23

SEQUENCE OF HISTOLOGIC ALTERATIONS IN PORCINE LIVER AND GALL BLADDER UNDERGOING POSTMORTEM AUTOLYSIS

BY

MICHAEL ADEKUNLE SALAKO
D.V.M., Ahmadu Bello University, Zaria, Nigeria, 1975

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1978

Approved by:

Samuel M Kruckenberg

Major Professor

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to his major Professor, Dr. S. M. Kruckenberg, for his advice, guidance and tolerance throughout his stay in the Department of Pathology at Kansas State University.

The advice and supervision of Dr. S. M. Dennis, Professor and Head, Department of Pathology is also appreciated. Sincere appreciation is extended to the Ahmadu Bello University, Zaria, Nigeria, and to the KSU/AID for the opportunity for this training. Dr. J. E. Cook and Dr. H. W. Leipold are complemented for their advice, and Dr. S. G. Lake for helping in the necropsy procedures.

Finally, the author wishes to thank his parents for giving to their children the education they did not have the opportunity to get, and to his wife for her endurance and perseverance.

INTRODUCTION

Postmortem decomposition or autolysis, is defined as enzymatic self-digestion of cells or tissues after death.[15] It is an important phenomenon that must be taken into consideration for accurately interpreting cellular and tissue changes.[30] Because it follows death of an animal as a whole, inflammatory cells are not expected to be present as in tissue necrosis.[30] Autolysis was first noted in 1871 by Hoppe-Seyler, and Jacoby introduced the term "autolysis" in 1900.[21] The extent of postmortem autolysis is influenced by how long an animal has been dead, environmental factors prior to and following death, body weight, external insulation, nutritional status, age and species of animal.[22,20,30,36,1,2]

Some work has been done on the study of postmortem decomposition,[4,5,6,10,11,19,23,24,27] but most investigations have been directed towards the later stages of the process, such as proteolysis, in the affected cells.[18] Berembom et al.[5,6] stated that autolysis and necrosis result from isolating a tissue from its normal supply of nutrients. This results in interruption of metabolism by toxic, physical and chemical agents. Bradley[7,8] claimed that autolysis can be prevented by good blood supply, and Wilhelmi et al.[37,38] demonstrated that glucose and good blood supply helped to prevent autolytic changes with anoxia.

The role of lysosomes can not be overemphasized. These inner cytoplasmic organelles contain hydrolytic enzymes,

including acid phosphatase.[28] These enzymes digest the tissues which produce them and hence the term "autolysis" or self-digestion.

Splitter and McGavin[33] noted that hepatocyte nuclei in guinea pigs disappeared almost entirely by karyolysis, and that nuclear fading accompanied by margination of nuclear chromatin was present by 6 hours after death at 26°C. Although Munger and McGavin[27] did not observe pyknosis in chicken hepatocytes, they observed karyorrhexis at 6 and 12 days postmortem at 4°C.

The concept of postmortem autolysis, simple as it appears, is important. Recent studies indicated that individual and species variation may be more significant than previously thought.[26,33] This experiment was designed to study the process in pigs.

The objectives of this study in pigs were to:

1. determine the sequence of histological changes in the liver and gall bladder resulting from postmortem autolysis;
2. estimate the time postmortem when tissues would not be useful under light microscopy;
3. determine the time postmortem giving the most readable sections;
4. study the sequential histological changes in liver and gall bladder in the intact carcasses maintained at -10°C, 4°C, and 26°C.

REVIEW OF LITERATURE

The importance of postmortem decomposition has been realized for over a century. For a complete and accurate histopathologic interpretation of cellular and tissue reactions, a pathologist must be able to differentiate between antemortem necrosis and postmortem decomposition.[22,20,31]

Since Hoppe-Seyler reported dissolution of dead tissue without previous putrefaction in 1871, several workers have investigated the concept of autolysis in man, guinea pigs,[33] chicken,[27] rat,[38,33] and other laboratory animals.[4,28] Jacoby introduced the term "autolysis" in 1900,[21] after Solkowski reported that postmortem decomposition was due to digestion of tissue by the enzymes it produced.[32] Bradley[7,8] claimed that autolysis can be prevented by good blood supply and Wilhelmi et al.[37,38] demonstrated that glucose and adequate blood supply helped to prevent autolytic changes from anoxia. It can be rightly assumed that, so far, most investigations on autolysis have been directed towards the later stages of the process, such as proteolysis in affected cells.[18]

The liver is the largest organ in the body and since it receives, through the portal blood, most of the absorbed products from the small intestine, it is clear that many of its functions relate to the metabolism of the absorbed products.[14] Among other functions of the liver are secretion of bile; metabolism of protein; carbohydrate and fat; detoxification of harmful substances; storage of vitamins; destruction of red blood cells; formation of blood proteins, i.e., synthesis of fibrinogen, albumin, globulin and prothrombin.

The liver is also responsible for phagocytosis of foreign particulate matter, conjugation of toxic substances and steroid hormones and finally, hemopoiesis *in utero* and potentially, in adults.[16]

In vitro and *in vivo* experiments have been performed with variable successes and setbacks. Results of *in vitro* experiments by Cruickshank[10] were influenced by inability to collect tissue samples aseptically, edema, rapid cellular plasmolysis, and retardation of nuclear changes in organs kept in physiological saline. Organs kept in moist chambers did not give accurate results since the nuclei degenerated rapidly, like necrotic tissue *in situ* would degenerate. Nevertheless, various observations were made regardless of whether the experiments were conducted *in vivo* or *in vitro*. These include Cruickshank's observation in 1912[10] that autolysis altered the histologic appearance of tissues, and that the process of autolysis was also modified by the presence of pathogenic bacteria. Gallagher *et al*. noted that the initial autolytic process in the liver of the rat resulted from failure of definitive enzyme systems.[18] The labile oxidative phosphorylation system, and the loss of respiratory co-factors were believed to trigger the series of reactions. It was also found that the addition of glucose and L-glutamate protected and reversed these failures.[18] Morrione *et al*. observed that microscopic sections prepared from livers fixed in Zenker's solution or formalin had little or no decrease in stainable glycogen, when fixation was delayed for

as long as 10 hours after death or surgical biopsy.[26] They concluded from necropsies performed as long as 10 hours post-mortem, that the microscopic appearance of glycogen in liver was a good index for stainable hepatic glucose content at time of death. However, chemical analysis for glycogen revealed marked decreases for corresponding periods.

Studies have also been done on the role of cellular components including deoxyribonucleic acid (DNA), mitochondria, and cell membrane. DNA studies by Stowell suggested that DNA was probably lost rapidly following death.[35] This was indicated by the rapid loss of the Fuelgen positive reaction. Ito,[19] in his investigations, concluded that mitochondria, granular reticulum, the nuclear envelope and cellular membranes were more resistant to autolytic changes than plasma membranes, Golgi complex and smooth surface reticulum.[19,20] However, Trump reported that mitochondria was the first to be affected by autolytic changes, and that various regions of the plasma membrane degenerated differently.[35,36] He noted loss of microvilli, interruptions in membrane continuity, bleb formation and enlargement of the space of Disse in the sinusoids, but not in bile canaliculi.

Following cell death, an early cytoplasmic change is increased eosinophilia due to increased binding of eosin by cytoplasmic protein as a result of pH change and loss of cytoplasmic basophilia.[9,23] Swelling of mitochondria can be observed as eosinophilic globules, and granulation and irregular spaces represent denatured and digested cytoplasmic organelles.[23]

Splitter and McGavin[33] noted in guinea pig hepatocytes undergoing autolysis that nuclei disappeared almost entirely by karyolysis, and that nuclear fading accompanied by margination of nuclear chromatin was present by 9 hours postmortem. They also observed that hepatocyte cytoplasmic basophilia started to disappear centrilobularly by 0.75 hours and was completely lost by 3 hours. Pyknosis of Kupffer cells was also observed to have begun as early as 1.5 hours. Perhaps the most significant observation was individualization of hepatocytes and the presence and prominence of the space of Disse. They also observed separation of the bile duct epithelium from the basement membrane at 18 hours.

Munger and McGavin[27] found that Kupffer cells and periportal lymph nodules became pyknotic and fragmented earlier than fading and karyolysis of nuclei of epithelial-type cells of the bile ducts and hepatocytes. They also indicated that pyknosis did not occur in chicken hepatocytes; and that karyorrhexis was observed only 6 and 12 days postmortem at 4°C. Because most periportal lymph node cells and most Kupffer cells either fragmented or disappeared by 12 hours, they suggested that Kupffer cells recognizable after long periods may actually have been different, perhaps "endothelial" cells.

Discussions about autolysis would be incomplete without mentioning hydrolytic enzymes, and in particular lysosomes, which are part of the inner organelles and contain the enzymes.[12]

The concept of lysosomes was first introduced in 1955.[3,13] Lysosomes contain great amounts of acid phosphatase and other hydrolytic enzymes. Pekkanen,[28] in studying the passage of acid phosphatase from lysosomes in bovine liver undergoing autolysis, found that the total acid phosphatase activity decreased with time. Sawant et al. also noted that the stability of lysosomal membranes decreased when the pH of their surrounding deviated towards acidity or alkalinity.[31] The activity of lysosomal enzymes seems to be closely associated with events resulting in or preceding physiological or pathological cell death, and hence are important in the autolytic process.

REFERENCES

1. Anderson, W. A. D., Pathology, Vol. 1, 7th ed., C. V. Mosby Co., St. Louis, Mo., (1972), pp. 94-95.

2. Anderson, W. A. D., Pathology, Vol. 2, 7th Ed., C. V. Mosby Co., St. Louis, Mo., (1972), pp. 1321-1324.

3. Appelmans, F., Wattiauz, R., and DeDeuve C., Tissue Fractionation Studies 5. The Association of Acid Phosphatase with a Special Class of Cytoplasmic Granules in Rat Liver. Biochem J. 59, (1955), pp. 348-445.

4. Baker, H. D. C., Ischaemic Necrosis of Mouse Liver Tissue in the Peritoneal Cavity. Lab Invest. 10, (1961): pp. 11-128.

5. Berenbom, M., Chang, P. I., Betz, H. E., and Stowell, R. E.: Chemical and Enzymatic Changes Associated with Mouse Liver Necrosis in vivo. Cancer Research, 15, (1955): pp. 1-5.

6. Berenbom, M., Chang, P. I., and Stowell, R. E., Changes in Mouse Liver Undergoing Necrosis in vivo. Lab Invest. 4, (1955): pp. 315-323.

7. Bradley, H. C.: Autolysis and Atrophy. Physiol. Review, 18, (1938): pp. 73-196.

8. Bradley, H. C., Studies of Autolysis, VII. The Nature of Autolytic Enzymes. J. Biol. Chem., 52, (1922): pp. 467-484.

9. Cheville, N. F., Cell Pathology, 1st Ed., Iowa State University Press, Ames, Iowa, (1976), p. 50.

10. Cruickshank, J. The Histological Appearances Occuring in Organs Undergoing Autolysis, J. Path., 16 (1912), pp. 167-183.

