COMPARATIVE AND EXPERIMENTAL STUDIES ON THE CYTOLOGY OF THE LIVER[1].

By

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With 7 figures in the text and plate VIII.

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Contents.

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Introduction.

Previous work by others and by myself has clearly indicated that the liver affords a promising source of material for investigation of the problems associated with mitochondrial form and function, and also that experimental cytological studies may point the way to a more complete analysis of the functions of the liver. At the same time it has become evident that only a prolonged series of correlated researches can significantly contribute to a solution of the central problems.

The observations reported in the present account constitute merely an extension of our earlier papers. Unfortunately much of the material is still difficult to interpret, in spite of the fact that an effort has been made to collect all possible data. However, as this phase of the work is complete it seems desirable to report it at the present time, even though we must await the results of other approaches before the obscure questions of hepatic function and of mitochondrial form are completely resolved.

Material and Methods.

This study is based upon the following material:

Animal	Total Number	Normals	Number receiving adrenalin	Number receiving bile	Others
Domestic cat	37	13	3	21	
Rabbit	38	9	22	5	2, starved 48 hours
Chicken	16	6	5	5	
Pigeon	19	6	5	5	3, 1—8 hours after eating
Guinea pig	20	5	6	5	4, $^1/_2$—3 hours after eating
White mouse	14	3	3	3	5, $^1/_2$—8 hours after eating
White rat	37	6	5	4	5. $^1/_2$—$1^1/_2$ hours after injection of 1,5 cc. 100% glucose solution. 5, starved 4 days, then injected with adrenalin. 12, starved 2—5 days.
Wood mouse (Peromyscus maniculatus)	9	4	5		
Hog	6	3	3		
Dog	9	3	4		2, starved 48 hours

All of the animals were mature except five of the cats, which weighed one and a quarter pounds each; fifteen of the rats, which were sixty

days of age; and all of the hogs, which were ten weeks of age. The immature rats were destributed among the normals and all experimental groups, and the cats between normal and bile injected groups.

The normal or control animals were killed twenty-four hours after feeding and the adrenalin chloride ($^1/_{1000}$ solution), bile (fresh beef bile), and glucose were so injected that the experimental animals could be killed after a similar period of starvation. The chickens, pigeons, mice and rats were killed by decapitation; the rabbits, guinea pigs and hogs by bleeding to death from the throat; the dogs by shooting through the head; and the cats either by a blow on the head or by bleeding from the throat.

The last food received by the animals consisted of the conventional diet for the respective species except in the case of the dog and cat. The former were given a porridge of meat, corn meal, bread and milk; and the latter bread and salmon.

The adrenalin was injected subcutaneously in the mice, rats, and guinea pigs, and intramuscularly, in the remaining species; the bile subcutaneously; and the sugar either subcutaneously of intraperitoneally. The amount of adrenalin that the various animals could tolerate was determined by the use of individuals not listed with the above numbers. The specimens were killed in periods varying from one-half to three hours after the injection of adrenalin, and in the case of bile injection this time was reduced to fifteen minutes to one hour. The mice received 1 cc. of bile; the rats, 3 cc.; the guinea pigs and pigeons, 5 cc.; and the rabbits, cats and chickens, 10 cc. Judging from the character of the mucous membranes there was no toxic effect. The amounts of adrenalin administered are given in another section.

Blood sugar determinations were made on all of the animals except the mice and a part of the cats. Accordingly, the normal and bile injected animals having abnormal blood sugar levels are considered separately from those in the proper range. FOLIN and WU's method was employed.

With the exception of the cat, slides were prepared from all livers by REGAUD's method of fixation and staining for mitochondria; by SCHRIDDE's fixation combined with ALTMANN's method of staining for mitochondria and fat; and by BEST's carmine method for glycogen. REGAUD's and BEST's were the only methods used on the cats.

The description of chondriosomal morphology is based primarily upon REGAUD sections, since this method gives more regular results than any other that has been employed [HAIR (1931); KATER and SMITH (1932)]. It is commonly recognized that it is difficult to get good iron-alum-haematoxylin stains after REGAUD fixation. However, I have found that a slight variation of the usual technique entirely overcomes this difficulty. After the slides are mordanted they are very thoroughly washed in distilled water and transferred to a three to five per cent

solution of haematoxylin, where they are permitted to remain for two or three days. This prolonged immersion in strong stain does not affect the ease of destraining the nucleus or ground cytoplasm, but so impregnates the mitochondria that they retain the black color with great avidity. Accordingly, highly differential preparations result.

BEST's Carmine method is generally regarded as very erratic in its behavior. In the beginning of this work I experienced considerable difficulty in its use, but have found that a few precautions will lead to uniform results. In the first place, the slides should be placed in the celloidin without removing the paraffin. After staining in haematoxylin, to such a degree that destraining is unnecessary, the slides should be placed in the usual proportion of stock carmine and ammonium hydroxide, omitting the methyl alcohol. After the celloidined sections are well permeated (about five minutes), add the methyl alcohol and permit the slides to remain for ten to fifteen minutes. If the carmine is completely precipitated by the alcohol more ammonium hydroxide should be added and the time of staining prolonged. I have tried other oils in place of clove, but without success.

All sections were cut at 4 micra in 58° paraffin. The REGAUD material was flattened in fifty per cent formalin in place of water. It is only very rarely that a section is lost after this procedure, even though the tissue is very hard.

Experiments and Observations.

Tolerance for Adrenalin and its Effect upon the Blood Sugar Level.

The animals used in this work show great variability in their ability to withstand injection of adrenalin without exhibiting marked neuro-muscular disturbance and without death resulting. This difference would be more striking if expressed in terms of body weight, but as exact data in that regard were not recorded the figures will be given for individual dosage.

The chicken can stand relatively larger amounts than any other animal employed. An intramuscular injection of 5 cc. of $^1/_{1000}$ solution has no noticeable effect on the neuro-muscular system. The dosage which the others received without fatal or even detrimental effects is as follows: dog, 10 cc.; hog, 10 cc.; cat, 3 cc.; rabbit, .5 cc.; guinea pig, .2 to .5 cc.; rat, .3 cc.; pigeon, .5 cc.; wood mouse, .1 cc.; white mouse, .05 cc. The striking difference in resistence to this drug may possibly be related to the results to be described later.

Likewise, the response of the blood sugar level to adrenalin is not constant for size or dosage, when different species are compared. The average percentage increase, in one-half to three hours, utilizing the above amounts for injection, are the following: chicken, 21%; dog, 100%; hog, 110%; cat, 200%; rabbit, 200%; guinea pig, 75%; rat,

100%; pigeon, 30%. Blood sugars were not determined for the mice as no centrifuge was available for use in the micro-method. By giving guinea pigs very small injections of adrenalin at frequent intervals for a period of four hours preceding bleeding, a rise of 125% can be effected.

The complete history of the blood sugar level has been followed in only one animal, the rabbit. The response is extremely rapid, as individuals killed ten minutes after injection showed a rise rangig from 30% to 100%. The peak of the sugar level is reached in two to three hours and returns to normal in five hours. It will be noted that the rates of liberation of sugar in the blood is extremely variable in different individuals. However, its disappearance from the blood stream during the three to five hour period is equally as striking in its regularity. This experiment has been performed with rabbits unfed for twenty-four hours and with individuals immediately after feeding, with no difference in results.

The Liver Glycogen.

It is well known that some animals carry their principal reserve of glycogen in the liver, while others utilize the muscles for storage of this substance. In the rat, white mouse, wood mouse, guinea pig and pigeon practically all glycogen disappears from the liver within twenty-four hours after eating, as measured by histological preparations. In the dog, cat, rabbit, hog and chicken an appreciable amount remains after that same period of starvation, the chicken possessing least. After forty-eight hours it disappears from the liver of the rabbit, but in the dog the same conditions produce an increase of glycogen over the amount present after receiving no food for twenty-four hours.

The Glycogen Pattern of the Hepatic Cell. SMITH (1931a) has described the deposition and withdrawals of glycogen in the hepatic cell as follows:

> During the early stages of digestive activity relatively large vacuoles are apparent in the hepatic cell, fixed in absolute alcohol. Glycogen is first found at the periphery of these more or less spherical vacuoles, enclosing them in an incomplete network. As deposition continues the vacuolar material gradually becomes displaced by glycogen until the latter substance forms a seemingly solid mass in the position of the earlier vacuole. In the withdrawal of glycogen the vacuoles surrounded by the network again make their appearance.

