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ABSTRACT

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By

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Research was conducted to improve a surgical technique for obtaining liver biopsies in cattle. The research involved devising appropriate instrumentation, a safe surgical technique and an evaluation of the technique in a series of 4 experiments in cattle. The technique was developed by obtaining 112 liver biopsies from 49 cows. Single biopsies to as many as 4 consecutive biopsies were obtained from cows.

The appropriate anatomical location for penetration of the abdominal wall for liver biopsy in mature Holstein cows was the right 12th intercostal space, 35 cm ventral to the dorsal midline. Injection of an anesthetic close to the paravertebral nerve trunks provided anesthesia of the surgical area. The liver area to be biopsied was seen by using a flashlight inserted through a large cannula or by using a fiber optic-adapted sigmoidoscope that was inserted through the abdominal wall. The appropriate site in the liver for biopsy was the area about 6 cm ventrally and anterior to the dorsal ligament of the liver. Samples of liver weighing 4 to 9 gm were obtained by using a specially devised biopsy instrument. The instrument was made of stainless steel material. The biopsy part was a cylinder to penetrate

the liver and at the distal end a knife blade was fixed so as to sever the core of liver.

In experimental work, liver biopsies were of value in indicating that there was poor correlation between vitamin A in the liver and the serum. It was established that 1 biopsy sample was representative of the entire liver. By liver biopsy it was determined that intramuscular injection of vitamin A provided liver stores equivalent to the stores from the same amount given orally. The technique was of value in establishing morphologic and chemical changes in the liver during starvation. Liver biopsy was of value in confirming the clinical diagnosis of fat cow syndrome.

The technique was safe and allowed collection of liver samples suitable for a wide variety of diagnostic and research work in cattle. This would have many applications in improving cattle production.

DEDICATED TO MY WIFE

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INTRODUCTION

A technique to routinely obtain liver samples from living cattle would have many applications in research on cattle diseases. In Brazil and other tropical countries, it would be especially useful because there are a number of important infectious diseases and toxicoses of cattle that primarily involve the liver. In the U.S.A., metabolic diseases that affect the liver are an important problem of dairy cattle. An improved liver biopsy technique would supply more precise information on the status of the liver than the indirect liver function tests that are currently used. Liver function tests tend to supply information as to the results of dysfunction, while a biopsy sample would supply information as to the cause of liver disorders. In clinical work a liver biopsy could supply information for an earlier and more accurate diagnosis and prognosis. Histopathologic evaluation of a liver biopsy could confirm and extend clinical diagnosis. It might reveal unexpected disorders that would pass undetected by liver functional tests. Treatment could thereby be initiated earlier and more appropriately. In cattle research the role of the liver in the pathogenesis of a disease could be evaluated over a period of time in the same animal. In addition, the amount of chemicals, drugs, hormones and nutrients in liver tissue could be determined and their role in health and disease evaluated.

In man and in laboratory animal models, liver biopsy techniques have supplied important information pertinent to infectious and metabolic diseases, nutrition and toxicology. In large animals, a liver biopsy technique was used in studies on copper metabolism in sheep (Dick, 1944, 1952). It was essentially a modification of the technique used previously in man. The current liver biopsy techniques, especially those used in man, are used to collect small (0.5 to 0.8 gm) samples primarily for histopathologic examinations. In some cattle investigations, larger samples, as much as 5 to 8 gm, are required to determine the content of specific biochemicals and drugs. In addition, in research it is important to know the location in the liver from where the sample was taken. An exploratory examination of the liver is not made when using the needle biopsy technique.

The value of a liver biopsy technique in cattle is not restricted to investigations of liver disease problems. It would also be of diagnostic value for a number of systemic and non-hepatic disorders. It could also be utilized in nutritional, physiologic and toxicologic research and in procedures such as biopsies of tumors.

This research was undertaken to devise a safe, surgical technique to collect suitable liver samples from cattle for clinical and research investigations. The research involved fabricating suitable instruments, perfecting a surgical technique and a chemical and histopathologic examination of biopsied liver tissue collected under different experimental conditions.

LITERATURE REVIEW

In clinical human medicine, needle liver biopsy has been used for over 100 years and is currently an excellent technique to collect specific information on the status of the liver (Pearson and Craig, 1980). It is useful as a routine procedure for establishing the presence of histologic changes in hepatic tissue associated with disease problems.

In man, the technique is rather standardized. A Vim-Silverman needle, 2.0 mm in diameter and fitted with a stylet, is used. The needle and stylet are inserted into the abdominal cavity, the stylet removed and the biopsy needle inserted into the liver. The needle is attached to a syringe, a negative pressure applied and the liver sample removed. The most severe complications have been biliary peritonitis, pneumothorax, hemoperitoneum and perforation of visceral organs (Raines et al., 1974). Among the advantages offered by this procedure are the obviation of general anesthesia, laparotomy, sophisticated and costly medical equipment and thereby the specialized medical training required for these procedures (Feldman and Ettinger, 1976).

In veterinary medicine, however, liver biopsy has been used as a technique primarily for research. In Australia, Dick (1944, 1952) adapted the liver needle biopsy technique as used in man and performed liver biopsy in sheep. The needle was inserted at the 9th intercostal space, through the pleural cavity and diaphragm and into the liver.

A negative pressure was applied with a syringe to remove the liver sample. He stated that "many hundreds" of liver samples from sheep were successfully collected for studies on copper metabolism.

Development of Liver Biopsy in Cattle

In England, Garner (1950) and Loosmore and Allcroft (1951) used essentially the same instrument as described previously by Dick (1944) for liver biopsy in research on copper metabolism in cattle. They inserted the needle at the 12th intercostal space, 15 to 20 cm from the dorsal midline, and did not penetrate the diaphragm. They reported no deleterious effects. In discussing the technique, Garner (1950) mentioned the advantage of not penetrating the diaphragm.

In Florida, Chapman et al. (1963) used a liver biopsy procedure to study mineral nutrition in beef cattle. Hepatic tissue values of copper and iron proved to be more reliable than either blood hemoglobin or total body copper in establishing the metabolism of copper in cattle. Copper values of liver samples varied among 4 areas of the liver.

Udall et al. (1952), at Cornell University, reported a simple liver biopsy technique to collect 0.7 gm samples from cattle which did not involve the use of negative pressure to remove the core of liver. A cannula with a trocar was used and the biopsy site was the 10th or 11th right intercostal space about a quarter of the way down from the vertebral junction of the rib and the costochondral junction. A large number of cattle were biopsied with 1 fatality caused by puncture of the thoracic duct. Seghetti and Marsh (1953), in Montana, obtained approximately 1 gm liver samples for vitamin A analysis from 15 steers by using a cannula and negative pressure from a syringe. This technique was further modified by a "clean out rod" and a syringe as a means of

aspiration by Bone (1954). Penetration of the abdominal cavity was made at the 11th intercostal space, about 25 to 30 cm ventrolateral to the spinal articulations of the ribs. The trocar and cannula were introduced through a skin incision and the intercostal muscles. The trocar was removed. The cannula was directed downward at an angle of about 20 degrees to the horizontal and slightly forward into the liver of pregnant cows. No harmful effects were mentioned.

Hughes (1962), in California, used a neoprene ring (to create a vacuum) at the base of a cannula, 0.95 cm in diameter, and collected 3 to 5 gm samples of liver for vitamin A analysis from 500 cattle. Three animals died. The possibility of fatal hemorrhage if a major vessel were accidentally injured was mentioned. They also cautioned that biliary peritonitis may result from injury to the large bile ducts and that puncture of a liver abscess would result in septic peritonitis.

In India, biopsy of the liver was conducted in 12 cattle while they were cast on their left side with the legs tied together (Narasimhamurty, 1966). A 1.2 cm incision was made at the right 12th intercostal space, 15 to 20 cm from the dorsal midline. The trocar and a cannula were introduced through the incision to gain access to the hepatic tissue. The trocar was withdrawn, and the cannula was pushed perpendicular to the body surface for a distance of 3 to 4 cm. The thumb was then placed firmly over the cannula and the cannula and liver sample removed. Uniform, consistent liver samples were obtained with a minimum of risk.

In general veterinary practice, liver biopsy was used to confirm a diagnosis of ragwort poisoning in a herd of Friesian cattle (Betty and Markson, 1954). The technique was also used to detect subclinical ragwort poisoning in animals. Liver biopsy was considered to be a convenient and practical technique for a more accurate diagnosis of cattle

diseases. In a clinic for treatment of cattle diseases, 115 biopsies were obtained for histopathologic diagnosis (Thoonen et al., 1967). The kidney was accidentally aspirated twice and the pancreas once. Degenerative changes in the liver were present in cases of metabolic disorders and an inflammatory process. Ketonemia was accompanied by fatty infiltration of the liver. Gibbons, using a liver biopsy technique (1956), mentioned that in aflatoxicosis there was liver cirrhosis and bile duct proliferation. Correlations between histological, clinical and biochemical changes were mentioned. Gibbons emphasized that cirrhosis of the liver resulting from the ingestion of poisonous plants such as crotalaria, senecio and lupines can be confirmed by biopsy.

In Sweden, Wallin and Pehrson (1965) described a liver biopsy technique that they claimed was risk-free to the patient to obtain liver samples for histologic examination. They used a small diameter needle (2.0 mm) and emphasized the importance of knowing the exact position of the liver. In 20 cows, they obtained adequate liver samples and on slaughter of the cows 24 hours later, hemorrhages caused by the biopsy were not evident. They also mentioned that anesthesia was not necessary. However, in ambulatory practice local anesthesia of the skin and subcutaneous tissue was beneficial. Moller and Simesen (1959), also in Sweden, mentioned that liver biopsy in conjunction with histologic examination was an important clinical-diagnostic procedure. They also emphasized the value of several consecutive liver biopsies to determine the pathogenesis of liver diseases and the application of the procedure for the evaluation of liver function tests.

The value of liver biopsies in investigations of metabolic diseases of dairy cattle was stressed by Raker and Rogers (1956) and Oxender et

al. (1971). Chemical analyses of liver samples obtained by needle puncture biopsy were as reliable as samples collected by laparotomy. These investigators mentioned peritonitis as one of the complications.

In research on Johne's disease in cattle, Patterson and Allen (1968) investigated liver biopchemical abnormalities by using a needle puncture biopsy to collect small samples and a laparotomy approach to collect more than 1 or 2 gm samples. The laparotomy-collected samples were more reliable. Blood contamination was considered to be a source of analytical error in biopsied liver tissue. Biopsies were also more susceptible to dehydration.

Additional reports are available (Erwin et al., 1956; Horvath et al., 1967; Lamothe et al., 1969; Ivascu et al., 1968; Turner and Green, 1976) confirming the general value of liver biopsy in cattle, especially for diagnostic application. In most of the reports, small (usually less than 1 gm) liver samples were collected. Many authors mentioned the need for improving the technique to avoid complications. The complications included injury to other tissues, peritonitis, and excessive hemorrhage. Many workers also mentioned the need to have more precise information as to the site for biopsy of the liver. In most reports, the need for a liver biopsy technique was precipitated by a specific research undertaking. Only a minimum amount of research emphasis has been devoted to details of the technique, such as surgery, anatomy, instrumentation, anesthesia, sample size, postsurgical complications, and collecting samples at specific intervals over a long period of time.

Collecting Larger Liver Biopsy Samples in Cattle

In an effort to collect larger biopsy samples (2 to 3 gm) and to minimize complications, Whitehair et al. (1952) described a technique used to obtain samples for vitamin A analysis in beef cattle. This was the first reported technique that examined the liver and the adjacent region in the peritoneal cavity before biopsy. The instruments consisted of a large rumen trocar with a tightly fitting cannula. The trocar and cannula were inserted into the abdominal cavity through an incision in the 12th intercostal space. The trocar was then removed and the surface of the liver was located and examined with a flashlight that had a flexible extension bulb. The biopsy sample was collected in a cylinder that was 1.2 cm in diameter x 2.6 cm in length. At the end of the cylinder, a wire was fitted so as to sever the attached core of liver. These workers mentioned that in collecting larger biopsies of liver, it was necessary to sever the distal end of the core of liver. These workers also indicated that the biopsy instrument they used was not entirely satisfactory because of the time involved in adjusting the wire to sever the core from the liver.

Liver Biopsy in Horses and Sheep

Sova (1965) used a needle liver biopsy technique in 299 horses. In 162 horses the biopsy permitted the diagnosis of various types of hepatopathies. In 9 horses the liver could not be biopsied. In biopsies from the left side, the stomach was sampled twice. Pulmonary hemorrhage developed in 3 horses, with a fatal outcome in 2. He concluded this procedure should be used with great caution in horses.

Petrovic et al. (1963) suggested that a liver biopsy provides valuable clinical data in horses with infectious anemia, especially in

the early stages of the disease, as well as during its subsequent course. The advantages of liver biopsy were straightforward clinical applications, low cost and rapid results, while at the same time entailing a minimal amount of injury. Liver biopsy had advantages over the usual biological tests and clinical manifestations in diagnosing infectious anemia.

In newborn lambs, Hogan et al. (1971), in Australia, reported that when using the aspiration biopsy technique there was a statistically significant trend towards an increase in copper values in samples obtained from the dorsal right lobe in comparison to the ventral lobe. However, this trend was less or totally lacking in older sheep.

Vulinec and Bauer (1970) determined the puncture site for liver biopsy in sheep by palpation and percussion and thereby located the caudal portion of the liver behind the curve of the last rib.

General Use of Liver Biopsy in Livestock

In a recent comprehensive review, Pearson and Craig (1980) emphasized that liver biopsy can be an extremely informative and useful procedure in livestock to confirm the presence of liver disease and in many instances for determining the etiology. These authors mention that,

> The cardiologist often finds heart disease such as valvular insufficiencies long before there is heart failure, and the urologist may find kidney disease such as glomerular nephritis long before there is renal failure. Unfortunately, there are usually no clinical signs of liver disease until there is failure of one or more of the liver's functions. Since the liver has so many functions, there are many possible signs of liver disease, depending somewhat on which functions are failing to meet the animal's need.

Pearson and Craig performed more than 200 needle liver biopsies on horses, cattle, sheep and goats without any untoward reaction. These

workers mentioned that in man the information from liver biopsies and the clinical history enabled pathologists to give a correct diagnosis in 98% of the cases submitted. They reviewed the techniques and rationale of liver disease diagnosis in large animal medicine and also discussed an evaluation of liver damage and functional failure. They concluded that veterinary practitioners have been reluctant to perform liver biopsy because of the risk to the animal and the time involved. However, with proper instruments and techniques, liver biopsy could be a safe and simple procedure.

