The Congress has the advantage of meeting in one of the great ports of Europe, and a visit to the docks at Antwerp would satisfy every inquirer on this subject that the matter can only be dealt with satisfactorily by international action. The question of uniformity of action in dealing with the dangers of invasion by cholera, yellow fever, and plague has been the subject of an International Conference which led to a common basis of action, and those regulations are now undergoing international revision. This fact proves that such subjects can be dealt with successfully, and the case for international regulations is much stronger when we consider marine hygiene, as it deals with the everyday life of those who live at sea under very depressing and unsatisfactory conditions which tend to lower the standard of work and to drive self-respecting individuals to seek employment ashore. Such a Conference should not be confined to Board of Trade officials, but should include all interests concerned, in order that the regulations might be satisfactory from the point of view of owners, masters, crew, the Board of Trade, and public health officials.

I sincerely trust, therefore, that this Congress will decide to further the appointment of a small international conference on the subject, and that the Permanent International Committee will, after the Congress, keep it under consideration until definite action is taken in the matter.

Newport, Mon.

THE DETECTION OF SMALL AMOUNTS OF GLUCOSE IN URINE.

BY SYDNEY W. COLE, M.A. CANTAB.,

DEMONSTRATOR OF PHYSIOLOGY, UNIVERSITY OF CAMBRIDGE.

IT has been repeatedly demonstrated that by the application of Fehling's test alone one cannot with any certainty demonstrate the existence of a small amount of glucose in a specimen of urine. I have elsewhere enlarged on the fallacies of the test.[1] Briefly stated, they are: 1. Urates reduce Fehling's solution. 2. Creatinine reduces Fehling's solution and also forms a soluble compound with cuprous oxide,[2] thus preventing the detection of a small amount of sugar. 3. Sodium hydroxide, if present in excess, destroys a small amount of glucose.[3] 4. Conjugated glycuronates are hydrolysed to reducing substances by sodium hydroxide. 5. The mixed solution is unstable, and if kept suffers auto-reduction on boiling. 6. The solution is reduced by lactose, a normal constituent of the urine of women during the period of lactation. 7. The solution is reduced by pentoses, which, however, are rarely found in urine.

In the case of a large number of samples of urine as much as 0·5 per cent. of glucose can be added without producing, when tried with Fehling's test, anything more than the greenish cloud that is seen with specimens that can be shown, by more suitable methods, to contain no more than the average normal amount of glucose. It cannot be too strongly urged that for the detection of small degrees of glycosuria Fehling's method is extremely unreliable, and the use of the reagent is almost certain to lead to erroneous conclusions.

In choosing a method to supersede Fehling's one must not lose sight of the fact that normal urine contains a small amount of glucose. The percentage calculated by different methods varies between 0·03 and 0·08. I certainly think that the latter is too high for the samples I have tried, but the exact figure is not material. It is of the greatest importance to be able to determine any excess of glucose, however small, above the normal. Macleod[4] emphasises this point. "If there really is an excess of dextrose, however small, it indicates that something is amiss with the utilisation of carbohydrates in the organism; it is a danger signal which if heeded and the proper treatment applied, may unquestionably enable us to stave off the incidence of what might afterwards prove a deadly diabetes."

[1] Practical Physiological Chemistry, S. W. Cole, third edition, 1913, p. 161.
[2] Hugh MacLean: Biochemical Journal, vol. i., 1906, p. 111.
[3] S. R. Benedict: Journal of Biological Chemistry, vol. iii., 1907, p. 101.
[4] J. J. R. Macleod: Diabetes: its Pathological Physiology, 1913, p. 16.

From an extensive series of experiments that I have made I am convinced that of the great number of tests that have so far been devised Benedict's[5] is the most satisfactory one to use in the great majority of cases. The substitution of sodium hydroxide by sodium carbonate overcomes the first four of the objections to Fehling's solution, and the use of sodium citrate instead of Rochelle salt renders the mixed solution perfectly stable. In fact, the only serious objection to it is that it gives a marked reaction with lactose.

