THE RELATION OF THE FAT AND LIPASE CONTENT
OF THE LIVER AND KIDNEY UNDER
VARIATIONS IN FEEDING.

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SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE
OF
MASTER OF ARTS
in the
GRADUATE SCHOOL
of the
UNIVERSITY OF MISSOURI

1917.

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Introduction.

In preceding years a large amount of reliable quantitative data has been obtained concerning the ester-splitting faculty of normal tissues, and it has been proven that practically every organ in the animal body possesses the ester-splitting property, which property is ascribed to the enzyme known to the scientific world as lipase.

Much research has been devoted to the study of lipases and their relation to the metabolism of fat, but as yet no true definite relationship has been established between the fat and lipase content of organs. Whether or not an increase or decrease in this tissue lipase causes a corresponding increase in the tissue fats or vice versa has not been definitely proven. Work during the past three years in this laboratory indicates that a definite relationship exists between the lipases and fats in the liver of animals fed on a diet rich in fat and in the liver of animals during periods of inanition.

Knowing that the lipase concentration per unit of tissue is characteristic of and relatively constant for each organ, it remains to be proven whether or not different physiological conditions, such as variation in feeding, will alter this relation; that is, to prove whether or not an increase or

decrease in the lipase content per unit of tissue will influence the fat content of this unit; and if so, what relation does this fatty change bear to the lipase concentration.

In this present work, by varying the diet of animals (litters of puppies) and by examining the liver and kidney of these animals we will try to present data to show whether or not the lipase concentration of these organs has decreased or increased per unit of tissue. In addition, we shall endeavor to determine whether there is a definite relationship existing between the tissue lipase and the fat content of the tissue.

Summary of Literature Bearing on Tissue Lipase Action. Catalysis.

It is interesting to note the manner in which highly stable bodies are broken down in living organisms; for instance, such chemical reaction as the hydrolysis of sugar to CO_2 and water and the hydrolysis of amino-acids takes place within the living organism and within the cells of the organism. Just such chemical reactions are known by the chemist to take place in the laboratory, although some of the reactions may require a long period of time to arrive at their final state. Any body, according to Bayliss, which will alter this hydrolytic reaction is known as a catalyst. The name catalysis is given to the accelerating process. Enzymes are known to possess all the properties and actions of catalysts, so it is thus necessary that one should understand their true meaning, properties and action.

¹
Bayliss states that a catalyst is not capable of initiating a reaction but merely changes the rate of the reaction already in progress. The catalyst does not appear chemically combined with the end product of the reaction but is found in the end products unchanged except in cases where it is destroyed by subsidiary reactions. The final result of catalysis depends upon the amount of the catalyst added. The degree of acceleration of a reaction is proportional to the concentration of the catalyst present although a very minute quantity is capable of perceptible activity.

Armstrong who has extensively studied the action of catalysts gives an excellent discussion of the older literature. He quotes Berzelius ² (1835) as one of the first to investigate the action of catalysts. Armstrong states that Berzelius came to the conclusion that many substances, simple as well as compound, have the property in the solid and also in the combined state of exercising an influence on compound substances which is quite different from that of ordinary chemical affinity, as they influence the occurrence of changes without their own constituents being necessarily concerned in the change, though there are cases in which this latter may happen. Berzelius finally calls attention to the possibility of thousands of catalytic processes being operative under vital conditions. According to the Oswald school, by a catalyst we understand a substance, which, without being required by the accelerated reaction or appearing among the final products, alters the velocity with which a chemical system strives to attain its final condition.

Armstrong,³ regarding chemical changes as electrical phenomena, for example, the interaction between Hydrogen and oxygen in the presence of platinum, defines a catalyst as the agent which brings about the inclusion in the circuit within which the change takes place so soon as the circuit is established. The electrolyte is the actual agent by which the change is effected.

Enzymes and Enzyme Action.

Armstrong states "that enzymes are peculiar as catalysts not only because they are agents derived from natural organic sources which determine the resolution of a variety of compounds by hydrolysis, but more particularly on account of their specific and limited activity; it is in this respect that they differ from most other catalysts. Each particular enzyme corresponds if not to a single hydrolyte, at most to a series of compounds of a particular type. But until their specific nature be deciphered it will be difficult to arrive at any final definition of enzymes."

Armstrong⁴ in earlier years (1904) brought forward the conception that not only is the amount of enzyme used not inconsiderable but that the rate of change is affected both by the alteration of the concentration of the hydrolyte and in the proportion which the hydrolyte bears to the catalyst; moreover, that the products of change are known to exercise an influence. He further states that the hydrolysis by enzymes, in the first instance, involves the combination of the enzymes with the hydrolyte. In this assumption, the rate at which the

enzyme action proceeds will depend upon the extent to which the combination of the enzyme with the hydrolyte takes place as well as on the degree of readiness with which the compound breaks down.

These views led Armstrong to form the assumption that an enzyme has a double function, "that of attracting or holding the hydrolyte and that of determining the hydrolysis; in other words, that the enzyme sustains the hydrolyte in circuit while the hydrolysis is being effected through the agency of the electrolyte itself formed from an acid radicle present in the enzyme." He attributes this two-fold action to the presence in the enzyme of an acceptor together with an agent; and thus bring forward the statement that an enzyme is a composite agent in which the functions of a catalyst, such as platinum black, are combined with those of an acid radicle. He interprets the acceptor as a radicle which is very closely allied to, if not identical with, a dominant group in the hydrolyte.

⁵ Bayliss states that at a very early date in the history of physiology bodies having the properties similar to those of inorganic catalysts were prepared from living organisms. As early as 1830 Dubrunfaut⁶ converted starch into sugar by means of a malt preparation. In 1833 Payer and Perzog⁷ isolated the enzyme maltase. As more and more of the catalysts were prepared from living organisms Kuhne⁸ introduced the word enzyme which was given to some of the better known substances, such as pepsin, trypsin, etc., called by many "unformed ferments." This name was later given to catalysts formed from living organisms.

Thus by the name enzyme as introduced by Kuhne we mean certain products of the chemical processes of the living cells which have the power of catalytic action after they have left the cell.

One of the most important of enzymatic reactions is the so-called hydrolytic cleavage process in which complex substances are divided into simpler substances. Such a process is of the greatest importance in the digestion of food stuffs in the alimentary canal as well as to the metabolic process of the body in general. It is obvious that in normal cell life the enzymes play an important role. We are coming more and more to accept the theory that the chemical transformation even in living cells are brought about by enzymes. Each enzyme is characterized by its action and by the condition under which the action is developed; and the substances, or related substances, or group of substances upon which a particular enzyme acts is called the substrate of that particular enzyme.

Synthetical as well as hydrolytical processes take place within the living organism. These processes are known as reversible, or balanced, reactions, or in other words, the same catalyst can influence the reaction in different directions according to the concentration of the substance present. Especially are such reactions noticeable in the cases of substances stored up in insoluble form as glycogen and starch. These substances are under certain conditions synthetized from sugars and under other conditions hydrolyzed back to sugar.

The first example of such a reaction was shown by

⁹
 Croft Hill who proved that maltose could be synthetized from
 glucose. Hill's work was later confirmed by Armstrong.¹⁰
¹¹
 Kastle and Loevenhart proved the synthesis of a fat, ethyl
 butyrate, from buytric acid and alcohol..

All these cleavage and synthetical processes produced by enzymes are of extreme importance to the metabolism of the body. The phenomenon of metabolism in terms of enzymatic processes is very accurately interpreted by Wells¹² in his Chemical Pathology when he states that "All metabolism may be considered as a continuous attempt at establishment of equilibrium by enzymes, perpetuated by prevention of attainment of actual equilibrium through destruction of some of the participating substances by oxidation or other chemical processes, or by removal from the cell or by entrance into it of materials, which overbalance one side of the equation." Bradley¹³ conveyed the same idea but in different terminology when he stated that "The intracellular enzymes which are present in every living cell's lipase, diastase, protease, etc. function as hydrolytic and synthetic agents. Under certain conditions dependent upon the active mass of the compounds involved, fats, carbohydrates, and tissue proteins may be built up and stored in the cell. Under other conditions the same substances will be hydrolyzed to simple compounds as an initial step in utilization and katabolism."

Lipase.

Before discussing the general literature on the enzyme lipase it is important that we should thoroughly understand just what rôle lipase plays in metabolism, or in other

words, just what has lipase to do with the digestion, absorption, assimilation and utilization of fats in the body.

As explained by Wells,¹⁴ the lipase in the intestine splits the fats into fatty acid and glycerol. These last two substances being diffusible are absorbed by the cells lining the intestine. The lipase which is ever present within the cells in turn combines with the absorbed fatty acid and glycerol and thus we have within these cells a mixture of fat, fatty acid and glycerol which will attain an equilibrium only when new additions of the last two substances cease to enter the cell. The tissue fluid continuously bathing these cells is relatively low in fatty acid and glycerol. Thus these diffusible fat constituents enter the tissue fluid where they are in turn resynthesized to fat by the lipase present in the fluid. By this diffusion of a portion of the cell contents into the tissue fluid, the chemical equilibrium of the cell is disturbed, so the cell lipase reverses its action and converts its newly formed fat again into fatty acid and glycerol which in turn is diffused into the tissue fluid. Thus we see that the whole process of fat absorption is a continuous process of hydrolysis and synthesis.

The process of fat assimilation is quite the same as that of its absorption. In the blood stream and bathing the cells is a mixture of fat, fatty acid and glycerol. If diffusible constituents enter a cell containing lipase, e. g., a liver cell, the whole process is quite the same as that which took place within the intestinal mucosa, the only difference being that "the fatty acid may be removed from the cell by being utilized

by oxidation or some other chemical transformation."

Discovery and Fundamental Properties of Lipase.

Claude Bernard ¹⁵ in 1846 discovered the enzyme now known as lipase, but there were also other early investigators. Leuckowitsch ¹⁶ in his Chemical Technology states that in 1855 Pelonze discovered in oil seeds a substance that is capable of hydrolyzing the oils that are contained in seeds.

The subject of lipolysis did not, however, attract ¹⁷ the attention of investigators very extensively until Hanriot's papers on serum lipase appeared in 1896. He found the serum lipase to be capable of hydrolyzing monobutyryne and stated that it would also hydrolyze oils and the natural fats. Later Arthus ¹⁸ questioned the statements made by Hanriot that serum would hydrolyze the oils and natural fats. The contention of ¹⁹ Arthus was upheld by Connstein and Michaelis, Umber and ²⁰ Brugsch, ²¹ Thiele and others. ¹¹ Kastle and Loevenhart experimenting with water extracts of different tissues were the first to make use of ethyl butyrate as an artificial substrate. In the same experiments they were able to convert alcohol and butyric acid into ethyl butyrate, thus proving the synthetical ²² property of tissue lipase. Hanriot ²³ in 1901 in the same manner obtained monobutyryne from butyric acid and glycerol with blood serum. Pottevin in 1903 confirmed the synthetical results of Kastle and Loevenhart, and Hanriot when he transferred oleic acid and glycerin into mono- and tri-olein by means of a pancreatic enzyme.

covery in their demonstration of the reversibility of lipase acting on the esters of the lower fatty acids. To quote the authors direction: "When a fresh aqueous extract of pancreas²⁴ is treated with a mixture of dilute butyric acid (0.1 to 0.05 N) and alcohol (sufficient to bring the whole up to 1.5 percent) the very characteristic odor of ethyl butyrate soon develops even at extraordinary temperatures, and in the presence of antiseptics, whereas if the pancreas extract is first boiled the mixture never develops the odor of the latter." Bayliss states that if this experiment were done on a large scale the ester can be separated by distillation and can be hydrolyzed back again by the same enzyme that produced it. When ethyl butyrate is hydrolyzed by lipase, the reaction is more complete and thus an equilibrium is established.

Further proof of the reversible action of lipase is²⁵ afforded by Dietz. Working with pancreatic lipase, in order to obtain an exact equilibrium, Dietz carried out experiments from both sides of the balanced reaction simultaneously. On the one side was a solution of ester in water plus amyl alcohol, on the other side equally concentrated solutions of butyric acid in water plus amyl alcohol. He found the final concentration of the acid to be the same whether approached from the side of the ester or from the side of the acid. When enzyme preparations of different activity were taken, the equilibrium was the same in all and different amounts of the same enzyme were without effect on this point.

²⁶

Wells summarizing the work of Kastle and Loevenhart

states that in each case the result is a mixture of fats, fatty acids, and glycerol, or in other words, that the lipase merely acts to establish an equilibrium between these substances. The effect of the enzyme is merely to hasten this equilibrium which would come about slowly without the aid of the enzyme.