Anatomical Considerations for Liver Biopsy

Getty et al. (1975) described the bovine liver as somewhat inconsistent in its anatomical position. In adults, it is located almost entirely to the right of the sagittal median plane, being rotated by about 90 degrees from its embryonic position. Thus, the right lobe is dorsal and the left is ventral. This displacement occurs because of the development of the rumen on the left side of the abdominal cavity. The long axis of the liver is directed downward and forward from the right kidney at the 13th rib, to a plane ventral to the 6th intercostal space. The diaphragmatic surface of the liver is for the most part in apposition to the right part of the diaphragm (right costal line of diaphragm), with only a small part in contact with the last 2 or 3 ribs and sometimes with the flank at the lumbocostal angle. The diaphragmatic line starts at the boundary of the dorsal third of the 12th rib. The line crosses through the middle of the 11th rib and proceeds ventrally to the ventral third of the 10th rib and ventral quarter of the 9th rib. It then crosses the costochondral junction of the 8th rib and extends along the 8th costal cartilage to the base of the xiphoid cartilage.

Occasionally, the diaphragm may project caudally and adhere to the last rib (13th) at the lateral end of the first lumbar transverse process. In the newborn calf, the liver is not yet displaced to its adult position, nor is it in complete contact with the right abdominal wall, because the rumen is not fully developed. Consequently, the right and left lateral lobes of the liver are located anteroventrally in the abdominal cavity.

In adult cattle, the right triangular ligament attaches the caudolateral angle of the right liver lobe to the dorsal abdominal wall. The left triangular ligament on the dorsal part of the liver extends from the esophageal impression on the diaphragm ventral to the esophageal hiatus. The coronary ligament attaches the liver to the diaphragm on a line from the right triangular ligament along the right side of the vena cava and around the ventral margin of the foramen venae cavae to the left triangular ligament. The triangular area on the dorsal surface of the liver is devoid of serous covering because of its diaphragmatic attachment. The hepatorenal ligament passes from the caudate process to the ventral surface of the right kidney.

As suggested by Whitehair et al. (1952), Narasimhamurty (1966) and Garner (1950), the logical operative site for liver biopsies in cattle is between the last 2 ribs on the right side at a point lower and posterior to the diaphragmatic line of pleural reflection. This location would avoid penetration of the diaphragm. It would provide close proximity to the caudate and dorsal lobes of the liver.

Gross Anatomy

Getty et al. (1975) and Popesko (1977) described the visceral surface of the liver as concave. On the visceral surface is the portal

area where the portal vein and the hepatic artery enter the liver and the common hepatic duct leaves it. The pancreas is also attached to the visceral surface. The gallbladder fossa extends from the portal area to the ventral border of the liver. The gallbladder fundus is in contact with the diaphragm opposite to the ventral parts of the 10th or 11th ribs.

The right border of the liver faces caudally and is short and thick. It presents a deep impression formed by the right lobe and caudate process of the right kidney and adrenal gland. The left border is a smooth curve continuous with the dorsal and ventral borders. The ventral border contains the gallbladder fossa and the notch for the round ligament. The dorsal border is almost median in its position and contains the caudal vena cava. The caudate lobe of the liver is between the vena cava and the left branch of the portal vein. The quadrate lobe is between the left branch of the portal vein and ventral border of the gallbladder fossa. The right dorsal lobe is an imaginary line from gallbladder fossa to the portal area. The left lobe is the part anterior to the falciform ligament.

Blood Vessels in the Liver

The portal vein divides immediately upon entering the liver into a short right branch and a long left branch (Julian and DeOme, 1949; Julian, 1952; Getty et al., 1975). Secondary branches from both are for the most part close to the visceral surface, but some arch through the liver and course near the diaphragmatic surface. The short right branch divides immediately into 4 or 5 secondary branches. These include branches to the caudate lobe, the right dorsal lobe, one or more to the right intermediate area, and branches to the right ventral lobe.

The long left branch runs at first along the long axis of the liver from the portal area towards the left lobe. It is very close to the visceral surface between the caudate and quadrate lobes and is covered by the papillary process, fat and hepatic lymph nodes. It bends sharply 45 to 90 degrees toward the notch for the round ligament and supplies branches to the quadrate, caudate and the left lobes.

The hepatic artery from the aorta usually gives off a left branch and 2 smaller right branches. These follow the branches of the portal vein previously discussed.

There are 3 main hepatic veins (right, medial and left) that generally run perpendicular to the branches of the portal vein and the hepatic artery. They extend from the vena cava toward the ventral border of the liver. They are deep in the liver parenchyma. The right hepatic vein joins the vena cava between the right and the caudate lobes and has 2 branches to the right lobe and 1 to the caudate process. The middle hepatic vein joins the vena cava close to the left hepatic vein. It runs obliquely through the caudate and quadrate lobes, with branches on each side. The left hepatic vein joins the vena cava near the esophageal impression. It has 3 or 4 branches to the left lobe.

Bile Ducts

The small bile ducts or ductules unite to form large intrahepatic ducts and leave the liver as the main hepatic ducts (Getty et al., 1975; Stinson and Calhoun, 1976). The extrahepatic bile passages are formed by the hepatic ducts, cystic ducts, the common bile duct and the gallbladder.

Microscopic Anatomy

The hepatic lobule is a structural unit organized around the central vein. The lobule is polygonal in shape on a cross section view. The sinusoids radiate from the periphery of the lobule to the central vein (Mall, 1906; Elias, 1949, 1953; Elias and Sherick, 1969; Stinson and Calhoun, 1976). The hepatic cells are arranged in a cord pattern around the central vein. Between the cords are the sinusoid channels, separated from the liver cells by a perisinusoidal space. The bile canaliculi are between opposing hepatocytes. The sinusoids are lined by discontinuous endothelial cells, some of which are phagocytic in nature and are a part of the reticuloendothelial system (Morgan and Hartroft, 1961; Stinson and Calhoun, 1976). These cells are called stellate reticuloendothelial cells. The boundary of usually 3 lobules is called the hepatic triad. Each central vein receives a portion of its blood from 3 to 4 or more hepatic triads.

The hepatic triad contains a branch of the hepatic artery, portal vein, a bile duct or ducts, connective tissue and the lymphatics. The triad is delineated by the limiting plates, which are nearly triangular in form on cross section but rather long on a longitudinal cut (Aterman, 1960; Elias and Sherick, 1969; Stinson and Calhoun, 1976). Hepatic triads may have different forms when sectioned through branches of the portal vein at different angles. The small ducts are lined by cuboidal or low columnar epithelium and the large ones by a high columnar epithelium with basal nuclei (Steiner and Carruthers, 1961; Elias and Sherick, 1969). The ducts and bile canaliculi of the parenchyma are connected by ductules and Hering canals, which are partially lined by cuboidal ductular cells and liver cells (Steiner and Carruthers, 1961; Wood, 1961). The connective tissue in a normal triad consists of small,

closely knit bundles of collagen fibers and a network of supporting reticular structures with very few fibroblasts. The picture is uniform throughout the liver. However, a few lymphocytes and histiocytes may be present. Lymph vessels are normally inconspicuous. The same is true of the portal nerve branches.

Nerve Supply to Thoraco-Costal Area

In the bovine, the majority of the thoracic nerves leave the vertebral canal through the lateral vertebral foramen caudal to the corresponding vertebra (Hall, 1971; Getty et al., 1975). After emerging from their respective foraminae, the spinal nerves immediately branch off into the dorsal and ventral branches. The dorsolateral branches of the dorsal branch of the 11th and 12th thoracic nerves pass between the longissimus thoracis and iliocostalis and give off branches to each muscle. The dorsolateral branch crosses the next rib at about the level of the lateral edge of the transverse process of the 2nd lumbar vertebra. It subsequently enters the serratus dorsalis and emerges from the thoracolumbar fascia accompanied by blood vessels and then branches off into the skin on the dorsal aspect at approximately the level of the tuber coxae. The ventral branches of most thoracic nerves emerge through the laterovertebral foramen in the bovine, terminating in the transversus thoracic muscle. Those of the 11th and 12th thoracic nerves transverse the rectus abdominis muscles, the aponeuroses of the oblique internus and externus abdominis and cutaneous trunci muscles and terminate in the skin cranial to the umbilicus.

Anesthesia for Liver Biopsy

Farquharson (1940), at the Veterinary Clinic, Colorado State University, suggested that the paravertebral method of anesthesia was the

best for laparotomy and rumenotomy in the bovine. Anesthesia of the 13th thoracic and first lumbar nerves was considered adequate for surgery in this area. The 13th nerve was located by palpating the head of the last (13th) rib. The location for paravertebral anesthesia was just posterior to the spinous process of the first lumbar and the 13th thoracic vertebra. The anesthetic solution was injected about 5 cm lateral from the dorsal midline at a depth of 5 cm. The advantages of this method, as opposed to local infiltration, were evident by the complete and uniform desensitization of the abdominal wall, including the peritoneum. Muscular relaxation was also observed. This method proved to be a simple and safe procedure. Another advantage was the rapidity with which it could be performed. The postsurgical convalescent period was short and no side effects were observed. According to Khatra and Tyagi (1969), the anesthetic solution is injected about 3 to 4 cm lateral to the dorsal midline. An 18 gauge, 5 cm long needle is inserted medial to the longissimus dorsi muscle until it touches the dorsal aspect of the posterior border of the transverse process of the 13th thoracic vertebra. The needle is then moved laterally to miss the vertebra and inserted about 1 cm deep to penetrate the intratransverse ligament. Eight milliliters of a 2% novocaine solution is infiltrated at this site. While removing the needle, an additional 5 ml of the anesthetic solution is injected to block the dorsal branch of the nerve. The anesthesia lasts from 30 to 115 minutes. Scoliosis towards the anesthetized side together with increased skin temperature at the injection sites were reported in cattle by Farquharson (1940) and were also observed in buffaloes after paravertebral anesthesia (Said et al., 1976).

Schreiber (1956), cited by Cakala (1961), reported that with the paravertebral thoracic and lumbar blocks described by Farquharson, the

needle may, in rare instances, puncture or lacerate the aorta or the thoracic longitudinal vein on the right side.

Other investigators used the same site for local anesthesia and the subcutaneous tissue was infiltrated with 5 to 10 ml of 2, 3 or 4% procaine hydrochloride, novocaine-adrenalin or xylocaine to produce anesthesia (Dick, 1944; Garner, 1954; Seghetti and Marsh, 1953; Bone, 1954; Chapman, 1963; Hojovcova and Kacafirex, 1967; Pearson and Craig, 1980). Whitehair et al. (1952) injected about 10 ml of local anesthetic solution just posterior to the caudal border of the ribs for regional nerve anesthesia. Deeper injections of the anesthetic solution into the tissue were advised against, since there is a risk of injecting the solution directly into the pleural cavity. They also induced anesthesia by local infiltration of the surgical area.

Narasimhamurty (1966), Erwin et al. (1956), Ivascu et al. (1968) and Holtenius (1961) considered it unnecessary to use anesthesia, since the entire procedure for collecting the liver sample took little time to perform.

Functions and Dysfunctions of the Liver

The liver is the largest gland in the body and in ruminants such as the cow it has important roles in maintaining physiologic "homeostasis" (Dougherty et al., 1965; Kronfeld, 1980; Bracken, 1980). One of the more important roles of the liver in cattle is the conversion of volatile fatty acids to energy. Disturbances in this latter activity are the basis for important metabolic diseases. The liver is a highly vascularized structure, receiving venous blood from the gastrointestinal tract and spleen in addition to its arterial blood supply (Swenson, 1970). The portal blood contains the absorbed products, such as the volatile fatty acids, from the gastrointestinal tract. Diseases

involving the liver are often not diagnosed until they become chronic and considerable parenchymal tissue has been replaced by fibrous connective tissue. It has been estimated that the liver is capable of maintaining basic functions even after 70% of the parenchyma has been destroyed (Bracken, 1980).

Primary diseases of the liver in cattle are not common (Bracken, 1980). The most common ones are parasitism, chemical and plant poisoning, and bacillary hemoglobinuria. Diseases that may involve dysfunction of the liver secondarily include hemolytic blood disorders, metabolic diseases, congestive heart failure, gastric foreign bodies, systemic infections and neoplasia. Dysfunction of the liver, whether primary or secondary, may influence the functioning capacity of other organs and are manifested by a generalized illness, loss of production and even death. For example, excessive ammonia absorption from the rumen or an inability to convert it to urea results in hyperammoniaemia, accompanied by clinical signs of a neurologic disease and, if severe, even death.

Liver degeneration is often a secondary, but occasionally a primary, disease in cattle (Bracken, 1980). It may be a combination of cloudy swelling or fatty changes and amyloidosis. The abnormal intracellular accumulation of fat in the liver is characterized on histopathologic examination by a vacuolation of the cytoplasm of hepatocytes. It is sometimes accompanied by necrosis and the presence of an inflammatory cell reaction, depending on the causative factor and severity of fat accumulation. It may have no effect on cellular function when mild. Severe fatty infiltration may impair cellular function, but unless some vital intracellular process is irreversibly impaired, as in carbon tetrachloride toxicity, fatty infiltration per se is reversible.

In addition to degenerative changes in liver cells, there may be substantial infiltration of the affected parts of the parenchyma by inflammatory cells. The infiltrate is composed mainly of mononuclear and polynuclear cells. Stellate reticuloendothelial cells are swollen.

Cirrhosis of the liver can develop by several possible mechanisms after acute hepatitis (Gibbons, 1970). The usual pathway for cirrhosis is chronic active hepatitis of greater or lesser severity. Extensive confluent necrosis may also produce cirrhosis rapidly without a striking inflammatory component. In cirrhosis, the structure of the liver is distorted by an increase in connective tissue, giving it a pseudolobular pattern. There may be some regenerated areas. Bile duct proliferation and inflammatory cell infiltration may be present. These liver lesions in cattle are an important problem. They are associated with enzootic diseases, especially plant poisoning, in various parts of the world, including the U.S.A.

Jensen et al. (1954) and Jensen and Mackey (1979) mentioned that liver abscesses are an important problem in beef and dairy cattle production. The incidence in slaughtered cattle is about 10%. The primary disturbance is an abnormal fermentation (acidosis) and resulting injury in the rumen. The liver abscesses are caused by the absorption of foreign substances and bacteria, usually *Fusobacterium necrophorum*. Liver abscesses are seldom diagnosed clinically. The disease is associated with feeding high-energy fattening-type rations and a minimum amount of roughage. Liver abscesses may also occur in cattle with metritis, traumatic peritonitis, mastitis, or navel ill.

The liver has an important role in carbohydrate and fat metabolism in cattle (Kronfeld, 1980). This role in the ruminant seems especially important in the conversion of the absorbed volatile fatty acids to

energy and in the synthesis of glucose. The maintenance of a proper energy "homeostasis" by the liver is significant in the prevention of important and common metabolic diseases such as ketosis. Glucose balance in the pregnant or lactating cow is often emphasized as precarious.

