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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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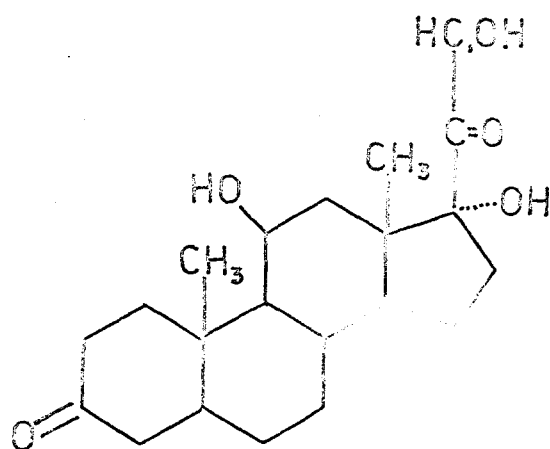
ABSTRACT

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-c¹⁴cortisol. The metabolite glucuronides were hydrolysed with β -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-c¹⁴cortisol, and after intraduodenal infusion of radioactive biliary metabolites obtained from I.V. administration of 4-c¹⁴cortisol. The metabolites collected at each stage were analysed both qualitatively and quantitatively.

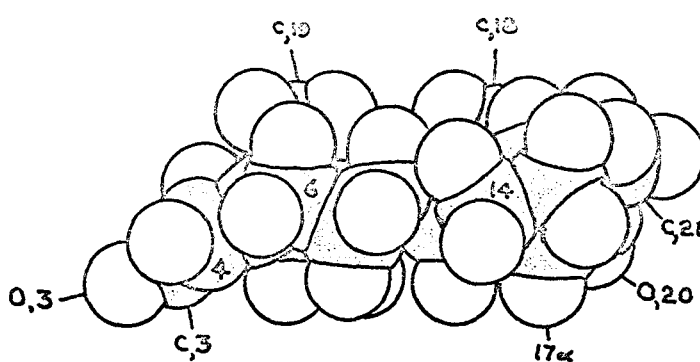
The urine collected each hour for 18 consecutive days from a normal sheep, was subjected to colorimetric determination for α -ketol and 17-ketogenic steroid content. The data obtained was analysed for diurnal variation in chromogen output, and the daily secretion rate of cortisol was estimated.

Fig 0.1a



CORTISOL

Fig 0.1 b



CORTISOL (side view)

NOMENCLATURE

Nomenclature used throughout this script is based on that evolved from the Giba 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 omitted. 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 ₂ OH
α-ketol side chain	- -CO-CH ₂ OH
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 kieselgur

SG silica gel

Steroid nucleus - structure based on the cyclopentano-
-phenanthrene skeleton with c-10 and c-13
methyl groups

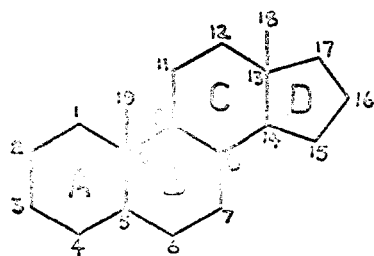
Tetrahydro derivative by complete hydrogenation of ring-A

T.L.C. thin layer chromatography.

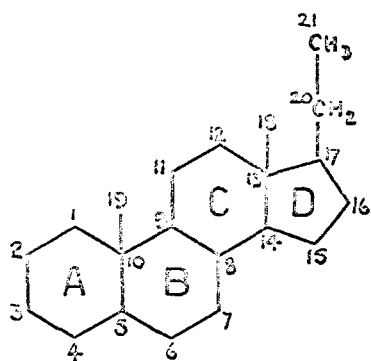
T.M.A.H. tetramethyl ammonium hydroxide.

<u>Trivial Name</u>	<u>Abbreviation</u>	<u>Mol. formula</u>
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

