Special Articles

The Journal DIABETES *is honored to present in this issue two articles by one of America's pioneer scientists, Dr. Franklin C. McLean of the University of Chicago. His eminence in the field of metabolic research stems from his early achievements in the development of the urea clearance test, his work with Hastings and Van Slyke, and for the past twenty years his important contributions to knowledge of bone and calcium metabolism.*

The first article is a reproduction of one of his earliest works, and of great interest to our readers in its treatment of the perennial subject—glucose regulation in the light of current knowledge. It was, we believe, the first documented report of clinical blood glucose determination in the United States. Students and technicians accustomed to microchemical methods will appreciate the difficulties of biochemical investigation fifty or more years ago when it is noted that the Bertrand method as employed by Dr. McLean required 10 to 15 cc. of blood, together with time-consuming steps, for each determination.

It is also a special reward for the Editor, on the grounds of personal friendship, to present as a companion piece the provocative application of cybernetics to the same problems of glucose homeostasis by the same investigator a half century later.

The Sugar-Content of the Blood and Its Clinical Significance

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The presence of a sugar-like substance in the blood was first shown by Dobson in 1775, in a case of diabetes. Seventy years later Claude Bernard[1] showed this substance to be a constituent of normal blood, and also demonstrated that the amount of this sugar was increased by his well-known "sugar-puncture" in the floor of the fourth ventricle. Since that time the occurrence of hyperglycemia, or an increase in the amount of the sugar found in the blood in diabetes and other conditions, has been recognized, but little attempt has been made to study it clinically until within the past three or four years. Recently newer methods for the determination of the sugar-content of the blood have been devised, putting these estimations within the reach of the clinician, and our knowledge on the subject is advancing rapidly. It is the purpose of this paper to attempt to demonstrate the value of such examinations particularly in cases of glycosuria, and to show what knowledge may be derived from this procedure that will help the clinician.

The blood of a normal adult, under average conditions of diet, exercise, etc., contains a fairly constant amount of sugar, this sugar being glucose, or the same sugar found in the urine in cases of diabetes mellitus. For the blood-plasma this amount averages between 0.08 and 0.11 per cent., the upper limit being about 0.12 per cent.[2] For the total blood the figures are slightly lower, and any content above 0.11 per cent. is to be regarded as a hyperglycemia. This upper limit is rarely exceeded in health except as a result of feeding a large amount of carbohydrates at any one time, when much higher figures may be obtained in perfectly normal individuals.[3] The sugar of the blood is derived mainly from the carbohydrates of the food, the liver storing up the sugar absorbed from the intestine and holding it in the form of glycogen as a reserve, to be delivered to the blood in the form of glucose as it is needed to supply

From the Laboratory of Pharmacology, Department of Medicine, University of Oregon. Read before the Portland City and County Medical Society, Jan. 21, 1914.

[1] Bernard, Claude: Referred to in MacLeod: Diabetes: Its Pathological Physiology, London, 1913, p. 23.

[2] Renal Diabetes, editorial. THE JOURNAL A.M.A., Nov. 1, 1913, p. 1632.

[3] Jacobsen: Biochem. Ztschr., 1913, lxvi, 471: referred to in The Variations in the Content of Sugar in the Blood, editorial, THE JOURNAL A.M.A., Jan. 10, 1913, p. 131.

