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HISTOCYTOLOGICAL STUDIES ON NORMAL BOVINE LIVERS AND ON BOVINE LIVERS EXHIBITING A FOCAL HEPATITIS AND TELANGIECTASIS

by

Robert Getty

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Veterinary Anatomy

Approved:

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I. INTRODUCTION

A knowledge of the normal histological and cytological picture of an organ, with its normal variations, is an essential prerequisite for the recognition of any pathological deviation from the normal. With this in mind, cytological and histochemical observations were made with the hope of establishing a definite microscopic picture of the normal bovine liver under the conditions of this investigation. Histochemical and cytological studies were also concurrently made of so-called sawdust[1] and telangiectatic bovine livers.

The writer has previously indicated (Getty, 1945) that the meat-packing industry has sustained a heavy monetary loss for years due to the condemnation, under federal inspection, of bovine livers affected with telangiectasis[2] and the so-called sawdust condition. Since the bovine liver has materially increased in monetary value and nutritional significance in recent years, it seemed advisable to first establish a definite normal microscopic picture of this organ. The establishment of such a picture may in turn facilitate further research and investigation pertaining to this organ.

During the course of this investigation, approximately 5,000 sections taken from 69 bovine livers, were examined both macroscopically and

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[1] Term applied by the Meat Inspection Service of the U.S. Department of Agriculture to bovine livers exhibiting a focal hepatitis.

[2] Hereafter referred to as telang.

microscopically. A complete history of the animals from which the material was taken was available and the writer personally collected all samples. The disposition of the carcass, the diet, the age, time of last feeding and watering were all known. The acid and alkaline phosphatase activity, the cell organelles, glycogen deposition and fat deposition were routinely studied. Since numerous investigators, Pappenheimer (1916), Bolt (1924), Seecof (1925), Cowdry (1926), Duthie (1935), Dalton (1941-42), and Steffens (1941), have demonstrated that mitochondrial and Golgi changes are often the most sensitive and earliest indications microscopically of cellular changes and damage, it was deemed advisable to study in detail the cell organelles. The phosphatases were also studied since alkaline and acid phosphatase have been observed to vary under various physiological and pathological conditions: Gomori (1941a 1941b), Greenstein et al. (1941-42-43-44-46), White et al. (1941-42), Kabat (1941), Wachstein (1945) and Sulkin (1948).

II. REVIEW OF LITERATURE

A. Phosphatase

Ever since the demonstration of phosphatase activity in living tissue by Grosser and Husler (1912), there has been considerable interest in the enzymes capable of hydrolyzing phosphoric esters. Plimmer (1913) and Robinson (1923) described experiments in which ground-up tissues or tissue extracts were shown to have had differing hydrolytic activity towards glycerophosphate or other phosphoric esters. Kay (1928) studied the quantitative distribution of the phosphatases in mammalian tissues analyzing those of the rabbit, cat and man. MacFarlane (1934) also studied the phosphatase activity of animal tissues. Davies (1934) first observed the presence of a phosphatase having an optimum pH of 4 to 5 in the human liver and spleen. Kutscher and co-workers (1935a, 1935b, 1936c) have shown that urine contains a similar phosphatase. Armstrong and Banting (1935) observed that removal of the viscera did not affect the serum phosphatase level and concluded that most of the serum phosphatase comes from bone. A. Bodansky (1937) studied the non-osseous origins of serum phosphatase and O. Bodansky (1937) showed that the phosphatases of bone, kidney, intestine, and serum differed.

However, it was Gomori (1939) who opened a new era in enzymatic histochemistry with his microtechnical demonstration of phosphatase in tissue sections. Simultaneously but independently Takamatsu (1939) also

published a similar method for the demonstration of phosphatase in microscopic sections. Both methods were devised on the basis that phosphatase is well preserved by alcohol fixation, (Martland and Robinson 1929). Since Gomori's original publication there has been an increasing interest in the phosphatase problem as evidenced by the papers published in recent years.

Gomori (1941) studied the distribution of alkaline phosphatase in normal organs and tissues and found that the liver presents a different picture in different species. The liver was practically negative in the guinea pig, ground hog and rat except for variable staining of the adventitia of the branches of the hepatic artery. The sinusoidal walls were positive, though not uniformly so, in man, the dog, the rabbit and the gopher. The liver cells were also positive in the same species, although the degree and extent were extremely variable. Usually the darkest staining was observed either around the central veins or in the periphery of the lobule, or in both places, leaving an intermediate area of the lobule paler. The bile capillaries were sharply outlined in black, especially in the rabbit, less so in man and in the dog. The bile capillaries were especially predominant near the periphery of the lobules. In practically all species, the epithelium of the bile ducts was positive.

Kabat (1941) examined adult human, chicken and mouse livers and found a conspicuous phosphatase reaction in the endothelium of the sinusoids and other vessels. The bile ducts and liver cells contained no phosphatase or only traces. Greenstein (1941-42) determined chemically the acid and alkaline phosphatase activity in tumors, normal tissues and

tissues of tumor bearing rats and mice. In the normal mouse liver and in the transplanted hepatic tumor, acid activity was much greater than alkaline. In fact, in each of the transplanted mouse hepatic tumors, the alkaline activity was zero or nearly so and was much less than that of normal mouse liver. Acid activity in each of the mouse tumors was either equal to or somewhat greater than that of normal mouse liver. In contrast, alkaline activity was very much greater than acid in the transplanted rat hepatic tumor, and both alkaline activity and acid activity for the tumor were much greater than the corresponding values for normal rat liver.

Wachstein (1945) studied the influence of dietary deficiencies and various poisons on the histochemical distribution of phosphatase in the liver. He noted that marked cytoplasmic changes took place in the liver of starved or protein depleted animals. The source of the increased amount of serum phosphatase noted concurrently with a damaged liver was also discussed. In 1946, the same author reported the distribution of alkaline phosphatase in tissue sections of the livers of human patients who had died with hepatic diseases, and also the livers of patients who had succumbed to other illnesses. The livers of the latter were normal grossly and microscopically. The cytoplasm stained faintly but the activity was greatest in the chromatin, the nucleoli and the nuclear membrane. A varying degree of phosphatase activity was noted in the walls of the sinusoids and in the Kupffer cells and in the nuclei of the epithelium of the bile ducts. Conspicuous activity was frequently noted in the

small arteries and arterioles. Lymphocytes in the portal fields showed inconstant amounts of phosphatase. The bile capillaries also showed considerable variation. A varying behavior of phosphatase activity in bile capillaries was noted in livers showing hepatocellular damage and biliary obstruction. None of the livers showing extensive necrosis of the hepatic cells showed an appreciable increase of alkaline phosphatase in the remaining undamaged liver cells nor was there an increase in enzymatic activity in the necrotic cells. In necrotic areas, most of the sinusoidal walls retained their activity. Infiltrating leukocytes and lymphocytes showed a varying degree of activity. Phosphatase activity was lacking where fat droplets had been present. The liver cells in various forms of cirrhosis usually showed normal phosphatase activity, but considerable activity was seen in proliferating connective tissues. A different behavior was observed in the staining reaction of lymphocytes in blood smears and those in tissue sections. Lymphocytes in blood smears were devoid of phosphatase but in chronic inflammatory foci, they showed variable activity. The reason for the difference was obscure.

Deane (1947) reported intense enzymatic activity at pH 10.0 to 9.0 in the nuclei, in the bile capillaries, in the circulating lymphocytes and in the endothelium of the small arterioles and capillaries in the portal canal area in the rodent liver. The cytoplasm of the hepatic cells was also readily demonstrated.

Gomori (1941) reported that the liver contains varying amounts of acid phosphatase in all species. Often the liver cells at the periphery

of the lobules were strongly positive, the lining of the bile ducts were usually negative or faintly positive, and the bile capillaries were not as predominant as those seen in sections stained for alkaline phosphatase.

Wolf, Kabat and Newman (1943) studied the distribution of acid phosphatase in human beings and monkeys. They observed that both the nuclei and cytoplasm of the hepatic cells were impregnated, the former more deeply. The nuclei and cytoplasm of the bile duct epithelium were stained and the cytoplasm and nuclei of the Kupffer cells also stained well. Nuclei of connective tissue cells were also impregnated, especially those in the portal spaces.

Deane (1947) described a slight reaction at pH 5.0 to 4.0 in the cytoplasm in rat livers and a more intense reaction in the hepatic cell nuclei, especially the nuclear chromatin. The most conspicuous reaction was in the bile capillaries and in the intracellular granules occasionally adjacent to them. Kupffer cells, and leukocytes, and the cells of the bile duct exhibited activity in all sites. However, the enzyme was most active at the periphery of the lobule.

Interesting observations have been made concerning the relationship between phosphatase and calcification under normal and under a variety of pathological conditions (Gomori, 1943). White et al., (1941-42) described an abundance of alkaline phosphatase in a transplantable induced hepatoma in the rat. Greenstein (1945-46) also described an increase in alkaline phosphatase activity in primary and transplanted rat hepatomas. However, Edwards et al., (1941-42) reported that little or no stainable alkaline phosphatase was present in transplantable spontaneous hepatomas in the

mouse. Greenstein (1941-42) also found low alkaline phosphatase values in mouse hepatomas. The authors, cited above, believed that the difference in alkaline phosphatase in the hepatomas was a species difference. Emmel (1946) recently verified the general validity of the various methods employed for fixation and phosphatase demonstration. Dense accumulation of alkaline and acid phosphatase was demonstrated in the Golgi regions of some intestinal and other epithelial cells by Deane and Dempsey (1945). Wislocki and Dempsey (1946) investigated the histochemical reactions of the placenta of the pig and Stafford et al., (1947) studied the acid and alkaline phosphatase in ovarian tissues of the rat during pregnancy and lactation.

Using Gomori's technique, Rabinovitch and Junqueira (1948) demonstrated the presence of "acid" phosphatase in human and animal bone-marrow smears. The granules of the eosinophiles stained intensely, neutrophile granules were negative. No basophiles were found. The cytoplasm of the red cell series stained poorly but the nuclear structure was evident. The mature red blood cells were entirely negative; however, the nucleus of the red blood cell of the chicken stained intensely.

Sulkin and Gardner (1948) reported phosphatase changes in the cytoplasm and nuclei of all hepatic cells during the first 15 days of restoration following partial hepatectomy. Increased alkaline phosphatase activity was noted in the cytoplasm, the nuclei and bile canaliculi during the first 15 days of restoration. Acid phosphatase activity was also increased in the nuclei of the hepatic cells but not in the cytoplasm.

B. Mitochondria

The literature on mitochondria was first completely reviewed by Duesberg (1911) in a summary which covered about 500 papers up to October 15, 1911. Another review from this period to July 1923 was published by Cowdry (1924). Bourne (1942) has summarized the literature, bringing up to date the various biochemical findings.

It has only been within comparatively recent years that the true characteristics of mitochondria have been revealed. Thurlow (1917) made the first quantitative studies on mitochondria. That author found a constant number of mitochondria per unit of cytoplasm in normal nerve cells. This constant differed for nerve cells of different types. Gatenby (1919) classified mitochondria and the Golgi apparatus as protoplasmic (living) inclusions; glycogen, fat, yolk, and pigment as (dead) inclusions. The effect of different hydrogen-ion concentrations on mitochondria in the liver cell was described by Scott (1924). Alterations in number and size were noted when rabbit's liver was incubated in physiological salt solution with a H-ion concentration ranging from pH 4.4 to pH 9.0. Cowdry (1924) stated that mitochondria in the form of granules, rods, and filaments could be seen in the living unstained cell. They were observed in organisms ranging from man to protozoa, to fungi, but their existence in bacteria was doubted. Using living amoebas, Horning (1926) made observations on the relation of mitochondria to the process of intracellular digestion. He concluded that the mitochondria brought about the digestion of the food by their enzymatic action. Ludford (1928) believed

that synthesis by enzymatic action occurred at the mitochondrial-cytoplasmic surface. Horning (1928), continuing his studies on the behavior of mitochondria within the living cell, observed mitochondria within the food vacuoles of living organisms and regarded this as a direct demonstration of the origin of digestive enzymes from mitochondria. Smith (1931) studied the ontogenetic history of the hepatic mitochondria of the white rat. It was revealed that feti of the same age presented the same mitochondrial picture as to number, type and size. Mitochondrial morphology characteristic of the adult was first observed in the 14 day young. Dalton (1934) made a similar study of the mitochondria and Golgi network of the hepatic cells of the chick. In recent years, considerable effort has been directed towards the function of the mitochondria of the hepatic cell; however, a wide variation of opinion still exists. The relationship between the mitochondria and glucose-glycogen equilibrium in the liver was studied by Hall and MacKay (1933). Histological examinations for glycogen and chemical determinations were made independently. They found that the quantity of glycogen in histological preparations compared very favorably with that found by chemical analysis. The shape, size, and location of the mitochondria were also studied in relation to glycogen content by the same two authors. Duthie (1935) investigated mitochondrial changes in autoplastic liver transplants noting that they were the most sensitive and earliest indications microscopically of cellular damage. Using the freezing-drying technique Bensley and Gersh (1933) were the first to demonstrate the true chemical nature of the

mitochondria in the hepatic cell. A year later, Beams and King (1934) determined the effect of ultracentrifuging on the mitochondria of the hepatic cells of the rat. In ultracentrifuged tissue, the mitochondria were displaced toward the centrifugal pole of the cell. They still retained their usual form after being moved through the cell. Thus it was concluded that the mitochondria in the rat liver cell were of a greater specific gravity than the cytoplasm. Bensley and Hoerr (1934) continued study of cell structure by the freezing-drying method. They obtained mitochondria for chemical analysis by differential centrifugation of an emulsion of guinea pig livers. Weighted quantities of mitochondria were found to contain an average of 43.6 per cent of fatty substance soluble in hot alcohol, ether, and chloroform. The remaining constituent portion was classified as protein. Bourne (1935) demonstrated vitamin C within the cells of various tissues noting that a portion of the vitamin C was present in the mitochondria and Golgi apparatus. He suggested that mitochondria were composed of an outer lipoidal cortex which contained vitamin A or cartenoid pigments and a water core containing vitamin C and glutathione. Thus, the mitochondria were believed to be ideally composed chemically to form an oxidation-reduction system which could function as respiratory centers of the cell. Kater (1937) stated that there were three possible interpretations advanced for changes in mitochondrial morphology, namely:

> (1) that they represent functional hypertrophy, (2) that they represent changes in the general metabolic level of the cell, (3) that they merely indicate definite changes in the ground cytoplasm in which they are not functionally involved.

Following a study of the liver-blood fluid exchange and the morphology of the hepatic cell mitochondria, Kater definitely felt the morphological changes were expressions of the water content of the tissue. The effect of temperature on mitochondria in liver cells of fish was studied by MacCardle (1937). It was noted that heat rigor and lethal temperature (37° to 42°) caused the mitochondria to fragment and dissolve. The Golgi apparatus also became swollen and dissolved. Bensley (1937) continued his chemical analysis of the mitochondria of the guinea pig liver. He believed that the fat of the mitochondria was not discrete but was dispersed in an ultramicroscopic form. Since the mitochondria also gave a Millon reaction in all parts, it was assumed that the proteins were equally dispersed. Bensley's analysis suggested that the mitochondrial unit was a mosaic of protein, glyceride, and cholesterol molecules forming a coacervate. Thus, any condition which might disturb the equilibrium of this coacervate, might result in dispersion of its molecular units. The disappearance of mitochondria from cells during fixation is thought to be in all probability such a reversal. McCurdy (1939) noted that starvation tended to produce definite changes in the mitochondrial picture in the livers of salamanders. Re-feeding tended to restore the normal mitochondrial picture. The author concluded that a close relation existed between the mitochondrial condition and the relative amounts of fat and glycogen in the cell. The shift from elongated mitochondria to granular paralleled the decrease in amount of fat and glycogen in the cell. The shift in the opposite direction paralleled the increase in amount of

these same components. Steffens (1941) in an experimental study of the mitochondria in the hepatic cells of the white rat also found that during starvation, the mitochondria of the liver tend to enspherulate. Feeding restored the filamentous character of the mitochondria. Steffens believed that the rhythmic activity of the liver was dependent upon the feeding regimen and was not correlated with time of day as suggested by Forsgren (1935). Deane (1942) made a study of the hepatic-cell mitochondria in the fatty liver produced by a high-sugar diet. The mitochondria in the cells of the peripheral and middle zones of the lobule became shortened and often swollen and vesiculated, while those in the central zone remained filamentous. Fat was initially deposited centrally and yet the swelling of the mitochondria began in the peripheral zone and proceeded centrally. The mitochondria were also enspherulated after starvation, especially in the peripheral zone. The mitochondria remained enspherulated and at times vesiculated when the high sugar diet was continued for a week. However, the mitochondria returned to their usual diameter when the diet was continued for more than two weeks. This observation was interpreted as an indication that the liver had become adjusted to the unbalanced diet. Deane concluded that there was no direct relationship between the mitochondria and the deposited fat. Two years later, Deane (1944) made a very complete cytological study of the diurnal cycle of the liver of the mouse in relation to storage and secretion. Definite zonation within the lobule was observed. The mitochondria were longer and shorter, the Golgi substance heavier, bile secretion greater, glycogen deposition greater, and fat deposition less marked at the periphery of

the lobule than centrally. Two factors were thought responsible for this zonation, namely, the greater supply of oxygen and food material at the periphery of the lobule. The author concluded that all zonation was probably dependent upon the character of the blood bathing the cells.

C. Golgi Apparatus

The literature pertaining to the Golgi apparatus and its significance has been periodically summarized: Pappenheimer (1916), Cowdry (1923), Bowen (1926-1928-1929), Kirkman and Severinghaus (1937-38), Bourne (1942), Baker (1944), and Worley (1946-47). Since many of the earlier reviews covered controversial problems which have since been clarified, reference will be made mainly to papers of comparatively recent years.

The Golgi apparatus has been described as a reticulum, network, vesicle, granular bodies, small spheres, rodlets, incomplete hollow spheres, fenestrated plates, group of vacuoles, and as a collection of discs. Kirkman (1937) stated that the only cells in which it has been impossible to identify the Golgi apparatus were short-lived or dying ones, such as mammalian erythrocytes, cornified epithelial cells and most mature sperm cells. Drew (1920) concluded that plants also possess a structure identical with or at least similar to, the internal reticular apparatus of animal cells. The literature on this apparatus in plant cells was more recently reviewed by Zirkle (1937).

Hill (1936) observed the Golgi apparatus, mitochondria, fat and glycogen granules of chick osteoblasts grown in vitro. The above author concluded that osteoblasts cultivated in vitro exhibited perfectly normal cytoplasmic inclusions and attributed the failures of earlier workers to unsuitable conditions of cultivation. Macdougald (1937) also demonstrated the Golgi apparatus of cells in tissue culture. Norminton (1937) by ultra-centrifuging, was successful in stratifying fat globules, Golgi bodies, and mitochondria into different layers. That author was also able to identify these organoids and inclusions in living, unstained, non-centrifuged material. Champy (1926) had also previously observed the Golgi network in unstained, and supra-vitally stained germinal epithelial cells of the rabbit ovary grown in tissue culture. Atwell (1932) and Severinghaus (1933) stated that although there was extreme morphological variability of the Golgi apparatus in animals and plant cells, there was also a marked constancy of form in a given type of cell. Kirkman and Severinghaus (1937) concluded that the apparatus may be present in a cell as a single organelle or as many individual segments. In the former, one would note a more or less compact appearance whereas in the latter, the apparatus would present a loose widespread appearance as frequently noted in many glandular cells, Worley (1946-47) observed that the Golgi apparatus was capable of existing in a number of forms both when examined in living tissue and fixed tissue. He stated that his observations on living material had confirmed theories advanced by previous investigators that used fixed and stained material. Baker (1944) observed that the Golgi apparatus of spermatocytes and spermatids of the common snail were visible

without staining in living cells. Ludford (1935) after vitally staining the cultures with methylene blue was the first to make a photomicrograph of the Golgi apparatus in a living fibroblast grown in vitro. Beams and King (1934) demonstrated by ultracentrifugation that the specific gravity of the Golgi apparatus was lower than that of the surrounding cytoplasm. This discovery is often referred to as the work that definitely marked the end of the Golgi apparatus-artefact controversy. It is now concluded by competent investigators that the apparatus exists and can be demonstrated in both living and fixed protoplasm.

Opinions vary not only as to the structure but also as to the chemical composition of the Golgi apparatus. It was suggested by Bowen (1920, 1926, 1928) that the Golgi system was of a duplex character, consisting of osmiophilic material and chromophobic material of a protein nature. Beams and King (1932a,1932b) described and illustrated both osmiophilic and osmiophobic portions of the Golgi apparatus, but ventured no opinion as to the osmiophobic portion. Since fat solvents destroyed the apparatus, Eastlick (1936) favored the lipoidal concept. Beams and King (1934) stated that the apparatus behaved as a lipoidal structure going to the centripetal pole of the cell during ultracentrifuging. Worley (1946-47) suggested that the Golgi elements act like a series of intracellular sponges which grow considerably due to their great absorbing or adsorbing ability. The Golgi substance according to Worley is constantly engaged in soaking up fat or protein from the surrounding cytoplasm. That author felt that the Golgi elements converted protein and fat forming substances brought to the cells by the blood into the

intracellular neutral fat and protein inclusions. In physical-chemical terms, the Golgi system may fulfill Taylor's (1943) prediction that coacervates may be active in living cells.

A nuclear origin of the Golgi apparatus was suggested by Hirschler (1929). Worley (1946-47) also reported as follows on the possible origin of the Golgi bodies:

> The evidence, at the present time, indicates that the new Golgi elements in all cells cannot be assumed to have arisen from pre-existing Golgi bodies of microscopically visible proportions. Since Golgi substance is probably always present, but it need not necessarily be so disposed as to make it recognizable by the morphologist as one or more Golgi bodies.

Worley tentatively suggested that the nucleolus may be thought of as discharging granules and droplets of protein or protein precursors into the cytoplasm. They may remain dormant or begin to absorb lipoidal substance. They will then, according to the author, blacken with osmic acid and may then be in reality homogeneous Golgi droplets. That author also suggested that the extruded nuclear material may remain in a submicroscopic form or the particles may aggregate to form visible elements or Golgi bodies. What the cytologist recognizes as the Golgi apparatus typically represents according to Worley:

> the more or less temporary aggregation of ultramicroscopic colloidal particles composed partly or largely of ribose nucleic acid or ribo-nucleoproteins, phospholipids and, probably frequently, vitamin C.

