5-1968

5 \( \beta \)-Pregnane-3\( \alpha \)-20\( \alpha \)-Diol Excretion in Urine of University Women During the Menstrual Period

Rita Shin-hui Liao

*Utah State University*

Follow this and additional works at: [https://digitalcommons.usu.edu/etd](https://digitalcommons.usu.edu/etd)

Part of the Molecular, Genetic, and Biochemical Nutrition Commons

**Recommended Citation**

Liao, Rita Shin-hui, "5 \( \beta \)-Pregnane-3\( \alpha \)-20\( \alpha \)-Diol Excretion in Urine of University Women During the Menstrual Period" (1968). *All Graduate Theses and Dissertations*. 5118.

[https://digitalcommons.usu.edu/etd/5118](https://digitalcommons.usu.edu/etd/5118)

This Thesis is brought to you for free and open access by the Graduate Studies at DigitalCommons@USU. It has been accepted for inclusion in All Graduate Theses and Dissertations by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.
5β-PREGNANE-3α, 20α-DIOL EXCRETION IN URINE OF UNIVERSITY WOMEN DURING THE MENSTRUAL PERIOD

by

Rita Shin-hui Liao

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Nutrition and Biochemistry

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1968
ACKNOWLEDGEMENTS

Sincere appreciation is expressed to my major professor, Dr. Ethelwyn B. Wilcox, Head of the Food and Nutrition Department, for her able guidance on this research and for her assistance in the preparation of this manuscript. Appreciation is also expressed to Dr. Harris O. Van Orden, Professor of Chemistry, Dr. C. Anthon Ernstrom, Professor of Dairy Science, and Mrs. Flora H. Bardwell, Associate Professor of Food and Nutrition, for their many helpful suggestions and serving as committee members.

This study was part of the Western Regional Research Project on Biological Interrelationships in Lipid Metabolism of Importance to Man. It was supported in part by W-44 funds obtained under the Research and Marketing Act.

The author wishes to express her gratitude to all others who gave their assistance and encouragement and to thank those persons who acted as experimental subjects.

Rita Shin-hui Liao
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>vii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>REVIEW OF LITERATURE</td>
<td>4</td>
</tr>
<tr>
<td>History, chemistry, and isolation</td>
<td>4</td>
</tr>
<tr>
<td>Biosynthesis of the hormonal steroids</td>
<td>5</td>
</tr>
<tr>
<td>Source and metabolism of progesterone</td>
<td>7</td>
</tr>
<tr>
<td>Effect of progesterone</td>
<td>12</td>
</tr>
<tr>
<td>Effect of sex steroids on ovulatory mechanism</td>
<td>14</td>
</tr>
<tr>
<td>Pregnanediol—as a test for ovulation</td>
<td>15</td>
</tr>
<tr>
<td>Recent research on pregnanediol</td>
<td>16</td>
</tr>
<tr>
<td>Analytical methods</td>
<td>16</td>
</tr>
<tr>
<td>Urinary pregnanediol level</td>
<td>19</td>
</tr>
<tr>
<td>METHOD OF PROCEDURE</td>
<td>23</td>
</tr>
<tr>
<td>Experimental design</td>
<td>23</td>
</tr>
<tr>
<td>Collection and storage of urine sample</td>
<td>24</td>
</tr>
<tr>
<td>Collection</td>
<td>24</td>
</tr>
<tr>
<td>Storage</td>
<td>26</td>
</tr>
<tr>
<td>Analysis of urinary $5\beta$-pregnane-3$\alpha$, 20$\alpha$-diol</td>
<td>26</td>
</tr>
<tr>
<td>Method</td>
<td>26</td>
</tr>
<tr>
<td>Recovery</td>
<td>27</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>28</td>
</tr>
<tr>
<td>Urinary pregnanediol level</td>
<td>28</td>
</tr>
<tr>
<td>Effect of menstrual cycle</td>
<td>28</td>
</tr>
<tr>
<td>Effect of two meals vs. three meals per day</td>
<td>34</td>
</tr>
<tr>
<td>Values obtained for subject J. B.</td>
<td>34</td>
</tr>
<tr>
<td>Relationships among serum cholesterol and urinary pregnanediol, total estrogen, and neutral 17-ketosteroids</td>
<td>41</td>
</tr>
<tr>
<td>Dietary intake</td>
<td>44</td>
</tr>
<tr>
<td>CONCLUSIONS AND COMMENTS</td>
<td>48</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>50</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>52</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>59</td>
</tr>
<tr>
<td>Analysis of urinary $5\beta$-pregnane-$3\alpha, 20\alpha$-diol</td>
<td>60</td>
</tr>
<tr>
<td>Equipment</td>
<td>60</td>
</tr>
<tr>
<td>Reagents</td>
<td>61</td>
</tr>
<tr>
<td>Procedure</td>
<td>62</td>
</tr>
<tr>
<td>VITA</td>
<td>67</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table                                                                 Page
1. Urinary pregnanediol values reported in the literature .... 20
2. Experimental design ............................................. 25
3. Age, weight and height of subjects ............................... 25
4. Recovery test of urinary pregnanediol ............................ 27
5. Urinary 5β-pregnane-3α, 20α-diol excretion values for the individual subjects consuming two meals and three meals per day by day of menstrual cycle ........... 29
6. Urinary pregnanediol excretion values for individual subjects by day of menstrual cycle ......................... 30
7. Mean values of pregnanediol in urine of subjects consuming two meals and three meals per day by day of menstrual cycle ........................................ 39
8. The relationships among serum cholesterol and urinary pregnanediol, total estrogen and neutral 17-ketosteroids by day of menstrual cycle .................................. 42
9. Mean daily intake of certain nutrients of subjects on self-chosen diets .................................................. 45
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Biosynthesis of the sex steroids</td>
<td>6</td>
</tr>
<tr>
<td>2.</td>
<td>Metabolic precursors of pregnanediol and its metabolism</td>
<td>11</td>
</tr>
<tr>
<td>3.</td>
<td>The effect of day of menstrual cycle on mean excretion values of pregnanediol of five subjects</td>
<td>31</td>
</tr>
<tr>
<td>4.</td>
<td>Mean values of pregnanediol in urine of five subjects by days of menstrual cycle</td>
<td>33</td>
</tr>
<tr>
<td>5.</td>
<td>Urinary pregnanediol excretion values for subject A. M., on two meals and three meals per day</td>
<td>35</td>
</tr>
<tr>
<td>6.</td>
<td>Urinary pregnanediol excretion values for subject C. L., on two meals and three meals per day</td>
<td>36</td>
</tr>
<tr>
<td>7.</td>
<td>Urinary pregnanediol excretion values for subject D. W., on two meals and three meals per day</td>
<td>37</td>
</tr>
<tr>
<td>8.</td>
<td>Urinary pregnanediol excretion values for subject P. B., on two meals and three meals per day</td>
<td>38</td>
</tr>
<tr>
<td>9.</td>
<td>The effect of number of meals on mean excretion values of urinary pregnanediol for five subjects</td>
<td>40</td>
</tr>
<tr>
<td>10.</td>
<td>The relationships among the serum cholesterol and urinary pregnanediol, total estrogen, and neutral 17-ketosteroi d levels</td>
<td>43</td>
</tr>
</tbody>
</table>
ABSTRACT

5α-Pregnane-3α, 20α-diol Excretion in Urine of University Women During the Menstrual Period

by

Rita Shin-hui Liao, Master of Science
Utah State University, 1968

Major Professor: Dr. Ethelwyn B. Wilcox
Department: Nutrition and Biochemistry

Five normal healthy young university women while on self-chosen diets and living under normal conditions served as experimental subjects in this study. Urinary pregnanediol was measured for 10 days of the menstrual cycle. Meal frequency (3 meals vs. 2 meals with no breakfast) was also included in the study.

A modification of the method of Eberlein and Bongiovanni on thin layer chromatography was used to determine pregnanediol in the urine sample.

Urine pregnanediol excretion was in the pattern as investigated by other workers. The values rose in the latter half of the menstrual cycle, and fell prior to the onset of the next period.

No relationship was found between pregnanediol level and meal frequency.

A larger number of subjects are recommended in the further work of this problem.

(74 pages)
INTRODUCTION

The high incidence of coronary heart disease in humans of middle-age and older in the United States prompted research in the many areas which are known to or seem to have possibilities in helping to decrease the incidence of coronary heart disease and atherosclerosis. At least nine factors are known to be involved in the control and regulation of atherosclerosis. One of these, serum cholesterol and its metabolism, was one of the first factors to be intensively studied. Scientists soon learned that the serum content of cholesterol is easily measured and that the serum level could be influenced by changes in the diet. In the study of the metabolism of cholesterol, its role as a precursor of hormones and other steroids of physiological interest, has prompted a systematic investigation of normal and disordered human sterol metabolism in various laboratories.