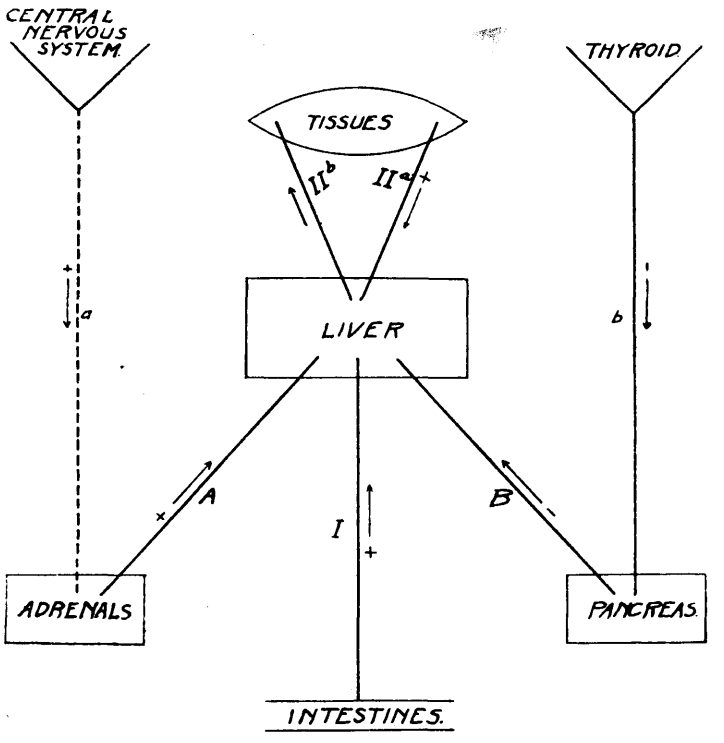

Fig. 1.—Schematic illustration (from von Noorden) of control of amount of sugar in blood; + = stimulation; — = depression; – – – – = nerve-paths; ———— = blood-paths; → = direction of action.

the tissues. It is then utilized to produce heat and energy, mainly by the muscles, which also contain a moderate reserve of glycogen. The sugar in the blood is mainly either free in solution, or in such loose combination that it has the chemical properties of free sugar, though some may exist in much firmer combination.[4] This *sucre virtuel,* however, may be disregarded for practical purposes.

In health the amount of sugar in the blood is to be regarded as a physiologic constant, just as the temperature, tension of carbon dioxid, etc., are physiologic constants.[5] The means by which the organism maintains this constant has been the subject of much study. Figure 1, taken from von Noorden,[6] illustrates schematically what we know of this regulation. The newer theory of diabetes[7] assumes that the amount of sugar in the blood depends on the rapidity of "mobilization" of glycogen in the liver, and that the hyperglycemia of diabetes depends on an abnormal increase in this mobilization, together with certain other factors. Normally, the glucose absorbed from the intestines is carried along Path I, and is built up to form glycogen. As the tissues call for sugar, sending the "call" back along Path IIa, the glycogen is mobilized, to form glucose, which reaches the tissues by way of Path IIb. This mechanism, however, is markedly influenced by other factors, and the rôle that these factors play is only imperfectly understood. The pancreas is to be regarded as having an inhibitory effect on the mobilization, through its internal secretion, so that an interference with this internal secretion, by removal of the pancreas or otherwise, results in the loss of this inhibition and an increased mobilization of the glycogen. On the other hand, the adrenals, through the liberation of epinephrin in the blood, are regarded as having a stimulating effect on this mobilization, an increase in the amount of epinephrin in the blood producing a hyperglycemia and glycosuria. It has been assumed, by von Noorden and others, that the hyperglycemia following removal of the pancreas is due to the fact that the epinephrin in the blood is allowed to act unopposed on the liver-cells, thus increasing the rate of mobilization of the glycogen. Both the pancreas and the adrenals are under the influence of still other factors. The activity of the pancreas is influenced by the activity of the thyroid, increased activity of the thyroid resulting in decreased activity of the pancreas and a corresponding increase in the rate of glycogen mobilization and a decreased tolerance to sugar. This explains the decreased tolerance to carbohydrates often observed in hyperthyroidism. The reverse occurs in hypothyroidism, these patients having a greater tolerance to sugar. The activity of the adrenals is influenced by the sympathetic nervous system, probably controlled by a center in the floor of the fourth ventricle. Irritation of this center by a "sugar-puncture," concussion, pressure from a tumor, etc., would cause an increase in the amount of epinephrin in the blood through the stimulation

[4] Lépine: Le diabète sucré. Paris, 1909, p. 59.

[5] MacLeod: Diabetes, p. 52.

[6] Von Noorden: Die Zuckerkrankheit. Ed. 6, Berlin, 1912, p. 171.