D. Glycogen

Since Bernard (1857) demonstrated the presence of glycogen in tissues, much has been published regarding its distribution and history in the

hepatic cell. Ehrlich (1883) established the diffuse distribution of glycogen in the liver cell and Lewis (1921) by means of tissue culture cells confirmed this work. However, evidence regarding glycogen distribution in the hepatic lobule and in the lobes of the liver is conflicting. Bartlett et al. (1914) believed that the relative amounts of glycogen in the different lobes of the liver must vary from time to time according to the stage of digestion. Injections of dilute copper sulfate solution were made into the lumen of the intestinal tract at various places, permitting normal absorption to continue over periods of from five days to three weeks. Later, final quantitative determinations of copper were made for the different lobes. From their experiments, they concluded that blood flowing into the portal vein from each of the smaller veins of the portal system does not blend diffusely into a common current but instead, blood from the stomach, spleen, duodenum, first part of the jejunem, and a portion of the rectum, flows mainly to the left lobes of the dogs liver and least to the right lobes. Blood from the lower jejunem, ileum, and first part of the large intestine flowed principally to the right lobes of the liver. However, Dowler and Mottram (1918) stated that the lobes of the liver of the dog, cat, and rabbit has a different and unpredictable glycogen content. The authors felt that the distribution of glycogen was irregular and according to no fixed plan, stating that no apparent relation existed between the time that elapsed after the meal and the lobe that had the largest amount of glycogen. They believed the probable factors that influenced the partition of metabolic products between the different lobes were localized constriction, incomplete mixing of the blood in the

portal vein, and potentiality variance in the cells for a certain type of work at a given time. Contrary results have also been reported regarding the glycogen deposition within the hepatic lobule. Noel (1923) found that glycogen appeared first in the cells adjacent to the central vein. Later, the other cells of the lobule gradually accumulated glycogen appearing last in the cells about the peripheral portion of the lobule. Although glycogen became rather evenly distributed throughout the cells of the hepatic lobule following ingestion of sufficient carbohydrates, those cells about the central vein usually contained the largest amounts. In general, the withdrawal of glycogen took place in a reverse order from the course of its deposition according to Noel. The cells around the central vein appeared to retain the glycogen longest. Forsgren (1935) and his associates also reported that the deposition of glycogen began in the interior of the lobule and advanced toward the periphery. They described a rhythmic cycle in the deposition of glycogen and in the secretion of bile. According to their observations, glycogen was present in large amounts during the night, whereas the largest amount of bile was secreted during the afternoon. This cycle was characteristic for the rabbit, the rat, and the mouse. Forsgren concluded that the quantity of the two substances and their distribution in the liver lobule varied inversely. The cells in the peripheral portion of the lobule in which production of bile began first and ceased last, were more secretory in character, while those in the interior, in which the production of glycogen began first and ceased last, were more assimulatory in character.

Higgins, Berkson, and Flock (1932) and Deuel et al. (1938) found that glycogen deposited in the liver in the diurnal cycle was largely alimentary in origin. Deane (1944) found that with both controlled and uncontrolled feeding, glycogen appeared to be deposited shortly after the time of eating. Deane felt that there was no evidence to support the conclusions of Forsgren that the time of glycogen deposition was controlled by factors other than alimentation. In disagreement with the descriptions of previous workers, Deane found that glycogen was deposited initially in the peripheral zone of the hepatic lobule, then more centrally. Deane's preparations showed that the storage of glycogen as well as the secretion of bile acids was greatest at the periphery of the lobule and thus contrary to Forsgren does not believe an inverse relationship between the storage of glycogen and the secretion of bile acids exists.

Although cytological methods have been valuable in determining various facts regarding glycogenesis and glycogenolysis in the liver, it was the studies of Mann and Magath's (1922a, 1922b, 1922c) that clarified the vital function of the liver in relation to carbohydrate metabolism. In a series of studies, the authors observed the effect of the total removal of the liver. Best (1934) in a series of articles also reviewed the role of the liver in the metabolism of carbohydrate and fat. The formation of glycogen from glucose in fed and fasted rats has recently been studied by Stetten and Boxer (1944). A constant concentration of deuterium oxide was maintained in the body fluids and the extent to which heavy hydrogen was incorporated into liver and carcass glycogen was determined. The general role of the liver in regulating the blood sugar

level has recently been reviewed by Soskin (1944). György (1944) brought up to date recent concepts dealing with experimental dietary hepatic injury. Freeman (1946) made a very comprehensive review of recent studies on the liver and bile.

E. Fat

Many workers have studied the problem of liver function in fat metabolism since Kölliker (1857) first demonstrated fat globules in the liver. Hartley's paper (1907-08) showed conclusively that liver fat had a higher iodine value than depot fat. A few years later, Mottram (1909) observed that there was an increase in liver fat when food was withheld from rabbits and guinea pigs for twenty-four hours. That author suggested that the increase was due to infiltration of the liver by depot fat and confirmed his results by a study of the iodine values. Dowler and Mattrom (1918-19) concluded from their study of the distribution of blood, glycogen, and fat in the lobes of the liver, that one should never assume that all parts of the liver are working at the same rate or upon the same material. Nöel (1923) noted that fat, which appeared about the portal spaces in the mouse following a meal, reached a maximum in quantity at four to five hours. It then diminished progressively to the tenth hour. The tissues and organs of beef animals prepared for slaughter and sale in the normal manner were analyzed by Bloor (1928) for the distribution of the unsaturated fatty acids. Bloor's work confirmed that of Hartley's in that the iodine value of the fatty acids of

the fat fraction were distinctly higher in the liver than in the stored fat of the animals. Two years later, Bloor and Snider (1930) studied the neutral fat of beef liver and other tissue taken from freshly slaughtered animals. Their results agree with previous findings that the neutral fat of liver is more unsaturated than that of the fat depots. The liver iodine number for all the samples averaged at least twenty points higher than the depot fat of the animal. Fat globules in the substance of spherical chondriosomes were described by Kater and Smith (1932). They believed that either the mitochondria served as a storage place for fats or that they acted as catalysts stimulating a synthesis of fats from the fatty constituents. Noël (1923) also concluded that fat was formed in the hepatic cell of the mouse under the influence of the mitochondria. This conclusion was based on the observation that hypertrophied mitochondria were surrounded by a ring of small fat globules. Cowdry (1932) stated that fat granules usually appear in the cells around the central vein. Best (1934) in a series of articles discussed the role of the liver in the metabolism of carbohydrates and fat in the rat. It was noted that choline added to the stock diet for some weeks previous and during the period of starvation prevented the accumulation of liver fat. When fat was provided in the diet of white rats, there was an accumulation of neutral fat in the liver. The addition of choline reduced the neutral fat to low levels. Hoerr (1936) in his histological studies, discussed osmic acid as a microchemical reagent with special reference to lipins. The author felt that two factors should be

considered in the preservation of lipin droplets for microscopic study, namely, completeness of reduction of the osmic acid itself and the mechanism by which the lipin droplets were rendered insoluble by the osmic acid. Hoerr believed that the techniques of Kater and Smith (1932) did not warrant their conclusions that mitochondria in the liver were concerned with the formation of fat within their centers. Hilditch (1937) studied chemically the composition of liver fat in the ox, cow, pig and chicken. Grafflin (1940) made a histological study of the fat distribution in guinea pig livers of chemically known fat content. A zonal deposition of fat was noted in the lobules of the liver. There was a tendency for the fat droplets to be largely confined to the central portion of the lobules. They were occasionally located in the intermediate zone but seldom in the peripheral zone. The cytoplasm of the hepatic and Kupffer cells contained a variable number of fat droplets. However, there was no relationship between the quantity of fat deposited in the Kupffer cells on the one hand, and that in the hepatic cells on the other. Fat droplets were also occasionally observed in the cells lining the bile ducts. At times, well stained sudanophil fat droplets were observed within the nuclei of the hepatic cell but they were never seen in the nuclei of the Kupffer cells or bile duct epithelium. The author concluded that the relative amounts of histologically demonstrable (sudanophile) fat in different livers were not an accurate index of the relative total lipid contents as determined by chemical methods. Grafflin (1940) had previously shown by differential analyses of guinea-

pig liver that only a small fraction of the total lipid was present as neutral fat. However, Hodge (1941) demonstrated that Sudan IV stain was specific for this neutral fat. He noted that the sudanophil content of the liver was closely related to the neutral fat content, but this was not the case in regards to phospholipid or cholesterol percentages. Hodge concluded that the histological classification and the chemical analysis compared very favorably for "neutral fat".

Some workers Kater and Smith (1932), Nöel and Pallot (1934) have described a direct relationship between mitochondria and fat droplets within the liver cells. Others, MacCardle (1937), McCurdy (1939) and Steffens (1941) have failed to find any direct relationship. Because of these conflicting views, Deane (1942) restudied the problem examining the fat converted from a high sugar diet and the fat mobilized during starvation. The high sugar diet of Barrett, Best, and Ridout (1938) which had previously been studied biochemically, was adopted. The livers of the mice on the high sugar diet became very fatty within a week, but when the animals were restored to a balanced diet, the fat was lost, and a normal liver picture returned. The fat tended to be concentrated in the central zone of the liver following a high sugar diet and after starvation. Fat droplets appeared in the liver within a day or two after the mouse had been placed on the high sugar diet. They were at first distributed uniformly in the lobule or slightly centrally. After two weeks, the fat was concentrated in the middle and peripheral zones of the lobule. However, during starvation, the fat was frequently more concen-

trated in the central half of the lobule in contrast to the more peripheral concentration seen in animals after two weeks on the diet. Stetten and Salcedo (1944) studied the source of the extra liver fat in various types of fatty livers. The authors labeled the newly synthesized fatty acids with deuterium by raising the level of the body fluids of animals on fat-free diets with respect to D_2O. Deane (1944) investigated the relationship between changes in the mitochondria and the Golgi substance on the one hand, and the secretion of bile acids, and the storage of glycogen and fat on the other. Whenever fat was present in the livers of the mice, it seemed to be of alimentary origin since it appeared soon after the time of feeding. The fat droplets were either limited to the central zone or more concentrated in this area than in the peripheral portion of the lobule.

III. METHODS OF PROCEDURE

A. Obtaining Liver Samples

The livers used in this study were obtained from sixty-nine animals slaughtered at the plant of the Bookey Packing Company, located at Des Moines, Iowa. A complete history of all the animals was available as to sex, age, breed, and type of feed and pasture.

Twenty-two 4H Club Cattle, both steers and heifers, were slaughtered on August 16, 1946. The animals were all approximately 1½ years of age and were in prime condition having been shown at the Iowa State Fair. They included the Hereford, the Aberdeen Angus and the Shorthorn breeds. The individual animals presented the following feeding records:

#111. (Three animals)

Project started December 31, 1945 - terminated August 15, 1946

Corn	6988.5 lbs.
Oats	2321.0 "
*Tarkio	706.0 "
Red clover	1362.0 "
Mineral	44.0 "
Beet pulp	68.0 "

*Protein	9%
Fat	1.5%
Fiber	8%

#32. (One animal)

Project started January 12, 1946 - terminated August 15, 1946

Corn	2013.0 lbs.
Oats	845.0 "
*Sweet Lassy	125.0 "
Mixed hay	645.0 "

*Protein 11%
Fat 1%
Fiber 13%

#232. (One animal)

Project started January 1, 1946 - terminated August 15, 1946

Corn	2938.0 lbs.
Oats	119.0 "
Soybean meal	70.0 "
Mineral	30.0 "
Alfalfa hay	307.0 "

#52. (One animal)

Project started January 1, 1946 - terminated August 15, 1946

Corn	3196.0 lbs.
Tarkio	228.5 "
Linseed oil meal	203.3 "
Red clover	?

#137. (One animal)

Project started December 28, 1945 - terminated August 15, 1946

Corn & cob meal	2338.0 lbs.
Oats	230.0 "
Linseed oil meal	140.0 "
Hay	579.0 "

#34. (Two animals)

Project started January 7, 1946 - terminated August 15, 1946

Ground corn and oats	5788.0 lbs.
Soybean oil meal	223.0 "
Molasses	196.0 "
Red clover	6377.0 "
Mineral	90.0 "

#121. (One animal)

Project started November 1, 1945 - terminated August 15, 1946

Ground corn	2680.0 lbs.
Oats	624.0 "
Tarkio	352.0 "
Timothy and clover	1105.0 "

#125. (Three animals)

Project started January 1, 1946 - terminated August 15, 1946

Ground corn or corn and cobmeal	5858.0 lbs.
Oats	1578.0 "
*Honeymead cattle pellets	651.0 "
Mixed clover & timothy	4822.0 "
Salt	50.0 "

*Protein	37%
Fat	1.5%
Fiber	7.5%

#225. (Two animals)

Project started November 22, 1945 - terminated August 15, 1946

Corn	1350.0 lbs.
Oats	1843.0 "
Linseed oil meal	224.0 "
Hay	3500.0 "

#38. (One animal)

Project started January 1, 1946 - terminated August 15, 1946

Corn	1896.0 lbs.
Oats	90.0 "
Oil meal	83.0 "
Tarkio	83.0 "
Salt	11.0 "

#38. (Two animals)

Project started January 1, 1946 - terminated August 15, 1946

Corn	4041.0 lbs.
Oats	220.0 "
Oatmeal	156.0 "
Tarkio	156.0 "
Mixed hay	750.0 "
Salt	22.0 "

#94. (Three animals)

Project started January 23, 1946 - terminated August 15, 1946

Corn	6985.0 lbs.
Oats	6985.0 "
Calfmeal	950.0 "
Pea silage	1350.0 "
Hay	1002.0 "
Straw	680.0 "

Forty-seven steers owned and fed under supervision of Mr. Lester Bookey were slaughtered on August 15, 1946, with the following history: The cattle were born in New Mexico in 1944 and by the spring of 1945 the average weight was 400 lbs. The animals grazed on grama grass and buffalo grass in New Mexico till winter and were then placed on wheat till April 1, 1946, when they averaged 686 lbs. On April 1, 1946, they

were shipped to Iowa where they were put on a blue grass pasture and fed ground ear corn for 60 days. The animals were then turned into dry lot for 60 days where they were fed corn silage, ground ear corn, chopped clover hay (new) and 1½ lbs. linseed meal per animal per day, plus salt. All the animals were between 2½ - 3 years of age and were in prime condition. Feed and water were removed from both the 4H cattle and the Bookey cattle at 3:00 o'clock in the afternoon of the previous day prior to slaughter. The animals were killed by a blow on the head and bled by way of the jugular vein. Both superficial and deep samples, usually one centimeter square, were taken from the peripheral and central portions of the livers. The specimens were immediately placed in the various fixatives employed and returned to the laboratory at Iowa State College for embedding, sectioning and mounting.

B. Cytological and Histochemical Methods

1. General fixative

For a general fixative, a 10 per cent solution of neutral formalin (equal to 4 per cent of formaldehyde) was used. The following stain techniques as described by Mallory (1938) were routinely employed: Harris' hematoxylin and eosin, Heidenhain's hematoxylin, Weigert's elastic tissue stain and Van Gieson's connective tissue stain. Knoefff's (1936) stain for collagenous connective tissue fibers and reticulum was also used.

2. Mitochondrial techniques

For mitochondrial studies, Regaud's technique as modified by Deane (1942) was followed. The tissue was fixed in 10 per cent neutral formalin for 24 hours and then post-chromed in 3 per cent potassium bichromate for three days at 37.5° C., washed for 24 hours in running water, dehydrated, imbedded in 56° C. paraffin, sectioned at 4 microns, mounted and stained by Heidenhain iron-alum hematoxylin. Some sections were counterstained with Babe's Aniline safranine following the technique of Mallory (1938).

Duplicate samples were also fixed in Bensley's (1938) acetic osmic bichromate solution for 24 hours, transferred directly into 50% alcohol, dehydrated in the usual manner and cleared in oil of cedarwood or dioxan Mossman (1937) and embedded. Sections were cut at 4 microns and mounted. The mitochondria appeared as red granules in those sections counterstained with Altmann's acid fuchsin-picric acid stain as described by Bowen (1928). Fat appeared as black droplets or granules in those tissues fixed in Bensley's acetic osmic bichromate solution.

3. Golgi apparatus techniques

For the demonstration of the Golgi apparatus, Nassonov's modification of Champy's fluid was used with post-osmication as suggested by Kolatchev and described by Bowen (1928). The blocks were osmicated at 35° - 37° C. for 3 to 7 days. The most favorable period was usually 5 to 6 days. At the conclusion of osmication, the blocks were washed thoroughly in running water for 24 hours, dehydrated by the alcohol series and

embedded as usual in paraffin. Sections were cut at 4 microns. Some sections were bleached by the hydrogen peroxide or the potassium permanganate-oxalic acid method as described by Bowen (1928). After bleaching, some sections were counterstained with Altmann's acid fuchsin-picric acid stain as modified by Hirschler (1915) and described in detail by Gatenby (1919) and by Bowen (1928). When counterstained with acid fuchsin, the mitochondria appeared red and the Golgi apparatus remained black.

4. Glycogen technique

For the demonstration of glycogen, the tissue was fixed in absolute alcohol, placed in xylol-xylol-paraffin and embedded in paraffin as recommended by Bensley (1938). The sections were cut at 4-6 microns and stained with Best's carmine following the staining methods of Mallory (1938).

5. Fat techniques

For the demonstration of fat, the tissue was fixed in 10 per cent neutral formalin for 48 hours and embedded in gelatin as described by Zwemer (1933) and modified by Deane (1942). The blocks were sectioned at 10-15 microns using the freezing microtome. Sections were fixed on the slide by the use of 1% gelatin and stained with sudan IV as described by Mallory (1938). A rapid fat stain employing oil red "O" was also used following the technique of Proescher (1927) as well as the Nile blue sulfate stain introduced by Smith (1907-1908). Sudan black was also used as described by Baker (1944).

6. Phosphatase techniques

For the histochemical demonstration of phosphatases, the methods of Gomori (1939, 1941a, 1941b) as modified by Deane and Dempsey (1945) were followed. Tissues were fixed in cold (0°C) acetone or cold (0°C) 80% alcohol for 24 hours. When acetone fixation was employed, the tissues were cleared through cedar oil followed by benzene or through benezene alone and then embedded in paraffin at 56-58°C. The tissues were never allowed to remain in the paraffin for more than three hours as a maximum. Following fixation in chilled alcohol 80%, the tissues were changed to 90% alcohol, to 95% alcohol, to absolute and cleared through cedar oil and several changes of benzene and embedded in paraffin. The blocks were sectioned at 4 and 5 microns, and the sections mounted by the use of Mayer's (1883) albumin-glycerine mixture. After deparaffinization, the sections were incubated in buffered solutions containing sodium glycerophosphate and either lead or calcium ions. The solutions were buffered either with acetate or sodium barbital depending upon the pH desired. All solutions were checked immediately before use with the aid of a Coleman pH Electrometer and adjusted for alkaline (pH 9.3) and acid (pH 4.8) phosphatases. The sections were incubated routinely for 2,8, 24,48,72,110, and 168 hours. Following incubation, the sections were dehydrated, cleared and mounted in the usual manner. The sites of phosphatase activity were made microscopically visible by converting the precipitated phosphatase into brown sulfides as described by Gomori

(1941a, 1941b). Control sections prepared by omitting the substrate in the incubating solutions were run on every section in order to reveal any possible preformed insoluble native phosphatases. The general validity of Gomori's methods for the demonstration of the intracellular distribution of phosphatases has recently been supported by Emmel (1946).

IV. RESULTS

A. Macroscopic Findings

Macroscopically, the normal bovine liver was reddish-brown in color, although in animals advanced in pregnancy and in fat animals, the color tended towards a yellowish-brown. The bovine liver presented but one large lobe, and two indistinct smaller caudate and papillary lobes. Its average weight varies between 10-12 lbs. according to Sisson and Grossman (1938).

Macroscopically, the typical telangiectatic liver appeared normal in size, weight, and shape. The lesions, which were purplish-black to reddish-blue in color varied in size from a pin-point to a centimeter or more. Usually, the involved area was rather definitely demarkated from the surrounding normal hepatic parenchyma; however, the very small purplish pin-point areas could be easily overlooked unless good lighting prevailed and careful observations were made. Upon incision, the lesions exhibited a mesh-like or net-like structure which was filled with blood. The lesions frequently were distributed throughout the entire organ or at times were confined largely to a single section or portion of the liver. The remainder of the hepatic parenchyma appeared normal. No cirrhosis, hypertrophy, or evidence of fascioliasis was noted.

Macroscopically, the sawdust livers appeared normal in size,

weight, and shape. The lesions were pinkish-white to yellow-grey in color and variable in their distribution. They were, at times, distributed throughout the entire liver or they were often found only in irregularly scattered areas. The lesions varied from pin-point size to one-half centimeter or more. The non-involved areas appeared normal in color and consistency. Upon incision, the cut surfaces approximated one another. The surface of the organ was smooth and no cirrhosis was evident by palpation.

Telangiectatic and sawdust lesions were frequently noted in the same liver. These lesions, which were variable in their extent and distribution, exhibited the combined appearance of the previously described telangiectatic and sawdust livers.

B. Microscopic Findings

1. Phosphatase

In the normal bovine liver, alkaline glycerophosphatase (pH. 9.5 to 9.0) was demonstrated as a dark brown to black precipitate. Alkaline phosphatase was observed to be present in the cytoplasm and nuclei of the hepatic cells, the bile canaliculi, the endothelial lining cells of the sinusoids and blood vessels, the cells of the bile ducts, and in variable amounts in the leukocytes and lymphocytes (Fig. 1). Dense concentrations of precipitate were noted in the nuclei of the normal liver cells throughout the hepatic

lobule. Intense activity was noted in the karyoplasm, in the nuclear membrane, nucleoli, and in the chromatin material (Fig. 2). Slight alkaline phosphatase activity was noted at the cell membrane; however, often this was obliterated or overshadowed by the intense alkaline phosphatase activity in the bile canaliculi (Fig. 3). A moderate concentration of enzyme was observed in the cytoplasm of all the hepatic cells. However, enzymatic activity was more intense in the portal canal area and in the peripheral cells of the lobule (Fig. 4). Extensive enzymatic activity in the peripheral portion of the lobule was also noted in the lining cells of the dilated sinusoids (Fig. 5). The bile canaliculi and the hepatic nuclei of the hepatic cells stained intensely when the tissues were incubated in a glycerophosphate solution at pH. 9.5 for 2 hours. The reaction was very slight in the cytoplasm of the cells with a 2 hour incubation period. Consequently, one obtained a distinct contrast between the cytoplasm and the bile canaliculi at 2 hours incubation. The contrast was gradually diminished as the incubation time was increased due to increased enzymatic activity in the cytoplasm. Occasionally, small foci of leukocytes and lymphocytes appeared in sections taken from so-called normal bovine livers (Fig. 6).

Figure 1.

Photomicrograph* of an hepatic lobule illustrating the distribution of alkaline phosphatase in the normal bovine liver. Gomori's technique. X 100, Specimen # 6d.

*All photomicrographs were taken from bovine liver specimens. For detailed description see "Results".

Figure 1. Distribution of alkaline phosphatase in the normal bovine liver.

Figure 2.

Note the intense enzymatic activity in the karyoplasm, the nucleoli, and in the chromatin material. Gomori's technique. X 400, Specimen # 6e.

Figure 2. Cytological distribution of alkaline phosphatase in the normal bovine liver.

Figure 3.

Reaction greatest in the nuclei and in the bile canaliculi. Gomori's technique, X 400. Specimen # 5e.

Figure 3. Alkaline phosphatase activity in the bile capillaries of the normal liver.

Figure 4.

Enzymatic activity most intense in the portal canal area and in the peripheral cells of the hepatic lobule. Gomori's technique. X 100, Specimen # 5b.

Figure 4. Alkaline phosphatase activity in the peripheral portion of the hepatic lobule.

Figure 5.

Note intense reaction in the walls of the hepatic artery and bile duct, and lining cells of the dilated sinusoids. X 400, Specimen # 5c.

Figure 5. Enzymatic activity in portal canal area.

Figure 6.

Note the focus of leukocytes and lymphocytes that were present in a section taken from an apparently normal bovine liver. X 400, Specimen # 4b.

Figure 6. So-called normal bovine liver revealing a focus of leukocytes and lymphocytes.

