MISCELLANEA

A Laboratory Trial of Some Modern Screening Tests for the Detection of Glucose and Protein in Urine

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(RECEIVED FOR PUBLICATION DECEMBER 17, 1958)

In recent years a number of simple diagnostic tests on urine have been developed which are less time consuming than the procedures previously used. The most important are those for the detection of protein and glucose which will be considered here.

The Ames Company has produced "albastix" and "clinitix" for the testing of urine for protein and glucose respectively. "Albastix" are strips of thick paper impregnated at one end with an indicator, tetrabromphenol blue, and a citrate buffer, approximately pH 3. Protein is adsorbed on the outer surface of the strip and produces a colour change in the indicator to green or blue-green caused by the phenomenon known as "protein error". This error occurs when a solution of protein is added to a pH indicator and a colour change takes place which is not due to change in pH.

The "clinitix" strips are similar in appearance to "albastix" but they are impregnated with the enzyme glucose oxidase, a peroxidase and orthotolidine. Glucose oxidase reacts with glucose in the urine to form gluconic acid and hydrogen peroxide. The hydrogen peroxide then reacts with orthotolidine to give a blue colour in the presence of peroxidase.

The same company produces "altest" tablets for the detection of protein in urine, a test based on the well-known salicylsulphonic acid reaction; and "clinitest" tablets for the detection of glucose, the principle involved being the reduction of alkaline copper sulphate by glucose.

Several workers have testified to the reliability and simplicity of these tests, especially the impregnated strips, when many specimens have to be dealt with as a routine. Hunt, Gray, and Thorogood (1956) obtained good results when comparing 170 specimens of normal and diabetic urines tested with "clinitix", Benedict's test, and an enzyme strip made by another company.

Luntz (1957) found, in comparing "clinitix" with "clinitest" tablets, that out of 942 specimens of urine 930 tests gave identical results, 916 being negative and 14 positive. Of the remainder he found that some of the urines contained reducing substances other than glucose which gave false positive results to the "clinitest", and others contained very small quantities of glucose which

gave positive "clinitix" readings but no reduction in "clinitest".

Carne (1958) recommends "albastix", particularly for screening purposes. He found no false negative results but reports that he occasionally saw a false weak positive test in urine from pregnant women. Baron and Newman (1958) performed comparative tests using "albastix" and the salicylsulphonic acid test on urine with known added amounts of protein. They obtained colour changes with "albastix" when the urine contained 5 mg. of protein per 100 ml. or more. Whittet (1956) records that the "altest" tablet method was very reliable.

It was decided to carry out a trial on the routine urine tests undertaken over a period of several months. The method was to carry out the salicylsulphonic acid test for protein and the qualitative Benedict's test for glucose on all urine specimens received and to test the same specimens with "clinitix", "altest", and "albastix". The "albastix" test was not available when the trial began but was introduced as soon as possible afterwards. When positive results were obtained, quantitative tests were performed, using standard opacity tubes for the salicylsulphonic acid test and the "clinitest" tablets for glucose.

METHODS Protein

Salicylsulphonic Acid Test.—One millilitre of urine is added to 3 ml. of 3% salicylsulphonic acid in a test tube, and the contents well mixed. Turbidity of the mixture denotes the presence of protein. This turbidity can be matched against standard opacity tubes in a range from 10 to 100 mg. per 100 ml. Should the reading be greater than 100 mg., suitable dilution may be carried out and the amount of protein present calculated accordingly.

"Altest" Tablets.—One "altest" tablet is added to 4 ml. of urine in a test tube and allowed to dissolve. The tube is gently shaken and any cloudiness appearing in the urine indicates the presence of protein.

"Albastix" Strips.—The impregnated end of the strip is immersed in the urine and removed immediately. If protein is present the moistened end turns green or blue-green usually in less than one minute.

Glucose

Benedict's Qualitative Test.—Urine, 0.5 ml., is added to 5 ml. of Benedict's qualitative reagent in a test tube. The tube is then placed in a boiling water-bath for five minutes and then cooled. If glucose is present the colour of the reagent changes to green, yellow, or orange according to the quantity of glucose present.