Such a reversible process of lipase activity is of very great importance in fat metabolism. This importance is pointed out by Loevenhart²⁷ when he asserts that there is no doubt that, during digestion and absorption of fat globules are found in the cells of the intestinal mucous membrane, and that fat taken as food is hydrolyzed in the lumen of the intestine. There must be some mechanism by which the products of hydrolysis are synthesized in the cells after absorption. It is obvious that if a lipase were present within these cells it would be capable of synthetic action, since the insoluble fat produced is deposited out of the "reacting system" in the form of droplets. Loevenhart obtains a lipase from the intestinal mucous membrane of a pig after thoroughly washing away the pancreatic enzyme. A similar enzyme was also obtained from the liver and other organs when fat storage occurs. In all these cases, when the blood and lymph bathing the cells becomes poor in fatty acid and glycerol, either owing to the fat being stored elsewhere or to its being used up by oxidation, as in starvation, the lipase restores equilibrium by effecting hydrolysis of the fat which has previously been stored.

Since the demonstration of the reversibility of enzyme action by Croft Hill, Kastle and Loevenhart, Pottevin, and

others, the general correspondence between enzyme action and catalysis by inorganic substances has been conceded. The true catalytic enzyme is then without question reversible. Under proper conditions it seems probable that all hydrolyze under other conditions.

Just what the chemical reactions are which are concerned in these synthetical processes has never been fully worked out, for the exact chemical structure of the enzyme is not known. Armstrong²⁹ and Gosney attempted to solve this problem when they put forward the following explanation of these processes. "The ethereal salts which are hydrolysed under the influence of lipase are all compounds of the type $R'.CO.OX'$. Since R' and X' may be varied within wide limits, it cannot well be supposed that the selective action of the enzyme is exercised with reference either to R' or X' ; consequently, the controlling influence must be attributed to the carboxyl radicle ($CO.O$): the enzyme must be so constituted that it can 'fit itself to this group.' Our experiments have led us to form the provisional hypothesis that the hydrolysis of the ethereal salt by Lipase involves the direct association of the enzyme with the carboxyl centre, and that such association may be prevented by the 'hydration' of this centre; Consequently, that those salts which are the more attractive of water will be the less readily hydrolysed."

Studies of the enzyme action made by Bradley in 1910 show that in the presence of an excess of water the hydrolysis of triolein is regularly increased by the increased amount of lipase present. The rate of increase suggests a mass effect of

lipase on the equilibrium of the reaction. He shows that by increasing the amount of lipase the reaction may be carried to completion; that a given amount of lipase may under optimum conditions liberate a definite amount of fatty acid from tri-olein irrespective of the mass of the latter. When water is present to the extent of more than fifty per cent of the mixture reversion is negligible and the reaction proceeds toward more complete hydrolysis, synthesis only reaches appreciable quantities under conditions of concentration so high as to approach desiccation. Bradley concludes that lipase, while important in the hydrolysis and absorption of fats, is not important in their synthesis and storage in the cells.

But the general law of the reaction of enzymes - and lipase comes in this group - may be stated as formulated by Hill ³¹ viz: "In the case of each enzyme there is in dilute solution a range of concentration, throughout which the activity increases approximately directly as the concentration."

Distribution of Lipase in the Body.

Since every organ and tissue in the body has its own part to play in the metabolism of fat one naturally expects that every part must contain its proportional share of the enzyme concerned in fat metabolism. It has been found by investigators that every organ and fluid of the body so far examined does contain some lipase.

Among the earlier workers who found lipase to be present in the blood are Hanriot, Michaelis, Umber and Brugsh.

Kastle and Loevenhart were among the first to study tissue lipases and to test the action of watery extracts on ethyl butyrate. They proved that the intestinal mucosa, mammary glands, liver, kidney, and muscles all contain lipase. They found that lipolytic activity of the pancreas, liver and kidney to stand in the following respective ratios: 1, 2.93, 0.5. Loevenhart, Magnus, Pierce, Bradley, Quinan and others working on the tissue lipase, and Bloor, Von Hess and others on the blood lipases have confirmed the work of Kastle and Loevenhart. Quinan studying the ester-splitting faculty of normal tissues determined a constant relation between liver, kidney and muscle of the normal guinea pig. In terms of the hydrolysis of ethyl butyrate per unit of tissue he expressed the proportion as 44.5, 25.5 and 13.5 for the three tissues named. He concludes that the enzyme concentration per gram of tissue is characteristic of and constant for each tissue; also that the relationship between the tissues may be proportional to their cellularities. Quinan finds the medulla and cortex of the kidney to differ in lipolytic activity in the ratio of 1.0 to 1.2 respectively.

Pagenstecher states that the existence of lipase in the brain was not known prior to 1909. Wroblewsky in 1911 isolated the ferment lipase from brain tissue. Later, English and McArthur found the following regional values; cerebrum 1.21, mid-brain 1.23, cerebellum 0.87, corpus callosum 0.56, medulla 0.36.

42

Quinan in 1916 studied the regional lipolytic activity of the normal human brain and concluded that all normal brain tissues, both gray and white, have lipolytic activity, that each main histological subdivision gives a constant and characteristic lipolytic value; and that the cerebellum is the most active lipolytically of the intra-cranial structures.

Granting the lipase concentration to be characteristic of and constant for each organ, the question arises whether or not this constancy always remains the same under varying conditions. It has been suggested that the age of the tissue might influence its lipase concentration to some extent. Buxton⁴³ and Schaffer state that very small embryos before there is much differentiation of the cells appear to be deficient in enzymes, but as the embryo develops the enzymes gradually appear and increase in amount. When the foetus is almost ready for expulsion the enzymes appear to be much less active than those in adult tissue.

44

Mendel and Leavenworth proved the early presence of lipase in liver and intestines of the embryo, but that the activity of the extracts of these tissues is decidedly less pronounced than that of comparable material of the full-grown animal.

45

Winternitz and Meloy confirmed the results of Mendel and Leavenworth and concluded that the nearer the organ approached the normal histologically the greater the lipolytic action; that the activity at birth is low but increases with extreme rapidity during the first few days of life; and that there is no decrease in activity during old age.

Greene and Carter state that the lipolytic activity of liver extracts from young dogs and cats increase with increasing age.

Besides the influence of age upon the lipase content of tissues there are various pathological conditions which might alter the normal lipolytic activity of tissues. Just such conditions as nephritis, chloroform poisoning, and toxins have been proven to have a detrimental effect on the lipolytic activity of tissue extracts. The process of autolysis might be considered in this instance as a pathological condition. ⁴⁷ Saxl asserts that during autolysis of tissues the splitting of natural fats in the cells is but slight; simple esters are attacked, more especially amy-salicylate.

Pesci makes the assertion that toxins increase the butyrase but not other lipases of the liver.

Poulain found the activity of the mesenteric and peripheral lymph glands of the same normal subject to be practically the same. In intestinal disease the lipolytic activity of the mesenteric glands diminishes while that of the peripheral glands remains the same. In diseases of the skin the glands draining the diseased area are lipolytically subnormal while the remote glands are normal. In general infections there is a decrease in ferment activity of all lymph glands.

Winternitz and Meloy found a decline in the lipolytic activity of all tissues in toxemia of pregnancy and in diabetes mellitus. They suggest that this decline might be due to an acid inhibition of the ferment. They also found a

decline in liver lipase in hepatic tuberculosis, chronic passive congestion and cirrhosis. The same conditions were true for the kidney in both acute and chronic nephritis. In extreme general tuberculosis with anemia the kidney, liver, lungs, and blood all show a loss in lipolytic activity.

35

Quinan working on Belgian hares with experimental nephritis from uranium intoxication found that both the liver and kidney suffer a profound decrease in lipolytic activity although the muscles are not appreciably affected. In observed cases of spontaneous nephritis there was a decrease in the enzyme activity of the kidney but practically no change in the other organs examined.

51

Flexner demonstrated the presence of lipase in the foci of fat necrosis.

52

Wells states that practically all cases of fat necrosis are produced by the action of pancreatic juice and he ascribes to lipase the cause of this production of fat necrosis.

53

Quinan in 1915 induced necrosis in the liver by means of chloroform poisoning and found that there is a loss of lipolytic ferment amounting to as much as thirty-eight per cent. The loss of lipolytic ferment precedes the morphological change known as necrosis. It was noticed that chloroform disturbs the lipase balance of tissues. In chloroform poisoning the liver substance shows an extensive loss of lipolytic activity per gram of substance while both the kidney and muscle substance at the same time show a corresponding increase.

Pathological conditions also influence the amount of lipase contained in the blood. As stated by Whipple, Mason and ⁵⁴Pieghthal in 1913, the lipase content of the blood is decidedly influenced by poison, such as chloroform, which causes destruction liver changes. They note a rise in lipase during the first few hours following chloroform anesthesia.

⁵⁵Sagal found that in bacterial diseases the sera show a slight increase in enzyme activity while sera from patients suffering from diseases of non-bacterial origin show a slight depression. Bile-stained sera and sera from hepatic cirrhosis show a marked decrease in enzyme activity.

Still another factor which might influence the ⁵⁶tissue content of lipase is food. Quinan states that in Belgian hares the large livers give lower values than smaller, dark colored livers. He attributes the variation in size to the glycogen content. Assuming this to be true and taking into account the fact that lipase seems to decrease as the livers increase in size, and vice versa, he thought it fair to assume that the ester-splitting faculty of a liver cell would be more apt to remain constant if the diet were strictly controlled by weight. Assuming the activity of a single cell to be fairly constant, the lipase value would become lower in proportion as the mass of the individual cell becomes greater. Thus an accumulation of glycogen in a cell would cause a corresponding relative diminution of the lipase figure per gram, because of the fact that the weighed test unit would contain fewer cells. Thus a uniform diet containing a fixed minimum amount of glycogen forming material should be fed.

46

Greene and Carter in this laboratory, working on the relation of fat and lipase of the liver during periods of fasting and feeding, found that after a meal rich in fat there occurs an initial fall two hours after feeding in the lipase content of the liver followed by a rise reaching its maximum at eight to eleven hours after feeding; followed by a gradual decrease in fourteen hours. In fasting puppies they found first an initial fall in the lipase content for the first two to six days followed by a rise lasting to the end of the fast. ⁵⁷ Greene and Summers at the same time found the lipase curve of the blood to run practically parallel to that of the liver lipase.

58

Abderhalden and Rona found that in dogs the feeding of large amounts of fat increases the lipolytic activity of the blood sera. ⁵⁹ One year later Abderhalden and Lampe showed that starvation in these animals produced similar conditions.

Summary of Literature Bearing on Tissue Fats.

It will be recalled that all living matter contains larger or smaller amounts of organic substances which are soluble in alcohol, ether and other fat solvents. These substances help give to protoplasm the property of retaining large amounts of water while not dissolving; also the power of taking up readily in large amounts chloroform, ether and other substances soluble in fats but not readily soluble in water. These organic substances are among the fundamental and ever present constituents of living matter. They are given the group name lipins - Greek lipos, fat.

The group lipins contains chemical substances such as the aromatic oils, true neutral fats, sterols, and the phosphatids,

but they all possess two or three properties by virtue of which they are called lipins. These properties are their greasy or fat-like feel, their solubility in chloroform, ether, and other fat solvents, and their insolubility in water. The lipins concerned in this paper are the ether extractible substances of the liver and kidney and are all grouped under the general term of fats.

The fats were the first of ⁶⁰three great classes of food stuffs to have their composition determined. Chevreul in 1814 saponified fat by alkalies into fatty acid and glycerol.

The fats and fatty oils are the neutral esters of the trihydric alcohol and contain the higher fatty acids, such as palmitic, stearic, oleic acids. They are, therefore, compounds of carbon, hydrogen and oxygen, but contain far less oxygen in proportion to the carbon than do the carbohydrates. The fats are solid at ordinary temperatures, while the oils are liquid; the fatty acids of the fats are, for the most part wholly saturated, while in the oils some of them are unsaturated. Neutral fats and oils are not crystalline in the cells, the temperature being high enough to keep the fats liquid. They are insoluble in water, the affinity of fat and water being very small; the molecular weight is large; they possess the power of reducing the surface tension of water, and spreading out over water in an extremely thin layer of molecular dimensions.

Physiological Value of Fats.

Before going into the discussion of fats and

their relation to and variation in the body tissues ~~views~~ will be presented as to their value to the general body metabolism. By their physical properties they contribute to the physical constitution of protoplasm. They are heat producing foods par excellence. Matthews states that fats burn in the body with ease, but outside the body the saturated fats are inactive at body temperatures. Whether the fatty acids are burned in metabolism only after they are set free from the glycerol or burned best in ester union is not known. But the general presence of lipase in all the cells, and the fact that the form of any substances which accumulates in cells is generally the stable form, and we know it is neutral fat that is stored, leads to the inference that they are hydrolyzed before oxidizing. The fatty acids are then probably united to some substance which hastens their oxidation.

Cellular Fat and Fat of Animal Organs.

The cells of all tissues contain fat. In many cells the fats may be seen in the form of fine droplets distributed throughout the cytoplasm. In adipose tissue the cell is almost composed of one large droplet of fat enclosed in a cell membrane. In the living protoplasm itself fat occurs in such a fine state of subdivision or union with other constituents that it can not be detected microscopically but its presence is shown by chemical examination.

