ASSESSMENT OF A NEW SIMPLE COLORIMETRIC TEST FOR PROTEINURIA

BY

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This communication describes the trial of a new test for proteinuria which is based on a principle not previously applied to the analysis of urine. Current tests for proteinuria are turbidimetric, and depend upon the precipitation of the proteins by heat or by various acids; they all have the disadvantage that urine which is already turbid requires filtration or centrifugation before testing.

The new test is colorimetric and may be performed upon turbid urines without filtration. It is based upon the "protein error of indicators" (Clark, 1928; Feigl, 1956). When a solution of protein is added to a pH indicator a colour change takes place which is not due to a change in pH.

The test material which we have investigated ("albastix") consists of a strip of paper. One end of this strip is impregnated with an inert base containing tetrabromphenol blue (tetrabromphenoltetrabromsulphophthalein) buffered to pH 3 with a citrate buffer. Saturation with a protein solution causes a change in colour, from yellow through green to blue, the amount of colour change varying with the concentration of protein in the solution.

Method

By the addition of normal human serum to normal urine (shown by ultrafiltration to contain about 1.5 mg. of protein/100 ml.) solutions of protein in urine were prepared at the following concentrations: 5, 10, 20, 30, 50, 100, 300, 500, 1,000, and 2,000 mg./100 ml. These protein concentrations were tested in urines at specific gravities of 1005, 1015, and 1025, and at pH 5, 6.5, 7.5, and 8.5. The specimens were tested on preparation and after standing at room temperature for 24 hours and 48 hours. We also tested 200 random urine specimens from normal subjects and hospital patients.

All of these specimens were tested by two methods: (1) *Standard salicylsulphonic acid test* (Harrison, 1957): 5 ml. of urine + 0.5 ml. of 25% salicylsulphonic acid. (2) *Albastix*: The whole treated end of the albastix strip was dipped into the urine, and withdrawn as soon as the paper was wet. The colour which developed was compared, after about five seconds, with the colour scale provided, which gives readings of 0, 30, 100, 300, and 1,000 mg./100 ml. The results could be read in daylight or by artificial light, but colour comparison was slightly easier in daylight.

Some specimens of normal urine were tested with albastix both before and after removal of all traces of protein by ultrafiltration.

Specimens of urine containing (1) tolbutamide, and (2) radio-opaque materials—sodium diatrizoate ("hypaque"), sodium acetrizoate ("diagnol"), iopanoic acid ("telepaque"), diiodone, iodoxyl ("pylectan"), "biligradin," "urogratin," and "endograftin"—were tested. As these substances are known to give false-positive results with the salicylsulphonic acid test, these specimens were also tested by the standard "boiling test" for protein (Harrison, 1957), as well as by albastix and salicylsulphonic acid.

*Employed under a whole-time grant from the Royal Free Hospital Endowment Fund.

To compare the accuracy of albastix and Esbach's method (Harrison, 1957) in quantitative measurement of proteinuria, specimens of urine from a nephrotic patient were used. Serial dilutions of these specimens were made with normal urine. The true protein concentrations in these diluted specimens were calculated from the results obtained by a micro-Kjeldahl assay (Harrison, 1957) performed upon the undiluted specimens. The range of protein concentrations so tested was from 15 to 700 mg./100 ml.

We have not investigated the manufacturers' suggestion that the test can be performed by the patient placing the albastix strip in the urine stream.

Results

A change in colour of the albastix strip was obtained with all specimens of urine which gave positive results with the standard salicylsulphonic acid test for proteinuria (147 specimens). The amount of colour change varied according to the protein concentration, but quantitation was not always reliable. Some normal urines also gave a colour change.

The results obtained with the specimens of urine of known protein content are summarized in the Table. In general, less accurate results were obtained in alkaline specimens of high specific gravity, when albastix often gave a reading greater than the true protein content.

Comparison of Actual Concentrations of Protein in Urine, and Albastix Results

Urine Protein Concentration (mg./100 ml.)	Concentration as Indicated by Albastix (mg./100 ml.)
0	0-less than 30
5	Trace-30
10	Trace-30
20	Trace-30
30	About 30
50	30-100
100	100-300
300	About 300
500	300-1,000
1,000	300-1,000
2,000	300-1,000

As shown in the Table, a proteinuria of 5 mg./100 ml. often gave the same degree of colour change as a proteinuria of 20 mg./100 ml. The lowest protein concentration at which a consistent degree of colour change was obtained was 30 mg./100 ml.

Of 161 random specimens judged to be protein-free by the salicylsulphonic acid test, 44 gave a detectable colour change with albastix. This false-positive colour change was usually slight and patchy, but was occasionally comparable to that obtained with urines known to contain 20 mg. of protein per 100 ml. After ultrafiltration the false-positive results were still obtained.

Little change in the albastix reading was observed when specimens were tested after standing at room temperature for 24 and 48 hours. In no instance did the reading change by one increment on the colour scale.

In comparing the value of Esbach's method and albastix as quantitative tests it was found that albastix gave more accurate results with low protein concentrations. It is known that Esbach's test is especially unreliable in the lower ranges. With higher protein concentrations (above 300 mg./100 ml.) albastix showed no advantage over Esbach's test.

Albastix did not give false-positive results with urine containing tolbutamide and the various radio-opaque materials, which all gave positive results with the salicylsulphonic acid test.

