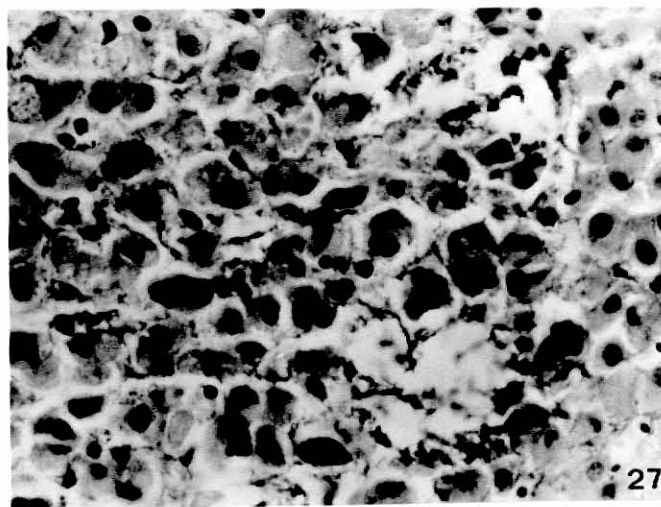
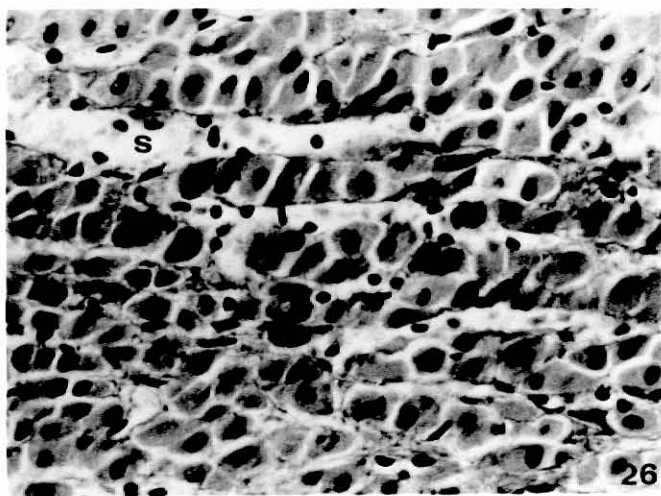


Fig 29. 0 hour: Zona reticularis. Cells arranged in a rete network lack distinct cellular borders. The cytoplasm stains deeply and has a granular appearance. Nuclei are round and vesicular, with clumped chromatin distributed marginally. Vascular sinusoids (s) are visible (410 X enlarged to 512X, PAS).

Fig 30. 24 hours: Zona reticularis at 24 C. Cell shrinkage and retraction from the reticular framework is conspicuous. Cellular individualization is also seen. The condensed granular cytoplasm stains darkly. Nuclei have become hyperchromatic to pyknotic and prominent with the vascular channels (s) (410 X enlarged to 512 X, PAS).

