

TABLE (continued).

Composition of Elements.	Difference of Potential in terms of	
	Standard Daniell.	Volt.
Copper in saturated solution of copper sulphate and copper in solution of potassium cyanide (1 part by weight of potassium cyanide and 5 parts by weight of distilled water)	1·102	1·189
Smee :— Platinized silver and amalgamated zinc in dilute sulphuric acid (1 volume of commercial sulphuric acid to 8 volumes of distilled water) ..	1·193	1·288
Leclanche :— Solution of ammonium chloride in distilled water	1·268	1·369
Grove :— Platinum in commercial nitric acid (sp. gr. 1·36) and amalgamated zinc in dilute sulphuric acid (1 volume of commercial sulphuric acid to 12 volumes of distilled water)	1·504	1·622
Platinum in acid solution of potassium bichromate (4 volumes of saturated aqueous solution of potassium bichromate to 1 volume of commercial sulphuric acid) and Amalgamated zinc in dilute sulphuric acid (1 volume of commercial sulphuric acid to 8 volumes of distilled water)	1·678	1·811
Carbon and zinc in acid solution of potassium bichromate (4 volumes of saturated aqueous solution of potassium bichromate to 1 volume of commercial sulphuric acid)	1·701	1·835

V. "The Physiology of Sugar in relation to the Blood."

By F. W. PAVY, M.D., F.R.S. Received June 12, 1877.

In a communication published in the 'Transactions of the Royal Society' (1860, p. 579) I gave the results of analyses showing that what had previously been looked upon, under Bernard's glycogenic theory, as the natural condition of the blood in relation to sugar was a fallacious representation due to a *post mortem* change being allowed to exert its in-

fluence. It had hitherto been asserted that the blood of the right side of the heart was in a notably different condition as regards the amount of sugar it contained from that of the arterial system, an error which I discovered arose from the non-observance of certain precautions in the mode of obtaining the blood for examination from the respective parts of the vascular system. Whilst the arterial blood had been collected during life, it was customary to collect that from the right side of the heart, without any special haste, after the destruction of the life of the animal. During the period thus allowed to elapse between the moment of death and the collection of the blood, an alteration occurs from the *post mortem* production of sugar in the liver, which causes the blood to assume an extent of saccharine impregnation which does not naturally belong to it during life, and which had failed to be recognized in its true light. I gave analyses which show that what was formerly taken as representing the natural condition of the blood of the right side of the heart furnished from .50 to .94 per cent., or, as it is more convenient to state it, 5.0 to 9.4 per 1000 of sugar, the blood from the carotid artery of the same animals, collected during life, having contained what I described as a trace of sugar. Other analyses, three in number, were given, representing the true condition of the blood belonging to the right side of the heart during life, and the results indicated from .47 to .73 per 1000 as the amount of sugar.

Bernard has recently published some communications entitled "*Critiques expérimentales sur la glycémie*," in the *Comptes Rendus de l'Académie des Sciences de Paris*. His statements are founded upon a method of analysis which is not only strikingly devoid of precision as a quantitative analytical process, but in itself of a nature calculated to give rise to a fallacious result.

The process adopted by Bernard is as follows:—He makes use of a Fehling's solution, titrated to render 1 c. c. equivalent to 5 milligrammes of sugar. He withdraws with a syringe, or collects as it flows from the vessel in a weighed porcelain capsule, a determined quantity—10, 15, or 25 grammes of blood. To this he adds an equal weight of sulphate of soda in small crystals, with a few drops of acetic acid, and heats immediately over the flame of a gas-burner or spirit-lamp, to coagulate the albuminous and colouring matters. On account of the relatively small quantity of sugar to be dealt with, he only uses for the analysis 1 c. c. of the standard copper solution. This he heats in a small glass flask, after having added 20 to 25 c. c. of a fresh concentrated solution of potash, and drops into it the liquid to be tested till decolorization is effected. From the suboxide remaining dissolved, and the liquid being thus free from precipitate, the attainment of the point of decolorization is easy to be perceived. Observation, he says, has shown that the relation of volume of liquid yielded to weight of a mixture of equal parts of blood and sulphate of soda is $\frac{4}{5}$; in other words, that 50 grms. of sulphate of soda and of blood give 80 c. c. of trial liquid. The estimation having

indicated how much sugar each c. c. of this liquid contains, the data are afforded for ascertaining the quantity of sugar in the volume corresponding with the weight of blood analyzed, and thence the ratio per 1000.

There are two seriously faulty points about the method as a quantitative process of analysis.