The concept that atherosclerosis is a metabolic disorder (Adlersberg, 1951) involving chiefly lipids and lipoproteins has stimulated extensive investigation of the endocrine influences on circulating lipids and on lipid metabolism. Age and sex differences in the distribution of serum lipoproteins have been reported by various laboratories. Normal young women have a relatively greater cholesterol concentration in the serum \( \alpha \)-lipoprotein fraction, and correspondingly smaller amounts of cholesterol in the \( \beta \)-lipoprotein fraction than normal men of corresponding ages. This difference is not apparent
after the menopause (Adlersberg, 1958). Other evidence has accumulated demonstrating that myocardial infraction occurs much more frequently in men than in women, particularly under the age of forty (Katz et al., 1953; Barr, 1955).

Naturally occurring estrogens corrected the abnormal lipid and lipoprotein pattern of survivors of myocardial infraction (Eilert, 1949). It is not unreasonable, then, to suppose that the effects of estrogens on lipid metabolism may be of therapeutic value in coronary artery disease. Androgen, on the other hand, may have an adverse effect. Because the incidence of estrogenic side effects is high, particularly in middle-aged man (Russ et al., 1951; Oliver et al., 1956; Robinson et al., 1956; Steiner et al., 1956), investigators have employed estrogen-androgen combinations to avoid the undesirable estrogenic action, yet the studies to date have not been useful (Russ et al., 1955; Oliver et al., 1956; Adlersberg, 1957).

Another female hormone, progesterone, has more recently been the object of intensive studies. The urinary titers of this compound may be taken as an index of corpus luteum function. The chief catabolic metabolite of the progesterone is $5\beta$-pregnane-$3\alpha, 20\alpha$-dil. Recently, investigators are working in the areas of relationships between the urinary pregnanediol excretion and breast cancer (Stern et al., 1964; Kim, 1965; Marmorston et al., 1965; Schwegge et al., 1967), endometrial cancer (Kelley et al., 1965), lung cancer (Marmorston et al., 1967), and the sex of fetus (Rawlings et al., 1964). There is only limited information on the relationship between the level of progesterone and the concentration of serum cholesterol in normal adult people while living under normal conditions.
This study is a part of a larger problem to determine relationships that exist among serum cholesterol, serum fatty acids, and concentrations of estrogens and degradation products of progesterone and the androgen and adrenal steroid hormones in urine as influenced by dietary factors. Simultaneous studies of these factors in human subjects while living in their homes under normal conditions have been very limited. Therefore, analysis of these biochemical metabolites and a study of their relationships should give basic data on the normal individual while eating and living as usual. One phase of the larger study, the urinary pregnanediol excretion in normal healthy young university women, at different days of the menstrual period, was the objective of the following report. Five university women were selected to cooperate in this study. All subjects were maintained on self-chosen diets for which the calorie, carbohydrate, fat, and protein content was calculated. They were on two or three meals a day (with and without breakfast). A 24-hour urine sample and blood sample were collected on day 1, 4, 7, 9, 11, 13, 15, 17, 20, and 26 of the menstrual cycle and at day 1 of the next cycle.

Urine pregnanediol was estimated by a modification of the method of Eberlein and Bongiovanni on thin layer chromatography (Chiang, 1966).
REVIEW OF LITERATURE

History, Chemistry, and Isolation

In 1929, Corner and Allen prepared an extract of corpora lutea with nonpolar solvents that effectively maintained pregnancy in ovariectomized rabbits and that worked together with estrogen to produce a proliferated uterus suitable for implantation of the blastocyst. They found that the proliferative effect on the rabbit's uterus could be used as a bioassay for following the purification of the active material that led to the isolation of progesterone, the hormone of the corpus luteum. Pregnanediol, urinary metabolite of the corpus luteum hormone, was discovered by Marrian (1929) in human pregnancy urine. Since pregnanediol was quite inactive biologically, no indication of the identity of progesterone was forthcoming until it was actually isolated from ovarian tissue. Four groups of workers in 1934 obtained progesterone almost simultaneously by extraction of sows' ovaries, and its structure was determined in the same year.

Butenandt (1930) isolated the same compound as Marrian's and showed the correct formula to be $\text{C}_{21}\text{H}_{36}\text{O}_2$, and later he provided clear evidence for its structural relation to the bile acid and named it "Pregnanediol." Odell and Marrian (1936) showed that pregnanediol was present in urine as a watersoluble acid-hydrolysable conjugate. Also in 1936, Venning and Browne reported the isolation of pregnanediol glucosiduronide (as its sodium salt from
pregnancy urine). This discovery was of considerably importance since it led to the development by the discoverers themselves of the first method for the quantitative determination of pregnanediol in urine which enabled them to prove that pregnanediol is the main end product of progesterone metabolism (Venning, 1937; Venning and Browne, 1937). This early phase of the pregnanediol story was brought to an end in 1944, when the late Head and his co-workers at McGill (Heard, Hoffman, and Mack, 1944) proved by an elegant piece of work that in pregnanediol glucosiduronide, the glucuronic acid moiety is combined with pregnanediol through the C-3 hydroxy group of the latter.

**Biosynthesis of the Hormonal Steroids**

The biosynthesis of the steroid hormones by adrenal, ovary, and testis appear to proceed through essentially identical pathways (Figure 1). The adrenal cortex may be thought of as the best potential. It can synthesize all of the steroids produced by ovary and testis. A common pathway for the formation of the steroid hormones can be defined for the adrenal cortex and the gonads. Variations depend on the relative specialization indicated for these glands. Beginning with activated two carbon fragments of acetyl-coenzyme A, a series of NADPH (TPNH) dependent condensation reactions has been demonstrated which ultimately yields the linear C_{30} compound squalene. Through cyclization and elimination of three methyl groups, cholesterol is formed. The liver and most other tissue, as well as the steroid producing endocrine glands, can synthesize cholesterol. Cholesterol is the most important, and may indeed be the sole precursor of the steroid hormones.
Figure 1. Biosynthesis of the sex steroids. Enzyme systems required:
(1) 3β-ol dehydrogenase. (2) 19-hydroxylase. (Segre and Labotsky, 1964, p. 137)
The adrenal and gonads have specialized abilities to convert this molecule into the biologically active hormonal steroids (Lloyd, 1964).

The DPN-dependent $\beta$-hydroxysteroid dehydrogenase system of the adrenal cortex catalyzes the conversion of pregnenolone to progesterone. The same dehydrogenation is accomplished by the corpus luteum and the placenta. Since the testis and ovary also effect the conversion of cholesterol to pregnenolone and progesterone, the biosynthesis of the C-19 and C-18 steroid hormones from cholesterol by these five glands seems to be the initial pathway.

The concept that cholesterol is a precursor of the adrenal steroid hormones was observed early (Long, 1947). He showed that the amount of cholesterol in adrenal glands is markedly decreased when the production and release of hormones is stimulated. It had also been shown by Bloch (1945) that the administration of deuterium-labeled cholesterol to a pregnant woman gave rise to labeled pregnanediol in the urine, presumably, the administered cholesterol was converted in the placenta to progesterone, from which pregnanediol was then formed. Subsequent work demonstrated that adrenal tissue can convert $^{14}$C-labeled cholesterol to labeled progesterone (Bligh et al., 1955), as well as to corticosterone and cortisol (Hechter et al., 1953). The cholesterol is a more efficient precursor of the hormones than is acetate.

**Source and Metabolism of Progesterone**

Progesterone is the most potent natural progestogen and has been isolated from the corpora lutea and placenta. It is also an intermediate in the biosynthesis
of the adrenocortical steroids and the testicular androgens. The study of Davis and co-workers (1952) and Klopper and associates (1957) have shown that after injection of ACTH (adrenocorticotropic hormone) in women the excretion of pregnanediol increased slightly. It was concluded that the adrenal cortex may produce progesterone. Balfour and colleagues (1957) isolated relatively large amounts of progesterone from the adrenal vein of the sheep and cow. However, there is no reason to assume that the human adrenal cortex produces significant amounts of progesterone during pregnancy.

Small amounts of progesterone are thought to be secreted by the pre-ovulatory follicle and the titers rise markedly during the course of pregnancy (Short and Eton, 1959). Pregnanediol excretion in pregnant women rises progressively after the second or third month and falls precipitously after loss of the placenta (Venning and Browne, 1937). The indications are that the corpus luteum of the human ovary produces progesterone during early pregnancy, whereas an additional supply is secreted by the placenta after the second month. It was indicated by Pearlman (1954) that the progesterone level is at 1-1.5 mg per kg of placenta. The source of the progesterone during pregnancy apparently varies with the species (Lloyd, 1964).

