NON FERMENTABLE REDUCING SUBSTANCES IN BLOOD.

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PART 1

Introduction

Due to the large amount of experimental work that has been done in the last few years on the quanitative determination of blood sugar, that has been advanced a correspondingly long series of quantitative methods in this field.

Until recently investigators have considered that results of blood sugar determinations obtained by fermentation and those obtained by copper reduction have been in quantitative agreement, and both of these methods have been regarded as being reliable. However, Aldo C. Castellani (1926) indicated that there may be fallacies in the various fermentation methods. His results showed that ordinary baker's yeast would ferment galactose, maltose, saccharose, lactose and many other carbon compounds other than glucose. Also, he found that Monilia Balanica, a bacterium, is the only organism found which fermented glucose, and did not ferment any of the other carbohydrates. Since this organism is never found in pure culture in baker's yeast, the ordinary fermentation methods of blood sugar determination are not dependable.

In order to gather some information bearing on this subject, it seemed desirable to determine the amount of copper reducing substance left in blood filtrate after complete fermentation with ordinary baker's yeast. The purpose of this investigation is to determine whether there are copper reducing substances remaining in the blood in considerable quantity after ordinary

fermentation by baker's yeast. If so, this would constitute a definite source of error in the ordinary yeast fermentation quantitative tests for the determination of sugar in the blood.[1]

PART II

History

Blood is a liquid tissue composed of two elements; one formed, the other unformed. The formed element is made up of red corpuscles (erythrocytes), white corpuscles (leucocytes), and the blood platelets. The unformed element consists chiefly of a solution of the various organic and inorganic salts commonly found in the animal body.

The blood contains substances (the reducing substance of Pflüger) which greedily appropriate any free oxygen which may be brought to the blood plasma, and are even capable of abstracting oxygen which is combined with hemoglobin. So arterial blood rapidly becomes converted into venous blood, when it does not have access to fresh oxygen. It is not known upon what substances in the plasma these properties depend. But since this is probably a general function of the protoplasm of cells, in the case of blood it may be associated with the protoplasm of the white corpuscles or with the protoplasm of the non-cellular portion of the blood plasma. The presence of copper reducing substances in the blood was suspected before the time of Dobson (1775); but he was the first to report the

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1. I am indebted to Dr. Arthur G. Mulder for this suggestion.

presence of such substances.

That blood may contain a sugar-like substance was first recognized by Dobson (1775). However, it was not until seventy years later that its presence in normal blood was demonstrated by the noted physiologist Claude Bernard. By means of his sugar piqure, he first noticed the association between hyperglycemia and glycosuria (glycuresis). Lewis and Benedict[1] in 1913 introduced a coloremetric method for the quantitative determination of blood sugar that was sufficiently simple to be employed in clinical as well as for research purposes. Earlier in the same year (1913) Bang[2] had described a gravimetric-volumetric method requiring only two or three drops of blood. However, the complexity of the gravimetric-volumetric method precluded extensive clinical application of the method.

The publication of these two methods of blood sugar determination served as a stimulus for extensive work in this field in the succeeding five years. Obviously, reference can be made here to only the more important of these investigations. Among the publications mentioned, the following papers are to be considered as the more important: Hopkins[3], Geyelin[4], Hamman and Hirschman[5], Denis, Aub[6], and

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1. Lewis and Benedict. Journ.Biol.Chem. 1913.
2. Bang. Zeitschr.f. Physiol. Chem 1913.
3. Hopkins. Journ. Phyiol. XX 134 1896.
4. Geyelin. Arch. f.d.Ges.Physiol. S286, 1873.
5.
6. Denis and Aub. Memoire sur le Samge. Journ Physiol.III,181.

Minot[6], Mosenthal, Clauser, and Hiller[7], Janney and Isacson[8], Bailey[9], Williams and Humphreys[10], Allen, Stillman and Fitz.[11] The work done by these men contributed in a large measure toward the advancement of and the accuracy of quantitative blood sugar determinations. The above methods also served to standardize the results as milligrams of sugar per one hundred cubic centimeters of whole blood.