The first is the assumption that the volume of the trial liquid corresponds in c. c. with $\frac{4}{5}$ of the weight in grms. of the mixture of sulphate of soda and blood. In reality, the actual relation between the volume of liquid obtained and the weight of the mixture employed must vary in each individual instance with the proportion of solid matter existing in the particular specimen of blood, and the loss of liquid by evaporation from the capsule during the process of coagulation by heat. It may be regarded as totally impossible to secure that the coagulation of the albuminous and colouring matters can be effected, in an open capsule at the high temperature necessary, with identically the same loss by evaporation in each individual instance; and as the amount of liquid derivable from the mixture is not large, a slight variation must alter to a decided extent the result, especially when it is worked out into the proportion in 1000 parts. With 20 grms. of blood as the quantity submitted to analysis, any error existing in the result yielded becomes multiplied 50 times when the representation of ratio per 1000 is made.

The other point is, that the process involves the influence of organic matter in preventing the deposition of the suboxide of copper. It is not the usual principle of applying the test that is appealed to. In the ordinary volumetric process of analysis with the copper solution, attention is given to the separation of suboxide till the whole of the copper has been removed from the liquid. In Bernard's process a large addition of potash is employed (20 to 25 c. c. of a concentrated solution to 1 c. c. of the copper test), the effect of which is to act upon some one or other organic principle left in the liquid obtained from the blood, and prevent a deposit of suboxide occurring. The presence of the potash does not by its own action interfere with the fall of suboxide; for when the process is carried out upon a simple solution of sugar, the suboxide falls in the usual way, whilst in the case of the liquid obtained from the blood, the removal of blue colour takes place without any signs of appearance of precipitate. Without the addition of the potash the usual behaviour occurs.

Actual observation shows that the results yielded by Bernard's process are very wide of those given by a process to be presently described, which is founded upon the precipitation of the suboxide and the subsequent collection of the copper by means of galvanic action upon a cylinder of platinum foil, as is now extensively done in the assaying of copper ores. This application of the copper solution yields a gravimetric instead of a volumetric process of analysis. There is nothing in it of a

doubtful nature. The result being given by the balance, there is no uncertainty belonging to it, as may be the case to a slight extent where the gradual fading of colour has to be watched and a decision formed with regard to the attainment of the precise point. If the reduction of the oxide of copper can be safely turned to account for effecting the quantitative determination of sugar (and analysts are agreed that it can), it is by such process that the most trustworthy information is supplied. The closeness attainable in the results of counterpart analyses shows that it there is not only susceptible of being carried out with great precision, but affords strong evidence of its reliability. Compared with this process, the results yielded by that of Bernard present the greatest discordancy. The figures given by Bernard's method are almost invariably too high, but there is no uniformity in the difference presented. The variance, indeed, shows no intelligible relation, and suggests that there is something fundamentally wrong in taking decolorization, without precipitation of the suboxide, as a means of estimating the amount of sugar. Subjoined are the results of the analyses of different specimens of blood in which Bernard's process has been compared with my own. In every case where my own process of analysis is put into practice I submit two samples of the blood to examination, in order that the results may be checked, and the same plan was adopted in some of the trials of Bernard's method. In these instances, therefore, we have the results derived from the analysis of four separate samples of the same blood, taken for examination at the same time:—

Source of Blood.	Sugar per 1000 parts.	
	Gravimetric process.	Bernard's volumetric process.
I. From bullock killed by Jewish method	{ a. .589 } .588 { b. .588 } (mean).	.975
II. From bullock killed by Jewish method	{ a. .510 } .499 { b. .489 } (mean).	{ a. .609 } .624 { b. .640 } (mean).
III. From bullock killed by Jewish method	{ a. .515 } .525 { b. .535 } (mean).	1.025
IV. From bullock killed by Jewish method	{ a. .698 } .703 { b. .709 } (mean).	.869
V. From bullock killed by pole-axe	{ a. 1.091 } 1.094 { b. 1.097 } (mean).	{ a. 1.311 } 1.357 { b. 1.403 } (mean).
VI. From jugular vein of dog, instantly after death.....	{ a. .795 } .803 { b. .811 } (mean).	.800
VII. From sheep	{ a. .509 } .517 { b. .526 } (mean).	.761
VIII. From case of severe diabetes, obtained by cupping.....	{ a. 4.990 } 4.970 { b. 4.951 } (mean).	{ a. 5.000 } 4.852 { b. 4.705 } (mean).

