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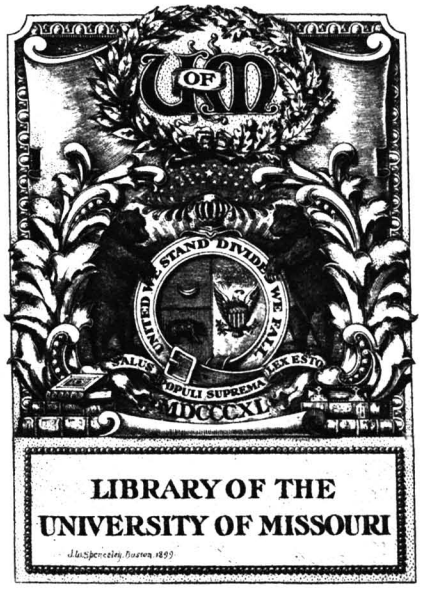

THE FAT AND LIPASE CONTENT OF THE LIVER

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THE FAT AND LIPASE CONTENT OF THE LIVER

Chapter I. Introduction

Kastle and Loevenhart[1] demonstrated the reversible action of animal lipase in 1900. This fact strengthened the conception of metabolism held by many biologists that all metabolism is a catalytic process effected by enzymes and perpetuated through the prevention of attainment of an actual equilibrium.

There are numerous works showing that under comparable conditions enzyme activity if not proportional to the quantity of enzyme present is very nearly so. We have found in the literature but little evidence showing whether with a fluctuation of the fat metabolism in an organ there is or is not a corresponding change in the lipase content of that organ.

The object of the present work is to ascertain if any broad correlation exists between the lipase content of an organ, and its state of fat metabolism. We have chosen the liver as an organ, with its lipase content and fat metabolism as the two factors under comparison.

It is expedient at the outset to review some of the properties of catalytic agents. Looked at chemically a striking characteristic of the changes taking place in the metabolism of the living organism is the ease with which bodies of highly stable nature are broken down. As an example,

glucose is oxidised to carbon dioxide and water, proteins to animo acids, and neutral fats to fatty acids and glycerine. Under ordinary laboratory conditions powerful reagents such as boiling hydrochloric acid are necessary to effect this type of hydrolytic splitting, in contrast with the facility with whic which it occurs in the living body, a fact towhich attention was called long since by Berzelius.

However it is known that under certain laboratory conditions, highly stable compounds can be greatly altered in their rate of reaction, either in their process of dis-association or splitting up, or in the converse of this reaction, synthesis or the building up. Oxygen and hydrogen, for instance, at ordinary temperatures combine so slowly that the product water cannot be detected by ordinary methods yet the addition of minute quantities of finely divided platinum is sufficient to greatly accelerate the reaction.

II. Review of Literature

A. Enzymes and Enzyme action.

I. Enzymes in General.

1. Early views.

Berzelius in 1835[2]* was one of the first to point out the possible biological significance of such accelerated reactions. He indicated the difficulty of explaining the complex phenomena of organic life with the aid of the chemical conceptions current upto that time, derived largely from the study or inorganic chemistry. He then drew attention to the discovery of certain reactions, in which one of the concerned members appeared to take no permanent part in the change but could be recovered unaltered in amount. Thus he refers to the formation of grape sugar from starch by the action of dilute acids as reported earlier by Kirchoff in 1814,[3] also the observation made for the first time by Dubrunfaut in 1830[4] that an extract of malt converted starch into sugar just as did the acid. Bayliss gives the quotation from Berzelius: "We have reasons, well founded on fact, to make the assertion, that in living plants and animals, there take place thousands of catalytic processes between tissues and fluids"

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* In reviewing the early literature we have summarized mainly from monograph by Balyiss "The Nature of Enzyme Action". In all such instances, note is made in bibliography.

(Berzelius coins the word catalysis from the Greek words κατα and λυο meaning loosening down.)

Three years after Dubrunfaut had reported on the activity of the malt extract, Payen and Persoz[5] precipitated by alcohol from such extracts a substance which could be dried and kept, this they called diastase.

As more substances with similar properties became known they were called "ferments", on account of the resemblance of their activity to the alcoholic fermentations. When Pasteur had shown that alcoholic fermentation was due to a living organism, diastase and other bodies like it were designated as "soluble" or "unorganized ferments" in contrast to the living organisms which were designated as "organized ferments".

The double use of the word "ferment" led to confusion, which induced Kuhne in 1878[6] to suggest a new name in an interesting passage, of which Bayliss gives the translation: "The later designations (e.g. organized and unorganized ferments) have not gained general acceptance, and in that on the one hand it was objected that chemical bodies like ptyalin, pepsin, etc., could not be called ferments, since the name had already been given to yeast-cells and other organisms (Brücke) while on the other hand it was said that yeast-cells could not be called ferment, because then all organisms, including man, would have to be so designated(Hoppe-Seyler) Without stopping to inquire further why the name excited so much opposition, I have taken the opportunity to suggest a new name, and I give the name enzyme to some of the better

known substances, called by many the "unorganized ferments". This is not intended to imply a particular hypothesis, but it merely states that enzyme something occurs (for example, in yeast) that exerts this or that activity, considered to belong to the class called fermentative. This name is not, however, intended to be limited to the invertin of yeast, but is rather intended to imply that more complex organisms, from which the enzymes, pepsin, trypsin, etc., can be obtained, are not so fundamentally different from the unicellular organisms, as some people would have us believe.

2. Definition of Enzyme

To realize the suggestion made by Berzelius, that is that there were close analogies between inorganic catalysis and organic ferments or enzymes, has been closely followed up and fairly established, we have only to refer to the opening lines of the late English edition of Euler's "General Chemistry of Enzymes".

"The name enzyme is given to animal or vegetable substances . . . which are able to accelerate chemical reactions. The term enzyme is thus included in the much more general term catalyst. By catalyst we understand a substance which, without being required by the accelerated reaction or appearing in the final compounds, alters the velocity with which a chemical strives to attain its final condition."

Bayliss[7] for convenience, divides chemical reactions into two classes: (1) Those which take place instantaneously, for example, silver nitrate and sodium chloride give at once a precipitate of silver chloride. (2) Those which take a measurable time to arrive at their final state, for example, the inversion or hydrolysis of cane sugar.

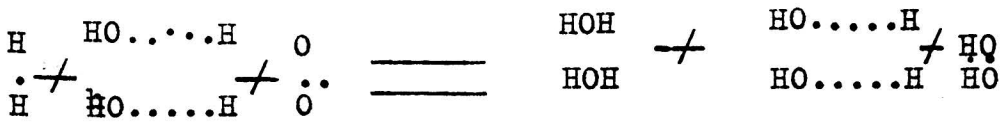
He then defines a "catalyst" as a body which alters the rate of reactions of this latter class. From the present status he defines an enzyme as the catalyst produced by the living organism.

Catalyst as originally defined by Ostwald[8] is of the same nature as those quoted above.

It should be pointed out that E. F. Armstrong and H. E. Armstrong[9] and others believe that a catalyst may start a reaction. These authors define a catalyst as "The agent which brings about the inclusion of the interacting substances in the circuit within which the change takes place so soon as the circuit is established, the electrolyte being the actual agent by which the change is effected". In their discussion they may be quoted as follows: "Obviously, however, the electrolyte may in some measure be regarded as the catalyst, and, as a matter of fact, it is generally regarded so in the case of the hydrolysis of ethereal compounds by acids.

"The distinction between the view promulgated by Ostwald and that which we advocate lies in the fact that we do not admit that action is either possible or ever takes

place between two non electrolytes, such as hydrogen and oxygen for example, but hold that action sets in only when a suitable electrolyte is present together with the two substances thought of as interacting directly, though in reality they interact only indirectly. Thus



(Electrolyte) (Electrolyte -2 H.OH)

"In such a case, the electrolyte is the catalyst, the occurrence of change being determined by and dependent on its presence: it does not merely accelerate the change but gives rise to it by making it possible. The change may be and is promoted, however, by the inclusion in the circuit of a substance, such as platinum, which by condensing or combining with the gases, promotes their association with the electrolyte. Therefore, if the term catalyst be restricted to materials which act merely by increasing the extent to which substances are brought into interaction and only as intermediaries, the definition given by the Ostwald school may be accepted as satisfactory: whereas, if the electrolyte be regarded as the effective catalyst, this is not the case, as the catalyst not only determines the occurrence of interaction, but contributes, ex-hypothesi, of its own substance, to the change and is only recovered unchanged, i.e. undiminished in amount because it is constantly being changed reversibly."

As to whether we are to consider that catalysts (enzymes) are able to initiate a reaction or not, is beside the question at point in this paper. In the above definitions, as will be noted, all agree on two points;- that a catalyst alters the rate of the initiated reaction, and that enzymes behave as catalysts. To the latter part of this statement there are certain apparent exceptions, to which we will refer later.

3. Terminology.

Before proceeding further with the question of enzymes, it is advisable to refer to the terminology of enzyme action in general. As previously mentioned, enzymes were first classed as ferments, later as unorganized ferments and later by Kühne in 1878 given the name enzyme. It is desirable to have a name for the substance on which the enzymes exert their activity. "Hydrolyte" would serve when the action is one of hydrolysis, but such terminology would exclude the synthetic processes such as the formation of neutral fat from the fatty acids and glycerine. Loevenhart and Peirce in 1906[10] suggest the use of the term "zymolyte". It has been objected to by Bayliss and others that the name applies only to the cases of enzymes and excludes other catalysts. The suggestion of difference is, we think,to be minimized. No really satisfactory name has as yet been suggested. On the whole, "substrate" as introduced by the German school, and now

used by the majority of writers, would seem to answer the purpose best, and will be used in this paper.

In following a uniform terminology in designating the different enzymes, it was suggested by Duclaux that the termination "ase" should be taken as denoting an enzyme, and that this ending should be added to the name of the substrate, e.g. lactase for the enzyme accelerating the hydrolysis of lactose, likewise, lipase, the enzyme accelerating the hydrolysis of fat lipoids. By far the great majority of the writers include in the term lipase, the enzymes which accelerate the hydrolysis of fat, also the enzyme which accelerates the hydrolysis of such other esters as ethyl buytrate, etc. Others use the term esteras for the enzyme which accelerates the hydrolysis of the lower esters, ethyl buytrate, etc., reserving the term lipase to designate those enzymes which accelerate the hydrolysis of what they term true fats. Since the true fats are also esters, it would seem that the term esterase can not be made specific. In this paper the term lipase will be used to include the enzyme which accelerates the hydrolysis of the lower esters such as ethyl buytrate. This involves the question of the identity of the enzymes which accelerate the hydrolysis of neutral fat and of such esters as ethyl buytrate.

Professor Armstrong[11] has pointed out that the terms "amylotic" and "protolitic", used to designate enzymes which attack starch and protein respectively are incorrectly formed. "Amylolytic in analogy with "electrolytic" should

mean a decomposition by means of starch. Professor Armstrong[12] advocates the use of the termination "clastic" instead of "lytic" in speaking of the action of any enzyme on its substrate. Professor Armstrong is also responsible for the introduction of the word "catalyst" as preferable to "catalyser" and he ventures to deprecate the use of the expression "to catalyse" - both because it appears to lack euphony and to be unnecessary if not undesirable."

II. Lipase

1. Hydrolytic and synthetic qualities.

The fat splitting enzyme now called lipase was discovered by Claude Bernard in 1856. The work on the lipases of the body tissues is reviewed by Connstein in 1904[12] and especially by C. Oppenheimer, 1909[13].

We are especially concerned with the lipase found in the liver. Hanriot in 1896[14] demonstrated the presence of a hydrolytic lipase in the extract of liver. Although the hydrolysis of starch into sugar by malt extract had been known since 1830, it was not until 1898 that Croft Hill[15] demonstrated the reversibility of this reaction. Using a 40 per cent solution of glucose, he demonstrated the synthesis of maltose in the presence of maltase. Kastle and Loevenhart[1] in 1900 were the first to demonstrate the reversible action of lipase. Fresh aqueous extract of liver, or of pan-

creas and of other tissues, were shown to synthesize with ethyl buytrate when placed with dilute buytric acid (N/10 to N/20) and ethyl alcohol (sufficient in quantity to bring the whole to 1½ per cent). The physiological importance of this reversibility of lipase action was justly emphasized by Loevanhart[16]. He explains the absorption of fat by this phenomena. Hanriot in 1901[17] obtained a buytric ester of glycerol, monobuytrin by means of lipase acting on their respective constituents. Pottevin in 1903[18] obtained synthetic mono- and tri-olein. Also Hammsick in 1909[19] and others have demonstrated the reversibility of enzyme action. It would seem that it is merely a question of proper conditions in order to be able to obtain synthesis from all enzymes.

2. Factors influencing the rate of action.

a. Coferment.

Magnus in 1904[20] working with the extract of beef liver, made the observation that its action on amyl salicylate, depended on the presence of two substances, one of the nature of an enzyme, non-dialyzable and destroyed by boiling, the other dialyzable and not destroyed by boiling, but soluble in water and absolute alcohol and not destroyed by ashing. This latter substance he called a coferment. Hewlett in 1905[21] was able to show that the pancreatic juice as secreted possessed comparatively little action toward the esters of the lower fatty acids. He holds that the principal function of the bile is to render the lipase more active.

A. S. Loevenhart in 1906[22] was able to confirm the observation made by Magnus, and further showed the so-called coferment to be bile salts. He also observed that when ethyl buytrate was used, no coferment was noted. He suggests that the action of the bile salts may have been to put the salicylate in solution. Therefore, he would deprecate the use of the term coferment as a general one.

b. Chemicals.

As regards other conditions, the first thing requiring attention is the part played by water. Hammsick[19] in particular has called attention to the importance that water plays. He employed a high degree of dessication and has succeeded in demonstrating a thirty per cent synthesis of tri-olein from its components.

That this fact is not antagonistic to the synthesis of fats in the cells, where it is known that the percent of water may be as high as 80-90 per cent, has recently been emphasized by Bradley in 1912[23]. He says:

"It has been shown similarly that pancreatic lipase tends toward the complete hydrolysis of triolein if water is present to the extent of 50 per cent of the reacting mixture, and the more water present the more rapid is the hydrolysis. If less than 50 per cent of water is present, equilibrium is reached before hydrolysis is complete. It requires, however, a relatively small amount

of water to secure a relatively large degree of hydrolysis and conversely a high degree of dessication is necessary to produce noteworthy syntheses. This fact, however, does not exclude the possibility of enzyme synthesis in cells where the total water content approximates eighty per cent, since there is abundant evidence of the fact that concentrations in localized portions of the cell may be far greater than in other adjacent portions."

Many chemicals are known to influence the rate of hydrolysis by an enzyme. Kastle and Loevenhart[10] first noted the inhibiting action of hydroflouric and sodium flouride on the ester splitting property of liver and pancreatic extracts.

George Pierce, working with Loevenhart, 1906-07[24] noted that very weak concentrations of sodium flouride, 1 to 500,000,000 accelerated, while solutions of a greater concentration inhibited. The greater the amount of enzyme present, the less the inhibiting effect of the given concentration of sodium flouride. He interprets this as proving that there exists a quantitative relation between the enzyme and the sodium flouride.

The same year C. G. Souder, working with Loevenhart[25] reports that bile salts, lecethin, and bile greatly accelerate the action of pancreatic juice on ethyl acetate, ethyl buytrate, ethyl propionate, diacetin, triacetin, and olive oil. They call attention to the fact that the effect of these accelerators on the hydrolysis of one ester, is no index to the degree of acceleration which will be noted on

other esters. They emphasize the fact that different experimental conditions alter greatly the degree of acceleration.

Oppenheimer in 1909[13] in speaking of experimental conditions, reports among other things too much toluol inhibits the enzyme action. The concentration of toluol employed by Kastle and Loevenhart was about two per cent.

Terroine in 1910[26] found that the amount of action of pancreatic lipase on olive oil was increased by sodium chloride, bromide, iodide and flouride in dilute solutions, but decreased in more concentrated solutions and in the order named.

Pekelharing in 1912[27] studied the action of a number of halides of the alkalies and the alkaline earths on the activity of pancreatic lipase in the hydrolysis of olive oil. He considered the increase to be due to the formation of insoluble soaps from the metallic elements added reacting with the oleic acid formed whereby the latter is removed from the sphere of action.

Falk in 1913[28] working with the castor bean lipase, reports on the action of various salts. Decreased activity as compared with aqueous solutions was shown by all the univalent salts observed (KI-NaF, K F; LiCl; Na $C_2H_3O_2$) and the decrease was proportional to the salt present. Since the potassium salts had a greater retarding effect thanthe sodium salts, he concludes that the metallic as well as the non-

metallic parts of the salts affect the activity of the lipase. Loevenhart and others having previously attributed the inhibiting action of NaF to the non-metallic element F. Increased activity was shown by dilute solutions of the chlorides of barium and calcium, by more concentrated solutions of sodium sulphate, by magnesium sulfate, and by the chloride and sulfate of manganese. He sums up by saying that "If an explanation of the retarding actions of the various salts be looked for, it may perhaps be found in the coagulation of the enzyme (either alone or together with other substances) by the addition of the salts, the ions of which produce their individual specific effects in each case. The unionized molecules may also take part in these reactions. The accelerations cannot be explained in as simple a manner except, perhaps, for the cases where increased formation of animal lipase (as by manganous salts) may be assumed."

In a later paper, Falk reports on the action of some organic substances on the activity of castor bean lipase. Methyl alcohol and ethyl alcohol show a marked retardation action. Glycerol shows no retarding action, except in very concentrated solutions. Methyl and ethyl esters show a small increase in lipolytic activity, with increased concentration of ester, while the glycerol esters show a marked increase in action with increase in the concentration of the ester. From the experimental evidence presented in these papers, he points out the possibility of explaining the selective action of lipases on different esters. This we quote:

"Now when these results are taken into consideration, it seems justifiable to extend the explanation advanced for the action of the simple alcohols to the action exerted by the simple esters; i.e. the ester causes a precipitation or coagulation of substances in the course of which the active lipase material is partially or wholly removed from the sphere of action. Methyl acetate and ethyl acetate show least increase in activity with increasing concentration of ester and therefore the greatest inhibiting action. Glyceryl acetate (triacetin) shows the greatest increase in activity with increasing concentration of ester and therefore the least inhibiting action. These results are exactly similar to those obtained with methyl and ethyl alcohols and glycerol. That the actions are not controlled entirely by the alcohol radicals is apparent from the fact that the triacetin, even the dilute solutions, do not show a proportionality between the amount of ester and the action. Falk advances chemical evidence which he believes to prove that enzymes are of a protein nature. In this view he is not followed by other authorities.

c. Radiations.

Enzymes are sensitive to certain radiations. Marshall Jr., and Rowntree in 1913[29] working on the theory that the increased growth in plants, noticed when they are subjected to radium emanation, and the reported therapeutic effect of

radium treatment in gout, was due to acceleration of an enzyme action. He found that no acceleration was experienced on the liver lipase (pigs) or the castor bean lipase, when these were subjected to radium emanations. On the contrary, inhibition of the activity is suggested.

Richards, 1914[30] finds that a short radiation with the X-rays has the effect of accelerating enzymatic activity, while a long radiation inhibits it. Between these two there is no effect.

d. Temperature.

Unlike other catalysts, lipase is destroyed by heat. Recently, Walker,Kendall and Day have observed a thermostable lipase, both in the filtrates of broth cultures of certain acid-fastbacilli and in the organisms themselves. An attempt should be made to choose an optimum where the proportional destruction of enzyme comes into consideration as little as possible. Euler[32] points out that optimum temperature is dependent on the amount of emzyme present, and the phase of the reaction considered.

Very ill-defined is the so-called "optimum temperature", the position of which depends entirely on the period or phase of the reaction considered. Indeed, even at the optimal temperature, the enzyme undergoes partial destruction during the reaction, so that if comparison is made of the time taken for the reaction to proceed to the extent of one-half, the optimum is apparently lower than if only the first fifth of the reaction is considered. For practical purposes

it is of interest to know the temperature at which the reaction proceeds most rapidly, and it would then be best to consider the time in which say, 90-95% of the substrate is decomposed. In any case in giving the optimum temperature, it must be stated for which stage of the reaction it holds.

Kastle and Loevenhart[1] left tubes containing 4 c.c. of water, 0.1 c.c. of toluene and 1 c.c. of a 10 per cent liver or pancreas-extract for five minutes in baths at 40°, 30°, 20°, 10°, and -10°C so that they assumed these temperatures. Ethyl buytrate (0.26 c.c.) was then added and the solutions titrated after 30 minutes. Their results are tabulated as follows:

Temperature	P Percentage hydrolysed	
	By liver-ext.	Pancreas-Ext.
40° C	11.29	2.82
30	5.96	3.16
20	5.27	2.51
10	3.89	1.88
0	2.26	1.25
-10	0.70	

The value obtained by Kastle and Loevenhart with liver-extract at 40° C has been questioned recently by Euler. Hanriot obtained similar results with his "esterases" from serum and pancreas.

It has been shown that lipase is much more resistant to heat in the presence of its substrate, expecially if the substrate be a fat. In 1890 Sullivan and Tompson[33] found that invertase would withstand uninjured, a twenty-five degree higher temperature in the presence of cane sugar than in its absence. As they point out, it is difficult to conceive how this is accomplished unless the enzyme enters into some kind of a combination with the sugar.

For lipase the optimum temperature is variously given in the recent taxtbook. Euler gives 38°C as an optimum with a maximum of 60° to 75°C. These factors, especially the latter will be influenced by the nature of the preparation. If in solution the presence and concentration of other substances will influence both the optimum and the maximum temperature.

e. Ratio of Enzyme and Substrate.

Aside from the numerous substances not true members of an enzymatic equation which may influence the rate of reaction, there are the true elements of the equation, as for example, the enzyme, the substrate, the products, and the inter-relations of the last two with the first, all of which are known to influence the rate of reaction. Already, it has been pointed out that the enzymatic reactions differ in certain details from the reactions in which an acid is the

catalyst. In the latter case the reaction progresses in accordance with the law of mass action. This law tells us that a reaction proceeds at a rate proportional to the concentration of the reacting molecules.

One of the most important physical properties of enzymes is their colloidal nature. This is shown by the fact that they do not pass through parchment, or do so very slowly. This property would seem as Bayliss states it the "only essential one which distinguishes enzymes from the other organic catalysts such as animo acids, etc. The distinguishing characteristic of a colloid is the enormous development of the surface. In enzymic action, the process seems to occur at the boundary surface between two phases, e.g. the enzyme and the substrate.

Thus, enzymatic reactions differ from those effected by inorganic catalysts, inasmuch as their velocity is determined not alone by the concentration of the catalyst (enzyme) but also by the concentration - ratio between enzyme and substrate. Given an excess of the substrate the velocity of reaction is approximately proportional to the concentration of the enzyme.

George Peirce in 1910[34] showed that in solutions of equal acidity a given amount of enzyme hydrolyses ethyl butyrate with the same absolute velocity, in other words above a certain concentration of the substrate with a given amount of enzyme the rate of acid production is nearly proportional to

the time.

If the enzyme be present in excess, the velocity will vary nearly proportional to the substrate present. If the substrate be present in excess, the velocity will vary with the proportion of enzyme present. This indicates that the enzyme in some way enters into a quantitative combination with the substrate. This is in no way adverse to our conception of catalytic action in general.

Clement and Desormes as early as 1806 in their "Theorie dela fabrication de l'acide sulfurique", in one of the first explanations of a catalytic process - the oxidation of sulphur dioxide to sulphuric acid by nitrous acid, formulate a theory of a temporary combination between the substrate and catalyst. The actual proof of such intermediate combinations was given by Brode in 1901.

Peirce showed that a large part of the so called "free enzyme" that not in combination with sodium flouride was in combination with the substrate, ethyl buytrate.

There is also evidence that the enzyme enters into combination with the products of the reaction as would be expected if both the hydrolytic and the syntheitc processes are catalysed. O'Sullivan and Tompson, in the work mentioned above showed invertase was protected from the action of heat by products of the inversion of cane sugar as well as by the sugar itself.

Frank Armstrong, in 1904[35] shows that the respec-

tive products of the "sucroclastic" enzymes,- lactose, maltose, invertase and emulsion, show to a certain degree a selective retarding influence on their respective enzymes. For example, fructose retards invertase, but has less effect on any one of the other enzymes. Likewise glucose has very little effect on the maltase but considerable retarding action on lactase.

An instance of one of the products of the reaction accelerating the reaction while the other product exerts a retarding influence is reported by . H. E. Armstrong and his co-workers in 1913[36]. Working with urase they observed that of the two products ammonia reduces the rate while carbonic acid accelerates the reaction. In the terminology of Euler[27] the two assumed molecules formed between the enzyme and substrate and the enzyme and product are called "enzyme-substrate" and "enzyme-reaction product." The enzyme appears as a rule to enter into a state of association with some particular molecular grouping in the substrate or in the reaction product. Now if this grouping is very uncommon, the enzyme will be very"specific".

