HISTOLOGIC CHARACTERIZATION OF BOVINE FETAL LUNG, LIVER, AND KIDNEY

by

GARY A. ANDERSON

B.S., South Dakota State University, 1975
D.V.M., Kansas State University, 1979

A MASTER'S THESIS

submitted in partial fulfillment of

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1979

Approved by:

Major Professor

DEDICATION

To Millie and Andy

ACKNOWLEDGEMENTS

I am grateful to my major professor, Dr. H. W. Leipold, for the help he has given throughout this study. I am also grateful to committee members, Drs. J. E. Mosier and S. M. Dennis, for their input.

The collection of fetuses and immunologic and bacteriologic surveys were carried out by the staff at the Animal Disease Research and Diagnostic Laboratory, Dr. M. W. Vorhees, Head, South Dakota State University, Brookings, South Dakota. Special thanks are extended to Drs. C. A. Kirkbride and Kurt Wohlgemuth for initiating this investigation, as well as their generous encouragement and assistance whenever needed.

TABLE OF CONTENTS

INTRODUCTION

Just as knowledge of normal function is essential for understanding pathologic processes, knowledge of normal structures and their development is essential for interpretating the microscopic appearance of tissues. Most histologic reports deal primarily with completely formed organs and treatment of embryologic or other developmental aspects is substantially ignored. Only organogenesis and early histogenesis receive considerable attention in embryologic reports. Consequently, knowledge of periods between embryologic and adult histologic consideration are grossly insufficient. This gap occurs when fetal changes are taking place rapidly and primitive patterns are converted to mature, complex arrangements.

Bovine abortion is a major economic concern for the livestock industry. Pathologists work daily with microscopic material from aborted fetuses, and reports on normal fetal histology are few and far-between. In fact, reports are essentially nonexistent for the bovine species. Since most organs change constantly and have microscopic differences throughout fetal development, familiarity with normal fetal histology is imperative for evaluating fetal tissues; because of these differences, fetal histology is often difficult to grasp.

The objectives of this investigation were to histologically follow the development of the lung, liver, and kidney in the bovine fetus. Lung, liver, and kidney sections were selected because they are the organs commonly examined for diagnosing abortions. The goal was to provide descriptions that would assist anyone microscopically

studying fetal tissues, particularly those persons attempting to identify abortive lesions.

I. HISTOLOGIC CHARACTERIZATION OF BOVINE FETAL LUNG, LIVER, AND KIDNEY: REVIEW OF LITERATURE

Lung

Volumes of literature record lung development in man, but few reports describe normal pulmonary development in other animals. Animals studied include dogs and cats,[1,2] mice,[3] rats,[4,5] guinea pigs,[6] rabbits,[7] pigs,[8,9] sheep,[10-12] and monkeys.[13] Most animal investigations, however, were concerned only with a small aspect of development and how it related to man. The human lung was accepted as a model because studies of other mammals followed similar patterns.[14]

Before viability, the lung is essentially a glandular organ with closed air spaces. Around the 24th day of embryonic life, the endodermal-lined gut forms a diverticulum from the caudal portion of the pharynx, thus beginning the pulmonary system. The endodermal tube proceeds to invade surrounding mesenchymal tissue by dichotomous branching. These divisions are accompanied by cartilage at 10 weeks gestation and new bronchi are complete by 16 weeks. However, cartilage continues to appear until the 24th week, reaching the same extent as found at term.[15] Paired segmental arteries arising from the dorsal aorta caudal to the aortic arches accompany the bronchial tree from its gut evagination and end in capillary plexuses in the developing lung.[16,17]

The epithelial mass invading mesenchyme early in embryonic life has a solid, glandular appearance, and canalization first appears around 4½ months gestation;[16] this concurs with similar periods of gestation in rabbits,[7] lambs,[10] and monkeys.[13] Boyden[13] called it the canalicular stage when columnar epithelium surrounding primitive air sacs became cuboidal and mesenchymal cells condensed to form primitive alveolar septa. The cuboidal lining cells are rich in

intracellular glycogen and differentiate into both Type I and Type II epithelial cells. In addition, these cuboidal cells make up most of the epithelial lung tissue at 6 months gestation.[16]

Between 6 and 7½ months, small, terminal air sacs give way to air spaces with branching patterns characteristic of alveolar ducts. Some epithelial cells flatten and extend the periphery of their cytoplasm, while interstitial tissue decrease and capillaries become incorporated into interstitial septa.[7,14,16]

Alveolar ducts continue to bud during the interval of 7½ months to near term; further septal thinning and epithelial flattening also occur. Kikkawa et al[7] reported that lung maturation in the rabbit proceeded from the center, so peripheral sections had slightly more primitive arrangements. Avery and Fletcher[15] classified epithelial cells and their locations during this period similar to mature lung. Type I alveolar lining cells covered anastomosing capillaries, and Type II great alveolar cells were cuboidal, glycogen-rich, and clustered at junctional sites. Clara cells were also cuboidal and glycogen-rich but lined respiratory bronchioles and alveolar ducts.

Concomitant development of rich pulmonary lymphatics occurred with development of pulmonary circulation. Lymphatic vessels surrounded bronchi down to alveolar ducts and were usually adjacent to pulmonary arteries and veins.[16] Intrapulmonary lymphoid tissue was not detected at any stage of development in the fetal pig.[14]

It must be stressed that architecture of the lung is never entirely uniform at any given stage of development, but general patterns are apparent and usually consistent among mammalian species.[7,14,15]

Liver

One characteristic unique to all vertebrates is a functional liver in adults. However, they demonstrate unbelievable diversity in development modes before attaining the final structure. Amphibians retain the most primitive hepatic developmental patterns, while mammals are more complex. Many accounts stating developmental differences exist and were summarized by Elias and Sherrick.[18] Although studies record various developmental aspects of the liver in the pig,[19] rat,[20] mouse,[21] rabbit,[22] and primates,[23] only one investigation concerned the bovine and did not involve microscopic descriptions of the fetus.[24] Most reports dealt either with developmental patterns in the embryo or histology of the mature organ, and omitted fetal development. Since higher vertebrates demonstrated striking similarities in final hepatic architecture and most information related to human development, human patterns were emphasized.

Elias and Sherrick[18] reported a large hepatic diverticulum that bulged from the cranioventral wall of the foregut-yolk sac junction early in embryogenesis. The diverticulum protruded into the septum transversum, and the septum was invaded by a plexus that arose from the vitelline veins and communicated with the umbilical veins. Visceral mesothelium also proliferated and extended between vitelline capillaries but was more caudally located. The hepatic muralium, a system of walls, is established by epithelial cells surrounding capillaries that become sinusoids.[25,26] Elias and Sherrick[18] also thought epithelial cells could originate from converted and recruited mesenchyme, similar to chick and pig embryoes.

The entire network of inter- and intralobular bile ductules develop from transformed liver cells. Epithelial vesicles develop

between hepatic cells and become separated by connective tissue. These vesicles flatten and proliferate until they anastomose with larger canals in the periportal spaces. Although most ductules were formed during the first trimester, DuBois[26] observed formation in all stages of fetal development.

A sinusoid was defined as a capillary lined by Littoral cells, or Kupffer cells; these cells were flat and demonstrated ability to engulf particulate matter as it flowed through the capillary. Capillaries originated from hepatic trabeculae advancing into lumina of umbilical and vitelline veins and pushing endothelium in front of them.[18] In addition, Elias[27] considered blood flow through the liver to be partially controlled by sinusoids. Kupffer cells were reported to bulge into the lumina of sinusoids and to slow the flow.

Hepatocytes were building blocks for the muralium and different sizes were apparent. Size and volume were dependent upon location of individual cells in the muralium.[18] Liver cells occupying corners of plates were larger than those in the middle of walls, while the smallest cells were adjacent to perforations in the liver plate. Although hapatocyte nuclei were usually large, Elias[25] believed size was related to chromosome numbers. Wilson and Leduc[21] encountered binucleated and multinucleated hepatocytes in mice and Doljanski[28] reported binucleated cells only in humans after birth. Cytoplasmic mass was small and finely granulated in young hepatocytes, but cytoplasm increased when the liver began to accumulate glycogen and lipid reserves.[26]

In man, hemopoiesis reaches its maximum around the 7th month of gestation and then decreased.[18] Hemopoietic cells appeared between endothelium and hepatocyte, that were extravascular in perivascular

mesenchyme.[18,29] Wilson et al[30] believed hemopoietic cells were descendants of proliferating mesothelium, but Thomas et al[31] followed a complete morphologic spectrum showing liver cells transforming into hemopoietic cells.

The muralium structure of the liver is basic and in accordance with a fundamental plan of vertebrate animals. Architectural patterns are established early and, unlike the fetal lung, change little throughout gestation.

Kidney

There are numerous reports of the different developmental aspects of the human kidney.[32-34] Investigations have been reported on the sheep,[35] cat,[36] mouse,[37] pig and apposum,[38] and rabbit.[39] Developmental patterns were similar in all mammals, except for a few minor species differences. Excellent accounts by DuBois[40] and Potter[41] stressing human development, were used for basic developmental patterns.

Development of the kidney is considered one of the most complicated and puzzling processes of histogenesis. Most organs evolved as smooth, direct processes from initial enlargements in the embryo, but is not true for the kidney.[41] The permanent kidney, metanephros, would not differentiate unless it was preceded by successive embryonic excretory organs, the pronephros followed by the highly differentiated mesonephros. Reptiles, birds, and mammals possessed a metanephros, while fish and amphibians developed only a functional mesonephros.[40]

The metanephros originated from 2 types of cells that had differing potentialities. One type, the ureteral buds, arose from the mesonephric ducts and divided into ureters, renal pelvis, calyces, and collecting

tubules. Actively growing portions of the buds were called ampullae, while remaining interstitial portions made up the tubules. These tubules were produced from forward growth of the ampullae. The first 3 to 5 generations of tubules from ampullary divisions dilated to form the renal pelvis, and the next 3 to 5 generations dilated to form minor calyces, papillae, and cribiform areas. Collecting tubules were formed from the last 6 to 9 generations of branching tubules.[41,43]

The second cell type that contributed to kidney development was the metanephric blastema. Because it had stromagenic and nephrogenic properties, it was responsible for the nephrons and connective tissue. Early in the embryo blastema, cells formed a zone 3 to 4 cells thick around the ampullae, and as ureteral buds divided, the blastema cells advanced in front of the ampullae. Interstitial portions between tubules were surrounded by differentiated connective tissue left behind after blastema advancement. This pattern of development occurred throughout life of the ampullae. Cells adjacent to interstitial portions of tubules were converted to connective tissue and those immediately next to ampullae were converted to nephrons.[41]

A discrete zone of nephrogenic cells adjacent to each ampulla was formed during ampullary activity. Nephrogenic vesicles were oval masses that formed near the junctions of ampullae and the remainder of the tubules. Vesicles were soon converted to structures resembling the letter S. Upper portions of the S differentiated into a renal corpuscle, while the remainder became tubular.[44] The S then touched the ampulla so communication was established between the nephron and collecting tubule.[44,45]

Immature renal corpuscles are unique. Their capillaries are covered by cuboidal or columnar cells instead of flattened endothelial

cells characteristic of mature glomeruli. Bowman's spaces were also lined by cuboidal cells that are somewhat flattened along the parietal layer.[29,46] The most immature glomeruli are always located just beneath and parallel to the kidney's capsule. This more or less distinct zone was called the nephrogenic or neogenic zone. Vernier and Birch-Andersen[47] studied fetuses up to 20 weeks gestation and reported the entire spectrum of glomerular development from the most immature to well-developed nephrons. They believed structural renal maturation was related to the relative number of mature nephrons.

Kidney development is complicated and progressive sequences are difficult to ascertain because so many involved processes occur simultaneously. However, mammals demonstrate remarkable similarities in fetal development. Good techniques in light microscopy, electron microscopy, and microdissection are important for a good understanding of the kidney.

This review of literature is not intended to be exhaustive. Although the basic interest was in the middle and late fetal periods, a background of embryonic and early fetal periods was necessary to appreciate and understand the more advanced developmental stages. Histologic descriptions are often absent from literature and seemingly irrelevant, but information and processes are compiled for reference to the microscopic features described in this thesis.

REFERENCES

1. Knight DS: Innervation of the pulmonary vessels in the dog and cat. Anat Rec 65:537-542, 1963.

2. Boyden EA: The postnatal growth of the lung in the dog. Acta Anat 47:185-215, 1961.

3. Alescio T, DiMichele M: Relationship of epithelial growth to mitotic rate in mouse embryonic lung development in vitro. J Embryol Exp Morphol 19:227-237, 1968.

4. Meyrick B, Reid L: The alveolar brush cell in rat lung, a third pneumocyte. Ultrastruct Res 23:71-80, 1968.

5. Blenkinsopp WK: Proliferation of respiratory tract epithelium in the rat. Exp Cell Res 46:144-154, 1967.

6. Reynolds SRM: The fetal and neonatal pulmonary vasculature in the guinea pig in relation to haemodynamic changes at birth. Am J Anat 98:97-127, 1956.

7. Kikkawa Y, Motoyama EK, Gluck L: Study of the lungs of fetal and newborn rabbit. Am J Path 52:177-207, 1968.

8. Baskerville A: Ultrastructural studies of the normal pulmonary tissue of the pig. Res Vet Sci 11:150-155, 1970.

9. Clements LP: Embryonic development of the respiratory portion of the pig's lung. Anat Rec 70:575-590, 1938.

10. Orzalesi MM, Moroyama EK, Jacobson HN: The development of the lung in the lamb. Pediatr 35:373-381, 1965.

11. Adams FH, Fujiwara R: Surfactant in fetal lamb tracheal fluid. J Pediatr 65:537-542, 1963.

12. Dawes GS, Mott JC, Widdicombe JG, Wyatt DG: Changes in the lungs of the newborn lamb. J Physiol 121:141-162, 1953.

13. Boyden EA: The development of the lung in the pigtail monkey (Macaca nemestrina L). Anat Rec 186:15-37, 1976.

14. Baskerville A: Histological and ultrastructural observations on the development of the lung of the fetal pig. Acta Anat 95:218-233, 1976.

15. Avery ME, Fletcher BD: The Lung and Its Disorders in the Newborn Infant. Philadelphia, W.B. Saunders Co, 1974, pp 3-37.

16. Stahlman MT: Acute respiratory disorders in the newborn, in Avery GB (ed): Neonatology. Philadelphia, J.B. Lippincott Co, 1975, pp 221-223.

17. Arey LB: Developmental Anatomy. Philadelphia, W.B. Saunders Co, 1965.

18. Elias H, Sherrick JC: Morphology of the Liver. New York, Academic Press Inc, 1969, pp 5-73, 189-261.

19. Elias H, Bond E, Lazarowitz A: The "normal" liver of the pig; is it an example of purely portal (and therefore subclinical) cirrhosis? A preliminary report. Am J Vet Res 15:60-66, 1954.

20. Gershbein L, Elias H: Observations on the anatomy of the liver of the rat. Anat Rec 120:89-98, 1954.

21. Wilson TW, Leduc EH: The occurrence and formation of binucleate cells and polyploid nuclei in the mouse liver. Am J Anat 82:353-392, 1948.

