Nettsonian Lectures

ON

CERTAIN POINTS CONNECTED WITH DIABETES.

By FREDERICK W. PAVY, M.D.,

PROFESSOR OF PHYSIOLOGY AND ASSISTANT-PHYSICIAN, GUY'S HOSPITAL.

LECTURE I.—(PART I.)

IN approaching the subject of "Sugar in the Animal Economy," it is necessary to place ourselves in the most guarded position. Fallacies arrive through such unexpected and multifarious channels, in researches on this point, that it is requisite to be especially cautious in dealing with the experimental results that may be obtained. The relations of sugar have certainly not yet been fully extricated from the deeper recesses of organic chemistry belonging to animal life; notwithstanding it seemed a short time since that our knowledge was complete, or nearly so, and that the whole pathology of diabetes was on the brink of disclosure. But a little more, indeed, than ten years back, the discovery of a sugar-forming function for the liver was announced. This discovery appeared to rest upon irrefutable grounds, and rapidly obtained an established footing in all our text-books on Physiology. The doctrine of gluco-genesis had become at last so familiar as an admitted fact, that one scarcely thought of stopping to question further its substantiality.

My own impression in favour of the gluco-genic theory was so strong that for some considerable time I did not give the importance that was due to a result I had obtained which did not accord with our preconceived views. I felt inclined to attribute what I observed, rather to some source of objection in the experiment, than to look upon it as controverting the position we held. At last, however, growing dissatisfied at not being able to account for what I saw, I resolved to commence a thorough investigation to discover, if possible, the cause of my result. I found there was not the source of fallacy I was contented before in supposing might exist. From that moment I have advanced step by step, as my experiments have directed me; and from the force of the evidence that has presented itself, I have been involuntarily led from the position of a strong advocate to that of an adversary of the gluco-genic theory. Disinclined, at first, to regard what I saw as constituting a valid objection, I have since been compelled to submit to evidence, which, I can only say, having convinced me, will I believe prove convincing to others. Much of this evidence, in as far as it is immediately connected with my subject, I shall have to advert to as I proceed, and it is my intention to show the experiments upon which my statements are founded.

I cannot refrain from mentioning at the outset that it is with the greatest deference I stand at variance on this subject of gluco-genesis with so distinguished a physiologist as Bernard. I am satisfied the liver is not specially intended as a sugar-forming organ; but whatever may ultimately prove to be the natural purport of the so-called glucogenic matter existing in it, to Bernard will always belong the credit of a great and important discovery.

Of the varieties of sugar, it is the glucose, or grape-sugar, that we shall have to deal with in these lectures. Now, as it is of the greatest importance that we should possess information about the means adopted for recognising our agent, I will first speak of the tests that are resorted to for determining its presence and its quantity. Happily, here we do not encounter any difficulty, for grape-sugar is almost as easily detected as any inorganic material, even when present in an exceedingly minute quantity. Numerous tests have been from time to time recommended, but the following are those that, on account of convenience or otherwise, are generally used:—

Moore's test consists in treating the specimen with a solution of potash and then boiling. The alkali decomposes the sugar,

resolving it into a dark-coloured substance, which renders the liquid more or less of a sherry or brownish hue. I do not consider this test a desirable one for physiological purposes. Where the quantity of sugar is large, as in diabetic urine, the effect is so strongly marked that there cannot be any mistake in recognising it; but where only a minute quantity of sugar is present, there is only a shade of difference to discriminate, and, with the best intentions, we may often be deceived, where only shades of difference in colour are concerned. What, in physiology, we require is something giving a more decided "aye" or "nay," and this we get with the various copper tests and with fermentation.

In using Moore's test for the detection of diabetes, a drachm, say, of urine is treated in a test-tube with about half its bulk of liquor potassæ, and boiled for a couple of minutes over a spirit lamp. It must be borne in mind that a fallacy—and I have frequently seen it—may occur. If the liquor potassæ have been kept in a white or flint-glass bottle, it becomes contaminated with lead. Lead may also be abstracted from the glaze of an earthenware dish, in which the potash may have been boiled or evaporated down. At all events, from whatever source lead may have been derived, on boiling liquor potassæ contaminated with it with any organic compound containing sulphur, the sulphur is liberated, and, combining with the metal, produces a more or less dark-coloured liquid, according to the amount of sulphuretted formed.

Amongst the copper tests we can have our choice; but in all, the principle of action is the same. Free protoxide of copper is present, which, on being boiled with grape-sugar, loses half its oxygen, and is thrown down as a yellow, orange-yellow, or orange-red precipitate, according to the amount of sugar present, which possibly determines the state of hydration of the deposit. A liquid containing a large proportion of sugar gives an orange-red deposit; whilst, when there is only a slight proportion of sugar present, the sub-oxide thrown down assumes a yellow colour.

