A Study of the Hepatic Cell Mitochondria of the Rat by the Perfusion Method

by

Donald Emerson Shay

Thesis submitted to the Faculty of the Graduate School
of the University of Maryland in partial
fulfillment of the requirements for
the degree of Doctor of Philosophy

1943

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

TABLE OF CONTENTS

I. STATEMENT OF PROBLEM

Studies related to the mitochondria of the hepatic cells have been numerous. In general workers have attempted to determine not alone the morphology of the mitochondria but the possible relationship existing between mitochondrial form and number and the physiological activity of the cell and the lobule. Outstanding among the contributions are those of Noel ('23), Kater ('31, '33, '37), Smith ('31), Kater and Smith ('32), Muggia and Masuelli ('32), Noel and Pallot ('33, '34), McCradle ('37), McCurdy ('39), and the most recent being that of Steffens ('41).

The writer earlier attempted an experimental study of the effect of inanition on the hepatic cell mitochondria (Shay '38). During the course of this study great difficulty was experienced in securing proper fixation of hepatic tissue. Immediate questions arose. "Does the usual fixation technique render an accurate picture of mitochondria, as in the living condition?" "If not, how may a technique be modified to assure uniform results?" "Is it not possible the discrepancies occurring between results of various workers on this subject may be due to a lack of uniformity in fixation technique?"

By a modification of the perfusion method a technique was devised by which the mitochondria of hepatic cells

may be fixed as they are in the living condition, that is fixed while in the living cell, thus eliminating artifacts due to faulty penetration and autolysis of cytoplasmic elements. This degeneration is in evidence when the lapse of time is too great between the killing of the animal and the removing of the tissue. The method devised by the author is a modification of the perfusion technique as applied by E. M. Shearer ('33), in his study of the development of arteries in the anterior limb of albino rats. A detailed account of this perfusion method as applied by the auther to the study of hepatic mitochondria and its practibility in general histological use will be discussed. Thus, with the development of a technique which would assure as perfect a cytological fixation as possible, the more important study of the morphology, distribution, and to a lesser extent, physiology of the mitochondria was instituted. It was felt that by the utilization of this technique a more accurate study of the mitochondria could be made, and the results analyzed and compared with earlier studies by other authors in which less adequate methods of treatment of the tissue were used.

Emmel's ('40) work on the effect of ischemia on the renal mitochondria has suggested to this author a possible correlation between the cytological picture and varying circulatory phenomena. Since the circulatory network is utilized in the perfusion method, a study of the correlation

between the circulatory phenomena and the cytological picture is only possible by this method. Because of this relationship, observations were made of hepatic mitochondria in the liver lobe during varying periods of vaso-occlusion.

II. ACKNOWLEDGEMENTS

I wish to thank Dr. W. L. Hard of the University of Maryland who suggested the problem, for his aid and many helpful suggestions during his supervision of this investigation.

Thanks are also due to Dr. J. V. Shankweiler for his valuable suggestions in preparing the photographs, and to the Department of Zoology of the University of Maryland and the Department of Biology of Muhlenberg College for facilities during the entire investigation.

III. HISTORICAL RESUME'

There is no one man who can be given the credit for discovering mitochondria. The "filia" of Fleming (1882), the "cytomicrosomes" of Strasburger (1882), and the "bioblasts" of Altman (1890) and others are all recognizable among the various structures which are regarded as being mitochondria of today.

In 1897 Benda, through new techniques, discovered these elements in many cells and applied the term "Mitochondria". The term mitochondria derived from the Greek Μίτος (mitos) a thread, and Χόνδρος (chondros) a grain, literally meaning "granular filament", was used from 1897 until 1919 when Duesberg (19) introduced the term chondriosome. This too, was derived from the Greek Χόνδρος (chondros) --grain, and σῶμα (soma) --body, hence, granular body. He substituted this new term for the term mitochondria, however today the terms are used by cytologists as synonyms. The work of Altman, Benda, Regaud, et al, offered a sharp impetus to the study of mitochondria; their distribution, morphology, and physiology. The results of mitochondrial investigations covering the many years of study since their discovery, are too voluminous to review here. The interested reader is referred to such reviews as those of Cowdry ('18), Wilson ('34), and Sharp ('34).

In general, results of previous work permit us to make the following conclusions regarding mitochondria. Mitochondria occur in practically all animal cells as well as plant tissues.

Knowledge as to the chemical make-up of mitochondria, though admittingly incomplete, indicates that mitochondria are probably composed of phospholipides and proteins in varying proportions. These structures stain black in Fe-hematoxylin. They show a characteristic affinity for stains such as Janus green and many other dyes commonly used with fixed material. They do not stain with neutral red. According to E. V. Cowdry (1918) mitochondria may be provisionally defined as "substances which occur in the form of granules, rods, and filaments in almost all living cells, which react positively to Janus green and which, by their solubilities and staining reactions, resemble phospholipins and to a lesser extent albumins."

Another problem which has greatly concerned the cytologist is the origin and multiplication of mitochondria. The author in his observations has not made an attempt to determine the cytogenesis of these elements. It would be too difficult to conclude or to establish an hypothesis on this matter until a further study could be made on the cytoplasm of those cells undergoing mitosis. Beams and King ('43), in their work on the origin of binucleate cells in the

hepatic cells of rats, report the same three predominating types of mitochondria in the cells undergoing division as I have observed in undividing mature hepatic cells. This might lead one to believe that they arise only from preexisting mitochondria and are therefore, permanent cytoplasmic structures.

Researchers on living material (Lewis & Lewis 1914, 15, 16), leave no doubt that mitochondria may appear anew and disappear, probably with some relation to metabolic processes in the cytoplasm.

Since mitochondria were first identified as permanent cytoplasmic structures many researchers have attempted to establish knowledge concerning their morpho-physiological significance.

One of the outstanding contributors to this knowledge is Noel ('33). He has divided the lobule of the mouse into three concentric zones based on mitochondrial deviation: (1) zone of permanent repose, (2) the intermediate variable zone, and (3) the zone of permanent function. These three zones contain rods, variable forms and granules respectively (fig. 16).

Kater ('31), in his work on the liver of the cat finds no intralobular zonation. He reports filamentous mitochondria in the periportal zone, and sperical mitochondria in both the perihepatic and intermediate zones.

Muggia and Sesulli (1932), from their report on the cytology of the rabbit liver cell, are of the opinion that the mitochondria are filamentous in all stages of the physiological activity of the animal. The appearance of granules is due to a transverse section across filamentous mitochondria.

Pallot (1933), on his study of the chondriosomes of the liver cells of cats, states that the mitochondria are filaments and small plaques oriented at right angles to nucleus and cell membrane.

Stephen ('41), made a study of hepatic mitochondria after hypophysectomy, pancreatectomy and thyroidectomy with conditioned feeding. In the first case the mitochondria were spherical; in the second, perinuclear clumping was evident. In the third and last case, thyroidectomy, no variation was observed.

With respect to the physiological activity of the mitochondria in the liver lobules various ideas have been suggested but no single theory is based on sufficient evidence to the exclusion of others. Kater and Smith (34) state that mitochondria are responsible agents for fat formation; Noel ('24), bile formation; Kater ('32), glycogen formation; Cowdry ('26), intracellular elements; and Ma ('23), secretion granules.

Kater and Smith ('34), and Smith ('34), in their investigation of the hepatic mitochondria, state that fat is

synthesized in the centers of these cellular elements.

In considering the possible methods of fixation of hepatic mitochondria, a factor which may not be ignored is the influence of the pH of the various fixatives on these structures. Scott ('35), states that mitochondria are best preserved as indicated by the degree of staining when the media is alkaline with a pH of 7.5 to 8.0. After incubation in an alkaline media a decrease in numbers is apparent while an increase in size is evident—some of the granules being as much as twice the diameter. Results must be based on living animals instead of test-tube cultures. In the test-tube they are not exposed to as complex a physiological aspect as when in the hepatic lobule.

The use of the perfusion technique suggested a study of the effect of variation of the circulatory flow on hepatic mitochondria. The literature on this hepatic circulation is voluminous. The conclusions of most of the investigators have been based on the evidence from perfusions and injection preparations of the excised livers and from experimental ligation of the hepatic artery and portal vein or both. Very few workers have perfused the tissues of the living animal while under anesthesia.

Myrtle (1884), by use of injected preparations of livers of amphibians and reptiles, concluded that the terminal branches of the hepatic artery do not form a capillary net-

work of their own, but empty directly into the large capillary network of the portal vein. He stated that the hepatic artery furnishes, together with the portal vein, a mixture of arterial and venous blood to the hepatic cells.

Leonard (1887), after studying injection of frogs' livers, did not agree with Hyrtle (1864), but concluded that the hepatic artery supplies blood only to the supporting tissue and bile ducts of the liver.

Cohnheim and Litten (1876) stated that in general exclusion of portal blood from the liver the hepatic artery could assume the function of the portal vein.

Mall ('06), after injecting defibrinated blood into the artery of a fresh liver observed three-fourths of it came out of the portal vein and one-fourth out of the hepatic vein. He concluded that the hepatic artery communicates more freely with the portal than with the hepatic vein.

Burton-Orpitz ('10, '11, '12) stated that about 75% of the total blood of the liver is furnished by way of the Portal vein. He stated that an intimate relationship between the venous and arterial blood is suggested by the fact that the fluid introduced into the hepatic artery escapes into the portal system.

Aunap ('31) by injection of carmine-gelatin into the portal veins and Berlin blue into the hepatic artery studied sections of the liver to determine the distribution of the two fluids. He found the sinusoids predominantly filled with

the fluid that had been injected through the artery. The interlobular branches of the portal vein contained, in some parts, fluid that had been administered by way of the artery and, in other parts, mixed fluid from both artery and vein. In the peripheral sinusoids, mixed fluid from both artery and vein was found.

Aumap ('31) then assumed that there was direct communications between the branches of the portal and hepatic artery in the interlobular spaces, but he could not find any. Accordingly, Aumap attributed the findings to the high pressure exerted on the arterial injections, a pressure which pushed the fluid through the lobules and central veins to the sublobular vein and back into the interlobular branches of the Portal vein. He said that the hepatic cells do not receive arterial blood, because embryologically speaking, the hepatic artery belongs not to the hepatic parenchyma, but to the gall-bladder and bile passages.

Jay ('34) in her study of the "intracellular blood canaliculi, inter and intracellular bile canaliculi" of the rat, disproves the theory of intracellular canaliculi as described by Shafer by use of the injection method of preparation of tissues. Using carmine gelatin and Berlin blue she demonstrated that by controlling the pressure at time of injection and the osmotic pressure of the solution, which-in this case-was Loche's solution. No intracellular injection material was visible.

Knisley ('39) studied intrahepatic circulation in the frog using his transillumination technique. He noted a sphincter at the exit of each sinusoid which regulates the amount of whole blood or red cells contained in the sinusoid. Knisley states that afferent hepatic arterioles and portal venules are contractile thus permitting the sinusoid to receive mixed blood or blood from only one source.

Two workers who contributed much to our knowledge of the vascular system in the liver are Wakim and Mann ('43), in their most recent work on "intrahepatic circulation of the blood", state that intrahepatic circulation manifests intermittent rhythmicity of irregular occurrence. About 75% of the hepatic circulation is in an inactive state under ordinary conditions.

Furthermore the above authors state the liver has a great functional reserve in the activity of its vascular system. The inactive sinusoids go through two phases-a non storage phase during which the sinusoids have scarcely any blood cells in their potential lumina. This suggests a blood storage function of the liver, as well as a storage phase during which the sinusoids are packed full of motionless cells.

Many authors have made studies of hepatic circulation and at the same time conducted studies of voluntary ischemia in various organs. Briefly reviewing this literature on vaso-occlusion Voegtlin et al ('34), on their observation in skeletal muscles of the rat, the author observed gradual rise

in acidity from a normal value of 7.55 to a range of from 6.8 to 6.6 in two hours.

White ('39) in his study or glomerular intermittance in normal dogs and rabbits found that varying response of different tubules may be related to circulatory condition at the time of vaso-occlusion.

The most outstanding contribution in the study of ischemia is the work of Emmel ('40), who studied the changes in mitochondria and tissue acidity following interruption and restoration of the renal circulation in the rats kidney. He found enspherulation and fragmentation of mitochondria occurring in a few proximal tubules within six minutes after enclosure of renal artery. As the ligation was prolonged the number of tubules involved increased. Mitochondrial changes in the distal tubules became apparent after thirty minutes. With ligation of the renal artery there was a rise in the tissue pH from 7.2 to 6.5 in the course of an hour. In view of the close correspondence between the appearance of mitochondrial changes and the rise in tissue acidity it might suggest that mitochondrial changes are to be associated with changes in tissue acidity.

IV. MATERIALS AND METHODS

The white rat, Mus norvegicus albinus, of the Wistar strain was used in this investigation. This laboratory specimen was chosen because of previous observations on the same species. The perfusion method has been tried by this worker on guinea pigs, cats and rabbits in the preparation of materials for general histological study. Many animals were used in carrying out this experiment as well as perfecting the technique.

The animals were of both sexes averaging in weight between 200-300 grams. The animals were kept in a room which had a constant temperature of from 65-75° F.

The animals were fed regularly with a balanced diet determined by known nutritional requirements of the laboratory rat yielding on a chemical analysis the following:

	Known Nutritional Requirement of Laboratory Rat	Analysis of Purina Dog Chow Checkers
Protein, %	20-25	22.50
Fat, %	5.00	5.50
Fibre, %	3.00	3.75
Ash, %	6.00	7.00
Carbohydrates		
Nitrogen free extract, %	50.00	50.25
Moisture, %		11.00

Calcium, %	1.00	1.75
Phosphorus, %	.50	1.00
Magnesium, %		.11
Potassium, %		.75
Soluable Clorides (as NaCl), %	.75	1.25
Maganese, parts per million		75.00
Iron, parts per million		180.00
Copper, parts per million		8.00
Cobalt, parts per million		.10
Vitamin, D, U.S.P. XI Units per gram	1.00	2.30
Vitamin A, U.S.P. XI Units per gram[1]	5.00	8.50

(1) Parts per million = U.S.P. $\frac{\text{Units per gram}}{4.5}$

Carotene parts per million		1.70
Thiamin Chloride (B_1) parts per million[2]	2.00	4.00

(2) International Units = $\frac{\text{Parts per million}}{3}$

Riboflavin (B_2), parts per million	3.00	4.75
Pantothenic acid, parts per million	5.00	10.00
Niacin, parts per million		35.50

Tissue designed to study the effect of fixation by the immersion method was prepared as follows: the animal was killed by a blow on the head; the body cavity was opened; and tissue was immediately removed from all lobes of the liver and placed in the following fixatives, Bensley's, Regaud's, Regaud's modification, Champy's and Mann-Kopsch. Further

information regarding the chemical make-up of these fixatives may be had by consulting Lee (37).

Tergitol and cellosolve in 1% solutions were added to the various fixatives in an attempt to increase the rate and intensity of the fixation as suggested by Hance ('36). These reagents did not produce any accreditable increase in penetration of the fixatives. Cellosolve has been recommended by the above worker as a catalyst to decrease the staining time. In an effort to get uniform staining over the entire slide they were placed in a coplin jar of pure cellosolve. Very little if any improvement was noted.

After fixation of the tissue it was washed for twenty-four hours, dehydrated, cleared in toluol and embedded in paraffin (m.p. 52°C.-58°C.). To overcome hardening, cedarwood oil and benzene were used as declochlixers, cedarwood oil giving the more satisfactory results in that the shrinkage was negligible.

The tissue was sectioned at a thickness of 2 to 4 u. The sections were applied to the slide with albumen fixative. Sections were stained with the Altman technique employing acid-fuchsin and picric acid or by the Heidenhain technique, directions for which may be had by consulting Lee ('37).

After the immersion method was used in attempts to obtain a true picture of the mitochondria, it was found that this technique did not offer a very satisfactory opportunity to study the entire liver lobule since the fixative did not

penetrate throughout the piece of tissue (fig's. 3 to 10). A technique had to be established by which the tissue could be fixed evenly throughout the entire block instead of the periphery as was the case above. Fixation of this type was essential in order to study mitochondria in the central as well as the peripheral cells of the hepatic cords.

The first attempt at perfusion was by the injection routine. The animal was anesthetized, and the body cavity was opened as previously explained. A sterile 5 cc. syringe was used with a 21 gauge needle. The post vena cava was nicked and the blood permitted to pass out into the body cavity. This was removed with cotton swabs. The needle was inserted in the hepatic portal and tied in place with a silk thread to permit only the fixative to pass into the liver. About 5 to 10 cc. of the fixative was injected directly into the hepatic portal vein. This method did not prove too satisfactory, primarily because the amount of the fixative injected was too small. Ample consideration will be given to this method in the discussion of this paper. Because of these encountered difficulties it was necessary to devise some method by which large amounts of the fixative could be passed through the liver at a minimum of pressure. Thus the perfusion method of fixation was attempted.

The apparatus consisted of two aspirator bottles of approximately (2000 c.c.). Each bottle was held to a

36 in. from laboratory stand (fig. 11). One bottle contained Ringer's solution, and the other Regaud's fixative.

Rubber tubing one eighth inch in diameter connected each aspirator bottle to the ends of a three way stop-cock. A piece of rubber tubing the same diameter was attached to the middle opening of the stop-cock. Attached to this rubber tubing was a glass cannula made by pulling one-fourth inch glass tubing to a fine point, (fig. 13a).

The animal was anesthetized with ether by being placed in a glass chamber with ether soaked cotton. After approximately three minutes the animal was completely anesthetized. The animal was then removed from the chamber and attached to a board by frog clips, dorsal side down. An ether cap was kept handy in case the animal showed signs of recovering from anesthesia. After the animal was securely attached to the board a longitudinal incision was made in abdominal cavity running from a point about 5 cm. anterior to the genitals to the diaphragm. A transverse incision was made in the region of the liver. The animal was sectioned in this way in order to expose the abdominal organs, especially the liver and the hepatic portal. The next step was to remove the connective and adipose tissue which surrounded the hepatic portal blood vessel. After this was completed a small light was a plastic hook on the end was placed under the hepatic portal. This instrument was

fashioned from a plastic tongue depressor (figs. 11,12). With the use of this lamp the author was able to observe the blood flowing through the hepatic portal blood vessel. After the light was placed in position under the blood vessel the glass cannula was forced into it. Immediately a silk thread was tied around the cannula in order to hold it in position and prevent the back flow of blood. The stopcock was turned in order to permit the Mammalian Ringer's to flow into the vessel. The dorsal aorta was nicked to permit the blood to flow from the animal. Cotton swabs were used to remove the collecting blood. The Mammalian Ringer's slowly replaced the blood in the heart and the liver. The first noticeable change in the liver was the change from a deep red color, characteristic of the liver, to a white color. This was due to the replacing of the blood within the lobules with Mammalian Ringer's. The gradual removing of blood from the liver and replacing it with Mammalian Ringer's is clearly visible (figs. 13, 14, 15). Upon close examination one will observe very small areas, 1 - 3 sq. cm. in diameter, from which the blood has not been removed. An explanation of this will be considered in the subsequent discussion. After the Ringer's was permitted to wash through the heart and lungs and was evident at the draining point in the dorsal aorta the stopcock was turned to permit the flow of the fixative through the animal. Complete perfusion was evident when

the liver turned from a white color to a deep yellow indicating that the fixative had passed throughout the liver lobe. This perfusion was continued for fifteen minutes, permitting complete fixation to take place. The liver at the time was very firm. These small reddened areas afore mentioned were still the same color indicating the persence of blood in these areas.

Alterations of this technique were used. Certain lobes were tied off and the remainder of the liver perfused after which comparisons were made between the tied off lobules and the perfused. Samples of tissue were taken from all areas of the various lobes and placed in the respective fixing solution.

Drawings were made with the aid of a monocular research scope, having a 10 X ocular and an oil immersion objective with a numerical aperature of 1.35, the combination of lenses giving a magnification of approximately 1100 diameters. The cells were drawn with the aid of a camera-lucida. The equipment used for doing the photographic work in this report was a Bausch and Lomb Horizontal-Vertical camera (Type A), and an E. Leitz Micro Camera attachment with a side inspection tube.

V. OBSERVATIONS

A. DESCRIPTION OF THE NORMAL LIVER LOBULE

The liver in the rat is divided into four parts: the median or cystic lobe, which bears a deep fissure for the ligamentum teres hepatis; a right lobe partially divided into an anterior and posterior lobule; a large left lobe; and the small caudate lobe (Spigelian lobe) which encircles the esophagus. The rat has no gall bladder; the ductus choledochus is made up of tributaries from the various lobes of the liver. The lobulation of the adult liver is diagrammed in figure 1.

The liver is surrounded by a connective tissue sheath composed mainly of white fibrous and reticular tissue. This sheath invaginates along all vessels forming trabeculae which enter the liver. They extend into the intra-lobular canals forming a delicate reticulum that supports the functioning hepatic cells and the intra-lobular capillaries and bile ducts.

Each lobe is divided into many smaller units called lobules (fig. 6). Wepfer (1664) was the first to recognize the lobule in the pig. Subsequent observations showed the lobule was composed of radiating bands of cells, called hepatic cords. The lobule in the rat is about.5 mm. in diameter. It has been estimated that there are about 480,000 in the liver of the rabbit.

Each lobule contains a central vein, a terminus for all the radiating cell cords, the sinusoids, bile capillaries and intralobular veins. Each lobule is supported by a reticular mesh work of connective tissue and cells which compose the reticuloendothelial system. Connective tissue is more evident in the regions of the peripheral veins, marking the boundry between the lobules. Individual lobules are not as easily identified in the rat as they are in the pig or camel.

Generally speaking, all hepatic cells appear similar, save for variation in size, number, and form of mitochondria, number of nuclei, and amount of inclusion materials present.