The ever presence of fat in the living cells has been demonstrated and proven beyond a doubt by many investigators. Among the first to notice the presence of fat in the

animal cells was Kolliker.⁶² He discovered the absorption of fat from the gastric mucosa of mammals in 1857.⁶³ Greene has noted that during the absorption of fat the epithelial cells of the intestinal mucosa of the king salmon is loaded with fat droplets.⁶⁴ Leathes showed that the red muscles of the rabbit like the diaphragm and heart contain a considerably greater store of fat than the white muscles of the cat.⁶⁵ Hill states that the fat is a normal tissue compound and is found in all organs and tissues even before birth; and that the percentage varies under different physiological conditions.

⁶⁶ Bell found fat in the cartilage cells, epithelial cells of all kinds, in the muscles, kidney, liver in both foetal and adult life. He states that fat in these tissues bears no relation to the fat cells of adipose tissues and unlike the fat in the fat cells, that in the epithelium, cartilage and muscle is independent of the nutritive condition of of the animal.

⁶⁷ Mendel fed fat stained with Sudan III and found the stained fat in the lacteals after four to five hours.

⁶⁸ Langley has pointed out that the fat in the liver cells of the frog varies with the time of the year, being at its maximum about February and March; and at its minimum about September and October. He states that warmth lessens the amount of fat in the liver in the winter frog, and that the decrease is in both number and size of the fat droplets, but that where fat is present in large quantities the amount is greater in the inner part of the cells.

⁶⁹ Mottram has demonstrated that there is an increase

in liver fat during pregnancy and that about the time of parturition total liver fat is markedly increased.

70

Noel Paton remarks that throughout the various parts of the liver there is a uniform distribution of the substances soluble in ether - the fats - and that in animals in the same condition, the percentage amount of fat is fairly uniform, being about five per cent of liver substance.

The formation of fat in the organism may occur in at least two ways; it may consist partly of fat absorbed from the food and deposited in the tissues; and partly of fat formed in the organism from other bodies, such as proteins and carbohydrates.

Fat from the food which is absorbed in the intestinal canal may be retained by the tissues. Radziejewski,⁷¹ Lebedeff,⁷² and Munk⁷³ fed dogs with various fats, such as linseed oil and rape-seed oil, and found the fat which they fed in the tissues.⁷⁴ Hofman starved dogs until they appeared to have lost all their fat, then fed them on a heavy fat diet with little protein. He found large quantities of fat in the tissues.

⁷³Munk states that fatty acids when fed are deposited in the tissues not as such but are transformed by synthesis with glycerin into neutral fats on their passage from the intestine to the thoracic duct.⁷⁵ Winternitz shows that iodized fats are absorbed in the intestine and deposited in the various tissues.

70

Noel Paton states that an excess of fat taken in the food is largely stored in the liver of certain animals, e.g., cat and rabbit.⁵⁵ Hill asserts that food fats are stored in the depots as body fats, and that an excess of one kind of food fat modified

the character of the depot fat toward that of the food fat.

⁷⁶
Whitehead fed melted butter stained with Sudan III to hungry cats and after four hours found the villi, lacteals, and lining epithelium to be gorged with fat. ⁷⁷
Raper fed cocoanut oil to cats and dogs and detected it in the liver after five to six hours.

⁷⁸
Greene and Skaer have described a definite cycle in the variation in fat content of the mucosa of the mammalian stomach following the taking of a meal rich in fat. They state that "the gastric epithelium in moderately fasting conditions contain traces of fat in the bases of the cells. The epithelial fat content following a meal rich in fat is expressed by a curve which at first shows a slight drop, then a rapid rise of the amount of fat in the cells, followed by a more gradual and slow decline to the normal."

⁷⁹
Mottram asserts that with a diet rich in fat, the kidneys of the cat are frequently seen to be infiltrated with fat, the amount at times being three times that of the minimal amount.

⁸⁰
McMeans finds on feeding cholesterol an accumulation of fat in the interstitial tissue in pyramids of the kidney. He also noticed fat cells in the endothelium of the aorta and arteries.

Definite proof of the conversion of carbohydrates ⁸¹
into fat was afforded by Meine and Strohmer. They fed a pig, weighing 140 kilos, two grams of rice per day, and collected the carbon and nitrogen of the excreta by means of a Pettenhofer-Voit apparatus. The results were as follows:

	Carbon	Nitrogen
Ingested in the food	765.37	18.67
Excreted	476.15	12.59
Balance retained in body	286.22	6.08

The nitrogen retained represented 38 grams of protein containing 20.1 grams of carbon, 209.12 grams of retained carbon were available for glycogen or fat construction. Since the amount of carbon retained exceeded the possible glycogen formation, fat must, therefore, have been added to the body. Had all the carbon retained been converted into fat it would represent a production of 343.9 grams of fat. This indicates a possible conversion of 21.5 per cent of starch ingested into fat.

Similar results as these were obtained and similar conclusions drawn by E. Voit and C. Lehmann when they fed rice to geese after a four and one-half days' fast. The same principles were shown by Rubner to be true in the case of a dog.

Pembrey describes how marmots previous to the winter hibernation instinctively devour large quantities of carbohydrates food, and how the respiratory quotient may rise even as high as 1.39. This indicates a fat production for use during winter.

Noel Paton states that a diet rich in carbohydrates tends to increase the amount of fat in the liver.

The question which has aroused great interest has been that concerning the production of fat from protein. Petten-

85

hofer and Voit found that after ingestion of large quantities of protein a part of the carbon was retained in the body and not excreted through the usual channels. They state that the carbon might have been retained either as glycogen or fat. Since the carbon was retained in such large quantities as to preclude the possibility of its retention entirely as glycogen they concluded that fat must have been prepared from protein and stored in the body.

86

Cremer starved a cat for several days and then fed it all the lean meat it would eat. The whole carbon retention for eight days was estimated to be fifty-eight grams, which corresponds to a glycogen production of one hundred and thirty grams. The cat, however, only contained thirty-eight grams of glycogen. The remainder of the carbon then must have been stored as fat.

84

Rubner has noticed a similar carbon retention after the ingestion of protein in excess. He explains the carbon retention by stating that protein may in part be converted first into glycogen and then into fat.

87

Chauveau, Gantier and Kaufmann consider the formation of fat from protein as positively proven.

88

Chauveau and Kaufmann state that in the direct formation of fat from protein the fat is formed besides urea, carbon dioxide and water, as an intermediary product in the oxidation of protein.

89

Gantier considers the formation of fat from protein as a cleavage without the taking up of oxygen. If fat is

formed from protein in the animal body, then such formation is not a splitting off of fat from the proteins, but rather a synthesis from primarily formed cleavage products of protein which are poor in carbon.

The effect of starvation or fasting is to set free the reserve foods. Thus there is a great decomposition of fats since they are called upon to supply the needs of the body for energy and fuel. The adipose tissue almost entirely disappears since there is a great mobilization of fat from the fat reserves.

90

Statkewitsch states that with a loss of body weight there is a great increase in liver fat. Paton finds no alteration in the amount of liver fat in a fast of four days' duration. The liver thus maintains its store of fatty acids during inanition.

91

Nicholoides confirmed the appearance of fat in the liver and other glandular organs during periods of fasting. In fasts extending over thirty days he found vacuoles which presumably once contained fat. Rosenfeld finds the percentage of fat to increase in the milk, the blood, liver and other organs during fasting periods. Gilbert and Jomier find a greater amount of fat on the average in hungry animals than in those of the fed. Smirnow also finds this same increase in liver fat during fasting periods and states it to be apparent both microscopically and macroscopically.

95

Greene and Carter have described a curve for the fat which shows a progressive increase up to the time approaching inanition when there occurs suddenly a great increase of fat in the liver.

Fatty Metamorphoses and Infiltration.

Fats after being hydrolyzed in the intestines, unlike other food stuffs, are rebuilt before they leave the intestinal wall. ⁹⁶ Bloor states that "there is a chemical transformation consisting in a rearrangement of the quantities of different glycerides, resulting in a mixture of different physical properties which may be more suitable for transport or storage than the fat fed; or a chemical change consisting of a rearrangement of the fatty acid in the molecule or some or all of the glycerides, which in addition to changing their physical properties would make them seemingly more adapted for use as tissue fats - for endogenous metabolism."

"The intestines seem to have the power to modify radically the composition of fats during absorption. The changes are apparently purposive in that they vary in kind and degree with the nature of the fat fed and also show in general a tendency toward the production of a uniform chyle fat, presumably, the characteristic body fat of the animal." Thus the mechanism serves to permit adaptive changes in fats during absorption. But it would seem that these purposive adaptive changes which take place during the absorption of fat according to views held by Leathes, Hartley, Meyer-Wedell and others, also take place during and are an important factor in the metabolism of fats.

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According to Leathes the one thing we can speak of with certainty is that the fat absorbed from the intestine in the first instance is taken to the liver. This fact was

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further proven by Leathes and Meyer-Wedell. They fed animals fats of high iodine values and found that the iodine values of the fat in the liver raised. It was also shown by Hartley ⁹⁹ that in animals which had not been treated by any specific fat that the fat in the liver under ordinary conditions, like that of the muscles, heart, spleen, kidney, and other organs differs from that of the stored reserve fat in that it is composed of fatty acids of much higher iodine values.

Hartley explains the occurrence of these highly unsaturated fatty acids in the liver and other organs by assuming that such acids represent some intermediate stage in the metabolism of fat. He found evidence that the fat contained in the heart, muscle and kidney are similar to and as unsaturated as those found in the liver. He, therefore, inferred it to be in the liver that the desaturation of fatty acids is effected and that the other organs are provided by the liver with the unsaturated acids which they require.

Leathes states that when there is an active mobilization of reserve fat, the fat in the liver by the low iodine value of the fatty acids of which it is composed, at once indicates that the stream of fat is directed to the organ. For instance, during chloroform poisoning the liver is found to be loaded with fats of low iodine value. The fat in the liver if it has not been recently loaded with mobilized fat is found to yield an amount of fatty acid similar to that yielded by the heart muscles and other organs. If there is an excessive amount of fat the percentage of fatty acid yielded on saponification

approximates to that which characterizes a mixture of simple glycerides as the fat contained in the connective tissue. "A normally functioning liver keeps pace with its work and contains fat with a similar iodine value, and about the same percentage of fatty acids as that which characterizes the fat of any active organ of the body in which fat is not merely stored in reserve in the inert unorganized form of the simple glycerides."

It seems thus that during normal states of fat metabolism the fat of the liver is of similar character to that found in other organs of the body, but that during excessive metabolism of fat there is an accumulation in the liver of fats of low iodine value mostly composed of simple glycerides. It is a natural inference to suppose that the alteration in fatty acids by which their iodine value is raised is effected by the liver. Thus it is the work of the liver to desaturate the fatty acids and provide the other organs with such.

Having thus noted the changes which occur in fat as promoted by the liver we are more able to understand the two forms of fatty changes "infiltration" and "degeneration" that may occur in pathological conditions.

100

Virchow in 1847 divided the fatty changes which may occur in pathological conditions into two large groups, namely, "infiltration" and "degeneration." Fatty infiltration he defines as an accumulation in the cells, from without of fat in the form of large droplets without any damage to the cell itself. Fatty degeneration he defines as an accumulation within the cell of fat in finely divided droplets with much disin-

tegration of the cell protoplasm seeming as though the fats were derived from the breaking down of the cell proteins.

For a long time fatty degeneration was looked upon as a chief evidence of the formation of fat from proteins for the cell protoplasm seemed to be changing directly into fat. This theory was disproven by Taylor when he established the fact that there is not true conversion of protein into fat during phosphorous poisoning.¹⁰¹

The question as to whether the cause of this fat accumulation might be in pathological conditions has been cleared up by Rosenfeld.¹⁰² He starved dogs poor in fat and then fed mutton tallow and linseed oil. On examination he found large quantities of these fats to be stored in the liver and subcutaneous tissues. Upon starving his dogs again for a few days he found that the foreign fat had left the liver but was still stored in the other store-houses. He then poisoned his dogs with phosphorus and noted a typical type of fatty degeneration in the liver and other viscera. Upon extracting the liver fat it did not prove to be the normal animal fat it would have been in case of cell disintegration but was the foreign type which was stored in the subcutaneous tissues and other fat depots.

It has also been shown by Leathes¹⁰³ that the iodine number of fatty human liver falls, as the amount of fat in the livers increases, to the iodine number of the adipose connective tissue.

¹⁰⁴
Rosenfeld argues that the accumulation of fat in

the liver which takes place when animals are kept in an overheated atmosphere is caused by a transportation of depot fat and is a case of fatty infiltration of the tissue and not one of degeneration.

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Mottram fasted guinea pigs and found a great accumulation of fat in the liver of the hungry animal, an increase amounting to about five times the amount of fat in the normal animal. He states that there is evidence which proves the accumulation to be not a pathological process but on the contrary a physiological infiltration from the depot fats. Mottram in a later article states that kidney fatty acids are more saturated than those of the depot fats - explained by assuming that the kidney infiltrates with fat circulating in the blood.