The liver has an important role in protein metabolism, especially in the conversion of nonprotein nitrogen to amino acids (Swenson, 1970; Stinson and Calhoun, 1976). It is the source of all serum albumin, globulin, fibrinogen and other blood-clotting factors. It has an essential role in the synthesis of factors involved in resistance to infectious agents. The liver is involved in the metabolism and storage of minerals and vitamins, and the content of these nutrients in the liver is often associated with the nutritional requirements by the animal.

Another important function of the liver is the secretion of bile (Swenson, 1970; Stinson and Calhoun, 1976). This is indispensable for fat and fat-soluble vitamin absorption and metabolism. Additional functions of the liver would include detoxification of harmful substances, regulation of circulation, temperature regulation, and the production of enzymes.

The Liver and Vitamin A Metabolism

Vitamin A (or its precursor, carotene) is the most important vitamin in cattle nutrition. The supplies of this nutrient may be limited during periods of droughts or in feeding regimens where there is a limited amount of forage fed (Perry et al., 1960; Hansen, 1980). A deficiency of vitamin A has been associated with a variety of clinical syndromes in cattle. These include respiratory, enteric and reproductive problems. Vitamin A is associated with maintaining resistance to infections. How this is accomplished is not clearly understood. In a

vitamin A deficiency, there is keratinization of columnar epithelial cells, especially epithelial cells that have a secretory function. This is an indirect method of providing resistance to microorganisms.

Because vitamin A is so important to cattle production, a considerable amount of research has been conducted to establish the requirements for this nutrient. During the late 1940s and early 1950s, considerable emphasis was given to the importance of vitamin A metabolism in cattle. Results from several laboratories suggested that blood values of vitamin A were not closely correlated with liver values. Hoefer and Gallup (1947), at the Oklahoma Agricultural Experiment Station, concluded that in lambs there was little relationship between the serum levels of vitamin A and the hepatic tissue levels when the serum levels exceeded 20 mcg per 100 ml. In England, Glover et al. (1947) reported that plasma levels of vitamin A in the rat were directly proportional to the concentration of vitamin A alcohol in the livers, but they were not correlated with the total stores of vitamin A, which consisted mainly of esters. Plasma vitamin A levels were maintained at levels of 35 to 40 I.U. per 100 ml when liver stores were approaching depletion. About this same time, Frey and Jensen (1947), at Colorado, reported that cattle fed a fattening ration depleted their vitamin stores at a constant rate regardless of dietary intake.

In an effort to clarify information on vitamin A and carotene metabolism in cattle, Oklahoma researchers (Whitehair et al., 1952) devised a technique (described previously) to repeatedly collect liver samples in the living animal and to correlate the liver vitamin A content with the carotene and vitamin A content of the feed and blood. Using this technique and collecting samples at monthly intervals for

1 year, Van Arsdell et al. (1951) reported that when cows and steers were fed a carotene-vitamin A deficient ration, the vitamin A in the plasma remained rather constant while liver stores became depleted. They concluded that there was a correlation between plasma and hepatic vitamin A values only at high levels of intake. No ill effects from repeated liver biopsy were apparent and on necropsy examination of the liver of biopsied animals there was only slight scarring of the liver tissue. In another long-term study (Baker et al., 1953) at Oklahoma, cows were fed different amounts of carotene and the levels of vitamin A and carotene were determined in the plasma, liver, and milk during reproduction and lactation. The vitamin A content of the livers of the calf was affected more by the carotene intake of the dam during lactation than by liver stores at parturition. This finding was confirmed by a more recent report (Diven et al., 1960). The latter 2 reports confirmed a previous suggestion by Wise et al. (1947) that supplemental vitamin A for cows during gestation did not prevent a characteristic decline in serum vitamin A values at parturition. In further studies on vitamin A metabolism, Kon et al. (1955) demonstrated that when the esters (acetate) of vitamin A were intravenously injected, vitamin A rapidly appeared as the alcohol (retinol) in the plasma.

Reliability of Biopsy Samples for Estimating Liver Vitamin A Content

Detailed research to obtain information on the reliability of a single biopsy sample as an estimate of the amount of hepatic vitamin A was conducted by Van Arsdell et al. (1951) and Anderson et al. (1962). These workers reported differences in the liver content of vitamin A between various sites, but these differences did not materially decrease

the accuracy of 1 sample. They concluded that the vitamin A content of a liver sample obtained by biopsy would not vary more than ± 17 to 21% from the true hepatic value. The distribution was more variable when the storage was either very low or unusually high. In more recent work in Poland, Honory (1974) found the highest content of vitamin A in normal cows in the right and caudate lobes and the lowest in the left lobe. The same distribution of vitamin A was noted in the liver of cows affected with leukosis and fascioliasis. Usui and co-workers (1960), at the Department of Veterinary Surgery and Obstetrics, University of Tokyo, compared different lobes of the liver and concluded that the vitamin A content of the middle lobe was the most representative of the vitamin A content of the liver. These workers also reported that a proliferation of connective tissue in the liver markedly reduced the storage of vitamin A. There was no correlation between vitamin A and glycogen values.

Methods of Administration of Vitamin A

Methods for administration of vitamin A to cattle to prevent a deficiency have been investigated by numerous workers. Hale and co-workers at Arizona (Hale et al., 1962) reported that a daily oral intake of 10,000 I.U. of vitamin A for 168 days failed to maintain the value of vitamin A content of the liver that was present in the beginning of the experiment. However, 40,000 I.U. daily maintained initial vitamin A content of the liver. These workers also noted that, with the exception of fat, tissues other than the liver had little capacity to store vitamin A.

Roberts and Stringham (1962), at the University of Manitoba, compared the intraruminal administration of 1 million I.U.of vitamin A

palmitate to feeding 10,000 I.U.daily during an 84-day period. Initial vitamin A liver values were low. Liver vitamin A values were markedly increased within 3 days after intraruminal injection of vitamin A and reached a maximum between 7 and 21 days postinjection. Thereafter, a gradual decline in liver vitamin A concentration occurred and the decline in concentration was the same for both methods of administration. In a later study (Phillips and Roberts, 1964), the value of 1, 2, 3 and 4 million I.U. of vitamin A given intraruminally was compared. Liver vitamin A values were higher with the higher dosage, but the higher amounts had no influence on weight gains or feed efficiency. In more recent studies at Michigan State University (Martin et al., 1971), intramuscular injections of 7 million units of vitamin A were much more effective than the same amount given intraruminally. These workers concluded that intraruminal injections produced no greater liver vitamin A stores than when no oral supplemental vitamin A was given. In a further study (Roberts et al., 1965), 1 million I.U.of vitamin A, whether given intramuscularly or intraruminally, provided the same concentration of vitamin A in the liver. The requirements and metabolism of vitamin A apparently depend on a number of nutritional, physiological and pathological factors. Whitehair (1980) mentioned a number of these factors, which include: 1) toxic substances, 2) other deficiencies, 3) hormones, 4) high environmental temperatures, 5) infectious diseases, and 6) high concentrate low fiber rations.

Liver Lesions and Metabolic Diseases of Dairy Cattle

The advent, in the early 1950s, of high energy rations to increase milk production was accompanied by an increase in the incidence of metabolic diseases in dairy cows, especially ketosis. In early

experimental work on ketosis, Scottish workers (Robertson and Thin, 1953) starved cows that were at peak milk production for 5 to 6 days. The effect of starvation was to produce a sharp decrease in volatile fatty acids in the rumen and an increase in rumen pH. This was accompanied by a decrease in milk production and body weight and an increase in ketone substances in the blood. In further studies (Robertson et al., 1960), a 6-day fast also produced clinical signs of milk fever. Danish workers (Simesen and Miller, 1959), using a liver biopsy technique, examined the livers of 20 cows with clinical ketosis and reported characteristic histopathologic changes. In clinical ketosis there was severe fatty infiltration and a pronounced reduction in glycogen. Concurrently with clinical improvement, there was a gradual reduction in fat deposits and an increase in the glycogen content. In severe cases of ketosis the liver cell nuclei had regressive changes characterized by "chromatotaxis"--a destruction of the nuclear chromatin. During the past decade British workers (Reid, 1973; Reid et al., 1977; Brumby et al, 1975; Baird et al., 1972) have experimentally produced ketosis by fasting high milk-producing cows for 6 days. The results of the latter studies supplied information on the sequence of metabolic changes occurring in the liver and other tissues. The 6-day fast caused an accumulation of fat in the liver, a reduction of liver glycogen and significant changes in the structure of liver cells. The alterations in the liver structure in induced ketosis were considered similar to those occurring in clinical cases. Grossly the liver was characterized by a pale yellow color and a soft, friable and greasy consistency. Liver sections stained with oil red O contained numerous fat droplets in the cytoplasm, particularly in the centrolobular area. The liver fat content increased almost 6-fold in induced ketosis, while glucose and

glycogen values decreased to 1/10 normal values. Ultrastructural examination revealed numerous alterations in cytoplasmic organelles. The pathogenesis of fatty liver is most likely due to an increased hepatic lipogenesis, enhanced mobilization of free fatty acids from the adipose tissue and decreased hepatic oxidation of fatty acids (Kronfeld, 1980). Additional factors that are apparently involved in clinical ketosis are thyroxin (Hibbitt and Baird, 1967) and the glucocorticoids (Sasaki et al., 1974). With the exception of decreased phosphorylase activity, most enzyme activity is not influenced by starvation (Manns, 1972). In most of the experimental work using lactating cows, a 6-day fast produced deleterious effects in the liver. In research (Jonsson et al., 1966) on young calves, a 24-hour fasting caused a significant (22 to 28%) reduction in liver weight. The liver loss in weight was due mainly to water and glycogen decrease.

Role of Dietary Protein and Energy in Ketosis

For many years, veterinary clinicians considered that dietary protein had an important role in causing ketosis and related metabolic diseases and also had a role in therapy. Swedish researchers (Hoflund and Hedstrom, 1948) suggested that the amount of protein in the ration had an important role in the digestion of cellulose. Excessive protein would retard cellulose digestion and reduce the amount of energy produced. At the other extremity, a deficiency would not provide the flora with the proper substrate for fermentation. The rumen microflora requires a minimum amount of nitrogen for an active and functional ruminal population (Moir and Harris, 1962). Researchers at Illinois (Leedle and Hespell, 1979) reported that when cows were starved the rumen bacteria had an extremely poor survival capacity. Within 2 hours

after starvation, 60% of an initial population failed to continue to form colonies. Cornell workers (Kinzella and Butler, 1970) reported that cows fed 24% protein during the first week of lactation had much lower fat in the liver (13.6% vs 7.8%) than cows fed a 15% protein ration.

Anorexia is a common clinical sign in metabolic diseases involving the liver (Morrow et al., 1979). The inappetence may vary from slight to complete feed refusal. Proper therapy to restore appetite and maximum production is therefore important.

In a comprehensive review on the management and nutritional aspects in ketosis, Schultz (1971) suggested that to prevent the disease cows should be fed at levels as near to optimum as possible within the ability of the cow to consume rations without going off feed. He also suggested to provide feeds containing adequate nutrients, following management practices which maximize intake during early lactation, avoiding intake of ketogenic materials, and minimizing prolonged mobilization of body fat. Most workers recommend glucogenic substances such as sodium propionate orally in treatment of ketosis. Feeding good quality roughage is important in stimulating the appetite. Appetite is closely associated with proper rumen function. Good quality roughage improves the palatability of a ration. Small amounts of molasses may not only improve the appetite but also provide energy for rumen microbes. Treatment should emphasize a balanced ration that considers microbial requirements to maintain health and production of cattle.

Summary

There is ample information from the literature that metabolic diseases are an important problem in cattle production. In most of these diseases the liver is involved in a primary or secondary way. A safe

technique to obtain suitable liver samples for research and diagnostic studies would be most helpful in supplying information to improve cattle production.

OBJECTIVES

1. To devise improved instruments and a safe surgical technique for liver biopsy in cattle.
2. To establish anatomical locations and a surgical procedure suitable for liver biopsy in cattle.
3. To determine the histologic appearance of the liver after repeated biopsy.
4. To determine if the content of specific substances such as vitamin A in liver biopsy samples is representative of the entire liver and to correlate the content in the liver with that in peripheral blood.
5. To determine the influence of a 6-day starvation on liver changes in lactating cows.
6. To evaluate the influence of a high-protein ration in comparison to a low-protein ration in recovery from starvation.

MATERIALS AND METHODS

Research on a reliable and safe procedure to collect suitable liver samples in cattle and an evaluation of its use in research and diagnosis was initiated in the fall of 1977. The investigation was conducted at the Veterinary Clinical Center, Dairy Barn, and Building F at the Veterinary Research Farms, Michigan State University. Analytic procedures were conducted in the laboratories in Fee Hall, the Veterinary Clinical Center, and the Department of Animal Sciences. The investigation was divided into (1) research on the development of the surgical liver biopsy technique and (2) the application of the technique to research germane to 4 disease problems in cattle.

Liver Biopsy Technique

A total of 112 liver biopsies were conducted on 49 cows. The cows were estimated to range in age from 2 to 8 years. They were cows that were routinely submitted to the Veterinary Clinical Center for diagnosis and treatment, cows on specific research projects in the Dairy Herd, or cows used previously in reproduction experiments by the Department of Pathology. Each time a liver biopsy was conducted on a cow, a detailed record was maintained on all aspects of the operation. In succeeding operations, improvements in various aspects of the procedure were evaluated. The criteria used to evaluate the operation were the general clinical response of the cow, evidence of lesions such as adhesions at the time the next biopsy was done or on cows at necropsy or

slaughter, and the analysis of the liver sample collected. The surgical technique was evaluated in a preliminary study on 2 cows that were later killed because they were contaminated with polybrominated biphenyls. Records from these cows are not included in the Results. A summary of the most appropriate procedure for each aspect of the operation will be given in the Results.

Restraint

A variety of restraints were used in doing liver biopsies. At the Veterinary Research Farm and at the Dairy Barn, a squeeze chute was used. At the Veterinary Clinical Center, the stall facilities for laparotomy and general examination of cows were used in most cases. At the Veterinary Clinical Center, 2 cows were restrained using a side-line. The value of a tranquilizer (xylazine) as an aid in restraint was appraised in 10 cows.

Location

The proper anatomical location for the surgical procedure was determined by suggestions in the literature, studying the liver anatomical relations in bovine cadavers at the necropsy laboratory, and by discussions with Dr. Al Stinson in the Department of Anatomy. The right costo-abdominal area, at the 12th intercostal space, seemed to be the appropriate location in which to enter the abdominal cavity. This provided access to an area on the liver that was not close to the gallbladder or major blood vessels.

Anesthesia

The appropriate area and products for anesthesia were suggested by the report of Farquharson (1940), the methods used in similar

surgical procedures in the Veterinary Clinical Center, such as laparotomies, and by the suggestions of Drs. Ames and Coy at the Veterinary Clinical Center.