In brief, this is apparently the true history of the glycogen pattern of the hepatic cell in many animals. I have found that account to be applicable to the white rat, cat, rabbit, white mouse, dog, and guinea pig. However, it seems desirable to add a few more details. Before the vacuoles become surrounded by the network of anastamosing filaments of glycogen the latter material appears at their periphery in the form of very tenuous strands. As these increase in number and size the network results, which is first replaced by a more or less complete sphere of glycogen enclosing the vesicle. Initially the glycogen forms a very thin

layer at the cortex of this structure, but gradually increases in width by growth in both directions, that is, towards the center of the sphere

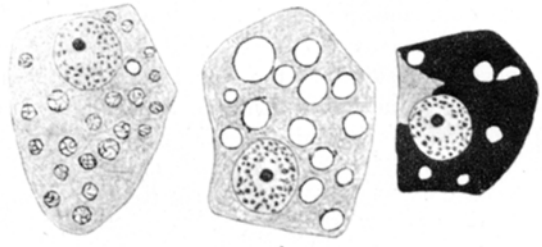

Fig. 1.

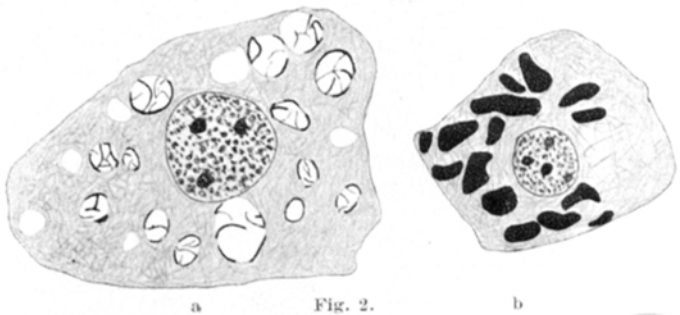

Fig. 2.

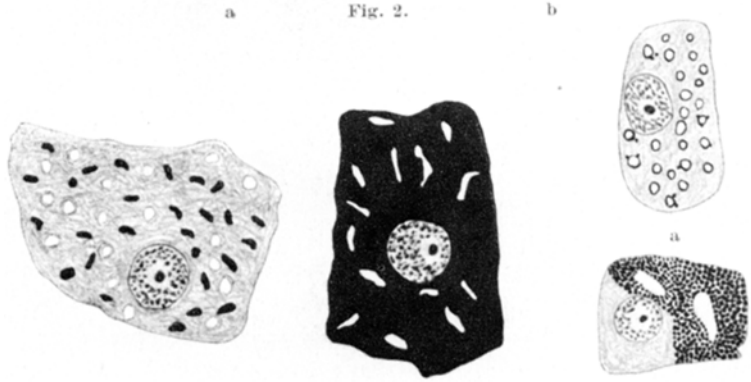

Fig. 3. Fig. 4.

Figs. 1—4. Drawings showing stages in the accumulation of glycogen in the hepatic cell. All from BEST's carmine preparations, 4 micra in thickness. Black indicates glycogen. Fig. 1, a to c are from the cat. Fig. 2, a and b are from the white mouse. Fig. 3, a and b are from the hog. Fig. 4, a and b are from the chicken.

and out into the surrounding cytoplasm (fig. 1, a to c). As described by SMITH, the vacuole is undoubtedly completely obliterated in many instances, but in some cases it partially remains until the maximum store of carbohydrate is obtained, as it can occasionally be seen in the midst of glycogen masses of large size.

In case sufficient carbohydrate food has been digested the individual bodies of glycogen lose their integrity by fusion with each other. Thus, the cell becomes filled with a very few large masses which completely obscure the cytoplasm except for very thin strands intervening between the aforementioned deposits. Of the animals used the mouse is the only exception to this condition as the vacuoles are always obliterated and the amount of glycogen stored in the hepatic cell never reaches such proportions as to cause the masses to fuse (fig. 2, a and b).

When the glycogen content of the hepatic cell of the hog is low the pattern is quite different. Relatively few small vacuoles occur and the glycogen is wholly unrelated, topographically, to them, this product first appearing as small granules which are scattered irregularly. When large amounts are present the cell is indistinguishable from that of the cat or similar animals (fig. 3, a and b).

The early history of glycogen in the liver of the domestic fowl is similar to that of the cat and when the cell becomes filled the same appearance is presented at low magnifications, but with the oil immersion objective it is observed that the large plaques are composed of minute granules (fig. 4, a and b).

The contents of the vacuoles around which the glycogen first forms cannot be stated. Comparison with osmic acid preparations shows that it cannot be fat, as the latter substance is less abundant and in smaller bodies in sections from the same livers. Accordingly, it seems probable that the vacuoles are composed of some aqueous solution.

The early stages of the deposition of glycogen or the late stages of its withdrawal give us little suggestion of the physical state of this substance during storage. However, when we consider that in some animals the cell can become so loaded with this substance that in haematoxylin-carmine preparations the entire cytoplasmic area is stained a deep red, without a trace of blue, and that the cell retains somewhere near its normal proportions it seems likely that the glycogen is affixed, by adsorption or otherwise, to the proteins of the cytoplasm and alters the affinity of the latter for stain, or merely obscures it.

Comparison of REGAUD and glycogen slides reveals that the cytoplasm is modified by the accumulation of glycogen, though not with perfect regularity in different species. In the rat, hog and dog the solution of the glycogen in the fixation of REGAUD material leaves large irregular vacuoles that apparently contain no coagulable material whatsoever. The ground cytoplasm and mitochondria are compressed into strands that enclose the nucleus and extend to the cell boundary. In the other animals one can observe in REGAUD sections the areas of greatest glycogen deposition by the displacement of the mitochondria, but some coagulable material remains so that vacuoles are not formed.

The picture of the glycogen in the hog is so different from that of the other animals tnat I am inclined to believe that the pattern is actually dissimilar in the living cell. It would appear much more likely that the aberrant condition observed in the chicken is a result of fixation, although, of course, neither can be definitely decided on a basis of sectioned material.

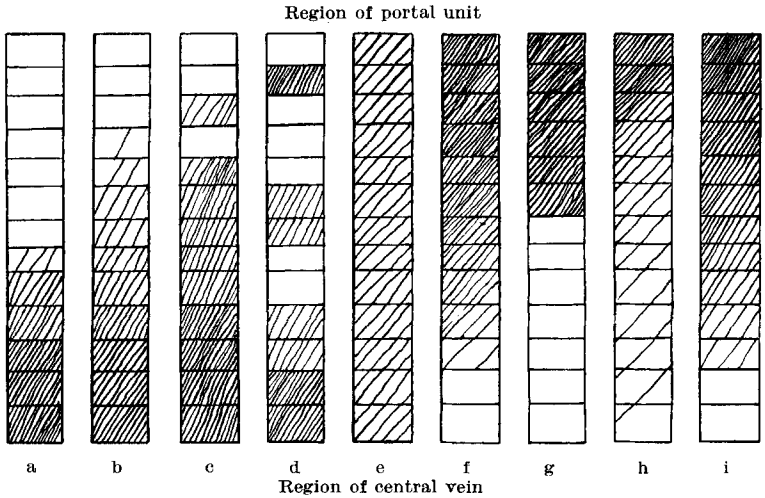

Fig. 5. Diagram showing deposition of glycogen in the hepatic lobule. Figures represent a cord of cells extending from the central vein to the periphery of the lobule. Oblique lines indicate glycogen, a guinea pig; b rabbit; c dog; d white mouse; e chicken; f hog; g cat; h cat; i rat.

The Glycogen Pattern of the Hepatic Lobule. In the white mouse, according to NOËL (1923), glycogen is first deposited in the cells bordering the central vein, is laid down in a decreasing gradient towards the portal vein, and is withdrawn in reverse order. We find exactly the opposite history in the white rat [SMITH (1931a)], glycogen first forming at the periphery of the lobule and gradually progressing towards the central vein. In withdrawal the center of the lobule is first to be deprived of its glycogen store.