As has been pointed out elsewhere,[6] a greyish precipitate of urates and phosphates may appear and lead to a slight amount of indecision.

Nylander's test when correctly applied[7] is also valuable in a negative sense—that is to say, a negative reaction indicates that the condition of glycosuria does not exist. But a positive test is yielded by other substances, and so cannot be relied upon as an indication of the condition of glycosuria.

The phenyl-hydrazine test for demonstrating the presence of glucose is very reliable when correctly performed. But it is almost too delicate. A large number of normal urines yield undoubted crystals of the glucosazone. In this connexion it may be pointed out that the most sensitive method of performing the test in my experience is as follows:

To 10 c.c. of the protein-free urine in a test-tube add six drops of glacial acetic acid, enough solid phenyl-hydrazine-hydrochloride to cover a shilling, and twice this amount of solid sodium acetate. Heat to dissolve and filter into another test-tube. Immerse this in a boiling water bath for 40 minutes. Turn out the flame and allow the tube to cool in the bath for an hour.

I find that the addition of the acetic acid markedly increases the ease with which crystals can be obtained. Binet[8] uses acetic acid after the use of lead acetate, but I find that the previous precipitation of the urine by lead is of very doubtful advantage.

During the course of another investigation I had occasion to use blood charcoal for the purpose of decolourising urine. I noted the fact that urates and creatinine are adsorbed with great readiness. In many of my experiments I obtained a filtrate containing only about 1 per cent. of the urates and 3 per cent. of the creatinine of the urine, and in some I apparently removed the whole of these substances that so markedly interfere with Fehling's test.

The adsorption of glucose from pure solution by blood charcoal has been studied by Rona and Michaelis.[9] They find that the addition of 10 per cent. of acetic acid or of 15 per cent. of acetone prevents the adsorption of glucose by charcoal. Andersen[10] confirms this for glucose in urine.

I have investigated the adsorption of glucose and lactose from water and urine under a variety of conditions. Some of the results are given below. The charcoal used was Merck's pure blood charcoal. The adsorptions were conducted at room temperature for 1½ hours in each case. The sugar was estimated by a sensitive polarimeter using both the sodium yellow and the mercury green.[11]

Glucose.

Per cent.	Per cent. charcoal.	Per cent. adsorbed from water.	Per cent. adsorbed from urine.	Per cent. adsorbed from 10 % acetic acid.	Per cent. adsorbed from urine + 10 % acetic acid.
1·78	6·35	55·5	50·2	1·1	1·7
0·91	4·5	52·5	27·9	0	0·8
0·49	5·0	63·8	31·2	—	—
0·49	5·0	65·0	44·9	—	—
0·36	4·6	65·0	42·2	0	0

Lactose.

1·80	6·35	88 9	77·5	17·9	6·6
0·91	4·5	97·5	76·5	5·3	4·8
0·51	5·0	100·0	86·5	—	—
0·51	5·0	100·0	92·5	—	—

[5] Stanley R. Benedict: Journal of Biological Chemistry, vol. v., 1909, p. 485.
[6] Macleod, loc. cit., p. 20.
[7] Cole, loc. cit., p. 161.
[8] P. Binet: Jahresbericht für Tierchemie, 1892, p. 506.
[9] Rona and Michaelis: Biochemische Zeitschrift, Band xvi., 1909, p. 491.
[10] Andersen: Ibid., Band xxxvii., 1911, p. 262.
[11] I am indebted to Professor Pope for his courtesy in allowing me to use his apparatus, and to his assistant, Mr. Williams, for help in making the observations.

It will be noted that the adsorption of both sugars in the absence of acetic acid is considerably less from urine than from pure aqueous solutions. Also that the adsorption of lactose is very much greater than that of glucose. Taking advantage of these facts, I have elaborated a comparatively simple method of detecting quite small amounts of glucose when present in urine.

There are two fundamental principles underlying my method: 1. Charcoal in a certain percentage adsorbs the greater part of the non-saccharine reducing substances of normal urine, the greater part of any lactose that may be present, and also a certain amount of the glucose normally present. 2. The filtrate is boiled with sodium carbonate, and thus converted to a reducing substance [12] which reacts with copper when subsequently added.

