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MEASUREMENT OF OVINE PLASMA ANDROGENS BY
A COMPETITIVE PROTEIN BINDING METHOD

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

by

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ABSTRACT

A method has been developed for measuring plasma testosterone in sheep using competitive protein binding (CPB) techniques. The procedure requires column chromatography on LH-20 Sephadex gel of a methylene chloride extract of plasma, and final determination of plasma testosterone by the CPB technique using salt precipitation of the protein bound fraction. The sensitivity of the assay has allowed application to the measurement of plasma testosterone levels in normal male, normal female, Klinefelter and freemartin sheep. It has also enabled the monitoring of the effect of stimulation - with intravenous pregnant mare serum gonadotrophin and human chorionic gonadotrophin (HCG) - on the plasma testosterone levels of normal male and Klinefelter sheep. A modification to the method, borohydride reduction of the initial methylene chloride extract, has enabled the plasma androstenedione levels to be determined, simultaneously with plasma testosterone, in two of the HCG stimulation studies.

NOMENCLATURE

Trivial names have been used throughout the text for most steroids. These trivial names together with the systematic names according to the International Union of Pure and Applied Chemistry - I.U.P.A.C. - see Biochemistry (Wash.) 8, 2227 (1969) are listed in the section on terminology and abbreviations.

TERMINOLOGY AND ABBREVIATIONS

CPB	competitive protein binding
T- ³ H	testosterone- ³ H, tritiated testosterone
TLC	thin layer chromatography
PC	paper chromatography
CC	column chromatography
TPNH	triphosphopyridine nucleotide, reduced form
PMSC	pregnant mare serum gonadotrophin
HCG	human chorionic gonadotrophin
FSH	follicle stimulating hormone
LH	luteinising hormone
17-ketosteroid	steroid with a ketone on carbon-17
CPM (cpm)	counts per minute
IU	international units
ng	nanogram
μg	microgram

<u>Trivial Name</u>	<u>Systematic Name</u>
Androstenediol	5 α -Androstan-3 β , 17 β -diol
Androstenedione	4-Androsten-3, 17-dione
Androsterone	5 α -Androstan-3 α -ol-17-one
Cholesterol	5-Cholesten-3 β -ol
Dehydroepiandrosterone (DHEA)	3 β -Hydroxy-5-androsten-17-one
Dihydrotestosterone	17 β -Hydroxy-5 α -androstan-3-one
Estradiol-17 β	1,3,5(10)-Estratrien-3,17 β -diol
17 α -hydroxypregnenolone	3 β ,17 α -Dihydroxy-5-pregnen-20-one
Lanosterol	5 α -Lanosta-8,24-dien-3 β -ol
Pregnenolone	3 β -Hydroxy-5-pregnen-20-one
Progesterone	4-Pregnene-3,20-dione
Squalene	2,6,10,14,18,22-Hexamethyl-2,6, 10,14,18,22-tetracosahexane
Testosterone	4-Androsten-17 β -ol-3-one

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