These two schemes seem to be the usual ones. The cat and hog have the same pattern as the rat (fig. 5, f to i); the dog, rabbit, and guinea pig conform to NOËL's description for the mouse (fig. 5, a to c). However, the white mice that I have studied do not exactly coincide with NOËL's account, as scattered cells throughout the lobule show glycogen at the same time that it appears around the central vein, though the general course of deposition is as described by that author (fig. 5, d).

The chicken again is in an isolated position. Apparently there is no gradient for the accumulation or disappearance of glycogen, as the cells throughout the lobule are equally supplied with this carbohydrate (fig. 5, e).

The perfection of the distribution gradients implied by the above general paragraphs should not be interpreted as absolute. Individual variation is common, as can be seen by comparing „g" and „h" in fig. 5, which are both from cats. This is the only animal in which something of a gradient is not always found. Though „h" represents the usual scheme for a cat, occasional specimens show abundant glycogen in the outer part of the lobule with a sharp line separating the interior, which contains none. Likewise, though the dog does not present the same irregularity as the mouse some specimens show a lack of uniformity in the presence of glycogen without the realm of general deposit.

Fat.

The Formation of Fat. SMITH (1931b) has pointed out the probability that the hollow spherical mitochondria found in REGAUD sections of the liver represent stages in the elaboration of intramitochondrial fat, the latter substance being dissolved by the alcohols. This has since been shown to be true by the use of SCHRIDDE and ALTMANN fixation followed by the ALTMANN stain [KATER and SMITH (1932)]. With this combination the fat globules show the deep black of the reduced osmic acid, the mitochondria are red and the cytoplasm a pale yellow. Most of the small fat globules in such slides are very clearly wholly inclosed in chondriosomes. It was pointed out that, in view of the non-diffusibility of fats, this observation clearly establishes the synthesis of fat within mitochondria. As all of the livers used in the present work, with the exception of the cat's, have been studied by the SCHRIDDE-ALTMANN technique the earlier observations on fat formation have been materially extended.

Before discussing this subject, however, it is imperative that we point out the reaction of hepatic tissue of the various animals to this fixation. As a general statement it can be said that this method is more effective on livers taken from animals shortly after feeding. The engorgement of the cells with glycogen leaves the mitochondria in rather widely spaced strands of coagulable ground cytoplasm, and in this condition it is very easy to obtain a highly differential stain. Otherwise the ground cytoplasm and mitochondria stain equally with the fuchsin. Accordingly, the technique is not very effective when an animal is killed twenty-four hours after feeding, except in the case of those that carry glycogen in their liver at that time. After that period of starvation the best results, so far as general fixation and differential character of the stain is concerned, have been obtained on the rabbit. The dog and hog respond satisfactorily in general fixation, but the stain obtained is not so highly differential as in the case of the rabbit. The chicken can be satisfactorily studied by this method, but less effectively than the other three. In the guinea pig, which carries no glycogen at twenty-four

hours after feeding, the fixation is poor, but the stain is good, and in dealing with the white mouse, wood mouse, pigeon and rat this method is wholly valueless. Thus, it is apparent that the technique is not one of general applicability. The high degree of success with which we met in the previous study of the rat was doubtless due to the fact that we utilized animals that had been fed cane sugar eight hours before the time of killing. The material from rats, white mice and pigeons, killed during the time of digestive activity, is equally as successful as that from the rabbit.

Fig. 6. Drawing of a SCHRIDDE-ALTMANN preparation, showing fat globules enclosed in mitochondria in the hepatic cell of the white mouse, two hours after feeding on corn.

The quality of this method for the general study of mitochondria in any specific liver is not wholly correlated with the lack of distortion of chondriosomes containing fat globules. In the animals that have been unfed for twenty-four hours it demonstrates intramitochondrial fat, in accordance with our previous description, in the dog, in the hog and in the chicken, these structures being wholly disrupted in the rabbit, and failing to show in the other animals. The best preparations, so far as this feature is concerned, were obtained from the rats, white mice, and pigeons whose livers were fixed during the early period of digestion (fig. 6). In the pigeon, rat, dog, hog, and chicken the fat is usually found within spherical mitochondria, but in the mouse it more frequently occurs in the ends of filaments, except in the periportal area.

The Deposition of Fat in the Hepatic Cell and Lobule. According to current belief globular fat first appears at the periphery of the lobule and is deposited in decreasing amount towards the center, and granular or pathological fat is formed in reverse order [MANN (1928)]. Only the globular fat has been considered in the present study and it has been found to conform to the above scheme in most of the animals used (fig. 7).

In the wood mouse and hog the first fat to appear is distinctly periportal (fig. 7, a and b). In the former, this area of fat synthesis includes about three-fourths of the lobule while in the latter it is restricted to two or three of the outer layers of cells. The central portion of the lobule of the wood mouse never contains an appreciable amount of fat, even though the liver is filled with this substance. Shortly after its apperance at the periphery of the lobule of the hog, deposition in the interior is also begun, though it is always dominant near the exterior.

The rabbit, pigeon, and dog belong, more or less, to this same group. There is always more fat near the portal unit than bordering the central

vein, but the two areas are not so sharply divided as in the hog and wood mouse and, almost invariably, a few scattered cells towards the center of the lobule receive their complement of fat at the same time as the outer ones (fig. 7, c to e).

Contrary to previous description, the white mice that I have studied present a bizarre picture, so far as fat is concerned. This product is first observed in scattered cells throughout the lobule, resulting in a random pattern. In later deposition the remaining cells receive fat, but never equal the initial loci (fig. 7, f).

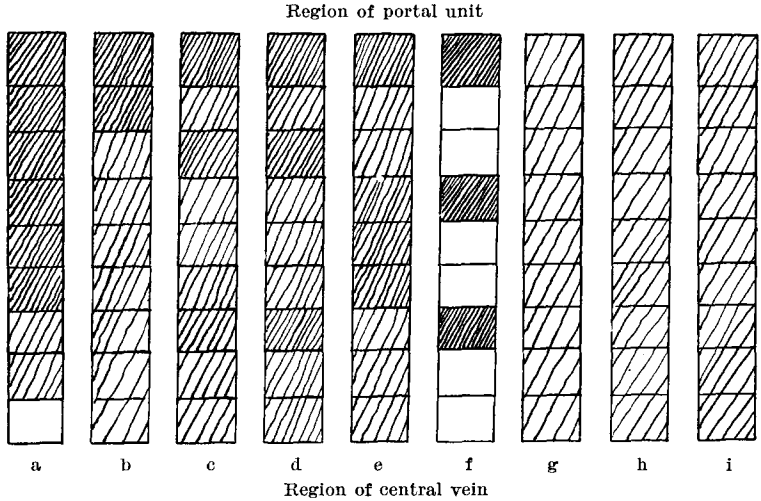

Fig. 7. Diagram showing the course of deposition of fat in the hepatic lobule. Figures represent cords of cells extending from the central vein to the periphery of the lobule. Oblique lines indicate fat. a wood mouse; b hog; c rabbit; d dog; e pigeon; f white mouse; g chicken; h white rat; i guinea pig.

In the chicken, rat and guinea pig there is, so far as microscopic examination can disclose, a perfectly even distribution of fat throughout the lobule (fig. 7, g to i).

With the exception of the rat and guinea pig the fat globules are not definitely restricted to any portion of the cell. In these two animals the early spherules always occur at or very near the periphery of the cell. The wood mouse is the only species in which there is a great accumulation of fat after starvation for twenty-four hours. In this animal, excepting the innermost part of the lobule, the cells contain so much fat that it becomes their most striking constituent, in osmium tetroxide preparations. The pigeon stands second in the quantity of fat and the hog, third, though both are far behind the wood mouse. The dog, rabbit and white mouse possess relatively little fat and the chicken, guinea pig and white rat even less.

Mitochondria of the Normal Liver.

Cat. I have previously described the mitochondria of the hepatic lobule of the cat [KATER (1931)]. The cats used in that study, sixteen in number, were all similar as to types of mitochondria and their distribution within the lobule. The chondriosomes of the cells near the periphery of the lobule were long filaments and those from cells near the central vein varied from short filaments to very short rods, with the intervening area presenting a gradual transition. Of the thirty-seven animals used in the present study only eighteen conform to that description. In direct contrast, the remaining nineteen exhibit absolutely no intralobular variability, the periportal cells being identical with those of the interior. The mitochondria of the individual cell include an assortment of all possible forms: spheres, beaded filaments, rods, and short smooth filaments, the last being predominant (pl. VIII, fig. 13).