22. Yamagishi M: Electron microscopic studies on the fine structure of the sinusoidal wall and fat-storing cells of rabbit livers. Arch Histol Jap 18:223-261, 1959.

23. Bearcroft WGC: Histological studies on the livers of West African primates inoculated with infectious hepatitis. J Path Bact 88:511-519, 1964.

24. Julian LM, deOhme, KB: Studies on the subgross anatomy of the bovine liver. I. Distribution of the blood vessels and bild ducts. Am J Vet Res 10:331-335, 1949.

25. Elias H: A re-examination of the structure of the mammalian liver. I. Parenchymal architecture. Am J Anat 84:311-334, 1949.

26. Du Bois AM: The embryonic liver, in Rouiller C (ed): The Liver. New York, Academic Press Inc, 1963, pp 1-39.

27. Elias H: Liver morphology. Biol Rev 30:263-310, 1955.

28. Doljanski F: The growth of the liver with special reference to mammals. Intern Rev Cytol 10:217-241, 1960.

29. Valdes-Dapena MA: An Atlas of Fetal and Neonatal Histology. Philadelphia, J.B. Lippincott Co, 1957, pp 75-87.

30. Wilson JW, Groat CS, Leduc EH: Histogenesis of the liver. Ann NY Acad Sci 111:8-24, 1963.

31. Thomas DB, Russell PM, Loffey JM: Pattern of hematopoiesis in fetal liver. Nature 187:877-879, 1960.

32. Rossi FG, Reale P, Reale E: Histochemical determination of acid and alkaline phosphate in the initial stages of the urinary apparatus during the prenatal development of man. Acta Anat 19:232-238, 1953.

33. Torrey TW: The early development of the human nephros. Contrib Embryol Carnegie Inst Wash Publ 35:175-197, 1954.

34. Ludwig E: Developmental history of the metanephros. Acta Anat 8:1-17, 1949.

35. Davies J: Correlated anatomical and histochemical studies on the mesonephros and placenta of the sheep. Am J Anat 91:263-299, 1952.

36. Fraser EA: The pronephros and early development of the mesonephros in the cat. J Anat 54:287-303, 1923.

37. Vetter MR, Gibley CW: Morphogenesis and histochemistry of the developing mouse kidney. J Morph 120:135-156, 1966.

38. Gersh I: The correlation of structure and function in the developing mesonephros and metanerphros. Contrib Embryol Carnegie Inst Wash Publ 26:33-58, 1937.

39. Leeson TS, Baxter JS: The correlation of structure and function in the mesonephros and metanephros of the rabbit. J Anat 91:383-390, 1957.

40. DuBois AM: The embryonic kidney, in Rouiller C, Muller AF (ed): The Kidney. New York, Academic Press Inc, 1969, pp1-50.

41. Potter EL: Normal and Abnormal Development of the Kidney. Chicago, Year Book Medical Publishers Inc, 1972, pp3-79.

42. Gruenwald P: The mechanism of Kidney development in human embryos as revealed by an early stage in the agenesis of the ureteric buds. Anat Rec 75:237-244, 1939.

43. Osathanondh V, Potter EL: Development of human kidneys as shown by microdissection; renal pelvis, calyces, and papillae, Arch Path 76:53-65, 1963.

44. Potter EL, Osathanondh V: Normal and abnormal development of the kidney, in Mostofi FKM, Smith DE (ed): The Kidney. Baltimore, Williams & Wilkins, 1966, pp1-16.

45. Jokelainen P: An electron microscope study of the early development of the rat metanephric nephron. Acta Anat 52: 1-71, 1963.

46. Fergusson AM: Studies of the renal blood supply in foetal and neonatal animals. J Anat 86:144-151, 1952.

47. Vernier RL, Birch-Andersen A: Studies of the human fetal kidney. Development of the glomerulus. J Ultrastruct Res 8:66-88, 1963.

II. HISTOLOGIC CHARACTERIZATION OF THE BOVINE FETAL LUNG

INTRODUCTION

Purves[1] stated that onset of respiration was essential for life to continue from intrauterine to extrauterine, and that fetal development must have evolved sufficiently for the successful transition. Viability of newborn humans was often limited by lung maturity;[2,3] other mammalian neonates cannot be considered exceptions.

Valuable information concerning behavior of neonatal and adult lungs was attributed to a better understanding of fetal histology that often provided clues to physiologic and pathologic processes.[4]

This study was undertaken to gather information about normal development of the bovine lung during the middle and late fetal periods.

MATERIALS AND METHODS

One-hundred twenty-four fetuses were collected from an abattoir in Huron, South Dakota during April and May in 1976. Gestational age was estimated according to the methods described by Hubbert et al[5] and any fetuses less than 120 days were discarded. All fetuses were removed from the uterus within 30 minutes after the cow was stunned by a captive bolt. A complete necropsy was performed and specimens were collected for bacteriologic, histopathologic, and radial immunodiffusion analysis (Appendix 1).

Lung, liver, and kidney were placed in 10% buffered neutral formalin for histologic examination, trimmed, embedded in paraffin, sectioned at 6 u, and routinely stained with hemotoxylin and eosin (H & E).

RESULTS

Radial immunodiffusion, bacteriologic, and histopathologic results are tabulated and summarized in Appendix 2.

Histologic Characterization:

A routine method was established for examining lung sections. To eliminate repetition, characteristics were considered consistent throughout gestation unless specifically described. The results are summarized in Table I and illustrated in Fig 1-28.

3½ to 4½ months gestation - Organoid architecture, representing potential air spaces, of the lung was most evident during this period. Future air passages appeared as simple, tubular spaces that branched frequently in loose mesenchymal stroma. Tubular spaces were lined by columnar epithelium with large, basophilic nuclei located adjacent to luminal surfaces. These lining cells, initial epithelium, were distinguished by clear cytoplasm, distinct cell boundaries, and prominent nuclei. Air spaces were separated by thick walls of abundant mesenchyme. Thin interlobular septa, composed of loosely arranged reticular tissue, separated the parenchyma into a lobular pattern. Prominent lymphatics were often present.

Nerves and blood vessels were usually located near large airways. However, small arterioles and capillaries were randomly situated within the mesenchymal stroma, lying between air spaces. Large bronchi were lined by pseudostratified ciliated, columnar epithelium. Cartilage, glands, and goblet cells were present.

Bronchioles were distinguished by a simple ciliated columnar to cuboidal epithelial lining. Goblet cells, mucus glands, and cartilage

were not usually present, and arterioles were often adjacent to bronchioles. Branching air passages began forming alveolar ducts and sacs later in this period. The lining epithelium was cuboidal with a centrally located nucleus in clear cytoplasm, but it was not nearly as prominent as the epithelium lining larger passages.

5 to 6½ months gestation - Original gland-like architecture of the lung became less apparent. Blood vessels began to appear in increasingly close relationship to air passages, that continued to form complex, saccular spaces. Blood capillaries often bulged into distal air passages and thinned out or disrupted the continuity of the lining epithelium. As spaces became more complex, mesenchymal stroma decreased so that only a thin partition remained between air sacs, that was gradually replaced by a thin network of fibers, fibroblasts, macrophages, and capillaries. Conversion from an immature organoid architecture to a complex system of air spaces for respiratory exchange began to become obvious during this period.

Lobules and interlobular septa increased in size, and lymphatic vessels became increasingly prominent within the interlobular septa.

Debris of many types within all air spaces began to appear with greater frequency and in varying quantities: mucus, meconium, keratinized squamous cells and vernix, epithelial cells, and unidentified material. Mucus was basophilic and arranged loosely in a fibrillar pattern, while meconium was amorphous, clumped, greyish to golden-brown, and had distinct borders. Vernix and squamae were lightly eosinophilic, thick, often spiculed, and had pleomorphic and meshing patterns. Epithelial cells were pleomorphic with faint outlines and nuclei that were usually pyknotic but sometimes absent.

7 months to near term - Few characteristics were unique to this period. Conversion to a complex structure was completed. Flattened, inconspicuous epithelium consistently separated vessels from air sacs, or alveolar spaces. Lobules continued to enlarge, but the overall architecture was not altered. Interlobular septa were wide and the lymphatics remained prominent. As term approached, so-called initial epithelium became difficult to find, even in the most terminal or peripheral air spaces. Lymphoid tissue continued not to be present around airways or vessels. Golden-brown meconium was present more often during this time. In general, the parenchyma was similar to that of lung tissue that had not yet inflated with air.

DISCUSSION

Estimation of fetal age according to Hubbert et al[5] was considered accurate within ± 5 days. The procedure was substantiated by mathematical expressions and statistical analysis.[6,7] Because of severe drought conditions, many farmers in South Dakota were required to extensively cull their beef herds. As a result many fetuses from normal, healthy cows became available. Prior and Lancaster[8] reported that fetal weight or composition were not influenced by maternal dietary levels. Only fetuses considered normal after laboratory evaluation were used for histologic characterization.

Histologic observations of lung sections revealed developmental patterns similar to that of other mammals. The lung was gradually transformed from a mass of mesenchymal tissue with endodermal channels to a highly vascularized system of irregular air sacs lined primarily

by simple squamous epithelium. Capillary migration, along with a progressive decline in mesenchymal stroma, and increased terminal air sacs were important stages necessary for efficient extrauterine respiration and existence. Respiratory function would be seriously impeded and independent existence prior to 6 months gestation slight.[9] The absence of lymphoid tissue around airways and blood vessels in significant when assessed in relation to diagnostic evaluation of abortions. Baskerville[4] considered migration of lymphoid cells from lymphoid organs to the lung occurred via blood after antigenic stimulation. He also thought that antigenic stimulation caused some lymphoid cells to develop from precursor mesodermal cells in the lung's connective tissue.

Various types of debris were common, particularly squamae and meconium. Whether this debris is significant depends upon the quantity and extent within air spaces. To be considered important, debris had to nearly fill the spaces. Fetal hypoxia and distress during routine slaughter collection could have been responsible for much of the debris observed.

Although maturation was not uniform throughout the lung at any one time, progression occurred so that early developmental stages coincided well with fetal age estimations. The findings should be of value to pathologists and diagnosticians involved in the evaluation of bovine abortions.

SUMMARY

One-hundred twenty-four bovine fetuses, between 3½ months and near term, were collected at a South Dakota abattoir from clinically

healthy cows. Histopathologic, immunologic, and bacteriologic findings indicated that the fetuses were normal and noninfected, and suitable for histologic evaluation.

Histologic data form lung sections were compatible with developmental stages described for other mammals. Organoid architecture was prominent through 4½ months gestation consisting mainly of endodermal channels of initial epithelium-lined spaces within masses of mesenchyme. This gland-like structure began to disappear and was not present by 7 months. During this transition, capillaries migrated next to air spaces and mesenchymal stroma decreased so more complex, saccular spaces were formed. Around 7 months, epithelium flattened, became inconspicuous, and separated capillaries from air sacs. Architectural changes were not prominent during the last 2 months of gestation. However, lobules enlarged and fetal lungs developed striking similarities to adult lungs. Lymphoid tissue was not observed in normal sections, but various types of debris were present, particularly meconium and squamae. Little significance was placed on debris unless it nearly filled air spaces. Maturation was not consistent at any one time, however general histologic patterns were apparent.

REFERENCES

1. Purves, MJ: Onset of respiration at birth. Arch Dis Child 49:333-343, 1974.

2. Avery ME, Fletcher BD: The Lung and Its Disorders in the Newborn Infant. Philadelphia, W.B. Saunders Co, 1974, pp3-37.

3. Lance JS, Latta H: Hypoxia, atelectasis, and pulmonary edema. Arch Path 75:373-377, 1963.

4. Baskerville A: Histological and ultrastructural observations on the development of the lung of the fetal pig. Acta Anat 95:218-233, 1976.

5. Hubbert WT et al: Recommendations for standardizing bovine reproductive terms. Cornell Vet 62:216-237, 1972.

6. Thomsen JL: Body length, head circumference, and weight of bovine fetuses: prediction of gestational age. J Dairy Sci 58:1370-1373, 1975.

7. Eley RM, Thatcher WW, Bazer FW, Wilcox CJ, Becker RB, Head HH, Adkinson RW: Development of the conceptus in the bovine. J Dairy Sci 61:467-473, 1978.

8. Prior RL, Laster DB: Development of the bovine fetus. J Anim Sci 48:1546-1533, 1979.

9. Noris RF, Kockenderfer TT, Tyson RM: Development of the fetal lung. Am J Dis Child 61:933-950, 1941.

TABLE I: Microscopic Features of Fetal Lung Sections

Fetal Age	3½ mo						4 mo							4½ mo								5 mo					
Case Number	76X-198	76X-200	76X-204	76X-225	76X-197	76X-199	76X-205	76X-223	76X-224	76X-226	76X-227	76X-228	76X-201	76X-206	76X-211	76X-216	76X-222	76X-229	76X-230	76X-163	76X-190	76X-207	76X-208	76X-209	76X-210
Microscopic Characteristic																									
Visceral Pleura	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Areolar/Elastic Capsule	1	1	2	1	2	3	3	3	3	1	2	2	3	2	2	2	3	1	3	3	2	2	2	3	3
Loose Reticular/Elastic Interlobular Tissue	1	2	1	1	2	2	3	2	1	1	2	1	2	2	2	2	2	2	3	3	2	2	2	3	3
Vasculature	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Organoid Architecture	4	4	4	3	3	3	4	4	3	4	3	4	3	4	4	2	3	3	3	2	2	2	2	2	2
Intrapulmonary Bronchi	Neg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pseudostratified Ciliated Columnar Epithelium	Neg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Goblet Cells	Neg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Areolar Lamina Propria	Neg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Muscularis Mucosae	Neg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubuloalveolar Mucous Glands	Neg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cartilagenous Rings	Neg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bronchioles	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Simple Ciliated Columnar/Cuboidal Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Goblet Cells	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mucous Glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cartilagenous Plaques	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Muscularis Mucosae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory Bronchioles	Neg	Neg	Neg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cuboidal Epithelium	Neg	Neg	Neg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lamina Propria	Neg	Neg	Neg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Smooth Muscle	Neg	Neg	Neg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar Ducts/Sacs	Neg	Neg	Neg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Debris	Neg	Neg	Neg	1	1	1	Neg	2	Neg	Neg	Neg	Neg	Neg	1	Neg	2	Neg	Neg	Neg	Neg	Neg	1	1	2	Neg

TABLE I: continued

Fetal Age	5 mo						5½ mo						6 mo					6½ mo				7 mo				
Case Number	76X-213	76X-214	76X-217	76X-218	76X-219	76X-221	76X-141	76X-203	76X-212	76X-215	76X-231	76X-140	76X-164	76X-179	76C-187	76C-189	76Z-202	76X-172	76X-182	76X-188	76Z-92	76X-99	76X-115	76X-131	76X-133
Microscopic Characteristic																									
Visceral Pleura	+	+	+	+	+	+	+	+	+	+	+	+	+	+	ND	+	+	+	+	+	+	+	+	+	+
Areolar/Elastic Capsule	3	2	3	2	2	2	2	2	3	2	2	2	2	2	ND	2	3	3	2	2	2	2	2	3	2
Loose Reticular/Elastic Interlobular Tissue	3	3	3	3	2	2	3	1	2	2	2	2	2	3	3	2	2	2	3	2	2	2	2	2	2
Vasculature	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Organoid Architecture	3	2	2	2	3	3	2	Neg	1	2	3	2	1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Intrapulmonary Bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pseudostratified Ciliated Columnar Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Goblet Cells	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Areolar Lamina Propria	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Muscularis Mucosae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubuloalveolar Mucous Glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cartilagenous Rings	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bronchioles	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Simple Ciliated Columnar/Cuboidal Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Goblet Cells	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mucous Glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cartilagenous Plaques	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Muscularis Mucosae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory Bronchioles	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cuboidal Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lamina Propria	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Smooth Muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar Ducts/Sacs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Debris	2	1	2	1	Neg	Neg	Neg	1	Neg	1	Neg	Neg	2	1	Neg	1	Neg	Neg	1	1	2	1	3	3	2

