DETERMINATION OF THE VARIATION OF THE FAT, THE LIPASE,
THE GLYCOGEN AND THE AMYLASE OF THE LIVER
AFTER FEEDING AND DURING FASTING

by

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Literature on Enzymes - General Points

Early in the history of Physiology substances were derived from living tissue which possessed properties quite similar to those possessed by inorganic catalysts. In 1830 Dubunfraut¹ effected the hydrolysis of starch into sugar by an extract of malt just as had previously been done by the use of strong acids in 1812 by Kirchoff.² Payen and Persoz,³ in 1833, were able to precipitate by alcohol the active principle of malt extract. They called the substance diastase.

In 1849 Claud Bernard discovered a substance in the pancreas with which he was able to hydrolyze fat. To this body he gave the name steapsin. This substance has now come to be known also as lipase.

Many bodies possessing properties similar to those just mentioned were discovered and because of the similarity of their action to that of alcoholic fermentation the substances were called ferments. After Pasteur showed that alcoholic fermentation was caused by the presence of living organisms, yeast, such bodies as diastase, lipase, etc., were known as "soluble" or "unorganized ferments" in contradistinction to living substances like yeast which were called "organized ferments".

To prove conclusively that an unknown substance is an enzyme it must present certain characteristics which are common to this group of bodies. The general properties possessed by enzymes are: first, that of simply accelerating a reaction already in progress and not of initiating it, and second, that of not appearing in the final product of the accelerated reaction.

Rettger,⁴ in 1909 showed that enzymes in general were destroyed by exposure to temperatures ranging from 60° to 100°. He thinks the destruction by heat is probably due to some change in the other components of the complex colloidal system of which the enzyme forms a part.

Enzymes in general manifest their greatest activity in an excess of water and are hydrolytic in reaction. As the temperature of water rises so does its concentration of hydrogen and hydroxyl ions which have hydrolytic action. Experimentation shows that cane sugar can be hydrolyzed by water at 100° at a fairly appreciable rate. It is therefore not entirely illogical to assume that this same process might proceed at room temperature, but at a greatly reduced rate. In support of this assumption Aggazzotti,⁵ in 1907, found starch solutions to be hydrolyzed spontaneously at comparatively low temperatures. Brailsford Robertson,⁶ in 1907, found the electrical conductivity of a solution of caseinogenate to increase on standing. Changes similar to this occur when this body is acted upon by trypsin.

So far as we know, the reactions catalyzed by enzymes are reversible; but since, as investigated in vitro, they take place in an excess of water, the equilibrium position is usually very near the stage of complete hydrolysis.

Owing to the reversible character of the reactions exhibited by enzymes, it follows that enzymes have synthetic properties as well as hydrolytic.

Literature on Lipase

In 1849 Claud Bernard⁷ discovered that the action of pancreatic juice in splitting fats is a process analogous to that of saponification, the neutral fats being decomposed by the addition of water into the fatty acid and glycerine. At a slightly later date in the same year the enzyme effecting this hydrolysis of fats was discovered and given the name, lipase. Practically all of the earlier work of Bernard, on this subject was corroborated by his contemporary Berthelot, working with him.

Not until some years after the discovery of the enzymes were its possibilities thoroughly developed. Croft Hill in 1898 and Kastle and Loevenhart working in 1900 contributed a great deal to our present knowledge of the action of lipase.

Kastle and Loevenhart⁸ determined the relative lipolytic activity of the pancreas, kidney, liver and sub-maxillary gland of pigs on 10% extracts of the respective tissues. The determinations were made by adding 1 c.c. of the 10% extract to tubes containing 4 c.c. of distilled water, 1 c.c. toluene and .26 c.c. ethyl butyrate, incubating for forty minutes at 40°C. At the expiration of the time the contents of the tubes were titrated with N/20 KOH. The results are as follows:

Tissue	c.c.N/20 KOH required	Percent of Hydrolysis
Pancreas	1.4	3.52
Kidney	0.7	1.76
Liver	4.1	10.29
Submaxillary gland	0.5	1.26

According to Kastle and Loevenhart, long before their time there existed a school which thought the hydrolysis of fats in the stomach was effected by the action of bacteria in the early stage of digestion. Further investigation of the question revealed the presence of a gastric lipase. It was found, however, that the splitting of the fat within the stomach occurred in the early stages of digestion because lipase does not resist the action of hydrochloric acid.

Results of other experiments performed by Kastle and Loevenhart, in 1909, show the lipolytic activity of the small intestine to be about 75% of that of the pancreas for equal amounts of tissue.

Previous to 1900 it was thought that unless extracts of pancreas were made immediately after the death of the animal the active principle could not be obtained. As compared to many enzymes lipase is an unstable body, but it does not disappear from the gland as soon as many commonly supposed. Kastle and Loevenhart⁸ found a portion of pig's pancreas to suffer a decrease of only 60% of its original lipolytic activity after refrigeration at 4°C. for seven days. Feeble lipolytic activity was found in a pancreas in

which putrefactive changes were already in progress. A ground sample of pancreas preserved for thirty days in a stoppered bottle with thymol was found to be nearly, if not quite, as active as it was originally. The lipolytic activity of pigs' pancreas refrigerated at 1°C . increased quite appreciably within forty-eight hours. A 10% aqueous extract increased from 4.09% hydrolysis to 4.44% hydrolysis within forty-eight hours. An increase in the lipolytic activity of tissues extracts of several different tissues has been consistently obtained, and according to Kastle and Loevenhart, some attribute this increase in lipolytic activity to the transformation of zymogen into enzyme.

The ability of lipase to hydrolyze fat is dependent on temperature. Hanriot,⁹ in 1894, gave the optimum temperature for lipase as 55°C . The action is retarded at 60°C . and at 72°C reduced to nil. Pancreatic lipase was found to increase the hydrolysis of ethyl butyrate more or less progressively from 0°C . to 40°C . Above 40°C . there was found to be a gradual decrease in the activity of the enzyme. Exposure of a 10% extract of pancreas, by Hanriot, to temperatures ranging between 65°C . and 70°C . for five minutes resulted in the destruction of the hydrolytic power of the enzyme.

Kastle and Loevenhart,⁸ in 1900, found the gradual increase in the lipolytic activity of hepatic extracts to be more variable than that of pancreatic extracts between the temperatures of 0°C . and 40°C . The following tabulations represent such a comparison.

Pancreatic Extract.

Temperature	c.c.N/20 KOH required	Percent of Hydrolysis
40°	1.125	2.82
30°	1.26	3.16
20°	1.00	2.51
10°	0.75	1.88
0°	0.50	1.25

Hepatic Extract.

Temperature	c.c.N/20 KOH required	Percent of Hydrolysis
40°	4.5	11.29
30°	2.375	5.96
20°	2.10	5.27
10°	1.55	3.89
0°	0.9	2.26
-10°	0.28	0.7

Hanriot,⁹ in 1894 observed that identical temperature variations do not exert the same influence on lipase obtained from different sources. He found that the pancreas exhibited the same amount of hydrolysis between 15°C. and 42°C., while the serum produced twice the amount of hydrolysis on monobutyrin at 42°C. as at 15°C.

Ethereal salts other than fats are hydrolyzed in the intestine. Baas,¹⁰ in 1890 discovered the hydrolysis of salol in dogs, and in one case 69% and in another 43.9%. He found

methyl salicylate hydrolyzed to 24.75% and ethyl salicylate to 21.2%. Heritsch,¹¹ in 1875, effected the hydrolysis of ethyl acetate by lipase. These results stimulated Kastle and Loevenhart to determine the possibility of hydrolyzing some of the commoner ethereal salts. Kastle and Loevenhart,⁸ using the usual amounts of extract and adding the salt in the proportion of twice the molecular weight of the salts in milligrams obtained the following results:

Ethereal Salt	Quantity of salt used - grams	N/20 KOH required-c.c.	Percent of Hydrolysis
Ethyl formate	0.148	0.65	1.6
Ethyl acetate	0.176	0.37	0.93
Ethyl propionate	0.204	0.42	1.05
Ethyl butyrate	0.232	1.26	3.13

In checks on this experiment the same order of stability was exhibited. Such results show that the higher the molecular weight of the acid the more readily is its ethyl ester hydrolyzed by lipase. The exception in the percentage of ethyl formate can probably be accounted for, according to Kastle and Loevenhart, by the fact that it is also very easily hydrolyzed by pure water.

The final conclusion to be drawn from data concerning the effect of antiseptics upon lipase is that the more germicidal the antiseptic the greater is its ability to inhibit and destroy the action of the lipase. The two extremes may be seen

in toluene and hydrofluoric acid. Toluene affects the lipolytic activity of the enzyme but little, while hydrofluoric acid almost completely destroys the activity of the enzyme.