The carbohydrates of blood plasma are divided into the following three types according to Hoppe-Seyler[12]: (1) glycogen, (2) animal gum, (3) and dextrose or grape sugar. Only traces of glycogen are found free in the blood plasma of fresh blood. The presence of any appreciable amount of glycogen in blood-plasma is most likely due to the disintergration products of leucocytes. Leucocytes as well as all other tissue cells of the animal body are known to contain glycogen, but this glycogen within the leucocyte is not usually found in the free state. Freund obtained from fresh blood a substance very similiar to that isolated and described by Landwehr[13] as "animal gum". This substance has come to be considered as a portion of the carbohydrate content of blood since that time. Animal gum ($C_6H_{10}O_5$) may be readily converted into dextrose

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7. Janney and Isacson. Arch. Int. Med. XXXL 1917.
8. Mosenthal, Clausen, and Hiller. Arch. Med. XXI, 1918.
9. Bailey. Arch. Int. Med. XXXI 1919.
10. Williams and Humphrey. Arch, Int. Med. XXXI, 1919.
11. Fitz. Arch. Int. Med. XX 1919.
12. Compt. Rend Acad.d.Sc. Hoppe-Seyler. Tome CXX, 1895.
13. Landwehr. Zeitschr.f.Physiol.Chem. VIII, 503.

by boiling it with dilute mineral acid. This substance is known to be non-fermentable as well as inactive optically, but apparently has the property of reducing alkaline copper solutions. Four liters of blood yield 0.82 mgs. which is a percentage of about 0.02. Dextrose is a constant constituent of the blood plasma of animals regardless of the nature of the diet (since 58% of all proteins and 12% of all fats are converted into carbohydrate, and the carbohydrates are ultimately absorbed and used as dextrose.) It occurs in the plasma of man to about 0.12%. Dextrose is always increased (normally) after a hemorrhage, this is most likely due to either the accession of lymph or to some nervous disturbance in the glucose-glycogen equilibratory apparatus. If upon being increased artificially, the increase in the blood sugar level results in more than 0.25%, the excess sugar is excreted in the urine. The percentage of blood sugar will not rise above 1.20% even in diabetics and depancreatized animals.

Substances in blood other than sugar which have reducing properties are: urea, uric, acid, creatin, creatinin, hippuric acid, xanthin, and hypoxanthin. However, the concentration of these substances as normally found in blood does not give appreciable results by the ordinary copper reduction methods of blood sugar determination. The reagents are so prepared as to not detect these substances in low concentrations. But in higher concentrations, they

have copper reducing power equal to or greater than that of blood sugar. Baldi in 1887 published a report concerning a substance normally found in blood, which was non-fermentable but which had copper reducing properties. This substance he called "jecorin". Jecorin is a rather complex chemical compound containing considerable amounts of sulphur and phosphorous. Phosphorous containing compounds practically always have coppery reducing properties. Possibly, then the reducing properties of jecorin are due to it's phosphorous content. Jacobsen[1] (1894) has shown that jecorin occurs in higher concentration in venous than in arterial blood. No satisfactory hypothesis has as yet been offered for this phenomena.

The following carbohydrates are regarded as reducing agents: dextrose, levulose, lactose, pentose, and glucuronic acid. Of these, only dextrose and levulose are capable of being fermented by ordinary baker's yeast. Dextrose, fructose, mannose, and invert sugar are fermented by all ordinary yeasts; while cane sugar, maltose, lactose, melibiose, and raffinose are fermented only after inversion by dilute acids or by appropriate enzymes. Of these, the most important are dextrose and levulose, since only these two are ordinarily held in the blood and stored in the tissues of the animal body. Other sugars are absorbed without entering into the metabolic processes; being quickly excreted in an unchanged form.

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1. Jacobsen. Centrabl. f. Physiol. 1894.

The ferments of the blood with which we are most concerned are: (1) diastase which is capable of converting amyloid materials into sugar; and (2) a clycolytic ferment which causes the disappearance of blood sugar, or, rather which causes a lowering of the concentration of the blood sugar. The latter has been demonstrated by Rohman,[1] who shows that blood sugar upon standing decreases.

LYMPH

The amount of sugar contained in lymph is very nearly the same as that found in blood. If dextrose be injected into the blood vessels, it soon appears in the lymph but in lesser concentration than in the blood. Lymph contains a distinct amount of glycogen, but it is wholly contained within the corpuscles in the blood and practically none exists in the free state in the plasma.[2] Dextrose is the only sugar which is unmistakably demonstrable in the circulatory fluids and in the tissues of the animal body.[3]

The most dependable as well as the test most suitable for laboratory and routine clinical work are those by Lewis-Benedict and by Folin-Wu. Various technical methods have been suggested by the many investigators working in this field. However, the two mentioned above, are the two most satisfactory because of their brevity and their utility and simplicity.