The hepatic cells of the rat are polyhedral and average 25 u in size (Shay '38)

In these cells the nucleus is centrally located and the cytoplasm appears granular. This is probably due to the cytoplasmic inclusions present, fat, glycogen and mitochondria. The uninucleate condition is predominant, however, binucleate cells are not uncommon averaging about 18% (Munzer '23). Binucleate cells are regional in that they are usually found in groups in the regions of the periphey of the lobule in young rats, thus indicating cellular multiplication in this area.

Cell membranes separate adjoining hepatic cells.

Some observers state that cell membranes are not present but that their apparent presence is due to highly concentrated regions of the ectoplasm of the cell.

A description of the hepatic mitochondria and the intracellular fat will be given in detail under the observations of the paper.

The following is a description of the arterial and venous blood supply of the liver.

The Coeliac Artery (fig. 2) is unpaired, arising from the lateral surface of the aorta at the level of the crus of the diaphragm. It has a short trunk which divides into three branches, as follows:

The Left Gastric - (coronary) - runs immediately toward the cardiac opening of the stomach from which it branches to both surfaces of the stomach; the Lineal Artery (Splenic) passes to the left and behind the stomach to the spleen, giving branches to the stomach and pancreas as well, and the Hepatic Artery which turns right sending a branch downward toward the duodenum, before continuing to the liver where it breaks up into many small anastomoses and terminating sinusoids.

The Hepatic vein (fig. 2) collecting branches from the liver, enters the vena cava inferior just as this vessel pierces the diaphragm. The Hepatic Portal System comprises those veins which receive blood from the digestive tract below the diaphragm, and from its associated organs,

the spleen and pancreas, and carry it to the liver where it passes through capillaries before entering the systemic system via the hepatic veins, and the vena cava inferior. The main tributaries of the hepatic portal vein are the Lienal (Splenic) which has its origin in several veins from the spleen, pancreas, greater curvature and fundus of the stomach (fig. 2); the Superior Mesenteric Vein which collects blood from the small intestine, the caecum, and from the whole length of the colon, (the superior mesenteric receives not only tributaries corresponding to the branches of the superior mesenteric artery, but also one corresponding to the inferior mesenteric artery); and the Pyloric Vein which collects blood from the region of gastroduodenal branch of the hepatic artery.

B. OBSERVATIONS OF MITOCHONDRIAL FORMS IN NORMAL PERFUSED HEPATIC TISSUE

Mitochondria as observed are of three general types, namely, rods, filaments and spheres. All other forms appear closely associated to the above types. The spheres are the predominating type localized in the normal liver tissue in the peripheral, intermediate and central parts of the lobule. They constitute nearly all the mitochondria in the peripheral region and about half in the intermediate area. (fig. 20). In tissue which is fixed with any other fixative other than Regaud's—the spherical mitochondria tend to increase in numbers in the respective cells and at the same time are present in larger areas throughout the lobule (fig. 21). In tissue which is not properly fixed (fig. 6) the mitochondria are spherical throughout the entire liver lobule. As to the size and contour of these spheres, they are very diverse, ranging in size from .5 to 2 microns (fig. 33). The outer surface of the larger mitochondria in some cases appears rather coarse giving an appearance of a somewhat distorted body (fig. 33A). The second most frequently occurring type is that of the rod shaped body, localized in most cases in the intermediate part of the lobule (fig. 34). The rod shaped mitochondria vary as to length, ranging from .5 to 5 microns, the outer surface usually being of a smooth nature. They are of variable diameters

ranging from fine structures, which require concentrated effort in order to see them, to swollen elements of a spheroidal nature (fig. 34). Some of the longer rods are curved in a quarter moon shape.

The third most frequent type is that of the filament. This form of mitochondria has been observed by the author only in the cells of the central and intermediate area. This type of mitochondria is more numerous in the cells of the central region than in any other part of the lobule. There is very little variation in this form of mitochondria.

Mitochondrial forms not entirely like the afore mentioned were visible in some areas of certain hepatic lobes. The presence of these forms is not consistant. Consider first the type shown in figure 33A which seems to be a form related to the spherical type. The size of these structures was always relatively larger than the spherical type. The edges were rough and the body of the mitochondrium was distorted. This form was present in a few cases in the peripheral region of the lobule, located within the cell closely associated with the smaller spherical forms. Another type, which appeared at various times in the peripheral tissue of the lobule and in the intermediate zone as well as when the tissue was exposed to ischemia, was that form of mitochondria with clear centers (fig. 34A). This varied form was usually spread evenly

among the mitochondria in the cells of the intermediate and the periportal zones. There was no visible relationship between the mitochondria of this type and the vacuoles. In cells that were fixed with Bensley's fixing solution the hollow spherical forms still persisted. Should the centers of these forms have been of a fatty consistency they would have blackened with osmic acid. In cells where the hollow spherical mitochondria were observed there usually appeared many other variations of this type in the same cell (fig. 37). In ischemic tissue this type of mitochondrial form was more prevalent in the peripheral area.

Another type was that of the rod with either one or both ends swollen (fig. 35A). This form was few in number and when present was found in the peripheral part of the lobule in other than normal tissue. They were most prevalent in the peripheral cells of the intermediate region of ischemic tissue. There were no clear centers in these club shaped forms. Not any of these varied types of mitochondria were very numerous with the exception of the hollow spherical type which was present in greater numbers in the intermediate and the peripheral regions of the lobule.

Additional observations were conducted on liver tissue that had been perfused with Ringer's solution which was hypertonic to the blood in the sinusoids. This hypertonicity was determined by the use of the hematocrit (fig. 36). The cells one to eleven represent a complete hepatic

cord. The cells of the central zone one, two and three, show fine granular mitochondria of variable sizes and hollow spherical types. In addition there are small rod shaped mitochondria. In the intermediate zone (cells 4,5,6,7) the mitochondria are granular with ovoid rods and a few hollow spheres. In the peripheral part of the lobule the mitochondria are all spherical of about like diameters. All the cells in the three zones show the presence of vacuoles. The vacuoles are not large enough to cause a bunching of the mitochondria. The blood cells in the active sinusoids exhibit crenation. Cells 12 to 16 were taken from a region boardering a central vein. These cells exhibit about all the forms (fig 33 to 35A) that have been observed up to this point. The blood cells in the sinusoids boardering these cells are crenated.

C. DISTRIBUTION OF FAT IN THE HEPATIC LOBULE

In normal liver tissue fixed with an osmic acid fixative fat globules may be seen to be spread throughout the entire liver lobule (figs. 37 and 39). This figure shows cells taken from an hepatic cord, cell one being adjacent to the central vein, and cell four being close to the peripheral vessel. Cells two and three have been taken from an intermediate area. The fat globules are more numerous in the cells nearest the central vein, raning from minute spheres to large vacuoles in size. In some cases the fat globules are so large that they cause the mitochondria to have a congested appearance. Cells in the intermediate area do not have as many fat globules as those cells of the central zone. In the peripheral cells the fat globules are fewer and show no relationship to the mitochondria (cell 4, fig. 37). There are hollow spherical mitochondria present in the intermediate and peripherial zones in which there was no sign of fat present. On closely focusing, the mitochondria appear to form bands around the fat vacuoles.

D. MITOCHONDRIAL SHAPE AND DISTRIBUTION RELATING TO FIXATION

1. By Immersion.

Hepatic tissue fixed with Benaley's solution--fixation by an osmic acid fixer of this type offers us a very specific picture (figs. 3 and 4). Fixation in the penetrable zone, was evident to a depth of approximately three cells (fig. 4). The cells show no distortion of the cell wall or intracellular elements (cell 1, fig. 3). The nucleus was ovoid and exhibited chromatin granules among the linen meshwork. There was a tendency on the part of some nuclei to retain the stain. The cytoplasm was smooth and showed no coagulative tendencies. The mitochondria in this area were of two types, rods and spheres. The rods appear about the same size in thicknesses and in lengths. The spheres were variable, ranging from small punctiform elements to large globular-like structures. There was a tendency toward grouping on the part of the mitochondrial elements in a few of the cells in this area, however, majority of the cells showed the elements evenly distributed throughout the cytoplasm. The number of mitochondria in this area were more numerous than in the transition (cell 2, fig. 3) or the central area (cell 3, fig. 3). In the transition area the cells did not show an even outline as in the former case. The

The nuclear elements are distinguishable and appear to be fixed better than the cytoplasm. The cytoplasm showed evidence of coagulation. The mitochondria tend to be all spheres, variable in shape; few rods were present. They were fewer in number as compared to the penetrable area. The mitochondria tended to be coaugmented, in majority of the cases, on the side of the cell adjacent to the penetrable area leaving the side toward the central area almost entirely free of mitochondria (cell 2, fig. 3). In the central area the tissue appeared to be inadequately fixed (fig. 4). Cell 3 in figure 3 was taken at random within the central area. The nuclear membrane and the nuclear contents showed evidence of degeneration and fragmentation. The cytoplasm was clumped in spots leaving large clear areas. Mitochondria were absent in this area with the exception of a few cases where only one or two mitochondria-like bodies were in evidence.

Fixation by the Mann-Kopsch method as in the preceding explanation with Bensley's, penetrates only a thin area of tissue (fig. 5). From the general appearance of the tissue one is led to believe that the cells fixed by this reagent are smaller than those fixed with Bensley's fluid. The cells were drawn from a region which appeared to be highly vascular having many blood capillaries blending with the hepatic cells (fig. 5). The penetrable zone

is very shallow indicating that the fixative entered the tissue to only a small extent (fig. 5). The cells in this zone appear to be fixed satisfactorily (cell 1, fig. 6). The nuclear and cell membranes are intact. The mitochondria are numerous and are of a small spherical nature. No rod shaped mitochondria are in evidence. The mitochondria are evenly dispersed throughout a smooth cytoplasm. In the transition zone (cell 2, fig. 6) the cells appear to be fixed satisfactorily, however, there is a difference as to the mitochondrial numbers as compared to the penetrable area.

There is no great line of demarcation between the penetrable area and the transition area (fig. 5). The two areas extended into the tissue to the extent of about twelve to fifteen cells. The mitochondria in the transition area are all of a spherical nature and are evenly dispersed throughout the cytoplasm. The transitional area is very easily distinguished from the central area (fig. 5). In this area the cells are shrunken; intercellular spaces and sinusoids are abnormally large. Intra-cytoplasmic and intra-nuclear structures are indistinguishable and peri-portal structures are fragmented. There is no evidence of mitochondrial elements in the central region.

Observations on hepatic tissue immersed in Flemings mitochondrial fixative (figs. 7 and 8) in the penetrable area (cell 1), the nuclear and cytoplasmic structures as

well as the nuclear membranes show evidence of slight overfixation. In the extreme outer row of cells, the mitochondria are of variable shapes and sizes including rods, spheres, and many intermittant stages of these (cell 1, fig. 7). They are numerous and are spread evenly throughout the cytoplasm. In the cells nearer the transition zone the mitochondria are generally spherical (cell 2, fig. 7). In only a few instances were short rods observed. The mitochondria in this area appeared slightly larger than in the extreme outer row. In the transition area, some of the cells have the same mitochondrial characteristics as the penetrable area. Because of this it is rather difficult to differentiate precisely where the one stops and the other begins (fig. 7). The only difference between these two areas is in the numbers of mitochondria and the location of these elements within the cell. In the transition area the mitochondria are fewer and are located centrifugally in respects to the tissue cube (cell 3, fig. 7). In the central area very little difference is noted from that condition found in Mann-Kopsch as given in the forgoing explanation (cell 3, fig. 6).

Observations on hepatic tissue immersed in Regaud's mitochondrial fixative (figs. 9 and 10), fixation was more satisfactory with Regaud's solution than with any other

fixative used for immersion. The penetrable area of the tissue was of great depth, in some cases to 15 or 20 cells (fig. 9). The cells were fixed satisfactorily. The nuclear structures were clearly distinguishable. The nucleoplasm was smooth with its delicate linen fibers clearly visable. The membrane showed no signs of shrinkage. Protein precipitation of the cytoplasm is entirely smooth and homogenous. Figure (10) shows a few cells taken from an hepatic cord that was in the region of the permeable area. The mitochondria in the cells nearest the central vein are of three types, spheres, filaments and small rods (cell 1, fig. 10). The mitochondria are fewer in number in the peripheral part of the cell cord and are of greater diameters (cell 3, fig. 10). In all the cells they appear to be evenly spread throughout the cytoplasm with no bunching or coaugmentation. In the transition area the mitochondria are all spherical and are larger than the spherical mitochondria in the penetrable area as when fixed with Flemming's reagent (cell 2, fig. 6). In the central area complete disintegration of the cell is in evidence as when fixed with Flemming's reagent (cell 3, fig. 6). No cytoplasmic or nuclear structures are distinguishable.

In addition to the above mentioned fixatives, Schridde's fixative was also used but without satisfactory results.

Of all the above mentioned fixatives used only three general types of mitochondria, filaments, small rods and spheres were viewed. The only variations of these forms were in size. No beaded mitochondria were in evidence.

2. By Injection. *

The amount of injected material forced into the hepatic portal blood vessel was too small (5 cc.) to give complete fixation of the entire lobule. As a result of this the immediate area surrounding the perihepatic vessels alone was fixed. Only the peripheral vessels of a large nature received the fixative. The smaller vessels of a higher order did not receive any of it. We shall for the present consider the perilobular canals, which contain the blood vessels and the bile ducts (figs. 18, 19). The supporting connective tissue trabeculae which pass through these canals are fixed satisfactorily. In all cases where there are large amounts of connective tissue present fragmentation appears. This presumably is due to the pressure exerted in the canal by the forcing in of the fixative. In regions where sinusoids were seen to be adjacent to the peripheral vessels they appeared abnormally large indicating a result of intra-sinusoidal pressure exerted

* For an explanation of the phraseology used in the observations and discussion see figure 16.

by the injection fluid. The blood cells have their characteristic shape showing no signs of flaccidity or turgidity. The region surrounding the inter-hepatic ducts may be divided into three areas similar to those reviewed in the regions of the periphery of the tissue prepared by the immersion method (fig. 17). In the layer of cells immediately surrounding the interhepatic canals the mitochondria appear only as spherical bodies or varying size. There is no evidence of grouping of the mitochondria in any particular area of the cytoplasm. The cytoplasm is not congealed, containing a nucleus with a smooth nuclear membrane and clearly distinguishable nucleor elements. There is no evidence of cellular variation. The numbers of mitochondria are fairly constant in all cells in this area. The extremely large mitochondria which were in evidence in some of the cells may have resulted from a fusion of a few smaller ones, since the large ones were usually in close association with the smaller forms. Moving centrifugally, the transition area is marked by a group of cells which appears to be satisfactorily fixed but has very few mitochondria within the cells (fig. 17). The mitochondria are localized in that part of the cell nearest the peripheral vessels. They are of one type, namely, spherical. Very little intra-cellular variation is noted in the size of the mitochondria. There is considerable variation, however, in the number of mitochondria

in the cells in this transitional area. Moving still further from the nodal point we find the area to which no part of the fixative has reached. These cells offer us a similar picture to that which was observed in the central area in tissues prepared by the immersion technique (figs. 3 to 10). This same state of the tissue continued throughout the entire lobule to the region of the central vein.

Only one morphological type of mitochondria was observed in this peripheral region, that of a spherical nature. There were slight variations in the size of these spheres. There was no indication of any rods, filaments or beaded filaments in this area.

3. By Perfusion.

Consider first the tissue prepared by using a modification of Regaud's fixing solution (figs. 20 and 23). The cells shown in these figures do not represent a complete hepatic cord. Because of the numerous cells which would have had to be represented and the similarity among the members of the hepatic cord, only type cells were drawn. Cells 1 are typical cells taken from the central vein region. These cells appear to be fixed satisfactorily since the cytoplasm shows no signs of coagulation. The mitochondria are of three general types: slightly curved rods, spheres, and filaments, one type appearing as frequently as the other in the cells. Only a sufficient number of cells were re-

presented in order to show the various forms and their locations. The mitochondria show no evidence of polarity or any nuclear association as reported by previous workers. Few vacuoles were observed in the cells surrounding the central vein. There was no visible relationship between the mitochondria and vacuolar genesis.

Moving peripherally, cells 2 figure 20 and cells 2 and 3 figure 22 are of the "intermediate zone" of the lobule. These cells have the same general characteristics as cells 1 with the exception that the mitochondrial elements of the three types are fewer in number and the rods are not as long as those of the "central Zone". In the intermediate zone there is a definite increase in cytoplasmic vacuoles. All mitochondrial forms are distributed evenly throughout the cytoplasm. In cell 3 figure 20 and cell 4 figure 22 we have cells typical of those in the peripheral zone or the "zone of permanent function". In this area a great number of the mitochondria are enlarged, the rods being more ovoid and the spheres increasing in size; few filaments are present. There is no evidence of any variation of form other than the rod, filament and spherical mitochondria in these hepatic cords. The mitochondria tend to decrease in number from the central to the peripheral zone. In the central zone the filamentous type predominates. In the intermediate region all three types are equally represented. In the

peripheral zone rods and spheres are equal in number and filamentous mitochondria are conspicuously absent.

A few examples of perfusion were completed with Bensley's fixative. In figure 21 are shown representative cells taken from an hepatic cord. In cell 1 of the central zone or "zone of permanent repose", the mitochondria are of three types, the filamentous type predominating. The mitochondria that are of spherical nature are smaller in cell 1 than in cells 2. In this zone the mitochondria are evenly dispersed throughout the cytoplasm. Fat vacuoles were present in several of the cells. In the intermediate zone (cells 2 and 3, fig. 21), the mitochondria were of variable forms: spheres, spheroids, short rods, curved rods and a few filaments. These various forms were evenly divided. The mitochondria in these cells show some evidence of grouping. The clear zones formed by the absence of mitochondria appear to be of the same nature as the vacuoles previously observed. In the peripheral zone (cell 4, fig. 21) the mitochondria are of two general types, spheres and rods with the filamentous type appearing occasionally. All the rods and spheres show a tendency to be swollen. Mitochondria in this area were fewer than in any other zone in the hepatic cord. There was no evidence of grouping within the cell boarders. The mitochondria did not fill up the entire cell; the region adjacent to the cell membrane was free of mitochondria.

C. ISCHEMIA

1. Natural Ischemia.

In livers which were being perfused there were areas ranging from .5 to 15 mm. which would not perfuse. Instead of permitting the blood in these areas to be replaced with Ringer's solution and then the fixative, the tissue retained the blood throughout the entire period of perfusion regardless of the length of the perfusive time. These areas which exhibit natural ischemia may be seen by observing figure 15. Consider first the extracellular elements.

The lobes of the liver while being perfused turn almost white due to the perfusion of them with Ringer's solution. They then turn yellow while the fixative is passing through, displacing the Ringer's solution. All this time the natural ischemic areas retain their red blood color. These islets of unperfused tissue were spread in various regions throughout the lobes, but variously located in each perfused animal (fig 15). On a microscopic examination of the entire lobule (fig. 29) we can see a section of one which has retained the blood in the sinusoids. All these sinusoids are in a storage phase. Sinusoids of this type appear to have a greater diameter than those that lack blood. In very few cases were serial sections

complete, thus making it difficult to follow through the entire length of these sinusoids.

Still other sinusoids within the same lobule may be cleared of all blood; these are thought to be in an active phase (figs. 29 and 30). The blood cells in these storage sinusoids in a majority of the cases were slightly crenated (fig 30). The mitochondrial and cytoplasmic picture was confusing in all cases where observations were made. In figure 31 we have cells representing an hepatic cord. In the central zone (cell 1) designated as such by the fact that this cell marks an area definitely different from all the other cells in the cord, the mitochondria are spread evenly throughout the cytoplasm with the exception of the area immediately surrounding the cell membrane. They are of varied forms, small spheres, ovoid-bodies, and swollen short rods. Cells 2 to 5, making up the remainder of the hepatic cord, show a great similarity as far as cytoplasmic elements are concerned; some have small vacuoles present. Others show the presence of larger vacuoles which probably resulted from fusion of the smaller vacuoles. These vacuoles influenced the position of the mitochondria within the cells. The mitochondria in both hepatic cords make up a large group of diverse elements. These elements include short swollen rods of varying size, and spheres including very large ones with clear centers. There seems to be no

formative relationship between these vacuoles and the mitochondria. Cell 5 was drawn from the outer part of the lobule from an intermediate region between two portal veins. It is considered as a cell of the "zone of permanent function". The mitochondria in this cell are all spheres, the larger ones being coarser in outline than the smaller.

Still another observation of natural ischemia is that of figure 32. Cells 1 to 4 are types from regions of an hepatic cord. Cell 1 was taken from the perilobular region; cell 4, in the region of the perihepatic canal. These cells contain few mitochondria, all of a spherical nature. There is no difference between the mitochondria of those in the region of the peripheral blood vessels and those merely lying on the margin of the lobule. All cells in this region appeared to be vesicular. The intermediate zone of the lobule is represented by cell 2. The mitochondria in this area are spherical bodies ranging from very small granules to large hollow spheres tending to localize in the center surrounding the nucleus and the intermediate part of the cell. Cell 3 is a member of the central zone. The mitochondrial forms in this cell consist of dumb-bell shaped bodies, curved rods, and spheres, some of which have clear centers. The mitochondria in this central zone are more numerous than in any other area of

of the entire lobule. In both the central and intermediate zones vesticulation is evident, however, not to such a great extent as in the perilobular region.

2. Induced Ischemia (figs. 23 to 28)

This phase of the study of mitochondria was undertaken in order to observe the variation in the mitochondrial forms during varying periods of induced ischemia. The observations of ischemia have been studied from tissue prepared by the immersion method. This method is inadequate for fixing the entire liver lobe, however, it fixes a sufficient number of cells in selected areas to permit a study of this type.