The position of progesterone in the conversion of acetate and cholesterol to C_{19} steroids has been indicated previously in Figure 1. This provided an explanation for the formation of 17\(\alpha\)-hydroxyprogesterone, androst-4-ene-3,17-dione, and testosterone on incubation of progesterone with testicular, ovarian, adrenal, or placental tissues. These three products, therefore, can be considered
as derived from progesterone metabolism. In addition, progesterone may give
rise to estrone and estradiol, via testosterone (Drill et al., 1956; Dorfman,
1957). Formation of these estrogens has been demonstrated experimentally in
studies in which progesterone has been incubated in vitro with human ovarian
tissue (Soloman et al., 1956).

Progestrone, unlike the estrogens, is not present in human urine or
feces. Many experiments have shown that progesterone disappears very rapidly
from the blood (Haskins, 1950; Butt et al., 1951; Zander, 1954; Zarrow et al.,
1954; Hinsberg et al., 1956). It has not been clear whether this is due to rapid
elimination through partition into the tissues or through a true inactivation by
chemical transformation of the molecule (Lloyd, 1959).

A number of C-21 metabolites related to progesterone are present in
the urine, but none of these has any progestational potency, and its excretory
metabolites seem to vary with the species. The feces of pregnant cows contain
large amounts of androgen, but very insignificant amounts are found in the
feces of bulls and nonpregnant animals. It is possible that the placental
progesterone of the pregnant cow is converted to androgen and excreted
through the feces. Rabbit liver can convert progesterone to pregnanediol;
pregnane-3, 20-dione and pregnane-3α,5β-3-one are also formed. On the
other hand, rat liver appears to be unable to reduce the 20-keto group of
progesterone, but this reaction can occur in some other rat tissues, since
eviscerated rats convert progesterone to 4-pregnen-20α,5β-3-one (Wiest,
1956).
In human, more significant quantitatively as a metabolite of progesterone is pregnane-3α, 20α-diol, a reduction product formed from progesterone chiefly in the liver, where it is coupled with glucuronic acid to form the chief urinary metabolite of progesterone, pregnanediol glucosiduronate. Reduction of the C-20 ketone group of progesterone to the 20α- and ϒ-hydroxy isomers also occurs hepatically. In the kidney (Wiest and Berliner, 1954; 1956; Ganis et al., 1956), the end product may be directly excreted in the urine. Pregnanediol formed in the liver can enter the intestinal tract via the bile, or be carried in the circulation to the kidney and excreted in the urine, as shown in Figure 2.

In addition to hepatic and renal inactivation it has been shown by these workers that inactivation of progesterone occurs also in peripheral tissues. According to Wiest (1957), the peripheral tissues have relatively few enzymes for the reduction of the A ring. These tissues reduce chiefly the side chain.

New information about the excretion of the metabolites of progesterone in the bile and stool came from the work of Wiest and co-workers (1955), Davis and associates (1956, 1957), and Sandberg and Slaunwhite (1958). Sandberg and Slaunwhite have postulated an enterohepatic circulation for the metabolites of progesterone in the human. The metabolites appearing in the bile and stool are not completely identified.

Although pregnanediol is not the sole metabolite of progesterone and is not derived entirely from progesterone, pregnanediol excretion in the urine is a convenient semiquantitative index of progesterone elaboration and metabolism. Pregnanediol determinations are of added significance if correlated with physiological
Figure 2. Metabolic precursors of pregnanediol and its metabolism. The heavy arrow indicates the major precursor of urinary pregnanediol. (Serge and Lobotsky, 1964, p. 144; White, Handler and Smith, 1964, p. 865)
status. In women with normal menstrual cycles the corpus luteum is the major source of progesterone; during pregnancy, progesterone rises chiefly in the placenta especially in the later period of gestation.

Two additional compounds with progestational activity and representing metabolites of progesterone have been isolated from human ripe follicles, corpora lutea, and placenta. These substances are preg-4-ene-3-one-20α-ol, with approximately one-third to one-half the progestational activity of progesterone, and the corresponding 20β-ol isomer, with one-tenth to one-fifth the activity of progesterone (Zander et al., 1958). Of interest is the observation that some samples of human milk may contain the 20β-ol isomer of urinary pregnanediol, and that this compound specifically inhibits in vitro the enzymic formation of bilirubin digluconurone. There appears to be an interesting correlation between the occurrence of this steroid in human milk and the appearance of hyperbilirubinemia in some breast-fed infants (White et al., 1964).

Effect of Progesterone

It is difficult to describe the physiology of progesterone because it normally acts in conjunction with estrogens and other steroids and it produces few specific changes when acting alone. In general, it may be said that estrogens promote primarily growth processes, whereas progesterone encourages tissue differentiation. At approximately the mid-point of the traditional 28-day cycle ovulation occurs. By this time a corpus luteum is formed which secretes progesterone (Corner and Allen, 1929; Allen, 1932; Favold and Hisaw, 1932;
Allen and Wintersteiner, 1934), free progesterone becomes demonstratable in plasma (Forbes, 1950; Forbes et al., 1950; Rock et al., 1957), and pregnanediol appears in increased amounts in the urine (Venning et al., 1937). Plasma progesterone levels reach a peak on about the twentieth or twenty-fifth day coinciding with the peak storage of glycogen in endometrial epithelium which begins on about the fourteenth day. Urinary pregnanediol may fluctuate during this time without evident pattern, though an increase is expected because of its derivation from progesterone (Danowski, 1962).

Progesterone acts upon the endometrium, previously prepared by estrogen, inducing mucus secretory activity indispensable for implantation of the ovum. If pregnancy ensues, continued secretion of progesterone is essential for completion of term. Progesterone also contributes to growth of the breasts and is thought to maintain the uterus quiescent during pregnancy (Astwood, 1939). Progesterone exerts an antiovulatory effect when given during days 5 to 25 of the normal menstrual cycle (Fried et al., 1958); this is the basis for the use of certain synthetic progestins (17α-Ethynyltestosterone, 19-Norprogesterone, 17α-Ethynyl-19-nortestosterone, 17α-Ethyl-19-nortestosterone, 17α-Ethynyl-17-hydroxy-5(10)-estren-3 one) as oral contraceptive agents.

Progesterone in very large doses will promote retention of salt and water, its action thus resembling that of certain adrenal cortical hormones (Fruton, 1953; White et al., 1964).
Effect of Sex Steroids on Ovulatory Mechanism

The long-term effects of androgens and estrogens on the gonadotrophin-releasing mechanism are in general inhibitory. They tend to suppress follicle growth to the point where ovulation is eliminated simply by the absence of competent follicles. A chronic effect of progesterone, on the other hand, is primarily to suppress the preovulatory release of luteinizing hormone. Short term effects of estrogen and progesterone are, by contrast, excitatory for the central ovulation mechanisms. There are indications that estrogens and progesterone can induce the discharge of luteinizing hormone during late diestrus in the rat, or it can greatly lower the neural threshold for triggering the hormone release in the estrous rabbit, either by reflex stimulation or by electrical stimulation for the brain. Injection of estrogen can also induce ovulation, but its full effect is less abrupt than that of progesterone, and release of ovulating hormone takes place only after a priming period of about twenty-four hours in the adult rat or two or three days in the immature rat or adult rabbit.

The hypophysis of a "masculinized" female rat is that the masculinity can be induced by release of the ovulating hormone after administration of progesterone followed by electrical stimulation of the tuber cinereum.

Progesterone, given concomitantly with the follicle-stimulating hormone, markedly decreased the follicle stimulating effect of the gonadotrophin, presumably by a direct effect on follicular tissues.
Pregnanediol—As a Test for Ovulation

There are three methods frequently used for determining the occurrence of ovulation. They are based on the assumption that the secretion of progesterone is by a corpus luteum that has formed following ovulation. Although there may be progesterone secretion by luteinizing thecal cells in the absence of ovulation, the amount of progesterone formed is small, and physiologically not very important. Significant progesterone during the latter part of the menstrual cycle is excellent evidence that ovulation has taken place.

One dependable method for estimation of physiological progesterone effect is by study of the endometrial biopsy obtained during the latter part of the luteal phase of the cycle. The optimal secretory response in the endometriam is generally at about the nineteenth to twenty-third day of the cycle of a woman who regularly menstruates in twenty-eight-day cycles. The secretory response is still present subsequent to this, but the levels of corpus luteum secretion of progesterone tend to decrease so the endometrial response may be somewhat difficult to assess.