It seems possible that this difference may be an expression of variation in nutrition, as seventeen of the nineteen presenting no intralobular variability had been kept in the animal house, located on the roof, for several months of very hot weather and had received rather scant attention by way of food and water. Most of them were somewhat emaciated. The remaining twenty animals, as well as the sixteen used in my earlier study, were brought to the laboratory in seemingly good condition, were well tended, and killed within one week. Only two of these thirty-six possess the mitochondrial pattern of the other group. Thus, I am led to conclude that twenty-four hours after feeding lobular variability is the normal condition in a cat, and that its absence is indicative of normal or induced pathological states. This individual difference within a single species emphasizes the necessity of knowing the physiological status of animals that are used in studies on mitochondria.

Hollow spheres, representing stages in the elaboration of intramitochondrial fat, were not evident in the cats formerly reported [KATER (1931)]. However, the entire thirty-seven utilized in the present work show some of these structures. They are especially abundant in the animals that possess no lobular variability. The fragmentation of the large hollow spheres has been described previously [KATER and SMITH (1932)].

Rabbit. The hepatic lobule of the normal rabbit is similar to that of the undernourished cat in the general absence of intralobular variability. The individual cell contains little else than long smooth filaments of equal width, with a slight admixture of hollow spheres, beaded filaments, rods, and spheres (pl. VIII, fig. 4). The last two are so few in number that they might well be ends of filaments removed by the knife. There is practically no size difference between individuals. In the majority of the thirty-eight rabbits all cells of the lobule contain exactly the same sizes, types, and proportion of mitochondria. However, in a few individuals a single layer of cells around some central veins is at variance

with the outer ones, possessing only spheres and short rods of almost twice the size of the chondriosomes in the remainder of the lobule (pl. VIII, fig. 5). In number of mitochondria these perihepatic cells seem to lag behind the others, when one considers the shortness of the rods. Some lobules in these same individuals are normal. This is the only case of interlobular variability noted in the rabbit. Although the animals were purchased in three lots from different sources several from each group exhibit this unusual state. Neither can the condition be correlated with glycogen store, blood sugar, or superficial appearance.

The hollow spheres occur very regularly in the hepatic tissue of the rabbit. In four micra sections very few cells are to be found that do not contain some of these elements. After a period of twenty-four hours without food they are all small and accordingly no stages of their fragmentation can be observed. During the late period of digestion larger ones can be seen, and their method of fragmentation is the same as in the rat [SMITH (1931b)].

Chicken. There is absolutely no regularity in the mitochondria of the liver of the domestic fowl with respect to any feature of their morphology. Some individuals posses a regular pattern of short, slender, beaded filaments, with neither inter- nor intra-lobular variability. Others are as consistent in that only relatively large spheres occur throughout the liver. One individual shows regularity in all lobules with long smooth filaments at the portal, spheres at the central, and beaded filaments in the intervening zone. About half of the chickens studied have a regular pattern of one of the above types in any individual lobule, but adjacent ones are very unlike and in two specimens opposite sides of the same lobule contain different types of chondriosomes. Excepting the last group, these conditions cannot be correlated with any known features of the condition of the birds, and, as no interpretation can be made, the mitochondrial morphology of the chicken is not being illustrated.

Hollow spheres are very scarce in this animal at twenty-four hours after feeding.

Pigeon. The mitochondria of the liver of the pigeon present neither inter- or intra-lobular variability. The pattern of all cells contains beaded filaments, smooth filaments, free spheres, and hollow spheres. All elements are short and both beaded and smooth filaments are frequently curved. The small number of free spheres would suggest that they result from the knife passing through the beaded filaments. The mitochondria found in the hepatic cell of the pigeon are more minute than those I have observed in the liver of any other animal. The only ones rivaling them, in this regard, are those of the guinea pig and of the perihepatic cells of the white mouse, and even the latter are appreciably larger.

Twenty-four hours after feeding this animal grain the liver contains an abundance of fat, the globules ranging from extremely minute spheres

to bodies as large as the nucleus. The fat retains its mitochondrial covering longer than in any other animal used in the present work. In the smaller spheres the chondriosomal cortex is complete, forming hollow structures of the same type observed in other animals; in the larger ones the mitochondrium becomes grooved from the outer surface or completely broken into curved segments in the same manner as has been described for the rat by SMITH (1931b). However, there is little tendency for the curved elements to migrate away from the fat globule (pl. VIII, fig. 11).

Guinea Pig. The mitochondria of the guinea pig liver are regularly filamentous. All of the lobules are alike and there is no polarization of types within the lobule. The majority of the cells contain long, slender, wavy filaments with sometimes a slight tendency towards polarization. The width of the filament is slightly greater than the width of those found in the pigeon. However, all cells within the lobule do not possess the same tenuous strands. Scattered at random one finds a minority containing filaments of more than twice the diameter of the common type. The occurrence of these cells cannot be associated with the position of biliary canals (pl. VIII, fig. 15).

Hollow spheres are only very rarely observed in the liver of the guinea pig after a period of twenty-four hours of starvation.

White Mouse. NOËL (1923) has described the hepatic mitochondrial pattern of the mouse. He divides the lobule into three zones: 1. the zone of permanent activity, 2. the zone of permanent repose, and 3. the intermediate variable zone. These areas include the periphery of the lobule, the center of the lobule, and the intervening portion, respectively. His description of relatively large spheres in the periportal region and of slender filaments in the perihepatic zone is entirely substantiated by my own observations. However, in the intermediate vicinity I find the dominant type of chondriosome to be the beaded filament. Towards the zone of permanent activity the beads are much larger than they are towards the center, so that there is a gradual transition from portal unit to central vein. The number of mitochondria found in the cells adjacent to the central vein is much smaller than that in those at the exterior of the lobule. The transition in this regard is less gradual than with reference to size (pl. VIII, fig. 3).

Hollow spheres occur only occasionally.

White Rat. The morphology of the mitochondria of the liver of the white rat has been adequately described previously [SMITH (1931b)]. For the sake of completeness a portion of that description is quoted.

> In the hepatic cells of the adult livers examined, the mitochondrial forms presented fell into two more or less distinct groups. While the mitochondria of one group are in the form of long, even, tangled filaments, free spheres and a few short rods, the other have no long even filaments, but only long, beaded filaments, free spheres and rods. Relatively few adult livers examined belong to the former group; the majority are either in the latter or in intermediary stages between the two (p. 497).

And:

Hollow spheres in various stages of fat formation may be found in abundance or may be entirely lacking, even though all individuals possess fat in their hepatic cells (pp. 497—498).

And again:

No lobular variation in mitochondrial size and type can be noticed. There is, however, a distinct lobular difference in relation to numbers. Cells immediately surrounding the central vein have about one half as many mitochondria as are contained in other cells of the lobule (p. 498).

The above quotations suffice for the description of the normal mitochondrial pattern of the rats which I have studied, except that I note a slightly greater preponderance of spheres in my material than occurred in the cells figured by SMITH (pl. VIII, fig. 18).

Wood Mouse. The size and configuration of the mitochondria of the liver of the wood mouse are identical with these same features of the white rat. However, there is no lobular variability even with reference to numbers nor is there interlobular variability. After a period of twenty-four hours starvation hollow spherical mitochondria are much more abundant than in the white rat, though most of the fat globules are free in the ground cytoplasm (pl. VIII, fig. 16).