Four specimens of urine from patients with myelomatosis gave positive results with albastix. These specimens contained Bence Jones protein only.

Discussion

The advantages of albastix over the standard protein tests are as follows. The test is simple, is quick to perform, and requires the minimum of apparatus. It is not necessary

to filter urines which are turbid. With albutix a positive result is indicated by a colour change; this is easier to assess than the change in turbidity obtained with the precipitation tests for proteinuria.

It is important to consider the sensitivity of albutix in comparison with other tests for proteinuria. It is generally agreed that normal subjects may excrete up to 50 mg. of protein in 24 hours (Rigas and Heller, 1951). In view of this, Fishberg (1954) has given as an acceptable definition of proteinuria: "When protein is present in urine in amounts sufficient to be detected by ordinary clinical tests which . . . are not sufficiently sensitive to detect normal amounts." The clinical tests for proteinuria (boiling, salicylsulphonic test) are generally accepted as sensitive to 5-10 mg. of protein per 100 ml. of urine when properly performed (King, 1951; Baron and Oakley, 1957; Rice, 1957). If albutix could be shown to detect 5-10 mg. per 100 ml. it would be acceptable as a clinical test for minimal proteinuria.

We have, in fact, found that albutix always gave a colour change with 5 mg. of protein per 100 ml. of urine, although the degree of colour change was often very slight. It may therefore be considered to have the sensitivity required. Unfortunately there was an appreciable proportion of apparent weak false-positive results with albutix. That these were false-positive results and were not due to physiological traces of protein is inferred by the observation that the same change in colour occurred with albutix before and after the removal of all protein by ultrafiltration. (We have not investigated the possible causes of the false-positive results.) A colour-change with albutix indicating less than 30 mg./100 ml. is therefore not specific for protein. If it is required to demonstrate proteinuria of less than 30 mg./100 ml. it is necessary to perform a salicylsulphonic acid or boiling test on urines giving these slight colour changes to verify the presence of protein.

The lowest protein concentration which is designated by the manufacturers on the colour scale is 30 mg./100 ml., and a colour change with albutix indicating a protein concentration of 30 mg./100 ml. or more always means proteinuria.

In the concentrations above 300 mg./100 ml. quantitation is unreliable. We feel that the colour scale should be modified to give four readings: "negative," "trace," "positive," "strong positive." These are respectively equivalent to protein concentrations of 0, approximately 30, approximately 100, and above 300 mg./100 ml.

In contrast to the salicylsulphonic acid test, albutix does not give false-positive results with urines containing radio-opaque materials or tolbutamide. This last fact may make albutix especially useful in diabetic clinics where patients are being treated with tolbutamide.

In view of these observations we make the following recommendations. Albutix is a convenient and reliable test for use by general practitioners, by nurses, and in large clinics when it is inconvenient to perform the boiling or salicylsulphonic acid test with sufficiently critical technique to ensure the highest sensitivity. Albutix provides a satisfactory test if unequivocal demonstration of minimal proteinuria is not required. The standard tests are preferred for laboratory use, except for screening.

In "albutest" the same principle is used in tablet form. An evaluation of this product has been made by Leonards (1957) and Rice (1957), both workers claiming that albutest could detect levels of 20 mg./100 ml.

Summary

A new manufactured colorimetric test for proteinuria, which involves dipping a prepared strip (albutix) into urine, has been investigated. The test is simple and convenient.

Colour changes were always obtained with specimens of urine containing 5 mg. of protein per 100 ml. or

more. Slight false-positive colour changes were sometimes given by protein-free urines. Colour changes indicating protein concentrations of 30 mg./100 ml. or more were specific for protein.

The test is roughly quantitative within the range 50-300 mg./100 ml. The clinical applications of this test are discussed.

We thank the Royal Free Hospital Endowment Fund for their support, the Ames Company (London) Limited for supplies of albutix; and Professor N. H. Martin and Dr. A. L. Miller for urines containing Bence Jones protein.

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LABORATORY TRIAL OF A PAPER STRIP TEST FOR PROTEINURIA

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A recent test for proteinuria employs impregnated paper sticks ("albutix") which remain yellow when dipped in normal urine but become green or blue if the urine contains protein. A five-step colour scale is provided, said to correspond to 0, 30, 100, 300, and 1,000 mg. of protein per 100 ml. of urine. The strips contain citrate buffer, pH 3 approximately, and an indicator, tetrabromophenol blue (tetrabromophenoltetrabromsulphonphthalein). The test is based on the so-called "protein error of indicators" and is not related in principle to either the heat-coagulation or the sulphosalicylic acid test for urinary protein.

Existing tests for urinary protein, although reliable and sensitive when carefully applied, are inconvenient to use outside the laboratory or consulting-room, particularly if a turbid urine requires filtration before the tests can be applied.

The new test, which requires no apparatus whatever, has obvious advantages in speed and simplicity. Since, however, it is based on an alteration in the effective pH range of an indicator, it was thought possible that differing conditions of pH, concentration, and buffering capacity of urine might cause some variation in the results of the test. The strips were therefore tested on a number of natural and modified urines in order to assess sensitivity and reproducibility, while normal and protein-containing urines were artificially varied in respect of pH and buffering capacity in order to assess the likelihood of errors from these sources.

Sensitivity and Accuracy of Calibration

Twelve protein-containing urines were each diluted with normal urine until they gave apparently negative indications with albutix, and these diluted urines were then tested for