

THE FAT AND LIPASE CONTENT OF THE BLOOD FOLLOWING
FAT FEEDING AND DURING INCREASED
MUSCULAR WORK.

By

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I. Introduction

Early in the history of physiology substances which accelerate chemical reactions were extracted from plant and animal tissues. To these agents which acted very much like inorganic catalyts the name "ferments" was applied. The first of these compounds was found in 1829 when Dubunfaut¹ converted starch into sugar by means of an extract of malt, and a number of such bodies were reported in the following few years.

The term ferments was already in use to denote living organisms like yeast. The confusion brought about by the new use of the term lead Kühne to suggest a new name for the class of "inorganic ferments". The name enzyme, which he coined, has been in the past and is today applied to the catalyts produced by the living organisms.

The great biological importance of the enzymes so widely distributed throughout the animal and plant kingdoms has stimulated investigation along the lines of enzyme action. The result is we now have a fairly definite idea of the general actions of these peculiar bodies, and to a limited extent the part they play in physiological processes.

Duclaux² suggested that the termination "ase" should denote an enzyme and should be added to the substrate, which is the name given to the substance on which an enzyme exerts

its activity. This suggestion has been followed and most enzymes are thus named. However, some of the old established names, such as, "pepsin" and "trypsin", remain. Following the recommendation of Duclaux, the fat splitting enzyme has become known as "lipase".

Enzymes are specific in their action, hydrolytic, amylotic, lipolytic, etc., and under favorable environment, optimum temperature, dilution and composition of the substrate, enzymes are able to accelerate the potential reaction which is present under such conditions. This reaction tends to bring about an equilibrium. An equilibrium may be established through the reversible reaction of the enzyme, a property first discovered by C. Croft Hill and later verified by many others and for other enzymes. When we introduce "lipase" into a fatty substrate following the general laws of enzyme action it sets into motion by its synthetic and its hydrolytic power a reaction which results in a balanced relation of the fatty constituents of the substrate.

Our modern theory of fat metabolism is based upon this foundation. We know that neutral fat, as such, can not pass through an animal membrane, but that the fatty acids and glycerine, into which it hydrolyzes, readily penetrate these membranes. Thus food fat in the intestinal canal hydrolyzes into fatty acids and glycerine.

The hydrolysis is complete, in as much as the removal of the products of the hydrolysis by absorption prevents the establishment of equilibrium. These products during their passage into the epithelial cells of the mucosa come in contact with the intracellular lipase. The enzyme of the epithelium, now in contact with fatty acids and glycerine only, can establish an equilibrium only through the synthesis of fat which occurs. When absorption ceases, the reaction reverses and the fat in the cells is hydrolyzed. Again the hydrolysis is preponderant and may be complete, for the cleavage products are constantly removed through diffusion into the lymph spaces and capillaries. Upon reaching the blood stream fatty fractions may be synthesized or fat hydrolyzed according to the total percentage in the blood, a balance determined by the relative rate of gain from the absorbing tissues and loss in other tissues. As the products pass into the general tissue cells they are synthesized again before being utilized or stored as fat.

This theory assumes the presence of lipolytic enzyme in all the tissues and fluids of the body which are involved in the rôle of fat metabolism. This universal presence has been demonstrated, and is brought out in the following review of literature on lipase. It is also shown that there are investigators who contend that the cycle is not brought about by enzyme action, but by the

living cell wherein there is a supply of external energy for the transformation. However, at present the experimental evidence favors the enzyme theory, and it is upon it that this research is based.

Lipase was discovered in the pancreatic juice in 1846 by Claud Bernard.³ Several years later, 1855, Pelouze⁴ demonstrated its presence in the castor oil seed. However, the subject of lipolysis did not attract the attention of investigators strongly until Hanriot's⁵ work on serum lipase appeared in 1896. Hanriot's work was based upon the hydrolysis of monobutyrim by the serum ferment, but he claimed that the action upon the higher fats was the same. Arthus⁶ then made Hanriot's assumption questionable when he showed that aseptic horse serum which hydrolyzed monobutyrim actively was devoid of action upon olive oil. However, the recent work of Rona and Michaelis⁷ has confirmed Hanriot's views, by proving that the sera of horses, cats, sheep and cattle have similar action on both monobutyrim and tributyrin.

Soon after the initial work of Hanriot, Connstein and Michaelis⁸ discovered that fat is changed into an easily reversible, soluble, filterable and dialysable form, by means of which change it is transported into the tissues, for the fat droplets which enter the blood from the thoracic duct can not wander out of the intact capillaries. They

were unable to give a satisfactory explanation of the phenomenon which they ascribed to a fat-dissolving or lipolytic function exerted by the red corpuscles. Their work has since been confirmed by Umber and Brugsch,⁹ and especially by Thiele.¹⁰

Kastle and Loevenhart¹¹ in 1900 found that ethyl butyrate is readily hydrolyzed and synthesized by the lipase contained in fresh pig's liver. By means of this substrate they demonstrated the almost universal presence of lipase in tissues. Their results were verified a little later when Pottevin¹² obtained a neutral oil, mono-olein, when a mixture of equal parts of glycerine, extract of fresh pancreas and pure oleic acid were incubated.

In 1901-02, Loevenhart¹³ confirmed his former work by again demonstrating the presence of lipase in all the tissues and fluids of the body. At this time he applied the reversible action of lipase to the various steps in fat metabolism, and by this application, according to Pflüger,¹⁴ "we are furnished with a chemical explanation for the digestion, absorption, transportation and storage of fat in the living body".

Because of the evidence given by Rona and Michaelis⁷ and Pottevin¹² there seems to be little doubt that the esterases are true lipases, and, therefore, that the action observed by Hanriot¹ in 1895 is a true lipolytic one, and

that it can be directly applied to the hydrolysis of natural fats.

The source of blood lipase was first investigated by Hanriot. Noting the similar actions of the lipase ferment and the pancreatic juice he removed the pancreas in a dog and found on the following day a great increase in the blood ferment. Autopsy, however, showed that part of the gland remained. Since then other investigators, Umber and Brugsch,⁹ Pagenstecher,¹⁵ and Thiele¹⁰ have recognized qualitative difference between the pancreatic enzyme and tissue lipases; and Loevenhartt¹² following up these investigations confirmed them, concluding that the lipase of the liver, kidney and blood are alike and differ from the pancreatic ferment.

Fiessinger and Marie¹⁶ believed that the lymphocytes are the chief source of sero lipase, but this as well as the views that hold that sero lipase is derived from any particular source seem untenable before the recent conclusion of Von Hess,¹⁷ that the blood ferment comes from the cells of the entire body and the variations in sera lipase is due to the general stimulation or depression of body cells. This theory is strengthened by Whipple,¹⁸ who finds that the blood lipase increases whenever there is injury to the liver, such as chloroform anesthesia.¹⁹ Bauer found that every human serum contains fat splitting

enzymes which are decreased in diseases lowering the general body "resistance", and are increased in the stage where the body tissues react with vigor. Further, the hypothesis of Pavlov and Voit²⁰ when applied to this phase of metabolism²¹ give Von Hess' theory a greater probability. Pavlov cites the responses of pancreatic secretion to various foods and asks why we should not expect body cells, which are capable of enzyme formation, to respond to different blood contents. Voit believes that the presence of abundant food increases the power of the cell to metabolize. Therefore, in light of our present knowledge it seems probable that all the tissue cells contribute a part of the blood lipase. The blood lipase content at a given time is a measure of the metabolism of the body under the existing conditions.

The lipase content of the blood has been studied under a number of physiological and pathological conditions, but as yet we know very little of the particular factors which determine the variations in either case.

²²Summers found in this laboratory that rich fat diet following fasts in both young and adult dogs cause an increase in the lipolytic activity of the blood. Preceding²³ the investigation of Summers, Abderhalden and Rona found an increase of blood lipase after fat feeding. Their work, however, is subject to criticism because they fasted their animals for several days before taking a normal. Never-

theless, their results are valuable in connection with the work of Summers, for both point to an increased enzyme content following the ingestion of fat. Summers²² found in the blood of fasting puppies that the lipase content remained practically constant for two days, rapidly fell to 45 per cent of the normal at four to six days, and sharply increased to 125 per cent on the ninth day. In adult dogs he reports a curve like that of the puppies, vis., a decrease to less than half the normal in four or five days, a low content for six or eight days, followed by a gradual rise. The lipase content in all tests ^{was} being higher at the end of the fast than at the beginning. Alderhalden and Lampe²⁴ state that the lipase content increases during fasting.

That lipase plays some part in pathological conditions has been pointed out by Bergel²⁵ who observed that sera of animals immunized to foreign red cells were about twice as active in splitting foreign fats as were the sera of untreated animals. The reports as to the blood content of lipase in disease are very conflicting. Carrière⁵⁴ found that its activity was normal in certain diseased conditions -- neurasthenia, hysteria, epilepsy, hemeplegia and compensated heart lesions -- lipase was increased in obesity, diabetes and pneumonia, and decreased in carcinoma, uremia and terminal tuberculosis. Fischer,²⁶ on the other hand, found a case of diabetes with marked lipemia in the serum

of which the presence of lipase was not demonstrable.

Archard and Clerc²⁷, from their investigations, conclude that the enzyme activity is lessened in most pathological conditions, while Pribram²⁸ found it increased during fevers. Saxl²⁹ reports no increase during phosphorus poisoning, and either a rise or fall in jaundice. Cetrou³⁰ and Plecher¹⁹ observed an increase of the ferment in syphilis, but Bauer¹⁹ found a decrease in that disease. The part played in abnormal conditions is thrown more in the dark³¹ by Sagal, who after a careful study of over 100 cases, concludes that the variation of lipase activity in diseased conditions is so small as to be devoid of clinical value.