Instruments

The initial liver biopsy samples were collected by using instruments essentially as described by Whitehair et al. (1952). The cannula was 22.5 cm long and 2.5 cm in diameter with a tightly fitting trocar that had a dull, diamond-shaped point on it. The biopsy instrument was modified and improved to meet the objectives of the surgery and research throughout the study. The instrument was revised about 10 times. Factors considered in improving the instrument were sample size, stainless steel construction for durability and sterilization, balance and weight, length, a knife at the end of the cylinder to cut the distal core of the liver, and a lever to control the knife blade to hold the sample in the instrument while it was removed. One of the final instruments designed was one that had interchangeable "heads" (cylinder) so samples of different sizes could be obtained. The description of the instrument most suitable for liver biopsy is given in the Results. The value of a fiber optic adapted sigmoidoscope as a cannula and light source to observe the biopsy location rather than the trocar, cannula and flashlight was determined in the last few operations.

Surgical Procedure

The general surgical procedure was adapted from the description for surgery for laparotomy in the bovine (Farquharson, 1940). Aseptic techniques and procedures were followed. The procedure most appropriate is given in the Results.

Application of Liver Biopsy in Research

To evaluate the use of liver biopsy in cattle research, a series of 4 experiments were conducted. The experiments were (1) to determine the correlation between blood and liver values of vitamin A and the correlation of vitamin A values in different areas of the liver, (2) to determine liver stores of vitamin A after oral or intramuscular injection, (3) an experiment on the liver changes due to the influence of starvation (experimental ketosis) and the influence on the liver of refeeding with either a low or a high protein ration, and (4) the value of the technique in the diagnosis of the fat cow syndrome. The general techniques and the specific procedures for each experiment are given.

General Techniques

Vitamin A. Liver and serum vitamin A values were determined using the technique of Dennison and Kirk (1977). Two, 1 gm samples of liver tissue were weighed. One was for dry weight determination and one for vitamin A extraction. For extraction, the liver sample was minced in a homogenizer with 5 ml of distilled water. A 0.5 ml aliquot was then pipetted into a 20 x 125 mm screw-cap tube (with a teflon liner in the cap). Extraction was with 4.5 ml hexane for 5 minutes on a Vortex[a] mixer. The tubes were then centrifuged at1000 x g for 10 minutes. The suspension was filtered through a 0.45 µ millipore filter. The filtrate was analyzed for vitamin A content (retinol and palmitate) by placing 100 µl of the hexane extract in chloroform solvent at 2.5 ml/min in an

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[a]Polytron, Kinematic GM BH, Type PT 10/35, Brinkmann Instruments, Westbury, NY 11590.

isocratic system. Fluorometry of vitamin A was done using wavelengths of 330 and 470 mm for the extraction and emission. The second 1 gm sample was used to determine the dry weight by drying in an oven at 56 C for 48 hr in a weighed aluminum container. The dry weight was calculated.

For vitamin A analysis, 1 ml of 100% ethanol was added to 1 ml of serum in a 16 x 100 ml tube and mixing for 5 seconds on a Vortex mixer. Two milliliters of hexane were added and extracted for 1 minute on the Vortex shaker. The tubes were centrifuged for 10 minutes at 1000 x g. The supernatant was pipetted off and filtered through a 0.45 μ millipore filter. The vitamin A values of the liver and serum were determined by chromatography recorded as μg/gm and ng/ml, respectively.

Liver Fat Analysis. The fat content of liver samples was determined by the method of Hara and Radin (1978). A 0.5 gm liver sample was homogenized with 9 ml of a 3:2 hexane:isopropanol mixture. The suspension was filtered through a sintered glass Buchner funnel of medium porosity under pressure into a 20 ml graduated tube. The extract was then washed 3 times with 2 ml 3:2 hexane-isopropanol (H:I) by resuspending the residue each time and letting the solvent soak for 1 minute before filtering. Solvent 3:2 H:I was added to bring the extract to 10 ml, then transferred to a 20 x 150 mm tube with a teflon lined screw cap. The graduated tube was rinsed with 2 ml 3:2 H:I and added to the solution. Six milliliters of 6.7% $NaSO_4$ were added to the extract and mixed in a Vortex for 1 minute. The 2 phases were allowed to separate. The supernatant was pipetted into tared 15 x 100 mm test tubes and 2 ml 7:2 H:I was added. Then the solvent was evaporated under N_2 in a heated sand bath until the solvent could no longer be detected by smell. The residue and tared tube were weighed to determine the fat weight.

Volatile Fatty Acid Analysis. Aliquots of rumen samples were analyzed for total volatile fatty acids (acetic, propionic, butyric and valeric) by gas chromatography.

Histopathologic Techniques. Liver for microscopic examination was fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 5 microns. Sections were stained with hematoxylin-eosin. Liver tissue for glycogen identification was fixed in Carnoy's fixative, embedded in paraffin, sectioned at 5 microns, and stained with periodic acid. Frozen sections were stained with oil red O for lipid determination. The procedures were as described by Luna (1968) and Ann Preece (1972).

Statistical Analysis. The results, where appropriate in each experiment, were analyzed using the Statistical Package for Social Sciences (SPSS-Northwestern University) at the Michigan State University Computer Center. Two-way analysis of variance for F distribution, comparison of means by Student's t-test, and correlation tests were performed.

Specific Procedures for Each Experiment

Experiment 1. Correlation between liver and blood values of vitamin A and content of vitamin A in different locations in the liver. The opportunity to evaluate and perfect the liver biopsy technique was done initially in conjunction with a research project in the Department of Dairy Science. The objective of the research was to collect liver samples from cows for enzyme analysis during various stages of lactation. For this research, liver samples 4 to 9 gm in weight were required.

In previous experiments Dr. Lee Shull had difficulties in collecting adequate liver tissue. The difficulties were that the samples were too small, various surgical problems occurred, and in some instances no samples were collectable. A total of 25 cows were used in this research and a total of 62 biopsies were conducted. In 6 cows there were 4 different biopsies at approximately 2-month intervals, in 1 cow there were 3 biopsies, in 17 cows 2 biopsies, and in 1 cow 1 biopsy. During the course of this research the opportunity existed to compare liver and blood vitamin A values. A total of 10 cows were used for this study. At the time the liver sample was collected for vitamin A, a sample of blood from the jugular vein was also collected. At approximately 2 months later, another liver and blood sample were collected. The blood samples were allowed to clot and the serum removed. The liver and serum samples were wrapped in aluminum foil and stored in a freezer at -24 C until analyzed. The serum samples were analyzed for total vitamin A, retinol and vitamin A palmitate. These cattle were all fed various amounts (depending on milk production) of rations used at Michigan State University.

To determine the vitamin A content in different locations in the liver and to compare the amount with the amount at the site of biopsy, 10 livers were obtained from cows slaughtered at the Van Alstine abattoir. Six of the livers were from cows previously biopsied for vitamin A content. There was no previous history available for 4 of the cows from which the livers were obtained. In each liver, samples were collected at 6 locations with the biopsy instrument. The samples were stored and all were analyzed at the same time.

Experiment 2. The content of vitamin A in the liver and serum as influenced by method of administration. Six nonpregnant cows were used that had been used in previous research. They were all fed the same amount of ration previous to and during the experiment. The ration was timothy hay and a concentrate mixture of corn, soybean meal and a complete mineral mixture. A liver biopsy and a serum sample were initially collected from each cow. Three cows were then each given 5,000,000 I.U. of vitamin A palmitate by 1 injection into the triceps femoralis muscle. Three other cows were each given 5,000,000 I.U. of vitamin A palmitate orally by gelatin capsule. One week after the first biopsy, a second biopsy and serum sample were collected. The liver and serum samples were stored and analyzed later. These cows were all sold to the Van Alstine Company approximately 1 month after the second biopsy sample was collected. At the time they were slaughtered, the operational area was examined and the livers collected for the study described in Experiment 1.

Experiment 3. Liver changes due to a 6-day starvation and evaluation of the liver after refeeding a high or low protein ration. Eight cows were used in this study to simulate the metabolic disturbance, ketosis. The histopathologic changes in the liver were determined after 6 days of starvation and after 15 days of feeding either a low protein or a high protein ration. The initial liver biopsy was collected 15 days after calving. This time was selected as a time when the cows were in full milk production. At the time the first liver biopsy was collected, a sample of rumen fluid was also collected for volatile fatty acid content. Food was then withheld from each cow for 6 days. Water was available at all times. At the end of the 6-day starvation

period, a second liver biopsy and rumen fluid sample were collected. Four of the cows were then fed a high protein ration and 4 cows a low protein ration for 15 days. The high protein ration was 20 pounds timothy hay and 12 pounds of soybean meal daily. This ration was calculated to supply a 23% total protein. The low protein ration was 20 pounds of hay and 12 pounds of ground corn daily. This ration was calculated to supply 8% total protein. After 15 days a final liver biopsy and rumen fluid sample were collected. Records were maintained on the cows as to weight changes, milk production and clinical signs. The urine of each cow was tested at selected intervals for ketone substances. This was done by using ketosis reagent strips.[b]

Experiment 4. The value of liver biopsy for histopathologic diagnosis of fat cow syndrome. Liver biopsies were collected from 10 cows submitted to the Veterinary Clinical Center with a history and clinical signs of the fat cow syndrome. In 7 cows, 1 sample was collected. In 2 cows, 2 samples and in 1 cow, 3 samples were collected. This study was done in cooperation with Dr. Herdt, who was determining the different types of fat in the liver of these cows. Liver biopsies from 4 of these cows were examined histologically to confirm the clinical diagnosis.

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[b]Ketostix, Ames Company, Elkhart, Indiana.

RESULTS AND DISCUSSION

During the course of this research there were numerous modifications and improvements in the instruments and in the surgical procedure. These results and specific discussion will be on (1) the liver biopsy technique most suitable and appropriate in consideration of obtaining suitable liver samples, the amount of time involved and the welfare of the cow and (2) each of the disease experiments involving the application of the technique.

Liver Biopsy Technique

Restraint

The most suitable restraint for cows for liver biopsy was the standing position with a minimum amount of physical restraint. The regular cattle squeeze chute seemed most appropriate (Figure 1). This type of restraint could be considered especially suitable for field conditions. Any additional restraint such as tying the head only seemed to cause more fighting and irritation. For restraint in the Veterinary Clinical Center, the cow was maintained in a standing position between 2 parallel bars and was tied loosely with a halter. This latter restraint was also satisfactory (Figure 2). A side-line with the cow against a firm wall could be used, but it allowed considerably more movement. The use of a tranquilizer might be helpful with excitable cows, but in most instances it was not advantageous. In

Figure 1. Restraint of cow for liver biopsy using a squeeze chute under field conditions. A previous liver biopsy had been done on this cow.

Figure 2. Restraint of cow for liver biopsy in Veterinary Clinical Center facilities.

general, restraint is not a problem in performing a liver biopsy. In some cases the cows continued eating hay during the operation.

Location

The most appropriate location for liver biopsy in the mature Holstein cow was the right 12th intercostal space about 35 cm lateral and ventral to the dorsal midline. This location was adjacent to the caudal-lateral part of the right dorsal lobe of the liver. This area of the liver provided a suitable site for biopsy because it avoided major blood vessels, the kidney, gallbladder, duodenum, and other vital organs. The appropriate anatomical location is illustrated in Figure 3. The anatomical location of the major vessels, as determined from Julian and DeOme (1952), is given in Figure 4. The location of the liver and adjacent organs in a cadaver is illustrated (Figure 5).

Surgical Preparation

The hair on the operative area was clipped close to the skin. The skin was cleansed thoroughly with soap and warm water and scrubbed several times with a stiff brush. The final preparation was with sterile gauze and Betadine[C] solution.

Anesthesia

The most satisfactory procedure for anesthesia of the abdominal wall and the peritoneum was to inject about 15 ml of a 2% solution of xylocaine immediately posterior to the costovertebral junction of T_{11} and T_{12} about 7 cm from the dorsal midline. This procedure anesthetized the dorsal and ventral nerve branches as they emerged from the lateral

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[C]Povidone-Iodine, The Purdue Frederick Company, Norwalk, Connecticut 06856.

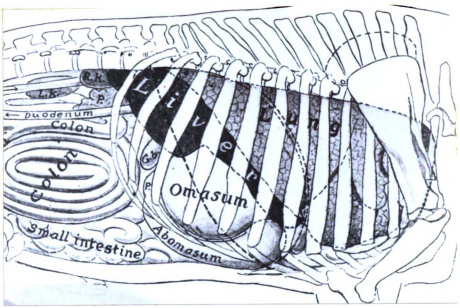

Figure 3. Anatomical location of liver. The diaphragmatic line is indicated by arrow. The (A) gallbladder, (B) kidney, (C) duodenum, and (D) dorsal lobe of liver and site for biopsy. (From Getty-Sisson and Grossman, 1975)

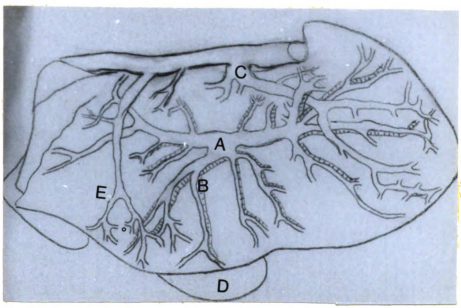

Figure 4. Schematic illustration of vasculature of liver. (A) Portal vein, (B) hepatic artery, (C) hepatic vein, (D) gallbladder. Absence of major vessels at site (E) of biopsy. (From Julian and DeOme, 1952)

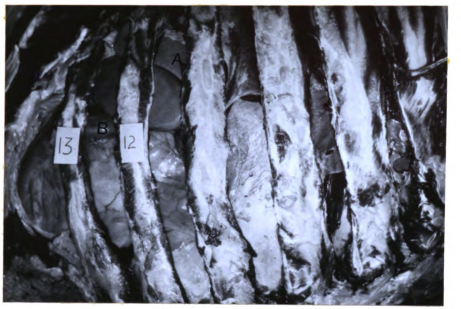

Figure 5. Photograph of liver and adjacent organs in cadaver. Dotted line indicates attachment of diaphragm, (A) caudal lobe of liver, (B) duodenum.

Figure 6. Photograph of cannula, diamond-pointed trocar and flashlight for examination of liver surface and adjacent region in peritoneal cavity.

foramina. The anesthetic solution was injected first into the skin and subcutaneous tissue with a 14 gauge needle. A smaller (16 gauge) needle was then inserted through the larger needle and the anesthetic solution deposited around the nerve trunk. The larger needle was used as a guide in locating the nerve trunk. An alternative method of anesthesia was to infiltrate 30 ml of a local anesthetic in the area of the incision. This latter technique was not as satisfactory, as the cows manifested pain when the peritoneum was penetrated. The paravertebral nerve block was not only more satisfactory for anesthesia but also seemed to relax the abdominal cavity during the surgery.