TABLE I: continued

Fetal Age	7 mo											7½ mo										8 mo						
Case Number	76E-137	76E-142	76E-154	76E-158	76E-161	76E-177	76E-181	76E-192	76E-193	76E-220	76E-93	76E-95	76E-132	76E-145	76E-150	76E-156	76E-159	76E-173	76E-174	76E-97	76E-129	76E-135	76E-139	76E-146	76E-147
Microscopic Characteristic																									
Visceral Pleura	+	+	+	+	+	ND	+	+	+	+	+	+	+	+	+	+	+	ND	+	+	+	+	+	+	+
Areolar/Elastic Capsule	2	2	1	2	3	ND	2	3	2	1	3	3	2	2	2	2	2	ND	2	2	3	2	1	1	2
Loose Reticular/Elastic Interlobular Tissue	2	2	2	2	2	2	2	3	3	1	2	2	3	2	2	3	2	2	2	2	3	2	2	1	1
Vasculature	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Organoid Architecture	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Intrapulmonary Bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pseudostratified Ciliated Columnar Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Goblet Cells	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Areolar Lamina Propria	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Muscularis Mucosae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubuloalveolar Mucous Glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cartilagenous Rings	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bronchioles	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Simple Ciliated Columnar/Cuboidal Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Goblet Cells	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mucous Glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cartilagenous Plaques	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Muscularis Mucosae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory Bronchioles	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cuboidal Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lamina Propria	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Smooth Muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar Ducts/Sacs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Debris	2	1	2	3	1	Neg	1	2	1	Neg	1	1	3	1	1	1	1	1	Neg	2	2	1	1	1	1

TABLE I: _continued

Fetal Age	8 mo								8½ mo										NT							
Case Number	76X-151	76X-155	76X-157	76X-171	76X-176	76X-178	76X-186	76X-194	76X-128	76X-134	76X-136	76X-149	76X-153	76X-160	76X-162	76X-175	76X-184	76X-138	76X-90	76X-91	76X-94	76X-96	76X-100	76X-101	76X-102
Microscopic Characteristic																										
Visceral Pleura	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Areolar/Elastic Capsule	2	3	2	1	2	2	2	2	2	2	2	2	3	3	2	2	3	2	3	2	3	1	2	2	2	
Loose Reticular/Elastic Interlobular Tissue	2	2	2	2	2	2	2	2	2	2	2	1	2	3	2	2	3	2	2	2	2	3	2	2	2	
Vasculature	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Organoid Architecture	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	
Intrapulmonary Bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Pseudostratified Ciliated Columnar Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Goblet Cells	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Areolar Lamina Propria	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Muscularis Mucosae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Tubuloalveolar Mucous Glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Cartilagenous Rings	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Bronchioles	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Simple Ciliated Columnar/Cuboidal Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Goblet Cells	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Mucous Glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Cartilagenous Plaques	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Muscularis Mucosae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Respiratory Bronchioles	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Cuboidal Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lamina Propria	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Smooth Muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar Ducts/Sacs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Debris	Neg	1	2	1	1	1	1	Neg	2	3	1	2	3	Neg	Neg	1	Neg	2	1	Neg	1	1	2	2	2	

TABLE I: continued

Fetal Age												NT										
Case Number	76X-103	76X-104	76X-105	76X-106	76X-107	76X-108	76X-109	76X-110	76X-111	76X-112	76X-113	76X-114	76X-116	76X-143	76X-144	76X-148	76X-152	76X-180	76X-183	76X-185	76X-191	76X-195
Microscopic Characteristic																						
Visceral Pleura	+	+	+	+	+	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Areolar/Elastic Capsule	2	2	2	2	2	ND	2	2	2	1	2	1	2	2	2	2	1	2	2	2	2	1
Loose Reticular/Elastic Interlobular Tissue	2	2	2	2	2	2	2	2	2	2	2	3	2	2	2	2	2	2	2	2	2	2
Vasculature	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Organoid Architecture	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Intrapulmonary Bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pseudostratified Ciliated Columnar Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Goblet Cells	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Areolar Lamina Propria	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Muscularis Mucosae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubuloalveolar Mucous Glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cartilagenous Rings	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bronchioles	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Simple Ciliated Columnar/Cuboidal Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Goblet Cells	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mucous Glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cartilagenous Plaques	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Muscularis Mucosae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory Bronchioles	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cuboidal Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lamina Propria	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Smooth Muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar Ducts/Sacs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Debris	1	1	1	1	2	1	2	1	1	3	1	Neg	1	1	Neg	1	2	Neg	1	1	2	2

The capsule and pleura of the lung are included in these photomicrographs. Note the capsule consists of coarse areolar connective tissue rich in elastic fibers. The most striking feature is the change in the overall pattern from an organoid architecture to a complex, saccular arrangement of potential air spaces.

Fig 1-The tubular spaces are lined by initial epithelium and separated by thick walls of mesenchymal stroma. Arterioles are present within the stroma between air spaces (arrow). 76X-204 (3½ months); H & E stain.

Fig 2-Note how branching air passages form air spaces reminiscent of alveolar ducts and sacs. Mesenchymal stroma decreases and the saccular spaces are separated only by thin interstitial spaces. 76X-163 (5 months); H & E stain.

Fig 3-Alveolar partitions continue to thin out and initial epithelium is difficult to find. 76X-177 (7 months); H & E stain.

Fig 1

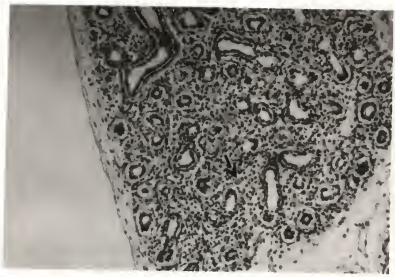


Fig 2

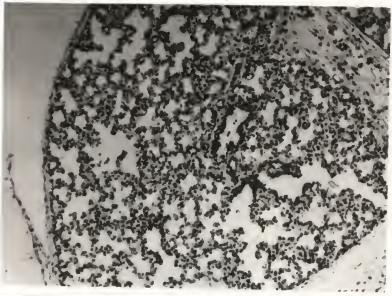
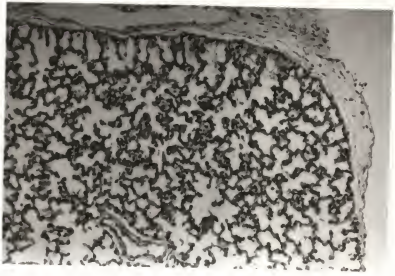


Fig 3



The next 5 photomicrographs demonstrate potential air spaces at higher magnifications.

Fig 4-The tubular spaces here are lined by columnar to cuboidal initial epithelium. These lining cells have clear cytoplasm, distinct cell borders, and prominent nuclei; the lumina are empty. 76X-204 (3½ months); H & E stain.

Fig 5-Original organoid architecture is not apparent in this section. Tubular spaces have branched into a saccular architecture and lining epithelium is not visible. Capillaries begin to bulge into air spaces, disrupting the lining continuity. 76X-163 (5 months); H & E stain.

Fig 6-Interstitial spaces continue to thin. 76X-177 (7 months); H & E stain.

Fig 4

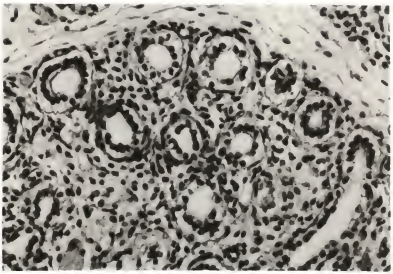


Fig 5

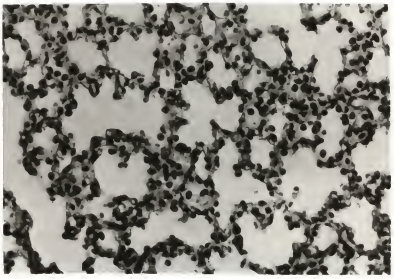


Fig 6

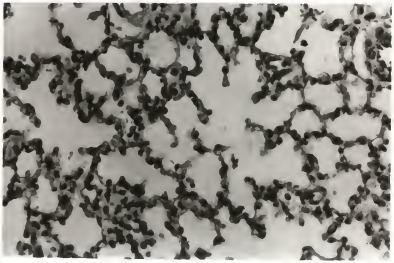


Fig 7-This is a higher magnification of maturing parenchyma. 76X-97 (8 months); H & E stain.

Fig 8-Note bronchiole lined by cuboidal epithelium that is only reminiscent of initial lining epithelium. 76X-111 (near term); H & E stain.

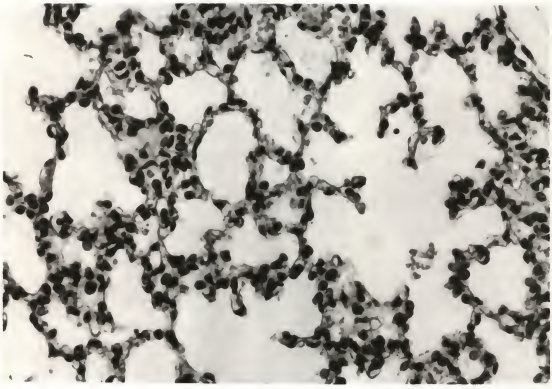


Fig 7

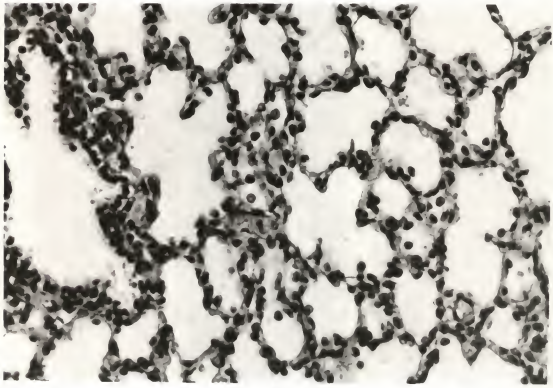


Fig 8

These photomicrographs represent smaller conductive portions of the lung.

Fig 9-This is a tertiary bronchus with pseudostratified ciliated, columnar epithelium. Goblet cells and lamina muscularis mucosae are present, while mucous glands and cartilage are absent.

Fig 10-Bronchioles are smallest divisions of the nonrespiratory portions of the lung. The lamina epithelialis is usually simple cuboidal but may be columnar in more proximal bronchioles. Cilia are present in this bronchiole, however, they diminish in distal bronchioles. It is important that cilia extend further down the respiratory tree than glands. 76X-111 (near term); H & E stain.

Fig 11-Various conductive portions of the lung are apparent here. Note diffences in lining epithelium and mural constituents of the large bronchus (L), tertiary bronchus (T), and bronchioles (B). Cartilage is present between the bronchi. 76X-204 (3½ months); H & E stain.

Fig 9

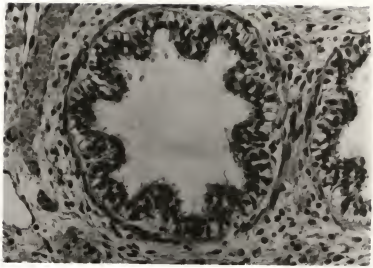


Fig 10

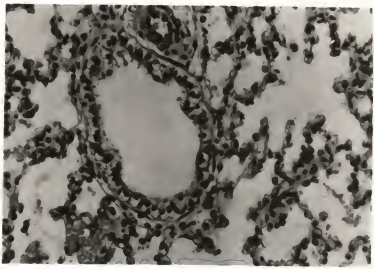


Fig 11

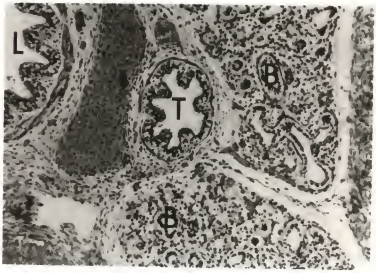


Fig 12-This is low power of a terminal bronchiole entering into an alveolar duct. The bronchiole is lined by cuboidal epithelium and is devoid of goblet cells and cartilage. 76X-97 (near term); H & E stain.

Fig 13-Entrance of a respiratory bronchiole into an alveolar duct at high power is present. Note the arteriole lying adjacent to the bronchiole. Cuboidal cells of the bronchiolar walls are interrupted by outpocketing alveoli. Lamina propria is indistinct, but fine collagenous and elastic fibers support lining cells. 76X-144 (near term); H & E stain.

Fig 14-These are distal pathways of the lung. Alveolar sacs are surrounded by alveoli. 76X-111 (near term); H & E stain.

Fig 12

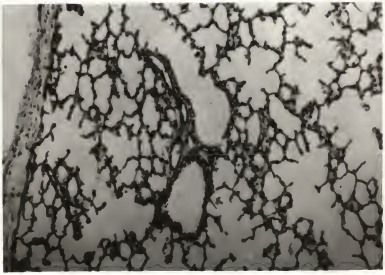


Fig 13

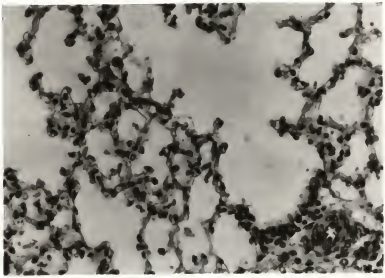


Fig 14

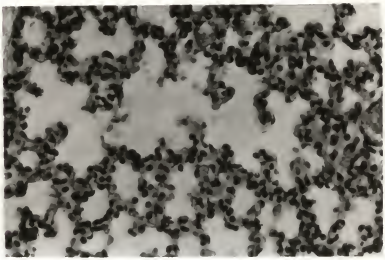


Fig 15-A pulmonary arteriole and secondary bronchiole are lying adjacent to each other. Note the wall of the bronchiole is lacking cartilage, but the lamina muscularis mucosae is present and continuous. The lamina propria is scant but consists of fine collagenous and elastic fibers. 76X-144 (near term); H & E stain.

Fig 16- This is another section of a pulmonary arteriole and bronchiole. 76X-111 (near term); H & E stain.

Fig 17-The pulmonary arteriole is alone in this section. However, the pulmonary artery and its peripheral subdivisions follow the distribution of airways to the level of respiratory bronchioles, where they continue as capillary beds associated with alveoli. 76X-111 (near term); H & E stain.