The fact that variations in the lipase content of the blood have occurred during different phases of fat metabolism and during diseases which affect the fat content of the body suggest that the two substances, lipase and fat, are correlated, and that observed variations in the fat may affect the amount of lipase. For this reason it seems necessary to review the literature on the blood fat along with that of the lipolytic enzyme.

It has been known since the time when blood letting was in common practice that the blood serum may become milky after fat feeding, but the quantitative aspect of fat changes in the blood with relation to fat metabolism was first studied by Newman.³² He counted the fat parti-

cles in the blood both before and after feeding. By means of the ultramicroscope he found that the number of particles greatly increased two hours after feeding.

³³ Neisser and Baurning, by the same method, found that fat particles began to appear in the blood serum in from one to two hours, reached the height of their concentration in six hours, and then diminished, the intensity of the changes varying with the animal and the kind of food used. The results by the above method would perhaps be unreliable if later observers, using chemical methods, had not found similar changes. ³⁴ Lattes, using the Kumagawa-Suto method of fat extraction, found that after feeding fat there was an increase in the blood fat up to double the ³⁵ normal value. Terroine observed a great rise in the concentration of the blood fat during the absorption of fat.

³⁶ ¹⁰ Other men, among them, Greenwald, Thiele, and Lusk ³⁷ and Murlin have demonstrated lipemia following the ingestion of foods rich in fat.

³⁸ Munk and Rosenstein have investigated the fat content of the human lymph and chyle and find that the fat increases during fat absorption the same as in dogs. They further note that the content is decreased in starvation.

³⁹ Bloor has noted its variations in the blood of dogs under the following approximately normal conditions:--
(1) after fat feeding, (2) after intravenous injections of

fat preparations, and (3) during fasting for short periods. In the first he verified previous findings by noting an increase in the fat content of the blood, beginning during the first hour and reaching a maximum in about six hours after food. No increase was found when the thoracic duct was tied, showing that the fat absorbed by the capillaries was not sufficient to cause lipemia. Intravenously injected fat preparations, enough to raise the fat content of the blood 100 per cent, disappeared from the circulation in less than five minutes. When much larger quantities, 0.8 gm. per kilo in the form of egg yolk, were injected some fat persisted for several hours. Fasting of from 5 to 7 days produced an increase in some cases and in others none. However, an animal which ordinarily showed no increase in blood fat during fasting was made to exhibit the "usual curve" by stuffing with fatty food for one week.

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Greene and Summers investigated the fat content of the blood following ordinary fat feeding and during fasting, using litters of puppies and adult dogs. Puppies from the same litter were fed rich diets and killed at different periods during absorption and the fat in their blood determined. During absorption a slight increase was noted followed by a sharp increase over normal during the 5 and 6 hours, and a smaller one from 11 to 14 hours. The blood of fasting puppies killed at successive intervals during

fasting showed the greatest increase of fat of 230 per cent on the eighth day and 185 per cent on the ninth day. The changes in the blood fat of adult dogs during fasting were very small with perhaps a tendency to decrease during the first 12 to 15 days followed by an increase after the 15th day.

⁴¹
Shutz, "examined the bloods of different animals during fasting and found that they contained 50 to 100 per cent more fat than fed ones"

⁴²
Daddi fasted animals over very long periods and reported an increased fat content during the first week, then a fall, and about two weeks before death a further decrease.

The effect of muscular work on the fat concentration of the blood has been studied by Murlin and Riche, ⁴³ who found a slight fall during the first half hour of work, sometimes in the first fifteen minutes, but this was followed by an increase of fat in the blood at the end of one hour. The content dropped below normal after one hour's rest.

The variations of the blood fat in many pathological conditions has been proven by a great number of investigators, and while there are many disagreements as to the actual changes, it is very probable that an explanation of the pathological fluctuations will be possible through a better understanding of the physiological changes.

From the investigations cited above there seems to be no doubt that variations occur in both the lipase and the fat content of the blood under different physiological conditions. But without correlation these variations do not help to solve the question of fat metabolism. Loeven-¹³hart in his original work found a certain degree of parallelism between them. Such a parallelism, when proved, would give positive evidence in favor of the present enzyme theory of fat metabolism. Bradley in 1913⁴⁴ repeated these observations, but failed to find "any broad correlation existing between the fat and lipase content of tissues". His work, however, has been criticized by Summers,²² who pointed out the fact that Bradley did not pay any attention to the general systemic nourishment of his animals. In 1915,²² Summers demonstrated a relationship between the lipase and fat contents of young and adult dogs when they were in comparable nutritional conditions. This relation was proved during fat feeding and during fasting. In the feeding experiments cream was fed so there is a chance that the absorption of lipase from it might have altered the amount in the blood; but since the relationship existed in the fasting experiments it is improbable that there was much cream lipase absorbed.

Although the relationship between the lipase and the "total fat" content of the blood gives some support to the

enzyme theory of fat metabolism the question remains an open one. There is a factor in the part played by the blood lipoids, which include fatty acids, cholesterol and lecithin. It is necessary that they should be taken into account while investigating the importance of sero-lipase.

In the past the increase in fat content of the blood under certain physiological conditions has been looked upon as a simple lipemia. There is at the present time experimental evidence which suggests that the process is more complicated, a "lipoidemia".

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Terrione found that the "total fat", fatty acid + cholesterol, was very constant in animals, and there was a constant relation between the fatty acids and the cholesterol. He suggested, "that the lipemia constant might be very much of the same order as the glycaemic constant and like it, was probably the expression of an efficient regulation".

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Csonka demonstrated a low cholesterol concentration in patients, with pernicious anemia, showing disturbed blood fat contents.

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Mayer and Schaeffer found that the relation between fatty acids and lecithin in the blood was remarkably constant for most animals.

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Müller observed that lecithin and cholesterol were often increased during lipemia; and Reicher in three experiments on dogs found great increases in lecithin, 82 per cent, and cholesterol, 65 per cent, during fat absorp-

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tion, while at the same time the average fat increases 53 per cent.

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Although Greenwald found no increase in lecithin during the different phases of fat absorption, the more recent and extensive researches of Bloor⁴⁹ seem to establish the importance of at least one of the lipoids, lecithin, in blood fat. He found, as before, that the total fat was greatly increased during absorption in both plasma and corpuscles. The changes in the cholesterol content were slight and inconstant, confirming the probability that cholesterol takes no part in the early stages of fat metabolism. This, however, disagrees with the results of the earlier investigators, but, because of his improved methods, it is perhaps more reliable. His conclusions in regard to lecithin are: that it increases in the blood, and that there is a definite relation between it and the fatty acids. The increase of lecithin is most marked in the corpuscles. From this fact and the recent investigations of Thiele and Fos⁵⁰ which are in agreement with it he concludes: "(a) that the blood corpuscles take up the fat from the plasma and transform it into lecithin; (b) that most if not all of the absorbed fat is so transformed; and therefore, (c) that lecithin is an intermediate step in the metabolism of fats".

The importance of the lipase in this step of the metabolism of fat is brought out by Bloor⁴⁹ when he writes in

regard to the lecithin formation in the corpuscles, "since the enzyme reactions in the living organism are generally regarded as reversible, the formation of lecithin in the presence of excess of fat in the blood, as exists in lipemia, is to be expected".

From the preceding literature review the variations in the blood fat content under normal conditions seem fairly clear. The lipase content is more obscure and the correlation existing between the fat and the lipase needs more experimental proof before the part and importance of the lipase in the blood can be definitely stated. A further study of the fat and the lipase contents of the blood after feedings of selected diets to animals in comparable nutritional conditions should give more conclusive evidence in regard to the relation of these two factors. In the present study we have undertaken an investigation of the lipid and lipase contents of the blood in animals during increased muscular exercise in an effort to determine whether during increased fat transportation and utilization there is any corresponding variation in the amount of blood lipase. Whatever the answer, the results of the experiment ought to throw more light on the important problem of fat metabolism.

III. Experimental Procedure

The experimental work is divided into two parts; (1) the effect of feeding upon the lipase and fat contents of the blood, and, (2) the effect of muscular work upon the lipase and lipid contents of the blood.

The feeding experiments were done in cooperation with Mr. Harvey, who studied the lipase and fat content of the liver and kidney. In order to secure the tissue samples it was necessary to kill the animals. For this reason the experiments were run on sets of animals. To secure the most uniform results litters of puppies were used. During a preliminary period, usually one week, the animals were placed in isolated compartments, and fed equal amounts based on grams per kilo., of a carefully prepared mixed diet. Then the animals were fasted for 24 hours to allow complete absorption of the food of the regular feedings. One animal of the series chosen as a normal was weighed and decapitated. The others were fed test meals, weighed and decapitated at different intervals during absorption. When drawing the blood, care was taken not to rupture the thoracic duct or allow any regurgitation from the stomach. The blood was collected in a casserole. Duplicate 2 cc. samples were taken for fat determination and the remainder was transferred to centrifuge tubes, allowed to stand three or four minutes, and centrifuged at a high rate of speed for fifteen

minutes. By this method a very clear serum was obtained which was used for lipase determinations.

In the experiments on the effect of muscular work dogs were used. These animals had been fed on a constant diet for several weeks and were in splendid condition. During the experimental period they were carefully tended to keep their nutritional states as normal as possible. For a period of twenty-four hours preceding the work tests no food was given in order to prevent complicating changes in the blood fat due to absorption. No water was given for a period of one hour before the test.

The muscular work was obtained by exercising the animals in a tread mill for given periods at a nearly constant rate of speed of about 3 miles per hour. The machine was carefully constructed and ran smoothly and with little difficulty. The dogs became accustomed to the treatment, and after a little training drove the mill intelligently.