At pH 4.7 to 5.0, less phosphatase activity occurred in the normal bovine livers than in the alkaline range. A slight reaction was noted in the cytoplasm of the hepatic cells and a moderate acid phosphatase activity was noted in the karyoplasm, the nuclear membrane, the nucleoli, and the chromatin. Intense enzymatic activity was usually always noted in the bile contained within the bile canaliculi (Fig. 7). A slight variation in enzymatic activity was observed in various lobules; however, the variation was in degree only, and in nearly all instances the greatest enzymatic activity was found in the peripheral portion of the lobule and in the portal canal areas. Acid phosphatase activity in the nucleus was quite constant throughout the lobule and section. The reaction was always more marked in the nucleus than in the cytoplasm and always intensive in the bile canaliculi and Kupffer cells (Fig. 8). The cells lining the bile ducts exhibited moderate enzymatic activity, whereas those lining the arterioles revealed a more marked reaction.

In the telangiectatic bovine liver, alkaline glycerophosphatase (pH 9.5 to 9.0) was demonstrated in similar sites and exhibited similar activity to that observed in the normal bovine liver. The intensity of the reaction in the cytoplasm, in the nuclei and throughout the hepatic lobule resembled that noted above in the normal liver sections. The typical telangiectatic section exhibited cavernous sinusoidal areas engorged with whole blood (Fig. 9). The hepatic cords that were adjacent

to the dilated sinusoids were greatly compressed but revealed average enzymatic activity. Alkaline phosphatase activity was observed in the cytoplasm of all the cells, but the most marked reaction was noted in the hepatic nuclei and Kupffer cells, and sinusoidal lining cells in the telangiectatic areas (Fig. 10).

Figure 7.

Reaction greatest in the bile within the bile canaliculi and in the small bile ducts. An intense reaction also occurred in the walls of the arterioles and in the Kupffer cells. X 400, Specimen # 5d.

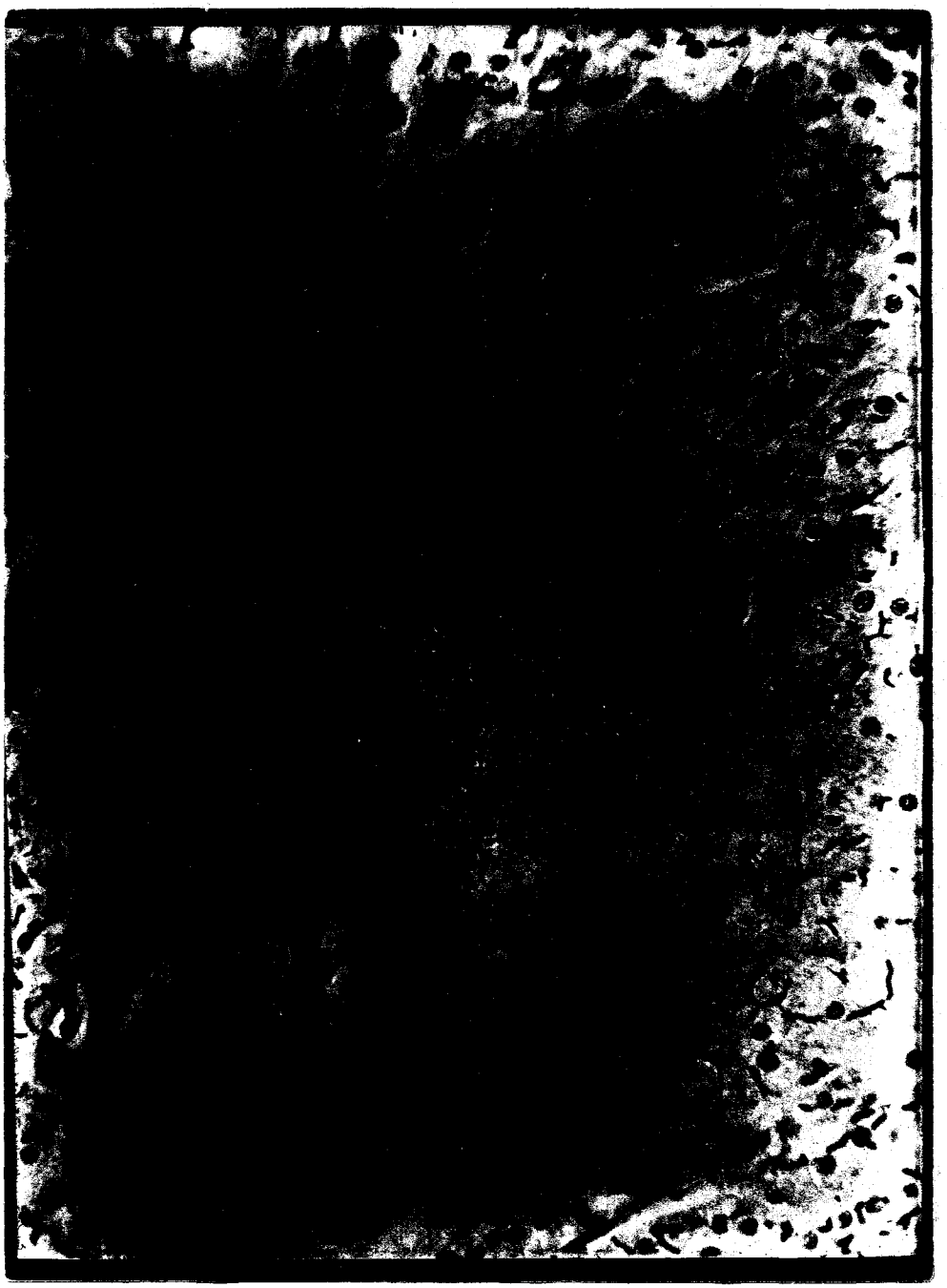

Figure 7. Enzymatic activity within the bile canaliculi.

Figure 8.

The reaction was always more marked in the nucleus than in the cytoplasm and always intensive in the bile canaliculi and Kupffer cells. X 100, Specimen # 4a.

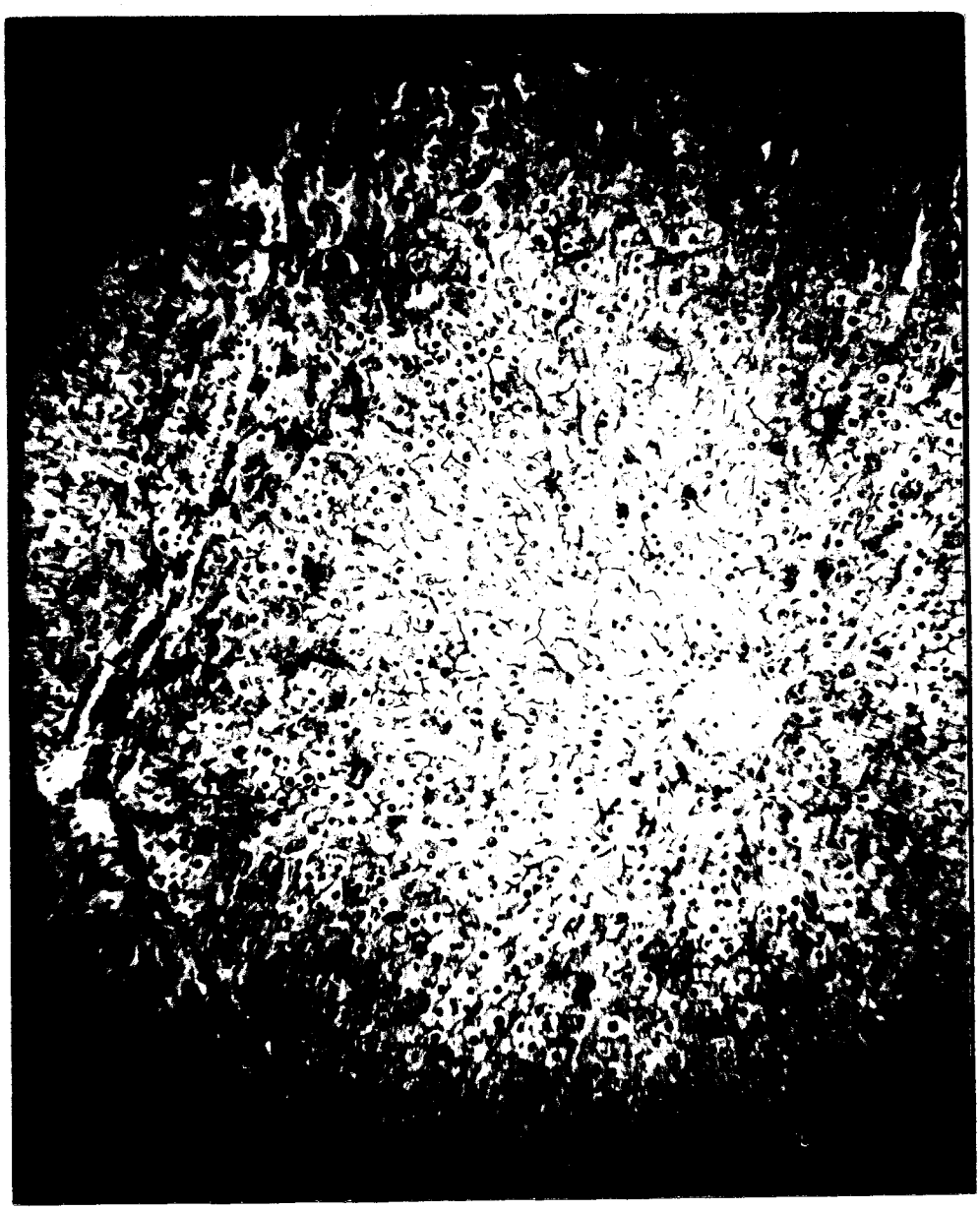

Figure 8. Acid phosphatase activity in the hepatic lobule of a normal bovine liver.

Figure 9.

The alkaline glycerophosphatase, in the telangiectatic liver, was demonstrated in similar sites and exhibited similar activity to that observed in the normal bovine liver. Note that this section revealed both a telangiectatic area and a sawdust focus. X 190, Specimen # 4350.

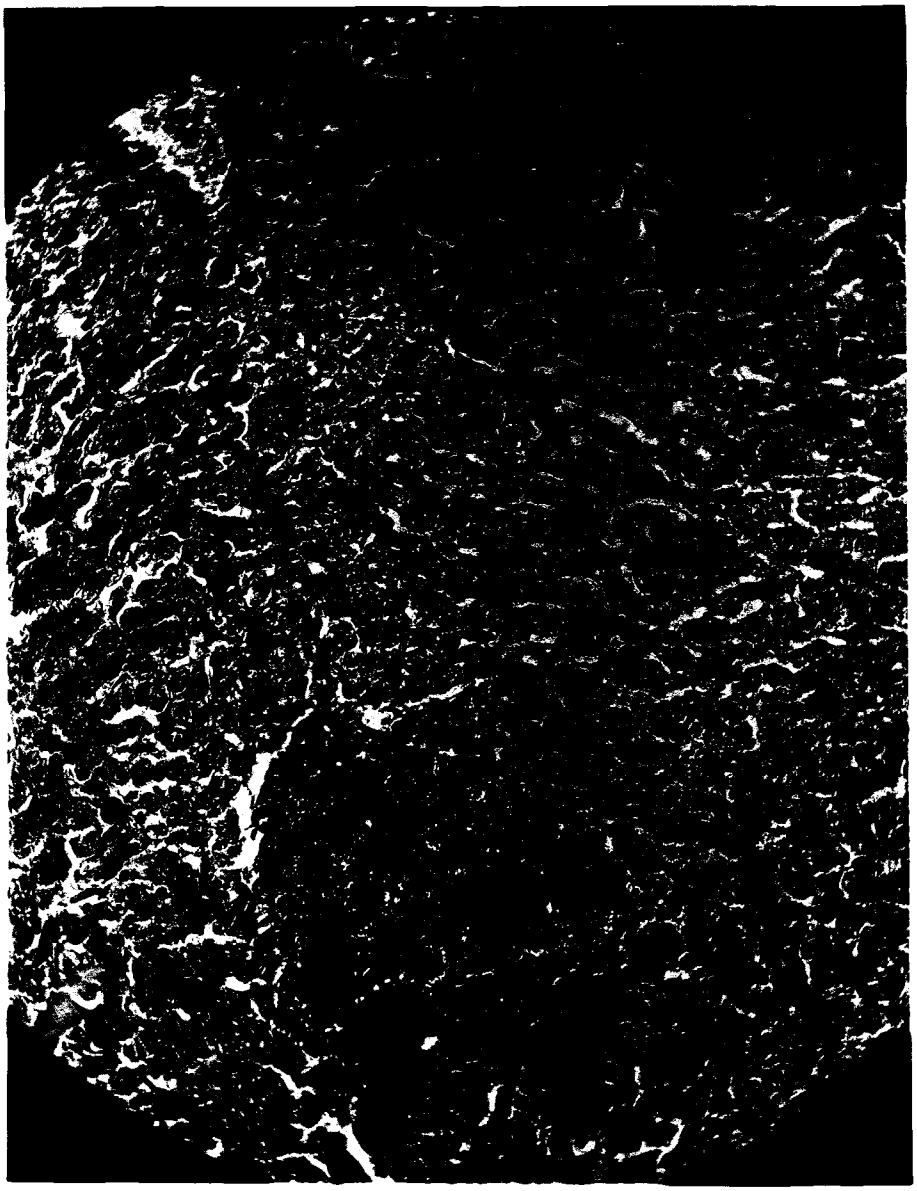

Figure 9. Alkaline phosphatase activity in the telangiectatic bovine liver.

Figure 10.

Note the average enzymatic activity in the cytoplasm of all the cells, the nuclei, and surrounding hepatic parenchyma contiguous to the telangiectatic area. The erythrocytes appeared as small white spheres within the cavernous sinusoidal areas. X 450, Specimen # 4351.

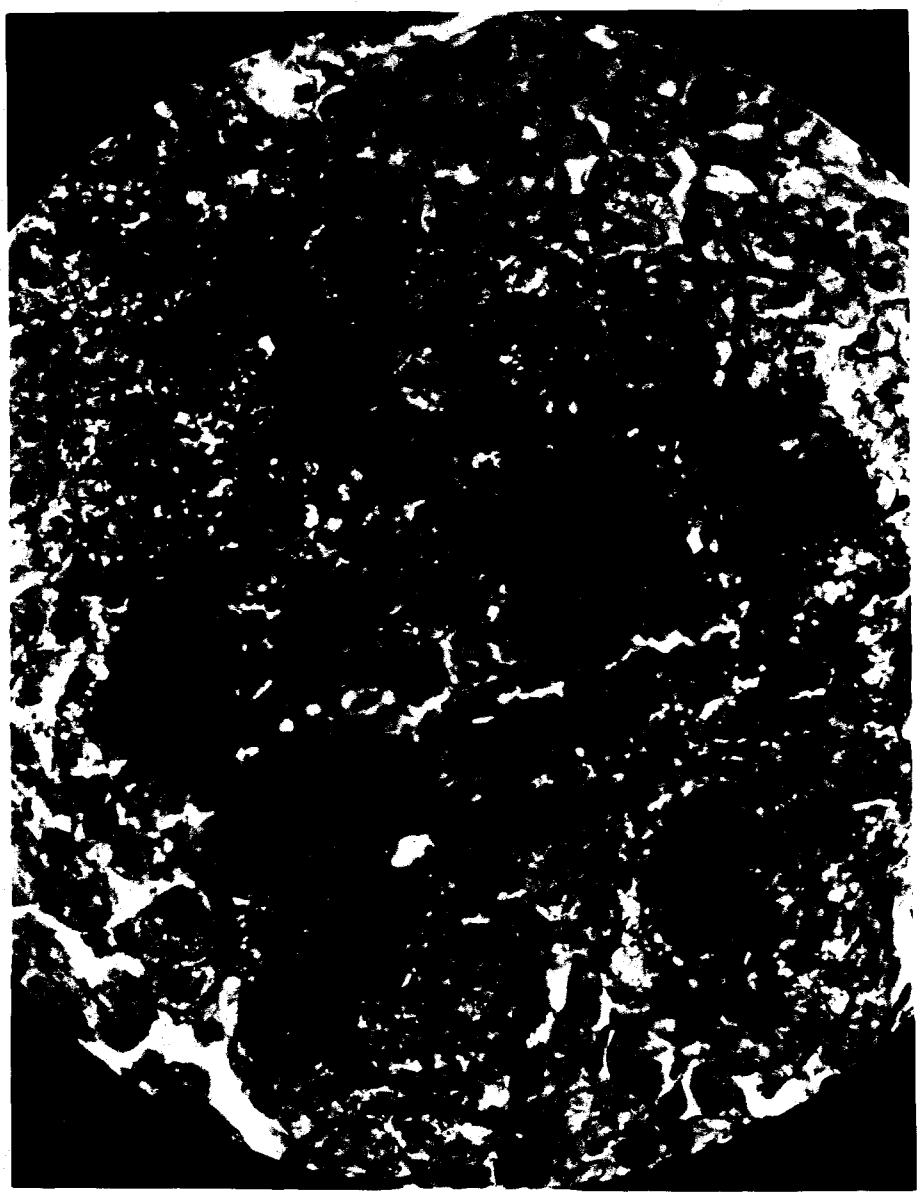

Figure 10. Enzymatic activity in the hepatic parenchyma adjacent to a telangiectatic area.

The distribution of acid phosphatase in the telangiectatic livers was similar to that described for the 9.0 alkaline range. However, the concentration or activity of the enzyme in the acid range was weaker. Only a very slight reaction was observable in the cytoplasm of the hepatic cells. The reaction was also less marked in the nuclei in the acid range than was noted in the alkaline range.

The bovine livers exhibiting a focal hepatitis (sawdust livers) revealed a very intense concentration of both acid and alkaline phosphatase both in and surrounding the foci of necrosis (Figs. 11, 12). The sinusoids and hepatic cords within the necrotic area were completely engorged with a black precipitate representing a very marked concentration of enzyme (Fig. 13). The leukocytes, lymphocytes, sinusoidal lining cells and Kupffer cells of the sinusoids surrounding the involved area also exhibited intensive enzymatic activity (Figs. 14, 15). The hepatic cells contiguous to the involved area were hypertrophied and vacuolated (Fig. 16). The nuclei and bile canaliculi, however, appeared normal in the adjacent surrounding tissue. However, a normal distribution and concentration of alkaline phosphatase was observed in the hepatic lobules, the cytoplasm and the nuclei throughout the sections except within, and contiguous to, the necrotic foci (Fig. 16). A normal distribution of acid phosphatase was also observed in those livers exhibiting a focal hepatitis excluding the necrotic areas. However, in all sites the acid enzyme reacted less

strongly than was observed in the alkaline range. One often observed simultaneously, telangiectatic and sawdust areas in the same section (Fig. 9). A study of approximately 300 sections as to cytological concentration and distribution of alkaline and acid phosphatase in the normal, sawdust and telangiectatic bovine liver was graphically recorded in Figs. 17 and 18 and Tables 1, 2, 3, 4, 5 and 6.

Figure 11.

Note the intense concentration of alkaline phosphatase both in and surrounding the focus of necrosis. X 190, Specimen # 4354.

Figure 11. Enzymatic activity in a bovine liver exhibiting a focal hepatitis (sawdust liver).

Figure 12.

Note that the increased enzymatic activity was due to an increase in number of leukocytes and lymphocytes in the delimited area. X 100, Specimen # 192.

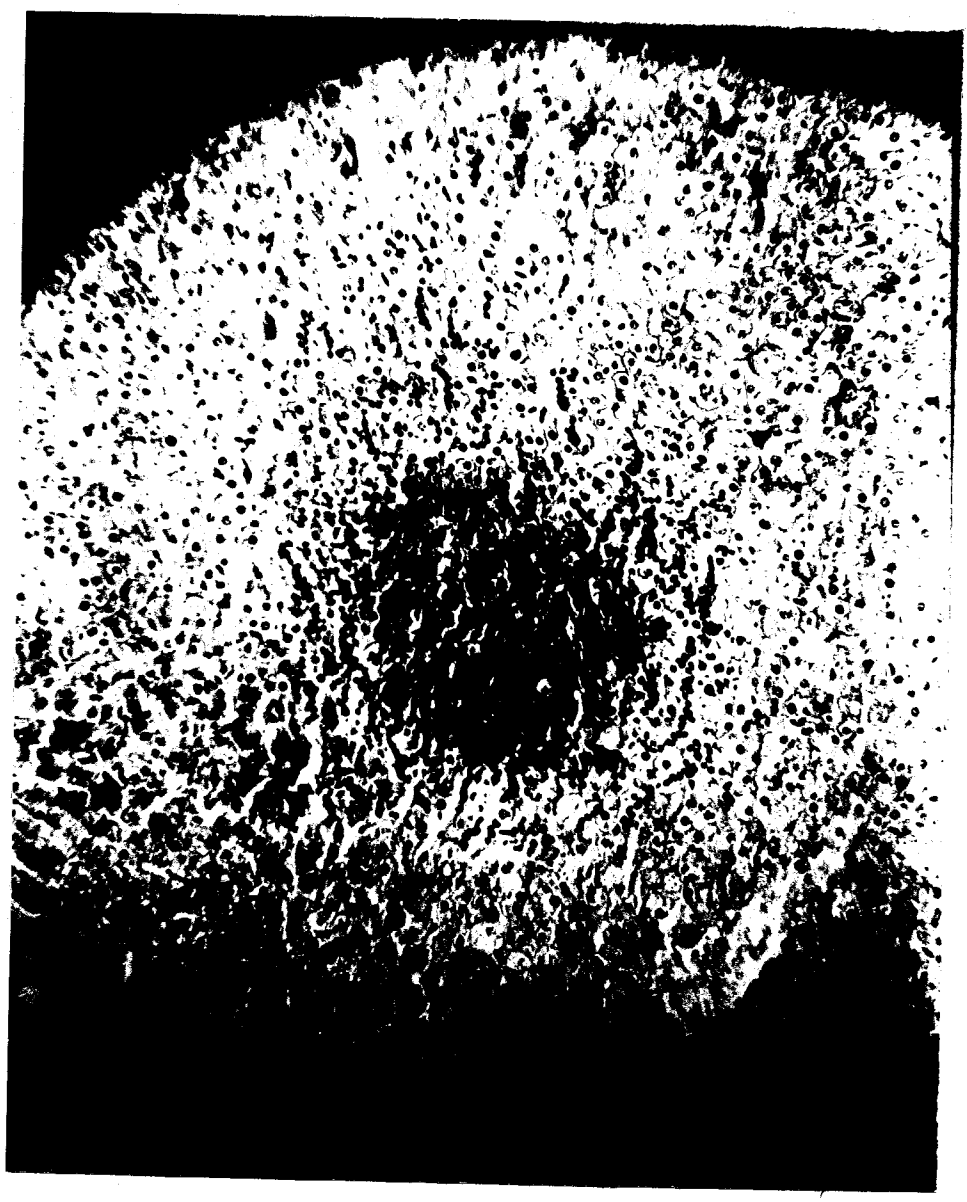

Figure 12. Enzymatic activity in a bovine liver exhibiting a focal hepatitis (X 100).

Figure 13.

Intense alkaline phosphatase reaction noted in the dilated sinusoids and adjacent hepatic cords within the necrotic focus. X 100, Specimen # 22b.

Figure 13. Enzymatic activity within a necrotic focus.

Figure 14.

Note the generalized enzymatic activity of the leukocytes, lymphocytes, sinusoidal lining cells and Kupffer cells of the sinusoids. X 400, Specimen # 16b.

Figure 14. Enzymatic activity within and adjacent to a focal necrosis.

Figure 15.

Note the sharp line of demarkation between the focal necrotic area and the contiguous normal hepatic parenchyma. Reaction particularly intense in the luekocytes, and in the wall of the bile duct. X 100, Specimen # 14a.

