0·2 mm was obtained by reducing a millimetre scale photographically. The areas (calculated using the formulae $A = \pi r^2$ and $A = \pi ab$, respectively) were then plotted against the corresponding concentrations of the standards. A new calibration must always be made when a new capillary or a new batch of membranes is used.

Fig. 2 shows the calibration curves of bovine serum, human γ-globulin and bovine serum albumin. The standard deviation of the mean $ts_{\bar{x}}$ was ±3 per cent ($P = 0{\cdot}01$). Within this limit each calibration curve was linear. Somewhat greater areas were found for whole serum compared with those for serum albumin or γ-globulin. This was probably due to the presence of various substances of lipoid character which might be adsorbed preferentially to the hydrophobic membrane, so that the proteins must migrate further to reach a free surface on which to absorb[1]. Some negligible inhomogeneity occurred at the start but was caused mainly by particles originating either from the sample or from the buffer or the paper wick.

Determinations of even lower protein concentrations were also possible when greater volumes of sample were applied. On the other hand, smaller volumes down to about 0·1 μl. could be applied with a suitable capillary. The areas of the protein spots were found to be proportional to the total amount of the protein regardless of its concentration in the given sample. Various concentrations of buffer in the range from 0·1 to 1 molar acetate did not influence the determination significantly. Other types of nitrocellulose membranes (*VUFS*, *AUFS*, *RUFS*) were also tested, but with less satisfactory results[1,4].

The method described here seems to offer the possibility of replacing methods in many cases more laborious, expensive or time consuming. Thus, it is suitable either for various microdeterminations[5] or for routine protein determinations; for example in the clinical laboratory and especially in emergency situations. It can also be used in following the course of various column fractionations of proteins with a minimal loss of material. Since different proteins behaved in a similar way to serum proteins during chromatography on nitrocellulose[1], it seems reasonable to assume a wider applicability of this simple and rapid technique.

Further experiments concerning this method, its applications and limitations will be published later.

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Metabolism of Oestrone and Oestradiol-17β in Human Liver *in vitro*

SINCE the discovery of the so-called "newer" oestrogens by Marrian *et al.* and by Gallagher and Fishman *et al.*[1], much work has been directed towards an explanation of their intermediary metabolism in human liver tissue[2]. In contrast, little information is available on the metabolism of the three "classical" oestrogens—oestrone, oestradiol-17β and oestriol—in human liver *in vitro*. Thus, Ryan and Engel[3] found a reversible transmutation of oestrone and oestradiol-17β in human liver tissue slices; Engel *et al.*[4] reported the formation of oestriol, 16-*epi*-oestriol and oestrone after incubation of oestradiol-17β by human foetal liver, and Breuer *et al.*[5], using a similar system, identified 6-hydroxyoestradiol-17β and oestriol as metabolites of oestradiol-17β. In view of the significance of oestrone and oestradiol-17β as precursors of the "newer" oestrogens, it seemed that a more intensive search for the metabolites of the two oestrogens in human liver tissue was justified. Accordingly, the present investigation was undertaken.

In a typical experiment, 100 mg of tissue slices from normal human liver were incubated for 60 min at 37° C with 2 μc. of [4-^{14}C]-oestrone or 2 μc. [4-^{14}C]-oestradiol-17β in 3 ml. of Krebs phosphate saline (*p*H 7·4), containing 20 mmoles of glucose/l. The incubation medium and the tissues were extracted twice with equal volumes of an ether–chloroform mixture (3 : 1 v/v), the extracts combined and evaporated to dryness. The metabolites formed from oestrone and oestradiol-17β were identified in the following way: (1) Paper chromatography in at least two of the systems formamide-monochlorobenzene, formamide-chloroform, formamide-monochlorobenzene–ethyl acetate (3 : 1), formamide-chloroform–ethyl acetate (5 : 1), or benzene–methanol–water (100 : 55 : 45); (2) reduction with sodium borohydride, removal of the elements of water and/or acetylation; (3) paper chromatography of the acetates in the systems propylene glycol–hexane or propylene glycol–heptane. By these methods, the radiochemical purity of the oestrogen metabolites was sufficiently established to enable definite identification.

Table 1. METABOLITES IDENTIFIED AFTER INCUBATION OF OESTRONE AND OESTRADIOL-17β WITH TISSUE SLICES OF NORMAL HUMAN LIVER

Steroid incubated	Metabolite formed	Identified as	Yield (per cent)
Oestrone	*N*-1	6α-Hydroxyoestriol	1·1
	N-2*b*	Oestriol	1·0
	N-3	16-*epi*-Oestriol	0·6
	N-4	7α-Hydroxyoestradiol-17β	1·5
	N-5	16α-Hydroxyoestrone	0·8
	N-7	Oestradiol-17β	1·6
	N-9	2-Methoxyoestrone	0·4
Oestradiol-17β	*L*-1	6α-Hydroxyoestriol	1·5
	L-2*a*	6α-Hydroxyoestradiol-17β	1·1
	L-2b_1	15α-Hydroxyoestradiol-17β	0·3
	L-2b_2	Oestriol	1·0
	L-3	16α-Hydroxyoestrone	0·8
	L-6	Oestrone	—

The results obtained after incubation of oestrone and oestradiol-17β with human liver slices are summarized in Table 1. As can be seen, the following reactions were found to occur in liver tissue of man: hydroxylations at positions 2, 6α, 7α, 15α, 16α and 16β, methylation of the 2-hydroxy group and oxidoreduction of the 17-hydroxy and 17-oxo-group.

There can be little doubt that the compounds formed from oestrone and oestradiol-17β are further metabolized; therefore, the yields in Table 1 may not represent the true rates of production of the oestrogen metabolites. Moreover, the amount of 6α-hydroxyoestriol was probably much higher, because only a small fraction of the steroid is extracted with ether-chloroform from an aqueous phase[6]. For these reasons, no definite conclusions can be drawn from results given in Table 1 with respect to the concentration of the various oestrogen metabolites in human urine.

Phenolic steroids substituted at positions 2, 16α, 16β and 18 have been isolated from the urine of pregnant women and, recently, 15α-hydroxyoestrone was isolated from the same source[7]. In view of the present findings, it seems reasonable to assume that 6α- and 7α-hydroxylated oestrogens are also present in human urine.

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