It is held by Oppenheimer that there are many specific lipases, each of which is responsible for the hydrolysis of a different ester or a different fat. One of the main reasons for his view is the different action of the bile salts in the various cases. Terroine showed in 1910[37] that in order to compare these actions properly esters of

the same acid must be taken. He took therefore triacetin, as a glycerol ester, also the acetic esters of amyl, propyl, ethyl and methyl. Under these conditions he found that the optimum concentration of bile salts was the same in all cases. He concluded that the enzyme concerned in the hydrolysis of the different lower esters and fats is the same.

Correlating this idea of the non-specificity of lipases and the nature of the association between enzyme and substrate, we shall quote the theory advanced by Professor Armstrong:[9]

"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.

It may well be that the configuration of the en-

zyme is such as specially to favour its association with glycerides of the higher fatty acids. But the association of enzyme and hydrolyte is doubtless determined by an intervening water film, i.e. the carboxylic centres in the two compounds are both to be thought of as hydrolated and as brought into contact through the agency of the attached water molecules. The number of molecules thus activated will depend on the osmotic conditions which prevail in the mixture undergoing change.

Loevenhart[38] reports certain apparent differences between the lipase of the liver and the lipase of the pancreas.

Dietz in 1907[39] was concerned with the action of pancreatic lipase. In obtaining an exact equilibrium the experiments were carried out from both sides simultaneously, on the one side a solution of ester in water and amyl alcohol was taken and on the other side an equally concentrated solution of buytric acid in amyl alcohol and water.

There are several points of importance to be noted in this experiment. First, the equilibrium position as indicated by the final concentration of acid is the same whether approached from the side of the acid or from the side of the ester. It was also shown that where preparations of different activity as shown by their initial velocity were taken the point at which they reached an equilibrium was the same. Similarly, varying the concentration

of the same preparation did not affect this point.

Arguing from the many known factors which influence the rate of enzyme action in vitro one may assume factors in vivo which will also influence the rate of enzyme action. It is conceivable that the rate of enzymic activity in metabolism can be altered without any material fluctuation in the amount of enzyme. A simpler and more probable assumption is that a fluctuation in metabolism of an organ takes place in correspondence with a variation in enzymic activity induced by the fluctuation in the enzymic content of that organ. A third assumption is that the organ is one of the enzyme producing organs and therefore has an excess of enzyme in it. With such conditions there might occur an increased metabolism in the organ without an increase in the enzyme content.

The physiological significance of a correlation between enzyme and metabolism was first pointed out by Loevenhart in 1902[16], He gives little or no data to show that the correlation does or does not exist. He reports some observations that are very suggestive, for example, that the inactive mammary gland has only one tenth the lipolytic activity of the active gland, that adipose tissue is more active than muscle or kidney.

A paper published in 1912, by Bradley[40] is the only one in which we find figures given not only for the

fat content of animal tissues but for the lipase as well. In his experiments Bradley deals with only one phase of metabolism, namely, the fat and enzyme content of the tissue in periods of plenty. He concludes:

"1. That no broad correlation exists between the fat and lipase content of tissue.

"3. Some of the most active fat producing tissues are relatively poorer in lipase than many other tissues which never normally contain or produce more than a small percentage of fat. . . . ."

Active mammary tissue affords the most striking example of this when compared with lung, kidney and muscular tissue, kidney having three times the lipolytic activity.

B. Fats and fat variations of the liver.

1. Mode of accumulation

A. Physiologic infiltration versus transformation.

Claude Bernard first showed that the liver was closely connected with the general metabolism of the body. Since that time the view of the older physiologists that the liver was simply a bile producing gland has gradually lost ground. At present, the liver must be regarded as having its predominating function in the regulation of the supply of the carbohydrates and the protein material of the body, rather than as a digestive gland. Just what is the function of the liver with reference to fat metabolism is not so well known.

The liver is on the direct channel for absorption of the carbohydrates and the proteids, but is not on so direct a route in the case of the absorbed fat. Numerous works have been published to show that at least the greater part if not all of the fats are absorbed by the lymphatics hence reach the liver only through the hepatic artery. One of the first to demonstrate this fact was Munk in 1891[41] and more recent the question is reviewed by Mendel and Daniels in 1912[42].

From an early date both the physiologist and the pathologist have granted that the liver was a fat con-

taining organ but as to the causes of the accumulation of fat in this organ they have disagreed. Generally speaking the pathologists have advocated the theory that an accumulation of fat in the liver is pathological a product of a degeneration occurring in the liver.

Opposing this view are those pathologists and physiologists who say that the accumulation of fat in the liver is not pathological and therefore not degenerative. They consider the accumulation of fat in the liver to be a normal phenomenon, i.e., a product of physiological activity. As to the nature of this activity they are not wholly in accord. One division explains the accumulation of fat in the liver as an infiltration or deposition of transported body fats. The other division explains the accumulation of fat in the liver not as an infiltration of extraneous fat, but as a transformation of the liver glycogen into fat.

Granting that the liver normally is a fat containing organ the question that interests us is, what are the factors influencing the fat content of the liver and what part does the organ play in fat mobilization and utilization.

Nosse in 1886[43] comes near suggesting what we now consider to be the probable function of the liver in fat metabolism. He compares the fatty condition of the liver to the conditions of sprouting in fat containing seeds.

in which the same high proportion of fatty acids exists. To account for the accumulation of fat in the liver, he suggests that there must be a transportation of the fat to the liver from other parts of the body. He is constrained to admit such a transportation of the fat of the body to the liver, and indeed an accumulation of the fat of the body in the liver has been proved by figures only in phosphorous poisoning. In other words, there were no actual figures showing that this accumulation of fat might occur as a physiological condition. Yet quite early there was histological evidence showing accumulation of fat in the liver, under conditions that were not universally considered pathological.

Thus Langley in 1885[44] gives a series of histological observations on the amount and distribution of fat in the liver of frogs under different conditions. He found the fat to accumulate during the cold months and to dissappear during the summer. Winter frogs exposed to a high temperature showed a decrease of fat in the liver. Immediately after the taking of food the hepatic fat decreased only to be later increased in amount and later still to fall to normal.

Such observations as these discredit the then predominating idea that the accumulation of fat in the liver was always a pathological condition a product of degeneration occurring in the liver.

Reviews of the literature of the period of

1885-1894 quote a number of papers reporting accumulation of fat in the liver in certain morbid conditions, phosphorus poisoning, etc. The greater number of these support the degeneration theory.

Opposing this theory there is considerable work published on the increase of fat visible in histological preparation during a fast and after feeding.

Rosenfeld and others point out "that heart and other tissues giving the recognized histological appearance of fatty degeneration, e.g. excess of fat, may by extraction actually have less fat than in the normal. Mattstrom[45] showed that this is not true in the case of the liver, rather he finds that the histological picture and the ether extraction show a close parallel.

Statkewitsch in 1894,[46] working on guinea pigs, dogs, cats and rabbits, reports that after a loss of the initial body fat amounting to ten to fifteen percent there was an appearance of fat globules in the inner cells of the lobule of the liver. As the fast was prolonged, the globules increased in size and number. The best results were obtained with guinea pigs.

Nikolaides in 1899[47] working with dogs without food for two to twenty days confirmed the appearance of fat in the liver and other glandular organs. In the extreme limit of the fast (30 days) instead of fat globules he found vacuoles that supposedly once contained fat.

Gilbert and Jamier in 1899[48] experimented on dogs and rabbits during fasts ranging from 25 hours to 8½ days and found a greater amount of fat in the livers of hungry animals on the average than in the livers of fed animals. No statement is made as to the relative time of feeding animals and their killing.

Noel Paton in 1895[49] published a paper on"Relations of the Liver to Fat". This work is especially of value for the breadth of view and scope of the investigation. Thus he shows:

"1. Throughout the various parts of the liver there is a uniform distribution of the substances soluble in ether, that is, so-called"fats".

"2. In animals in the same condition the percentage amount of substances soluble in ether is fairly uniform."

In a small number of experiments reported on the influence of feeding, he says:

"These experiments prove that an excess of fat taken in the food is largely stored in the liver cells, from which it is gradually got rid of."

Noel Paton found that the liver after a fat meal returned to normal in 70 hours. (Animals used were pups kittens, and young rats.)

Still other experiments on feeding were performed by Noel Paton in which the animals were given a diet rich in

carbohydrate. The animals were then fasted and killed on successive days. It was found that there was a marked increase of fat in the liver at the period when the glycogen had reached a low level. That there was an actual accumulation of fat is shown by the fact that the fat increased in relation to the liver solids deprived of glycogen and of fat.

Is this increase due to an increased formation of fat from the glycogen in the liver, or is it due to a transportation of fat to the liver from the body. Paton favors the latter view. He calls attention to the well known fact that the liver fats have a higher melting point than the body fats. He then makes the assumption that if the increase of fat in the liver is due to a transportation of the body fats, which have a lower melting point, there should be a lowering of the liver fats' melting point.

In support of his assumption he gives figures showing that after a fatty meal of low melting point, there is a rise in the amount of fat in the liver accompanied by a lowering of the melting point of the fat in the liver.

Noel Paton's experimental data showed that during a fast there occurred an increase in the liver fats in the rabbit with no change in the melting point. He concluded that the rise in the fat in the liver during a fast is due to a transformation of the liver's glycogen into fat, and not to a transportation of fat to the liver from the body.

Bloor's[50] more recent work demonstrates the most significant fact that as the fats of a meal are absorbed

their melting points may be materially changed. It is evident that the melting point of a fed fat can not be an index to the melting point of the absorbed fat which reached the liver. This invalidates the analogy drawn by Noel Paton and weakens his argument for the sources of the liver fats in the liver glycogen.

Further, it is argued, that if the fat accumulations during hunger is derived from glycogen, we should expect the accumulation to be most active during the period of rapid dissappearance of glycogen, and not some time after the glycogen has reached a low level, a time at which experimentally the liver fat is shown to rise.

Nosse[43] favors the transportation theory and in support of this says that in starvation the serum may become milky from the taking up of the fat from the body. This Noel Paton tested by feeding three rabbits on turnips from the 5th to the 7th of February, then killing one at once as a normal which showed 1.75% fat in the liver, the second after 36 hours which had 2.36% of fat, and the third after 52 hours with 5.26% of fat in the liver. In no case was he able to observe the milky serum reported by Nosse. He concludes that the evidence as he finds it tends to militate against the transportation theory of Nosse and in favor of the transformation theory.

b. Pathological - Degeneration.

There is a third possibility, one which the adherents to the fatty degeneration theory advance, that is, that the fat which is known to accumulate in the liver during hunger comes from the proteins of the organ. They assume that normally a large percent of the fat of the liver and of other organs is held in a chemical combination with the protein of the organ and that such a compound does not respond to the usual methods of demonstrating fat. This compound is broken down during the fast and the fat is therefore liberated and becomes evident. If there is an actual destruction of liver-protein or liver substance, we should expect to find a decrease in the other solids with the rise in fats, a fact which will be checked in the experimental work of this thesis.

More recent work of Mettram[45] showing a marked fatty infiltration in rabbits after so short a fast as twenty-four hours lends discredit to the degeneration theory. The fast is too short to assume that any degeneration would occur.

If the fat that accumulates in the liver during a fast is due to a transportation of the depot fats to the liver, then we should expect an increase in the total solids of the liver, at least this should occur after the first twenty-four hours during which time any increase in the

solids which might occur with the transportation of the depot fats would be offset by the rapid depletion of the liver glycogen.

Further since Hartley in 1909[51] shows conclusively that the fat of the liver has a high iodine value, whereas the depot fats have a low iodine value, in the transportation of the depot fats to the liver we might expect a fall in the iodine value of the liver fats. Just such conditions as these are reported by Mottram[45] that is, during a fast, there is a lowering of the iodine value of the fat of the liver showing a transportation of the fat of the body to the liver has accrued. Similarly, there is an increase in the percent of the total solids in the liver. The solids fall below normal for the first twenty-four hours, due to the depletion of the liver glycogen.

2. Chemical changes induced in fats.

Had Mottram's results shown no change in the iodine value it would not have weakened the theory that the depot fats are transported to the liver during a fast, since it is highly probable that the liver alters the iodine value of fats transported to it. Indeed Mottram concludes that there is chemical and histological evidence that fats transported to the liver under go changes and that the changes are of the nature of unsaturation of the fatty acids.

Leathes and Wedell[52] fed oils of a high iodine value to animals (rats and cats). It was found on exam-

ination of the fat from the various organs that the liver fat had a higher iodine value ~~that~~ that of the oils administered, while other organs such as the kidney, etc. showed little fluctuation in the iodine value of their fat. From this it was concluded:

"1. That the liver as well as the connective tissues takes up fat conveyed to it by the blood. The other organs do not at any rate to the same degree.

"2. But whereas the connective tissues store the fat as brought to them, the liver changes the fatty acids in such a way as to increase their power of absorbing iodine. This maybe interpreted as due either to the introduction of new unsaturated linkages, or possibly to the transposition of existing ones from situations in which they are less liable to saturation by halogens (or hydroxyl groups) to others in which they are more so."

Indeed, we may refer to Hartley[51] for evidence of the shifting of the double linkage of a fat stored in the liver. He reports that the double linkage in the oleic acid of the liver lies between carbon 6 and 7 recovering from the methyl group of the chain, while the storage oleic acid is known to have the double link exactly in the middle of the chain.

Raper in 1913[58] attempts to prove or disprove a third possibility, namely that the liver has a particular affinity for the fats of a high iodine value and rejects the saturated ones. He administered highly saturated oil

(cocoanut oil) to animals by mouth, by introducing soap solutions into the small intestine and intravenous ingestion of a fine emulsion of cocoanut oil. When the emulsion was given intravenously, 25-60% of the oil which enters the systemic circulation was found in the liver. When the iodine values of the volatile acids obtained from the liver in these experiments were examined, it was found that they absorbed more iodine than the volatile acids from normal livers. To quote the author: "The increase was not great but it probably indicates that saturated fatty acids containing 10, 12, or 14 carbon atoms may become unsaturated in the liver."

Cocoanut oil administered to cats and dogs by the mouth was detected in the liver in five or six hours, the amount present not exceeding six per cent of that absorbed. If a cat be anesthetized (urethane or ether) and a solution of cocanut soaps containing glycerine and bile salts be run into the small intestine, then about thirty percent of the absorbed fatty acid is found in the liver. Raper suggests that it is probable that the greater retention of oil by the liver when it is administered in the form of soap of a fine emulsion is partly due to the anesthetic and partly to the rapidity of the administration. He apparently did not consider another factor, namely, the rate of absorption.

Thus we have a suggestion that the fat content of the liver fluctuates with the fat content of the blood,

e.g. with a sudden rise in blood fat there is a corresponding rise in liver fat. That the fat variation in the liver will be superposed on the fat variation in the blood.

Mottram[45] using fasting rats found that there existed a double wave of variation in the fat infiltration of the liver, the first crest coming at the end of twenty-four hours and the second at the end of four days. The author points out that if this durable variation is normal then it is extremely necessary that experiments be run in series. There is also the possibility that the curves may be lengthened either vertically or longitudinally with changes in the seasons, or, as Perls[55] suggests, the power of accumulation of fat in the liver may vary in different animals. He states that fatty infiltration is not easily produced, even by phosphorus poisoning.

Mendel and Daniels[42] in 1912 showed that the fat-soluble dyes are more soluble in the bile than in the fat. This explains why these experimenters who have used stained fats as a means of testing infiltration have not been able to confirm the fatty infiltration in the liver either during fasting or after a fatty meal.

Smirnow[54] in 1913 reports that the amount of water ingested influences the rate of the fatty change in the livers of fasting rabbits. Fasting unwatered rabbits for ten days and upwards showed a decided fatty infiltration of the liver, apparent both on gross examination and microscopically. Fasting animals that have been allowed all the

water they will take may show similar changes in the liver but the percent of incident is much less. He was not able to confirm Mottrams observations[45] of an increase of the fat in the livers of rabbits after twenty-four hours fast.

Coope and Mottram, 1915[55], one of the latest papers, reports both histological and chemical data showing that in late pregnancy and early lactation there occurs an accumulation of fat in the liver. Coope and Mottram emphasize that accumulation of fat in the liver does not indicate a morbid condition of that organ. They may be quoted:

"Fat infiltrations of the liver are usually regarded as being pathological and not physiological. Thus, they accompany many pathological states of the body: e.g. diabetes, mellitus, pancreatic and phloridzin diabetes, alcohol and chloroform poisoning, pernicious diarrhoea, cyclical vomiting, and typically phosphorus and arsenic poisoning. Against this view, however, Rosenfeld has persistantly protested, and Leathes with his theory of the function of the liver in fat metabolism, explains it as a normal phenomenon. According to Resenfeld, the liver is calling up the last reserves of the body to meet an emergency, presumably in its own tissue, and so may be regarded as functioning normally. Leathes suggests that the fat is being mobilized in the liver, there to be desaturated and passed on to the tissues for consumption wherever metabolism is active. both regard the increased amount of fat in the liver as

a sign that the liver is functioning normally. We have a further confirmation of the theories of Rosenfeld and Leathes that a fat infiltration of the liver does not indicate a morbid state of that organ, but that it is functioning normally."

3. Mechanism of Transference of Fat.

Both Rosenfeld and Leathes regard the accumulation of fat in the liver during a fast as a normal physiological process. But neither of them make any definite statement as to the nature of the mechanism which is concerned in eroding away the depot fats, or how once in the circulation the fats are caught up and transferred to the liver cells, nor how the fat is again released from the liver cells to passon to the tissues for consumption wherever the fat catabolism is active. The broad biological significance of the reversible action of lipase was first pointed out by Loevenhart in 1902. The theory there advanced offered biologists a most attractive explanation of the chemical mechanism involved in the digestion, absorption, transportation, disposition and utilization of fat. Kastle and Loevenhart had proved that the intestinal and gastric mucosa possessed lipolytic activity. In their discussion they may be quoted thus: "In the epithelial cells there is undoubtedly a synthesis of fat from the absorbed fatty acids and glycerine, and

and it is believed that this synthesis is occasioned by an enzyme contained in the cells which is capable of effecting fat synthesis or fat decomposition according to conditions."

Greene and Skaer[56] made a study of the absorption of fat in the gastric mucosa and observed that there is a synthesis in the gastric mucosa and that it occurs with a definite cycle of variation in the fat content of the gastric epithelium and gastric glands which they explain on the theory of lipase variation in relation to the reversible lipase reaction during fat digestion and absorption. To quote from their summary:-

"5. The gastric epithelium and gastric glands of puppies and kittens both peptic and pyloric show a marked increase in the stainable fat after the taking of the first meal. Under ordinary conditions this fat is never reduced to the quantity and characteristic arrangement of that in the embryo.

"6. The amount of fat in the gastric glands runs a definite and characteristic cycle of variation in relation to the taking of fatty foods. The cycle is marked by an initial slight drop with a slow and prolonged rise to a maximal and fall to the normal. The extremes of the fat content of the glands vary much less than in the epithelium.

"9. In medium to late fasting there is occasionally an increased quantity of fat in the gastric gland cells.

This has no relation to absorption fat but is explained as due to mobilization of body fats."

The initial drop in fat content, they explain as related to the great increase in lipase produced by the activity of the gastric glands. This disturbs the balance of the fat in the gastric glands and in the mucosa leading to its disassociation and dissappearance. The increased quantity of fat observed in medium to late fasting is also explained as a disturbance as between lipase production and fat mobilization. "If one assumes that an excessive production of lipase takes place in these glands at the time during fasting when the fats are being dissolved from the storage tissues and are present in a relatively high per cent in the circulating fluids, it follows that there will be an increased synthesis of fats in the lipase producing tissues themselves"

Greene[57] has presented a unique condition of affairs existing in the salmon. The long period of fast which the animal undergoes during its migration is a perfectly normal process. Thus he is able to make observation on the phenomena of fat transference in the salmon's body under normal fasting conditions. His histological and chemical data on the fat in the different types of muscles at the various periods of the salmon's fast indicate that there occurs a synthesis of fat in the pink muscle fibers. In describing the microscopic picture he says: "The intramuscular fat after the beginning of the spawning

migration makes its appearance throughout the substance of the pink muscle fibers of all sizes. It appears in short chains of very small liposomes that are quite evenly interspersed among the groups of fibrillae of the muscle cells. This intracellular fat is present within the pink muscle fibers throughout the migration and at the time of death after the spawning." He considers the accumulation of fat in the muscles to be a normal process, the result of synthetic activity on the part of the muscles' lipase. He formulates a detailed working hypothesis concerning fat metabolism as related to lipase during a fast which may be quoted in part as follows:- "The transition from a feeding to a fasting state is associated with numerous tissue changes in other parts of the body, changes which are accompanied by equally important functional readjustments. Among the functional changes the one that most concerns the present argument is the increased production of the fat splitting enzyme, lipase."

"There is an abundant store of fat in the intermuscular depot, great quantities of it, and a lipogenesis comes to the support of the salmon in a way quite comparable to the glycogenesis of the mammal as conceived by Claude Bernard. Under these conditions the activity of the muscular tissue is directly dependent on the fat as a source of energy. The muscle oxidizes fatty bodies in the salmon, just as it oxidizes carbohydrate bodies in certain other well known animals.

"It is to be assumed that the muscle fibers absorb the fatty bodies from the lymph and blood, presumably as soluble fatty acids and glycerin. The fatty bodies of the blood and lymph are derived from the stored fat by a process of lipolysis. To that extent to which the store of intermuscular fat of the pink muscle is eroded by this process of lipolysis will the percentage concentration of the cleavage products of the pink muscle lymph be high. From the lymph the fat cleavage products dialyse directly into the pink fibers and become available for oxidation. In the early stages of the fast there are numerous tissues besides the muscle containing an excess of stored fat; the digestive tube, the pancreas, the liver, the skin, etc., as well as the connective tissue and the muscles.. . . . Hence with increasing production of lipase in the blood there is an ever-increasing percentage of fatty bodies thrown into solution in the blood and lymph.

"Hand in hand with the increase of fatty bodies in the blood and lymph will go an increase in fatty products in the substance of the muscle fibers. Muscular oxidations are not rapid enough to keep down the increasing quantity of fatty bodies, hence they will diffuse into and be present in the muscle fiber, a fact demonstrated for other muscle tissues. Under the law of reversible lipase action this excess of fatty cleavage bodies is bound to be reconverted into and deposited as

neutral fat. Thus arise the chains of microscopic liposomes of the pink muscle at the beginning of the salmon fast."

4. The specific problem in this work, with a resumé of the salient points in the literature concerned.

In summing up the review of the literature the facts that have been determined and which are recognized to play an essential part in fat metabolism are as follows: Fat metabolism is inseparably bound with the presence of a fat enzyme, lipase. Lipase is a reversible reacting enzyme. It is present throughout the animal tissues in varying quantities, but less in resting than in active organs, for example in resting mammary glands versus active glands. Certain particular tissues very active in fat metabolism show a very rich fat content. For example, the liver shows a variation of the percentage quantity of fat present in relation to feeding and fat metabolism. The problem of fat and fat distribution has been determined in numerous cases.

Concerning the lipase content of the tissues, less is known. The variation of lipase content under different physiological conditions such as feeding and hunger has been inadequately studied, and the relationship between the variation in lipase content and varia-

tion in fat content is practically an unexplored field. Theoretically, therefore, we should expect a relationship of lipase content to the fat content of a tissue. The question is, what is the nature of this fat lipase relationship? Is there any broad correlation between the factors that influence the lipase content and the factors that influence the fat content of an organ. To determine this question calls for consistent and parallel study of both the fat and the lipase content of some favorable organ. We have undertaken the solution of this problem by the determination of the fat and of the lipase content of the liver during fat feeding and during fasting.

III Materials and Methods

The animals used in the experiments were young puppies of the same litter, young kittens of the same litter, adult dogs and adult cats.

The method of observation rests on the determination of the weight of the ether extractable substance in the dry liver tissue of 10 grams of fresh sample of liver for the fat. The lipase 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 liver residue, actimg on .5 c.c. of ethyl butyrate and 4 c.c. of distilled water incubated at 38°C for one hour (or other time when specifically stated. The manipulation is as follows:- In each experiment a series of animals, usually of the same litter, were fed on a constant diet to secure relative stability of conditions, then food withheld for a definite time to insure complete dissappearance of previous food, usually 40 hours. At the end of this time one animal was decapitated and the liver taken as a normal. If the experiment was intended to make observation on the liver following a fat meal, the remaining animals were fed a meal of very rich cream and were decapitated at varying times during digestion and absorption. Where the experiment was planned to make observa-

tion on the liver during a fast the remaining animals were carefully isolated from all food. They were decapitated at different intervals of the fast. The liver was quickly excised and the fresh weight taken -- in most cases this was minus the gall bladder. In all cases at least the initial and final body weights were taken. Observations were made on the subcutaneous, omental, renal and pericardial fat; the color and quantity of the bile; the material contained in the stomach, the small and the large intestine and the lacteals.

W. S. Summers determined the fat and lipase content of the blood on these animals. For the sake of convenience and correlation of results, the designation of experiment and number of animal is the same in this paper as has been used in his work.

The Technique of the Fat Determination.