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Mendel and Daniels state that when animals are fed with food stained with Sudan III and then phosphorus poisoning is produced, the constant finding of Sudan III in the blood and bile makes it evident that the dye migrates from the stained adipose tissue and is brought to the liver where it is eliminated in the bile. This gives additional evidence that a fatty liver in phosphorus poisoning is produced by infiltration.

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Hill explains the infiltration process as a physiological occurrence. He states that in conditions of intoxications and mal-nutrition the cells increase their energy at first by oxidation of glycogen and finally they fall back upon the fats and take up increasing quantities of this from the depot through the blood. The probability of this view is established by the fact that feeding glycogen-producing foods lowers the out-

put of fat in the liver, and restoration of a fatty liver after being poisoned by phosphorus is signalized by the return of glycogen.

"Thus," according to Wells,¹⁰⁸ "it seems evident that fat accumulating in the liver during fatty degeneration ~~is~~ not derived by a transformation of cell protein into fat but is rather an infiltrated fat brought in the blood from the fat depots of the body to the disintegrating organ."

¹⁰⁹Rosenfeld did not entirely clear up the subject of fatty degeneration for he found that in the course of his analyses of organs, both microscopically and macroscopically, fatty degeneration showed, but the chemical analyses of these same organs showed no correspondence to the amount of fat that appeared to be present by microscopic examination. This is found to be particularly true of the kidney.

¹¹⁰Wells,¹¹¹ reviewing the work of Rosenbloom and Gies¹¹² on the lipins of organs in phosphorus poisoning; that of Bondi¹¹³ on the lipo-peptids; Bell on the intracellular lipoids; and¹¹⁴ Taylor,¹¹⁵ with the results obtained by Rosenfeld on the relation of the morphological to the chemical change of the amount of fats in organs during phosphorus poison came to the following conclusion: "That fatty degeneration of an organ means in case of the liver, myocardium and pancreas, an infiltration of neutral fat from without into the cells which have been degenerated by the action of the poison or other injurious influences, plus a certain amount of apparent increase in fat because of the setting free of previously invisible fats and lipoids normally present in the effected cells. In the kidney,

spleen and muscles an increase of fat seldom occurs from these causes, but the cells may show a marked fatty metamorphosis through the setting free of the invisible intracellular fats and lipoids by autolytic changes."

"Each cell contains a large amount of fat and lipoids which is so combined that it cannot be detected microscopically; this may be liberated during the autolytic processes of cell disintegration and become visible, constituting a macroscopical and microscopical degeneration but without any actual increase in fat - this condition occurs particularly in the kidney and nervous tissues."

G. Inter-relation of Tissue Fat and Tissue Lipase. ✓

From the review of the literature certain factors have been noted which must play an important part in the metabolism of fats. It is a striking fact that there is always associated with fat metabolism the ever presence of the enzyme, lipase. In the active glands of the body, e.g., the liver, where there is a large assimilation of fat there is always associated with this assimilation large amounts of lipase. Also it may be noted that in the kidney where the fat content is lower than that of the liver there also occurs a lower lipolytic activity. There seems to be no doubt that variations in both lipase and fat content of organs under varying physiological conditions do occur, but there needs to be some correlation between these lipase and fat variations before the present lipase theory of fat metabolism can be positively accepted.

¹³
Bradley states positively that "there is no broad

correlation between the lipase and fat content of tissues." But since Bradley paid no attention to the general systemic nourishment of his animals his results can not be taken as any definite proof. ⁴⁶ Carter demonstrates a definite correlation and relationship between the lipase and fat content of the liver in fat feeding, in fasting, in increasing age, and in ether anesthesia. But yet further investigation needs to be made and further proof afforded before we can accept the existing lipase theory of fat metabolism as conclusively proven. We have undertaken the further solution of the problem by determining the relation of the lipase and fat content of the liver and kidney under variations in feeding.

MATERIALS AND METHODS OF EXPERIMENTATION.

The method of attack on the problem before us was to feed a number of animals a determined amount of food of given composition. All were fed individually and at the same time. At definite intervals during digestion and absorption individuals were killed and examined for fat and for lipase. These observed results were then compared with a normal killed for a comparison standard just before feeding the experimental set.

In all our experiments puppies of the same litter or of practically the same age and species were to secure a series of animals of as nearly a uniform condition as possible and to eliminate as far as possible from the experimental results the chances of a wide individual variation.

Before beginning each experiment, in order that the animals used might be in an equally nutritive condition, they were fed a carefully mixed diet based on grams per kilo for a period of time lasting about one week. Then food was withheld for a definite time to secure a disappearance of the previous food, usually about thirty hours. At the end of this period one animal was selected as a normal, weighed and decapitated. The blood, liver and kidney of this animal were taken as the normal tissues. The remaining animals were at the same time and each one individually fed test meals, weighed and decapitated at different intervals during absorption. A post mortem examination was made in each case and particular notice taken of the omental fat, the quantity and condition of the food contained in the stomach and intestines, and of the condition of

the lacteals.

The determination of the amount of fat was made by determining the weight of the ether-extractible substances in the dry tissue of 10 gram samples of the fresh tissue. The lipolytic activity was measured in terms of the number of cubic centimeters of N/20 NaOH required to neutralize the acidity developed by 1 c.c. of a 10 per cent aqueous solution of the fat free tissue acting on .5 c.c. of ethyl butyrate and 10 c.c. of distilled water incubated at 38°C. for 24 hours.

The method of determining the blood fat and lipase given in detail in Mr. D. A. Robnett's Thesis was followed by me in all experiments except the first which was determined for me by Mr. Robnett.

METHOD OF FAT DETERMINATION.

In all cases duplicate 10 gram samples of the liver were taken; and in most cases one 10 gram sample of the kidney. The samples were immediately ground with 10 grams of purified sand; the mixture then transferred to a watch glass and dried to a constant weight in a glass desiccator over H_2SO_4 or in the hot sun. The dried sample was then transferred to a mortar, re-ground and removed to a paper extracting thimble. Care was taken that all the sample was removed to the thimble and the ether rinsings of the watch glass and mortar were poured into the thimble.

A straight ether extraction was made by means of Greene's modification of the soxhlet. The extraction was continued for six hours, the ether running over the sample on the

average of once per minute. The ether was then distilled from the fat and the flask plus the ether extractible substances dried to a constant weight. This weight minus the dry weight of the flask was taken as the weight of the ether extractible substances of the liver and kidney tissues.

The extracting thimble containing the sand plus the fat free solids - was removed from the extractor and termed the fat free sample.

METHOD OF LIPASE DETERMINATION.

Each of the fat free samples was transferred to a mortar and ground for about four minutes. The fine powder was then transferred to a 100 c.c. glass stoppered graduated cylinder. The mortar was rinsed well with distilled water and the entire contents transferred quantitatively to the glass cylinder. The cylinder was then filled to the mark and 4 c.c. extra of distilled water added to make up for the water displacement of the ten grams of sand.

The straining method as has formerly been employed by Carter was not used for in that case the protein material remaining as a residue must contain some lipase within its cells and thus one does not have a true 10 per cent extract. The entire quantity of the fat free sample including the sand was transferred to the cylinder. These 10 per cent watery extracts were placed on an incline and shaken several times during twelve hours to procure an equally distributed colloidal suspension of the lipases. The samples were then placed in an upright position and the bulk of the protein and sand allowed

to settle in the bottom, leaving a clear watery lipase suspension above.

The procedure for the quantitative work on the hydrolytic activity of the extracts was as follows:

Into each of three medium sized weighing bottles was placed 10 c.c. of distilled water, .5 c.c. of ethyl butyrate, .08 c.c. - 3 drops - of toluol, and .5 c.c. litmus solution. Into each of two bottles of the first set up and into one bottle of the second set up was placed 1 c.c. of the 10 per cent watery extract of the fat free tissue to be tested for lipolytic activity. The contents of each bottle was then thoroughly stirred for about a minute's time so as to produce a very fine emulsion. Each bottle was then tightly stoppered to prevent evaporation and placed in an incubator at 38°C . for 24 hours. After 24 hours incubation the bottles were quickly chilled in cold tap water and the developed acidity determined by neutralizing with $\text{N}/20 \text{ NaOH}$.

The two bottles of the first set up which contained the watery extract measured the lipolytic activity, the autolytic acidity and also the free acidity. The third bottle of the first set up measured the free acidity and the bottle of the second set up measured the autolytic acidity. Then by determining the autolytic and free acidity the lipolytic activity could be easily calculated.

Determination of the lipolytic activity made from the same extract nearly always correspond within .02 to .1 c.c. of $\text{N}/20 \text{ NaOH}$.

EXPERIMENTAL RESULTS.

Fat and Lipase content of the Blood, Liver and Kidney at different periods following a meal rich in Fat.

Two feeding experiments were run on two groups of six puppies each. The first group was about six months old, the second group about three months old. In the sets of puppies, the sixth of the first group was of a different litter of apparently about the same age as the others. All the pups were in good condition, the average weight for the first litter was about five and one half kilograms, that of the second litter about three kilograms. All were given the usual preliminary feeding which lasted in the first case over a period of about one week, in the second case about four days. Their nutritional condition was observed constantly and they were all apparently in the same general systemic condition at the time of the test. All of the first series were fasted 24 hours, number one was taken for a normal and the remaining five were fed 90 grams per kilo, of a bread consisting of corn meal and cotton seed oil about 25 per cent of fat. All of the second series were fasted 35 hours, number one was taken for a normal and the remaining five were fed each 100 grams of cream containing 22 per cent fat plus 10 grams of butter fat, plus 20 grams of dextrose.

The fed puppies were killed at intervals during the digestion and absorption of the fatty meal. The first was decapitated two and one half hours after feeding, and the others at the fifth, eighth, eleventh, and fourteenth hours respectively.

PROTOCOLS OF THE AUTOPSIES.

Series No. 1.

The stomach of pup number one taken for the normal was partially filled with soft food, containing much cracklin fat, showing that not all the previous food had been digested and absorbed as was assumed. The lacteals were slightly visible and the intestines practically empty. The stomach of pup number two, two and one half hours after feeding, was gorged with partially digested bread; the lacteals were slightly prominent and the intestines empty. The stomach of pup number three, five hours after feeding, was filled with semi-digested food; the lacteals were very prominent and milky in appearance; the intestines contained much chyle. The stomachs of number four eight hours after feeding number five eleven hours after feeding, and number six fourteen hours after feeding, were one half, one third, and one fourth filled with emulsified food respectively. All the lacteals were filled and the intestines contained chyle.

Two unforeseen facts revealed by the autopsy findings may have some significance in influencing the interpretation of the results. Since the meal of the preliminary feeding had not been entirely digested, as the findings in the standard pup shows, there must have been some absorption still in progress. We are forced to assume that fat metabolism had not as yet reached a state of stable equilibrium as between the absorbing tissues, the blood and the storage tissues. The second significant point is the fact that the rate of digestion and absorption of the test meal was very slow and not complete at the end of the fourteen hours allowed, an allowance of time

previously found in this laboratory to be quite adequate for the meal of milk and cream.

Series No. 2.

The stomach and intestines of number one taken for normal were empty, the lacteals were not prominent but were clear showing that practically no absorption of fat was in progress. The stomach of number two, two and one half hours after feeding, was about three fourths filled with curdled milk and cream; the intestines also contained some semi-digested food; the lacteals were slightly prominent and milky in appearance indicative of fat absorption. The stomach of number three, five hours after feeding, was half filled with semi-digested cream; the intestines contained much chyle; and the lacteals were milky in appearance. The stomach of number four, eight hours after feeding, and of number five, eleven hours after feeding, were about one third and one fourth filled with well digested food, respectively. The intestines were well filled with chyle, and the lacteals milky in appearance. The stomach of number six, fourteen hours after feeding, was practically empty; and the intestines contained less chyle than that of the two preceeding animals. The lacteals were more clear, showing less fat absorption to be in progress.

On the whole, the findings at the autopsies of this second fat series indicate everything to be in absolutely perfect condition; and eliminating the chances of experimental error point toward absolutely comparable results.

FEEDING EXPERIMENT NO.1:

Fat Series No. 1.

Feeding experiment number one, April 20, 1917, showing variation in the fat and lipase content of the blood, liver, and kidney following a meal of cotton seed oil and carbohydrates. The determinations were made on the blood, liver, and kidney of a series of six pups, about six months old, in comparable nutritional condition. One was killed for a normal; the others were decapitated at two and one half, five, eight, eleven, and fourteen hours respectively after the test meal.

* TABLE NO.1. Blood

Animal Number	Time of Sample	Total Fat per cent	Lipase N/20 NaOH
1	Before the test meal	2.45	0.94 c.c.
2	2½ hours after test meal	2.95	0.90 c.c.
3	5 hours after test meal	3.64	1.12 c.c.
4	8 hours after test meal	2.29	1.03 c.c.
5	11 hours after test meal	2.83	1.07 c.c.
6	14 hours after test meal	2.88	1.22 c.c.