Fig 15

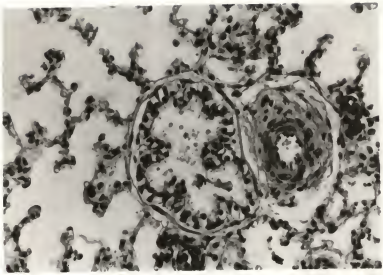


Fig 16

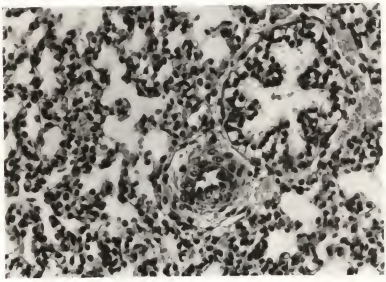


Fig 17

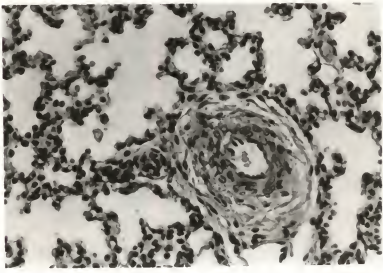


Fig 18-This is a tangential cut through a secondary bronchus. The lamina epithelialis is pseudostratified ciliated epithelium containing numerous goblet cells. The lamina propria is areolar connective tissue with numerous elastic fibers continuous with connective tissue of the hilus.

Fig 19-This is a cross-section of a large bronchus. Note the cilia and the cartilagenous plaque in the lower right. The lamina muscularis mucosae is present beneath the lamina propria. The tunica submucosa consists of areolar connective tissue and has branched, coiled tubuloalveolar mucous glands. 76X-144 (near term); H & E stain.

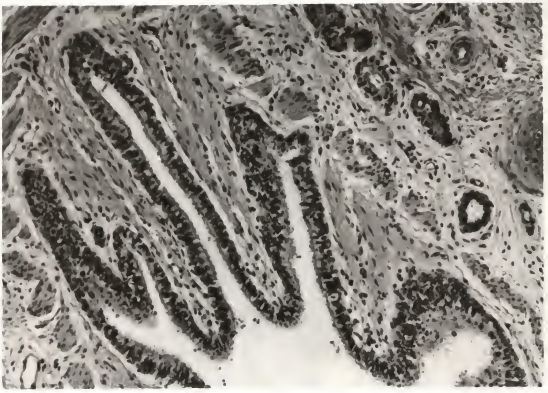


Fig 18

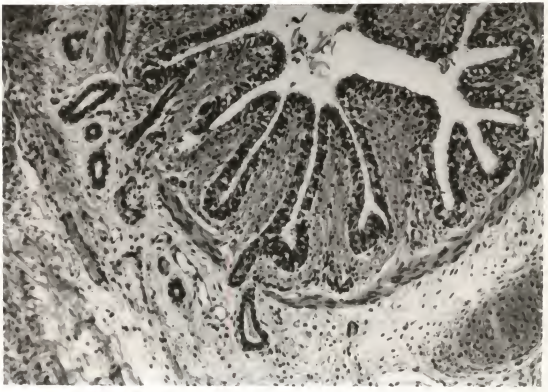


Fig 19

These photomicrographs demonstrate the lung capsule and its lining of visceral pleura.

The capsule consists of loose areolar connective tissue that is usually rich in elastic fibers. Note how interlobular septa are similar to and continuous from the capsular tissue. Blood and lymph vessels and nerves are present in the capsule.

A single layer of mesothelial cells covers the lung. This serous membrane follows the surface contours of lobes.

Note potential air spaces and thickness of their walls.

Fig 20-76X-204 (3½ months); H & E stain.

Fig 21-76X-177 (7 months); H & E stain.

Fig 22-76X-224 (3½ months); H & E stain.

Fig 20

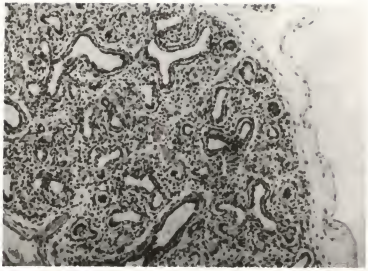


Fig 21

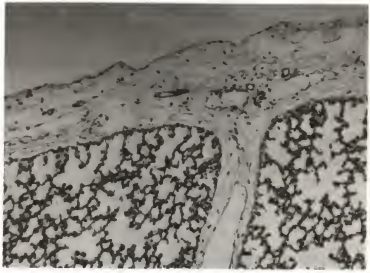
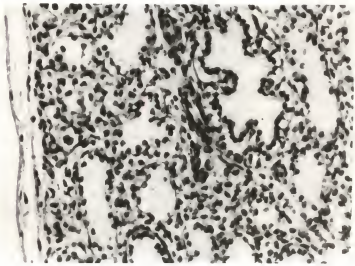


Fig 22



The next 6 photomicrographs illustrate various types of debris.

Fig 23-This bronchiole contains stringy, granular material and a few cells. This type of material was quite common. 76X-191 (near term); H & E stain.

Meconium was common within all air passages and alveoli. It is dark staining (arrows) and is closely associated with other material. Freshly aspirated meconium is usually golden-brown and easy to recognize.

Fig 24-76X-209 (5 months); H & E stain.

Fig 25-76X-209 (5 months); H & E stain.

Fig 23

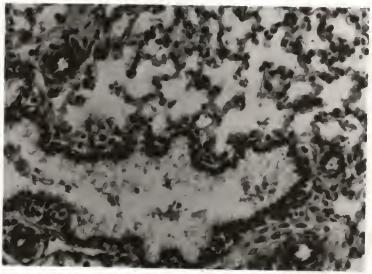


Fig 24

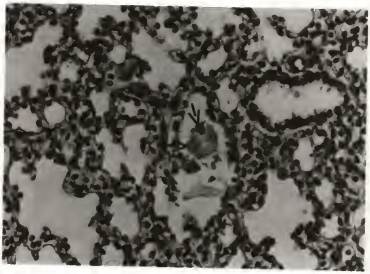


Fig 25

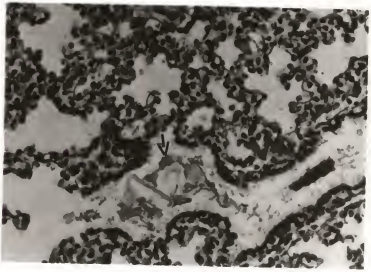


Fig 26-Two keratinized squamous cells (squamae) from the skin are associated with granular material and 2 clumps of meconium (m). 76X-225 (3½ months); H & E stain.

Fig 27-Note the typical aggregate of squamae. Clumps like this were common and seldom completely fill air spaces in normal fetuses. 76X-209 (5 months); H & E stain.

Fig 28-A single-celled, spiculed squame is present in an alveolar sac. The lower right bronchiole contains a clump of sloughed lining cells. 76X-155 (8 months); H & E stain.

Fig 26

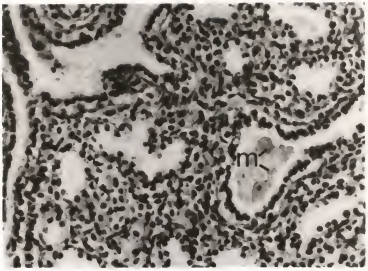


Fig 27

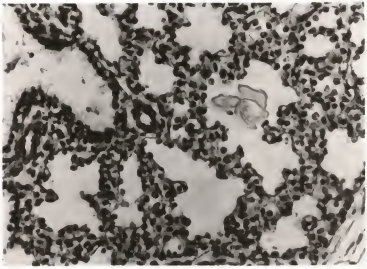
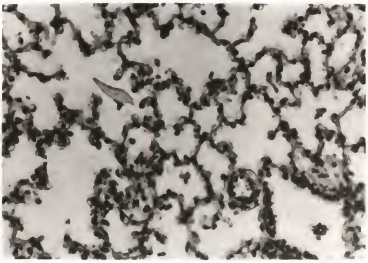


Fig 28



III. HISTOLOGIC CHARACTERIZATION OF THE BOVINE FETAL LIVER

INTRODUCTION

The liver is reported to be an organ unique to vertebrates and to have unequaled functional versatility. Embryologically, the liver is defined as a substitute yolk sac. Development occurs in close association with the yolk sac in all vertebrates and originated directly from the sac in Amphibia. Histologically the liver is described as a continuous muralium of parenchymal cells tunneled by capillaries that are lined by Kupffer cells. It conveys venous blood from the gastrointestinal system to the heart.[1]

Embryologic development and characterization of the mature liver has been reported often for various animal species; microscopic descriptions of development in the fetal bovine are lacking. Consequently, this investigation was concerned primarily with histologic description unique to the middle and late gestation in the bovine.

MATERIALS AND METHODS

The methods were described in Part II of this thesis and in Appendix 1.

RESULTS

The results from immunologic, bacteriologic, and histopathologic surveys on the fetuses are tabulated and summarized in Table I and in Appendix 2.

Histologic Characterization:

A routine method of examining and evaluating fetal liver was

established. Results of microscopic features are tabulated in Table I and illustrated in Fig 1-17.

3½ to 5½ months gestation - Histologic features during this period were fairly consistent and straightforward. Liver sections were covered by a thin coat of peritoneum, and the capsule itself was usually a thin, loose connective tissue layer. Lobular architecture was not outstanding. Central veins were prominent in relationship to the portal areas, but were close together and randomly placed. Central veins were endothelium-lined spaces, usually containing blood cells, with scant amounts of perivascular connective tissue and not accompanied by ducts or arteries.

The portal areas contained bile ducts and ductules, a branch of the portal vein, a branch of the hepatic artery, lymphatic vessels, and nerves. These structures were surrounded by reticulum fibers, elastic fibers, and collagen fibers. The portal areas normally contained some histiocytes, but no lymphocytes or leukocytes. Some bile ducts were lined by low cuboidal epithelium, while others were lined by low columnar epithelium.

Parenchymal cells were polygonal and had reasonably distinct cytoplasmic membranes. The cytoplasm was generally amphophilic, ranging from more acidophilia to more basophilia, and was uniformly finely granular and lacy. Most hepatocytes had a single, large oval to round nucleus with a prominent nucleolus and little chromatin. Many megakaryocytes were present, but multinucleated hepatocytes and mitotic figures were rare. Liver cells were cordlike and radiated from central veins.

Sinusoids were identified but the space of Disse was not visible. The sinusoids were lined by flattened stellate cells with darkly

staining Kupffer cells occasionally projecting into the sinusoidal lumen. Entrance of sinusoids into central veins could usually be seen. Bile canaliculi were not observed.

Extramedullary hemopoiesis was extensive and scattered diffusely throughout the liver. The pattern varied from one or two cells to large aggregates distributed variably within the capsule, portal areas, sinusoids, and between hepatocytes.

6 to 7½ months gestation - This period was characterized by transitional rather than abrupt changes. The capsule remained a loose connective tissue layer that generally increased in thickness. Portal areas became more prominent and lobular architecture was slightly more distinct. Megakaryocytes decreased during this stage. The most dramatic change involved extramedullary hemopoiesis. It decreased and became focally intense but was scattered randomly throughout the liver.

8 months to near term - Lobular architecture was more consistent during this time, and the connective tissue within portal areas matured. Megakaryocytes continued to decrease and often were not observed. Hemopoiesis became indistinct and when observed, was always focal. As the fetus progressed to term, hepatocyte cytoplasm usually became more coursely granular with concurrent droplets that were large and lipid-like.

DISCUSSION

Microscopic architecture in the bovine fetal liver did not change significantly from 3½ months to term. All structures were

easily identified at all developmental stages. The connective tissue matured and the lobules increased insize. Polygonal hepatocytes were generally consistent in shape and size. The cytoplasm was finely granular and became more vacuolated as development progressed to term. This phenomenon was also reported by Valdes-Dapena[2] and attributed to glycogen deposition. Megakaryocytes were prominent early and decreased significantly as term approached. Multinucleated hepatocytes were not common, a finding consistent with a previous report[3] that stated that binucleation increased after birth in rabbits, guinea pigs, cattle, and human. Hemopoiesis was altered and decreased by the seventh month. Along with decreased quantities of hemopoietic cells, patterns changed to more focally intense aggregates. Thomas et al[4] considered hepatic hemopoiesis involved erythropoiesis much more than granulopoiesis. To the diagnostician, however, differentiation between generalized hemopoiesis and inflammatory processes is more important. Portal areas became more prominent as gestation progressed; lymphocytes and leukocytes were not normal findings here. Fetal maturation of the liver was histologically apparent but not striking.

SUMMARY

After laboratory evaluation, 124 abattoir fetuses were deemed normal for histologic characterization. The fetuses varied in age from approximately 3½ months to near term. Histologic data of the fetal liver were remarkably consistent throughout the middle and late development. Essentially all microscopic structures were present to the end of the fetal periods, and tissue maturation and lobular sizes did increase. Significant alterations involved decline in megakaryocyte

number and hemopoietic cells around 7 months. Hemopoietic patterns also changed from diffusely scattered to focal aggregates about the same time. Over-all liver architecture of fetuses did not vary significantly from that expected in adult livers.

REFERENCES

1. Elias H: Liver morphology. Biol Rev 30:263-310, 1955.

2. Valdes-Dapena MA: An Atlas of Fetal and Neonatal Histology. Philadelphia, J.B. Lippincott Co, 1957, pp 75-87.

3. Beams HW, King RL: The origin of binucleate and large mononucleate cells in the liver of the rat. Anat Rec 83:281-298, 1942.