Blood samples were taken before work, after thirty minutes exercise, after one hour's exercise, then after resting thirty minutes and one hour. The lipolytic activity of the serum was determined and the whole blood was examined for fat, cholesterol and lecithin. The blood was drawn directly from the small saphenous vein in the hind leg. After considerable practice, by using a properly sharpened needle and applying a light

tourniquet or by compressing the vein with the thumb, it was possible to obtain an 8 or 10 cc sample very quickly. The blood was collected directly from the needle in a centrifuge tube, both the needle and tube having been previously rinsed with 3 per cent potassium oxalate. A 2 cc. sample was run into the alcohol-ether mixture for fat determination, and the rest of the sample, after standing three or four minutes, was centrifuged at a high rate of speed for ten minutes. The serum was pipetted off and used for determining the lipolytic activity.

Lipase Determination

Duplicate 1 cc. samples of the serum from the centrifuged blood are placed in 30 x 60 mm. glass stoppered weighing bottles. To each is added 10 cc. distilled water, 0.5 cc. ethyl butyrate, 0.4 cc. litmus, and 0.08 cc. toluol to prevent hydrolytic action of bacteria and the whole immediately neutralized with N/20 butyric acid. The bottles are tightly corked, shaken fifty to one hundred times to insure complete emulsion and placed in an electric incubator at 38°C for 24 hours. The butyric acid liberated by the lipase action in the sample is titrated with N/20 NaOH. The quantity of the alkali required to neutralize the butyric acid set free during the period of incubation is taken as the measure of the lipolytic activity of the serum.

Fat Determination

The fat content, total fatty acids plus cholesterol, was determined by a modification of Bloor's⁵¹ method which is based on a comparatively new principle, i. e., the determination of the fat by precipitation in a water solution and comparison of the cloudy suspension so obtained with that of a similarly prepared standard fat solution. The comparison was made by the use of the nephelometer. The procedure is as follows: Extraction. About 2 cc. of freshly drawn blood are run with stirring into a weighed graduated flask containing about 40 volumes of a mixture of 3 parts alcohol and 1 part ether. After again weighing to find the amount of blood added, the solution is raised to boiling in a water bath, cooled under the tap, made up to volume with alcohol-ether mixture, mixed and filtered. The filtrate is water clear and almost colorless.

Determination. 10 cc. of the filtered extract (containing about 2 mgm. fat) are measured with a pipette into a 150 cc. beaker and saponified by evaporating just to dryness in a water bath with 2 cc. N/1 sodium ethylate. The dried residue is gently warmed with 5 cc. alcohol-ether mixture until all but the flakes of alkali are dissolved. To the contents of the beaker 50 cc. of distilled water are slowly added with stirring.

Five cc. of a standard fat solution (containing 2 mgm.

oleic acid in alcohol-ether mixture) are measured into a similar beaker and 50 cc. water added with stirring. To the standard and the test solutions are added simultaneously 10 cc. portions of dilute (1:4) hydrochloric acid and the solutions allowed to stand for five minutes, after which they are transferred to the comparison tubes of the nephelometer.

The nephelometer used was a modification of the Duboscq colorimeter. The metal screen and the solution cups were discarded and the reflection mirror was not used. The screen was replaced by one having a partition separating the two prisms to prevent reflection of light from the two tubes, and with two narrow slits (1 cm. wide), one in front of each prism so placed as to allow the light to pass in from directly in front only. At the same time the screen cut off the light at the lower edge of the prism. This last precaution does away with the necessity of blacking the prisms of the colorimeter, since the only light reflected through the prisms must come in through the ends. The tubes were replaced by black metal frames which fit snugly into the tube supports. In these were placed clear, carefully matched white glass tubes made from 18 mm. test tubes. A constant source of light was maintained by placing a well screened 100 watt condensed filament "Mazda" lamp about 1/2 meter in front of the nephelometer. With these changes it was

found that with similar solutions in the two cups the field was homogeneous when the tubes were reading the same on their respective scales.

When making the comparisons the two tubes filled to the same height were placed in the frames, the standard tube always on the same side. The standard was set at 10, and comparison was made by raising or lowering test solutions until the images of the two solutions showed equal illumination. The average of not less than five readings, taken alternately from above and below, was used as the recorded reading. Determinations were made in duplicate, and repeated if the results failed to check closely. The readings were calculated to percentages.

Cholesterol Determination.

Cholesterol was determined by the new method of Bloor⁵² Ten cc. of the alcohol-ether blood extract used in the fat determination are measured into a 50 cc. beaker and evaporated just to dryness on a water bath. Any heating after dryness produced a brownish color which passes into the chloroform and renders the subsequent determination difficult or impossible. The cholesterol is extracted from the dry residue by boiling out three or four times with successive 5 cc. portions of chloroform and decanting into a 10 cc. glass stoppered graduated cylinder which had been previously calibrated. The

combined extracts when cooled are made up to 5 cc. The solution was colorless but not necessarily clear, since slight turbidity cleared upon adding the reagents.

Five cc. of a standard cholesterol solution in chloroform (containing 0.5 mg. of cholesterol) were measured into a similar 10 cc. cylinder.

To each of the solutions are added 2 cc. acetic anhydride and 0.1 cc. concentrated sulphuric acid. The solutions are then mixed by inverting several times, and set away in a cool dark place for 15 minutes, after which they are transferred to the cups of a Duboscq colorimeter set in plaster of Paris. The average of several readings was taken and calculated to percentages.

Lecithin Determination.

The lecithin content was found by the strychnine molybdate method of Kober,⁵³ applied as follows: 10 cc. alcohol-ether blood extract are measured into a 200 x 25 mm. Jena test tube, previously etched at 25 cc. mark, three glass beads added and the solution evaporated to dryness in a water bath. The tube should be shaken frequently until boiling commences, after which the evaporation will proceed to dryness without further attention. The residue is dried for 15 minutes to remove the last traces of alcohol which might interfere with complete oxidation. For oxidation,

1.5 cc. of equal parts of HNO_3 and H_2SO_4 are added to the dry residue in test tube, and digested by heating with a micro-burner. The heating is done in two stages. During the first 15 minutes the mixture is gently boiled with a very low flame until the red fumes cease to come off, care being taken not to drive off HNO_3 before complete oxidation has taken place. The tubes are inclined at an angle of about 30° to prevent loss by spattering. The heat is gradually raised until the nitric acid is completely driven off, after which the solution is boiled for 10 minutes. The mixture is then cooled and two drops of .25 per cent cane sugar are added (to destroy nitric-phosphoric acid combination), after which the mixture is heated for 1 minute. The sugar causes a slight browning of the liquid, which disappears on heating. The tube is then cooled and the sides washed down with about 3 cc. H_2O . To the solution in the tube is added 1 drop phenolphthalein, and it is then neutralized with 20 per cent NaOH , noting the amount added. The solution is made just acid with one or two drops of dilute hydrochloric acid (1-1), cooled and made up to mark.

Five cc. of a standard phosphate solution (containing .025 gm. per cc.) are measured into a similar graduated tube, 0.70 cc. H_2SO_4 , concentrated, a drop of phenolphthalein and the amount of 20 per cent NaOH used in sample are run in. The contents are then neutralized with 1-1 HCl , made

slightly acid, and made up to volume.

Precipitation was carried out as follows: To each of two 50 cc. flasks are added 25 cc. distilled water, 5 cc. of 1-1 HCl and 5 cc. of molybdate reagent; then 10 cc. of the phosphate solutions are added with a pipette, keeping the flasks gently rotating during the addition. The flasks are then made up to volume with distilled water, allowed to stand for 3 minutes, and then compared in the nephelometer. The values obtained, multiplied by eight, give a close approximation to the lecithin value.

III. Experimental Results

1. The Fat and the Lipase Content of the Blood

Following a Meal Rich in Fat.

A single feeding test was run on a group of six pups about six months old. We had the misfortune to lose by infection all the other large litters collected for the test. In this set of puppies the first five were of the same litter, while the sixth was an odd pup of apparently the same age. All were in good condition, the average weight being about five and one-half kilograms. The six pups had been together for several weeks, and had been fed a carefully prepared mixed diet. Their nutritional condition was observed constantly, and they were all in practically the same condition at the time of the test. All were fasted for 24 hours, pup number one was killed for a normal and the remaining five were fed 90 grams per kilo of bread consisting of corn meal and cotton seed oil, containing 25 per cent of fat. The fed pups were killed at intervals during digestion and absorption of the fatty meal. The first was killed two and one-half hours after feeding, and the others at the fifth, eighth, eleventh and fourteenth hours respectively.

Protocols of the Autopsies. - The stomach of the pup taken for the normal was partially filled with soft food,

showing that all of the previous day's food had not passed into the intestine. The food also contained a good deal of undigested cracklin fat, all indicating that the previous meal had not been digested and absorbed as assumed, but that the process was still going on. This may account for the high percentage of fat in the blood of this pup. The lacteals were scarcely visible and the intestines were practically empty. The stomach of pup number two, two and one-half hours after feeding, was gorged with the undigested bread mixture. The lacteals were visible, but not prominent, and the intestines were nearly empty. The stomach of pup number three, five hours after feed, was full of semidigested food. The lacteals stood out clearly, and were milky in color, and the intestine contained chyle. The stomachs of pup number four, eight hours after feeding, of number five, eleven hours after feeding, and number six, 14 hours after feeding, were about one-half, one-third, and one-fifth full of emulsified food respectively. In all three the lacteals were full, and the intestines contained chyle.

The autopsy findings reveal two unforeseen facts that complicate the results. The first is that the normal animal killed twenty-four hours after the last feeding had not completed the digestion of the previous meal as was assumed. It is only fair to assume that some degree of absorption was still going on and that the blood fats had not yet reached

stable equilibrium as between the absorbing tissues, the blood and the storage tissues. Its fat content, and possibly the lipase content also, is probably higher than the real normal. This reduces by so much the percentage of increase to be expected from the experimental feed. The second significant fact is that the rate of digestion and absorption of the test meal was slow for the series, and not complete at the end of the fourteen hours allowed, an allowance of time previously found in this laboratory to be quite adequate for a test meal of milk and cream.