[7] The Changing Theories of Diabetes, editorial. THE JOURNAL A.M.A., Aug. 2, 1913, p. 348.

of this mechanism, and thereby result in a hyperglycemia and glycosuria. Other factors, such as the internal secretion of the posterior lobe of the hypophysis, the internal secretion of the parathyroids, etc., have also an influence on carbohydrate metabolism, but our knowledge of this influence is too imperfect to permit us to draw any conclusions. It may be seen from the diagram that the primary disturbance in diabetes may be in any one of numerous situations. According to von Noorden,[8] primary anomalies of the liver-cells are the least common, and disturbances of the function of the pancreas the most common, other cases coming possibly from interference with the nervous control of the adrenals or from abnormalities of the adrenals themselves. Disturbances of the thyroid probably do not cause true diabetes.

The nature of the stimulus to the mobilization of glycogen from the liver, sent back by the tissues in need of sugar, or rather the intermediary regulator of the whole mechanism, is not known. The possibility of "hormone" control has been suggested by numerous authors (MacLeod[9]), but the nature of the hormones has not been proved. It seems reasonable to suppose that this hormone might be a product of the metabolism of dextrose, just as the oxygen intake of the body is regulated by the carbon dioxid content of the blood. This product may be carbonic acid, lactic acid, or some other end-product of the utilization of sugar in the muscles, the use of an increased amount of sugar resulting in an increased amount of lactic acid, for example, and the increased lactic acid content of the blood either acting directly on the liver-cells or at some point on the regulatory mechanism to increase the mobilization of glycogen in the liver.

The amount of sugar present in the circulating blood at any time may be determined by one of a number of methods, all of which are a trifle complex for the average clinical laboratory, in that they require special apparatus, standardized solutions and some knowledge of volumetric analysis. The method used by us in all of our determinations is one of the older methods, but is accurate, not time-consuming, and gives constant results. The method of precipitating the proteins from the blood was described by Schenck.[10] The method of determining the amount of sugar in the clear filtrate was described by Bertrand,[11] and his method is the most accurate method for the determination of glucose in small amounts.

The combined method is as follows:

Five c.c. of a 1 per cent. potassium oxalate solution are accurately measured from a pipet into a dry 50-c.c. volumetric flask. The blood is withdrawn from the patient's arm vein by letting it flow through a needle directly into the oxalate solution, which prevents clotting. From 10 to 15 c.c. are collected in this manner, no attempt being made to measure the amount withdrawn directly. The flask is now filled to the mark with water from a buret. The amount of blood withdrawn is then determined by difference, subtracting the amount of water used from the buret from 45, the capacity of the flask after 5 c.c. of oxalate solution had been put into it. The amount is recorded and is later used to calculate the percentage of sugar. The mixture in the flask is then poured into an Erlenmeyer flask of about 250-c.c. capacity, the measuring flask then filled with 2 per cent. hydrochloric acid to the mark, the hydrochloric acid poured into the blood mixture, and the same process repeated with 5 per cent. mercuric chlorid. The amount of the mixture now totals 150 c.c. and an aliquot part of this amount is later used in determining the total amount of sugar in the mixture.

After being throughly mixed, and allowed to stand about five minutes, the mixture is filtered through a dry filter into a second dry flask. It should filter rapidly and be perfectly clear. As the excess of mercury interferes with the sugar determination it must be removed, which is done by passing hydrogen sulphid through the liquid until precipitation is complete, which requires about ten minutes. After the precipitate is filtered off the excess of hydrogen sulphid is removed from the filtrate by a current of air passed through it for from ten to fifteen minutes, and the clear solution is now ready for the sugar estimation by Bertrand's method. Fifty c.c. of the solution are carefully measured from a pipet into an Erlenmeyer flask of about 250-c.c. capacity, the acid is neutralized with a strong solution of potassium hydroxid, and 20 c.c. each of Bertrand's copper and alkali solutions are added. These solutions have the following formulas:

I

Crystalline copper sulphate, C. P.	40
Distilled water to	1,000

II

Potassium sodium tartrate, C. P.	200
Sodium hydroxid (sticks)	150
Distilled water to	1,000

The mixture is now brought to a boil in about two minutes, and is allowed to boil rather gently for exactly three minutes. It is allowed to cool and then filtered through a specially prepared asbestos filter which collects the cuprous oxid reduced by the sugar. The cuprous oxid in the filter and that remaining in the flask after being washed well are dissolved in 20 c.c. of Bertrand's iron solution, having the following formula:

III

Ferric sulphate (not ferrous)	50
Sulphuric acid (concentrated)	200
Distilled water to	1,000

The whole solution is washed through a filter into a clean filter flask below with from 50 to 100 c.c. of water, and the mixture saved for titration. The amount of copper present in the solution is determined by titration with a standard solution of potassium permanganate, 1 c.c. of which represents 2 mg. of cuprous oxid. This solution is made by dissolving 5 gm. of potassium permanganate in 1,000 c.c. of water, and adjusting the solution until 22.5 c.c. are just decolorized by 0.250 gm. of ammonium oxalate.