"Clinitest" Strips.—The impregnated end of the strip is dipped into the urine and removed immediately. If

glucose is present in the urine, the moistened end of the strip turns blue, usually within one minute.

"Clinitest" Tablets.—Five drops of urine and 10 drops of water are delivered into a test tube and one "clinitest" tablet added. As the tablet dissolves heat is generated and the fluid in the tube boils. Fifteen seconds after boiling has ceased the tube is shaken and the colour noted and compared with a colour chart which gives the approximate quantity of glucose in the urine. If no glucose is present the colour remains blue.

RESULTS

Three thousand seven hundred and six specimens of urine were tested for glucose by the Benedict's and "clinistix" methods. If either or both of these gave positive results, then the "clinitest" method was carried out. The results are recorded in Table 1.

TABLE 1
RESULTS FOR GLUCOSE

Benedict's Test	"Clinistix"	"Clinitest"	Number of Specimens
Negative	Negative	—	3,534
Positive	Positive	Positive	80
Positive	Positive	Negative	71
Positive	Negative	Negative	8
Negative	Positive	Negative	13

It is claimed by the manufacturers that the "clinistix" test is sensitive to about 0.1 g. per 100 ml. whereas "clinitest" is sensitive to 0.25 g. per 100 ml., and this contention is supported by the work of Hunt, Gray, and Thorogood (1956). This probably explains why the 84 specimens of this series which gave positive reactions with "clinistix" were negative to "clinitest". The eight specimens which gave positive reactions to Benedict's reagent and negative reactions to both "clinistix" and "clinitest" may have contained very small quantities of reducing substances other than glucose or they may have contained very small quantities of glucose. The former explanation is more likely as Benedict's reagent can be reduced by such substances as creatinine, protein, sugars other than glucose, drugs such as aspirin, and uric, glucuronic, ascorbic, and homogentisic acids.

The total number of specimens tested for protein was 3,617. The trial was divided into two series, the first in which only the salicylsulphonic acid test and "altest" were performed and the second when "albustix" strips were used in addition. The results are given in Table 2.

In the first series it will be seen that very close correlation was obtained between the salicylsulphonic acid

TABLE 2
RESULTS FOR PROTEIN

Salicylsulphonic Acid Test	"Altest"	"Albustix"	No. of Specimens
<i>First Series</i>			
Negative	Negative	—	1,574
Positive	Positive	—	39
Trace	Positive	—	2
Trace	Trace	—	6
Trace	Negative	—	1
<i>Second Series</i>			
Negative	Negative	Negative	1,958
Positive	Positive	Positive	31
Positive <10 mg. per 100 ml.	Positive	Negative	3
Trace	Positive	Negative	1
Trace	Trace	Negative	1
Trace	Negative	Negative	1

method and "altest". In the larger second series correlation was again very close, with "altest" showing as a slightly more sensitive test than "albustix". But as the "albustix" strips are said to be sensitive to 5 mg. per 100 ml. (Baron and Newman, 1958), the slightly superior sensitivity of "altest" is of no practical value.

The manufacturers issue a colour chart for "albustix" and it is claimed that approximate quantitation may be achieved by comparing the colour change on the strip with the chart which gives colours for 30 mg., 100 mg., 300 mg., and over 1,000 mg. of protein per 100 ml. of urine. This rough estimation of quantity may be useful in the field but it is considered that where laboratory facilities are available, the quantitative salicylsulphonic acid test should be performed on all urine specimens showing a positive reaction to "albustix".

CONCLUSIONS

The results of this trial show that the detection of significant amounts of glucose and protein in specimens of urine received for routine examination may be rapidly and accurately carried out by the use of the impregnated strips "clinistix" and "albustix" respectively. If preferred "altest" tablets are eminently suitable for detecting protein.

I am grateful to Dr. P. R. Boyd for his encouragement and permission to undertake this trial, and Messrs. R. L. Harding, A. Hickson, and F. N. Hine for their technical assistance.

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