The toxicity of acids towards lipase was found by Kastle and Loevenhart to be directly proportional to the strength of the acid.

The data obtained by Kastle and Loevenhart on hepatic and pancreatic lipase shows the rate of hydrolysis by lipase to be proportional to the concentration of the enzyme present. This is particularly true of hepatic lipase. Unless the amount of enzyme present is relatively high the hydrolysis is incomplete. The decreasing velocity of the action of lipase on an ethereal salt is due to the establishment of a chemical equilibrium between the ethereal salt and water and the products of the reaction, the acids and alcohol. Besides this, there is also an increase in the acidity of the fluid which contributes its part inward inhibiting the hydrolytic activity of the enzyme.

Croft Hill¹² in 1898 proved the reversibility of the action of maltase by demonstrating the synthesis of maltose from glucose and by showing that the action of maltase on maltose tends to an equilibrium which is the same whether starting with glucose or with an equivalent quantity of maltose.

Ethyl butyrate is unmistakably detected by its odor. To synthesize it by tissue extract the extract does not have to be fat free. Since from the chemical standpoint neutral fat and

ethyl butyrate are analogous, Kastle and Loevenhart,⁸ in 1900, attempted the synthesis of ethyl butyrate by lipase. They were successful in the attempt to synthesize ethyl butyrate from butyric acid and alcohol by the use of a diluted glycerine extract of pig's pancreas. Control experiments in which the extract was boiled gave negative results. These results showed that lipase action is reversible at least upon ethyl butyrate.

Lipase exists not only in animal organisms, but its presence has been demonstrated by many in both resting and germinating seeds. Sachs,¹³ in 1887, pointed out that "the presence of fats in the seedling can only be explained by assuming that glycerine and the fatty acids travel from cell to cell and are continually becoming reunited for the formation of fat." Charabot,¹⁴ concluded that the ethereal salts in plants are the result of direct action of free acids on glycerine or other alcohols.

In the section on the review of the literature on the fat content of the liver the relations of lipase will be further discussed. It will be shown there that fat may be digested, absorbed, transported, deposited and utilized in the organism by the agency of the reversible action of lipase.

Literature on the Fat Content of the Liver.

We recognize that the liver plays an important role in urea formation, the transformation of purine bases into uric acid, in ridding the blood of uncombined blood pigments, the transformation of glycogen into sugar and vice versa, the storage and probable chemical transformation of fats, as well as in other possible body activities. However, we shall here interest ourselves only in the question of the presence of fats in the liver through the simultaneous existence of fat and lipase in that organ.

H. G. Wells¹⁵ holds that fat accumulation may occur through two processes, physiological or normal, and pathological or abnormal. Wells states that Rosenfeld¹⁶ demonstrated that one of the principal sources of fat to the body is the fat of the ingested food. To demonstrate this point he starved a dog until it was as free from fat as possible and then fed fat of a type greatly different from that normally found in the animal. The accumulated fat exhibited properties characteristic of the fat which was fed. In this same investigation Rosenfeld showed carbohydrate to be a source of fat to the body. The fat derived from carbohydrate is quite different from that found after a mixed diet. It has a preponderance of palmitin and stearin. Very little is deposited in the liver as compared to that deposited in the subcutaneous and mesenteric tissues. Fats are

not readily formed from carbohydrates in man. In swine and geese the opposite condition obtains, fat being readily formed from carbohydrates. In the formation of fat from carbohydrate a synthesis must occur because the fatty acid radical of the ordinary fat is much larger than the carbohydrate radical. Laws and Gilbert,¹⁷ sixty years ago showed that animals could synthesize fats from carbohydrates contained in their food. Magnus Levy¹⁸ found acetic acid, lactic acid, butyric acid and hydrogen to be produced by samples of liver effecting the synthesis of fat from carbohydrate under the proper conditions. He was unable to determine the source of the butyric acid but the simultaneous evolution of hydrogen suggested an analogy with butyric fermentation of sugar. For the explanation of the synthesis of this butyric acid from carbohydrate Magnus Levy accepted the hypothesis proposed by Nencki. According to Leathes¹⁷ this same hypothesis may be amplified to explain the formation of the higher fatty acids from carbohydrate. The essentials of the hypothesis are: lactic acid is first formed from sugar, and possessing the characteristics of all alpha hydroxy acids, readily splits into formic acid and the aldehyde with one less carbon atom than the hydroxy acid, in the case of the lactic acid and acetic aldehyde. The aldehyde formed condenses to form aldol, which is the beta-oxybutyric aldehyde. It is easily conceivable that butyric acid can be formed from

this compound. This hypothesis is not limited to just the formation of butyric acid but, by similar means, caprylic acid with eight carbon atoms, and so forth, may be formed. That the acids formed must possess some multiple of two carbon atoms is the only limitation to such an hypothesis. This is coincident with the fatty acids found in nature, according to the same investigator, Leathes. If butyric acid can be formed by the liver from glucose according to the above hypothesis, it is quite probable, according to Leathes, that the liver can effect the synthesis of oleic acid and stearic acid by similar reactions. In the commercial production of butyric acid by butyric fermentation of sugar, enough six carbon caproic acid is produced to be worth saving. Raper¹⁹ at the Lister Institute, showed that along with the production of the butyric and caproic acids there is enough eight carbon caprylic acid produced to detect and identify.

From experimental results, Leathes¹⁷ says there are grounds for designating the liver as the locus of the synthesis of fatty acids. He further thinks that perhaps this may in some manner account for the fact that there are greater variations in the amount of fat in the liver than in any other organs of the body. In this connection it is not to be forgotten that the fatty acids found in the liver are more saturated than those found in the connective tissue of the organism.

Virchow¹⁵ in 1847 divided the changes which may occur in pathological conditions into two groups: "infiltration" and "degeneration". According to his view the fat entered the cell

from without in the case of infiltration, although there was an enormous accumulation of fat within the cell in the form of large droplets without causing any irreparable damage to the cell or nucleus. If, on the other hand, the fat remained in the form of small droplets and did not coalesce and the cells themselves exhibited evidence of disintegration, the cells were considered to have undergone fatty degeneration or the protoplasm had suffered a transformation into fat. Voit¹⁵ and his school supported this early view of Virchow concerning fatty degeneration. Pflüger¹⁵ and his pupils were able to point out what seem to be the essential errors in these investigations, so that now Physiologists are supporting the view that proteins are a possible source of fat. It has not been established, however, that proteins are either a common or an important source of fat in either physiological or pathological conditions, or indeed that they ever do form fat.

The question concerning the source of fat in certain types of fatty degeneration has been largely cleared by Rosenfeld.¹⁵ He starved animals until they were almost destitute of fat. He then fed them foreign fats of high and low melting points and high and low iodine numbers respectively. These fats were accumulated by the animals in the fat depots. After starving a few days the liver was again rendered fat free still leaving fat in the depots, however. These animals were then poisoned with phosphorus or other poisons, which induce so called fatty degeneration

in the liver and other viscera. On analyzing the liver, the fat which was found was not true dog fat but was of practically the same type as the foreign fat which the dogs had deposited in their fat depots. Had this been fat derived from the disintegration of cellular protein, it would have possessed the characteristics of native dog fat, according to Rosenfeld. He observed also that dogs which had undergone fasts to the extent of almost depletion of the supply of body fat do not develop the characteristic fatty liver in the case of phosphorus poisoning. Quite contrary to Virchow's idea, fatty degeneration in the liver is fat brought from the storehouses of the organism by the blood and deposited in the liver rather than a transformation of cell proteins into fat.

Rosenfeld¹⁶ did not entirely clear up the point of the sources of fat in degenerating tissue. In the course of his analyses on organs he found that there is not always a correspondence between the amount of fat found by chemical methods and that found by microscopical methods. This was found to be particularly true of the kidney. He observed that a kidney might contain less fat per unit of dry weight than the normal kidney, yet it would show fatty degeneration. It might also contain more fat per unit of dry weight and yet show no signs of fatty degeneration.

The appearance of a large amount of a substance in an organ is due either to that substance having been produced within the organ, or to its transportation to the organ from some other part of the body. Owing to the fact that the liver in the case of the absorption of fats, unlike that of carbohydrates and proteins, is not on the direct channel of absorption, Noel Paton raises the question, "What is the role played by the liver in the metabolism of fats?" The enormous accumulation of fat in the liver during certain seasons and in other animals under varying conditions of diet has provoked the idea that the liver must play some important role in this connection.

The probable connection of the liver with fat metabolism was suggested by Nasse²⁰ in a lecture to the Rostock Naturalists' Society. He reported that Hofmann found an excess of free fatty acids in the liver which condition he compared to a similar condition in sprouting seeds, where the same high proportion of free fatty acids exists. From this evidence it would seem that there must be a transport of fat to the liver from the body.