For the standardization, 0.25 gm. of ammonium oxalate is weighed out carefully and warmed with 100 c.c. of water and 2 c.c. concentrated sulphuric acid to from 60 to 80 C. This is titrated with the potassium permanganate solution until a rose color appears, and the strength of the solution adjusted, if necessary, until it is of the right concentration so that 22.5 c.c. will just cause a rose color in the oxalate solution. This

[8] Von Noorden: Die Zuckerkrankheit, p. 173.

[9] MacLeod: Diabetes, p. 83.

[10] Schenck: Arch. f. d. ges. Physiol. (Pflüger's), 1894, iv, 203.

[11] Bertrand: Bull. Soc. chim. de Paris, 1906, xxxv, 1285.

TABLE 1.—FOR SUGAR ESTIMATION[12]

Copper in mg. =	Sugar in mg.	Copper in mg. =	Sugar in mg.
1.1	0.5	11.5	5.5
2.2	1.0	12.5	6.0
3.3	1.5	13.5	6.5
4.4	2.0	14.5	7.0
5.5	2.5	15.5	7.5
6.5	3.0	16.5	8.0
7.5	3.5	17.5	8.5
8.5	4.0	18.5	9.0
9.5	4.5	19.5	9.5
10.5	5.0	20.5	10.0

solution keeps well if tightly stoppered and kept in a dark place. Just before titration of the copper it is diluted 1:5 volumetrically, and the diluted solution represents 2 mg. copper to each cubic centimeter. The permanganate is now run carefully into the iron-copper solution, until a permanent rose color just appears, and the amount of copper is then determined directly by multiplying the number of cubic centimeters of permanganate solution used by 2. The amount of glucose is then calculated from Table 1, and the total amount found is divided by the number of cubic centimeters of blood withdrawn to determine the amount in each cubic centimeter. One mg. per cubic centimeter of blood represents 0.1 per cent.

The results obtained by this method are said to be slightly lower than those obtained by other methods, but the methods said to be superior are much more difficult and time-consuming. Other methods, such as that of Kowarsky, are somewhat simpler and require less blood, but are correspondingly less accurate.

In pathologic conditions the amount of sugar found in the blood may vary within wide limits. As already mentioned, the content of normal blood rarely exceeds 0.11 per cent., and then only as the result of feeding a large amount of carbohydrates at one time. In diabetes, however, this amount may be increased by several hundred per cent. and results of 0.36 per cent. and higher are very common. Rolly and Oppermann[13] have found amounts exceeding 1 per cent. in diabetic coma. In our series results of practically 0.7 per cent. have been obtained in two cases. The amount in the blood in diabetics is very susceptible to changes in diet, because of the disturbance of the physiologic adjustment. Thus a greater increase in the blood-sugar content asserts itself in the abnormal than in normal persons after an identical intake of carbohydrate.[3]

The relation between the sugar in the blood and that in the urine, while not constant, shows certain tendencies. The appearance of sugar in the urine is to be regarded as an over-

[12] Moeckel and Frank: Ztschr. f. phys. Chem., 1910, lxv, 325.

[13] Rolly, F., and Oppermann, F.: Biochem. Ztschr., 1913, xlix, 278: referred to in The Blood-Sugar in Human Diabetes, Current Comment, THE JOURNAL A.M.A., Aug. 9, 1913, p. 418.