Table I.

Feeding Experiment 1, April 20, 1917, showing variations in the fat and lipase contents of the blood following a meal of cotton seedfat and carbohydrates. The determinations were made on the blood of a series of six pups, about six months old, in comparable nutritional condition. One was killed without food for a normal. The others were decapitated at two and one-half, five, eight, eleven and fourteen hours, respectively, after the test meal.

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

Chart I.

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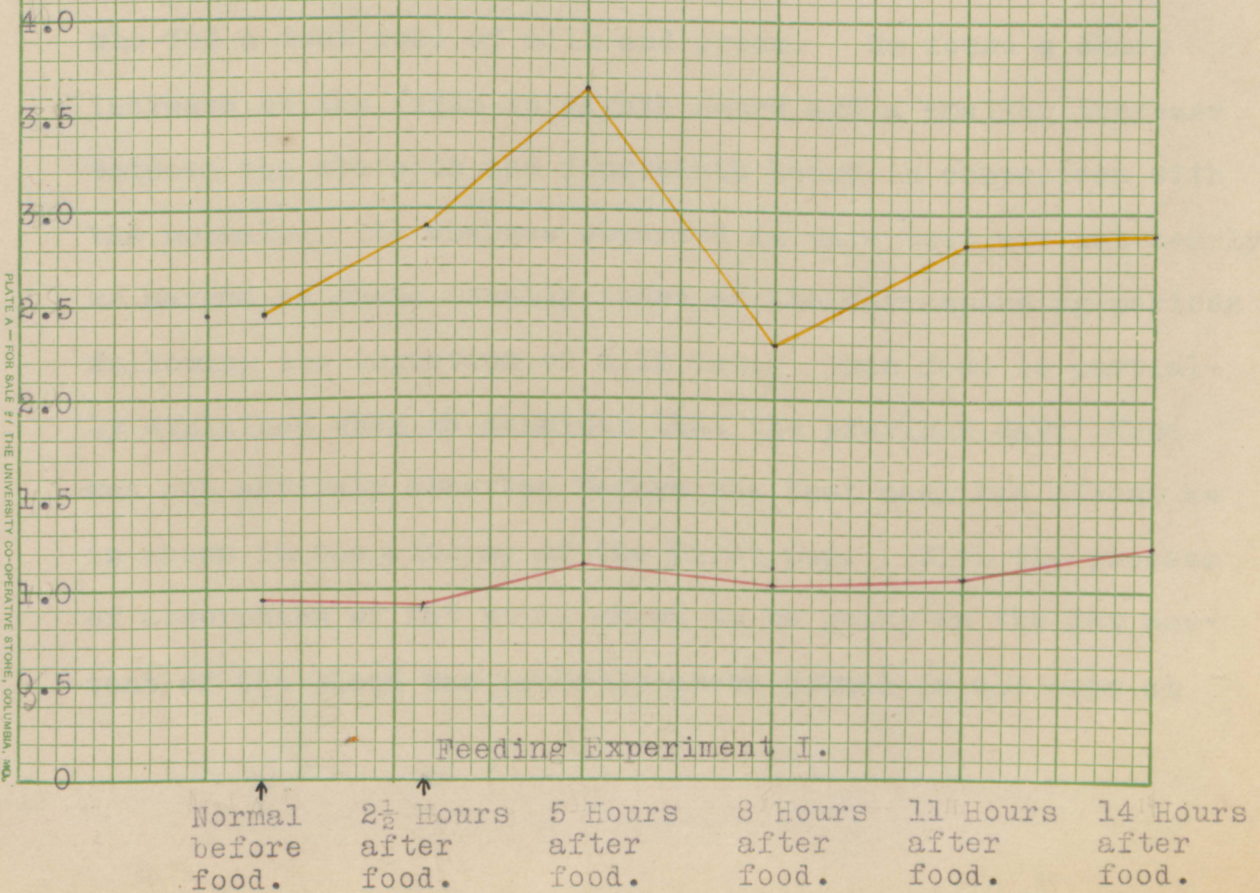


PLATE A - FOR SALE AT THE UNIVERSITY CO-OPERATIVE STORE, COLUMBIA, MO.

The changes in the "total fat" content of the blood in the above experiment are in the main those long known to take place in the blood during alimentary lipemia. There was a marked increase in the blood fat content two and one-half hours after the test meal, 20 per cent above the normal. This was followed by an increase of 48 per cent over the normal at the fifth hour. At the eight hour interval the blood contained less fat than that of the normal. The samples taken at the eleventh and fourteenth hours showed increases in the blood fat content of 20 and 21 per cent above the normal, respectively.

These results of the test on the ingestion of cotton seed oil fat with carbohydrate admixture are in accord with the variations in the blood fat content reported by Summers,²² who fed a test meal of milk and cream. He found a sharp increase at the fifth to eighth hours and a smaller increase between the eleventh and fourteenth hours in comparison with the normal. The changes observed in this test are not nearly so marked as those usually found at the corresponding periods following the ingestion of milk fat. This fact is partially explained when we consider that the previous days' food was not entirely absorbed before the test meal was given, as is shown in the autopsy of the first pup. With the process of absorption of fat still shown to be going on the fat content of the blood was probably above normal in all pups at

the time of the test feeding. It is obvious that the marked change from a normal low fat per cent, to the high ones usually reported at the two and five hour periods will not be observed.

The low per cent in the fat content of the blood sample eight hours after the test meal may be assumed to be an experimental exception. In this pup the fat percentage of the blood during digestion is below the figure found in the blood of the normal check animal before the meal. However, this pup was of a very nervous temperament, and the surmise is, since the results at the other five digestion periods correspond with previous findings, that there was some abnormality in his digestion and absorption of the test meal or that his blood fat content before feeding was lower than the standard.

The rise noted in the 11 and 14 hour animals shows clearly that the process of absorption was still going on at those periods. If we were permitted to omit animal number four and fill in the curve from the five hour period to the eleven hour period, Chart I, the whole series would make a comparatively symmetrical graph. The terminus of the graph could only be shown by later stages of digestion than were available in our experiment.

The sero-lipase does not vary greatly but presents a fairly symmetrical curve in the animals of the above experiment.

There is a very slight fall in the lipase content at the end of the two and one-half hours of digestion, a variation well within the limits of the error of determination. This was followed by a rise of 19 per cent at the fifth hour. There is a small relative decrease, though 10 per cent above the normal, at the eight hour period, the animal with unusual fat content. The sample taken 11 hours after the meal shows a small relative increase of lipase, 14 per cent above the normal. The blood at the 14 hour shows the highest lipase content of the series. The blood fat at this period is still 17 per cent greater than normal, while the lipase has increased to 30 per cent of the normal. While the 14 hour blood sample was from the odd pup and the marked rise may be due to that fact, it is more probable that the variation is bound up with a changed lipase production, in which sero-lipases have their source.

The most noticeable fact in regard to the sero-lipase is its apparently slight variation. Nevertheless, with the single exception of the $2\frac{1}{2}$ hour period the lipase content remains constantly higher than the normal - 19 per cent, 10 per cent, 14 per cent and 30 per cent in the series. The gradual rise thru the eighth, eleventh and fourteenth hour is associated with the later phases of the digestion period. The fat curve indicates that it is at least past the maximum phase of fat absorption. It is possible that the 30 per cent

rise at the 14 hour period may be due to a resorption of digestive lipases no longer wholly consumed in the intestine. Von Hess has suggested that the pancreas is one of the sources of sero-lipase. The higher level of blood lipase would seem at least to be favorable in keeping the increased influx of fats in solution while they are being absorbed in excessive amount and while disappearing in the fat storage tissues.

The results of this test on cotton seed oil fat feeding show that during the absorption of this fat the blood fat increases, reaching a maximal at five hours or later, and continues above the normal for more than fourteen hours; that the time relations of the curve are greater for cotton seed oil than for milk fat; that the lipase content of the blood increases during the period of cotton seed fat absorption; the highest lipase content is in the late hours of the digestion when the fat absorption curve has passed its maximum. Further, the changes in the fat and lipase content of the blood following fat feeding are definite and characteristic even when there is a high per cent of fat in the blood preceding the test meal, though less marked than when the blood has a low fat content preceding the meal.

2. The Fat and Lipase of the Blood During a
Period of Muscular Work Followed
by Rest.

In the execution of this series of experiments on dogs it is assumed that the fat and lipase is in a relatively constant state of equilibrium in regard to the variations due to food intake on the one hand, and to fat utilization and storage on the other. The effort has been to produce only one new variant, namely, vigorous muscular work for a definite and constant period.

Three sets of tests were run on each of three different dogs, an old male, Number 1; a three-fourths grown female, Number 2; and a full grown, but young female, Number 3. The varying ages are presented on the basis of the known variations in the responsiveness of animal tissues in reaction to age as shown by previous fat and lipase studies in this laboratory by Summers.²²

The experimental procedure previously given in detail, is as follows: After a given fast period, of from 24 to 43 hours, a normal blood sample was drawn. The animal was then permitted to run for 60 minutes in the tread-mill, and then allowed to rest for 60 minutes, in one case the rest period was 120 minutes. Samples of

blood were taken every half hour. The blood fat content in grams per 100 cc. and the lipolytic activity of the serum in terms of N/20 NaOH were determined in all the samples. In four tests the lecithin content of the blood was determined in grams per 100 cc. of blood, while in five the cholesterol fraction in grams per 100 cc. was found.

