CORTISOL METABOLISM IN THE SHEEP

(Romney breed)

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science

by

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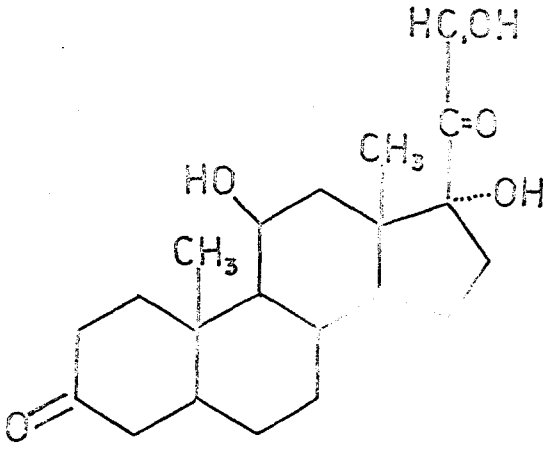
ABSTRACT

The metabolism of cortisol in the normal Romney ewe was investigated by analysis of the radioactive metabolites excreted in the urine following intravenous (I.V.) administration of 4-c^{14}cortisol. The metabolite glucuronides were hydrolysed with b-glucuronidase and extracted from the aqueous medium with ethyl acetate. The neutral fraction was divided into c-19 and c-21 metabolites by sequential elution from a florisil column. Extensive use was made of T.L.C. for the separation and analysis of each fraction before the quantitation of individual components.

A series of experiments was performed with surgically modified sheep involving collection of bile and urine both after I.V. injection of 4-c^{14}cortisol, and after intraduodenal infusion of radioactive biliary metabolites obtained from I.V. administration of 4-c^{14}cortisol. The metabolites collected at each stage were analysed both qualitatively and quantitatively.

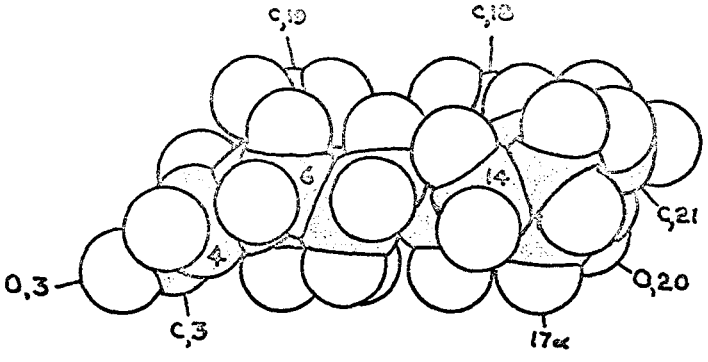
The urine collected each hour for 18 consecutive days from a normal sheep, was subjected to colorimetric determination for a-ketol and 17-ketogenic steroid content. The data obtained was analysed for diurnal variation in chromogen output, and the daily secretion rate of cortisol was estimated.

Fig 0.1a



CORTISOL

Fig 0.1 b



CORTISOL (side view)

NOMENCLATURE

Nomenclature used throughout this script is based on that evolved from the Ciba Conference in London (1950) and is generally in accordance with that approved by the International Union of Pure and Applied Chemistry (I.U.P.A.C.-see Biochemistry (Wash.) 8, 2227 (1969).

The steroid skeleton is numbered as shown in fig.0.2 . For compactness the terms Androstan- and Pregnan- are reduced to A- and P-, respectively. Double bonds in the parent structure are positioned by the suffix -ene-, eg. P-4-ene-. The configuration of the hydrogen at c-5 is indicated by a prefix 5a- or 5b-. The prefix allo- when used, refers to a 5a- configuration (fig. 0.2).

Substituents are prefixed by numerical position and Greek letter (when pertinent), but numerating particles are omnitted. Substituents attached to the nucleus on the same side as the angular methyl groups at c-10 and c-13, are designated b- and drawn with a heavy line; those below the plane of the ring are a- and drawn with a broken line (fig. 0.1).

TERMINOLOGY & ABBREVIATIONS

ACTH adrenocorticotrophic hormone

Adrenocortical steroid, corticosteroid - steroid from adrenal cortex, usually a c-21.

B.T.Z. blue tetrazolium (chloride)

c-19 steroid with no side chain at position 17.

c-21 steroid with a two carbon side chain.