Instruments

The large (22.5 x 2.5 cm) cannula and trocar (Figure 6) as described by Whitehair et al. (1952) worked satisfactorily to allow observation of the liver and the site to be biopsied with the flashlight. The fiber optic-adapted sigmoidoscope as a cannula and a continuous source of light (Figure 7) was a major improvement in that more detail and much clearer vision of the liver and adjacent region could be seen. This instrument obviated the need for the flashlight in the cannula and thereby improved the vision.

The biopsy instrument was modified on about 10 different occasions and the instrument most suitable is illustrated (Figure 8). The "head" of the instrument is 1.5 cm in diameter and 5 cm long. This size head enables one to obtain a 4 to 9 gm sample of liver. A knife was recessed in the wall at the distal end of the cylinder. The position of the knife was controlled by a rod on a lever at the handle end of the instrument. When in the open position, the blade was recessed in the wall (Figure 9A). When in the closed position, the blade was more than

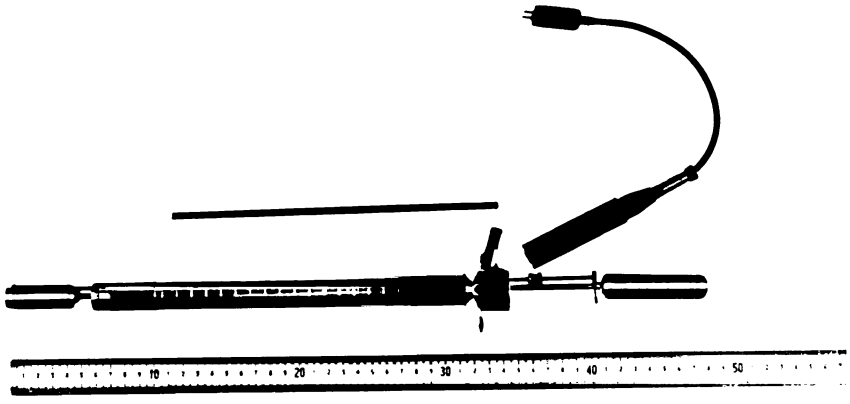

Figure 7. Fiber optic-adapted sigmoidoscope with trocar and biopsy instrument inserted. The electrical source supplies continuous light.

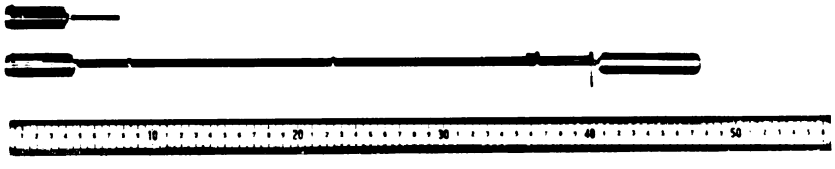

Figure 8. Liver biopsy instrument (with an additional smaller exchangeable head). The lever at the handle manipulates the cutting blade in the head.

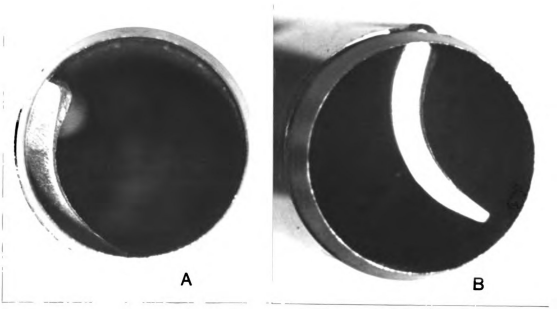

Figure 9. Illustration of the blade (A) recessed (open position) in a slot in the cylinder wall while penetrating the liver and (B) in the closed position and more than one-half across the cylinder to fully cut and hold the liver sample.

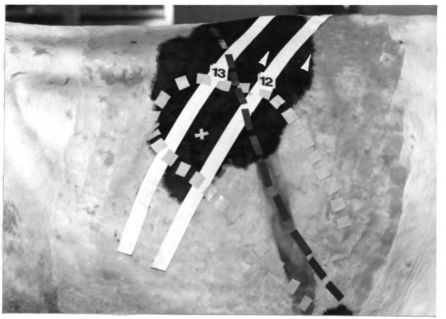

Figure 10. Locations for anesthesia (arrows), incision and insertion of trocar and cannula (X). Dark dotted line indicates line of diaphragm. Twelfth and thirteenth ribs and outline of liver (white bars).

halfway across the instrument (Figure 9B). This was essential to completely cut the center of the distal core of the liver sample and hold it in the head while the instrument was removed. The instrument was of all stainless steel construction. To meet the needs of an instrument that would collect samples of different sizes, it was adapted so as to accommodate heads of different sizes. A 1 to 2 gm sample of liver would be sufficient size for histopathologic analysis, while a 5 to 8 gm sample would be required to be sufficient tissue for chemical analysis.

Surgical Procedure

With proper anesthesia, a skin incision about 5 cm in length was made in the right 12th intercostal space posterior to the diaphragm about 35 cm lateral and ventral to the dorsal midline (Figure 10). The trocar with cannula was placed in the incision and with a sharp thrust forced slightly backward and ventrally through the costo-abdominal wall (Figure 11). After reaching the abdominal cavity, the trocar was removed and replaced by the light source. The cannula was directed forward and the dorsal ligament of the liver located. In some instances, when the liver was more anterior, the ventral surface of the liver was observed at first. The blood vessels, gallbladder and duodenum were clearly visible. From the dorsal view of the liver, the dorsal ligament, the posterolateral edge of the liver, the visceral attachment of the diaphragm and about 1/3 of the right lobe were visible. After examination of the surrounding tissue, the cannula was redirected ventrocranially to reach the biopsy area in the dorsal liver lobe in front of and about 6 cm ventral to the triangular ligament. The cannula was gently pressed against the liver while the biopsy instrument was inserted through the

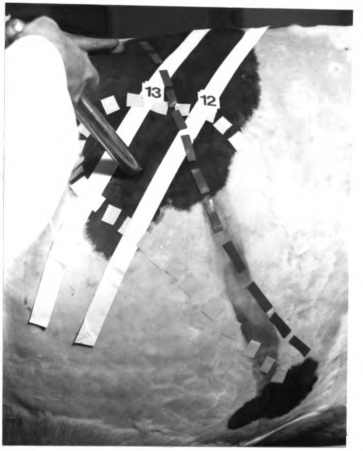

Figure 11. The trocar and cannula are inserted through abdominal cavity at first in a ventral forward direction. After penetration of the abdominal cavity, a posterior direction is followed.

cannula. The biopsy instrument was placed on the site and pressed with a clockwise twisting movement into the liver parenchyma (Figure 12). When the proper depth in the liver was penetrated, the knife lever was pressed to the closed position to cut and hold the core of liver within the instrument while it was withdrawn (Figure 13). After removing the instrument with the liver core (Figure 14), a piece of gelfoam[e] was placed where the sample was removed to control hemorrhage. The peritoneum and muscular layers were sutured with simple chromic catgut. The skin was sutured with Vetafil[d] in an interlocking pattern. Skin sutures were removed after 8 to 10 days, and the cows were given no further attention until the next biopsy. The total number of biopsies, number of biopsies in individual cows, and summary of complications are given in Table 1. One cow in the dairy herd died 3 hours after the biopsy. This cow had a previous biopsy done by personnel in the Department of Dairy Science. This cow died because a large branch of the portal vein was severed when a second biopsy was taken during this operation. In 4 cows there were adhesions from previous biopsies. In 2 of these cows there was 1 previous biopsy and in 2 cows there were 2. In 3 cases the hemorrhage was due to injury of the liver when the trocar was inserted. In the other, the hemorrhage was due to cutting large vessels, and this was due in part to a more dorsal approach (closer to the midline). Subcutaneous emphysema around the incised area was observed in 24 hours in 6 cows. In 1 of the 6, emphysema spread up to the scapula. This was caused by just suturing the skin. After suturing

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[d]Suprylon Pfrimmer, Haver-Lockhart Dist. Bayvet Div., Cutter Lab, Shawnee, Kansas 66201.

[e]Upjohn Company, Kalamazoo, Michigan 49001.

Figure 12. The biopsy instrument is inserted into liver parenchyma with small amount of pressure and clockwise twisting movement.

Figure 13. After penetration into parenchyma at proper depth, lever placed in closed position and instrument turned several times.

Figure 14. Removal of biopsy instrument with core of liver. This biopsy weighed 8 gm.

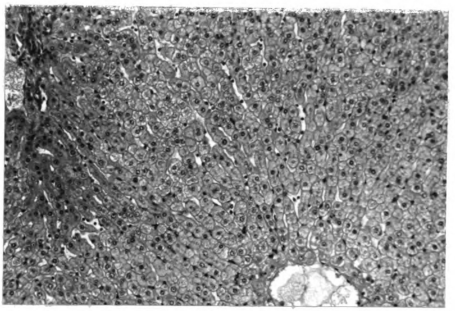


Figure 15. Photomicrograph of section of liver sample in Figure 14. Liver cords for the most part are 1 or more cells thick. They appear to radiate from the centrolobular area. H&E stain; X160.

Table 1. Summary of total number of biopsies, number of biopsies in individual cows and complications

No. of cows	Number of Biopsies				Complications					Total
	1	2	3	4	A	B	C	D	E	
7	X						2		2	7
27	X	X			1	2	3		2	54
9	X	X	X		1*		1	2	1	27
6	X	X	X	X		2				24
49	49	42	15	6	2	4	6	4	5	112

A = death
B = adhesion
C = subcutaneous emphysema
D = incision infection
E = peritoneal detachment
* = euthanatized for necropsy

as indicated this did not occur. After the technique was perfected, there were no further complications.

Histologic Features of Liver Specimens Obtained During Surgery

The liver samples collected (at locations other than previous biopsy) while developing the surgical technique, especially in Experiments 1 and 2, were essentially normal (Figure 15). This applied to samples taken from livers that had been biopsied as many as 4 times. Microscopically, each hepatic triad was relatively sharply delineated by the limiting plate. The triad consisted of connective tissue which contained at least an artery, a portal vein branch, and bile ducts in addition to lymphatics and nerves. Interlobular bile ducts appeared to be lined by cuboidal or low columnar epithelium, while septal bile ducts were lined by tall columnar cells with basal nuclei. Those bile ducts usually occupied the central area of the hepatic triad. The smallest efferent veins, the centrolobular veins, were at the center of the conventional lobule.

The hepatic parenchymal cells formed interconnecting walls or plates separated from each other by the sinusoidal system. The sinusoids were lined by an incomplete layer of flattened endothelial cells together with the stellate endothelial cells. The main hepatic phagocytes appeared to be normally distributed throughout the sections. The hepatic cells, arranged in plates, were normally one cell thick. The cells were usually polygonal in shape and with well defined borders and had one or more rounded nuclei containing nucleoli. The cells surrounding the triad appeared to be slightly smaller and more deeply stained than the other hepatic cells.

In 3 cows, microscopic examination of sections of liver where previous biopsies were taken had a mixed population of mono- and polymorphonuclear cells and increased amounts of fibrous connective tissue (Figures 16 and 17). The fibroblasts appeared to be active. Thrombi were observed at the margin of the previous biopsy. Bile duct proliferation was present. The smallest proliferating ducts had cuboidal epithelium, while the larger ducts had cuboidal or columnar epithelium. Bile duct proliferation was not present in first biopsies.

Use of Liver Biopsy in Experiments on Metabolic Diseases

Experiment 1. Correlation Between Liver and Blood Values of Vitamin A and Content of Vitamin A in Different Locations in the Liver

Liver and serum values of vitamin A in individual cows and at 2 months later are summarized (Table 2). There was no consistent relationship between liver and serum vitamin A values ($p<0.10$) when the samples were collected at the same time. The value of liver vitamin A in the second biopsy sample was correlated with the value of vitamin A in the first liver sample ($p>0.10$). There was considerable variation between individual cows as to both liver and serum vitamin A values. This information in general is in agreement with previous literature reports (Glover et al., 1947; Hoefer and Gallup, 1947; Van Arsdell et al., 1951; Baker et al., 1953). Liver vitamin A values for cow 32 (2nd biopsy) would be considered low for cows fed these rations, as Whitehair (1980) considered liver values below 5 µg/gm to be indicative of a deficiency.

The liver vitamin A content at 6 different locations in the liver was compared with the content at the location where the biopsy was

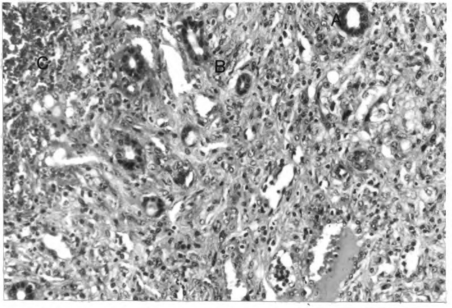


Figure 16. Photomicrograph of section of liver where biopsy was taken 4 days previously. There are (A) bile duct proliferation, (B) fibroplasia, and (C) congestion appears to be present. Sample collected at postmortem. H&E stain; X160.

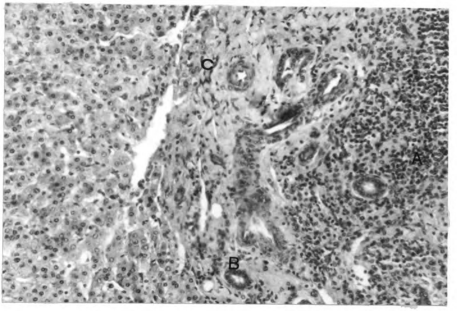


Figure 17. Photomicrograph of section of liver taken by biopsy from area of biopsy 30 days previously. Note (A) inflammatory cell infiltration, (B) bile duct proliferation, and (C) connective tissue infiltration. The liver cords appear to radiate from the centrolobular vein. Punch biopsy. PAS stain; X160.

Table 2. Comparison of liver and serum vitamin A values (as retinol or palmitate) in 10 individual cows and at approximately 2-month intervals

Cow No.	First Biopsy				Second Biopsy			
	Liver (µg/gm) dry wt		Serum (ng/ml)		Liver (µg/gm)		Serum (ng/ml)	
	Palm.	Ret.	Palm.	Ret.	Palm.	Ret.	Palm.	Ret.
86	11	1	84	137	9	11	54	125
32	44	2	70	42	6	1	42	78
77	104	1	108	75	103	1	114	169
59	85	2	164	268	44	3	51	112
38	124	4	59	119	61	5	89	195
53	242	1	96	219	87	1	62	77
65	52	1	84	147	37	1	115	229
82	78	1	60	129	21	2	165	222
93	90	1	99	107	45	1	187	222
69	38	1	72	95	28	1	41	121
Mean ± SE	87 ±20	1.5 ±0.31	90 ±9.7	144 ±21	44 ±10	2.7 ±1.0	92 ±16.5	155 ±18

Palm. = palmitate form of vitamin A. Ret. = retinol form of vitamin A.

collected (Figure 18). The results are summarized in Table 3. There was a high correlation (.939) between the liver content of vitamin A where the biopsy was collected and the content at other locations. This suggests that a single biopsy would be representative of the entire liver.

