A NOTE ON THE PHENYL-HYDRAZINE TEST
FOR SUGAR.By G. LESLIE EASTES, M.B., B.Sc.,
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IN the BRITISH MEDICAL JOURNAL of January 19th, 1901, in answer to a query concerning the application of this test, the statement is made that the test can be performed on 5 c.cm. of the urine. This is perfectly true if the specimen contains more than 20 parts per 1,000 of sugar. But the chief use of the test is as confirmation or otherwise of the presence of sugar in urines, of which the total reducing power is equivalent to 5 parts per 1,000 or less. Urines containing a higher proportion of glucose than this reduce Fehling's solution while being boiled with it, if this latter test is properly applied. In view of the importance of the problem and the uncertainty which appears to exist as to the exact application of confirmatory tests, I take this opportunity of detailing the procedure which should be adopted. I am indebted to my predecessor and late colleague, Mr. F. G. Hopkins, for the method I am about to describe, and must disclaim any originality for it.

Using perfectly fresh Fehling's solution, boil 5 c.cm. of it in a clean test tube. When boiling, add 3 to 4 c.cm. of the urine, but not more. Again boil, and keep the mixture in constant agitation and ebullition for at least one minute. If reduction occur immediately or within thirty seconds, the urine contains glucose. If by the end of a minute reduction is only commencing, as shown by the prevalence of a dirty greenish colour with opacity, set the mixture aside to cool. The greenish colour will gradually change to a dirty yellow, and some yellowish deposit will settle. The reaction is probably due to glucose, or, if the patient is a female, lactose may be present. There is yet another class of urines with which no reduction occurs during the minute of boiling, but after cooling a distinct greenish-yellow colour supervenes, with some flocculence. This reaction may be due to the presence of one or more of the various reducing substances normally and abnormally present in urine. It is in these two classes of urine that the phenyl-hydrazine test is of use for diagnostic purposes.

Before proceeding, however, to apply confirmatory tests the presence or absence of albumen should be ascertained by the application of heat to some of the filtered urine to which a drop of dilute acetic acid has been added. If more than a cloud is observed the coagulated proteid should be filtered off and Fehling's test again applied. This is essential, as I have occasionally met with proteid-containing urines which react with Fehling's solution, the reaction not occurring after the separation of the proteid by heat and filtration. Should the reduction still take place we can then proceed with the phenyl-hydrazine test.

Take of the filtered urine about 60 c.cm. in a beaker of 100 c.cm. capacity. Add 1 gram of sodic acetate and rather less of the phenyl-hydrazine hydrochlorate. Stir with a glass rod, which remains in the beaker throughout the entire operation. The beaker is then placed on a water bath and the urine gradually evaporated down to 10 to 15 c.cm. During this process the beaker should be occasionally removed, and any sediment collected on the sides of the vessel scraped off with the glass rod into the fluid. In this way none of the sugar is left by evaporation on the sides of the beaker. When reduced to the bulk above indicated the flame should be removed and the beaker remaining on the bath the whole should be allowed to cool. This will take two hours or longer. When quite cold stir up all sediment, and with a pipette place some on a glass slide for microscopical examination. Osazone crystals will have formed if there is one part per thousand or more of sugar in the urine. If no crystals are found it may be safely concluded that sugar (that is glucose) is absent.

The method may be used to differentiate lactose from glucose, and it is my experience that the crystals of lactosazone as formed from urine are short, pointed at one or both ends, and somewhat broad, and frequently curved in the longitudinal plane. They also show less tendency to sheaf or rosette formation than the crystals of glucosazone.

The simplicity and inexpensiveness of the requisites, and

the very slight technical skill required combine to make this a test which might with advantage be employed by practitioners, nearly all of whom may be assumed to possess a microscope with optical combinations for magnifying up to 300 diameters. The delicacy of the test when applied as described above is equal to that of the fermentation test, while the latter is by no means so easily employed.

A NOTE ON THE PHENYL-HYDRAZINE TEST
FOR SUGAR.By WALKER HALL, M.B., CH.B.(VICT.),
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THIS test gives reliable information as to the absence of sugar from a solution, and also offers valuable confirmatory evidence when sugar is present. In practice, however, its use has been considerably minimised by the varied descriptions of its procedure and the divergent statements in regard to the reagents employed. A few of these may be cited:

	Phenyl- Hydrazine Hydro- chlorate.	Sodium Acetate.	Urine.	Ratio.	Time of Boiling.
Moritz ¹ ...	0.5 gram	1 gram	10 c.cm.	1:2:20	60
Stewart ² ...	0.1 "	0.2 "	5 c.cm.	1:2:50	20-60
Halliburton ³ ...	0.1 "	0.2 "	5 c.cm.	1:2:50	30
BRIT. MED. JOURN., p. 191, 1901	0.5 "	0.75 "	5 c.cm.	1:1.5:10	20-30
Taylor ⁴ ...	0.14 (2 grs.)	0.28 (4 grs.)	4 c.cm.	1:1.5:40	20-30
V. Jaksch ⁵ ...	2 parts	3 parts	6-8 c.cm.	1:1.5:?	20-30
Williamson ⁶ ...	$\frac{1}{2}$ -in. t. tube	$\frac{1}{2}$ -in. t. tube	$\frac{1}{2}$ -in. t. tube	1:?:?	2
Hutchinson ⁷ ...	" "	" "	" "	1:?:?	2

Saundby, Allbutt, and many others give only approximate figures. It will excite little surprise that unequal results have been obtained and the test disregarded clinically. As Williamson⁶ remarks, there are two distinct methods of performing the test. One involves laboratory utensils, skilled manipulation, is very sensitive and accurate, but the crystals obtained are frequently amorphous; the other is simple, easy and reliable enough for all clinical purposes. In relation to the above table I have again repeated a series of previous experiments to determine the exact times and quantities necessary to produce good crystals, and at the same time be sufficiently sensitive. From these it appears that 0.5 gram (7 to 8 grs.) of phenyl-hydrazine, 1.5 gram (23 grs.) of sodium acetate, and 5 c.cm. (2 drachms) of urine give the most rapid and satisfactory results with glucose, but that 10 c.cm. should be taken if the solution contains maltose or lactose. These quantities are approximately Williamson's directions, and answer with 0.1 to 5 per cent. solutions; if a higher percentage is present the solution or urine should be diluted. Since these proportions have been used in this laboratory, out of a large number of students, [very few have failed] to obtain crystals at the first trial.

Before adding the urine, the reagents should be dissolved by gently warming in a few cubic centimetres of water. When the urine is added the mixture is brought to the boiling point, and there maintained for fully three minutes with strong, and five minutes with weak solutions. After some practice, however, a change in the manner of ebullition will be noted as an indication when to stop the boiling. The test tube need not be placed in cold water; it should be simply placed at rest. Within two to ten minutes the crystals will be formed.

A very delicate reaction, based upon an extension of this test, is suggested by Professor Riegler (Jassy)⁸: 0.1 gram of phenyl-hydrazine hydrochlorate, and 0.5 gram of sodium acetate are dissolved in a few drops of water and then boiled during several seconds with 1 c.cm. (20 drops) of urine or sugar solution; 1 c.cm. (20 drops) of caustic soda (10 per cent.) is added, and within one second to five minutes, a deep-red violet colour appears. If performed in a porcelain dish instead of a test tube the colour change is well-marked, and is developed in rings or streaks that are both striking and distinctive. From careful examination I con-