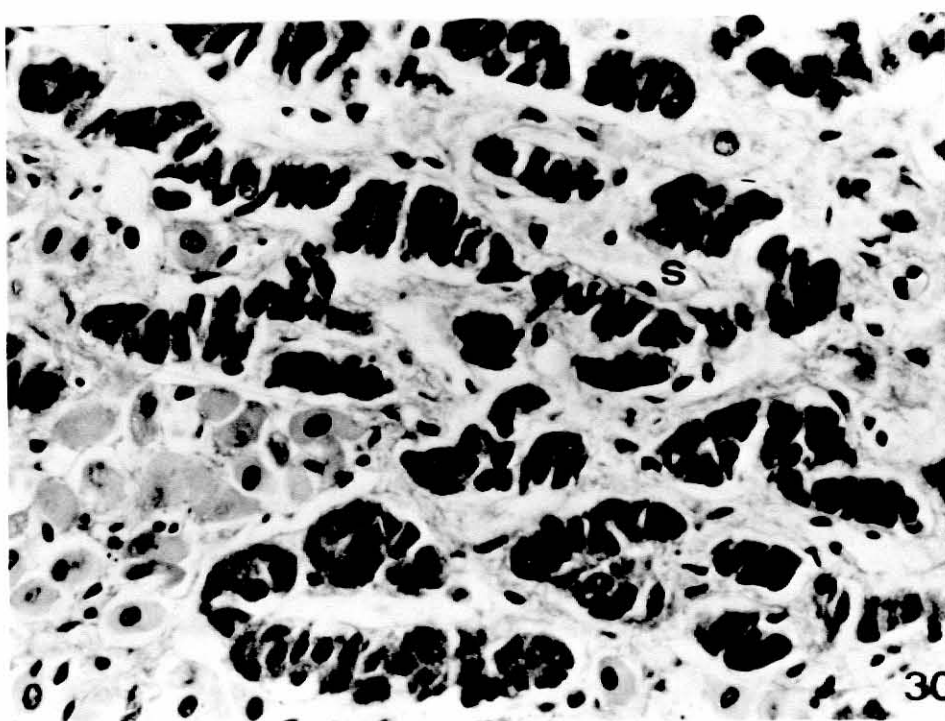
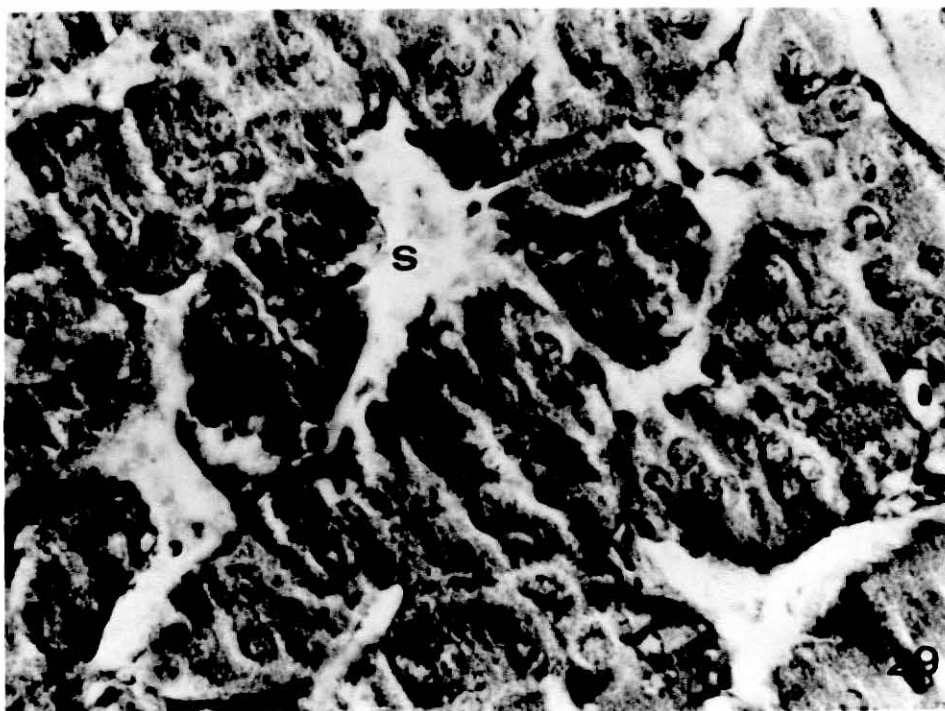


Fig 31. 0 hour: Adrenal medulla. Medullary cells form nests separated by vascular channels. The cells lack distinct cytoplasmic outlines, have stippled cytoplasm, and large, rounded vesicular nuclei. Nucleoli are conspicuous in some nuclei (410 X enlarged to 512 X, PAS).

Fig 32. 48 hours: Adrenal medulla at 24 C. Cells have shrunk and retracted from their reticular framework. Some cellular individualization is present, but most cell outlines are indistinct. Nuclei are pyknotic. Sinusoids with pyknotic endothelial cells are visible (410 X enlarged to 512 X, PAS).