Experiment 2. The Content of Vitamin A in the Liver and Serum as Influenced by Method of Administration

The influence of injecting 5 million I.U. of vitamin A palmitate in comparison to orally giving the same amount on the liver and serum vitamin A is summarized (Table 4). The number of cows used in this experiment was considered too small for meaningful analysis of the data. In reviewing the data, it would appear that the intramuscular injection of vitamin A gave slightly higher liver and serum values for vitamin A than the same amount given orally. The injection of vitamin A increased the mean total vitamin A in the liver 47%, while the same amount given orally increased the liver content only 30%. While the data are limited, the mean serum values were slightly increased due to the injections and slightly decreased when the same amount was given orally. This information on the method of administration agrees with previous work at Michigan StateUniversity (Martin et al., 1971). In the report of Martin and associates, using the same number of cows, the intraruminal injections of 7 million I.U. of vitamin A resulted in liver storage of about 40% of the same amount given intramuscularly. In Experiment 2, the oral administration resulted in liver storage of 45% of the amount given by injection. In previous research at Kentucky (Mitchell et al., 1967), it was determined that about 50% of orally administered vitamin A underwent destruction in the rumen of steers.

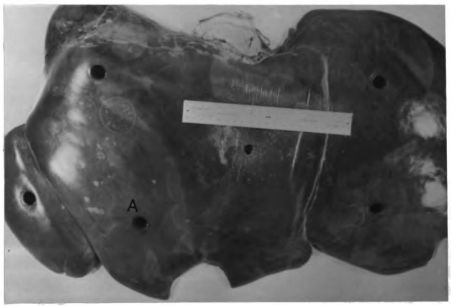


Figure 18. Liver locations where samples were taken for vitamin A analysis. (A) is the site where biopsy is usually taken.

Table 3. Vitamin A content of 10 livers at 6 different locations (µg/gm dry weight)

Liver No.	Location on the Liver: Biopsy Site 1	Other Sites 2	3	4	5	6	Mean ± SE
1	343	170	218	309	217	154	235 30.8
2	160	107	162	218	125	262	172 23.7
3	503	932	235	526	239	450	564 98.7
4	1561	1042	687	385	919	848	907 160.0
5	1098	424	397	546	592	468	587 106.0
6	724	382	446	256	687	531	504 73.5
7	373	330	289	143	173	116	237 47.7
8	247	133	245	208	186	116	189 22.6
9	113	117	196	170	204	193	166 16.6
10	196	99	168	105	234	183	148 23.0
Mean	522	374	304	287	408	332	371
SE of mean ±	151	109	52	49	93	75	81

Table 4. Comparison of oral vs intramuscular injection of 5 million I.U. of vitamin A on liver and serum values 1 week later

	Cow No.	At First Biopsy						At Second Biopsy					
		Liver (µg/gm) dry wt			Serum (ng/ml)			Liver (µg/gm) dry wt			Serum (ng/ml)		
		Palm.	Ret.	Total	Palm.	Ret.	Total	Palm.	Ret.	Total	Palm.	Ret.	Total
IM	14	159	2	161	76	192	268	124	5	129	91	199	290
	8	227	9	236	87	143	230	284	6	290	85	159	244
	16	210	9	219	91	193	284	463	23	486	90	185	275
Mean		199	7	205	85	176	261	290	11	302	89	181	270
Oral	4	217	9	226	78	166	254	162	3	165	99	145	244
	34	89	3	92	69	152	221	154	5	159	70	131	201
	15	212	5	217	85	193	278	359	9	368	69	175	264
Mean		173	6	178	77	170	251	225	6	231	79	150	236

Palm. = palmitate form of vitamin A. Ret. = retinol form of vitamin A.

This research, as well as previous studies at Michigan State University (Martin et al., 1971), would suggest that the intramuscular injection of vitamin A would be an effective way of assuring adequate vitamin A in cattle nutrition.

Experiment 3. Liver Changes Due to a 6-Day Starvation and Evaluation of the Liver after Refeeding a High or Low Protein Ration

The liver fat values before and after 6 days of starvation and after 15 days of refeeding a high or low protein ration are summarized (Table 5). The 6 days of starvation caused a significant ($p<0.01$) increase in liver fat content in comparison to initial values. After 15 days of refeeding a high or low protein ration, liver fat content was reduced to close to the initial values. The low protein ration reduced liver fat content more than the high protein ration. The difference was not statistically significant ($p>0.10$). Cow No. 1 became very weak due to starvation and had to be euthanatized 2 days after the starvation period ended. The value of liver fat content is not included in the refeeding.

Liver samples examined histologically correlated closely with the chemical analysis of fat in the liver. Initially the liver was morphologically normal (Figure 15). However, there was more evidence of cellular vacuolation in the liver sections examined initially in this experiment than in tissue from a nonlactating cow that was considered normal (Figure 15). This was attributed to the influence of milk production at this time. At the end of 6 days of starvation, the amount of vacuolation was increased and glycogen was depleted (Figures 19 and 20). Staining sections with oil red O showed the vacuolation was due to fat (Figure 21). After refeeding, glycogen was increased (Figure 22)

Table 5. Liver fat content of biopsy samples initially, after 6 days of starvation, and 15 days after refeeding either a high or low protein ration

Cow No.	Percent Fat Content of Biopsy Sample		
	Initial Sample	After 6 Days of Starvation	After 15-Day Refeeding
			High Protein Ration
1	6.26	16.79	died
3	9.96	19.31	15.30
10	6.73	14.26	6.49
17	6.69	14.68	3.81
			Mean 8.53±3.47
			Low Protein Ration
7	5.35	13.90	5.72
9	5.99	9.81	5.31
11	4.39	11.11	4.07
12	10.36	17.54	5.85
			Mean 5.23±0.41
Mean fat	6.97±0.75	14.68±1.13	

± = standard error of mean

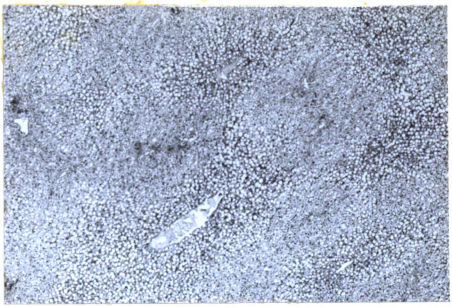


Figure 19. Photomicrograph of liver biopsy section from cow after 6-day starvation. Diffuse vacuolation of hepatic cells. There is also depletion of glycogen. PAS stain; X64.

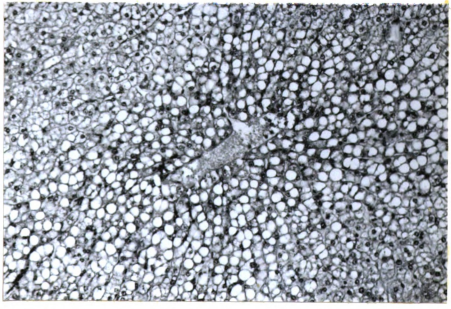


Figure 20. High power magnification of Figure 19. PAS stain; X160.

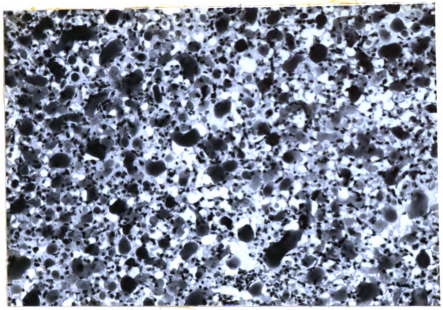


Figure 21. Photomicrograph of liver biopsy section from cow after 6-day starvation stained with oil red O. Oil red O stain; X160.

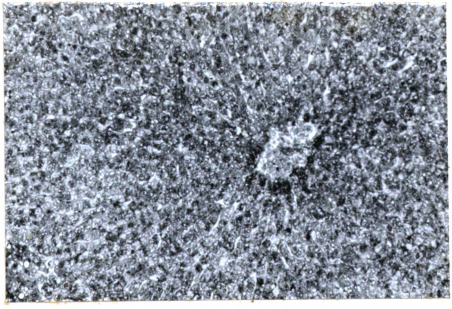


Figure 22. Liver. Presence of glycogen after refeeding. Punch biopsy. PAS stain; X160.

and the amount of vacuolation was reduced (Figures 23 and 24). In 1 cow (No. 3), peritonitis developed and the liver vacuolation (and liver fat content) remained about the same as at the end of the starvation period (Figure 25). This cow had a rather poor appetite after the starvation period.

Weight changes during starvation and refeeding a low or high protein ration are summarized (Table 6). The 6-day starvation resulted in mean average weight loss of 204 pounds, or 18% of their initial weight. This weight change, in comparison to the initial weight, was highly significant ($p<0.01$). On refeeding, the cows fed the high protein ration gained an average of 83 pounds during the 15 days, or 8.5% over the starvation weight, while the cows refed the low protein ration gained an average of 39 pounds, or 4%. This difference was not statistically significant ($p>0.10$).

The mean daily milk production for 3 cows in each group is summarized (Figure 26). During the 6-day starvation period there was a decline in milk production in both groups. Milk production was increased slightly during the refeeding and was higher in the cows fed the high protein ration.

The rumen content of total volatile fatty acids for 3 of 4 cows decreased during starvation (Table 7). The volatile fatty acid values were increased 24 hours after refeeding and, with one exception (No. 7), at the end of the refeeding period. These values are qualitative in that the amount of water consumed prior to collection of the samples could not be accurately controlled. Thus, there was a dilution effect.

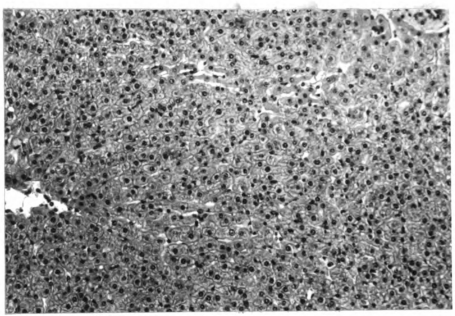


Figure 23. Photomicrograph of liver biopsy section of cow after refeeding high protein ration for 15 days. Amount of vacuolation reduced from amount present at end of 6-day starvation. H&E stain; X160.

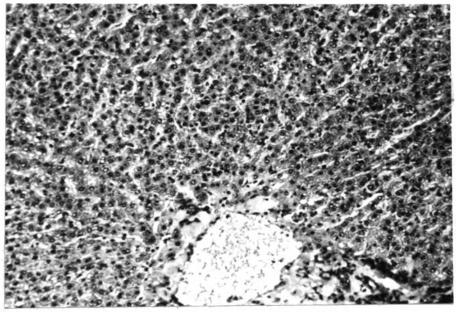


Figure 24. Photomicrograph of liver biopsy section of cow after refeeding a low protein ration for 15 days. Amount of vacuolation reduced from amount present at the end of 6-day starvation. H&E stain; X160.

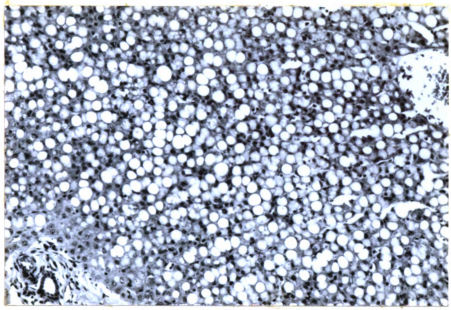
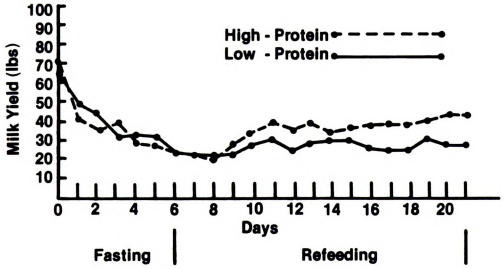


Figure 25. Photomicrograph of liver biopsy section from cow No. 3 that had a peritonitis. Most of the hepatocytic cytoplasm is replaced by fat globules. H&E stain; X160.



Daily milk production during fasting and refeeding periods (3 cows, each group)

Figure 26. Daily milk production during 6-day starvation period and 15-day refeeding either a high protein or low protein ration.

Table 6. Weight changes due to starvation and refeeding either a high or low protein ration for 15 days

Cow No.	Cow Weights (lbs) Initial	After 6 Days of Starvation	After 15-Day Refeeding
			High Protein Ration
1	1050	876	---
3	1228	1052	1128
10	980	838	960
17	1180	1020	1070
			Mean gain 83±21.07
			Low Protein Ration
7	1200	1020	1028
9	1060	770	870
11	1418	1128	1165
12	1150	928	938
			Mean gain 39±21.5
Mean	1158.3±47.73*	954.0±42.78	

*± = standard error of mean

Table 7. Total volatile fatty acids* in aliquots of rumen fluid initially, after 6 days of starvation, and at 24 hours and 15 days of refeeding a high or low protein ration

Cow No.	Total Fatty Acid Values			
	Initial Value	Value after 6 Days of Starv.	Refeeding 24 hr	Refeeding 15 da
			High Protein Ration	
17	534	230	610	534
10	411	301	506	550
			Low Protein Ration	
7	243	379	386	221
9	526	236	436	544

*Qualitative comparative values - gas chromatography values

Experiment 4. The Value of Liver Biopsy for Histopathologic Diagnosis of Fat Cow Syndrome

In cows submitted to the Veterinary Clinical Center, the liver biopsy technique was of assistance in confirming a diagnosis of the fat cow syndrome. Sections of liver from these cows had a great increase in vacuolated liver cells (Figures 27 and 28). Almost every liver cell was affected. Some inflammatory cells were also present. In some sections the fat droplets seemed to coalesce to form a large fatty cyst. In these cows, as well as in the cows after 6 days of starvation, on visual examination of the liver through the cannula, the liver appeared enlarged with rounded borders and had a yellowish color. In addition, it cut much easier with the biopsy instrument. In cows with the fat cow syndrome, there was also less bleeding at the site of the biopsy.

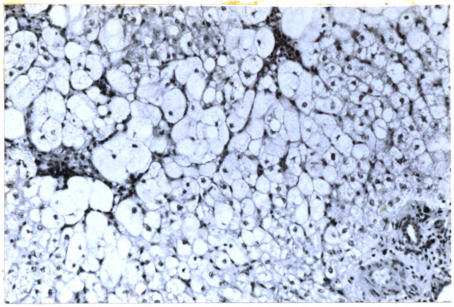


Figure 27. Photomicrograph of biopsy liver section from cow with history and clinical signs of fat cow syndrome. Most of the cell cytoplasm is replaced by fat vacuoles. Many vacuoles coalesced in a cystic-like formation. Mononuclear cells are present. H&E stain; X160.

