remain at the start. Therefore, the use of solvent system II followed by solvent system I represents a suitable combination for studying certain problems of nucleotide metabolism.

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## Simultaneous separation of common mammalian $\Delta^4$-3-oxosteroids and oestrogens using two-dimensional thin-layer chromatography

Many paper and thin-layer chromatographic (TLC) systems are available for separating particular groups of $\Delta^4$-3-oxosteroids and oestrogens[1–3]. However, the literature does not appear to contain a simple, rapid and effective method for simultaneous separation of the common $\Delta^4$-3-oxosteroids and oestrogens of adrenal, testicular, ovarian, and placental origin. The method described in this paper was first developed to permit fractionation of such steroids produced in *in vitro* incubations with $^{14}$C-labelled 3$\beta$-hydroxysteroid substrates, after the $\Delta^4$-3-oxosteroids (and oestrogens) had been isolated (from 3$\beta$-hydroxysteroids) as a group using the method of TAYLOR[4].

The system involves the two-dimensional development of a 20 × 20 cm TLC plate. Fig. 1 depicts the separation (visualized under ultraviolet light at 254 nm) of thirteen steroids obtained using thin layers, 0.5 mm thick, of silica gel, "Kieselgel $GF_{254}$ nach Stahl" (Merck, Darmstadt). A similar pattern is obtained on 0.1-mm layers of "Kieselgel $HF_{254+366}$ nach Stahl" (Merck, Darmstadt) prepared, using starch as a binder, according to the method of TAYLOR[4].

The 0.5-mm layers readily permit separation of 10-$\mu$g quantities of each of the thirteen steroids listed. This occurs even when these quantities are applied together with the ethereal phase from an ether/water partition of an 80% aqueous alcoholic extract of 1 g human foetal adrenal tissue. If a large amount of tissue is extracted, the simple expedient of using several plates to fractionate the extract overcomes problems arising from the large volume of unwanted material present. Even in this circumstance, the system allows rapid primary fractionation without need for prior "cleaning up" procedures.

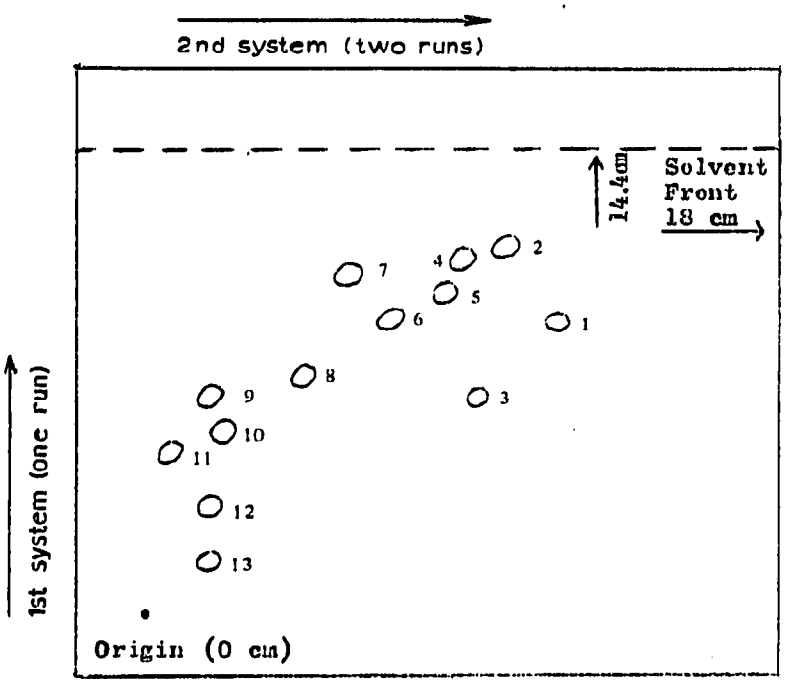


Fig. 1. Two-dimensional thin-layer chromatogram of steroids. Solvent systems: (1) chloroform–methanol–water (94:6:0.5); (2) cyclohexane–ethyl acetate (50:50). 1 = Oestrone (3-hydroxy-1,3,5(10)-estratrien-17-one); 2 = progesterone (4-pregnene-3,20-dione); 3 = oestradiol (1,3,5(10)-estratriene-3,17$\beta$-diol); 4 = androstenedione (4-androstene-3,17-dione); 5 = 17$\alpha$-hydroxyprogesterone (17$\alpha$-hydroxy-4-pregnene-3,20-dione); 6 = testosterone (17$\beta$-hydroxy-4-androsten-3-one); 7 = deoxycorticosterone (21-hydroxy-4-pregnene-3,20-dione); 8 = 11-deoxycortisol (17$\alpha$,21-dihydroxy-4-pregnene-3,20-dione); 9 = corticosterone (11$\beta$,21-dihydroxy-4-pregnene-3,20-dione); 10 = cortisone (17$\alpha$,21-dihydroxy-4-pregnene-3,11,20-trione); 11 = aldosterone (18,11-hemiacetal of 11$\beta$,21-dihydroxy-3,20-dioxo-4-pregnen-18-al); 12 = cortisol (11$\beta$,17$\alpha$,21-trihydroxy-4-pregnene-3,20-dione); 13 = oestriol (1,3,5(10)-estratriene-3,16$\alpha$,17$\beta$-triol).

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