Comparisons can be made with the perfused tissue, since lobes were tied off for the same length of time that another lobe was being perfused. In not all cases were the same lobes tied off. Every time the perfusion was used another lobe was occuled; by doing this interlobular variations could be studied. The liver lobes that were perfused appeared as those in figure 15, assuming a hardened consistency as perfusion progressed. In the lobes that were tied off during perfusion the only visible change was that in color from a red to a dark red.

(1) Ligation 8-10 minutes (fig. 23). The left and Spigelian lobes were tied off.

The cells shown here are taken from various parts

of an hepatic cord. In the central zone or "zone of permanent repose" (cell 1, fig. 23) the mitochondria are similar to those in the same areas in normal liver tissue, with the exception that there are fewer spherical mitochondria present. In the "intermediate zone" there appears to be a general thickening of the rods and filaments (cells 2, fig. 23). In the "zone of permanent function" (cells 3, fig. 23) the mitochondria are enspherulated and show rods which are swollen and slightly curved. Mitochondria with clear centers were observed in the "intermediate zone" of the lobule (cells 2, fig. 23).

(2) Ligation - 15 minutes (fig. 24). The left lobe was tied off.

The cells were taken from a short hepatic cord. Little difference is noted in these cells from those reported above that were ligated for ten minutes. Short rods and few filaments are seen.

(3) Ligation - thirty minutes (fig. 25). The right lobe was tied off.

Some cells of the "central zone" still have short filamentous mitochondria (cell 1, fig. 25). Mitochondria are more numerous in this area than in the intralobular zones. The large mitochondria appear closely associated as fusion elements in the cytoplasm (cell 3, fig. 25). Clear areas are visible in the zone of the nucleus.

The majority of the mitochondria are spherical in cells of the intermediate zone (cell 2, fig. 25) and are smaller than those observed in the "central zone" (cell 1, fig. 25). Greater amounts of vacuolization are evident. The mitochondria are grouped in the periphery of the cell and large clear vacuoles are seen in the region of the nucleus. The nuclei show evidence of distortion in the cell type taken from the "periportal zone"; the nucleus shows some evidence of distortion similar to the condition viewed in the "intermediate zone". The mitochondria are slightly larger than in the "intermediate zone", and are grouped in some areas of the cytoplasm showing a tendency to fuse. Only one type of mitochondria is present and it is spherical (cell 3, fig. 25).

(4) Ligation - thirty-five minutes (fig. 26). The caudal lobe was tied off.

There are a few filamentous mitochondria in the "central zone", spheres predominating (cell 1, fig. 26). In the "intermediate or variable zone" (cell 2, fig. 26) and the "parihepatic zone" (cell 3, fig. 26) only spheres are present. Generally there is very little noticeable difference from the lobe ligated for thirty minutes (fig. 25) and this one for thirty-five. Cells taken from the "intermediate and the periportal zones" show a greater number of mitochondria near the nucleus. This may be due

to the location of the vacuoles in the mid-area of the cell.

(5) Ligation - forty-six minutes (fig. 27). The lateral lobe was tied off.

The figure shows a group of cells in the region of the central vein and in the region of the perihepatic blood vessel. The blood cells show no signs of swelling or shrinkage. The mitochondria in the cells in the "central zone" (cells 1, fig. 27) are of a small granular nature, distributed rather evely throughout the cytoplasm. All cells of this group have about the same number of mitochondria. In the cell which represents a typical "intermediate type" (cell 2, fig. 27) the mitochindria all all fine granules showing a tendency to group in spots. The cytoplasm showed evidence of vacuolization. This vacuolization is also evident in the "perihepatic zone" (cells 3, fig. 27). The mitochondria are spherical and are of all sizes. In some of the cells there seems to have occurred a fusion of the smaller mitochondrial granules to form large, swollen, irregular masses. Generally it may be said that the mitochondria are more numerous in the "central zone" than in any other zone in this type of lobule.

(6) Ligation - one hour (fig. 28). The median ventral lobe was tied off.

After vaso-occlusion for a period of one hour the cells in the "central zone" (cells 1, fig. 28) show fine

granular mitochondria embedded in a precipitated cytoplasm localized in the intermediate part of the cell leaving large clear vacuoles in the region of the nucleus and the cell membrane. Cells of the "intermediate zone" are not shown because of their similarity to those drawn from the "perihepatic zone" (cells 2, fig. 28). Members of this group show evidence of striking cytoplasmic changes taking place. The cells show some degree of separation from the connective tissue in the region of the portal canal. The cytoplasm is a precipitated disintegrating mass with large vacuoles. The cytoplasmic elements are coagumented making it impossible to observe separate elements. Ligation for a total of ninety minutes was conducted but results were similar to those as of sixty minutes afore mentioned.

VI. DISCUSSION

This investigation of the mitochondria in rats' livers differs from previous studies in that the perfusion of livers through the hepatic portal was attempted while the animal was still alive, thus giving a true picture of the mitochondria in the hepatic cells of the albino rat while they are in an active physiological state. Before this method of perfusion was adopted, the effects of etheral anesthesia on mitochondria had to be determined. This was accomplished by comparing the mitochondrial forms of the anesthetized rat with those of the animal that was rendered unconscious by a blow on the head, and those that were anesthetized by the use of chloroform. On comparing these cells no visible effects of ether or chloroform anesthesia were noted. These observations seem to agree with those of Krupp and May (1927), in their study of the effects of ether, oxygen, and carbon dioxide inhalation on the mitochondria in white cells.

Notable inconsistencies among previous workers dealing with mitochondrial forms may be due to the technique of fixation used by them. All these workers have used the regular method of immersing the tissue in the fixative, which after observation and experimentation has proven to be unsatisfactory for accurate determination of

the Mitochondrial picture. When this immersed tissue is compared with the perfusion technique tissue, it is possible to identify areas which are probably a true picture; but-at best-they must be carefully interpreted. From the results obtained from immersion of the tissue in an osmium fixative, such as Benaley's solution, it was noted that the fixative had very little penetrating power (fig. 3 and 4), as insufficient material was fixed in the superficial area for an adequate observation of the entire hepatic lobule (fig.4). Miggia and Masuelli (1931) in their investigation of the hepatic cells of rabbits in which they used only an osmic acid fixer, state that the mitochondria throughout the entire liver lobule were of a filamentous nature. In my experience this observation cannot be substantiated. Many trials with this fixative demonstrate that the penetrating power, in the liver, is confined to only a few cell layers at the periphery (fig. 4). The remainder of the tissue is either/too poorly fixed to demonstrate mitochondria or if these structures are stained they are so altered as to render interpretation impossible or at least inaccurate. In some of the cells in/the periphery of the tissue, rod shaped mitochondria are present. Since these forms are not found in the cells in the deeper layers we have reason to believe the presence of spherical mitochondria is not due to transverse sectioning of the filamentous mitochondria as stated

by these workers, but is caused by faulty fixation. From these observations it would lead one to believe that osmic acid fixatives are inadequate and are only practicable for use on isolated elements.

The combination of chemicals used in osmium fixatives, such as Bensley's and Mann-Kopsch, have varying degrees and rates of penetration when applied to the surface of the tissue (figs. 3 to 8). The osmium tetroxide fixes the mitochondria unevenly, but causes no shrinkage of these elements. The potassium dichromate has a slow acting power of penetration but in time penetrates to great depths, and has little or no power to precipitate cellular elements, Baker ('33). The other reagent, acetic acid, does not precipitate cytoplasmic protein, but does precipitate the nucleor protein. It has been stated that the acetic acid penetrates faster than any other fixative which may account for the fact that the nucleor elements in the central and transition areas in tissue fixed with osmium fixatives containing acetic acid show nucleor elements clearly distinguishable, while the cytoplasm is a coagulated unfixed mass. Possibly the reason for the absence of mitochondria in cells in the transition and central zones is because of the dissolving action of the acetic acid on the mitochondria as prescribed by some authors. However, the work of Champy (1911) and that of Baker (1933) reports that with using acetic acid on

the livers of newts and mammalian kidneys the mitochondria are still clearly visible. Young ('28) substantiates this conclusion by reports that 5% acetic acid in combination with a chrome salt does not dissolve mitochondria. The rate of penetration of osmium fixatives is too slow to reach sufficient depths in the short time which is required by cells to change their cytoplasmic picture during moribundity and death (fig. 4). By noting the specific action of the reagents making up these fixatives little doubt remains as to the great influence exerted on the mitochondria, causing a variation in shape, size and number within the lobule. The visible mitochondria at the periphery of the tissue, being saturated with the fixing agent, are fixed satisfactorily. The tissue in the transition area receives only the benefit of the potassium dichromate and acetic acid; the osmic acid does not penetrate that deep. These two chemicals exert a different action on the mitochondria. The penetration of only the acetic acid to the center of the tissue results in cells with fixed nuclei and the cytoplasm a coagulated mass but no preservation of the mitochondria. From the descriptions given of the various reagents one has little difficulty in ascertaining the reason for the varied pictures of the hepatic mitochondria. Thorough saturation by an osmic fixer is required if proper fixation is to be realized.

When tissue was immersed in Regaud's solution there was a somewhat different mitochondrial picture (fig. (9). Greater tissue penetration was evident, presenting a penetrable zone of greater depths than when tissue was immersed in an osmic acid fixative. This may be due to the fact that there are only two chemical reagents to be considered in using the fixative: namely, formalin and potassium dichromate. The formalin has a high degree of penetration and is not too chemically active, giving hardened cytoplasmic structures of elastic nature. It fixes cytoplasmic elements of a conjugated lipid nature; consequently, the mitochondria, being of this same constituency, are like-wise fixed.

By observation one can see that shrinking is negligible (figs. 9, 10.). The potassium dichromate penetrates slower than the formalin and as a result its effectiveness is not as immediate. However, it penetrates to greater depths and fixes the cytoplasmic elements without any precipitation, resulting in satisfactory fixation. From the afore going facts concerning the penetrating power and its ability to fix the nuclear and cytoplasmic elements, one is lead to believe (fig. 10) that fixation of the mitochondrial elements is complete to a greater extent when using Regaud's fixing solution.

From the results attained in using these various fixatives by the immersion method, one may conclude that

Regaud's is definitely better as a mitochondrial fixative than those of an osmic acid nature. However, not any of these when used in immersion give an adequate picture of an entire hepatic lobule.

By applying the perfusion method the difficulties encountered were overcome. By perfusing into the hepatic portal sufficient amounts of fixative, under not too great a pressure, the blood is forced out of all the vessels and sinusoids with the exception of those sinusoids in the storage phase. By removing this blood and replacing it with a fixative, such as Regaud's in nature, adequate fixation throughout the liver lobule can be procured. The following discussion will be based entirely on observations attained by the perfusion method.

In the liver tissue of the rat, in accordance with the reports by Kater and Smith ('31), and the previous report of this author (Shay '38), the hepatic lobule of the rat resembles that of the mouse as to zonation, but not as to the various mitochondrial elements as reported by Noel ('23). He divides the lobule into 3 zones based on the morphology of the mitochondria, the central zone, the intermediate zone and the periportal zone (fig. 16). According to Noel in the central zone, surrounding the central vein, the mitochondrial elements possess a filamentous appearance, and the cells are constantly at rest. It is the zone of permanent repose. Around the periportal veins, the mitochondria appear as spheres. The periportal zone is the

zone of permanent function. The part between these two represent the intermediate variable zone, in which the mitochondria appear as both spheres and rods. The results attained by the perfusion method do not confirm the results of Noel as to the specific type of mitochondria in the various regions. All the mitochondria of the lobule seem to be of three general types: rods, spheres and filaments. In the central zone the three types are represented, the filamentous type predominating. In the intermediate zone the mitochondria are of all three types, and are about equal in number. In the peripheral zone the spheres predominate. The rods appear swollen, some assuming an ovoid shape. Noel ('33) took exception to the conclusions of Kater and Smith and stated that although the three zone he himself described are not too clearly defined in the liver of the rat, nevertheless the general zonation which exists in the mouse is present also in the rat. I am inclined to agree with Kater and Smith that the forms are variable in the three zones. The reasoning for this conclusion is simply that since the mitochondrial morphologies show signs of a stoichiological significance it might be presumed that they vary in accordance with their particular function. The mitochondria in the peripheral part of the lobule show the greatest amount of variation in form. If a change in shape of mitochondria is determined to be

a reflection of physiological activity, then we may believe that the peripheral part of the lobule is the most active, and the name as given by Noel, "the zone of permanent function", is very appropriate.

The author is in accordance with Noel ('23) as to the relative number of mitochondria present in the three zones. In the cells in the center of the lobule, the mitochondria are so numerous that clear views of the individual elements are difficult. Because of this great number only a few of the representative types were shown in the sketches (figs. 30, 31, 32). In the periphery of the lobule, the mitochondria are fewer in number. With any mitochondrial fixative used there is a progressively decreasing number of mitochondria from the central vein to the periportal region (fig. 30). Deane ('42), in her studies of hepatic mitochondria in controlled animals (fasted six hours) states that mitochondria in the central zone are few in number and are of a fine, long, tangled, beaded, filamentous nature. The results of the writer are not in accordance with those of Deane and the discrepancy may only be explained at present on the basis that my animals were in a full nutritional state, not fasted. The hepatic mitochondria tend to enspherulate in animals that are starved for a period of five hours after the allotted time for digestion (Shay '38) which would suggest the possibility that the cells observed by Deane as normal cells are those suffering from inanition.

Intracellularly the mitochondria are evenly distributed throughout the cytoplasm with the exception of those regions where the vacuoles of a fatty nature are abundant (cell 2, fig. 31). In cases like these the mitochondria are bunched on account of intra-cytoplasmic pressure caused by these vacuoles. Pallot ('33), on his study of the mitochondria of liver cells of mammals, states that mitochondria are filamentous and are at right angles to the nuclear and cellular membranes. In normal liver cells I have observed mitochondria to be evenly distributed throughout the cytoplasm. However, in those cells where ischemia has persisted for sometime the mitochondria are bunched around the nucleus with no special arrangement. The author has been able to observe mitochondria which exhibit polarity only in those cells which are columnar in nature, such as those in the digestive tract of the roach, Periplaneta americana, (Shay '39). In the liver cells, on which most all observations were based, there was no evidence of polarity. This leads one to believe that the arrangement mentioned above is due to the turgescense within the cell caused by the inter-cellular pressure among cells of this type and has nothing to do with the physiological action of these elements.

Recently in the work of Smith ('31) on the ontogenetic history of mitochondria of the hepatic cells

of the white rat, she reveals the presence of beaded filaments in conjunction with rods and spheres.

The beaded filaments are supposed to represent a type of mitochondria present in the 15 day fetus. In the adult animal she claims no lobular variation. Observations were conducted specifically to observe any cells that might contain these beaded forms in the mature liver cell, but none were identified. In addition, observations were conducted on young cells, those that showed evidence of a recent mitotic change. These cells, which lie near the generative area near the nodal point of the hepatic lobule and which we are led to believe are in the same physiological condition as a young cell in the foetus, show no evidence of beaded mitochondria. Instead they are of the same type as those of the mature cell. These observations are confirmed by those of Beams and King ('42), on their observations of binucleate cells in the liver of white rats. I am led to believe the results obtained by Smith and Deane may possibly be due to inadequate fixation with an osmic acid fixing solution.

Kater ('31), from his investigations on the cat, reports zonations similar to those reported by Noel ('23)

in the liver lobule of the mouse, but the zones are marked by an entirely different mitochondrial form than in the mouse. In contrast to Noel's work, he found spherical mitochondria in the region of the central zone, rods in the intermediate, and filaments in the periportal zone.

In the author's own observations there appears to be a certain degree of zonation within the hepatic lobule of the rat, but in the majority of the cases the zones are not clearly distinct, nor are they to be identified by a specific morphological type of mitochondria. Since the type of zonation as reported by Kater ('31) is the reverse of Noel, et al, and the results of the current study are of a marked discrepancy with either of these workers some explanation should be evident, but at present the varying results can only be interpreted as due to such factors as the nutritional condition of the animal, the type of fixation, or the differences in the circulatory phase of the lobule at the time of fixation.

Another factor to be considered when attempting to ascertain the influence of environmental changes on mitocondria is that of osmotic pressure. The author has been unable to find any specific work on the influence of solutions of varying osmotic pressure on mitochondria with the exception of the work of Kater ('37). In his article on the liver blood fluid exchange and morphology of the

hepatic mitochondria Kater states that the shape and size of the mitochondria are dependent on the water content of the tissue. With the application of this perfusion method on living animals it offers one splendid opportunity to observe the results of fluids which were not osmotically correct. Some of the tissue prepared by the perfusion method showed the influence of hypertonic solution (fig.38). In this figure the blood cells as well as the hepatic cells show the probable effects, the red cells to the extent that the majority of them in the sinusoids show a definite crenation, some more than others, the degree of crenation varying with the extent of hypertonicity of the injection mass. The cells in the adjoining hepatic cord, show evidence of shrinkage. In sinusoids that were in the storage phase there were a few cases of crenated red cells (fig.38). An explanation of this seems to be that the injection mass was of such a hypertonicity that it extracted water not only from the cells surrounding the active sinusoids carrying the perfusion mass, but from blood and hepatic cells of the surrounding area as well. The results of investigations by this author tend to agree with Kater who reports that by altering the water content of the hepatic cell the change in shape of the mitochondria is in evidence. This change in shape is purely an osmotic phenomena. This is not hard to understand when too often one may experience

in the laboratory the crenation of blood cells when working with physiological saline solution which is supposed to be osmotically correct. The influence of the osmotic pressure results in the spherical mitochondria becoming smaller in diameter and the rod shaped mitochondria changing to spherical and ovoid shapes with an increase in their diameters.

By use of the perfusion technique the possibility suggested itself of studying the effect of varying ischemic conditions on mitochondria. Within the last few years results of research have offered a more complete knowledge of liver circulation than heretofore has been available. By use of the transillumination apparatus the functional hepatic circulatory system has been studied (Knisley, Wakim and Mann). The author has dissected with great care and exactness the route which the hepatic blood takes through the liver. In an attempt to determine the type of anastomoses between the collecting branches and the post caval vein (fig. 3). The hepatic sinusoids are supplied with blood from the ramifications of the portal vein and hepatic artery, and are drained by tributaries to the hepatic veins and thence to the post caval.

By macroscopic examination areas of natural ischemia are noted on the surface of the liver lobule, indicating an intermittancy of circulatory activity in various

regions of the lobe (fig. 15). In some areas where this condition exists not just one but several lobules are included as being inactive in an ischemic condition.

By microscopic examination of the lobule, regions of varying degrees of ischemia were observed. The number of these sinusoids as well as the number of areas showing natural ischemia is highly variable. This is also true of hepatic lobules in different animals. In these swollen sinusoids there are many blood cells in the lumen, causing them to have a bulging appearance. These sinusoids are in the storage phase (figs. 29, 30). Still other sinusoids have no blood whatsoever; they are thought to be in the active phase. Nearly all the blood was removed by the injection of Ringer's solution.

These observations are similar to those as reported by Knisley ('33) who noted in the frog's liver a sphincter at the exit of each sinusoid which regulates the amount of whole blood or red cells contained in the sinusoid. He states that the afferent hepatic arterioles and portal venules are contractile thus permitting the sinusoid to receive mixed blood or blood from only one source.

Wakim and Mann ('42) in their studies of the intrahepatic circulation of the blood in rats, reports that the sinusoids draining into the ramification of the hepatic vein are slightly sphincterated just before they join the

the draining vein. An explanation by this author as to factors which might influence the amount of sinusoidal blood storage is not forth coming at this time since this condition even though it might have a definite influence on the mitochondria in the surrounding cells was not a conclusion that could be established in this paper.

From the work of Mall ('03) in his study of the structural unit of the liver states that the formation and growth of new lobules and the flow of blood through the portal anastomoses is altered to meet the increase in the number of new lobules and new cells being formed in the region of the nodal point. Because of this nodal point reorientation the blood supply to a group of lobules in a local area may be stopped for a time resulting in zones of ischemia, the amount of ischemia depending on the extent of the reorientation in the nodal region.

In an effort to determine the effect of ischemia, and resulting anaxia for variable periods, conditions of voluntary ischemia were produced by tying off various lobes of the liver for varying periods of time while the remainder of the liver was being perfused. When circulation was stopped in the various lobes the sinusoids, the periportal and central veins contained large amounts of blood.

The mitochondrial picture of the areas influenced by natural and that of induced ischemia are very similar.

Mitochondrial changes take place very quickly when the liver lobe is deprived of its blood.

Within 10 minutes after the left and Spigelian lobes were tied off the mitochondria in the central regions of the lobule show a tendency to shorten and thicken (fig. 23). Many of the mitochondria have a ring form. In the periportal area all the mitochondria are ensphaerulated (fig. 22). If the mitochondrial picture is followed throughout, from a period of vaso-occlusion for ten minutes up to that of sixty minutes, it is noted that the mitochondria in all regions of the lobule and in all the later stages tend to ensphaerulate, later becoming smaller and fragmenting. In the later stages the mitochondria completely disappear or if present, they are embedded in a coagulated cytoplasm to such an extent that identification is impossible. Where vaso-occlusion was permitted to continue for sixty minutes some cells in the periportal region showed a tendency to pull away from the connective tissue trabeculae which supported the periportal canals (fig. 27).

In some of the hepatic cords taken from liver lobes occluded for ten, fifteen, thirty, and thirty-five minutes the cells in the central zone showed mitochondria which still retained their rod-shaped characteristics. In the other lobules there was a difference in the size of the mitochondria within the central and periportal regions in tissue

occluded for ten and fifteen minutes. Even in cases where ensepherulation was evident, variation in the size of the sphere persisted. This variation may be explained on the basis that in the normal tissue a considerable variation in mitochondrial forms is noted in the zonation of different lobules. Even though the stimulus,-ischemia,-at present is the same for each cell in the hepatic cord variations in mitochondrial shapes and distribution do exist.

