BLOOD SUGARS.*

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THIS paper attempts to collect the information available concerning a small but important factor in human metabolism—the blood sugars.

Except where otherwise stated, all the facts mentioned will refer to human blood, since experiments on other animals may give divergent results owing to differences in the animals' metabolism. Further, the term "blood" refers to the blood of the general circulation and not to that of the portal system.

The subject will be discussed in four sections :

1. The Quality of the Blood Sugars.
2. Their Sources.
3. Their Fate, and
4. The Quantitative Estimation of Blood Sugar.

(i) *The Quality of the Blood Sugars.*

The writer has been unable to obtain any original articles on the *quality* of the sugars found in the blood. The standard text-books on Physiology agree that glucose, fructose and galactose may all be found in the circulation, that mannose can be assimilated if administered, and that all these may be utilised in the body.

Starling¹ states that maltose will be assimilated if given slowly by hypodermic injections, while sucrose and lactose—two other common di-hexoses—are excreted unchanged if administered in the same way.

Any ordinary hexose then may be found in the blood, but it remains to be discovered what conditions govern the presence and the amount of each variety.

This is not surprising, since, for their discovery or estimation,

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large quantities of absolutely fresh blood are required, to which the phenyl hydrazine test may be applied.

The difficulty is aggravated by the fact that both glucose and fructose give the same osazone. Their determination in extracts of a fluid which already contains many optically active substances (amino-acids) present difficulties so great as to dismay the most ardent chemist.

From the observations of de Bruyn² on hexose mutation in weak alkali solutions—observations more recently confirmed by the work of Nef—it would appear probable that the three common mono-saccharides in blood are interchangeable in form. Heuberg and Meyer³ show that rabbits can partially transform mannose to glucose. The same transformation can be produced in fructose or galactose.

One is probably right in saying that hitherto no quantitative estimation has been made of blood sugars separately. The figures given in this paper are the amounts of the blood sugars estimated as glucose.

(ii) *The Sources of the Blood Sugar.*

The sources of blood sugar may be grouped under three headings :

1. The carbohydrates of the diet after their hydrolysis by the enzymes of the alimentary tract ;
2. The stored carbohydrates of the organism ; and
3. The carbohydrates of nucleo-protein metabolism.

The last-mentioned do not concern us here as they probably only account for a comparatively small proportion of the circulating sugar.

As regards the other sources, there exists normally a balance between the carbohydrate store and supply, excess sugar being removed either by polysaccharide formation or by renal excretion, and deficiency being remedied by fresh supplies from the alimentary tract or from the glycogen stores of the organism.

Of special significance in metabolism is the sugar assimilation limit. The arrival of excessive sugar from the alimentary tract causes this limit to be passed and an alimentary glycaemia results. The limit is usually 200-250 gms. of glucose for ordinary

people, though Taylor and Hulton⁵ describe five cases in which 500 gms. were administered and only in one case did glycuressis occur. No doubt the rate of sugar fixation of the organism is the important factor in the problem though the renal threshold level is also involved. In the case of higher saccharides, such as starch, there appears to be no assimilation limit for most people; the relatively slow hydrolysis giving the tissues sufficient time to fix the sugars.

Before passing from this section we may refer to the unsolved problem of sugar storage as polysaccharide. Four questions remain uncertain:

1. Whether the liver alone can form polysaccharide, or whether muscles have a similar power;
2. Assuming that polysaccharide formation only occurs in the liver, how does it reach the other tissues?
3. Whether polysaccharide is hydrolysed to sugar in the liver, in the circulating blood, in the muscles, or in more than one of these tissues; and
4. What factors govern the interaction between polysaccharide formation and breakdown.

(iii) *The Fate of Blood Sugar.*

If we assume that the muscles have power to form polysaccharide then part of the blood sugar is removed in that way, and the blood sugar disappears in three ways:

1. By storage as polysaccharide;
2. By direct use as a source of energy, either (a) in the muscles; (b) in the formation of proteins; or (c) in the oxidation of fats; and
3. By excretion through the kidneys.

As a rule the kidney excretion is very slight; but, as is well known, the kidney threshold may be so lowered that, in the absence of hyperglycæmia, glucose appears in the urine.