Table II

Muscular Work Experiment 1, April 5, 1917. Dog 1, an old male, mongrel bird-dog, weight 20.5 kilograms. The normal sample was taken 22 hours after regular feeding. He drove the mill at a fairly constant rate of speed for the hour, averaging about 3 miles per hour. The work was interrupted 5 minutes at the end of the first work period for the second sample. At the end of the period he was tired but not exhausted.

Time of Sample	Total Fat Percent	Lecithin Percent	Cholesterol Percent	Lipase N/20 NaOH
Normal before work	1.83	.33	.18	1.21 cc.
After 30 min. work	1.69	.30	.18	1.21 cc.
After 60 min. work	1.06	.23	.17	1.24 cc.
After 30 min. rest	2.23	.38	.24	1.04 cc.
After 60 min. rest	1.51	.29	.18	1.16 cc.

Table III.

Muscular Work Experiment 2, April 12, 1917. Dog 1, Normal sample taken 24 hours after regular feeding. Drove the mill very fast for first 30 minutes, but slowed down to the regular speed in the last one-half hour. The work was interrupted 2 minutes for the second sample. He showed more fatigue at the end of the run than he did at the close of the first experiment.

Time of Sample	Total Fat Percent	Lecithin Percent	Cholesterol Percent	Lipase N/20 NaOH
Normal before work	1.71	.35	.21	1.03 cc.
After 30 min. work	1.28	.28	.17	1.02 cc.
After 60 min. work	1.16	.25	.20	1.11 cc.
After 30 min. rest	----	--	--	.92 cc.
After 60 min. rest	1.33	.29	.18	1.03 cc.

Table IV.

Muscular Work Experiment 3, May 4, 1917. Dog 1.
 Normal sample taken 20 hours after a very heavy feed of the regular diet. During the entire period he traveled at a faster speed than in either Experiment 1 or 2. The work was interrupted 4 minutes during the first work period and 5 minutes for the second sample.

Time of Sample	Total Fat Percent	Cholesterol Percent	Lipase N/20 NaOH
Normal before work	2.74	.43	.95 cc.
After 30 min. work	2.54	.41	.78 cc.
After 60 min. work	2.09	.45	.82 cc.
After 30 min. rest	2.44	.35	.89 cc.
After 60 min. rest	1.95	.40	.85 cc.

Table V.

Muscular Work Experiment 4, April 17, 1917. Dog 2.
 A three-fourths grown female pup, mongrel breed, apparently part bull-dog. Weight 11.3 kilograms. The normal sample was taken 24 hours after the regular feeding. Drove the mill at about two and one-half miles per hour for the entire period. The work was interrupted two minutes during the first period, and seven minutes for the second sample. At the end of the first 30 minute period she was very tired, and at the close of the run practically exhausted.

Time of Sample	Total Fat Percent	Lecithin Percent	Cholesterol Percent	Lipase N/20 NaOH
Normal before work	2.71	.36	.27	1.12 cc.
After 30 min. work	1.95	.31	.24	1.06 cc.
After 60 min. work	2.40	.34	.41	1.13 cc.
After 30 min. rest	2.15	.30	.27	1.20 cc.
After 60 min. rest	1.48	.27	.27	1.10 cc.

Table VI.

Muscular Work Experiment 5, April 26, 1917. Dog 2.
 Normal sample was taken 24 hours after regular feeding.
 She traveled a little faster than in Experiment 3. The
 work was interrupted 2 minutes for the second sample, and
 3 minutes during the second period. She was exhausted at
 the end of the run.

Time of Sample	Total Fat Percent	Lecithin Percent	Cholesterol Percent	Lipase N/20 NaOH
Normal before work	2.13	.37	.37	1.81 cc.
After 30 min. work	2.02	.35	.35	1.69 cc.
After 60 min. work	2.45	.40	.24	1.73 cc.
After 30 min. rest	1.63	.26	.20	1.57 cc.
After 60 min. rest	1.56	.24	.22	1.34 cc.

Table VII.

Muscular Work Experiment 6, May 5, 1917. Dog 2.
 Normal sample was taken 42 hours after regular feeding.
 Ran at about the same experiment as in Experiment 1.
 The work was interrupted 2 minutes during the first period,
 and 2 minutes at the time of the second sample, and three
 minutes in the last period.

Time of Sample	Total Fat Percent	Lipase N/20 NaOH
Normal before work	1.63	1.36 cc.
After 30 min. work	1.56	1.15 cc.
After 60 min. work	1.77	1.23 cc.
After 30 min. rest	1.28	1.12 cc.
After 60 min. rest	1.15	1.07 cc.

Table VIII.

Muscular Work Experiment 7, May 3, 1917. Dog 3.
 Adult female, weight 16.6 kilograms. The normal sample was taken 25 hours after feeding. She traveled at a rather slow constant speed for the entire hour, about two and one-half miles per hour. The work was interrupted 5 minutes for the second sample. The animal was not very tired at the end of the exercise.

Time of Sample	Total Fat Percent	Lipase N/20 NaOH
Normal before work	1.53	1.82 cc.
After 30 min. work	1.20	1.84 cc.
After 60 min. work	1.62	1.91 cc.
After 30 min. rest	1.83	1.99 cc.
After 60 min. rest	1.49	1.85 cc.

Table IX.

Muscular Work Experiment 8, May 8, 1917.

Dog 3. Normal sample was taken 22 hours after the regular feeding. She ran at a very fast constant speed for the hour, averaging about three and one-half miles. The work was interrupted 2 minutes during the first period and 3 minutes for the second sample. At the end of the test she was very tired.

Time of Sample	Total Fat Percent	Lipase N/20 NaOH
Normal before work	2.68	1.98 cc.
After 30 min. work	2.48	2.03 cc.
After 60 min. work	1.52	2.57 cc.
After 30 min. rest	2.22	3.18 cc.
After 60 min. rest	1.73	3.05 cc.

Table X.

Muscular Work Experiment 9, May 12, 1917. Dog 3.
 The normal sample was taken 43 hours after a light feeding of bread and meat scraps. She ran at a relatively constant speed for the entire hour, about 3 miles per hour. The work was interrupted 6 minutes for the second sample. In addition to the usual samples one was taken after 90 minutes rest, and a final one after two hours rest.

Time of Sample	Total Fat Percent	Lipase N/20 NaOH
Normal before work	2.03	1.81 cc.
After 30 min. work	1.63	1.88 cc.
After 60 min. work	2.17	2.14 cc.
After 30 min. rest	1.95	2.20 cc.
After 60 min. rest	1.42	2.20 cc.
After 90 min. rest	1.80	2.14 cc.
After 120 min. rest	1.83	1.97 cc.

Chart II.



Chart III.



Normal before work. 30 Min. after work. 60 Min. after work. 30 Min. after rest. 60 Min. after rest.

Chart IV.



Chart V.

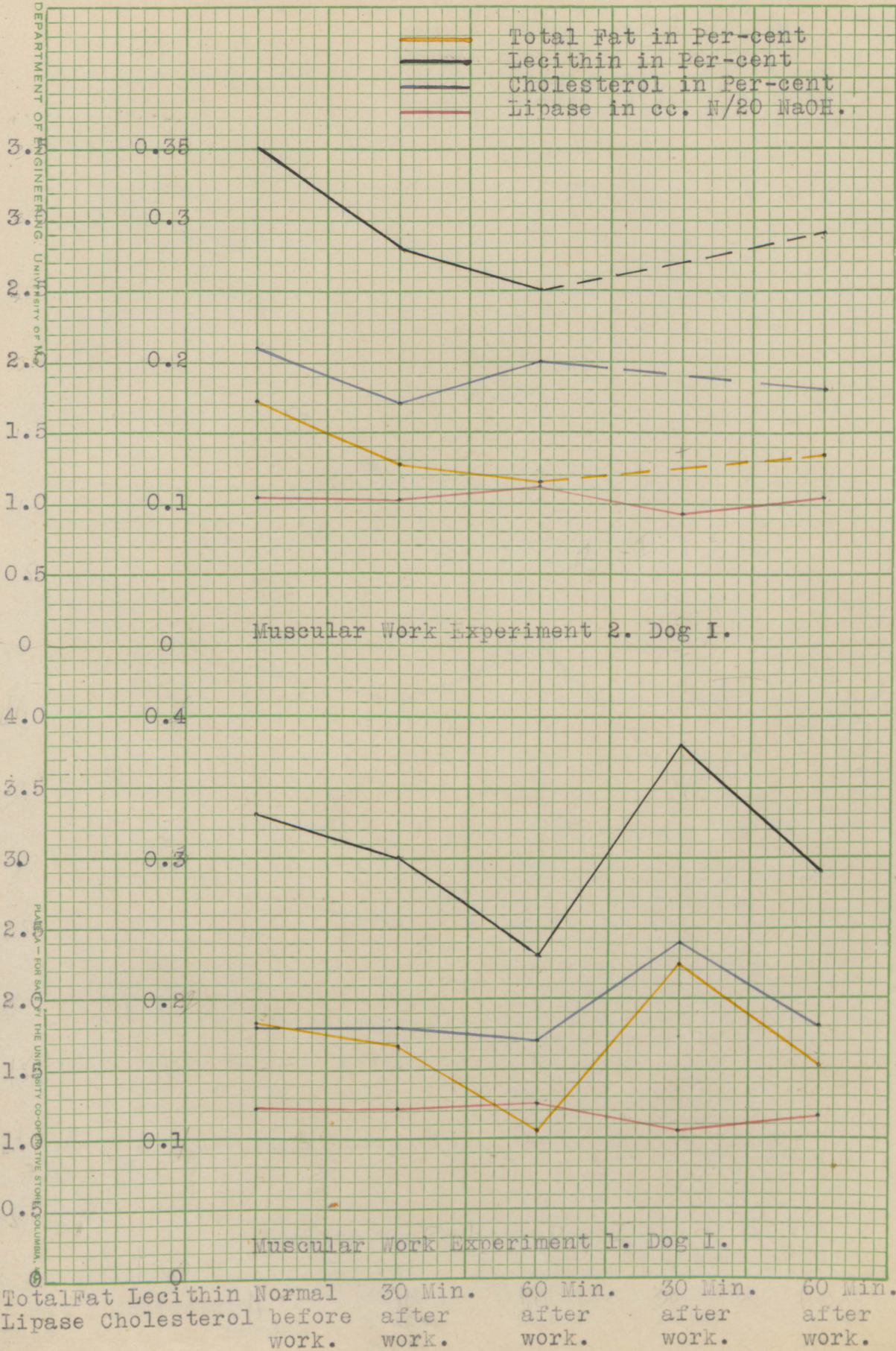


Chart VI.

