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VARIATION IN THE CARBOHYDRATE AND DIASTASE
CONTENTS OF THE TISSUES AFTER FEEDING

by

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SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF ARTS

in the

GRADUATE SCHOOL

of the

UNIVERSITY OF MISSOURI

1918

378.7M71

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THE DISCOVERY AND DISTRIBUTION OF AMYLASE

The literature of carbohydrate hydrolysis dates from 1912 when Kirchhoff (1) hydrolyzed starch to sugar with strong acids. Dubunfraut (2) in 1830 got the same result with an extract of malt. The active principle of malt was precipitated by Payen and Persoz (3) in 1833, and again three years later by Linter. This opened for investigation the new field of specific organic catalysts. The term enzyme was first applied by Kuhne in connection with yeast fermentation and soon came to be used in reference to all organic catalysts.

The specificity of enzymes was soon recognized and proteolytic or protein splitting, lipolytic or fat splitting, amylolytic or carbohydrate splitting, oxidizing or reducing, etc., enzymes were found. In fact, enzymes, it was discovered, were responsible for a great many of the complex changes, both analytical and synthetical, upon which the catabolic processes of life depend.

Wittich (4) in 1873 demonstrated for the first time a substance in the liver which hydrolyzes glycogen. His work followed Bernard's (5) discovery of glycogen in 1857, who a little later independently produced similar evidence of glycogenase. Pavy (6) in 1879 corroborated these results of Wittich in 1873, and of Bernard in 1877, extracting the enzyme from the liver and demonstrated its independent function.

Among the earlier workers, Leuchs (7) discovered in 1831, and Bernard (8) in 1856 demonstrated the presence of amylase in the pancreas. Widdecombe (9) was one of the first to demonstrate the diastatic activity of the blood; he also showed that extracts of the mucous membrane of the intestine invert cane sugar freely. He later in 1901 demonstrated a sugar splitting enzyme in the gastric mucosa which was active only in an acid medium. McLean (10) in 1909, while investigating the diastatic activity of mammalian tissues found more or less hydrolyzing power in the liver, heart, lungs, kidney, stomach, bladder, and skeletal muscle.

In 1902 Vernon (11) investigating the nature of diastatic activity showed that the enzyme of malt differed from animal diastases, that salivary diastase differed from pancreatic diastase, and the pancreatic diastases of various animals were different from each other. The action of any of the enzymes is to accelerate a particular reaction which is in progress, while they do not appear as a part of the final product.

The reversibility of enzyme action is best illustrated by the work of Kastle and Loevenhart (12). To quote: "When a fresh aqueous extract of pancreas is treated with a mixture of dilute butyric acid (.1 - .05 N) and ethyl alcohol (sufficient to bring the whole to 1.5 %), the very characteristic odor of ethyl butyrate soon develops even at ordinary temperatures and in the presence of antiseptics, whereas if the pancreatic extract is first boiled the mixture never develops the odor of the ester." The ester can be separated

by distillation and hydrolized back again by the same enzyme that produced it. So in such actions of enzymes an equilibrium must be reached.

Croft Hill (13) in 1898 working earlier found that the reversible action held true for maltase as well, altho the picture he presents is not so simple. When maltase acted upon a strong solution of flucose, simple maltose did not result from the synthesis as was to be expected; instead, two forms of maltose isomers resulted. The synthetic products are almost completely hydrolized back by the same enzyme preparation which produced them.

Within the organism the point of equilibrium reached in any tissue depends upon many factors; the enzyme action changes accordingly from hydrolysis to synthesis or back to hydrolysis to maintain a metabolic equilibrium.

Bayliss (14) found that at least three enzymes are necessary for the rapid hydrolysis of starch; that amylase hydrolized starch to dextrans, dextrans are split to maltose by dextrinase, and maltose in turn is simplified to glucose by maltase, following the nomenclature suggested by Duclaux. Carbohydrates when hydrolized to the simpler sugars are in soluble form and easily transported by the blood to the tissue cells throughout the body. There these simple sugars are oxidized directly or by the reverse action of the same or similar enzymes are resynthesized into higher or more complex carbohydrates. The action of the enzymes in relation to carbohydrate metabolism is mainly one of hydrolysis. The excess of water found in most tissues would tend to favor enzyme

activity. The balance or equilibrium which the reaction reaches depends upon numerous factors and conditions which determine the direction of the action, whether it is hydrolytic or synthetic.

Some tissues are found to have a greater affinity for carbohydrates than others; for example, the liver and the muscle tissues. These tissues seemingly have a greater absorbing power. The simple sugars are built up by their enzyme, glycogenase, into the complex carbohydrate glycogen. The reverse action occurs to meet later tissue conditions, that is, this higher form stored away as a part of the cells of a particular tissue is again hydrolized to the simpler form and redistributed by the blood and lymph.

The diastases found in the tissues and employed in the utilization of blood sugars may have their origin within the tissues themselves, but more probably they are immediately derived from the blood diastases. The blood in turn would seem to receive its supply mainly from the pancreas rather than the liver as was suggested by Pugliese and Domenichine (15) in 1907. This was brought out by Boldyreff (16) in a recent work, 1916, upon hunger contractions. He finds that hunger contractions have accompanying them increased periodic pancreatic and intestinal secretion, suggesting that what has been partly called the internal secretion of the gland is really the periodic functioning of the pancreas to produce the external secretion, which is absorbed and acts as an internal secretion as the term is ordinarily applied. The enzymes are taken up by the blood and transported constantly

to the tissues, supplying them with diastases as a means of utilizing nutriment received.

Storage of glycogen in the liver and muscles takes place by direct synthesis. Natcher and Wolf (17) in 1907 perfused the hind leg of a dog, finding that saccharose is converted to glycogen just as is glucose under similar conditions. This glycogenesis in muscle according to Cohnheim (18) is controlled by an activator or coenzyme which is produced by the pancreatic gland. The possibility remains that this activation was really the direction action of the pancreatic diastases since the extract of the gland was used. Cohnheim further finds that sugars are not oxidized by muscle juice alone but only disappear in a mixture of muscle juice when pancreatic extract is added. Then too, Knowlton and Starling (19) record experiments to show that the addition of pancreatic extract to the blood restores the power of the muscles to utilize glucose.

As reviewed by Paton (20) the pancreas seems to exert a twofold function in this phase of metabolism, (1) checking mobilization of sugar by the liver and (2) facilitating the utilization of the sugars in the tissues. The possibility remains that the activating substance from the pancreas may be a periodic supply of the diastases which is carried to the tissues in general by the blood.

The gland known to be most active in the production of diastase is the pancreas. The diastatic activity of the tissues may depend upon the enzymes carried to them by the blood from the pancreas, which is in harmony with the periodic

activity and the failure of the pancreatectomized animal to utilize sugars.

The liver is concerned in the control of the supply of sugar within the organism. When sugar for combustion throughout the body is low, the deficiency affects the liver through changes in the proportion of sugar-enzyme in the blood. The result is an increase of the glycogenolytic utility, that is, dehydration of glycogen and the liberation of hepatic sugar into the blood stream.

The sugar content of the blood is relatively constant. As the tissues take up sugar from the blood, the liver mobilizes sugar to make up the loss. Conversely, the liver conserves sugar by the storage of any excess in blood sugar content through the law of reversible enzyme reaction.

The sources of sugar for the body are found in the carbohydrates, the proteins, and possibly the fats of the foods. That the proteins are a source of sugars was first shown by Bernard. He found that the livers of dogs fed exclusively upon lean meats held large quantities of glycogen. This was confirmed by Kulz (21) and by C. Voit (22) on hens. Dakin (23) recently has by feeding experiments shown that glycine, alinine, aspartic, and other amino acids are converted to sugar and glycogen within the organism. Lusk (24) in 1903 found that forty per cent of ingested amino acids are excreted as sugar in the diabetic dog. Janney and Blatherwick (25) present data showing that 57.5 to 58 per cent of human muscle proteins are excreted as glucose by the diabetic animal. Cheminers (26) showed that lactic and propionic

acids may be converted to glucose. This was confirmed by Hockendorf (26) who also demonstrated alanine to be the intermediary compound between glycocoll and glucose. Isidor Greenwald (27), in 1913, demonstrated the change of propionic acid to glucose in human diabetes. From such evidence it would seem that considerable sugar is derived from ingested proteins.

In the metabolism of fats, the facts that their hydrolysis in glycerine and fatty acids are formed, and that glycerine can be converted to glycogen, would indicate that the body carbohydrates may have their origin in fats. Cremer (28) found on feeding diabetic animals glycerine in the absence of proteins and carbohydrates that the typical hyperglycemia and glycosuria persisted. Bloor (29) in 1911 gave evidence that the carbohydrate esters of the higher fatty acids could be formed, for example, mannite distearate. The fats, however, are not to be considered a ready source of body carbohydrates.

The question arises, is there a normal relationship between the carbohydrate and amylase contents of a given tissue? What effect would the diet have upon these contents? Or would the relation of the enzyme and the substratum change? Lewis (30), in 1908, demonstrated that the diastatic activity of saliva in a given individual remains constant upon a mixed diet and decreases upon a carbohydrate diet. Bradley (31), in 1911, was unable to demonstrate any definite relationship between diet and the character of the pancreatic secretion in man, although a meal of milk, bread, or meat

will affect the rate and amount of the secretion. Luckhart (32), as confirmed by Carlson (33), finds that protein or carbohydrate diets do not affect the diastases of the blood serum. Allen (34) is of the same opinion. McGuigan and Von Hess (35) give positive evidence that the effect of a meat diet or the absence of carbohydrates in the food is to increase the diastases of the blood. McGuigan and Ross (36) show that there may be hyperglycemia after either albuminose or peptone injections without any effect on the blood diastases. They hold that this is a disturbance confined to the liver.

Does a condition of hyperglycemia accompany a lack of diastases or activating substances in the blood? Or does hyperglycemia indicate a high diastatic activity of the blood? Does any direct relationship exist between the enzyme and the substratum in the various types of tissue? Does the response of the various organs and tissues concerned differ after various kinds of foods?

The blood as the conveyor of the carbohydrates, the liver and the muscles to a less extent as a depot or storehouse, and the kidney as an excretory organ form the chain concerned in metabolism. These tissues seem to be the logical point of attack to show evidence of any relationship which might exist between the sugar and the amylase contents.

In the present study an attempt is made to collect data upon the normal variations of the carbohydrate and amylase contents in the blood, liver, muscle, and kidney after various test meals.