A second means of estimating the physiological effects of progesterone is by measurement of its thermogenic effect. Progesterone increases body temperature, probably through the action of its degradation products. A rise in the basal body temperature after the estrogen peak that is sustained throughout the latter part of the menstrual cycle is circumstantial evidence that progesterone is present and, therefore, that ovulation has taken place.

The demonstration of the excretion of increased amounts of urinary pregnanediol during the latter phase of the menstrual cycle is excellent evidence
of progesterone formation by the corpus luteum. When the normal woman
excretes 5 or 6 mg of pregnanediol daily, her own corpus luteum probably
synthesized about 50 mg of progesterone each day (Lloyd, 1964).

Recent Research on Pregnanediol

Recently, investigators are working in the areas of relationships between
the urinary pregnanediol excretion and the breast cancer, benign breast disease
(Stern et al., 1964; Kim, 1965; Marmorston et al., 1965; Schweppe et al., 1967),
endometrial cancer (Kelley et al., 1965), lung cancer (Marmorston et al., 1965),
bacteriostatic action on Staphylococci and other organisms (Yotis et al., 1966),
neonatal hyperbilirubinemia (Ramos et al., 1966), and/or the sex of the fetus.

The excretion of pregnanediol in the urine of pregnant women is an index
of the progesterone in the blood. Review of pregnancy in a large series of
abortion-prone women confirmed the observation of a high male-to-female ratio
in babies of patients with high pregnanediol excretion, and a reversed ratio in
those with low pregnanediol excretion. When the pregnanediol excretion was
interzone, the number of male and female babies was equal. It would appear
that the excretion of pregnanediol shows a distinct variation depending on whether
the placenta is of "male" or "female" type (Rawling et al., 1964).

Analytical Methods

The determination of progesterone in blood would be helpful in diagnosis
of clinical states such as corpus luteum insufficiency and threatening abortion.
However, the methods are too complex and time-consuming for the assay to be useful in a clinical laboratory.

Urinary assays for progesterone metabolites are useful. Although pregnanediol (5β-pregnane-3α, 20α-diol) is not a unique metabolite of progesterone (it is derived from desoxycorticosterone as well), it has proved to be a useful measure of progesterone production. When progesterone is injected, the mean recovery of material as urinary pregnanediol is 13 per cent. In the majority of cases, the conversion falls into quite a narrow range of 11 to 14 per cent. It has proved useful in studying abnormalities of menstruation and fertility as well as in studying abortion early in pregnancy and placental function in late pregnancy.

The development of methods for determining urinary pregnanediol originated with Venning (1937) and involved the gravimetric assay of pregnanediol glucosiduronate. In this procedure sodium pregnanediol glucosiduronate was extracted with butanol and purified by an acetone precipitation technique. Among the disadvantages of this method were the occurrence of emulsions during the butanol extraction and the difficulty of purifying the extract (Sinowara, 1940). Astwood and Jones (1941) attempted to overcome the difficulties of the Venning method by hydrolyzing the conjugate with mineral acid in the presence of toluene. This method has gained wide acceptance, although many modifications have been reported. deWatteville (1951) and Stimmel et al. (1952) introduced alumina column chromatography of the crude urinary extracts to improve the specificity of earlier methods. The advent of gas chromatography of steroids prompted many investigators to apply this technique to the determination of pregnanediol.
in biological extracts. In most cases the toluene-acid hydrolysis procedure has been utilized (Seegar Jones et al., 1962; Cox, 1963; Jansen, 1963; Turner et al., 1963; Wotiz, 1963; Chamberlain et al., 1964) and the extract submitted to analysis either directly or following prepurification and acetylation. Enzyme hydrolysis has been reported by some investigators. The extract is purified by means of thin layer or column chromatography (Klopper, Michie and Brown, 1955) and acetylated, converted to the trimethylsilyl ether, or analyzed directly (Patti et al., 1963; Kirschner et al., 1964; Marmorstan et al., 1965; Raman et al., 1965).

The methods employed usually comprise the following steps: (1) hydrolysis of the pregnanediol glucuronide, the form in which most of the pregnanediol is excreted in the urine, (2) extraction of the free pregnanediol, (3) separation of the pregnanediol from other compounds simultaneously extracted, and (4) some sort of quantitative or semiquantitatively estimation of the separated pregnanediol.

Since elution of pregnanediol from the column without being contaminated by other compounds, especially the other chemical related corpus luteum hormone metabolites, is very difficult, it was found to be more advantageous to carry out the separation by thin-layer chromatography (TLC). By this method it is possible to localize the pregnanediol spot precisely and separate it from any other compound. Waldi et al. (1962) developed a method using this principle, which gave a semiquantitative estimation of the amount of pregnanediol in urine. They proposed use of the method as a pregnancy test.
A method which uses paper chromatography described by Eberlein and Bogiovanni (1958) is probably the most specific and most sensitive method for estimation of pregnanediol in a small volume of urine. A modification of the method of Eberlein and Bongiovanni in which paper chromatography was changed to thin-layer chromatography was successfully used by Chiang (1966) in this lab, and was used in this study.

**Urinary Pregnanediol Level**

Since the development of analytical methods for pregnanediol, various studies have been done on urinary pregnanediol excretion in normal and abnormal human-beings.

In Table 1, differences in pregnanediol excretion between normal men and women are shown. Pregnanediol excretion is low in the follicular phase, increases in the luteal phase and pregnancy, and drops after the parturation and in post-menopausal women.

Information has been sought from the studies of the excretion of the newborn and amniotic fluid. Pregnanediol has thus far not been detected in urine of the newborn, at least by the usual methods having a sensitivity of as little as 1 mg. Philipp (1936) and Hoffman and Unde (1954), using biological tests, found up to 4 mg of progesterone in the urine of newborn babies in the first 24 hours after delivery. It has been isolated also from the amniotic fluid (an average value of 0.14 mcg per ml). On the basis of these findings, it was thought that the progesterone is not metabolized in the fetus and that some of the progesterone
Table 1. Urinary pregnanediol values reported in the literature

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of subjects</th>
<th>Value (mean) mg/24 hours</th>
<th>Condition</th>
<th>Investigator</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>-</td>
<td>0.1-0.8 mg/1000 ml urine. Normal</td>
<td>Astwood et al., 1941</td>
<td>Gravimetric method</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>Preovulatory phase</td>
<td>Grollman, 1941</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Late post ovulatory phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>9</td>
<td>0.38-1.42 (0.92) Normal</td>
<td>Klopper et al., 1955</td>
<td>Column chromatography</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>4</td>
<td>0.78-1.50 (1.12) Proliferative phase</td>
<td>Eberlein et al., 1958</td>
<td>Paper chromatography</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.4-4.2 (3.3) Luteal phase</td>
<td>Ehrlich, 1965</td>
<td>Thin layer chromatography</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.28-0.86 (0.63) Post menopause</td>
<td>Hawk, 1965</td>
<td>Paper chromatography</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>1</td>
<td>0.45-0.56 Normal</td>
<td>Eberlein et al., 1958</td>
<td>Paper chromatography</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>9</td>
<td>0.29-3.70</td>
<td>Ehrlich, 1965</td>
<td>Thin layer chromatography</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>-</td>
<td>0-1.5 Normal</td>
<td>Eugene et al., 1964</td>
<td>Column chromatography</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>-</td>
<td>0.1-2.0 Proliferative phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0-7.0 Luteal phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>4</td>
<td>0.90-1.18 Normal</td>
<td>Ehrlich, 1965</td>
<td>Thin layer chromatography</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>6</td>
<td>11.73-41.52 Pregnant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>-</td>
<td>0.2 Normal</td>
<td>Hawk, 1965</td>
<td>Paper chromatography</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>-</td>
<td>0.2 Follicular phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-10 Luteal phase and from fertilization to the end of the ovarian phase of pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>No. of subjects</td>
<td>Value (mean) mg/24 hours</td>
<td>Condition</td>
<td>Investigator</td>
<td>Method</td>
</tr>
<tr>
<td>-----</td>
<td>----------------</td>
<td>--------------------------</td>
<td>-----------</td>
<td>--------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>F</td>
<td>-</td>
<td>1</td>
<td>Preovulatory phase</td>
<td>Bang, 1964</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>Late post ovulatory</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mg/1000 ml urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>2</td>
<td>0.13-0.29</td>
<td>Normal</td>
<td>Raman et al., 1965</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>0.60</td>
<td>Follicular phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>7.0</td>
<td>1st trimester pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>31.0</td>
<td>3rd trimester pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>4</td>
<td>0.15-2.46 (0.77)</td>
<td>Normal</td>
<td>Chiang, 1966</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>F</td>
<td>5</td>
<td>0.14-1.85 (0.87)</td>
<td>Proliferative phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20-5.76 (1.77)</td>
<td>Luteal phase</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
is excreted unchanged, it may be that some is returned to the mother (Lloyd, 1959).