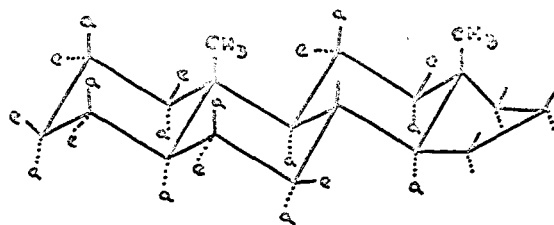
Fig 0.2



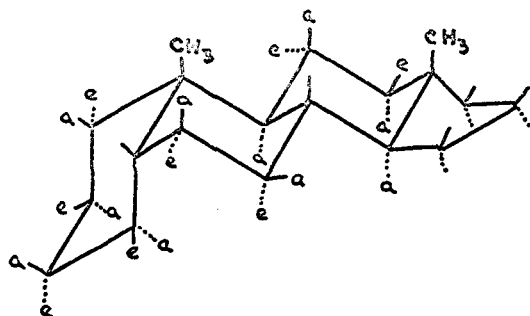
(a) Androstane



(b) Pregnane



(c) 5 α -



(d) 5 β - Androstane

TABLE OF CONTENTS

Abstract	page
Nomenclature	iii
Terminology and Abbreviations	iv
Chapter	
1 Review of Literature	
1.1 Metabolic effects of cortisol	1
1.1.1 Glucocorticoid properties of cortisol	1
1.1.2 Mineralocorticoid properties of cortisol	7
1.2 The adrenal cortex	8
1.2.1 Chemistry and anatomy	8
1.2.2 Nature of adrenocortical secretion	10
1.2.3 Rate of adrenal hormone secretion	13
1.2.4 Biosynthesis of corticosteroids	15
1.2.5 Cyclic secretion from the adrenal	15
1.3 Chemistry of the corticosteroids	17
1.3.1 Derivation of the corticosteroid structure	17
1.3.2 Structural features of the adrenal steroids	19
1.3.3 Stereochemistry of biological activity	21
1.4 The metabolism of cortisol	22
1.4.1 Reduction in ring-A	23
1.4.2 Side chain cleavage	27
1.4.3 Reduction of the 20-ketone	28
1.4.4 Hydroxylation	29
1.4.5 The cortisol-cortisone equilibrium	30
1.4.6 Reduction of 17-ketones	32
1.5 Steroid conjugation	33
1.6 Steroid-protein association in blood	36
1.7 Ultimate distribution	40
2 Materials and Experimental Methods	
2.1 Materials	42
2.2 Preparation and care of experimental sheep	42
2.3 Collection and preservation of samples	43
2.4 Extraction of steroid conjugates	43
2.5 Hydrolysis of steroid conjugates	44
2.5.1 Assay of b-glucuronidase	44
2.5.2 Optimum pH of paua b-glucuronidase	45
2.5.3 Protein precipitation from paua stomach	45
2.5.4 Partial purification of paua b-glucuronidase	46
2.5.5 Partial purification of limpet b-glucuronidase	47
2.5.6 Enzymic hydrolysis of conjugates	47
2.5.7 Acid hydrolysis of conjugates	49

2.6	Extraction of neutral free steroid from aqueous solution	50
2.7	Steroid separation techniques	51
2.7.1	Column chromatography	51
2.7.2	Thin layer chromatography	53
2.7.3	Detection of corticosteroids on thin layer plates	57
2.8	Methods of corticosteroid analysis	59
2.8.1	Recording of I.R. and U.V. spectra	59
2.8.2	Chemical modification of the corticosteroids	60
2.8.3	Colorimetric methods	64
2.9	Radiochemical Procedures	66
2.9.1	Radioactive materials	66
2.9.2	Quantitative determination of radiochemicals	66
3	Experimental and Results	69
3.1	The -3,17-ol-11-one group	70
3.1.1	Characterisation of c-19-6, c-19-8	70
3.1.2	Characterisation of c-19-9	73
3.1.3	Characterisation of c-19-7	74
3.2	The -3,11,17-ol group	75
3.2.1	Characterisation of c-19-2, c-19-3	76
3.2.2	Characterisation of c-19-1, c-19-4	77
3.2.3	Characterisation of c-19-5	77
3.3	The 17-ketosteroid group	78
3.4	Quantitation of the c ¹⁴ -19-metabolites	79
3.5	Characterisation of the c-21-B series	81
3.6	Characterisation of the c-21-A series	83
3.7	Quantitation of c ¹⁴ -21-metabolites	85
3.8	Investigation into the pathways of cortisol metabolite excretion	85
3.8.1	Expt.-1 Urinary excretion of c ¹⁴ -F metabolites in whole sheep	86
3.8.2	Expt.-2 Excretion of c ¹⁴ -ketosteroid in bile and urine	87
3.8.3	Pathways of cortisol excretion	88
3.9	Investigation of diurnal variations in urinary metabolite excretion	90
3.9.1	Quantitation of B.T.4. chromogen	91
3.9.2	Quantitation of Zimmerman chromogen	92
4	Discussion	
4.1	Hydrolysis of steroid conjugates	93
4.2	Excretion of steroid conjugates	95

