Separation of 17-oxosteroid conjugates by thin-layer chromatography

In a recent communication KAY AND WARREN[1] have reported the extraction of urinary 17-oxosteroid conjugates as pyridinium salts and their electrophoretic separation.

The method to be described here uses thin-layer chromatography for the separation of these steroid conjugates and may be suitable for screening purposes in a survey of the pattern of 17-oxosteroid conjugates in normal and pathologic urines.

Silicagel GF 254 (Merck) containing an inorganic fluorescent dye and calcium sulphate as binding material was used as adsorbent.

A layer of 200 μ on microscope slides was prepared with the apparatus consisting of one glassplate 2.5 × 30 cm, thickness 4.45 mm, four microscope slides 2.5 × 7.5 cm, approximately 1.20 mm thick, and two 5.85 mm diameter rods. The rods are fixed to the glassplate with plasticine in order to hold the slides in place. The adsorbent, 10 g in 20 ml water, is poured on to the slides and spread with the 4 mm wide polished edge of a glassplate (2.5 × 20 cm).

The coated microscope slides are separated from each other with a scalpel blade, allowed to dry at laboratory temperature (18–23°) for 2 h and then heated in an electric oven at 105–110° for 1 h. They are kept in a desiccator over Silicagel until used.

An ethanolic extract of the urinary steroid conjugates, containing 8–10 μg in 10–20 μl is applied to the adsorbent layer with a Drummond disposable pipette 1.2 cm from the bottom edge of the slide and dried with a jet of N_2. The slide is placed in a small glass jar (7 × 9 cm) which is lined with filterpaper, covered with a glassplate and protected from draught by standing in a cardboard box.

The solvent system, chloroform–methanol–ammonia (19:1:0.2) is poured into the jar several hours before development starts to saturate the atmosphere. Time of development is approximately 7 min when the solvent front reaches about 6 cm from the origin. Under a Chromatolite Short Wave Lamp which has maximum radiation at 2536 Å the Silicagel GF 254 layer shows a yellow-green fluorescence while the steroids having a quenching effect appear as dark spots.

For quantitative work larger plates 10 × 20 cm or 20 × 20 cm, a glasstank of suitable size, and a layer of 400 μ can be used.

A separation of steroid pyridinium salts extracted from the urine of a normal male, age 35, shows 5 spots. Paper electrophoresis[1] resolved the extract into three zones only.

A parallel separation was performed of steroid pyridinium salts extracted from the urines of a normal male, age 35, and of a young female, age 23, afflicted with hirsutes for which no endocrine abnormality could be found. There is a striking difference in these two patterns.

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