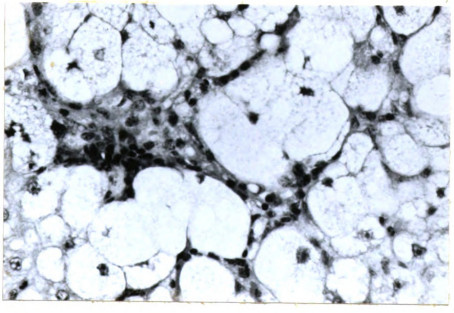


Figure 28. High power of Figure 27. H&E stain; X400.

GENERAL DISCUSSION

The general objectives of this research were accomplished. Information was obtained that was new and its use could improve diagnosis and research in cattle. The research also provided the author valuable training and experience in bovine surgery and medicine, pathology and experimental procedures. The liver biopsy technique in cattle can be an extremely informative and useful procedure in confirming the presence of liver disease and in many cases for determining its etiology. The technique also has many applications in infectious and noninfectious disease research in cattle. It is a safe and relatively simple technique, if surgical procedures and proper precautions are followed. It is a technique that can be easily performed under conditions of general cattle practice. This esteem for the value of a liver biopsy technique in cattle agrees with veterinary clinicians at Cornell and Oregon (Pearson and Craig, 1980).

The main contributions of this research were that (1) an instrument was devised that could regularly obtain a reliable sample of liver from a desired location in the liver, (2) improvements were made in anatomical and surgical procedures that avoided complications described by previous authors, and (3) the technique could be used in collecting a consecutive series of liver samples from the same animal in experimental work.

The technique has many advantages of the previously used "needle" biopsy technique. It can be assumed that as the technique is used more routinely in clinical and research activities there will be additional modifications and improvements. It is anticipated and desired that the opportunity will be available in Brazil to continue this research.

The Liver Biopsy Technique

A minimum amount of restraint was required to perform liver biopsy in cows. The facilities used routinely for handling cattle for vaccinations, minor surgery or clinical examination would be sufficient restraint. The use of tranquilizers for the operation does not seem justified unless the cattle are extremely nervous and excited.

The proper anatomical location for liver biopsy in cattle is very important. The right costo-abdominal cavity is posterior to the diaphragmatic attachment and thus avoids complications from penetrating the pleural cavity. This location is somewhat more ventral than that suggested by previous workers (Whitehair et al., 1952). The more ventral approach avoids the right kidney, vena cava and duodenum. There is also less subcutaneous tissue and musculature in the abdominal wall at the more ventral location. In preliminary trials (not counted in the total), the kidney was accidentally biopsied twice when using the more dorsal approach. The more ventral location still provides access to the proper location on the liver. This location is not too close to the gallbladder and major blood vessels in the liver. Additional research on the vasculature of the liver would be very worthwhile. This would provide more detailed information as to areas to avoid to prevent hemorrhage. Early in this research an attempt was made to determine the distribution of the major blood vessels in the liver.

However, the resin (methyl acrylate) was not satisfactory for the study.

The paravertebral nerve anesthesia was very satisfactory. It supplied complete anesthesia for the surgery, was effective in a few minutes, and was free of any complications. There was no evidence of pain manifested in any of the cows when the liver was penetrated for biopsy. While some previous investigators (Erwin et al., 1956; Holtenius, 1961; Narasimhamurty, 1966; Ivascu et al., 1968) considered that anesthesia was not necessary, for humane reasons and better surgery, anesthesia seems proper. It is simple and can be done during routine surgical preparation.

The instruments as devised, while satisfactory for collecting liver biopsies in cattle, might still be modified and improved by further work. The modifications would be based on the size of liver sample desired, type of research, and age and status of the animal (calf vs pregnant cow).

The cannula (as used with the trocar) could be approximately 10 cm longer. This would provide for holding it more firmly while examining the abdominal cavity and at the site on the liver to be biopsied. In pregnant cows the liver seemed to be located more forward and was difficult at times to reach with the cannula as described. The end of the trocar should be rather blunt than sharp so as not to injure the liver (or other tissues) when it is thrust through the abdominal wall. This blunt end would make it somewhat more difficult to penetrate the abdominal wall; however, later injury would be less likely.

The fiber optic-adapted sigmoidoscope was a definite advantage in viewing the liver and other tissues in the abdominal cavity. The light source and the elimination of the flashlight provided for a much clearer

view of internal structures. This instrument would be improved if it had a diameter of 2 or 2.5 cm (same as cannula for trocar) rather than 1.9 cm. The larger diameter would improve vision and more easily accommodate the biopsy instrument. The fiber optic-adapted sigmoidoscope would seem to have use in general examination of abdominal tissues in addition to its use in liver biopsy. This instrument seems rather expensive ($300).

A significant advantage of the improved biopsy instrument was the mechanism for severing the distal core of liver. Practically all previous workers (Whitehair et al., 1952; Udall et al., 1952; Seghetti and Marsh, 1953; Bone, 1954) mentioned the difficulty when using the needle biopsy (and aspiration) in getting good liver samples. It was essential that the knife blade goes more than halfway so as to cut the center of the core. Otherwise, as the biopsy instrument is twisted, this center section is not cut. With the instrument as described, samples were collected on every occasion. The size of the cylinder "head" was arrived at by compromise in that if it were longer it would penetrate too deeply into the liver and be more likely to sever large vessels. The larger diameter, necessary to collect sufficient tissue, was less injurious than a longer one. Previous workers (Udall et al., 1952; Hughes, 1962) mentioned fatalities when major vessels were severed. Udall and co-workers penetrated the entire liver with a biopsy needle and injured the thoracic duct. At the location suggested, the liver is approximately 7 cm thick. It would seem most logical to have an instrument with 2 sizes of heads, one to collect biopsy samples for histopathologic investigation (2 or 3 gm) and one for chemical or biochemical analysis (5 to 8 gm). The described instrument has a slit in the wall to accommodate the rod for the blade. This also allows air

to escape from the cylinder head as it penetrates the liver. The stainless steel structure provides durability, allows sterilization, and maintains a cutting edge. The lever for manipulating the knife fits in a ratchet that maintains the knife either in the "open" or "closed" position. This greatly facilitates the surgery and helps in removing the core of liver.

Inserting the trocar and cannula into the abdomen in a posteroventral direction is a precaution to avoid injury to the liver. In 5 cows the liver was lacerated by the trocar and cannula. Adhesions were present at this site at succeeding biopsies. This was avoided in subsequent biopsies by the initial posteroventral thrust. The most dependable way to locate the area for biopsy on the liver was to locate the dorsal ligament of liver and insert the instrument in front and 6 cm ventrally. The insertion of gelfoam where the core of liver was removed seemed helpful to control hemorrhage. The amount of hemorrhage from the liver varied considerably and in many cows the hemorrhage was minor. In 5 cows an attempt was made to collect 2 samples during one operation. In collecting a second sample, the hemorrhage from the first biopsy made locating the site for a second one difficult. Collecting 1 larger sample would be better than 2 small samples.

Application of Liver Biopsy in Research

The liver biopsy technique for collecting larger (5 to 8 gm) samples of liver would have a special value in research. The needle biopsy technique (usually less than 1 gm) only provides enough tissue for diagnostic work. Larger samples are required for chemical analysis in most research. The technique described in this dissertation of examining

the liver site to be biopsied avoided the complications mentioned by previous authors who used the needle biopsy procedure.

In Experiment 1, the liver biopsy technique established the variability between liver and serum content of vitamin A in individual cows. It also established that there was considerable variation between cows, even though they were fed the same type of ration. These results agreed in general with previous reports (Hoefer and Gallup, 1947; Glover et al., 1947; Frey and Jensen, 1947; Van Arsdell et al., 1951; Baker et al., 1953). The liver values for vitamin A are suggested as a better indication of the vitamin A status of cows than serum values. If this would be true of other nutrients as well, then the technique would have use in establishing the nutritional status for a variety of nutrients. Serum values of nutrients such as vitamin A are perhaps more of a reflection of dietary intake. One biopsy seems to supply a reliable indication of the content of a nutrient such as vitamin A in the entire liver.

In Experiment 2, the liver values supplied information that vitamin A could be given by intramuscular injection as well as orally. This information agrees with previous work at Michigan State University (Martin et al., 1971).

The starvation experiment (Experiment 3) provided information on the value of the technique to conduct research on the pathogenesis of ketosis. This research provided some evidence that the quality and quantity of the ration at parturition have an important role in initiating ketosis. Liver changes resulting from starvation in cows was indicated by both chemical (content of fat) and morphologic changes. Weight gains suggested high protein rations were more conducive for recovery from starvation than low protein rations. This research was

conducted mainly with the role of energy in ketosis. The role of additional factors, such as protein, vitamins and minerals, needs further investigation. During the past 2 decades much information has been obtained on reactions in the rumen by surgical procedures such as fistulas (Dougherty et al., 1965). Liver biopsy would allow an enormous amount of research on reaction in the liver. Combining the 2 techniques would provide important information in cattle.

Experiment 4 illustrated the value of the liver biopsy technique in confirming a diagnosis of the fat cow syndrome. The fat cow syndrome is difficult to diagnose at times. The technique could be expanded to evaluate various therapeutic measures. Many sick cows are submitted to clinics daily in which a definitive diagnosis is not available. These cows are treated symptomatically. The liver biopsy technique would have an advantage not only in diagnosis but also in evaluation of proper therapy.

SUMMARY

Research was conducted to improve a surgical technique for obtaining liver biopsies in cattle. The research involved devising appropriate instrumentation, a safe surgical technique and an evaluation of the technique in a series of 4 experiments in cattle. The technique was developed by obtaining 112 liver biopsies from 49 cows. Single biopsies to as many as 4 consecutive biopsies were obtained from cows.

The appropriate anatomical location for penetration of the abdominal wall for liver biopsy in mature Holstein cows was the right 12th intercostal space, 35 cm ventral to the dorsal midline. Injection of an anesthetic close to the paravertebral nerve trunks provided anesthesia of the surgical area. The liver area to be biopsied was seen by using a flashlight inserted through a large cannula or by using a fiber optic-adapted sigmoidoscope that was inserted through the abdominal wall. The appropriate site in the liver for biopsy was the area bout 6 cm ventrally and anterior to the dorsal ligament of the liver. Samples of liver weighing 4 to 9 gm were obtained by using a specially devised biopsy instrument. The instrument was made of stainless steel material. The biopsy part was a cylinder to penetrate the liver and at the distal end a knife blade was fixed so as to sever the core of liver.

In experimental work, liver biopsies were of value in indicating that there was poor correlation between vitamin A in the liver and the

serum. It was established that 1 biopsy sample was representative of the entire liver. By liver biopsy it was determined that intramuscular injection of vitamin A provided liver stores equivalent to the stores from the same amount given orally. The technique was of value in establishing morphologic and chemical changes in the liver during starvation. Liver biopsy was of value in confirming the clinical diagnosis of fat cow syndrome.

The technique was safe and allowed collection of liver samples suitable for a wide variety of diagnostic and research work in cattle. This would have many applications in improving cattle production.

REFERENCES

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Anderson, T. A., Taylor, R. E., Diven, R. H., Hubbert, F., Jr., and Hale, W. H.: Reliability of the liver biopsy technique for estimating hepatic vitamin A. J. Anim. Sci., 21, (1962): 369-372.

Ann Preece, H. T.: *A Manual for Histologic Technicians*, 3rd Ed., Little, Brown and Company, Boston, (1972): 428p.

Aterman, K.: The dark and the light cells of the liver. Anat. Rec., 136, (1960): 157.

Baird, G. D., Heitzman, R. J., and Hibbitt, K. G.: Effects of starvation on intermediary metabolism in the lactating cow. A comparison with metabolic changes occurring during bovine ketosis. Biochem. J., 128, (1972): 1311-1318.

Baker, F. H., MacVicar, R., Pope, L. S., and Whitehair, C. K.: Placental and mammary transfer of vitamin A and carotene by beef cows. Proc. Soc. Exp. Biol. Med., 83, (1953): 571-574.

Betty, R. W., and Markson, L. M.: Liver biopsy in the diagnosis of ragwort (*Senecio jacobea*) poisoning in a herd of cattle. Vet. Rec., 66(28), (1954): 398-400.

Bone, J.F.: A technic for aspiration liver biopsy in dairy cattle. North Am. Vet., 35, (1954): 747-752.

Bracken, F. K. (ed.): *Bovine Medicine and Surgery Diseases Affecting Liver Function*, 2nd Ed., Vol. II. H. E. Amstutz-Am. Vet. Publ. Inc., Santa Barbara, California, (1980): 683-696.

Brumby, P. E., Anderson, M., Tuckley, B., Storry, J. E., and Hibbitt, K. G.: Lipid metabolism in the cow during starvation-induced ketosis. Biochem. J., 146, (1975): 609-615.

Cakala, S.: A technic for the paravertebral lumbar block in cattle. Cornell Vet., 51, (1961): 64-67.

Chapman, Jr., H. L., Cox, D. H., Haines, C. E., and Davis, G. K.: Evaluation of the liver biopsy technique for mineral nutrition studies with beef cattle. J. Anim. Sci., 22, (1963): 733-737.

Dennison, D. B., and Kirk, J. R.: Quantitative analysis of vitamin A in cereal products by high speed liquid chromatography. J. Food Sci., 42(5), (1977): 1376-1379.

Dick, A. T.: Aspiration biopsy of the liver in sheep. Aust. Vet. J., 20(5), (1944): 298-303.

Dick, A. T.: Improved apparatus for aspiration biopsy of the liver in sheep. Aust. Vet. J., 28(9), (1952): 234-235.

Diven, R. H., Pahnish, O. F., Roubicek, C. B., Erwin, E. S., and Page, H. M.: Vitamin A and carotenoid inter-relationship in bovine plasma and liver. J. Dairy Sci., 43, (1960): 1632-1638.

Dougherty, R. W., Allen, R. S., Wise, B., Jacobson, N. L., and McGilliard, A. D.: *Physiology of Digestion in the Ruminant,* 1st Ed. Butterworths Inc., Washington, (1965): 408p.

Elias, H.: The liver cord concept after one hundred years. Science, 110(4), (1949): 470-472.

Elias, H.: Dependence of the lobular architecture of the liver on the porto-hepatic blood pressure gradient. Anat. Rec., 115, (1953): 71-85.

Elias, H., and Sherrick, J. C.: *Morphology of the Liver.* Academic Press, New York, (1969): 390.

Erwin, E. S., Dyer, I. A., Meyer, T. O., and Scott, K. W.: Uses of aspiration biopsy technique. J. Anim. Sci., 15, (1956): 428-435.

Farquharson, J.: Surgery and obstetrics. Paravertebral lumbar anesthesia in bovine species. J. Am. Vet. Med. Assoc., 97, (1940): 54-57.