Normally sugar begins to appear in the urine when its concentration in the blood reaches 0.15 to 0.16%. A patient under the writer's observation was excreting several ounces of sugar daily, yet her blood sugar estimated by MacLean's method was only 0.16%.

(iv) *The Quantitative Estimation of Blood Sugar.*

The following figures are typical of the amounts given :

Osler and McCrea : <i>Principles and Practice of</i> <i>Medicine</i>	0.06—0.11%
Starling : <i>Principles of Human Physiology</i>	0.1 —0.15%
Hawk : <i>Practical Physiological Chemistry</i> —	
Normal Blood	0.08—0.12%
Uræmia	0.10—0.2%
Early Diabetes	0.14—0.3%
Late Diabetes	0.3 —1.2%

MacLean ⁵ gives results varying from 0.096 to 0.110% in venous blood, and 0.112 to 0.127% in capillary blood from the finger whose glucose content might be expected to be higher. The present writer found 0.89—1.15% in normal blood.

The concentration of sugar in the corpuscles is usually a little lower than that in the plasma according to Wishart ⁶, and a little higher according to De Wesselow ⁷, using MacLean's method of estimation. The difference however is slight.

In normal individuals the blood sugar value rises to about 0.15% one hour after a meal and returns to normal after two hours. Pathological metabolic conditions greatly alter this curve. In hyperthyroidism, pancreatic diabetes and nephritis the return to normal may be greatly delayed. Such conditions will tend to be accompanied by glycosuria in proportion to their severity and to a low renal threshold for sugar.

The small concentrations of sugar in blood reflect the difficulty of estimating accurately its amount. Any methods must satisfy the following requirements :

1. Sufficient quantities of blood must be obtainable without injury to the patient. This necessitates the careful use of a delicate micro-method.
2. The result must not be vitiated by interfering substances. Thus polarisation methods cannot be applied, acetone will interfere with reduction tests ⁸ and protein emulsoids, unless removed, both increase the amount of sugar found by reduction

tests and render opaque the liquid which is to be analysed. The thorough removal of protein is necessary.

3. Glycolysis which occurs rapidly during the first three hours after the blood is drawn must be prevented. In one sample of diabetic blood which the writer divided into three parts the amount of sugar found was as follows : 0.26% and 0.27% where glycolysis was prevented, but 0.197% where glycolysis proceeded for about two hours.
4. Finally, re-oxidation must be prevented in reduction methods.

Among the methods for estimating blood sugar Benedict's method ⁹, or its modification by Epstein ¹⁰, are those most generally used. In the former, 2 c.c.'s of blood are laked. The proteins are precipitated with picric acid and removed by filtration. The filtrate is treated with standard sodium carbonate solution. After incubation the colour produced is compared with a standard. In Epstein's modification, which has much to recommend it, only 0.2 c.c.'s of blood are used. Unfortunately no results are given in the article quoted to show the consistency of the method. After seeing the effects produced by altering the time of heating in reduction experiments, the present writer is disinclined to adopt for research a method in which a few cubic centimetres of fluid are "concentrated to a small volume equal to about two or three drops."

Criticisms levelled at Benedict's method affect the Epstein modification. We have referred to the effect of acetone in the blood. More important is the fact that, as shown by De Wesselow ⁷, interfering substances which are concentrated in the blood corpuscles rendered results on five samples of blood from 28% to 41% higher when estimated by Benedict's method than when estimated by that of MacLean. De Wesselow discusses the results and shows conclusively that the results obtained by Benedict's method are too high.

MacLean's Method.

Under these circumstances, and because the original descrip-

tion of the method ⁵ is now out of print, MacLean's method ought perhaps to be described. The steps are as follows :

1. 0.2 c.c.'s of blood are measured by a special pipette applied to a prick in the cleansed finger-tip.
2. The blood is deproteinised by heating in an acidified 15% sodium sulphate solution, after which dialysed iron is added to complete the precipitation. The heating also prevents subsequent glycolysis.
3. After cooling, the mixture is filtered and a perfectly clear protein-free filtrate is obtained.
4. An aliquot part of the filtrate is boiled with an alkaline copper solution containing potassium iodide and iodate. The boiling is carried out for six minutes over a standard flame in a flask which has a capillary tube passing through its cork.
5. After boiling, the solution (which contains suspended cuprous oxide in proportion to the sugar present) is rapidly cooled and is treated with a slight excess of hydrochloric acid.

