Screening Test for Hydrindicuria

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A screening test is described for the detection of a type of disorder associated with the metabolism of melanin and manifested by mental symptoms. The test employs the conversion of the urinary melanogens to hydrindic acid by acidification. By extracting the pigment with iso-butanol and purifying the extract by washing, the urinary excretion levels may be assayed by photometry.

Ever-growing evidence indicates that the indole metabolism has a major role in mental illness. In previous publications (1, 2) we reported the identification, isolation, and the purification of some unusual indolic substances from the urine of a mentally ill child. It was found that the chromogen of the end product was hydrindic acid, and the intermediate substances were derived from aberration of the melanin biosynthesis. This paper describes a screening test, which may be useful for the detection of disorders of similar or identical etiology.

The test consists of the acidification of the urine sample followed by isobutanol extraction of the pigment, removal of the impurities by washings of the extract and by reextraction into the alkaline phase. Quantitative determination is made by photometry. In all consistently positive cases a more thorough investigation is indicated, as noted previously (1).

**Methods**

**Preliminary Screening Test**

To 5 ml. of a urine sample 0.5 ml. of concentrated sulfuric acid is added. If after approximately 10 min. no strong red or violet color forms, the test is considered negative and no further examination is necessary.

One must exclude the possibility that positive tests are not the result of medication, especially phenothiazine drugs. The preliminary screening test is repeated on 5 or 6 successive days and when a consistent positive reaction is found, the quantitative screening test is performed.

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Quantitative Screening Test

A 24-hr. urine sample is collected. (No special care for preservation is required).

Step 1

To 5 ml. of the urine, which is placed in a centrifuge tube of 15 ml. capacity, 0.5 ml. of concentrated sulfuric acid is added. After 10-15 min. 5 ml. of isobutanol is added and the contents of the tube are vigorously shaken, then centrifuged at about 4000 rpm for 5 min.

The organic layer is quantitatively removed and its absorbance is measured at 540 m\(\mu\) in a suitable instrument (\(R_2\), total pigments). The aqueous phase is discarded.

Step 2

To the organic phase a solution of 10% (w/v) NaOH is added until a definite alkaline reaction is obtained. The tube is again shaken well and centrifuged. The two phases are separated.

The upper organic layer is acidified by the addition of 10% (v/v) \(H_2SO_4\), drop by drop. After shaking and centrifuging, the excess acid is separated and discarded. The extract is washed twice with 5-ml. portions of water saturated with isobutanol and its absorbance is measured at 540 m\(\mu\) (\(R_2\), red-pigment).

Step 3

The aqueous layer from step 2 is reacidified, then 5 ml. of isobutanol is added and, after shaking and centrifugation, the absorbance of the organic phase is read, at the same wave length as in the previous steps (\(R_2\), brown pigment). Calculation is made either by preparing a reference standard solution from the substance obtained from the urine of an established case of hydrindicuria as previously described (1), or by the DOPA degradation method which is performed as follows:

DOPA (DL-3,4-dihydroxyphenylalanine*), 10 mg., is dissolved in 5 ml. of 0.5M phosphate buffer of pH 8. The solution is allowed to stand, away from direct sunlight, for 16-18 hr., is acidified by 0.5 ml. of 40% (v/v) sulfuric acid, and then extracted with 5 ml. of isobutanol.

The absorbance of the solvent layer equals 12.3 mg./100 ml. of hydrindic acid pigment.

For a simpler method of calculation, the calibration curve (Fig. 1) obtained in our experiments may be used.

Discussion

More than fifty years ago Ross (3) reported that in the urine of mentally ill patients a significantly high "urorosein" (4) reaction may be generally found. He also presented evidence that in some of his cases the reaction was not influenced by dietary factors or intestinal putrefaction (5). The significance of Ross's observation was obscured in later years by the accumulation of data on the "ur!orosein" reaction in a wide variety of conditions.

However, recently, the diversity of the urinary indole acids was demonstrated by Armstrong et al. (6) and at least two compounds (No. 14 and 15) were found significantly increased in the urine of the mentally ill. Thus, when in the urine of a mentally retarded child we encountered a reaction similar to the urorosein reaction, we isolated, purified, and identified some of the components responsible for the reaction (1).

Since the major part of the isolated substances could be experimentally produced by the nonenzymatic degradation of DOPA (7, 8) and also by our corroborative analytic data we concluded that the substances involved were produced by the erratic metabolism of melanin. In order to facilitate the discovery of identical or similar disorders a simple method for the screening of a large number of urines has been evolved. The principle of the test involves the liberation of the chromogen from possible conjugates, its conversion to the pigment by acidifi-
cation, extraction into isobutanol, partial purification of the extract, and photometric determination of the pigment levels. In a mass survey, now in process, we have tested the urine of several hundred patients with various disorders, and in only 1 patient, a 13-year-old hermaphrodite suffering from severe psychosis but not mentally retarded, could we demonstrate true hydrindicuria. In many other cases (about 1-2%) we obtained a positive reaction by the screening test, but such results proved to be either due to uorosein or to nonspecific pigments of an inconsistent nature.

References