* Table taken from Robnett's Thesis, page 29.

TABLE NO. 2 Liver.

Animal Number	Time of Sample	Total Fat per cent	Lipase N/20 NaOH
1	Before test meal	3.03	4.57 c.c.
2	2½ hours after test meal	3.65	8.61 c.c.
3	5 hours after test meal	3.32	6.24 c.c.
4	8 hours after test meal	2.76	2.54 c.c.
5	11 hours after test meal	2.94	2.97 c.c.
6	14 hours after test meal	3.12	2.64 c.c.

TABLE NO. 3 Kidney

Animal Number	Time of Sample	Total Fat per cent	Lipase N/20 NaOH
1	Before test meal	3.33	1.15 c.c.
2	2½ hours after test meal	3.51	1.84 c.c.
3	5 hours after test meal	3.96	1.21 c.c.
4	8 hours after test meal	3.34	2.45 c.c.
5	11 hours after test meal	3.43	1.22 c.c.
6	14 hours after test meal	4.09	0.81 c.c.

FEEDING EXPERIMENT NO.2

Fat Series No. 2.

Feeding experiment number two July 21, 1917, showing variations in the fat and lipase content of the blood, liver, and kidney following a meal of cream and butter fat. The determinations were made on the blood, liver, and kidneys of a series of six pups, about six months old, in comparable nutritional condition. One was killed for a normal; the others were decapitated at two and one half, five, eight, eleven, and fourteen hours respectively after the test meal.

TABLE NO. 4 Blood.

Animal Number	Time of Sample	Total Fat per cent	Lipase N/20 NaOH
1	Before the test meal	1.39	0.96 c.c.
2	2½ hours after the test meal	2.15	1.00 c.c.
3	5 hours after the test meal	2.25	1.10 c.c.
4	8 hours after the test meal	2.10	0.95 c.c.
5	11 hours after test meal	1.51	0.94 c.c.
6	14 hours after test meal	1.82	1.02 c.c.

TABLE NO. 5 Liver

Animal Number	Time of Sample	Total Fat per cent	Lipase N/20 NaOH
1	Before the test meal	3.48	2.75 c.c.
2	2½ hours after test meal	6.56	2.68 c.c.
3	5 hours after test meal	9.31	4.26 c.c.
4	8 hours after test meal	10.11	4.52 c.c.
5	11 hours after test meal	7.08	4.18 c.c.
6	14 hours after test meal	5.94	4.02 c.c.

Table No. 6 Kidney

Animal Number	Time of Sample	Total Fat per cent	Lipase
1	Before the test meal	3.26	1.06 c.c.
2	2½ hours after test meal	3.29	1.14 c.c.
3	5 hours after test meal	3.31	1.08 c.c.
4	8 hours after test meal	3.34	1.10 c.c.
5	11 hours after test meal	3.50	1.34 c.c.
6	14 hours after test meal	4.06	1.45 c.c.

Fat and Lipase of the Blood
Following a Test Meal Rich in Fat,

Hours After a Test Meal Rich in Fat.

Fat and Lipase of the Liver

Following a Meal Rich in Fat.

Fat in Per-cent. Lipase in c.c. of N/20 NaOH

PLATE B - FOR SALE BY THE UNIVERSITY CO-OPERATIVE STORE, COLUMBIA, MO.

DEPARTMENT OF ENGINEERING, UNIVERSITY OF MISSOURI

Hours After a Meal Rich in Fat.

Chart No.3. Experiment No.1.

Fat and Lipase of the Kidney

Following a Meal Rich in Fat.

Fat in Percent. Lipase in c.c. of N/20 NaOH.

Hours After a Meal Rich in Fat.

Fat in Percent. Lipase in c.c. of N/20 NaOH.

EXPERIMENT NO. 1

Fat Series No. 1.

The Blood.

The changes in the total fat content of the blood (table No. 1) as shown in this experiment are similar to those previously shown to take place after a fatty meal. There is a marked increase in blood fat during the first two and one half hours of absorption, 20 per cent above the normal blood fat. This is followed by a still higher rise to 48 per cent above the normal at the fifth hour. In the eighth hour animal the blood fat is lower than the normal. But the samples taken at the eleventh and fourteenth hours show an increase above the normal of 15.5 and 17.5 per cent respectively.

The findings are in accord with the variations of blood fat shown by Summers. He found a sharp increase at the fifth and eighth hours and a smaller increase at the eleventh and fourteenth hours of absorption in comparison with the normal. The changes are not nearly so marked as those found by Summers. The lower volume of change may be partially explained when we consider the fact that the previous days' food was not entirely digested and absorbed in this set of animals before the test meal was given. Thus, with the absorption of fat still in progress at the time the test meal was given, the fat content of the blood was probably above normal in all the pups. There can not be the marked change in fat content of the blood usually observed at the two and five hour periods of absorption.

The low per cent of the fat content of the eight hour blood sample may be assumed to be an experimental exception. In this pup the fat per cent of the blood during absorp-

tion is below the figure found for the blood of the normal before the meal. However, this puppy was of a very nervous temperament, and the surmise is, since the results of the other five digestion periods are consistent and correspond with previous findings, that there was some irregularity in his digestion and absorption of the test meal, or that his blood fat content before feeding was lower than the standard.

The high fat content of the blood noted in the eleventh and fourteenth hour animals shows clearly that the process of absorption was still going on at those periods at a rate above that of its removal by storage. If we were permitted to omit animal number four and fill in the curve from the five hour period to the eleven hour period, Chart No. 1, the whole series would make a comparative^{ly}/symmetrical graph. The terminus of the graph could only be shown by later stages of digestion than were available in our experiment.

The sero-lipase (table No..1) presents a fairly symmetrical curve of slight variation. There is a very slight fall in the lipase at the end of the two and one half hour period of digestion, a variation well within the limits of experimental error. This is followed by a rise of 19 per cent at the fifth hour. There follows a small relative decrease, though two per cent above normal at the eighth hour in the animal with the unusual fat content. The eleventh hour animal shows a small relative increase of lipase, 14 per cent above normal. The blood of the fourteenth hour animal shows the highest lipase content of the series, 30 per cent above the normal.

THE LIVER.

The changes in the total fat content of the liver (table No. 2) following a fatty meal, as shown by experiment number one, are the following: a marked increase in the fat content during the first two and one half hours of absorption, - 20 per cent above the normal, a slight rise of 9 per cent above the normal at the fifth hour period. At the eight hour the liver fat like that of the blood was below the normal. At the eleventh hour period the liver fat is about the same as the normal and there is a slight increase above the normal, 3 per cent. These findings are not in accord with those of Carter's, who found a sharp rise above the normal at the two, five, and eight hour periods. My maximal increase in liver fat comes earlier, i.e. at the first period. The variation in results may be partially explained by the fact that the previous day's food was not entirely digested and absorbed before the test meal was given. The fat content of the liver was probably above normal in all the animals, hence we do not get the marked increase in liver fats during the first eight hours of digestion and absorption.

The low fat content of the liver of the eight hour puppy may be explained in the same manner as the low blood content of this animal was explained, i.e. as an experimental exception.

The slight rise at the eleventh and fourteenth hour periods shows that the process of absorption and infiltration into the liver of fat was still in progress.

There is an unusual rise in liver lipase (table No. 2)

in experiment number one at the two and one half hour period following the test meal of fat, - a rise of 88 per cent. This rise in lipase would seem out of ^{the} question if it were not for the fact that the lipase curve remains high at the fifth hour period - almost 36 per cent above the normal. At the eight hour period the lipase drops far below the normal by 44 per cent, another instance of irregularity of this animal. The lipase figures for the eleventh and fourteenth hour pups also fall far below the normal.

THE KIDNEY.

The kidney fat (table No. 3) increases gradually to 19 per cent above the normal up to the fifth hour after the test meal, then drops back to normal at the eighth hour; at the eleventh hour it is slightly above the normal, about 3 per cent, and rises at the fourteenth hour period to a height of 23 per cent above normal reaching the highest crest in the curve at this period. The graph indicates a slower process of infiltration in the kidney as compared with the liver.

The lipase of the kidney (table No. 3) like that of the liver rises during the first two and one half hours of absorption, 60 per cent above the normal, but falls again at the fifth hour period to 5 per cent above the normal. At the eighth hour the lipase curve reaches its highest at 113 per cent above normal, falls again at the eleventh hour to five per cent above, and a further fall at the fourteenth hour to 19 per cent below normal. This lipase curve follows the liver lipase curve except in the case of the fourth animal which

has been assumed to be an experimental exception.

SUMMARY OF EXPERIMENT 1.

A most noticeable fact in regard to the sero-lipase is its apparently slight variation. Nevertheless, with the exception of the two and one half hour period the sero-lipase remains constantly above the normal; 19 per cent, 2 per cent, 14 per cent, and 30 per cent in the respective tests. The rise through the eighth, eleventh, and fourteenth hour periods is associated with the later phases of fat metabolism. The fat curve at these periods indicate that it is at least past the maximum phase of absorption. Whether the 30 per cent rise at the fourteenth hour is due to a resorption of digestive lipases as suggested by Robnett or to some other lipase source will be discussed later.

The striking feature of the liver lipase curve is its sharp rise at the first period followed by a progressive fall to a figure below the normal during the eighth, eleventh, and fourteenth hour periods. The low lipase figures of the eighth, eleventh, and fourteenth hour periods are undoubtedly associated with the later stages of fat metabolism. The fat content describes a curve which, except in the last period, is parallel to the curve formed by the lipase. This last drop in the lipase at the fourteenth hour is undoubtedly associated with the later phases of fat metabolism as will be described later.

The kidney lipase and kidney fats both describe an ascending curve during the first two and one half hours following the fatty test meal but there is practically no parallelism in the

kidney fat and lipase curves during the later stages of fat metabolism.

The apparent variation in the parallelism of the kidney fat and lipase curve throws much light on the subject of the later metabolism of the fats in the animal body.

Leathes⁹⁷ states that fat from the intestines is first taken to the liver, and that during an excessive mobilization of fat a great stream of fat is directed to the liver from the fat storage tissues. Hartley⁹⁹ states that the fat of the liver, in ordinary conditions is similar to that of the muscles, heart, spleen, and kidneys and differs from that of the stored reserve fat in that it is composed of fatty acids of much higher iodine values. He explains the occurrence of these unsaturated fatty acids in the liver and other organs as some intermediate stage in the metabolism of fats. He inferred it to be in the liver that the desaturation of the fatty acids is effected and that the active organs are provided with the unsaturated acids which they acquire by the liver. Thus it seems to be the work of the liver to desaturate the fatty acids and provide the other organs with such.

Since there is such a great transportation of fat into the liver there must be some mechanism at work in the liver whereby this fat is taken up from the blood and stored there. With such an enormous infiltration into the liver it only seems fair to assume that the liver is a lipase producing organ. Based on the assumption that the liver is a lipase producing organ, we can safely state that the liver does store fat in proportion as it is transported to that organ by the blood. Thus,

assuming that the liver is a lipase producing organ, the enzyme production and similarly the lipase content of this organ, as explained by Carter, will most likely not represent the amount of lipase necessary to carry on its own metabolism. It will represent the lipase required for lipogenesis accruing within the liver tissue plus an excess which is to be utilized in transporting and laying down of fat elsewhere in the animal body.

Thus when the liver receives a stimulus to lipase production as it undoubtedly does during the first two and one half hours of fat absorption, there will be a production of lipase in excess of the amount necessary for its own lipogenesis. This excess is undoubtedly carried by the blood to the heart, muscle, kidneys, and other less active lipase producing organs.

Since the blood, and through the blood, the kidney receives a portion of the excess liver lipase production, the kidneys also, through its recently absorbed excess lipase content, takes an increasing portion of fat from the blood but in the later stages of the process. As a matter of fact, there is a great increase in kidney lipase during the period preceding the eleventh hour, and with this excess of kidney lipase the fat sharply increases in the kidney at the end of the experimental series. This is in contract with the observations on the liver.

With the liver and kidney nearing equilibrium at the fourteenth hour as regards fat content it is possible that the excess of lipase is resorbed by the blood, which would cause the rise in blood lipase during the period preceding the

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fourteenth hour.

Chart No.4. Experiment No,2.

Fat and Lipase of the Blood

Following a Test Meal Rich in Fat.

Fat in Per-cent. Lipase in c.c. of N/20 NaOH.

PLATE B — FOR SALE BY THE UNIVERSITY CO-OPERATIVE STORE, COLUMBIA, MO.

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Fat in Per-cent. Lipase in c.c. of N/20 NaOH.

Hours After a Test Meal Rich in Fat.

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Chart No.5. Experiment No.2.

Fat and Lipase of the Liver
Following a Test Meal of Fat.

Fat in Per-cent. Lipase in c.c. of N/20 NaOH.

Hours After a Test Meal Rich in Fat.

Chart No.6. Experiment No.2.

Fat and Lipase of the Kidney
Following a Test Meal Of Fat.

EXPERIMENT NO. 2.