In most cases, three 10 gram samples of the liver were taken, - number one from the left lobe, number two from the right lobe and sample number three was taken at random. Sample number one and sample number two were immediately ground with ten grams of purified sand. The mixture of liver and sand was then transferred to a watch glass upon which the sample of liver had been originally weighed. It was then placed in a glass dessi-

cator over H_2SO_4, a partial vacuum created by use of the water pump and dried to a constant weight. The drying process was usually complete after 36 hours of such treatment. The dried sample of liver plus the sand was then transferred to a mortar, reground for two minutes, and then removed to a paper extracting cup. The watch glass on which the sample of liver plus the sand had been dried, and also the mortar in which the sample of liver plus the sand had just been ground, were rinsed with ether and the rinsings poured on top of the sample of liver and sand in the paper extracting cup.

For the extraction, Greene's modification of the soxhlet was used and a straight ether extraction made. The extraction was continued for six hours, the ether running over the sample on an average of once per minute. At the end of this time the paper cup now containing the fat free residue - the sand plus the fat free solids of the liver sample - was removed from the extractor and from now on the sample will be designated as the fat free sample. The ether was now removed from the fat by distilling and catching the ether in the plain glass cup. The ether flask which now contained only the fat was removed, placed in a vacuum incubator and held at 90°C for six hours. At the end of this treatment the ether flask plus the fat was removed and weighed, then returned to the vacuum incu-

bator and dried to a constant weight. The difference between the weight of the flask and the flask plus the ether extract was taken as the weight of the ether soluble fat content of the sample of liver. Two such determinations were made on the liver of each animal, number one from the left lobeand number two from the right lobe. The accuracy of this method is shown by the checks made on the respective livers. With careful work two determinations were made on the same liver usually checked within .2 per cent and never differed more than 1.0 per cent, even when the fat ran as high as 55 per cent.

The Technique of Lipase Determinations.

In making the fat free aqueous extract of the liver tissue the proceedure is as follows:- Each of the fat free samples - not later than 12 hours after the fat extraction -- were then transferred to a mortar and ground for two minutes, 35 c.c. of distilled H_2O was then added to the mortar and the grinding continued for thirty seconds. The fat free sample plus the water was allowed to stand 2 minutes, the solution was then filtered through a fine cloth into 100 c.c. volumetric cylinder. 35 c.c. more H_2O was added to the mortar and the sample transferred quantitatively to the cloth filter. The filtering was allowed

to extend over five minutes when it was completed by squeezing the cloth. The 100 c.c. cylinder was filled to the mark and this will be designated as the 10 per cent fat free extract.

With the great majority of the experiments a 10 per cent fresh turbid extract was also used. This was made up as described by Loevenhart. Ten grams of the fresh liver was ground with 10 grams of purified sand, extracted with water, and made up to 100 c.c.

The procedure in the quantitative work on the hydrolytic activity of the extracts was as follows:- In a medium sized weighing bottle was placed 4 c.c. of distilled water, .5. c.c. of ethyl butyrate, and about .08 c.c. -- three drops -- of toluol. The tops were then placed firmly so as to avoid evaporation, and the bottles with their contents were allowed to stand in the incubator for 1 hour at 38°C. They were then removed and to each of three bottles was added 1 c.c. of the well mixed fat free extract made from fat free sample number 1 -- taken from the left lobe. To a second set was added 1 c.c. of the thoroughly mixed fat free extract made from fat free sample number 2, taken from the right lobe. For each set two checks were run - check number one consisted of 4 c.c. of the distilled water and 1 c.c. of the fat free extract. Check number two consisted of 4 c.c. of the distilled water and.5 c.c. of ethyl butyrate. All tubes were then placed in the

incubator for the time indicated in the tabulations.

At the end of the incubation period the bottles were removed and 2 c.c. of 95 per cent alcohol added to each bottle. Titrations were made immediately against N/20 NaOH. Corrections were made for check number 1, and check number 2. These checks were always very small, number 1 never exceeding .14 and number 2 was usually so small as to be negligible. In the cases where double strength lipase was used, 2 c.c. of the 10 per cent fat free extract was added instead of 1 c.c. the remainder of the set up being the same as that just described.

Where the 10 per cent fresh turbid extract was used, the procedure was the same as that for the 10 per cent fat free extract, with the exception that 1 c.c. of the fresh turbid extract was added instead of 1 c.c. of the fat free extract. The fat free extract was in most of the cases more active than the fresh turbid. The exceptions being in those livers having a very high percentage of fat, 25 to 55 per cent fat.

The fat free extract, as here described by us, lends itself in this problem of correlation of lipase and fat content of the liver in a unique manner. Ether boils at 36° C. Lipase has as its optimum temperature 38°C and its maximum as 50 to 55°C. Thus by making a straight ether extraction of the dried tissue we were able to determine

both the fat and lipase content on the same sample, making the results more strictly comparable than where the fat content is determined on one sample and the lipase content on another. Further in any event it is not desirable to have in the system, in addition to the single pure fat or ester selected, a mixture of the different fats and lecethins of the liver, also the extract is least turbid and insures a greater degree of accuracy in titrating. Determinations made from the same extract correspond within .04 to .1 c.c. of N/20 NaOH. Taylor[58] has pointed out the advantage of a fat free extract in work on the castor bean lipase. He finds that "ferment is lost in this process -- i.e., the fat extraction by ether --.....the different fractions of ether employed in the successive extractions contains quantitities of ferment proportional to the fat contant."

We have evidence that some of the lipase is carried over in ether-fat solution, but the percentage is very small, as is shown by these figures. The whole of the ether-fat obtained from a 10 gram sample, pup number 1, feeding experiment number 1, when neutralized and incubated for 2 hours with 8 c.c. of distilled water and 1 c.c. of ethyl butyrate gave a titration of .40 c.c. N/20 NaOH. 1 c.c. of the fat free extract from the same animal incubated at 38°C with 4 c.c. H_2O and 5 c.c. ethyl butyrate for one hour gave a titration of 4.59 c.c. of N/20 NaOH.

Observation on the rate of lipase action was made in some dozen instances. The procedure was as follows:- After bringing a considerable number of bottles of the usual set up -- 4 c.c. H_2O and .5 c.c. ethyl butyrate, and three drops of toluol to temperature--38°C for one hour--1 c.c. of the fat free lipase extract was added and incubated. They were removed at 15 minute intervals, 2 c.c. of alcohol added and the titration made against N/20 NaOH. The results obtained show the greatest rate to occur in the first 15 minutes of the incubation. In the second 15 minutes the reaction proceded at practically the same rate as was observed for the third 15 minutes. The fourth 15 minutes shows a marked decrease in the rate of acid formation. Similarly the titration following the fourth 15 minutes showed progressive decrease in the rate of action.

Fasting Experiment Number 1.

The lipolytic activity shown by 1 c.c. of the fat free extract of the liver in terms of c.c. of N/20 NaOH at 15 minute intervals.

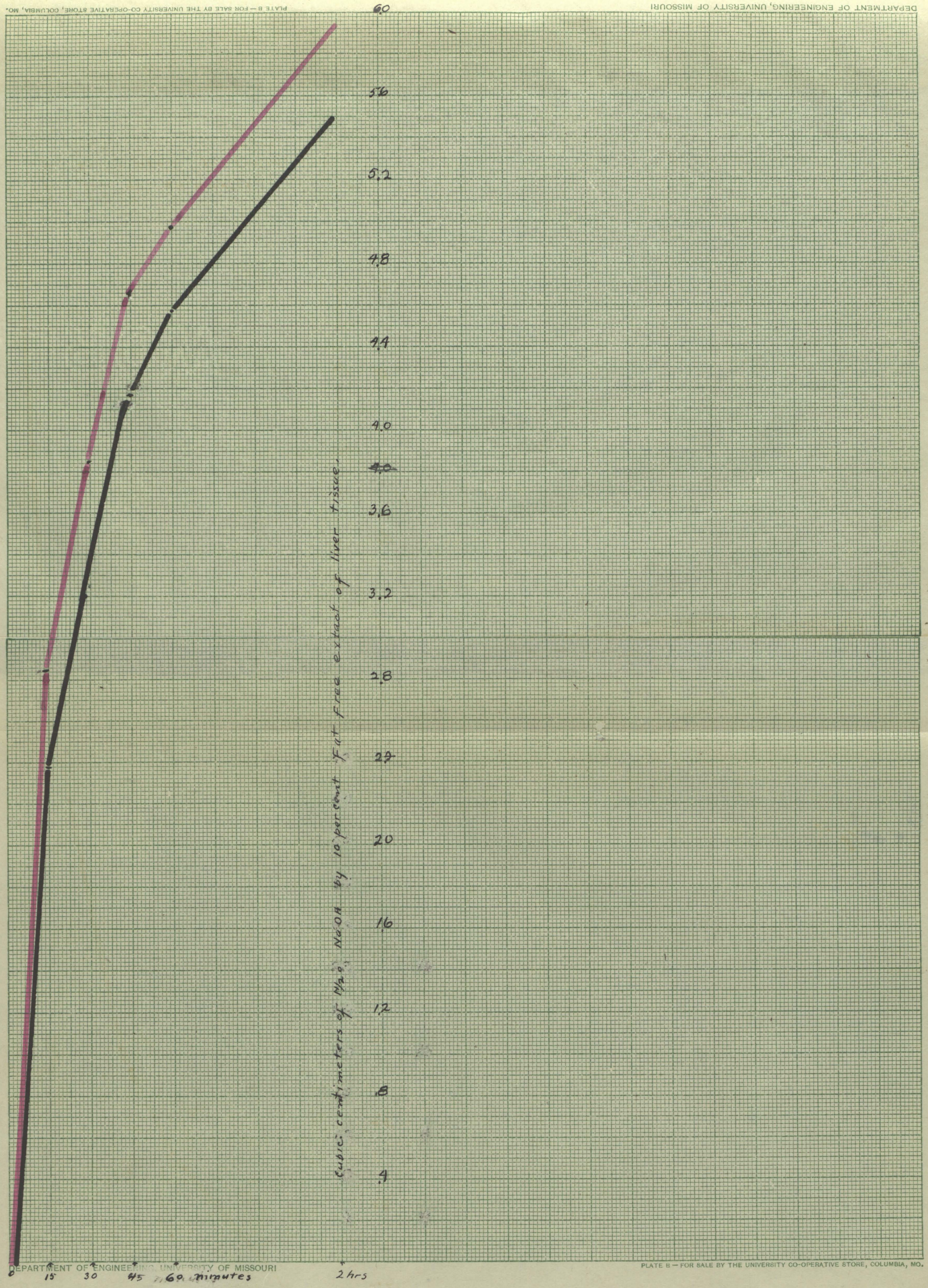
	1st 15	2nd 15	3rd 15	4th 15	2nd hr.	4th hr.
Normal pup	.94	.43	.42	.02	.19	
Pup fasted 6 days	1.24	.60	.65	.26	.60	.23

Fasting Experiment Number 2.

	1st 15	2nd 15	3d 15	4th 15	2nd hrs	4th hrs.
Normal pup: Number one:	2.38	.87	.92	.42	.94	
Pup fasted: two days	2.86	.99	.82	.31	1.00	

For the curves obtained from this data see charts number 1A. The lipase of fasting animal in red, the normal in black.

It is evident that the great proportion of the lipase activity occurs within the first hour and that the rate of activity within this period is quite regular. For these reasons most of our incubations were made for one hour.



Red — lipase per fasting pup.

Black — " " Normal " .

A. Experiments in the Feeding of Fat.

a. The determination of the amount of fat and of lipase in the liver in relation to the taking of a meal rich in fat.

We have taken pups of the same litter and withheld food for a definite time, usually 40 hours, in order to establish relative stability of fat metabolism. After this time a meal very rich in cream was fed. The animals were then decapitated at varying intervals during digestion and absorption, and the lipase and fat content of the liver determined on these animals. This test we have made on three series of animals with the view of throwing light on the lipase and fat variations during the normal cycle of digestion. We present our results in the following tables and corresponding charts.

Protocol of Feeding Experiment Number 1,

Series Number 1.

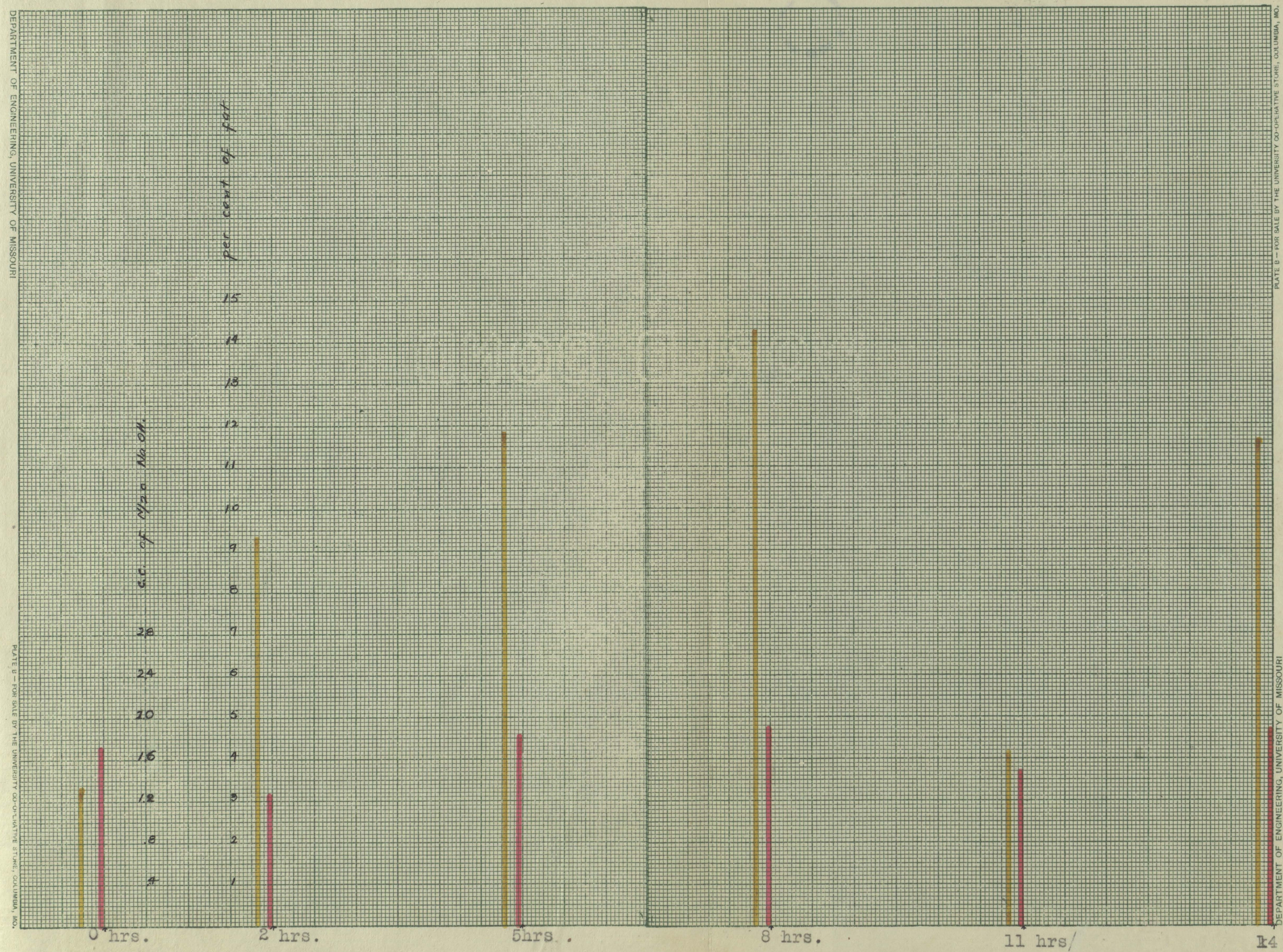
January 13, six pups of the same litter, about five weeks old in fine condition average body weight 750 grams, withheld food 44 hours for normal, at the end of which one animal was killed by decapitation as a normal standard and the remaining animals each fed 90 grams of 43 per cent cream. They were then decapitated at intervals of 2, 5, 8, 11 and 14 hours with the results shown in the following table.

Table number 1, giving the lipolytic activity in terms of cubic centimeters of N/20 Na OH - shown by one cubic centimeter of the 10 per cent fat free aqueous extract acting on .5 cubic centimeters of ethyl butyrate, 4 cubic centimeters of water, .08 cubic centimeters of toluol at 38°C for a period of six hours. The fat content is given in terms of per cent weight of the ether extractable substance in the liver tissue.

Hours after feeding	0-Normal	2	5	8	11	14
Cubic centimeters of N/20 Na OH	1.74	1.27	1.86	1.92	1.52	1.93
Per cent fat in liver tissue	3.55	9.40	11.55	14.32	4.13	11.60

Chart number I represents graphically the data recorded in the above table number 1. The yellow indicates the percentage fat and the red the percentage lipase content of the liver. The curve for the fat shows a progressive accumulation of fat in the liver to a maximum at the 8 hour period after the taking of the meal rich in fat. The curve for the lipase shows the percentage lipase content of the liver to remain relatively constant after the taking of the fat food.

CHART NUMBER I



After taking a meal rich in fat.
Yellow - per cent of fat
Red c. c. of N/20 Na OH- Lipase

Protocol of Feeding Experiment Number 2,

Series Number 2.

January 22, a series of four pups, three pups of the same litter about 10 weeks old in excellent condition and one odd pup of an odd litter not quite so well cared for, average body weight 2700 grams, withheld food for 60 hours at the end of which time the normal was decapitated; the remaining animals were each fed 270 grams of 33 per cent cream and decapitated at the times indicated.

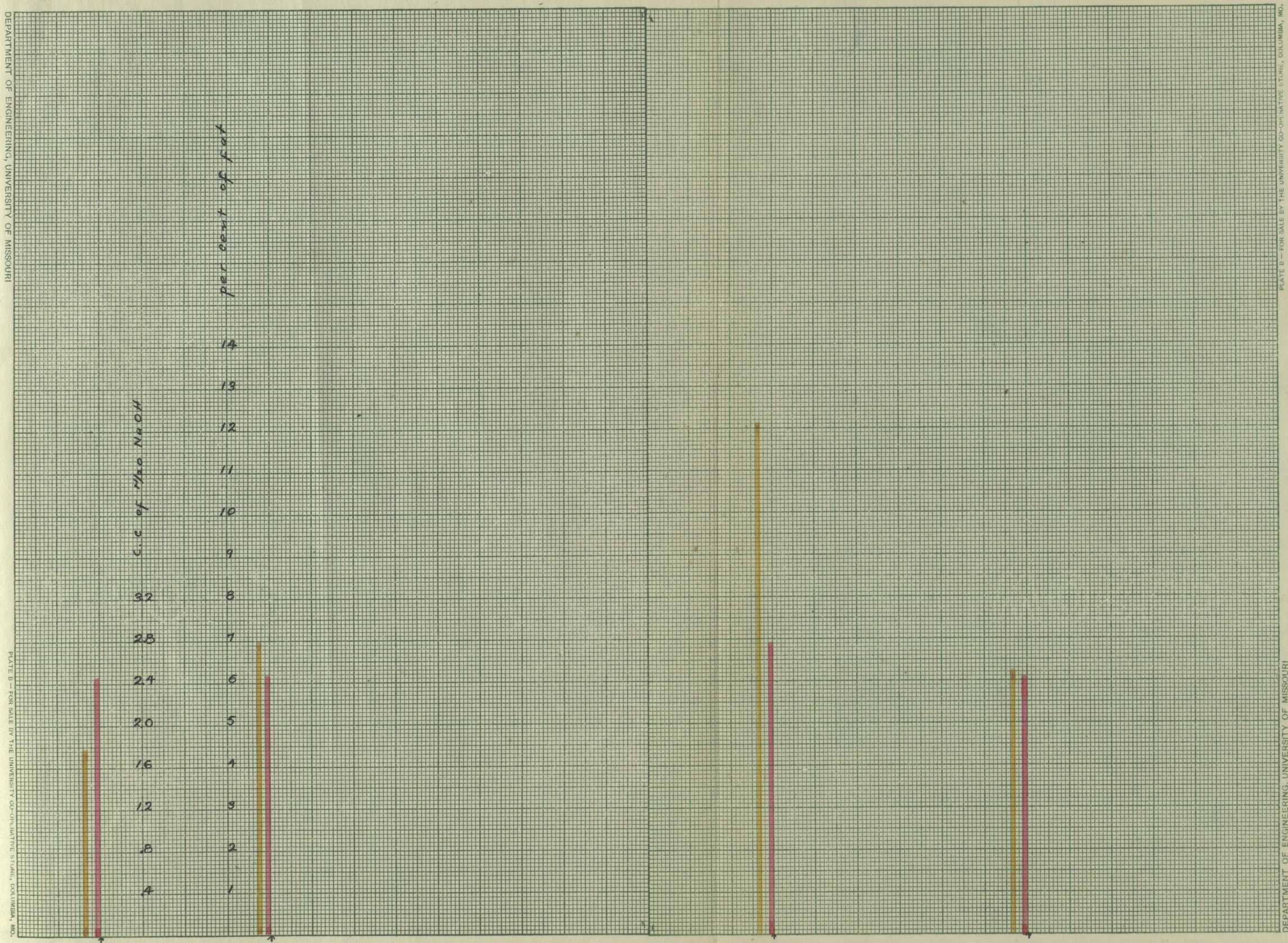
Table number 2, giving the lipolytic activity in terms of cubic centimeters of N/20 Na OH - shown by one cubic centimeter of the 10 per cent fat free aqueous extract acting on .5 cubic centimeters of ethyl butyrate, 4 cubic centimeters of water, .08 cubic centimeters of toluol at 38° C for a period of six hours. The fat content is given in terms of per cent weight of the ether extractable substance in the liver tissue.

Hours after feeding	0-Normal	2	8	11
Cubic centimeters of N/20 Na OH	2.47	2.51	2.77	2.45
Per cent fat in liver tissue	4.40	7.00	12.15	6.25
				Odd pup

Chart number II gives a graphic representation of the data cited in table number 2. The percentage of fat is represented in yellow, the percentage lipase in red. The fat curve shows an increase in the percentage of fat content of the liver to a maximum at the 8 hour period following the taking of the meal rich in fat. This maximum is followed by a decline in the percentage fat content at the 11 hour period. The curve for the percentage lipase is comparatively constant, showing only slight variation after taking the fat food.

The animals in experiment number 2 were kept without food longer than in other experiments the object being to obtain as normal a liver of lower fat content. As is seen, the normal animal fasted 60 hours in series 2 has a higher fat content than the animal fasted for 44 hours in series 1, 4.40 percent fat as compared with 3.35 per cent, the significance of which is developed in later experiments.

CHART NUMBER II.



After taking a meal rich in fat.

Yellow-percentage of fat. Red - c.c. of N/20 NaOH

Protocol of Feeding Experiment Number 3,

Series Number 3.

March 28, three pups of the same litter in good condition about six months old, one odd pup somewhat larger, average body weight 1266 grams, withheld food 43 hours at the end of which time the normal was taken; remaining animals fed 75 grams of 33 per cent cream and decapitated as indicated.

Table number 3, giving the lipolytic activity in terms of cubic centimeters of N/20 Na OH - shown by one cubic centimeter of the 10 per cent fat free aqueous extract acting on .5 cubic centimeter of ethyl butyrate, 4 cubic centimeters of water, .08 cubic centimeters of toluol at 38° C for a period of one hour. The fat content is given in terms of per cent weight of the ether extractable substance in the liver tissue.

Hours after feeding	:O-Nor-: mal :	5 :	8 :	11 :
Cubic centimeters of: N/20 Na OH	4.20:	4.16 :	3.88 :	3.81 :
Per cent fat in liver tissue	3.46:	4.40 :	7.45 :	10.71 :

Chart number III represents in a graphic manner the percentage fat and lipase content of the liver as given in table number 3 at the periods 5, 8 and 11 hours after the taking of the fat food. The curve for the fat in yellow shows a gradual increase in the percentage of fat up to and including the last period - eleven hours. The curve does not show a fall following the maximum. This we explain as due to the short period over which the experiement is extended. The percentage lipase content in red is again shown to remain comparatively constant after the taking of a fat meal.

CHART NUMBER III.

The most striking thing from the point of view of lipase content of the liver is the rigid constancy in the percentage of lipolytic action shown by animals of the same litter, or even between animals comparable as regards condition and age but of different litters. This constancy of the percentage of lipase content of the liver remains throughout the normal cycle of digestion and absorption of a meal of fat.

It may be objected that the percentage of lipase content aside from other data means nothing. Thus it may be argued that the infiltration of a non-lipase containing material would mask an increase in the lipase production of an organ. Further if the infiltration of this material occurred at a rate proportional to the lipase produced, the percentage lipase content would seem to remain constant though as a matter of fact increasing.