Hog. There are striking individual differences in the mitochondria of the liver of ten weeks old hogs. In one of the three control animals the mitochondria of the hepatic lobule are very distinctly polarized. At the portal unit the cells contain long, smooth, tangled filaments, the length of many of them exceeding the greatest distance from the nucleus to the periphery of the cell. At the central vein the chondriosomes are almost entirely free spheres with only occasional dumb-bells and a few short, beaded filaments. The diameter of the spheres found at the center of the lobule is somewhat greater than the width of the filaments occurring in the periportal area. The intermediate zone contains every possible mitochondrial type, with free spheres as the predominant configuration nearest the central vein, and dumb-bells, beaded filaments, rods, and smooth filaments progressively increasing in direct proportion to the distance from the central vein. Each of the three zones includes about one-third of the radius of the lobule. The transition from the inner to the middle zone is extremely gradual but the division between the outer and the intermediate ones is rather sharp. The numbers of mitochondria in the cells containing filaments are much smaller than in the cells possessing spheres. In the two other controls the general pattern of mitochondria is the same except that the periportal area contains only short beaded filaments. The same tendency towards dissociation of beads to form free spheres, in progressing towards the central vein, is observed (pl. VIII, fig. 2).

Hollow spheres occur rather regularly throughout the liver of the hog. In the periportal cells, in slides prepared by REGAUD'S method,

there are extremely large vacuoles, apparently those left by the dissolution of glycogen. Accordingly, the mitochondria are restricted to the slender intervening spaces.

Dog. The dog excels in the number of cells found along a radius of any lobule that present distinctly different mitochondrial patterns. A few layers of cells immediately surrounding the central vein contain spherical mitochondria, of distinctly variable size. Next to this zone comes a somewhat narrower one in which the chondriosomes are, for the most part, rod-like. The width of these rods is usually almost equal to the diameter of the spheres towards the interior. Adjacent to this thin layer comes the widest zone within the lobule of the dog, wherein the chondriosomes are long slender filaments that are generally straight or only slightly curved. This area covers about half the radius of the lobule, and, accordingly, reaches almost to the portal vein. The fourth zone is of about the same width as the second. It is also characterized by filamentous chondriosomes, but is distinguished from the third zone by a much greater tenuosity of filaments. The fifth and last usually includes only a single layer of cells around the portal unit. Contrary to the custom in other animals this tier does not entirely surround the lobule, but is found only in the vicinity of the portal unit. The mitochondria of these cells are extremely large spheres, being about twice the size of those occurring near the central vein (pl. VIII, fig. 1).

Hollow spheres are regularly found throughout the lobule. Glycogen vacuoles are somewhat more numerous in the inner and middle zones than in the outer ones.

WEATHERFORD (1932) has briefly described the hepatic mitochondria of the dog. His description is almost the reverse of that given herewith. As no data was included on the treatment his animals had previously received it is useless to endeavor to harmonize the two accounts.

Reaction of the Mitochondria to Injection of Adrenalin.

Cat. I have recently shown that induced hyper- or hypoglycemia is associated with a relatively great increase in the size of the mitochondria of the hepatic cell of the cat [KATER (1931)]. This enlargement is also accompanied by a very pronounced tendency for these elements to become spherical. In reporting those observations, I was slightly perplexed by the variability exhibited by different individuals subjected to the same experimental conditions. Nevertheless, I drew the following conclusions:

Since the control is not absolute, we would anticipate that, if there is any relationship between mitochondrial morphology and carbohydrate metabolism, the mitochondria of the hepatic cell would not only differ in normal animals, but also in animals representing any specific sugar level, and this has been found to be true, as described above. Such variability does not detract at all from the significance of the observations recorded, because in no case does the tendency toward

enspherulation and hypertrophy of normal mitochondria exceed that of any experimental animal. In other words, the largest and most sphere-like mitochondria of a normal animal are smaller and less sphere-like than are those of the least modified case of hypo- or hyper-glycemia (pp. 284—285).

In later considerations, however, I felt that the case would be more clearly established if some procedure could be devised whereby an animal subjected to an experimental condition could be checked against itself in a normal state, rather than against another individual in a normal state. In this way, individual variability would not have to be considered, and one would consequently not be dealing merely with averages. This investigation would naturally prohibit the use of anaesthetics that would affect the sugar level of the blood. Amytal would seem to be the logical one, but, as there is some question of it having a slight effect on the blood sugar, the work has been done without a general anaesthetic.

The first step taken was to determine whether or not the necessary surgical method alone would modify the morphology of the mitochondria of the hepatic cell. Cats, twenty-four hours after feeding, were tied out on a dissecting board, using all possible care to avoid excitement, and a short incision was made in the midventral line, just beneath the posterior end of the right lobe of the liver. The tip of this lobe was then tied off with a broad bandage and the small distal portion excised and fixed. The opening was clamped shut and the animals left on the board for fifteen minutes. At the end of this time a small piece was cut from the left lobe of the liver and another one from the right, and fixed as before.

The livers of five cats were studied in this manner, and all gave exactly the same results. Of course, the five animals exhibit variability when compared with each other, that is, some possess mitochondria that are much larger and more spherical than do others, but in every case the second piece, taken fifteen minutes after the abdominal cavity was opened, shows exactly the same mitochondrial picture presented by the first piece. Thus, it is clear that this surgical procedure does not perceptibly influence the morphology of the mitochondria of the hepatic cell. In view of this, it is evident that my previous work on hypo- and hyperglycemia can be checked, using a single animal as control and experimental material.

Three more cats were studied in the same manner, except that after the removal of the first piece of liver the animal received a very large intravenous injection of adrenalin, 2 cc. of $^1/_{1000}$ solution. In these cases, the second piece of liver possesses mitochondria that are larger and more inclined to be spherical than are those of the first piece (pl. VIII, figs. 13 and 14). The difference is in no case as great as in some of the cats that I employed previously. This was probably caused by the short time that the adrenalin was permitted to work, only fifteen minutes, as compared with several hours in my former study. The figures of that report

represent about the degree of difference found in the three animals studied in this manner. Accordingly, it would seem that I was justified in my earlier work in concluding that a disturbance of the glycogen-glucose equilibrium modifies, or is associated with, the morphology of the mitochondria of the hepatic cell of the cat.

Rabbit. After my initial observations on the rabbit it was selected as the animal to be used in following the complete series of mitochondrial changes throughout the period in which a single injection of adrenalin creates an abnormal blood sugar level. This choice was based upon the lack of individual variability in the normal and the decided alteration of the mitochondria observed during the time of pronounced hyperglycemia. No effort has been made to time with great exactitude the first mitochondrial response, since the objective of this feature of the work was to determine the general relationship of the alterations of mitochondrial morphology to the blood sugar level.

The earliest material taken is from animals killed ten minutes after injection. Fig. 6, pl. VIII, is from one of this group, the one that attained the highest blood sugar, namely, 200 mg per 100 cc. of blood, in a period of ten minutes. The alteration in the normal mitochondrial pattern is also more pronounced than in the other animals killed in a similar length of time. Examination of the drawing referred to will disclose that the first reaction to adrenalin involves a tendency for the chondriosomes to clump in a few masses, leaving the remainder of the cytoplasm either free from these inclusions or only sparsely supplied with them. Besides the clumping the mitochondria becomes converted into spheres and rapidly enlarge so that in size they equal those of the single layer of cells around the central vein in the normal individuals that present this feature of intra-lobular variability.

Consideration of the number and size of the mitochondria in the normal liver and in the initial hyperglycemic stage obtained suggests that the spheres of the latter are formed by fragmentation of the filaments of the former. However, the increase in numbers is hardly sufficient to indicate that the normal filaments fragment without an initial contraction. Accordingly, I believe that the course of chondriosomal reaction involves a contraction of filaments followed by fragmantation of the resulting thick rods. After the chondriosomes become completely converted into spheres the latter again become rather evenly dispersed through the cytoplasm, concomitant with their hypertrophy. The enlargement during the next stage is more pronounced than was the enlargement during the period of sphere formation, though a few of the mitochondria remain small (pl. VIII, fig. 8).

In the adrenalized rabbits the cells near the central vein are distinctly smaller than those near the portal, a difference which cannot be noted in normals (pl. VIII, fig. 7). It would seem likely that this is due to the removal

of a certain amount of glycogen from that area, though the same feature is not so pronounced in the other species which were studied. Of course, a definite conclusion in this regard cannot be made upon personal judgment without the substantiation of a very large number of measurements.