Fat Series No. 2.

THE BLOOD.

The changes in the total blood fat in this experiment, like the first, are similar to those previously shown to take place after a fatty meal (see table No. 4, 1.11). There is an increase in blood fat of 54 per cent above the normal during the first two and one half hours of absorption. This is followed by a higher rise to 61 per cent above the normal at the fifth hour. This rise is followed by a gradual decline through the eight hour period to the eleventh hour period of 9 per cent above the normal. In the final or fourteenth hour period there is a final rise of 30 per cent above the blood fat of the normal animal.

The sero-lipase curve (table No. 4) in this experiment presents a very symmetrical, though slight, curve. There is a rise during the first two and one half hours of digestion of 4 per cent above the normal, which is followed by a further rise at the fifth hour of 14 per cent. Following the fifth period there is a gradual decline of the curve through the eighth hour period to normal at the eleventh hour, and a 6 per cent increase above the normal at the fourteenth hour.

The curves of fat and of lipase variation in the blood are practically identical and present a marked parallelism.

THE LIVER.

The fat content of the liver (table No. 5) describes a curve which shows a progressive increase in fat through the two

and one half hour period to the fifth hour period of 167 per cent above normal only to be followed at the eighth hour by an increase of 190 per cent above normal. This crest in the liver fat curve is succeeded by a gradual and progressive decline through the eleventh hour period to 71 per cent above the normal at the fourteenth hour.

The liver lipase curve (table No. 5) shows an initial drop in the lipase content of 28 per cent below the lipase figure for the normal animal. This sudden drop is followed by a rise at the fifth hour to 13 per cent above that of the normal animal. This is followed by a further rise at the eighth hour to 20 per cent above the normal. This ascending curve is in turn followed by a gradually descending one through the eleventh hour period to the fourteenth hour period to a figure 7 per cent above that for the normal animal.

THE KIDNEY.

The kidney fat (table No. 6) presents a curve of practically no change in the fat content during the first three periods other than a slow progressive rise to 2.5 per cent above the normal at the eighth hour period. This gradual rise is followed by a more sharp rise through the eleventh hour period to 24 per cent above the normal at the fourteenth hour period, comparing favorably with experiment one in this feature.

The kidney lipase (table No. 6) presents a curve of practically no variations whatever. With the exception of a very slight decline at the fifth hour period, the curve represents a perceptible rise in the lipase content from the

normal animal to the fourteenth hour animal. The whole rise amounts to 37 per cent above that of the figure for the normal animal.

SUMMARY OF EXPERIMENT NO. TWO.

In experiment two, as in the first experiment, the sero-lipase forms a curve of only slight variations. Nevertheless there is a distinct increase to a maximum at five hours.

The fat on the other hand gives a relatively symmetrical graph of rise and final decline during the course of absorption. The increase in blood fat is out of all proportion to the increase in lipase, yet the maximal increase of fat coincides with the greatest variation in lipase.

The liver fats and liver lipases present curves which, with the exception of the low liver lipase at the two and one half hour period, are parallel, and show clearly a definite relationship to exist between the amount of liver fat and lipase.

But how can the decreased lipase content at the first period be accounted for? The lipase of experiment one increases along with the fat increase in the liver. So does the lipase at the later periods in experiment four. The drop below the normal at this one period is not explained. Of course there is always the uncertainty of the assumed resting stage of the normal. However, aside from this one test, the two series give a strikingly similar set of graphs showing the correlation of liver lipase and fat content during absorption.

The kidney fat and lipase curve remain practically constant and without variation for the first three periods. At the eleventh and fourteenth periods there is a sharp increase in both the lipase and fat of the kidney. Since these ascending curves in the kidney are associated at the same time with descending curves in the liver, it only seems fair to conclude that the kidney and possibly the other active organs of the body, do acquire their fats through the liver as Leathes implies.

EXPERIMENTAL RESULTS.

FAT AND LIPASE CONTENT OF THE BLOOD

LIVER AND KIDNEY FOLLOWING A

MEAL RICH IN CARBOHYDRATES.

A single feeding experiment was run on a group of six puppies. The pups were about three months old and all were in excellent condition. Three were of one and three of another litter, but they were all practically of the same age and were in the same nutritional condition at the time of the experiment. Their average weight was about two and one half kilograms. All were given the usual preliminary feeding which lasted over a period of about one week. All fasted for 48 hours. Number one was taken for a normal and the remaining five were fed each equal portions of a bread consisting of 900 grams corn meal plus 300 grams of dextrose. The fed puppies were decapitated at intervals during the digestion and absorption of the test meal. The first pup was killed

four hours after the feeding and the other four were decapitated at the eighth, twelfth, sixteenth and twentieth hours, respectively, after the feeding of the test meal.

PROTOCOLS OF THE AUTOPSIES.

Carbohydrate Series No. 1.

The stomach of pup number one taken for the normal was practically empty. The intestines were also empty. The lacteals were clear and not prominent, showing that fat was not being absorbed. The stomach of pup number two, four hours after the test meal, was about three-fourths filled with partially digested bread; the intestines were practically empty. The lacteals were clear, showing that no fat was being absorbed. The stomach of number three, eight hours after the test meal, was about one-third filled with partially digested food; the intestines contained much food. The lacteals were slightly cloudy showing very little fat absorption. The stomach of animal number four, killed twelve hours after the test meal, was about one-fourth filled with the partially digested food, and the intestines filled with chyme. The lacteals were only slightly cloudy. The stomach of pup number five still contained food, was about one-fifth filled with food and the intestines contained much chyme. The lacteals were only slightly visible. The stomach of pup number six, killed twenty hours after the test meal, contained only five to ten grams of fluid-like material. The small intestines were practically empty but the large intestines were filled

with semi-solid material. The lacteals were scarcely visible, showing little, if any, fat absorption.

The findings at the autopsies indicate nothing that would interfere with the experimental results.

FEEDING EXPERIMENT NO. 3.

Carbohydrate Series No. 1.

Feeding experiment number three, June 25, 1917, shows the variations in the fat and lipase content of the blood, liver and kidney following a meal of corn bread containing thirty-three and one-third percent dextrose. The determinations were made as a series of six pups, about three months old, in comparable nutritional condition. One was killed for a normal; the others decapitated at four, eight, twelve, sixteen, and twenty hours respectively after the test meal.

TABLE NO. 7 (Blood.)

Animal Number	Time of Sample	Total fat per cent	Lipase		
			N/20	Na	OH
1	Before the test meal	1.19	.61 c.c.		
2	4 hours after test meal	1.40	.85 c.c.		
3	8 hours after test meal	1.35	.44 c.c.		
4	12 hours after test meal	1.07	1.13 c.c.		
5	16 hours after test meal	1.06	.86 c.c.		
6	20 hours after test meal	1.03	.607c.c		

TABLE NO. 8 (Liver.)

Animal Number	Time of sample	Total fat per cent	Lipase N/20 Na OH
1	Before the test meal	2.86	2.63 c.c.
2	4 hours after test meal	4.14	3.53 c.c.
3	8 hours after test meal	1.74	3.20 c.c.
4	12 hours after test meal	1.64	2.23 c.c.
5	16 hours after test meal	1.22	2.83 c.c.
6	20 hours after test meal	1.54	1.99 c.c.

TABLE NO. 9 (Kidney.)

Animal Number	Time of Samples	Fat per cent	Lipase N/20 Na OH
1	Before the test meal	4.91	.25 c.c.
2	4 hours after test meal	6.05	.30 c.c.
3	8 hours after test meal	4.13	.39 c.c.
4	12 hours after test meal	2.22	.34 c.c.
5	16 hours after test meal	3.84	.75 c.c.
6	20 hours after test meal	3.83	.27 c.c.

Fat and Lipase of the Blood

Following a Meal Rich in Carbohydrate.

Fat in Per-cent. Lipase in c.c. of N/20 NaOH.

Hours After a Meal Rich in Carbohydrate.

PLATE B - FOR SALE BY THE UNIVERSITY CO-OPERATIVE STORE, COLUMBIA, MO.

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Fat in Per-cent. Lipase in c.c. of N/20 NaOH.

Chart No.8. Experiment No.3.

Fat and Lipase of the Liver

Following a Meal Rich in Carbohydrate.

Chart NO.9. Experiment No.3.

Fat and Lipase of the kidney

Following a Meal Rich in Carbohydrates.

Hours After a Meal Rich in Carbohydrate.

Fat in Per-cent. Lipase in c.c. of N/20 NaOH.

EXPERIMENT NO. 3.

Carbohydrate Series No. 1

THE BLOOD.

There is a marked rise (table no. 7) in total blood fat during the first four hours following the test meal:- a rise amounting to 17 per cent above total blood fat content of the normal animal. The blood fat then remains well above normal, 13 per cent, at the eight hour period but falls at the twelve hour, to 10 per cent below that for the normal animal. From this period on the blood fat remains below normal, reaching a figure 13 per cent below at the twentieth hour.

The sero-lipase (table No. 7) at the fourth hour also presents a marked increase above that of the normal animal, rising 29 per cent above normal at this period. This rise is followed by a fall of 28 per cent below normal at the eighth hour, only to be succeeded by a rise even more marked than that of the first period. This second rise at the twelfth hour period presents a figure 85 per cent above that of the normal animal. From this period on the curve is marked by a gradual and progressive fall of sero-lipase to a figure practically normal at the twentieth hour.

THE LIVER.

The liver fat (table no. 8) describes an ascend-

-ing curve parallel to that of the blood fat during the first four hours following the test meal. Following this rise there is a fall of the liver fat at the eighth hour period to a content 38 per cent below that of the normal animal. There is a decrease in liver fat through the twelfth to the sixteenth hour period to a point in the curve, 57 per cent below that of the normal animal. This extended decline in liver fat is followed by a rise during the period preceding the twentieth hour, but still 46 percent below the normal.

The liver lipase (table No. 8) during the first period similar to that of the blood lipase, describes a curve parallel to that formed by the blood lipase. There is a marked rise at the fourth hour of 34 per cent above the figure shown for the normal animal. This marked rise is followed by a gradually declining curve through the eighth hour reaching a figure at the twelfth hour which is 15 per cent below that given for the normal.

This decline is followed by a rapidly ascending curve reaching a figure at the sixteenth hour which is 8 per cent above that of the normal. But this rise does not persist; it falls again during the succeeding period to a figure 24 per cent below normal at the twentieth hour.

THE KIDNEY.

The kidney fat (table No. 9) during the first period presents a curve similar to that of the blood fat.

This curve is marked by rise of 23 per cent above the fat content of the kidney of the normal pup. Following this period there is a marked decline of the kidney fat curve through the eighth hour period, arriving at a figure 54 per cent below normal at the twelfth hour. The fat then rises at the sixteenth hour period to a figure 72 per cent above that of the preceding animal but still remains 21 per cent below normal at this period and at the twentieth hour period also.

The kidney lipase (table No. 9) presents a curve of no marked variation up to the twelfth hour period. There is a gradual rise in lipase of 36 per cent above that of the normal animal up to the twelfth hour period. But here the lipase curve rises and at the sixteenth hour reaches a height 200 per cent above that of the normal; only to be followed by a decline to normal at the twentieth period.

A most noticeable observation in the accompanying charts is the fact that there occurs at the end of the first period, fourth hour animal, a marked rise in both fat and lipase of the three tissues examined. The fact that this rise in fat and lipase as noted in all the three tissues, occurs at the fourth hour; the time near which, as shown in the two preceding fat experiments, is the period at which fat absorption is at its height, leads us to infer that this marked rise in fat is caused not by a conversion of the carbohydrates into fat, which will undoubtedly occur in a longer period of time, but to a direct absorption from the

alimentary tract of the traces of fat found to be contained in the test meal of corn bread, not to an absorption from the alimentary tract of fat formed there by a reduction of the carbohydrate intake.

Following this period there is a marked decline in fat of both liver and kidney while the blood fats still remain high above the normal. Just at this period the liver and kidney are undoubtedly being infiltrated with and are storing in large quantities sugar from the test meal. Since sugar, as glycogen, is being stored in such large quantities and since it is the first ingredient to be used in the general metabolism of the body, the liver and kidney at this time have no need of their large fat contents. The fat then, which they hold, must be stored in the fat reserve depot for future use. The fat therefore is given up by these two organs and distributed to the various fat depots in the body. Thus there occurs a fall in the fat content of these two organs associated with a blood fat which remains well above the normal caused by the eliminated liver and kidney fat. The blood is transporting this kidney and liver fat, and hence its fat content remains well above the normal.

The liver, since it has stored large quantities of sugar as glycogen and has eliminated a large portion of its fat, has no need for the excess lipase production and there occurs also an elimination of its lipase content. This large elimination of lipase taken up by the blood, having caused

a temporary rise in blood lipase as seen in the chart at the sixteenth hour is distributed to the fat storing tissues to be used in the storage of the eliminated liver and kidney fat.