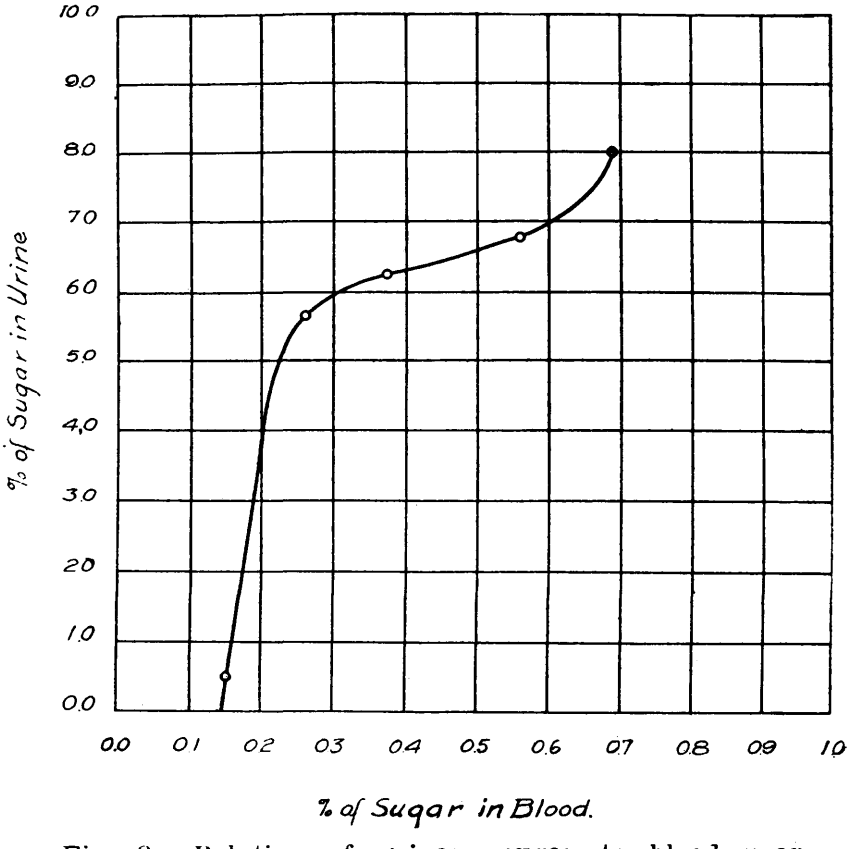

Fig. 2.—Relation of urinary sugar to blood-sugar.

TABLE 2.—RELATION OF URINARY SUGAR TO BLOOD-SUGAR

Case No.	Percentage of Sugar in Blood	Urine	Case No.	Percentage of Sugar in Blood	Urine
Normal	0.076	0	4	0.19	+
Normal	0.082	0	15	0.198	0
Normal	0.087	0	5	0.26	5.3
Normal	0.099	0	14	0.275	6.4
Normal	0.10	0	7	0.29	1.5*
10	0.111	0	12	0.36	6.4
9	0.127	0	11	0.38	4.8
13	0.133	0	1	0.39	6.8
3	0.14	0	8	0.40	6.3
1	0.15	0.8	6	0.56	6.85
11	0.158	0.1	16	0.63	8.0
9	0.182	0	14	0.69	8.0
2	0.19	0	5	0.72	8.0

*Coma.

flow from the blood when the concentration in the blood reaches a certain point. That is, the kidneys, while normally excreting practically no sugar, act as a safety-valve to prevent the accumulation of large amounts of it in the blood. Usually this overflow occurs when the sugar of the blood reaches a concentration of about 0.15 per cent., although this threshold may be altered in certain cases of diabetes, being either raised or lowered.[14] This is illustrated in three of our cases, in which values of 0.19, 0.198 and 0.182 per cent., respectively, were obtained in the blood when no sugar was found in the urine, though sugar had previously been found in all three cases. In these cases the results of treatment must be watched entirely by the blood-sugar, as the sugar appears in the urine only when a marked hyperglycemia is present, and does not serve as a guide to dietetic treatment. The threshold is also somewhat higher in normal persons, as Jacobsen[3] has shown that the content of sugar in normal persons may be raised by overfeeding with carbohydrate to 0.16 or 0.17 per cent. without inducing an elimination of glucose.