In our preliminary work there is experimental evidence which seems to show that lipase is only very slightly if at all soluble in fat. If we assume that the fat of the liver contains no lipase then following a fat meal a time at which there occurs an accumulation of fat in the liver there are just such conditions as would mask an actual increase of lipase in the liver at that time. In such a case it becomes necessary to state the lipolytic activity in terms of fat free fresh tissue, i.e. the weight of the fresh sample minus the weight of fat obtained which gives the weight of the fat free fresh sample, ~~i.e.~~ the

active tissue. The lipolytic activity per unit of fat free tissue is recalculated on this basis.

Computed on such a basis the lipase content of the liver in two out of three of our feeding experiments shows an increase per unit of fat free fresh tissue corresponding in a degree to the increase in fat in that organ. Or in other words with the increasing fat content following a meal of fat, there is an increasing total liver lipase per unit of active liver tissue.

The total lipase content may be conveniently indicated by the quotient obtained by dividing the total calculated ~~liver~~ lipase content of the liver as computed per unit of/fatfree sample, or active tissue, together with the lipase quotient and the percentage of fat of the liver for our three feeding experiments is given in the following table.

Series Number 1.

Feeding Experiment Number 1.

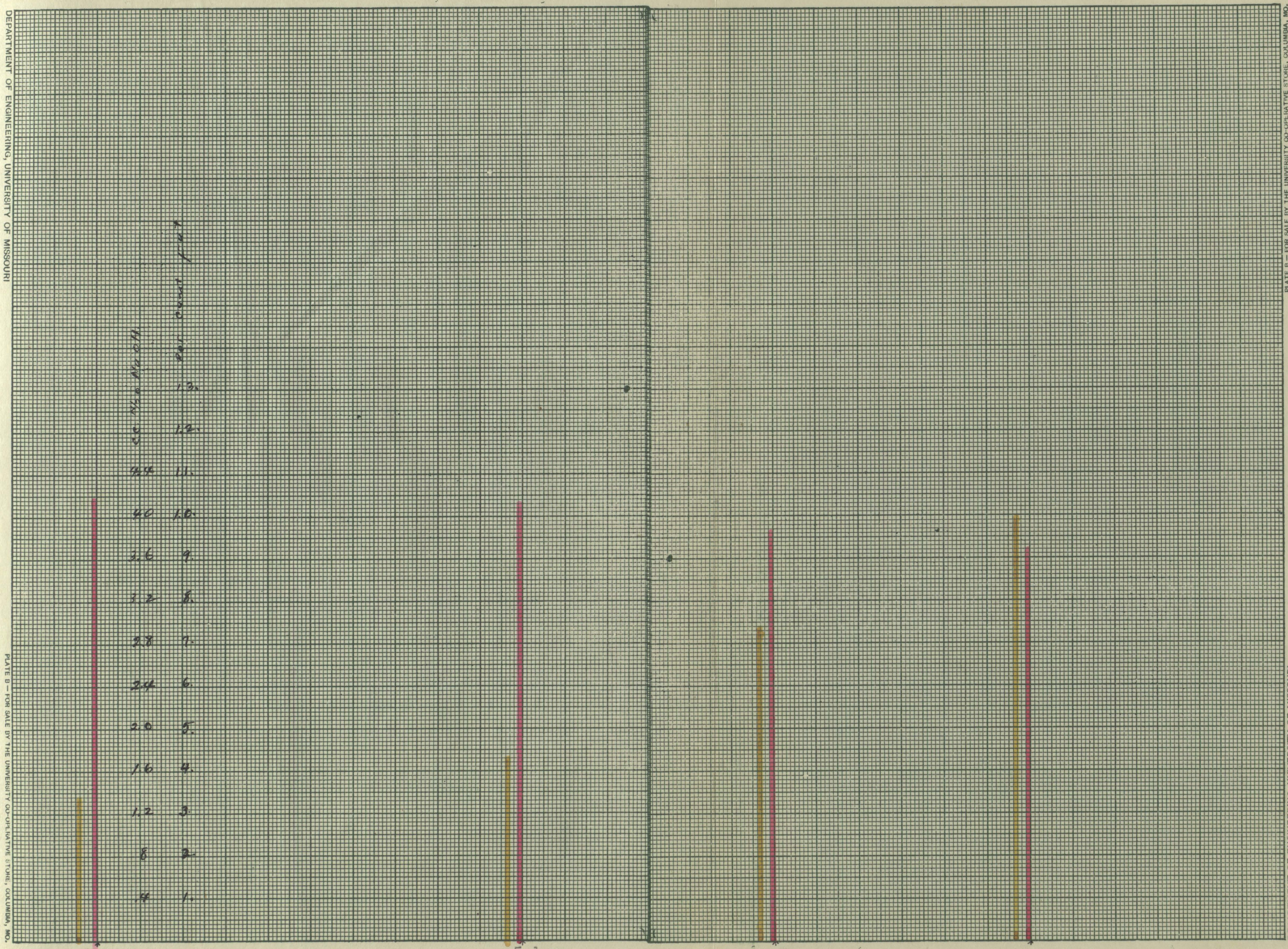
Table Number 4.

This table gives at the periods 5, 8, 11 and 14 hours after the taking of the fat meal, the lipase content as computed on the fat free fresh liver sample basis in terms of cubic centimeters of N/20 NaOH (x10'); also the percentage fat in the liver and the lipase quotient - total calculated liver lipase in terms of N/10 NaOH - divided by the initial body weight. The lipase quotient given in the tabulation is the actual quotient (x10').

Hours after feeding	0-Normal	2	5	8	11	14
cc N/20 NaOH per unit fat free fresh tissue x10'	1.79	1.40	2.11	2.24	1.58	2.18
Percent fat in liver	3.35	9.40	11.95	14.32	4.13	11.60
Lipase quotient (x10)	4.03	3.03	4.54	4.45	3.28	3.60

Chart number IV is a plot of the data given in the above table number 4. The curves for the lipase content of the liver as indicated by the lipase quotient (in red) and the lipase calculated on the fat free fresh sample (in green) correspond, showing an initial fall followed by rise to a maximum to later fall again. The lipase quotient and the per cent fat, as well as the lipase per unit of fat free fresh sample, correspond in a degree throughout their cycle of variation, the one exception being that the fat does not show the initial fall, as is seen in the lipase.

CHART NUMBER III.



After taking a meal rich in fat.
Yellow - percentage of fat.
Red - c.c. of N/20 NaOH

Series Number 2.

Feeding Experiment Number 2.

Table Number 5

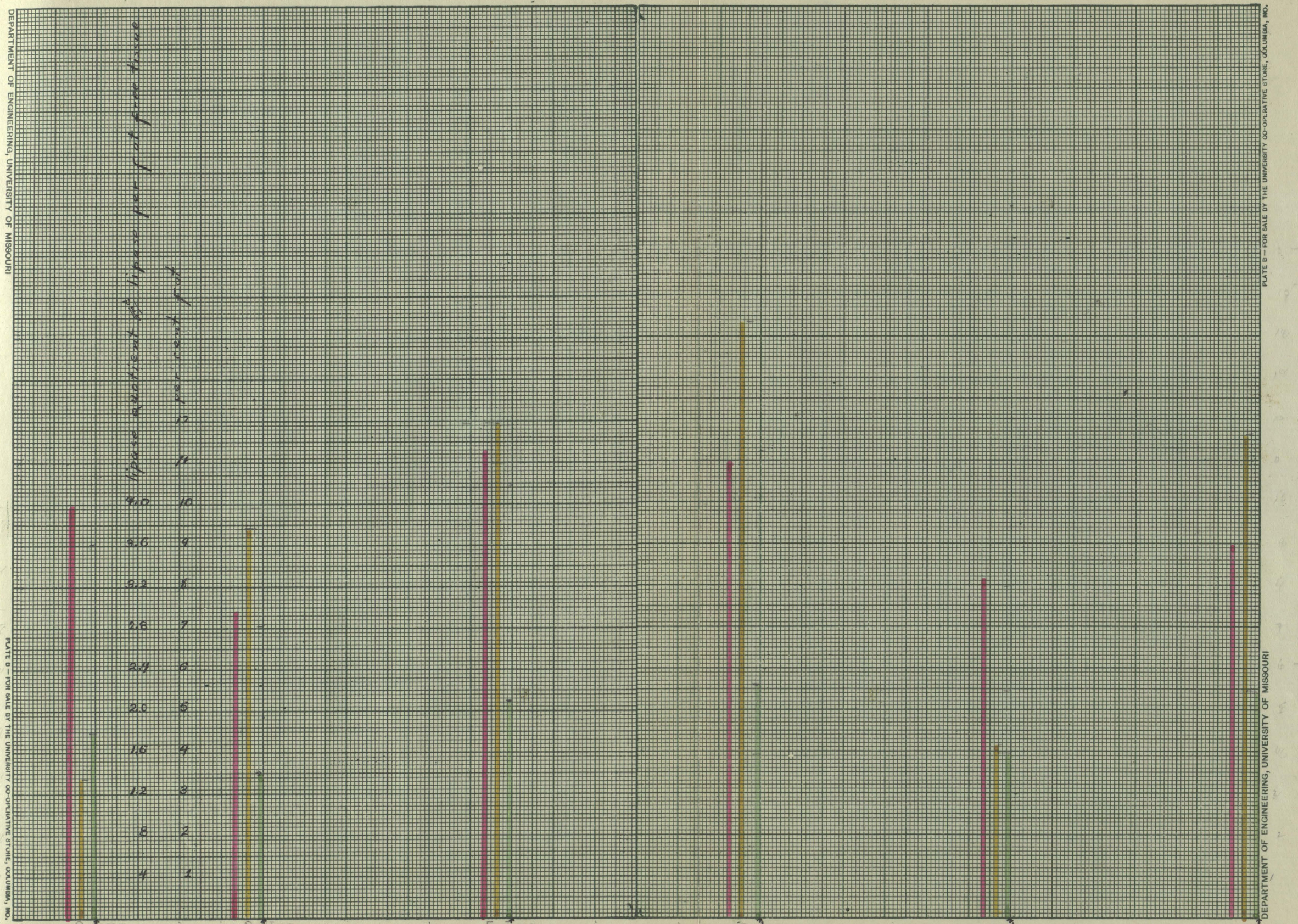
This table gives at the periods 2, 8 and 11 hours after the taking of the meal rich in fat, the lipase content as computed on the fat free fresh liver sample basis in terms of cubic centimeters of N/20 NaOH ($\times 10^{-1}$); also the percentage fat in the liver and the lipase quotient - total calculated liver lipase in terms of N/10 NaOH - divided by the initial body weight. The lipase quotient given in the tabulation is the actual quotient ($\times 10^{3}$).

Hours after feeding	:O-Nor-: mal	2	8	11
c.c. N/20 NaOH per unit fat free fresh tissue ($\times 10^{-1}$)	2.58	2.69	3.15	2.61
Percent fat in liver:	4.40	7.00	12.15	6.25
Lipase quotient($\times 10$)	5.80	3.86	6.23	3.96

Chart Number 5.

This chart gives a concrete representation of the data given in the above table number 5. The curve for the lipase quotient(in red) shows as it did in chart number 4 an initial fall followed by a rise to later fall again. Similarly the lipase quotient and the percent fat correspond in a degree in their cycle of variation.

CHART NUMBER IV.



After taking a meal rich in fat
Red - lipase quotient.
Yellow - percent of fat.
Green - lipase per unit of fat free fresh tissue.

Series Number 3.

Feeding Experiment Number 3.

Table number 6

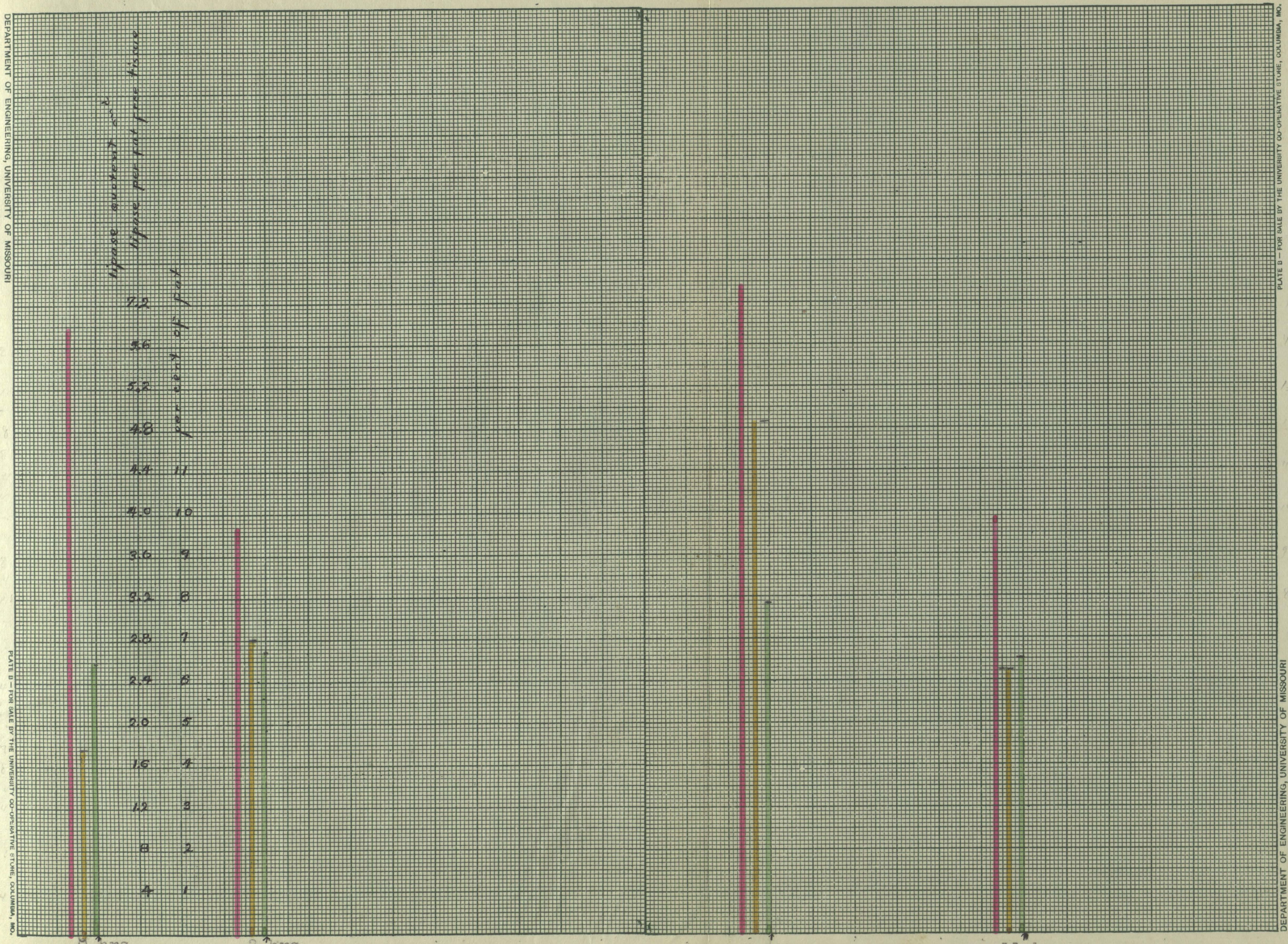
This table gives at the periods 5, 8 and 11 hours after the taking of the meal rich in fat, the lipase content as computed on the fat free fresh liver sample basis in terms of cubic centimeters of N/20 NaOH (x 10^{-1}); also the percentage fat in the liver and the lipase quotient - total calculated liver lipase in terms of N/10 NaOH - divided by the initial body weight. The lipase quotient given in the tabulation is the actual quotient (x 10^{1}) .

Hours after feeding	0-Normal	5	8	11
c.c. N/20 NaOH per unit fat free tissue (x 10^{-1})	4.35	4.33	4.19	4.26
Per cent fat in liver	3.46	4.40	7.45	1.071
Lipase quotient(x10^{1})	7.83	7.85	7.27	7.50

Chart Number 6.

This chart figures graphically the data given in the preceding table number 6. The variation in the percent fat is seen to be least marked in this series of either of the three reported, and likewise the variation in the lipase quotient as well as the lipase per unit of fat free fresh sample is very slight.

CHART NUMBER VI



After taking a meal rich in fat.

Red - lipase quotient.

Yellow - per cent of fat.

Green - lipase content per unit of fat free fresh tissue.

It would seem that the first two feeding experiments afford evidence that there is an increasing lipase content per unit of fat free fresh liver tissue following the cycle of digestion and absorption of a fat meal. The third feeding experiment shows the least variation in fat content, and similarly shows only slight variation in lipase content.

In the determination of fat in the liver in relation to a fat meal the point to be emphasized is the progressive accumulation of fat in this organ. Assuming that the transportation of fat is normally associated with an infiltration of fat in the liver, we would expect an increase in the fat content of the liver following the digestion and absorption of a fat meal. The observations made on the total of fourteen pups following a fat meal show that the fat content of the liver increases during the absorption of fat without exception, and the greatest increase comes at a period when the absorption is occurring at the greatest rate. This is evident from both the percentage of fat in the liver tissue, and from the quotient obtained when the total liver fat is divided by the normal body weight, i.e. the fat quotient, as is shown by the following tables number 7, 8 and 9.

Series Number 1.

Feeding Experiment Number 1.

Table Number 7.

Tables numbered 7, 8 and 9 give the percentage of fat in the liver as compared with the total calculated liver fat divided by the initial body weight, the fat quotient, following the taking of a fat meal. The fat quotient as tabulated is the actual quotient x 10^4.

Hours after feeding	0 Normal	2	5	8	11	14
Percentage fat	3.35	9.40	11.05	14.32	4.13	11.60
Fat quotient x 10^4	15.75	44.95	58.36	64.12	17.89	45.20

Series Number 2.
Feeding Experiment Number 2.
Table Number 8.

Hours after feeding	0 Normal	2	8	11
Percentage fat	4.40	7.00	12.15	6.25
Fat quotient x 10^4	20.64	25.52	56.61	20.30

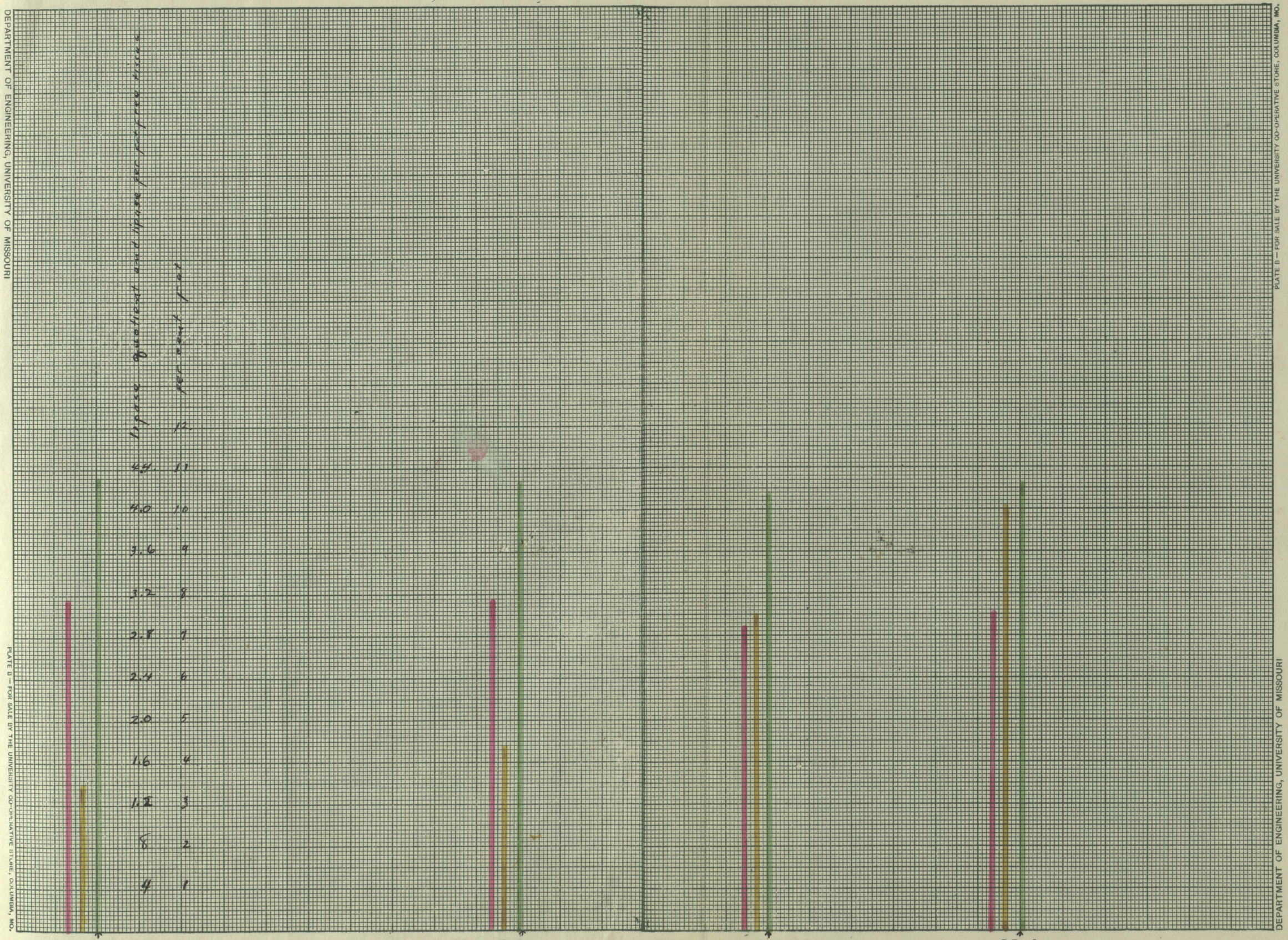
Series Number 3.
Feeding Experiment Number 3.
Table Number 9.

Hours after feeding	0 Normal	5	8	11
Percentage of fat	3.46	4.40	7.45	10.71
Fat quotient x 10^4	12.90	16.62	27.93	42.17

It is evident that the true comparative index of the fat and lipase content of the liver is their respective quotients. It remains to compare the fat and lipase quotients and see their relationship. This comparison is made graphically in charts number 7, 8 and 9. The variations in the lipase and fat content of the liver at periods following the taking of a meal rich in fat show a definite and in a degree corresponding cycle. The lipase quotient shows a slight fall followed by a rise to a maximum later to fall again. The fat quotient shows an increase to a maximum, then a decreasa.

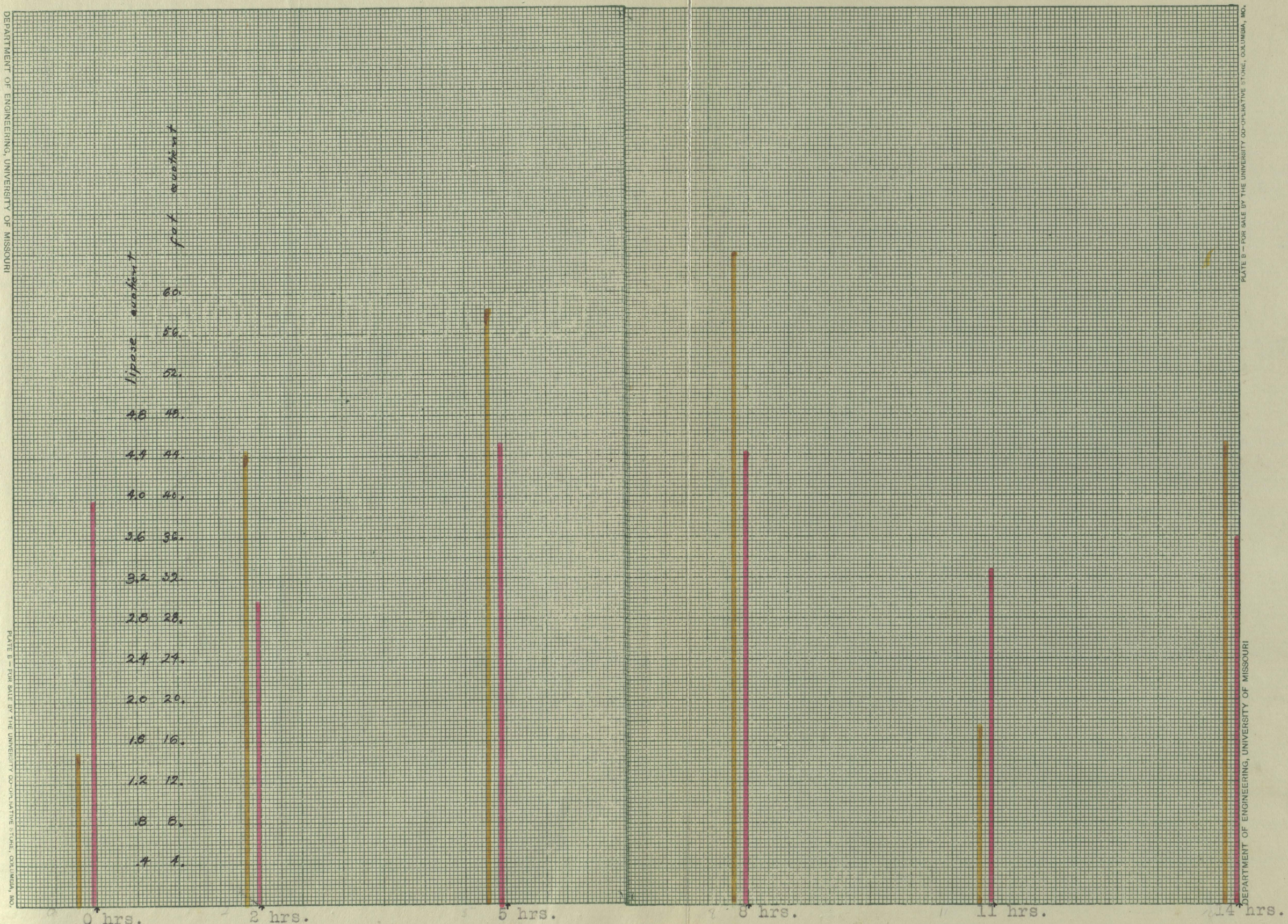
The fat results show that the curve for the increasing total fat content of the liver after a fat meal follows the curve for fat absorption in the blood as given by Bloor. He may be quoted thus: ".....in general the curve of variation in blood fat in fat feeding appears to follow that of normal fat absorption . . . . . slight changes during the first two hours, an increase to a maximum at about the sixth hour, then a decrease." In our experiments the fat in the liver, as in the blood, two hours after the fat meal, shows a slight increase over the normal. Still later there is a continued gradual increase of fat in the liver until a maximum is reached at the end of 8 to 12 hours after the taking of the meal rich in fat. Following the maximum, there is evidence of a gradual decrease in the fat content of the liver corres-

CHART NUMBER VI.