The intermittency of circulation in the liver in cases of natural ischemia, and in sinusoids in the active and storage phase, may favor the interpretation that local differences in circulatory activity may have a direct influence on the mitochondria in these areas.

The mitochondrial changes in this experiment observed under a condition of natural or induced ischemia are very much in common with those of Emmel (1940) in the ischemic kidney. In his observations Emmel noted during varying periods of ischemia a rise in tissue acidity suggesting the possibility of a close relationship between the two.

In earlier work (Shay '38) attempts were made to determine the pH of the fixing solutions used, in an effort to determine on the basis of Scott's work (1935) if enlargement of the mitochondria was due to a difference in pH.

With determinations made on a Hellige pH meter, the resulting hydrogenion concentration for the fixing solutions used was approximately as follows: Regaud's - 4.37 pH, Bensley's - 2.97 pH. It appeared from the observations that cells fixed with, a low pH (2.97) Bensley's, the mitochondria are smaller in size than with Regaud's which has a slightly higher pH (4.37). In view of the work of Scott (1935) who incubated liver cells in a slightly alkalin tissue culture media, a decrease in numbers and an increase in size was noted; results similar to those the author obtained with Regaud's fixative. Whether the slight differences in pH was sufficient to cause the observed changes in mitochondria may only be conjectured. It is pertinent to point out that Scott's results were obtained from cells grown in an artificial media and not subject to normal environmental conditions. Without doubt the increase in acidity of the environment appears to have a decided influence on the morphology of hepatic mitochondria during natural and induced ischemia. Reformation of the mitochondria in response to alteration in tissue acidity has been observed by other workers, Lewis and Lewis ('15) as well as Scott ('35). Their investigations suggest that mitochondria are in osmotic equilibrium with the surrounding cytoplasm. Since the distribution of water in a colloidal system can be altered by changes in hydrogen ion

activity, it is possible that the changes in the morphological aspect of these structures may be due to increased acidity occurring during ischemia.

The results of Abell and Clark ('30) in their experiment with using indicator dyes introduced into a transparent chamber in the rabbit's ear, it was observed that by compression of the blood vessels to that region injected there was a rise in tissue acidity from pH 7.2 to 6.8 in fifteen minutes. The tissue acidity observed in these experiments on ischemia is probably due to the formation of carbonic acid resulting from an increasing amount of CO_2, a biproduct of cellular respiration. Voegtlin ('34) reports from his work on ischemia in skeletal muscle, states that the lactic acid content of the tissue increases during an oxygen deficiency.

Contrary to the observed results of this author, and others afore mentioned, Nicholson (1934) in his work on the thyroid gland, find that with ligation of the large arteries of the thyroid no alteration was noted in the mitochondria after three days when the blood supply was reduced. At the end of this period mitochondrial filaments became shorter and more granular, eventually passing through the same stages that were seen in the transformation of the hepatic mitochondria. But not until after sixty days of reduced blood supply did the mitochondria

completely disappear. In addition to Nicholson (1934) there are other authors whose observations are at variences with the reports as included within this paper. Emmel (1940) reports that Israel (1891) Mannehl (1898), Takaki ('08), and Oka ('20) who have studied postmortem autolysis and anemic necrosis in the kidney by crude cytological means, report that mitochondrial changes are not apparent until six to twenty-four hours after ischemia. Emmel ('40) takes issue with these results stating that the renal mitochondria might undergo postmortem changes more rapidly. As evidence he cites the work of Pappenheimer ('16) who noted that thirty minutes after death the mitochondria in the rat's kidney broke into granules. The hepatic mitochondria from the present study have been observed to undergo these same changes (fig. 25). An explanation for the formation of granules in ischemic tissue may not be complete at this point because of insufficient experimental evidence. However, there are reasons to believe that because of the cessation of the blood flow the process of metabolism is replaced by katabolism and the process of autolysis begins, resulting in a degenerative type of mitochondria, the granule.

A condition which may produce ischemia in animals as well as in humans is that of fibrosis and vascular obstructions. The results of ischemia induced by fibro-

sis in the region of the hepatic blood supply to the extent of causing death to the patient, may be seen in figure 40.* In the low power photograph a section of the liver lobule from the central vein to the peripertal canals may be seen. It shows the complete absence of blood and fibrillar degeneration of the tunica of the vein taking place. In the peripheral region the connective tissue trabeculae supporting the bile ducts and the peripertal vessels show a sign of deteriation to the extent that fragmentation may be seen. In the high power photograph the connective tissue fibers are seen separating the functional hepatic cells. It also shows a region of the peripertal canal and hepatic cells which show signs of intussusceptive connective tissue growth. The character of the cells shown in figure 41 are similar to those in the hepatic tissue of the rat in the later stages of ischemia, with the possible exception that ischemia in the human liver has progressed to a further extent. It would be most desirable to secure tissue from such pathological ischemic conditions as described above for analyses and comparison of the mitochondrial picture with that of experimentally induced ischemia. Such acquisition of tissue has not been possible to date.

* The material for these observations was procured through the cooperation of Dr. M. Wreitz, pathologist, Sacred Heart Hospital, Allentown, Pennsylvania.

The nature of mitochondrial activity is naturally the goal of all studies of mitochondria. Up to this time, this primary objective has proven to be one of an illusive nature. Thus far the interpretations of the general functional significance varies from the denial for grounds of any kind of conclusions to assignment of specific functions to these elements. In the case of the liver where the functions are so varied as to include both exocrine and endocrine activity we encounter great difficulty in establishing perfectly controlled conditions for experimental work.

An organ like the liver with varied functions presents a greater source of error in attempted controlled experiments. In the following phase of the discussion the author will attempt to utilize his own observations, which are in no way complete, as well as the work of predecessors on mitochondria function. It is necessary to give due consideration to the work of previous and contempory workers for otherwise we would be likely to regard their work unjustly. Needless to say, the results thus far attained by my colleagues are far from complete. Two of the earliest workers Meves ('08) and Regaud ('11) suggested the possible connection between the mitochondria and the formation of secretion granules by utilizing materials from the cytoplasm. The secretory nature of mitochondria has been contested by Meyer (11-'20) who

states that they are merely ergastic products of an albuminous nature. He compounds his views in that their action is just what would be expected of reserve substances necessary as a source of energy. The view that mitochondria are products of metabolic activity rather than distinct protoplasmic organs has been held in one form or the other by many workers and appears to be well supported by observations on both living and fixed cells.

One of the problems that has demanded much attention in this field is that of the synthesis of fat by hepatic cell mitochondria. Kater and Smith ('32) describe the fat as being formed within the vesiculated mitochondria, while Noel and Pallot ('34) consider it elaborated on the surface of the larger mit. In the work of MacCradle ('37), McCurdy ('39), Steffens ('41) as well as this author all conclude that no direct relationship exists between the fat droplets and mitochondria within the liver cells. Because of these conflicting views the previous work begun by this author (Shay '38) was extended to include additional observations to profitably restudy the problem.

In normal liver, fat is accumulated as droplets in the cells surrounding the central vein, some appearing very large and confluent (fig. 37). The fat is present in depreciating amounts when moving peripherally in the

lobule. Kater and Smith ('31) used various fixatives in the investigation of fat formation within the mitochondria in normal hepatic cells of the white rat from fetal to adult life. At a later age these fat vacuoles become large and coincidently their covering becomes thin and fragmented by a process of furrowing from the outer surface. This results in the formation of short, crescent-shaped, rod-like mitochondria and the liberation of the vacuolar appearing structure in the ground cytoplasm.

I have observed vesiculated mitochondria in normal liver tissue (fig. 34a). The idea of Kater and Smith that only rats at birth show small fat vacuoles surrounded by mitochondria is not substantiated by my results. Minute fat droplets are present in the hepatic cells of adults as well as in the fetal liver. From a thorough study of these preparations, there is no apparent relationship between vesiculated mitochondria to the deposition of fat. With a normal diet, the mitochondria in the cells of the peripheral and middle zones are rod shaped, ovoid and elongated and spherical, some spheres being vesiculated. The greater deposits of fat are largely present in the central zone yet the vesiculated mitochondria in which the fat is to be synthesized are more numerous in the peripheral and intermediate zones. Consequently, the zone relationship does not substantiate

the claim of Kater and Smith ('33) that the vesiculated mitochondria synthesize and deposit fat within themselves or the statement by Noel and Pallot ('34) that the larger mitochondria elaborate fat on their surfaces. As previously stated, where large spheres are present, the idea of Kater and Smith is plausible. One would conclude that with an increase in size of the fat vacuoles and no increase in the intracellular space the cell becomes congested. As a result the mitochondria are viewed as a covering of the individual fat vacuoles (fig. 37). In addition I observed these vacuoles with tissue perfused with an osmic acid fixative which should oxidize all fat within the cell. However, the centers of the mitochondria remains clear. According to Nath ('34) this is not impossible, if the fat was present in an unsaturated state.

In animals fed on a normal diet, as well as starved animals, the mitochondrial transformations such as formations of spheres and rods take place. Whether or not this reaction of the mitochondria is associated with a specific function of the cell is difficult to ascertain, but seems doubtful that it is related to the synthesis of fat. This transformation and vesticulation might be associated with the deposition or secretion of glycogen or bile formation as reported by Noel ('23), or it might merely be the response of the mitochondria to

a change in the cytoplasm which may be effected by many different causes as yet undetermined. The appearance of the hollow spherical mitochondria in the outer lobular regions as contrasted with areas adjacent to the central vein referred to above might indicate that this region is in the secretory stage. According to the work of Forgacsen (1939), the rabbit's liver has a rhythmic function with alternating assimulatory and secretory stages, secreting bile in one region and assimilating glycogen in the other. To confirm this, sections would have to be prepared to determine the presence of bile and glycogen and their specific distribution. Perhaps again these factors may be interpreted in terms of the rhythmic circulatory changes as previously discussed (pg. 64). Many cytologists have suggested that enspherulation of mitochondria is an indication of a reduced rate of cellular oxidation (Cowdry '24), Joyet-Lavergne '38) and thus the enspherulation following the normal and starvation diets might in both instances be attributed to a lowered rate of metabolism of the liver cells.

Additional attempts to alter mitochondria by experimentally changing the physiological state of the animal have recently been made by Steffens ('41). This worker noted perinuclear clumping of mitochondria in a pancreatectomized rat fed on glucose similar to those in

cell 2 in figure 26, and empherulation of the mitochondria after hypophysectomy. The question of the significance of these changes in the mitochondria under these experimental conditions remains unsolved.

This author is undertaking further experiments with the aid of the perfusion technique for the purpose of attempting to more accurately determine the relationship of experimental diets, and consequent modification of liver function, to mitochondrial form and distribution. It is felt that first the perfusion technique offers a more accurate means for cytological study than has heretofore been used for a study of this organ. Secondly, by carefully controlling and modifying the dietary condition of the animal, resulting changes in the mitochondrial picture may offer a better index to hepatic cell function than we are now aware of.

VII CONCLUSIONS

1. Liver tissue prepared by the immersion method for the study of mitochondria in majority of the cases is inadequate because of the lack of penetration of the fixative.

2. When using the injection method fixation is attained only in the areas surrounding the perilobular veins. The mitochondria in these areas are spherical.

3. Adequate fixation of liver tissue is attained by using the perfusion method.

4. Zonation appears to a certain degree in the liver lobule. In majority of the cases these zones are not clearly distinct. Mitochondria of the central zone are of three types, filaments, spheres and rods; intermediate zone - rods and spheres appear in equal numbers; peripheral zone rods and spheres(some being vesiculated) with the later perdominating.

5. In perfused tissue areas of natural ischemia persist. The mitochondria of which are spherical and rod shaped including variable forms such as, sphere with distorted borders and hollow centers, and rods and filaments with one or both ends enlarged.

6. Mitochondrial forms, rods, spheres and filaments, as seen in the normal tissue, changes to variable forms when the tissue is deprived of its blood from

lo to 60 minutes. The areas influenced by induced ischemia and that of natural ischemia are very similar.

7. The intermittency of circulation in the liver in cases of natural ischemia, and in sinusoids in the active and storage phase, may favor the interpretation that local differences in curculatory activity may have a direct influence on the mitochondria in these areas.

8. When a hypertonic Ringer's solution is perfused throughout the hepatic lobule espherulation of the mitochondria is noted. The hepatic cells are vaculated and the blood cells in the adjoining sinusoids are crenated.

9. Fat is localized in the cells surrounding the central vein. There is no relationship between the mitochondria and the fat vacuole.

10. The perfusion method offers a more accurate means of cytological study than has heretofore been used for a study of this organ.

11. By carefully controlling and modifying the dietary condition of animals, resulting changes in the mitochondrial picture may offer a better indix to hepatic cell function than we are now aware of.

VIII. BIBLIOGRAPHY

Abell, R. G. and E. R. Clark. 1939. Studies on intracellular substance in the living rabbit. Anat. Rec., vol. 73 (Suppl. 2), p. 1.

Altmann, R. 1890. Die Elementarorganismen, usw. Leipzig.

Anusp, E. 1931. Ueber den Verlauf der Arteria hepatica in der Leber. Ztschr. f. mikr.-anat. Forsch., vol. 25, pp. 238-251.

Baker, J. R. 1933. Cytological Technique. Methuen's Monographs on Biological subjects.

Beams, H. W. and R. L. King. 1942. The Origin of binucleate and large mononucleate cells in the liver of the Rat. Anat. Rec., Vol. 83 (2), pp. 281-297. 2 pl.

Burton-Opitz, Russell. 1910. The vascularity of the liver. I. The flow of the blood in the hepatic artery. Quart. J. Exper. Physiol., vol. 3. pp. 297-313.

------ 1911. The vascularity of the liver. IV. The magnitude of the portal inflow. Quart. J. Exper. Physiol., vol. 4, pp. 113-135.

------ 1912. The vascularity of the liver. VI. The influence of the greater splanchnic nerves upon the venous inflow. Quart. J. Exper. Physiol., vol. 5, pp. 189-196.

Champy, C. 1911. Recherches sur l'absorption intestinale et le role des mitochondries dans l'absorption et la secretion. Archives D'Anatomie Microscopique, Tom. 13, pp. 55-170.

Cohnheim, Jul., and M. Litten. 1876. Ueber Circulationsstorungen in der Leber. Virchows Arch. f. path. Anat., vol. 67, pp. 153-165.

Cowdry, E. V. 1924. Cytological constituents--mitochondria, Golgi apparatus, and chromidial substance. E. V. Cowdry (editor), General Cytology, Univ. Chicago Press, pp. 311-382.

Cowdry, E. V. 1926. Surface film theory of the function of mitochondria. American Naturalist, vol. 60, pp. 157-165.

Cowdry, E. V. 1918. The mitochondrial constituents of protoplasm. Contributions to Embryology. Carnegie Inst. of Wash., vol. 8, no. 25.

Duesberg, J. 1919. On the present status of the chondriosome problem. Biol. Bull. 36 (2).

Emmel, V. M. 1940. Mitochondrial and pH changes in the rat's kidney following interruption and restoration of the renal circulation. Anat. Rec., vol. 78, pp. 361-381.

Flemming, W. 1882b. [illegible] Arch Mikr. Anat., vol. 20, pp. 1-86, pls. 1-4.

Forsgren, E. A. 1929. The anatomical qualities of the liver during the various stages of its functional activities. J. Morph. and Physiol., vol. 47, pp. 519-530.

Greene, E. C. 1935. Anatomy of the Rat. Transactions of the American Philosophical Society, vol. 27.

Hance, T. 1938. Microscopic Technique. Report read at A. A. A. S. meetings, Richmond, Va. 1939.

Hyrtle, Joseph. 1864. Ueber das Verhalten der Leberarterie zur Pfortader bei Amphibien und Fischen. Sitzungsb. d.k. Akad. d. Wissensch. Math.-naturw. Cl., vol. 49, pp. 167-175.

Israel, O. 1891. Die amamische Nekrose der Nierenepithelium. Virchows Arch. f. path. Anat., vol. 123, p. 310.

Jay, E. M. 1934. A cytological study on the liver of the rat, with special reference to the intracellular blood canaliculi, inter and intracellular bile canaliculi, mitochondria and golgi apparatus. Jr. Morph., Vol. 56, 2.

Joyet- Lavergne, P. 1928. Chondriome in Sarcolegnia. Comot. Rend. Acad. Sci. Paris, vol. 186, pp. 595-597.

Kater, K. McA. and D. McL. Smith. 1932. The formation of fat in the hepatic cell. Anat. Rec., vol. 52, no. 1, Feb. 25.

Kater, J. McA. 1937. The liver-blood fluid exchange and the morphology of hepatic cell mitochondria. J. of Morphology, vol. 61, p. 473, no. 3.

Knisley, M. H. 1938. A method of illuminating living structures for microscopic study. Anat. Rec., vol. 64, pp. 493-534.

---- 1938. An approved fused quartz living tissue illuminator. Anat. Rec., Vol. 71, pp. 503-508.

---- 1939. Microscopic observations of the circulatory conditions in the living frog liver lobules. Anat. Rec., vol. 73 (Suppl. 2), pp. 69-70.

Kropp, B. and May R. 1934. A study of Mit. in Knit cells following gaseous inhalation. Anat. Rec., vol. 26-27, pp. 389-392.

Lee, A. B. 1938. The microscopists' vade-mecum. 10th ed. Edited by J. Bronte Gatenby and T. S. J. Painter and A. Churchill Ltd. xi + 734 pp.

Leonard, Alice. 1937. Der Einfluss der Jahreszeit auf die Leberzellen von Rana temporaria. Arch. f. Physiol., suppl.-Bd., pp. 38-47.

Lewis, M. R., and W. H. Lewis. 1915. Mitochondria (and other cytoplasmic structures) in tissue cultures. Am. J. Anat., vol. 17, pp. 339-401.

MacCardle, R. C. 1937. The effect of temperature on the mitochondria in the liver cells of fish. J. Morph., vol. 61, pp. 613-639.

McCurdy, Mary B. D. 1939. Mitochondria in liver cells of fed and starved salamanders. J. Morph., vol. 64, pp. 9-35.

Ma, W. 1924. The changes in the pancreatic cell of the guinea pig during inanition and re-feeding. Anat. Rec., vol. 27, pp. 47-60.

Mall, F. P. 1906. A study of the structural unit of the liver. Am. J. Anat., vol. 5, p. 227.

Meves, F. 1918. Chondriosomes. Arch. Mikr. Anat., vol. 92: II, pp. 41-136. 18 figs.

Meyer, A. 1911-20. Chondriosomes. Ber. Deu. Bot. Gessell, vol. 29, pp. 158-160.

Muggia, G. and L. Masuelli. 1932. La citologia della cellula epatica in rapporto alla qualita dell'alimento introdotto. Zeit fur Zell. und mihro. Anat., vol. 16, pp. 659-673.

Munzer, F. T. 1923. Uber die Zweikernigkeit der Leberzellen. Arch. f. Mikr.-Anat. u. Entwickelungsmechn., Bd. 98, S., pp. 249-282.

Nath, Visbwa. 1934. Microchemical tests for fat, lipoids, and vacuoles with special reference to oogenesis. Quart. J. Micro. Sci., vol. 76, pp. 139-143.

Nicholson, F. M. 1924. An experimental study of the mitochondrial changes in the thyroid gland. Jr. Exp. Med., vol. 39, Jan.-June.

Noel, R. 1922. Theses de l'universite de Paris, Pais Masson et Cie. 158 pp. (282) Divisions of areas in liver and changes in mitochondria with the feeding of fat, protein and sugar.

Noel, R. 1923. Recherches histo-physiologique sur la cellule hepatique des mammifires. Arch. d' Anat. Microscopique, T. 19, pp. 1-158.

Noel, R. and G. Pallot. 1933. Research on the Chandriome of the liver cells of Mammifires. The Mitochondria after Chez rat. Bull. Hist. Appl. Physiol. and Path., vol. 10 (6), pp. 169-186, 6 fig.

Oka, 1920. Zur Frage der postmortalen Autolyze der Zellengranula. Virchows Arch. f. path. Anat., vol. 228, p. 200.

Pappenheimer, A. M. 1916. The Golgi apparatus. Anat. Rec., vol. 11, p. 107.

Regaud, C. 1908-11. Chondriosomes. Compt. Rend. Soc. Biol. Paris, vol. 65, pp. 660-662.

Scott, W. J. 1918. Experimental mitochondrial changes in the pancreas in phosphorus poisoning. Am. J. Anat., vol. 20, pp. 237-253.

Sharp, L. W. 1934. Introduction to Cytology. McGraw Hill Co. 3 ed.

Shay, D. E. 1938. Studies of the hepatic cells of mus norvigicus albinos during various periods of inanition, with especial reference to the mitochondria. Unpublished.

Shay, D. E. 1939. Observations on the mitochondria in the mid-gut of Periplaneta americana. Unpublished.

Shearer, E. M. 1933. Development of arteries in the anterior limb of albino rats. Am. J. Anat., vol. 53, pp. 427-467.

Smith, D. M. 1931. The Ontogenetic history of the mitochondria of the hepatic cell of the white rat. J. Morph. and Physiol., vol. 52, pp. 485-511.

Strasburger, E. 1882 b. Chondriosomes Uber Zellbildung und Zeltheilung. 3d ed.

Takaki, M. 1907. Uber die Stabchenstrukturen der Niere. Arch. f. mikros. Anat., vol. 70, p. 245.

Voegtlin, C., and H. Kahler. 1932. The estimation of the hydrogen-ion concentration of the tissues in living animals. Sci., vol. 75, p. 362.

Voegtlin, C., R. H. Fitch, H. Kahler and J. M. Johnson. 1934. The hydrogen ion concentration of mammalian voluntary muscle under various conditions. Amer. J. Physiol., vol. 107, p. 539.