In the return to normalcy the first alteration is the reverse of the last step during the period of increasing modification, namely, contraction of the spheres. In fig. 9, pl. VIII, most of the mitochondria are still spherical but are appreciably smaller than at the peak of the blood sugar level. The chondriosomes that have departed from this contour, for the most part, have assumed a pyriform or spear-like configuration. It is these elements that give the clearest picture of the association, followed by fusion, of separate mitochondria. Two similar structures come in contact, with the small end contiguous. The actual fusion is apparently effected by one or more slender pseudopodial strands which, if laterally placed and double, give the two elements the appearance of an hour-glass with a clear area at its narrowest point. This figure was taken from an animal killed four and one-half hours after injection and which had a blood sugar of 150 mg per 100 cc. of blood. From this point onward the return to the normal state is very rapid, particularly the decrease in size. In fig. 10, pl. VIII, the mitochondria have regained their normal size though most of the spheres are still separate. This illustration also is taken from an animal four and one-half hours after infection, but which has a blood sugar of 125 mg per 100 cc. of blood. At five hours after injection, with a blood sugar of 105 mg per 100 cc. of blood the mitochondria are normal. From this time to six and one-half hours after injection, the latest time at which material was fixed, they remain normal.

The tendency for the mitochondria to clump, seen in the initia. reaction to adrenalin, is again evident during the stages of recoveryl In fact, this feature is pronounced after the mitochondria have regained the normal size.

Pigeon. Despite the fact that the blood sugar level of the pigeon is modified only slightly by the injection of large doses of adrenalin the mitochondria of its liver show a very clear-cut response to this experimental condition. The beaded filaments of the normal hepatic cell are broken up to form separate spheres which enlarge, relatively, to almost as great an extent as do those of the rabbit, though, of course, the final size attained is much less than the greatest state of hypertrophy of the chondriosomes of the rabbit. The five pigeons which received adrenalin responded to varying degrees. Fig. 12, pl. VIII, represents the greatest case of hypertrophy. There is a slight tendency for the mitochondrial covering of the largest fat globules to become disrupted, leaving the latter free in the ground cytoplasm.

Wood Mouse. In this animal the injection of adrenalin leads to the conversion of the beaded filament and smooth rods into free spheres which hypertrophy sufficiently to attain twice the size of the spheres of the normal cell. Also, there is a slight tendency for the mitochondria to become aggregated so that portions of the cell are almost free of them.

Hog. The reaction of the mitochondria of the liver of the hog to injection of adrenalin is not at all clear-cut. All elements are converted into spheres, but there is practically no enlargement. Study of the three adrenalin injected and three controls indicates that there is hypertrophy, but that it is so slight that it can be regarded as negligible. However, the consistent enspherulation justifies the conclusion that in the hog mitochondrial morphology is related to experimental hyperglycemia, though less strikingly than in the case of the other animals showing response.

Chicken, Guinea Pig, White Mouse, White Rat, Dog. With the exception of the chicken the above named animals very clearly show no response to the injection of adrenalin. It is difficult to make any statement regarding the chicken because of the irregularity of the mitochondrial pattern of normals, so it has been included with those having no reaction.

In view of the belief that the synthesis of sugar from proteins can occur only in the liver it was deemed desirable to study the effect of induced hyperglycemia on mitochondrial morphology in starving animals of the type showing no response to adrenalin. Accordingly, five rats, ranging from sixty days of age to large specimens, were given adrenalin after four days starvation. The response of the blood sugar was typical for rats deprived of food for only one day and the mitochondria were unaffected, the younger animals having spheres of irregular size, identical with the controls, and the older ones presenting the usual picture of a normal rat.

Reaction of Mitochondria to the Injection of Bile.

As noted in my earlier article [KATER (1931)] the mitochondria of the liver of the cat exhibit relatively great individual variability, which naturally makes it difficult to draw definite conclusions regarding the effect of any experimental condition. The initial group of cats killed for the study of the effect of utilizing bile as a cholagogue consisted of nine animals, five of which received bile subcutaneously, the remaining four being used as normals. The results were somewhat variable, but seem to suggest, when considered on a basis of average mitochondrial size, a slight hypertrophy of the chondriosomes. The case, however, was not sufficiently clearly established, so additional animals were killed, and again, the difference in normals and experimentals was not

sufficient for decision. A third group was then scarificed, which also failed to elucidate the problem. It was concluded that it would be better to cease utilizing the cat and endeavor to solve the problem on other animals.

The five rabbits which received subcutaneous injections of bile possess mitochondria that appear to be very slightly larger than those of the normal rabbit, though the difference, if any, is so insignificant that it is almost impossible for personal judgment or outline sketches to be depended upon sufficiently to permit any conclusions to be drawn. However, one reaction of the hepatic cell to stimulation by this cholagogue is unmistakable, namely, aggregation of the mitochondria, usually around the nucleus. Thus, plaques of cytoplasm, generally peripheral, become devoid of mitochondria. This clumping of the chondriosomes adds to the difficulty of comparing their sizes to those of the mitochondria of the normal cell.

In the case of the chicken, pigeon, guinea pig, white mouse, and white rat, if any reaction occurred, with respect to mitochondrial size, it was so slight that one could not determine it. The same clumping tendency observed in the rabbit is again evident, but very much less pronounced.

Reaction of the Mitochondria to the Injection of Glucose.

When 1.5 cc. of 100% glucose solution is injected into a rat, either subcutaneously or intraperitoneally, sufficient glycogen appears in the liver in one half hour to make it easily demonstrable histologically. The amount of this storage material continually increases up to the latest time the animals were killed, that is, one and a half hours after injection. When the sugar solution is injected subcutaneously the rat apparently experiences no difficulty resulting from osmotic phenomena However, when injection is intraperitoneal the abdominal cavity continually accumulates more water until the animal actually dies, despite the fact that enough of the sugar is removed for striking quantities of glycogen to appear in the liver.

When the sugar is injected subcutaneously no modifications of the morphology of the mitochondria of the liver result. Likewise, when an animal receiving intraperitoneal injection is killed before general weakness is evident there is no modification of the chondriosomes. However, after intraperitoneal injections, if the animal is permitted to live until the dehydration of the body is sufficient to cause general weakness, indicated by staggering, the mitochondria are distinctly altered. All of the chondriosomes are converted into spheres and these elements hypertrophy to as striking a degree as in response to adrenalin injections in the cat and pigeon. This enlargement is of the same character, also, as that found in adrenalin injected animals since it is not an effect of fusion of chondriosomes.

Mitochondria in the Hepatic Cell in Starving and Pathological Individuals.

The effect of starvation on the mitochondria of the liver is apparently quite variable. They become slender filaments in those animals capable of hibernation, even though the specimens are deprived of food for long periods of time [TAKAGI (1929); DAWSON (1931)]. In the rabbit the reaction is quite different, as little effect is observed until after the animals lose one-third of their body weight, at which time the mitochondria become converted into spheres of irregular diameters [MARTIN, CROIZAT, and GUICHARD (1930)]. I have determined the reaction of hepatic mitochondria of the rat to starvation, in the present work, and find the initial mitochondrial response to be directly related to the blood sugar level. Rats of sixty days of age generally die after three days of starvation. Mature individuals ordinarily do not die under five days and some are quite active and normal at that time. The initial symptoms of impending death are general weakness and staggering, at which time the sugar concentration of the blood is diminished. In such moribund individuals I have obtained readings ranging from 46 mg to 85 mg per 100 cc. of blood. The mitochondria of the liver remain perfectly normal until the blood sugar begins to fall and then they very rapidly fuse, forming a few large mitochondrial masses in the cell. This reaction must occur rather suddenly since no difference is observed in an individual with 46 mg of sugar per 100 cc. of blood and in one with 85 mg per 100 cc. of blood or in one at any intermediate level. It should be emphasized that the large mitochondria found in starving animals are not resultants of hypertrophy of elements, but of fusion, as a consideration of relative numbers clearly indicates (pl. VIII, fig. 20).

A few of the animals used as normals were found to have abnormal blood sugar levels. One pigeon has a blood sugar of 165. Its mitochondria were perfectly normal. One of the dogs that was starved for two days was evidently sick since it refused food and had a blood sugar of 65 mg. Its hepatic lobules appeared quite normal except for the absence of variability of mitochondria, the type found in the middle zone of the normal occurring throughout. The mitochondria of the other dog that was unfed for two days and whose blood sugar was normal were the same as those of the animals starved only twenty-four hours. Three of the chickens studied possessed livers in which a large number of the hepatic cells had been replaced by connective tissue. One of these had the customary blood sugar and the mitochondria of its remaining hepatic cells were the usual type, so far as normalcy can be determined in this bird. The other two had 75 mg of sugar per 100 cc. of blood, a very low blood sugar for the domestic fowl. These are the two animals referred to in the descriptive section as showing distinct variability on two sides of the same lobule.