In Trommer's test, the oxide of copper is set free at the time of application. A drop, or a couple of drops, of a moderately concentrated solution of sulphate of copper are let fall into the specimen to be tested, and then liquor potassæ is added in considerable excess. The precipitate at first thrown down on the addition of the alkali is redissolved if sugar be present, and a deep-blue liquid is the result, which, on boiling, deposits the reduced or sub-oxide precipitate. The objection to this test is, that it is not so convenient in practice as the copper solutions to which I am about to refer; and, where only traces of sugar are present, they may escape detection, on account of undissolved protoxide obscuring a slight production of the reduced oxide.

Of the solutions of copper, Barreswil's liquid is by far the most celebrated. It is this form of copper test that has been employed by Bernard in his researches. Reduced to our English scale of weight and measurement, it is thus composed:—

Bitartrate of potash (cream of tartar)	960 grains.
Carbonate of soda (crystallized)	... 960 "
Caustic potash (potassa fusa)	... 640 "
Sulphate of copper	... 320 "
Distilled water	... 20 fluid oz.

These materials are to be dissolved, mixed, and the resulting blue liquid to be filtered.

Barreswil's liquid is thus a rather complex solution. Essentially it consists of oxide of copper dissolved in an alkali by the presence of an organic material (tartaric acid), which does not occasion its reduction at the temperature of ebullition. It is the property of the protoxide of copper not to be soluble in an alkali unless some form of organic matter be present. Hence the necessity of the tartaric acid, or some such agent, in the cupro-potassic solutions. But why use the bitartrate of potash, which calls for the employment of the carbonate of soda to neutralize its excess of acid? It is more simple to take at once the neutral tartrate of potash, and then the carbonated alkali may be dispensed with. Fehling's solution is of this description, and consists of sulphate of copper and tartrate of potash, with soda as the alkali.

I can recommend the following as a simple method of preparing a copper solution for use in clinical practice in the detection of saccharine urine. Take five grains of sulphate of copper and ten grains of tartrate of potash, and dissolve in two drachms of liquor potassæ. A clear deep-blue liquid is formed, which is quite as efficient as any other kind of cupro-potassic test. The liquid, however, that I use and keep ready prepared in my laboratory is made with a larger proportion of potash than what is contained in the liquor potassæ—a condition

necessary to enable the test to be preserved in an efficient state. The following are the proportions of the ingredients used:—

Sulphate of copper	320 grains.
Tartrate of potash (neutral)	640 „
Caustic potash (potassa fusa)	1280 „
Distilled water	20 fluid oz.

Dissolve each in a separate portion of the water, then mix the sulphate of copper and tartrate of potash, and afterwards add the alkali.

The precipitation of sub-oxide from any of these copper solutions on boiling must not be looked upon as affording an infallible indication of the presence of sugar; neither, on the other hand, must the absence of a precipitate be regarded as absolutely proving the absence of sugar. Such a remark must seem materially to detract from the value of testing with a copper liquid; but, bearing in mind certain considerations, I do not think there is much chance of falling into error, and certainly the copper is the most useful test that we have.

It is said that glycerine, tannine, cellulose, leucine, uric acid, and chloroform are each capable of producing, in different degrees, a reduction of the oxide of copper. Chloroform certainly exerts a strong reducing effect. Uric acid I have observed to occasion some deposit of red oxide; and from cotton I have obtained just a trace of precipitate. But the glycerine I have tested has not yielded the slightest vestige of reaction. A circumstance, however, of great importance is, that if a copper solution be kept for any considerable period, and particularly if exposed to light, it will of itself deposit some red oxide on boiling. Hence, unless the solution have been recently prepared, it should be tested, by boiling a little, alone, from time to time; and if it should be found to give a precipitate, a fresh addition of potash will restore it, rendering it again as fit as ever for use.

But under certain circumstances no deposit of sub-oxide will take place, although sugar may be indisputably present. Ammoniacal salts have the property of occasioning this. I have frequently noticed where an ammoniacal odour is evolved on boiling a specimen with the blue liquid, that at first there has been no perceptible change; then a change of colour, without any precipitation, has taken place; and if the boiling has been continued so that the potash of the blue liquid has expelled the whole of the ammonia, a considerable deposit of sub-oxide has afterwards fallen. The presence of albumen interferes with the proper reaction of our copper test: I expect, on account of the ammonia evolved as the result of destruction by the potash in the test. In a case of apoplexy where the urine was albuminous I tested for sugar, expecting to find it, because there was sudden and great congestion of the circulation. The action of the test was obscure; but on boiling the urine and filtering before applying it, a neat precipitation of sub-oxide occurred.

