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Note on the detection of hexoestrol, stilboestrol, dienoestrol and the *p*-hydroxy metabolites of phenobarbitone and phenytoin in urine

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A procedure for the identification of the synthetic oestrogens, *p*-hydroxyphenobarbitone and 5-(*p*-hydroxyphenyl) 5-phenylhydantoin in urine by use of two dimensional paper chromatography is described.

THE examination of the phenolic acids of human urine by two dimensional paper chromatography has been described previously (Tompsett, 1961). The results of such an examination could be confused by the presence of certain drugs, for example the synthetic oestrogens or of the *p*-hydroxy metabolites of drugs such as phenobarbitone (Butler, 1954, 1956; Curry, 1955; Algeri & McBay, 1956) and phenytoin (Butler, 1956, 1957) which may be included in treatment schedules.

Experimental

Essentially the same procedure was used as that described previously (Tompsett, 1961). Before ether extraction of the urine, the conjugates were hydrolysed by adding hydrochloric acid to give a N solution; the mixture then being placed in a boiling water-bath for 1 hr. Two dimensional paper chromatography using two systems (1) isopropanol ammonia, 0·88 : water (8:1:1) and (2) benzene: propionic acid: water (2:1:1) was employed and the same detecting agents used.

Since the substances under examination are *p*-hydroxylated compounds, the 1-nitroso 2-naphthol-nitric acid reaction described for the detection of tyrosine on paper chromatograms (Block, Durran & Zweig, 1955) was also used. The paper chromatograms were sprayed with a 0·1% solution of 1-nitroso 2-naphthol in ethanol and allowed to dry. After spraying with an aqueous 10% solution of nitric acid, the chromatograms were heated at 100° for 3 min. A positive reaction is indicated by the development of a red colour.

Experimental results are shown in Table 1.

Results and Discussion

In the first solvent system, the synthetic oestrogens show Rf values very much different from those shown by the phenolic acids. Little confusion can therefore result and the use of a second solvent system is unnecessary. Two dimensional paper chromatography is however necessary to indicate the presence of *p*-hydroxyphenobarbitone and 5-(*p*-hydroxyphenyl) 5-phenylhydantoin.

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TABLE 1. CHARACTERISTICS OF SUBSTANCES EXAMINED

	Rf values solvent (1)	Rf values solvent (2)	Colour reactions on paper chromatograms *A*	*B*	*C*	*D*
Stilboestrol	0·85	—	yellow	brown	brown	negative
Dienoestrol	0·85	—	purple	brown	brown	negative
Hexoestrol	0·85	—	red-brown	brown	brown	positive
p-Hydroxyphenobarbitone.. ..	0·60	0·00	orange	deep purple	purple	positive
5-(*p*-Hydroxyphenyl)-5-phenyl-hydantoin	0·65	0·25	yellow	brown purple	orange	negative

A Pauly Reaction
B Diazotised *p*-Nitroaniline
C Diazotised Diethylaminoethyl *p*-aminophenylsulphone
D 1-Nitroso 2-naphthol/Nitric Acid Reaction

The use of differential detecting agents is of value in the detection of these substances. By the use of the Pauly reaction, at least 5 μg of each of these substances can be detected on paper chromatograms. This reaction can also be used for approximate determination, using the technique previously described (Tompsett, 1961). 5, 10, 20, 40 and 80 μg represent a useful range of standards. When positive, the 1-nitroso 2-naphthol-nitric acid reaction can detect 5 μg of these substances on paper chromatograms. Approximate determination can be made by comparison against a series of standards, 5, 10, 20, 40 and 80 μg representing a useful range. Amongst the phenolic acids, only *p*-hydroxyphenylacetic acid gives this reaction.

It has been suggested (Kolzelka & Hine, 1943) that phenytoin is also metabolised to α-aminodiphenylacetic acid (10 to 27%). In this investigation, no evidence to support this could be found.

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