Wakim, K. G. and Mann, F. C. 1941. The intrahepatic circulation of the blood. Anat. Rec. Vol. 82. no. 2 and supplement 1. Feb. '42.

White, H. L. 1939. Observations indicating absence of glomerular intermittence in normal dogs and rabbits. Amer. J. Physiol., vol. 128, p. 159.

Wilson, E. B. 1925. The Cell in Development and Heredity. 3rd ed. MacMillan Co.

Young, R. T. 1928. A note on Mitochondria. Anat. Rec., vol. 40, 3, Nov. 25.

XVI PLATES

41 - FIGURES

PLATE I

Figure 1 - This figure represents the liver as removed from a normal healthy rat, showing the point where the portal vein enters and where the ductus choledochus leaves the liver. Note the absence of the gall bladder and the relationship between the oesophagus and the liver lobules.

Figure 2 - The arterial and venous blood supply of the rat's liver are here represented. The intricate anastomoses of the intrahepatic sinusoids and capillaries are not shown.

PLATE-I

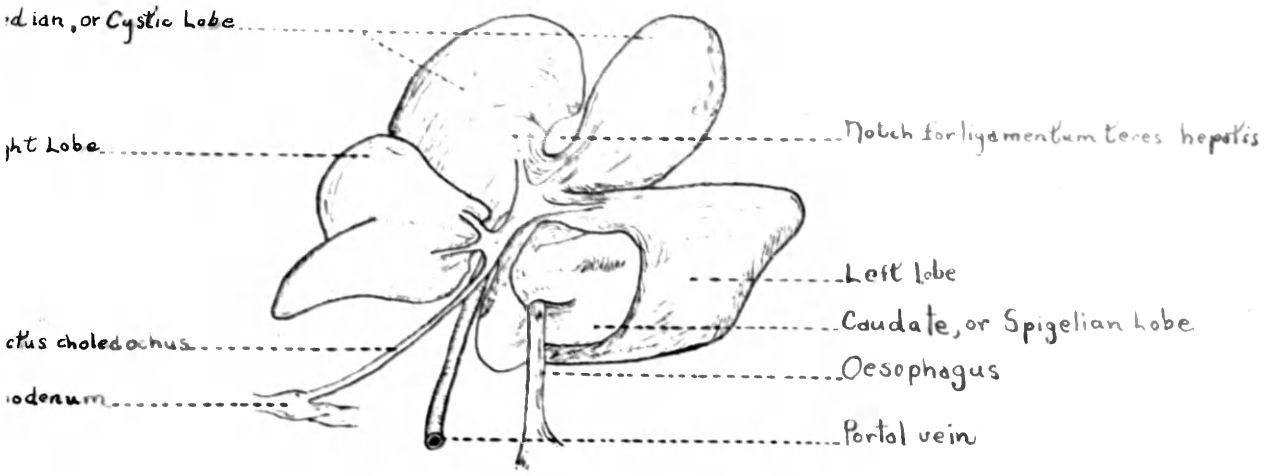


FIGURE- 1

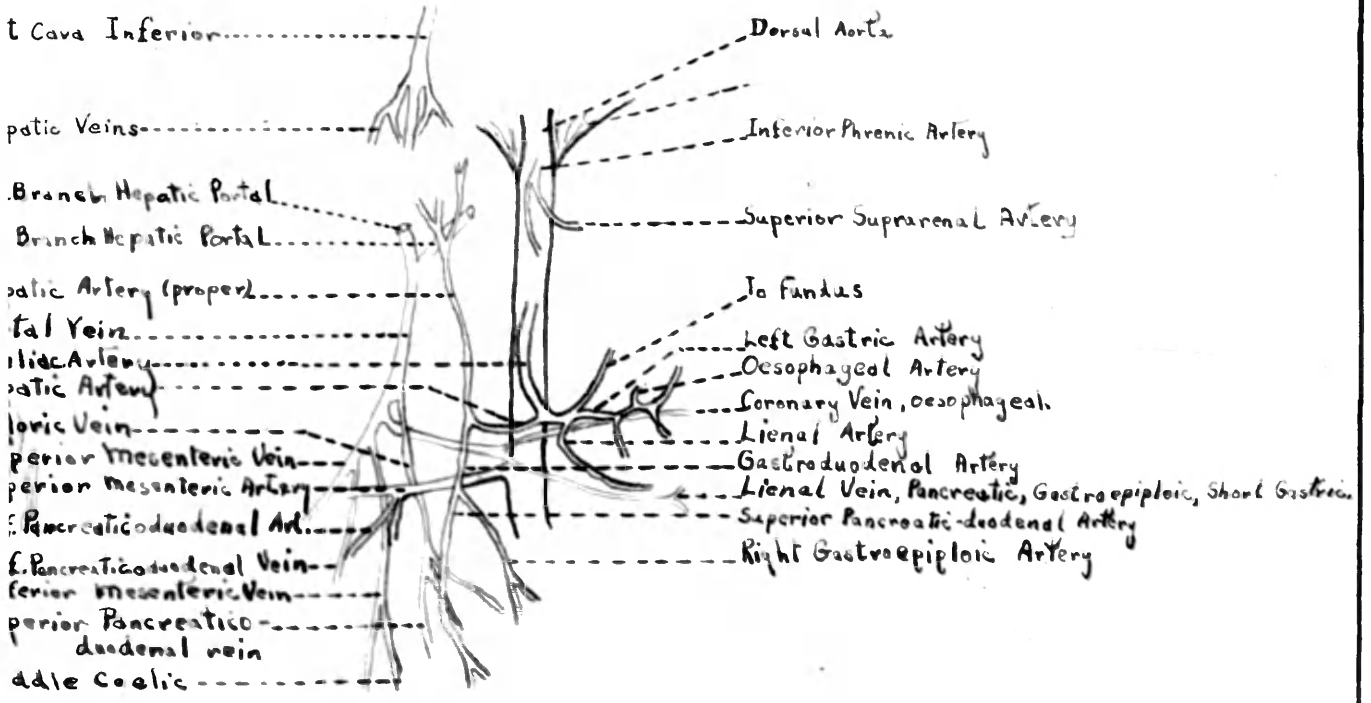


FIGURE- 2

PLATE II

Tissue Prepared by the Immersion Method

Figures 3 and 4 - represent tissue prepared using Bennley's fixative. Cell 1 was drawn from the penetrable zone, the clear area on the right of figure 4. The mitochondria are of two general types, rods and spheres. There is a tendency toward grouping of the mitochondria in cells in this area. Cell 2 was drawn from the transitional zone, the dark central portion in figure 4. The mitochondria in these cells are grouped on the side of the cell nearest the penetrable zone and are spherical with only a few short rods present. Cell 3 was drawn from the central zone, the area on the extreme left of figure 4. No mitochondrial elements are present. The cell is unfixed and the cytoplasm is clumped. Note the large intercellular spaces in this tissue.

Figures 5 and 6 - are a photograph and sketches of tissue prepared by using Zenn-Kopsch fixative. Cell 1 was drawn from the penetrable area, the light zone to the right in figure 5. The mitochondria are all spherical and are evenly spread throughout the cell. Cell 2 was drawn from the transitional area, the dark central zone in figure 5. The mitochondria are spherical and tend to clump on the side of the cell nearest the penetrable zone. Cell 3 was drawn from the central unfixed zone, the area on the extreme left in figure 5. The cytoplasm is clumped and is separated by large clear spaces. No true mitochondrial elements were observed. Note the large sinusoids in this area.

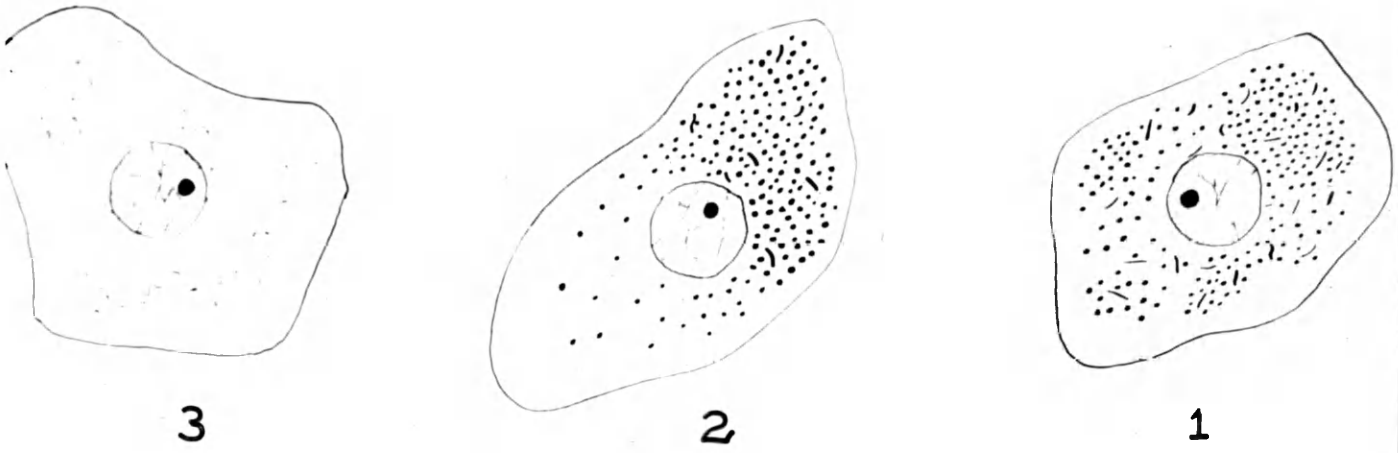


FIGURE-3

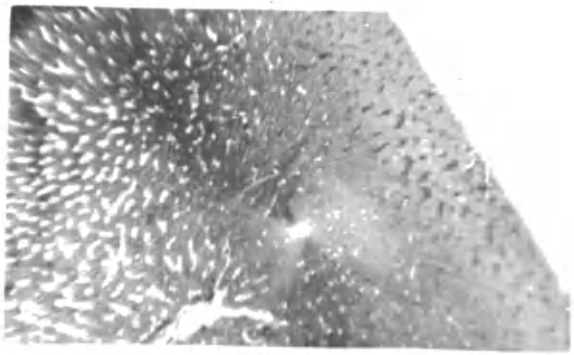


FIGURE-5

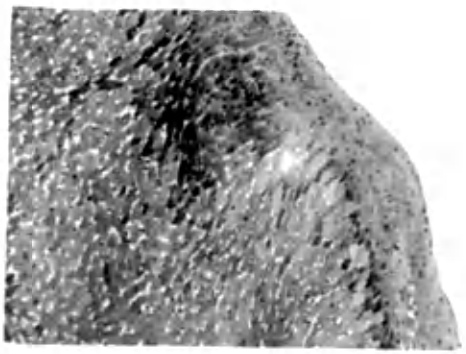


FIGURE-4

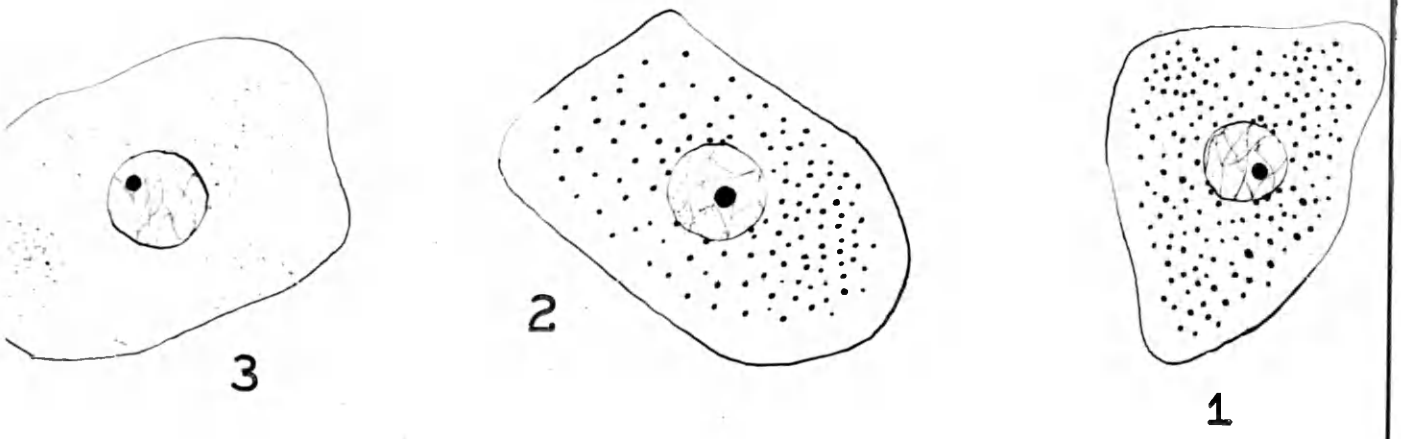


FIGURE-6

PLATE III

Tissue Prepared by the Immersion Method

Figures 7 and 8 - represent a photograph and sketches of tissue fixed by the use of Fle ming's solution. Cell 1, drawn from the penetrable zone, the light area to the right in figure 8, shows mitochondria of variable shapes evenly distributed throughout the cell. Cell 2 was drawn from the transitional area, the dark central zone in figure 8. Most all the mitochondria in this zone as shown in this cell are spherical with few short rods present. Cell 3 was drawn from the central unfixed zone on the extreme left of figure 8. The tissue is not fixed satisfactorily. The mitochondria shown are of a spherical nature and are localized in one corner of the cell boardering the transitional area. Note the large intercellular spaces in this area and the uneven cytoplasm of the cells.

Figures 9 and 10 - represent a photograph and sketches of tissue fixed with Regaud's solution. Cell 1 was drawn from the penetrable area shown in figure 9 on the extreme right. The mitochondria in this area are of three types, rods, spheres, and filaments. Cell 2 was drawn from the transitional area shown in the center of figure 9. The mitochondria are in the form of spheres and short rods, spheres predominating. Cell 3 was drawn from the central area shown on the extreme left of figure 9. The mitochondria are of two types, spheres and rods and are swollen to twice the size of the mitochondria in the other two areas. There are unfixed areas in the central zone shown in the upper left corner of figure 9.

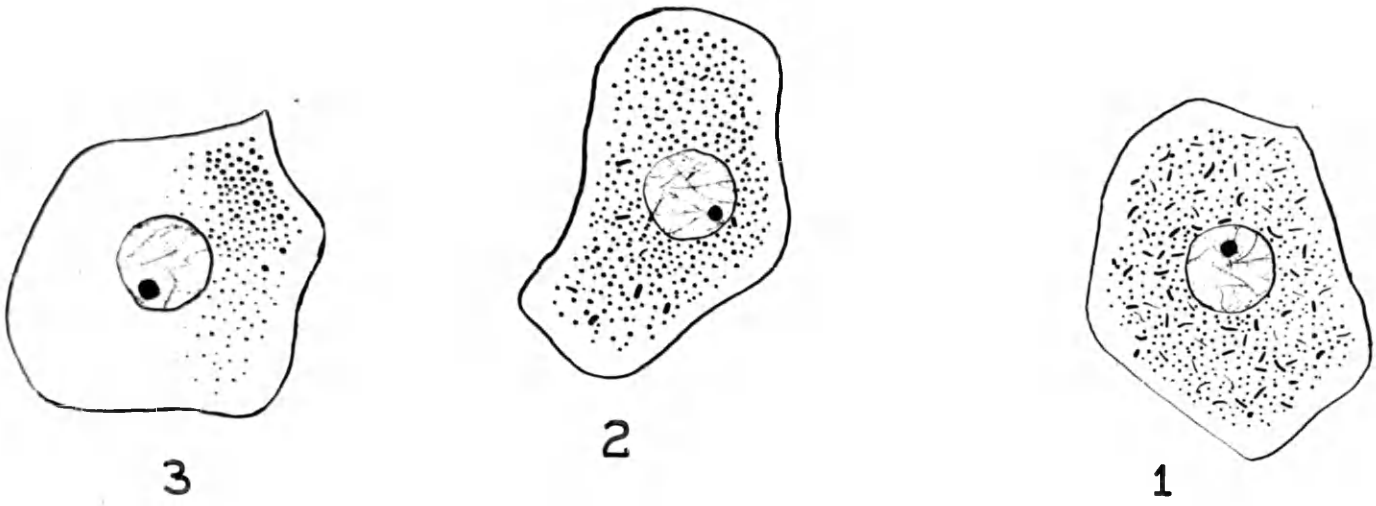


FIGURE - 7

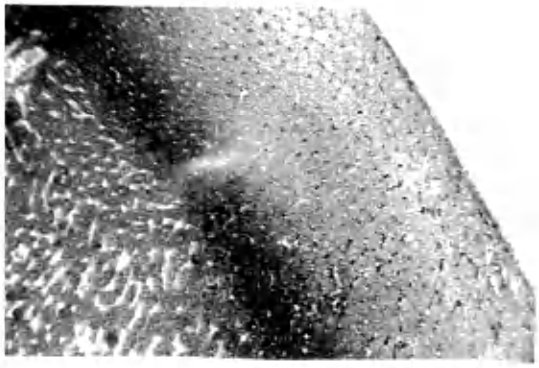


FIGURE- 8

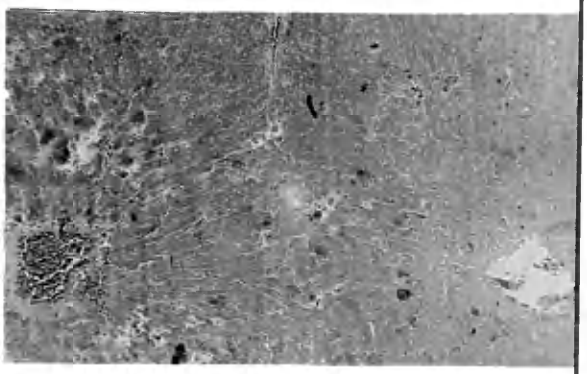


FIGURE- 9

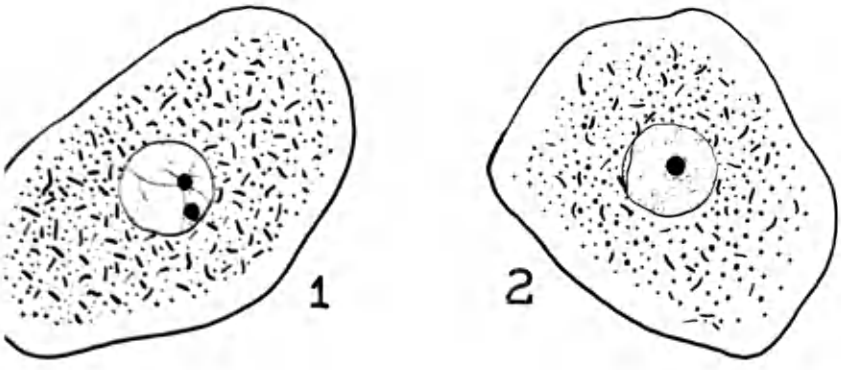


FIGURE-10

PLATE IV

Apparatus

Figure 11 - Materials in position to begin perfusion.

Figure 12A - Glass cannula used for perfusing into the hepatic portal vessel and plastic light used for illuminating areas and showing boundaries of the hepatic portal vein.

Figure 12B - Close-up of the animal in position for perfusion showing the cannula and light in place.

FIGURE-12A

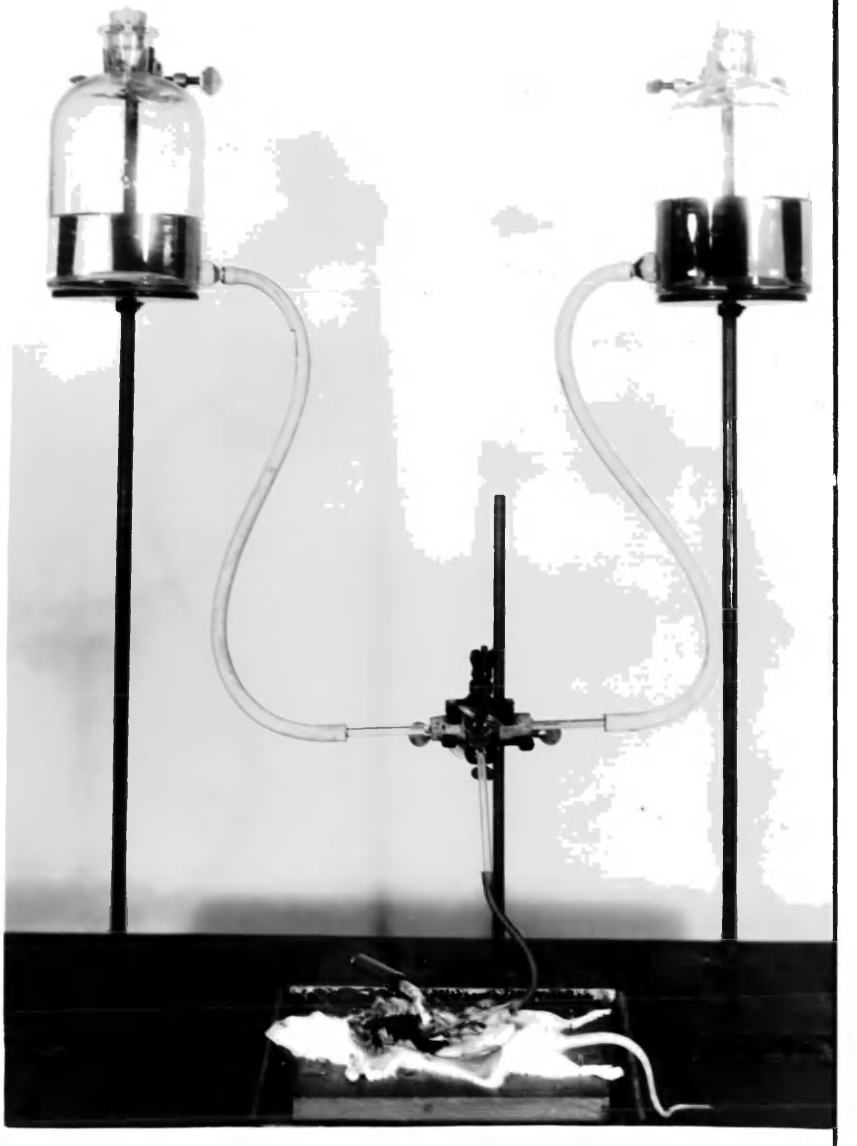


FIGURE-11

FIGURE-12B

PLATE V

Figure 13 - viscera of rat before perfusion. Note the exposed hepatic portal vein.