Figure 15. Enzymatic activity within the portal canal area of a liver exhibiting a focal hepatitis.

Figure 16.

Note that there is definitely an altered enzymatic picture within the hepatic cords of an area undergoing cellular changes characteristic of beginning necrosis. The enzymatic picture clearly delimits the area from the surrounding normal hepatic parenchyma. X 190, Specimen # 4352.

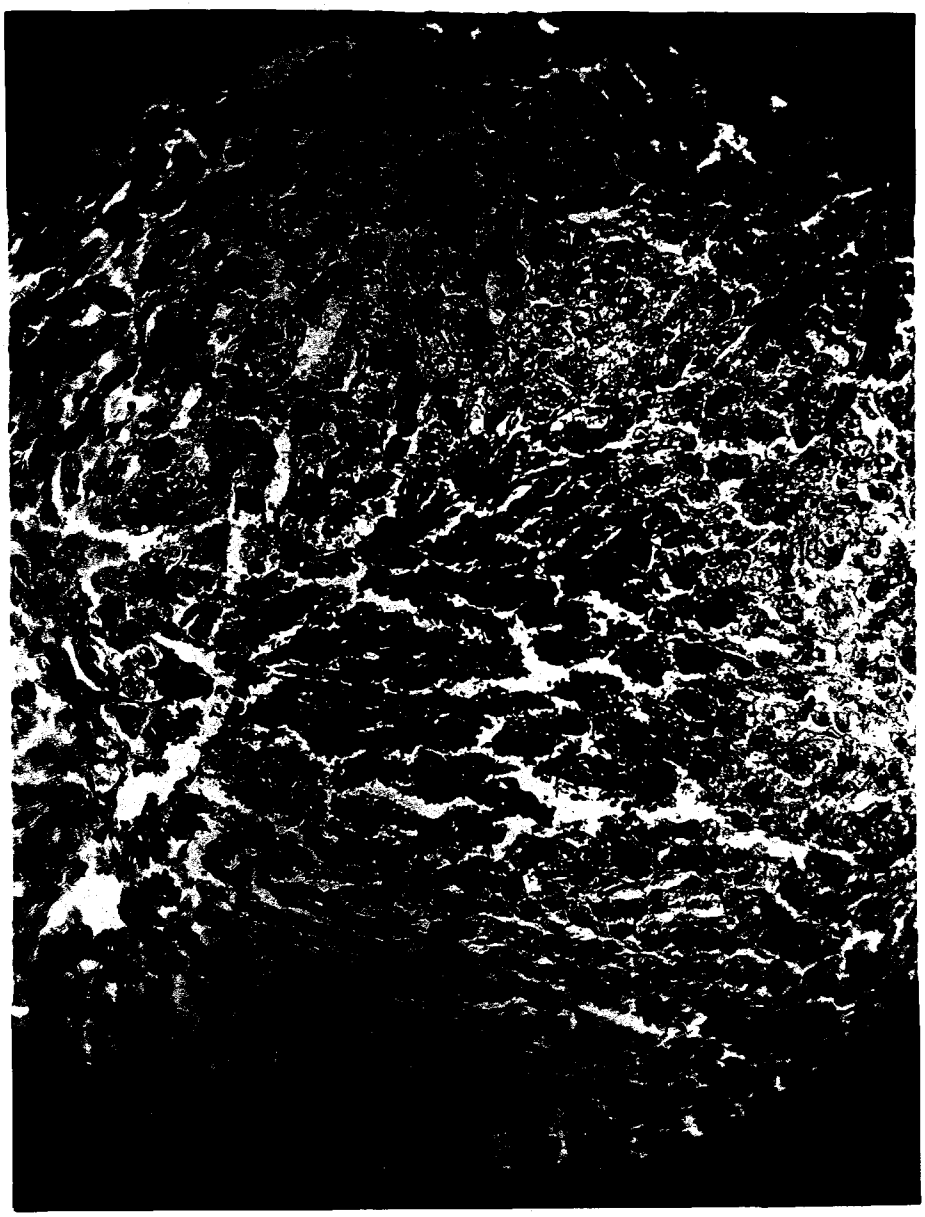

Figure 16. Enzymatic activity in the hepatic cords of an area undergoing cellular changes characteristic of beginning necrosis.

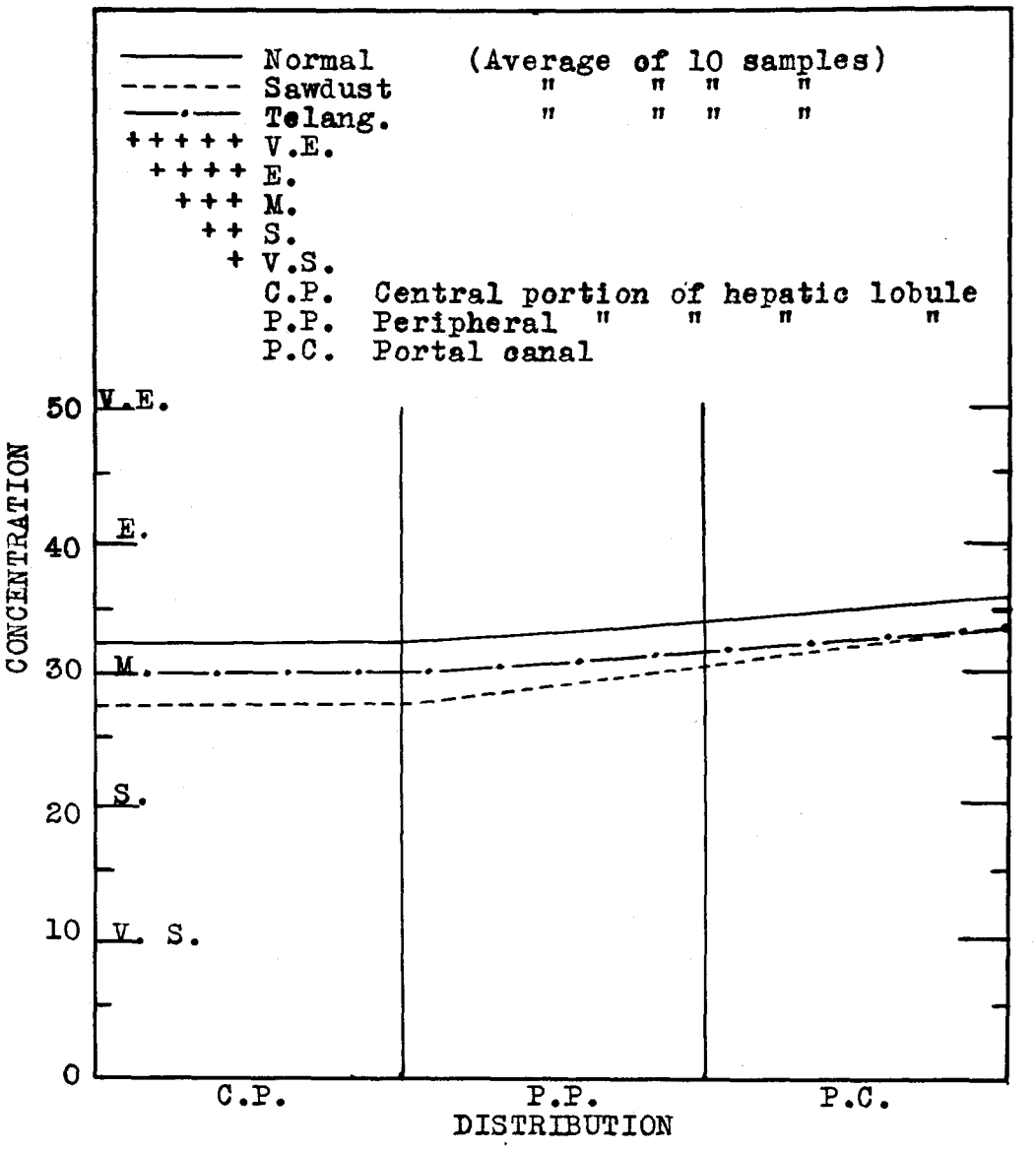

Fig.17. Distribution and concentration of alkaline phosphatase in the hepatic lobule*

V.S. Indicates that scattered cells in the lobule exhibited very slight enzymatic activity
V.E. Indicates that all cells in the hepatic lobule exhibited intense enzymatic activity
* The plus signs have been converted to a numerical basis

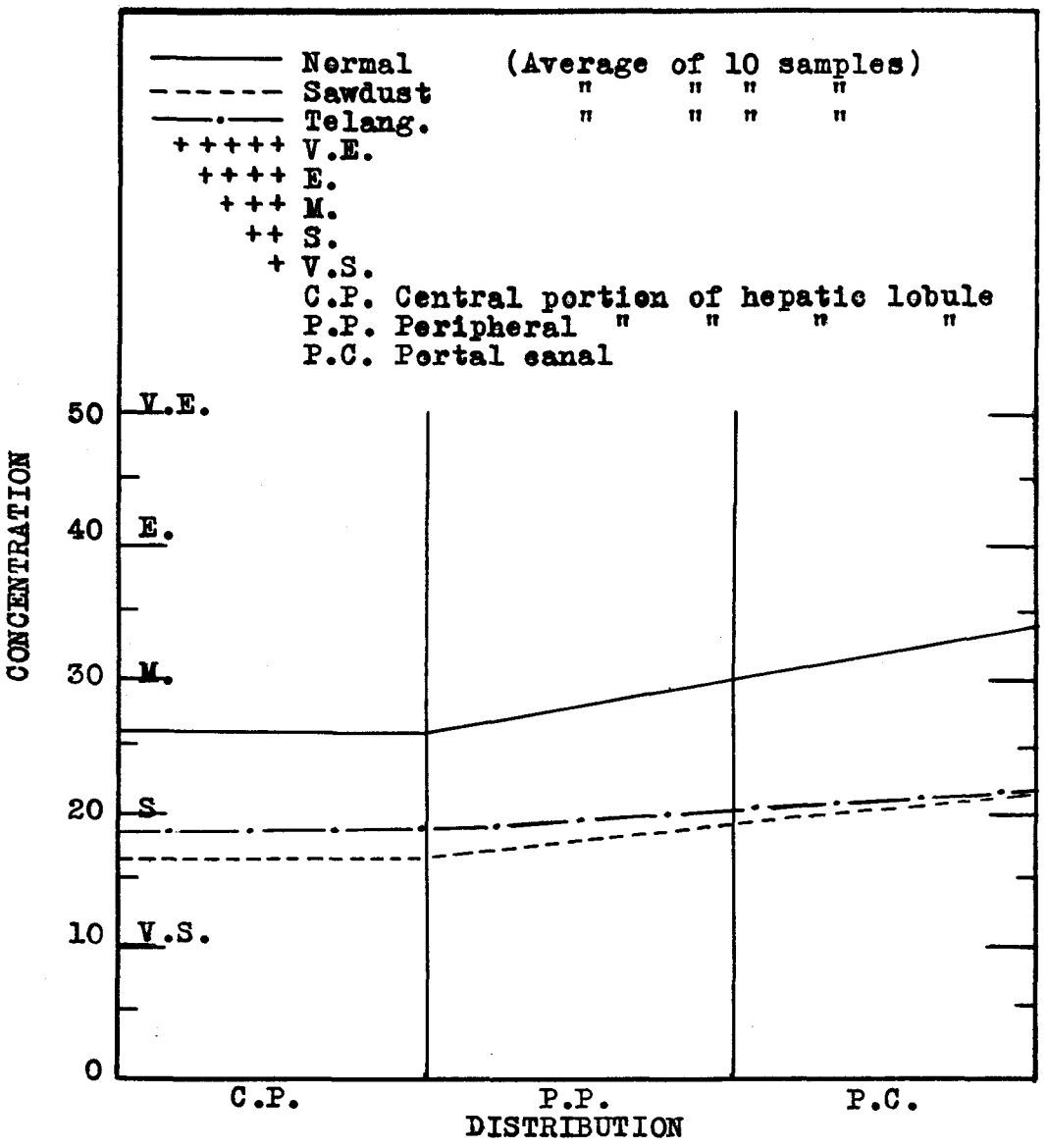

Fig.18. Distribution and concentration of acid phosphatase in the hepatic lobule*

V.S. Indicates that scattered cells in the lobule exhibited very slight enzymatic activity
V.E. Indicates that all cells in the hepatic lobule exhibited intense enzymatic activity
* The plus signs have been converted to a numerical basis

Table 1

Cytological concentration and distribution of alkaline phosphatase in "normal" bovine livers

	Cytoplasm	Cell Membrane	Karyoplasm	Nuclear Membrane	Nucleoli	Chromatin	Bile Canaliculi
1.	++	+	+++	++++	++++	+++	+++
2.	++	+	+++	++++	++++	+++	++
3.	++	+	+++	++++	+++	+++	++
4.	++	+	+++	++++	++++	++++	++
5.	+++	+	+++	++++	++++	++++	++++
6.	++	+	+++	++++	++++	++++	+++
7.	++	+	+++	++++	++++	++++	+++
8.	+++	+	+++	++++	++++	++++	+++
9.	+++	+	+++	++++	++++	++++	+++
10.	++	+	+++	++++	++++	++++	+++

\+ Slight concentration +++ Extensive concentration
++ Moderate concentration ++++ Very extensive concentration

Table 2

Cytological concentration and distribution of alkaline phosphatase in "sawdust" (Focal hepatitis) bovine livers

	Cytoplasm	Cell Membrane	Karyoplasm	Nuclear Membrane	Nucleoli	Chromatin	Bile Canaliculi
13.	+++	+	++++	++++	++++	+++	+++
14.	+++	+	++++	++++	++++	+++	+++
15.	+++	+	++++	++++	++++	+++	+++
16.	+++	+	++++	++++	++++	+++	+++
17.	+++	+	++++	++++	++++	+++	++
18.	+++	+	++++	++++	++++	+++	++
19.	++	+	+++	+++	+++	++	++
20.	++	+	+++	+++	+++	++	+++
21.	++	+	+++	+++	+++	++	+++

\+ Slight concentration +++ Extensive concentration
++ Moderate concentration ++++ Very extensive concentration

Table 3

Cytological concentration and distribution of alkaline phosphatase in "telangiectatic" bovine livers

	Cytoplasm	Cell Membrane	Karyoplasm	Nuclear Membrane	Nucleoli	Chromatin	Bile Canaliculi
22.	+++	+	++++	++++	++++	++++	+++
23.	++	+	++++	++++	++++	++++	+++
24.	++	+	+++	++++	++++	+++	++
25.	++	+	+++	++++	+++	+++	++
26.	+	+	++	+++	+++	+++	++
27.	++	+	+++	++++	++++	+++	++
28.	++	+	+++	++++	++++	++++	++
29.	++	+	+++	++++	++++	++++	++
30.	+++	+	++++	++++	++++	++++	++
31.	++	+	+++	++++	++++	+++	++

\+ Slight concentration
++ Moderate concentration
+++ Extensive concentration
++++ Very extensive concentration

Table 4

Cytological concentration and distribution of acid phosphatase in "normal" bovine livers

	Cytoplasm	Cell Membrane	Karyoplasm	Nuclear Membrane	Nucleoli	Chromatin	Bile Canaliculi
1.	*	-	+	+	+	+	++
2.	+	-	++	++	++	++	+++
3.	++	-	++	++	++	++	+++
4.	+	+	++	++	++	++	+++
5.	+	+	++	++	++	++	+++
6.	+	+	++	++	++	++	+++
7.	+	+	++	++	++	++	+++
8.	+	+	++	++	++	++	+++
9.	+	+	++	++	++	++	+++
10.	*	-	+	+	+	+	++

\- Negative
* Very slight concentration
\+ Slight concentration
++ Moderate concentration
+++ Extensive concentration
++++ Very extensive concentration

Table 5

Cytological concentration and distribution of acid phosphatase in "sawdust" (Focal hepatitis) bovine livers

	Cell			Nuclear			Bile
	Cytoplasm	Membrane	Karyoplasm	Membrane	Nucleoli	Chromatin	Canaliculi
13.	*	-	*	*	*	*	++
14.	*	*	*	*	-	-	++
15.	*	*	*	*	-	-	++
16.	*	-	*	*	-	-	++
17.	*	+	+	++	+	+	+++
18.	+	+	++	++	++	++	+++
19.	*	+	+	++	+	+	+++
20.	*	+	+	+	+	++	+++
21.	*	+	+	++	++	+	+++

\- Negative
* Very slight concentration
\+ Slight concentration
++ Moderate concentration
+++ Extensive concentration
++++ Very extensive concentration

Table 6

Cytological concentration and distribution of acid phosphatase in "telangiectatic" bovine livers

	Cell			Nuclear			Bile
	Cytoplasm	Membrane	Karyoplasm	Membrane	Nucleoli	Chromatin	Canaliculi
22.	*	*	*	*	*	*	++
23.	*	*	*	*	-	-	++
24.	+	-	++	+++	++	++	*
25.	*	-	++	++	++	++	+
26.	*	-	*	+	+	+	-
27.	*	-	+	++	++	+	-
28.	*	-	+	++	++	+	-
29.	*	+	+++	+++	++	++	-
30.	*	+	++	++	++	++	+
31.	*	+	+	++	++	+	-

\- Negative
* Very slight concentration
\+ Slight concentration
++ Moderate concentration
+++ Extensive concentration
++++ Very extensive concentration

2. Mitochondria

It was observed, upon low power examination of sections made from macroscopically normal livers, that the cells about the peripheral portion of the hepatic lobule appeared darker in color than those centrally located (Fig. 19). Figure 20 represents the lighter appearing central region of the hepatic lobule whereas Figure 21 illustrates the darker appearing hepatic cells and cords in the portal canal area and peripheral portion of the hepatic lobule. High power examination (2000 x) of the central lobular area, revealed that the mitochondria were peripherally located in the hepatic cells (Fig. 22). Thus the peripheral location of the mitochondria within the cells imparted a lighter, vacuolated appearance to the cells located in the central portion of the hepatic lobule. However, it was observed that not all cells in all lobules consistently exhibited this definite zonal mitochondrial pattern. The mitochondria of the central zone may appear as well stained granules or may only be lightly stained or may apparently be absent (Fig. 23). The cells that were located about the portal canal and the peripheral portion of the lobule frequently appeared darker in color than those cells centrally located. High power examination (2000 x) revealed that the mitochondria were diffusely distributed as small spherical black granules throughout the cytoplasm of the hepatic cells (Fig. 24). Thus, the mitochondrial pattern produced a darker appearing hepatic cell in the peripheral portion of the hepatic lobule.

Figure 19.

The hepatic cells appeared darker in the peripheral portion of the lobule than those cells located centrally. Counterstained with safranine. X 100, Specimen # 1 F.

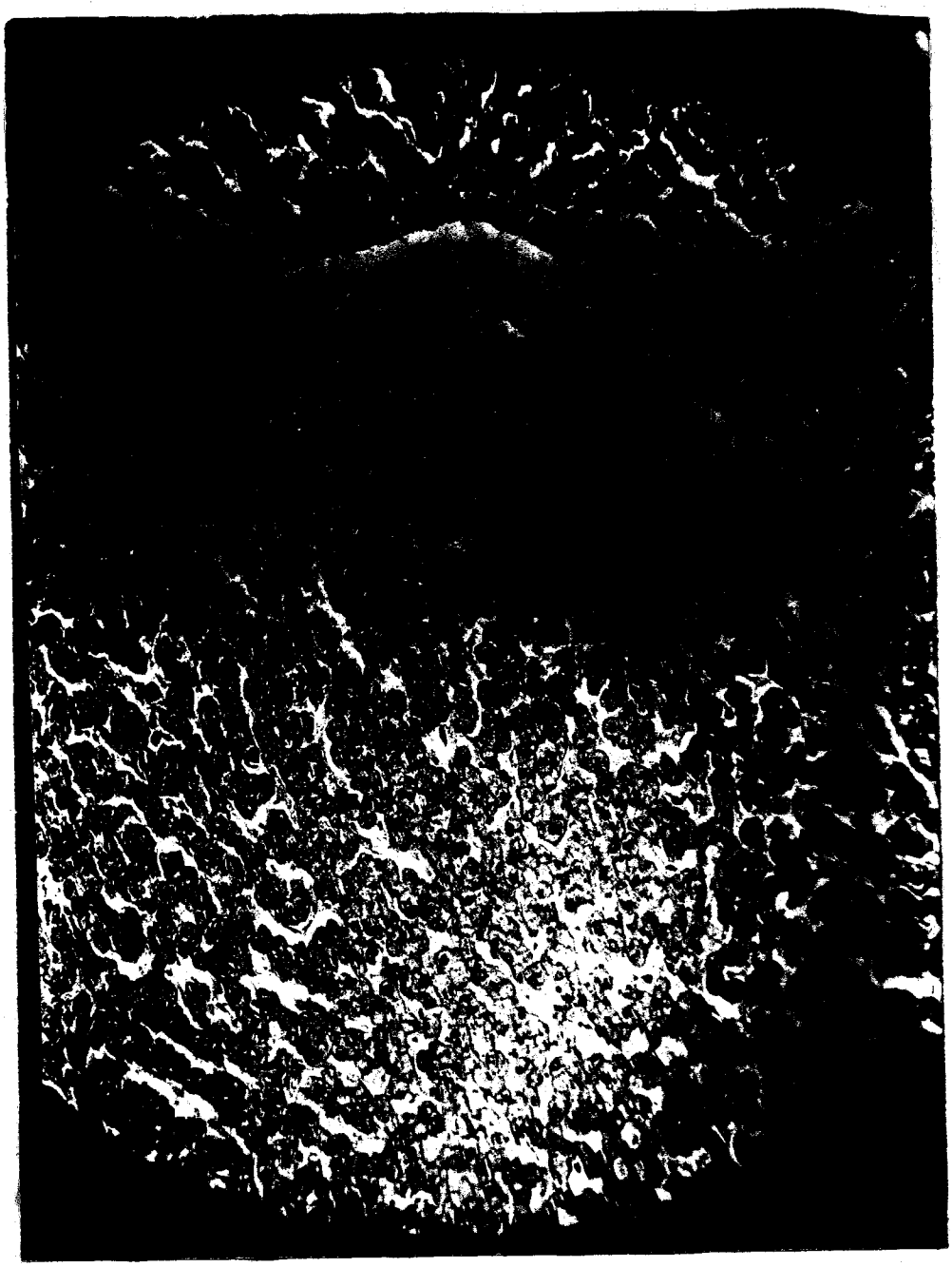

Figure 19. Mitochondrial distribution pattern in the hepatic lobule of a normal bovine liver.

Figure 20.

The cells centrally located appeared lighter in color than those located at the peripheral portion of the hepatic lobule. Counterstained with safranine. X 400, Specimen # 1 F.

Figure 20. Distribution of mitochondria in the hepatic cells located centrally in the hepatic lobule.

Figure 21.

Photomicrograph illustrates the darker appearing hepatic cells and cords observed in the portal canal area and peripheral portion of the hepatic lobule. The difference in density was due to the granular mitochondria which were diffusely distributed throughout the hepatic cells located peripherally in the lobule. Counterstained with safranine. X 400, Specimen # 1 F.

Figure 21. Distribution of mitochondria in the hepatic cells located peripherally in the hepatic lobule.

Figure 22.

High power examination revealed that the mitochondria were peripherally located in the hepatic cells. Thus, under low power, the cells located in the central portion of the hepatic lobule appeared lighter in color and vacuolated. Counterstained with safranine. X 2000, Specimen # 1 F.