The earlier methods gave results which were stated in terms

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1. Rohman. Arch. f. d. ges. Physiol. 1892.
2. Dastu. Compt. rend. acad. d. sc. 1895 tome CXX.
3. Shafer. Text bk. Physiol. Vol. I, pp. 917.

of grams of sugar per one hundred cubic centimeters of blood or percentage of glucose in whole blood. That is the reduction of the various reagents employed in the processes was presumed to be wholly due to glucose. This was generally accepted until the rechecking of the work of Folin-Wu, and that of Lewis-Benedict by themselves as well as by others. First Folin found that the results given by the Lewis-Benedict method were entirely too high for glucose. Discussions between Folin and Benedict have been responsible for the great improvements made in the development of the quantitative blood sugar determinations. The majority of the important work done in the past few years in this field has been done by these two men.

Recently there has arisen a question as to whether all of the reducing substance in blood is due to glucose, or whether there are other substances present which are partly responsible for this reduction. If so, then according to one hypothesis, the nitrogenous substances in the blood might be responsible for this action; while according to another the reducing properties are due to carbohydrate substances other than glucose.

Rockwood[1] (1926) says, "Obviously, there is some reducing substance in the blood which is not glucose, which gives a positive reduction under the conditions present in the Folin-Wu method but not with the Benedict method." He concludes that this reducing substance is not nitrogenous in nature. Support-

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1. Rockwood,Reed. A study of the New Benedict Method for the Determination of blood sugar. Journ. Biol. Chem. LXIX 1926.

ing this theory, he cites a number of cases of uremia in which the blood shows no appreciable increase in sugar by the Folin-Wu method of quantitative determination. He also concludes that this substance occurrs in higher concentration in the cell than in the plasma of blood, and that the reducing action is not due to the organic phosphorous compounds.

Lyttle and Hearn (1926) have shown that creatin and creatinin and other reducing substances not carbohydrate, in order to show reduction must be in higher concentration than normally found in blood. That is, when in the same concentration as found in the blood these substances do not show appreciable reduction by the Folin-Wu method of quantitative blood sugar determination.

Folin (1926) reported that the blood of nephritic patients gave abnormally high glucose values; and that reducing substances other than carbohydrates did not occurr in the spinal fluid in as high concentration nor so constantly as they do in the blood of the same subjects. Folin and Swedberg (1926) found that blood contained a non-fermentable reducing substance which maintained a remarkably constant level independent of the level of blood sugar.

PART III

Experimental Methods

Among the methods of quantitative blood sugar analyses published since 1923 are: Hagedorn and Jensen,[1] Haskin, Holbrooks,[2]

1. Hagedorn and Jensen. Biochem. Zeitschr.CXXXV 46, 1923.
2. Haskins and Holbrooks. Journ. Lab. and Clin. Med.VIII,1923.

Paton[1], Calvert[2], Milroy[3]. A modification of Bang's method has been proposed by Coppans[4], while Gilbert and Bode[5] have adapted micro methods to the Folin-Wu determinations. Lynch makes a comparison of methods including those of Bang, Folin-Wu, McKenzie, Wallace and Gallagher, Calvert, Benedict, Hagedorn, and Jensen, and concludes that the results given by the Folin-Wu and that given by Benedict's methods are the most accurate as in terms of blood sugar.

From a view of simplicity and ease of performance as well as a shorter period of time required in the proceedure, the technique of either the Folin or Benedict methods is suitable for this type of work. The new Folin Modification of the Folin-Wu methods was chosen for this experimental problem because of its simplicity and because of the fact that less work has been done in checking this than any of the other methods.

The method of fermentation chosen by us was the routine incubation of the specimen with ordinary Fleischman's yeast at thirty-seven and one-half degrees centigrade for not less than fifteen minutes. Ten minutes additional was allowed after the solution was placed in the incubator for an even distribution of heat throughout the blood filtrate solution.

Treatment of the blood filtrate after incubation with Lloyd's

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1. Paton. Biochem Journ. XVIII 965, 1924
2. Calvert. Ibid. XVIII 839, 1924.
3. Milroy. Journ. Physiol. LX 26, 1925.
4. Coppans. Ned. Tijd. Gebeesk. LXVIII, I. 153
5. Gilbert and Bode. Journ. Biochem. LXII 364, 1924.

reagent was selected because it appeared a most satisfactory method of completely removing nitrogenous substances from the solution. However this was done in an acid medium, and it is known that Lloyd's reagent in the presence of acids produces a calcium salt which reduces copper. These objectionable calcium salts were removed from the solution by treating it with permutit. Premutit combines with the calcium salts and being heavier than the solution proper, carries it down as a solid.