Figure 14 - same animal as in figure 13. The cannula is in position in the hepatic portal and perfusion has progressed for five minutes. Note the change in color of the lobes of the liver due to the removal of the blood.

Figure 15 - animal in figures 13 and 14 after 10 minutes of perfusion. Note the areas of ischemia in the various lobes. By continued perfusion more blood is removed but some ischemic areas remain.

PLATE- V

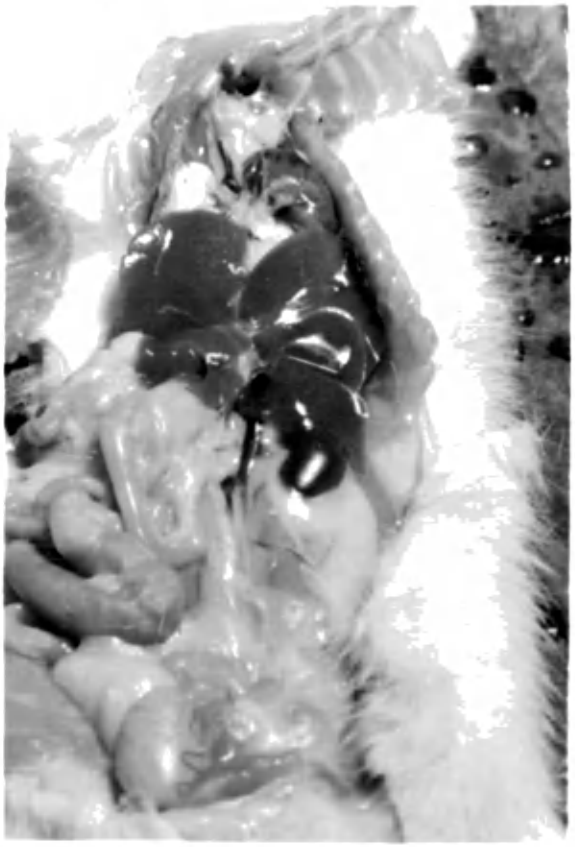
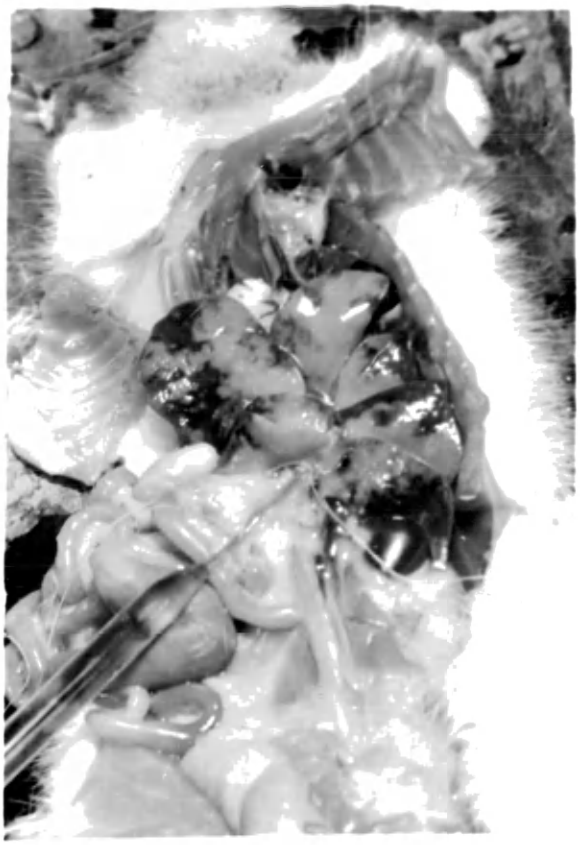


FIGURE- 13

FIGURE-15

FIGURE-14



INTERPRETATION OF THE HEPATIC LOBULE

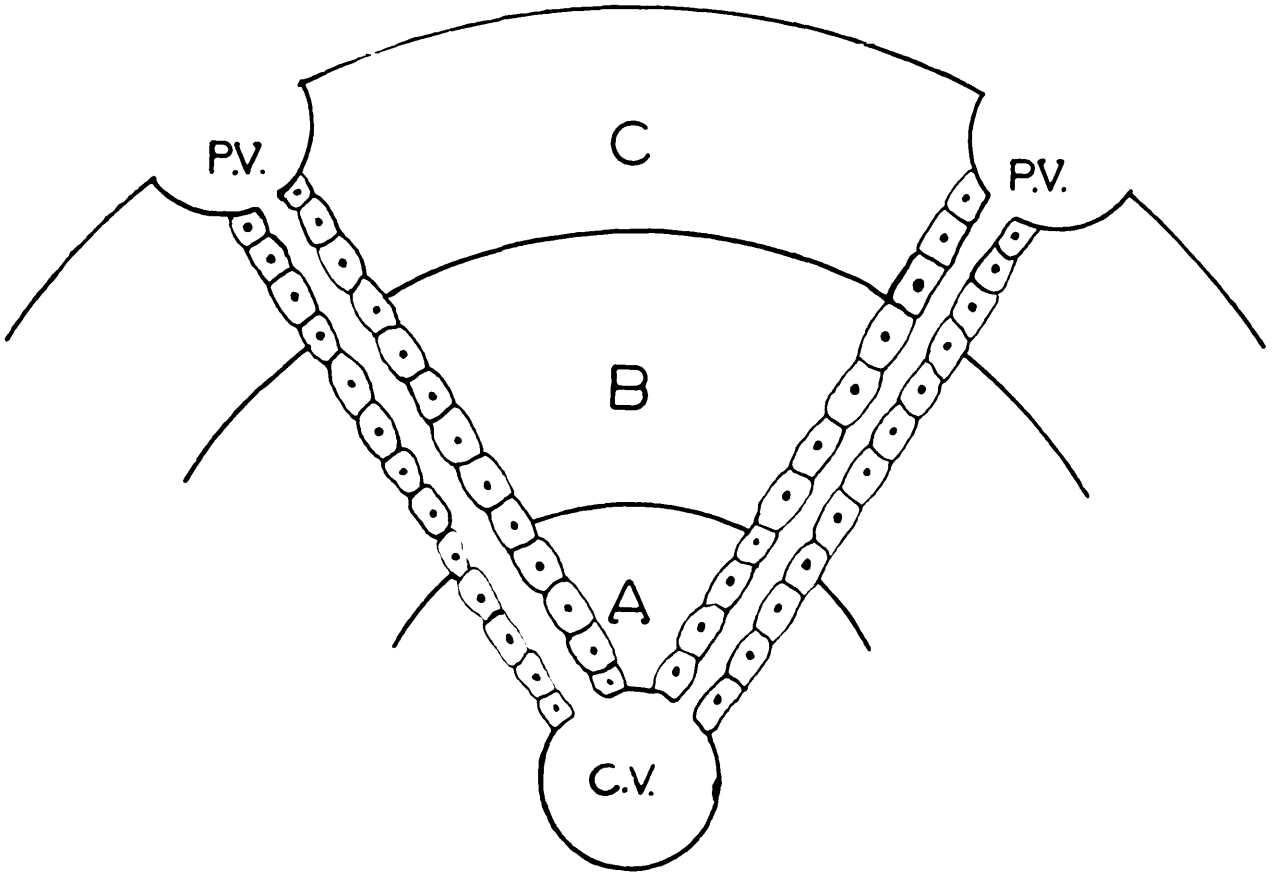


FIGURE-16

A-CENTRAL ZONE, THE ZONE OF PERMANENT REPOSE

B-INTERMEDIATE ZONE, THE VARIABLE ZONE.

C-PERIPHERAL ZONE OR PERILOBULAR REGION, ZONE OF PERMANENT FUNCTION.

PLATE VII

Tissue Prepared by the Injection Method

Figure 17 - drawn from an area surrounding the peripheral vessels of the lobule on the slide photographed in figure 19. The connective tissue trabeculae and the cells immediately surrounding the ducts and vessels are fixed.

The area which shows mitochondria may be divided into three zones; the penetrable area where the mitochondria are fixed and spread evenly throughout the cytoplasm; the transitional area where only a few of the mitochondria are fixed, usually on the side of the cell nearest the peripheral vessel; and the unfixed zone where the cells show no mitochondria present. All the mitochondria shown here are of a spherical nature.

Figure 18 - The dark area surrounding the peripheral vessel shows the mitochondria in the fixed cells. Moving centrifugally, the lighter transitional area contains few mitochondria. The extreme outer area is the central zone in which fixation was not possible.

Figure 19 - Same description as that of figure 17.

PLATE- VII

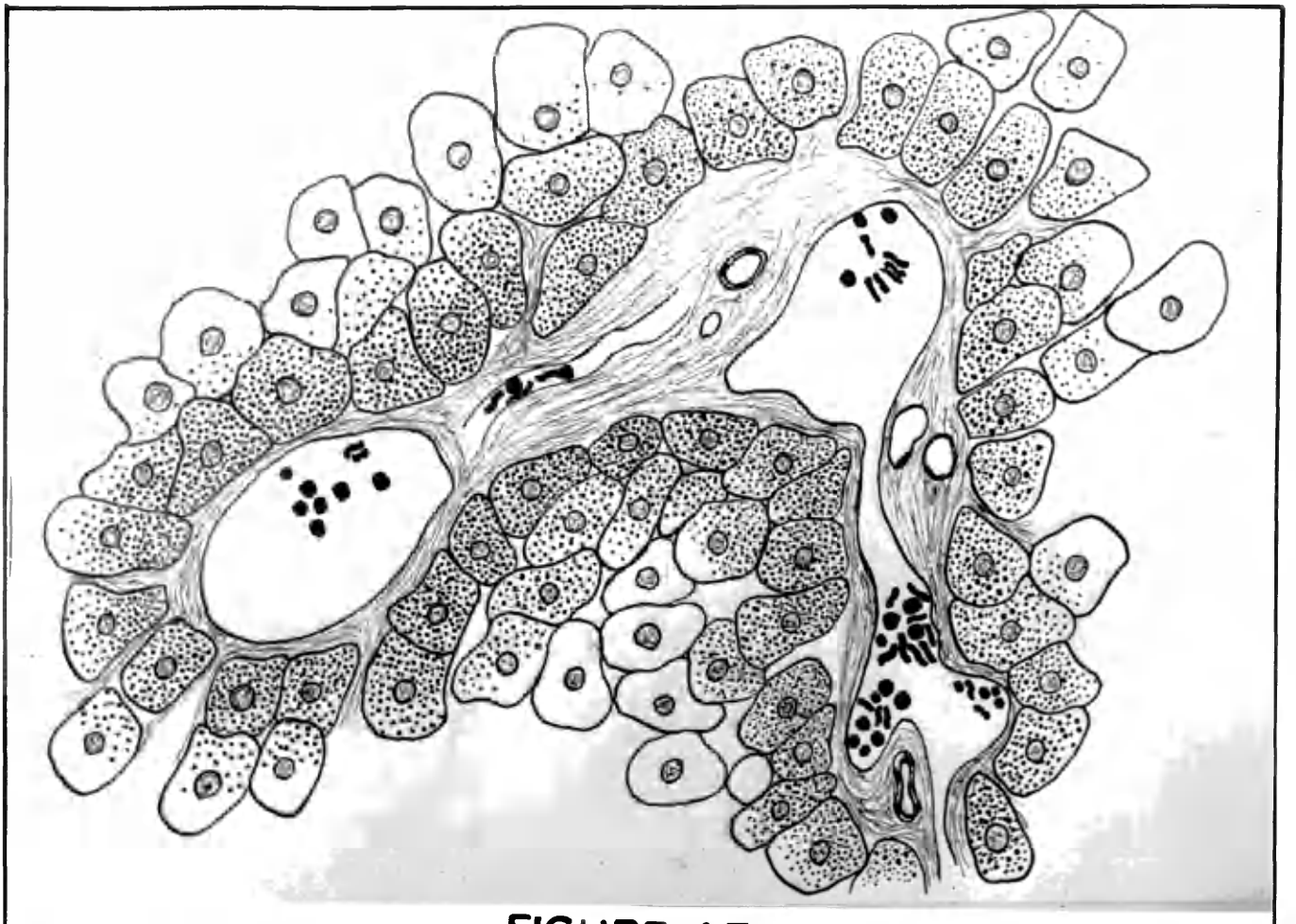


FIGURE-17

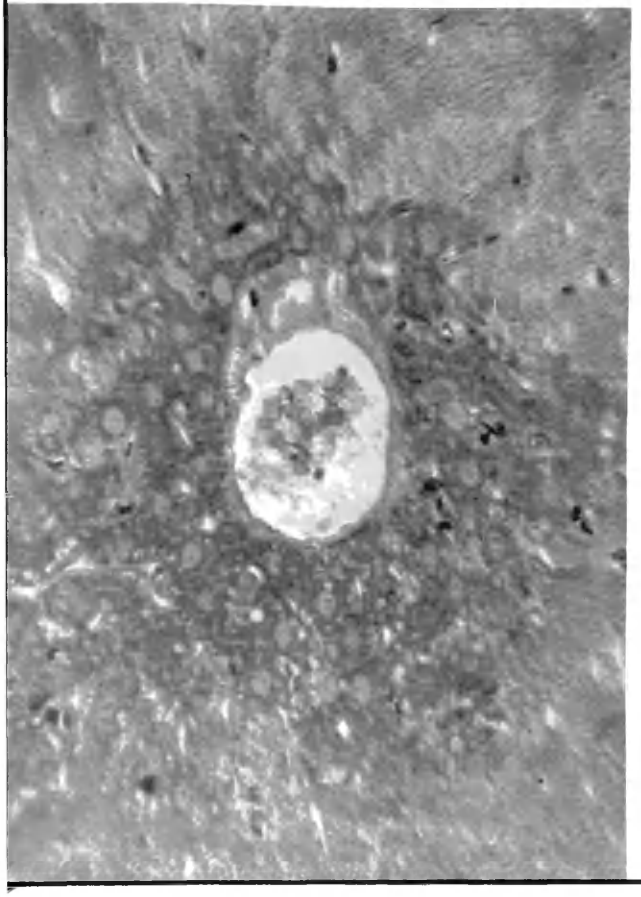


FIGURE-18

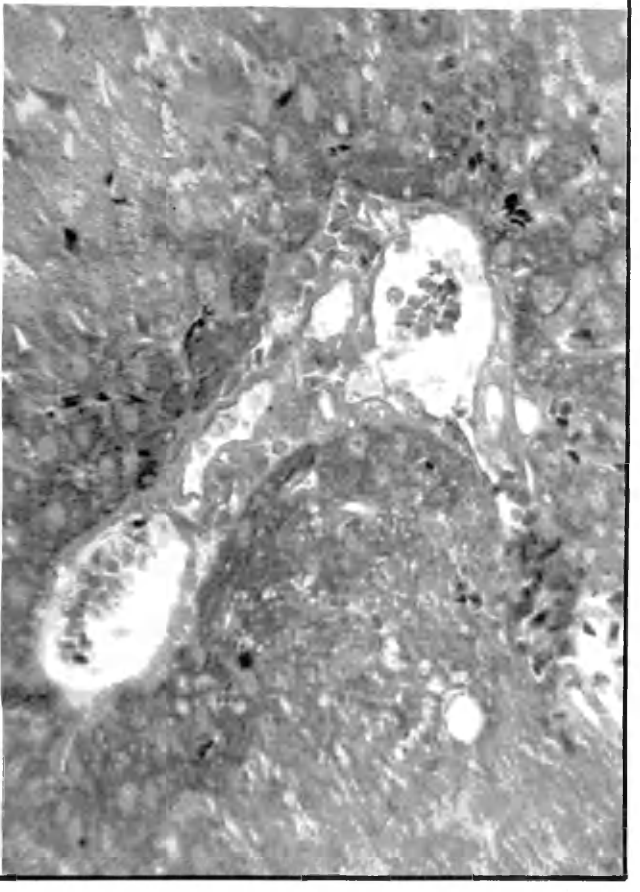


FIGURE-19

PLATE VIII

Tissue Prepared by the Perfusion Method

Figure 20 - Cells fixed with Regaud's solution. Cell 1 of the central region shows three types of mitochondria, rods, spheres and filaments in equal numbers. Cell 2 of the intermediate region, has the same general types of mitochondria, however they are somewhat greater in diameter and are fewer in number. Vacuolization was evident in the central region. Cell 3 of the peripheral region shows fewer mitochondria of two general types, rods and spheres.

Figure 21 - Cells using Bensley's fixative drawn from the same areas as in figure 20. The same types of mitochondria exist in tissue prepared by this method as when using Regaud's. Cells fixed by Bensley's solution contain mitochondria which are more swollen than those prepared by Regaud's solution.

Figure 22 - Another group of cells fixed with Regaud's solution. Cell 1 drawn from the central zone, 2 and 3 from the intermediate zone, and 4 from the peripheral zone. The description for these cells is similar to the one given for figure 20.

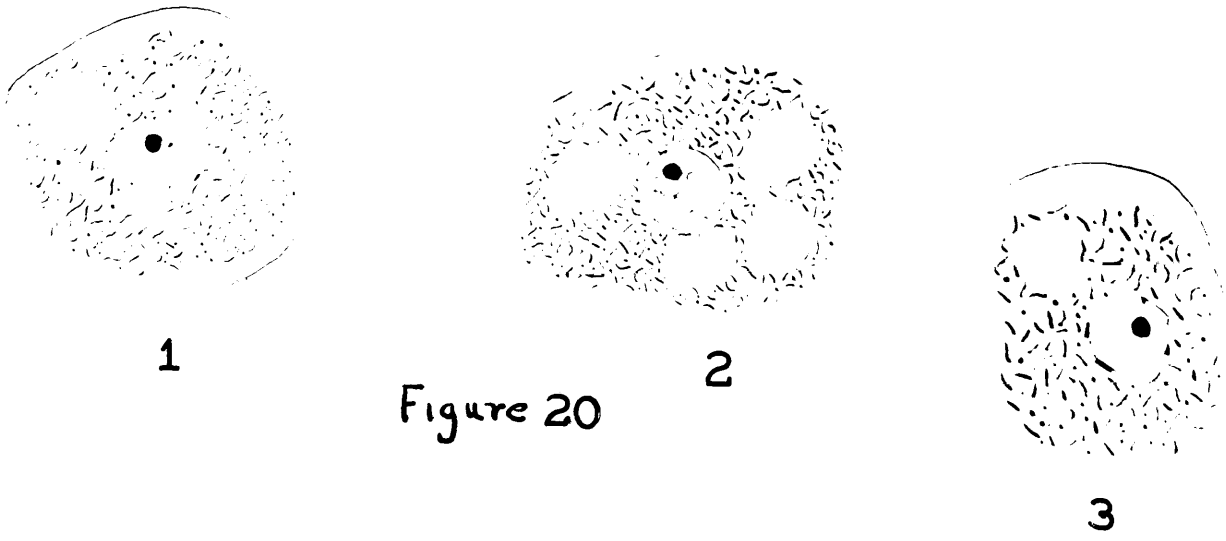


Figure 20

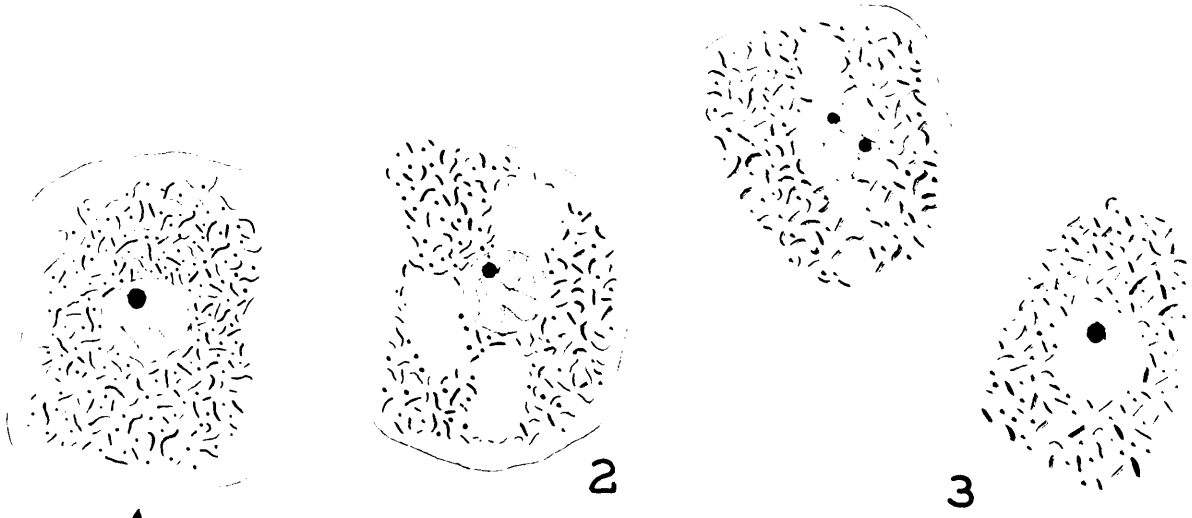


Figure-21

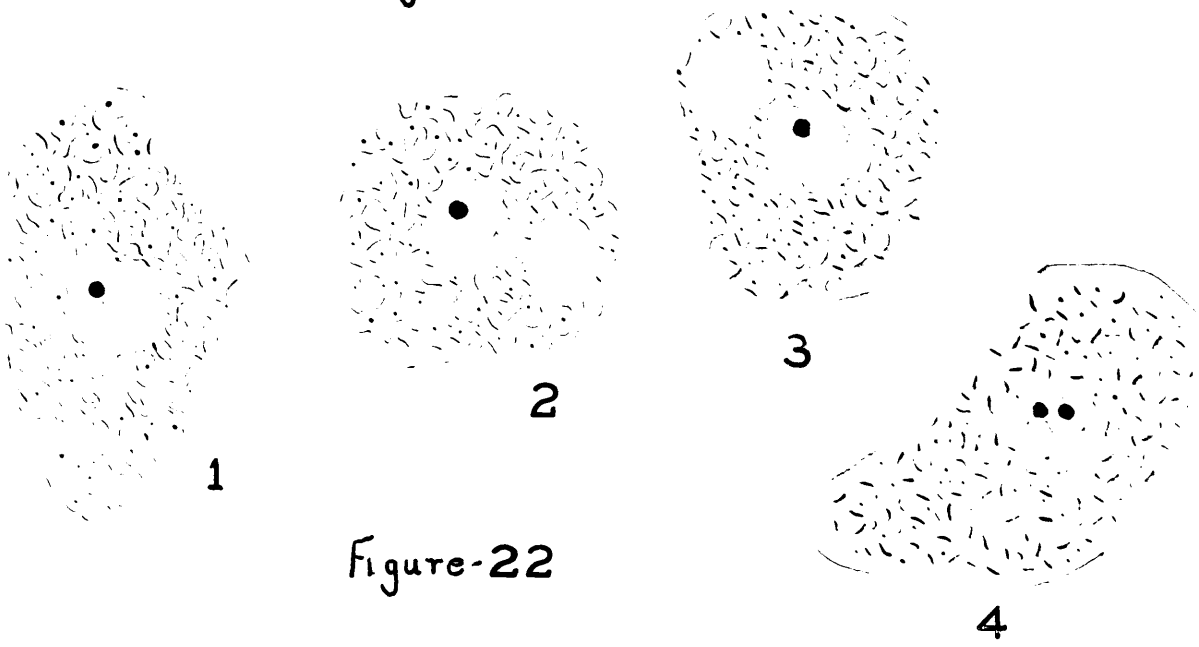


Figure-22

PLATE IX

Induced Ischemic Tissue

Figure 23 - From the left and Spigelian lobes ligated from 8 to 10 minutes. In cell 1 of the central zone the mitochondria are small spheres and rods. In cells 2 of the intermediate zone there is a general thickening of the mitochondrial forms; variations of the hollow spherical type are visible. Cells 3 from the zone of permanent function, show mitochondria in the forms of spheres as well as swollen and slightly curved rod

Figure 24 - From the left lobe ligated 15 minutes. Little difference is noted between these cells and those described in figure 23.