METHODS EMPLOYED

For small quantities of blood sugar, the older gravimetric and volumetric methods depending upon the reduction of copper salts are inadequate. The recent developments of new colorimetric methods along all lines has furnished a sugar method which seems to be an accurate method for estimating variations in the small quantities found in blood. The procedure recently worked out by Lewis and Benedict (37) lends itself admirably to rapid and accurate determinations of small quantities of reducing sugars. The method depends upon the reduction of picric acid in the presence of sodium carbonate to picramic acid, as indicated by the probable equation:-

A reducing sugar in a saturated solution of picric acid reduces the yellow picric to red picramic acid. The amount of reduction is proportional to the amount of sugar present. The resulting solution, a mixture of picric and picramic acids, is compared colorimetrically to a standard solution of picramic acid in a saturated solution of picric acid, equivalent in color to that produced by a known amount, 0.64 mg., of dextrose. The sugar in the unknown solution is then calculated by the following formula:

Set of Standard x .64

= mg. in unknown

Reading of Unknown

The range of the colorimeter was determined by setting the standard solution at 10, 15, 20, etc., on the scale and reading against it various and numerous strengths and dilutions of the standard and many known solutions containing various amounts of dextrose. The data so gained was checked with mathematical data, e.g., a 10 cc sample containing .96 mg. when diluted to 20 cc, read to contain .48 mg., or diluted to 30 cc, read to contain .32 mg., etc.

The results of checking trials tabulated as follows: -

Standard set at 10.

Scale Reading	Value Read	Known Value
Units	mgs.	mgs.
5.0	1.28	1.28
6.25	1.0253	1.00
6.75	.9487	.96
7.0	.9128	.90
8.0	.800	.80
8.5	.7529	.77
8.75	.7320	.72
9.75	.6588	.66
10.0	.640	.64
10.5	.6095	.60
11.5	.5560	.54
12.5	.5120	.51
12.75	.5021	.50
13.25	.4831	.48
14.0	.4571	.45
15.0	.4266	.42
16.0	.4000	.40
18.5	.3440	.34
19.0	.3333	.33
20.0	.3200	.32

Similar data was taken when reading to the standard set at 10, 15, and 20 on the scale. The plot below shows the comparative figures:-

The Standard solution (a 10 cc amount) was placed in the left cup of the Duboseq Colorimeter and 10 cc of the unknown solution in the right. The left cup was set and the right cup moved up and down, and three readings taken going in each direction. To check, the right cup was set at the average of these readings and the standard left cup moved. In all determinations duplicate samples of blood and tissues were taken and duplicate analyses made upon each sample.

If the sample to be read, when made up to 10 cc for comparison, was darker than the standard solution, it was diluted to 20, 30, 50, etc., cc until it was approximately the same intensity or a little lighter and the read value multiplied by 2, 3, 4, 5, etc., according to the dilution. It will be noticed from the curves in the above plot that the most accurate readings are possible when the unknown solution is equal to or lighter than the standard solution, because when readings are made below the standard set, a greater change of scale is required per milligram of sugar detected than when the reading of the unknown is made above the standard. When determinations were made upon solutions containing proteins, the latter were precipitated out of the solution by the picric acid which was added to saturation. The readings were then made upon the supernatant fluid after centrifuging or filtering to get a perfectly clear solution.

Technique on Blood

Two cubic centimeters of whole blood were measured accurately from an Oswald pipette, which had been washed in a

potassium oxalate solution, into test tubes containing 8 cc of water and immediately laked by shaking. Enough dry picric acid to saturate the water present was added with 10 cc of saturated picric acid solution and a drop of alcohol to prevent foaming. The tube was thoroughly shaken, the precipitated protein was filtered off, leaving the sugar in a saturated solution of picric acid. To 9 cc of this filtrate, (holding the sugar of $9/10$ of a cubic centimeter of blood) 1 cc of ten per cent sodium carbonate was added and the whole heated carefully over a free flame in a Jena Test tube, then boiled vigorously until precipitation occurred. The resulting solution was then made up to the original volume of 10 cc (or diluted if needed) and compared to the standard solution. Readings were made in milligrams and calculated to milligrams per cubic centimeter of blood or tenths of a per cent.

Carbohydrate determinations on tissues

As an index to the relative carbohydrate content of the tissue under examination, the glycogen was converted to sugar by the enzymes normally present. The optimum period of incubation was questioned by incubating the samples (prepared according to the technique below) and testing the sugar content at intervals, care being taken to remove with each sample a proportionate amount of the tissue undergoing hydrolysis and keeping the concentration of the extract constant throughout the incubation period.

The samples were taken from the livers and kidneys

of normal animals of various sizes, ages, weights, and conditions. Below is recorded a typical result, of tissues incubated at 38 degrees Centigrade.

Incubation Period	Sugar present in
in hours	milligrams
0	8.00
$\frac{1}{2}$	10.66
1	12.80
$1\frac{1}{2}$	14.22
2	16.00
$2\frac{1}{2}$	18.20
3	21.37
$3\frac{1}{2}$	20.68
4	19.00
$4\frac{1}{2}$	18.20
5	17.06
$5\frac{1}{2}$	16.00
6	14.22
$6\frac{1}{2}$	12.80

Plotted the following curve resulted:-

It should be noted that fermentation occurred in the above samples decomposing the sugar; this destruction of sugar increases per hour and soon overbalances the sugar formed by the enzyme and the curve shows a fall in sugar content. The above would be the resultant figures of the two processes as shown by the hypothetical curves below:-

(D)

As the destruction curve rises it makes the actual rise in sugar due to the hydrolysis of glycogen (curve E) less and less and soon a negative quantity. Then the fall in the former table does not mean absence of hydrolysis for the enzyme is still active as could be demonstrated with a substratum of starch and teluol as a preservative.

In an attempt to bring the process of conversion of glycogen to sugar to a final equilibrium, the samples were incubated at 20°C with toluoline and determinations made at intervals as long as any change occurred. Below is tabulated a typical set of results from this procedure:

Interval of incubation at 20°C	Sugar present in milligrams
0 hours	10.00
12 hours	17.75
1 day	22.80
$1\frac{1}{2}$ days	27.43
2 days	30.52
$2\frac{1}{2}$ days	32.00
3 days	33.81
$3\frac{1}{2}$ days	34.70
4 days	35.83
$4\frac{1}{2}$ days	36.02
5 days	36.23
6 days	36.80
7 days	36.79

Plotted the curve below shows the course of hydrolysis and the approach to equilibrium:-

The curve approaches a straight line as a limit. The time required varies with the amount of enzyme present and the amount of glycogen present. The type of the curve remains the same, however. In every case the final ratio of glycogen to sugar would be the same and a constant.

A reading taken as the equilibrium is approached, in this sample 36.8 mg., would give a most accurate basis for the comparison of a series of samples.

Since the rate of hydrolysis depends upon the concentration of glycogen found under normal conditions, the amount of change would give a further check upon the diastase present in the samples under consideration. In all the samples active diastase was demonstrated by incubating at 38° C with starch added as a substratum.

Upon being hydrolyzed in a one per cent solution of hydrochloric acid for two hours with a reflux condenser, the tissues - liver and kidney - showed an increase of equal proportions over the sample as it tested when equilibrium was reached in the above series. The final figures after the acid treatment were found to be in direct proportion to those of normal equilibrium. The total glycogen in the liver equals 1.2 times observed equilibrium readings, and in the kidney 1.3 times the equilibrium reading.

Tabulated:-

Sample	Equilibrium Reading mg.	HCl in mg.	Ratio
Liver 36	7.5	18.0	1:2.4
Liver 27	10.5	24.9	1:2.38
Liver 28	16.0	34.0	1:2.17
Liver 29	34.13	68.3	1:2.01
Liver 30	24.1	48.6	1:2.17
Liver 32	19.0	43.0	1:2.26
			Mean 1:2.23
Kidney	2.2	6.8	1:3.09
Kidney	2.1	6.3	1:3.00
Kidney	3.2	9.6	1:3.00
			Mean 1:3.03

The figures found after the acid treatment would give exactly the same comparative results as the equilibrium figures, as the foregoing table demonstrates; they would, however, be nearer the true total carbohydrate content of the tissues examined, yet on the other hand, they would be high since the acid would also break up all the carbohydrate-protein compounds, e.g., the mucoid substances and probably some of the proteins to liberate sugar which would play no part in this work where only the carbohydrates susceptible to enzyme action are to be considered. So this last step in analysis is to be discarded as it is both inapplicable and unnecessary.

Technique for tissues

A 10 gm. sample, weighed fresh, upon a Sartorius balance accurate to milligrams, was ground with a 4 cc volume of pure cleaned white sand, sugar free, to a fine pulp and diluted to 104 cc of pure distilled water free of ammonium and carbon dioxide. Each cubic centimeter of this extract would contain 100 mg. of tissue, making 1 mg. read per sample = 1%. In tests for the approach of the equilibrium 2cc samples were found to be accurate enough. However, the final determinations were made upon a 10 cc sample of the extract and the measurements made in volumetric flasks in the place of pipettes and burettes. The state of equilibrium was usually approached after 4 to 6 days incubation at 20° C. Sometimes sooner, depending in the cases of the liver upon the amount of glycogen held by the tissue, and in all cases upon the amount of enzyme present. The extract was treated as blood and the technique given there followed.

Determinations of Diastatic Activity

An index to the diastatic activity was determined by the amount of starch reduced to sugar by a unit volume of the fluid, to be tested, in a 15 minute period of incubation at 38° C.

Technique for Blood Diastases

The recent method of Meyer and Killian (38) was employed. Two samples of whole blood, 2 cc each, were pipetted into tubes containing 7 cc of water. The blood so laked and in-

cubated at 38°C until their temperature was constant (usually about 10 minutes). To one, the control, 1 cc of water was added; to the other, 1 cc of a one per cent starch solution (10 mg. starch), with both solutions at 38°C and both tubes reincubated at that temperature for exactly 15 minutes. When the tubes were removed, about one gram of dry picric acid and 10 cc saturated solution of picric acid were added and the sugar present in each was then determined according to the technique for blood sugar. The difference in the sugar content of the test and the control tubes represents the sugar from the reduced starch. The results were tabulated in terms of per cent of the 10 mg. of starch reduced.