Although C. Voit²¹ produced the evidence to show clearly that fat is formed from carbohydrates he leaves unanswered the question of whether this transformation is effected by all of the organs of the body or in a special organ, for example, the liver.

Langley,²² although his evidence is solely histological, has given a series of observations on the amount and distribution of fat in the liver of frogs under varying conditions. His evidence gives weight to the view that fat is metabolized in the liver whenever the general metabolism is active.

In the summer all of the fat disappears that was accumulated during hibernation. The hibernating frogs when exposed to high temperatures exhibit a decrease in the fat content of the liver, the individual globules becoming smaller and disappearing finally. A decrease in hepatic fat follows immediately after taking of food to be later increased in amount and later still to fall again to normal.

Examinations of livers of man and other animals reveal a wide variation in their normal fat content. Hartley⁸ at the Lister Institute bears out this statement in the case of the fatty acids.

Notwithstanding the fact that Rosenfeld's²³ work on the determination of fat in the livers of abnormal fat content has been criticised the fact cannot be evaded that the range in the variations in the amount of the fat in the liver that he was successful in producing experimentally was so much wider than that in the case of other organs that the uncertainty of his methods is not to be considered. The liver is the only organ in the body that becomes excessively flooded with fat.

According to Leathes¹⁷ there are still better reasons for thinking that the transportation of fat from the storage places in the connective tissue to the liver is responsible for the large amounts of fats sometimes found in the liver. One of the most marked cases of fatty liver is presented by Rosenfeld²⁴ Dogs were starved for five days and then given phlorrhizine on

the sixth and seventh days. He observed that this extraordinary accumulation did not occur until after the fortieth hour and eight hours later 70% of the dry weight of the organ was fat. If the animal were allowed to live 24 hours longer without more phlorrhizing the liver would contain only a normal amount of fat.

These marked and transitory accumulations are due apparently to migration of fat from adipose tissue to the liver. The character of the fat produced by the liver is determined by that of the stored fat which fact in itself points to the migratory hypothesis. Leathes says that since the liver seems to experience the most rapid and greatest changes in fat content it is easy to suppose that the migration of the fat from the adipose tissue is to the liver. The increase in the fat content of the liver may be as high as 300 to 400 per cent above the normal figures as compared to a maximum increase of 100% occurring in other parts of the body. There are certain essential differences in the fats obtained from the liver, heart and adipose tissue. Leathes made determinations of the fatty acids in the tissues he investigated. He too found the fatty acids of the heart and liver to differ greatly from the fatty acids of adipose tissue. Rosenfeld²⁴ found the power of extracts of liver and heart tissue to absorb iodine to be greater than that possessed by the extract of adipose tissue, in spite of the

fact that they contained only 16% of fatty acid. There is perhaps more than one explanation of that fact but it is more probable that the fatty acids of the fatty substances obtained from the liver and heart were more unsaturated than the fatty substances of the adipose tissue.

Hartley²⁵ is responsible for a great deal of work relative to the nature of the fat in different tissues of the body. The first point he undertook was the determination of the relative saturation of fatty acids obtained from the different tissues of the organism. For such determinations a modification of Lieberman's method was used. Leathes²⁶ had found a larger amount of fatty acid soluble in ether but insoluble in petroleum ether in the organs than in the adipose tissue. If the acids soluble in petroleum ether are exposed to the air long enough or are heated, they will be rendered insoluble in petroleum ether. Farnsteiner³ proposed a method which when applied to separating acids of the linoleic and linolenic series from acids more saturated gave evidence that acids even less saturated than oleic acid were present in considerable amount. This seemed to indicate that there was even a difference in the unsaturated acids stored in the connective tissue and the organs of the body. With this knowledge in mind, Hartley set about to elucidate this difference and thereby throw some light on the nature of the changes fat undergoes when it leaves the connective tissue, and enters the active organs of the body. He found the mixture of

fatty acids of the heart, liver and kidneys of all species tested to absorb more iodine than that from adipose tissue. The average iodine value of the organs was found to be about 120. The determinations, however, showed a range from 110 to 140, whereas the iodine values of the adipose tissue varies from 40 in the case of the goat to 65 in man.

Hartley and Mavrogordalo,²⁷ in 1908, published a series of results, which have been corroborated since, which fully confirm the fact that the more fat there is in the liver the more it approaches the chemical nature of the fat stored in the adipose tissue. This can be explained by supposing that when the liver becomes flooded with fat such a condition may be due to an excessive amount of stored fat having been transported to the liver. Leathes' view is that this supposition admits the inference that the normal liver receives fat containing a large percentage of saturated fatty acids but these acids are prepared for use through a process of desaturation accomplished in the liver. Connective tissue fat can undoubtedly be traced to the liver. If this importation from the connective tissue is not excessive the iodine value of the fat is similar to the iodine value of the fat of the other working organs of the body. This indicates that the liver possesses a mechanism which is able to raise the iodine value of the fats it obtains from the connective tissues.

From a series of experiments performed by Leathes¹⁷ he concluded that the liver has something to do with the fats absorbed from the intestines even if the absorbed fats are not thrown directly into the portal stream from the intestine. Because of the fact that the fat from the intestines is discharged into the systemic circulation all parts of the body have an equal chance with the liver to arrest a portion of it, yet it is found that the connective tissue is the only tissue which derives any fat¹⁰⁰ from the systemic circulation besides the liver. The fat arrested by the connective tissue is stored unchanged. On the other hand the quota of fat taken up by the liver undergoes a desaturation. It is supposed that the changes wrought in fat by the liver are merely preparatory. With the final oxidation of fats there is a great liberation of energy. It is likely that this oxidation occurs in tissues where the liberation of energy is most pronounced, as in the muscles, kidneys, etc.

Leathes raises the question of whether or not the only change effected in fats by the liver is in desaturating the fatty acids. The unorganized fat of adipose tissue is the unaltered fat of the ingested food. The iodine value of the newly stored fat is determined by the iodine value of the fat already stored there. This variety of fat contains no phosphorus. The organized fat found in the heart, liver, etc., is very largely composed of phosphatide lipoid substance. Leathes further ex-

presses the idea that perhaps the liver effects the conversion of unorganized fats into organized fat and the desaturation of the fatty acids is a subsequent reaction. Kenneway and Leathes²⁸ have made a number of observations to ascertain whether or not such a change is veiled by the influence of the complex phosphatide and nitrogenous basic constituents of the more complex molecules such as lecithin. The results of the examinations of the different varieties of fatty substance of the liver by these observers tend to show that this desaturation is effected in the liver in the simple glycerides. The phosphatides are not the only factors involved.²²

Noel Paton²⁰ performed a series of analyses of livers for changes in fat content under the influence of fasting. He found that during starvation the liver not unlike the other organs of the body, loses weight, the glycogen rapidly disappears, but the metabolic changes are continued as is shown by the constant production of glucose, of urea and of the various bile constituents. That the activity of metabolism is diminished is shown by the low temperature of the blood leaving the organ and by the diminished manufacture of the various products of the liver. This diminution in the hepatic metabolism in starvation is further demonstrated by the experiment of Schroeder³¹ and the more recent observations of Schöndorff³² on urea production.

He then raises the question, "Does this diminution in the metabolism lead to an increased accumulation of the liver fats or do the needs of the organism produce a withdrawal of fats from the liver?" To elucidate this point he starved three kittens for 56 hours and found no diminution in the amount of fat, as compared to the usual storage of fat from ordinary food-cats' milk.

In other experiments Paton produced an abnormally high fat content in the liver. After a fast of sixty-eight hours he found it to be got rid of to a large extent, i.e., practically a return to the normal level.

In the case of fasts extending over four days Paton found the normal amount of fats in the liver to be unaltered. He was unable to demonstrate, however, whether this was indicative of a non-metabolism of the fats, or whether it shows a correlated decreased accumulation and metabolism maintaining the normal amount. By determining simultaneously the glycogen content of the liver he was able to show that there is no such disappearance of fat as of glycogen from the liver in starvation.

What the source of the fats in the liver is seems as yet not to have been determined. Paton³⁰ thinks that the results obtained by phosphorus poisoning and phlorhizin on starving animals indicates that they might be derived from the body. He suggests that they might be derived from the sugar which sugar is certainly derived from the proteins during inanition, although he has no experimental proof.

Experiments of Paton³⁰ and Carter³³ conclusively show that fat accumulates in the liver subsequent to a meal excessively rich in fat. Perls³⁰ observed that this power of accumulating fat varies in different animals, and states that in the case of the pig's liver this fatty infiltration is not easily produced even by phosphorus poisoning. Paton's³⁰ experimental results bear out Perls' observations.