The gravimetric process of analysis to which I have referred consists

of three stages. The blood is first mixed with the sulphate of soda and heated to separate the albuminous and colouring matters; the liquid is then separated and the coagulum well washed to remove all the sugar. Boiling with an excess of the copper solution is next performed, and the reduced oxide afterwards collected and dissolved by the agency of an acid. In this solution a cylinder of platinum foil is immersed, for the purpose of receiving the copper removed by means of galvanic action. Weighing the platinum foil before and after the operation gives the weight of the deposited copper; and from this may be calculated the amount of sugar which has effected the reduction of the cupric oxide. Such is an outline of the process; but a special description of the manipulation is required, as success is dependent upon certain details being closely followed. In the application of the process many difficulties at different stages presented themselves; but it is satisfactory to be able to state that they have all been overcome, and there is reason to believe that no source of fallacy now exists in any part of the operation.

Forty grms. of sulphate of soda in small crystals are weighed out in a beaker of about 200 c. c. capacity. About 20 c. c. of the blood intended for analysis are then poured upon the crystals, and the beaker and its contents again carefully weighed. In this way the precise weight of the blood taken is ascertained. The blood and crystals are well stirred together with a glass rod, and about 30 c. c. of a hot concentrated solution of sulphate of soda added. The beaker is placed over a flame guarded by wire gauze, and the contents heated till a thoroughly formed coagulum is seen to be suspended in a clear colourless liquid, to attain which actual boiling for a short time is required. The liquid has now to be separated from the coagulum, and the latter washed, to remove all the sugar. This is done by first pouring off the liquid through a piece of muslin resting in a funnel into another beaker of rather larger capacity. Some of the hot concentrated solution of sulphate of soda is then poured on the coagulum, well stirred up with it, and the whole thrown on the piece of muslin. By squeezing the liquid is expressed; and to secure that no sugar is left behind the coagulum is returned to the beaker, and the process of washing and squeezing repeated.

The liquid thus obtained may be fairly regarded as containing all the sugar that existed in the blood. From the coarse kind of filtration and the squeezing employed, it is slightly turbid, and requires to be thoroughly boiled to prepare it for filtration through ordinary filter-paper. A perfectly clear liquid freely runs through; and to complete this part of the operation, the beaker in use and the filter-paper are washed with the concentrated solution of sulphate of soda before referred to.

The next step is boiling with the potassio-tartrate of copper test solution. The liquid is again placed over a flame, and brought to a state of ebullition. A sufficient quantity of the copper solution to leave some in excess is now poured in, and, from the commencement of boiling again, brisk ebullition is allowed to continue for the full space of one minute.

This suffices for all the sugar to be oxidized, and accordingly for all the required action upon the copper solution to occur. There is no risk during this time of spontaneous change occurring in the copper solution; but observation has shown that should boiling be continued for a lengthened period (by which I mean ten minutes or a quarter of an hour) the copper solution undergoes alteration, and no longer possesses the power of resisting spontaneous reduction. As regards the amount of copper solution, 10 c. c. of the test of ordinary strength are found to be more than sufficient for 20 c. c. of the blood of animals in a natural state. Where from any cause an extra quantity of sugar exists, more in proportion of the test-solution is of course required.

The precipitated suboxide of copper has now to be separated from the excess of copper solution. Experience shows that filtration through filter-paper cannot be resorted to for the purpose. In the first place, the pores of the paper tend to become blocked up and filtration to be stopped; and in the next (and this is a fatal objection) the paper absorbs and so tenaciously holds some of the copper solution that it cannot be effectually washed out. A plug of asbestos, in a filter-funnel, may be used instead; but it is not always easy to procure the asbestos with fibres of the medium state of fineness to answer well for speedy and, at the same time, delicate filtration. A material, however, which has recently been introduced, viz. glass-wool, exactly furnishes what is wanted. Properly packed in the neck of a funnel, it permits filtration to be effectively and speedily performed; the state of the filtrate readily shows if the plug has not been sufficiently closely packed to keep the whole of the precipitate back.

Should the crystallization of the sulphate of soda in this or the preceding filtration interfere with the process of filtration, the funnel may be lodged upon a beaker containing fluid kept in a state of ebullition. Through the heat thus applied, the liquid is prevented from assuming a crystalline form.

The suboxide having been collected and washing with distilled water performed, it is returned to the beaker in which the reduction was effected, to secure that whatever precipitate may have been adhering to the sides of the vessel is retained. The plug is simply pushed with a glass rod from the funnel inverted over the beaker, and the funnel washed and its surface cleaned from all adhering precipitate. We have now the suboxide in a fit state to dissolve; and until I resorted to the use of peroxide of hydrogen to effect its oxidation a difficulty presented itself in this part of the operation, the precipitate requiring an amount of acid to dissolve it which interfered with the subsequent deposition of copper by galvanic action. After the addition of a few drops of peroxide of hydrogen, a very small quantity of nitric acid (a few drops only) is sufficient to lead to instantaneous solution; and after boiling to decompose the excess of peroxide of hydrogen, the contents of the beaker, consisting of

the filter-plug and dissolved precipitate, are poured into a funnel containing a loose plug, to obtain the liquid in a separate form. The requisite washing with distilled water having been performed, there only remains the final stage of the process to be conducted.