One is led to infer, therefore, that the elimination of glucose through the kidney-cells causes certain alterations to their susceptibility to the presence of increased amounts of sugar in the blood. After the presence of sugar in the urine has become established, the amount rises rapidly in proportion to the amount in the blood. This is shown in Figure 2, the curve being plotted from the comparative amounts of sugar in the blood and in the urine in our series. In preparing this curve atypical results were disregarded, the average typical results being used. MacLeod[15] has shown that the fall of the blood-sugar curve following stimulation of the splanchnics is much more rapid than the fall of the urinary-sugar curve. This is probably due to the fact that the kidneys become accustomed to excreting large amounts of sugar, and react slowly to the decrease in the amount in the blood. The same occurrence is noted in certain cases of diabetes. For example, in Cases 5 and 14 of our series, after marked reduction in the blood-sugar, the amount in the urine remained disproportionately high. Table 2, from which the curve was plotted, shows the relation of the urinary sugar to the blood-sugar in our cases. The first five were normal persons, the others all cases of glycosuria, though numerous examinations were made during aglycosuric periods. All estimations of urinary sugar were made simultaneously with the blood-sugar, the polarimeter being used in every case.

The detailed report of these cases will form the subject of a later communication, but Table 2 illustrates much of what is to be derived from the study. It may readily be seen that the sugar-content of the urine does not give a true index of the amount in the blood, and that a direct determination of the amount in the blood must be made if one desires to have knowledge of it. The retention of sugar in coma is illustrated in Case 7, in which, with a relatively high blood-sugar content, the urinary content is low. The daily excretion of sugar is not shown in this table, and except from the point of view of loss in caloric value by excretion of sugar, the daily excretion is unimportant if blood-sugar estimations are made.

Besides the interest attached to the foregoing observations from the experimental point of view, we find that the estimation of the sugar in the blood may be of very great value from the practical point of view. The procedure finds its greatest clinical value in the diagnosis, prognosis and control of treatment in cases of diabetes mellitus. The finding of hyperglycemia alone does not prove the existence of diabetes mellitus, as we have shown that a transient increase in the amount of sugar in the blood may occur as a result of overfeeding with carbohydrates, or as a result of sudden discharge of glycogen from the liver. If, however, a hyperglycemia remains persistent after the withdrawal of carbohydrates from the diet for twenty-four hours or more, the case should be regarded as diabetes, even if the glycosuria disappears under these conditions. For example, in Case 2 the patient had glycosuria only at times, the sugar entirely disappearing from the urine after twenty-four hours on a carbohydrate-free diet. The blood after such a period showed 0.19 per cent. of sugar, which demonstrates that the case was not merely a transitory alimentary glycosuria, so-called, but a true case of diabetes. In Case 10, however, in which the patient had a glycosuria due to a cerebral disturbance, the blood twenty-four hours after sugar had been present in the urine showed a content of 0.111 per cent., or practically a normal content, even on a mixed diet. This would of course rule out a true diabetes mellitus. In any case of glycosuria, when there is doubt as to the true nature of the glycosuria, an estimation of the sugar in the blood, under proper control of the diet, should be made to make or disprove the diagnosis of diabetes mellitus.

As a guide to treatment and to the results of dietetic measures the estimation of this sugar-content of the blood is also of great value. We have long been accustomed to follow the excretion of sugar in the urine as an index to the progress of the case, though, as we have shown, the sugar-content of the blood, which is the primary condition, is not accurately indicated by the excretion in the urine. In cases in which it is possible, and this has been adopted as a routine in many European clinics, the results of treatment should be followed by blood-sugar estimations. Especially is this true when the sugar has disappeared from the urine. The time-honored method in such cases has been to determine the tolerance to carbohydrate by determining the amount necessary to cause a reappearance of sugar in the urine, and to give all that can be tolerated without glycosuria. We find that in many cases this

[14] MacLeod: Diabetes. Diefmann and Stern: Biochem. Ztschr., 1906, i, 299.

[15] MacLeod: Diabetes, p. 47.

is unjustifiable, as a patient may have constantly a blood-sugar content of 0.18 per cent. or even higher without the appearance of sugar in the urine.