4. Thomas DB, Russel PM, Yoffey JM: Pattern of hematopoiesis in fetal liver. Nature 187:876-877, 1960.

TABLE I: Microscopic Features of Fetal Liver Sections

Fetal Age	3½ mo				4 mo							4½ mo													5 mo								
Case Number	76X-198	76X-200	76X-204	76X-225	76X-197	76X-199	76X-205	76X-223	76X-224	76X-227	76X-228	76X-201	76X-206	76X-211	76X-216	76X-222	76X-229	76X-230	76X-163	76X-190	76X-207	76X-208	76X-209	76X-210	76X-213	76X-214	76X-217	76X-218	76X-219	76X-221
Microscopic Characteristic																														
Visceral Peritoneum	+	+	+	ND	+	+	+	+	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	ND	+	+
Fibrous Capsule	1	1	2	ND	2	2	1	1	ND	2	2	3	1	1	1	1	1	1	2	3	1	1	2	2	2	2	1	ND	1	1
Hexagonal Lobular Structure	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	1	1	Neg	1	Neg	1	Neg	Neg	Neg	Neg	Neg	Neg
Areolar Interlobular Tissue	Neg	Neg	Neg	Neg	Neg	1	Neg	Neg	Neg	Neg	1	1	1	1	Neg	Neg	1	1	Neg	1	1	1	Neg	Neg	1	Neg	1	1	Neg	1
Vasculature	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Extramedullary Hemopoiesis	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	2	2	3	4	4	4	3	4	4	4	3	4
Megakaryocytes/LPF	15	9	8	11	6	6	15	6	13	11	8	12	11	10	16	7	10	5	3	3	8	10	5	8	7	6	6	8	12	4
Central Veins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocytes																														
Polygonal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Amphophilic Cytoplasm	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vacuolization	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	1	1	1	1	1
Cord Arrangement	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Round Vesicular Nuclei	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Kupffer Cells																														
Stellate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flattened Nuclei	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sinusoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Portal Areas																														
Portal Vein	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatic Artery	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile Duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphatics	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE I: continued

Fetal Age	5½ mo					6 mo					6½ mo		7 mo														
Case Number	76X-141	76X-203	76X-212	76X-215	76X-231	76X-140	76X-164	76X-179	76X-187	76X-189	76X-202	76X-182	76X-188	76X-92	76X-99	76X-115	76X-131	76X-133	76X-137	76X-142	76X-154	76X-158	76X-161	76X-177	76X-181	76X-192	76X-193	76X-220
Microscopic Characteristic																											
Visceral Peritoneum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	ND	+	+	+	+	+	+	+	+	+	+
Fibrous Capsule	3	3	2	1	1	3	3	1	2	1	2	2	2	3	3	3	3	ND	1	3	2	2	3	2	2	3	2	3
Hexagonal Lobular Structure	Neg	Neg	Neg	Neg	Neg	2	2	1	2	2	Neg	2	2	1	1	2	1	2	2	2	2	2	2	2	2	1	2	1
Areolar Interlobular Tissue	1	2	1	Neg	1	1	2	1	2	2	1	2	3	1	2	2	1	2	1	2	1	3	2	2	2	2	2	1
Vasculature	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Extramedullary Hemopoiesis	2	4	3	4	4	2	2	3	Neg	Neg	4	Neg	1	2	2	1	2	1	1	1	1	Neg	Neg	1	1	2	Neg	2
Megakaryocytes/LPF	7	7	6	10	6	4	2	7	1	Neg	6	Neg	2	5	2	2	3	1	1	5	5	Neg	Neg	4	Neg	4	Neg	2
Central Veins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocytes																												
Polygonal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Amphophilic Cytoplasm	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vacuolization	1	1	1	1	1	1	2	1	3	2	1	2	1	1	1	2	1	1	1	2	1	1	1	3	4	1	2	1
Cord Arrangement	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Round Vesicular Nuclei	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Kupffer Cells																												
Stellate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flattened Nuclei	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sinusoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Portal Areas																												
Portal Vein	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatic Artery	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile Duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphatics	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE I: continued

Fetal Age	7½ mo									8 mo												8½ mo						
Case Number	76X-93	76X-130	76X-132	76X-145	76X-150	76X-156	76X-159	76X-173	76X-174	76X-97	76X-129	76X-135	76X-139	76X-146	76X-147	76X-151	76X-155	76X-157	76X-171	76X-176	76X-178	76X-186	76X-194	76X-128	76X-134	76X-136	76X-149	76X-153	76X-160	76X-162
Microscopic Characteristic																														
Visceral Peritoneum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous Capsule	1	3	2	2	3	3	2	3	4	2	2	2	3	2	4	2	3	3	3	2	2	3	4	2	1	2	2	2	2	3
Hexagonal Lobular Structure	3	2	2	2	1	2	2	3	3	2	2	2	3	3	3	2	3	2	2	2	3	2	2	3	2	2	2	2	2	2
Areolar Interlobular Tissue	2	2	2	2	2	3	2	2	3	2	3	3	3	3	3	2	3	3	3	2	3	2	2	2	3	2	3	3	3	3
Vasculature	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Extramedullary Hemopoiesis	Neg	2	2	1	1	Neg	2	1	1	1	1	Neg	1	1	1	1	Neg	1	Neg	1	2	1	1	1	2	Neg	1	2	1	1
Megakaryocytes/LPF	1	Neg	5	1	1	Neg	1	2	Neg	3	1	Neg	1	Neg	Neg	Neg	1	Neg	Neg	1	Neg	2	Neg	Neg	1	Neg	Neg	2	1	1
Central Veins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocytes																														
Polygonal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Amphophilic Cytoplasm	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vacuolization	2	1	1	1	2	3	2	3	2	2	2	2	1	1	1	2	1	2	3	3	1	2	3	2	1	3	1	1	2	1
Round Vesicular Nuclei	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Kupffer Cells																														
Stellate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flattened Nuclei	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sinusoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Portal Areas																														
Portal Vein	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatic Artery	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile Duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphatics	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE I: continued

Fetal Age	8½ mo																NT													
Case Number	76X-175	76X-184	76X-90	76X-91	76X-94	76X-96	76X-98	76X-100	76X-101	76X-102	76X-103	76X-104	76X-105	76X-106	76X-107	76X-108	76X-109	76X-110	76X-111	76X-112	76X-113	76X-114	76X-116	76X-143	76X-144	76X-148	76X-152	76X-180	76X-183	76X-185	76X-191	76X-195
Microscopic Characteristic																														
Visceral Peritoneum	+	+	+	+	+	+	+	+	+	+	+	+	ND	+	+	+	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous Capsule	3	2	4	1	3	2	3	2	2	2	3	1	ND	4	2	1	ND	2	4	1	2	1	3	3	4	2	4	2	2	3	3	4
Hexagonal Lobular Structure	1	1	2	2	2	1	2	1	3	3	3	3	1	3	1	2	1	2	1	2	1	1	1	3	2	2	2	2	2	3	2	3
Areolar Interlobular Tissue	3	3	3	2	3	2	2	3	3	3	3	3	2	2	2	2	1	2	2	3	2	2	3	3	3	3	3	3	3	2	2	3
Vasculature	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Extramedullary Hemopoiesis	Neg	1	1	1	2	1	1	1	1	1	Neg	2	Neg	1	3	2	1	1	2	1	3	1	1	Neg	Neg	Neg	1	Neg	Neg	Neg	Neg	1
Megakaryocytes/LPF	Neg	1	Neg	Neg	1	1	1	1	1	1	Neg	Neg	Neg	Neg	2	3	Neg	1	4	1	3	2	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Hepatocytes																																
Polygonal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Amphophilic Cytoplasm	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vacuolization	3	3	2	1	2	1	1	1	2	2	3	1	4	1	1	2	3	1	2	1	2	1	2	3	3	2	3	4	3	3	2	1
Cord Arrangement	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Round Vesicular Nuclei	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Kupffer Cells																																
Stellate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flattened Nuclei	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sinusoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Portal Areas																																
Portal Vein	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatic Artery	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile Duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphatics	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

These photomicrographs illustrate the liver parenchyma covered by the capsule (Glisson's capsule) and mesothelial layer of peritoneum. Extramedullary hemopoiesis is prominent as black speckling, and it diminishes as development progresses.

Fig 1-76X-206 (4½ months); H & E stain.

Fig 2-76X-140 (6 months); H & E stain.

Fig 3-76X-139 (8 months); H & E stain.

Fig 1

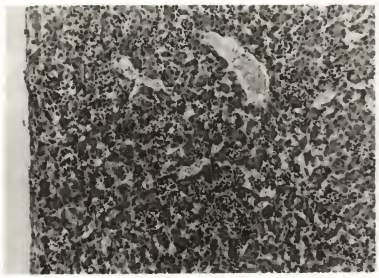


Fig 2

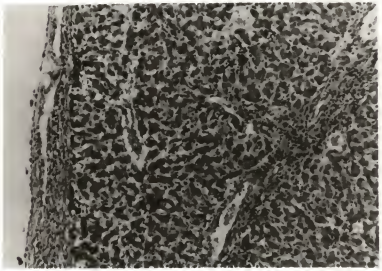
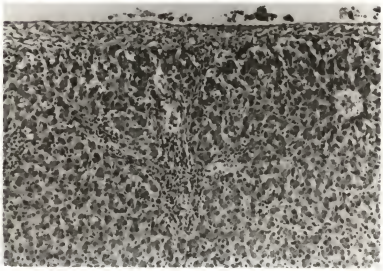


Fig 3



These are a higher magnification of the 3 previous photomicrographs.

Peritoneum is a single-cell layer lining the liver. The loose connective tissue of the capsule is well developed and is continuous with interstitial connective tissue.

Extramedullary hemopoiesis is evident as cells with small, dark, round nuclei with little cytoplasm. These cells usually lie within sinusoids, but may be associated with portal areas and the capsule.

Fig 4-Hemopoiesis is extensive and scattered diffusely throughout the section. Megakaryocytes (m) are present and occur with greatest frequency early in gestation. 76X-206 (4½ months); H & E stain.

Fig 5-Note the lymphatic vessel and area of extramedullary hemopoiesis within this capsule. 76X-140 (6 months); H & E stain.

Fig6-Connective tissue of Glisson's capsule is more dense here. The section is almost devoid of hemopoiesis. 76X-139 (8 months); H & E stain.

Fig 4

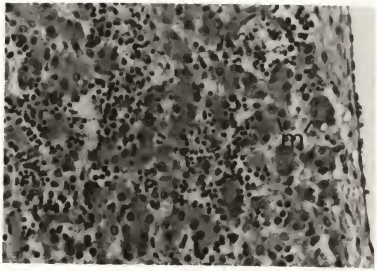


Fig 5

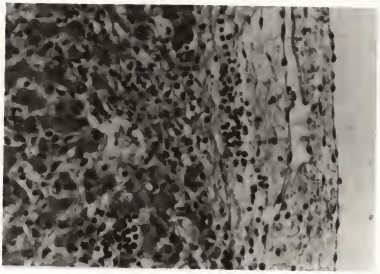
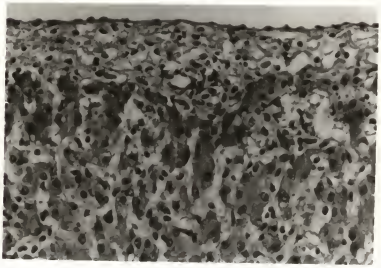


Fig 6



These photomicrographs illustrate prominent megakaryocytes. These giant cells were common during early stages of development, and the number decreased considerably during the 7th month.

Fig 7-76X-133 (7 months); H & E stain.

Fig 8-76X-139 (8 months); H & E stain.

Fig 9-76X-195 (near term); N & E stain.

Fig 7

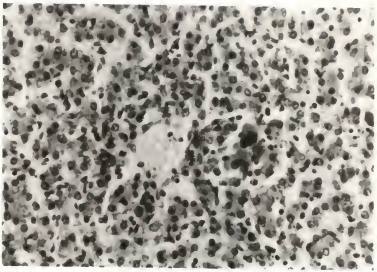


Fig 8

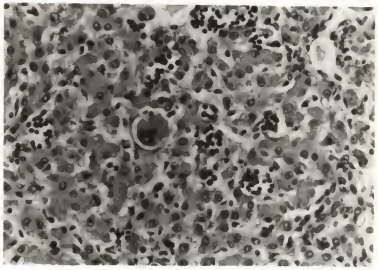
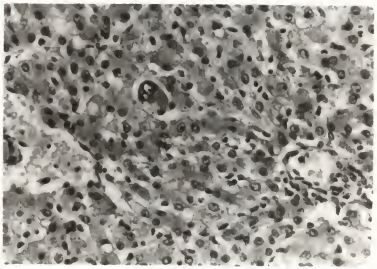


Fig 9



Portal areas are located between lobules and contain branches of the bile duct (b), portal vein (p), hepatic artery (a), and lymph vessels (l). Nerves are present but difficult to identify consistently. The structures are surrounded by reticulum fibers, elastic fibers, and collagen fibers.

Fig 10-Note the extramedullary hemopoiesis present in this portal area. 76X-195 (4½ months); H & E stain.

Fig 11-Cuboidal epithelium with prominent nuclei lining the bile duct; it is sometimes columnar. 76X-217 (5 months); H & E stain.

Fig 12-The fine, dark granules in this section are acid hematin. 76X-217 (5 months); H & E stain.

Fig 10

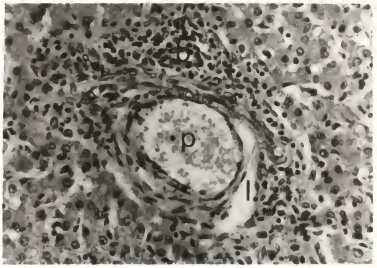


Fig 11

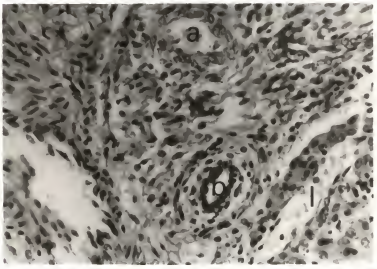
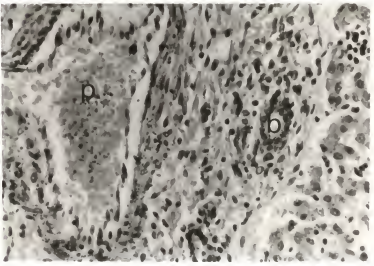


Fig 12



Two photomicrographs of sinusoids emptying into central veins. Central veins are lined by endothelium and usually contain blood cells.

Fig 13-Arrangement of liver cells is cord-like, and they radiate from central veins. Hepatocytes have large nuclei and finely granular cytoplasm. 76X-147 (8 months); H & E stain.

Fig 14-Note the prominent endothelium lining this central vein. 76X-134 (8½ months); H & E stain.

Fig 15-Cells making up walls of sinusoids are present. These Kupffer cells (k) are flattened and stellate. 76X-147 (8 months); H & E stain.

Fig 13

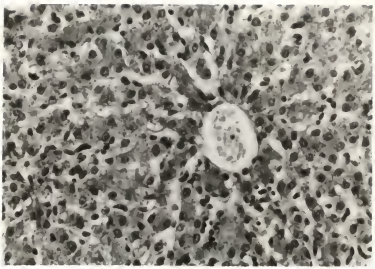


Fig 14

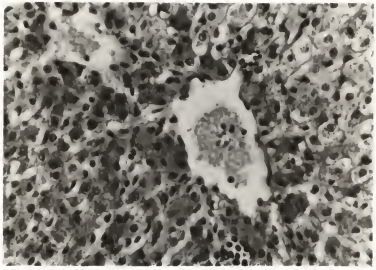


Fig 15

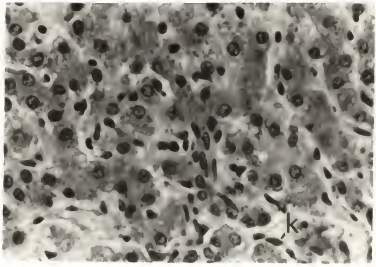


Fig 16-Megakaryocytes and abundant hemopoietic cells are prominent. 76X-206 (4½ months); H & E stain.

Fig 17-Generalized vacuolization of the cytoplasm of hepatocytes becomes more conspicuous as development progresses to term. The vacuoles have distinct borders, are clear, and vary in size. 76X-103 (near term); H & E stain.