Technique for Tissue Diastases

The fresh aqueous extract of tissue was treated by the method for laked blood in the above. The glycogen normally present in the tissues would serve as substratum for the enzyme along with the starch added, but being equal in the test and the control tubes it would not affect the accuracy of the test. This is especially true since the amount in the richest livers is so much less than the starch added and that in the kidney as negligible as is the glycogen held in the red blood cells in the sugar determinations. The diastase determinations were made after the extracted tissue had stood for at least 12 hours at 20°C, having been shaken several times at intervals to insure a thorough and equal

distribution of the enzyme throughout the extract. The determinations were again made at the approach of equilibrium of the glycogen and sugar in the tissue extract for the two-fold purpose (1) to check the first set of determinations, and (2), to demonstrate the presence of active enzyme in the extract at the time of equilibrium. In all tests for diastase duplicate samples both of tests and controls were made. In Experiment No. 1, Series No. 1, diastase determinations were made also upon fat-free samples of the tissues. This procedure was later deemed unnecessary as the values checked throughout with determinations made upon the fresh samples.

Technique for Diastase in Urine

Two samples, 2 cc each, were pipetted into tubes; an equal known amount, 2 mg., of dextrose was added to each and the whole made up to an 8 cc volume. The tubes were incubated at 38° C until their temperature was constant. To one, the control, 2 cc water was added, to the other 2 cc of a one per cent starch solution; both solutions at 38° C and both tubes reincubated at that temperature for 30 minutes. When the tubes were removed, the diastatic activity was determined as in the case of laked blood. Creatinine in the urine would reduce picric acid as dextrose does but the amounts being equal in the control and the starch tube their comparison would remain the same.

The amount of diastase present would be represented by the difference in sugar content of the two tubes, i.e., the sugar resulting from the reduction of starch.

The figures gained are offered only as preliminary determinations or rough estimates.

RESULTS OF EXPERIMENTS

The effect of diet upon the carbohydrate and diastase contents of the liver, kidney, and blood

Since tissue samples were to be taken, it was, of course, necessary to kill the animals since each experiment was run upon a series of animals.

In using a series of animals for comparative determinations of the sugar contents of tissues, the factor of individual differences will, of course, enter. The question arises to what extent will this factor influence the results? To determine this point the attempt was made to determine the range of variations of content in normal condition.

The amount of glycogen in the livers depends upon the factor of the state of depletion and the activity of each particular animal. The content of the kidney would vary with uncontrolled environmental conditions, as with the liver. The blood, however, is known to remain at a relatively constant level as to its sugar content, except during the periods of active digestion.

The extent of the variation in normal animals under constant conditions should give an index as to the accuracy of comparisons made between the contents of a series of animals in a diet test. Determinations of the blood sugar were made upon dogs of various age, sex, breed, weight, and nutritional condition. The animals used in this preliminary experiment were experimental animals used in the routine labora-

tory work in Physiology and Pharmacology. All the animals had been fasted for at least 40 hours and not more than 48 hours before the samples were taken for analysis. The determinations were made according to the technique described later in this paper. The tabulated results appear as follows:

Table No. x

Animal	Number	Sex	Age	Weight	Blood Sugar in mg. or .1 % per cc.
Dog	3	male	1½ yrs	6.0 k	2.07
Dog	4	male	2 yrs	8.0 k	2.16
Dog	5	female	1 yr	5.0 k	2.22
Dog	7	female	1 yr	4.5 k	2.45 *
Dog	7a	female	1 yr	4.5 k	3.61 ''
Dog	17	male	1½ yrs	7.9 k	2.74 *
Dog	17a	male	1½ yrs	7.9 k	3.58 ''
Dog	18	female	6 mos.	7.0 k	2.50 *
Dog	25	female	3½ mos.	3.0 k	2.40
Dog	24	female	6 mos.	3.0 k	2.28
Dog	10	female	8 mos.	4.0 k	2.22
Dog	11	female	6 mos.	3.0 k	2.39
Dog	12	male	6 mos.	3.5 k	2.34
Dog	13	female	6 mos.	4.0 k	1.96
Dog	14	female	10 mos.	5.5 k	2.40
Dog	19	female	6 mos.	2.8 k	1.94
Dog	20	female	6 mos.	2.9 k	2.07
Dog	21	female	3 mos.	2.3 k	1.98
Dog	22	female	6 mos.	3.1 k	2.41
Dog	23	male	3 mos.	2.25k	2.21

* Samples taken after 1 hour of ether anesthesia.

'' Samples taken after 3 hours of ether anesthesia and
intravenous injections of adrenalin hydrochloride.

The blood sugar content for this series, excepting those which had been subjected to the influence of drugs, averages 2.16 mg. per cc. The greatest variation lies between 2.40 and 1.94, a difference of .46 mg. per cc. or less than .05 per cent. The extremes are to be found among animals of the same age and weight. The weight, age, and sex seems to have little or no effect upon the blood sugar content. The experimental error also considered, this small variation would seem too small to prohibit the use of sets of animals in the feeding experiments contemplated where comparative results are important. However, to make the results as truly comparable as possible, the animals used for each experiment were, whenever possible, taken from a single litter.

Preliminary to the feeding of the test meal, the animals were placed in isolated compartments and fed equal portions, based upon grams per kilo, of a carefully prepared mixed diet. The meals consisted of cornmeal, meat, and cracklins; mixed, cooked, and baked. The feedings were made twice a day and daily records kept of animal weights and general conditions. This preliminary treatment lasted usually a week and never less than four days, after which the series was fasted for 24 to 48 hours to insure complete digestion and absorption of the previous meal. One animal was taken as the group normal, was weighed and decapitated, the others were weighed and fed equal portions of the test meal, then weighed and decapitated at various intervals during the period of digestion. In drawing the blood, care was

taken not to rupture the thoracic duct or to allow any regurgitation from the stomach. The blood was collected from the carotid artery in a large casserole. Duplicate samples were taken for sugar and diastase determinations and the analyses were made immediately.

Complete postmortem examinations were made upon each animal. The pancreatic and bile ducts were ligated and the liver and kidneys excised and weighed. Ten gram samples of the tissues were taken, two from different lobes of the liver, and when possible, one sample from each kidney. Where the kidney weighed less than ten grams, eight or nine gram samples were taken and extracted with 80 or 90 cc of water instead of 100 cc, keeping the whole series of samples upon the same basis. Samples were ground and extracted immediately after they were taken and in the same order.

The following four series of feedings consisting of seven group experiments were carried forward in this investigation:

Series No. I. Fats plus Carbohydrates

Experiment No. 1. Cornmeal and Cracklins

Experiment No. 2. Milk, cream, butter, and dextrose

Series No. II. Pure Carbohydrates.

Experiment No. 3. Cornmeal and dextrose

Experiment No. 4. Cornmeal and dextrose

Series No. III. Proteins

Experiment No. 5. Cooked Beef

Experiment No. 6. Cooked Beef

Series No. IV. Carbohydrates plus Proteins

Experiment No. 7. Cornmeal, cooked beef, & dextrose

Series I, Experiment 1

The carbohydrate and diastase content of the liver, kidney, and blood following a meal rich in fats and carbohydrates.

Six puppies about six months of age were used for this experiment. The first five were of the same litter, the sixth of the same age, type, and condition. All were in good condition with an average weight of about five and one-half kilos. The series had been together for several weeks and had been fed a constant mixed diet based upon grams per kilo of body weight. Their nutritional condition was observed constantly and all were in the same condition at the time of the experiment. After a fast of 24 hours, No. 1 was killed as a normal; the remaining five were each fed 90 gms. per kilo of the test meal consisting of cornmeal and cotton seed oil containing 25 per cent fat. The pups were then killed at various periods during digestion and absorption, as follows: No. 2 after 2.5 hours, No. 3 after 5 hours, No. 4 after 8 hours, No. 5 after 11 hours, and No. 6 after 14 hours.

Autopsy records:

No. 1 Normal: The stomach contained some soft semi-digested food, showing that the previous meal had not yet been completely digested. This would indicate that the normal reading would be high.

No. 2 - 2.5 hours: The stomach was gorged with digesting food, the intestines were nearly empty. This super-imposed test meal would for a time inhibit the passage

of food from the stomach into the intestine, possibly allowing a nearer approach to normal than No. 1 would show.

No. 3 - 5 hours: The stomach was full of semi-digested food, the intestines held chyle, and the lacteals were plainly visible. Absorption of fat at least is occurring and the carbohydrates are in the intestine in the process of being digested.

Nos. 4, 5, and 6: Stomachs were $1/2$, $1/3$, and $1/5$ full of partially digested material, respectively. The intestines of all indicated that active absorption was taking place.

The presumably high normal will decrease the percentage of change found. The rate of digestion was slower than anticipated, hence the curves do not show the complete digestive cycle.

Table No. 1.

Series, I, Experiment 1. Variation in the carbohydrate content of the liver, kidney, and blood after a meal rich in carbohydrates and fats.

Animal No.	Time of sample After test meal	Carbohydrate as sugar mg. per gm.		
		Liver	Kidney	Blood
1	Before test meal	7.2	1.08	1.84
2	2½ hrs after test meal	10.4	1.19	2.04
3	5 hrs after test meal	15.8	1.34	1.77
4	8 hrs after test meal	33.9	1.64	2.80
5	11 hrs after test meal	24.1	1.59	1.88
6	14 hrs after test meal	22.0	1.45	1.88

Table No. 2

Series I, Experiment 1. Variation in the carbohydrate content of the liver, kidney, and blood after a meal rich in carbohydrates and fats.

Animal No.	Time of sample After test meal	Diastatic Activity Percent starch hydrolyzed in 15 minutes at 38° C.		
		Liver	Kidney	Blood
1	Before test meal	19.0	2.0	17.0
2	2½ hrs after test meal	15.6	2.0	12.0
3	5 hrs after test meal	13.9	4.5	9.5
4	8 hrs after test meal	39.9	15.1	20.5
5	11 hrs after test meal	19.9	5.0	14.0
6	14 hrs after test meal	11.5	5.5	14.0

Chart No. 1.

Chart No. 2.

Series I. Experiment 2

The effect of a meal rich in fat and carbohydrates upon the carbohydrate and diastase contents of the liver, kidney, and blood.

Six pups, three months old, average weight of three kilos, all of the same litter and in very good nutritional condition were used. The preliminary treatment was the same as in Experiment No. 1. They were kept in isolated compartments and fed regularly upon the same mixed diet. Their weights and conditions were carefully followed for four days, then after fasting for 36 hours, their stomachs on aspiration appeared empty. The test meal, consisted of 100 gms per kilo of cream testing 22% fats to which was added 10 gms of melted butter fat and 20 gms dextrose, making a food which was 20% dextrose and 32% fat. The milk proteins are very low, that held in 48 grams of skim milk per kilo.