The kidney assumed to be a non-lipase producing organ, receives the lipase needed to carry on its own fat metabolism through the blood; and receives lipase in proportion as it is needed and brought to it through the blood. This relatively constant lipase content of the kidney being active in eliminating the excess kidney fat in the presence of increasing glycogen content does not decrease in amount as that of the liver does, whose amount is always produced in excess of its own needs.

The kidney being the most active organ of the excretory group requires large amounts of energy producing materials and thus oxidation of its stored sugar and glycogen occurs in a comparatively short period of time. The excess sugar and glycogen presumably being in a large proportion used up at the twelfth hour, there is need of an increased fat content to furnish the substances required for its own active metabolism. Thus, at this period, the kidney absorbs from the blood large amounts of lipase and simultaneously large quantities of fat, - hence the increase lipase and fat of the kidney noted in the charts at the sixteen hour period.

The liver being an active organ has at this period also presumably used up a large proportion of its excess of stored glycogen and sugar. It only follows then that more fat

is needed for its metabolic processes. Thus, with a need of fat occurs a stimulus to new lipase production which is naturally followed by an increased fat content as shown in the liver.

Yet there is another possible explanation for this sharp increase in kidney fat between the twelfth and sixteenth hour periods; and for the small increase in liver fat at the twentieth hour period. This increased liver and kidney fat may be assumed to be a formation of fat from the excess glycogen and sugar which is stored.

It is known that a large proportion of the carbohydrates taken into the body are oxidized and used up in setting free heat and energy. But there is a possibility of a smaller portion of these carbohydrates being reduced, not oxidized, and fat thus formed by the reduction. Thus this rise in fat may be termed a reduction of the excess stored glycogen and sugars, but very little is known of the chemistry of such a reduction.

Since it has been assumed by Leathes, and by Hartley, that the liver infiltrates with fat from the store houses and prepares them for transportation to the other organs, it seems that if such were the case there would occur previous to a rise in kidney fat, a rise in the liver fat. This previous rise in liver fat does not occur in this experiment as in the two previous fat experiments. Thus it seems more plausible to accept the latter explanation for the late rise in kidney and liver fat in this experiment as being a carbohydrate reduction process rather than a fat infiltration process.

FEEDING EXPERIMENT NO. 4

Carbohydrate Series No. 2.

A second carbohydrate feeding experiment was run on a group of six puppies. The puppies were about three and one half months of age and all were in excellent condition. All were of the same litter and in the same nutritional condition at the time of the experiment. Their average weight was about two and three-fourths kilograms. All were given the usual preliminary feeding which lasted over a period of four days. All fasted 48 hours. Number one was taken for a normal and the remaining five were fed each equal portions of a bread consisting of 900 grams of cornmeal plus 300 grams of dextrose. The fed puppies were decapitated at intervals during digestion and absorption of the test meal. The first pup was killed four hours after the feeding and the other four were decapitated at the eighth, twelfth, sixteenth, and twentieth hour, respectively, after the feeding of the test meal.

PROTOCOLS OF THE AUTOPSIES.

Carbohydrate Series No. 2

The stomach of pup number one, taken for the normal, was practically empty. The intestines were also empty. The lacteals were clear and not prominent, showing that fat was not being absorbed. The stomach of number two, four hours after the test meal, was about one half filled with partially digested bread. The intestines contained some chyme. The

lacteals were clear, showing that fat was not being absorbed. The stomach of number three, eight hours after the test meal, was about one-fourth filled with partially digested food; the intestines contained much chyme. The lacteals were clear, showing that fat was not being absorbed. The stomachs of animals four, five, and six killed twelve, sixteen, and twenty hours, respectively, after the test meal, were one-fourth, one-fifth, and one-tenth filled with partially digested food. The intestines in each pup contained large amounts of chyme. The lacteals in each case were perfectly clear, showing that fat was not being absorbed.

Chart No. 10. Experiment No. 4.

Fat and Lipase of the Blood

Following a Meal Rich in Carbohydrate.

Fat in Per-cent. Lipase in c.c. of N/20 NaOH.

Hours After a Meal Rich in Carbohydrates.

PLATE B - FOR SALE BY THE UNIVERSITY CO-OPERATIVE STORE, COLUMBIA, MO.

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Fat in Per-cent. Lipase in c.c. of N/20 NaOH.

Chart No.11. Experiment No.4.

Fat and Lipase of the Liver

Following a Meal Rich in Carbohydrate.

Chart No.12. Experiment No.4.

Fat and Lipase of the Kidney

Following a Meal Rich In Carbohydrates.

FEEDING EXPERIMENT NO. 4.

Carbohydrate Series No. 2

Feeding experiment number four, July 23, 1917, shows the variations in the fat and lipase content of the blood, liver and kidney following a meal of cornbread containing thirty-three and one-third per cent dextrose. The determinations were made on a series of six puppies, three and one half months old, in comparable nutritional condition. One was killed for a normal and the others decapitated at four, eight, twelve, sixteen and twenty hours respectively, after the test meal.

Table No. 10. BLOOD.

Animal Number	Time of sample	Total Per cent	Lipase N/20 Na OH
1	Before the test meal	1.21.	0.88 c.c.
2	four hours after test meal	1.10	0.74 c.c.
3	eight hours after test meal	1.15	0.85 c.c.
4	twelve hours after test meal	1.11	1.21 c.c.
5	sixteen hours after test meal	1.13	0.92 c.c.
6	twenty hours after test meal	1.10	0.85 c.c.

TABLE NO. 11 LIVER

Animal Number	Time of Sample	Total fat per cent	Lipase N/20 Na. OH
1	Before the test meal	3.46	3.13
2	four hours after test meal	3.23	2.54
3	eight hours after test meal	2.45	1.76
4	twelve hours after test meal	2.05	1.88
5	sixteen hours after test meal	2.64	2.81
6	twenty hours after test meal	3.37	2.49

TABLE NO. 12 KIDNEY.

Animal Number	Time of Sample	Total fat per cent	Lipase N/20 Na. OH
1	Before the test meal	3.21	0.72 c.c.
2	four hours after test meal	3.47	0.77 c.c.
3	eight hours after test meal	2.71	0.82 c.c.
4	twelve hours after test meal	2.08	0.90 c.c.
5	sixteen hours after test meal	2.99	1.40 c.c.
6	twenty hours after test meal	3.12	0.84 c.c.

EXPERIMENT NO. 4.

Carbohydrate Series No. 2

THE BLOOD.

There is a slight fall in total blood fat (table No. 10) during the first four hours following the test meal, - a fall amounting to 9 per cent below the total blood fat content of the normal animal. The blood fat then rises to 5 per cent below at the eighth hour but falls again at the twelfth hour to 8.2 per cent below normal. At the sixteenth and twentieth hour periods the blood fat is 6.6 per cent and 9 per cent below normal respectively.

The sero-lipase (table No. 10) at the fourth hour also presents a slight decrease below that of the normal animal, - 16 per cent. The slight fall is followed by a rise through the eighth hour and presents a figure at the twelfth hour 37 per cent above normal. The rise precedes a gradual decline to 3.4 per cent below at the twentieth hour.

THE LIVER.

In the liver fat (table No. 11), as in the blood fat, we do not see an initial rise as in the first carbohydrate experiment, but there is a gradual decline in liver fat approaching a figure at the twelfth hour 40 per cent below the standard. From this period there is a marked rise in the liver fat to 2.6 per cent below normal.

The liver lipase (table No. 11) during the first two periods describes a curve parallel to the liver fat. At the eighth hour the lipase content is 43 per cent below that of the normal animal. There is only a slight variation in the succeeding period but at the sixteenth hour the lipase curve has risen to a point 10 per cent below normal. This rise is followed by a slight fall to 20 per cent below at the twentieth hour.

THE KIDNEY.

A very slight variation in the case of the kidney fat (table No. 12) curve is shown during the first period. There is a slight rise in kidney fat of 8 per cent above normal at the fourth hour. Then follows a successive fall to 35 per cent below normal at the twelfth hour. The next period, the sixteenth hour period, marks a rise in kidney fat to 6.8 per cent below normal. From this period the curve gradually approaches toward the normal, being only 2.7 per cent below at the twentieth hour period.

The kidney lipase (table No. 12) presents a curve of very marked similarity to the kidney lipase curve of the first carbohydrate feeding experiment. There is a very gradual rise through all the earlier periods to 25 per cent above normal at the twelfth hour. The succeeding period, the sixteenth hour period, marks an abrupt rise to 94 per cent above normal. But preceding the twentieth hour period there is a marked fall to 16.6 per cent below the figure for the normal animal.

The tissues examined in the second carbohydrate feeding experiment present curves which, with the exception of the first period, are in general of the same character and variation as those described by the tissues of the first carbohydrate series. This second experiment seems to bear out the assumption that the rise in fat and lipase noted in the four hour animal was undoubtedly due to an individual variation.

This experiment brings out no new points but forcibly emphasizes those brought out in the first carbohydrate feeding experiment.

EXPERIMENTAL RESULTS.

FAT AND LIPASE CONTENT OF THE BLOOD, LIVER AND KIDNEY FOLLOWING A MEAL RICH IN PROTEIN.

A single feeding experiment was run on a group of six puppies. The pups were about two and one half months of age and all were in excellent condition. Two were of one litter and four of another, but were all practically the same age. Pups number one and number three were slightly heavier than the other four and slightly more obese. The average weights were about two kilograms. All were given the usual preliminary feeding for a period of four days. All were fasted 48 hours; pup number one was taken as a normal and the remaining five fed each 150 grams of cooked lean meat. The fed puppies were decapitated at different intervals during the digestion and absorption of the test meal. The first was

killed four hours after the feeding and the other four were decapitated at the eighth, twelfth, sixteenth, and twentieth hour respectively, after the feeding of the test meal.

PROTOCOLS OF THE AUTOPSIES.

Series No. 1.

The stomach of pup number one taken for the normal was practically empty; the intestines were also empty. The lacteals were clear, showing no fat absorption to be in progress. The stomach of number two was three-fourths filled with semi-digested meat and the intestines contained a large amount of chyme. The lacteals were clear, showing no fat absorption. The stomach of the third pup was about three-fourths filled with semi-digested meat and the intestines contained much chyme. The lacteals were also clear in this pup. The stomachs of pups number ^sfour, five, and six were one-half, one-third, and one-fourth filled with semi-digested meat. The intestines of all three pups were filled with chyme and each showed clear lacteals, indicating no fat absorption to be in progress.

FEEDING EXPERIMENT NO. 5.

Protein Series No. 1.

Feeding experiment number five, July 6, 1917, shows the variation in the fat and lipase content of the blood, liver and kidney following a meal of cooked lean meat. The determinations were made on a series of six pups, about two and one half months old, in comparable nutritional

condition. One was killed for a normal, the others decapitated at four, eight, twelve, sixteen, and twenty hours respectively, after the test meal.

TABLE NO. 13 BLOOD.

Animal Number	Time of Sample	Total Fat per cent	Lipase N/20 Na OH
1	Before the test meal	1.47	.45 c.c.
2	four hours after test meal	1.04	.39 c.c.
3	eight hours after test meal	1.23	.41 c.c.
4	twelve hours after test meal	1.27	.26 c.c.
5	sixteen hours after test meal	1.21	.40 c.c.
6	twenty hours after test meal	1.15	.35 c.c.

TABLE NO. 14. LIVER.

Animal Number	Time of Sample	Total Fat per cent	Lipase N/20 Na OH
1	Before the test meal	2.92	2.67 c.c.
2	four hours after test meal	2.17	1.80 c.c.
3	eight hours after test meal	3.49	3.87 c.c.
4	twelve hours after test meal	2.93	1.64 c.c.
5	sixteen hours after test meal	2.79	2.10 c.c.
6	twenty hours after test meal	2.87	2.59 c.c.

TABLE NO. 15 KIDNEY.

Animal Number	Time of Sample	Total fat per cent	Lipase N/20 NaOH
1	Before the test meal	2.55	.15 c.c.
2	four hours after test meal	1.96	.05 c.c.
3	eight hours after test meal	1.65	.15 c.c.

TABLE NO. 15 KIDNEY. (Continued.)

Animal Number	Time of Sample	Total fat per cent	Lipase N/20 NaOH
4	twelve hours after test meal	2.03	.50 c.c.
5	sixteen hours after test meal	2.48	.20 c.c.
6	twenty hours after test meal	1.98	.12 c.c.

Chart No.13. Experiment No.5.

Fat and Lipase of the Blood

Following a Meal Rich in protein.

Fat in Per-cent. Lipase in c.c. of N/20 NaOH.

Fat in Per-cent. Lipase in c.c. of N/20 NaOH.

PLATE B - FOR SALE BY THE UNIVERSITY CO-OPERATIVE STORE, COLUMBIA, MO.

DEPARTMENT OF ENGINEERING, UNIVERSITY OF MISSOURI

Hours After a Test Meal Rich In Protein.

Chart No.14. Experiment No.5.

Fat and Lipase Of the Liver

Following a Meal Rich in Protein.

Chart No.15. Experiment No.5.