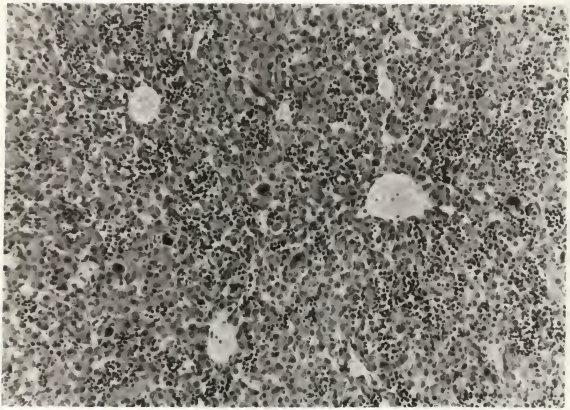


Fig 16

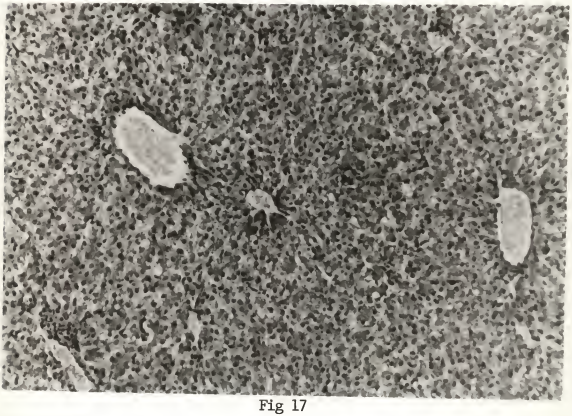


Fig 17

IV. HISTOLOGIC CHARACTERIZATION OF THE BOVINE FETAL KIDNEY

INTRODUCTION

The kidney was no exception when information was gathered concerning development and histologic characterization of later fetal periods. Reports often involved only embryonic periods and detailed investigations into specific developmental abnormalities occurred.[1,2] The purpose of the study reported here was to describe the microscopic structures and features during the midddle and late bovine fetal development.

MATERIALS AND METHODS

The procedures followed were described previously in Part II of this thesis.

RESULTS

Immunologic, bacteriologic, and histopathologic findings are summarized in Appendix 2.

Histologic Characterization:

A routine method of examination of fetal kidney sections was established. The findings are summarized in Table 1 and illustrated in Fig 1-21.

3½ to 5½ months gestation - The most striking feature during this period was the prominent nephrogenic zone just beneath and parallel to the capsule. These zones had nephrogenic vesicles and ampullae located peripherally that differentiated to maturing renal corpuscles and

urinary tubules and the excretory system. Nephrogenic zones were mesenchymal in origin, they were deeply basophilic, and had numerous mitotic figures. Bowman's spaces were initially lined by columnar to cuboidal epithelium that progressively flattened to simple squamous. Parietal layers were always more flattened than visceral layers. Immature glomerular capillaries were lined by cuboidal epithelium, that tended to flatten as the glomeruli matured. Nephrogenic zones were most prominent up to 4½ months gestation and decreased slightly in activity and magnitude toward the end of this period.

Renal corpuscles were composed of tufts of capillaries that connected afferent and efferent arterioles that were surrounded by Bowman's capsules. Maturing Bowman's capsules, within the cortex deep to nephrogenic zones, usually had parietal, simple squamous epithelium and sometimes visceral, simple squamous epithelium. When visceral squamous epithelium was present, it was difficult to distinguish the visceral lining from capillary endothelium. Stellate mesangial cells were occasionally located between capillary loops of glomeruli and also within the juxtaglomerular apparatus of the vascular poles. Parietal, simple squamous epithelium gradually became cuboidal at the urinary poles, where it continued into proximal convoluted tubules. Renal corpuscles, including nephrogenic vesicles and maturing corpuscles, observed per low power field varied in number but demonstrated a trend that was consistent throughout this stage of development.

A loosely adherent capsule of dense, irregular connective tissue covered the kidney and some smooth muscle fibers were occasionally observed toward the parenchyma. Where visible, capsular thickness and connective tissue development were uniform. Trabeculae were absent,

but interstitial tissue consisted of reticular fibers around small vessels and the collecting duct system. Loose connective tissue was located around large blood vessels.

Uriniferous tubules were lined by simple epithelium but had different characteristics in each segment. Definitive identification of each segment was often difficult. However, basic judgements were made and segmental differences noted. These differences became more apparent as fetal age increased. Early in this period, differences between convoluted tubules and the excretory duct system were most evident, but differentiation into other segments soon became apparent.

Proximal convoluted tubules were lined by granular to vacuolated, pyramidal cells that nearly filled the lumen. Cell boundaries were indistinct and usually not visible. Each cell contained a single spherical nucleus irregularly located within eosinophilic cytoplasm. Brush borders on cell surfaces were not readily apparent. Proximal tubules made up the bulk of the kidney cortex.

Descending and ascending portions of the loops of Henle had cuboidal epithelium resembling proximal tubules. These segments were identified primarily by their presence within medullary rays. Thin segments of loops were lightly stained and contained flattened elongated cells with translucent cytoplasm and spheroid nuclei, that bulged into lumina. Thin loop segments were found deep in the medulla that often extended into medullary papillae. These segments were frequently arranged in nests within medullary mesenchyme and reticular stroma. Since numerous capillaries were present in the medulla, it was necessary to carefully distinguish the thin segments of loops of Henle.

Epithelim of distal convoluted tubules was lower and lumina were larger than the proximal counterparts. Although distal tubules were not as large as the proximal tubules, they were lined by more cells and their lateral borders were more clearly defined. In addition, these cells were less acidophilic than the proximal counterparts and had nuclei central to basal in location.

Connecting and collecting tubules of the excretory duct system had irregular cuboidal cells with central nuclei. Cell boundaries were distinct, and the cytoplasm was clear. Epithelial cells became columnar with eccentrically located nuclei in the larger tubules. Papillary duct and renal pelvis epithelium was transitional and had clear cytoplasm.

Golden-brown pigment was frequently located within tubular epithelium, particularly the distal tubules. This pigment was round and had distinct borders but varied in size. Tubules at the cortico-medullary junctions most often contained the pigment, but it was occasionally observed in other tubules throughout the sections. Small arterioles adjacent to pigment-laden tubular cells often had the same pigment within their walls. Pigment granules filled the cytoplasm but were not associated with other cellular changes. Although the degree of pigmentation varied and usually involved only a few random tubules, it was observed consistently as fetal age increased.

6 months to near term - Very few changes occurrred as fetuses matured to term. Nephrogenic zones continued to become less obvious and were only occasionally present after 7½ months. Medullary rays became more prominent and tubular segments more readily differentiated.

Birefringent crystals were observed within tubules of 3 near term fetuses, but there were no associated changes. The crystals were not obvious without polarized light. They varied in size and appeared as clumps or rosettes.

DISCUSSION

Microscopic patterns in the bovine fetal kidney were similar to those described in other mammals.[1,2] Although Gersh[1] believed the human fetal kidney to function as an excretory organ by the end of the third month, Ivemark[2] reported enzymatic localization differences between fetal and adult nephrons that indicated excretory function was not the same. Histologically, only juxtamedullary nephrons with large, maturing renal corpuscles were considered functional so early in fetal development. The nephrogenic zone was usually prominent during early fetogenesis but regressed significantly by midgestation. Renal corpuscles varied considerably as they matured, particularly the visceral and parietal layers of Bowman's spaces. The older the fetus, the greater the distance between the renal pelvis and subcapsular area where the nephrons originated. As a result, the point any loop of Henle reached was dependent upon kidney size when the nephron originated. Complete maturation, however, was not always attained until adult life.[3]

Capsular development was difficult to evaluate because it was often absent. Valdes-Dapena[4] reported fetal capsules were more easily stripped than those of adults, and that was compatible with the findings here. Capsules observed, however, always consisted of dense connective tissue.

Tubular segments of nephrons were as difficult to assess as those in adult kidney sections. Differences between segments were noted and characterized but they did not vary significantly from adult descriptions. Little significance was attached to the golden-brown pigment within tubular epithelium, as it was not associated with other cellular changes. No special procedures were utilized to identify the pigment. It was interesting that pigment was most often observed in the corticomedullary regions during the fetal period studied.

Birefringent crystals in this study were typical for those reported as oxalates.[5] Gopal et al[6] reported congenital anomalies or various other congenital lesions associated with 54 of 56 calves with renal oxalosis. In addition, oxalate crystals were reported in 53.3% of cases without etiologic diagnoses and in 66.7% of cases with anomalies.[5] Only 10.3% of near term and 2.4% of all fetal kidneys examined here had crystals, and none of the fetuses had significant laboratory findings. Schiefer and Moffatt[5] speculated that nearly 20% of all pregnancies may have renal oxalosis without causing fetal death. They felt, however, that crystals predisposed calves to postnatal diseases, viral or other infectious agents. Renal oxalosis in this investigation was probably not significant, but it was interesting that all fetuses involved were near term.

SUMMARY

One-hundred twenty-four normal bovine fetuses between 3½ months and near term were collected at an abattoir

Nephrogenic zones were prominent and consistently observed until 5½ months gestation. After this, the zones rapidly regressed

and were only occasionally observed after 7½ months. Nephrogenic vesicles and ampullae were present within mesenchymal tissue of these proliferating zones. Renal corpuscles in various stages of development were observed, with the most mature ones being present at the corticomedullary region. Tubules were identified but not significantly different from those of mature kidneys. Two features were found associated with the tubules. A golden-brown pigment within epithelial cells, unassociated with lesions, was readily observed. Little significance was placed on this pigment. Oxalate crystals were present within tubules of 3 near term fetuses. Little significance was placed on their presence also, as no other microscopic changes were present. Kidney section characterized in this study were similar to those reported for human fetuses.

REFERENCES

1. Gersh I: The correlation of structure and function in the developing mesonephros and metanephros. Contrib Embryol Carnegie Inst Wash Publ 26:33-58, 1937.

2. Ivemark BI: Histochemical studies on the human foetal kidney (metanephros). Non-specific phosphate-splitting enzymes. J Anat 92:98-109, 1958.

3. Potter EL: Normal and Abnormal Development of the Kidney. Chicago, Year Book Medical Publishers Inc, 1972, pp 3-79.

4. Valdes-Dapena MA: An Atlas of Fetal and Neonatal Histology. Philadelphia, J. B. Lippincott Co, 1957, pp 59-73.

5. Schiefer B, Moffatt RE: Bovine abortion associated with renal oxalosis in the fetus. Can Vet J 15:57-65, 1974.

6. Gopal T, Leipold HW, Cook JE: Renal oxalosis in neonatal calves. Vet Pathol 15:519-524, 1978.

TABLE I: Microscopic Features of Fetal Kidney Sections

Fetal Age	3½ mo				4 mo							4½ mo								5 mo							
Case Number	76X-198	76X-200	76X-204	76X-225	76X-197	76X-199	76X-205	76X-223	76X-224	76X-227	76X-228	76X-201	76X-206	76X-211	76X-216	76X-222	76X-229	76X-230	76X-163	76X-190	76X-207	76X-208	76X-209	76X-210	76X-213	76X-214	76X-217	76X-218	76X-219	76X-221
Microscopic Characteristic																														
Capsular Development	2	2	1	1	1	ND	2	1	1	ND	1	1	1	1	2	2	2	2	2	ND	3	1	ND	ND	1	ND	2	1	1	2
Nephrogenic Zone	4	4	4	4	3	4	4	4	3	3	3	3	3	3	4	2	3	2	2	ND	3	2	2	2	3	4	4	3	2	4
Vasculature	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal Corpuscles																														
Number/LPF	33	26	35	36	34	45	24	46	32	30	27	36	48	37	36	37	45	38	46	37	40	35	38	41	40	41	43	40	45	30
Endothelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesangial Cells	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vascular Pole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Juxtaglomerular Apparatus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary Pole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial Connective Tissue	1	2	2	2	2	3	1	3	2	2	3	1	3	2	1	2	1	1	1	1	2	1	2	2	1	2	2	2	3	3
Proximal Tubules																														
Granular Cytoplasm	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cuboidal Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Loop of Henle																														
Descending Limb (cuboidal)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thin Segments (squamous)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ascending Limb (cuboidal)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Distal Tubules (cuboidal)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Excretory Duct System	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Calyces and Pelvis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Birefringent Crystals	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Brown Tubular Pigment	Neg	Neg	Neg	+	+	Neg	Neg	+	+	+	Neg	Neg	Neg	+	+	Neg	Neg	+	+	+	+	+	Neg	+	+	+	Neg	+	+	+

TABLE I: continued

Fetal Age	5½ mo					6 mo						6½ mo		7 mo												
Case Number	76X-141	76X-203	76X-212	76X-215	76X-231	76X-140	76X-164	76X-179	76X-187	76X-189	76X-202	76X-182	76X-188	76X-92	76X-99	76X-113	76X-131	76X-133	76X-137	76X-142	76X-154	76X-158	76X-161	76X-177	76X-181	76X-192	76X-193	76X-220
Microscopic Characteristic																												
Capsular Development	ND	1	3	2	1	1	ND	ND	ND	ND	ND	1	ND	1	1	ND	1	ND	1	1	1	ND	1	2	1	1	1	1
Nephrogenic Zone	ND	2	3	3	2	1	ND	1	1	ND	2	1	ND	1	1	ND	2	ND	1	1	1	1	1	1	1	1	2	1
Vasculature	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal Corpuncles																												
Number/LPF	49	54	45	39	37	48	34	29	36	42	46	38	40	30	38	30	42	31	25	28	21	32	28	30	40	41	35	42
Endothelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesangial Cells	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vascular Pole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Juxtaglomerular Apparatus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary Pole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial Connective Tissue	1	3	3	2	3	3	2	2	3	3	2	3	2	3	2	2	2	1	3	2	3	3	4	3	2	3	3	2
Proximal Tubules																												
Granular Cytoplasm	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cuboidal Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Loop of Henle																												
Descending Limb (cuboidal)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thin Segment (squamous)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ascending Limb (cuboidal)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Distal Tubules (cuboidal)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Excretory Duct System	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Calyces and Pelvis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Birefringent Crystals	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Brown Tubular Pigment	+	+	+	+	+	+	Neg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE I: continued

Fetal Age	7½ mo										8 mo												8½ mo				
Case Number	76X-93	76X-95	76X-130	76X-132	76X-145	76X-150	76X-156	76X-159	76X-173	76X-174	76X-97	76X-129	76X-135	76X-139	76X-146	76X-147	76X-151	76X-155	76X-157	76X-171	76X-176	76X-178	76X-186	76X-194	76X-128	76X-136	76X-138	76X-149	76X-153
Microscopic Characteristic																													
Capsular Development	1	ND	2	ND	1	1	1	ND	3	ND	2	1	3	1	ND	1	1	ND	1	3	1	1	3	2	1	ND	ND	3	ND
Nephrogenic Zone	1	ND	1	ND	Neg	Neg	Neg	Neg	1	ND	Neg	Neg	Neg	Neg	ND	1	Neg	Neg	1	Neg	1	Neg	Neg	Neg	1	ND	ND	Neg	Neg
Vasculature	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal Corpuscles																													
Number/LPF	30	36	31	33	26	32	26	23	32	31	41	28	17	30	43	26	28	29	36	20	36	29	31	27	34	27	19	22	33
Endothelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesangial Cells	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vascular Pole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Juxtaglomerular Apparatus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary Pole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial Connective Tissue	3	3	2	3	3	3	3	3	2	3	3	2	3	2	3	3	2	3	3	4	3	3	2	4	4	2	3	2	2
Proximal Tubules																													
Granular Cytoplasm	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cuboidal Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Loop of Henle																													
Descending Limb (cuboidal)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thin Segment (squamous)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ascending Limb (cuboidal)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Distal Tubules (cuboidal)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Excretory Duct System	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Calycea and Pelvis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Birefringent Crystals	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Brown Tubular Pigment	+	+	+	+	+	Neg	+	+	+	+	+	+	Neg	Neg	+	+	+	+	+	+	+	+	+	Neg	Neg	+	+	+	+

TABLE I: continued

Fetal Age	8½ mo															NT															
Case Number	76X-160	76X-162	76X-175	76X-184	76X-90	76X-91	76X-94	76X-98	76X-100	76X-101	76X-102	76X-103	76X-104	76X-105	76X-106	76X-107	76X-108	76X-109	76X-110	76X-111	76X-112	76X-113	76X-114	76X-116	76X-143	76X-144	76X-148	76X-152	76X-180	76X-183	76X-185	76X-191	76X-195
Microscopic Characteristic																																	
Capsular Development	I	I	ND	I	I	I	I	I	I	I	2	I	ND	I	ND	I	I	I	I	I	I	I	ND	ND	3	ND	I	ND	2	I	ND	ND	ND
Nephrogenic Zone	I	I	ND	Neg	I	Neg	Neg	Neg	Neg	I	Neg	Neg	Neg	Neg	Neg	I	I	Neg	Neg	I	Neg	I	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	I
Vasculature	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal Corpuscles																																	
Number/LPF	36	48	21	26	30	19	29	36	43	38	21	20	30	26	30	37	33	19	30	44	26	29	36	22	20	19	28	27	28	34	33	14	30
Endothelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesangial Cells	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vascular Pole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Juxtaglomerular Apparatus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary Pole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interatitial Connective Tissue	2	3	3	3	3	2	3	4	3	3	3	2	3	3	2	2	3	4	3	1	4	2	3	2	3	3	4	3	3	2	3	3	3
Proximal Convoluted Tubules																																	
Granular Cytoplasm	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cuboidal Epithelium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Loop of Henle																																	
Descending Limb (cuboidal)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thin Segment (squamous)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ascending Limb (cuboidal)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Distal Tubules (cuboidal)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Excretory Duct System	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Calyces and Pelvis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Birefringent Cryatals	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	5	Neg	Neg	6	Neg	4	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Brown Tubular Pigment	+	+	Neg	+	+	+	+	+	+	Neg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Neg	+	+	+	Neg	+	+	+	+

These photomicrographs demonstrate the total width of kidney parenchyma, including capsule, cortex, and as much of the pyramid as possible. They are all at the same magnification.

