Notes

A paper chromatographic system for estrogens*

Removing interfering chromogens is a constant problem when identifying steroid metabolites from bovine urine[1,2]. Various chromatographic systems have been used successfully with estrogens in human urine and plasma[3,4]; however, none of these systems was found to work satisfactorily with bovine urine.

A system using triethyleneglycol (stationary phase) and *o*-dichlorobenzene (mobile phase) was chosen to separate the chromogens remaining after chemical extraction, followed by gradient-elution chromatography on a silica gel column[5].

This system retains strong polar chromogens at the starting point, while allowing the weaker polar and neutral steroids to separate.

TABLE I

R_F AND R_E VALUES OF SOME STEROIDS

Steroids	R_F	R_E	*Detection*
Estriol	0.0	—	1, 3
Estradiol-17β	0.13	0.17	1, 3
Estradiol-17α	0.16	0.22	1, 3
Equilenin	0.45	0.58	1, 2, 3
Estrone	0.76	1.00	1, 2, 3
Hexestrol	0.0	—	1, 3
Diethylstilbestrol	0.0	—	1, 3
2-Methoxyestrone	0.92	1.28	1, 2, 3
Testosterone	0.90	1.10	2
Desoxycorticosterone	0.65	0.79	2

1 = FOLIN AND CIOCALTEU[8]; 2 = ZIMMERMAN[9]; 3 = $FeCl_3$ and $K_3Fe(CN)_6$[10].

Experimental

A source of natural bovine chromogens was obtained by refluxing 1 l of late pregnancy urine with 5% (v/v) of concentrated sulfuric acid and extracting with 300 ml of ether for 24 h. Chemical separation of the estrogens was the same as employed by BEER *et al.*[5]. After the ether was evaporated with diminished pressure, the residue was dissolved in a small amount of benzene. The solution was placed on a silica gel column (12 g, 10–200 mesh, Davison Chemical Co., 1.5 cm o.d.) and eluted with a gradient eluant of 250 ml benzene and 250 ml of (1:1) benzene and ethyl acetate[6]. The first 200 ml of eluate was collected and evaporated, then the residue was dissolved in methanol and applied as described below. This eluate may contain estrone, estradiol, estriol, and 2-methoxyestrone from the natural source[6].

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The steroids listed in Table I were applied individually to methanol-washed[7] 15 cm × 45 cm precut Whatman No. 1 strips. Starting lines were placed 13 cm from the top of each chromatograph before the strips were drawn through the stationary phase of 33% triethyleneglycol and methanol (w/w) and blotted between Whatman No. 1 paper. The solutes, dissolved in methanol, were applied with a micropipette and evaporated with a hair dryer, then dried for several minutes to allow any excess methanol to evaporate. Sheets of filter paper were used to line each chamber and thoroughly wetted with the mobile phase, an excess of the mobile phase was also added to the bottom of the chamber (12 in. × 12 in. × 24 in. glass jar). All the chromatograms were developed by the descending technique.

The system was allowed to equilibrate for at least 2 h (or overnight) in an insulated room maintained at a temperature of 16° ± 1°. After equilibration the mobile phase saturated with triethyleneglycol was added to the trough for chromatography from 4 to $4^1/_2$ h, when the solvent front usually was within several cm of the end of the strip. The chromatogram was removed and allowed to lie several minutes on blotting paper to absorb the excess mobile phase, then developed with the appropriate reagent.

Results and discussion

Movement of the mobile phase is approximately 7 cm/h, which allows rapid separation. The areas of separation are well defined and chromogens still present after silica gel elution remain 1 or 2 cm from the starting line. Two terms are used to describe the movement of the solute: R_F and R_E. The R_F is defined as the ratio of the distance moved by the solute to the solvent front. R_E is defined as the ratio of the distance moved by a particular solute to that of the standard estrone. The expression R_E is used as a relative value and is independent of the effects of temperature, saturation by the stationary phase, humidity, etc. These variables influence both standard and other solutes by the same degree.

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