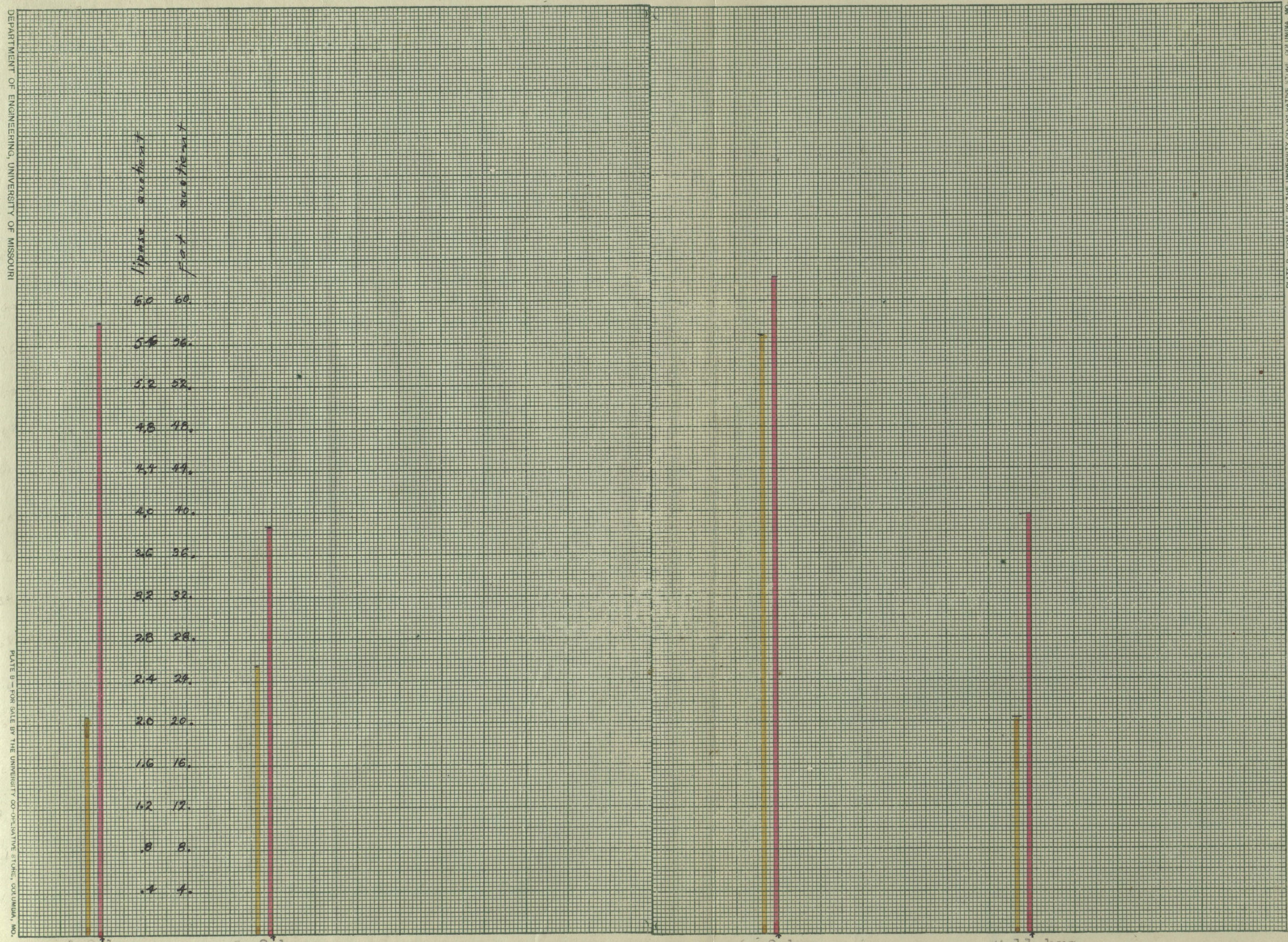
After taking a meal rich in fat.
Red - lipase quotient.
Yellow- per cent of fat.
Green - lipase per unit of fat free fresh tissue.

CHART NUMBER VII.



After taking a meal rich in fat.
Yellow - fat quotient.
Red - lipase quotient.

CHART NUMBER VIII.



After taking a meal rich in fat.
Yellow - fat quotient.
Red - lipase quotient.

ponding to the decrease in the fat content of the blood. But to the last statement there is one apparent exception.

In experiment number 1, the 11 hour animal shows a very low fat content of the liver- a content only slightly greater than the normal and far less than either of the animals preceding and following it in the series. It should be pointed out that the lipase content in this animal bears a similar relationship. This animal was the smallest of the series, at autopsy the stomach was practically empty and the intestine contained only a small amount of food. The pups on either side of it in the series showed an abundance of digesting cream both in their stomach and small intestine. It was suspected since the 11 hour pup showed a lack of food in the stomach and small intestine that it had lost the food soon after being fed. If so, this would account for the low fat content in this liver. It is noticed that the eleven hour pup in experiment number 2 also has a fat content somewhat lower than that of the eight hour pup. It is a third higher in fat content than the normal. If we assumed the maximum absorption in Exp. No. 2 to fall at eight hours, then the data obtained here would seem quite normal showing the decrease in fat content following the maximum. at 11 hours.

The eleven hour pup in experiment 3 shows the greatest content of fat in the liver of this series. The appareht irregularities in the time at which occurs the maximum fat content of the liver after the fat meal is

easily explained if we refer to the work of Vaughan Harley[58] on the"Normal Absorption of Fat . . . ." He reports that although the quantity of milk given per kilo be the same in each case, the quantities of fat in the various parts of the alimentary tract may differ very materially, and likewise the amount absorbed varies with the individual. Further, the maximum rate of absorption of fat normally occurs about 7 hours after taking a fat meal. The maximum rate of absorption as shown by the fat content of the liver in two out of three experiments falls at the eight hour period after taking food, and in the third series it occurs at the eleventh hour test. When one considers that these animals had all been without food for 43 hours or more previous to the feeding, the curves here obtained would seem to demonstrate ~~that~~ an infiltration of fat in the liver following the digestion and absorption of the fat meal. Further, the degree of this infiltration is proportional to the absorption occurring in the blood.

This relation of the liver fat to the blood fat is most strikingly represented if the fat content of the liver shown in our feeding experiment number 1 for the periods 2, 5, 8 and 11 hours after the taking of a fat meal is compared with the fat content of the blood during normal fat absorption as shown by Bloor in his feeding experiment number 1, which is an approximate average of the four ex-

periments he reports. The data for such a comparison is given in the following tabulation.

Table Number 10.

This table gives the percentage blood fat following a fat meal (cited from Bloor), also the fat in per cent by weight of either extractable substance in the dry liver tissue cited from our experiment number 1.

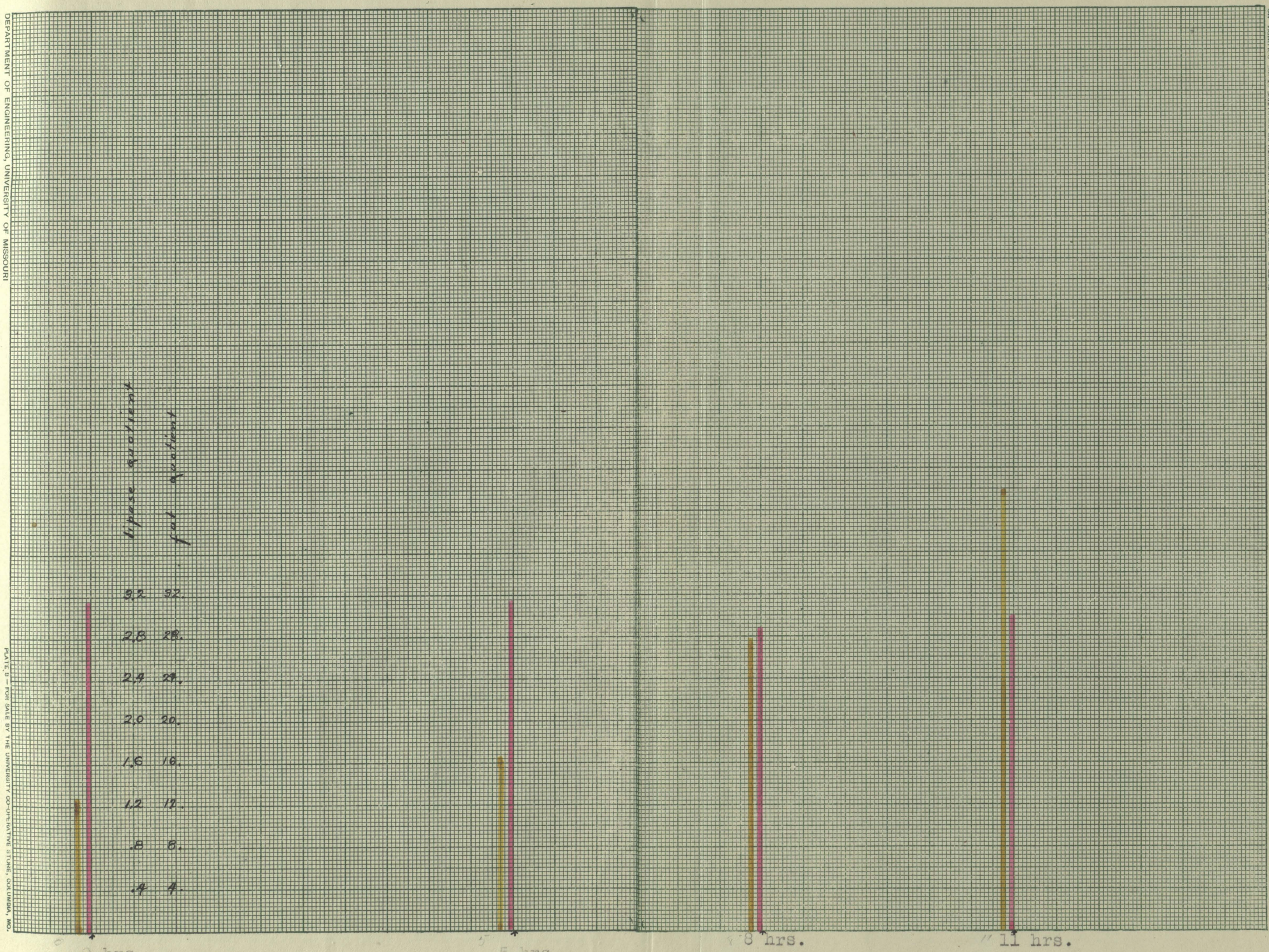
Hours after feeding	:0-Nor-mal	2	5	8	11	14	
Per cent fat in blood Bloor'sfeeding exp.#1	.58		1.2	.9			
Percent fat in liver: our feeding exp.# 1	3.35	9.40	11.95	14.32		11.60	

Chart Number X.A

This chart represents the change in the percent fat content of the blood during normal absorption - as given by Bloor and the changes occurring in the per cent fat content of the liver at corresponding periods in our feeding experiment Number 1. The blood fat is in black, the liver fat in yellow. The form of the curve for the fat in the liver and for the fat in the blood correspond throughout their cycle of variation.

The ratio of the fat in the liver to the fat in the blood is roughly shown to be 10 to 1. Thus at the 5 hour period in feeding experiment number 2 Bloor gives the fat

CHART NUMBER IX.



0 hrs.
After taking a meal rich in fat.
Yellow - fat quotient.
Red - lipase quotient.

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

content as 1.2% as compared to 11.95% the figure for the percentage of fat in the liver at the 5 hour period in our feeding experiment number 1. This inter- relation between the blood fat and the liver fat is most strikingly shown by a comparison of the average blood fat reported by Bloor as compared with the average fat content of the liver in our feeding experiments number 1 and number 2 at corresponding periods after the taking of a meal rich in fat. This data is given in the following table.

Table Number 11.

This table gives ~~graphically~~ the changes in the fat content of the blood and of the liver during normal absorption.

Hours after feeding	Ø Normal	2	5	8	11	14
Feeding Exp. #1, per cent fat in liver	3.35	9.40	11.95	14.32		11.60
Feeding Exp. #2, per cent fat in liver	4.40	7.00		12.15	6.25	
Average per cent fat in liver	3.87	8.10	11.95	13.23	6.25	
Bloor's Exp. #1 per cent fat in blood, read from curve	.58		1.2	.9		
Bloor's Exp. #2 per cent fat in blood	.67	.83	1.15	.		
Bloor's Exp. #3 per cent fat in blood	.6		1.0	.85		
Bloor's Average per cent fat in blood	.63	.83	1.12	.87		

In comparing the above averages it is seen that the time of the variation in the blood fat has a tendency to run ahead of the liver fat, both during the rise and the fall, i.e. the variation in the blood fat seems to initiate or precede the variation in the liver fat.

This evidence seems to me to militate consistently in favor of Leathes'[52] theory of fat variation in the liver, namely, "The character of the fat in the liver . . . . is determined in the first instance by the character of the fat offered it by the blood."

If we take this view in explanation of our results, it means that the quantity of fat in the liver is determined by the quantity of fat offered it by the blood. The fall in fat content following the maximum may be taken to indicate that the liver is not a place to which the fat comes to stay or in which it is permanently stored but to indicate that the liver has work to do in preparing fat for the metabolic processes in which fat is concerned, - possibly a desaturation as Leathes has shown to occur in the liver.

Further it may be taken to show that the liver acts as a regulator of the quantity of fat in the blood, holding the percentage of blood within a certain maximum and minimum as it is known to do in the case of blood sugar. The late work of Bloor[59] showing the rapid dissappearance of large quantities of fat injected into the blood stream would lend strength to such a hypothesis. The findings of Mr. Summers in his studies of the blood in our cooperative work on these

animals are in harmony with and support this view. It may well be that the liver performs both of these functions, regulating the fat content of the blood, and also prepares the fat for the metabolic process in which fat is concerned, a true lipogenesis of Loevenhart.

In making the fat and lipase determinations sample number 1 was always taken from the left lobe and sample number 2 from the right lobe. Thus we hoped to determine the uniformity of the fat and lipase content throughout the organ. The variation in the lipase content of the two lobes was always within the limit of experimental error, likewise, as is reported by Noel Paton, the fat content was uniform through the liver.

The feeding experiments performed with pups net four results, namely:

1. The fat and lipase content of the liver runs a definite and characteristic cycle of variation in relation to the taking of a meal rich in fat.

2. This cycle is characterized by a gradual increase in the fat and lipase content of the liver to a maximum, followed by a fall. The lipase cycle differs from the fat cycle in that the lipase shows an initial fall which is not shown by the fat.

3. The accumulation of fat in the liver after fat food is proportional to the absorption of fat in the blood.

4. The fat and lipase is uniformly distributed throughout the liver.

B. Experiments on Fasting.

a. The determination of the amount of fat and of lipase in the liver in relation to early and late fasting.

The lipase and the fat relationship during the normal cycle of fat intake is complicated by the mere fact of the fat additions to the body supply from the feeding, i.e. from external sources. This condition is eliminated during fasting when the body can consume only such fat as is already on hand in some one or other of the sources of body fat. This test we have made on three series of animals with a view of throwing light on the lipase and the fat variation during the metabolism of the bodies own tissues and stores. For these experiments we have taken pups of the same litter, fed them on a general and constant diet to secure uniform conditions. Twenty four hours after the last feed the normal was decapitated and samples of the liver taken. Food was withheld from the remaining animals of each series the individuals of which were killed at intervals for the tests. The lipase and the fat content of these livers was determined.

Two series of experiments along this line are presented in the following tables and charts.

Series Number 4.

Fasting Experiment Number 1.

February 12, four female bull pups about three weeks old in the very best of condition, average body weight 1500 grams, 18 hours after feeding the normal was killed, the remaining animals were fasted and killed at the times indicated.

Table Number13.

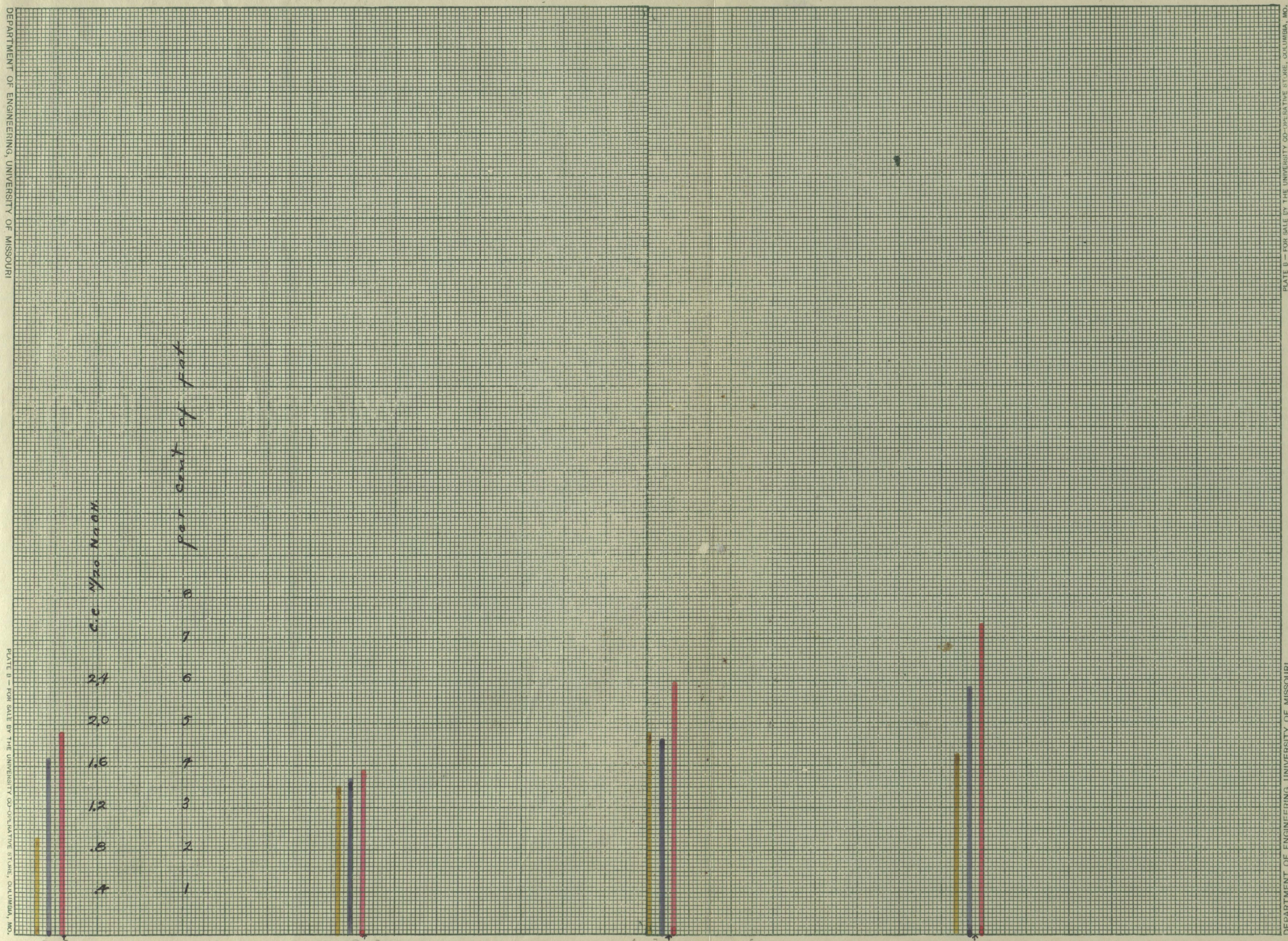
This table gives the lipolytic activity shown by 1 cubic centimeter of the 10 per cent fat free aqueous extract of the liver acting on .5 cubic centimeters of ethyl butyrate 4 cubic centimeters of water, .08 cubic centimeters of toluol at 38°C for a period of one hour in terms of cubic centimeters of N/20 NaOH. The fat content, i.e. the ether extractable substance in the dry liver tissue is given in terms of per cent by weight of the fresh sample.

Days fasted	0	2	4	6
Cubic centimeters of N/20 NaOH	1.95	1.56	2.40	2.86
Percentage fat in liver	2.30	3.50	4.75	4.27

Chart Number X.

This chart shows graphically the variations in the per cent fat and lipase content of the liver during a fast as given in the above table number 13. The percentage lipase content as was made evident by the fat free extract is indicated in red, the percentage lipase content as shown by the turbid extract is pictured in blue, and the percentage fat in yellow. A comparison of the two lipase curves shows the fat free extract to be the more active. The cycle of variation in the lipase is characterized by an initial fall in the percentage content at the 2 day period followed by a continued rise at the 4 and 6 day periods. The curve for the fat shows an increase in the fat content of the liver in the fasting animals over the normal.

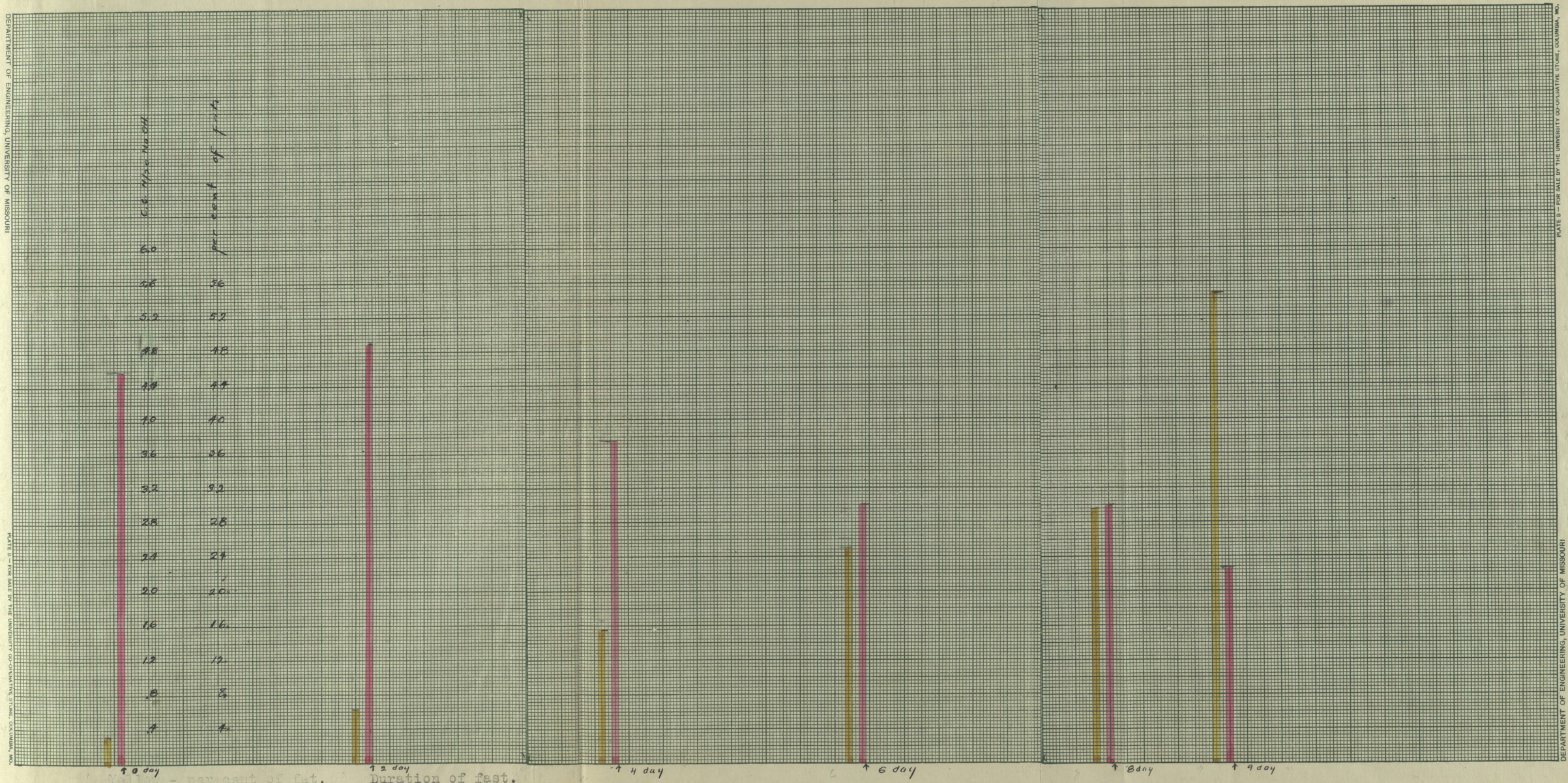
CHART NUMBER X.