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- Total Fat in Per-cent
- Lecithin in per-cent
- Cholesterol in Per-cent
- Lipase in cc. N/20 NaOH.

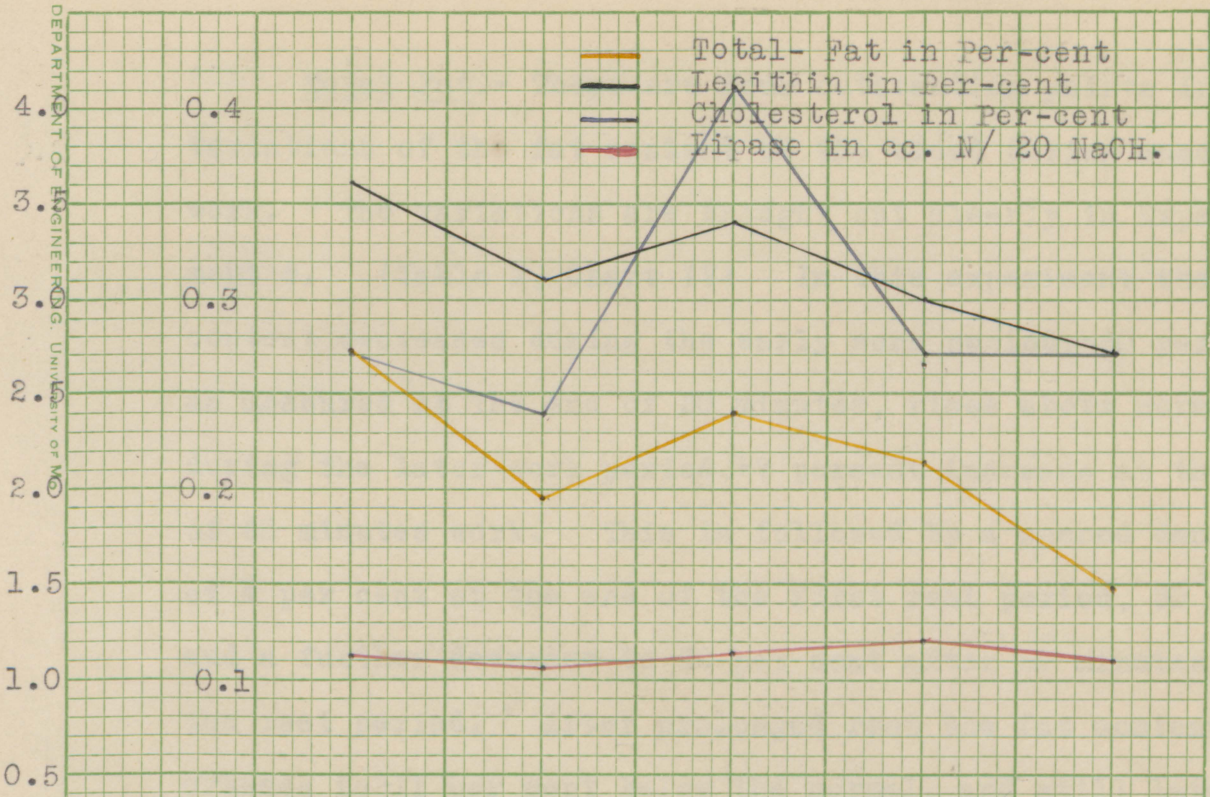


Muscular Work Experiment 3. Dpg I.

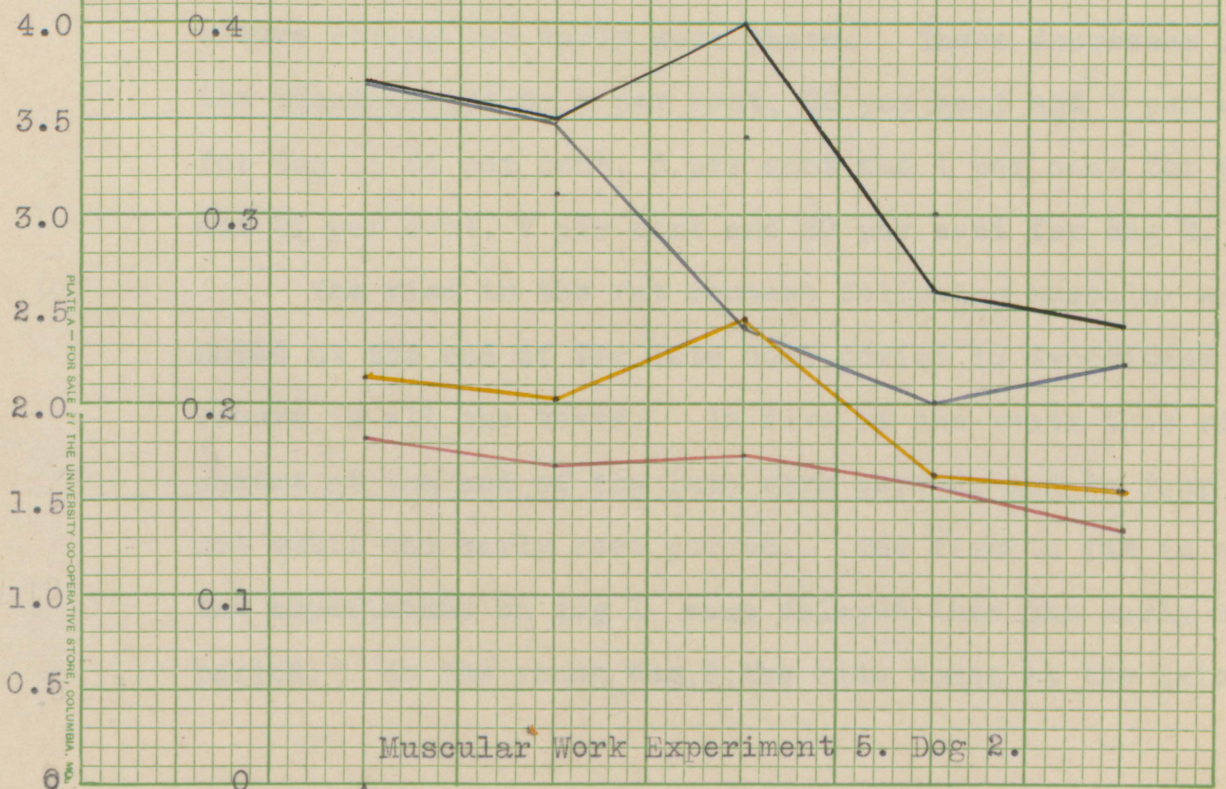
Total Fat
Lipase
Cholesterol
Normal
30 Min. after work.
60 Min. after work.
30 Min. after work.
60 Min. after work.

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

Chart VII.



Muscular Work Experiment 4. Dog 2.



Muscular Work Experiment 5. Dog 2.

Total Fat Lecithin Normal 30 Min. 60 Min. 30 Min. 60 Min.
Lipase Cholesterol before after after after after after
work. work. work. rest. rest.

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 PATENT FOR SALE BY THE UNIVERSITY CO-OPERATIVE STORE, COLUMBIA, MD.

Total Fat.- There are four outstanding points in the variations of the fat content of the blood during muscular work. The variations are brought out clearly in Experiment 9. (Table X, Chart IV) In this experiment the fat content decreases during the early work period, -20 per cent as measured at the end of 30 minutes, rises above normal during the later work period, +7 percent increase at the end of 60 minutes work. This is followed by a second decrease of 4 per cent at the end of the first rest period, 30 minutes, and a further decrease to -30 per cent after 60 minutes in the rest period. After prolonged rest the fat content increases to -11 per cent below the normal, and still later it is only -10 per cent under the original normal percentage.

In every experiment a decrease in the fat content occurs during the early work phase. These decreases in the blood amount to 8 per cent, 23 per cent and 7 per cent for dog 1; 24 per cent, 5 per cent and 4 per cent for dog 2; and 21 per cent, 7 per cent and 20 per cent for dog 3.

The rise in the fat content following muscular work, noted at the end of the late work period in Experiment 9, occurs in all the tests. In five of the tests this rise occurs at the end of the late work period, and in four not until the early rest period. The rises in fat in all the experiments on dog 1 were delayed into the early rest period. In the young dog, 2, the rise comes at the late

work period. In dog 3 two rises are shown/^{one}at the late work period and one at the early rest phase.

The secondary decrease in all the tests is marked after one hour's rest. The fall amounts to 12 per cent, 22 per cent and 29 per cent in the tests on dog 1; 45 per cent, 27 per cent and 29 per cent on dog 2; and 21 per cent, 35.7 per cent and 30 per cent in dog 3.

The fats constitute one of the main sources of energy in the animal body. During ordinary metabolism, i. e., when there is no fat absorption from the alimentary canal, and the animal is in a resting condition, there is an equilibrium between the fat in the storage tissues and the tissues of fat utilization. The fat content of the blood is the expression of this condition.