11. Dawkins, M. J. R., Judah, J. D., and Rees, K. R.: Factors Influencing the Survival of Liver Cells During Autolysis. J. Path. Bact., 77 (1959), pp. 257-275.

12. DeDuve, C.: The Lysosome Concept. In Lysosome, Ciba Foundation Symposium. Ed. V. S. de Reuck and M. P. Cameron. J. A. Churchill, Ltd., London, (1963), pp. 1-28.

13. DeDuve, C., Presman, B. C., Gianetto, R., Wattiaux, R., and Appelmans, F.: Tissue Fractionation Studies 6. Intracellular Distribution Patterns of Enzymes in Rat Liver Tissue: Biochem. J., 60 (1955), pp. 604-607.

14. Dellman, H., Brown, E. M.: Textbook of Veterinary Histology, 1st Ed. Lea and Febiger, Philadelphia, PA, (1976), pp. 253-260.

15. Dorland's Illustrated Medical Dictionary, 25th Ed., Saunders. Philadelphia, PA.

16. Duke's Physiology of Domestic Animals, 8th Ed., Cornell University Press; Ithaca, NY, (1970), pp. 369-397.

17. Florey, H.: General Pathology, 4th Ed., W. B. Saunders Co., Philadelphia, PA, (1970), pp. 444-448.

18. Gallagher, C. H., Judah, J. C., and Rees, K. R., Enzyme Changes During Liver Autolysis: J. Path. Bact., 72, (1956): pp. 247-256.

19. Ito, S.: Light and Electron Microscopic Study of Membranous Cytoplasmic Organelles. In the Interpretation of Ultrastructural, Ed. by Harns, R. J. C., (1962): pp. 129-148.

20. Ito, S.: Postmortem Changes of the Plasma Membrane. In Electron Microscopy. Fifth International Conference for Electron Microscopy, Philadelphia, PA, August 29-September 5, 1962. Ed. by Breese, S. S., Vol. 2, pp. 1-5, New York, Academic Press, Inc. 1962.

21. Jacoby, M., cited by Florey, H., General Pathology, 4th Ed., W. B. Saunders Co., Philadelphia, PA, (1970), pp. 444-448.

22. Jubb, K. V. F., and Kennedy, P. C., Pathology of Domestic Animals, Vol. 2, Academic Press, NY, (1963), p. 247.

23. Lavia, M. F., and Hill, R. B., Principles of Pathobiology, 2nd Ed., Oxford University Press. New York, (1975), p. 13.

24. Majno, G., LaGattuta, M. L., and Thompson, T. E.: Cellular Death and Necrosis: Chemical, Physical and Morphologic Changes in Rat Liver. Virch. Arch. Path. Anat., 333, (1960), pp. 421-465.

25. Minjer, A., Histological Examination of the Breakdown of Hepatic Glycogen by Postmortem Glycogenolysis and by Action of Saliva, J. Path. Bact., 73, (1957), p. 11.

26. Morrione, T. G., and Mamelok, H. C., Observations on the Persistence of Hepatic Glycogen After Death, Am. J. Path., 28, (1952), pp. 497-502.

27. Munger, L. L., and McGavin, M. D., Sequential Postmortem Changes in Chicken Liver at 4, 20, or 37°C. Avian Diseases, 16, No. 3, (1972), pp. 587-605.

28. Pekkanen, T. J., Distribution of Acid Phosphatase During Autolysis in Bovine Liver, Acta., Vet. Scand., 11, (1970), pp. 283-294.

29. Runnels, R. A., Monlux, W. S., and Monlux, A. W., Principles of Veterinary Pathology, 7th Ed., The Iowa State University Press, Ames, Iowa, (1965), pp. 236-239.

30. Sawant, P. L., Desal, I. D., and Tappel, A. L., Factors affecting the lysosomal membranes and availability of enzymes. Arch. Biochem., 105, (1964), pp. 247-253.

31. Smith, H. A., Jones, T. C., and Hunt, R. D., Veterinary Pathology, 4th Ed., Lea and Febiger, Philadelphia, PA, (1974), pp. 21-23.

32. Solkowski, E., cited by Florey, H.: General Pathology, 4th Ed., W. B. Saunders Co., Philadelphia, PA, (1970), pp. 444-448.

33. Splitter, G. A., and McGavin, M. D., Sequence and Rate of Postmortem Autolysis in Guinea Pig Liver. Am. J. Vet. Res. Vol. 35, No. 12, (1974), pp. 1591-1596.

34. Stowell, R. E., Chang, J. P., and Berenbom, M., Histochemical Studies of Necrosis of Mouse Liver Tissue in Peritoneal Cavity. Lab. Invest. 10, (1961), pp. 111-128.

35. Trump, B. F., Goldblatt, P. J., and Stowell, R. E., An Electron Microscopic Study of Early Cytoplasmic Alterations in Hepatic Parenchymal Cells of Mouse Liver During Necrosis in vitro (Autolysis). Lab. Invest. 11, (1962), pp. 986-1015.

36. Trump, B. F., Goldblatt, P. J., and Stowell, R. E., Studies on Necrosis of Mouse Liver in Vitro, Ultrastructural Alterations in Mitochondria of Hepatic Parenchymal Cells. Lab. Invest., 14, (1965), pp. 343-371.

37. Wilhelmi, A. E., Engel, M. G., and Long, C. N. H., Influence of Feeding on the Effects of Hepatic Anoxia on the Respiration of Liver Slices in Vitro. Am. J. Phys., Vol. 147, (1946), p. 181.

38. Wilhelmi, A. E., Russell, J. A., Engel, F. L., and Long, C. H. H. The Effects of Hepatic Anoxia on the Respiration of Liver Slices in Vitro. Am. J. Phys., Vol. 144, (1947), p. 669.

PAPER I

SEQUENCE OF HISTOLOGIC ALTERATIONS IN PORCINE LIVER AND GALL BLADDER UNDERGOING POSTMORTEM AUTOLYSIS AT 26°C.

INTRODUCTION

Autolysis is the enzymatic self-digestion of tissues after death. The rate of autolysis is influenced by how long an animal has been dead, environmental factors prior to and following death, body weight, external insulation, nutritional status, age and species of animal.[4,7,8]

The first changes observed are loss of cytoplasmic basophilia and granularity. Cytoplasmic eosinophilia becomes evident due to increased affinity for eosin by the cytoplasmic proteins. In the nucleus, typical changes are pyknosis, karyolysis, and karyorrhexis.[7,8] Occasionally, margination of nuclear chromatin are observed, particularly in guinea pigs.[9]

Karyolysis is secondary to pyknosis in Kupffer cells. Swelling and pyknosis are the most constant autolytic changes in Kupffer cells.[9] Gall bladder epithelial sloughing is rapid.

MATERIALS AND METHODS

Sixteen pigs of both sexes weighing about 17 kg and 6 to 8 weeks old were electrocuted by connecting one electrode behind an ear and another on the tail and a current of 110 volts was passed for 10 seconds between the 2 electrodes. Two pigs were necropsied as quickly as possible following electrocution, and 1 x 6 x 3 cm sections of liver were removed from the lateral aspects of the left and right lateral

lobes and immediately placed in 10% buffered neutral formalin (BNF) to serve as controls. A piece of gall bladder was also taken. The other pigs were kept in a chamber at a constant temperature of 26°C and relative humidity of 50%. Two pigs were necropsied at 3, 6, 12, 24, 48 and 96 hours postmortem, respectively, and sections of liver and gall bladder were taken and placed in 10% BNF.

Another set of control tissues was collected by anesthetizing 2 pigs by Rompun[a] intramuscularly. Blood was allowed to flow by cutting the femoral vein and 10% BNF was perfused under pressure by connecting a tube containing the solution to the left ventricle. Perfusion was stopped when the clear color of 10% BNF drained from the femoral vein instead of blood. The pigs were necropsied and similar liver and gall bladder sections were taken and fixed in 10% BNF.

The tissues were dehydrated through graded ethanols, cleared in xylene, and cut at 6 microns. They were then stained with Harris' hematoxylin and eosine Y (H&E). Some sections were stained with periodic acid-Schiff (PAS) for glycogen and control slides digested by diatase (PAS-diatase) were also prepared to rule out glycoproteins which also accept this stain.[1] Sections were also stained with Spicer's stain for cytoplasmic RNA and nuclear DNA.

---

[a]5,6-Dihydro-2-(2,6-xylidino)-H-1,3-thiazine; Haver-Lockhart, BAYVET Division, Cutter Laboratories, Inc., Shawnee, Kansas 66201.

Microscopic observations were under magnifications of 40 and 400 and scored as "present" or "absent", negative (-) or positive (+), or adequately described. The degree of positivity ranged from plus one (+1) to plus three (+++), as indicated below.

Grade	Remarks
+	Slightly present
++	Moderately present
+++	Distinct

RESULTS

Gross Findings

The perfused liver appeared lighter than non-perfused which was mahogany red, the difference being attributed to the effect of formalin flushing erythrocytes from the perfused liver. The color was retained for about 3 hours postmortem after which it became increasingly lighter and irregular. Texture and firmness decreased from 6 hours postmortem and by 96 hours, the liver sections were virtually "poured" into the 10% BNF. Bile imbibition started before 3 hours postmortem and increased with time depending on the amount of bile present in the gall bladder.

Microscopic Findings

General Architecture

Persistence of the lobular pattern throughout the entire experiment was remarkable. Even at 96 hours postmortem, the interlobular fibrous connective tissue was still discernible. Both sets of control (zero hour perfused and zero hour non-perfused) had few erythrocytes within the sinusoids and blood vessels, though the sinusoids were dilated and interlobular connective tissue was widened due to formalin in the perfused sections (Fig. 1). The portal triads were prominent and all vessels were dilated in the controls (Fig. 3).

As time between death and necropsy increased, the amount of blood pooling in the sinusoids increased until between 12 and 24 hours when hemolysis was advanced enough to mask reasonable observations. Glycogen depletion also occurred with time, but there appeared to be lobular selectivity regarding the extent of depletion. Glycogen depletion was most marked in the midzonal region of the affected lobules.

Individualization of hepatocytes started at about 6 hours and increased progressively until 48 hours (Table 1). At 96 hours postmortem, many lobules contained clear air or gas spaces. Aggregates of extramedullary hematopoietic centers were observed along some interlobular septa. These cells were more hyperchromatic than hepatocytes in the controls, but slowly lost their staining ability with time. They were still observable as dark basophilic, round cells even at 96 hours postmortem.