Depending on the method used the result of the determination of glucose for normal blood varies over a wide range of from twenty to one hundred milligrams per one hundred cubic centimeters of whole blood. But, with any particular method the normal variation covers about thirty milligrams. That is, for the new Folin-Wu method[1] ninety to one hundred and twenty milligrams may be regarded as normal limits; and for Folin's modification of this method, eighty to one hundred and ten; for Benedict's copper reduction method, seventy to one hundred and twenty milligrams. The lower values in each instance are thought to be more accurate determinations of sugar because the various methods have been revised to make the conditions more specific for the reduction of alkaline copper solution by glucose. In mild diabetes values from one hundred and forty to three hundred milligrams per one hundred

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1. Folin. Journ. Biol. Chem. LXVII 357, 1926.

cubic centimeters of blood have been obtained, and in severe diabetes values up to twelve hundred milligrams. Hyperglycemia is also observed in nephritis, hyperthyroidism, pancreatic disease, and in certain hepatic disorders. Hypo-endocrine disorders (excluding those of the pancreas) may result in lowered levels of blood sugar. Among these conditions are: Addison's disease, hypopituitarism, cretinism, myxedeman and muscular dystrophy.

Folin's modified method of the Folin-Wu quantitative blood sugar method of determination involves the use of the ordinary copper sulphate solution using sodium bicarbonate instead of some caustic alkaline substance. He also uses the phosphomolybdic solution to promote oxidization of the glucose. His method, as do all other methods, using copper sulphate solution depends upon the property of glucose to reduce cupric sulphate to cuprous sulphate.

PART IV

Experimental work

The experimental work described in this paper was conducted as a double series, using two samples of blood for each determination; the one being de-fibrinated, the other being oxalated. Ther was a delay of not less than thirty minutes between the time of actually obtaining the blood from the animal and the beginning of the precipitation proceedure. This delay was due to the distance between the packing house and the laboratory. Ox blood was used throughout the experiment.

PROCEEDURE

(1) The first step in the laboratory proceedure consisted of preparing a protein-free blood filtrate according to the Folin-Wu method, using fifty cubic centimeters of freshly drawn blood, three hundred and fifty cubic centimeters of distilled water (seven volumes), fifty cubic centimeters of ten per cent sodium tungstate (one volume), and fifty cubic centimeters (one volume) of two thirds normal sulphuric acid. The distilled water is added to lake the corpuscles, the sulphuricacid acts on the sodium tungstate liberating tungstic acid. The tungstic acid combines with the protein of blood in the ratio of one molecule of acid to two molecules of protein. This method, according to the leading investigators in this field, leaves less protein in the filtrate than any other published method. Particular care must be taken to accurately neutralize the sodium tungstate solution. The excess liberated tungstic acid, if there be any, is neutralized as near as possible without delay by adding the required amount of dilute sodium hydroxide at once to the solution. The precipitated samples of blood-filtrate are then double-filtered, that is, the filtration apparatus was set up in such a way that one funnel was directly above the other. The uppermost funnel draining into the expanded portion of the lower. See fig.1.
(2) The resulting filtrate is then examined as to neutrality, and after the proper adjustments are made, a small portion of the filtrate is used for a sugar determination by the Folin's modified Folin-Wu quantitative determination of sugar.

The hydrogen ion concentration of the remaining solution is adjusted to correspond to the optimum acidity for yeast fermentation, and one fresh cake of Fleischmann's yeast was dissolved in each the oxalated and the defibrinated samples of blood. The resulting solutions were then heated to thirty seven degrees centigrade over a water bath and placed in an incubator. The temperature of the incubator had been previously adjusted and checked at thirty seven and one half degrees cantigrade. The same thermometer was used in the two steps, that is, in both the water bath and the incubator. The solution was left undisturbed in the incubator for twenty-five minutes.

(3) After twenty five minutes fermentation the solutiom is removed from the incubator and again double-filtered. The filtering, this time is done through quantitative paper.