Raman et al. (1965) could not detect any pregnanediol excretion in urine of babies (male and female, from 3 days to 3 years old) with congenital adrenal hyperplasia, obese and hirsute, and after adrenal suppressions.

No data can be found for the difference between young boys and girls prior to puberty, and no study has been conducted in the field or pregnanediol excretion an old-aged men.

From a clinical point of view, although there may be variation in the estimations of total pregnanediol output according to the method used, the significance of the value will be assessed from the range of normality found using that particular method.
METHOD OF PROCEDURE

Experimental Design

Five normal healthy young university women were selected to cooperate in this study. All subjects were given a physical examination by the University Health Service before starting the experiment and were found to be in excellent condition. Blood pressure and hemoglobin were normal, urine analysis for sugar and albumin were negative.

All subjects were allowed to continue on their self-chosen diets but were asked to maintain constant weight and to go without breakfast for one of the two test periods. Dietary records were kept of all foods eaten each day before urine was collected. The content of calories, protein, fat, carbohydrate, saturated fatty acids, unsaturated fatty acids (oleic and linoleic), and cholesterol was then calculated from the food tables in the U. S. D. A. Agricultural Handbook No. 8 by Watts and Merrill (1963), and additional cholesterol values from a table by Okey (1945).

Two menstrual cycles period of 28-34 days were studied, one was in the fall from November 5, to December 19, and the other was in the winter between January 2, and February 12. In order to minimize the effect of environmental factors such as class work, examination, emotion, weather, etc., three of the

1Doctor S. M. Budge
subjects received their regular three meals a day, while two went without breakfast for the first test period, and the eating pattern was reversed for the two groups of subjects in the second period as shown in the experimental design in Table 2.

The subjects were weighed before breakfast each morning that blood was obtained, and then served breakfast (if allowed) in the Food and Nutrition Research Laboratory. Age, height, and body weight of subjects are presented in Table 3.

One of these five subjects, J. B., was married. As she became pregnant in the second test period her values obtained in the second period were not included in the average but are presented separately.

**Collection and Storage of Urine Sample**

**Collection**

A 24-hour urine sample and blood sample from the finger-tip (for lipid determination) were collected on day 1, 4, 7, 9, 11, 13, 15, 17, 20, 26, of the menstrual cycle and at day 1 of the next cycle. Two menstrual cycles were studied for each subject. One hundred and eight urine samples were analyzed.

Instructions for collecting 24-hour urine specimens were given to each subject, that is, to discard the first morning specimen on the day of collection and to collect all urine voided up to and including the first morning specimen of the next day. Containers of adequate size (two one-quart mason jars) labeled with name and date were used for 24-hour urine collection of each subject.
Table 2. Experimental design

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Two meals with no breakfast</th>
<th>Three meals with breakfast</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st: Nov. 5–Dec. 19</td>
<td>D. W.</td>
<td>J. B.</td>
</tr>
<tr>
<td></td>
<td>P. B.</td>
<td>A. M.</td>
</tr>
<tr>
<td>2nd: Jan. 2–Feb. 12</td>
<td>J. B.</td>
<td>D. W.</td>
</tr>
<tr>
<td></td>
<td>A. M.</td>
<td>P. B.</td>
</tr>
<tr>
<td></td>
<td>C. L.</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Age, weight, and height of subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>J. B.</th>
<th>C. L.</th>
<th>P. B.</th>
<th>D. W.</th>
<th>A. M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>21</td>
<td>21</td>
<td>22</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>Weight (pounds)</td>
<td>116.5</td>
<td>145.5</td>
<td>131</td>
<td>168</td>
<td>115.5</td>
</tr>
<tr>
<td>Height (feet)</td>
<td>5'4.5&quot;</td>
<td>5'9&quot;</td>
<td>5'6.7&quot;</td>
<td>5'8.5&quot;</td>
<td>5'4&quot;</td>
</tr>
</tbody>
</table>
Creatinine values were determined on the fresh urine to check the accuracy of the completeness of the total urine for the 24-hour collection. These values have been shown to be nearly constant for a given individual. Folin's method (Hawk et al., 1954) was used in this analysis. Results were all within the range of creatinine values for normal subjects.

Storage

A mixture of penicillin and streptomycin solution (1000 units of penicillin and 5 mg of streptomycin for each 24-hour collection) was used as a preservative to prevent both bacterial contamination and deterioration upon frozen storage. The volume of urine was recorded, the urine mixed well, and 4 small bottles (about 120 ml of urine per bottle) of the urine were then stored at \(-10^\circ\) C until the analyses for various steroid hormone end products could be made.

**Analysis of Urinary \(\beta\)-Pregnane-3\(\alpha\), 20\(\alpha\)-diol**

Method

The method used in this study was a modification of the method of Eberlein and Bongiovanni (1958) as described by Chiang (1966), which uses thin layer chromatography for purification of the sample (see Appendix).

Duplicate aliquots were analyzed for each sample. Those samples that did not peak at 425 mp, and/or those showing a large difference in the results from duplicates were repeated again.
Recovery

The precision of the method was established by a recovery test. The recovery test was run in duplicate, and it was made on each subject's urine. To a 10 ml of urine sample was added 5 mcg of standard $5\beta$-pregnane-3$\alpha$, 20$\alpha$-diol, which was run at the same time with the 10 ml urine sample by following the routine procedure. The results were shown in Table 4.

Table 4. Recovery test of urinary pregnanediol

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Pregnanediol mcg</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 ml urine</td>
<td>Std. a + 10 ml urine</td>
</tr>
<tr>
<td>A. M.</td>
<td>12.11</td>
<td>16.88</td>
</tr>
<tr>
<td>C. L.</td>
<td>14.29</td>
<td>19.08</td>
</tr>
<tr>
<td>D. W.</td>
<td>36.87</td>
<td>41.46</td>
</tr>
<tr>
<td>J. B.</td>
<td>25.13</td>
<td>29.91</td>
</tr>
<tr>
<td>P. B.</td>
<td>14.44</td>
<td>19.14</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a4.95 mcg standard $5\beta$-pregnane-3$\alpha$, 20$\alpha$-diol
RESULTS AND DISCUSSION

Urinary Pregnanediol Level

Values of urinary $\beta$-pregnane-3, 20 $\alpha$-diol excretion for the five subjects, with and without breakfast, during the two experimental periods, are presented in Table 5 by day of menstrual cycle and the mean concentrations for each individual in Table 6.

The daily excretion of pregnanediol in urine found in this study ranged from 0.24 mg to 1.69 mg for the first half of the menstrual cycle, and 0.48 mg to 3.10 mg for the latter half of the cycle.

The data showed that individual difference existed in the pregnanediol excretion. In Table 5, the data showed that the pregnanediol daily output of subject C. L. was higher than for subjects A. M., and D. W. This difference also was shown clearly in the mean value for each subject (Table 6). However, the values of pregnanediol excretion by subject C. L. was still within the normal range as reported by other research workers.

Effect of menstrual cycle

The variation among individual data of the excretion of pregnanediol in the urine of the subjects by days of menstrual cycle is presented in Table 6 and Figure 3. The excretion of pregnanediol rose in the expected fashion during the second half of the menstrual cycle, and precipitously fell prior to the onset of the next cycle.
Table 5. Urinary 5\(\beta\)-pregnane-3\(\alpha\),20\(\beta\)-diol excretion values for the individual subjects consuming two meals and three meals per day by day of menstrual cycle

<table>
<thead>
<tr>
<th>Subject</th>
<th>Meals</th>
<th>Cycle (days)</th>
<th>Days of menstrual cycle</th>
<th>mg/24 hour urine</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>A. M.</td>
<td>2</td>
<td>32</td>
<td>0.78</td>
<td>0.67</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>31</td>
<td>0.81</td>
<td>-</td>
<td>0.35</td>
</tr>
<tr>
<td>C. L.</td>
<td>2</td>
<td>34</td>
<td>0.77</td>
<td>0.94</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>29</td>
<td>0.30</td>
<td>0.48</td>
<td>1.58</td>
</tr>
<tr>
<td>D. W.</td>
<td>2</td>
<td>37</td>
<td>0.71</td>
<td>0.56</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>32</td>
<td>0.76</td>
<td>0.37</td>
<td>0.57</td>
</tr>
<tr>
<td>P. B.</td>
<td>2</td>
<td>26</td>
<td>0.77</td>
<td>0.92</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>31</td>
<td>0.44</td>
<td>-</td>
<td>0.80</td>
</tr>
<tr>
<td>J. B.</td>
<td>2(\text{a})</td>
<td>32</td>
<td>0.83</td>
<td>0.56</td>
<td>0.74</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>0.69</td>
<td>0.62</td>
<td>0.89</td>
</tr>
</tbody>
</table>

\(\text{a}\)Subject J. B. was found pregnant on Feb. 9 in the 2nd test period. The following values are for period 2.