Erythrocytes

Erythrocytes were scanty in both sets of controls in the sinusoids and vascular tissue (Fig. 2). This was expected in perfused pigs, and in recently killed pigs blood flowed freely from the small sample collected in 10% BNF. Erythrocytes tended to be present in greater numbers from 3 till 12 hours (Table 11). Clumping of erythrocytes within sinusoids and vessels, as well as loss of acidophilic staining, was observed at 6 hours. At 12 hours, most erythrocytes had disappeared as result of hemolysis and some dark-brown pigments of acid hematin was visible in some sinusoids. Ghost cells of erythrocytes were observed at 48 hours.

Sinusoids

Sinusoids were narrow in the zero hour non-perfused sections but dilated in perfused tissues though blood cells were not visible. At 3 hours postmortem, the sinusoids were observed to contain blood and the width or extent of dilation depended on the amount of blood present.

Portal Areas

The bile duct epithelium was preserved for as long as 12 hours postmortem. However, it contained pyknotic nuclei and the cytoplasm was slightly eosinophilic. At 24 hours, sloughing and separation of the bile duct epithelium was observed. The endothelial cells lining the veins were still intact, though swollen, and with pyknotic nuclei at 24 hours (Fig. 4). At 48 hours, the portal triads were more

discernible than at 96 hours postmortem, and some pyknotic nuclei were observed in the bile duct epithelium, though most cells had sloughed and separated from the lamina propria.

Hepatocyte Cytoplasm

The cytoplasm was faintly basophilic and finely granular in both sets of control at zero hour. Large clear spaces or vacuoles were observed in most hepatocytes that were PAS positive, and negative for PAS-diatase, indicating glycogen. Cytoplasmic basophilia started to disappear diffusely at about 3 hours. At 6 hours, the cytoplasm was more eosinophilic, with aggregation of basophilic material in "clumps" in most hepatocyte cytoplasm. These basophilic clumps persisted until 12 hours when the cytoplasm became mainly eosinophilic and the granularity was completely lost. At 96 hours, part of the cytoplasm was replaced by vacuoles (Fig. 6). The fate of glycogen, determined by PAS and PAS-diatase, indicated independent lobular activity. The disappearance was greatest in most cases in the midzonal region, and less so in the centrolobular and periportal areas. In some lobules, glycogen disappearance was mainly centrolobular, while in some, the pattern was periportal. Changes in glycogen concentration was first noted at 3 hours and became prominent by 6 hours. Total glycogen loss was not achieved throughout the entire experiment, since PAS positive materials were present in hepatocyte cytoplasm even at 96 hours postmortem.

Hepatocyte Nucleus

In both sets of control, the hepatocyte nuclei were centrally placed and relatively large. Typically, one nucleus was present per cell, but many hepatocytes had two nuclei. The chromatin granules were mostly one or two, but few cells had more than two. At 3 and 6 hours, the number of chromatin granules per nucleus increased to an average of 5 per nucleus. They were mostly scattered within the nuclei, though many hepatocyte nuclei had marginally arranged chromatin. At 12 hours postmortem, some nuclei were pyknotic and some karyorrhexic, but most appeared swollen and fading. With increased cytoplasmic eosinophilia, nuclear fading was more prominent at 24 hours (Table III). Some nuclei appeared fragmented. At 96 hours, only ghost-like appearance of nuclei was visible, as most had disappeared (Fig. 6).

Kupffer Cells

The Kupffer cells appeared mostly flattened with hyperchromatic nuclei. The zero hour perfused controls, however, had swollen Kupffer cells. Swelling and pyknosis of nuclei of the Kupffer cells were observed to increase with time and at 12 hours, they were oblong shaped (Table IV). At 96 hours, nuclear fading was noted, though the nuclei were still more hyperchromatic and discernible than hepatocyte nuclei. Swelling of the entire cell was so marked in some, that they resembled cells of the extramedullary hematopoietic centers.

Gall Bladder

The amount of sloughing of gall bladder epithelium was remarkable by 3 hours postmortem (Table V). Most of the epithelial cells were separated from the lamina propria and only spared in places where epithelial mucus was abundant on the surface (Fig. 9). In such areas, the nuclei were pyknotic and the cytoplasm eosinophilic. Exposed lamina propria displayed vacuoles resembling pox vesicles in areas where the epithelium had sloughed. The cells of the lamina propria immediately below the epithelium were vacuolated, empty and devoid of nuclei (Fig. 10). The changes were less severe progressively towards the muscular layer, the nuclei being present and pyknotic. The serosal surface appears swollen and edematous.

DISCUSSION

The perfused liver sections were included in the experiment to eliminate the possibility of autolysis between death and collection of specimens in the zero hour non-perfused control. Except for dilation of sinusoids and widening of the interlobular spaces in the perfused pigs, no other microscopic changes were detectable with the light microscope.

Gross findings were in accordance with the basic principles of pathology[7,8] namely, progressive loss of color and strength, increased odor of putrefaction, and bile

imbibition. Double nucleated hepatocytes are frequently encountered in young, growing animals.[2] Extramedullary hematopoietic centers are also commonly encountered in young and sometimes in adult animals.[2,3] The persistence of fibrous connective tissue reflects the fact that they are probably the last to undergo postmortem autolysis.[8]

Glycogen was not completely lost, since stainable glycogen was present even at 96 hours. Morrione and Mamelok[6] and later, Splitter and McGavin[9] made similar observations. Although centrolobular and periportal glycogen depletion were observed, most lobules had midzonal depletion, the picture being similar to necrosis presented by pigs fed aflatoxin and horses fed moldy hay.[5] Splitter and McGavin also observed similar glycogen loss in guinea pigs.[9]

Hepatocyte individualization was observed at 6 hours, the time reported by Splitter and McGavin.[9] They observed hepatocyte individualization as early as 6 hours postmortem at 26°C. It is possible that the thick fibrous connective tissue, responsible for the unique lobulation in porcine liver, is also responsible for holding the cells together and hence delaying individualization. It was also observed that while hepatocyte nuclei either faded or became pyknotic, the Kupffer cell nuclei became primarily pyknotic. Fading was secondary.

Loss of cytoplasmic basophilia and hence an increase in acidophilic staining is due to loss of nucleoproteins ribonucleic acid and deoxyribonucleic acid from the

cytoplasm and nucleus.[8] However, the persistence of basophilia in the form of cytoplasmic clumps in one area of the cell while the rest of the cells gradually took to acidophilic staining indicated that the cytoplasmic RNA was lost gradually within 12 hours postmortem. The clumps stained positive for Spicer's stain, indicating that they contained RNA.

The rate of gall bladder epithelial sloughing is worth mentioning. Total sloughing was achieved within 3 hours. Since the porcine gall bladder does not have a submucosa,[2] the lamina propria was always exposed following epithelial desquamation. The cells of the lamina propria were devoid of nuclei and cytoplasm and appeared like pox vesicles. This was the most consistent feature of this study as varying reactions were displayed by different cells. For example, only about 70% of the nuclei examined revealed fading, the rest pyknosis and fragmentation; 50% of the lobules presented midzonal glycogen depletion, 25% centrilobular, 15% periportal and about 10% diffuse glycogen depletion. It is, however, significant that mucus appears to play a preservative role, as gall bladder changes were less severe where epithelial mucus was abundant.

SUMMARY

Sixteen pigs were electrocuted to study the sequence of histologic changes in the liver and gall bladder.

Twelve pigs were maintained at 26° and 50% relative humidity, and four served as controls and were necropsied immediately. Liver and gall bladder sections were taken from 2 pigs at 3, 6, 12, 24, 48, and 96 hours postmortem, respectively, and fixed in 10% BNF.

In hepatocytes, loss of cytoplasmic basophilia was first observed at 3 hours and cytoplasmic clumping was prominent by 6 hours. Both loss of cytoplasmic granularity and individualization of hepatocytes were observed by 6 hours postmortem. Karyorrhexis of hepatocyte nuclei was not observed throughout the test period of 96 hours, but fragmentation of nuclear chromatin started as early as 3 hours postmortem. Hemolysis of erythrocytes began at 6 hours and was advanced at 12 hours.

Unlike hepatocytes, Kupffer cells nuclei became pyknotic rather than karyolytic. These changes were observable by 3 hours postmortem and became progressively more prominent with time. Loss of cytoplasmic glycogen was not of any particular pattern, though midzonal depletion was most prominent.

Sloughing of gall bladder epithelium was complete by 3 hours postmortem. Exposure of the underlying lamina propria was observed and cells were vacuolated. The epithelium was preserved only where epithelial mucus was abundant. The conventional porcine hepatic lobulation was maintained throughout the entire experiment.

REFERENCES

1. Cheville, N. F., Cell Pathology, 1st Ed., Iowa State University Press, Ames, Iowa, (1976), p. 50.

2. Dellman, H., Brown, E. M.: Textbook of Veterinary Histology, 1st Ed., Lea and Febiger, Philadelphia, PA, (1976), pp. 253-260.

3. Duke's Physiology of Domestic Animals, 8th Ed., Cornell University Press, Ithaca, NY, (1970), pp. 369-397.

4. Jubb, K. V. F., and Kennedy, P. C.: Pathology of Domestic Animals, Vol. 2, Academic Press, New York, NY, (1963), p. 1247.

5. McGavin, M. D. and Kanke, R.: Hepatic Midzonal Necrosis in a Pig Fed Aflatoxin and a Horse Fed Moldy Hay, Vet. Path., 14: 182-187, (1977).

6. Morrione, T. G., and Mamelok, H. C.: Observations on the Persistence of Hepatic Glycogen After Death, Am. J. Path., 28, (1952), pp. 497-502.

7. Runnels, R. A., Monlux, W. S., and Monlux, A. W., Principles of Veterinary Pathology, 7th Ed., The Iowa State University Press, Ames, Iowa, (1965), pp. 236-239.

8. Smith, H. A., Jones, T. C., and Hunt, R. D.: Veterinary Pathology, 4th Ed., Lea and Febiger, Philadelphia, PA, (1974), pp. 1-23.

9. Splitter, G. A., and McGavin, M. D.: Sequence and Rate of Postmortem Autolysis in Guinea Pig Liver, Am. J. Vet. Res., Vol. 35, No. 12, (1974), pp. 1591-1596.

TABLE I

Summary of histopathologic changes with time in hepatocytes at 26°C.

Time Postmortem	Cytoplasmic Basophilia	Cellular Outline	Cytoplasmic Clumping	Cytoplasmic Granularity	Individualization of Hepatocytes
Zero hour Perfused	++	++	Extremely slight	++	-
Zero hour Non-perfused	++	++	None	++	-
3 hrs.	+	++	+	+	?
6 hrs.	-	+	++	+	+
12 hrs.	-	+	?	-	+
24 hrs.	-	+	-	-	++
48 hrs.	-	-	-	-	++
96 hrs.	-	-	-	-	++

++ Distinct
+ Observable
? Questionable
- Absent of not observed

TABLE II

Summary of histopathologic changes with time in red blood cells at 26°C.

