Accuracy was assessed by adding known quantities of each oestrogen (50–2500 pg) separately to 2.5 ml samples taken from a pool of male plasma and determining the masses of each oestrogen present. The equation of the regression line for the recovery of oestradiol-17 β was $y = 1.03x + 24$ (endogenous concentration, determined separately, 33 pg/2.5 ml) and for the recovery of oestrone was $y = 0.99x + 79$ (endogenous concentration 74 pg/2.5 ml). No interference by one oestrogen in the determination of the other was detected.

A high degree of specificity was achieved by a combination of the insensitivity of the cytosol binding 'protein' to non-oestrogenic steroids (Korenman, 1969; Corker & Exley, 1970; and our observations) and selective chromatography.

Application of the method to the determination of oestrone and oestradiol-17 β throughout the menstrual cycle will be presented.

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The Intracellular Distribution of Adrenal 3 β -Hydroxy Steroid Dehydrogenase and 3-Oxo Steroid Δ^4 – Δ^5 -Isomerase Studied with the Zonal Rotor

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The conversion of pregnenolone into progesterone during steroid biosynthesis in the adrenal cortex requires two enzymes, an NAD⁺-dependent 3 β -hydroxy steroid dehydrogenase and a 3-oxo steroid Δ^4 – Δ^5 -isomerase. It is well established that these enzymes are located in the microsomal fraction obtained by differential centrifugation after tissue homogenization (Beyer & Samuels, 1956). The observed association of dehydrogenase and isomerase activities with the mitochondrial fraction has been attributed to contamination by microsomal enzymes (for discussion see Hayano *et al.* 1956). This view has recently been questioned and the possibility of a bimodal distribution of these enzymes in the adrenal cell has been suggested (McCune *et al.*, 1970). Experiments involving rate-zonal centrifugation of a crude mitochondrial preparation on sucrose gradients in the HS zonal rotor (Measuring and

Scientific Equipment Ltd.) have now indicated that the dehydrogenase and isomerase are firmly bound to the mitochondria, as well as to the endoplasmic reticulum, of the bovine adrenal cortex.

The crude mitochondrial fraction prepared from 5 g of bovine adrenal cortex by conventional differential centrifugation was loaded on to a continuous sucrose density gradient (10–55%, w/v) linear with rotor radius. Separation was allowed to proceed for 45 min at 7500g, after which 15 ml fractions were collected and centrifuged at 24000g for 15 min. The pellets were assayed for marker enzymes as follows: for mitochondria, cytochrome oxidase, succinate dehydrogenase, steroid 11 β -hydroxylase and cholesterol side-chain-cleavage enzyme system; for microsomal fraction, 5'-nucleotidase and steroid-21-hydroxylase; for lysosomes, acid phosphatase. In addition, the protein and cholesterol contents of each fraction were determined. Certain fractions were also examined by electron microscopy.

Biochemical and electron-microscopic examination of the fractions show the microsomal particles to be concentrated at the light end of the gradient, whereas the mitochondria sediment as a broad band through the gradient. The width of this band reflects considerable variation of mitochondrial size.

A comparison of the distribution of dehydrogenase and isomerase enzymes with that of the marker enzymes revealed that the enzymes responsible for the conversion of pregnenolone into progesterone are firmly associated with mitochondria and microsomal fraction. The significance of this observation in relation to pathways of steroid biosynthesis in the adrenal cortex will be discussed, together with the advantages of the zonal rotor for preparing mitochondria with minimal microsomal contamination.

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Phosphorylation of the Erythrocyte Membrane: Stimulation by Adenosine 3':5'-Cyclic Monophosphate

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Cyclic AMP (adenosine 3':5'-cyclic monophosphate) is thought to be implicated in the control of cation permeability of membranes. It is possible