Conjugate formed by esterification with a small molecule eg. glucuronic acid (glucuronide).

C.R.F. corticotrophin releasing factor.

EtAc ethyl acetate

EtOH ethanol

Glycerol side chain - $=C,OH-CH,OH-CH_2OH$

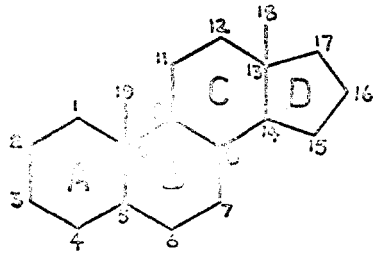
a-ketol side chain - $-CO-CH_2OH$

17 ketogenic - c-21 corticosteroid attacked by mild oxidising agents to give a 17-ketosteroid.

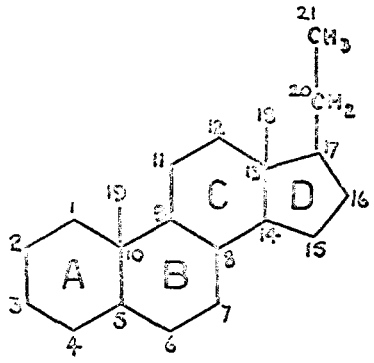
17 ketosteroid - steroid with a ketone on carbon-17.
Kg kieselghur
SG silica gel
Steroid nucleus - structure based on the cyclopentano--phenanthrene skeleton with c-10 and c-13 methyl groups
Tetrahydro derivative by complete hydrogenation of ring-A
T.L.C. thin layer chromatography.
T.M.A.H. tetramethyl ammonium hydroxide.

Trivial Name	Abbreviation	Mol. formula
cortisol	F	P-4-ene-11b,17a,21-ol-3,20-one
urocortisol	THF	5b-P-3a,11b,17a,21-ol-20-one
allotetrahydrocortisol	allo"	5a-P-3a,11b,17a,21-ol-20-one
cortisone	E	P-4-ene-17a,21-ol-3,11,20-one
urocortisone	THE	5b-P-3a,17a,21-ol-3,20-one
cortol	-	5b-P-3a,11b,17a,20a,21-ol
b-cortol	-	5b-P-3a,11b,17a,20b,21-ol
cortolone	-	5b-P-3a,17a,20a,21-ol-11-one
b-cortolone	-	5b-P-3a,17a,20b,21-ol-11-one
11-OH-Androsterone	11OHA	5a-A-3a,11b-ol-17-one
11-keto-Androsterone	11KA	5a-A-3a-ol-11,17-one
11-OH-Etiocholanolone	11OHE	5b-A-3a,11b-ol-17-one
11-keto-Etiocholanolone	11KE	5b-A-3a-ol-11,17-one
corticosterone	B	P-4-ene-11b,21-ol-3,20-one
deoxycorticosterone	11DOC	P-4-ene-21-ol-3,20-one
dehydroepiandrosterone	DHEA	A-5-ene-3a-ol-17-one
testosterone	-	A-4-ene-17b-ol-3-one

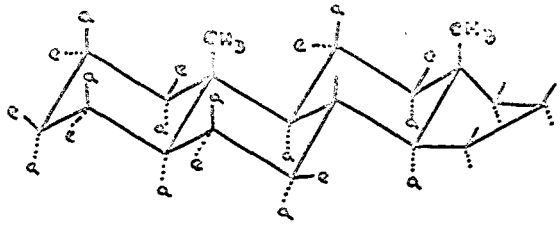
Fig 0.2



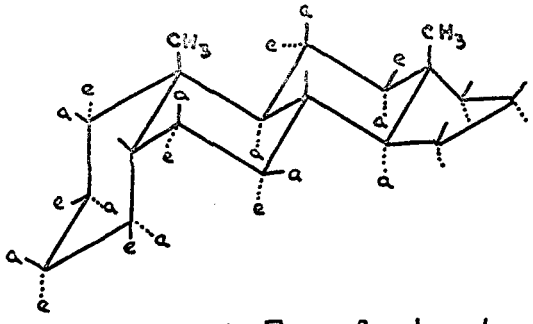
(a) Androstane



(b) Pregnane



(c) 5α-



(d) 5β-Androstane

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