Note the progressive increase in breadth of the cortex, as well as the entire organ. There is also an increase in the number of glomeruli, and the medullary rays become increasingly prominent. The medullary rays contain descending and ascending portions of the loops of Henle and the collecting ducts.

Nephrogenic zones (z) of the first 2 cases are apparent even at this power, just beneath and parallel to the capsules.

Fig 1-76X-225 (3½ months); H & E stain.

Fig 2-76X-219 (5 months); H & E stain.

Fig 3-76X-194 (8 months); H & E stain.

Fig 1

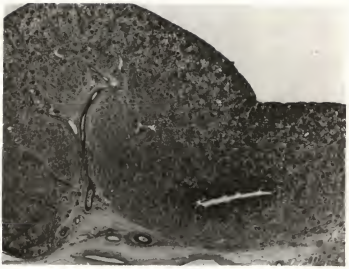


Fig 2

Fig 3

These photomicrographs illustrate the development of the renal cortex. Note the disappearance of a clear-cut nephrogenic zone by 7 months gestation. Glomeruli increase in size and distance between each other throughout gestation. The tubules constantly increase in number during development, and the proximal tubules make up the bulk of the renal cortex.

Fig 4-76X-219 (5 months); H & E stain.

Fig 5-76X-158 (7 months); H & E stain.

Fig 6-76X-194 (8 months); H & E stain.

Fig 4

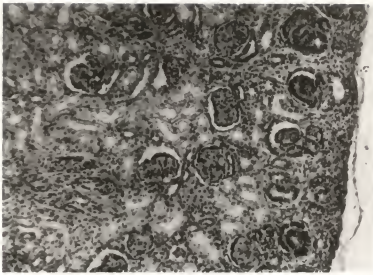


Fig 5

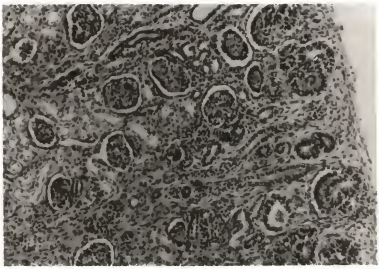
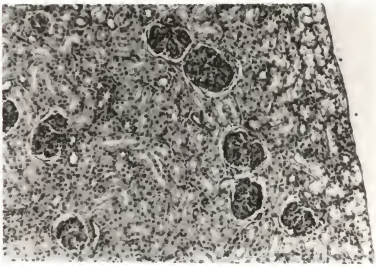


Fig 6



These photomicrographs are all from the same case and illustrate an active nephrogenic zone. Note the differences between visceral and parietal epithelium of Bowman's capsule. Fig 7-The lumina of the vesicles (V) broaden and basal cells become slightly less columnar. Most lower cells flatten and become precursor cells of Bowman's capsule. Some cells temporarily enlarge (arrows), that eventually become the epithelial portion of the glomerulus. Ampullae (A) divide into tubules. 76X-97 (4 months); H & E stain.

Fig 8-Cells of Bowman's capsule begin to flatten, and the ampullae (A) divide into tubules. Note that the epithelial cells of the glomerulus have large nuclei. A vesicle (V) is present, with enlarging cells (arrow). 76X-97 (4 months); H & E stain.

Fig 9-Primitive S-forms (S) may be seen. 76X-97 (4 months); H & E stain.

Fig 7

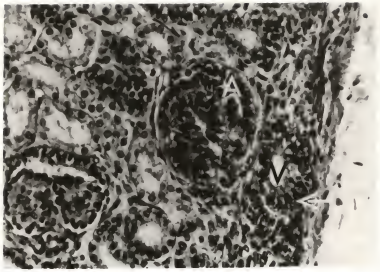


Fig 8

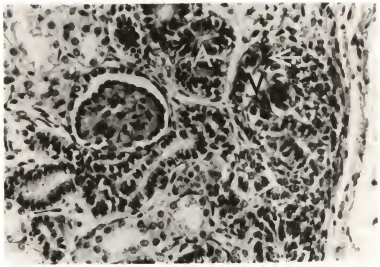
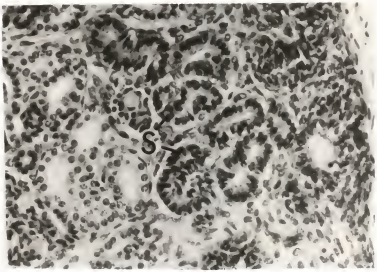


Fig 9



This series of photomicrographs illustrate the detailed changes in the subcapsular renal corpuscles.

The epithelium covering the glomerular capillaries and lining Bowman's capsule is striking. The parietal layer flattens to squamous epithelium early, while the visceral layer often remains cuboidal throughout later developmental stages.

Note how stromal tissue changes from mesenchymal to reticular.

As gestation progresses, there is an increase in the number of tubules.

Fig 10-76X-197 (4 months); H & E stain.

Fig 11-76X-207 (5 months); H & E stain.

Fig 12-76X-194 (8 months); H & E stain.

Fig 10

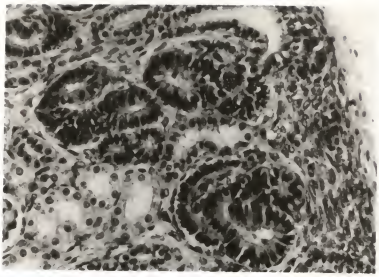


Fig 11

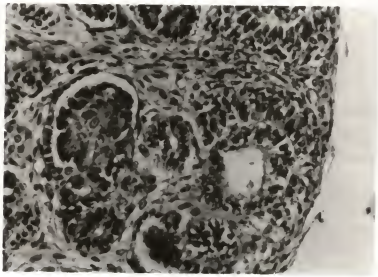
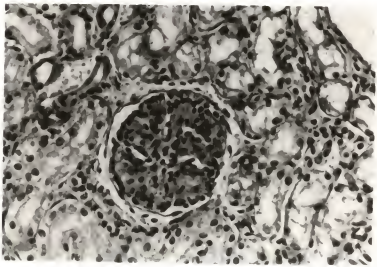


Fig 12



Changes in this group of juxtamedullary glomeruli are not nearly so striking as those in the subcapsular region. Probably the most significant feature is how the cuboidal epithelium covering the glomerular capillaries often flattens out later in gestation. This flattening was neither consistent nor obvious in all fetuses. Glomerular size may be significant in these cases.

Note the differences between proximal (p) and distal (d) tubules.

Fig 13-76X-197 (4 months); H & E stain.

Fig 14-76X-141 (5½ months); H & E stain.

Fig 15-76X-194 (8 months); H & E stain.

Fig 13

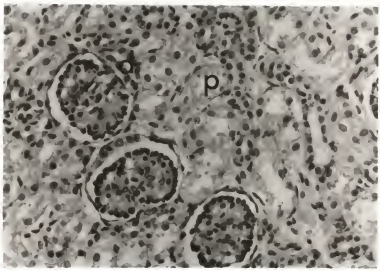


Fig 14

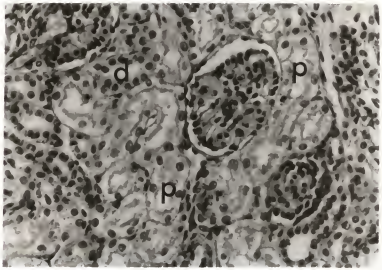
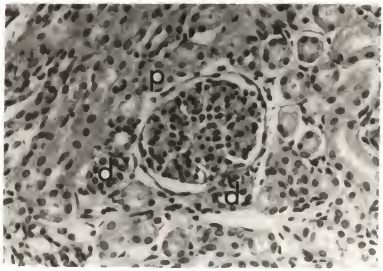


Fig 15



These photomicrographs were taken at the tip of pyramids. It is interesting to note an increase and maturity of interstitial tissue as development progresses.

During the early stages of gestation, transitional epithelium lines the renal pelvis and often lines the distal collecting ducts. Note differences in sizes of ducts.

Fig 16-Note how tubules are forming nests within the mesenchyme and reticular stroma. 76X-225 (3½ months); H & E stain.

Fig 17-Transitional epithelium is lining the renal pelvis and some ducts. 76X-141 (5½ months); H & E stain.

Fig 18-Note the similarity between capillaries (c) and thin segments of loops of Henle (h) deep in the medulla. 76X-105 (near term); H & E stain.

Fig 16

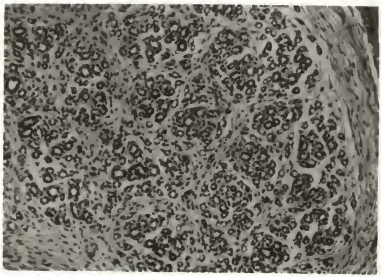


Fig 17

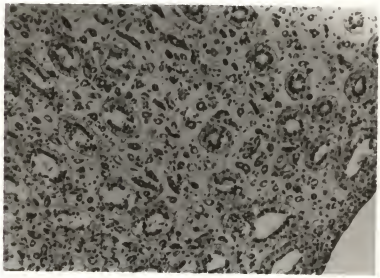
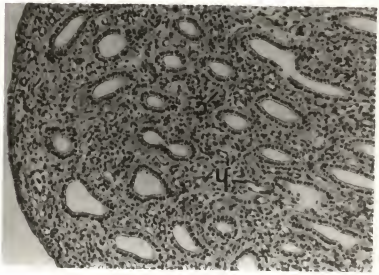


Fig 18



The first 2 photomicrographs were taken high in pyramids, to demonstrate the character of the epithelium lining collecting ducts. Note the prominent nuclei within clear cytoplasm.

Fig 19-76X-158 (7 months); H & E stain.

Fig 20-76X-105 (near term); H & E stain.

Fig 21-This is an example of the birefringent crystals present in tubules of near term fetuses (semi-polarized). There is no evidence of accompanying degeneration or necrosis. 76X-108 (near term); H & E stain.

Fig 19

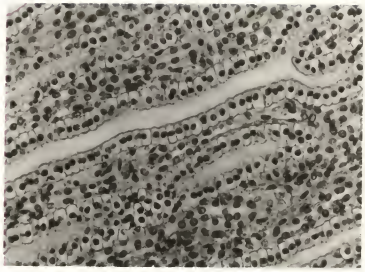


Fig 20

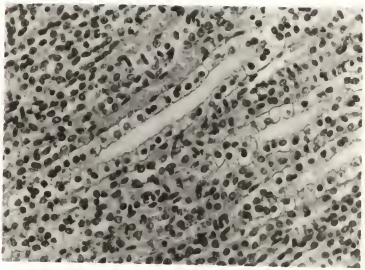


Fig 21

APPENDIX

1. Procedures performed at South Dakota State University

Necropsy Procedure

Each fetus was placed in lateral recumbency on its left side. A midline incision was made through the skin, extending from the mandibular symphysis to the pubis and the skin and right limbs reflected dorsally. The thoracic and abdominal cavities were opened by incising through the right costochondral junctions and the abdominal wall, respectively. Reflection of these allowed the thoracic and abdominal viscera to be examined in situ. The tongue was cut free of adjacent tissues and the hyoid bones disarticulated, allowing the tongue to be pulled posterio-ventrally, so that the trachea and esophagus were freed from the adjacent cervical tissues. The tongue, larynx, esophagus, thymus, heart, and lungs were released upon incision of the mediastinum and thoracic-esophagus at the esophageal hiatus of the diaphram. Abdominal viscera was removed by incising the abdominal esophagus at the esophageal hiatus of the diaphram, the colon at the pelvic inlet, and the mesenteric attachments. Spleen, liver, and gallbladder were examined separately, and the urinary tract was removed intact.

Portions of lung, liver, spleen, and kidney were collected as aseptically as possible for bacteriologic examination and placed in sterile plastic bags. The abomasal wall was punctured with a sterile needle and a portion of the contents drawn into a sterile disposable syringe. Lung, liver, and kidney were placed in 10% buffered neutral formalin for histologic examination.

Blood was collected from the umbilical veins for immunoglobulin determination.

Bacteriologic Examination

The portions of lung, liver, spleen, and kidney to be cultured were flamed, opened with sterile scissors, and smeared onto 5% sheep blood agar plates. A drop of abomasal contents was streaked on blood agar. The plates were incubated at 37 C in an atmosphere containing 10% CO_2 and were examined at 24 and 72 hours, and 7 days. In addition, a drop of abomasal contents was streaked on McConkeys agar and incubated aerobically. McConkey plates were also examined at 24 and 72 hours, and 7 days. When numerous similar colonies appeared, the organisms were isolated and biochemically characterized.

A drop of abomasal contents was placed on a slide, covered with a coverslip, and examined under darkfield illumination at X400.

Radial Immunodiffusion

The fetal blood samples were allowed to clot and the sera stored at -20 C until tested. Radial immunodiffusion was performed with commercially prepared kitsets.* To increase the sensitivity of the test for IgG concentrations less than 100 mg/100 ml, 5.0 µl of serum or standard IgG solution was placed in each well rather than 2.5 µl as recommended in the kit directions. IgM test kits were used as directed.

IgG solutions of known concentrations provided with the kits were diluted with buffered isotonic saline to produce standard solutions of 12.5, 25, 50, and 100 mg/100 ml, and the known IgM solutions were diluted to concentrations of 15, 30, 60, 130, and 250 mg/100 ml.