When the resting animal is suddenly thrown into violent exercise the metabolic conditions are disturbed. In the above tests the result of the initial work was always a decrease in the blood fat content. Murlin and Riche⁵⁵ have shown that during the early stages of increased muscular activity and heat production there is a decrease in the blood fat content. They attribute this fall to the oxidation of blood fat in the body tissues. It is plausible in view of the above experimental results to conclude that the tissues of the body during the early

phase of increased muscular work require more food than is normally coming into the blood during a period of rest and that some of the food in the blood is oxidized; surely fat is used.

The increase in the fat content of the blood which sooner or later always follows the initial decrease is the response of the fat regulating mechanism to the changed metabolic conditions. It appears from the results that when the equilibrium existing between the fat storage tissues and the utilizing tissues is thrown out of balance by the oxidation of fat in the latter, that a compensatory rise in the blood fat occurs. This replenishes the loss of blood fat, and supplies the tissues with the extra fuel required.

In the blood of the younger animals this rise occurs promptly within the hour of the work period, but in the old dog it always comes late, during the early rest phase. This brings out the point previously mentioned that the responsiveness of tissue to changed conditions of metabolism is more delicate in young animals than it is in old.

When the animal stops work the amount of fat required for tissue metabolism is certainly less than the amount required during active exercise, but the oxidation rate is more rapid than in the resting animal. So the increased

fat in the blood is quickly oxidized and soon falls below normal. This secondary decrease continues until the regulating mechanism stimulates the storage tissues and causes them to free more fat. Then the fat content increases back toward a normal, and since the normal rate of metabolism is reestablished, due to the prolonged rest, the oxidation in the tissues soon equals the amount of fat coming in under normal conditions. At this time the normal equilibrium existing between the fat storage tissues and the tissues of fat utilization is reached, and the blood fat returns to its original normal.

Experiments 3, 4 and 8 show variations from the rule. In the first one, dog 1, the normal fat content is very high, 39 per cent higher than the other normals on the same animal. Yet the initial percentage decrease during the early work phase is less than in either of the other two tests. The high normal fat content is no doubt due to the ingestion of an abnormally large amount of fatty food 20 hours before the test. The high amount of the normal fat content indicates that absorption was still going on so that the small decrease during the first half of the experiment may be accounted for by an inflow of fat from the intestine. In addition the rate of speed in this experiment was less than in the other two, so besides the increased inflow into the blood there was a possible decreased use by the muscles.

In Experiment 4, dog 2, the blood shows a much higher normal fat content than is found in the other two experiments, 5 and 6, on this animal. However, the changes in the blood fat in Experiment 4 due to muscular work are qualitatively the same as the changes found in Experiments 5 and 6.

Experiment 8 shows a very marked variation. The normal fat per cent was 33 per cent and 75 per cent higher than it was in the other two tests on the dog. The initial drop in the first work stage occurs but it is only 7 per cent compared with the 20 per cent and 21 per cent falls in the seventh and ninth experiments on this animal. The late work stage shows a further fall, 43 per cent below the normal, while at the same periods in Experiments 7 and 9 there are rises of 6 per cent and 7 per cent respectively. At the end of the run the dog vomited quite a bit of semi-digested food. The absorption of fat from the intestine, as in Experiment 3, dog 1, perhaps accounts for the slight initial fall. The great drop in the next period, which in reality is a drop not much below the previous normals found for this dog is perhaps due to the fact that thruout the entire run she maintained a higher rate of speed than

in the preceding experiments. The explanation for the unusual curve would be that fat absorption from the intestine prevented a marked initial drop during the first work phase, but that during the second work phase the fat flow into the blood was not enough to replace the loss to the tissue. The fall during this last period to the point where the compensatory mechanism would release stored fat to replace the deficiency was unusually great due to the high original content. The curve from then on shows the usual work rise and fall after one hour's rest.

From these variations it appears that the nutritional condition of the animal plays an important part in the changes of blood fat during muscular work; that when the blood fat content of a given dog drops to a certain point the fat regulating mechanism replaces the loss; and that the degree of work plays an important factor in the fat changes.

The charts show that the changes in the individual animals are fairly constant. The variations that do occur can be accounted for by changed nutritional and experimental conditions. But the three different animals show broad variations. These different responses can not be accredited to changed variations in the animals them-

selves or to changes in methods, but must be ascribed to individual responses. The mechanism of each animal for the control of the fat content of the blood during muscular work is characteristic.

Lecithin. - The changes in the lecithin content of the blood due to muscular work are marked, and directly parallel to the changes in the total fat content of the blood. At the end of the early work phase the percentage of fat decreases, the lecithin shows a corresponding fall. The increase of fat in the late work or early rest phase is accompanied with an increase of lecithin, and in the late rest phase both the fat and the lecithin are below the normal percentages found at the beginning of the experiment. The decrease in the lecithin in the early work and the late rest phase shows that lecithin is utilized by the tissues. The increase following this initial fall proves that the stored lecithin in the tissues is freed into the blood, or that it is formed from the neutral fat in the blood. In view of the recent conclusion of Bloor,⁴³ that absorbed neutral fats pass thru the phosphatide state before being utilized or stored, it seems very likely that at least part of the neutral fats when reabsorbed into the blood are transformed into lecithin before being utilized by the tissues.

Cholesterol. - The variations found in the cholesterol content are more irregular than those found in the lecithin fraction. In three of the experiments the percentage of cholesterol is lower at the end of the test than it was at the start, and in two the final percentage is the same as that found at the beginning. The changes found in the cholesterol content amounts to 50 per cent in one case, and in the others, Charts V, VI and VII, the percentage variations are very marked. The irregularities in the variations make it impossible to draw definite conclusions regarding the changes in the cholesterol content of the blood due to muscular work, but the wide variations found indicate that cholesterol takes some part in the increased fat metabolism brought about by muscular work.

Lipase. - The changes in the lipase content of the blood following muscular work are for the most part slight, but the constancy of the variations indicate that sero-lipase is one factor in the fat regulating mechanism in the body.

For the variations in the lipase content of the blood, Experiment 9, dog 3, (Table X, Chart VII) may be described as an example. In this experiment the lipase shows a slight rise, 0.3 per cent above normal, at the early work period. This is followed by a further rise of 17 per cent

during the late work period, and a further rise to 17.3 per cent at the early rest period. In the extreme rest period there is a decrease to 0.9 per cent above normal.

In all the other experiments the lipase content rises in either the late work period or early rest period, and in all the tests the lipase content at the end of the late rest period is either below the normal or decreasing toward it.

In dog 1, Chart II, the rise in all three experiments occurred in the late work period. In this animal the variations were very small, being less than 5 per cent in all cases. The fall which appeared in the lipase during the rest period in the three experiments on this dog was constant amounting to 15 per cent, 10 per cent and 10 per cent respectively.

In dog 2, Chart III, the rise occurred during the late work phase and in each case the percentage was below normal at the end of the late rest period.

One of the experiments on dog 3 has been described and the others are practically the same. The changes in the lipase in Experiment 7 are less marked than those in Experiment 9, but the crest of the rise is at the early rest period and the lipase content was back almost to normal at the last rest phase. In Experiment 8 the

variations in the lipase content are the most marked for the series. The rise at the early rest period, amounting to 60 per cent of the normal, and the fall toward normal in the last period was 9 per cent of the normal.

From the above results it appears evident that the variations in the fat and the lipase contents of the blood due to muscular work are correlated. During the initial drop in the fat content the changes in the lipase are quite within the range of experimental error. In four cases there is a slight fall, in four a slight rise and in one the content remains unchanged. But in the late work or early rest phase when the fat content shows a marked rise there is also an increase in the lipase content, indicating that when the mechanism for freeing the fat from the storage tissues is active there is an increase in blood lipase. The secondary fall in the blood fat content in the later rest period is always followed by a slight decrease in the sero-lipase. In Experiment 9, Chart IV, which shows the extreme rest period the fat content slowly increases back up to the normal, while the lipase content slowly falls to its normal at the same time.

Because of the changing conditions prevailing in

the muscular work experiments, such as variations in fasting periods preceding the tests, the different amounts of exercise in each; and the limitations of the methods employed for the analyses, it is hardly expected that the changes in the fat and the lipase contents of the blood would be exactly the same in all the tests. But there is a surprising degree of constancy in the sets. Since the changes in the sero-lipase occur coincident with the changes in the blood fat content, the two must be related. The parallelism occurring between the lecithin and the total fat bears out Bloor's contention that lecithin is an intermediate step between the resorption and the utilization of fat. From the changes which occur in the cholesterol content we may conclude that cholesterol also takes some part in fat metabolism. This evidence justifies the conclusion that sero-lipase is at least one part of the mechanism governing fat metabolism during muscular work, and that lecithin and cholesterol are both active factors in the process of fat utilization.

IV. Summary.

1. The fat content of the blood increases after a meal rich in cotton seed oil fat.
2. Cotton seed oil fat is more slowly digested than cream fat.
3. The lipase content of the blood increases after a meal of cotton seed oil fat.
4. The fat and lipase content of the blood show marked variations from the normal during muscular work and in the period of rest following work.
5. The changes in the fat and lipase occur so correlated in the various animals that they justify the conclusion that lipase is part of the mechanism controlling the distribution and utilization of fat during increased metabolism due to muscular work.
6. The variations in both fat and lipase are smaller and occur at later stages of exercise in old animals.
7. The variations in the fat content are affected by the nutritional condition of the animal and the degree of exercise.
8. The lecithin variations in the blood are parallel to the blood fat variations during work.
9. The cholesterol in the blood shows marked percentage changes during work.