Feldman, E. C., and Ettinger, S. J.: Percutaneous transthoracic liver biopsy in the dog. J. Am. Vet. Med. Assoc., 169(8), (1976): 805-810.

Finger, M. G., Gibbons, W. J., Mayer, K., and Park, S. E.: Liver biopsy. In *Diseases of Cattle,* 1st Ed. Am. Vet. Publ. Inc., Evanston, CA, (1956): 28-29.

Frey, P. R., and Jensen, R.: Depletion of vitamin A reserves in the livers of cattle. Science, 105, (1947): 313.

Garner, R. J.: Aspiration biopsy of the liver in cattle. Vet. Rec., 62(48), (1950): 729-730.

Getty, R., Sisson, S., and Grossman, J. D.: *Anatomy of Domestic Animals,* 5th Ed. W. B. Saunders Co., Philadelphia, (1975): 1211.

Gibbons, W. J.: Disease of the Liver. In *Bovine Medicine and Surgery,* 1st Ed. Am. Vet. Publ. Inc., (1970): 442-450.

Glover, J., Goodwin, T. W., and Morton, R. A.: Studies in vitamin A. 2. The relationship between blood vitamin A levels and liver stores in rats. Biochem. J., 41, (1947): 97-100.

Hale, W. H., Hubbert, Jr., F., Taylor, R. E., Anderson, T. A., and Taylor, B.: Performance and tissue vitamin A levels in steers fed high levels of vitamin A. Am. J. Vet. Res., 23(96), (1962): 992-996.

Hall, L. W.: *Wright's Veterinary Anaesthesia and Analgesia,* 7th Ed. Bailliere and Tindall, (1971): 83-101.

Hansen, P.: Feeding practices may cause vitamin A deficiencies. Feedstuffs, Dec. 8, (1980): 18-20.

Hara, A., and Radin, N.S.: Lipid extraction of tissues with a low-toxicity solvent. Analyt. Biochem., 90, (1978): 420-426.

Hibbitt, K. G., and Baird, G. D.: An induced ketosis and its role in the study of primary spontaneous bovine acetonemia. Vet. Red., 81(20), (1967): 511-517.

Hoefer, J. A., and Gallup, W. D.: The comparative value of a carotene concentrate, alfalfa meal, and a fish liver oil in maintaining the vitamin A content of the blood and liver of fattening lambs. J. Anim. Sci., 6, (1947): 325-333.

Hoflund, S., and Hedstrom, H.: Disturbances in rumen digestion as a predisposing factor in the appearance of acetonemia. Cornell Vet., 38, (1948): 405-417.

Hogan, K. G., Money, D. F. L., and Walker, R. S.: The distribution of copper in the liver of pigs and sheep and its effect on the value of chemical analyses made on biopsy samples. New Zealand J. Agric. Res., 14, (1971): 132-141.

Hojovcova, M., and Kacafirek, A.: Liver biopsy in cattle. Acta Univ. Agr. Fac. Vet. Brno., 36, (1967): 513-520.

Holtenius, P.: Cytological puncture. A new method for the study of bovine hepatic disease. Cornell Vet., 51(1), (1961): 57, 63.

Honory, K.: Distribution of vitamin A and carotenoids in the liver of cattle in different life periods, seasons of year and in various diseases. Polskie Arch. Wet., 17, (1974): 131-145.

Horvath, Z., Papp, L., and Kapp, P.: Liver biopsy tests in cattle. Magyar Allatorvosok Lapja., 22(5), (1967): 205-208.

Hughes, J. P.: A simplified instrument for obtaining liver biopsies in cattle. Am. J. Vet. Res., 23(96), (1962): 1111-1112.

Ivascu, I., Ivascu, V., and Cociu, A.: Recherches experimentales sur la biopsie hepatique chez les bovins. Arch. Tierhelk., 110(62), (1968): 414-419.

Jensen, R., Connell, W. E., and Deem, A. W.: Ruminitis and its relation to change of ration and the proportion of concentrate in the ration of cattle. Am. J. Vet. Res., 15(56), (1954): 425-428.

Jensen, R., and Mackey, R. D.: Ruminitis, liver abscess complex. In *Diseases of Feedlot Cattle*, 3rd Ed. Lea and Febiger, Philadelphia, (1979): 80-84.

Jonsson, G., Hassler, L., and Ostlund, K.: The effect of a 24-hour fast and glucose-feeding on the weight and composition of the liver in sucking calves. Acta Vet. Scand., 7, (1966): 143-156.

Julian, L. M.: Studies on the subgross anatomy of the liver. III. Comparative arrangement of the blood vessels of the livers of the bovine and equine fetuses. Am. J. Vet. Res., 13(47), (1952): 201-303.

Julian, L. M., and DeOme, K. B.: Studies on the subgross anatomy of the bovine liver. I. The distribution of the blood vessels and bile ducts as revealed by the vinylite-corrosion technique. Am. J. Vet. Res., 10(37), (1949): 331-335.

Khatra, G. S., and Tyagi, R. P. S.: Regional anesthesia of paralumbar fossa - a study in Buffalo calves. Indian Vet. J., 49(3), (1969): 286-295.

Kinsella, J. E., and Butler, T. F.: Liver lipids of lactating bovine: fatty acid composition. J. Dairy Sci., 53(5), (1970): 604-606.

Kon, S. K., McGillivray, W. A., and Thompson, S.: Metabolism of carotene and vitamin A given by mouth or vein in oily solution or aqueous dispersion to calves, rabbits and rats. Brit. J. Nutr., 9(3), (1955): 244-267.

Kronfeld, D. S.: Metabolic disorders - homeostatic disorders associated with lactation. In *Bovine Medicine and Surgery*, 2nd Ed., Vol. I. H. E. Amstutz, Am. Vet. Publ. Inc., Santa Barbara, California, (1980): 537-596.

Lamothe, P., Guay, P., Gote, M. G., Panisset, J. C., Brodeur, J., and Alary, J. G.: Biopsie hepatique chez le bovin. Can. Vet. J., 10(9), (1969): 248-249.

Leedle, J. Z., and Hespell, R. B.: Some responses of rumen bacteria to starvation. Feedstuffs, July 30, (1979): 38-40.

Loosmore, R. M., and Allcroft, R.: Technique and use of liver biopsy in cattle. Vet. Rec., 63(24), (1951): 414-416.

Luna, L. G. (ed.): *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, 3rd Ed. McGraw-Hill Book Company, New York, (1968): 32-46.

Mall, F. P.: A study of the structural unit of the liver. Am. J. Anatomy, 5, (1906): 227-308.

Manns, E.: Effects of starvation on enzymes, glycogen and neutral fat in livers of sheep and cattle: a histochemical study. Res. Vet. Sci., 13, (1972): 140-145.

Martin, F. H., Ullrey, D. E., Miller, E. R., Kamp, K. E., Geasler, M. R., and Henderson, H. E.: Vitamin A status of steers as influenced by corn silage harvest date and supplemental vitamin A. J. Anim. Sci., 32(6), (1971): 1233-1238.

Mitchell, Jr., G. E., Little, C. O., Sewell, H. B., and Hayes, B. W.: Mobilization of liver vitamin A in sheep. J. Nutr., 91, (1967): 371-374.

Moir, R. J., and Lorin, E. H.: Ruminal flora studies in the sheep. X. Influence of nitrogen intake upon ruminal function. J. Nutr., 77, (1962): 285-297.

Moller, A. T., and Simesen, M. G.: Liver biopsy in cattle. I. Technique and clinical-diagnostical application. Nord. Vet. Med., 11, (1959): 719-730.

Morgan, J. D., and Hartroft, W. S.: Juvenile liver age at which one cell thick plates predominate in the human liver. Arch. Path., 71, (1961): 86-88.

Morrow, D. A., Hillman, D., Dade, A. W., and Kitchen, H.: Clinical investigation of a dairy herd with the fat cow syndrome. J. Am. Vet. Med. Assoc., 147, (1979): 161-167.

Narasimhamurty, G.: Liver biopsy in cattle. Indian Vet. J., 43, (1966): 114-119.

Oxender, W. D., Askew, E. W., Benson, J. D., and Emery, R. S.: Biopsy of liver, adipose tissue and mammary gland of lactating cows. J. Dairy Sci., 54, (1971): 286-288.

Patterson, D. S. P., and Allen, W. M.: Chemical analysis of bovine liver biopsy specimens: a comparison of data obtained using two biopsy techniques and blood contamination as a source of analytical error. Vet. Rec., 82(1), (1968): 35-37.

Pearson, E. G., and Craig, A. M.: The diagnosis of liver disease in equine and food animals. Modern Vet. Pract., part 1-2, (1980): 233-237, 315-320.

Perry, T. W., Beeson, W. M., and Holz, R. C.: Levels of supplemetnal vitamin A for fattening beef steers. J. Anim. Sci., 19, (1960): 1283-1284.

Petrovic, D., Kralj, M., Miklaavsic, B., Marzan, B., and Cvetnic, S.: The value of aspiration biopsy of the liver in the diagnosis of equine infectious anemia. Vet. Arch., 7-8, (1963): 178-185.

Phillips, G. D., and Roberts, W. K.: Intraruminal injection of vitamin A in steers. Am. J. Vet. Res., 25(107), (1964): 1070-1073.

Popesko, P.: *Atlas of Topographical Anatomy of the Domestic Animals,* 2nd Ed. W. B. Saunders, Philadelphia, (1977): 205p.

Raines, D. R., Van Heertum, R. L., and Johnson, L. F.: Liver physiology and disease. Intrahepatic hematoma: a complication of percutaneous liver biopsy. Gastroenterology, 67(2), (1974): 284-289.

Raker, C. W., and Rogers, J. A.: Reports of liver glycogen determinations by biopsies on dairy cows. Am. J. Vet. Res., 17(63), (1956): 205-206.

Reid, M.: An ultrastructural and morphometric study of the liver of the lactating cow in starvation ketosis. Exp. Molec. Pathol., 18, (1973): 316-330.

Reid, M., Stark, A. J., and Isenor, R. N.: Fasting and refeeding in the lactating dairy cow. I. The recovery of milk yield and blood chemistry following a six-day fast. J. Comp. Path., 87, (1977): 241-251.

Roberts, W. K., Findlay, G. M., and Stringam, E. W.: Liver vitamin A storage in fattening cattle following intraruminal or intramuscular injection of vitamin A. Can. J. Med. Vet. Sci., 29, (1965): 43-45.

Roberts, W. K., and Stringam, E. W.: Note on liver vitamin A stores and weight gains in beef cattle following intraruminal injection or oral administration of vitamin A. Can. J. Anim. Sci., 42, (1962): 110-111.

Robertson, A., and Thin, C.: A study of starvation ketosis in the ruminant. Brit. J. Nutr., 7(1), (1953): 181-195.

Robertson, A., Paver, H., Barden, P., and Marr, T. G.: Fasting metabolism of the lactating cow. Res. Vet. Sci., 1(117), (1960): 117-124.

Said, A. H., Shoukry, M. M., and Fouad, K.: Paravertebral anesthesia in buffaloes. Zbl. Vet. Med., A23, (1976): 85-88.

Sasaki, Y., Kumazaki, K., and Ikeda, O.: Relationship between plasma glucocorticoids and the maintenance of blood glucose level in the fasted animal. Jap. J. Zootech., 45(2), (1974): 81-87.

Schultz, L. H.: Management and nutritional aspects of ketosis. J. Dairy Sci., 54(6), (1971): 962-973.

Seghetti, L., and Marsh, H.: Liver biopsy in cattle. Am. J. Vet. Res., 14, (1953): 9-11.

Simesen, M. G., and Moller, T.: Liver biopsy on cattle. II. The patho-morphological picture of ketosis. Nord. Vet. Med., 11, (1959): 787-790.

Sova, V. Z.: Die leberbiopsie beim pferd, ihre technik, ergebnisse und bedeutung in der labordiagnostik der hepatopathien. Der Praktische Tierarzt., 46(10), (1965): 498-499.

Steiner, J. W., and Carruthers, J. S.: Studies on the fine structure of the terminal branches of the biliary tree. 1. The morphology of normal bile canaliculi, bile pre-ductiles (ducts of Hering) and bile ductules. Am. J. Path., 38(6), (1961): 639-648.

Stinson, A. W., and Calhoun, M. L.: Digestive system. In *Textbook of Veterinary Histology*. Horst-Dieter Dellmann, Esther M. Brown, ed. Lea and Febiger, Philadelphia, (1976): 207-264.

Swenson, M. J. (ed.): *Duke's Physiology of Domestic Animals*, 8th Ed. Comstock Publ. Assoc., Cornell Univ. Press, Ithaca, New York, (1970): 396-404.

Thoonen, H., Oyaert, W., Hoorens, J., and Muylle, E.: De diagnostiche waarde van de leverbiopsie. Bij het klinisch onderzdek van het rund. Vlaams Diergeneeskundig Tijdschrift, 36, (1967): 1, 3-15.

Turner, J. C., and Green, R. S.: The use of a liver biopsy technique for investigating the effects of hexachlorobenzene on some drug-metabolising enzymes in sheep. Res. Ret. Sci., 20, (1976): 155-157.

Udall, R. H., Warner, R. G., and Smith, S. E.: A liver biopsy technique for cattle. Cornell Vet., 42, (1952): 25-27.

Usui, K., Fujita, T., and Inoue, K.: Studies on vitamin A deficiency in domestic animals. I. Storage of vitamin A in the liver of dairy cattle in Japan with special reference to its seasonal fluctuation. Jap. J. Vet. Sci., 22, (1960): 159-166.

Van Arsdell, W. J., Whitehair, C. K., and MacVicar, R. W.: A liver biopsy technique and observations of its use in vitamin A studies with beef cattle. J. Anim. Sci., 10, (1951): 1064-1065.

Vulinec, M., and Bauer, M.: Aspiration biopsy of the liver in sheep. Veterinarski Archiv., 3-4, (1970): 114-117.

Wallin, O., and Pehrson, B.: A new method for liver biopsy in cattle. Nord. Vet. Med., 17, (1965): 594-598.

Whitehair, C. K.: Nutrition and disease. In *Bovine Medicine and Surgery*, 2nd Ed., Vol. I. H. E. Amstutz, ed. Am. Vet. Publ. Inc., Santa Barbara, California, (1980): 511-536.

Whitehair, C. K., Peterson, D. R., Van Arsdell, W. J., and Thomas, O. O.: A liver biopsy technique for cattle. J. Am. Vet. Med. Assoc., 121, (1952): 285-287.

Wise, G. H., Atkeson, F. W., Caldwell, M. J., Parish, D. B., and Hughes, J. S.: Effects of high vitamin A intake on milk and fat yields and on vitamin A constituents in milk, blood, and livers of dairy cows. J. Dairy Sci., 30(5), (1947): 279-291.

Wood, R. L.: The fine structure of the junction between bile canaliculi and bile ducts in mammalian liver. Anat. Rec., 139, (1961): 287.

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