For example, in Case 9, in Table 2, at the first examination of the blood after the patient had been rendered aglycosuric by a carbohydrate-free diet, a sugar-content of 0.127 per cent. was found. Following this the tolerance was tested, and it was found that the patient could take about 200 gm. of carbohydrate daily without a glycosuria. After six weeks on such a diet a second examination of the blood was made, and a sugar-content of 0.182 per cent. was found. It would manifestly be wrong to allow such a patient to continue on such a liberal allowance of carbohydrate, for though the urine was sugar-free, the presence of 0.182 per cent. of sugar in the blood may lead to various complications, and is almost certain to lead to a diminished tolerance to sugar. The diet regulation in such cases should aim at keeping the concentration in the blood at approximately normal, and not merely at keeping the urine sugar-free. In other cases, too, of very mild diabetes, unnecessary starving of the patient may often be avoided. The effect of the dietetic measures of the blood-sugar also affords a valuable key to the prognosis. In cases in which a normal concentration is easily reached and maintained, the prognosis should be good, though the other factors of acidosis, age of patient, etc., must always be taken into account. The actual amount of sugar found in the blood, as also the amount found in the urine, gives no guide to the severity of the case unless these observations are made after careful regulation of the diet.

In other forms of glycosuria the chief value of the blood-sugar determination lies in the ability of the clinician to rule out diabetes mellitus, and thus to find a better prognosis and often to allay the fears of the patient, who has in many cases been told that he has an incurable and rapidly fatal disease. For example, it has recently been shown[16] that in most cases of glycosuria occurring in pregnancy the blood-sugar remains normal or even slightly diminished, showing that the glycosuria is entirely of renal origin, and that it is by no means to be regarded as diabetes mellitus. In these cases, of course, the prognosis is good, and it is unnecessary to burden the patient with the dietetic regimen of a diabetic. Cases of cerebral glycosuria, so-called, are usually transitory, and occur after injury, or from pressure of a tumor, etc. As shown above, they are due to a disturbance of the center in the floor of the fourth ventricle. In these cases, as in Case 10, the blood-sugar rapidly falls to normal following the excretion of the sugar set free in the circulation from the liver. In such cases it may be of value to determine whether the glycosuria is a result of a cerebral condition or whether the cerebral symptoms are a result of diabetes, and this may readily be done by blood-sugar estimations under proper control. In cases of alimentary glycosuria[17] the hyperglycemia due to overfeeding with carbohydrates rapidly diminishes after the ingestion of carbohydrates is stopped, and such a test should be made when a transitory glycosuria is accidentally discovered. This transitory hyperglycemia and glycosuria are not to be regarded as diabetic symptoms, and one may often reassure the patient after a careful study of the case. This feature may come to be of value in life-insurance examinations, for though some of these patients later develop true diabetes, it is quite probable that an injustice is done in some cases by refusing insurance to them.

In conclusion I wish to thank those physicians who have so kindly cooperated in furnishing assistance and material for this work.

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[16] Frank, Erich: Arch. f. exper. Path. u. Pharmakol., 1913. lxxii, 387, referred to in Renal Diabetes, editorial, THE JOURNAL A.M.A., Nov. 1, 1913, p. 1632.

[17] The Variations in the Content of Sugar in the Blood, editorial, THE JOURNAL A.M.A., Jan. 10, 1914, p. 131.

The Homeostasis of Blood Sugar—1914-1964

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The title of this paper is taken from a chapter heading in Walter B. Cannon's *The Wisdom of the Body.*[1] The term homeostasis had been introduced by Cannon in 1926, but the concept of self-regulation goes back to Claude Bernard, who wrote in 1878[2] that "all the vital mechanisms, however varied they may be, have only one object, that of preserving constant the conditions of life in the internal environment."

When the paper which this is written to accompany was published in 1914, it drew chiefly on the teachings of MacLeod and of von Noorden for the idea that the glucose content of the blood is a physiologic constant, and the scheme illustrating the regulatory mechanism, reproduced in that paper, was adapted from von Noorden. The role of the central nervous system, acting through the sympathetic system, and mediated by the adrenal medulla, to control the mobilization of glucose from the liver, while subject to modification in detail, is still described substantially as it was by Claude Bernard. The other half of the mechanism, however, concerned with control of the internal secretion of the islands of Langerhans of the pancreas, and with the mode of action of insulin, has had a long history of investigation and speculation, with the end not yet in sight. It is the purpose of this note to call attention to the evolution that has taken place during the past half

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