With a fluid like blood, a process of preparation for testing is of course absolutely indispensable. Boiling does not get rid thoroughly of the albuminous and colouring matters; its alkalinity enables the liquid to retain some of these. The cautious addition of acetic acid to neutralization will effect their entire precipitation; but a little too much acid produces the condition that was intended to be removed. I consider this process troublesome, and always resort to the use of the sulphate of soda—a material which does not in the slightest degree interfere with our test, and which furnishes us with a perfectly clear liquid from the blood. The blood is simply mixed with about an equal weight of sulphate of soda, and heated to effect coagulation in a small porcelain capsule. Thrown on a filter, a limpid transparent liquid immediately runs through.

Bernard has more recently recommended animal charcoal for separating albuminous and colouring matters from a liquid, where we are desirous of applying the cupro-potassic test. According to Bernard, it is the property of animal charcoal to abstract certain materials and not others from a liquid. Glucose is a substance which it does not remove, but the albumen and colouring matters are completely separated by it from the blood, albumen and uric acid from urine, and even the caseine and fatty matter from milk. It is sufficient, therefore, to treat an animal fluid with the necessary quantity of animal charcoal; and upon filtration a clear liquid will run through, which retains any sugar that might have been present, although deprived of many other substances.

A solid substance may be prepared for testing, either by making a plain decoction of it, or else by pounding it in a mortar with the sulphate of soda, heating, and filtering in the same manner as with blood.

Although just a point of doubt might be attached to the in-

dications afforded by the other tests, yet with the fermentation test we have been hitherto accustomed to look upon its reaction as perfectly characteristic of the presence of sugar. It seems, however, from the researches of a French chemist, M. Berthelot, who has recently devoted much time to the chemistry of the sugars, and whose statements are entitled to our greatest consideration, that there are other substances besides sugar—viz., glycerine, mannite, dulcine, and sorbine, which are capable of undergoing the alcoholic fermentation with yeast. Alcoholic fermentation, therefore, can no longer be regarded as affording an infallible indication of sugar.

In applying the fermentation test, it is necessary that the yeast should be thoroughly washed before use. Yeast, as purchased, will undergo a considerable fermentation alone, and even after being well washed, I have found it give rise to the evolution of a slight amount of carbonic acid. In fact, the fermentation test in my hands has not proved susceptible of that delicacy and absolute certainty I could have desired. The nature of the gas evolved may be determined by the action of potash, and the presence of alcohol in the liquid may be shown by distillation, and then mixing the distilled product with unslaked lime, and heating in a convenient apparatus for igniting the vapour that passes over.

It now only remains for me in this preliminary part of my subject, to speak of the mode of conducting the quantitative determination of sugar. The best process I know of is with the copper solution, and this, I am satisfied from considerable experience with it, is susceptible of the utmost delicacy and precision. We cannot separate and weigh the sugar as the chemist does with an inorganic material; but we estimate its amount by its reducing or deoxidizing effect on a copper solution of determined strength. The liquid I employ—that composed of potash, tartrate of potash, sulphate of copper, and water, in the proportions I have already given—is of such a strength, that 100 minims of it are exactly decolorized by half a grain of the purest well-dried grape sugar that I have been able to obtain. In other words, half a grain is the exact amount of sugar required to convert the oxide into the sub-oxide contained in 100 minims of my blue liquid. To give an example, I will describe how I proceed in the case of diabetic urine. The quantities are measured in graduated tubes, drawn at one end to a point so that liquid may be dropped as required. 100 minims of the blue liquid are taken, and a mixture of one part of urine with five of water (because the urine is too concentrated alone) allowed to fall into it drop by drop whilst kept boiling in a small porcelain capsule over the flame of a spirit-lamp or gas. As soon as the blue colour of the copper solution has been completely removed, the operation is at an end. The amount of diluted urine employed can be read off, and will contain an equivalent to half a grain of glucose.

Should the fermentation-test be used quantitatively, according to Dr. Christison, every cubic inch of carbonic acid evolved corresponds, in round numbers, to a grain of sugar; or, more precisely, 47 cubic inches of gas are equivalent to 45 grains of sugar.

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By HENRY THOMPSON, Esq., F.R.C.S., &c.

GENTLEMEN,—It is in accordance with a time-honoured custom that we suspend, on one of the fortnightly meetings of the year, that discussion of purely scientific topics which forms the ordinary business of our Society, and devote an evening to the contemplation of some of the larger and higher relations which connect our noble profession with the rest of the great human family. On those ordinary occasions, we are accustomed to meet here frequently throughout the season, in the spirit of a pure but practical philanthropy, and to occupy ourselves mainly in communicating the result of our investigations respecting the various means of a therapeutic character, by which we seek to avert disease and to relieve pain, and to submit those results to the consideration and friendly criticism of our brethren.