Duration of fast.
Yellow = per cent of fat.
Blue - lipase - turbid extract.
Red - lipase-fat free extract.

CHART NUMBER XI.

CHART NUMBER XI.



Yellow - per cent of fat. Duration of fast.
Yellow. per cent of fat. lipase.
Red - c.c. N/20 NaOH - lipase.

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

Series Number 5.

Fasting Experiment Number 2.

March 28. Six pups of the same litter about 6 weeks old and in good condition, average body weight 1200 grams, 22 hours after feeding, the normal was decapitated, the remaining five pups were allowed water but no food, and decapitated as indicated.

Table Number 14.

This table gives the lipolytic activity shown by 1 cubic centimeter of the 10 per cent fat free aqueous extract of the liver acting on .5 cubic centimeters of ethyl butyrate, 4 cubic centimeters of water, .08 cubic centimeters of toluol at 38°C for a period of one hour in terms of cubic centimeters of N/20 NaOH. The fat content, i.e. the ether extractable substance in the dry liver tissue is given in terms of per cent by weight of the fresh sample.

Days fasted	0	2	4	6	8	9
Cubic centimeters of N/20 NaOH	4.59	4.85	3.84	3.00	3.13	2.27
Percentage fat in liver	3.32	6.35	15.57	25.05	29.60	54.75

Chart Number XI.

This chart represents graphically the variation in the percentage fat and lipase content as given in the above table Number 14, yellow, the percentage fat;red, the percentage lipase as indicated by the fat free extract. The curve for the percentage fat shows a progressive increase up to and including the 8 day period of the fast and on the 9th day period there occurs a sudden increase in the fat. The curve for the percentage lipase shows a gradual decrease throughout the fast in contrast to fasting experiment number 1 which shows an initial fall followed by a rise. Compare chart number X.

The two fasting series demonstrate without exception an increase in the percentage of fat content in the liver of the fasting pups over the normal. The percentage of lipase determinations in the two series do not harmonize to so striking a degree. Fasting experiment number 1 shows a fall in the percentage of lipase content in the 2 day animal, followed by a gradual and continued rise in the percentage of lipase content in the 4 and the 6 day animals. While in the fasting experiment number 2 following the two day period there is a decrease throughout the fast in the lipolytic activity of the 10 gram sample of liver which means of course a decrease in the percentage content of liver lipase.

It may be objected that the percentage of lipase aside from other data means nothing. Assume that the lipase is contained only in the fat free protoplasm. Then if the lipase remains constant per unit of fat free fresh liver tissue, e.g. per unit of vital protoplasm a sudden increase in non-protoplasmic substance - fat in this instance-would show an apparent decrease in the lipase content of the liver when in reality there was no change. If the increase of fat is sufficiently great the lipase content would seem to decrease, though, as a matter of fact it might actually be increasing. Further a variation in the liver mass during the fast would render the percentage of lipase apart from other data of less value.

It is evident - data to follow- that there is a variation in the liver mass during the fast. If the cells

decrease in volume by the loss of some substance, the effect would be to allow a greater proportion of cells per unit mass of liver. Assume that the lipase remains constant per cell, then the depletion of glycogen or water from the cells would send up the percentage lipase in a unit of liver mass thus giving an apparent increase in the lipase content of the liver. The glycogen content of the liver of young pups may be up to 40 per cent of the dry mass. If the glycogen suddenly dissappears with no other compensating factor the ~~percentage~~ lipase content of the liver would apparently be greatly increased.

To satisfy these objections the percent lipase in the liver must be compared to some standard. The following figures will show that the variation in the liver mass of fasting pups does not occur at the same rate as the variation in the body weight.

Series Number 5.

Feeding Experiment Number 2.

Table Number 15.

This table gives the comparison as between the mass of the liver in fasting animals and their initial and also their final body weight.

Days fasted	0	2	4	6	8	9
Fresh liver weight ÷ final body weight x 10'	4.89	3.58	4.14	4.91	6.11	9.28
Fresh liver weight ÷ initial body weight x10!	4.89	3.23	3.39	3.47	4.33	6.23

Pups it would seem lose heavily in liver weight on the first day, and at a much greater rate than they lose body weight. Following this initial fall there seems to be a steady increase in the weight of the liver -- see weight of liver divided by initial body weight. This of itself would point to a possible accumulation of substance in the liver during a fast. It is evident that if we wish to refer the per cent lipase in the liver to some standard, it must be either to the initial body weight or to the mass of active liver tissues. For the present we will make the comparison to the latter, i.e. the lipase per unit of vital protoplasm. It thus becomes necessary to state the lipase content of the liver in terms of fat free fresh liver tissue, i.e. the weight of the fresh sample minus the weight of the fat obtained which gives the weight of the fat free fresh

sample or the active tissue. From the fat free fresh sample the lipolytic activity per unit of active tissue as calculated for our two series of fasting pups is given in comparison with the percentage fat content of the liver in the following tables number 16 and 17.

Series Number 4.

Fasting Experiment Number 1.

Table Number 16.

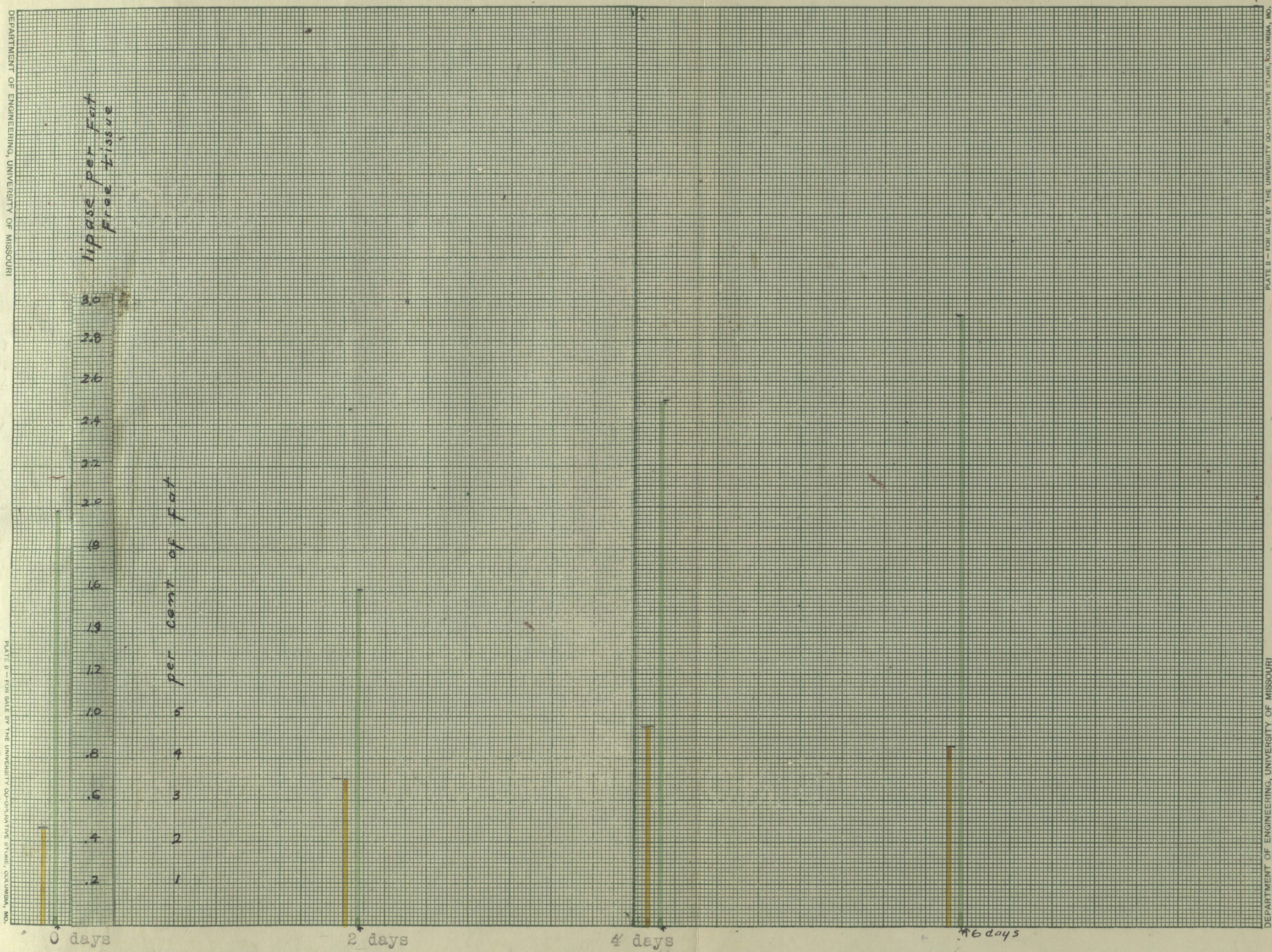
This table gives for the period 2, 4 and 6 days of a fast the percentage fat content of the liver also the lipase content as computed per unit of the fat free fresh liver sample in terms of cubic centimeters of N/20 NaOH x 10'.

Days fasted	:Normal:	2 :	4 :	6 :	8 :
c.c N/20 NaOH per unit fat free fresh tissue	: 1.99:	1.61:	2.51:	2.92:	
Percent fat	: 2.30:	3.50:	4.75:	4.27:	

Chart Number XII

This chart presents in a graphic manner the data given in table number 16, yellow the percentage of fat, green the lipase per unit of fat free fresh tissue. The cycle of variation in the fat and lipase corresponds in that there is an increase in each at the medium to the lat fasting periods. In this series the form of the curve for the lipase per unit of

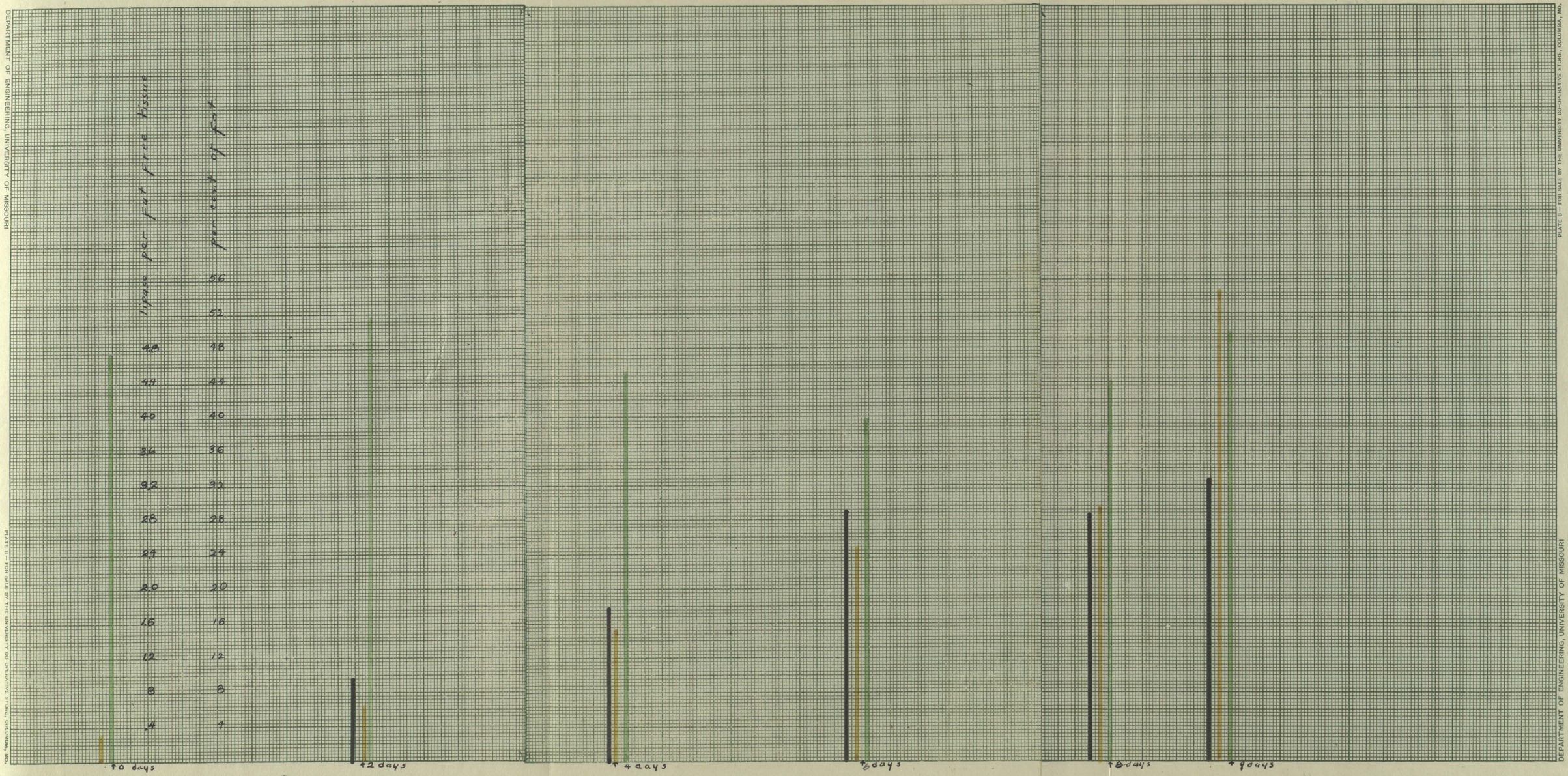
CHART NUMBER XII.



Duration of fast.
Yellow - per cent of fat.
Green - lipase per unit of fat free fresh tissue.

CHART NUMBER XIII

CHART NUMBER XIII.



Black - per cent loss body wt.
Yellow - " " of fat in the liver
Green - lipase per fat free tissue

PLATE 8 — FOR SALE BY THE UNIVERSITY CO-OPERATIVE STORE, COLUMBIA, MO.
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active protoplasm does not differ materially from the curve for the percentage lipase of the entire 10 gram sample, thus in each there is an initial fall at the 2 day period followed by a rise at the 4 and 6 day periods of the fast. Compare chart number X.

Series Number 5.

Fasting Experiment Number 2.

Table Number 17.

This table gives for the periods 2, 4 6, 8, and 9 days of a fast the percentage fat content of the liver, also the lipase content as computed per unit of the fat free fresh liver sample in terms of cubic centimeters of N/20 NaOH x 10'.

Days fasted	Normal	2	4	6	8	9
c.c. N/20 NaOH per unit fat free fresh tissue x 10'	4.74	5.17	4.54	4.00	4.44	5.01
Per cent of fat	3.32	6.35	15.57	25.05	29.60	54.75

Chart number XIII.

This chart represents gfaphically in part the data presented in table number 17, black the percentage loss of body weight ~~in black~~, yellow the percentage of fat in the liver green the lipase content per unit of active tissue calculated on the fat free fresh sample basis. The curve for the lipase

following the second day shows a fall in lipase content at the 4 and 6 day tests, followed by a continued rise in the 8 and 9 day tests thus differing from the curve for the percentage lipase of the 10 gram sample which shows a continued fall throughout the fast. Compare chart number XI.

In each of the fasting experiments the lipase content as figured on the fat free fresh sample basis, shows an initial fall followed by a rise in lipase content of the liver. Or in other words in the transition from a feeding to a fasting state there occurs an initial fall in the total of the lipase content of the liver, to be followed by an increase in the total of the lipase content of that organ. The total lipase content is conveniently represented in a comparative form by the total calculated lipase content of the liver divided by the initial body weight of the animal. This we have termed the lipase quotient and present the same in comparison with the percentage lipase and also the fat quotient - total for calculated liver fat divided by the initial body weight - in the following tables 18 and 19.

Series Number 4.

Feeding Experiment Number 1.

Table Number 18.

This table gives at the 2, 4 and 6 day periods of a fast, the lipolytic activity shown by one cubic centimeter of a 10 per cent fat free aqueous extract of the liver acting on .5

cubic centimeters of ethyl butyrate, 4 cubic centimeters of water, .08 cubic centimeters of toluol at 38°C for a period of 1 hour, i.e. the percentage lipase content of the liver in terms of cubic centimers of N/20 NaOH. In comparison with the above is presented the total calculated liver lipase in terms of cubic centimeters of N/10 NaOH - divided by the initial body weight - the lipase quotient. The figure as tabulated for the lipase quotient is the actual quotient x 10^1. The fat content of the liver is given as total calculated liver fat in grams divided by the initial body weight - the fat quotient. The figure as tabulated for the fat quotient is the actual fat quotient x 10^4.

Table Number 18.

Fasting Experiment Number 1.

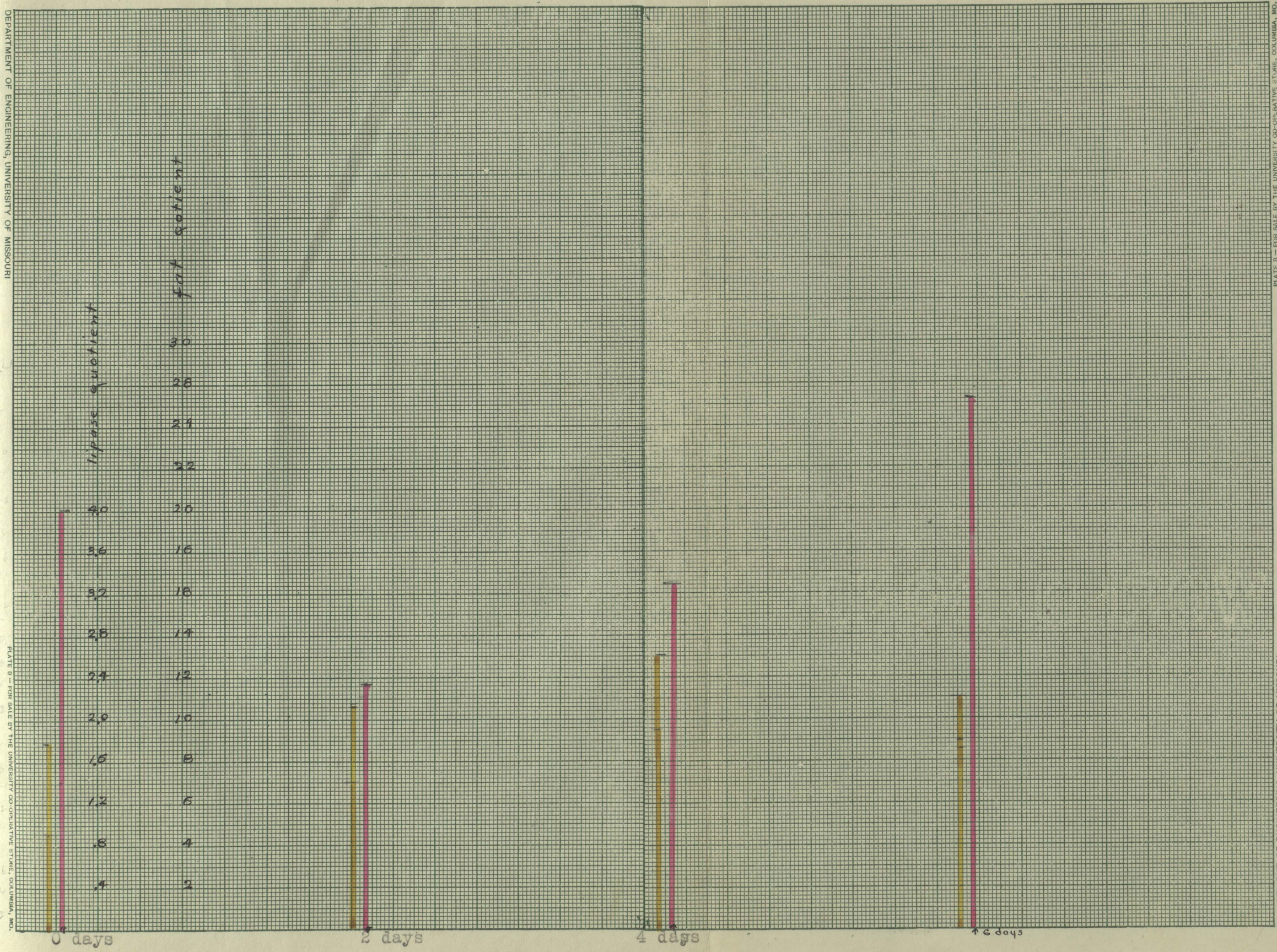
Pups 3 weeks old.

Days fasted	Normal	2	4	6
Lipase quotient x 10^1	4.03	2.33	3.29	5.05
Fat quotient x 10^4	8.90	10.61	13.04	11.00
Per cent lipase	1.95	1.56	2.40	2.86

Chart Number XIV.

This chart presents in part the data given in the above table number 18. Yellow the fat quotient, red the lipase quotient. The curve for the fat quotient shows without exception an actual increase in the total fat content in the liver of

CHART NUMBER XIV



Duration of fast.
Yellow - Fat quotient
Red - lipase quotient.

CHART NUMBER XIV

fasting pups over the normal. The curve for the lipase quotient resembles in form the curve for the percentage lipase and most especially does it correspond in form to the curve for the lipase content per unit of fat free fresh tissue, i.e. an initial fall followed by a rise. Compare charts number X and XII.

Series Number 5.

Fasting Experiment Number 2.

Table Number 19.

This table gives at the 2, 4, 6, 8, 9 day periods of a fast, the lipolytic activity shown by one cubic centimeter of a 10 per cent fat free aqueous extract of the liver acting on .5 cubic centimeters of ethyl butyrate, 4 cubic centimeters of water, .08 cubic centimeters of toluol at 38°C for a period of 1 hour, i.e. the percentage lipase content of the liver in terms of cubic centimeters of N/20 NaOH. In comparison with the above is presented the total calculated liver lipase in terms of cubic centimeters of N/10 NaOH divided by the initial body weight - the lipase quotient. The figure as tabulated for the lipase quotient is the actual quotient x 10^4. The fat content of the liver is given as total calculated liver fat in grams divided by the initial body weight the fat quotient. The figure as tabulated for the fat quotient is the actual fat quotient x 10^4.

Table Number 19.

Days fasted	Normal	2	4	6	8	9
Per cent lipase	4.59	4.85	3.84	3.00	3.13	2.27
Lipase quotient x 10^4	11.23	7.84	6.52	5.26	6.80	7.00
Fat quotient x 10^4	16.24	20.53	52.90	86.90	150.18	342.18

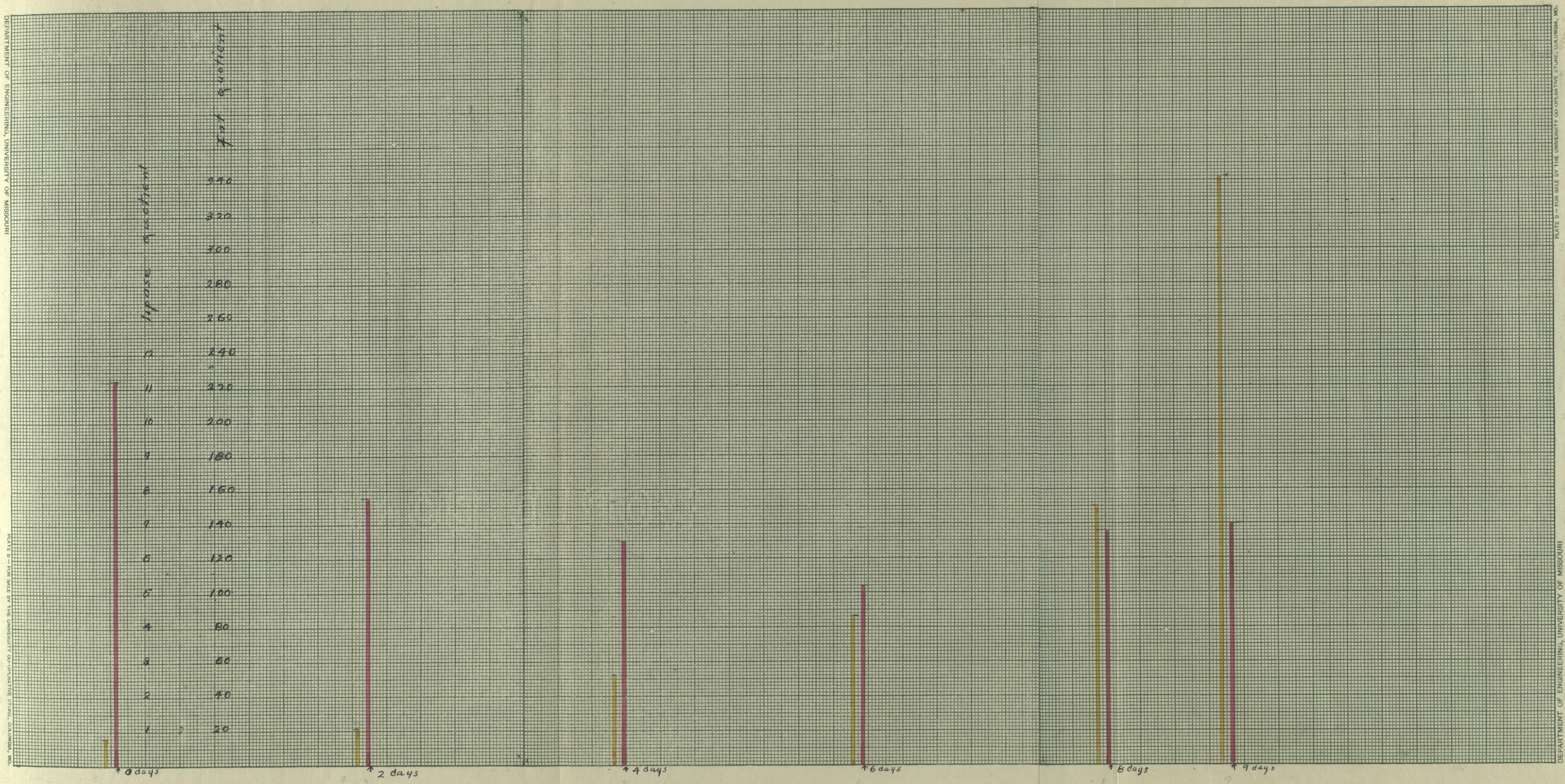
Chart Number XV.