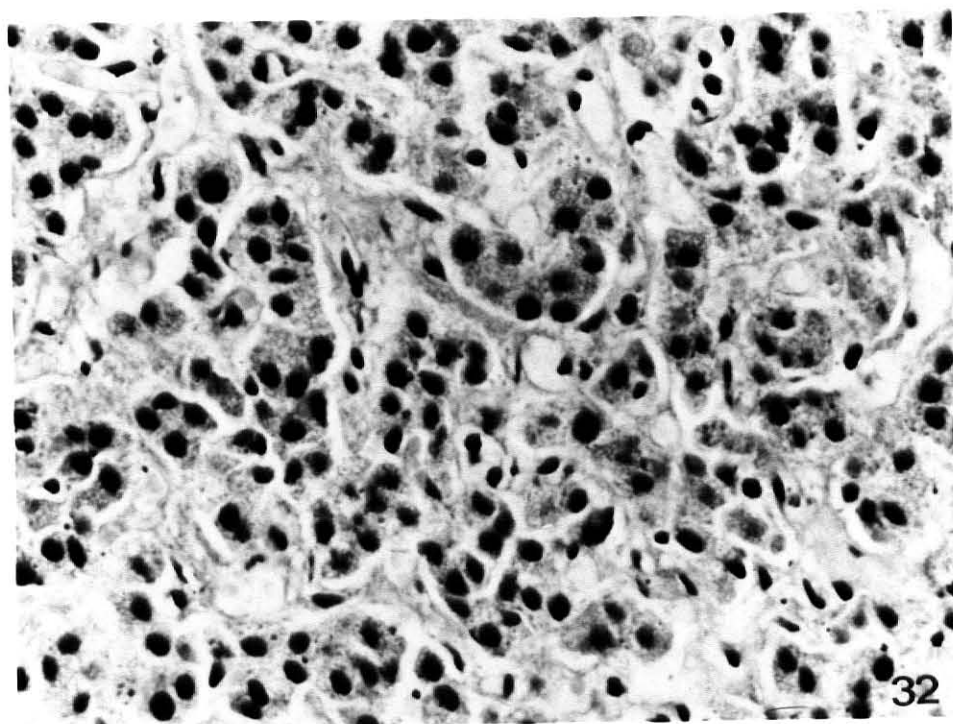
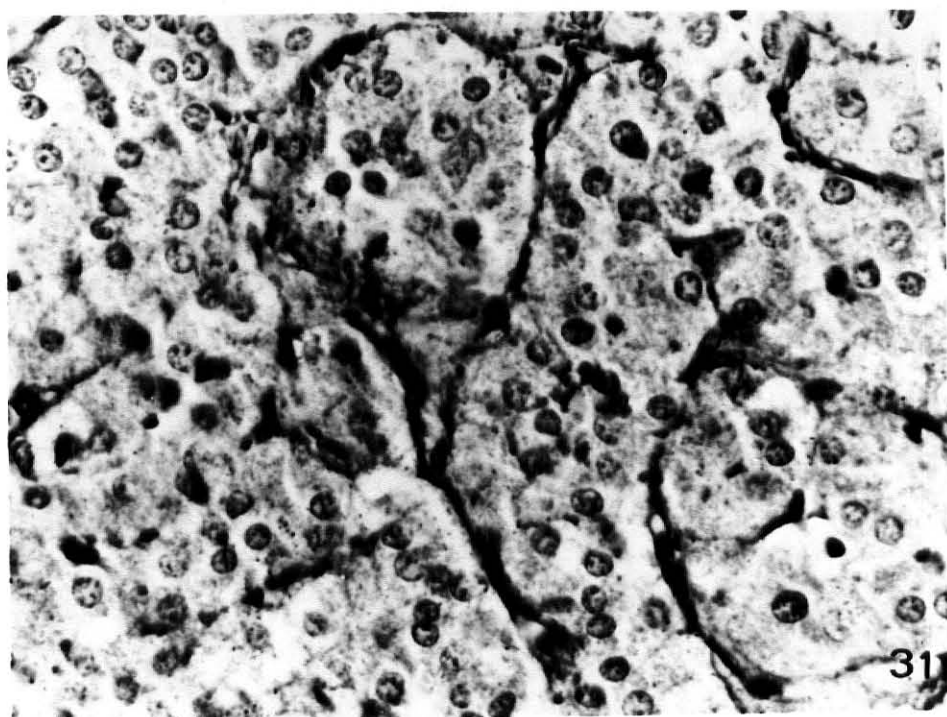