Figure 25 - From the right lobe ligated 30 minutes. Cell 1 of the central zone in which large vacuoles appear around the nucleus, contains spherical and a few rod shaped mitochondria. Cell 2 of the intermediate zone with large vacuoles occupying the space surrounding the nucleus, shows spherical mitochondria crowded in the peripheral part of the cell. Cell 3 with large vacuoles present in the region of the nucleus is drawn from the peripheral region and illustrates mitochondria as large irregular shaped bodies grouped along the cell membrane. The nuclei are somewhat distorted.

Figure 26 - From the lateral lobe ligated 33 minutes. In cell 1, drawn from the central region, the mitochondria are small rods and spherical bodies distributed evenly throughout the cytoplasm. Cell 2 drawn from the central region, contains only spherical mitochondria. In cell 3 representing the peripheral zone, the mitochondria are larger than in the other two zones and are grouped around the nucleus. Vacuoles are present in all three areas of the lobule.

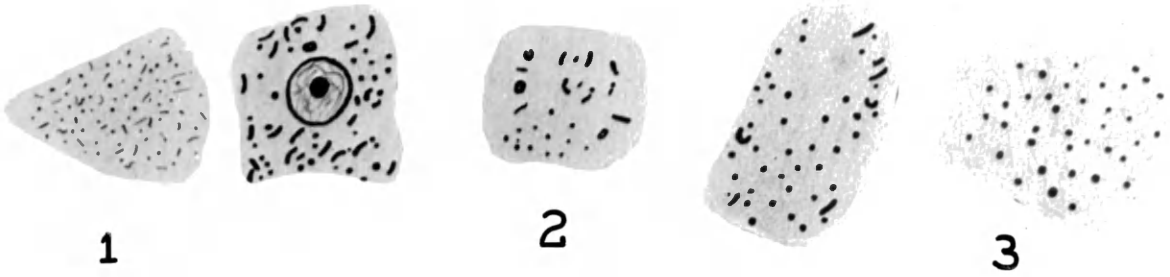


FIGURE-23

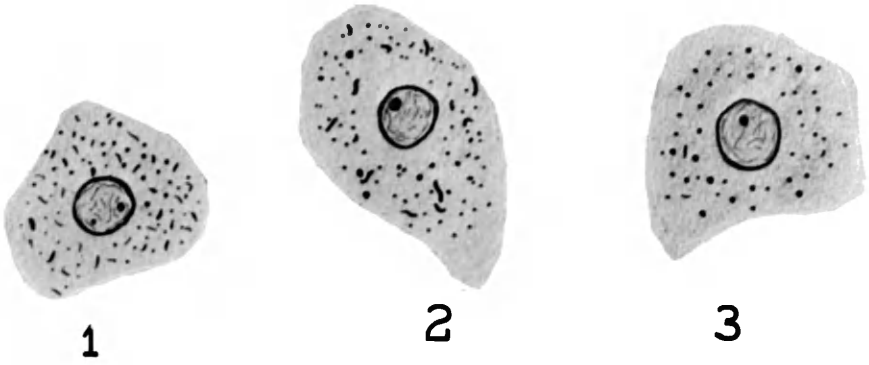


FIGURE-24

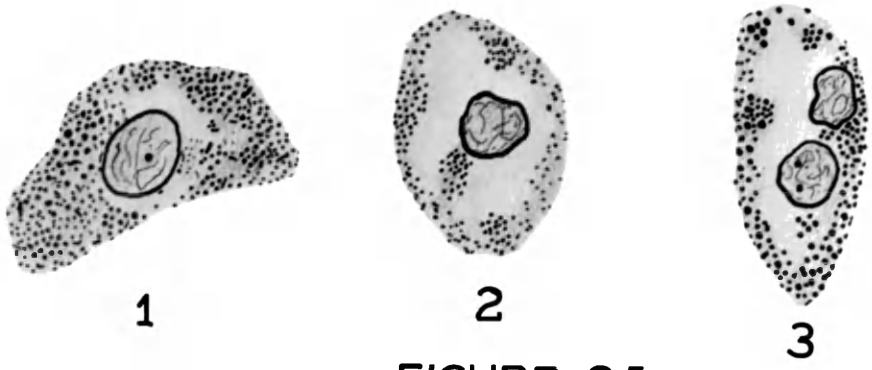


FIGURE-25

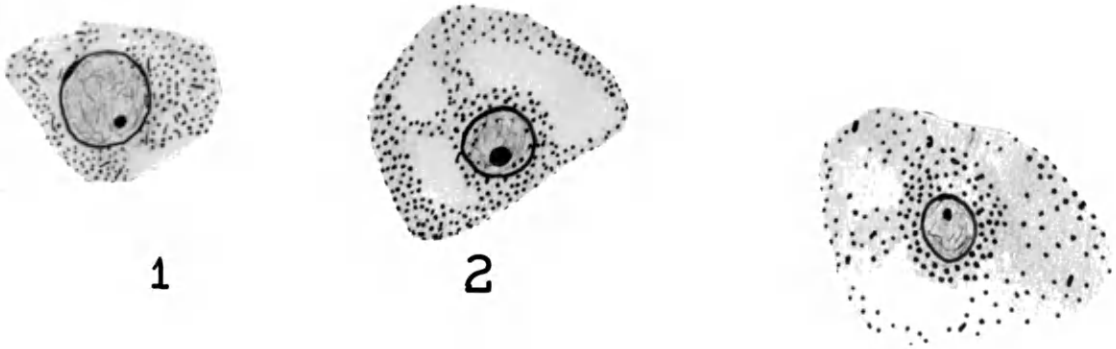


FIGURE-26

PLATE X

Induced Ischemic Tissue

Figure 27 - From the lateral lobe ligated 45 minutes. In cells 1 from the central vein region, the mitochondria are of a spherical nature and are well distributed throughout the cell. Cell 2 from the intermediate zone, exhibits perinuclear clumping. Cells 3 drawn from the peripheral region, show perinuclear clumping and mitochondria larger than those of the intermediate region. Large vacuoles are present in these cells.

Figure 28 - From the median ventral lobe ligated 60 minutes. The mitochondria in cells 1 are fine granules grouped in the cytoplasm. The cells show clumping of the intracellular elements. In cells 2 of the peripheral region, the cytoplasm was a coagulated mass with large vacuoles present. Mitochondrial identification was impossible and the nuclei show signs of distortion. In the peripheral region the cells have pulled away from the perilobular vessels. Type cells from the intermediate area are not shown because of their similarity to those of the peripheral region.

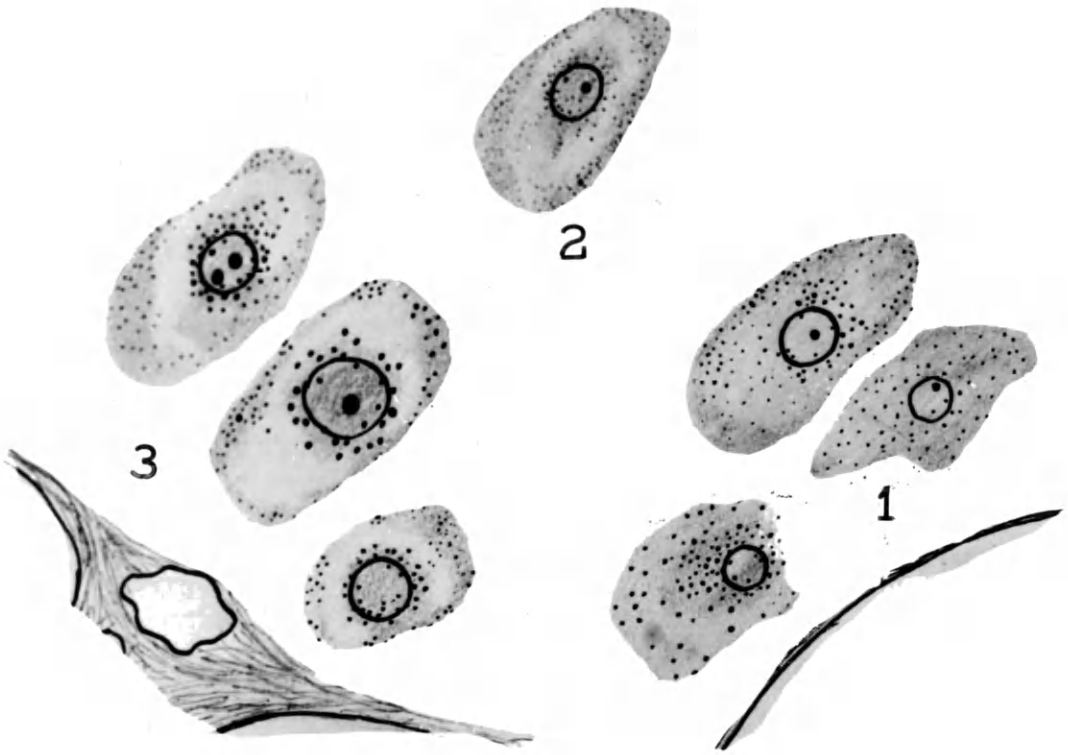


FIGURE -27

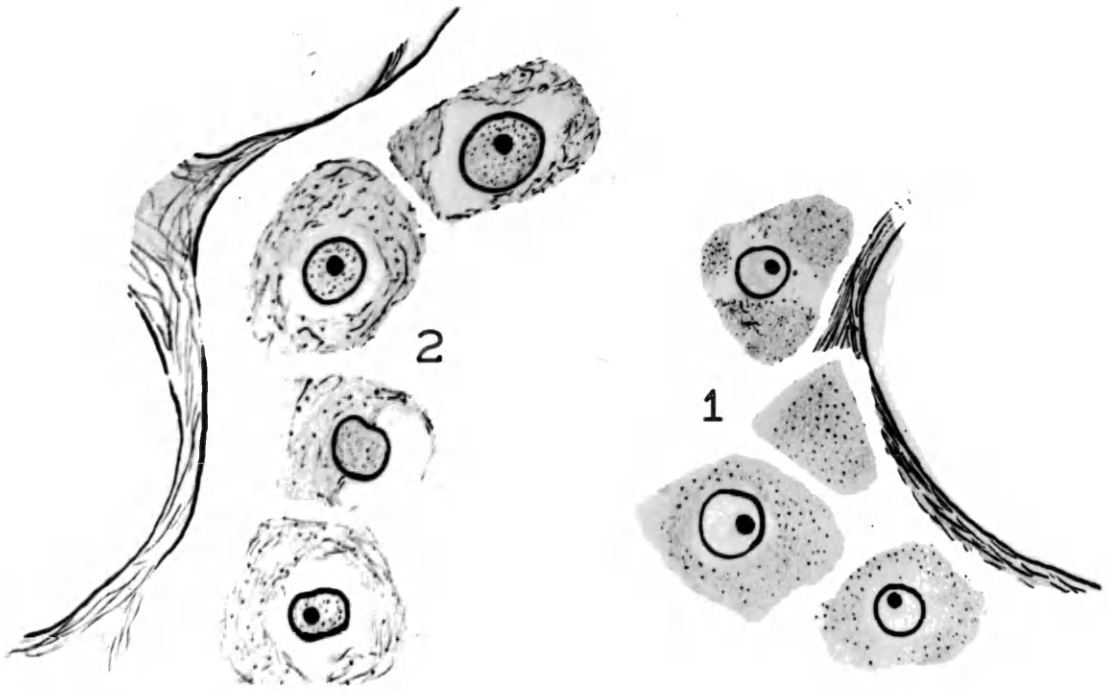


FIGURE-28

PLATE XI

Figure 29 - A greater portion of an entire hepatic lobule with one-third of the sinusoids in the storage phase. Note in the lower right corner of the photograph the blood cells packed in the sinusoids and in the upper region of the lobule the empty sinusoids.

Figure 30 - Another section of an hepatic lobule in the region of the central vein. Note in the lower left corner of the figure the sinusoids in the storage phase and in the upper left corner the open sinusoids.

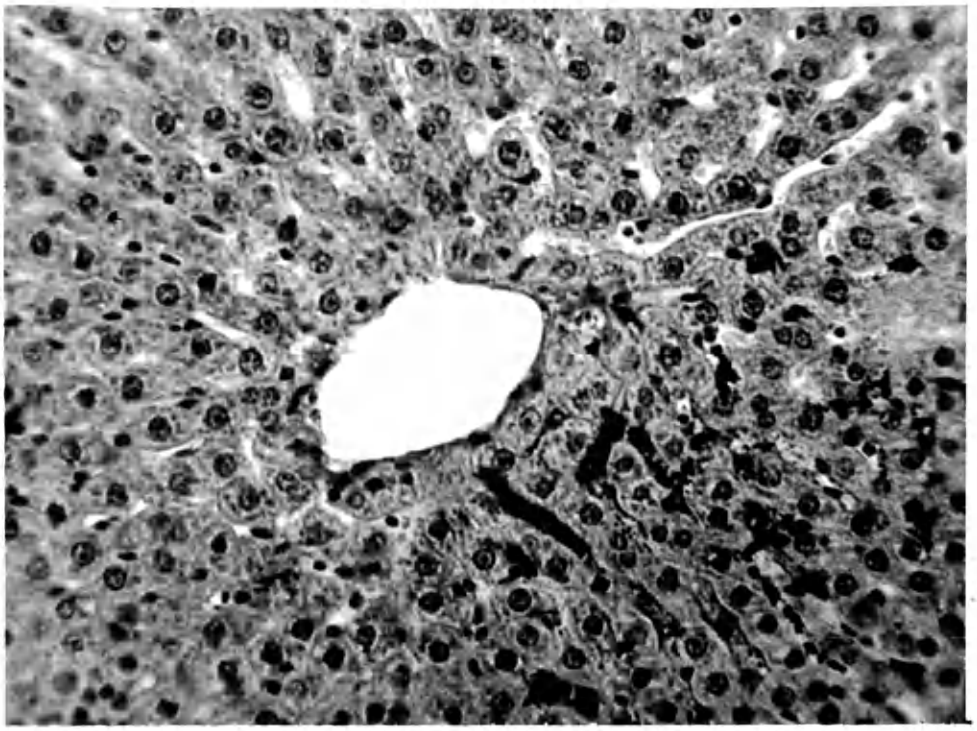


FIGURE-29

FIGURE-30

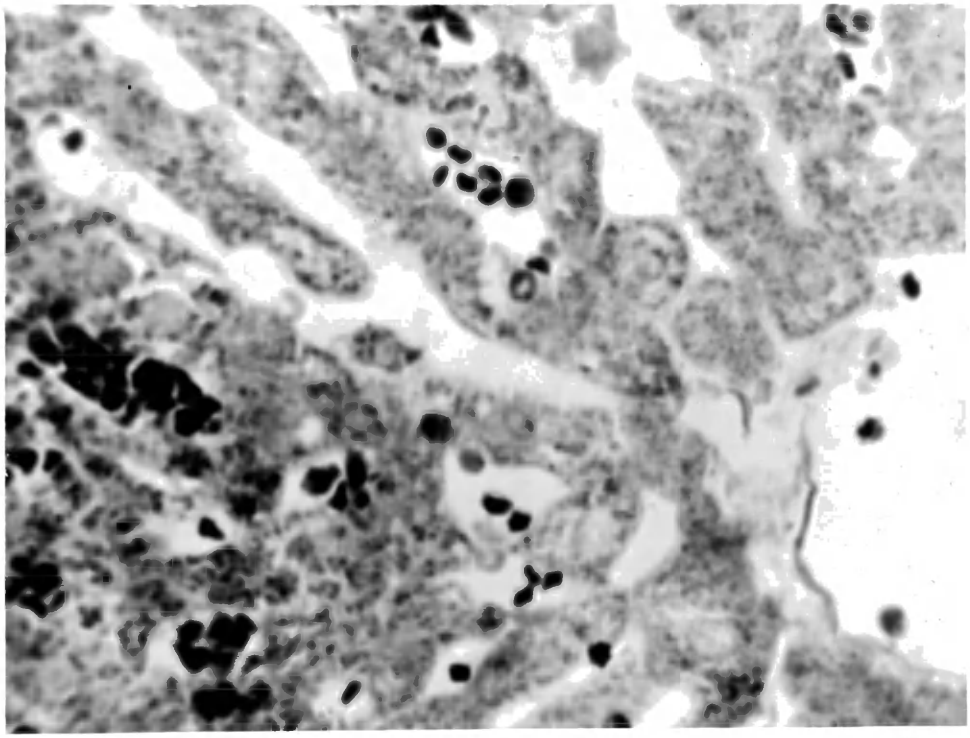


PLATE XII

Natural Isohemia

Figure 31 - Cell 1 was drawn from the central zone of the lobule and shows spherical and rod shaped mitochondria. Cells 2, 3 and 4 are taken from the intermediate region of the lobule moving peripherally. The mitochondria in cell 2 and 3 are all spherical, some having clear centers. The cells show grouping of the mitochondria due to many vacuoles in the cytoplasm. Cell 4 from this same region shows all forms of mitochondria from curved rods to a fine granular type. Cell 5 was drawn from the peripheral part of the lobule and contains only spherical mitochondria which are evenly spread throughout the cytoplasm.

Figure 32 - Cell 1 was drawn from the perilobular region; cell 4 from the region of the perihepatic canal. These cells contain few mitochondria all of which are spherical in nature. All the cells in this region appeared vesticulated. The intermediate area of the lobule is represented by cell 2. The mitochondria in this are spherical bodies ranging from very small granules to large hollow spheres. Cell 3 of the central zone exhibits mitochondria of various types, rods, dumb-bell shaped rods, spheres and granules some of which have clear centers. The mitochondria in the central zone are more numerous than in any other region of the lobule.