Summary of Observations.

Two hundred and five animals, representing eight species of mammals and two of birds, have been studied to determine, primarily, the effect of alteration of the glucose-glycogen equilibrium and of stimulation by cholagogues upon the mitochondria of the hepatic cell.

All animals were starved for a period of twenty-four hours before being killed.

The blood sugar was determined on most of the animals and all of the livers were prepared for study by REGAUD's method for mitochondria, and BEST's carmine method for glycogen, and most of them by SCHRIDDE's fixation and ALTMANN's stain for fat and mitochondria.

The sugar concentration of the blood was modified by use of adrenalin. The different animals show little consistency in the amount of this drug tolerated or in the height to which the blood sugar rises.

The blood sugar level of the rabbit was followed from ten minutes after injection of the drug to six and one-half hours after its administration. The blood sugar returns to normal five hours after the adrenalin is given.

Histological preparations show appreciable quantities of glycogen in the liver of the dog, cat, rabbit, hog, and chicken when killed twenty-four hours after ingesting the last food. The white rat, white mouse, wood mouse, guinea pig and pigeon show practically no glycogen under the same conditions.

When small amounts of glycogen are present in the hepatic cell it occurs as small granules, in the case of the hog. In all other species it is found as a reticulum surrounding clear vacuoles. When great amounts are present it is in the form of large masses, which may appear to fill the cell completely.

In the cat, hog, and rat glycogen is first deposited around the portal vein and the region about the central vein is the last to receive its complement of carbohydrate. The dog, rabbit, guinea pig, and white mouse show the reverse course of deposition, and in the chicken the glycogen occurs evenly throughout the lobule.

Slides prepared by the SCHRIDDE-ALTMANN method show fat globules within mitochondria.

In the wood mouse, hog, rabbit, pigeon, and dog the initial deposition of fat is at the periphery of the lobule; in the white mouse, the first fat appears in irregularly scattered cells; in the chicken, rat and guinea pig fat accumulates evenly throughout the lobule.

In most cats the mitochondria range from filaments at the periphery of the lobule to short rods at the central vein. However, in some individuals there is no variability within the lobule. The dominant form of chondriosomes in the liver of the rabbit is the filament. There is no variation within the lobule, in most individuals. The mitochondrial pattern of the liver of the chicken is so irregular that nothing can be

regarded as normal. The chondriosomes of the hepatic cell of the pigeon are very small; most of them are beaded filaments. In the guinea pig the mitochondria of the liver are typically filamentous. Adjacent cells may vary in the width of these filaments. In the white mouse the mitochondria at the outer margin of the lobule are spherical, the middle portion of the lobule contains beaded filaments, and the central area smooth filaments. A mixture of even filaments, beaded filaments, rods, and spheres occurs in the hepatic cell of the white rat. There is no variability within the lobule. The mitochondrial pattern of the hepatic cell of the wood mouse is almost indistinguishable from that of the white rat. At the outer margin of the hepatic lobule of the hog, long smooth or beaded filaments occur and a gradual transition is found from this area to the central vein, where the mitochondria are spherical. There are five zones within the lobule of the dog which, from central vein outward, contain, respectively, spheres, rods, filaments, filaments of a more tenuous character, and very large spheres.

Adrenalin hyperglycemia is accompanied by pronounced enspherulation and hypertrophy of the mitochondria of the hepatic cell in the cat, rabbit, pigeon, and wood mouse, and by enspherulation alone in the hog. The same experimental condition has no effect whatever on the mitochondria of the chicken, guinea pig, white mouse, white rat, and dog.

The injection of bile has little or no effect on the morphology of the mitochondria of the liver cell. However, it appears to be associated with clumping of the chondriosomes.

Storage of glycogen after glucose injection is not associated with any change in mitochondrial morphology until osmosis has largely dehydrated the body.

In prolonged starvation there is no change in the mitochondria of the liver of the rat until the concentration of the sugar in the blood begins to decrease. At that time the mitochondria show a pronounced tendency to fuse.

Discussion and General Conclusions.

The Relation of Mitochondrial Morphology to Carbohydrate Metabolism.

The observations on the mitochondria of the liver of the cat and rabbit herewith described clearly establish that in these two animals mitochondria vary directly with induced hyperglycemia. I have previously pointed out that the identity of the reaction of the hepatic mitochondria to adrenalin injection, etherization and insulin injection suggests that the change in the morphology of the chondriosomes is associated with the alteration of the sugar level and is not a direct effect of the therapeutic treatment. The present work on the rabbit very

clearly indicates the same conclusion, since the return to normalcy on the part of the mitochondria coincides with the reversion of the sugar level to the normal concentration. The reaction of the mitochondria of the hepatic cell of the pigeon, wood mouse and hog is less convincing because the investigation has not been as comprehensive, though in view of the identity of results, so far as the studies have been carried, it is justifiable to consider these five animals in a single group. Therefore, the conclusion is unavoidable that the morphology of the mitochondria of the hepatic cell of these animals is directly associated with the sugar level of the blood. The leopard frog can also be classed with this type, according to the work of CLARK and HAIR (1932).

The failure of the other five species of animals to show any chondriosomal response to adrenalin is above question. The number of individuals used, the number of species represented, together with the regularity of the mitochondrial patterns of their hepatic cells, leave no room whatever to doubt that in many vertebrates there is no relationship between the morphology of the mitochondria occurring in the liver and induced alterations in the sugar content of the blood. Since the ten animals employed in the present investigation are equally divided between the two groups it would seem likely that vertebrates in general may be likewise divided.

Unfortunately, it is impossible to associate this difference in reaction with any known features of hepatic structure or function. The blood sugar of the cat and rabbit is more greatly modified by the injection of adrenalin than that of any of the other animals. Yet in this regard the pigeon is very unresponsive, so it is impossible for the mitochondrial reaction to be related to the degree of responsiveness of the blood sugar level. Three of the animals whose mitochondria become modified after adrenalin is injected carry sufficient glycogen in the liver, after twenty-four hours of starvation, for it to be histologically demonstrable; two of those showing no reaction likewise carry glycogen in the liver. Thus, the response cannot be related to the glycogen content of the liver. It is also evident that this grouping is wholly irrespective of so-called taxonomic relationships.

The interpretation of the significance of the relationship of mitochondrial form to the blood sugar level would seem, at first thought, to be rendered more difficult by the fact that other species show no modification. However, I believe that very difference in the reaction of closely related animals affords the best possible basis for interpretation. The same processes relative to carbohydrate metabolism occur in both groups; hyperglycemia is effected in both; the liver is essential for the maintenance of the blood sugar level in both; yet the mitochondrial reaction is distinctly different. Accordingly, I believe we are justified in concluding that, in the liver, the morphological transformations of the

mitochondria that accompany alterations of the glucose-glycogen equilibrium in some animals are not related causally to the chemical processes involved, but are merely parallel reactions. It also seems justifiable to go further and conclude that the mitochondrial transformations are an expression of the physical state of the ground cytoplasm, a conclusion which I have previously intimated [KATER (1931)] and that has been clearly stated by CLARK and HAIR (1932) in the following sentence. "We wish to suggest further that morphological alterations of mitochondria are not a purposive factor related to functional activity, but are brought about as a result of the alterations of the physical state of the ground cytoplasm associated with cellular activity." If we assume this interpretation to be true it seems quite reasonable that innate differences in the architecture of the ground cytoplasm might readily permit the same chemical processes to occur in that substratum without alteration of mitochondrial morphology in one case, whereas in another modification would result. The study of glucose injected rats makes this conclusion unavoidable, since the incoming carbohydrate effects no change of the mitochondria, but the dehydration caused by intraperitoneal injection alters chondriosomal morphology in the same manner as hyperglycemia does in the cat and rabbit.

I also think that the observations of the present work are sufficiently distinct to justify discarding the possible significance of the topographical relationship of mitochondria to areas of glycogen formation, which has led to the suggestion that these elements are involved in the utilization of carbohydrate [DAWSON (1931)].