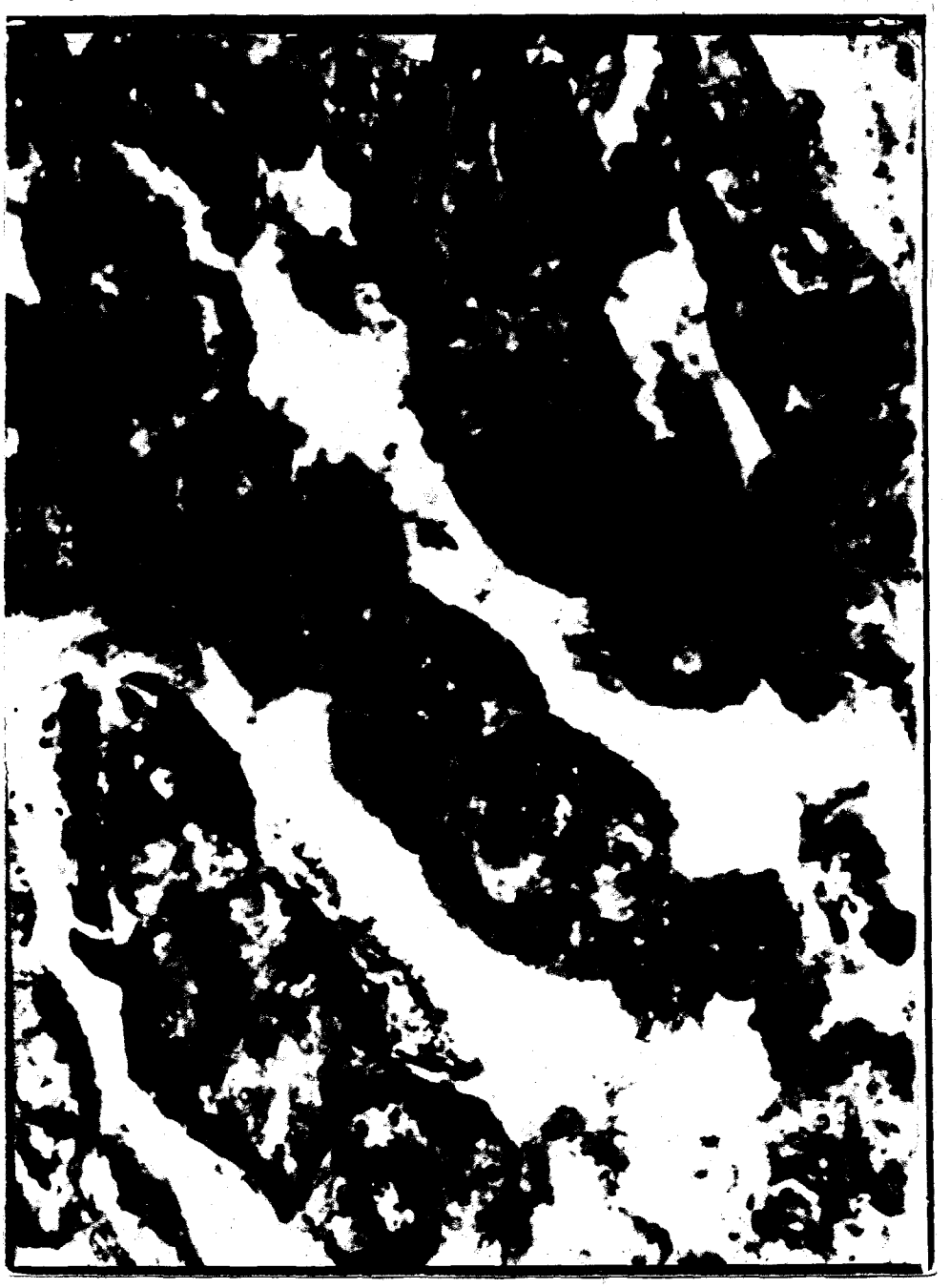

Figure 22. Mitochondrial distribution pattern within the hepatic cells centrally located in the hepatic lobule.

Figure 23.

The mitochondria of the central zone of the hepatic lobule may appear as well stained granules or may only be lightly stained or may apparently be absent. Not all cells exhibited a definite zonal mitochondrial pattern. X 2000, Specimen # 94.

Figure 23. Mitochondrial pattern in cells about central vein of normal bovine liver.

Figure 24.

The diffuse granular mitochondrial pattern produced a darker appearing hepatic cell in the peripheral portion of the hepatic lobule. Counterstained with safranine. X 2000, Specimen # 1 F.

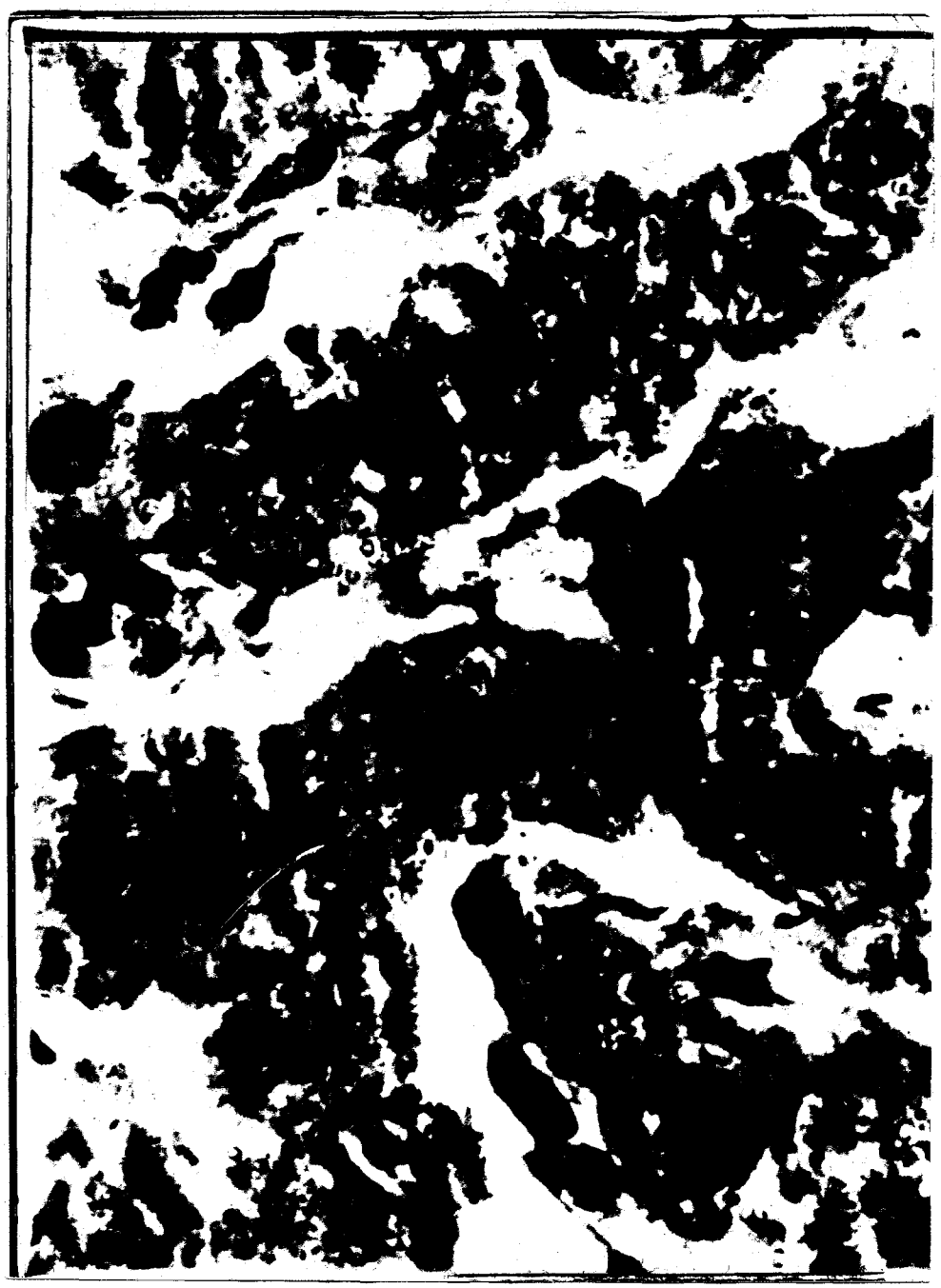

Figure 24. Mitochondrial pattern within the cells in the peripheral portion of the hepatic lobule.

Low power examination of sections made from livers exhibiting a focal hepatitis presented the following picture. The cytoplasm of the hepatic cells in and contiguous to the necrotic foci appeared very dark and conspicuous as compared to the cytoplasm of the surrounding normal hepatic cells (Fig. 25). Upon high power examination (2000 x), it was noted that the dark appearance of the cytoplasm was due to the clumping of the mitochondria into large osmiophilic masses. These large black masses at times completely obliterated all cellular detail (Figs. 26, 27). The hepatic cells adjacent to the necrotic foci presented small mitochondrial spheres diffusely scattered throughout the cell (Fig. 28).

The microscopic sections made from telangiectatic livers frequently revealed dilated sinusoids engorged with blood (Figs. 29, 30). However, it was noted, at higher magnification (2000 x), that the cytoplasm, the nuclei and the mitochondria presented a picture comparable to that previously described for the normal hepatic cell (Fig. 31). Telangiectatic lesions frequently presented cavernous, sinusoidal areas which at times were free of blood (Fig. 32). At both 400x and 2000x magnification, the mitochondria, cytoplasm, and nuclei appeared normal (Figs. 33, 34). No mitochondrial clumping was observed in the hepatic cells or cords lining the cavernous sinusoidal telangiectatic areas.

Figure 25.

The cytoplasm of the hepatic cells in and contiguous to the necrotic focus appeared very dark and conspicuous as compared to the cytoplasm of the surrounding normal hepatic cells. X 400, Specimen # 11 F.

Figure 25. Section from liver exhibiting a focal hepatitis and stained for mitochondria.

Figure 26.

The dark appearance of the cytoplasm noted in Figure 25 was due to the clumping of the mitochondria into large osmiophilic masses. X 2000, Specimen # 11 F.

Figure 26. Mitochondrial pattern in cells within a necrotic focus.

Figure 27.

Mitochondria clumped into large osmiophilic masses which at times completely obliterated all cellular detail. Counterstained with safranine. X 2000, Specimen # 11 F.

Figure 27. Clumping of mitochondria in the hepatic cells within a necrotic focus.

Figure 28.

The normal appearing mitochondrial spheres were diffusely scattered throughout the hepatic cells. The bile canaliculi were also well illustrated. X 2000, Specimen # 11 F.

Figure 28. Mitochondrial pattern within the hepatic cells adjacent to a necrotic focus.

Figure 29.

The telangiectatic area was largely located in the peripheral portion of the hepatic lobule. The dilated sinusoids were engorged with blood which appeared as black masses. Counterstained with safranine. X 100, Specimen # 20 F.

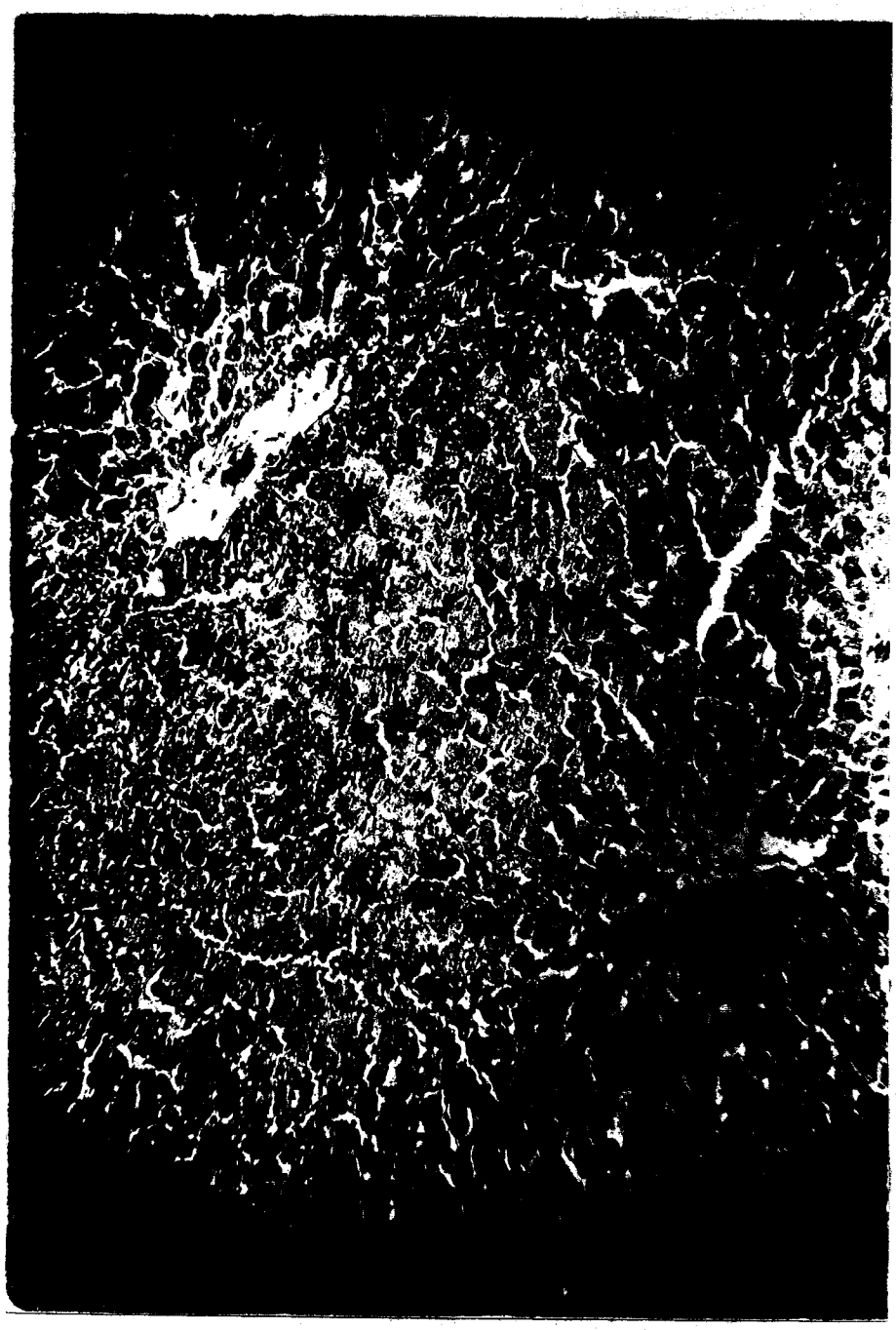

Figure 29. Photomicrograph of a telangiectatic area. (x 100)

Figure 30.

The cavernous sinusoidal areas were engorged with whole blood which appeared as black masses. Counterstained with safranine. X 400, Specimen # 20 F.

Figure 30. Photomicrograph of a telangiectatic area. (x 400)

Figure 31.

The hepatic cords and cells adjacent to the dilated cavernous sinusoids appeared normal. The blood elements contained within the sinusoids appeared as black masses. X 2000, Specimen # 20 F.

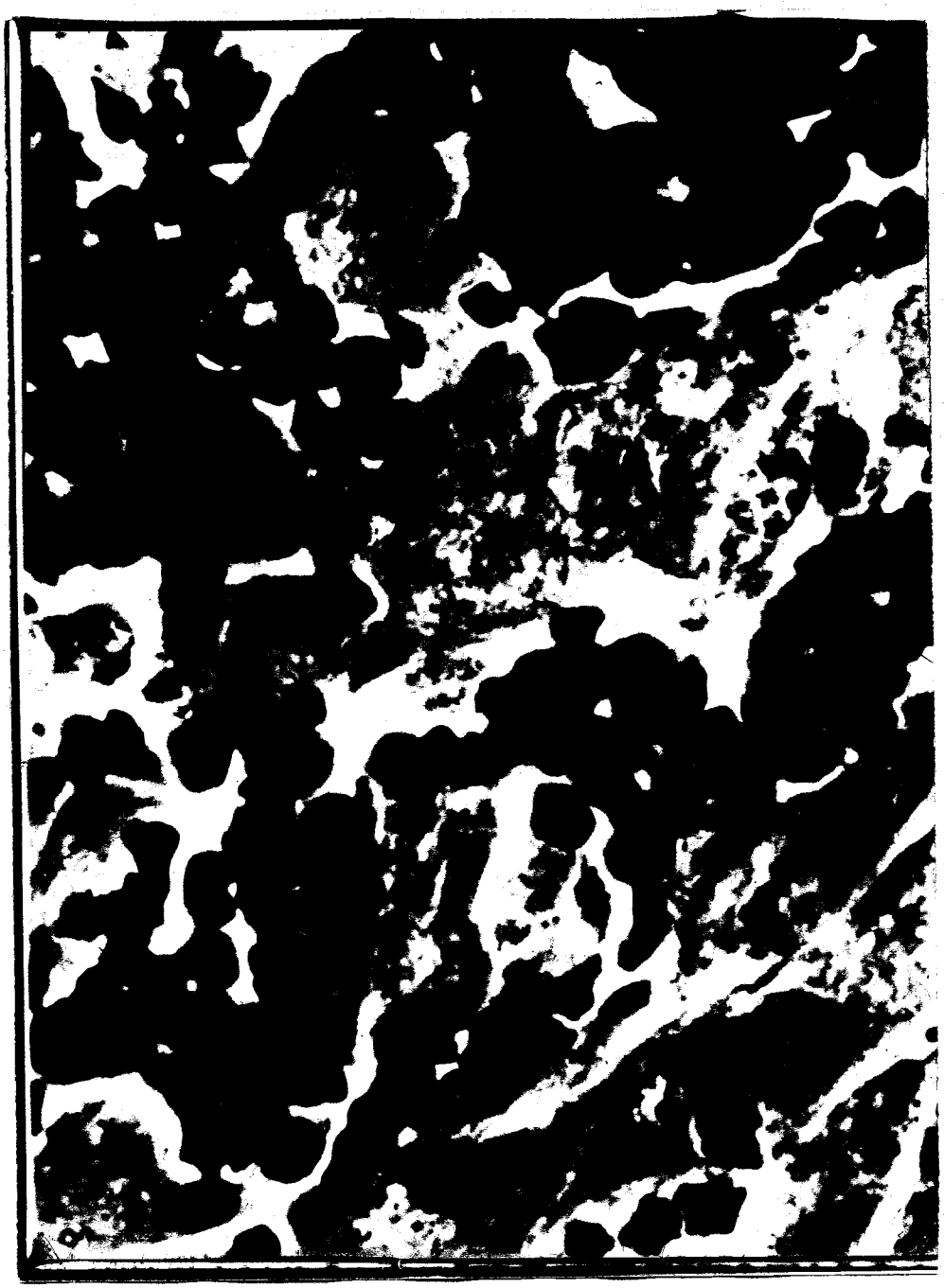

Figure 31. Photomicrograph of a telangiectatic area. (x 2000)

Figure 32.

The telangiectatic livers presented at times, sinusoidal areas which were free of blood. X 100, Specimen # 38.

Figure 32. Photomicrograph of a telangiectatic area. (x 100)

Figure 33.

The hepatic cords and cells contiguous to the cavernous sinusoidal areas appeared normal. X 400, Specimen # 38.

Figure 33. Photomicrograph of a telangiectatic area. (x 400)

Figure 34.

No mitochondrial clumping was observed in the hepatic cells or cords lining the cavernous, sinusoidal telangiectatic areas. The sinusoidal lining cells and hepatic cells appeared normal. X 2000, Specimen # 38.

Figure 34. Photomicrograph of a telangiectatic area. (x 2000)

3. Golgi apparatus

The Golgi substance, in most instances, was fused into the form of tight networks. These strongly osmiophilic networks were both juxtanuclearly and peripherally located (Figs. 35, 36). The relative amount of Golgi material demonstratable within the hepatic cell varied. A variation within the hepatic lobule as well as within the cell was noted. However, in many instances, the Golgi material seemed more massive, darker, and less fragmented in the peripheral cells of the hepatic lobule than in the cells of the central portion of the lobule. The mitochondria appeared red in those sections that were counterstained with acid fuchsin following the technique described under cytological procedures. The mitochondrial granules which were diffusely distributed throughout the hepatic cytoplasm could easily be differentiated from the Golgi apparatus which remained as black heavy strands forming a tightly woven network.

The Golgi apparatus was also readily observed in telangiectatic liver sections. The Golgi material of the hepatic cells in the telang. area was arranged in a continuous network (Fig. 37). This lace-like network imparted a fenestrated appearance to the cell (Fig. 38). A fenestrated appearance was also observable under low magnification (Fig. 39). The mitochondria were granular in form and similar in size and shape to those noted in the normal hepatic cell.

The Golgi substance in the cells contiguous to and within a sawdust focus was fused into a tight strongly osmiophilic mass which

at times completely obliterated all other cytoplasmic detail (Fig. 40). Figure 41 illustrates a typical sawdust focus with dilated sinusoids adjacent to the necrotic area. The hepatic cords were dark adjacent to the necrotic focus due to the clumping of the Golgi material (Figs. 42, 43). Frequently, the mitochondria and the Golgi substance could not be differentiated in those cells undergoing necrosis within the sawdust foci (Fig. 40).

Figure 35.

The Golgi material, demonstrable as osmiophilic networks, was both juxtanuclearly and peripherally located. X 2000, Specimen # 52.

Figure 35. The Golgi apparatus of the normal bovine liver cell.

Figure 36.

The relative amount of Golgi material demonstrable within the hepatic cell varied. The mitochondria appeared red in those sections counterstained with acid fuchsin and photographed as small dark bodies. Counterstained with acid fuchsin. X 2000, Specimen # 71.

Figure 36. The Golgi substance and mitochondrial granules within the normal hepatic cytoplasm.

Figure 37.

The Golgi material of the hepatic cells in the telangiectatic area was arranged in a continuous network, thus imparting a fenestrated appearance to the cell. Counterstained with acid fuchsin. X 2000, Specimen # 59.

Figure 37. The Golgi apparatus in telangiectatic livers.

Figure 38.

Photomicrograph illustrating fenestrated appearance of hepatic cells imparted by the Golgi network. Counterstained with acid fuchsin. X 2000, Specimen # 53.

Figure 38. The Golgi material and mitochondrial granules in telangiectatic livers.

Figure 39.

The Golgi material imparted a fenestrated appearance to the hepatic cells in the telangiectatic area. However, the mitochondria and Golgi substance were clumped into black osmiophilic masses in those hepatic cells contiguous to a necrotic focus. Counterstained with acid fuchsin. X 400, Specimen # 59.

Figure 39. The Golgi material in a bovine liver exhibiting both telangiectasis and a focal hepatitis.

Figure 40.

The Golgi substance in the cells contiguous to and within a necrotic focus was fused into a tight strongly osmiophilic mass. All cytoplasmic detail, at times, was completely obliterated. Counterstained with acid fuchsin. X 2000, Specimen # 53.

Figure 40. The Golgi substance in the bovine liver exhibiting a focal hepatitis.

Figure 41.

A necrotic focus (delimited by dotted lines) was seen surrounded by dilated sinusoids. The hepatic cells and cords contiguous to the necrotic focus were dark due to the clumping of the Golgi material and the mitochondria. Counterstained with acid fuchsin. X 400, Specimen # 53.

Figure 41. The Golgi apparatus of the hepatic cells in and adjacent to a necrotic focus.

Figure 42.

Under low power observation, the darkened hepatic cords contiguous to the dilated sinusoids that were adjacent to the necrotic focus, were readily demonstratable. The Golgi material was clumped into black homogenous masses, the details of which have been previously described in Figures 39, 40 and 41. Counter-stained with acid fuchsin. X 100, Specimen # 53.