Days of menstrual cycle | 13 | 15 | 17 | 28 | 31 | 39 | 48 | 66 |
Pregnanediol mg/24 hour  | 0.30 | 0.34 | 0.42 | 1.42 | 1.62 | 4.06 | 5.14 | 4.67 |
Table 6. Urinary pregnanediol excretion values for the individual subjects by day of menstrual cycle

<table>
<thead>
<tr>
<th>Subject</th>
<th>Days of preovulatory phase</th>
<th>Days of luteal phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>A. M.</td>
<td>0.79</td>
<td>0.67</td>
</tr>
<tr>
<td>C. L.</td>
<td>0.53</td>
<td>0.71</td>
</tr>
<tr>
<td>D. W.</td>
<td>0.74</td>
<td>0.47</td>
</tr>
<tr>
<td>P. B.</td>
<td>0.61</td>
<td>0.92</td>
</tr>
<tr>
<td>J. B. a</td>
<td>0.83</td>
<td>0.56</td>
</tr>
<tr>
<td>Mean</td>
<td>0.69</td>
<td>0.62</td>
</tr>
</tbody>
</table>

*Value of only one experimental period*
Figure 3. The effect of day of menstrual cycle on mean excretion values of pregnanediol of five subjects.
Although the mean pregnanediol values appeared to be similar from days 7 to 11, they showed a slight tendency to rise on day 13 (Figure 4). Further increases were noted through days 15 and 17, which reached its peak by day 20. The peak between day 17 and 20 may have been missed because analysis was not done every day. The concentration of pregnanediol decreased prior to the onset of the next menstrual period. Pregnanediol excretion in the urine for the subjects with normal cycle of 28 days reached its peak during days 17 and 20. The peak would appear on a later day between day 20 to 26 with the cycles longer than 28 days (30 to 35 days). However, differences in mean pregnanediol excretion between day 20 and 26 were not significant.

Smaller amounts of pregnanediol were detectable during the first half of the cycle. Possibly this pregnanediol was derived from the metabolism of adrenal progesterone and desoxycorticosterone.

The overall means of urinary pregnanediol output per day were found to be 0.84 mg from day 1 to 14 (preovulatory phase), and 1.53 mg from day 15 to 28 luteal phase), of the menstrual cycle. Thus, the pregnanediol excretion values and range of preovulatory phase and luteal phase of this study showed close agreement with values reported previously by other investigators in the literature (Table 1).

Because of the variation between subjects, it would be highly desirable to obtain similar basic data on a larger number of subjects to observe the significant of the effect of the menstrual cycle on this metabolite.
Figure 4. Mean values of pregnanediol in urine of five subjects by days of menstrual cycle.
Effect of two meals vs. three meals per day

Urinary pregnanediol excretion values for the individual subjects with and without breakfast by day of menstrual cycle are given in Table 5, and Figures 5 to 8, for four subjects. The mean excretion values by meals for the five subjects are presented in Table 7 and Figure 9.

There existed no apparent difference of mean pregnanediol excretion when the subjects were with or without breakfast (0.80:0.88) at the preovulatory phase, yet the values were slightly higher with breakfast (1.64 vs. 1.42) at the luteal phase.

No difference was found in the pregnanediol excretion values of specific days of the menstrual cycle and the mean values of subjects of A. M., C. L., and D. W. either with or without breakfast. Subject P. B. showed a definite increase of pregnanediol output from day 7 through to the end of the cycle during the period with breakfast.

It appeared that meal frequency did not influence the pregnanediol output of most subjects. More subjects and greater differences in meal frequencies are needed to draw conclusions on this phase of the problem.

In this study, pregnanediol excretion values were influenced by the menstrual cycle to a greater extent than by the frequency of meals per day.

Values Obtained for Subject J. B.

Subject J. B. was married. She became pregnant during the second experimental period. The last urine sample for analyses was collected on day
Figure 5. Urinary pregnanediol excretion values for subject A. M. on two meals and three meals per day.
Figure 6. Urinary pregnanediol excretion values for subject C, L. on two meals and three meals per day.
Figure 7. Urinary pregnanediol excretion values for subject D.W. on two meals and three meals per day
Figure 8. Urinary pregnanediol excretion values for subject P, B. on two meals and three meals per day.
Table 7. Mean values of pregnanediol in urine of subjects consuming two meals and three meals per day by day of menstrual cycle

<table>
<thead>
<tr>
<th>Meals</th>
<th>Days of menstrual cycle</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td>13</td>
<td>15</td>
<td>17</td>
<td>20</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.76</td>
<td>0.76</td>
<td>0.97</td>
<td>0.88</td>
<td>0.75</td>
<td>1.15</td>
<td>0.88</td>
<td>1.23</td>
<td>1.08</td>
<td>1.52</td>
<td>1.82</td>
<td>1.42</td>
</tr>
<tr>
<td>3</td>
<td>0.63</td>
<td>0.47</td>
<td>0.81</td>
<td>1.00</td>
<td>1.02</td>
<td>0.89</td>
<td>0.80</td>
<td>1.27</td>
<td>1.51</td>
<td>2.10</td>
<td>1.65</td>
<td>1.64</td>
</tr>
<tr>
<td>Mean</td>
<td>0.69</td>
<td>0.62</td>
<td>0.89</td>
<td>0.94</td>
<td>0.89</td>
<td>1.02</td>
<td>0.84</td>
<td>1.25</td>
<td>1.29</td>
<td>1.81</td>
<td>1.74</td>
<td>1.53</td>
</tr>
</tbody>
</table>
Figure 9. The effect of number of meals on mean excretion values of urinary pregnanediol for five subjects.
66 after day 1 of the last menstruation. Assuming that she became pregnant at time of ovulation the data on day 66 represented approximately one and one-half months pregnant.

During the first menstrual period, the pregnanediol excretion pattern was found to be in agreement with other investigators. During the second period of study, the influence of the pregnancy was great; the higher pregnanediol excretion values for her was 5.14 mg for day 48. The data of this study period was not included in the calculation with the other four subjects.

Relationships Among Serum Cholesterol and Urinary Pregnanediol, Total Estrogen, and Neutral 17-Ketosteroids

Serum cholesterol, neutral 17-ketosteroids, and total estrogen were determined in this laboratory by Sang (1967), Yam (1966), and Lee (1967) on the same subjects. The pattern of these three components and of pregnanediol are shown in Table 8 and Figure 10. Serum cholesterol was low on day 1 and then rose to high level during the first half of the menstrual cycle, and dropped to lower levels for the second half of the cycle. In contrast, pregnanediol was low in the first half of the cycle, and rose for the latter half of the cycle, especially, on days 20 and 26. Neutral 17-ketosteroids reached its peak on day 15, and total estrogen climbed twice, on the ovulation and on the luteal phase of the cycle. It appeared that, the concentration of serum cholesterol had a reciprocal relationship to pregnanediol, neutral 17-ketosteroids, and total
Table 8. The relationship among serum cholesterol and urinary pregnanediol, neutral 17-ketosteroids, and total estrogen by day of menstrual cycle

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>4</th>
<th>7</th>
<th>9</th>
<th>11</th>
<th>13</th>
<th>15</th>
<th>17</th>
<th>20</th>
<th>26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>165</td>
<td>191</td>
<td>189</td>
<td>189</td>
<td>191</td>
<td>188</td>
<td>186</td>
<td>188</td>
<td>181</td>
<td>181</td>
</tr>
<tr>
<td>mg/100 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnandiol</td>
<td>0.69</td>
<td>0.62</td>
<td>0.89</td>
<td>0.94</td>
<td>0.89</td>
<td>1.02</td>
<td>1.25</td>
<td>1.29</td>
<td>1.81</td>
<td>1.74</td>
</tr>
<tr>
<td>mg/24 hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral 17-ketosteroids</td>
<td>4.45</td>
<td>5.16</td>
<td>5.51</td>
<td>5.88</td>
<td>5.61</td>
<td>5.95</td>
<td>7.24</td>
<td>5.42</td>
<td>4.69</td>
<td>4.02</td>
</tr>
<tr>
<td>mg/24 hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mcg/24 hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 10. The relationships among serum cholesterol and urinary pregnanediol, total estrogen, and neutral 17-ketosteroids levels.
estrogen. This result would be in agreement with the knowledge that cholesterol served as the precursor of sex hormones and adrenal cortical steroids.