Although it has been shown that carbohydrates are a possible source of fat in the body it is not an established fact that fat is formed from carbohydrate in the liver. C. Voit³⁰ suggests that carbohydrates might be a source of fat in the liver and cites his experiments on Strassburg geese, which when fed on maize showed an appreciable accumulation of fat in the liver.

Hoppe-Seyler³⁰ is credited with the statement, "The formation of fat in the normal liver is undoubtedly connected with the formation of glycogen though it may occur later." Paton³⁰ says: "Tabulations of the results clearly show that there is no truth in the statement that the amount of fat and glycogen in the liver in any way correspond to one another." Paton,³⁰ feeding strictly carbohydrate diets got results which seem to indicate an increase in liver fats due to the carbohydrates. When carbohydrates first enter the body they are converted into glycogen, so that if fats are derived from glycogen there will be an increase in the hepatic fat at a period when the glycogen content

is being decreased. Further experiments of Paton revealed a marked increase in the fatty acids in the liver at the time glycogen was disappearing. If the hepatic fat is derived from glycogen it is to be expected that the most sudden rise in fat content would be concomitant to the most rapid decrease in glycogen content. Paton's³⁰ results show this increase in fat to occur shortly after the relative depletion of glycogen. Evidence has established the facts that food fats are transferred to the liver and stored, but it isn't established that body fats can be so transported.

In fasting animals Paton³⁰ found the fat of the liver to remain nearly uniform through the period of inanition, also a persistent absence of fat from the blood serum. He holds that such results militate against Leathes' view of a transportation of adipose fat to the liver. He favors the conclusion that by the metabolism of glycogen fatty acids are synthesized from carbohydrate within the liver.

The results of Croft Hill,¹² Kastle and Loevenhart,⁸ Taylor,³⁶ Pottevin,³⁷ and Hamsick³⁸ are adequate to explain the variations in the fat content in the liver during feeding and fasting. Carter,³³ in this laboratory found a relation to exist between the fat content and the lipolytic efficiency in the liver during the processes of absorption and of fasting. These facts invite the suspicion that such a relation obtains in the liver in the case of glycogen and amylase, a relation to be investigated in the experiments of this paper.

Literature on Amylase

Wittich,³⁹ in 1873 demonstrated for the first time the enzyme in the liver which hydrolyzes glycogen. Claud Bernard's⁴⁰ discovery of the glycogen in 1857 furnished the stimulus for the investigation of Wittich. Shortly after Wittich's discovery Bernard advanced the same evidence, independently.

Pavy⁴¹ about 1897 corroborated the results of Wittich in 1873 and of Bernard in 1877 and showed further that this substance from the liver which converts glycogen and starch into sugar performs its function independent of even the presence of the tissue cells from when it comes.

Although the presence of this enzyme in animal and plant tissue is universally admitted its exact role is a subject of discussion. It is a known fact that amylase (diastase) is able to hydrolyze glycogen. Glycogen is also known to be present in body tissue. These facts according to McLean⁴² rather suggest that the function of amylase in the body is associated directly with the cleavage of glycogen into the simpler carbohydrate-sugar.

On the other hand, Pavy was not reconciled to this view and in turn suggested that the enzyme is the product of dead or dying cells which is derived from an inactive pre-existing zymogen. If an analogy exists between amylase and other intracellular enzymes^{the} evidence would indicate that the only difference in the influence of amylase on glycogen during life and in vitro

is one of degree and that its activity is initiated by some not yet understood body condition. (McLean⁴²)

Mendell and Leavenworth⁴³ in 1907 reported the absence of glycogen from the livers of pig embryos ranging in size from 85 to 230 m.m. and in 1908 Mendel and Saiki⁴⁴ report that the embryo liver gradually acquires its diastatic power with growth.

The fact that the diastatic power of embryonic liver and muscle is greater than embryonic blood tends to show the digestion by the tissues is not referable solely to the blood which they contain. Some have held that the transformation of glycogen is due primarily to the action of the blood and lymph. The results of Mendel and Saiki attribute a great portion of this diastatic action to the enzymes of the cells themselves. The following tabulation is the findings of F. Pick⁴⁵

100 grams blood digested 0.31 grams of glycogen in 3 hours.

100 grams liver digested 0.69 grams of glycogen in 3 hours.

100 grams kidney digested 2.37 grams of glycogen in 3 hours.

These results show the relative amylolytic activity of adult tissues. Liver is again found to be more active than blood. Borchardt⁴⁶ in 1909, believes the enzymes of the liver and blood to be identical but thinks the activity of hepatic ferment is greater than that in the case of the blood. That the amylolytic

enzymes are produced by the liver and poured into the blood stream was maintained by Pugliese and Domenichine⁴⁷ in 1907.

Mendel and Saiki⁴⁴ found developing muscle tissue which contained glycogen during embryonic life to give evidence during the earlier embryonic stages of a relatively greater power of digesting glycogen than was found in the case of liver tissue free from glycogen. Although both of these embryonic tissues show a gradual increase in amylolytic power with increasing age, the increase on the part of the liver is relatively greater than that of the muscular tissue. Lowe, in 1904 noted the amylolytic power of blood of different individuals of the same species to be approximately constant. He also points to the variations in amylolytic power associated with age, but does not attempt any explanation.

Kish,⁴⁹ in 1906 showed that the amylolytic power of adult muscle tissue is not dependent on nutritive or functional conditions of the animal. Some factor other than a demand for sugar by the organism regulates the glycogen conversion within the organism.

Nasse,⁵⁰ in 1875, found the activity of diastatic ferments to depend greatly on the presence of salts. Addition of chlorides, nitrates, and sulphates of sodium, he found the diastatic power of pancreatin to increase from 7 to 31 percent. From experimental results throughout the literature it is evident that salt not only helps the diastatic action of the ferment but is essential for its action.

Kendal and Sherman,⁵¹ working with pancreatic amylase in 1909, found that "with one per cent starch solution, however favorable the conditions of salt and alkalinity and however large the amount of enzyme, the hydrolysis tended to come to equilibrium when the weight of maltose reached about 85 per cent of the initial weight of starch." Also, "with a given amount of enzyme, properly activated, the initial rate of hydrolysis was not dependent upon the amount of starch; as the reaction proceeded its rate diminished but less rapidly the greater the amount of starch present."

It has been proven that glycogen is formed from dextrose in the liver. Since the liver is known to possess the enzyme diastase, it is to be expected that this enzyme is responsible for the synthesis of glycogen from dextrose. Wittich was the first to show that the liver possessed a material which was able to hydrolyze glycogen and starch, thus providing the hydrolytic action of the hepatic amylase.

Literature on the Glycogen Content of the Liver

Glycogen, which is the carbohydrate with which we are chiefly concerned in the liver, is closely related to the starches and dextrans, having the general formula $C_6H_{10}O_5$. Claud Bernard discovered and isolated glycogen in 1857. He showed the presence of sugar in the liver as early as 1848.

In examining certain specific tissues for the presence of glycogen he noted particularly that he failed to find it in the liver in early embryonic existence.

Pflüger⁵³ reports having found glycogen in all of the embryonic livers he examined. These analyses were made on cow, sheep, and pig embryos. In accounting for the low values, sometimes obtained, he says: "One must not draw the conclusion that in the earlier embryonic period the liver has a behavior different from that of later life, after birth." The low content of glycogen in the fetal liver is ascribed by Pflüger to the deficient feeding of the mother animal; and he suggests that the animals killed for sale which furnish the embryos for analytical study often fail to receive food before being slaughtered. ✓

Lockhead and Cramer⁵⁴ in 1907, reported their results of quantitative estimations of carbohydrate in the placenta, fetal liver, etc. in series of pregnant rabbits, ranging from the

eighteenth day to the end of gestation. "The results show that at the earlier dates the maternal placenta possesses a considerable store of glycogen, its percentage amount being quite comparable with that of a normal adult liver. It remains constant until the twenty-fourth day, and then a distinct and progressive decrease occurs each day until the end of gestation. The results present a striking relation to those obtained from the analyses of fetal livers. In the latter, though definite amounts of glycogen are found at dates when histological examination proves negative, the percentage is very low up to the twenty-fifth day, when for the first time it rises above that of the rest of the fetal body. This, therefore, represents the date at which the liver assumes its adult glycogenic function. It will be noted that the time coincides accurately with the decrease in the placental glycogen. After this date the percentage of liver glycogen rapidly increases, but even at birth it falls short of the average found in the liver of an adult rabbit.

"Neither the placental store of glycogen nor that of the fetal liver is affected by feeding the animal on a diet rich in carbohydrates."

The quantity of glycogen in the adult liver is dependent essentially on the food, rest and activity. During hibernation this factor is found to decrease while during activity it diminishes. The quantity of liver glycogen may amount to from 12% to 16% after partaking of large quantities of carbohydrate. Ordinarily the glycogenic content of the liver ranges from 1.2% to 4%.