The pups at the time of the experiment were in excellent condition and the digestion of the previous meal was known to be complete. As before, one was taken as the normal and decapitated, the others fed and decapitated at intervals of 2.5 hours, 5 hours, 8 hours, 11 hours, and 14 hours after the test meal. The same routine in taking the samples was followed as in Experiment No. 1.

Autopsy Results:

The autopsy of No. 1 confirmed the test that digestion of the previous meal was complete. The stomach was empty, the intestines were empty, the lacteals were not visible, the viscera were normal.

Upon examinations of Nos. 2, 3, 4, and 5, the stomachs were found to be $3/4$, $1/2$, $1/4$, and $1/5$ full of fluid material respectively; none of them showed the presence of solid food from the previous meals of the preliminary feedings. The intestines all showed evidence of active absorption, lacteals being plainly visible in all. No. 6 on autopsy showed the stomach practically empty, as were the intestines. The lacteals were just barely visible, indicating that digestion was nearly complete. As expected, this meal was much more easily digested and absorbed than that of Experiment No. 1.

Table No. 3.

Series I, Experiment 2. The variation in the carbohydrate content of the liver, kidney, and blood following a meal rich in fats and carbohydrates.

Animal No.	Time of sample after test meal	Carbohydrates as sugar in mg.		
		Liver	Kidney	Blood
1	Before test meal	11.8	1.2	2.1
2	2½ hrs after test meal	17.4	1.4	2.55
3	5 hrs after test meal	28.9	1.99	3.30
4	8 hrs after test meal	39.2	2.4	3.55
5	11 hrs after test meal	32.8	1.8	2.25
6	14 hrs after test meal	27.9	1.5	2.15

Table No. 4.

Series I, Experiment 2. The variation in the diastase content of the liver, kidney, and blood following a meal rich in fats and carbohydrates.

Animal No.	Time of sample after test meal	Diastatic Activity - per cent starch (10 mg) hydrolyzed in 15 minutes at 38° C.		
		Liver	Kidney	Blood
1	Before test meal	20.4	10.5	22.1
2	2½ hrs after test meal	33.6	11.0	28.4
3	5 hrs after test meal	52.1	15.0	38.2
4	8 hrs after test meal	38.2	19.2	28.9
5	11 hrs after test meal	31.2	21.1	26.4
6	14 hrs after test meal	19.6	20.0	21.5

Series No. I, Experiment No. 2.

Carbohydrates plus Fats

Chart No. 3.

Series No. I, Experiment No. 2.

Carbohydrates plus Fats

Chart No. 4.

Discussion of Series I

Of the two experiments, the second gave the more consistent results. This diet was more digestible, hence the cycle of absorption was more complete. The pre-feeding food was entirely removed, which was not the case in Experiment 1. Accepting Experiment 2 as the type, it is evident that a distinct rise and final fall of the amount of both sugar and diastase takes place in the blood and the tissues examined as the absorption of a meal of carbohydrates and fats proceeds. The rapidity of the absorption and the crest of the curve in terms of time vary with the digestibility of the food given.

In Experiment 1, the blood after 2.5 hours shows a slight increase; this may be due to the fact that the previous meal was in the process of being absorbed when the test meal was fed, so sugar was being absorbed from the intestine. The subsequent fall is possibly the true return to normal as absorption is for the moment arrested by the test meal in the stomach which holds the pylorus closed until the mass becomes acid. The blood sugar shown then by Dog No. 3 is probably closer to the normal than Dog No. 1. The sugars from the previous meal at the fifth hour have been absorbed and stored in the liver and muscles or other tissues, while the carbohydrates of the test meal have not yet reached the blood stream in the process of digestion; at least not enough of them have been converted to soluble form to hold the blood sugar level above normal. In the eighth hour of digestion the blood sugar increases nearly 100 per cent, indicating that the absorption is at its height, that soluble sugars are entering

the blood stream much faster than they can be stored up or used up in the tissues. As the storage continues and the amount being absorbed lessens, the blood sugar content falls to normal and is at a constant level. From the eleventh hours this constant level is maintained at the expense of the sugar stored up in the liver as glycogen.

The liver content increases continuously, markedly from the fifth to the eighth hour; the slow initial increase may be due to the previous meal. Tissue "demands" begin to "call out" the stored up carbohydrates from the liver, so a gradual decrease in content is evident throughout the eleventh and fourteenth hour periods. It would be necessary to have continued Experiment 1 over a longer period to show the complete withdrawal of the available glycogen from the liver to meet the expenditures of the organism.

The kidney as a type peripheral organ shows an absorption of sugar from the blood from the beginning. This increase is a gradual one and maintained until the eleventh hour period, that is, after the liver's decline has begun and the blood level has become constant. It would seem that the kidney normally uses carbohydrates in the process of functioning; this supply is absorbed from the blood, which is in turn kept constant at the expense of the stored up material, as in the liver. The kidney has no or little power of storage but the tolerance limit is soon reached, and it reassumes absorption when its own supply is depleted, and its own functional demands necessitate a new supply.

Then in the course of digestion of a carbohydrate meal the tissue demands then evident are first supplied

and the excess poured into the blood is temporarily stored in special depots; the liver is the notable example.

The kidney shows no change in diastase until the fifth hour period when its hydrolytic power is increased at the expense of the blood diastase. At the eighth hour period this increase is most marked and absorption is at its height, the liver and blood both showing a great increase in diastatic activity. At the eleventh hour the kidney diastase content becomes constant at a higher level than the original.

The blood and liver show parallel curves. During the first five hours of digestion period they show a decrease of diastatic activity. This may be due to the fact that they both were being held higher by the previous meal which autopsy showed to be still in the process of absorption in the normal pup, and by the diastase being poured into the blood stream from the pancreas and intestine. When this supply is cut off or checked by the test meal, these tissues approach their normal levels before a second installment comes from absorption of the test meal. The cottonseed oil seems to have made digestion slow, giving time for this conservation of enzymes of the pancreas in the intestine for hydrolysis of the chyle to be felt in the tissues. As absorption of the soluble sugars and the accompanying enzymes are absorbed from the intestine into the blood stream after the fifth hour, the diastase of the blood rises. This rise would in turn then affect the liver content, but in addition, the presence of the high blood sugar content at this time stimulates the

liver to the production of enzymes to resynthesize and store up the sugar as glycogen, so at the eighth hour period the liver shows an enormous increase of three times that of the fifth hour period. During the first part of the digestive period the sugar falling fails to stimulate the liver to the production of glycogenase, so the lower reading at the fifth hour period is probably the truest approach to normal. This is supported by the final fall of enzyme content through the eleventh and fourteenth hour periods reaching a level a little below that of the fifth hour period. The blood diastase rises with the active absorption of sugar from the test meal and falls to a constant level when storage in the liver is complete.

In Experiment 2, the dextrose was already in soluble form, so absorption begins as soon as it reaches the intestine, although being mixed with the fat which must undergo hydrolytic cleavage may slow the process somewhat. However, the absorption is well under way at the fifth hour period, increasing regularly to the eighth hour period, whereupon the stored up material in the liver is yielded to the tissues throughout the organism. The sugar curve of the blood is similar but of less variation. Experiment 1 gives a like curve.

The diastase content behaves somewhat differently. The blood shows an increase of 150 per cent of the normal as against a 100 per cent increase of the normal in Experiment 1. This high level is more persistent in the blood than in Experiment 1 and more consistent. The increase of diastatic

activity in the liver is practically the same in the two experiments. In the kidney the diastase increases with the blood to its crest at the eighth hour and continues to increase in diastase notwithstanding the fall in blood content. This increase continues through the eleventh hour period and falls very slightly in the next three hours. It approaches the blood level in the last period of the digestion. It is suggested that the passive tissue, the kidney, represents the type that derives its diastases from the blood. The kidney seems to absorb diastase in a way similar to which the liver stores sugar or fat. Whenever diastase appears in the blood above the normal level, it is distributed to the peripheral tissues. Whether the pancreas alone is responsible for the increase in blood diastase will be brought out later.

Series II, Experiment 3

The effect of a pure carbohydrate meal upon the carbohydrate and diastase content of the liver, kidney, and blood.

For this experiment a series of six pups was used. They were three each from two litters, both sets from shepherd mothers. All were of the same build, weight $2\frac{1}{2}$ kilos, and age, three months. They had been together two or three weeks previous to the test and were in the same nutritional condition. The preliminary treatment was the same as in the previous experiments. They were fasted for 48 hours after the last mixed diet feeding, some stomach contents being evident on aspiration at the end of 36 hours fasting. Since

the digestion of cornmeal seemed to occur rather slowly, this series was made to cover a period of 20 hours, decapitating at the end of every four hours after the feeding of the test meal.

Autopsy results:

No. 1 showed an empty stomach, the intestines practically so, and the viscera normal. Evidently digestion of the previous meal was complete at the time of the experiment.

No. 2. The stomach was $3/4$ full of semi-digested food, intestines nearly empty.

No. 3. The stomach was $1/3$ full of food, small intestine containing considerably chyle.

No. 4. The stomach was $1/4$ full of semi-digested material which is rather fluid. Entire small intestine was well loaded with digested contents.

No. 5. The stomach was $1/5$ full of material as above. The small intestine was the same as in No. 4 and the large intestine was partially full.

No. 6. The stomach was practically empty; the small intestine was empty except the lower 15 cm. The large intestine was full of solid fecal matter. Absorption was complete.

Table No. 5.

Series II, Experiment 3. Variations in the carbohydrate content of the liver, kidney, and blood following a meal of carbohydrates.

Animal No.	Time of sample after test meal	Carbohydrates as sugar in mg. per gram of tissue		
		Liver	Kidney	Blood
1	Normal, before test meal	10.0	1.87	2.45
2	4 hrs after test meal	23.0	1.80	3.33
3	8 hrs after test meal	48.0	1.90	3.1
4	12 hrs after test meal	44.5	2.20	2.76
5	16 hrs after test meal	41.0	1.75	2.8
6	20 hrs after test meal	38.0	1.75	2.67

Table No. 6.

Series II, Experiment 3. Variations in the diastase content of the liver, kidney, and blood following a meal of carbohydrates.