Owing to the great ease with which sugar can be detected in the filtrate after adsorption with charcoal, I had considerable difficulty in finding the exact conditions so that the normal sugar of urine should not give a positive test. I believe that I have succeeded in my object and have also found a simple method for distinguishing lactose from glucose. The details of the method are as follows:—

In a dry boiling tube or large test-tube place about 1 gm.[13] of Merck's pure blood charcoal.[14] Add 10 c.c. of the urine and shake from side to side to mix thoroughly. Heat to boiling point, shaking the whole time. Cool thoroughly under the tap and shake at intervals for about five minutes. Filter through a small paper (9 to 11 cm. in diameter) into a rather wide test-tube containing about half a gramme of anhydrous sodium carbonate.[15] When the fluid has filtered through add 6 drops of pure glycerine,[16] shake and heat to boiling.[17] Note the time when boiling commences. Maintain active boiling for 50 seconds, shaking from side to side to prevent spurting. Immediately add 4 drops of a 5 per cent. solution of crystallised copper sulphate.[18] Shake for a moment to mix the solutions, and allow the tube to stand without further heating for one minute. With normal urine the fluid remains blue, with a variable amount of a greyish precipitate of the earthy phosphates. If glucose is present to the extent of 0·02 per cent. or more above the average normal amount the blue colour is discharged, and a yellowish precipitate of cuprous hydroxide forms.

The rapidity with which the precipitate forms is a rough measure of the amount of glucose present. With 0·05 per cent. it appears in a few seconds. With 0·02 per cent. it may not appear till 50 seconds. A yellowish precipitate or colouration appearing after 60 seconds must not be taken as evidence of any degree of abnormal glycosuria. It may be due to the normal amount of sugar in urine.

I have experimented with the urine passed by a large number of apparently healthy individuals. Only once have I obtained a positive result, and in that case the yield of osazone crystals was so large that I am convinced that a slight degree of glycosuria existed. It is interesting to note that the sample in question gave a negative result with Benedict's and Nylander's methods. But my method, in my hands, is more sensitive than either of these.

Chloroform does not give a positive reaction, even when present in considerable excess. I have repeatedly tried the urines of patients treated with relatively large doses of chloral. In no case have I obtained a positive result, though I was able to demonstrate the presence of glycuronates by Tollen's and Bial's tests. And in all cases the characteristic slow reduction of Fehling's solution was obtained.

There is no necessity to remove albumin, but it is advisable to do so by boiling and filtering; otherwise the coagulation of the albumin when boiled with the charcoal interferes somewhat with filtration. Should the specific gravity of the urine exceed 1025 it is advisable to dilute it with an equal volume of water and to take 10 c.c. of the diluted urine.

[12] Benedict: Journal of Biological Chemistry, vol. iii., 1907, p. 101.

[13] This can be approximately measured by means of a spatula or the large blade of a pen-knife. A spatula three-eighths of an inch broad, well piled up with the charcoal for just over an inch, carries about ½ gm.

[14] The only charcoal that is free from suspicion is Merck's "Blutkohle, mit säure gereinigt." It can be obtained from Baird and Tatlock's.

[15] I recommend the use of Baird and Tatlock's "extra pure anhydrous." A quarter of a gramme is carried by about one-half of an inch of the large blade of an ordinary pocket-knife.

[16] Baird and Tatlock's pure glycerine—sp. gr. 1·260—is to be recommended, since it gives no reduction when boiled with alkalies and copper sulphate. But all the samples that I have tested lose their reducing power when boiled with alkalies for the time I mention.

[17] It is advisable to use a test-tube holder, which can be improvised by folding stiff writing paper.

[18] This can be obtained approximately by diluting a cold saturated solution with five times its volume of distilled water.

Test for glucose in pure solution.—Provided that pure glycerine can be obtained that does not give a reduction on boiling with alkalies and copper sulphate, a very sensitive reduction test can be performed as follows:—

To 5 c.c. of the solution add 6 drops of glycerine, 2 drops of 40 per cent. sodium hydroxide, and a drop of a saturated solution of copper sulphate. Shake to mix, and boil for a minute. A yellow or red precipitate separates out.