4.3 Extraction of free steroid	95
4.4 Chromatographic analysis of corticosteroids	96
4.5 Route of disposal of cortisol	97
4.6 Metabolism of cortisol in whole sheep	99
4.7 Metabolism of cortisol in surgically modified sheep	102
4.8 Methods of colorimetric estimation of corticosteroids	106
4.9 Colorimetric estimation of 17-OH-CS in the sheep ^{in the sheep}	108
4.10 Cortisol production rate in the sheep	111
4.11 Diurnal variations of corticosteroid metabolites in sheep urine	111
Summary	116
Literature cited	119

LIST OF TABLES

	page
1-1 Cortisol secretion rates	14a
1-2 Plasma corticosteroid binding capacity . . .	37a
1-3 Distribution of total plasma cortisol . . .	37a
2-1 Extraction of conjugates from urine . . .	45a
2-2 Elution characteristics of florisil . . .	52a
3-1 List of c-19 metabolites (whole sheep) . . .	69a
3-2 Digitonin precipitation of c-19-6,8 . . .	72a
3-3 Borohydride reduction of c-19-6,8 . . .	72a
3-4 Borohydride reduction of c-19-8,9 . . .	73a
3-5 AR_{Mr} values for 11=O reduction . . .	73a
3-6 Borohydride reduction of c-19-7 . . .	73a
3-7 T.L.C. of c-19-2,3	76a
3-8 T.L.C. of c-19-2	76a
3-9 CrO_3 oxidation of c-19-2,3	78a
3-10 Comparison of c-19-7,8 with c-19-2,3 . . .	77a
3-11 T.L.C. of c-19-1,4	77a
3-12 R_M and AR_M values for 17b-17a epimerisation	78a
3-13 T.L.C. of 17-ketosteroids	78a
3-14 CrO_3 oxidation of 17-ketosteroids . . .	78b
3-15 Borohydride reduction of 17-ketosteroids . .	78b
3-16 T.L.C. of reduced 17-ketosteroid . . .	78b
3-17 T.L.C. of 2,4-dimethyl derivatives . . .	78b
3-18 Quantitation of c-21 metabolites . . .	79b
3-19 T.L.C. of acetylated cortols	84a
3-20 Mild oxidation of c-21 fraction	84a
3-21 AR_M values for 11=O reduction	84a
3-22 Urinary excretion of I.V.-cortisol . . .	87a
3-23 Excretion of ketosteroid	87a
3-24 Excretion of F in modified sheep	87a
3-25 Quantitative analysis of experiment-3 . . .	89a
4-1 Diurnal excretion of ketonic chromogen in the cow	112

LIST OF FIGURES

	page
0.1 Structure of cortisol	iiia
0.2 Nomenclature of steroids	va
1.1 Biosynthesis of cortisol	15a
2.1 Optimum pH of paau b-glucuronidase activity	45a
2.2 Protein precipitation from paau stomach .	45b
2.3 Methanolic fractionation of b-glucuronidase	46a
2.4 Hydrolysis of steroid conjugate - buffer .	48a
2.5 Hydrolysis of steroid conjugate - urine .	48b
2.6 Extraction of free steroid	50a
2.7 Florisil column fractionation (2% MeOH). .	52b
2.8 " " " (2.5% MeOH) .	52c
2.9 " " " (5% MeOH). .	52d
2.10 Fractionation of 4-c ¹⁴ -F metabolites . .	52e
2.11 Bismuthate side chain cleavage	63a
3.1 Scan of c-19 A.L.C. separation	69b
3.1a Scan of 17-ketosteroid separation . . .	69c
3.2 Autoradiograph of c-19 A.L.C. separation .	69d
3.3 Separation of c-19 fraction into components	79a
3.4 Scan of c-21 separation by T.L.C. . . .	81a
3.4a Autoradiograph of c-21 A.L.C. separation .	81b
3.5 Quantitation of c-21 metabolites . . .	79b
3.6 a,b,c,d, - scan of IVB, IVU, IFB, IFU T.L.C. chromatograms respectively .	89b-
3.7 Diurnal excretion of a-ketol	91a
3.8 Diurnal excretion of 17-KGS	92a
3.9 Urine volume variations	92b
4.1 Comparison of a-ketol and 17-KGS excretion .	114a
4.2 Diurnal excretion of reducing corticoids in rams	114b
Overall metabolism of cortisol in the sheep	118a