

A Rapid Spot Test for the Determination of Cystinuria and Aminoaciduria

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A simple test for the determination of cystinuria and of aminoaciduria is described. The basis of the test is the color reaction between nitroprusside and thiol groups. The reagent is a dry mixture to which the urine samples are applied. The ease of performance of the test makes it ideal for use as a screening procedure. The reaction is nonspecific, and positive tests require further investigation by more elaborate laboratory procedures.

CYSTINURIA is a relatively rare metabolic disorder, although during the last 2 years, on routine microscopic examination of urinary sediments we found 7 individual cases. We think it not unlikely that some cases are overlooked.

Since, even in proved cases of cystinuria, the typical hexagonal cystine crystals are not always present in the urine, especially when dilute or fresh specimens are examined.

The pathologic manifestation of cystinuria is the formation of urinary calculi. Fortunately, as reported by Albright (1), the cystine calculi respond to medical treatment and may be dissolved successfully if discovered at an early phase of development. Therefore we examined routinely every submitted urine specimen for the presence of increased amounts of cystine, by a simple method which is based on principles similar to those of the rapid spot tests described by Fischl and Pinto (2).

The test is based on the reaction between nitroprusside and thiol groups, the interference of electrolytes being prevented by cyanide. The reagent can be compounded into a dry mixture since it keeps

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well, and a large number of tests can be performed easily. The reaction was found useful also for the detection of increased excretion of amino acids.

Methods and Results

Reagent

Mix thoroughly in a mortar 200.0 gm. of anhydrous ammonium sulfate with 2.0 gm. of sodium nitroprusside. Transfer to a wide-mouth jar (preferably polyethylene). Then similarly mix 200.0 gm. of anhydrous sodium carbonate with 1.0 gm. of sodium cyanide and add the mixture to the same jar. Close the jar and mix its contents thoroughly by shaking. Remove the amount needed for daily use, but otherwise keep the container tightly closed. Test the reagent with a cystine solution (100 mg./100 ml.) and discard if it does not give a clear-cut positive reaction. Failure to observe such a reaction may occur 3-4 weeks after preparation.

Procedure

Place a pinch (0.3-0.5 gm.) of the reagent on a porcelain spot plate and add a few drops of urine so as to wet the reagent thoroughly. The development of a cherry-red color in a few seconds is indicative of cystinuria. The color fades quite rapidly unless a large amount of cystine is present.

Aminoaciduria produces an intense brick-red color, the reaction paralleling that caused by cystine. With some experience, the two can be differentiated easily by the color.

Acetone interferes with the reaction but can be recognized by the more violet tint of the color and the fact that the reaction develops at a slower rate and lasts longer. Normal urine produces no color change, or only a light pink which turns to yellow.

Our method of investigation was as follows: Every urine specimen submitted to the laboratory for routine urinalysis was tested with the reagent. The negative samples were discarded and the positive samples divided into two parts. One portion was refrigerated and centrifuged on the following day, and the sediment was examined microscopically for the presence of cystine crystals. An aliquot of the second part was chromatographed on paper and the relative concentration of the amino acids determined.

Whenever abnormalities were discovered, the patient was registered and his clinical condition noted. After 3, and again after 6

days, new urine specimens were requested and examined. A final examination was performed on the day before the patient was discharged from the hospital. The patients whose specimens were repeatedly a positive for either cystinuria or aminoaciduria were considered to be permanently affected, and those whose specimens were negative on subsequent examination, to be transiently affected.

During a 3-month period 2194 urine specimens were screened; 53 of these were found to be pathologic. Of the positive reactions, 32 were transient, and 21 permanent; of the transient reactions, 21 showed aminoaciduria and 11, cystinuria; of the permanent reactions, 9 revealed aminoaciduria and 12, cystinuria. Three cases of congenital cystinuria and 1 of cystine storage disease were diagnosed.

Discussion

The reagent described above has the advantage over the fluid reagents based on similar principles that it does not deteriorate for 3-4 weeks, in contrast to solutions of nitroprusside and cyanide. Moreover, it may be compounded into one reagent, and it is always ready for use. The test can be employed routinely, and every specimen may be screened for amino acid abnormalities without much loss of working time. By widespread use of the test, much information concerning the etiology and incidence of cystinuria can be collected, as well as valuable data on aminoaciduria.

In a relatively short time (although indeed more than 2000 patients were examined) we discovered 3 cases of cystinuria (to be reported by Dr. Pruzanski) and 1 case of the rare metabolic disorder of cystine storage disease (to be reported by Dr. Wallis). That diagnosis of cystine storage disease in a living subject is highly unusual is shown by the statement of Peters and Van Slyke (3), in a discussion of cystinosis, that "Cystinuria has not been reported in most cases, but it has not always been sought because the nature of condition has not been suspected during life." In our case the cystine storage disease was suspected after the positive test result and was verified later by isolation of cystine from the bone marrow and by chromatographic studies of the patient's blood and urine.

The color reaction obtained in the test is not specific for cystine, but it serves well for the screening of suspicious cases. However, every positive result must be verified by additional procedures. Acetone and its related compounds, as well as all sulfhydryl-contain-

ing metabolites in the urine, react with the nitroprusside. In a few instances, unsuspected ketonuria was revealed by the test.

Aminoaciduria is readily diagnosed with the test because proportionally more sulfhydryl groups are present in the specimen either as a result of increased production or of faulty reabsorption. According to the studies of Soupart (4), methionine, cysteine, homocysteine, cystine, and homocystine represent a relatively small portion (2 per cent) of the total excretion in normal subjects. Taurine, on the contrary, is excreted in considerable amounts (13 per cent). Therefore, increase in urinary taurine levels seems to be a good index of total amino acid excretion, provided that patient does not have a condition in which the relative proportions of the various amino acids are altered.

We found an abnormally high total amino acid output in 21 specimens giving a positive reaction. On the other hand, 14 samples submitted for amino acid determination gave negative results which also were found to be negative on subsequent quantitative and chromatographic examination.

Congenital cystinuria is usually accompanied by a greatly increased excretion of lysine, arginine, and ornithine (5), in addition to the increase in cystine excretions. Of the four amino acids, only cystine contains sulfhydryl groups, and as a result the reaction is positive and clear-cut in all cases of cystinuria.

Transitory cystinuria has been reported in pyelonephritis (6), and it is probable that it may be present in many other disorders. A wider use of the test may increase the number of cases of cystinuria recognized as well as clarify the pathologic importance of transitory cystinuria.

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