The preceding review shows that there are numerous articles in the literature giving information concerning the fat and glycogen content of the liver. The lipolytic content of the liver has been measured in comparison with other organs, as has also its amylolytic efficiency. Last year in this laboratory was the first time that the relation of the fat content to the lipolytic efficiency of the liver was studied under constant conditions with a fixed variant.

Consistent studies of the variations in liver amylase content to glycogen content have not been made, and to the present it is not determined whether there is any correlation between the fluctuation of the carbohydrate content of the liver and its amylolytic efficiency either following feeding or during fasting. Neither is it determined whether or not there is an interrelation of the fat and lipase content to the carbohydrate and amylase relation in the liver.

In this study the attempt has been made to collect data on the four varying factors in liver composition, - in fat, in lipase, in carbohydrate, and in amylase under conditions of one variant influencing metabolism with the hopes of discovering any interrelation of these factors in body metabolism. The variants chosen for the setting of the series of tests are (1) fasting and (2) feeding.

Experimental Procedure

In this study puppies were used exclusively. In order to secure animals of stable conditions of nutrition, animals of the same age were chosen. The animals in all of the series except one were from the same litter. Quantitative determinations of the liver were made for fat, carbohydrate, lipase and amylase.

The puppies in each series were fed equal quantities of mixed food for a period of at least three days, in most instances five days, to establish a relatively stable condition of metabolism in the different members of the group.

At the end of the preparatory diet the sets of pups which were to undergo inanition received no food. These animals were decapitated consecutively at intervals of one and two days until the entire series was disposed of.

After the puppies undergoing the feeding experiments had established relatively stable conditions of nutrition, they were fasted for 24 hours. At the expiration of this time they were given a diet consisting of an excess of fat and sugar in milk. These animals were killed at varying intervals throughout the period of digestion and absorption, namely, three, five, eight, eleven, and fourteen hours.

The livers were excised, and after post mortem examination were divided into samples of ten grams each. The samples were weighed on a Sartorius balance, which is sensitive to tenths of a milligram. These ten gram samples were then ground separately

with ten grams of sand and the resulting paste was used for the quantitative determinations of fat, carbohydrate, lipase and amylase.

Samples for fat determination were dried in a desiccator to constant weight. They were then ground in a mortar and transferred to the fat extractor. The form used was Greene's modification of the Soxhlett's fat extractor. The extraction by ether proceeded for eight hours in each case. The resulting extract was dried to constant weight. This final weight represents the ether extractable fat from a ten gram sample of fresh liver tissue. The portion of liver which remained in the extraction cup to be dealt with later is designated as the fat free sample.

For the determination of carbohydrate the method of Robt. C. Lewis and Stanly R. Benedict⁵⁷ for the "Estimation of Sugar in Small Quantities of Blood" was modified so that it could be used in the case of the liver.

The following is the detail of the procedure: The ten gram sample of liver which had been ground with sand was transferred to a 100 c.c. volumetric flask and made up to the mark. After this sample had stood for twenty-four hours to make sure that all of the glycogen of the liver was converted into dextrose, a 10 c.c. aliquot of the "brei" was transferred to a 50 c.c. volumetric flask. 25 c.c. of a saturated aqueous solution of picric acid was added and the flask filled to the mark with dis-

tilled water. This was filtered to rid the solution of the protein precipitated by the picric acid. 8 c.c. of the filtrate was transferred to a large Jena test tube. To this was added 2 c.c. of saturated picric acid, 1 c.c. of a ten per cent sodium carbonate (together with two drops of mineral oil and two glass heads). The contents of the test tube were then evaporated over a free flame until precipitation occurred. After 3 c.c. of distilled water was added the mixture was again heated to boiling when the precipitate was completely dissolved. The resulting solution was cooled, diluted until the intensity of its color approximated that of the standard solution. The color of this solution was compared to a permanent standard solution of picramic acid.⁵⁷ This standard solution possesses the same intensity of color as that obtained by the proposed method of Lewis and Benedict with .64 mgs. of dextrose when the final volume of the reaction fluid is made up to 10 c.c. The Dubosque Colorimeter was used.

For calculating the amount of sugar in the sample it is to be remembered that in the original 100 c.c. volumetric flask there was only 96.4 c.c. of liver extract, the sand occupying the remainder of the volume.

The method used for the quantitative determination of lipase is essentially the same as that used by Kastle and Loevenhart.³⁵ The results given in this dissertation are derived from turbid extracts of "brei" of the fresh liver tissue also from tissue extracts of the fat-free liver tissue. Such an extract will be denoted as a fat-free tissue extract of liver.

The turbid extract was prepared by transferring the ground sample of liver to a 100 c.c. volumetric cylinder and made up to the mark with distilled water. 1 c.c. samples of the extract were incubated with 4 c.c. of water and three drops of toluol for an hour at 40°C. At the expiration of the hour the tubes, with tight fitting tops were removed and the contents were titrated against N/20 NaOH. With each sample run there were two checks run. Check No. 1 contained 1 c.c. of the extract and 4 c.c. of water. Check No. 2 contained only water and ethyl butyrate. As soon as the first titration was completed .5 c.c. of ethyl butyrate was added to all of the tubes except Check No. 1. The tubes were then incubated at 40°C. for 24 hours, after which their contents was again titrated against N/20 NaOH. The figures in terms of c.c. N/20 NaOH then represents the amount of alkali required to neutralize the fatty acid liberated by ethyl butyrate on hydrolysis with lipase. In all but the last series this extract gave a higher figure for the same series than did that from the fat free tissue.

Lipase determinations were made on the tissue remaining after the extraction of fat. This tissue was ground for two

minutes. 35 c.c. of water was then added and the mixture ground for thirty seconds. This was then strained through a cloth (standard bleached domestic) after another addition of 35 c.c. of water. The whole was allowed five minutes in which to strain. At the end of five minutes that liquid which had not passed through the cloth was squeezed through. This extract was then made up to 100 c.c. The rest of the determination is the same as that in the case of the turbid extract except that 1 c.c. of the fat free extract was used.

Determinations of amylase were made only on the turbid extract, but checks were run on each sample. For work such as this where a comparison of amylolytic power suffices, the method recently devised by Wolgemuth⁵⁸ is very reliable. A series of Jena test tubes are arranged containing successively increasing amounts of the turbid extract. To this extract in the tubes, which are immersed in ice water to arrest the action of the enzyme, 10 c.c. of a .1% soluble starch solution is added. These are now placed in a water bath and incubated at 40°C. for thirty minutes. At the end of the incubation period the tubes are removed to the ice bath where three drops of N/10 iodine was added to each tube. All of the tubes were next diluted to the same volume with water and their colors compared. "The last tube to show the starch iodine blue was taken as the "limes" and the "D" calculated. The "D" means the number of c.c. of .1% starch carried to the endpoint by 1 c.c. of the enzyme in the incubation period."⁵⁹ In making

up the solution of soluble starch (Mercks) the neutral salt sodium chloride was used (.9%) as a solvent and dilutant.

III Experimental Results.

1. Determination of variation of fat, lipase, glycogen and amylase content of the liver in relation to fasting.

Two sets of puppies were used for these determinations. Both sets were composed of two broken litters of different ages. Each animal was fed the same quantity of a constant mixed diet for a period of five days to insure uniformity of metabolism. One animal of each series was killed at the expiration of 24 hours after the last feeding. This animal was regarded as the standard or normal against which the others of the series were compared. The remaining animals were decapitated at varying durations of fasting.

Since no consistent studies have been made on the relation of the amylase content to the glycogen content of the liver during fasting, we may hope by this study to determine if such a relation exists between these two factors as has been found to exist between the fat and lipase in the liver under similar conditions. Also we hope to determine if any interrelation exists between the fat-lipase relation and the carbohydrate-amylase relation during a period of fasting.

Fasting Experiment No. 1.

Series No. 1.

For this experiment a litter of three terrier puppies in prime condition and of average body weight of 2330 grams was used. They were fed equal amounts of a constant mixed diet for five days. The first puppy (normal) was killed at the end of the first twenty-four hours of the fast. The other two were killed at 48 and 96 hours after the last feeding. The liver was analyzed for fat, lipase, glycogen (determined as sugar) and amylase.

In comparing variations in fat, lipase, glycogen, and amylase in this set of experimental animals their ages and relative sizes must be considered. The first animal of this set was at least a month older than the remaining members of the group. It was also practically double the other members in weight. This same animal had a much larger liver than the rest of the puppies.

Table No. 1.

Giving the analytical data from
the puppies of Series 1.