The liquid to be now dealt with contains the copper in the form of nitrate, which experiment has shown to be the most suitable for yielding a pure metallic deposit by galvanic action. For the purpose of collecting the deposit, a cylinder of platinum foil soldered to a platinum wire, for hooking on to the negative pole of the battery, is employed. This is immersed in the liquid so as nearly to touch the bottom of the vessel, and inserted within it is a spiral coil of platinum wire, made to form the positive pole of the battery. In order to secure a good continuous connexion, the platinum spiral is closely bound to the copper conducting-wire of the battery, and the other pole is provided with a platinum hook, for the suspension of the cylinder. This precaution has been found necessary from the ready manner in which copper exposed ends become oxidized and rendered imperfect conductors by the oxygen escaping from the liquid underneath. It may be also mentioned that the platinum spiral, after several days' use, presents a brown surface, and requires to be occasionally cleaned by immersion in hydrochloric acid. In the case of an analysis of blood containing an ordinary amount of sugar, and therefore yielding a limited amount of copper to be deposited, twenty-four hours have been usually found to thoroughly suffice for complete removal to occur; but it is necessary that there should be no uncertainty upon this point; and to provide against this the liquid must be tested before the operation is regarded as finished. A small quantity is taken from the *bottom* of the vessel by means of a pipette, and a little ammonia added to it in a test-tube. Should a decided blue colour be produced it should be returned to the vessel; should no blue colour be perceptible the testing must be carried further. Acetic acid is added in excess to the contents of the test-tube to supersaturate the ammonia, and then a small quantity of a solution of yellow prussiate of potash dropped in. In the absence of copper, nothing but the faint yellow colour of the test is perceptible; but with the presence of copper a brownish hue is produced, and the galvanic action must be carried on till, on testing, this is no longer brought out. When it is thus found that the whole of the copper has been thrown down, the cylinder is lifted quickly out of the liquid and instantly plunged, first into distilled water and then into spirit. After drying in a water-oven, it is ready for weighing, and it need hardly be said that a delicate balance is required for the purpose. The weight of the cylinder before and after the operation indicates the amount of copper deposited.

The galvanic action requires to be steadily and continuously maintained, and a modification of Fuller's mercury-bichromate battery has been found to be highly suitable for use. The arrangement that has been

employed in my experiments consists of an outer cell charged with bichromate of potash dissolved to saturation in dilute sulphuric acid. In this two carbon plates are immersed. The inner porous cell contains a little mercury at the bottom, but is otherwise filled up with water. An amalgamated zinc rod is inserted, and dips down into the layer of mercury. This battery, it is found, gives a steady current, and, used every day, will remain in good working order for at least a fortnight, all that is necessary being to pour out the liquid in the porous cell when it has become green from reduction of the diffused bichromate solution and replace it with water. Attention is of course necessary to secure that the proper battery-power exists to effect the deposition of the copper; and when the current becomes weak the zinc rod must be cleaned and the bichromate of potash solution replenished.

The relation existing between sugar oxidized and cupric oxide of the copper test solution reduced is, that one atom of the former reduces five atoms of the latter. This is the foundation upon which the action of the test is based, and the calculation made in estimating by its agency the amount of sugar present. Taking 63.4 as the atomic weight of copper, and 180 as that of glucose ($C_6H_{12}O_6$), 317 parts of copper will stand equivalent to 180 parts of glucose. Thus one part of copper corresponds to .5678 of glucose; and in calculating the amount of sugar in the blood analyzed, the weight of the copper deposited has only to be multiplied by .5678 to give its equivalent in glucose. The quantity of sugar in the amount of blood taken for analysis being thus determined, the data are furnished by which the proportion per 1000 parts may be ascertained.

I have entered thus minutely into the description of the gravimetric process of estimating sugar, as I feel that it supplies a new mode of investigation which is calculated to materially advance our position with reference to the physiological relations of sugar in the animal system. It has hitherto always seemed to me that in giving numerical representations of minute quantities of sugar they could only be regarded as approximate results. Now, however, sufficient precision is attainable to enable minute differences to be ascertained with the requisite certainty for definite physiological conclusions to be drawn.

In a further communication, to be presented for the next meeting of the Society, I propose to give the results I have already obtained bearing on (1) the natural state of the blood; (2) the comparative states of arterial and venous blood; and (3) the spontaneous change ensuing after the removal of blood from the system.