**Dietary Intake**

Diet records were kept for all subjects on each day before urine was collected. The total dietary content of calories, protein, carbohydrate, fat, saturated fatty acid, unsaturated fatty acids (oleic and linoleic acids), and cholesterol as calculated is presented in Table 9.

The mean caloric intake of the four subjects was 1885 calories per day; 1986 and 1781 calories for 3 and for 2 meals per day, respectively. Thus, the caloric intake was 205 calories or 10 per cent less on 2 meals a day than on 3 meals. This difference was due to the higher percentages of calories from protein (15.2 vs. 14.7 per cent) and fat (38.4 vs. 37 per cent) and a slight increase from carbohydrate (46 vs. 48 per cent) on 3 meals vs. 2 meals per day.

All the subjects had a lower caloric intake than the 2100 recommended by National Research Council (1964), except for one subject who was consuming 2348 calories on 3 meals. However, this difference in the caloric intake is not great enough to effect the study.

The diets appear to be well balanced. Nutritionists recommend for a well balanced diet that the total calories eaten as protein should be 10 to 15 per cent; as carbohydrates, 48 to 53 per cent; and the rest as fat. When individual dietary intakes were considered, all subjects were consuming diets that were within the upper range for protein (13 to 17 per cent of total calories
Table 9. Mean daily intake of certain nutrients of subjects on self-selected diets

<table>
<thead>
<tr>
<th>Name of subjects</th>
<th>Total calories</th>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Total fat</th>
<th>Total saturated fatty acid</th>
<th>Unsaturated fatty acids</th>
<th>Dietary cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gm.</td>
<td>Gm.</td>
<td>Gm.</td>
<td>Gm.</td>
<td>Gm.</td>
<td>Mg.</td>
</tr>
<tr>
<td>Two meals per day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. M.</td>
<td>1711</td>
<td>192</td>
<td>65</td>
<td>76</td>
<td>28</td>
<td>28</td>
<td>11.3</td>
</tr>
<tr>
<td>D. W.</td>
<td>1862</td>
<td>259</td>
<td>62</td>
<td>64</td>
<td>21</td>
<td>26</td>
<td>12.6</td>
</tr>
<tr>
<td>C. L.</td>
<td>1730</td>
<td>207</td>
<td>65</td>
<td>71</td>
<td>29</td>
<td>32</td>
<td>11.4</td>
</tr>
<tr>
<td>P. B.</td>
<td>1821</td>
<td>205</td>
<td>70</td>
<td>83</td>
<td>32</td>
<td>28</td>
<td>11.5</td>
</tr>
<tr>
<td>Mean</td>
<td>1781</td>
<td>216</td>
<td>65</td>
<td>74</td>
<td>27</td>
<td>29</td>
<td>11.7</td>
</tr>
<tr>
<td>Percentage of total calories</td>
<td>----</td>
<td>48.5</td>
<td>14.7</td>
<td>37</td>
<td>13.6</td>
<td>14.6</td>
<td>5.9</td>
</tr>
<tr>
<td>Three meals per day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. M.</td>
<td>2348</td>
<td>253</td>
<td>85</td>
<td>109</td>
<td>36</td>
<td>52</td>
<td>22.4</td>
</tr>
<tr>
<td>D. W.</td>
<td>1745</td>
<td>218</td>
<td>74</td>
<td>64</td>
<td>30</td>
<td>37</td>
<td>5.3</td>
</tr>
<tr>
<td>C. L.</td>
<td>2013</td>
<td>244</td>
<td>71</td>
<td>84</td>
<td>34</td>
<td>34</td>
<td>10.9</td>
</tr>
<tr>
<td>P. B.</td>
<td>1847</td>
<td>205</td>
<td>73</td>
<td>82</td>
<td>36</td>
<td>32</td>
<td>7.2</td>
</tr>
<tr>
<td>Mean</td>
<td>1986</td>
<td>230</td>
<td>76</td>
<td>85</td>
<td>34</td>
<td>34</td>
<td>11.5</td>
</tr>
<tr>
<td>Percentage of total calories</td>
<td>----</td>
<td>46.3</td>
<td>15</td>
<td>38.4</td>
<td>15.4</td>
<td>15.4</td>
<td>5.7</td>
</tr>
<tr>
<td>Name of subjects</td>
<td>Total calories</td>
<td>Carbohydrate</td>
<td>Protein</td>
<td>Total fat</td>
<td>Total saturated fatty acid</td>
<td>Unsaturated fatty acids</td>
<td>Dietary cholesterol</td>
</tr>
<tr>
<td>------------------</td>
<td>----------------</td>
<td>--------------</td>
<td>---------</td>
<td>-----------</td>
<td>---------------------------</td>
<td>------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>2 meals</td>
<td>1686</td>
<td>174</td>
<td>71</td>
<td>79</td>
<td>33</td>
<td>30</td>
<td>8.0</td>
</tr>
<tr>
<td>3 meals</td>
<td>1829</td>
<td>199</td>
<td>79</td>
<td>85</td>
<td>34</td>
<td>34</td>
<td>8.7</td>
</tr>
<tr>
<td>Mean</td>
<td>1757</td>
<td>187</td>
<td>75</td>
<td>82</td>
<td>33.5</td>
<td>32</td>
<td>8.4</td>
</tr>
<tr>
<td>Percentage of total calories</td>
<td>----</td>
<td>42</td>
<td>17</td>
<td>41</td>
<td>17.1</td>
<td>16.3</td>
<td>4.3</td>
</tr>
</tbody>
</table>
as protein); adequate in carbohydrate for two subjects (48 to 56 per cent), somewhat low carbohydrate intake for two subjects (43 and 44 per cent); and medium to high fat intake (31 to 42 per cent). The linoleic content of the diet was 5.9 and 5.7 per cent of the total calories on 2 and 3 meals, respectively. The ratio of polyunsaturated fatty acids to saturated fatty acids was 0.45. The dietary cholesterol intake ranged from 289 to 434 mg and 238 to 439 mg daily, on 2 and 3 meals per day, respectively.
CONCLUSION AND COMMENTS

Basic information of pregnanediol excretion was obtained on specific days during the menstrual cycle of normal young healthy women living and eating under normal condition.

As was expected, the data showed that the pregnanediol excretion in the urine was greatly affected by ovulation and the luteal phase during the menstrual cycle. The excretion value would reach its peak during the days of 17-20 for the subjects with a normal period of 28 days; with a longer cycle the peak would appear later than the twentieth day. The variation between the individual subjects was quite great in this study. Since the number of subjects in this study was only five, one subject with high or low values would influence greatly the pregnanediol mean value.

There existed a reciprocal relationship between the urinary pregnanediol and serum cholesterol.

No definite relationship was established between the urinary pregnanediol and the frequency of meals (with or without breakfast).

One subject (J. B.) became pregnant in the second test period. Her data were not included with the other four subjects. Her highest value was 5.14 mg on day 48 compared to 3.1 mg on day 17 for another subject with the next highest value. The high values were also sustained for a longer period of time (days 39, 48, and 66).
Because of the wide variations between subjects, it is recommended that the study be repeated using a larger number of subjects, as well as analyze for more days from day 15 to the end of the cycle. Relationships between biochemical indices would thus be more valid. Also, it would be desirable if the subjects selected for the study had a similar length of the menstrual cycle (normal 28 days is desirable).
SUMMARY

Five healthy university women, while living at home and on self-chosen diets, were selected to cooperate in the study of obtaining the basic data of $5\beta$-pregnane-3\alpha, 20\alpha-diol excretion in urine during the menstrual cycle. Effect of meal frequency (3 meals vs. 2 meals with no breakfast) was also included in this study.

Two complete menstrual cycles were included in the test periods for each subject. A 24-hour urine sample and blood sample were collected on day 1, 4, 7, 9, 11, 13, 15, 17, 20, 26 and at day 1 of the next cycle. Urinary pregnanediol was determined by a modification of the method of Eberlein and Bongiovanni on thin layer chromatography.

A diet record was kept for each subject on the day prior to urine collection. The mean caloric intake was 1885 calories per day, the percentages of calories consumed as protein was 12 to 17, carbohydrate, 48; and fat, 31 to 42.

The overall means for urinary pregnanediol output were found to be 0.84 mg for the first half of the menstrual cycle, and 1.53 mg for the latter half of the cycle. The range of pregnanediol excretion values was 0.24 mg to 1.69 mg during the preovulatory phase, and 0.48 mg to 3.10 mg for the luteal phase. Excretion values showed quite wide variations between individual subjects in this study.
The pregnanediol excretion was greatly influenced by the menstrual cycle. It rose in the second half of the menstrual cycle and fell precipitously prior to the onset of the next period, as reported by other investigators. There was no definite evidence that the pregnanediol excretion was influenced by the meal frequencies.