Days fasted :	1	2	4
Lipase-in terms of c.c. N/20NaOH per ten gram sample :	732	1008	236
Percent of fat :	4.5	4.9	6.5
Units of amylase in a ten gram sample :	321	321	2410
per cent of glycogen and sugar:	1.7	.75	.74

CHART NUMBER I.

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Duration of fast.

Table 1 shows the lipase content to rise quite appreciably during the first 48 hours of the fast. At 96 hours the lipase content fell to less than what it was in the 24 hour animal. The percent of fat gradually increases from 4.5 in the normal to 6.5 in the animal undergoing the 96 hour fast. During the first 48 hours the amylase content does not change, but at the 96 hour interval it shows an enormous increase, rising from 321 units to 2410 units. The sugar decreases about half during the second 24 hours, but does not seem to decrease during the last 48 hours. Perhaps the most striking change to be noticed in the comparisons of this series are: first the great increase in amylolytic content in the final stages of the experiment, notwithstanding the decrease in carbohydrate content, and second, the gradual increase in the percentage of fat.

Fasting Experiment No. II.

Series No. 2.

Four mongrel puppies from two litters were used for this experiment. Animals number 1 and 2 referred to in the table were about 18 weeks old and number 3 and 4 were about 14 weeks old. Their average body weight was about 3900 grams. The diet preceding the fast lasted five days. The normal animal was decapitated 24 hours after the last feeding. The three remaining animals were decapitated at intervals of 4, 7, and 11 days after feeding was discontinued.

It must be borne in mind that the animals 1, and 2 in this experiment were four weeks older than numbers 3 and four. Numbers 1 and 2 were considerably larger than 3 and 4 as their weights indicate.

At autopsy cracklin bread was found in the stomach of number 4.

The following table will show the data obtained from analyses on the liver for fat, lipase, carbohydrate and amylase.

Table No. 2.

Days fasted :	1	4	7	11
Lipase-c.c.N/20 NaOH per 10 gram sample :	1118	1118	515	684
Per cent of fat :	2.5	4.1	4.1	3.8
Units amylase per 10 grams	200	209	178	803
Per cent of glycogen :	1.6	.36	.54	.87

The lipase content hardly changes during the first four days. The 7th day shows a decrease to about half the original amount and finally there is an appreciable rise in the 11 day animal. There is an increase of 1.5 per cent in the fat content of the liver during the first 4 days. At the 7 day period there is no change from the four day period. After the 11 days of fasting there is a slight decrease in the fat content. The amylase content rises between the first and fourth day of the experiment. On the 7th day the amylase is low, but

more than quadrupled at the 11th day. Finally the carbohydrate decreases as the fast progresses though the results are not very consistent.

The data of this series shows a tendency for the lipase content to diminish during the earlier and middle stages of the fasting and of the fat to gradually increase during the same period. The amylase on the contrary, exhibits an enormous final increase while the carbohydrate content on the whole decreases.

A true comparison of the variations of fat, lipase, glycogen and amylase of the liver during fasting rests on the assumption that we are measuring the content in terms of units of active protoplasm. Both fat and sugar are inert substances in the liver, and since neither lipase nor amylase is soluble in them a relative increase in these inert substances per sample of fresh tissue would apparently cause a decrease in the quantity of enzymes present, whereas there might be an increase in the quantity of enzymes per unit of active tissue. To eliminate the possibility of controversy over this point we have calculated the fat, lipase, glycogen and amylase in terms of fat free tissue. The quantity of sugar found in the series of puppies used for the fasting experiments was so small that we have disregarded its presence in calculating the factors in terms of active tissue.

- Lipase-c.c. per 10 gn. sample.
- Fat- Per cent.
- Amylase- Units per 10 gn. sample.
- Glycogen-Per cent.

Duration of fast.

Tables 3 and 4 give the data of fasting experiment numbers 1 and 2 recalculated in terms of fat free liver.

Table No. 3.

The analytical data of fasting experiment Number 1 calculated in terms of fat free liver.

Days fasted	: 1	2	4
Lipase - c.c. N/20 NaOH per 10: gram fat free liver	: 761	1058	252
Fat- per cent of fat free liver	4.7	5.2	6.9
Amylase- Units per 10 grams of: fat free liver	: 333	337	2578
Sugar- per cent of fat free liver	: 1.78	0.79	0.74

Such corrections as table 3 shows are given graphically in chart 3. The curves of variation are only slightly accented in comparison with the total tissue basis of calculation.

Table No. 4.

The data of fasting experiment number 2, calculated on a fat free liver basis.

Days fasted	1	4	7	11
Lipase- c.c. N/20NaOH per 10 grams fat free liver	1140	1152	536	704
Fat- per cent of fatfree liver	2.5	4.2	4.2	3.9
Amylase- units per 10 grams fat free liver	204	217	185	827
Sugar- per cent of fat free liver	1.67	0.38	0.57	0.9

Chart 4 gives a graph exhibiting the results stated in Table 4.

CHART NUMBER III.

2600
2500

- Lipase-c.c. N/20 NaOH per 10 gm. fat free liver.
- Fat-Per cent of fat free liver.
- Amylase- Units per 10 gm. fat free liver.
- Glycogen-Per cent of fat free liver.

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Duration of fast.

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2. Determination of the variation of fat, lipase, glycogen, and amylase content of the liver in relation to the taking of a meal rich in fat and carbohydrates.

For obtaining data on this point two litters of six puppies each were used. To establish relatively stable metabolism throughout the groups each animal was fed the same quantity of a constant mixed diet for five days. The standard or normal animal was killed 24 hours after the last feeding. The remaining five animals of the set were then fed a meal rich in fat and carbohydrate. They were then killed at varying intervals during digestion and absorption.

Feeding Experiment No. I.

Series No. III.

The average body weight of the six 5 weeks old puppies used in this experiment was about 1670 grams. Apparently they were all in good condition as was judged by their alertness. The animal used for the standard was killed 24 hours after the last meal of the five day preparatory diet. The remaining five puppies were each fed 30 c.c. of milk containing 19.5% fat and 10% dextrose and killed at intervals of 3, 5, 8, 11, and 14 hours during digestion and absorption.

Autopsy on series 3 revealed the presence of gastric and intestinal worms in each puppy. In one average case the worms taken from the small intestine alone were sufficient to

CHART NUMBER IV.

fill a one ounce bottle.

Table 5 gives the data showing variations in the lipase, fat, glycogen and amylase content of the liver per 10 gram sample of fresh tissue.

Table No. 5.

Hours after feeding	0	3	5	8	11	14
Lipase- c.c.N/20 NaOH required for 10 gram sample	983	742	645	838	886	877
Fat - per cent	2.9	4.0	4.8	3.8	4.7	4.59
Amylase- units per 10 gram sample	482	318	241	602	267	370
Sugar- per cent	7.4	8.2	7.2	7.19	7.09	9.99

The variations in the lipase and amylase are almost identical, see chart 5. Both enzymes decrease during the first five hours of digestion and increase during the middle and later periods of the test. In the 8 hour animal the amylase was unusually high, a variation for which no obvious exceptional condition was found. At the 14 hour interval the lipase does not change, but the amylase rises appreciably.

Both the fat and carbohydrate increase during the first 3 hours of absorption. The fat rises in the 5 hour animal, falling and rising alternately throughout the rest of the period but always greater than the normal. In the 5, 8 and 11 hour

CHART NUMBER V.

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

- Lipase-c.c. N/20 NaOH per 10 gm. sample.
- Fat-Per cent.
- Amylase-Units per 10 gm. sample.
- Glycogen-Per cent.

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Feeding Experiment No. 2.

Series No. 4.

The six mongrel bull puppies used in this experiment were from the same litter and of an average body weight of 1133 grams. After they were fed a uniform mixed diet for five days, they were fasted for 24 hours. The normal animal was then killed. The others of the set were given 30 c.c. of milk containing 19.5% fat and 10% dextrose and killed at intervals of 3, 5, 8, 11, and 14 hours after the test meal. At autopsy they were found to be badly infested with intestinal and gastric parasites, a condition that may have a bearing on the variations shown in the analytical data of the series. The resulting analytical data is tabulated in table 6.

Table No. 6.

Hours after feeding	0	3	5	8	11	14
Lipase- c.c. N/20 NaOH required	342	260	342	183	216	163
Fat - per cent	4.8	6.8	4.6	6.13	4.8	10.9
Amylase - units	535	321	229	385	482	241
Sugar - per cent	5.89	0.77	5.17	8.36	9.15	3.22

The lipase content varies but little in this series. The normal is high in lipase, possibly for some exceptional reason. The other determinations show a curve increasing at the 3 and 5 hour absorption periods and decreasing later. The fat also shows great variation, amounting to nearly 11 per cent at 14 hours.

CHART NUMBER VII.

CHART NUMBER VII.