Bibliography

1. Dubunfaut: Ueber Verwandlung des Staerkmehls in Zucker durch Malz. Fr. Bayliss', Monograph, "The Nature of Enzyme Action". Green & Co., Second Edition, p. 2
2. Duclaux: Chemie Biologique, Paris, 1883. Fr. Bayliss.
3. Bernard: Compt. rend. xli, 461, 1855.
4. Pelouze: Fr. Lewkowitsch, Chemical Technology, Fifth Edition, 1.
5. Hanriot: Sur un nouveau ferment du sang. Compt. rend. Acad. des. Sci., cxxiii, 753, 1896.
Compt. rend. de Biol., xlvi, 925, 1896.
6. Arthus: Compt. rend. Soc. de Biol., 381, 1902.
Fr. Von Hess, Jour. Biol. Chem. x, 382, 1911-12.
7. Rona and Michaelis: Uber Ester-und Fettspaltung im Blute und im Serum. Biochem. Zeitschr., xxxi, 345, 1911. Fr. Wells' Chem. Path., 76.
8. Connstein and Michaelis: Weitere Mittheilungen über die lipolytische Function des Blutes. Pflüger's Archiv., lxix, 1898. p. 76.
9. Umber and Brügisch: Archiv. f. exp. Pathol., lv, 164, 1906.
10. Thiele: On the lipolytic action of the tissues. Biochem. Jour., vii, 275, 1913.

11. Kastle and Loevenhart: Concerning lipase, the fat-splitting enzyme, and the reversibility of its action. Amer. Chem. Jour., xxiv, 491, 1900.
12. Pottevin: Compt. rend. Acad. des Sci., cxxxvi, 1152, 1903. Fr. Sagal.
13. Loevenhart: On the relation of lipase to fat metabolism - Lipogenesis. Amer. Jour. Physiol., vi, 331, 1901-02.
14. Pflüger: Fr. Bradley, Jour. Biol. Chem., xiii, 407, 1912-13.
15. Pagenstecher: Das Vorkommen von Lipasen in den Geweben. Biochem. Zeitschr., xviii, 285, 1909.
16. Fiessinger and Marie: Compt. rend. Soc. Biol., lxvii, 177, 1909. Fr. Wells' Chem. Path., 78.
17. Von Hess: The relation of the pancreas to the lipase of the blood and the lymph. Jour. Biol. Chem., x, 381, 1911-12.
18. Whipple: Tests for hepatic function and disease under experimental conditions. Johns Hopkins Hosp. Bull., xxiv, 207, 1913.
19. Bauer: Wien. klin. Wochnschr., xxv, 1376, Fr. Wells' Chem. Path., 66.
20. Pavlov: Fr. Hammarstan's Physiological Chemistry. Trans. by Mandel, 284.

21. Voit: Fr. Graham Lusk, Jour. Biol. Chem., xiii, 38, 1912.
22. Summers: Some factors influencing the lipase content of the blood. Thesis for Master's degree, University of Missouri, 1915.
23. Abderhalden and Rona: Studien ueber das Fettspaltungsvermoegen des Blutes und ^{des} Serums des Hundes unter verschiedenen Bedingungen. Zeitschr. f. Physiol. Chem., Bd., 75, 30, 1911.
24. Abderhalden and Lampe: Weitere Versuche über das Fettspaltungsvermoegen des Blutes und des Plasmas unter verschiedenen Bedingungen. Zeitschr. f. Physiol. Chem., lxxviii, 397, 1912.
25. Bergel: Deutsch. Archiv. f. klin. Med., cvi, 47, 1912. Fr. Sagal.
26. Fischer: Ueber Lipämie u. Cholesterämie, sowie über Veraenderungen des Pankreas und der Leber bei Diabetes mellitus. Virchow's Archiv. f. path. Anat., clxxii, 30, 1903. Fr. Imrie, Jour. Biol. Chem., xx, 87, 1915
27. Archard and Clerc: Nouvelles recherches cliniques sur le pouvoir lipasique du serum. Compt. rend Soc. de Biol., lvi, 1144, 1902.
28. Pribram: Zur Kenntnis der Blutlipase. Zentralbl. f. Inn. Med., xxix, 81, 1908.

29. Saxl: Uber Fett-und Esterspaltung in den Geweben.
Biochem. Zeitschr., xii, 343, 1908.
30. Cetrone and Reicher: Berl. klin. Wochenschr.,
xlv, 1398, 1908. Fr. Sagal.
31. Sagal: Blood lipase in disease. Jour. Med. Re-
search, xxxiv, No. 2, 231, 1916.
32. Newman: Wien. klin. Wochenschr., 851, 1907. Fr.
Bloor, Jour. Biol. Chem., xix, 1, 1914.
33. Neisser and Brauning: Zeitschr. f. exper. Path.
u. Therap., iv, 747, 1907.
34. Lattes: Ueber den Fettgehalt des Blutes des
Hundes usw. Archiv. f. exp. Path. u. Phar.,
lxvi, 132.
35. Terroine: Variations lipo-cholesterinemiques au
cours de l'inanition et de l'alimentation.
Jour. Physiol. et Path. gen., xvi, 386, 1914.
36. Greenwald: Estimation of the phosphorus content
of the blood. Jour. Biol. Chem., xxi, 29,
1915.
37. Lusk and Murlin: The cause of increased heat pro-
duction following pancreatectomy in the dog.
Amer. Jour. Physiol., xlii, 582, 1917.
38. Munk and Rosenstein: Zur Lehre von der Resorption
in Darm nach Untersuchungen an einer Lymph
(Chylus) fistel beim Menschen. Virchow's
Archiv., Bd., cxxiii, 484. Fr. Hammarstan.

39. Bloor: Variations in the fat content of the blood under approximately normal conditions. Jour. Biol. Chem., xix, No. 1, 1, 1914.
40. Greene and Summers: The fat and lipase content of the blood in relation to fat feeding and fasting. Amer. Jour. Physiol., xl, No. 1, 146, 1916.
41. Schutz: Uber den Fettgehalt des Blutes beim Hunger. Pflüger's Archiv., lxxv, 299, 1897.
42. Daddi: Sur le poids de l'extract étheré du sang et de la lymph dans le jeune de courte duree, Archiv. ital. de Biol., xxx, 437, 1898.
43. Murlin and Riche: The fat of the blood in relation to heat production, narcosis and muscular work. Amer. Jour. Physiol., xl, No. 1, 148, 1916.
44. Bradley: Lipase and fat of animal tissues. Jour. Biol. Chem., xiii, 407, 1912-13.
45. Csonka: A critique of certain data on the content of cholesterol and fatty substances in the blood, together with a modification for the estimation of cholesterol. Jour. Biol. Chem., xxiv, 431, 1916.
46. Mayer and Shaeffer: Jour. physiol. et path. gen., xv, 984, 1913. Fr. Bloor, Jour. Biol. Chem., xix, No. 1, 1, 1914.
47. Mueller: Uber Meskierung des Blutfettes und der Blutlipoide sowie über verdauungsplipämie beim Menschen. Zeitschr. Physiol. Chem., lxxxvi, 469, 1912.

48. Reicher: Zeitschr. klin. Med., lxxv, 235.
49. Bloor: Fat Assimilation. Jour. Biol. Chem.,
xxiv, No. 4, 447, 1916.
50. Foa: Recherches sur le metabolisme des Graisses,
etc. Archiv. ital. Biol., lxxiii, 239, 1915.
Fr. Bloor, Jour. Biol. Chem., xxiv, 447, 1916.
51. Bloor: Determination of fats in small amounts in
the blood. Jour. Biol. Chem., xvii, No. 2,
377, 1914.
52. Bloor: Determination of cholesterol in the blood.
Jour. Biol. Chem., xxiv, No. 3, 227, 1916.
53. Kober: Nephelometric estimation of phosphorus.
Jour. Amer. Chem. Soc., xxvii, July -- December,
2373, 1915.
54. Carrière: Compt. rend. Acad. d. Sci., 1899.
Fr. Sagal.
55. Murlin and Riche: Blood fat in relation to heat
production and depth of narcosis. Proc. Soc.
Exper. Biol. and Med., xiii, No. 1, 7, 1915.

UNIVERSITY OF MISSOURI
COLUMBIA

DEPARTMENT OF PHYSIOLOGY

May 23, 1917

Dean Walter Miller,
The Graduate School,
University of Missouri.

Dear Dean Miller:-

The thesis of Mr. Dudley Anderson Robnett is forwarded herewith. The thesis is a study of the lipase-fat relation in the blood as influenced by muscular work for a definite period. The strictly new contribution in the work is the determination of lipase variations. The crucial and most significant deduction is expressed in item "5" of the summary.

The thesis is approved.

Very truly,

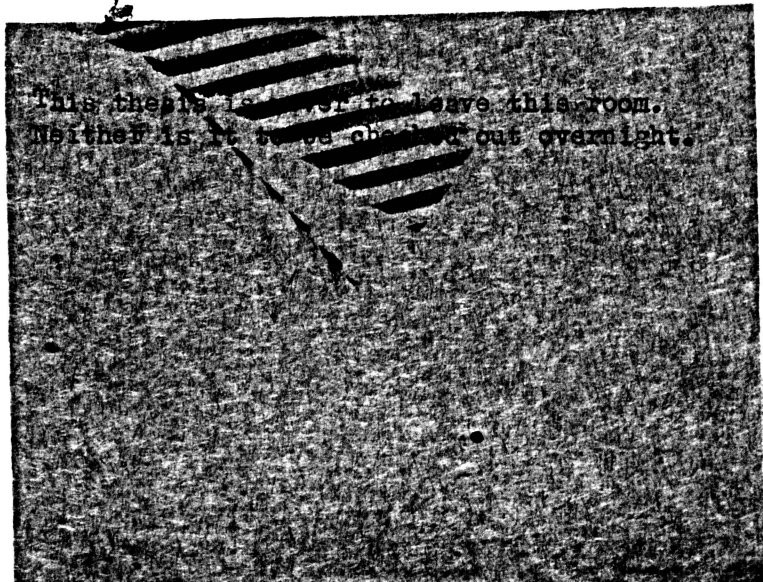
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