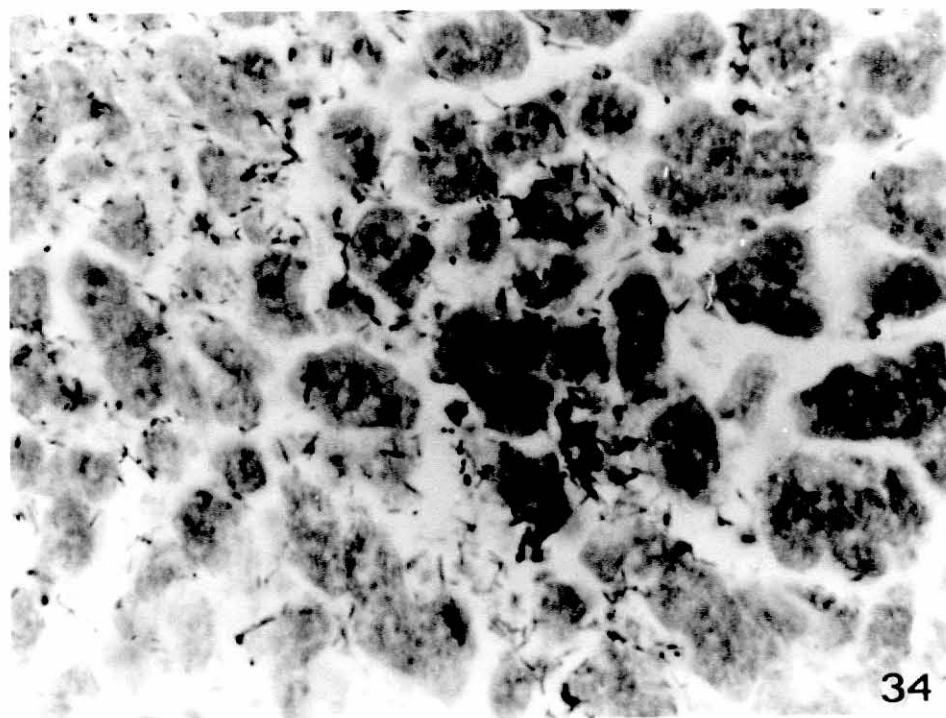
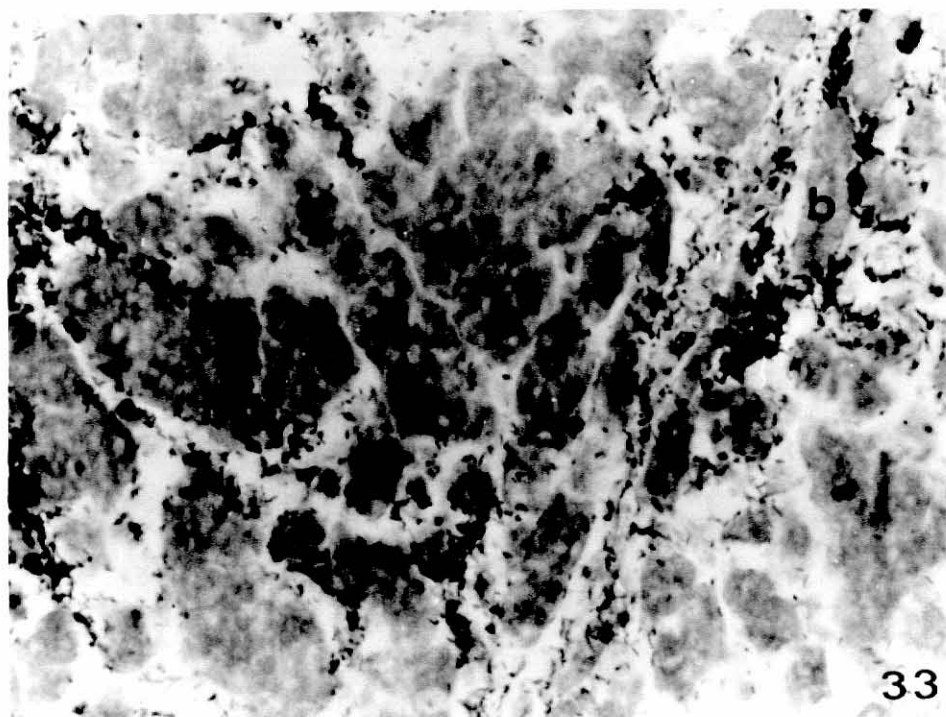