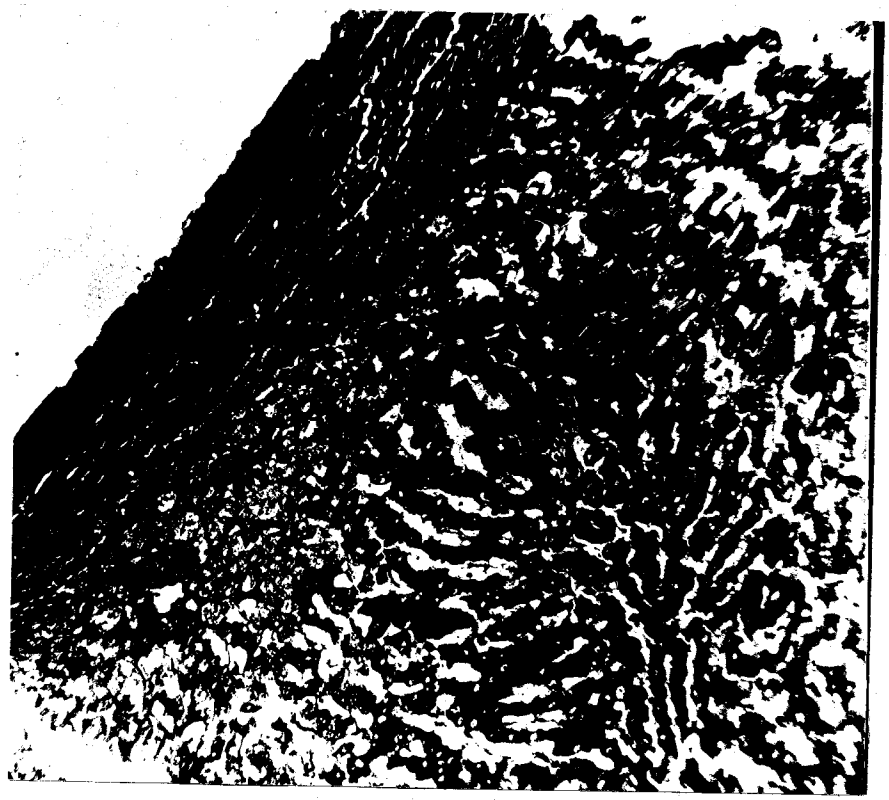

Figure 42. The Golgi apparatus in the hepatic cells in and adjacent to a necrotic focus. (x 100)

Figure 43.

Illustrates the clumping of the Golgi material and mitochondria in the hepatic cells (see arrows) adjacent to a necrotic focus. Counterstained with acid fuchsin. X 2000, Specimen # 53.

Figure 43. The Golgi apparatus in the hepatic cells in and adjacent to a necrotic focus. (x 2000)

4. Glycogen

Glycogen granules were extensively distributed throughout the sections taken from normal liver blocks (Table 7). In many instances, the glycogen was quite evenly distributed within the lobule (Fig. 44). However, some sections revealed the granules of glycogen to be slightly more concentrated in the central zone. The granules of glycogen were also, as a rule, diffusely and irregularly distributed throughout the cytoplasm of the individual cells (Fig. 45).

In the telangiectatic and sawdust liver, the distribution of glycogen granules both within the cells and within the lobule resembled that described for the distribution in sections taken from normal liver (Tables 7, 8, 9, 10). A normal concentration and distribution of glycogen was also noted in the cells and hepatic cords contiguous to the sawdust and telang areas (Figs. 46, 47).

Table 7

Histological distribution of glycogen in the hepatic lobule in "normal bovine livers*

Blocks # "Normal"	Central zone	Middle zone	Peripheral zone	Distribution in section
1	+++	+++	++	+++
2	+++	+++	++	+++
3	++++	+++	+++	+++
4	+++	+++	+++	+++
5	++++	+++	+++	+++
6	++++	+++	++	+++
7	++++	+++	+++	+++
8	+++	++	++	++
9	+++	+++	+++	+++
10	++++	++++	+++	+++

\+ Slight concentration +++ Extensive concentration
++ Moderate concentration ++++ Very extensive concentration

\# Five slides were made from each block
* The symbol + indicates that only scattered cells in the hepatic lobule contain glycogen; one or more +'s signifies that an increasing quantity is present

Table 8

Histological distribution of glycogen in the hepatic lobule in "sawdust" (Focal hepatitis) bovine livers*

Blocks # Sawdust	Central zone	Middle zone	Peripheral zone	Distribution in section
1	++	++	++	++
2	+++	+++	+++	+++
3	+++	+++	+++	+++
4	++	++	++	++
5	+++	+++	+++	+++

\+ Slight concentration +++ Extensive concentration
++ Moderate concentration ++++ Very extensive concentration

\# Five slides were made from each block
* The symbol + indicates that only scattered cells in the hepatic lobule contain glycogen

Table 9

Histological distribution of glycogen in the hepatic lobule in "telangiectatic" bovine livers*

Blocks # Telang	Central zone	Middle zone	Peripheral zone	Distribution in section
1	++	++	++	++
2	++	++	++	++
3	+++	+++	+++	+++
4	+++	+++	+++	+++
5	+++	+++	+++	+++

\+ Slight concentration +++ Extensive concentration
++ Moderate concentration ++++ Very extensive concentration

Five slides were made from each block
* The symbol + indicates that only scattered cells in the hepatic lobule contain glycogen

Table 10

Histological distribution of glycogen in the hepatic lobule of bovine livers exhibiting both telangiectasis and a focal hepatitis

Blocks # Telang & Sawdust	Central zone	Middle zone	Peripheral zone	Distribution in section
1	++	++	++	++
2	+++	+++	+++	+++
3	+++	+++	+++	+++
4	+++	+++	+++	+++
5	+++	+++	+++	+++

\+ Slight concentration
++ Moderate concentration
+++ Extensive concentration
++++ Very extensive concentration

\# Five slides were made from each block
* The symbol + indicates that only scattered cells in the hepatic lobule contain glycogen

Figure 44.

The glycogen granules were diffusely distributed within the hepatic cells throughout the entire hepatic lobule. No counterstain. X 100, Specimen # 10, Slide # 86.

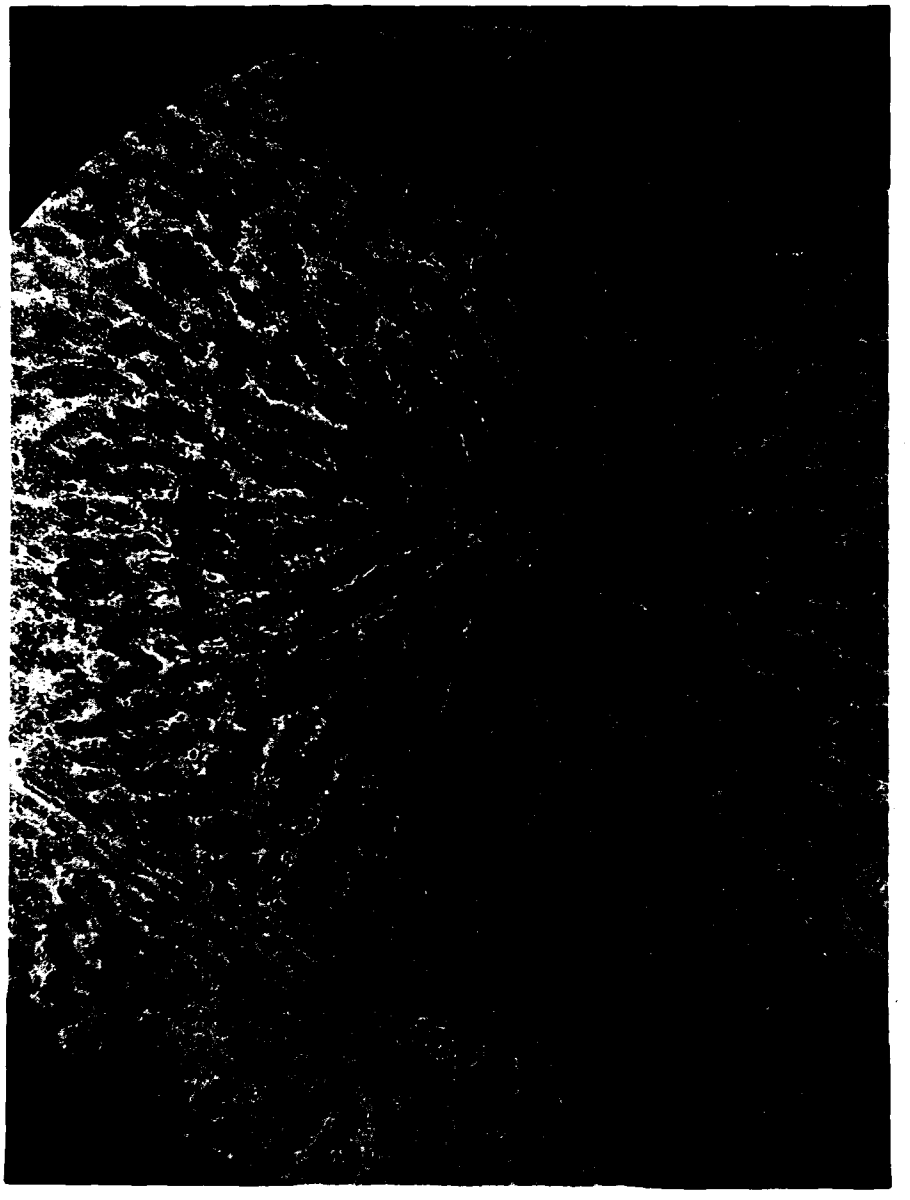

Figure 44. Glycogen distribution within the normal hepatic lobule.

Figure 45.

The granules of glycogen were diffusely and irregularly distributed throughout the cytoplasm of the individual cells. No counterstain. X 400, Specimen # 10, Slide # 86.

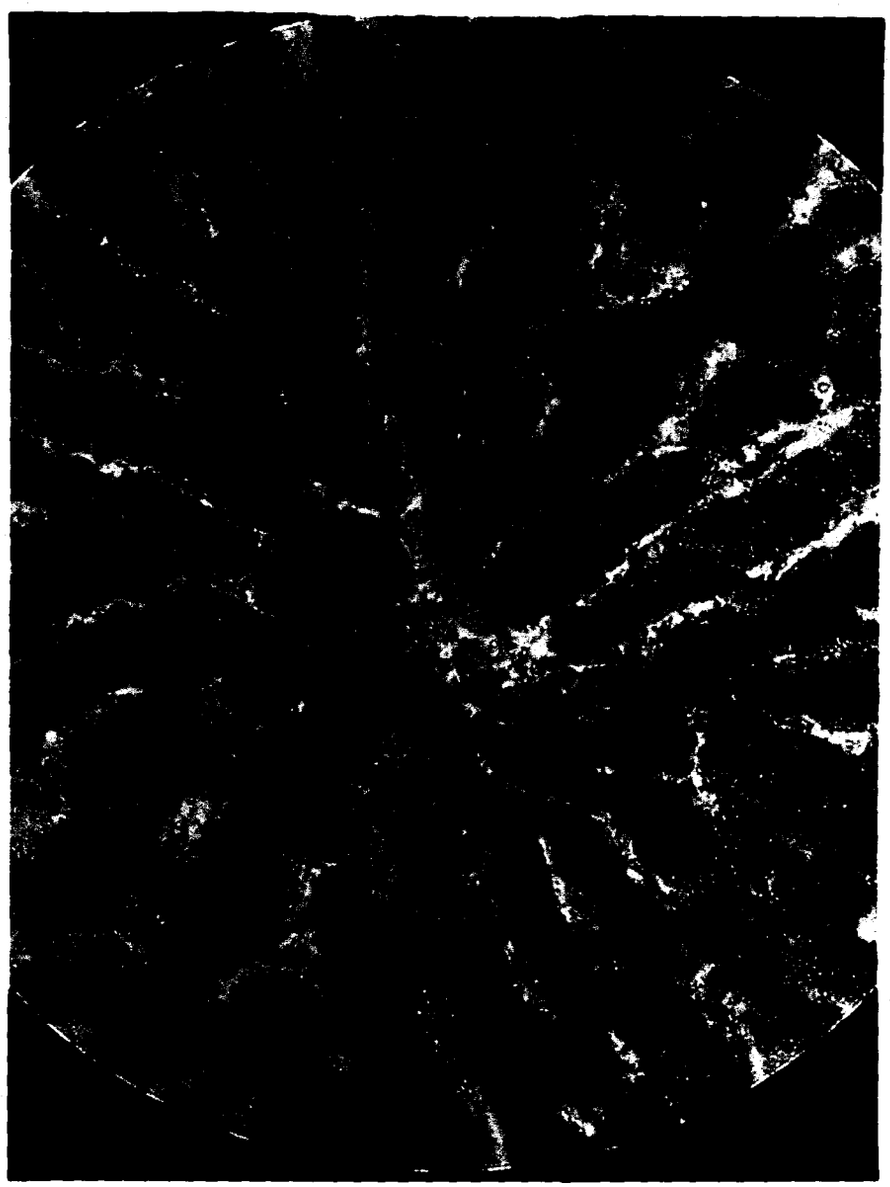

Figure 45. Glycogen distribution within the hepatic cell.

Figure 46.

A normal concentration and distribution of glycogen was present in the hepatic cells and cords contiguous to the telangiectatic areas. No counterstain. X 100, Specimen # 5, Slide # 39.

Figure 46. Glycogen distribution within the bovine liver exhibiting telangiectasis.

Figure 47.

A normal concentration and distribution of glycogen was noted in the cells and hepatic cords adjacent to the necrotic foci. X 100, Specimen # 5, Slide # 39.

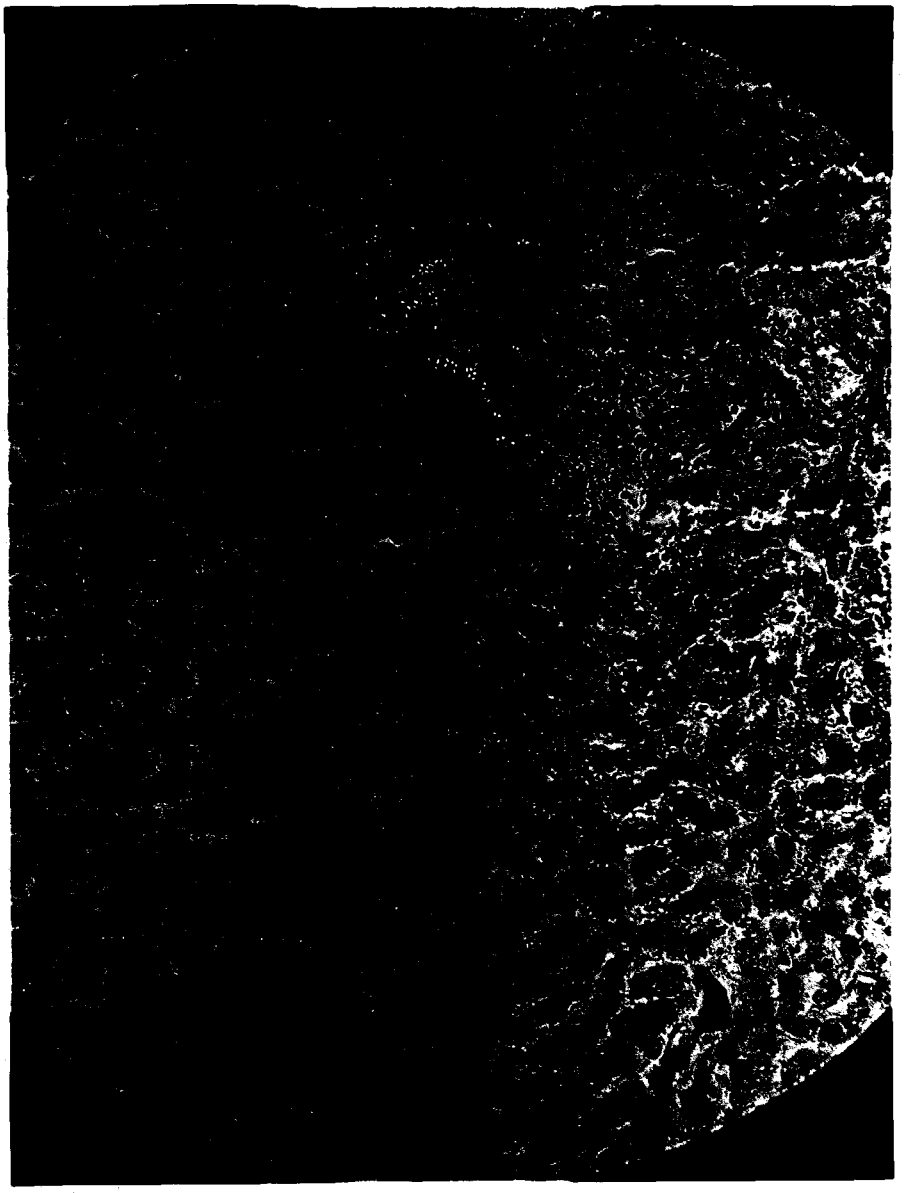

Figure 47. Glycogen distribution within the bovine liver exhibiting a focal hepatitis.

5. Fat

As was mentioned previously under cytological procedures, Bensley's acetic osmic bichromate fixative was used in order to demonstrate mitochondria and fat. The mitochondria were stained with Altmann's aniline acid fuchsin, Mallory (1938), and were found to present a similar picture to that previously described under "Results" when Deane's (1942) mitochondrial technique was used.

The osmiophilic lipids appeared black when Bensley's acetic osmic bichromate fixative was employed. Sixteen normal liver blocks were sectioned and five slides were made from each block. It was observed that very few lipid globules were demonstrable within the normal liver sections when osmic acid was used as the histochemical reagent (Fig. 48).

Bovine livers exhibiting both a focal hepatitis and telangiectasis were also fixed in Bensley's A.O.B. solution. Of the ten blocks sectioned and studied, eight were observed to reveal numerous lipid globules which were blackened in varying degrees. The osmiophilic lipids of the hepatic cells in the livers presenting a focal hepatitis appeared as numerous black granules which varied in number and size (Fig. 49). Some of the cells were hypertrophied and vacuolated. In many instances the fat globules appeared only as a ring of black surrounding a clear light area (Fig. 50).

Figure 48.

When osmic acid was used, very few lipid globules were demonstrable within the hepatic cells of the normal liver section. The speckled appearance of the hepatic cells was due to the mitochondria which were stained red with Altmann's aniline acid fuchsin. X 400, Specimen # la.

Figure 48. Normal bovine liver stained with osmic acid.

Figure 49.

Numerous lipid globules were blackened in varying degrees. The black granules varied in size and number. Some of the cells were hypertrophied. Counterstained with Altmann's aniline acid fuchsin. X 400, Specimen # 11x1.

Figure 49. Bovine liver exhibiting a focal hepatitis, stained with osmic acid.

Figure 50.

The fat globules were numerous and variable in size. Some fat globules appeared as clear vacuoles surrounded by a black halo. See arrow. X 400, Specimen # 11z.

Figure 50. Bovine liver exhibiting a focal hepatitis, stained with osmic acid but not counterstained.

The hepatic cells and cords adjacent to a telangiectatic focus also presented numerous black osmiophilic granules which varied in size and number (Fig. 51). The smaller granules and dots which appear black in the photomicrograph Figure 51 appeared as red mitochondrial granules in the original slide.

The sudanophilic substance stained well with Sudan black following a modified technique of Baker (1944). All of the cells in the normal bovine liver were stained a light blue when Sudan black was employed. However, it was noted that those cells about the peripheral portion of the hepatic lobule and portal canal area frequently stained a very intense blue-black which at times delimited the area (Fig. 52).

The hepatic cells and cords contiguous to the telangiectatic areas when stained with Sudan black revealed a staining reaction similar to that described for the normal bovine liver (Fig. 53).

The bovine livers exhibiting a focal hepatitis revealed necrotic areas which varied in color from light blue to an intense dark blue-black (Fig. 54).

Sudan II and Sudan IV revealed a few diffusely scattered very faint orange lipid droplets which usually were negligible in amount in the normal, the telangiectatic, and the sawdust liver.

Figure 51.

Numerous black osmiophilic granules which varied in size and number were seen within the hepatic cells. The small dots which appear black in the photomicrograph appeared as red mitochondrial granules in the original slide. X 400, Specimen # lly.

Figure 51. Hepatic cells and cords adjacent to a telangiectatic focus stained with osmic acid.

Figure 52.

All of the cells stained a light blue. However, those cells about the peripheral portion of the hepatic lobule and the portal canal area, frequently stained a very intense blue-black. X 100, Specimen # 698.

Figure 52. Normal bovine liver stained with Sudan black.

Figure 53.

The hepatic cells contiguous to the telangiectatic areas revealed a staining reaction similar to that described for the normal bovine liver. See Figure 52. X 100, Specimen # 701.

Figure 53. Telangiectatic liver stained with Sudan black.

Figure 54.

The necrotic focus stained blue-black. The delimited area (see dotted lines) was surrounded by normal appearing hepatic cords stained a light blue. X 100, Specimen # 702.

Figure 54. Bovine liver exhibiting a focal hepatitis stained with Sudan black.

When sections of bovine liver were stained with the Lorrain Smith Nile blue sulfate technique, two sharp color reactions were obtained. When Nile blue sulfate was used on normal liver sections, it was observed that those cells peripherally located in the hepatic lobule appeared dark blue in contrast to the lighter, more pink appearing hepatic cells centrally located within the lobule. The cells about the portal canal and the collecting veins also appeared dark in color (Fig. 55).

Bovine livers exhibiting a focal hepatitis and telangiectatis, when stained with Nile blue sulfate presented the following picture. The necrotic foci observed in those livers revealing a focal hepatitis appeared pink to light violet in color, in contrast to the surrounding dark blue hepatic cells (Figs. 56, 57). However, the hepatic cells and cords adjacent to the cavernous telangiectatic areas presented a blue color comparable to the color observed in the normal bovine hepatic cell Figs. 56, 57).

Figure 55.

Tangential section of collecting vein (C.V.). The cells about the portal canal and the collecting veins appeared dark in color in contrast to the lighter more pink appearing hepatic cells centrally located within the lobule. X 100, Specimen # 699.