A gradual fall from 535 units in the normal to 229 units in the 5 hour test marks the initial change in the amylase content. It rises through the 8 and 11 hour periods and again falls in the 14 hour animal. Barring the 8 hour period in feeding series 3, the two curves in liver amylase agree in showing a decrease during the active stage of food absorption. The percentage of sugar in the normal is 5.89 per cent. In the 3 hour period it is inexplicably low, only 0.77 per cent. It rises more or less gradually to the 11 hour stage where it is 9.15 per cent. The 14 hour animal shows again a low sugar content. A variation suggested in chart 6 is the general tendency for the sugar content to decrease when the fat increases and vice versa.

To forestall the objection that the absolute and possibly the relative variation in one factor during this series of comparisons might be hidden by the relative increase or decrease in the amount of the inert factors of fat and glycogen, we have referred all of our data derived from the feeding experiments to the same standard, namely a unit of active fat and glycogen free tissue. Tables 7 and 8 present this data.

Feeding Experiment No. 1.

Series No. 3.

In this table, the fat, lipase, glycogen and amylase factors are calculated in terms of 10 grams of fat and sugar free tissue.

Table No. 7.

Hours after feeding : 0	3	5	8	11	14
Lipase- c.c.N/20 NaOH: per 10 gram sample :1091	846	729	.939	1001	1026
Fat - per cent : 3.2	4.6	5.5.	:4.3	5.3	5.3
Amylase - units per 10 gram sample : 535	362	272	674	301	433
Sugar - per cent : 8.3	9.3	8.1	8.0	8.0	11.6

The cycle of variations shown on this basis of computation differ from those showing the computations based on the total tissue chiefly in the later stages of absorption in which the stored fat and glycogen is greatest.

Chart 7 shows the lipase and amylase to exhibit very similar variations. It also shows the fat and glycogen content to vary more nearly simultaneously.

Feeding Experiment No. 2.

Series No. 4.

Table 8 gives the results of the analyses of the livers of series 4 in terms of fat and sugar free tissue.

Table No. 8.

Hours after feeding : 0	3	5	8	11	14
Lipase- c.c. N/20 NaOH per 10 gram fat and sugar free tissue : 379	281	376	212	251	190
Fat - per cent of fat: and sugar free tissue: 5.3	7.3	5.0	6.4	5.5.	12.7
Amylase- units per 10: gram fat and sugar free tissue : 593	346	252	447	559	279
Sugar- per cent of fat and sugar free tissue: 6.5	0.8	5.6	8.7	19.6	3.7

This method of calculation produces little variation in the form of the curve of variation of the factors of this series though the absolute values are sharply increased by the relatively high content of fat and glycogen.

CHART NUMBER VIII.

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CHART SHOWING COMPARISON OF CURVES FOR FASTING EXPERIMENT.

CHART SHOWING COMPARISON OF CURVES FOR FEEDING EXPERIMENTS.

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OUR RESULTS COMPARED TO THOSE OF CARTER FOR FAT AND LIPASE AFTER FEEDING.

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OUR RESULTS COMPARED TO THOSE OF CARTER FOR FAT AND LIPASE DURING FASTING.

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IV Discussion and Summary.

In entering upon a discussion of the variation and relation of fat, lipase, glycogen and amylase of the liver during fasting and after feeding, it is necessary that we establish certain premises. Under certain conditions of our experimental procedure we have produced both an increase and a decrease in the amounts of the factors we are studying. Since in definite pathological states these same substances are known to undergo similar variations in quantity, it is expedient that we ascertain whether or not these conditions with which we are dealing here are pathological or normal. Studies made in this laboratory last year by Carter on the fat and lipase content of the liver under conditions of feeding and fasting suffice to show that the condition of our experimental animals was at all times within physiological limits. Granting then that we are dealing with animals all of which are normal it is our problem to account for the variations we have found in our experimental animals on a purely physiological basis.

Throughout this entire study we are hampered in drawing any but tentative conclusions by the lack of consistent experimental results. In the first fasting experiments we find an initial rise in lipase content at the 48 hour period and a fall at 96 hours while in the second series of tests there is a slight initial fall in this factor at the 96 hour period. The fat content in both series rises during the

earlier part of the fasting test, but at the end of eleven days fast instead of rising, as we supposed it would, decreased slightly. Granting that our methods of arriving at these results are correct the two series agree. But it will evidently require many additional series to determine the typical physiological reaction to fasting. Here it is that we find our data to be inadequate.

Leathes has pointed out that the fat in the liver might be an intermediate step between the source of the fat and its fate in oxidation. Hartley has indicated that when fat is taken into the system a part of it is used immediately and that which is not immediately oxidized is laid down as the fatty constituent of adipose tissue. It has been further suggested that during fasting after the supply of oxidizable carbohydrate has been depleted the fat which is laid down in the fat depots is in some manner utilized. Now if we consider that the fat as it is found in the fat depots is not organized or not of the proper chemical composition for oxidation, but that the liver is able to organize or prepare such fat for oxidation we must also explain a mechanism by which this unorganized fat can be transported to the liver for organization or desaturation as Leathes calls the process. For a long time lipase has been known as the substance in the body which is able to effect the hydrolysis of fat and thereby render the non-diffusible substance diffusible. Also it was shown by Kastle and Loevenhart that this enzyme is reversible in its action. Before the unorganized fat of the fat depots can

enter the blood stream to be transported to the liver for desaturation it is necessary for it to first be converted into fatty acid forming soluble soaps and glycerine, both of which substances are diffusible. Whatever the source of lipase is the erosion of this unorganized depot fat occurs and it is transported to the liver by the blood where through the agency of the lipase of the liver cells an infiltration of fat into this organ occurs. Using the results of Carter's work on this question, with the first stage of fast there is a decrease in the amount of lipase in the liver. To explain this initial fall in the lipase content we must assume a decrease in lipase coming to the liver or decreased production in the liver cells. We have no data to determine this question of source of lipase. The liver had not yet responded to the stimulus to produce more lipase. The subsequent rise in the lipase content is attributed to an over production of lipase in response to the stimulus within the organism for more oxidizable material. During fasting fat is generally thought to be one of the last of the body materials to be burned for energy, carbohydrate being the first. The lipase which is eroding the depot fats will, under our assumption, produce more fat in the blood stream than will be used immediately by the body and hence we will have an infiltration of fat into the liver since the amount of lipase is at all times presumably in excess of the immediate needs of the tissue.

Both of our fasting experiments tend to substantiate this hypothesis as far as the fat content is concerned. The results obtained for the lipase factor in one series is at variance with the above explanation of results. We attribute certain of our non-typical results to varying conditions of age and size of the individual animals within the sets of experimental animals. With this sort of material we cannot hope to obtain the happiest results and it is very difficult to obtain large litters of puppies for experimentation.

The explanation for the variation of the two factors in animals after a meal rich in fat is easier on practically the same basis as that offered for fasting. In feeding, the fat is absorbed by the blood and is thus transported to the liver. Since this happens rather rapidly digestion lipemia occurs. Thus there is produced an initial decrease in the lipase content of the liver. For the rest of the period of absorption we would expect the lipase content of the liver to recover to at least the normal and remain practically unchanged assuming that the liver in reaction to this stimulus produces an excess of lipase.

Our results show a tendency towards these variations although they are not entirely in keeping with other results obtained from similar experimentation. Throughout our studies there was a tendency for the fat content of the liver to increase during the entire period of absorption. The lipase curves for both series showed the primary depression, and throughout the remainder of the test they present a relative

constancy. The presence of such a great number of gastric and intestinal parasites in the young puppies quite possibly produced variations in physical condition among individuals, that we could not check.

The results we have gotten for the variation of glycogen and amylase are not entirely consistent. However, the results for glycogen are in accord with the results obtained by other investigators. In series 2 of fasting animals the analysis of the liver of the last animal revealed a slight increase in the glycogen content. This animal showed bread in its stomach at the autopsy. Since carbohydrate is the first material burned in the body we would expect the supply of carbohydrate to be depleted quite suddenly to supply energy for the organism. The liver being one of the principal storage places for glycogen we would expect the glycogenic content of the liver to decrease rather suddenly. In one set of experiments this is what actually happened. In the other fasting series the glycogen decreased, but it was more gradual and prolonged, the probable better picture. Experimentation by others has shown that glycogen is impossible to remove completely, but that it drops to a low level and remains there. Corresponding to the initial decrease of glycogen there was a tendency towards only a very slight rise in the amylase content. That sugar in the blood is taken from the blood and stored in the liver and other tissues of the body is a discovery of Claude Bernard. Later he discovered the enzyme which is able to effect this transformation. Pugliese and Domenichine⁴⁷ maintain that

the liver is an amylase producing organ. We have here a case analogous to that of fat and lipase in many respects. In response then to the stimulus of the need of oxidizable material we would naturally expect an initial decrease to occur due to an outflow from the liver of the enzyme to other parts of the body where glycogen is stored. Since our first analysis was not made until at least 24 hours after the fast started and glycogen is often sharply depleted within that time we could not expect our curve to show an initial decrease in the amount of this factor if it were present. I think we are justified in saying that glycogen and amylase in general each run a definite and characteristic cycle of variation during fasting. The results, however, are not consistent throughout the entire period of experimentation as we are again dealing with animals of varying age and size.