Time Postmortem	Present	Color	Shape	Hemolysis
Zero hour Perfused	rare	red	normal	no
Zero hour Non-Prefused	very few	red	normal	no
3 hrs.	yes	red	normal	no
6 hrs.	yes	red	normal	slight
12 hrs.	yes	red	abnormal	yes
24 hrs.	yes	orange	abnormal	yes
48 hrs.	yes	orange	abnormal	yes
96 hrs.	yes	orange	abnormal	yes

TABLE III

Summary of histopathologic changes with time in hepatocyte nuclei at 26°C.

Time Postmortem	Chromatin Margination	Nucleolar Particles	Karyorrhexis	Karyolysis	Pyknosis
Zero hour Perfused	none	1-3	-	-	-
Zero hour Non-Perfused	none	1-3	-	-	-
3 hrs.	scattered	3-6	-	-	-
6 hrs.	marginal	3-6	-	-	+
12 hrs.	marginal	3-6	-	+	+
24 hrs.	?	3-6	-	+	+
48 hrs.	-	-	-	++	?
96 hrs.	-	-	-	++	?

++ Distinct
+ Observable
? Questionable
- Absent or not observed

TABLE IV

Summary of histopathologic events with time in Kupffer cells at 26°C.

Time Postmortem	Staining Quality	Shape	Karyorrhexis	Karyolysis	Pyknosis
Zero hour Perfused	++	elongated	-	-	-
Zero hour Non-Perfused	++	elongated	-	-	slight
3 hrs.	++	oblong	-	-	+
6 hrs.	++	oblong	-	-	+
12 hrs.	+	oval	-	-	++
24 hrs.	+	round	-	-	++
48 hrs.	+	round	-	+	++
96 hrs.	?	round	-	+	++

++ Distinct
+ Observable
? Questionable
- Absent or not observed

TABLE V

Summary of histopathologic events with time in the gall bladder at 26°C.

Time Postmortem	Separation of Gall bladder Epithelium	Vacuolization of Lamina propria	Intactness of Muscular Wall
Zero hour Perfused	-	-	++
Zero hour Non-Perfused	-	-	++
3 hrs.	+	++	++
6 hrs.	++	++	++
12 hrs.	++	++	+
24 hrs.	++	++	+
48 hrs.	++	++	?
96 hrs.	++	++	?

++ Distinct
+ Observable
? Questionable
- Absent or not observed

Fig. 1. Zero hour perfused liver. Note the effect of 10% BNF perfusion on Kupffer cells. H&E stain; x 400.

Fig. 2. Zero hour non-perfused control liver. H&E stain; x 400.

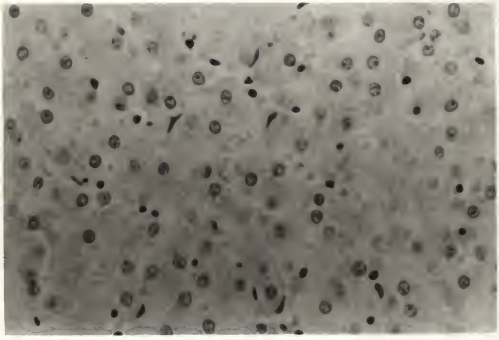

Fig. 1.

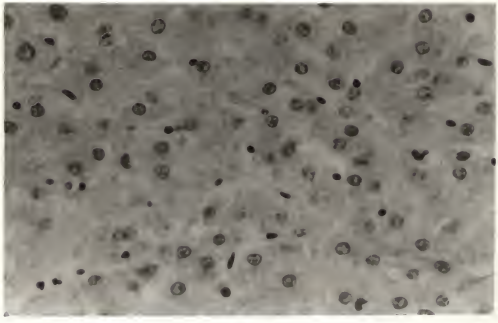

Fig. 2.

Fig. 3. Zero hour perfused liver. Note dilation of vessels of the portal triad and the interlobular septa. H&E stain; x 40.

Fig. 4. 12 hours liver, 26°C. Note loss of staining ability, nuclear fading and early separation of bile duct epithelium. H&E stain; x 400.

Fig. 3.

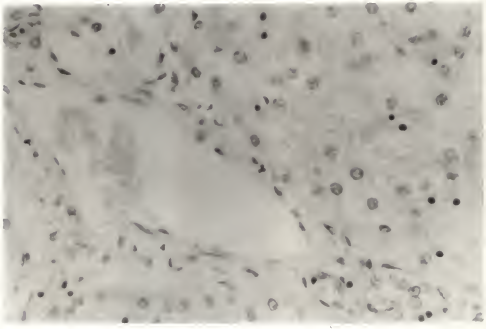

Fig. 4.

Fig. 5. 24 hours liver, 26°C. Note hepatocyte individualization, acid hematin from Hemolyzed blood and the presence of glycogen in hepatocyte. PAS stain; x 250.

Fig. 6. 96 hours liver, 26°C. Note complete absence of nuclei, hepatocyte individualization, loss of staining ability and lack of cellular outline. H&E stain; x 250.

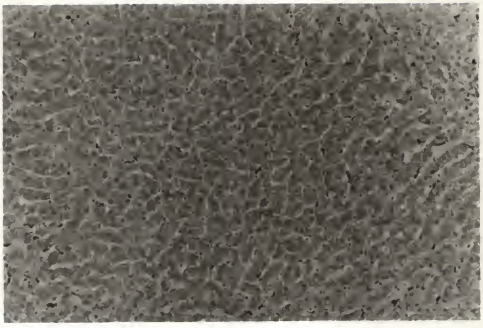

Fig. 5.

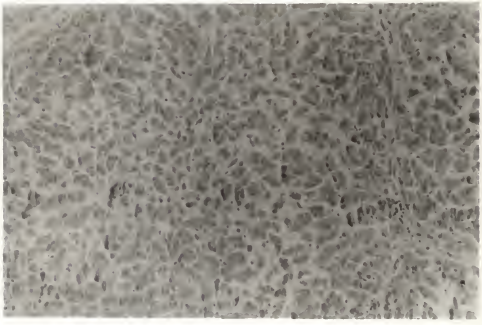

Fig. 6.

Fig. 7. Zero hour control gall bladder, non-perfused. H&E stain; x 40.

Fig. 8. Zero hour control gall bladder, perfused H&E stain; x 100.

Fig. 7.

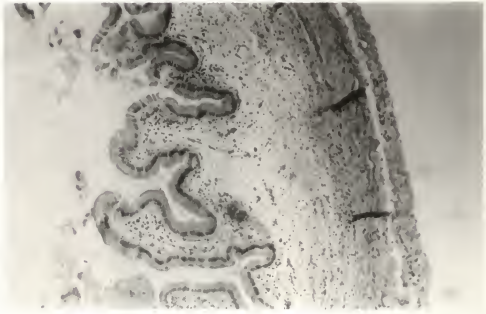

Fig. 8.

Fig. 9. 3 hours gall bladder, 26°C. Note total absence of epithelium and vacuolization of lamina propria. H&E stain; x 100.

Fig. 10. 12 hours gall bladder, 26°C. Note also autolysis in the musclaris. H&E stain; x 100.

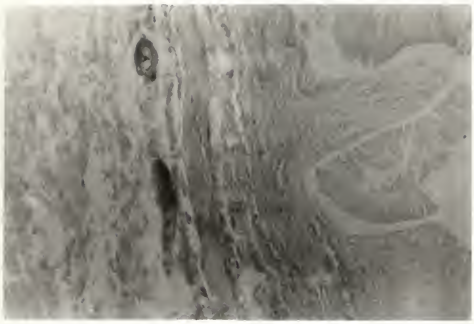

Fig. 9.

Fig. 10.

PAPER II

SEQUENCE OF HISTOLOGIC ALTERATIONS IN PORCINE LIVER AND GALL BLADDER UNDERGOING POSTMORTEM AUTOLYSIS AT 4°C.

INTRODUCTION

Though numerous factors such as, how long an animal has been dead, environmental factors prior to and following death, body weight, external insulation, nutritional status, age and species of animal are known to affect postmortem autolysis,[4,8] not all factors affecting this phenomenon are known.[2] External insulation, such as fleece and subcutaneous fat are known to affect the rate of postmortem autolysis.[8] These tend to keep the body temperature high and so accelerate autolysis.

In studies on guinea pigs by Splitter and McGavin, it was found that postmortem autolysis occurred twice as rapidly at 37°C than at 20°C.[10] Such morphologically altered findings in the liver included bile duct epithelial sloughing, hepatocyte individualization, loss of cytoplasmic granularity and basophilia, pyknosis of Kupffer cells and sloughing of gall bladder epithelial cells. This experiment was designed to study the morphological histopathologic findings in porcine liver and gall bladder undergoing postmortem autolysis at 4°C.

MATERIALS AND METHODS

A total of 16 pigs, weighing about 17kg and between 6 and 8 weeks of age, were used. They were electrocuted by

passing a current of 110 volts for 10 seconds between electrodes placed behind an ear and the tail. They were then kept at 4°C and two were necropsied at 3, 6, 12, 24, 48 and 96 hours postmortem, respectively. Liver sections taken from both the right and the left lateral lobes were fixed in 10% buffered neutral formalin (BNF) immediately after removal.

For controls, 2 pigs were necropsied immediately and fixed in 10% BNF. A second set of controls was obtained by anesthetizing 2 pigs by Rompun[a] intramuscularly. Blood was allowed to flow by cutting the femoral vein and 10% BNF was perfused under pressure by connecting a tube containing the solution to the left ventricle. Perfusion was stopped when the clear color of 10% BNF drained from the femoral vein instead of blood. The pigs were necropsied and similar liver and gall bladder sections were taken and fixed in 10% BNF.

The tissues were dehydrated through graded ethanol, cleaned in xylene, and cut at 6 microns. They were then stained with Harris' hematoxylin and eosine Y (H&E). Some sections were stained with periodic-acid Schiff (PAS) and PAS-diatase. Spicer's stain was also used, to detect changes in cytoplasmic RNA and nuclear DNA.

---

[a]-5,6-Dihydro-2-(2,6-xylidino)-H-1,3-thiazine; Haver-Lockhart, BAYVET Division, Cutter Laboratories Inc., Shawnee Mission, 66201.

RESULTS

Gross Findings

There was progressive loss of color and strength and progressive increase in odor and bile imbibition. These changes were not prominent until after 24 hours. The livers were more friable than those of the control but were not liquified as a result of autolysis by 96 hours postmortem.

Microscopic Findings

General Architecture

There appeared to be little difference in the changes observed in the interlobular connective tissue between the controls and 96 hour livers at 4°C. The lobular pattern remained relatively unaltered. The presence of erythrocytes in the sinusoids was remarkable and hemolysis at both 12 hours and 24 hours postmortem was not pronounced at 4°C. Glycogen was observed to decrease in amount as time postmortem increased. However, from the extent of reaction with PAS and PAS-diatase, it decreased only slightly with time postmortem.