(4) The refiltered blood filtrate is then treated with Lloyd's reagent in the presence of one tenth normal and one tenth volume of oxalic acid. As previously explained, this step in the proceedure removes the nitrogenous constituents of the blood. The filtrate is then treated with permutit to remove the calcium salts formed from Lloyd's reagent and oxalic acid. The Loyd's reagent and treatment with permutit was carried on in accordance with plans reccomended by Folin-Swedberg.[1] After the treatment with permutit, the supernatent fluid is decanted off and not filtered.

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1. O.Folin and A. Swedberg. Journ. Biol. Chem. Lxx 420.

(5) The solution was then placed in vessel a of the apparatus as set up in figure two. The apparatus consisted of a low pressure and low remperature evaporation arrangement. Evaporation was continued until the solution was approaching dryness. Care was taken to not allow the solution go above thirty nine degrees centigrade during the process of evaporation.
(6) The resulting concentrate was taken up in double distilled water, and another sugar determination was made. These results were interpreted as blood glucose. Later, a control consisting of a solution of glucose of known concentration aws carried through the proceedure in exactly the same manner as the blod filtrate samples. Polariscope readings were madeon the concentrated blood filtrate solution.
(7) The portion of the blood filtrate examined in the polariscope was then tested by the ordinary qualitative proceedures for properties of carbohydrates, proteins, and fats. The results were negative for each of these, as well as for the phenylhydrazine test.

The above outlined proceedure was followed in series I, III, and V. In series II, IV, and VI the concentrated filtrate was treated with equal volumes of eighty five per cent ethyl alcohol, filtered and Folin's modification of the Folin-Wu quantitative sugar determination was made.

Later, a control consisting of a standard glucose solution was carried through the entire proceedure.

PART V

Results

The following tabulated results repersent milligrams of reducing substance per one hundred cubic centimeters of blood.

Oxalated

Series no.	Original blood filtrate	Filtrate after fermentation abd concentration	Coloremetric determinations	Blood Filtrate after fermentation and 85% ethyl alcohol
I(a)	96.70	7.774	14.31	--
(b)	96.72	7.770	14.33	--
(c)	96.74	7.740	14.36	--
II(a)	96.80	7.769	14.19	6.004
(b)	96.60	7.750	14.19	6.000
(c)	96.40	7.700	14.38	6.006
III(a)	92.41	7.630	13.90	--
(b)	92.46	7.400	13.83	--
(c)	92.48	7.400	13.88	--
IV(a)	92.40	7.628	13.92	5.950
(b)	92.60	7.700	13.87	5.950
(c)	92.75	7.710	13.78	5.950
V(a)	88.82	7.461	13.32	--
(b)	88.80	7.461	13.50	--
(c)	88.76	7.460	13.43	--
VI(a)	96.64	7.763	14.10	6.001
(b)	96.60	7.770.	14.10	6.004
(c)	96.73	7.764	14.30	6.003

Defibrinated

I(a)	90.00	2.421	1.120	--
(b)	90.24	2.440	1.132	--
(c)	90.06	2.453	1.126	--
II(a)	90.60	2.400	1.100	1.923
(b)	90.54	2.421	1.110	1.928
(c)	90.70	2.445	1.120	1.936

Series no.	Original blood filtrate	Filtrate after fermentation and concentration	Coloremetric determinations	Blood Filtrate after fermentation and 85% ethyl alcohol.
III(a)	88.24	2.386	1.100	--
(b)	88.24	2.386	1.100	--
(c)	88.30	2.388	1.103	--
IV(a)	88.20	2.377	1.165	1.930
(b)	88.27	2.386	1.159	1.947
(c)	88.22	2.358	1.156	1.909
V(a)	84.61	20.60	1.096	--
(b)	88.55	20.49	1.078	--
(c)	88.57	20.56	1.075	--
VI(a)	89.96	24.90	1.128	1.928
(b)	89.90	24.00	1.120	1.926
(c)	89.84	24.30	1.120	1.937.

Each series represents the blood from one ox, that is a sample of that blood. Three readings were made on each sample and three samples were analyzed from each series.

Comparison of the above results will show that approximately eight per cent expressed in milligrams of the total blood sugar represents some substances which are nonfermentable and which have copper reducing properties. Approximately seventy seven per cent of this non-fermentable substance is precipitable by eighty five per cent ethyl alcohol. These values are applicable only to the samples of oxalated blood filtrate. Results from the defibrinated blood filtrate are given merely to show that the non-fermentable substance(copper reducing) is more abundant in the corpuscles than in the plasma of blood. Seven and sevent-seven one hundreths milligrams per one hundred cubic centimeters of blood is in non-fermentable form

and has the property of reducing alkaline copper solution. Five and eighty-seven miligrams of this is precipitable by eighty-five per cent ethyl alcohol.