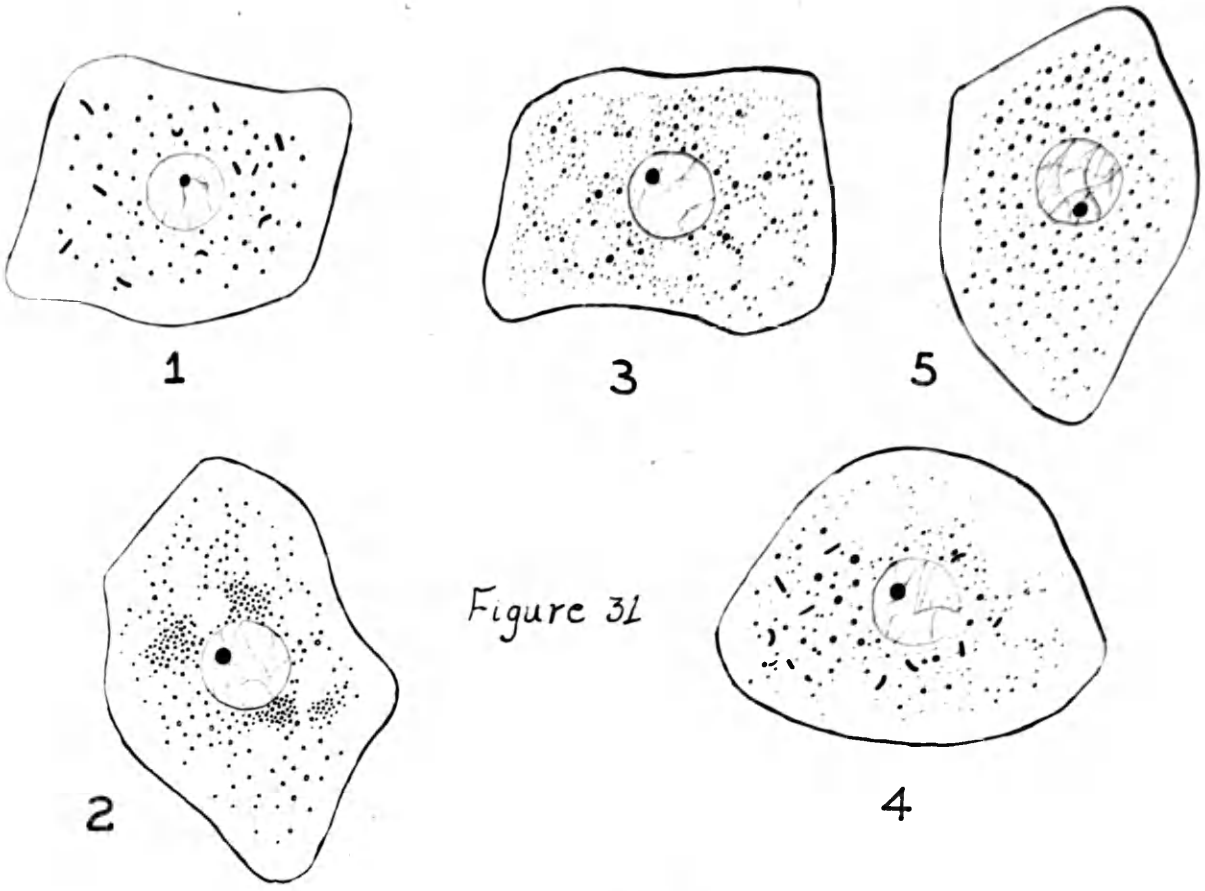


Figure 31

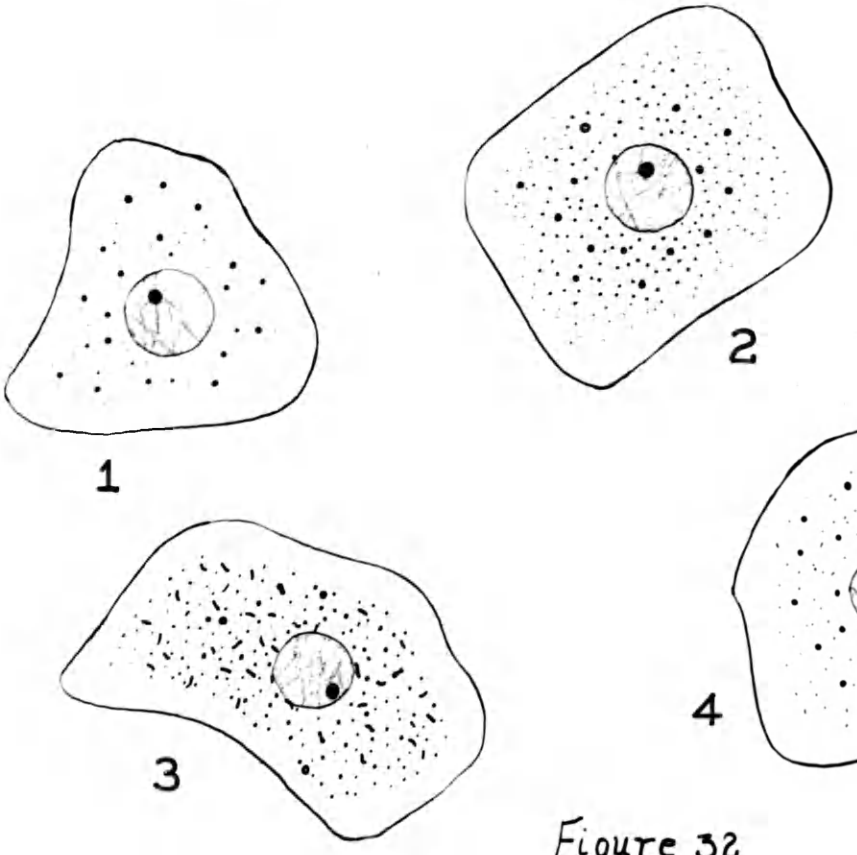


Figure 32

PLATE XIII

Mitochondrial forms

Figure 33 - Spherical mitochondria found most abundantly in the peripheral part of the lobule, and are more numerous there than in either the intermediate or central zone. Their diameters range from .5 to 2 u.

Figure 33A - Varies from the spherical type mitochondria in that it is larger, has rougher edges, and distorted bodies. Is most evident in ischemic tissue.

Figure 34 - Rod shaped mitochondria of this type are usually smooth, vary from .5 to 5 u in length, vary in diameter, and are sometimes curved in a quarter moon shape.

Figure 34A - Mitochondria with clear centers. Evident in normal and ischemic tissue in the peripheral part of the lobule. The vacuoles are of variable sizes as illustrated in this figure.

Figure 35 - This filamentous type has been observed to be more abundant in the central than in the intermediate areas of the lobule. It does not appear in the pheripheral area. The only visible variations of this form are the curvature and length which ranges from 2 to 12 u; the diameter is fairly constant.

Figure 35A - Filamentous and rod shaped mitochondria with enlarged ends. They occupy the peripheral part of the lobule in normal tissue and the peripheral and intermediate areas of ischemic tissue.

Variations In Mitochondrial Forms

Figure 33

Figure 33A

Figure 34

Figure 34A

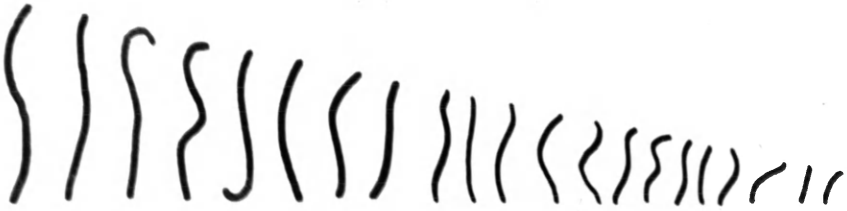


Figure 35

Figure 35 A

PLATE XIV

Influence of Osmotic Pressure on Mitochondria

Figure 36 - Tissue prepared by the perfusion method showing an hepatic cord which exhibits the influence of an hypertonic Ringer's solution on hepatic mitochondria. Cells 1 to 11 represent a complete hepatic cord. Cells 1, 2 and 3 are from the central area and show fine granular mitochondria of variable sizes. Hollow spherical mitochondria and a few rod shaped mitochondria are present. Cells 4 to 7 are taken from the intermediate region of the lobule, the mitochondria of which are granular with ovoid rods present in some of the cells. Cells 8 to 11 are from the peripheral part of the lobule, the mitochondria all of which are spherical of like diameter. The cells in the entire cord show vacuolization. Cells 12 to 16 were taken from another lobule in the region of the central vein and show mitochondria of all the types thus far observed. Note the crenated blood cells in the sinusoids boardering these cells.

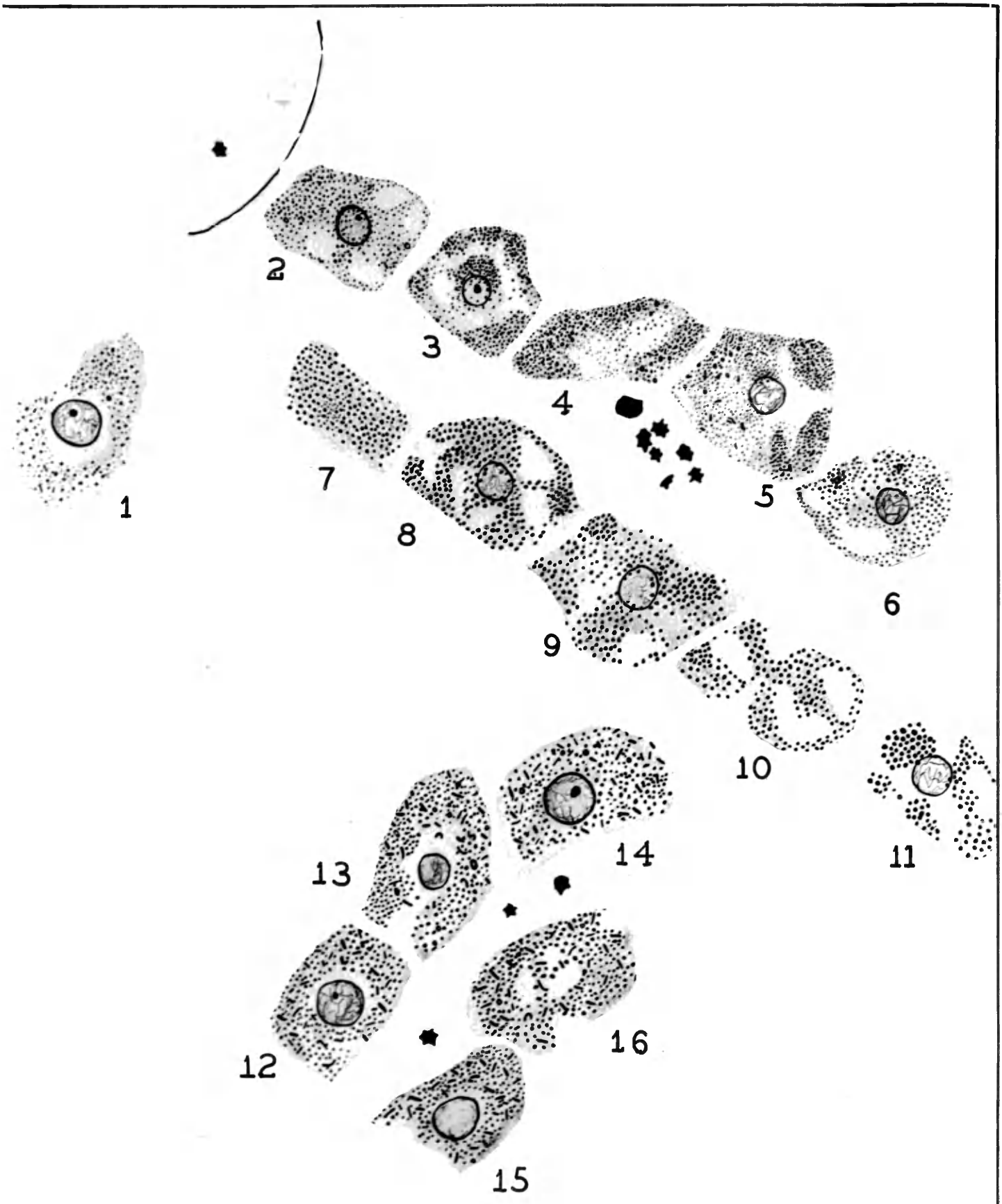


FIGURE - 36

PLATE XV

Fat in the Hepatic Lobule

Figure 37 - Tissue prepared by the immersion method using Bensley's fixative and stained with acid-fuchsin and Picric acid. Cell 1 drawn from the central zone of the lobule shows fat globules spread throughout the entire cell. The globules vary in size. The mitochondria are of three types, rods, spheres, and filaments. Cells 2 and 3 were taken from the intermediate area. The mitochondria are of three types, rods, spheres and hollow spheres. There is a grouping of the mitochondria in cell 2 due to large fat vacuoles localized in one area of the cell. In cell 4 of the peripheral zone few fat bodies are in evidence. The mitochondria are spherical, many of which have clear centers.

Figure 38 - Section of lobule showing the relationship of fat to mitochondria as drawn in figure 37.

Figure 39 - Photograph showing intracellular distribution of fat.

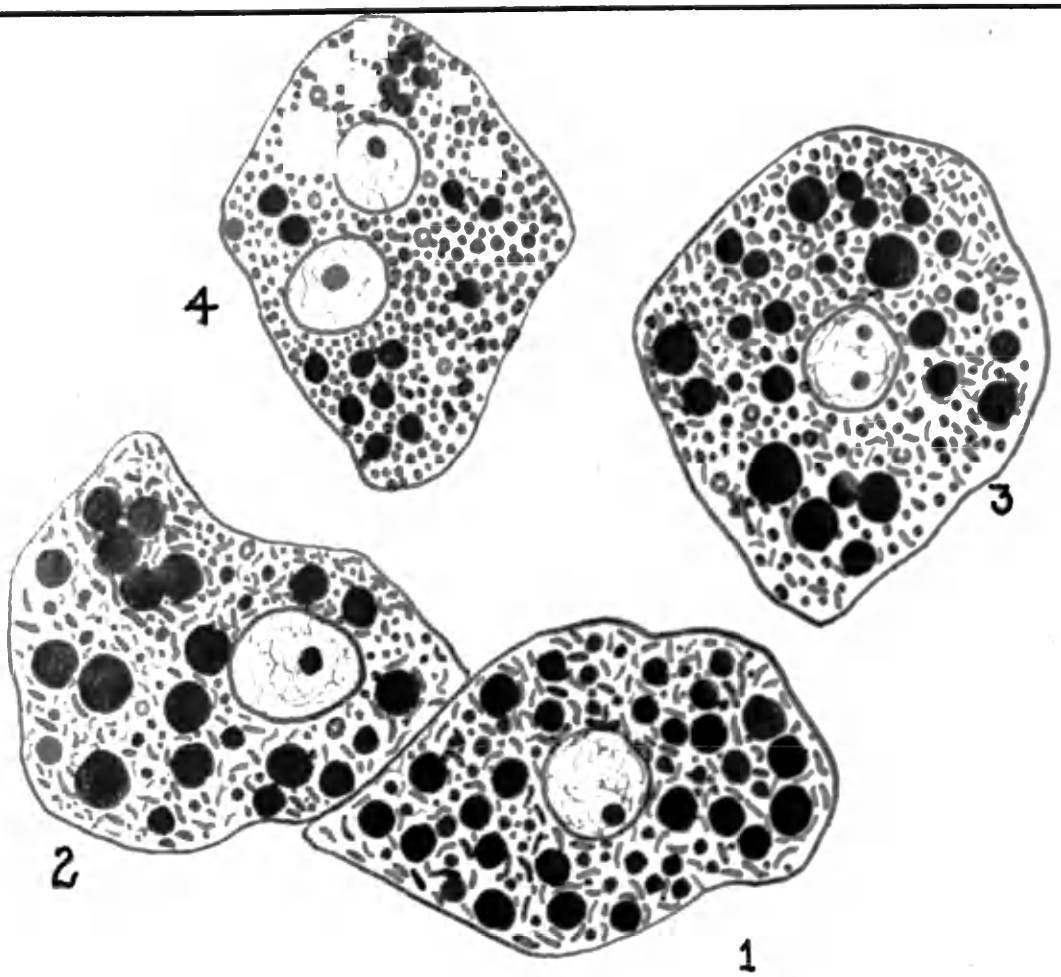


FIGURE -37

FIGURE -38

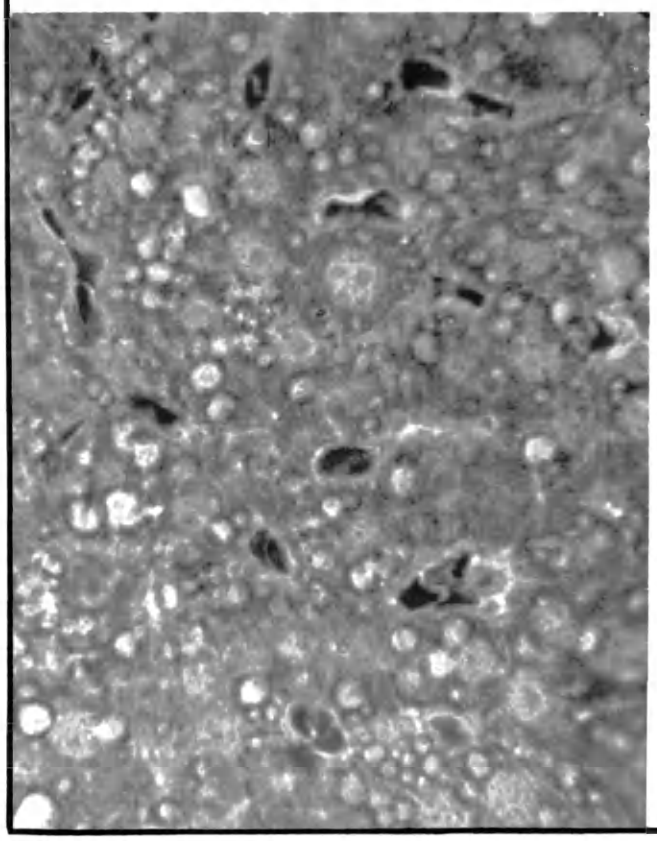


FIGURE -39

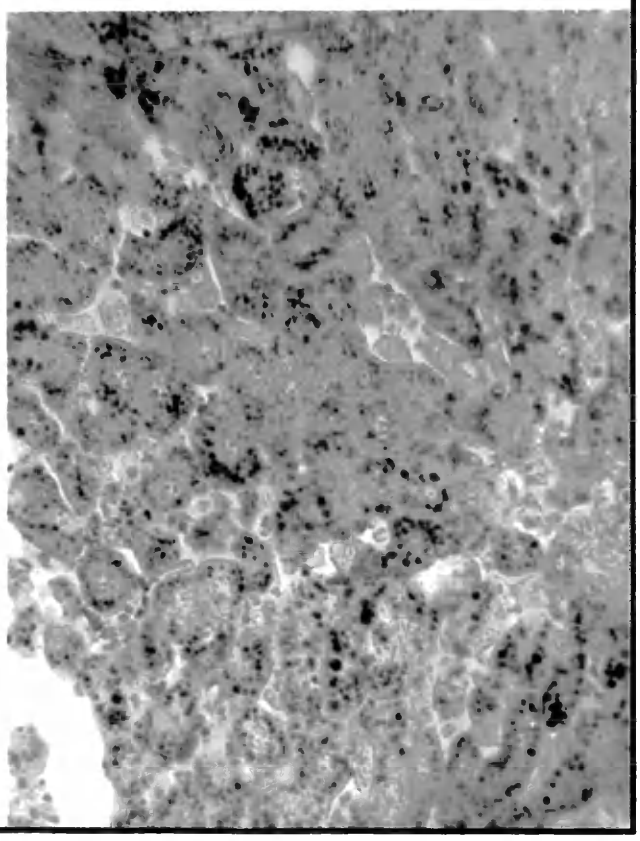


PLATE XVI

Natural Ischemia in Human Liver Tissue

Figure 40 - Low power photograph. Note the complete absence of blood cells in the sinusoids and the fibrillar degeneration of the tunica of the veins. Fragmentation of the connective tissue trabeculae is evident in the periphery of the lobule to the right of the figure.

Figure 41 - High power photograph of the peripheral region of figure 40. The connective tissue may be seen separating the individual hepatic cells. In the center of the photograph intussusceptive connective tissue growth may be seen. To the left of the figure the liver cells still retain their cord-like arrangement. Note the complete absence of blood

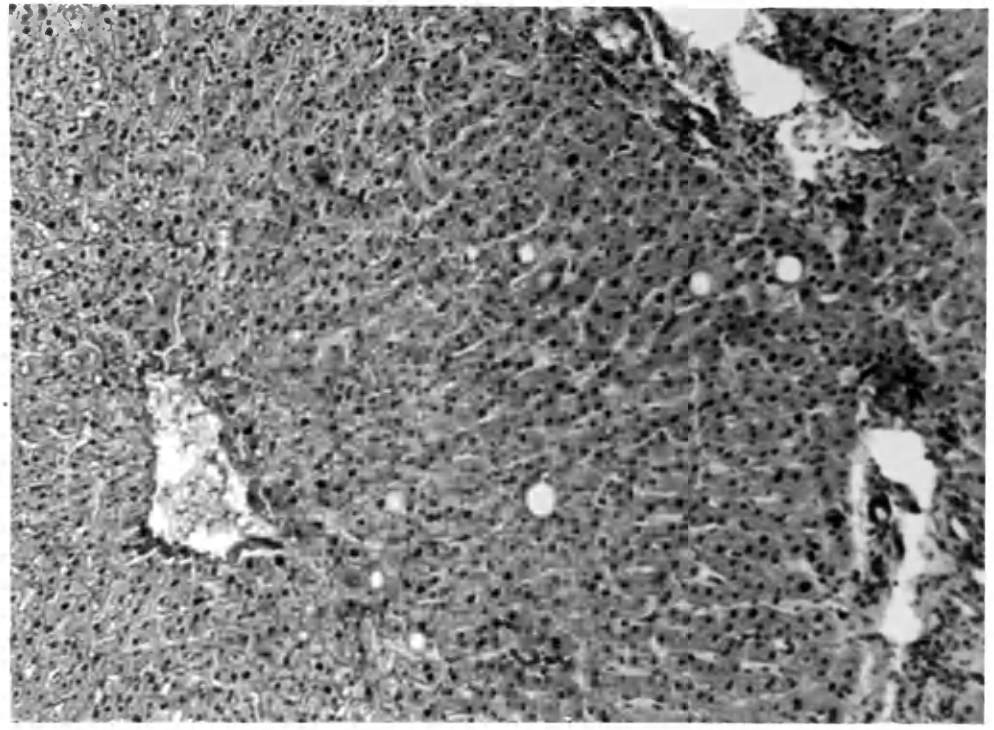


FIGURE - 40

FIGURE - 41