Animal No.	Time of sample after test meal	Diastase in per cent of starch (10 mg) in 15 minutes at 38° C.		
		Liver	Kidney	Blood
1	Normal, before test meal	8.0	12.5	22.5
2	4 hrs after test meal	15.0	11.2	26.0
3	8 hrs after test meal	80.0	18.9	47.1
4	12 hrs after test meal	90.0	21.8	35.0
5	16 hrs after test meal	57.2	19.1	27.2
6	20 hrs after test meal	11.0	14.95	27.2

Chart No. 5.

Chart No. 6.

Series II, Experiment 4

The effect of a carbohydrate meal upon the carbohydrate and diastase content of the liver, kidney, and blood.

Six pups of one litter were used for this experiment. They were $3\frac{1}{2}$ months of age, average weight $2\frac{3}{4}$ kilos. The preliminary treatment was similar to the preceeding experiment. They series were kept upon the constant mixed diet for four days and the experiment started after a fast of 48 hours. The test meal consisted of equal portions of a bread containing 900 grams of cornmeal and 300 grams dextrose. The samples were taken as before at four hour intervals during the digestive period.

Autopsy results:

No. 1. The stomach was empty, intestines practically empty. Absorption of the previous meal was complete at the time of the test meal.

No. 2. The stomach was $\frac{3}{4}$ full of the food mass. There was some chyle in the intestines.

No. 3. The stomach was $\frac{1}{2}$ full of semi-digested material. The intestines contained a large amount of chyle.

No. 4. The stomach was $\frac{1}{4}$ full and the intestines as the preceeding animal.

No. 5. The stomach was $\frac{1}{5}$ full of material. The small intestine was well loaded throughout its length.

No. 6. The stomach was practically empty. Intestine was empty for the most part, the large intestine well filled. There is probably a little absorption going on but the amount is very small and this animal shows the digestion of

the test meal to have been practically completed by the twentieth hour.

Table No. 7.

Series II, Experiment 4. Variations in the carbohydrate content of the liver, kidney, and blood after a meal of carbohydrates.

Animal No.	Time of sample after test meal	Carbohydrates as sugar mg. per gm.		
		Liver	Kidney	Blood
1	Before test meal	8.2	1.79	2.88
2	4 hrs after test meal	17.45	1.75	3.21
3	8 hrs after test meal	40.00	1.95	4.96
4	12 hrs after test meal	38.65	2.60	2.88
5	16 hrs after test meal	36.01	1.85	2.32
6	20 hrs after test meal	33.33	1.86	2.30

Table No. 8.

Series II, Experiment 4. Variations in the diastase content of the liver, kidney, and blood after a meal of carbohydrates.

Animal No.	Time of sample after test meal	Diastase in per cent starch hydrolyzed in 15 minutes at 38° C.		
		Liver	Kidney	Blood
1	Normal, before test meal	9.1	10.2	20.1
2	4 hrs after test meal	17.97	11.0	25.8
3	8 hrs after test meal	54.93	19.41	47.6
4	12 hrs after test meal	87.78	22.00	36.67
5	16 hrs after test meal	53.99	20.56	27.32
6	20 hrs after test meal	20.15	14.11	24.68

Series II, Experiment 4.

Carbohydrate Meal

Chart No. 7.

Series II, Experiment 4.

Carbohydrate Meal

Chart No. 8.

Autopsy results:

The autopsy showed the absorption of the last meal of the routine mixed diet to be complete within the 48 hours fast before the test meal was given. The normal for liver content of glycogen is lower than any of the previous normal determinations, which is accounted for by the fact that this set was fasted 12 hours longer than in the previous experiments, as greater inroads had been made upon the stored up glycogen.

At the eighth hour period the absorption rate is the greatest; sugar is pouring into the blood stream faster than the liver and other tissues can resynthesize it into glycogen, increasing from a normal of 2.48 to 5.1 mgs. per cc. at the eighth hour.

The liver shows the greatest storing activity between the fourth and eighth hours of the digestive cycle. The pancreas, it is assumed, has continued to produce enzymes in response to the presence of a rather slowly digesting mass of food. This diastase tends to speed up the rate of hydrolysis as digestion continues and the blood sugar increases rapidly. At the twelve hour some absorption is still occurring, as the higher blood sugar content indicates, but the storage in the liver is growing less and is overbalanced by that which is being carried away.

The kidney as the passive tissue concerned, continues to absorb sugar as long as the blood sugar remains above the normal level, then gradually decreases as this small storage is being used up in the normal functioning of the organ.

The blood diastases follow the blood sugar content but end at a higher level than the normal, a condition to be expected when the amount of enzyme which has been produced to take off the carbohydrates present is considered. In general, the whole complex mechanism of carbohydrate metabolism undergoes quite an upheaval, a much greater effect than a mixed diet would produce.

The liver diastase is the most striking in its enormous increase of eleven times the normal, reaching its height at the twelfth hour. The normal diastase content in this series was low, as has been stated of the glycogen content. Throughout this and all the other series, liver diastase always follows the liver sugar content, indicating that the liver produces diastase in proportion to its activity. The lower normal value than usual is accounted for by the fact that in this series the 48 hour fast practically depleted the available carbohydrates stored within the liver from the previous meal and the activity of the liver would be expected to be small. On the other hand, when, after a large meal of carbohydrates, sugar continues to pour into the blood stream, its presence in the blood continues to stimulate the liver to produce diastases to take care of it and accomplish the storage of all that is in excess of the blood level and that above the tolerance limit of the peripheral and passive tissues. The high value in liver diastase then follows, the value is four to five times the normals in the series subjected to a shorter fast period, and three times greater than in the experiments where a mixed diet and less carbohydrates were given in the test meal.

The amount of liver diastases produced seems to be in direct proportion to amount of carbohydrates to be taken care of; i.e., the presence of an excess of sugar in the blood seems to stimulate the liver under these conditions, produce glycogenase, and to store up the excess sugar.

The kidney's diastase content follows that of the blood, and at the end is constant at a higher level than normal. The exception to this is a slight decrease during the first four hour period. This may be an individual variation. But if the pancreas is a source of tissue diastase, it is ineffectual at this time; possibly all its products are being used within the intestine and a shortage of tissue diastase would accompany such a condition at this period.

Whether this is selective secretion will be discussed in the next series, but the more favorable opinion is that it is merely a stimulation of longer duration. In Experiment 1, Series I, selective secretion would call out pancreatic lipase too, for the fat is also stored within the liver, possibly at the expense of the diastase production of the same organ. But here again the quantitative element producing the stimulation and controlling the length of the action would seem the more plausible view.

The results in this experiment check with the preceeding one. The diastase curves demonstrate more clearly that the liver function is a thing apart from the condition of the blood, ~~wh~~its action continues regardless of the condition of the blood, whether its diastase and sugar

contents are increasing or decreasing. The diastase content of the passive organs seems to depend directly upon the blood.

The liver is shown as being very active during the first eight to twelve hours of the digestive period. At the eighth hour sugar is beginning to decrease in the liver, to be mobilized from the liver into the blood to supply tissues of the body. The diastase of the liver is still high and absorption is still taking place from the intestines. The stimulus to the liver is persisting even though the excess of sugar to be stored up is growing less and less. The duration of this stimulus must be related to the fact that active digestion of the carbohydrates is continuing. The pancreatic secretions, the increase in blood sugar, and the products of carbohydrate digestion are present, all of which in some way seem to influence the activity of the liver. The liver seems to supply enzymes in accordance with the amount of carbohydrates being transferred in the process of digestion, and independent of the blood or tissue diastase. The tissues seem to depend upon the blood and pancreas for their diastases, for the kidney content falls with the blood in diastase at a time when the liver shows a very high and even a rising content, which is really an overabundance for its own needs. Yet, it does not seem to go out into the blood stream and into the tissues, but is probably used up in the mobilization of sugar from the liver. The noticeable and striking fact brought out by the experiment is the tremendous activity shown by the liver. Any meal of equal weight and digestibility presumably might call out the same amount of secretions from the pancreas, yet when the meal consists of carbohydrates alone, the stimulation of the liver

is stronger or of a greater duration when its response is taken as the measure. The product of the pancreas alone does not seem to constitute the whole of the factors concerned in the control of the liver in carbohydrate metabolism.

Series III, Experiment 5

The effect of a protein meal upon the carbohydrate and diastase content of the liver, kidney, and blood.

Six pups, four of one litter and two of another, $2\frac{1}{2}$ months old, were used in this experiment. Their average weight was two kilos, and all were in good nutritional condition. The four were of a hound breed and rather quiet, while the others were of a terrier breed a week older and very lively and playful. The two latter were taken as Numbers 1 and 3 in the series.

The preliminary treatment was the same as in previous experiments. After a fast of 48 hours the test meal of lean beef, the meat having been stewed and drained, was fed 75 grams per kilo. No. 1 was decapitated at the time of the feeding and taken as the normal. Numbers 2, 3, 4, 5, and 6 were decapitated at 4, 8, 12, 16, and 20 hours respectively after the feeding. Analyses were made on the blood for diastase and sugar immediately. Diastase determinations were made upon aqueous extracts of the tissues 12 hours after decapitation allowing the diastases present to become thoroughly distributed throughout the extract. Samples were taken from time to time until the equilibrium between the sugar and glycogen seemed established, the final determinations made, and the diastases determined again, checking

the 24 hours determinations.

Autopsy Results:

No. 1. Good condition, stomach practically empty, intestines empty. Previous meal completely digested.

No. 2. Good condition, stomach $\frac{3}{4}$ full of semi-digested meat. Intestines contain chyle.

No. 3. Good condition, stomach $\frac{3}{4}$ full, content more fluid than above. Intestines the same, though well filled.

No. 4, and 5. Stomachs $\frac{1}{2}$ and $\frac{1}{3}$ full of partially digested matter. Intestines as above.

No. 6. Stomach $\frac{1}{4}$ full of material. Chyle in the intestines. Absorption was not quite complete.

Table No. 9

Series III, Experiment 5. Variations in the carbohydrate content of the liver, kidney, and blood following a meal rich in proteins and containing little or no carbohydrate material.

Animal No.	Time of sample after test meal	Carbohydrates as sugar in mg.		
		Liver	Kidney	Blood
1	Normal before test meal	10.8	1.55	2.44
2	24 hrs after test meal	17.5	2.46	2.77
3	8 hrs after test meal	14.2	2.80	4.2
4	12 hrs after test meal	14.6	2.98	3.33
5	16 hrs after test meal	14.2	2.68	2.8
6	20 hrs after test meal	14.2	2.24	2.6

Table No. 10

Series III, Experiment 5. Variations in the diastase content of the liver, kidney, and blood following a meal rich in proteins and containing little or no carbohydrate material.