This chart presents in part the data given in the foregoing table number 19. Yellow the fat quotient, red the lipase quotient. The fat curve for the fat quotient shows a progressive accumulation in the total fat content of the liver in fasting pups over the normal. The curve for the lipase quotient shows a decrease in the total lipase content at the 2, 4 and 6 day periods of the fast followed by an increase in the 8 and 9 day periods, thus corresponding in a degree to the curve for the lipase content per unit of fat free fresh tissue, and differing from the curve for the percentage lipase which shows a fall throughout the fast. Compare charts number XI and XIII.

In the lipase determinations it is evident as it was after the taking of a fat meal, that in fasting animals the percentage of lipase of a 10 gram sample is not a true index of the total lipase content of the liver. The variance in the lipolytic activity of the liver tissue, as is made evident by the percentage of lipase content compared to the lipolytic activity shown by the total lipase content divided by the initial body weight, is most marked in those livers showing the highest percentage of fat. In the fasting experiment number 1 there is not at any time a great increase in the percentage of fat and in all cases the percentage of vital or fat free fresh tissue is of course correspondingly high. There is no sudden increase in the non-lipase containing substance - fat. For these reasons the form of the curves for the percentage of lipase, and the curve for the total lipase as well as the lipase per unit of fat free fresh tissue correspond in a degree throughout the fasting experiment number 1. But in fasting experiment number 2 the changes occurring in the liver are shown to be more radical in nature, namely an increase from 3.32 per cent fat in the liver to 54.45 per cent fat in that organ. It is under such conditions as these that the perdentage of lipase in a 10 gram sample of liver shows an apparent decrease in lipase content when in reality there is an increase in the total lipase content of the organ. Thus the curve for the percentage of lipase shows a fall in the lipase content throughout the 9 day fast while the total lipase as

CHART NUMBER XV.

CHART NUMBER XV.



Duration of fast

Yellow - fat quotient
Red - lipase quotient

DEPARTMENT OF ENGINEERING, UNIVERSITY OF MISSOURI
PLATE D — FOR SALE BY THE UNIVERSITY CO-OPERATIVE STORE, COLUMBIA, MO.

made evident by both the lipase quotient and the lipase per unit of fat free fresh sample shows an increase at the 8 and 9 day periods of the fast. The constant and striking observation concerning the total lipase content of the liver during a fast may be stated thus: with the functional readjustment occurring at the transition from the feeding to the fasting state there occurs a fall in the total lipase content of the liver. This is followed by a rise in the total lipase content of this organ.

The observations made concerning the fat content of the liver during a fast are even more striking and constant. From early to late fasting there occurs a gradual increase in the percentage fat as well as an increase in the total fat of the liver. An animal of a corresponding series fasted for a longer period shows the higher percentage fat content. To this last statement there is one exception to be mentioned. In fasting experiment number 1, the animal fasted 6 days has a slightly lower per cent of fat in the liver tissue than has the 4 day animal. This is the only case in the entire series of experiments where a pup of the same litter which had fasted for a greater number of days did not show a greater per cent of fat in the liver tissue.

Fasting experiment number 2 shows a progressive accumulation of fat in the liver throughout the entire fast. In this experiment the increase in fat is approximately proportional to the length of the fast. Thus at the normal and

the 2, 4, 6 and 8 day periods the percentage of fat in the liver is 3.32, 6.35, 15.57, 25,05, and 29.60 respectively while the 9 day period shows a sudden increase in the percentage of fat content - 54.75. The fat quotients for this experiment represent in a still more striking manner that the relation of quantity of fat in the liver is in a degree proportional to the length of the fast. Thus the normal and the 2, 4, 6 and 8 day periods give a fat quotient giving a fat quotient ($\times 10^4$) of 16.24, 20.53, 52.92, 86.90, 150.18 in the order given. The fat quotient for the 9 day period is exceedingly high - 342.18. What is the significance of this accumulation of fat in the liver during a fast?

There is ground for thinking that the large amounts of fat found in the liver of fasting animals is due to the importation of fat from the various storage places. This accumulation of fat in the liver may be taken to indicate that the storage fats are first carried to the liver to undergo some intermediate step or preparation before being passed on to the tissue active in metabolism. As the fast is prolonged the animal becomes more completely dependent on its stores of fat which necessarily means that the longer the fast the more the fat that will be carried to the liver by the blood. In our feeding experiments we present data which seems to indicate that the amount of fat in the liver in proportion to the amount of fat offered

that organ by the blood. If this assumption be correct the accumulation of fat in the liver is the evidence we would expect to obtain from the examination of that organ in fasting animals. During a fast the quantity of fat carried to the liver will most likely not be intermittent, or varying as it is known to be after the taking of a fat meal. For this reason the fat content of the liver in fasting animals will not show a decreased content following the maximum, as was observed to occur after the taking of the fat food. Thus it is seen that the observations made on the fat content of the liver of fasting pups support strongly the hypothesis advanced in explaining the fat determinations obtained in fat feeding, namely that the quantity of fat in the liver in a degree is determined by the quantity of fat offered that organ by the blood.

The unusually high percentage of fat in the liver at the late periods of the fast does not indicate that the liver is holding the fat for its own metabolism or storing it permanently. It is not of course to be expected that the body's active metabolism of fat should necessarily in any event outrun the powers of the liver for working up and passing on the fat to other parts. Rather it is to be expected that the liver will follow the general law of over compensation and have on hands an overly adequate store of fat to be passed on as needed. Also the accumulation of fat in the liver during the period of resorption

of stored fats into the blood would indicate that the liver holds the fat content of the blood within a certain minimum and maximum. The slight variation in the percentage of fat of the blood during a fast as reported by Bloor and others would strengthen such a hypothesis. Of course it is entirely possible that in the case of inanition the liver may become clogged with fat; in such a case there will occur a sudden increase in the percentage fat in the blood. As an example of such a phenomena we may cite the 8 and the 9 day animals of feeding experiment number 2, both of which showed profound constitutional changes. The blood serum was creamy and the blood fat as determined by Summers was indeed very high.

It is to be concluded that with the functional variations occurring at the transition from a feeding to a fasting state there occurs a fall in the lipase content of the liver to later Be followed by a rise, and that this lipase cycle is associated with a gradual increase in the fat content of the liver.

b. Fat in the liver during hunger.

Is this observed increase of fat in the liver during a fast pathological? - the product of a degeneration of the liver cells. Or is this accumulation of fat in the liver a normal process? Is it not a fatty infiltration of the body fat being transported from the storage depots to the liver, there as Leathes suggests to be desaturated and then passed on to the other tissues for consumption.

If the increase of fat in the liver during a fast is occasioned by a breaking down of the organ's own protoplasm, it will necessarily follow that an increase in fat will accompany a decrease in liver protoplasm. Or in other words, as the fat increases the fat free solids of the liver will decrease. By taking the difference between the total dry weight of the sample and the weight of the fat obtained from that sample, we have a figure which gives us the weight of the fat free solids of vital protoplasm in that sample. Where the sample taken is 10 gram, as it has been in our experiments, the fat free weight gives the percentage of vital protoplasm in the fresh liver. The percentage of vital protoplasm in the 10 gram sample of liver as calculated on the above basis for the two fasting experiments performed on pups is presented in comparison with the percentage of fat in the liver in the following tables number 20 and 21.

Series Number 4.

Fasting Experiment Number 1.

Table Number 20.

Tables number 20 and 21 give the percentage of fat free solids in the 10 gram liver sample, i.e. the total dry weight of the 10 gram sample minus the weight of the fat obtained is tabulated in terms of percentage of fat free solids in the fresh liver sample; this is given in comparison with the percentage of fat in the corresponding sample, i.e. the grams of ether extractable substance in the dry liver tissue in terms of per cent by weight of the fresh liver sample.

Days fasted	0	2	4	6
Percentage fat free solid	22.23	21.82	23.77	21.87
Percentage fat In dry liver tissue	2.30	3.50	4.75	4.27

Series Number 5.
Fasting Experiment Number 2.
Table Number 21.

Days fasted	0	2	4	6	8	9
Percentage fat free solid in liver	27.28	20.20	19.64	17.07	15.80	10.52
Percentage fat in dry liver tissue	3.32	6.35	15.57	25.05	29.60	54.75

In fasting experiment number 1 the percentage of fat free solids in the liver throughout the 6 day fast remains quite constant. Thus the normal and the 2, 4 and 6 day tests give 22.23, 21.82, 23.77, and 21.89 percentage of fat free solids by weight of the fresh sample in the order given. This constancy of the percentage of fat solids is associated with a moderate increase in the percentage of fat in the liver during the fast. But in fasting experiment number 2 the percentage of fat free solids in a 10 gram sample shows a progressive decrease throughout the 9 day fast thus the normal and the 2, 4, 6, 8 and 9 day periods respectively show a percentage of fat free solids in the 10 gram sample of 27.65, 20.20, 19.64, 17.07, 15.80 and 10.50. This decrease in the percentage of fat free solids is associated with a remarkable increase in the percentage of fat content in the liver, 3.32 per cent normal as compared with 54.75 per cent at the 9 day test. On first observation of the fat free solids in the livers of the fasting experiment number 2 it would seem that with the accumulation of fat in the liver of a fasting animal there is a decrease in the percentage of fat free solids, and necessarily a decrease in the total active liver tissue, or in other words a degeneration of the liver protoplasm. But upon closer study this evidently is not what has happened.

The percentage of the fat free solids in a 10 gram sample of liver can not be taken as a true index of the total fat free solids in that organ.

It is conceivable that when the liver becomes heavily loaded with fat the cells will be larger, and a unit of liver mass will contain less than the normal number of liver cells. Assume that the fat free solids, or vital protoplasm is constant per cell then a sudden increase in the fat content of the liver on the percentage basis would show a decrease in the fat free solids of the liver when actually there had been no change in the mass of vital protoplasm.

That we may have a true index of the fat free solids, they must be referred to our standard, the initial body weight; i.e. the total calculated fat free solids of the liver divided by the initial body weight gives us a fat free solid quotient. The fat free liver solids quotient as calculated for our two fasting experiments together with the fat quotient for the corresponding sample of liver is tabulated below.

Series Number 4.

Fasting Experiment Number 1.

Table Number 22.

Tables Number 22 and 23 give the fat free solid quotient of the liver, i. e. the total calculated fat free

solids of the liver divided by the initial body weight. The figure as tabulated is the actual quotient (x 10^3). This is given in comparison with the fat quotient of the liver, i.e. the total calculated liver fat divided by the initial body weight. The quotient as tabulated is the actual quotient (x 10^4).

Days fasted	Ø Normal	2	4	6
Fat free solid quotient of liver: x 10^3	9.8	6.8	6.5	6.0
Fat quotient of liver (x10^4)	8.90	10.61	13.04	11.00

Series Number 5.

Fasting Experiment Number 2.

Table Number 23.

Days fasted	Ø Normal	2	4	6	8	9
Fat free solid quotient of liver (x 10^3)	13.0	6.4	6.4	6.1	6.8	6.3
Fat quotient of liver (x 10^4)	16.24	20.53	52.90	86.90	150.18	342.18

The fat free solids , i.e. vital protoplasm of the liver is shown to remain remarkably constant at the 2, 6, 8

and 9 day periods of the fast. Further the quotients obtained for the two fasting experiments correspond in value. Thus fasting experiment number 1 gives the fat free solid quotient for the 2, 4, and 6 day periods as 6.8, 6.5, 6.0, and fasting experiment number 2 for corresponding periods gives the fat free solid quotient as 6.4, 6.4, 6.1, and the 8 and 9 day periods as 6.8 and 6.3 in the order given. True these figures are lower than those obtained for the normal. Fasting experiment number 2 gives 9.88 as the fat free solid quotient of the normal liver and fasting experiment number 2 gives 13.0. This variance is explained as due to the store of glycogen contained in the normal livers, which factor of course was not present in the liver of the fasting pups. In other words as soon as the liver has lost its store of glycogen the fat free solids as made evident by the fat free solid quotient, remain relatively constant from early to late fasting. This constancy of the liver's vital protoplasm during a fast is associated with an increase in the fat content of that organ. In no case is there an increase in the total fat of the liver accompanied by a decrease in the total active tissue of the liver. This would seem to be strong evidence in opposition to the hypothesis that the accumulation of fat in the liver during a fast is due to pathological changes - the product of a degeneration of the liver's own protoplasm.

If we may assume that the fat of the liver has no water associated with it, and allow time for the disappearance of glycogen, we may then consider all the water contained in the fresh sample as being associated with the fat free or active protoplasm of the liver. If the accumulation of fat in the liver during a fast is accompanied by a degeneration of that organ's own protoplasm we should get an increase in the percentage of water associated with the active tissue. On the other hand if the infiltration of fat in the liver during a fast is the expression of a normal function of the liver, there should occur no radical change in the percentage of water in the active tissue during this accumulation of fat. The percentage of water contained in the active tissue for our two fasting experiments is tabulated in tables number 24 and 25.

Series Number 4.

Fasting Experiment Number 1.

Table Number 24.

Tables number 24 and 25 give the percentage of water in the active liver tissue, i.e. the free protoplasm during a fast. - In comparison with this is given the fat quotient for the corresponding samples of the liver, i.e. the total calculated liver fat ÷ the initial body weight. This table also gives the fat free solid, or active tissue quotient, i.e. the calculated total fat free solids ÷ the initial body weight.

Days fasted	Normal	2	4	6
Per cent water in active liver tissue	77.2	77.3	75.1	77.1
Fat quotient for liver ($\times 10^4$)	8.90	10.61	13.04	11.0
Fat free solids quotient for liver ($\times 10^3$)	9.8	6.8	6.5	6.0

Series Number 5.
Fasting Experiment Number 2.
Table Number 25.

Days fasted	Normal	2	4	6	8	9
Per cent water in active liver tissue	72.4	76.7	76.9	77.3	78.1	77.3
Fat quotient for liver ($\times 10^4$)	16.24	20.52	52.90	86.90	150.90	342.18
Fat free solid quotient for liver ($\times 10^3$)	13.0	6.4	6.4	6.1	6.8	6.3

Chart Number XVI.

This represents graphically the data given in the above table number 25. Blue, percentage of water in the active tissue of the liver, olive, fat free solid or active tissue quotient. and yellow, the fat quotient for the liver. The curve for the percentage of water in the active tissue of the liver shows little variation throughout the 9 day fast. Likewise as soon as the glycogen has dissappeared the curve for the fat free solid quotient is comparatively constant throughout the experiment. This constancy in the percentage of water and of the fat free solids or active tissue is associated with a profound increase in the fat content of the liver during hunger.

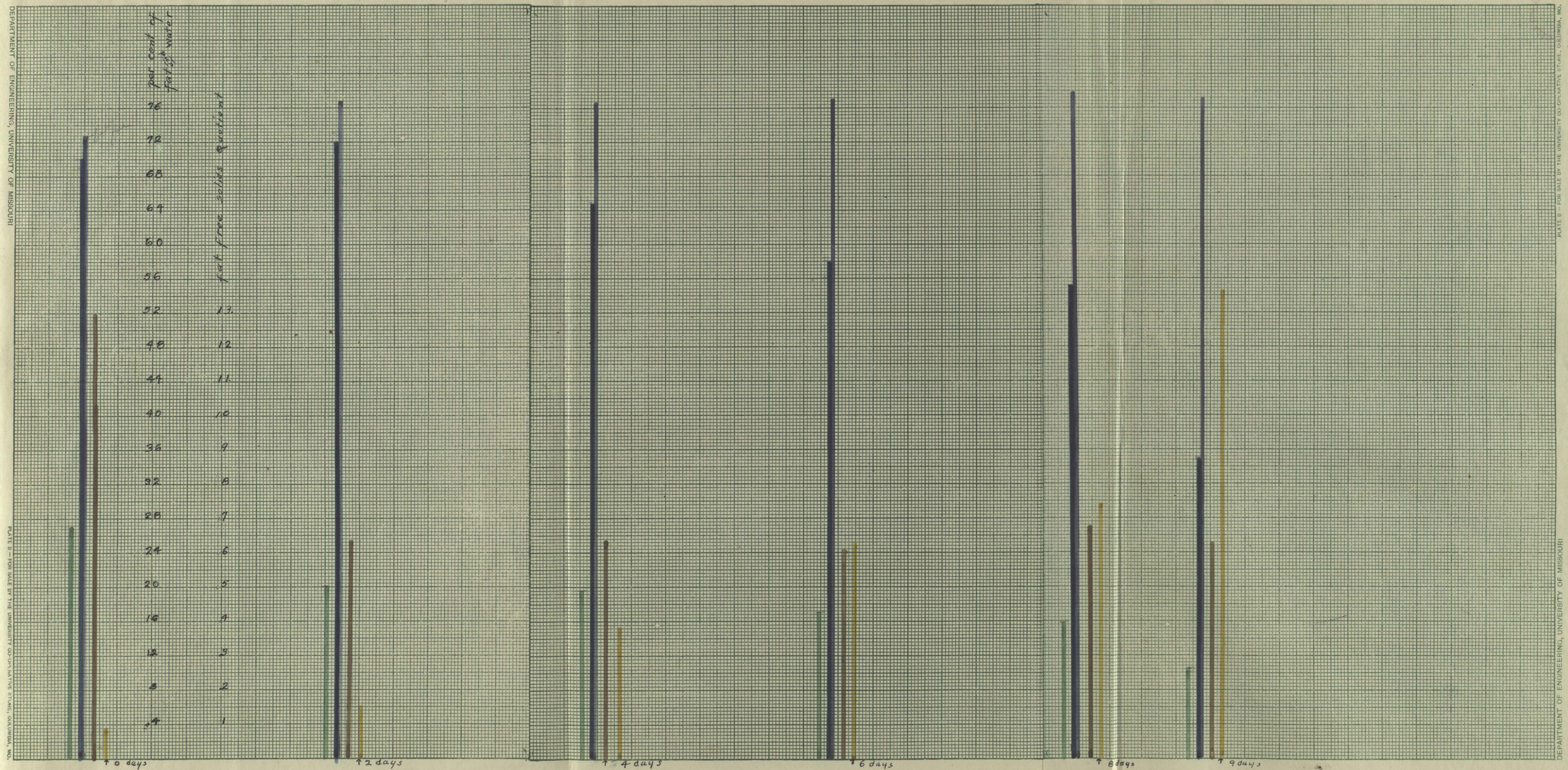
Chart Number XVII.

Taken from Feeding Experiment No. 3.

This chart is given in comparison with chart number XVI. Blue, percentage of water in 10 grams sample of liver, olive, the percentage of fat free solids, and yellow, the percentage of fat in the corresponding sample of liver. A comparison of the two charts shows the conditions to be very similar as to the relation of the fat to the fat free solids and water content of the liver in fed and fasted animals .

CHART NUMBER XVI.

CHART NUMBER XVI.



Olive - per cent of fat free solids Duration of fast
Blue - per cent of water in fat free solids = Hedge, per cent of water in fresh sample = Ledge
Brown - fat free solids quotient
Yellow - per cent of fat

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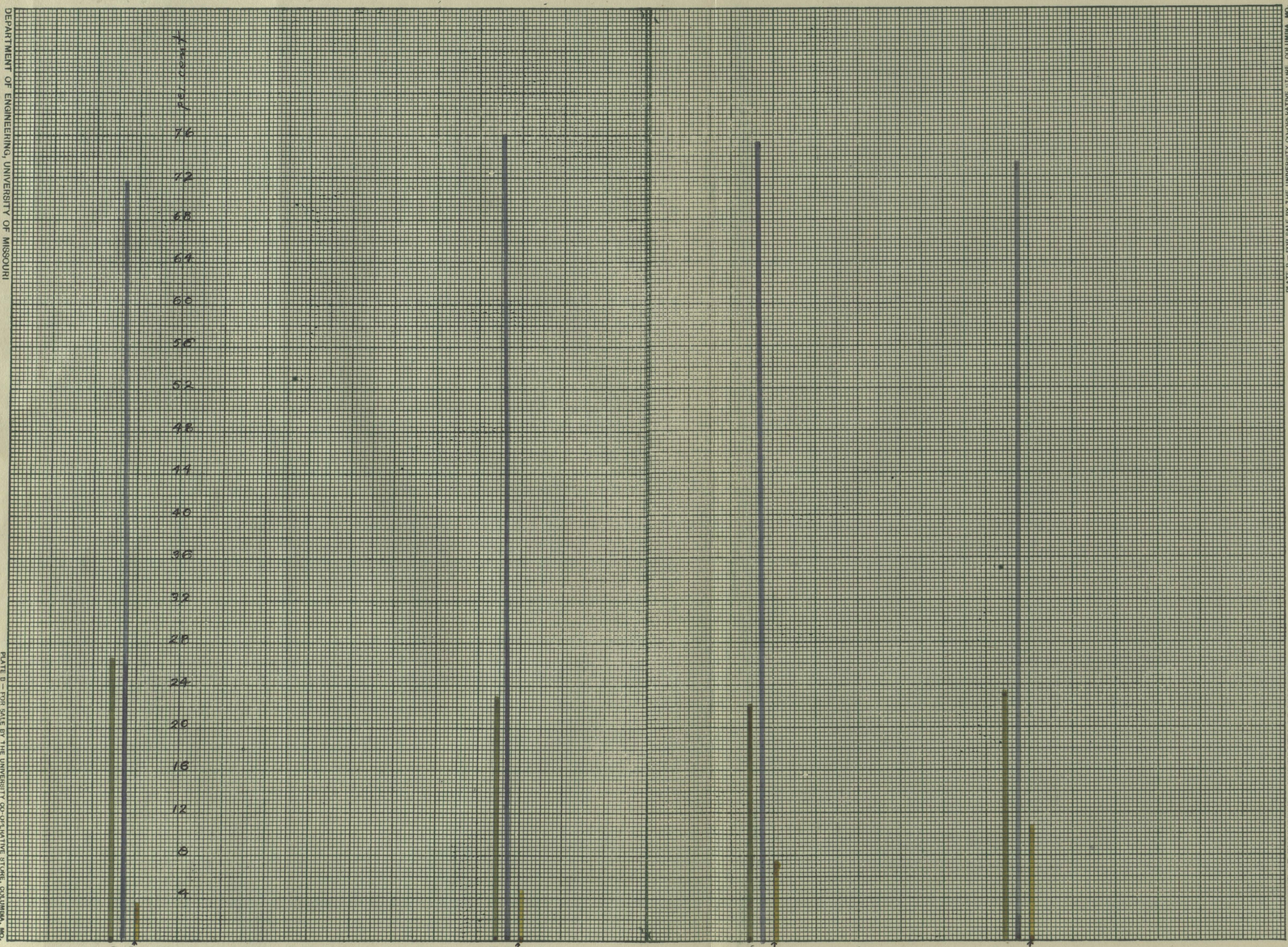
PLATE B — FOR SALE BY THE UNIVERSITY CO-OPERATIVE STORE, COLUMBIA, MO.

CHART NUMBER XVII.



After taking a fat meal.
Olive - per cent of fat free solids.
Blue - per cent of water in fat free solids.
Yellow - Per cent of fat.