Hepatocyte individualization was not prominent until 12 hours postmortem (Table I), the number observed at 24 and 48 hours was small (Fig. 4). Staining ability of the extramedullary hematopoietic centers were also less than observed for the controls with time postmortem.

Erythrocytes

The number of sinusoidal erythrocytes was higher than the controls from 3 hours onwards. At 12 hours postmortem, hemolysis was not significant as erythrocyte clumping was marked (Table IV). Loss of acidophilic staining was observed, but only slightly. Little acid hematin was seen since hemolysis was slight. These changes were more pronounced at 24 hours postmortem.

Sinusoids

Sinusoidal dilation was marked as a result of congestion. Except for the non-perfused control, sinusoidal collapse was not observed throughout the study.

Portal Areas

Preservation of bile duct epithelium was observed for as long as 24 hours. The nuclei were pyknotic and the cytoplasm slightly eosinophilic. Slight epithelial sloughing was observed. The nuclei of endothelial cells lining the veins were swollen at 24 hours, but the endothelial cells were still intact. The cytoplasm, however, was slightly eosinophilic. Sloughing, pyknosis and loss of cytoplasmic basophilia were less severe at 96 hours postmortem than would be expected at higher temperatures.

Hepatocyte Cytoplasm

The significant observations included delay in loss of cytoplasmic basophilia. Traces of eosinphilia in the cytoplasm was not observed until 6 hours postmortem (Table I).

Condensation of cytoplasmic RNA was pronounced and persisted until about 24 hours postmortem. At this period, cytoplasmic granularity was almost completely lost. Glycogen depletion also presented independent lobular activity. Centrolobular loss of glycogen appeared to be predominant over midzonal and periportal depletion. It was also observed that the rate of depletion first detected at 3 hours postmortem by PAS and PAS-diatase, was slow, since concentration of glycogen granules within the cytoplasm only slightly altered with time postmortem.

Hepatocyte Nuclei

The major observation was the intactness of nuclear chromatin. Starting from 3 hours to 96 hours postmortem, it was observed that most hepatocytes contained 1 or 2, or occasionally 3 nuclear chromatin as in the controls (Fig. 2). Fragmentation was not severe, and only observed in a few hepatocyte nuclei. The few nuclei with fragmentation of chromatin did not present marginally arranged chromatin, but diffuse scattering within the nuclei.

Kupffer Cells

Nuclei of the Kupffer cells had swelling and pyknosis early (Fig. 5). From 6 hours postmortem, rapid swelling was observed. Many cells were rounded at 12 hours, and by 24 hours postmortem, pyknosis was a major change. Fading of Kupffer cells was observed at 48 hours and 96 hours postmortem, but this appeared to be more important in cells with swollen rather than pyknotic nuclei (Table III).

Gall Bladder

Sloughing of gall bladder epithelium was not prominent until 6 hours postmortem (Fig. 10) (Table V). At 3 hours, pyknosis of nuclei, swelling of epithelial cells and increased cytoplasmic eosinophilia of these cells were observed (Fig. 9). At 6 hours postmortem, the epithelium was preserved only where overlying mucus was abundant. The exposed lamina propria was also vacuolated, simulating pox vesicles. These lamina proprial cells were devoid of nuclei immediately beneath the epithelium. These changes were less severe progressively towards the muscular layers. The serosal surface appeared swollen and edematous at 12 hours postmortem.

DISCUSSION

In comparing autolysis in the liver at higher temperatures with that of 4°C, the effect of temperature on postmortem autolysis is well elucidated. Since the rate of autolytic changes increase with temperature,[5,8] the reverse also may be true. Hepatocyte individualization was significantly delayed since it was observed 12 hours postmortem at 4°C. Hemolysis of erythrocytes, prolongation of cytoplasmic basophilia, sloughing of the bile duct epithelium, pyknosis of the Kupffer cells and sloughing of the gall bladder epithelium were changes also delayed. Glycogen depletion preferred a centrolobular pattern. This observation is worth mentioning, though hepatocytes did not react

uniformly in the amount of glycogen contained initially, and the rate or pattern of glycogen depletion.

Of significance is the condensation of RNA of the nucleoproteins in the cytoplasm. The reactions with Spicer's stain were intense. Liver cell death will be accompanied by inhibition of RNA synthesis which is accompanied by fragmentation of the nucleolus,[6,7] and inhibition of protein synthesis by some fat accumulation, and the appearance of large aggregates containing ribosomes between the stacks of rough endoplasmic reticulum.[1,6] The condensation of these ribosomes probably increases at lower temperatures and hence is responsible for the increased cytoplasmic "clumping" observed at $4^{o}C$. This may also explain the fragmentation of nuclear chromatin observed to be more prominent at $26^{o}C$ than at $4^{o}C$.

Although all conditions determining the rate of postmortem autolysis are not known,[2] temperature plays an important part. Morphological differences were also observed in this study and these changes were visible by light microscopy and the naked eye. Retarding the rate of autolysis by lowering the temperature may be explained by assuming that temperature drop reduces the rate or extent of deviation of pH from neutral towards acidity and this in turn affects the stability of lysosomal membranes which are responsible for releasing lysosomal enzymes.[5] Since the activity of lysosomal enzymes are closely related with the external and internal environment of the cell,[3] a slight deviation in pH

and the preservation of RNA at lower temperatures will affect their activity less than at higher temperatures. Similarly, the effect of temperature on nuclear chromatin may be attributed to the preservative effect of lower temperatures on DNA, since DNA is believed to be rapidly lost following death.[9]

SUMMARY

Sixteen pigs were electrocuted to study the sequence of histologic changes in the liver and gall bladder at 4°C. Two of them were perfused with 10% BNF prior to death and necropsied immediately after death with two others that were not perfused to serve as controls. The rest were maintained at 4°C and two each were necropsied 3, 6, 12, 24, 48 and 96 hours after electrocution, respectively. Their livers and gall bladder were removed and fixed in 10% BNF.

Significant microscopic findings included delay of hepatocyte individualization till 12 hours postmortem. Hemolysis was not severe and erythrocyte clumping and crenation were more pronounced. Anisocytosis was also observed. Loss of hepatocyte cytoplasmic granularity and basophilia were significantly delayed, and centrilobular glycogen loss appeared predominant over midzonal and periportal loss. Fragmentation of nuclear chromatin was not observed.

Swelling and pyknosis of Kupffer cells was observed. Gall bladder epithelial desquamation was noted at 6 hours. Lower temperatures probably reduced the activity of lysosomal enzymes by preventing rapid pH changes from neutral towards acidity. The characteristic porcine hepatic lobulation was preserved throughout the time period of this study.

REFERENCES

1. Faber, J. L. and El-mofty, S. K., The Biochemical Pathology of Liver Cell Necrosis, American J. of Path., Vol. 81, No. 1, (1975), pp. 273-250.

2. More, H. R., and Crowson, C. N., Glomerulotublar Nephrosis Correlated with Hepatic Lesions, Arch. Path, 60, (1950): pp. 63-84.

3. Pekkanen, T. J., Distribution of Acid Phosphatase During Autolysis in Bovine Liver, Acta Vet. Scand., 11, (1970), pp. 283-294.

4. Runnels, R. A., Monlux, W. S., and Monlux, A. W., Principles of Veterinary Pathology, 7th Ed., The Iowa State University Press, Ames, Iowa, (1965), pp. 236-239.

5. Sewant, P. L., Desal, I. D., and Trappel, A. L., Factors affecting the lysosomal membranes and availability of enzymes. Arch. Biochem. 105, (1964), pp. 247-253.

6. Shinozuka, I. I., Farber, J. L., Kornish, Y., Anakarahanonta, J.: D-galactosamine and Acute Liver Injury. Fed. Proc. 32: (1973), pp. 1516-1526.

7. Shinozuka, I. I., Martin, J. T., Farber, J. L., The induction of fibrillar nucleoli in rat liver cells by D-galactosamine and their subsequent re-formation into normal nucleoli. J. Ultrastruc. Res. 44, (1973), pp. 279-292.

8. Smith, H. A., Jones, T. C., and Hunt, R. D., Veterinary Pathology, 4th Ed., Lea and Febiger, Philadelphia, PA, (1974), pp. 21-23.

9. Stowell, R. E., Chang, J. P., and Berembom, M., Histochemical Studies of Necrosis of Mouse Liver Tissue in Peritoneal Cavity, Lab. Invest. 10, (1961), pp. 111-128.

10. Splitter, G. A., McGavin, M. D.; Sequence and Rate of Postmortem Autolysis in Guinea Pig Liver; Am. J. Vet. Res., 12, (1974), pp. 1591-1596.

TABLE I

Summary of histopathologic changes with time in the hepatocyte at $4^{o}C$.

Time Postmortem	Cytoplasmic Basophilia	Cellular Outline	Cytoplasmic Clumping	Cytoplasmic Granularity	Individualization of Hepatocytes
0 hour Perfused	++	++	very slight	++	-
0 hour Non-Perfused	++	++	none	++	-
3 hrs.	+	++	+	+	-
6 hrs.	slight	++	++	+	-
12 hrs.	-	+	++	slight	+
24 hrs.	-	+	+	-	+
48 hrs.	-	+	-	-	++
96 hrs.	-	?	-	-	++

++ Distinct
+ Observable
? Questionable or indistinct
- Absent or not observed

TABLE II

Summary of histopathologic changes with time in the heaptocyte nucleus at $4^{o}C$.

Time Postmortem	Chromatin Margination	Nucleolar Particles	Karyorrhexis	Karyolysis	Pyknosis
0 hour Perfused	none	1-3	-	-	-
0 hour Non-Perfused	none	1-3	-	-	-
3 hrs.	none	1-3	-	-	-
6 hrs.	none	1-3	-	-	-
12 hrs.	none	1-3	-	-	-
24 hrs.	none	1-3	-	+	+
48 hrs.	?	1-3	-	++	+
96 hrs.	?	?	?	++	?

++ Distinct
+ Observable
? Questionable
- Absent or not observed

TABLE III

Summary of histopathologic events with time in the Kupffer cells at $4^{o}C$.

Time Postmortem	Staining Quality	Shape	Karyorrhexis	Karyolysis	Pyknosis
0 hour Perfused	++	elongated	-	-	-
0 hour Non-Perfused	++	elongated	-	-	-
3 hrs.	++	oblong	-	-	slight
6 hrs.	++	oblong	-	-	+
12 hrs.	++	oval	-	-	+
24 hrs.	++	round	-	-	+
48 hrs.	+	round	-	-	++
96 hrs.	+	round	-	-	++

++ Distinct
+ Observable
? Questionable
- Absent or not observed

TABLE IV

Summary of histopathologic events with time in the red blood cells at $4^{o}C$.