Variations in the Mitochondria and in the Accumulation of Storage Products in the Hepatic Lobule.

In an organ presenting the structural regularity of the lobular type of liver it is very difficult to understand or interpret very great irregularities in the more minute features of its structure and activities. Differences in the amount of glycogen carried in the liver are not so disconcerting as a complete reversal of the course of deposition, when we consider that the relationship of the lobule to the portal unit and to the central vein is the same in all cases. Thus, I feel that the initial deposition of glycogen around the central vein in some animals and around the periphery of the lobule in others introduces a significant problem, with reference to hepatic function, which at present is wholly enigmatic. The fact that opposite types are found in the same genus is very stimulating, and makes one wonder if the conventional taxonomic criteria are of any significance in the determination of actual relationships.

The variability exhibited by different species in the accumulation of fat is less pronounced than in the case of glycogen, but to the extent that it is found it also suggests wholly unsolved problems.

Even more striking than the interspecific irregularities in glycogen storage are the mitochondrial differences. Most of the animals studied show no intralobular variability in mitochondrial morphology. Of the others, three show a distinct polarization of variable mitochondrial types with the largest and most sphere-like near the central vein. The other species, the mouse, also regularly show such polarization with the largest and most spherical at the periphery of the lobule. It is self-evident that the presence and arrangements of gradients of mitochendrial form within the hepatic lobule cannot be related to quantity or course of storage of glycogen or fat within the liver nor even to the character of the connective tissue elements of that organ. In a word, they cannot be related to any known feature of the constitution of the liver. Accordingly, it would seem better to refrain from applying names with kinetic implications to zones within the lobule.

This study of the mitochondria of the liver clearly establishes interspecific mitochondrial irregularity in this organ, in direct contradiction to the rather general belief that tissues with similar functions have similar mitochondrial types. Perhaps we would be justified in assuming that in substrata of similar physical states mitochondria of the same type would be found, the idea which affords the primary stimulus for the general conclusion which has just been mentioned. If that be true then we are compelled to conclude that the physical state of the hepatic cell exhibits great differences in related species.

The essential absence of individual differences in one species under similar physiological conditions, with the exception of the cat and chicken, suggests a problem relative to the physiology of these two animals.

Activity of Mitochondria in Fat Formation.

The significance of the facts revealed by the use of the SCHRIDDE-ALTMANN technique in studying fat formation in the liver has been previously discussed [KATER and SMITH (1932)], and need not be repeated here. Nevertheless, it is necessary to add a few considerations.

The failure of this method successfully to fix and stain the hepatic tissues of many animals and, likewise, its distortion of the mitochondria containing fat globules within themselves, in many instances, might deter one from attaching weight to any observations based upon its use. However, its perfect regularity in the successful preparation of tissues of one specific type would incline one to accept it as valid in those cases. In addition, it is much easier to conceive of a disruption of mitochondria inclosing fat globules than of a general dispersal of chondriosomal material followed by its precipitation around fat globules in such a manner as to form normal spherical and filamentous mitochondria. Accordingly, I believe that one is justified in concluding that in the liver fat is formed within mitochondria and that the hollow spheres observed in REGAUD

sections are the elements in the process of sythesizing fat, a conclusion that is clearly supported by sections from animals that have been fed butter and lard.

General Aspects of the Question of Mitochondrial Form and Function.

It now behooves us to consider in a more general manner the specific conclusions with reference to mitochondrial form and function advanced in previous sections of this discussion. One might easily conceive three distinctly different interpretations of hypertrophy or other morphological alterations of mitochondria: 1. that it represents functional hypertrophy or atrophy of an element active as a catalyst, or otherwise, in the synthesis of cellular constituents or storage products, 2. that the mitochondrial state varies with the general metabolic activity of the cell, and 3. that the configuration of the chondriosomes represents specific alterations of the ground cytoplasm in which they are not functionally involved. The first of these conclusions is now clearly established with reference to fat formation in the liver, but only with reference to fat formation, and most of the structural transformations which occur are unrelated to this process. It has been shown above that there is no evidence that most of the transformations which accompany alterations in the activity of the liver, with reference to carbohydrate metabolism, are functionally related to those alterations. Accordingly, it would seem desirable to consider the possibilities of verity of the two remaining explanations. The absence of any clear-cut relationship between mitochondrial form and bile secretion would deter one from concluding that heightened or lowered metabolic activity with reference to any physiological process can be represented or measured by the condition of the chondriosomes. Therefore, I believe that it is highly probable that most morphological changes of mitochondria observed in specific tissues are merely expressions of certain alterations of the ground cytoplasm, and bear no relationship either to functional activity or to general metabolic level.

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Explanation of Plate VIII.

All figures are from 4 micra sections prepared by REGAUD's method. Drawings were were made with the aid of ABBÉ model camera lucida under LEITZ fluorite oil immersion objective ($^1/_{16}$) and compensating oculars (8 × und 15 ×). Abbreviations: cv-region of central vein; pu-region of portal unit. Magnification: figures 1—3, 844 ×; figures 4—20, 1600 ×.

Fig. 1. Normal dog. Cord of cells extending from the central vein to a portal unit. Observe that the lobule is divided into five zones, on a basis of mitochondrial morphology.

Fig. 2. Normal hog. Note that in this animal the lobule is divided into three zones.

Fig. 3. Normal white mouse. Here the lobule contains the same number of zones as that of the hog, but the mitochondrial types are reversed.

Fig. 4. A group of four cells from the mid-region of the lobule of a normal rabbit.

Fig. 5. Two cells from the border of the central vein of the lobule of a normal rabbit. Observe that the mitochondrial types around the central vein extend through only one layer of cells.

Fig. 6. Two cells from the hepatic lobule of a rabbit killed ten minutes after the injection of .5 cc. adrenalin. Note the enspherulation and clumping of the chondriosomes. Blood sugar, 200 mg per 100 cc.

Fig. 7. Two cells bordering the central vein in a rabbit killed two and one-half hours after injection of .5 cc adrenalin. Observe the pronounced hypertrophy of the chondriosomes and absence of clumping. Blood sugar, 300 mg per 100 cc.

Fig. 8. A group of cells from the periphery of the same lobule from which fig. 7 was taken.

Fig. 9. Cells from the hepatic lobule of a rabbit, four and one-half hours after the injection of .5 cc. adrenalin. Observe that the some mitochondria are becoming pyriform and fusing with similarly shaped elements. The blood of this animal contained 150 mg of sugar per 100 cc.

Fig. 10. Hepatic cell from another rabbit killed four and one-half hours after injection of .5 cc. adrenalin and whose blood sugar was 125 mg per 100 cc. of blood. Observe that the size of the mitochondria is the same as in a normal rabbit, but they have not so completely returned to the filamentous state.

Fig. 11. Hepatic cells from a normal pigeon. The mitochondria are predominantly beaded filaments.

Fig. 12. The same, one and one-half hours after injection of .5 cc. adrenalin. Observe the enspherulation and hypertrophy of the mitochondria, and the disruption of the mitochondrial covering of the larger fat globules.

Fig. 13. Hepatic cells from the mid-region of the lobule of the normal cat. This block of tissue was removed without killing the animal. The mitochondria are predominantly short filaments.

Fig. 14. Hepatic cells from the same cat illustrated in fig. 13 but removed from the animal fifteen minutes later, after injection of 2 cc. adrenalin. Note the large spherical character of the mitochondria.

Fig. 15. Two cells showing the cellular variability of mitochondria in the normal guinea pig.

Fig. 16. Hepatic cell from a normal wood mouse. Observe the numerous types of mitochondria.

Fig. 17. Hepatic cell from a wood mouse killed one hour after injection of .1 cc. adrenalin. Note the striking enlargement of the mitochondria, as compared to fig. 17.

Fig. 18. Normal rat with variable mitochondrial form.

Fig. 19. Hepatic cell of a rat killed one and one-half hours after injection of 1.5 cc. of 100% glucose solution. Observe that the mitochondria are numerous and large.

Fig. 20. Hepatic cell from a sixty days old rat that was starved for four days before killing. Blood sugar, 65 mg per 100 cc. The mitochondria are scarce and vary from minute structures to excessively large ones.