Animal No.	Time of sample after test meal	Diastatic Activity in per cent starch hydrolyzed in 15 minutes at 38° C.		
		Liver	Kidney	Blood
1	Normal before test meal	24.33	27.6	18.00
2	4 hrs after test meal	26.8	59.64	12.70
3	8 hrs after test meal	23.42	92.90	73.63
4	12 hrs after test meal	27.60	98.85	45.57
5	16 hrs after test meal	26.40	75.10	34.70
6	20 hrs after test meal	24.90	64.80	27.20

Series III, Experiment 5.

Protein Test Meal

Chart No. 9

Chart No. 10.

While the stomach of the normal pup, No. 1, appeared empty, the slight possibility remains that there may still be a small quantity of sugar from the previous meal entering the blood stream. There will, too, be a small amount of glycogen in the lean beef fed in the test meal, but the usual depleted condition of an animal for slaughter considered together with the amount of lean beef consumed and the manner of preparation of the meal would undoubtedly make this source of sugar too small to influence the results.

The blood shows a slight increase in sugar in the first four hours of digestion and a marked rise up to the eighth hour period, after which the sugar content falls to normal. For the source of this sugar we must look to the proteins ingested.

The liver glycogen content has been nearly depleted by the 48 hour fast which preceded the experiment. The liver, in fact, shows a slight fall at the eighth hour period, but the decrease is so small it is probably accounted for by the marked activity of this animal, No. 3. At any rate the fall is too slight to account for the doubling of the blood sugar content. The liver content throughout the remainder of the digestive period remains constant. The difference shown in No. 1 and No. 3 at the first and eighth hour period is due to the individual characteristics of these animals and the functional activity of the liver of No. 3 in regards to protein metabolism. If carbohydrate is derived from proteins it is not stored as glycogen in the liver.

The liver certainly is active in function during the digestion, assimilation, and distribution of a mixed carbohydrate-protein-fat meal. The striking point of the protein meal test is that even though there is an excess of sugar in the blood stream, the liver is not stimulated to store up this sugar to the extent that it does when there is the same or even a less excess of blood sugar derived from a carbohydrate meal. The question arises, is sugar of protein derivation different from that from hydrolyzed carbohydrates, in that the glycogenase of the liver is not capable of resynthesizing this material to glycogen? The distribution of enzymes concerned and the other products of protein digestion may throw some light upon this point.

The kidney as a type of passive tissue shows a marked increase through the eighth hour and continuing through the twelfth hour where it increases 100 per cent of the normal content, then a gradual fall. It continues throughout the digestive period to remain high but just below the blood sugar level.

Liver diastase content seems to follow the liver glycogen content. As the liver gives up the excess of stored glycogen, its activity decreases as the limit of depletion is approached. The diastase content diminishes with the activity. After a protein meal there is either a lack of the substances which stimulate the liver to increased activity, or there is an excess of the substances which inhibit the activity of the liver in its function in relation to carbohydrate metabolism and glycogen storage.

The secretion of the pancreas is evidently not selective. This is shown by the large rise in blood diastases presumably derived from the pancreas, as the liver is relatively inactive at this period. This rise in blood diastases is at once transferred not to the liver but to the passive tissues. The kidney shows a truly remarkable increase. The increase in blood diastase would indicate that as long as the pancreas is active all its enzymes are produced equally regardless of the exact nature of stimuli and whether they are needed or not. Diastases then coming from the pancreas throughout the period of digestion are unused and absorbed into the blood stream and distributed throughout the organism, the liver receiving little or none. The fact that the diastases are unused is evident in the increase of three and a half times the normal blood level. The kidney shows a continuous and sharp rise in diastase, at the eighth hour reaching three times the normal and continuing to rise during the next four hours to a maximum of three and seven-tenths times the normal level, finally remaining above the normal blood level. The blood after the twelfth hour falls rapidly to normal while the diastases in the kidney decrease slowly but remain at a relatively high level. So the pancreas seems to be the source of supply of the tissues for their diastases but the liver is selective in its secretion and produces its own diastases after receiving certain stimuli.

What controls the amount of liver activity in regard to sugar storage? The amount of diastase in the blood is not the only factor it would seem. Neither is sugar in

in the blood above the normal the controlling factor alone at least. The activity of the liver and its production of diastases seems to correspond, however, to the quantity of carbohydrates taken into the body involved in the process of being stored up by the liver. The complex which controls the activity of the liver is very involved and not clearly understood. The pancreas produces a secretion, internal or external, the lack of which causes the liver to give up sugar rapidly; in this series there is an excess of pancreatic diastase in the blood. The converse seems to hold, in that the liver gives up little or no glycogen as sugar but it does not stimulate persistent glycogenesis even when an excess of sugar and diastase continue to be present in the blood.

The secretion of the pancreas is usually accepted as containing a hormone which exerts an inhibitory influence upon the liver, for conservation of the glycogen, opposite to the effect of the epinephrin of the adrenal glands which accelerates the output of sugar from the liver. Whether this substance is the so called internal secretion of the pancreas or not, it is reasonable that it would be produced whenever the pancreas is stimulated to activity. After the protein meal, fed in this series, the pancreas is stimulated to activity and this controlling factor would be present, yet the liver is not stirred to activity adequate to storing up the present sugar, not on the other hand does it lose in glycogen, but soon remains passive to carbohydrate metabolic processes. Yet the liver is active, however, in the digestion of proteins. Could the by-products of protein

metabolism inactivate the normal excitor of the liver to glycogenesis, or is there absent some factor due to the hydrolysis of carbohydrates? In the liver, in the process of deamination of the proteins, there will be ammonium carbonate and urea formed but these are carried in the blood to the tissues and exert no influence upon the transfer of carbohydrates as the kidney shows active diastase and sugar utilization while at the time is known to be saturated with and actively excreting urea.

A test meal of proteins and carbohydrates would probably show both activities of the liver occurring at the same time. Then it would seem that something more than excess blood sugar and increased pancreatic secretion is necessary to stimulate the liver to active glycolysis. The fact remains that the liver shows selective secretions and functions, the complete control of which as yet is not well understood.

The only other explanation satisfying the results of this series is that the sugar present is not more completely taken up by the liver because the oxidases, hydrolases, and other conditions present in the liver during protein absorption, prevent resynthesis. Either the complex glycogenetic activator is absent or its action is inhibited by some other fact. Diastases are, it is ^{us}ually accepted, always found holding within their complex colloidal structure a carbohydrate; this arrangement might so react with the products of protein metabolism in the blood and the liver as to render stimulation of glycolysis in the liver improbable, but does

not interfere with the utilization of sugar by the kidney and other tissues. Whether the protein metabolism going on in the liver has any effect is not clear, but the critical point seems to be located in the liver cells and their reaction to the influences of protein digestion.

The pancreas in general affects carbohydrate metabolism in two ways; (1) favors storage in the liver, and (2) favors the utilization in the tissues. After a protein meal the activity of the latter is greatly increased while the former is not. It may be that the liver is more difficult to stir to activity and the excess blood sugar is adequately taken care of by the tissues after a fast of 48 hours. The part played by protein metabolism going on at the same time is not clear, but as a peptone solution injected intravenously is known to produce mobilization of sugar in the liver and increases the blood sugar, even to glycosuria, it is reasonably to suppose that as the liver already is depleted in glycogen, the peptones in the blood from a protein meal would, if not producing the usual hyperglycemia, at least inhibit storage of sugar as glycogen. By the time the proteins are assimilated and the blood content of proteins returns to normal the sugar is no longer present in excess in the blood to be stored up because of the abundance of pancreatic secretions facilitating absorption of sugar within the tissues other than the liver.

Series III. Experiment 6

To determine the effects of a protein meal upon the carbohydrate and amylase contents of the liver, kidney,

muscle, and blood.

The series of animals consisted of five pups of the same litter, $2\frac{1}{2}$ months of age, average weight $2\frac{1}{2}$ kilos, and in good nutritional condition. The preliminary treatment was the same as in the preceeding experiments covering a period of eight days. The test meal was composed of lean beef as in Experiment 1, Series III.

The procedure of the previous experiment was followed in the taking and treatment of the samples.

Autopsy results:

No. 1. Stomach empty, small intestine empty except the last six cm, large intestine filled; urine sample taken.

No. 2. Stomach $\frac{3}{4}$ full of semi-digested food. First fourteen cm. of small intestine contained chyle. Large intestine full.

No. 3. Stomach $\frac{1}{2}$ full of rather fluid contents, small intestine well loaded with chyle. Fecal matter in the large intestine. Urine sample taken.

No. 4. Stomach $\frac{1}{4}$ full of fluid contents, little of the test meal remaining. Lower small intestine well loaded with rather solid contents.

No. 5. Samples not taken.

No. 6. Stomach entirely empty. Upper intestine empty. Lower intestine filled with solid fecal matter. Urine sample taken.

Table No. 11

Series III, Experiment 6. Variations in the carbohydrate content of the liver, kidney, muscle, and blood after a meal of proteins.

Samples taken at the time of autopsy in the manner described in the previous experiments. In Series III, in addition, to the samples of liver and kidney tissue, duplicate samples of muscles of the hind leg were taken and determinations made upon them for diastase and sugars.

Animal No.	Time of Sample after test meal	Carbohydrates as sugar			
		Liver	Kidney	Blood	Muscle
1	Normal before meal	7.79	1.23	2.75	2.80
2	4 hrs after test meal	14.23	1.33	3.12	6.84
3	8 hrs after test meal	10.98	1.515	3.95	3.65
4	12 hrs after testmeal	10.05	2.15	3.39	3.55
5	16 hrs after test meal				
6	20 hrs after testmeal	4.02	1.16	2.559	2.98

Table No. 12

Series III, Experiment 6. Variations in the amylase contents of the liver, kidney, muscle, and blood after a meal of proteins.

Animal No.	Time of sample after test meal	Amylase in per cent starch hydrolyzed				
		Liver	Kidney	Muscle	Blood	Urine
1	Normal before meal	2.0	27.0	21.0	12.49	9 %
2	4 hrs	10.0	36.6	19.02	34.1	
3	8 hrs	33.1	45.5	22.26	33.5	26 %
4	12 hrs	36.0	50.0	55.45	30.3	
5	16 hrs	No samples				
6	20 hrs	10.15	36.0	61.58	12.2	93 %

Series III, Experiment 6.

