Acknowledgements

This work was supported in part by grants from the National Institutes of Health (HE 05085-03 MET) and the American Heart Association (61 G 77).

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Received June 17th, 1963

Separation of equol from oestrogens by thin-layer chromatography

Equol may be present in hen's urine in relatively large amounts[1,2]. It can be separated from steroid oestrogens by thin-layer chromatography (TLC). The procedure involves (i) separation by TLC of the phenols into (a) oestrone, (b) oestradiol-17β plus equol and (c) oestriol; (ii) methylation of (b); and (iii) subsequent TLC of the methyl ethers of (b), which affords excellent separation of these two compounds.

A mixture of oestrone, oestradiol-17β, oestriol and equol was chromatographed on silica gel G (Merck) in benzene–methanol (85:15)[3]. The two terminal strips of the chromatoplates were sprayed with 1 % (w/v) *p*-nitrobenzenediazonium fluoborate in acetic acid–water (1:1 v/v) while the middle section was protected by a plastic plate. The respective R_F values were 0.63, 0.41, 0.24 and 0.41. The blank area corresponding to the oestradiol-17β plus equol spot was removed, eluted with ethanol and methylated[4]. The methyl ethers were chromatographed in benzene–methanol (95:5) and the spots detected by spraying with 2 % (v/v) sulphuric acid in aqueous ethanol[3]. Methylation renders equol less "polar" than oestradiol in consequence of the formation of the dimethyl ether[5], which moves far ahead of the 3-methyl ether of oestradiol-17β in this solvent system (R_F: 0.78 and 0.37 respectively).

Acknowledgements. We thank the National Research Council of Canada for a Postdoctoral Fellowship held by one of us (F.H.) and Professor W. KLYNE, Westfield College, London, N.W. 3, for a sample of reference equol.

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Received June 7th, 1963