The observations made in fasting experiment number 1 show the percentage of water in the active liver tissue to remain very constant throughout the 6 day fast. The normal and the 2, 4 and 6 day periods show a percentage of water of 77.2, 77.3, 75.1 and 77.1 in the active liver tissue. This constancy of water content is associated with a moderate increase in fat content. Inasmuch as the increase in the fat content of the liver was comparatively small and also the length of the fast relatively short, it may be objected that sufficient time was not allowed for a degeneration to occur. But fasting experiment number 2 shows a similar constancy in the percentage of water and of fat free solids and in this experiment the 8 and 9 day animals showed profound constitutional changes and the increase in the fat content of the liver is very great - 3.32 per cent in the normal as compared with 54.75 per cent for the 9 day animal. The slight variations in the percentage water content in the active tissue together with the constancy of the total fat free solids of the liver during a fast would seem to justify the conclusion that the accumulation of fat in the liver in our fasting experiments is not pathological in nature, i.e. the product of a degeneration of the liver's own protoplasm.

Noel Paton believes this accumulation of fat in the liver during a fast satisfactorily explained on the basis of glycogen being transformed into fat. If the glycogen is the sole source of fat in the liver during a fast, we should ex-

pect to get the greatest increase in the liver fat at a time when the liver is being most rapidly depleted of its store of glycogen. In other words the maximum increase in fat should occur at the second day or at least before the end of the third day. But our observations show the maximum increase in the fat content of the liver to occur at a time considerably after the liver has been depleted of its store of glycogen, for example the maximum fat content of the liver in fasting experiment number 2 occurs at the 9 day period. In no case does the maximum increase in the fat content of the liver of fasting animals coincide with the time at which the glycogen is a factor to be considered. Our data does not disprove the hypothesis that the glycogen of the liver is transformed into fat during a fast, but the observations made in our experiments seem to furnish strong evidence that the glycogen at least is not the only source of fat in the liver during a fast.

If the increase of fat in the liver during a fast is due to an infiltration of the transported depot fats we would expect an increase in the total liver solids. Our experiments show that after the second day of the fast there is an increase in the percentage of solids in the liver and this increase corresponding very closely to the increase in percentage of fat in the same organ. This is shown in a most striking manner by the data presented in the following tables number 26 and 27.

Series Number 4.

Fasting Experiment Number 1.

Table Number 26.

Tables 26 and 27 give the percentage of total solids in the 10 gram sample of the liver. For comparison the percentage of fat for the corresponding sample is also tabulated here.

Days fasted	Normal	2	4	6
Percent of total liver solids	24.5		25.3	26.1
Percent of fat in the liver	2.30	3.50	4.75	4.75

Series Number 5.

Fasting Experiment Number 2.

Table Number 27.

Days fasted	0	2	4	6	8	9
Per cent of total liver solids	30.6	26.6	34.7	42.1	45.4	64.9
Per cent of fat in liver tissue	3.32	6.35	15.57	25.05	29.60	54.75

The least variation observed in the percentage of fat in the liver during a fast is shown by fasting experiment number 1. Similarly this experiment shows only slight variations in the percentage of total solids of the liver. While fasting experiment number 2 shows a decided increase in the percentage of total solids in the liver and ~~similarly~~ there is a corresponding increase in the percentage of fat in the liver. In all cases the period showing the higher percentage of fat in the liver has also a higher percentage of total solids in the 10 gram sample.

It may be objected that the increase in the percentage of solids in the liver is due to the decrease in the percentage of water in the sample. And therefore it could not be cited as evidence of an infiltration of fat into the liver.

That there is a decrease in the percentage of water in the fresh sample, accompanying the increase in percentage of fat is shown by the following figures.

Fasting Experiment Number 2.

Table Number 28.

Days fasted	: 0 : 2 : 4 : 6 : 8 : 9
Percentage H_2O in fresh sample	: 70. : 72. : 65. : 58. : 55. : 35.
Percentage fat in: liver tissue	: 3.32: 6.35:15.57:25.05:29.60:54.75

(See Chart Number XVI)

To meet these objections we must refer the total solids of the liver to our standard, the initial body weight Thus the total calculated liver solids divided by the initial body weight gives us a quotient which we have designated as the total liver solid quotient. As calculated on the above basis this quotient is given for fasting experiment number 2 in comparison with the fat quotient of the liver at corresponding periods.

Series Number 5.

Fasting Experiment Number 2.

Table Number 29.

This table gives total liver solids as divided by the initial body weight, i.e. total liver solid quotient and also the fat quotient of the liver for corresponding periods.

Days fasted	0	2	4	6	8	9	
Total liver solids ÷ initial body weight:	14.9:	8.6 :	11.8 :	14.5 :	19.7 :	40.5 :	($\times 10^3$)
Total liver fat ÷ initial body weight:	16.24:	20.53:	52.9:	86.9 :	150.1:	342.1:	($\times 10^4$)

The fall in liver solid quotient on the second day is explained as due to the loss of glycogen from the liver cells, following this initial fall it would seem that there is quite conclusive evidence of an actual increase in the total liver solids during a fast, and that this increase is approximately proportional to the increase in fat in the liver of fasting animals. In no case in our experiments is an increase in the fat content of the liver associated with a decrease in the liver solids. There always occurs an increase in the liver solids with an increase in the fat content of the liver.

To summarize, in hunger there occurs in the liver an increase in the fat content which corresponds to an increase in the total liver solids. Further the fat free solids and also the percentage ofwater with which they are associated remains constant from early to late fasting . These results are in harmony with and support the observations made in fat feeding, or in other words the manner in

which an accumulation of fat in the liver occurs during a fast is not in any way materially different from the manner of the accumulation of fat in the liver during the normal absorption of fat into the blood. These observations we believe to consistently show that the increase of fat in the liver during a fast is the expression of a normal function of the liver in relation to fat metabolism, i.e. there occurs an infiltration of the transported body fats into the liver where the fat undergoes one of the intermediate steps in its catabolism.

C. Experiments with varying age.

a. The determination of the amount of fat and lipase in the liver in relation to varying age.

Observation was made on the fat and lipase content in the liver tissue of four kittens of the same litter. These were killed at different ages. One 30 minutes and another 40 minutes were killed without being allowed to take any food. A third kitten remained with the mother two days and a fourth six days before testing. The mother was also killed at the same time as the last kitten. Determinations of the fat and of the lipase content of the livers were made. The results as indicated by the fat quotient, i.e. the total calculated fat content of the liver divided by the initial body weight, in comparison with the lipase quotient, i.e. the total calculated lipase content of the liver divided by the initial body weight, are presented in the following table and chart.

Series Number 6.

Table Number 30.

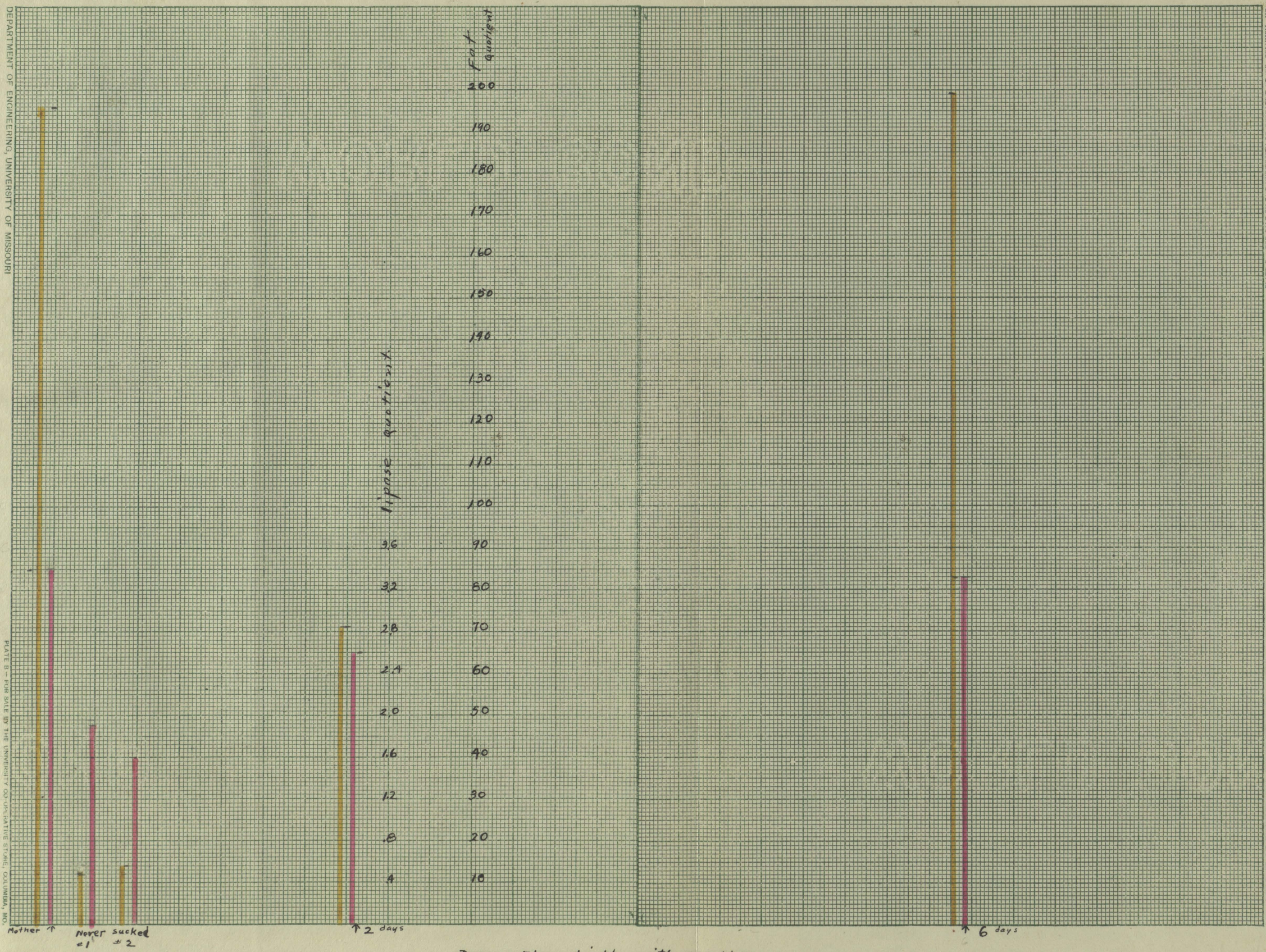
Showing increasing fat and lipase content of the liver with increasing age of young kittens

	Never sucked: No. 1	Never sucked: No. 2	Days after birth with mother: 2 da.	Days after birth with mother: 6 da.	Mother.
Total liver lipase divided by the initial body fat (x 10)	30' 1.9	40' 1.6	2.6	3.3	3.41
Total liver fat divided by the initial body weight (x 10^4)	11.9	14.	71.5	199.5	195.8

Chart Number XVIII

This chart represents graphically the data presented in the above table number 30. Yellow represents the fat content and red the lipase content. The curve for the liver fat as indicated by the fat quotient shows a gradual increase in the fat content of the liver with increasing age. The curve for the lipase quotient also shows an increase and this increase corresponds in a degree to the increase in the fat content of the liver.

CHART NUMBER XVIII.



The total lipase content of the liver, as is made evident by the total lipase content of that organ divided by the initial body weight, shows a progressive increase with the advancing age of the young kittens. Thus the newly born and the 2 and 6 day animals have a lipase quotient of 1.6, 2.6 and 3.3 respectively while the mother has a liver lipase content of 3.41. This increase in the lipase content of the liver is associated with an increase in the fat content of that organ. Thus the newly born and the 2 and 6 day animals have a fat quotient of 14, 71.5 and 199.5 while the mother has a fat quotient of 199.8. That newly born kittens which have not sucked, consistently have a low lipase and similarly a low fat content in the liver is shown by two animals. Number 1 has a lipase quotient of 1.9 and a fat quotient of 11.9 and number 2 has a lipase quotient of 1.6 and a fat quotient of 14, while the 6 day animal has a lipase quotient of 3.3 and a fat quotient of 195.8.

It should be pointed out that the increase in the lipase quotient of the liver with the increasing age of the kittens is paralleled by an increase in the lipase content of the blood. A study of the lipase content of the blood of this series of cats has been made by Mr. Summers.

The percentage of lipase in the liver of the 6 day kitten shows a decrease as compared with the percentage of lipase in the 4 day kitten. This decrease in percentage of lipase is associated with a high lipase quotient as is shown by the following table.

Table Number 31.

Showing that the percentage of lipase content may differ materially from the lipase quotient - total calculated lipase content of the liver divided by the initial body weight.

	Never sucked: No. 1	Never sucked: No. 2	Days after birth with mother: 2	Days after birth with mother: 6
c.c. NaOH 1 c.c. fat free extract	.83	.68	1.14	.76
Total liver lipase divided by initial body weight (x 10')	1.9	1.6	2.6	3.3
Percentage of fat in liver tissue	2.40	3.00	15.30	22.80

The lack of correlation between the percentage of lipase in the liver and the lipase quotient in the 6 day animal may be explained by the high percentage of fat in this liver. That is to say if the lipase is associated only with the fat free solids, the heavy infiltration of fat into the liver would lower the percentage of lipase, when in reality there might be an increase in the total lipase production and in

the percentage of lipase per unit of fat free solids. Unfortunately in this experiment, we have not the necessary data to enable us to figure the percentage of lipase on a fat free solid basis. There are the other instances in the work, in which there is a lack of correlation in the percentage of lipase and the lipase quotient, as example, fasting experiment number 2. From the results obtained in such cases we are possibly justified in believing that the percentage of lipase as figured on the fat free solid basis would have shown, as the lipase quotient does, an increase in the lipase content of the liver with increasing age. This increase in lipase content of the liver is associated with an increase in the fat of the same organ. This affords a most striking instance of the correlation of lipase and fat. With increasing age the animals are daily receiving and utilizing more and more fat, this is met by an increased lipase production by the lipase producing organs.

Further evidence of an increase in the lipase content of the liver with increasing age of young animals is shown upon making a comparison of the normal pups used in this work.

Table Number 32.

Showing increasing fat and lipase content of the liver with increasing age of puppies.

Experiment	Date of Decapitation	Lipase in c.c. N/20 NaOH	Percentage fat	Time fasted	Age
Fast.Exp.2:	2-12-15	1.95	2.30	18hrs	3 wks.
Feed.Exp.1:	12-13-14	1.78	3.35	44 hrs	5 wks.
Feed.Exp.2:	1-19-15	2.47	4.40	60 hrs	10 wks.
Fast.Exp.2:	4-1-15	4.59	3.32	22 hrs	7 wks.
Feed.Exp.3:	4-5-15	4.20	3.46	43 hrs	7 wks.

It may be objected that the variation here is due to the season. The pups of a low lipase content being taken in the midwinter, and those of high lipase in the early spring. The weight of this objection is depreciated when we consider that all animals were kept in a well heated animal house. The high fat content of the normal in feeding experiment number 2 is to be explained as due to infiltration into the liver of transported body fats, occasioned by the 60 hour fast. The low enzyme content of the normal of this same series may be due to the initial fall in the lipase content of the liver which is shown to occur in our work on fasting pups.

In general it would seem that there is an increasing lipase and fat content of the liver with increasing age in

young animals. This increase does not occur with increasing old age. This last statement is demonstrated by the slight variance in the lipase content of the liver of the 6 day kitten and the mother. The offspring having a lipase quotient of 3.3. while the mother is only slightly higher- 3.41.

The increasing lipase content of the liver observed with increasing age points strongly to the liver as a lipase producing organ. With advancing age the organism receives an increasing amount of fat, the transportation of which requires a proportionately large quantity of lipase. To meet this demand we should expect an increasing lipase content in a lipase producing organ.

D. Experiments with ether anesthesia.

a. The determination of the amount fat and of lipase in the liver in relation to different lengths of ether anesthesia before death.

Observation was made on the percentage of fat and lipase content of the liver in two-thirds grown dogs, food withheld 24 hours and kept under deep ether anesthesia. In one instance the animal was anesthetized for four hours, then rested 24 hours and then anesthetized again for 7 hours. Determinations were made of the fat and of the lipase content of the liver. Our results are presented in the following table.

Series Number 7.

Dogs etherized for different lengths of time before death.

Table Number 33.

This table gives the lipolytic activity shown by 1 cubic centimeter of the 10 per cent fat free aqueous extract of the liver acting on .5 cubic centimeters of ethyl butyrate, 4 cubic centimeters of water, .08 cubic centimeters of toluol at 38°C for a period of one hour in terms of cubic centimeters of N/20 NaOH. The fat content, i.e. the ether extractable substance in the dry liver tissue, is tabulated in terms of per cent by weight of the fresh sample.

Dog Number	N/20 NaOH	Per cent fat	Hours of ether anesthesia
1	2.96	16.6	4
2	1.90	5.08	6
3	2.00	3.00	6
4	1.93	2.30	8
5	1.42	3.12	4, rested 24, ran 7
6	1.98	2.08	12
7	----	2.64	14

Dog number 1 was subjected to ether for the shortest period of time - 4 hours - and shows the highest percentage of fat in the liver - 16.6 per cent, while those animals etherized for long periods of time show a low percentage of fat in the liver. For example, animals number 4,5,6, and 7, etherized for 8, 11, 12 and 14 hours in the order given have a percentage of 2.30, 3.12, 2.08, 2.64 fat in the liver.

The highest percentage of fat occurs in those livers having the greatest lipase content while the lower fat contents are associated with a lower lipase content Thus it would seem that even in ether anesthesia there is a correlation as between fat and lipase.

E. Fat-Lipase Relationship in the Liver.

A hydrolytic cleavage of the fat must precede its infiltration into the liver cells. This hydrolysis is known to be accomplished in vitro by an enzyme lipase. The rate of hydrolysis with an excess of fat is proportional to the lipase present, or vice versa, with an excess of lipase the hydrolysis is proportional to the fat.

If these two factors are normally in a state of equilibrium we should expect an increase in one to lead to an increase in the other. What is the explanation of this rise following a fall in the lipase content of the liver of fasting pups? If the rate of infiltration is proportional to the lipase content of the liver cells, how are we to account for the initial decrease of lipase content in the liver being associated with a rise in the fat content of the liver?

Let us assume that the liver is a lipase producing organ. Then the enzyme production and similarly the lipase content of this organ will most likely not represent the amount of lipase necessary to carry on its own fat metabolism. It will represent the lipase required for lipogenesis occurring in the liver tissue plus an excess which is to be utilized in the transporting and laying down of fat. For the sake of

clearness, let us assume further that the lipase required for the liver's own metabolism be represented by two units of lipase. The excess of lipase produced by the liver also be represented by two units. The entire normal lipase production of the liver is represented then by four units. The variation occurring in the normal four units of lipase may be governed by stimulations. The stimuli for the lipase production coming to the liver let us assume to vary with the condition of fat metabolism in the animal's body.

In pups whose diet consisted largely of milk, one showed a large excess of lipase in an enzyme producing organ, since the organism is receiving daily large quantities of fat. Also it is evident that the quantity of fat received will be in excess of the organism's immediate needs. In growing pups this excess of fat is laid down in the fat depots. The function of the two assumed units of excess lipase produced in the liver is normally utilized in the transportation and laying down of the recently absorbed fat.

The transition from a feeding to a fasting state is associated with numerous changes in the tissues of the body, changes that are accompanied by corresponding functional readjustments. In the functional changes, the one which will most concern this argument is the variation in the stimuli for the formation of the fat-splitting enzyme, lipase. Let

us assume that the last products of digestion have been absorbed and utlized by the tissues. When this point has been reached, what is the source of energy in the organism? It is obvious that the body stores will be called upon. In young pups the glycogen may be abundant. It is this source of material that comes first to the support of the body in this crisis. The other stores that are called upon will depend entirely upon those present. Assume that the fat store is one of the last to be utilized. Then at the beginning of the fast, at a time when the non-fatty stores are being used, we would expect a decrease in the lipase content of an enzyme producing organ. Later when the glycogen and other stores of the body have been exhausted by the tissues and the fat stores are being used, we would expect an increase in the lipase content of the enzyme producing organ. Further, it will be evident that the time at which this increase in lipase during a fast will occur depends on the extent and availability of the glycogen and other stores of the body. This would seem to be a possible explanation of the initial fall followed by an increase in the lipase content of the liver observed in our fasting experiments. It still remains to offer a possible solution of the observation that there occurs an increase in the fat content of the liver associated with a fall in the lipase content of that organ.

It is evident that with absorption the transported fat will be in excess of the amount of fat oxidized in the body tissues. This excess of fat will utilize a proportionally large excess of lipase. In a fasting animal where the stored fats are being used, it would be reasonable to expect that the transported fats would not be greatly in excess of the needs of the body tissues. Similarly, to transport this proportionally small amount of fat would require a smaller production of excess lipase. If the excess production of lipase in the liver is decreased to one half the normal excess, i.e. is represented by one unit over and above the two units assumed to be associated with the liver's own fat metabolism, this unit is available for transporting and laying down of fat in the liver and later passing it on to the body tissues for oxidation.

By this view it would seem that in fasting animals it is still possible for an infiltration of fat in the liver to occur even where there is a decrease in the lipase content of that organ.

If the liver as a lipase producing organ normally has an excess lipase content, the rate of infiltration of fat into the liver will be proportional to the fat carried to that organ. With such conditions following a fat meal, we might expect the increase in the infiltration of fat in the liver to be associated with no radical change in the enzyme content of that organ. Also as the infiltration

of fat increases an equilibrium between the fat and lipase content should be approached. Our feeding experiments show that after a fat meal there occurs first a slight fall to be followed later by a slight rise in the lipase content of the liver and that this cycle is associated with a steady increase in the fat content of this organ.

The observed increasing fat and lipase content of the liver with increase in the age of young pups and kittens is a most striking example of a definite relationship between fat and lipase. Thus with increasing age of young animals they receive and utilize daily more and more fat. This increase in fat is necessarily associated with an increase in lipase.

In conclusion let us emphasize the main contribution in this thesis, namely, that there is a correlation between the fat and the lipase content of the liver as shown by the definite relationship observed as between the fat and lipase content of that organ in fat feeding, in fasting, in increasing age, and in ether anesthesia.

SUMMARY

1. In young puppies the fat and the lipase content of the liver each run a definite and characteristic cycle of variation following a meal rich in fat.

a. In the lipase cycle there occurs in the early periods of digestion, two hours, an initial fall in the lipase content of the liver. This is followed later by a rise in the lipase content reaching its maximum at the height of absorption, eight hours.

b. The fat cycle is characterized by a progressive increase in the fat content of the liver to a maximum at eight to eleven hours after feeding, followed by a gradual decrease, to 14 hours.

2. In young puppies both the fat and the lipase content of the liver have a definite and characteristic curve of variation in relation to fasting.

a. The lipase curve is marked by an initial fall in the lipase content of the liver for the first two to six days and is then followed by a rise to the end of the fast.

b. The curve for the fat shows a progressive increase up to the time approaching inanition when there occurs suddenly a great increase of fat in the liver.

3. The fat and the lipase content of the liver is uniformly distributed in the different lobes of that organ.

4. Following the second day of a fast there is an increase in the total liver solids associated with the increase in the fat content of that organ.

5. Following the depletion of the liver's glycogen the ratio of the total fat free solids of the liver to the initial body weight of puppies is practically constant throughout a fast.

6. The percentage of water associated with fat free solids of the liver varies but little from early to late fasting.

7. The accumulation of fat in the liver during hunger is physiological and not pathological in nature.

8. With increasing age of young pups and kittens there occurs an increase in the fat and the lipase content of the liver.

9. There is a definite correlation between the fat and the lipase content of the liver as shown by the definite relationship observed with feeding, with fasting, with increasing age and with ether anesthesia.

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UNIVERSITY OF MISSOURI
COLUMBIA

DEPARTMENT OF PHYSIOLOGY

May 21, 1915.

Dean Walter Miller,

Chairman of Graduate Committee.

Dear Dean Miller:

I am sending you herewith the thesis of Mr. John H. Carter who is a candidate for his A. M. degree in the Department of Physiology.

Very respectfully,

Chas. W. Greene

The thesis is approved
C. W. Greene
May 21, 1915.

May 24.

Dear Dr. Trowbridge:

It is customary for the Graduate Committee to refer dissertations, submitted by candidates for the degree of Master of Arts, to some member of the Group who is not connected with the Department in which the candidate's work has been done. I am sending you herewith a dissertation which has been submitted by J. M. Carter I shall be greatly obliged if you will kindly examine the same at your earliest convenience and report to us for the Graduate Committee whether in your opinion the dissertation meets the general standard which has been established in this University for the Master's dissertation.

Very truly yours,

J. M. Miller

Chairman, Graduate Committee.

UNIVERSITY OF MISSOURI
COLUMBIA

DEPARTMENT OF AGRICULTURAL CHEMISTRY

May 26, 1915.

Dean Walter Miller,

Graduate School.

My dear Dean Miller:

In accordance with your request of the 24th I have read the thesis of Mr. John M. Carter and report that in my opinion it is a most excellent thesis and well worthy of fulfilling that requirement for the degree of Master of Arts. My only criticism would be that it is too long. I believe the data might have been fully presented in less space.

Yours truly,

P. F. Trowbridge