Protein Test Meal

Chart No. 11.

Chart No. 12

In Animal No. 2 of the preceding series the liver shows an increase in sugars similar to that in the preceding experiment. The blood and kidney contents are almost perfectly parallel. The decided drop in liver sugars between the fourth and eighth hour periods agree. The liver is known to be active at this time in concerned with the protein products of digestion; whether this activity accounts for the loss of liver sugar is not clear. In Animal No. 3 the muscle is seen to be active in storing up sugar. The source of this sugar is not determined.

The results on diastatic activity, while agreeing rather closely for the most part, are not so striking as in the preceding series which may be partially accounted for by the difference in age of the two series of pups used.

The liver's diastase content as usual follows very closely its sugar content, showing a slow and then a sharp initial rise to a maximum at eight hours. This level is almost maintained at twelve hours, after which a rapid fall occurs. The crest comes a little earlier than that of either the blood or the kidney.

The kidney shows the same remarkable rise of diastase content, reaching a crest at twelve hours. The curve follows closely that of the blood.

If the determinations made upon the urine are to be relied upon, the kidney seems very active in excreting the excess diastases of the blood after the tissue needs for the enzymes are adequately supplied. Following a protein meal where little or no use is made of the abundant pancreatic

diastases at hand, this excretion is large.

The disposal of this excess diastase after a protein meal as suggested by Experiment 6 is possibly made clearer by results of tests made upon muscle tissue. The diastase content of this tissue is seen to first fall slightly during the first four hours of digestion, possibly because the periodic supply from the pancreas is for the time cut off, after which a steady and sharp increase occurs. The muscle seems to fairly store up diastases far in excess to the immediate needs. This picture would point to the view that the muscles especially use pancreatic diastases in their functional activities. If the muscle be taken as a type of peripheral tissue (the kidney being excretory in this case), the tissue needs for diastase are supplied by the pancreas and the excess above this amount is eliminated through the kidneys and the urine.

Series IV. Experiment 7.

The effect of a meal of proteins and carbohydrates upon the carbohydrate and amylase contents of the liver, kidney, muscle, and blood.

This series was made up of five hound pups of the same litter, $2\frac{1}{2}$ months of age, average weight $2\frac{1}{2}$ to 3 kilos, all in very good nutritional condition. The series was fed upon the usual mixed diet for a period of six days, the feedings being equal, based upon the individual weight of each animal. The series fasted for 48 hours, allowing water, 100 cc per kilo, in five installments during the fast up to within 12 hours of the feeding of the test meal. One animal taken as the normal, the others decapitated at four hour intervals

after the feeding. The test meal was composed of 100 grams of cooked lean beef and 50 grams of cornmeal, 50 grams dextrose, the whole being thoroughly mixed, boiled, and baked together.

Autopsy results:

No. 1. Stomach empty, small intestine empty, except the last 10 cm. Large intestine filled with fecal matter. Urine sample taken.

No. 2. Stomach $3/4$ full of semi-digested material. Some chyle present in the duodenum. Large intestine filled with fecal matter.

No. 3. Stomach $1/2$ full of rather fluid contents, chyle plentiful in the small intestine, large intestine nearly full of fecal matter.

No. 4. Stomach $1/4$ full of fluid contents. Small intestine contains chyle. Large intestine full.

No. 5. No sample taken.

No. 6. Stomach empty excepting about 5 gms of fluid containing little or none of the test meal. Lower intestine holds solid matter. Urine sample taken.

From the autopsies digestion and absorption seem almost complete.

Table No. 13.

Series IV, Experiment 7. Variations in the carbohydrate content of the liver, kidney, muscle, and blood following a meal of proteins and carbohydrates.

Animal No.	Time of sample after test meal	Carbohydrate content as sugar			
		Liver	Kidney	Muscle	Blood
1	Normal before test meal	7.74	1.23	2.75	2.8
2	4 hrs after test meal	15.58	2.22	8.00	5.12
3	8 hrs after test meal	23.26	2.259	9.34	5.43
4	12 hrs after test meal	28.47	1.92	6.06	4.80
5	16 hrs after test meal				
6	20 hrs after test meal	12.196	1.16	5.74	3.55

Table No. 14.

Series IV, Experiment 7. Variations in the amylase content of the liver, kidney, muscle, and blood following a meal of proteins and carbohydrates.

Animal No.	Time of sample after test meal	Amylolytic Activity - percent starch hydrolyzed				
		Liver	Kidney	Muscle	Blood	Urine
1	Before test meal	22.3	27.0	21.0	12.4	9.
2	4 hrs	45.34	33.3	49.1	28.2	
3	8 hrs	15.3	34.1	80.0	60.0	
4	12 hrs	35.1	30.8	15.2	50.7	
5	16 hrs					
6	20 hrs	20.69	21.2	7.57	30.3	18.

Series IV, Experiment 7.

Protein and Carbohydrates

Chart No. 13.

Chart No. 14.

This series was run to investigate the liver's activity in particular after a meal of both carbohydrates and proteins.

The liver is active in the storing up of sugars as glycogen and at the same time in playing its part in the digestion, assimilation, and distribution of proteins. That this latter activity influences the former is very evident from the curve of the liver diastases; the rather checked increase of content, though more rapid after the eighth hour period, reaching a maximum at the 12th hour period, four hours later than is common to the digestion of a meal of carbohydrates and fats or of carbohydrates. (Series I and II)

The muscle tissue displays its property of sugar storage as long as the blood sugar content is above normal, unaffected by the protein products present in the blood stream, increasing at the usual eighth hour period to a maximum, an increase of 240 per cent of its original content.

The liver diastase although high normally shows a moderate and steady increase. The increase, persisting throughout the first eight hours of digestion, however, is relatively a small one, demonstrating the lessened or checked activity of the liver during its dual functioning.

The muscle tissue again shows itself very active in extracting blood diastases for its own tissue needs. The fall in content continuing after the eighth hour indicates muscular activity of the animal and points to the fact that of the supply received by the blood from the pancreas was partially depleted, being used up in the digestion of the carbohydrates

ingested.

That the final excess diastase was small is indicated by the urine of the last animal of this series showing only 18.5 per cent as the figure for its diastatic activity. The excretion of excess diastases in the blood stream was small. The diastatic content of the kidney shows about the usual increase after a mixed meal and indicates the presence of little excess diastase above tissue needs.

DISCUSSION AND SUMMARY

The series of responses of the organism to a meal is, first, that of digestion and absorption, second, distribution and utilization, and, third, the reestablishment of a relative metabolic equilibrium within the organism. For example, following by way of illustration the results of Series II, an animal is after a fast of 48 hours in a state of metabolic equilibrium. The digestive tract is free of food, the blood sugar and diastase content is constant, the tissue diastase and sugar content is also in equilibrium; the liver is low in amylase, below the other ~~and other~~ tissues, and its glycogen is depleted or at a comparatively low level and the organ as a whole is displaying minimum activity. Upon the mastication and swallowing of the food the gastric and salivary secretions begin and there is the production of a general hyperemia alimentosis. With the first opening of the pylorus, the hydrochloric acid of the gastric juice upon the intestinal mucosa activates the secretion which in turn stimulates the pancreas to pour out its secretion into the intestine which there meets the carbohydrates of the chyle and hydrolysis begins. The hydrolysis continues and the resulting sugars are absorbed by the blood together with the diastases, causing a marked rise in the content of the blood in diastase and sugar. This process continues as long as the carbohydrates remain in the alimentary tract.

The peripheral tissues immediately respond to the disturbed equilibrium produced by the excess of sugar and

diastase in the blood and "stock up" by absorbing sugar so long as their tolerance limits are not reached and the blood content remains above normal. The liver, as the organ of high sugar storing capacity, manifests an enormous increase in carbohydrate metabolism, that is, glycogenesis. It apparently produces its own amylase, resynthesizes and stores glycogen in excess during the time of influx of sugars in the blood. This conservation of sugar continues until upon the reduction of the blood income the reverse process occurs, that is, mobilization of the liver sugar by hydrolysis and redistribution through the blood stream, thus holding the blood sugar level constant in spite of the ravages of the passive tissues made upon the blood content.

In terms of osmotic pressures and densities the greatest concentration of sugar is to be found in the liver cells during mobilization and so it is forced out into the less dense blood stream. The concentration in the passive tissues, e.g., the kidney, being ever less than the blood, sugars pass from the blood into their cells. The tissue content of sugar is governed by the ^{content in the} blood and the physico-chemical laws maintaining equilibrium, hence, it can never rise higher than that in the blood as Series III shows in the later stages of digestion. If such a higher blood sugar level persists, as in the diabetic condition, the tissue tolerance limit would soon be reached and excretion begin. In the kidney, and probably in all secreting cells, as the concentration approaches that of the blood, sugar will be given up into the secretion, as evidence by the glycosuria in the diabetic.

The tissues such as the blood and kidney seem to follow relatively simple physical and chemical laws, while the liver is seen to be a more specialized tissue and reacts in response to a varied and complex set of stimulations, the full nature of which is not entirely understood. For this reason, the liver's activity seems rather separate and distinct from the other digestive and assimilative processes.

The salient observations made in this paper may be categorically stated as follows:

1. After a carbohydrate meal, either alone or in combination with other food principles, the amount of sugar in the blood increases to a maximum in from eight to twelve hours, then gradually returns to the preceding normal, the whole cycle occurring in from twenty to forty hours according to the digestibility of the food.

2. After a carbohydrate meal, the liver content of glycogen is enormously increased, by as much as 100 - 380 per cent of the original normal. The storage reaches a maximum in from eight to twelve hours after which a rapid decline occurs to a somewhat higher level than the preceding normal after twenty hours.

3. The kidney as a passive tissue exhibits a similar but a very much lower curve than that of the blood or liver, the crest occurring a little later than that of the liver.

4. The amylase of the blood, the liver, and the kidney in every case is augmented in quantity after a carbohydrate meal. The curve reaches a maximum in about twelve hours, or a little later than the crest of the sugar curve,

then decreases to an approximate normal after twenty hours.

5. The liver amylase is great in quantity, showing an increase of the maximum content of the blood and kidney. This suggests active amylase production by the liver.

6. A protein meal without fats or sugars does not lead to glycogen storage in the liver though a slight increase was noted in the blood and the kidney.

