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Use of Toluidine Blue for sequential staining of urinary amino acid chromatograms

Two urine screening tests in current use for detecting inborn errors of metabolism are paper chromatography for amino acids[1] and the BERRY spot test for mucopolysaccharides[2]. These tests are usually run separately, but this note describes a means of combining both tests in a single chromatogram using urine volumes containing known quantities of creatinine. The two advantages of this method are that duplicate spotting of urine is eliminated and the screening for mucopolysaccharides is performed on comparable amounts of urine.

The creatinine content of the urine is measured by the Jaffé reaction; the volume of urine applied to the chromatogram is then calculated from the following equation:

$$\text{Volume of urine applied } (\mu\text{l}) = \frac{215}{C^{0.67}}$$

where C = mg creatinine per 100 ml of urine.

In this equation, the numerator is derived empirically by us, since it provides chromatograms of good quality under our conditions. The denominator is the same as that used by WOOLF[3] in order to correct for the apparent aminoaciduria of dilute urine.

One dimensional chromatography is carried out using O'BRIEN's system[1], but the staining procedure is modified in the following manner. After development of the ninhydrin stain (Fig. 1A), a strip containing the points of origin (R_F 0–2) is cut from the proximal section of the chromatogram and overstained with 0.02 % Toluidine

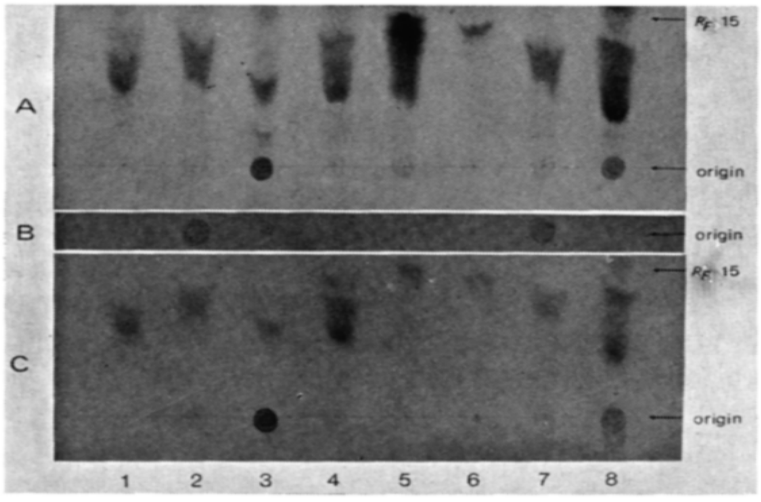

Fig. 1. Sections of chromatograms of urine stained with (A) ninhydrin; (B) ninhydrin overstained with Toluidine Blue; and (C) ninhydrin overstained with Pauly's reagent. Urines are from (1) a normal child; (2) a patient with Hurler's syndrome; (3) a patient with the nephrotic syndrome and proteinuria; (4) a child with histidinemia; (5) a cystinuric child; (6) a normal adult; (7) a patient with Hurler's syndrome; and (8) a patient with amino aciduria and proteinuria due to Lowe's syndrome.

Blue* as in the direct BERRY spot test (Fig. 1B). The middle section of the chromatogram (R_F 2–16) is overstained with Pauly's reagent for the histidines and the distal section (R_F 16–100) with Ehrlich's reagent for proline, hydroxyproline and other Ehrlich positive compounds in the usual manner.

Since neither proteins nor mucopolysaccharides move from the origin in this system, it is possible to detect the presence of both types of compound by this method. Proteins stain with the initial ninhydrin stain (Fig. 1A) and with the conventional Pauly's overstain (Fig. 1C), but are decolorized by the Toluidine Blue. Sulfated mucopolysaccharides stain only with the Toluidine Blue (Fig. 1B). Since Pauly's stain of the origin serves only to confirm the presence of proteinuria, we only use it to stain the middle section of the chromatogram (R_F 2–15) for the histidines.

We now have used this modification in over 1000 urine samples without finding any false results.

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* Toluidine Blue, 0.2 g; glacial acetic acid, 10 ml; 95% ethanol, 20 ml; water to 1000 ml.

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Paper chromatography of some aurones

Aurones (2-benzalcoumaranones) represent one of the minor classes of flavonoid compounds. They were discovered as naturally occurring pigments by GEISSMAN AND HEATON[1,2]. Since that time only a few such compounds have been found in nature. Physical and chemical characteristics of these compounds have been recorded by GEISSMAN AND HARBORNE[3], HARBORNE[4], and FARKAS AND PALLOS[5]. More recently HUKE and coworkers[6–9] have published a series of papers describing various analytical methods applied to polyhydroxyaurones including thin-layer chromatography[8].

During the course of systematic studies in our laboratory several aurones were encountered which prompted us to look into paper chromatographic separation of