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Studies Relating to the Ovarian Monitor

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

This thesis is dedicated to

Mervyn George Blackwell

Abstract

Hormonal data contained on the Melbourne Women's Hospital Menstrual Cycle Database and data collected in the Palmerston North Centre of the World Health Organisation trial were analysed and compared. The analysis of the two sets of data showed that the utilisation of a threshold excretion rate for urinary Pregnanediol or Pregnanediol Glucuronide of 7 μmol 24 hr^{-1} was an acceptable marker for the end of the fertile period. The data collected by the women participants in the World Health Organisation Trial also showed that the Ovarian Monitor, a home fertility test, provided the most simple, comprehensive and accurate marker of fertility status available.

Lysozymes from several sources were examined as possible replacements for the hen egg white lysozyme in the Ovarian Monitor as a means of reducing the Estrone Glucuronide assay time. Unfortunately, although they were all found to possess a faster initial rate, the clearing curves were also more biphasic making them unsuitable for use in the current end-point assay. These differences were attributed to the presence of electrostatic fields on both the enzyme and the substrate. However, the human lysozyme obeyed second order kinetics for a significant percentage of the twenty minute clearing curve. Thus, the Estrone Glucuronide assay time could be significantly reduced by adapting the Ovarian Monitor to linearise the human lysoyzme clearing curve with an appropriate algorithm.

Human lysozyme is very expensive thus, it was necessary to optimise conjugation condition for Estrone Glucuronide using the more economical hen egg white lysozyme. Also, chromatographic conditions for conjugate purification had to be established before the human lysozyme could be conjugated and the viability of the above proposal could be tested.

Both the mixed anhydride and active ester conjugation methods were optimised. The most effective purification scheme involved pH 4.3 phosphate buffers using a Mono-S column followed by an Alkyl Superose column.

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Merry Christmas!!

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Abbreviations

α	Smoothing Constant
A_{280}	Absorbance at 280 nm
Abs	Absorbance
BBT	Basal Body Temperature
BIP	Basic Infertility Pattern
bp	Base Pairs
CM	Carboxy-methyl
c type	Chick Type
DCC	Dicyclohexylcarbodiimide
DCU	Dicyclohexylurea
DMF	Dimethylformamide
DMSO	Dimethylsulphoxide
E_o	Total Enzyme Concentration
e	Charge on an electron
E. coli	*Escherichia coli*
E1G	Estrone Glucuronide
E1G-(H)	Estrone Glucuronide (acid form)
E1G-(Na)	Estrone Glucuronide (Na form)
EDTA	Ethylenediamine Tetra-acetic Acid
ESA	Exponentially Smoothed Average
FE	Forecast Error
FPLC	Fast Protein Liquid Chromatography
FSH	Follicle Stimulating Hormone
GEWL	Goose Egg White Lysozyme
GLC	Gas Liquid Chromatography
g type	Goose type
HEWL	Hen Egg White Lysozyme
HuL	Human Lysozyme
Hz	Hertz
IBC	Isobutylchloroformate

I.D.	Internal Diameter
IEP	Iso-electric Point
k	Boltzmann's constant
kb	Kilobase Pairs
LB	Luria-Bertani Broth
LH	Luteinising Hormone
MAD	Mean Absolute Deviation
M. lysodeikticus	*Micrococcus lysodeikticus*
NAG	N-acetylglucosamine
NAM	N-acetylmuramic Acid
NFP	Natural Family Planning
NHS	N-hydroxysuccinimide
NMR	Nuclear Magnetic Resonance
PAGE	Polyacrylaminde Gel Electrophoresis
Pd	Pregnanediol
PdG	Pregnanediol Glucuronide
SD	Standard Deviation
SDS	Sodium Dodecyl Sulphate
SEn	Substrate Molecule with n Number of Lysozyme Molecules Attached
SFE	Smoothed Forecast Error
std. dev.	Standard Deviation
T	Absolute Temperature
T1	Initial Transmittance
T2	Final Transmittance
ΔT	Change in Transmission
T4L	T4 Phage Lysozyme
TAE	Tris-Acetate Buffer containing EDTA
TE	Tris-HCl Buffer containing EDTA or Total Estrogens
TEPDDE	Total Estrogen and Pregnandiol Data Entry
TEWL	Turkey Egg White Lysozyme
TS	Tracking Signal
TLC	Thin Layer Chromatography

TNB	Tri-n-butylamine
WHO	World Health Organisation