



Urinary excretion of estrogenic substances by the bovine in the estrous cycle
by Robert Kaye Bergman

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Dairy Production at Montana State College
Montana State University
© Copyright by Robert Kaye Bergman (1957)

Abstract:

The urine of sixteen open cows from the college herd was collected and analysed for estrogenic substances in terms of micrograms of estrone per hundred pounds of body weight. It was found that the urinary estrogen excretion of the cow which did not conceive was higher than that of the cows which did conceive. Method of analysis was a chemical extraction of the urine for the estrogenic substances and a measurement of the fluorescence of the urine extract compared with the fluorescence of a standard of estrone.

In order to gain greater accuracy in measuring only estrogenic substances of the urine extracts, samples of urine extracts were passed through a Celite chromatographic column. By passing pure crystalline estrone and estradiol through the Celite columns it was found that the estrone was eluted out in the first 10 milliliters of benzene and estradiol in the next 140 milliliters of benzene. The addition of crystalline estrone and estradiol to the urine of a bilaterally ovariectomized cow with subsequent extraction of the urine by the chemical process and passing 0.1 milliliter of urine extract through the Celite column gave the same recovery pattern. This was also true when the urine of cows in late pregnancy (250 days) was analysed by the same procedure.

The urine of eleven virgin heifers was extracted for estrogenic substances and chromatographed. In some cases half of the eluate was bioassayed with immature female rats and the results compared with the fluorimetric assay of the other half of the eluate. In all cases, injection of rats with extracts of eluates gave an estrogenic effect on a crude uterine weight basis. The coefficient of correlation between the two methods on estrone was 0.4483 while on estradiol it was -0.133. This difference in the correlations was probably due to the carry over of some estrone into the estradiol fraction. Estrone gives a higher fluorescence than does estradiol, but it is less potent in its estrogenic activity. Therefore, a small amount of estrone in the estradiol fraction would give a false high measurement of estradiol by fluorimetric assay which would not be proved out in bioassay.

The results of the fluorimetric assays of the urine from the heifers gave results quite similar to those of the cows. The estrogen excretion of the heifers which conceived was lower than was that of the heifers which did not conceive. These results are taken to support the theory that some of the infertility problems in dairy cattle are caused by hormone imbalances. It is felt that the high estrogen level in the cattle which did not conceive was partially responsible for preventing pregnancy.

1957

URINARY EXCRETION OF ESTROGENIC SUBSTANCES
BY THE BOVINE IN THE ESTROUS CYCLE

by

ROBERT KAYE BERGMAN

A THESIS

Submitted to the Graduate Faculty

in

partial fulfillment of the requirements


for the degree of


Master of Science in Dairy Production


at

Montana State College

Approved:


Head, Major Department


Chairman, Examining Committee


Dean, Graduate Division

Bozeman, Montana
June, 1957

RECEIVED
MONTANA STATE COLLEGE
BOZEMAN
JUN 19 1957

N378
B454u
cop. 2

1094

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS	4
LIST OF TABLES	5
ABSTRACT	6
INTRODUCTION	7
REVIEW OF LITERATURE	9
ISOLATION AND ASSAY OF ESTROGENS	9
DEFINITION OF ESTROGENS	9
STRUCTURE OF ESTROGENS	10
EXTRACTION OF ESTROGENS	11
METHODS OF BIOASSAY	12
CHEMICAL METHODS	15
METABOLISM OF ESTROGENS	16
FATE OF ESTROGENS	17
<u>IN VITRO</u> STUDIES ON TISSUE SLICES	18
METABOLISM IN THE LIVER	19
PROGESTERONE INTERACTION ON ESTROGEN DEACTIVATION	23
INTESTINAL METABOLISM	23
EFFECT OF NUTRITION	23
CARBON-TETRACHLORIDE FEEDING EFFECTS ON ESTROGEN METABOLISM	24
PHYSIOLOGICAL ACTIONS OF ESTROGENS	25
SEX ORGANS AND SECONDARY SEX CHARACTERISTICS	26
EFFECT OF ESTROGEN TO BRING ON "HEAT"	27

TABLE OF CONTENTS (CONTINUED)

VAGINAL CELLULAR CHANGES IN ESTROUS CYCLE	28
EFFECT OF ESTROGENS ON PREGNANCY	29
INFLUENCE OF ESTROGEN ON UTERINE MUSCLE	31
ESTROGEN AND MAMMARY DEVELOPMENT	32
ESTROGEN AND MAMMARY CANCER	32
ESTROGEN AND GROWTH	33
EXPERIMENTAL PROCEDURE	36
RESULTS	43
DISCUSSION AND CONCLUSIONS	56
SUMMARY	58
LITERATURE CITED	59

LIST OF ILLUSTRATIONS

<u>Figure</u>		<u>Page</u>
1	Chromotography of Estrone From Celite Chromatographic Columns	45
2	Chromotography of Esdradiol from Celite Chromatographic Columns	46
3	Chromotography of Estrone and Estradiol from Celite Chromatographic Columns	46
4	Chromotography of Estrone Added to Urine of Ovariectomized Cow. Urine Extract Added to Celite Chromatographic Column	47
5	Chromotography of Estradiol Added to Urine of Ovariectomized Cow. Urine Extract Added to Celite Chromatographic Column	48
6	Chromotography of Estrone and Estradiol Added to Urine of Ovariectomized Cow. Urine Extract Added to Celite Chromatographic Column	48
7	Fluorescence Obtained from Urinary Extracts Added to Celite Chromatographic Columns	49
8	Crude Uterine Weight Response Curve of Immature Female Rats Given Four Different Dosage Levels of Pure Crystalline Estradiol in Peanut Oil Injection	51
9	Crude Uterine Weight Response Curve of Immature Female Rats Given Three Different Dosage Levels of Pure Crystalline Estrone in Peanut Oil Injection	52

LIST OF TABLES

<u>Number</u>		<u>Page</u>
I	THE EFFECT OF INTERCHANGING THE GONADS BETWEEN THE TWO SEXES ON THE ULTIMATE BODY WEIGHT OF GUINEA PIGS	33
II	THE EFFECT OF OVARIAN IMPLANTS ON THE LENGTH OF BONES IN THE CASTRATED MALE GUINEA PIG	34
III	FLUORESCENCE PRODUCED BY URINARY EXTRACTS OBTAINED DURING THE ESTROUS CYCLE OF COWS THAT CONCEIVED	43
IV	FLUORESCENCE PRODUCED BY URINARY EXTRACTS OBTAINED DURING THE ESTROUS CYCLE OF COWS THAT FAILED TO CONCEIVE	44
V	COMPARISON OF THE RESULTS OF FLUORIMETRIC ASSAY WITH BIOASSAY ON URINE EXTRACTS OF VIRGIN HEIFERS WHERE ESTIMATED POTENCY OF INJECTION WAS MADE BY FLUORIMETRIC DETERMINATION	53
VI	FLUORESCENCE PRODUCED AFTER CHROMATOGRAPHIC SEPARATION OF URINARY EXTRACTS FROM VIRGIN HEIFERS WHICH CONCEIVED AFTER BREEDING	54
VII	FLUORESCENCE PRODUCED AFTER CHROMATOGRAPHIC SEPARATION OF URINARY EXTRACTS FROM VIRGIN HEIFERS WHICH DID NOT CONCEIVE AFTER BREEDING	55