The results we have obtained for the glycogen and amylase content of the liver after a meal rich in fat and carbohydrate are disappointing as regards the glycogen content. The liver is regarded as a regulator of the amount of sugar in the blood and tends toward keeping this factor constant. If then a large supply of carbohydrate is absorbed by the blood we would expect the glycogen content of the liver to increase proportionally. This happens normally, especially as is shown for rabbits. One of our series showed this rise to occur, but the other showed a comparative enormous decrease in the 3 hour animal (possibly an exceptional case), later to rise and ultimately to fall. Since the liver is so situated in the

portal system that the absorbed sugar passes through it first, it is natural to suppose that a great deal of the blood sugar of absorption would be stored as glycogen in this viscus. The amylase shows a consistent fall in both series during the period of digestion and absorption, the 8 hour animal in series 2 being the one exception.

The puppies used in these two feeding experiments were so badly infected with gastric and intestinal parasites that disturbed metabolism must have been inevitable. It is for this reason that we consider these experiments as tentative only. We expect to experiment further on this problem at a later date.

In fasting we find the fat content of the liver to increase until just before the supply of depot fat is exhausted. This was Carter's finding though my results are not so extreme as his. Carbohydrates as we explained before, are practically all used up at an early period. When the fat content of the liver shows its initial increase we find the carbohydrate to have undergone a relatively greater decrease. It is hardly probable that this glycogen is converted into fat for the fat content continues to rise long after the carbohydrate is used up. We should also expect the lipase and amylase to run corresponding cycles of variation in fasting, the changes occurring in the amylase cycle earlier than in the lipase cycle.

In concluding, let us say that although the results of our experiment are not adequate to demonstrate that an inter-relation exists between fat-lipase and glycogen-amylase, they

at least suggest that such a relationship does exist. Further experimentation on this question is necessary before any definite conclusions can be submitted.

General Protocol for Fasting Experiment No. 1.

Series 1.

	24	48	96
Hours duration of fast	24	48	96
Initial weight of animal - grams	2310	1103	2550
Final weight of animal - grams	2300	1095	2500
Loss of weight - grams	10	8	50
Loss of weight - per cent	0.4	0.8	2.0
Liver - total weight - grams	125.3	90.7	95.7
Water in sample - per cent	75.3	74.2	72.6
Solids, sugar and fat free- per cent	26.2	28.0	29.9
Fat in sample - grams	0.454	0.496	0.652
Fat in whole liver - grams	5.68	4.49	6.23
Fat - per cent	4.54	4.95	6.52
Fat in fat free tissue - per cent	4.75	5.22	6.98
Dextrose in sample - milligrams	171.4	75.2	74.2
Dextrose in 10 gm.fat free liver - milligrams	178.3	79.0	77.9
Dextrose in sample - per cent	1.71	0.75	0.74
Dextrose in fat free sample- percent	1.78	0.79	0.77
Lipase- c.c.N/20NaOH required per 10 gram sample	732	1008	236
Lipase - c.c. N/20NaOH required per 10 gram fat free sample	761	1058	252
Lipase- c.c. N/20 NaOH required per whole liver	9186	9125	2260
Amylase- units per 10 gram sample	321	321	2410
Amylase- units per 10 gram fat free liver	333	336	2578
Amylase- units per whole liver	4022	2911	23065

General Protocol for Fasting Experiment No. 2.

Series 2.

Days duration of fast	1	4	7	11
Initial weight of animal- grams	4012	4386	3605	3595
Final Weight of animal - grams	4000	4300	3500	3450
Loss of weight - grams	12	86	105	145
Loss of weight - per cent	.3	1.6	3.0	4.2
Liver - total weight - grams	155	128	90	97
Water in sample - per cent	78.2	75.5	75.6	77.4
Solids, sugar and fat free -percent	20.1	21.9	22.5	21.7
Fat in sample - grams	.253	.411	.412	.38
Fat in whole liver - grams	3.92	5.26	3.70	3.68
Fat - per cent	2.53	4.11	4.12	3.8
Fat in fat free tissue - per cent	2.58	4.29	4.29	3.96
Dextrose in sample- milligrams	163.3	36.6	54.3	87.0
Dextrose in 10 gm. fat free liver- milligrams	167.6	38.2	57.0	90.8
Dextrose in sample - per cent	1.63	0.36	0.54	0.87
Dextrose in fat free sample-percent	1.67	0.38	0.57	0.90
Lipase- c.c.N/20NaOH required per 10 gram sample	1118	1108	515	684
Lipase- c.c.N/20NaOH required per 10 gram fat free sample	1140	1152	536	704
Lipase- c.c. N/20NaOH required per whole liver	17332	14190	4641	6639
Amylase - units per sample	200	209	178	803
Amylase - units per 10 gram fat free liver	204	217	185	827
Amylase - units per whole liver	3107	2677	1605	1799

General Protocol for Feeding Experiment No.1

Series 3.

Hours after feeding	0	3	5	8	11	14 :
Weight of animal - grams	2000	1500	1600	1750	1650	1600:
Liver, total weight - grams	81	101	95	78	78	93 :
Fat in sample - grams	.291	.404	.485	.384	.470	.459:
Fat in whole liver - grams	255	4.08	4.6	2.99	3.66	4.26:
Fat - per cent	2.91	4.04	4.85	3.84	4.74	4.59:
Fat in fat and sugar free sample - per cent	3.2	4.6	5.5	4.3	5.3	5.3 :
Dextrose in sample -milligrams	746.1	824.3	721.0	719.4	709.8	999.5
Dextrose per whole liver- mgms	6225	8916	6849	5611	5536	9283.8
Dextrose - per cent	7.4	8.2	7.2	7.1	7.0	9.99:
Dextrose per fat and sugar free sample - per cent	8.3	9.3	8.1	8.0	8.0	11.6:
Lipase - c.c. N/20 NaOH required per sample	983	742	645	838	886	887 :
Lipase- c.c. N/20 NaOH required per 10 gm. fat and sugar free liver	1091	846	729	939	1001	1026:
Lipase- c.c. N/20NaOH required per whole liver	7964	7496	6135	6541	6917	8158:
Amylase - units per sample	482	318	241	602	267	370 :
Amylase - units per 10 gm. fat and sugar free liver	535	362	272	674	301	433 :
Amylase - units per whole liver	3904	3213	2289	4699	2082	3442:

General Protocol Feeding Experiment No. 2

Series Number 4.

Hours after feeding	0	3	5	8	11	14
Weight of animal - grams	1200	1200	1200	1250	1050	900
Liver, total wt.- grams	43	37	42	38	34	33
Fat in sample - grams	.480	.682	.464	.613	.480	1.09
Fat in whole liver - grams	2.06	2.52	1.94	2.33	1.63	3.62
Fat - per cent	4.8	6.8	4.6	6.1	4.8	10.9
Fat per fat and sugar free sample - per cent	5.3	7.3	5.0	6.4	5.5	12.7
Dextrose in sample - milligrams	589	77.7	517	836	915	322
Dextrose per whole liver- mgms	2536	287	2172	3178	3112	1064
Dextrose - per cent	5.89	0.77	5.17	8.36	9.15	3.22
Dextrose per fat and sugar free sample - per cent	5.5	0.8	5.6	8.7	10.6	3.7
Lipase - c.c. N/20 NaOH required per sample	3.42	260	342	183	216	163
Lipase - c.c. N/20 NaOH required per 10 gm. fat and sugar free sample	379	281	376	212	251	190
Lipase - c.c. N/20 NaOH required per whole liver	1163	963	1437	737	737	540
Amylase- units per sample	535	321	229	385	482	241
Amylase - units per 10 gm.fat and sugar free liver	593	346	252	447	559	279
Amylase - units per whole liver	2300	1187	963	1465	1638	795

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UNIVERSITY OF MISSOURI
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DEPARTMENT OF PATHOLOGY

May 26, 1916.

Dean Walter Miller,
Graduate School,
University.

Dear Professor Miller:-

I beg to report
that I have examined the thesis of Mr.
Proviso V. Prewitt, and consider it to
be fully up to the general standard set
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Yours very truly,

DHD/O.

D. H. Dally.

University of Missouri - Columbia

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