Time Postmortem	Present	Color	Shape	Hemolysis
0 hour Perfused	very few	red/brown	normal	no
0 hour Non-Perfused	none	red/brown	normal	no
3 hrs.	yes	red/brown	normal	no
6 hrs.	yes	red/brown	normal	no
12 hrs.	yes	red/brown	normal	slight
24 hrs.	yes	red/brown	abnormal	yes
48 hrs.	yes	orange	abnormal	yes
96 hrs.	yes	orange	abnormal	yes

TABLE V

Summary of histopathologic events with time in the gall bladder at $4^{o}C$.

Time Postmortem	Separation of Gall bladder Epithelium	Vacuolization	Intactness of Muscular Wall
0 hour Perfused	-	-	++
0 hour Non-Perfused	-	-	++
3 hrs.	-	+	++
6 hrs.	+	++	++
12 hrs.	++	++	++
24 hrs.	++	++	+
48 hrs.	++	++	+
96 hrs.	++	++	?

++ Distinct
+ Observable
? Questionable
- Absent or not observed

Fig. 1. Zero hour non-perfused liver. H&E stain; x 400.

Fig. 2. Zero hour perfused liver. The sinusoids are dilated due to perfusion by 10% BNF. H&E stain; x 100.

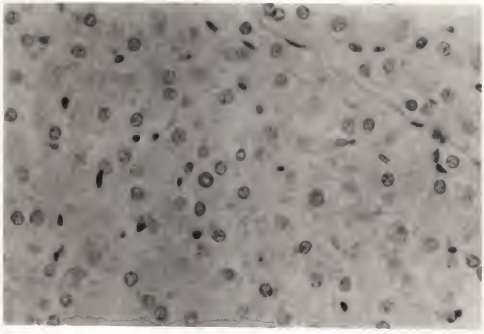

Fig. 1.

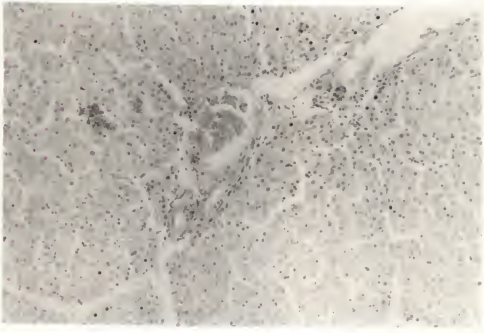

Fig. 2.

Fig. 3. Six hour liver, 4°C. The Kupffer cells have undergone early pyknosis even at this temperature. H&E stain; x 400.

Fig. 4. 12 hours liver, 4°C. Hepatocyte individualization is prominent at this magnification. H&E stain; x 100.

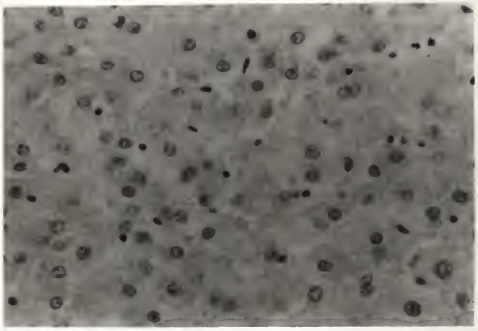

Fig. 3.

Fig. 4.

Fig. 5. 24 hour liver, 4°C. Nuclear fading is prominent as well as Kupffer cell pyknosis. Note loss of staining ability. H&E stain; x 400.

Fig. 6. 48 hour liver, 4°C. Pyknosis is more distinct and hemolysis more advanced. H&E stain; x 400.

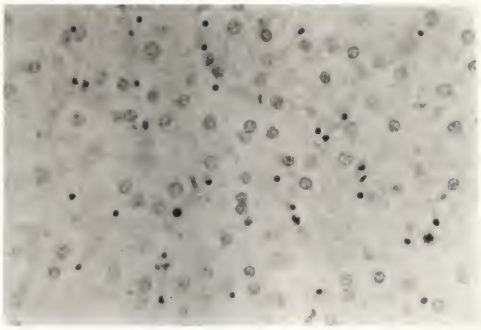

Fig. 5.

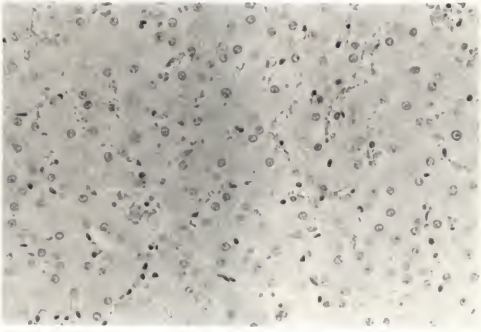

Fig. 6,

Fig. 7. Zero hour non-perfused gall bladder. H&E stain; x 400.

Fig. 8. Zero hour perfused gall bladder. H&E stain; x 400.

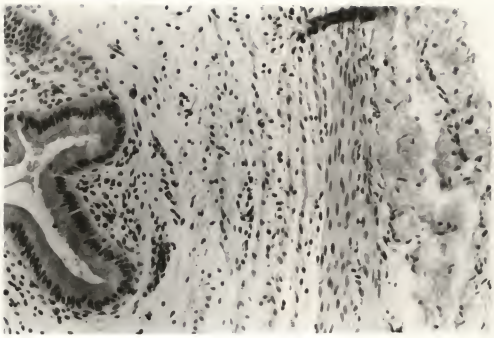

Fig. 7.

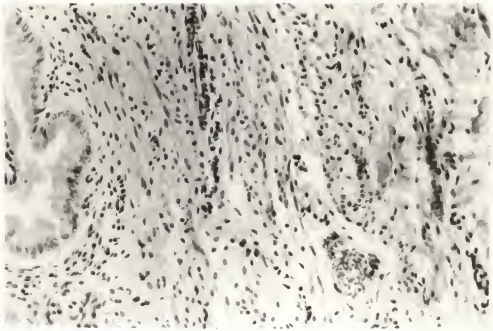

Fig. 8.

Fig. 9. 3 hours gall bladder, 4°C. Note the incomplete desquamation of the epithelium and very little vacuolization of the lamina propria. H&E stain; x 400.

Fig. 10. 6 hours gall bladder, 4°C. Note total desquamation of epithelium and vacuolization of lamina propria. H&E stain; x 400.

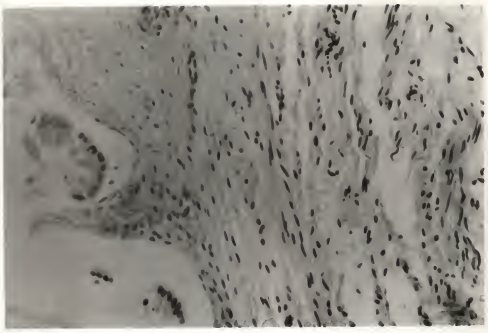

Fig. 9.

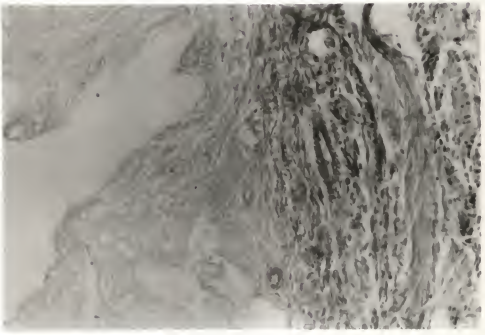

Fig. 10.

PAPER III

SEQUENCE OF HISTOLOGIC ALTERATIONS IN PORCINE LIVER AND GALL BLADDER UNDERGOING AUTOLYSIS 24 AND 48 HOURS POSTMORTEM AT -10°C.

INTRODUCTION

It is known that postmortem autolysis is slower at lower than at higher temperatures. It is also known that tissues are frozen during transportation and storage to minimize autolytic changes following death. This experiment was designed to study what histologic alterations occur when porcine liver and gall baldder are kept at -10°C for 24 hours and 48 hours.

MATERIALS AND METHODS

A total of 8 pigs, between 6 to 8 weeks of age and averaging 17kg, were used. For controls, 2 were electrocuted by passing a current of 110 volts for 10 seconds between 2 electrodes, one placed behind an ear and the other on the tail. Another set of controls was obtained by administering Rompun[a] intramuscularly and then allowing blood to flow by cutting the femoral vein while 10% buffered neutral formalin (BNF) was perfused under pressure by connecting a tube containing the solution to the left ventricle. Perfusion was stopped when clear 10% BNF drained from the

---

[a] 5,6-Dihydro-2-(2,6-xylidino)-H-1,3-thiazine, Haver-Lockhart BAYVET Division, Cutter Laboratories, Inc., Shawnee, Kansas 66201.

femoral vein instead of blood. All four control pigs were necropsied immediately and liver and gall bladder sections were taken and fixed in 10% BNF.

The remaining four test pigs were similarly electrocuted and the intact cadavers stored in a freezer maintained at -10°C. Two pigs each were necropsied at 24 and 48 hours after freezing, and liver and gall bladder sections taken and fixed in 10% BNF.

All tissues collected were then dehydrated through graded ethanols, cleared in xylene, and cut at 6 microns. Four sections of each liver and gall bladder samples were cut and stained with hematoxylin and eosin Y (H&E), Periodic-acid Schiff (PAS), PAS-diatase and Spicer's stains. Microscopic observations were made under light microscopy.

RESULTS

Gross Findings

Bile imbibition was slight but present, and all four livers frozen for 24 and 48 hours presented little color difference compared with those of the controls. The livers frozen for 48 hours appeared lighter in color than the controls and the 24 hour frozen livers. Shedding of gall bladder epithelium was observed grossly at 48 hours.

Microscopic Findings

General Architecture

The characteristic porcine lobules were retained in both sets of frozen tissues. Erythrocytes were numerous in the sinusoids and hemolysis was virtually absent even at 48 hours (Fig. 3). The cord-like arrangement of hepatocytes was retained and individualization was not observed. Hepatocyte cytoplasmic basophilia and granularity were persistent even at 48 hours. The cells of the extramedullary hematopoietic centers were neither altered structurally nor in their staining ability. Some heaptocyte cell membrane rupture was observed at both 24 and 48 hours. These observations were more discernible with the PAS stain and when predigested with diatase. Occasional freezing artifacts were present at 48 hours.

Sinuoids

The sinusoids were narrow at both 24 and 48 hours compared with the controls. Erythrocytes were present and clumped together. The narrowing of the sinusoids were attributed to the swelling of the hepatocytes due to freezing of intracytoplasmic water which increases in volume when it forms ice.

Erythrocytes

Erythrocytes were most numerous around the portal areas. There was observable clumping and crenation, as well as

anisocytosis (Fig. 3). Hemolysis was not observed throughout the entire experiment. The acidophilic staining ability of the erythrocytes was retained for as long as 48 hours.