The acid interacts with the iodate and iodide liberating iodine to the known amount of the iodate in the solution. At the same time any cuprous oxide is dissolved and cuprous chloride is formed. A solution of cuprous chloride is very instable, but oxidation is prevented by the free iodine present with which the chloride immediately reacts, thus reducing the amount of free iodine in proportion to the amount of copper which the sugar reduced.

Since the cuprous oxide present at an earlier stage is in suspension, no re-oxidation occurs during the cooling before the hydrochloric acid is added ; while still earlier during the boiling there is no air in the flask, and at the beginning of heating air is driven off before the reduced copper can be oxidised to any extent.

6. The solution to which the acid has been added is a perfectly clear one and the free iodine in it is titrated against N/400 thiosulphate, using two drops of a 1% starch solution as indicator. The end point can be read without difficulty to within 0.04 c.c.'s of thiosulphate.

7. A blank thiosulphate value having been obtained for the copper solution, the difference of thiosulphate used is noted and the results are read off from the tables, the difference being due to the amount of iodine absorbed by the cuprous chloride formed from the cuprous oxide.

MacLean's method undoubtedly requires the use of fresh thiosulphate solution—which should be made up once or twice a week from stock N/10 solution carefully prepared. In working with the method one finds that MacLean's system of obtaining the thiosulphate value of the copper solution without heating it does not give quite the same result as if it is boiled for the six minutes. The writer, too, prefers a reflux

condensor on the boiling flask to the capillary tube. But these are minor points, and the method successfully overcomes the difficulties of working with a small quantity of blood, of de-proteinising, of preventing both glycolysis and re-oxidation and of obtaining a very delicate end-point. No interfering substances have so far been discovered. The method gives very consistent results. MacLean quotes the following figures for three samples of blood :

	1st sample.	2nd sample.	3rd sample.
1st Estimation ...	0·112%	0·127%	0·114%
2nd „ ...	0·116%	0·126%	0·119%

In working with the method the writer has obtained the following results :

1. Estimation of a glucose solution prepared from Kahlbaum's specially prepared pure glucose, dried and weighed after being powdered :

1st estimation ...	...	...	...	0·107%
2nd „ ...	...	...	...	0·101%
Calculated strength ...	...	...	...	0·100%
2. Blood sugar in healthy male adult :

12.0 noon ...	...	...	...	0·110%
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 After a light mixed lunch at 1 p.m. :

2.30 p.m. ...	...	...	...	0·115%
4.0 p.m. ...	...	...	...	0·104%

 showing a rapid disappearance of sugar excess from the blood.
3. Case of glycuresis mentioned ... 0·160%
4. Case of true diabetes, 1st estimation ... 0·26%

2nd „ ...	...	...	0·27%
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5. At the suggestion of Dr. Fearon that saliva might be a channel for the excretion of sugar in diabetes, samples of saliva were examined :

Saliva, healthy male ...	...	...	0·036%
Saliva, alleged diabetic without hyperglycæmia ...	...	...	0·040%
Saliva, true diabetic ...	...	...	0·043%

In conclusion it may be said that, from the point of view of further research, MacLean's method for blood sugar estimation,

by its superior accuracy combined with its comparative simplicity, promises to be a valuable aid in solving the problems of abnormal carbohydrate metabolism.

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References.

1. Starling : *Principles of Human Physiology*, 3rd edition.
 2. De Bruyn : *Rec. Trav Chim* 14, 156, 1895 ; Ditto, with van Eddenstein, 204.
 3. Heuberg and Meyer : *Zeitschrift fur Physiol. Chemie*, 37.
 4. Taylor and Hulton. *J. Biol. Chem.*, 22, 63, 1915.
 5. MacLean : *Biochem. Jour.*, xiii., Oct., 1919.
 6. Wishart : *Jour. Biolcg. Chem* 44, 563, 1920.
 7. De Wesselow : *Biochem. Jour.*, xiii., Oct., 1919.
 8. Guy : *Biochem. Jour.*, xv., 575, 1921.
 9. Benedict : *Jour. Biolog. Chem* 34, 203, 1918.
 10. Epstein : *Jour. Amer. Med. Assn.*, 63, 19, 1167-1914.
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