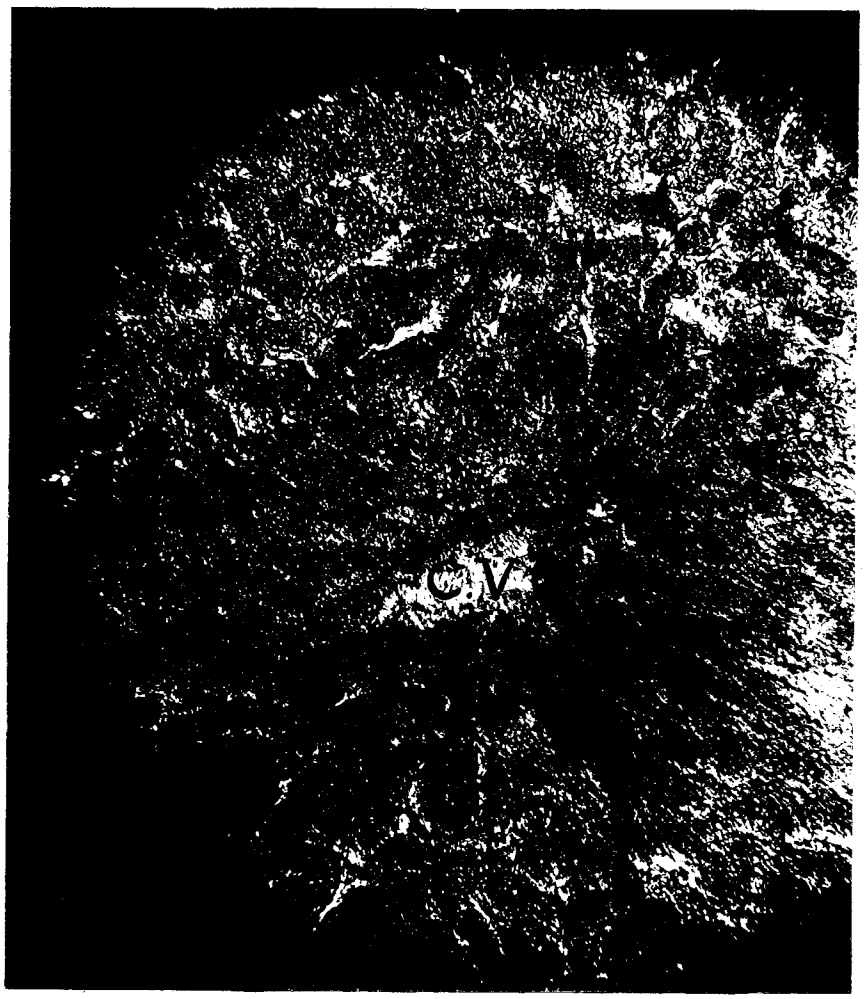

Figure 55. Normal bovine liver stained with Nile blue sulfate.

Figure 56.

The necrotic foci (delimited by dotted lines) appeared pink to light violet in color in contrast to the surrounding dark blue hepatic cells. T. telangiectatic area. C.V. central vein. X 100, Specimen # 700a.

Figure 56. Bovine liver exhibiting telangiectasis and a focal hepatitis stained with Nile blue sulfate.

Figure 57.

The hepatic cells and cords adjacent to the cavernous telangiectatic areas presented a blue color comparable to the color observed in the normal bovine hepatic cell. X 100, Specimen # 700b.

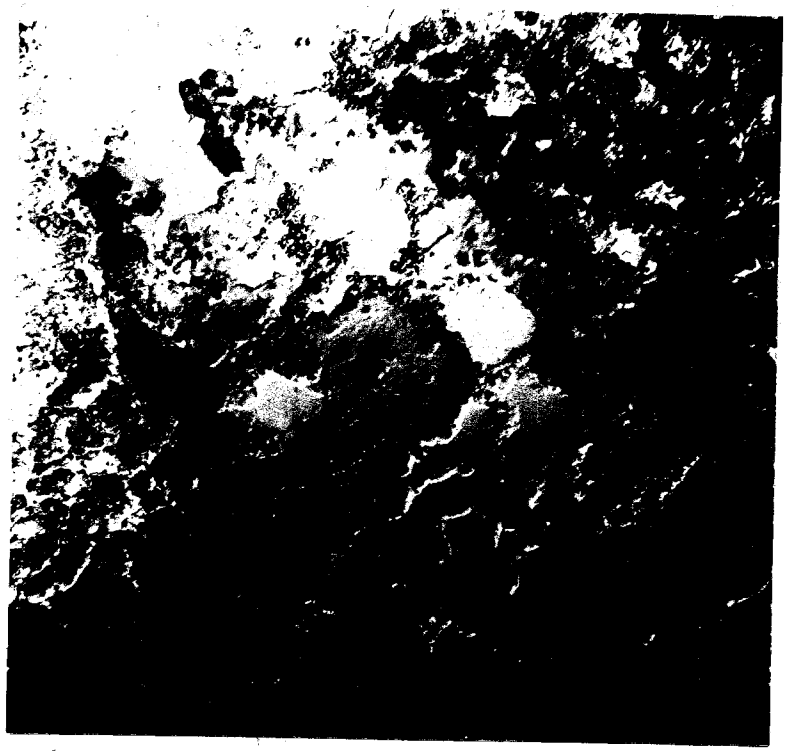

Figure 57. Bovine liver exhibiting telangiectasis stained with Nile blue sulfate.

6. Cytoplasm, reticulum, and collagenous connective tissue

Since the cytoplasm of the liver cell reflects to some extent the functional state of the cell (Maximow, 1938), it was deemed advisable to note the microscopic picture of the hepatic cell as revealed by routine stains. Twenty specimen blocks were removed from normal bovine livers and at least ten slides were made from each block. The liver sections stained with routine hematoxylin and eosin stain revealed the following microscopic picture.

Liver sections stained with hematoxylin and eosin presented a variable picture. The hepatic cell frequently appeared vacuolated and fenestrated. The fenestrated appearance imparted by the vacuolation of the cytoplasm was often noted in the hepatic cells located centrally within the hepatic lobule (Fig. 58). High power examination (x 400) disclosed enlarged hepatic cells (Fig. 59). The cytoplasm presented a granularity which one could mistakenly believe to be cloudy swelling. Many of the nuclei appeared pyknotic and the cellular outline was frequently vague. The hematoxylin and eosin stain also revealed many hypertrophied hepatic cells which were irregularly distributed throughout the section. The cells were frequently twice the size of those hepatic cells classified as normal. The cytoplasm appeared vacuolated and the nuclei enlarged. However, high power examination (x 400) revealed normal chromatin distribution and nucleoli, nuclear membrane and cell membrane (Fig. 60).

Figure 58.

A fenestrated appearance imparted by the vacuolation of the cytoplasm was often noted in the hepatic cells located centrally within the hepatic lobule. X 100, Specimen # 6.

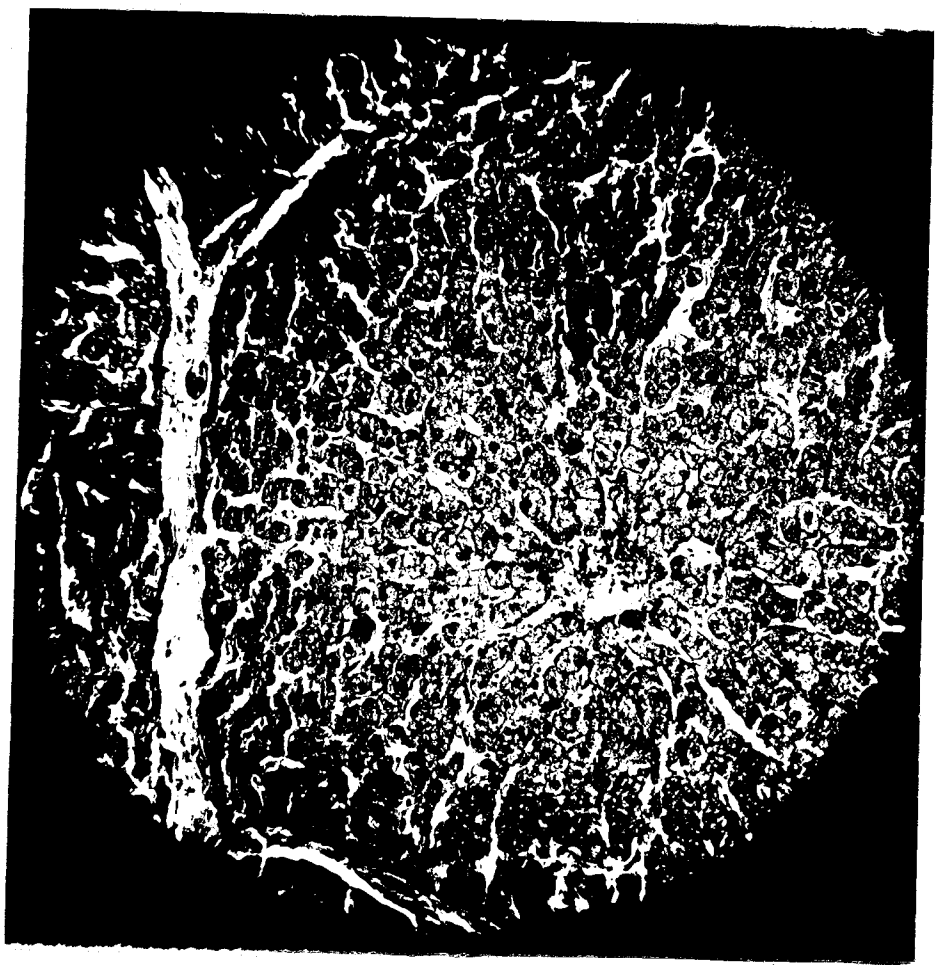

Figure 58. Normal hepatic lobule stained with hematoxylin and eosin.

Figure 59.

Normal physiological variation of cytoplasmic structure within the normal bovine liver. Many hepatic cells were often hypertrophied. The cytoplasm appeared granular and vacuolated and the nuclei pyknotic. This apparently normal variation in size and structure was noted in many of the 200 sections examined. Stained with hematoxylin and eosin. X 400, Specimen # 1, Slide # 15.

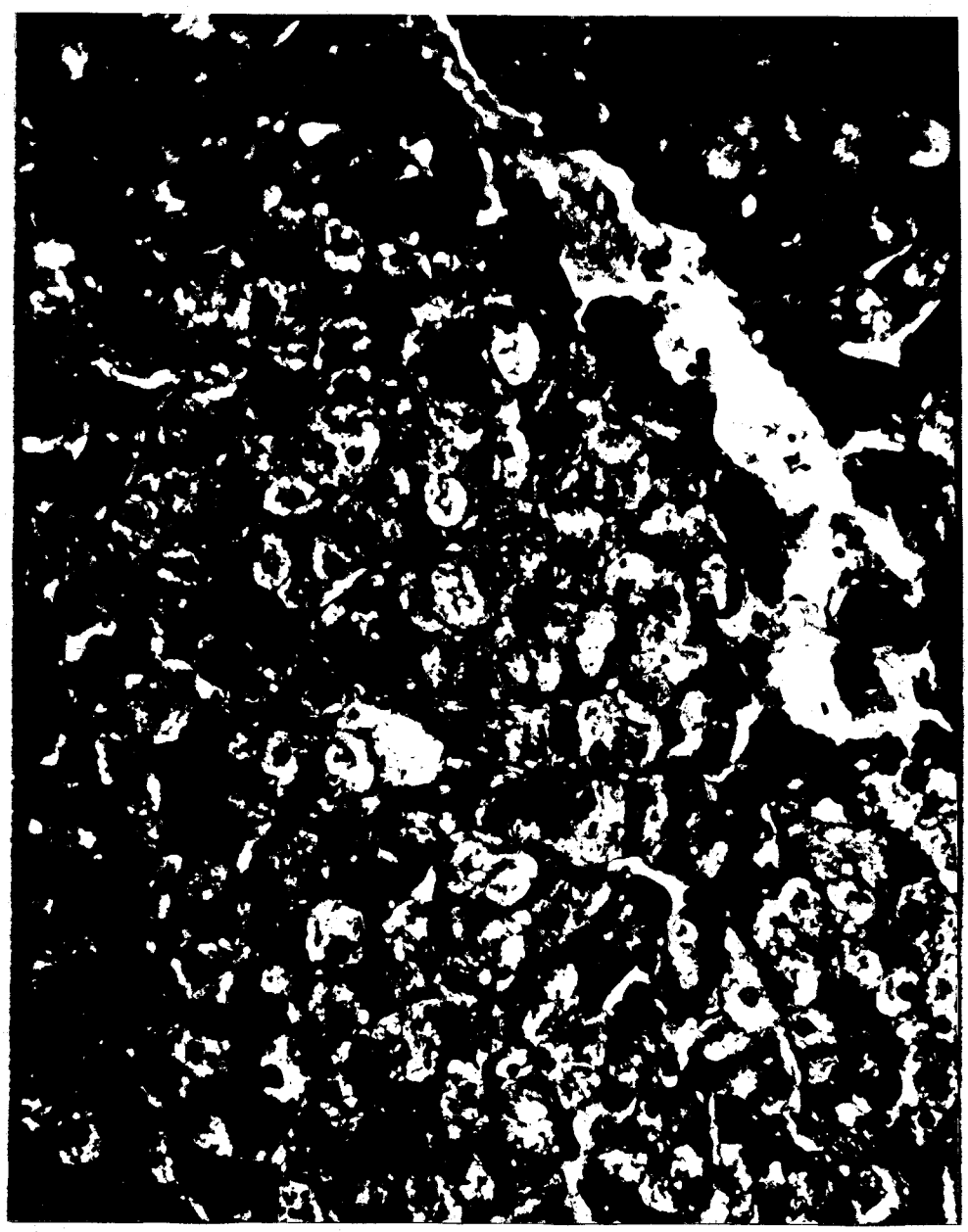

Figure 59. Enlarged hepatic cells within apparently normal hepatic lobule.

Figure 60.

Numerous hepatic cells were often enlarged to variable dimensions. However, high power examination revealed normal chromatin distribution and nucleoli, nuclear membrane and cell membrane. Stained with hematoxylin and eosin. X 400, Specimen # 1, Slide # 17.

Figure 60. Apparently normal physiological variation in size and appearance of the bovine hepatic cell.

Occasionally one noted small foci of leukocytes and lymphocytes in sections taken from liver sections classified as normal macroscopically (Fig. 61). So-called normal livers also revealed beginning telangiectatic foci (Fig. 62). High power examination (x 400), revealed normal liver cells contiguous to the dilated sinusoids which were engorged with whole blood.

The bovine livers exhibiting a focal hepatitis and telangiectasis that were stained with Heidenhain's iron hematoxylin and Van Gieson's picric acid and acid fuchsin stain for collagen and reticulum showed no increase in collagenous fibers in or about the telangiectatic areas or necrotic foci. Sections were also stained with Weigert's elastic tissue stain and Knoeff's iron-hematoxylin-anilin-blue stain. A normal distribution of elastic fibers were also present (Fig. 63). Thus, no apparent increase or decrease in connective tissue or elastic tissue fibers was noted in those livers exhibiting a focal hepatitis and telangiectasis.

Figure 61.

A focus of leukocytes and lymphocytes was noted approximately one-half way between the central vein and the periphery of the lobule. See arrow. Stained with hematoxylin and eosin. X 100, Specimen # 6.

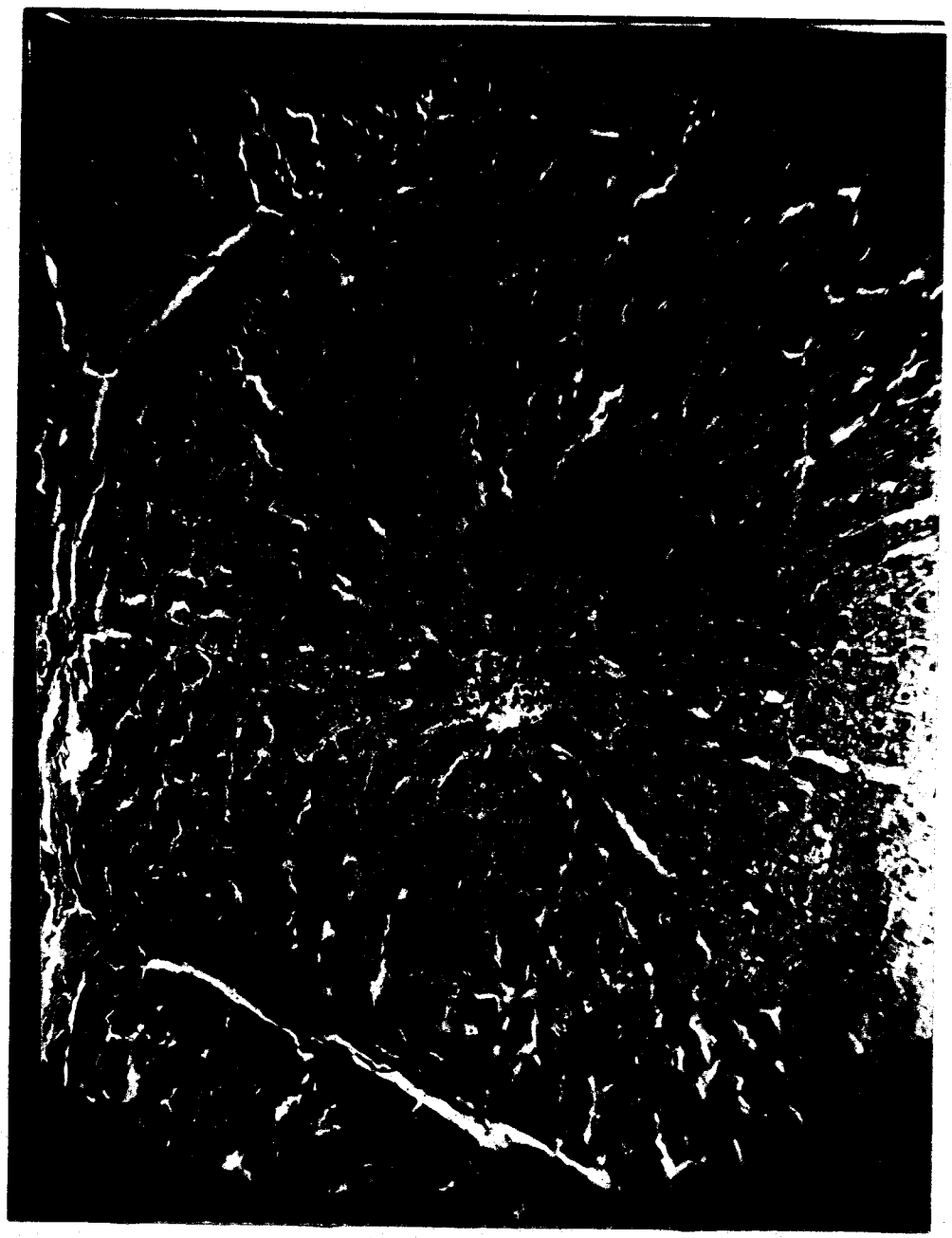

Figure 61. Leukocytic focus within an apparently normal liver.

Figure 62.

Normal hepatic cells were noted contiguous to the dilated sinusoids which were engorged with whole blood. Stained with Heidenhain's hematoxylin and Van Gieson's connective tissue stain. X 400, Specimen # 94x, Slide # 41.

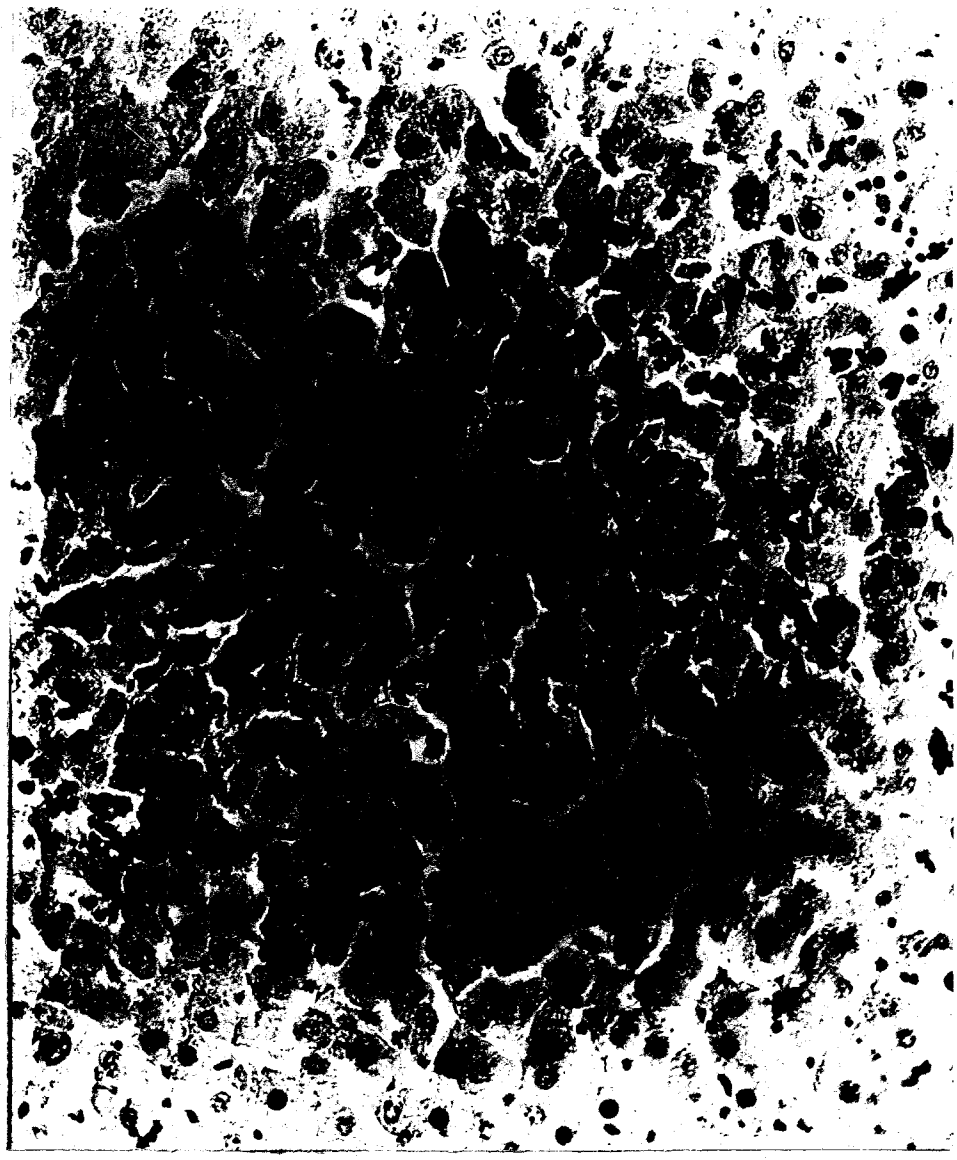

Figure 62. Beginning telangiectatic focus within an apparently normal bovine liver.

Figure 63.

No apparent increase or decrease in elastic fibers was noted in those livers exhibiting a focal hepatitis and telangiectasis. Cavernous sinusoids surrounded the necrotic focus. R. reticular fibers. C.V. central vein. N. necrotic focus. Knoeff's stain. X 400, Specimen # 13.

Figure 63. Distribution of the reticular fibers in a liver exhibiting a focal hepatitis and telangiectasis.

V. DISCUSSION

The macroscopic examination of sixty-nine bovine liver specimens and the microscopic examination of approximately five thousand tissue sections give the following impressions. The macroscopic observations were identical to those discussed previously (Getty, 1945). It should be reemphasized that the microscopic findings described under "results" are particular and significant only for the described conditions of this investigation. In other words, a definite time interval elapsed between the time of last feeding and watering and the killing time, the details of which were given under "results". This time interval was the same for all 69 animals examined. The writer wishes to emphasize that a complete history as to diet, age, sex, time of last feeding and watering, and method of killing should always be known and considered when evaluating microscopic findings of any parenchymal organ.

Previous investigators, Deane (1944), Deuel et al. (1938), Higgins, Berkson and Flock (1932), as well as the present author, have been cognizant of the normal cyclic cytological and histochemical changes correlated with the processes of storage and secretion within the hepatic cell and lobule. Thus a variable microscopic picture is frequently within the realm of normal physiological variation. Before discussing in detail the microscopic results which were given under six sub-headings, it should be stated that the findings of this investigation have confirmed the previous impression of the writer, Getty (1945), that the so-called "sawdust" and "telang" lesions represent different stages of a focal hepatitis and its termination.

1. The principal sites in which phosphatase occurs in the animal body and in the liver have been surveyed by a number of investigators (Gomori, 1941; Kabat and Furth, 1941; Greenstein, 1941; Wolf, Kabat and Newmann, 1943; Stafford et al., 1947; Deane, 1947). A marked species variation in the concentration and kind of phosphatase has also been observed by these investigators. Although it is known that phosphatases are concerned with carbohydrate metabolism, nucleotide metabolism, phospholipid metabolism and calcium deposition (Sumner and Somers, 1943), the exact biochemical role of the enzyme in its many and varied sites has, as yet, not been clearly defined.