Hepatocyte Cytoplasm

Cytoplasmic basophilia and granularity was persistent for 48 hours. Eosinophilic staining was also evident at both 24 and 48 hours after freezing; it was more intense at 48 hours than at 24 hours. Clumping of cytoplasmic RNA as basophilic globules was present but slight. Glycogen was present in hepatocytes throughout the entire study though the intensity of staining with PAS decreased with time postmortem (Fig. 4).

Hepatocyte Nucleus

The nuclei were characteristically large and round. There was negligible fading in a few hepatocyte nuclei at 48 hours. Typically only one or two nucleoli were present and these were centrally located. Fragmentation and margination of nuclear chromatin was not observed throughout the entire study. Pyknosis was also not observed.

Kupffer Cells

The Kupffer cells were swollen, pyknotic and oblong-shaped both at 24 and 48 hours postmortem. At 48 hours, many Kupffer cells were almost round. Their nuclei were round and swollen and the cytoplasm could hardly be delineated.

Portal Areas

The endothelial lining of the arteries and veins, and the bile duct epithelium were preserved as long as 48 hours. The bile duct epithelial cells were swollen, and the endothelial cells of veins were pyknotic and swollen at 48 hours. The walls of the vessels of the portal triad appeared edematous.

Gall Bladder

The epithelium of the gall bladder was swollen and nuclei were pyknotic at 24 hours after freezing. At 48 hours, the epithelial lining was mostly desquamated and the wall of the gall bladder edematous. However, the lamina propria was not vacuolated.

DISCUSSION

Since freezing reduces the rate of autolytic changes, the observations were less severe than those present at higher temperatures. Occasional cell membrane rupture occurred in some cells. This may be attributed to freezing of water content within the cytoplasm. Water, when frozen, increases in volume and this increase may be responsible for rupture of the cell membrane.

The Kupffer cells presented more noticeable changes as they were swollen and pyknotic throughout the entire test period. The alterations observed might have proceeded

before the freezing process involved the visceral organs, as porcine subcutaneous fat is a good insulator and interferes with heat exchange.

Epithelium of the gall bladder is probably the most vulnerable structure, as there was separation of the epithelium from the lamina propria by 24 hours after freezing. In work by Splitter,[1] it was found that the gall bladder epithelium separated from the lamina propria in the guinea pig as early as 9 hours at 4°C. This is rapid in view of the fact that lowering the temperature has a preservative effect on tissues.

Hepatocyte nuclear changes were minimal. The number of chromatin remained the same as in the control. Pyknosis was virtually absent in the hepatocytes.

SUMMARY

Eight pigs were electrocuted to study the sequence of histologic changes in porcine hepatocyte and gall bladder at -10°C. For control, two perfused and two non-perfused samples were utilized. Two pigs were necropsied 24 and 48 hours after freezing and immediately fixed in 10% BNF.

The cytoplasmic basophilia and granularlity was persistent for 48 hours. Clumping and crenation of erythrocytes was marked and hemolysis absent. Hepatocyte nuclear fragmentation and margination was not observed and pyknosis

was not prominent. The Kupffer cells were swollen, pyknotic and oblong. Bile duct epithelial cells were swollen, while the endothelial cells of the hepatic vein were swollen and their nuclei pyknotic.

The gall bladder epithelium presented swollen, pyknotic nuclei at 24 hours and desquamation of the epithelial cells was observed at 48 hours. Concentration of erythrocytes was highest around the portal triad. The typical porcine hepatic lobulation was preserved. Fibrous connective tissue is most resistant to autolysis. This may be attributed to the fact that they contain few lysosomes and a corresponding low level of hydrolytic enzymes.

REFERENCES

1. Splitter, G., Sequential Changes in the Guinea Pig Liver and Gall Bladder Undergoing Postmortem Decomposition: M.S. Thesis, KSU, 1970.

GENERAL DISCUSSION

The most consistent observations made in this series of experiments include loss of cytoplasmic granularity and basophilia, sloughing and separation of gall bladder epithelium from the lamina propria, fragmentation of nuclear chromatin, nuclear fading and hepatocyte individualization.

Cytoplasmic granularity and basophilia are lost early following death. In these experiments, it was observed that loss of cytoplasmic basophilia was noted after 3 hours postmortem at 26°C; 6 hours postmortem at 4°C, and 24 hours after freezing at -10°C (Table I). These findings were similar to those of Splitter and McGavin,[4] who first noted loss of cytoplasmic basophilia centrilobularly in the guinea pig liver at 0.75 hours, and total loss of basophilia at 3 hours when the guinea pigs were kept at 20°C. Sloughing and separation of gall bladder epithelium also occurred early. It can be concluded that the gall bladder is useful for histopathologic study after 3 hours postmortem only if frozen, and even then, it is useful only if studies are made before 24 hours after freezing.

Nuclear changes were fairly consistent. Fragmentation and margination of nuclear chromatin were observed only at 26°C. These changes were not observed at 4°C and -10°C, indicating that they were temperature dependent. Typical nuclear changes were fading or lysis and to a lesser extent pyknosis. The Kupffer cells in contrast to hepatocytes had pyknosis and swelling, and fading was only secondary, even under freezing conditions.

Hemolysis, which was observed at 26°C before 12 hours was replaced at 4°C and -10°C by anisocytosis and crenation. These findings were similar to the findings of Willot et al.[5] who noticed distortion and anisocytosis in guinea pig liver erythrocytes after 4 hours postmortem. Although glycogen was observed in all samples throughout the entire experiment, midzonal loss of glycogen was most prominent at 26°C, while at lower temperatures, glycogen loss had no particular pattern. This is in agreement with the findings of Morrione and Mamelock,[2] who found no relationship of glycogen disappearance to hepatic lobule topography. At 4°C, however, in this study more lobules had centrolobular loss of glycogen than either midzonal or periportal.

Fibrous connective tissue is the most resistant to autolysis, as there was persistence of interlobular connective tissue throughout the entire experiment. This reflects the fact that they have few lysosomes and a corresponding low level of hydrolytic enzymes.[1,3]

REFERENCES

1. Anderson, W. A. D., and Kissane, J. M., Pathology; 7th Ed., C. V. Mosby, St. Louis, MO (1977), p. 94.

2. Morrione, T. G. and Mamelok, H. L., Observations on the Persistence of Hepatic Glycogen After Death., Am. J. Path., 28, (1952): pp. 497-502.

3. Smith, H. A., Jones, T. C. and Hunt, R. D., Veterinary Pathology, 4th Ed., Lea & Febiger, Philadelphia, PA, 1972.

4. Splitter, G., and McGavin, M. D., Sequence and Rate of Postmortem Autolysis in Guinea Pig Liver; Am. J. Vet. Res. 35, 12 (1974), pp. 1591-1596.

5. Willot, M., Debarge, A., and Muller, P.; Aspects histologiques: I'Autolyse du foie de cobaye. Acta Med Leg et Soc (Liege), 20, (1967): pp. 21-64.

TABLE I

Comparison of the Microscopic Findings in 10% BNF Fixed Liver and Gall Bladder of the Intact Porcine Cadavers Undergoing Autolysis at 26°C, 4°C, and -10°C.

Histologic Alterations	Time Postmortem to Prominence of Alterations		
	26°C	4°C	-10°C
Nuclear Changes			
Chromatin fragmentation	3 hrs.	12 hrs.	none
Fading	24 hrs.	48 hrs.	none
Absence	96 hrs.	-	-
Cytoplasm			
Loss of Basophilia (total).	6 hrs.	12 hrs.	48 hrs.
Cytoplasmic Clumping	6 hrs.	12 hrs.	
Hepatocyte			
Individualization	3 hrs.	6 hrs.	24 hrs.
Gall Bladder			
Epithelial Sloughing (total)			

TABLE I (continued)

Histologic Alterations	Time Postmortem to Prominence of Alterations		
	26°C	4°C	-10°C
Erythrocytes			
Anisocytosis	none	12 hrs.	24 hrs.
Hemolysis	12 hrs.	24 hrs.	-
Loss of Acidophilic staining	12 hrs.	24 hrs.	-
Kupffer Cells			
Pyknosis	6 hrs.	12 hrs.	24 hrs.
Swelling	6 hrs.	12 hrs.	24 hrs.

Fig. 1. Zero hour perfused liver. Note swelling of Kupffer cells and some cells of the extramedullary hematopoietic center. H&E stain, x 400.

Fig. 2. 24 hour liver, -10°C. The cells of the extramedullary hematopoietic center are still more hyperchromatic than the hepatocytes and Kupffer cells. Note also the red blood cells in the sinusoids. H&E stain; x 400.

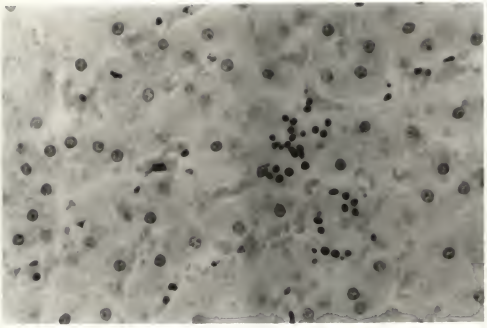

Fig. 1.

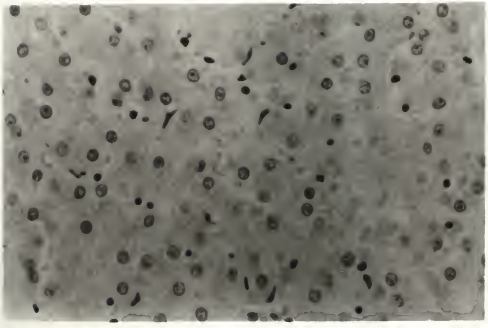

Fig. 2.

Fig. 3. 24 hours liver, -10°C. Erythrocytes are crenated in sinusoids and portal vein. H&E stain; x 40.

Fig. 4. 48 hours liver, -10°C. Note persistence of glycogen in hepatocytes. PAS stain; x 40.

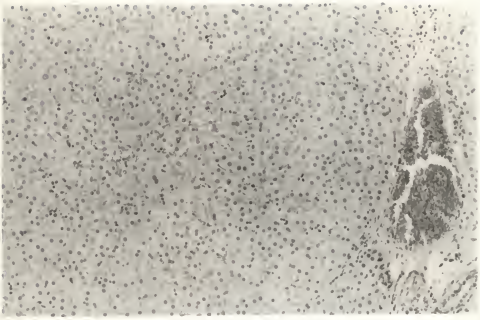

Fig. 3.

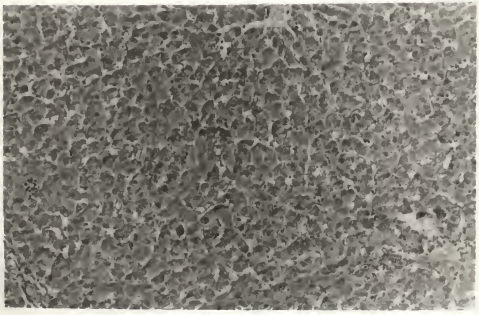

Fig. 4.