7. After a protein meal little amylase augmentation occurs in the liver, though a persistent rise occurs in the blood and the kidney, reaching a maximum at the eighth hour and twelfth hour respectively. It is inferred that this amylase comes from the pancreatic secretion, which, if correct, implies an independent secretion of amylase by the liver during carbohydrate absorption.

8. Emphasis is laid on the close relation in time and in type of curve of the variation in amylase and sugar content of the blood and tissues and in particular the liver during the absorption of a carbohydrate meal. This is the major contribution of this investigation.

BIBLIOGRAPHY

1. Kirchoff, C., *Über die Reinigung Getreide Stark.*
Schweigler's Jour. of Chem. u. Physik., Volume 14,
p. 385, 1815, from Bayliss, p.9.
2. Dubunfrant, *Über Verwandlung des Starkmehls in Zucker
durch Malz*, Jour. of tech. u. ölung Chem. Erdmann,
Vol. 9, pp. 156, 1830. Trans. from; Bull. des
Sciences Technol., Paris 1830, fr. Bayliss.
3. Persoz and Payen, *Memoir sur la diastase*, Annales de
Chemie et Physiol., 1883, vol. 53, p. 73.
4. Wittich, from Levene, P.A., and Meyer, G. M., *The com-
bined action of Muscle, Plasma, and Pancreatic ex-
tract on Glucose and Maltose*, Jour. Biol. Chem.,
1911, vol. 9, p. 97, 1912, vol. 11, p. 347.
5. Bernard, C., *Comptes Rendus*, 1877, vol. 85, p. 519.
6. Pavy, Ref. from Bayliss (14)
7. Leuch-Kastners, *Arch. f.d. ges. Naturlehre*, 1831, fr.
Bayliss.
8. Bernard, C., *Lecons sur la diabete et glycogenesis
animale*, 1877, Paris; Reference from Mathews.
9. Widdecombe, J. H., *Digestion of Cane Sugar*, Jour.
Physiol., 1902, vol. 28, p. 175.
10. McLean, R.M., *Diastatic Activity in Mammalian Tissue*,
Jour. Biol. Chem., 1907, vol. 4, p. 467.
11. Vernon, H. M. *Pancreatic diastase and its zymogens*,
Jour. Physiol., 1902, vol. 28, p. 226; 1901, vol. 27,
p. 174.

12. Kastle, J. H. and Loevenhart, A. S., On Lipase, the Fat Splitting Enzyme, and the reversibility of its action., Amer. Chem. Jour. 1900, vol. 24, pp. 491-525.
13. Hill, C., Reversible Zymohydrolysis, Jour. Chem. Soc., 1898, vol. 73, p. 634. Reference from Bayliss.
14. Bayliss, Wm., The Nature of Enzyme Action, Monograph, Ed. 2, 1911.
15. Pugliese & Domenichini, Archives italiennes de biologie, vol. 47, p. 1, 1907, from McLean, H. Biochemical Journal, vol. 4, p. 467, 1909.
16. Boldyreff, W., Function periodique de l'organisme les l' Homme et les animaux D'ordre superieur, (Pancreas comme principal agent du processus de assimulatione D'aus tout le corps) Quar. Jour. Physiol., vol. 10, p. 175, 1916.
17. Knowlton and Starling, 1912, Jour. Physiol., vol. 45, p. 101.
18. Cohnheim, O., Zeit. schr. f. Physiol. Chem., 1904, vol. XLII, Reference from (4)
19. Hatcher, R.A. and Wolf, C. G. L., The Formation of Glycogen in Muscle, Jour. Biol. Chem., 1907.
20. Paton, Noel D., Regulators of Metabolism., 1913, Ed. Macmillan and Company, Limited.
21. Kulz, Beitrage zu der Lehre von der Glykogenbildung in der Leber, Arch. f.d. ges. Physiol., 1881, vol. 24, p. 24.
22. Voit, C., Uber die glycogenbildung nach Aufnahme verschieden Zuckerarten, Zeit. f. Biol., 1891, vol. 28, p. 245.

23. Dakin, H. D., Studies on the intermediary metabolism of the Amino Acids, Jour. Biol. Chem., 1913, vol. 14, p. 321.
24. Lusk, G., Amer. Jour. Physiol., 1903, vol. XLIX, p. 123, vol. XVI, p. 129.
25. Janney and Blatherwick, Glucose formation from Human proteins, Jour. Biol. Chem., vol. 23, p. 77.
26. Cheminiers - from
Hockendorf - from
Ringer - The chemistry of glucogenesis, Jour. Biol. Chem., vol. 14, p. 43, 1913, vol. 15, p. 145, 1913.
27. Greenwald, L., Blood Glycolysis, Jour. Biol. Chem., 1913, vol. 15.
28. Cremer, Ergebnisse der Physiol., Abtheilung, 1902, vol. 1, p. 803.
29. Bloor, W. R., Studies in Fat Metabolism., Jour. Biol. Chem., vol. 8, p. 126.
30. Neilson, C. H. and Lewis, D. H., Effect of diet upon the Amylolytic power of saliva, Jour. Biol. Chem., 1908, vol. 4, p. 53.
31. Bradley, H. C., Human Pancreatic Juice, Jour. Biol. Chem., 1909, vol. 6., p. 172.
32. Luckhart, W., Amer. Jour. Physiol. 1908-9, vol. 23, p. 148.
33. Carlson, A. C., Experimental Glycosuria, from Lewis, H.B. and Frankel, E. M., Inulin on the glucose output in phlorizin diabetic dog., Jour. Biol. Chem., 1914, vol. 17, p. 365.

34. Allen, Glycosuria and diabetes, Boston Ed., 1913.
35. McGuigan, H. and Vos Hess, C. L., Dextrose and
Diastase of the Blood, Jour. Biol. Chem. 1915,
vol. 22, p. 417, 1916, vol. 25.
36. McGuigan, H. and Ross, E. L., Peptone Hyperglycemia,
Jour. Biol. Chem. 1915, vol. 26, p. 417.
McGuigan, H. and Von Hess, C. L., Dextrose and diastase
of the blood as affected by the ether on animals fed
on different diets, Amer. Jour. Physiol., vol. 30, p.
341.
37. Lewis, R. C. and Benedict, S. R., Blood Sugar Determinations,
Jour. Biol. Chem, 1915, vol. 22, p. 63.
38. Meyers, V. B. and Killian, J. A., Diastatic activity
of the Blood in diabetes, 1916, Jour. Biol. Chem.,
vol. 29, p. 179.

A P P E N D I X

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PROTOCOLS

Weight charts of each experimental series on preliminary diet

Series I, Experiment 1.

April 25, 1917.

Age 6 months

Weights before experiment, in grams.

Animal	Sex	6/19	6/20	6/21	6/22	6/23	6/24	6/25
1	female	3760	3210	3230	3110	3200	3150	3000
2	female	3225	2900	2975	3000	2995	3000	2900
3	female	3000	2590	2590	2515	2475	2525	2500
4	male	2555	2160	2230	2145	2100	2125	2035
5	female	2450	2135	2145	2135	2140	2150	2125
6	male	2865	2445	2440	2440	2400	2450	2360

Average 2486.33

Series I, Experiment 2.

June 12, 1917.

Age 3 months

Weights before experiment, in grams.

Animal Sex

1	male	3000	3100	3155	3140	3135	3100	3020
2	female	3260	3290	3200	3310	3285	3220	3190
3	male	3040	3110	3150	3120	3190	3100	3000
4	female	2790	2800	2810	2785	2760	2775	2695
5	female	2990	2975	3000	2985	2895	2945	2865
6	female	2985	2965	2975	2940	2985	2940	2880

Series II, Experiment 3.

June 23, 1917

Age 3 months

Weights before experiment, in grams.

Animal Sex

1	female	2500	2510	2535	2545	2500	2420	2400
2	female	2710	2700	2690	2700	2695	2635	2600
3	female	2450	2500	2510	2500	2580	2525	2470
4	male	2350	2320	2365	2375	2370	2345	2300
5	female	2480	2490	2475	2500	2485	2455	2435
6	male	2380	2400	2410	2400	2435	2400	2370

Series II, Experiment 4.

June 25, 1917.

Age 3½ months

Weights before experiment, in grams

Animal Sex

1	male	3000	3000	3100	3100	3065	3020	2990
2	female	2755	2790	2785	2780	2790	2765	2740
3	female	2550	2560	2550	2490	2500	2475	2435
4	male	2500	2455	2400	2450	2465	2430	2410
5	female	2885	2800	2810	2800	2815	2800	2780
6	female	2600	2610	2635	2620	2645	2530	2605

Series III, Experiment 5.

July 6, 1917

Age $2\frac{1}{2}$ months

Weights before experiment, in grams

Animal Sex

1	male	2000	2065	2085	2090	2095	2075	2045
2	female	1850	1885	1910	1900	1920	1900	1875
3	male	2010	2000	2020	2025	2045	2020	2000
4	female	1675	1700	1710	1710	1730	1700	1685
5	female	1790	1835	1830	1825	1845	1830	1810
6	female	1800	1800	1840	1835	1830	1815	1805

Series III, Experiment 6.

July 30, 1917

Age $2\frac{1}{2}$ months

Weights before experiment, in grams.

Animal Sex

1	female	2510	2550	2535	2565	2575	2555	2500
2	male	2400	2400	2410	2440	2445	2430	2415
3	female	2365	2380	2400	2410	2400	2385	2360
4	male	2800	2750	2765	2760	2790	2780	2755
5	female	2660	2660	2665	2675	2670	2660	2640

Series IV, Experiment 7.

July 30, 1917

Age $2\frac{1}{2}$ months

Weights before experiment, in grams.

Animal Sex

1	female	2510	2550	2535	2565	2575	2555	2500
2	male	2775	2775	2785	2795	2780	2770	2760
3	female	2800	2850	2845	2840	2845	2830	2815
4	female	3100	3200	3210	3215	3200	3160	3245
5	female	3235	3265	3280	3280	3275	3255	3240

UNIVERSITY OF MISSOURI
COLUMBIA

DEPARTMENT OF PHYSIOLOGY

May 18, 1918

Chairman, Graduate School,
University of Missouri.

My dear Sir:-

The thesis of Mr. Egbert
Eugene Brown on, "Variation in the Carbo-
hydrate and Diastase Contents of the
Tissues after Feeding", presented in
relation to his candidacy for the Degree
of Master of Arts is approved.

Very truly yours,

Charles W. Greene

CWG

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