Fig 33. 96 hours: Adrenal tissue at 24 C. Adrenal cells are autolyzed beyond recognition. Complete karyolysis of nuclei, and extensive bacterial invasion (b) predominate in this section. The architecture is destroyed, making positive identification of this zone impossible (410 X enlarged to 512 X, PAS).

Fig 34. 96 hours: Adrenal tissue at 24 C. Loss of zonal architecture and staining characteristics, total karyolysis, and bacterial colonies (b) are primary features of autolytic adrenal glands. (410 X enlarged to 512 X, PAS).



SEQUENTIAL HISTOLOGIC POSTMORTEM CHANGES IN
PORCINE KIDNEYS AND ADRENAL GLANDS

by

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AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

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1980

ABSTRACT

To study histologic changes in porcine kidneys and adrenal glands, 28 clinically normal six-to-eight-week-old pigs were electrocuted and tissue samples were collected at 3, 6, 12, 24, 48, and 96 hours after death. For each postmortem interval, two unopened carcasses were refrigerated at 4 C and two were maintained at 24 C until necropsy. Four pigs served as controls: two were necropsied at 0 hour, and two were anesthetized, perfused with 10% buffered neutral formalin, and necropsied immediately. All tissue samples were fixed in 10% buffered neutral formalin. Histologic changes were evaluated on tissue sections stained with hematoxylin and eosin, and by periodic acid Schiff.

In kidneys, glomeruli swelled and obliterated Bowman's space in all except the perfused specimens. Refluxed debris from proximal convoluted tubules was present in the space. An increasing number of affected glomeruli were seen with increased postmortem interval. Glomerular tufts began to shrink and nuclear pyknosis was conspicuous within 24 hours at 4 C, and 12 hours at 24 C. Distal convoluted tubules were severely affected within 3 hours. Collecting tubules were moderately changed within 12 hours at 4 C, and by 3 hours at 24 C.

In the adrenal glands, cells of the zona arcuata became shrunken and individualized, with pyknotic nuclei within

6 hours at both temperatures. Large bacterial rods were present in the zona fasciculata at 24 and 48 hours at 24 C. No bacteria were observed in refrigerated specimens. Moderate disruption of the parallel cord architecture of this zone appeared within 12 hours and increased in severity through 96 hours at 24 C. Moderate to marked changes were noted in the zona reticularis within 12 hours at both temperatures. Medullary cells were moderately shrunken with hyperchromatic nuclei in adrenals at 4 C; these changes were marked within 24 hours, at 24 C.

With the exception of the distal tubules, renal structures were still useful for diagnostic purposes after 96 hours at 4 C, and up to 24 hours at 26 C. The adrenal glands were diagnostically useful 48 hours after death in carcasses cooled to 4 C. They could only be used for up to 12 hours at 24 C.