Fat and Lipase of the Kidney

Following a Meal Rich in Protein.

THE BLOOD.

The blood fat (table No. 13) and blood lipase both decline during the entire experiment. The curve of fat marks a fall of 30 per cent below that of the normal during the first four hours following the test meal of lean meat. This fall is less marked at the eight hour period but at the twelfth hour is still 15 per cent below the standard. There is a progressive fall through the sixteenth hour period to a figure at the twentieth hour period 22 per cent below normal. The blood fat falls below normal at the beginning period and remains below throughout the entire experiment.

The sero-lipase (table No. 13) describes a curve fluctuating somewhat but declining throughout the experiment. During the first eight hours it declines to a point 9 per cent below normal. A greater drop of 42 per cent below normal occurs during the period preceding the twelfth hour, followed by a rise to 12 per cent below at the sixteen hour period, and 22 per cent at the twentieth hour.

The blood fat and sero-lipase curves each remain below the level of the normal animal. The findings undoubtedly show that fat and lipase are both gradually disappearing from the blood.

THE LIVER.

The curve for liver fat marks a fall of 25 per

cent during the first four hours of digestion and absorption of the protein meal. But during the next period there is a rise in liver fat reaching a figure 19 per cent above that for the normal animal. There occurs a slight fall to normal during the period preceding the twelfth hour. The liver fat falls slightly below the normal at the sixteen hour period but again approaches the normal at the twentieth hour.

The liver lipase (table No. 14) like the liver fat, decreases in amount during the first four hours, a decrease of 33 per cent. But following this fall there is a marked rise of 45 per cent above the normal animal. This rise is followed by a greater decline at the twelfth hour period to a figure 39 per cent below that given for the standard. This second decline is succeeded by a gradual and progressive rise through the sixteen hour period to the twentieth hour which is only 3 per cent below the normal.

THE KIDNEY.

The kidney fat shows (table No. 15) a gradual and progressive decline during the first eight hours of the experiment, reaching a figure at this period which is 35 per cent below that given for the normal. This curve shows a variation from the ones described by the liver and blood fat. There is not the rise at the eight hour period which characterizes the blood and liver fat curves but this rise occurs at a later period. From the eighth to the sixteen hour periods there is a gradual and progressive rise in kidney fat to a point at the sixteen period which is some 3 per cent below normal. Then follows a slight fall at the twentieth hour period.

The kidney lipase, (table No. 15) is very small in amount, i.e. 0.15 cc for the normal. Like that of the liver and blood, it falls below normal at the first period. This temporary fall is followed by a gradual rise through the eighth hour period, passing the normal here to the highest point in the curve at the twelfth hour period, which is three times the normal. The next two periods describe a gradual and progressive decline, i.e. from 0.5cc to 0.2 and 0.12cc, the curve declining to a point at the twentieth hour which is practically the same as that given for the standard animal.

A very noticeable fact is the fall in both fat and lipase of all the three type tissues during the first four hours following the test meal of lean meat. Since this meat was practically free from fat no fat was being absorbed through the alimentary tract. The usual rise in blood fat during the first few hours following a meal that contained much fat did not occur in this case. Assuming the blood fat to be on a decline at the beginning of the experiment and since very little fat was being absorbed in the first animal, the decline should continue through the experiment, unless fat had been produced from the lean meat. The later rise in blood fat does not come from fat absorbed from the intestinal tract but from some other source altogether. For this same reason we might explain the slight fall in blood lipase during the first period; for since no fat was being absorbed a large percentage of lipase was not needed in the blood.

The liver and kidney fats present curves of different character than those noted in the previous experiments. But such a decline in fat at this period may be expected when we base our expectations on the views put forward by Mathews. He states that there is no doubt but that very little, if any, protein storage occurs, but that the excess protein is torn to pieces and the nitrogen eliminated as urea. Heat production is at the same time increased, and there appears to be a stimulated decomposition of the fats. Thus, with an enormously increased absorption of amino -- acids from the digestive tract there is an increased heat production and expenditure of energy in the body caused by the increased oxidative decomposition of the excess of amino-acids. Since the liver and kidney have a large part to play in this increased oxidative decomposition of amino-acids, their fat stores will be called upon to furnish the fuel for such oxidative processes. With this increased oxidative and burning of fats there would naturally occur a decrease in the fat content of the liver and kidney at this period.

But with this increased oxidative decomposition in progress in the organs the normal fat content is soon used below the normal level. Possibly this may lead to a process in the liver productive of lipase. At any rate there is a sharp rise in liver lipase at the eighth hour.

The kidney, by this time having used up its normal store of fats in the oxidative decomposition processes, has need of more fat. Since it is known, or rather thought to be true, that the kidney receives its fat from the storage depots by way of the liver, we may explain this large rise in liver fat occurring at the eighth hour period as an infiltration of fat from the fat depots for its own metabolic use. Such an infiltration seems all the more true when we notice the progressive increase in the kidney and blood fat up to the sixteenth hour period associated with a gradual and progressive decline in liver fat through the same periods.

This experiment yields increasing evidence on the lipase theory of fat metabolism. The kidney at the twelve hour period, just at the time the liver is decreasing in lipase, shows an increasing portion of lipase.

Since the whole metabolic process is nearing an equilibrium and the organs are being adjusted and adapted to the excess protein intake, this second rise in liver fat and lipase is not nearly so marked as the first rise; for during the first periods the whole metabolic equilibrium was disturbed by the large excessive intake of protein and the marked increased metabolism may be explained as a compensatory reaction to bring about, as quickly as possible, the re-establishment of a metabolic equilibrium.

SUMMARY OF DISCUSSIONS.

In the process of fat metabolism, as far as can be demonstrated by the extent of our experiments, we may describe two separate and distinct phases; the one, an absorptive and liver assimilating phase; the other, a resorptive and fat distributing phase. Especially in the two fat experiments are the two distinct phases brought out forcibly. During the first five hours following the intake of a fatty meal the process is purely an absorptive one. There is always a rise in liver fat associated with a rise in blood fat and there seems to be no doubt that the liver does store fat in proportion as it is brought to this organ by the blood. All doubt concerning the justification of the above division of the phases of fat metabolism is cleared away when we contrast the curves formed by the two fat experiments and that one formed by the protein experiment during the first five hours following the test meals. In the protein experiment the first phase of the fat metabolism, the absorptive phase, is completely eliminated and we see only the later stage in a more distinctive and striking form. There is no fat absorption in the experiment where a protein diet was fed, no fat to be absorbed.

The second phase of fat metabolism, the resorptive and distributing, is not nearly so simple a process as the first, but is more complete and confusingly bound up with the perplexities and uncertain knowledge of interval metabolism.

One assumed fact does stand out more prominently than any other. It does seem, when comparing the curves, that the kidney acquires a large portion of its fat later than the liver, possibly through the liver. During the first phase of fat metabolism the kidney takes little part, remains practically dormant with reference to an increased fat content. But it is a most noticeable fact that the greatest rise in kidney fat does not occur until after the height of the blood and liver fat increases have occurred and passed. The liver, having assimilated large portions of the absorbed fat, portions far in excess of its own needs, later eliminates this excess. Just at the period at which the re-sorption of liver fat is in progress the blood fat content remains relatively higher than normal and at this period and not until the period of liver fat elimination is there any marked increase in kidney fat whatever.

The infiltration of the liver with fat for its own use is more forcibly shown in the protein experiment than in the fat experiments. This is associated with an increased oxidative metabolism in progress in the body caused by a large intake of protein. We assume that fat is the agency that furnishes the energy in the process of oxidation of amino-acids. The fat storage depots are the only sources of this supply of fuel. The liver undoubtedly infiltrates with this storage fat, not absorbed fat. The infiltration

of an excess of fat is, I think, beyond a doubt, proven by the facts of the present experiment. The rise in kidney fat takes place always later than the rise of digestion lipemia and after the fall of the wave of liver absorption of fat. Associated with this fall in liver fat is the rise in kidney fat, and a marked rise does not occur until then in either experiment of the series except the carbohydrate feeding.

first
The carbohydrate feeding experiment presents a picture slightly different from this. The marked rise in fat of the blood, liver and kidney during the first few hours of digestion are difficult to explain. Whether this rise is due to an absorption of fat which might have been contained in the test meal; whether it is due to an absorption of fat from the alimentary canal formed there by a reduction of the carbohydrates in the tract; or whether this rise is due to a greater individual variation than has been noted heretofore, I am unable to say. I am inclined to believe that it is the latter, for I do not think that the small amount of fat in the test meal would cause such a rise and I am very doubtful that there is any carbohydrate reduction in progress within the intestinal tract. If it were not for the rise in blood fat also, at this first period, one might assume that the increase in liver and kidney fat at this period is due to a carbohydrate reduction within the organs themselves, but this increase in blood fat at the same period

forbids one making any such surmise.

The large decrease in kidney and liver fat during the second and third periods associated with a high blood fat content undoubtedly signifies a distribution of the fat in excess of the liver and kidneys metabolic oxidations to other storage places.

The second rise in kidney fat in the first carbohydrate experiment is not due to any infiltration of fat from the blood by way of the liver, for just at this period the liver fat shows no marked variation and there is not the usual previous rise of liver fat. Also the blood fat is exceedingly low at this period, showing that no liver fat is being resorbed. This increased kidney fat is then possibly a carbohydrate reduction and not a fat infiltration. The kidney is known to receive large amounts of sugar and to eliminate large amounts of sugar through the urine. Part of the sugar it receives in excess of its own metabolic needs is undoubtedly reduced and stored as fat. This hypothesis would explain the increased fat content at the sixteenth hour period.

The second carbohydrate series presents curves of no marked variation from those of the first series, excepting in the case of the first period, where a marked rise in the fat of all the tissues was noticed. This rise is assumed to be an individual variation. The increase in the liver and kidney fat in the second series at the sixteen and

twenty hour periods is possibly a carbohydrate reduction and not an infiltration.

The lipase content of the blood and animal organs presents a problem more difficult to solve than the fat problem. But one fact stands out above all others and that is the fact that there is a definite relationship existing between the lipase and fat of the blood and animal organs. With only a very few exceptions, there is always associated with the rise and fall in liver and blood fat a corresponding and parallel rise and fall in liver and blood lipase. This suggests a function association.

The kidney fat and lipase present parallel curves during the absorptive phase of fat metabolism but during the later stage the curves are not parallel to the same degree as the parallelism shown by the blood and liver curves. Yet there is a noticeable close relation of the curves. With each fall in liver lipase there is accompanying this fall, or immediately following it, a rise in kidney lipase. This leads one to conclude that the kidney acquires lipase through the blood from the eliminated excess liver lipase. Its increased fat content generally accompanies, or more often follows, its increased lipase content. These facts enable one to draw the conclusion that the liver is an organ for the interval secretion of lipase, and this lipase, being secreted in excess of its own needs, is distributed to the other active organs, absorbed by them from

the blood and taken up in excess of their own normal content.

With the animal body in a state of nutritional equilibrium the ingestion of any test meal is a compensatory reaction on the part of the active body tissues and the body enzymes to reestablish and maintain this metabolic equilibrium.

By the experiments reported in this paper some light is thrown upon the theory of the relation of lipase to the metabolism of fats. The absorption, assimilation, distribution, and storage of fat is certainly due in a large measure to the ever-presence of the enzyme lipase and not alone to some internal characteristic mechanism of the animal cell itself as is thought by some to be the case.

CONCLUSIONS.

1. There is a fairly definite correlation between the fat and lipase contents of the blood, kidney, and liver as shown in animals after a meal rich in fat, carbohydrate, or protein.

2. Indications are that the liver is an organ of internal secretion producing lipase.

3. There are two distinct phases in the metabolism of fat: the one, an early absorptive and liver assimilating phase; the other, a later resorptive and distributing phase.

4. The height of fat absorption and liver assimilation occurs at a period between the fourth and eighth

hour following a meal of fat.

5. A large increase in kidney fat does not occur until after the height of the absorptive and liver assimilating phase of fat metabolism has been reached. The liver infiltrates with an excess of absorbed fat; also with an excess of storage fat.

6. There is evidence of an increase in fats in the liver and kidney following a meal rich in carbohydrates.

7. The rise in liver fat at the eighth hour and increase in kidney fat following, succeeding a meal of lean meat may be looked upon as a liver infiltration from the fat store houses,-an infiltration compensatory to the fat used for fuel in the oxidative decomposition of the excess of amino-acids.

8. The increase in kidney fat occurs when the liver store is decreasing.

9/ During absorption the liver infiltrates with fat in excess of its permanent store,

10. During the fall in liver lipase, after the initial increase, there is accompanying this fall or immediately following it, a rise in kidney lipase.

11. The absorption, assimilation, distribution, and storage of fat is certainly due in large measure to the ever presence of the enzyme lipase and not alone to some internal characteristic mechanism of the animal cell.

12. The whole process of fat metabolism following an excess ingestion of any test meal is a compensatory reaction on the part of the active tissues and the animal enzymes to re-establish and maintain general metabolic equilibrium.

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