Wislocki and Dempsey (1945) demonstrated that the presence of phosphatase and the deposition of glycogen was correlated, noting that in the epithelial cells of the uterine glands of the cat dephosphorylation by the action of phosphatase occurred in the same cells that also stored the glycogen. The zonal location of phosphatase as described in detail in "results" in both the alkaline and acid range in the bovine livers studied in this investigation lends support to the hypothesis that the presence of phosphatase and the deposition of glycogen are correlated. Deane (1947) has previously demonstrated that the phosphatase enzyme reacted more strongly, at the periphery of the lobule of the rodent liver. The preparations of Deane (1944) demonstrated that glycogen was deposited initially in the peripheral zone of the lobule of the rodent liver.

Wachstein (1945) reported that the fat-laden liver cells in experimentally produced phosphorous and carbon tetrachloride poisonings showed

a decrease in both alkaline and acid phosphatase. Since Deane (1944) observed that fat droplets are more numerous in the central portion of the hepatic lobule of mice, the lessened enzymatic activity noted within the central portion of the hepatic lobule in the normal bovine liver may in part be due to normal physiological deposition of fat. The explanation advanced by Deane (1944) that the peripheral cells, with their greater supply of oxygen, are better able than the central cells to oxidize or otherwise dispose of fat brought to them by the blood seems quite plausible.

The intense enzymatic activity at pH 9.5 that was noted in the nuclei, the bile canaliculi, the lymphocytes and the endothelium of the small arterioles and capillaries in the portal canal area, similated the observations reported by Deane (1947) for the rodent liver. Similarly, the cytoplasm of the hepatic cells was also readily demonstrated in the alkaline range. The slight reaction in the cytoplasm at pH 4.7 to 5.0 noted in the bovine liver also is in accord with the observations described by Deane (1947) for the rat liver. The moderate concentration in the acid range, of the enzyme in the nuclei, the bile canaliculi, lymphatics, Kupffer cells, and the endothelial lining cells of the bovine liver similates the observations reported by Deane (1947) for the rat.

A normal distribution and concentration of both the alkaline and the acid phosphatase was observed in the undamaged liver cells of bovine livers exhibiting a focal hepatitis. Similarly, Wachstein (1946) observed no appreciable increase in alkaline phosphatase in the normal hepatic

parenchyma in human livers showing extensive necrosis of the hepatic cells. The infiltrating leukocytes and lymphocytes, however, showed a varying degree of activity, but the necrotic cells did not show an increase. Gomori (1941) studying the distribution of acid phosphatase in tissues under normal and under pathological conditions also reported no increase in necrotic or caseating areas, but he did observe a marked reaction in chronically inflammed tissue. A similar observation was noted in the bovine livers exhibiting a focal hepatitis. The necrotic foci were surrounded by leukocytes and lymphocytes undergoing marked enzymatic activity. The apparent increase in enzymatic activity within the foci was in most instances due largely to the very marked increase in number of leukocytes and lymphocytes in the circumscribed area. However, altered and increased enzymatic activity within the cytoplasm of the hepatic cell did occur in some foci indicating a variance in enzymatic activity. Wachstein (1945) likewise reported that necrotic liver cells, damaged by phosphorus, chloroform or carbon tetrachloride, presented either normal or occasionally moderately increased alkaline and acid phosphatase activity. The above author also observed a marked increase in phosphatase activity in the livers of starved or protein depleted animals which he believed to be due to the intensification of metabolic processes which are not taking place to the same degree under normal conditions. These changes were fully reversible when the animals were placed on a normal diet. No significant change in phosphatase activity was noted, however, in areas of necrosis, but the infiltrating cells and Kupffer cells revealed strong activity. Thus, in the bovine liver, the variance

in cytoplasmic activity within and contiguous to the areas exhibiting a focal hepatitis is in agreement with Wachstein's findings.

A record of the approximately 300 sections, graphically presented in Figs. 17 and 18, and Tables 1, 2, 3, 4, 5 and 6, reveals that the normal bovine livers and livers exhibiting a focal hepatitis and telangiectasis present a similar or comparable enzymatic pattern. It is of interest to note that Greenstein (1941-42) determined that both the alkaline and acid phosphatase activity in transplanted rat hepatomas was much greater than the corresponding values for normal rat liver. White et al. (1941-42) also observed that there was an increase in alkaline phosphatase in rat hepatomas particularly in the necrotic zones. Thus, the findings of this investigation would not seem to reveal an enzymatic picture characteristic of malignant or transplanted tumors as described for other species.

The normal bovine liver presented a characteristic mitochondrial pattern. As was noted under results, the cells about the peripheral portion of the hepatic lobule appeared darker in color than those centrally located. High power examination (2000 x) of the central lobular area, revealed that the mitochondria were peripherally located in the hepatic cells. Thus, the peripheral location of the mitochondria within the cells imparted a lighter, vacuolated appearance to the cells that were located in the central portion of the hepatic lobule. However, it should be emphasized that not all cells in all lobules consistently exhibited this definite zonal mitochondrial pattern.

Since changes in mitochondrial morphology often represent functional hypertrophy or changes in the general metabolic level of the cell (Kater, 1937), one would not expect to observe the same mitochondrial pattern in all the hepatic cells within a hepatic lobule at a given time. The cells that were located about the portal canal and the peripheral portion of the lobule frequently appeared darker in color than those cells centrally located. High power examination (2000 x) revealed that the mitochondria were diffusely distributed as small spherical black granules throughout the cytoplasm of the hepatic cells. Thus, the mitochondrial pattern produced a darker appearing hepatic cell in the peripheral portion of the hepatic lobule. The conclusions of McCurdy (1939) that a relationship exists between mitochondrial morphology and the relative amounts of fat and glycogen within the cell seems plausible. The rhythmic activity of the liver which is dependent upon the feeding regime (Steffens, 1941) could readily account for the altered mitochondrial pattern in the hepatic cells located centrally and peripherally within the hepatic lobule. Then too, the conclusions of Deane (1944) that all mitochondrial zonation is probably dependent upon the character of the blood bathing the cells, would presuppose a changing mitochondrial pattern rather than a static one. Since the hepatic cells located peripherally in the hepatic lobule and about the portal canal area are bathed with blood high in oxygen and nutrient material, one would expect a different but a characteristic mitochondrial pattern in the peripheral and central portions of the hepatic lobule.

It is of interest to note that the characteristic mitochondrial pattern observed in the normal bovine liver was altered, however, in those livers exhibiting a focal hepatitis. The cytoplasm of the hepatic cells in and contiguous to the necrotic foci appeared very dark and conspicuous, when stained for mitochondria, as compared to the cytoplasm of the surrounding normal hepatic cells. Upon high power examination (2000 x), it was noted that the dark appearance of the cytoplasm was due to the clumping of the mitochondria into large osmiophilic masses (Fig. 26). These large black masses at times completely obliterated all cellular detail. Thus, these observations are in accord with the findings of Duthrie (1935), that mitochondrial changes are the most sensitive and earliest indications microscopically of cellular damage.

The telangiectatic livers revealed a mitochondrial picture comparable to that previously described for the normal hepatic cell. Since no mitochondrial clumping was observed in the hepatic cells or cords lining the cavernous sinusoidal telangiectatic areas, and since high power examination (2000 x), revealed normal nuclei and cytoplasm, the findings would indicate that the hepatic cells are unimpaired within and contiguous to the telangiectatic areas.

Thus, the mitochondrial findings within the normal, telangiectatic and sawdust livers, as referred to above, are in accord with the belief that altered mitochondrial patterns, are expressions of anabolic or catabolic activities.

As was previously noted in the review of literature, a vast amount of controversial material can be found pertaining to the Golgi apparatus. Opinions still vary not only as to the structure, but also as to the chemical composition of this Golgi substance. At present, the Golgi apparatus is thought to consist of two parts, an outer which absorbs osmium and an osmio-phobic inner portion: Bowen (1920, 1926, 1928), Bourne (1934), Weier (1933). Beams and King (1932a, 1932b) described and illustrated both osmiophilic and osmiophobic portions of the Golgi apparatus, but ventured no opinion as to the osmio-phobic portion. Beams and King (1934) stated that the apparatus behaved as a lipoidal structure. However, Bourne (1942) has concluded that the Golgi apparatus like the mitochondria is composed of protein and lipoidal or fatty substances. Bourne believes the Golgi apparatus and the mitochondria possess a superficial adsorbed layer of protein. Bourne's explanation could thus account for the fact that neither mitochondria nor the Golgi apparatus normally react with fat stains. In physical-chemical terms, the Golgi system may fulfill Taylor's (1943) prediction that coacervates may be active in living cells. Worley (1946-47) recognizes the Golgi apparatus as a temporary aggregation of ultramicroscopic colloidal particles composed of ribose nucleic acid or ribonucleoproteins, phospholipids and, probably frequently, vitamin C. Observations by Bourne (1934-35) also suggest very strongly that vitamin C may frequently be associated with the Golgi apparatus of a wide variety of cells.

All investigators concede that the Golgi apparatus varies considerably in size and shape. The problem of making accurate comparative estimations of the amount of Golgi material is almost impossible, as with mitochondria, even with the most accurate standardization of methods. The Golgi apparatus varies in shape and consistency in different cells of the body and also in individual cells according to their physiological condition. It is also almost certain that in the living cell it is constantly but slowly changing its conformation. However, as a general rule, a simple Golgi pattern can be established for the cells of an organ. This pattern can be, and is, often altered under various physiological and pathological conditions. Bourne (1942) believes that there may be a mitochondrial-Golgi surface to cell volume ratio for which the cell has its optimum function. Bourne suggests that

> An alteration of this surface volume ratio by bacterial toxins, undue amounts of hormones or drugs, or deficiency of minerals or vitamins may be one of the factors involved in the state known as ill health.

In the present investigation, the Golgi substance in the apparently normal bovine liver was fused into the form of tight networks. These strongly osmiophilic networks were both juxtanuclearly and peripherally located. However, the relative amount of Golgi material demonstratable within the hepatic cell varied. The Golgi apparatus was also readily observed in telangiectatic liver sections. The Golgi material of the hepatic cells in the "telang" area was arranged in a continuous network which imparted a fenestrated appearance to the cell. In the

bovine liver exhibiting a focal hepatitis, a characteristic Golgi pattern was also observed. The Golgi substance in the cells contiguous to and within a necrotic focus was fused into a tight strongly osmiophilic mass which at times completely obliterated all other cytoplasmic detail. The clumping of the Golgi material imparted a dark black appearance to the cells and cords adjacent to the necrotic foci. As has been previously mentioned, an altered Golgi pattern frequently is seen concurrently with various physiological and pathological conditions. Bourne (1942) cites the studies of Chang (1932) who noted an increase in the amount of Golgi material in the nerve cells in beriberi. Bourne (1935) also noted that the mitochondria clumped in large masses in scurvy. Gatenby (1931, 1931a) who studied the effect of phosphorus poisoning and irradiation on spermatogenesis of Abraxas also noted that the Golgi apparatus was the most sensitive of the cell organs.

Thus, the observations that both the mitochondria and the Golgi material are clumped in the hepatic cells in and contiguous to, the necrotic foci seen in the sawdust liver, would lend support to the belief that normal metabolic processes have been altered in the bovine liver exhibiting a focal hepatitis. However, the mitochondrial and Golgi pattern in the telangiectatic liver remains within the realm of normal physiological variation.

Contrary results have frequently been reported in the literature regarding glycogen deposition within the hepatic lobule. Deane (1944) found that with both controlled and uncontrolled feeding,

glycogen appeared to be deposited shortly after the time of eating. Deane also found that glycogen was deposited initially in the peripheral zone of the hepatic lobule, then more centrally. The above author pointed out that one must at all times be cognizant of the fact that a cyclic variation in glycogen deposition exists. The discrepancy in our present literature regarding glycogen deposition is no doubt due to the lack of conformity to the different time intervals that various investigators have allowed to elapse between feeding time and killing time. Thus, it is again emphasized that the diffuse distribution of glycogen throughout the hepatic lobule as reported in this present investigation (Tables 7, 8, 9, 10) would no doubt be different at different time intervals.

In discussing the results noted when the various fat stains were used, it should be mentioned that Baker (1944) stated that the "rose" color forms in sites of unsaturated glycerides, while the blue color has no histochemical significance when Nile-blue sulfate is used. However, Mallory (1938) stated that neutral fats are pink and fatty acids and other fatty substances are blue to violet with Nile-blue sulfate. It is of interest to note that Sudan II and Sudan IV revealed only a few diffusely scattered very pink orange lipid droplets which usually were negligible in amount in the normal, the telangiectatic and the "sawdust" liver.

When sections of bovine livers were stained with the Lorrain-Smith Nile blue sulfate technique, two sharp color reactions were obtained.

When Nile blue sulfate was used on normal liver sections, it was observed that those cells peripherally located in the hepatic lobule appeared dark blue in contrast to the lighter more pink appearing hepatic cells centrally located within the lobule. Thus, if one uses Mallory's interpretation of the Nile blue sulfate stain the results would indicate a deposition of neutral fats centrally within the normal hepatic lobule. With Nile blue sulfate, the necrotic foci observed in those livers revealing a focal hepatitis appeared pink to a very light violet color, in contrast to the surrounding darker blue hepatic cells. However, the hepatic cells and cords adjacent to the cavernous telangiectatic areas presented a blue color comparable to the color observed in the normal bovine hepatic cells. As was noted under "results", the sudanophilic substances stained well with Sudan black. All of the cells in the normal bovine liver were stained a light blue color. The hepatic cells and cords contiguous to the telangiectatic areas revealed a staining reaction similar to that described for the normal bovine liver. However, the bovine livers exhibiting a focal hepatitis revealed necrotic areas which varied in color from light blue to an intense blue-black.

Although osmic acid lacks specificity as discussed by Bensley and Bensley (1938), it was deemed advisable to study those sections fixed in Bensley's acetic-osmic bichromate fixative for fat deposition. The above authors believed that the unsaturated fatty acids blacken much more readily than the saturated fatty acids when osmic acid is used. Consequently, the observation that both the "telang" and the "sawdust"

liver sections revealed more numerous black granules than the normal bovine liver when osmic acid was used, would indicate according to Bensley, that less unsaturated acids are present in the normal liver than in the sawdust or telang liver.

Summarizing the results noted when various fat stains were used, it can be stated that various lipoidal substances were observed in all the hepatic cells of the normal bovine hepatic lobule. However, the neutral fats appeared centrally whereas the fatty acids and other fatty substances which appeared in lesser amounts, were seen peripherally in the lobule.

Although the lobule of the liver was first recognized grossly by Wepfer (1664), its true histological architecture is still debatable today and questioned by some investigators. Much has been written regarding the livers morphological organization: Kierman (1833), Mall (1906), Mann (1920), Mann and Magath (1922), Opie (1925), Higgins and Murphy (1930). Cowdry (1932) made an excellent review of the literature. The classical description of the liver cells as being arranged more or less regularly in cords which form columns extending radially from the central vein, may in the light of recent findings, (Hans Elias, 1949) of necessity require revision. However, this present investigation was concerned primarily in the cytology of the hepatic cell rather than the lobule. The present writer would like to emphasize that perhaps the contradictory observations of many investigators is largely due to the failure to standardize properly the physiological

conditions of the histological and cytological investigations. The variability of the structure of the hepatic cell due to its functional activity, makes the description of the so-called normal hepatic cell difficult especially if the diet, age, sex, withdrawal of food and water are not considered. These functional variances have been considered in the present investigation and the findings recorded under "results" are peculiar only to the described limitations of this investigation. Eighteen hours following the withdrawal of food and water, the animals were slaughtered as described under method of procedure. The hepatic cell frequently appeared vacuolated and fenestrated when stained with hematoxylin and eosin. The vacuolation of the cells about the central vein was no doubt due to the xylol removing the neutral fat droplets. However, many cells were hypertrophied frequently to twice the size of those hepatic cells classified as normal (Fig. 60). Cowdry (1932) also has emphasized that the hepatic cell increases in size and undergoes intracellular alterations depending upon food intake, character of food, etc. Similarly, Cowdry further noted various nuclear and cytoplasmic changes which varied in character and degree, which were definitely classified as normal physiological variances.

The bovine livers exhibiting a focal hepatitis and telangiectasis that were stained with Heidenhain's hematoxylin and Van Gieson's connective tissue stain showed no increase in collagenous fibers in or about the telangiectatic areas or necrotic foci. Sections were also stained with Weigert's elastic tissue stain and Knoeff's iron hematoxylin-anilin-blue stain. A normal distribution of elastic

fibers were also present. Thus, no apparent increase or decrease in connective tissue fibers or elastic tissue fibers was noted in those livers exhibiting focal hepatitis and telangiectasis.

In view of the macroscopic and microscopic findings of this investigation and the availability of a complete known history as to diet, age, etc. of the sixty-nine animals studied, it would next seem advisable to consider this problem from a nutritional basis. Since no proof to date has been revealed that telangiectatic and sawdust livers are of an infectious origin, and since the recognition of purely nutritional factors as important agents causing hepatic disturbances is well confirmed, the attempt to prevent telangiectatic and sawdust livers by dietary means appears to be fully warranted. Sullivan et al. (1932) and Lillie (1932), while making preliminary studies on amino-acid toxicity and amino acid balance, noted that periportal coagulation necrosis and hemorrhagic necrosis was produced in the liver of the rat by the addition of various amino acids. The fact that dietary factors alone can initiate liver injury was demonstrated by Weichselbaum in 1935, and by Györgi and Goldblatt in 1939. Earle and Victor (1941) and Earle et al. (1942) noted that excess dietary cystine or cysteic acid produced a portal hemorrhagic necrosis, a focal necrosis and cirrhosis in the liver of the rat within two weeks. In the same year, Earle and Victor (1942) studied the effect of nine different diets on the liver lesions resulting from excess dietary cystine. György (1944) brought up to date recent concepts dealing with experimental dietary hepatic injury. Himsworth (1944) also demonstrated the influence of nutritional factors in liver disease. Glynn et al. (1945)

made an excellent study of the various pathological conditions seen in the liver of the rat due to deficiency of the sulfur containing amino-acids. Two years later, Glynn (1947) stated that cysteine had a three-fold action upon the liver. Follis in 1948 cited cystine deficiency as a specific cause for liver necrosis and hemorrhage in the rat. Since many of the hepatic lesions noted in the rat by the various authors cited above were comparable to those observed in telangiectatic livers and livers exhibiting a focal hepatitis, a controlled dietary feeding experiment for the bovine species would seem indicated, particularly in view of the fact that the anatomy and physiology of the ruminant is considerably different from that of the rat.

VI. SUMMARY AND CONCLUSIONS

1. Sixty-nine bovine liver specimens were studied macroscopically and microscopically.
2. Observations have been made on the location of phosphatases in the normal bovine liver, and in the bovine liver exhibiting a focal hepatitis and telangiectasis.
3. Both alkaline and acid phosphatases were demonstrable in the normal bovine liver, and in the bovine liver exhibiting a focal hepatitis and telangiectasis.
4. In the normal liver and in the liver exhibiting a focal hepatitis and telangiectasis, the alkaline and acid phosphatase enzymatic activity was more intense in the portal canal area and in the peripheral cells of the lobule than in the central portion of the hepatic lobule.
5. Alkaline and acid phosphatase was observed to be present in the cytoplasm and nuclei of the hepatic cells, the bile canaliculi, the lining cells of the sinusoids and blood vessels, the cells of the bile ducts, and in variable amounts in the leukocytes and lymphocytes.
6. Less phosphatase activity occurred in the normal bovine liver, the telangiectatic liver and the liver exhibiting a focal hepatitis, at pH. 4.7 to 5.0 than in the alkaline range.

7. In the telangiectatic bovine liver, alkaline glycerophosphatase (pH. 9.5 to 9.0) and acid glycerophosphatase (pH. 4.7 to 5.0) was demonstrated in similar sites and exhibited similar activity to that observed in the normal bovine liver.

8. The bovine liver exhibiting a focal hepatitis revealed a very intense concentration of both acid and alkaline phosphatase within and about the foci of necrosis.

9. In the livers exhibiting a focal hepatitis, the hepatic parenchyma exclusive of the necrotic areas, revealed a normal distribution of alkaline and acid phosphatase.

10. The observations as to the distribution of alkaline and acid phosphatase in those livers exhibiting a focal hepatitis and telangiectasis, would not seem to reveal an enzymatic picture characteristic of malignant tumors as described for other species.

11. A characteristic mitochondrial pattern was noted in the hepatic cells located in the peripheral and central portions of the normal hepatic lobule.

12. In the normal bovine liver, the mitochondria were peripherally located in the hepatic cells of the central lobular area in contrast to a diffuse distribution within the hepatic cells of the peripheral portion of the lobule.

13. The cytoplasm of the hepatic cells in and contiguous to the necrotic foci seen in livers exhibiting a focal hepatitis appeared very dark due to the clumping of the mitochondria into large osmiophilic masses.

14. The telangiectatic liver revealed throughout a mitochondrial pattern comparable to that observed for the normal hepatic cell.

15. The Golgi substance in the apparently normal bovine liver was fused into the form of tight networks both juxtanuclearly and peripherally located.

16. The Golgi material of the hepatic cells within the telangiectatic area was arranged in a continuous network which imparted a fenestrated appearance to the cells.

17. The Golgi substance in the cells contiguous to and within the necrotic foci of the "sawdust" liver was fused into a tight strongly osmiophilic mass which at times completely obliterated all other cytoplasmic detail.

18. The observation that both the mitochondria and the Golgi material are clumped in the hepatic cells in and contiguous to, the necrotic foci seen in the "sawdust" liver, would lend support to the belief that normal metabolic processes have been altered in the bovine liver exhibiting a focal hepatitis.

19. The mitochondrial and Golgi pattern in the telangiectatic liver remains within the realm of normal physiological variation.

20. The discrepancy in our present literature regarding glycogen deposition in the hepatic lobule is no doubt due to the lack of conformity to a definite time interval between feeding time and killing time.

21. Various lipoidal substances were observed in all the hepatic cells of the normal bovine hepatic lobule.

22. The neutral fats appeared centrally, whereas the fatty acids and other fat-like substances which appeared in lesser amounts were seen peripherally in the normal hepatic lobule.

23. One must at all times be cognizant of the variability in histochemical and cytological structure of the hepatic cell due to its functional activity.

24. No apparent increase or decrease in connective tissue fibers or elastic tissue fibers was noted in those livers exhibiting focal hepatitis and telangiectasis.

25. Both telangiectatic areas and necrotic foci were noted macroscopically in the same liver and microscopically in the same section.

26. Many livers, designated as "normal" grossly, exhibited a focal hepatitis and telangiectasis microscopically.

27. Since the phosphatase activity, the mitochondrial pattern, the Golgi pattern, the glycogen deposition and the fat deposition within the livers exhibiting telangiectasis resembled that observed histologically in the normal bovine liver, it is concluded that normal metabolic processes are unimpaired in this condition.

28. Since the phosphatase activity, the mitochondrial pattern, and the Golgi pattern, in those livers exhibiting a focal hepatitis, presented a different histological picture than that observed in the normal bovine liver, it is concluded that normal metabolic processes are altered in bovine focal hepatitis.

29. All carcasses of which the livers showed the lesions discussed were passed for human food; no other concurrent lesions were evident grossly.

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