The alcohol precipitable and non-fermentable reducing sub-substance was tested and proven to be neither, carbohydrate, protein, nor fat. It was found to be water soluable and was slightly soluable in fat solvents. It has been planned to do more work on the determination of the physical and chemical properties of this substance soon, or at least in the near future. Lack of sufficient time prevents this work's being done at this time.

PART VI

Discussion

In normal ox blood eight per cent,(seven and seventy- seven one hundreth milligrams) of the total blood sugar as determined by Folin's modification of the Folin-Wu method of quantitative blood sugar determination, is non-fermentable by fresh Fleischmann's yeast. This substance gives negative results with the Biuret test, agreeing with the work of Folin-Swedberg (1926). It is soluable in water and slightly soluable in fat solvents, and when precipitated by eighty five per cent ethyl alcohol, appears as a finely divided amorphous precipitate. This precipitate gives a negative osazone crystal test. Folin-Swedberg concluded that the non-fermentable substance of blood is constant and does not vary with the level of the blood sugar. Our results support the hypothesis that

this substance is found in higher concentration in the corpuscles than in the plasma of blood.

The fermentable sugar usually expressed as glucose appears to be only ninety-two per cent of the results given by the Foline modification of the Folin-Wu quantitative blood sugar determination. The removal of the fermentable sugar from the blood filtrate is to us particularily interesting, in that the remaining copper reducing substance is apparently isolated. Yeast fermentation removes only glucose or glucose plus other reducing sugar with reducing properties simiiliar to those. Then the amount of fermentable blood sugar should be practically independent of the copper method by which the sugar is determined before fermentation. On the other hand, if yeast fermentation also takes other sugars with weaker reducing properties than glucose from the blood filtrate, then amount of fermentable blood sugar should depend in part, on the copper method used, and in this particular case, the fermentable blood sugar as determined by Folin-Wu methods should be greater than that found by the new method advanced by Folin. This is a new problem, of course, only in relation to the fact that various copper methods yield such different values for total blood sugar.

The fermentable sugar may be the same or even a little lower than the values obtained by direct reduction, where the blood sugar level is normal.[1]

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1. and 2. Folin and Swedberg. Journ. Biol. Chem. LXX 420 1926.

The fermentable sugar of blood is not maltose or any other di- or poly- saccharide. Presumably it is produced within the organism and represents some phase of intermediary metabolism of carbohydrates.[2]

Embden and his coworkers[3] concluded that hexose-phosphoric acid in the carbohydrate metabolism of muscle yielded fructose upon hydrolysis. It has been previously pointed out that fructose was readily fermented by ordinary bakers yeast. Tests for the presence of fructose in blood are not at all sufficiently positive to warrant the statement that it is one of the constituents of normel blood.

PART VII

Conclusions

(1) For comparison between glucose values for blood as given by fermentation and by copper reduction methods, Folin's modification of the Folin-Wu quantitative method for the determination is the most accurate of the various copper reduction methods.

(2) The Folin-Wu method of preparing a protein free blood filtrate more nearly removes all protein from the blood than any other methods tried in the preliminary investigations of this experiment.

(3) Treatment with Lloyd's reagent, oxalic acid, and permutit it is a very satisfactory method for removing the nitrogenous constituents from protein free blood filtrate.

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2. Folin and Swedberg. Journ. Biol. Chem. LXX 420, 1926.
3. Embden and his Associates. Zeitschr. f. Physiol. ChemLIX 1-2.

(4) Folin-Wu blood sugar expressed in milligrams represents only ninety two per cent-fermentable and eight per cent non-fermentable reducing substance.

(5) Seventy-seven per cent of the non-fermentable reducing substance found in blood is precipitated when treated with eighty-five per cent ethyl alcohol.

(6) The non-fermentable reducing substance is more abundant in the corpuscles than in the plasma of blood.

(7) The non-fermentable reducing substances of blood do not have the characteristics of carbohydrates, protein, or fats. They are soluable in water and slightly soluable in fat solvents.

PART VIII

Resume

Folin's modification of the Folin-Wu mwthod of quantitative blood sugar analysis yields results which show too high an amount as glucose. The error is approximately eight per cent of the total glucose content of whole blood as expressed in milligrams.

PART IX

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