---

*Miles Laboratories, Kankakee, Ill.

The loaded IgG plates were incubated at room temperature for 18 hours and the IgM plates for 22 hours. Before measuring the precipitin rings, the plates were flooded 3 to 5 minutes with 1% tannic acid solution and then washed with a gentle stream of distilled water. Rings were examined with a magnifying glass using oblique light and a dark background. The diameters of the rings were read from the scale printed on the plates.

2. Results of immunologic, bacteriologic, and histopathologic findings of the fetuses

TABLE I: Fetal Immunologic, Bacteriologic, and Histopathologic Findings

Case Number	Fetal Age	IgM (mg/100ml)	IgG (mg/100ml)	Bacteriology	Histopathology
76X-198	3½ mo	Neg	Neg	Enterica* & alpha-Streptococci	Nil
76X-200	3½ mo	ND	ND	alpha-Streptococci	Nil
76X-204	3½ mo	Neg	Neg	Enterica & alpha-Streptococci	Nil
76X-225	3½ mo	Neg	Neg	Enterica	Nil
76X-197	4 mo	Neg	Neg	Neg	Nil
76X-199	4 mo	Neg	Neg	Enterica & alpha-Streptococci	Nil
76X-205	4 mo	Neg	Neg	Few E coli & alpha-Streptocci	Lung-RBC Extravasation
76X-223	4 mo	Neg	Neg	Neg	Lung-RBC Extravasation
76X-224	4 mo	Neg	Neg	Neg	Nil
76X-226	4 mo	Neg	Neg	Enterica	Nil
76X-227	4 mo	Neg	Neg	Neg	Lung-RBC Extravasation
76X-228	4 mo	Neg	Neg	Neg	Nil
76X-201	4½ mo	Neg	Neg	Few Enterica & Few alpha-Streptococci	Nil
76X-206	4½ mo	Neg	6.5	alpha-Streptococci	Nil
76X-211	4½ mo	Neg	Neg	Enterica	Nil
76X-216	4½ mo	Neg	Neg	Neg	Nil
76X-222	4½ mo	Neg	Neg	Neg	Nil
76X-229	4½ mo	Neg	Neg	Enterica	Nil
76X-230	4½ mo	Neg	Neg	Neg	Nil
76X-163	5 mo	Neg	Neg	Bacillus sp	Nil
76X-190	5 mo	Neg	Neg	Neg	Nil
76X-207	5 mo	88.0	Neg	Few alpha-Streptococci	Nil
76X-208	5 mo	Neg	Neg	Enterica & Proteus sp	Nil
76X-209	5 mo	Neg	Neg	Few E coli	Kidney-Mononuclear Focus
76X-210	5 mo	Neg	Neg	Few E coli	Nil
76X-213	5 mo	Neg	6.5	Enterica	Lung-RBC Extravasation
76X-214	5 mo	Neg	Neg	Enterica	Nil
76X-217	5 mo	Neg	Neg	Neg	Nil
76X-218	5 mo	Neg	Neg	Enterica & alpha-Streptococci	Nil
76X-219	5 mo	Neg	Neg	Enterica & Proteus sp	Nil
76X-221	5 mo	Neg	Neg	alpha-Streptococci	Lung-RBC Extravasation
76X-141	5½ mo	Neg	Neg	Neg	Nil
76X-203	5½ mo	Neg	Neg	Enterica	Nil
76X-212	5½ mo	Neg	150.0	Enterica	Nil
76X-215	5½ mo	Neg	Neg	Enterica & alpha-Streptococci	Nil
76X-231	5½ mo	Neg	Neg	Enterica	Nil
76X-140	6 mo	Neg	Neg	Neg	Nil
76X-164	6 mo	45.0	52.0	Neg	Lung-RBC Extravasation
76X-179	6 mo	Neg	6.5	Proteus sp	Nil
76X-187	6 mo	Neg	18.0	Few Enterica	Nil
76X-189	6 mo	Neg	6.5	Few Bacillus sp & Few Enterica	Nil
76X-202	6 mo	Neg	Neg	E coli & alpha-Streptococci	Nil
76X-172	6½ mo	Neg	8.0	Neg	Nil
76X-182	6½ mo	Neg	Neg	Few Enterica	Lung-RBC Extravasation
76X-188	6½ mo	Neg	35.0	1 Staphylococcus sp & 1 Enteric	Nil
76X-92	7 mo	ND	ND	Neg	Nil
76X-99	7 mo	ND	ND	Neg	Nil

TABLE I: continued

Case Number	Fetal Age	IgM (mg/100ml)	IgG (mg/100ml)	Bacteriology	Histopathology
76X-115	7 mo	Neg	8.0	Few Enterica	Lung-RBC Extravasation
76X-131	7 mo	Neg	80.0	Neg	Lung-RBC Extravasation
76X-133	7 mo	Neg	8.0	Neg	Lung-RBC Extravasation
76X-137	7 mo	Neg	12.0	Few Enterica & Few Bacillus sp	Nil
76X-142	7 mo	Neg	Neg	Few Staphylococcus sp	Nil
76X-154	7 mo	Neg	Neg	Few Staphylococcus sp	Lung-RBC Extravasation
76X-158	7 mo	Neg	4.0	Bacillus sp	Lung-RBC Extravasation
76X-161	7 mo	Neg	Neg	Neg	Nil
76X-177	7 mo	Neg	10.0	Few Enterica	Nil
76X-181	7 mo	Neg	6.5	Few Enterica & Few Bacillus sp	Nil
76X-192	7 mo	Neg	10.0	Neg	Nil
76X-193	7 mo	Neg	120.0	Neg	Nil
76X-220	7 mo	2.5	Neg	Enterica	Nil
76X-93	7½ mo	ND	ND	ND	Nil
76X-95	7½ mo	Neg	44.0	Few Enterica	Lung-RBC Extravasation
76X-130	7½ mo	Neg	6.5	Few Enterica	Nil
76X-132	7½ mo	Neg	8.0	ND	Lung-RBC Extravasation
76X-145	7½ mo	Neg	Neg	Few Enterica	Lung-RBC Extravasation
76X-150	7½ mo	Neg	Neg	Neg	Lung-RBC Extravasation
76X-156	7½ mo	Neg	Neg	Neg	Nil
76X-159	7½ mo	Neg	Neg	Few Enterica	Lung-RBC Extravasation
76X-173	7½ mo	Neg	Neg	Enterica & alpha-Streptococci	Nil
76X-174	7½ mo	Neg	10.0	Few Bacillus sp	Liver-Triad Mononuclear Infiltration
76X-97	8 mo	Neg	8.0	Few Enterica	Nil
76X-129	8 mo	Neg	8.0	Few Bacillus sp	Lung-RBC Extravasation
76X-135	8 mo	Neg	10.0	Neg	Nil
76X-139	8 mo	Neg	15.0	Few Enterica	Nil
76X-146	8 mo	Neg	12.0	Neg	Nil
76X-147	8 mo	Neg	10.0	Neg	Nil
76X-151	8 mo	Neg	4.0	Few Staphylococcus sp	Nil
76X-155	8 mo	Neg	Neg	Neg	Nil
76X-157	8 mo	Neg	Neg	Neg	Nil
76X-171	8 mo	88.0	18.0	Neg	Nil
76X-176	8 mo	Neg	6.5	Neg	Nil
76X-178	8 mo	Neg	150.0	Few Staphylococcus sp & Few Enterica	Nil
76X-186	8 mo	Neg	42.0	Neg	Nil
76X-194	8 mo	Neg	12.0	Few Enterica	Nil
76X-128	8½ mo	Neg	12.0	Neg	Lung-Lymphoid Nodules, Kidney-Mononuclear Focus
76X-134	8½ mo	Neg	15.0	Neg	Lung-RBC Extravasation
76X-136	8½ mo	Neg	8.0	Neg	Nil
76X-138	8½ mo	Neg	28.0	Neg	Lung-RBC Extravasation, Liver-Dilated Veins
76X-149	8½ mo	Neg	Neg	Neg	Lung-RBC Extravasation
76X-153	8½ mo	Neg	Neg	Few Enterica	Nil
76X-160	8½ mo	Neg	Neg	Few Enterica	Nil
76X-162	8½ mo	Neg	Neg	Neg	Nil
76X-175	8½ mo	Neg	8.0	Neg	Nil
76X-184	8½ mo	Neg	Neg	Few Enterica	Liver-Triad Mononuclear Infiltration
76X-90	NT	ND	ND	Neg	Nil
76X-91	NT	ND	ND	Neg	Nil
76X-94	NT	Neg	Neg	Neg	Nil
76X-96	NT	Neg	8.0	Enterica & alpha-Streptococci	Nil

TABLE I: continued

Case Number	Fetal Age	IgM (mg/100ml)	IgG (mg/100ml)	Bacteriology	Histopathology
76X-98	NT	Neg	Neg	Neg	Nil
76X-100	NT	Neg	7.0	Few Enterics	Nil
76X-101	NT	Neg	90.0	Neg	Nil
76X-102	NT	Neg	55.0	Few Pseudomonas sp	Nil
76X-103	NT	Neg	8.0	Neg	Nil
76X-104	NT	Neg	8.0	Few Enterics	Nil
76X-105	NT	Neg	Neg	Neg	Nil
76X-106	NT	Neg	Neg	Few Enterics	Nil
76X-107	NT	Neg	Neg	Neg	Nil
76X-108	NT	ND	ND	Few Enterics and Pseudomonas sp	Nil
76X-109	NT	Neg	Neg	Few Enterics and Bacillus sp	Lung-RBC Extravasation
76X-110	NT	Neg	8.0	Few Enterics	Nil
76X-111	NT	Neg	13.0	Neg	Nil
76X-112	NT	Neg	8.0	Neg	Lung-RBC Extravasation
76X-113	NT	Neg	Neg	Neg	Nil
76X-114	NT	Neg	Neg	Neg	Kidney-Mononuclear Focus
76X-116	NT	Neg	150.0	Neg	Lung-Lymphoid Nodules
76X-143	NT	Neg	Neg	Few Enterics	Nil
76X-144	NT	Neg	Neg	Neg	Nil
76X-148	NT	Neg	4.0	Neg	Nil
76X-152	NT	Neg	Neg	Few Enterics	Nil
76X-180	NT	Neg	Neg	Few Enterics	Nil
76X-183	NT	Neg	Neg	Neg	Nil
76X-185	NT	Neg	6.5	Neg	Nil
76X-191	NT	Neg	Neg	Neg	Nil
76X-195	NT	Neg	10.0	Few Enterics	Nil

* Enterobacteriaceae, excluding E coli

Radial Immunodiffusion

Results of 117 abattoir fetuses are presented as immunoglobulin results were incomplete for 7 of the original 124 fetuses. Immunoglobulins were detected in sera from 54 (46.2%) of the 117 fetuses. Two fetuses demonstrated only IgM and 2 had both IgM and IgG levels, while 50 fetuses demonstrated only IgG levels. Thirteen (11.1%) fetuses had more than 20 mg/100 ml IgM, IgG, or both. The mean concentration of IgM was 55.9 mg/100 ml (range 0-88.0) and the mean concentration of IgG was 28.7 mg/100 ml (range 0-150.0)

Bacteriology

Results of bacteriologic examination are listed in Table I. Bacteriologic results were not obtained for 2 fetuses. Bacteria were cultured from 66 (54.1%) of the 122 fetuses. Mixed bacterial cultures were isolated most often; 51 (41.8%) cultures were mixed and always included an enteric (Enterobacteriaceae excluding E coli) or alpha-hemolytic streptococci. Genera cultured alone and/or concurrent with others are listed: 49 (40.2%) enterics, 14 (11.5%) alpha-hemolytic streptococci, 8 (6.5%) Bacillus sp, 5 (4.1%) Staphylococcus sp, 4 (3.5%) Escherichia coli, 3 (2.5%) Proteus sp, and 2 (1.6%) Pseudomonas sp.

Histopathology

Tissue changes were observed in 29 (23.4%0 of the 124 fetuses, and 14 (48.3%) had concurrent immunoglobulin levels. Additionally, bacteria were cultured in 14 (48.3%) of the 29 fetuses. Red blood cell extravasation into lung parenchyma and interlobular septa was observed in 20 fetuses, while peribronchial lymphoid cuffs were noted only twice. An interstitial focus of mononuclear inflammatory cells was seen in 3

separate kidney sections. Two liver sections had portal areas infiltrated by mononuclear inflammatory cells and one section had extremely dilated central and portal veins. Results are tabulated with immunologic and bacteriologic findings in Table I.

HISTOLOGIC CHARACTERIZATION OF BOVINE FETAL LUNG, LIVER, AND KIDNEY

by

GARY A. ANDERSON

B.S., South Dakota State University, 1975

D.V.M., Kansas State University, 1979

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1979

The objectives of this study were to follow the histological development of the bovine lung, liver, and kidney through the middle and late fetal stages. Fetuses were collected from clinically healthy cows at slaughter in South Dakota and removed from the uterus within 30 minutes post mortem. Fetal specimens were also collected for bacteriologic, histopathologic, and radial immunodiffusion analysis.

Histologically, lung maturation was not uniform throughout the sections at any one fetal period. Organoid architecture gradually transformed from a mass of mesenchymal tissue with endodermal channels to a highly vascularized system of irregular air sacs lined by simple squamous epithelium. This transition was essentially complete at 7 months gestation. Capillaries migrated adjacent to air spaces, mesenchymal stroma decreased, and epithelium flattened as development progressed. Consequently, more complex saccular spaces were formed for efficient extrauterine respiration. Major architectural changes did not occur during the last 2 months. Lymphoid tissue was not observed around airways or vessels in any normal lung sections. Squamae and meconium were observed often, but little significance was placed on any debris unless it blocked air spaces. Early developmental stages of lungs coincided well with fetal age, and it is doubtful if independent bovine extrauterine existence is possible prior to 6 months gestation.

Histologic features in the liver did not alter as much as that of the lung. Microscopic structures were identified at all developmental stages. Cytoplasm of hepatocytes became more vacuolated near term and this was attributed to extensive glycogen depositions. Megakaryocytes were prominent early and were only occasionally observed after 7

months gestation. Hemopoietic activity decreased as development progressed and distribution became more focal after 7 months. Lymphocytes and leukocytes were not normally observed in portal areas. Architecture of fetal livers was remarkably consistent throughout middle and late gestation.

The most striking microscopic feature of kidney development was the nephrogenic zone beneath and parallel to the capsule. Nephrogenic vesicles and ampullae were active within the mesenchyme of the zone until midgestation. Although nephrogenic zones were not common after 7 months gestation, renal corpuscles continued to mature throughout gestation. Visceral and parietal layers of Bowman's capsule changed from cuboidal or columnar to simple squamous epithelium, but the parietal layer changed much earlier. Golden-brown pigment was often present within tubular epithelium. It was most common in distal convoluted tubules and the corticomedullary junctions, and special procedures were not done to identify the pigment. Birefringent crystals, believed to be oxalates, were present within the tubules. Little significance was placed on either of these findings because other microscopic changes were not present.

Fetal development and histologic characterization of bovine lung, liver, and kidney did not differ significantly from reports of other mammals. In addition to these observations, bacteriologic, immunologic, and histopathologic findings were made from this group of abattoir fetuses by the Animal Disease Research and Diagnostic Laboratory, South Dakota State University.