With dilute solutions of sugar it is necessary to decrease the concentration of the alkali and the copper. By using a single drop of 5 per cent. soda and 2 or 3 drops of 1 per cent. copper sulphate, I can detect one part of glucose in a million parts of water.

Distinction between lactose and glucose.—In the case of urine from a pregnant or nursing woman the following procedure should be adopted:—

Treat 20 c.c. of the urine with 1 gm. of charcoal as described above. Treat the whole of the filtrate with another gramme of charcoal and repeat the process. To 5 c.c. of the filtrate from this add ½ gm. of sodium carbonate and 6 drops of glycerine and boil for 50 seconds. Now add to the hot solution 4 drops of the 5 per cent. copper sulphate and set the tube aside for one minute. A reduction occurring within the specified time indicates the presence of at least 0·04 per cent. of glucose in the urine.

If less than 0·3 per cent. of lactose is present it is entirely adsorbed by 10 per cent. of charcoal, as in the routine method. By using 5 per cent. of charcoal, as in the special modification, a considerable amount of lactose is removed, and that left is entirely adsorbed by the second treatment. I find that the adsorption of lactose from the filtrate from decolourised urine is practically identical with that from water. By the addition of as much as 1 per cent. of lactose to urine I have failed to get a positive test when tried in this manner; whereas 0·04 per cent. of glucose gives a distinct reaction. Should this test give a negative result, though the original urine responds to Benedict's test, the urine almost certainly contains lactose.

The identification of lactose in urine.—I do not know of any simple method hitherto published of demonstrating the existence of lactose in urine. The osazone, probably owing to its relative solubility, does not usually separate when the osazone test is applied direct to the urine. (I do find, however, that the addition of 10 per cent. of acetic acid results in a small yield with 0·5 per cent. of lactose added to the urine.) The mucic acid test is a good one, but the evaporation with the nitric acid is inconvenient. The failure to obtain fermentation with yeast also involves much delay in diagnosis and is not very certain. By taking advantage of the difference in the adsorption of lactose from urine in the absence or presence of acetic acid I can obtain typical lactosazone crystals from urine containing as little as 0·15 per cent. of lactose. The method is as follows:—

To 1 gm. of charcoal add 25 c.c. of the suspected urine, mix by shaking, boil for a few seconds, cool thoroughly, and shake at intervals for 10 minutes. Filter through a small paper or use a filter pump. When the charcoal has completely drained transfer it to a porcelain dish containing 10 c.c. of water and 1 c.c. of glacial acetic acid. This is best done by opening the paper, holding it by the clean half and moving it about in the liquid. The greater part of the charcoal is thus removed from the paper. Stir the charcoal with a glass rod and transfer the mixture to a boiling tube. Heat to boiling for about 10 seconds and filter the hot solution through a small paper into a test-tube containing as much solid phenyl-hydrazine-hydrochloride as will lie on a shilling, and twice this amount of solid sodium acetate. Mix thoroughly and filter from any insoluble oily residue. Place the tube in a boiling water bath and leave it there for 45 minutes. Remove the tube and allow it to stand at room temperature for at least one hour. It is advisable to allow it to stand longer if possible. Pipette off a little of the deposit, if any, and examine it on a slide under the high power of a microscope.

Lactosazone crystallises in characteristic clumps with projecting spines ("hedge-hog" crystals). It can be recrystallised by filtering through a small paper, washing with a small amount of distilled water, and then passing about 4 c.c. of boiling water through the paper into a clean tube. The filtrate is boiled and passed through the paper two or three times, boiling between every filtration. On allowing the solution to stand, typical crystals of the osazone separate out. They can be filtered off, dried, and the melting point taken (200° C.).

I trust that the publication of these methods will encourage the routine examination of all specimens of urine for small amounts of glucose. In that way, I believe, valuable information as to the limits of tolerance to carbohydrates in various pathological conditions would come to hand.