APPENDIX

TABLE I - SUMMARY OF SEQUENTIAL HISTOLOGIC CHANGES IN PIG LIVER AND KUPFFER CELLS HELD AT 26°C POSTMORTEM

	HEPATOCYTES											KUPFFER CELLS				
	CYTOPLASM					NUCLEUS						NUCLEUS				
Time (Hours)	Cytoplasmic granularity	Cytoplasmic basophilia	Cytoplasmic clumping	Cell border	Hepatocyte individualization	Chromatin fragmentation	Number of chromatin granules	Karyolysis	Karyorrhexis	Pyknosis	Distinctness of lobulation	Pyknosis	Staining ability	Shape	Karyolysis	Karyorrhexis
0 hour-																
Perfused	+++	+++	-	+++	-	-	1-3	-	-	-	+++	-	+++	N	-	-
Non-perfused	+++	+++	-	+++	-	-	1-3	-	-	-	+++	-	+++	N	-	-
3	++	+	+	++	-	S	3-6	-	-	-	+++	+	+++	N	-	-
6	+	+	++	++	+	M	3-6	-	-	+	++	++	++	A	-	-
12	-	-	++	+	++	M	3-6	+	-	+	++	++	A	-	-	-
24	-	-	-	-	++	?	3-6	+	-	+	++	+++	++	A	-	-
48	-	-	-	-	+++	-	-	++	-	+	+	++	+	A	+	-
96	-	-	-	-	+++	-	-	++	-	+	+	+	A	+	-	++

KEY - A = Abnormal
N = Normal
S = Slight
P = Present
R = Red
O = Orange
- = Absent or not observed
+ = Slightly present
++ = Moderately present
+++ = Very distinct
? = Questionable

TABLE II - SUMMARY OF SEQUENTIAL HISTOLOGIC CHANGES IN PIG ERYTHROCYTES AND GALL BLADDER HELD AT 26°C POSTMORTEM

	PORTAL AREAS		GALL BLADDER			ERYTHROCYTES			
Time (Hours)	Separation of bile duct epithelium	Pyknosis of vascular epithelium	Sloughing of epithelium	Vacuolization of lamina propria	Edema	Presence	Color	Shape	Hemolysis
0 hour-									
Perfused	-	-	-	-	-	S	R	N	-
Non-perfused	-	-	-	-	-	S	R	N	-
3	-	-	++	++	S	P	R	N	-
6	-	+	+++	+++	S	P	R	N	S
12	++	++	+++	++	-	P	O	A	+++
24	+	++	+++	-	-	P	O	A	+++
48	++	++	+++	-	-	P	O	A	+++
96	++	+++	+++	-	-	-	-	A	+++

KEY - A = Abnormal
N = Normal
S = Slight
P = Present
R = Red
O = Orange
- = Absent or not observed
+ = Slightly present
++ = Moderately present
+++ = Very distinct
? = Questionable

TABLE III - SUMMARY OF SEQUENTIAL HISTOLOGIC CHANGES IN PIG HEPATOCYTE AND KUPFFER CELLS HELD AT 4°C POSTMORTEM

	HEPATOCYTES											KUPFFER CELLS				
	CYTOPLASM					NUCLEUS						NUCLEUS				
Time (Hours)	Cytoplasmic granularity	Cytoplasmic basophilia	Cell border	Hepatocyte individualization	Cytoplasmic clumping	Chromatin fragmentation	Number of nuclear chromatin granules	Karyolysis	Karyorrhexis	Pyknosis	Distinctness of lobulation	Pyknosis	Staining ability	Shape	Karyolysis	Karyorrhexis
0 hour-																
Perfused	+++	+++	+++	-	-	-	1-3	-	-	-	+++	-	+++	N	-	-
Non-perfused	+++	+++	+++	-	-	-	1-3	-	-	-	+++	-	+++	N	-	-
3	++	++	+++	-	+	-	1-3	-	-	-	+++	S	+++	A	-	-
6	++	S	+++	-	++	-	1-3	-	-	-	+++	+	++	A	-	-
12	S	-	++	+	++	-	1-3	-	-	-	+++	+	++	A	-	-
24	-	-	++	+	+	-	1-3	+	-	+	++	+	++	A	-	-
48	-	-	+	++	-	?	1-3	+	-	+	++	++	+	A	-	-
96	-	-	-	++	-	?	1-3	++	?	?	+	+++	+	A	-	-

KEY - A = Abnormal
N = Normal
S = Slight
P = Present
R = Red
O = Orange
- = Absent or not observed
+ = Slightly present
++ = Moderately present
+++ = Very distinct
? = Questionable

TABLE IV - SUMMARY OF SEQUENTIAL HISTOLOGIC CHANGES IN PIG ERYTHROCYTE AND GALL BLADDER HELD AT 4°C POSTMORTEM

	PORTAL AREAS		GALL BLADDER			ERYTHROCYTES			
Time (Hours)	Separation of bile duct epithelium	Pyknosis of vascular endothelium	Sloughing of epithelium	Vacuolization of lamina propria	Edema	Presence	Color	Shape	Hemolysis
0 hour-									
Perfused	-	-	-	-	-	S	R	N	-
Non-perfused	-	-	-	-	-	S	R	N	-
3	-	-	-	+	-	P	R	N	-
6	-	-	+	++	S	P	R	N	-
12	-	+	++	++	S	P	R	N	S
24	++	++	++	++	-	P	R	A	++
48	++	++	++	++	-	P	R	A	++
96	++	++	++	++	-	P	R	A	++

KEY - A = Abnormal
N = Normal
S = Slight
P = Present
R = Red
O = Orange
- = Absent or not observed
+ = Slight present
++ = Moderately present
+++ = Very distinct
? = Questionable

TABLE V - SUMMARY OF SEQUENTIAL HISTOLOGIC CHANGES IN PIG HEPATOCYTE AND KUPFFER CELLS HELD AT -10°C POSTMORTEM

	HEPATOCYTES											KUPFFER CELLS				
	CYTOPLASM					NUCLEUS						NUCLEUS				
Time (Hours)	Cytoplasmic granularity	Cytoplasmic basophilia	Cell border	Hepatocyte individualization	Cytoplasmic clumping	Chromatin fragmentation	Number of nuclear chromatin granules	Karyolysis	Karyorrhexis	Pyknosis	Distinctness of lobulation	Pyknosis	Staining ability	Shape	Karyolysis	Karyorrhexis
0 hour-																
Perfused	+++	+++	+++	-	-	-	1-3	-	-	-	+++	-	+++	N	-	-
Non-perfused	+++	+++	+++	-	-	-	1-3	-	-	-	+++	-	+++	N	-	-
24	++	++	++	-	+	-	1-3	-	-	-	++	++	++	A	-	-
48	+	+	++	+	+	-	1-3	-	-	-	++	++	++	A	-	-

KEY - A = Abnormal
N = Normal
S = Slight
P = Present
R = Red
O = Orange
- = Absent or not observed
+ = Slightly present
++ = Moderately present
+++ = Very distinct
? = Questionable

TABLE VI - SUMMARY OF SEQUENTIAL HISTOLOGIC CHANGES IN PIG ERYTHROCYTE AND GALL BLADDER HELD AT -10°C POSTMORTEM

	PORTAL AREAS		GALL BLADDER			ERYTHROCYTES			
Time (Hours)	Separation of bile duct epithelium	Pyknosis of vascular epithelium	Sloughing of epithelium	Vacuolation of lamina propria	Edema	Presence	Color	Shape	Hemolysis
0 hour-									
Perfused	-	-	-	-	-	S	R	N	-
Non-perfused	-	-	-	-	-	S	R	N	-
24	-	+	+	-	++	P	R	A	-
48	+	+	++	-	++	P	R	A	-

KEY - A = Abnormal
N = Normal
S = Slight
P = Present
R = Red
O = Orange
- = Absent or not observed
+ = Slightly present
++ = Moderately present
+++ = Very distinct
? = Questionable

SEQUENCE OF HISTOLOGIC ALTERATIONS IN PORCINE LIVER AND GALL BLADDER UNDERGOING POSTMORTEM AUTOLYSIS

BY

MICHAEL ADEKUNLE SALAKO
D.V.M., Ahmadu Bello University, Zaria, Nigeria, 1975

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1978

The aim of this study was to determine the sequence and rate of histologic changes resulting from postmortem autolysis in the porcine liver and gall bladder, as well as determining the effect of temperature on these changes.

Thirty-two pigs between 7 and 8 weeks of age with an average weight of 17kg were used in the study. Thirty were electrocuted with 110 volts for 15 seconds and 2 were immediately necropsied and their liver and gall bladder fixed in 10% buffered neurtal formalin (BNF) as controls. Another set of control was obtained by perfusing 2 pigs with 10% BNF after administering rumpum intramuscularly and their liver and gall bladder were removed and fixed in 10% BNF. Twelve were maintained at $26^{o}C$ and 12 at $4^{o}C$. From these two test groups, 2 pigs were necropsied at 3, 6, 12, 24, 48 and 96 hours postmortem (PM), respectively. The remaining 4 pigs were frozen at $-10^{o}C$ and 2 were necropsied at 24 and 48 hours PM, respectively. Sections cut from the livers and gall bladder were stained with hematoxylin and eosin.

The earliest changes were observed 3 hours PM at $26^{o}C$ and included sloughing of gall bladder epithelium, and loss of hepatocyte basophilia and granularity. Fragmentation and margination of nuclear chromatin were prominent at 12 hours. Fading was the most typical nuclear change. Glycogen loss was rapid but presisted as long as 96 hours. Lobular pattern was retained.

At 4°C, the changes were less rapid. Fragmentation of nuclear chromatin was not observed and hepatocyte basophilia and granularity was significantly delayed till 6 hours PM. Sloughing of gall bladder epithelium was observed at 6 hours. Hepatocyte individualization was marked at 12 hours. It was noted that changes at 4°C occurred half as rapidly as they did at 26°C. Glycogen loss was noted to be less rapid and to follow no particular pattern. Hemolysis was less severe than at 26°C and clumping, crenation and anisocytosis were observed.

At -10°C, hemolysis was absent. Clumping of erythocytes and anisocytosis were prominent. There was pyknosis of Kupffer cells and rupture of some cell membrane walls due to freezing. The gall bladder epithelium was preserved at 24 hours but had sloughed at 48 hours after freezing. Fragmentation and pyknosis of hepatocytes were not observed.

The most rapid alterations observed in this study were loss of cytoplasmic basophilia and sloughing of gall bladder epithelium. However, following death, the Kupffer cells lost their elongated, slender form and became swollen and pyknotic at the temperatures employed. Bearing these alterations in mind, good histologic studies of the liver can be made within 24 hours postmortem at 4°C and up to 48 hours at -10°C.