A reciprocal relationship in this study was observed between the pregnanediol excretion values and the serum cholesterol.
LITERATURE CITED


Analysis of Urinary $5\beta$-Pregnan-3α, 20α-diol

A modification of the method of Eberlein and Bongiovanni (1958) as used by Chiang (1966).

Equipment

1. Mounting board for glass plates, 22 x 113 cm with retaining ledges 1.8 cm wide along a short and a long side.¹
2. Desaga standard applicator, Model S-11, for mechanically producing a standard thickness.¹
3. Glass plates of uniform thickness: 5 x 20 cm and 20 x 20 cm.¹
4. Labeling template, graduated.¹
5. Developing tank.²
7. Oven, 110°C.
8. Hamilton microliter syringes, 10, 20, and 50 lambda.
9. Hot plate.
10. Filter paper, Whatman No. 1, 45 x 60 cm sheets.
11. Pouring funnels, 2.5 inches in diameter.
12. Erlenmeyer flasks, 50 ml.

¹Brinkmann Instruments, Inc., 115 Cutter Mill Road, Great Neck, Long Island, New York.

14. Water bath, 37°C.

15. Test tubes, 1.5 x 15 cm.

16. Razor blades, single edge.

17. Conical centrifuge tubes, 50 ml, and 15 ml.

18. Glass rods.

19. Serum lifter.

20. Beckmann spectrophotometer, and 3 ml silica cuvettes.

Reagents

1. Silica Gel G obtained from Brinkman.

2. Iodine.

3. Acetone, reagent grade, distilled.

4. Chloroform, reagent grade, distilled.

5. Benzene, reagent grade, distilled.


8. 5β-pregnane-3α,20α-diol, 0.1 per cent standard in distilled chloroform.

9. 1M, pH 4.5 acetate buffer.

3 Obtained from University of Utah Machine Shop.
10. Beta-glucuronidase, 5000 unit per ml.\(^4\)

11. 1N NaOH.

12. Sulfuric acid mixture: 30 gm sodium bisulfite is cautiously added to 200 ml of concentrated sulfuric acid in a 1000 ml beaker (fumes), with stirring under the hood, and allowed to cool. The acid is decanted into a small bottle in which it may be stored at room temperature.

**Procedure**

**Hydrolysis.** Duplicate 20 ml samples of urine were used. To 20 ml of urine in a 50 ml centrifuge tube was added 2 ml of 1M, pH 4.5 acetate buffer, and 5000 units (1 ml) beta-glucuronidase. Each tube was stoppered with glass stoppers to prevent any loss of the sample during hydrolysis. Hydrolysis was performed by incubating the tubes in a \(37^\circ\) C water bath overnight (12 to 18 hours). Two standard samples, 10 microliters of pregnanediol plus 20 ml water, buffer, and ketodase, were also incubated under standard condition.

**Extraction.** After hydrolysis, the samples were extracted with 10 ml benzene by the beating technique, which used a footed stirring rod for the beating. The two layers separated readily on standing, however, if emulsion formed, a short centrifugation separated the layers. The benzene layer was transferred by means of a serum lifter to a conical 50 ml centrifuge tube. The extraction was repeated with another 10 ml of benzene and the extracts were combined.

\(^4\)Sigma Chemical Company, 3500 Dekalb Street, St. Louis, Missouri.
Washing and drying. The combined extracts were washed with 8 ml of 1N NaOH three times, and then 8 ml of water three times, the water layer was removed, and then dried over anhydrous sodium sulfate. The benzene extracts were transferred to a conical 15 ml centrifuge tube and evaporated to dryness at 45-50°C under a gentle stream of filtered nitrogen gas.

Preparing the glass plates. The glass plates were coated with silica gel G as follows:

1. Preparation of glass plates. The glass plates were thoroughly cleaned with a cleaning agent, rinsed well with tap water, distilled water, and air dried on a drying rack at room temperature. Just before use, the plates were further cleaned by washing with acetone to remove any dust or contaminating materials.

2. Preparation of the suspension of silica gel G and filling the spreader. Silica gel G was used as the absorbent for coating the glass plates. The volume of slurry required to coat five 20 x 20 cm plates contained 27 gm silica gel G and 75 ml redistilled water in a 250 ml stoppered flask. The flask was shaken vigorously for 30 to 45 seconds, and immediately transferred to the open spreader. The spreader was held with both hands and drawn across the plates without applying much pressure. It is important to finish this step within 4 minutes.

3. Drying the plates. The plates were left in a flat position for 10 minutes, and then dried in air overnight. This gave layers that adhered particularly well.

4. Activation. The plates were placed in a drying cabinet. Time and temperature of heating were determined by the required activity of the layer.
Heating to 110° C for 2 hours gave the correct adsorption activity. Layers with greater adsorption activity can be obtained by heating for longer times at higher temperatures.

5. Storage. Since active plates became deactivated in moist air, they were stored over a desiccant in a desiccator, or in a plate cabinet. If hot plates were placed in the desiccator the stopcock must be left open, which has been provided with a short drying tube filled with silica gel. The layers were thus protected as well as possible against laboratory fumes and mechanical damage.

Chromatography. Just before applying the sample, impurities were removed from the plates by placing them in a glass tank, which had been saturated with chloroform.

The dried sample of pregnanediol was dissolved in 50 microliters of chloroform and was applied to the plate with a 20 microliter syringe at a point approximately two centimeters from the edge of the plate at right angles to the direction in which the plates were coated. A standard solution of 5β-pregnane-3α, 20β-diol prepared for thin layer chromatography was applied to the plate as a mark for identification. Immediately after the sample was applied, the plate was placed in a developing tank which previously had been saturated by adding 150 ml of developing solvent. The developing solvent was chloroform:acetone: absolute alcohol = 85:15:10. Saturation was accomplished by lining the tank with a filter paper wick. When the solvent front had reached a predetermined mark (15 cm), the plate was removed from the chamber, air dried, and quickly placed in an iodine atmosphere until the pregnanediol components become visible as yellow spots.
The plate was removed from the iodine atmosphere, the area of pregnanediol was located, and the portion of silica gel G containing the pregnanediol component was immediately scraped off into an Erlenmeyer flask (50 ml) containing the eluting solvent (chloroform, 20 ml). The silica gel G–pregnanediol mixture was filtered through a sintered glass filter into a 1.5 x 15 cm tube. The flask was rinsed several times with solvent to insure quantitative transfer of the pregnanediol. The solvent was evaporated just to dryness in a nitrogen atmosphere while the tube was held in a warm water bath. Although thin layer chromatographic analysis of the components (pregnanediol and steroids) extracted from urine samples showed good retention, it also showed tailing of the neighboring steroid classes. In order to further purify the pregnanediol it was re-chromatographed by the thin layer procedure and isolated a second time as described above.

Spectrophotometry. To the dried sample, was added 2.6 ml of 15 per cent sodium bisulfate in sulfuric acid solution. In this mixture the absorbance of pregnanediol at 425 μm is more than twice that in sulfuric acid alone. An acid blank was prepared simultaneously. (A silica gel blank is not necessary.) The spots of the standard solution of pregnanediol were treated in the same way as the urinary extracts. The tubes were rolled to distribute the acid evenly and then placed in a boiling water bath for four minutes. After cooling at room temperature for fifteen to twenty minutes, the contents of each tube were transferred to 3 ml silica cuvettes and read in a Beckman D. U. spectrophotometer at 390 μm, 425 μm, and 460 μm against the acid blank. The corrected reading does not appreciably change over a period of several hours. The intensity of the color is maximum at
425 mu. Many factors would influence the color density, such as, the length of time permitted for color developed by the individual pregnanediol, and the concentrations of sodium bisulfite and sulfuric acid. Therefore, Allen’s correction was followed to reduce the error (Allen, 1950).

**Calculation.** The corrected absorbance was calculated by the Brown modification of the Allen’s correction:

\[
2 \text{ absorbance}_{425} - (\text{absorbance}_{460} + \text{absorbance}_{390})
\]

The excretion of \(5\beta\)-pregnane-3\(\alpha\), 20\(\alpha\)-diol in urine for mg per 24-hour = corrected absorbance unknown/corrected absorbance known x 24-hour urine volume/volume of urine sample x mcg of standard used x 1/1000.
VITA

Rita Shin-hui Liao

Candidate for the Degree of

Master of Science

Thesis: 5β-Pregnane-3α,20α-diol Excretion in Urine of University Women During the Menstrual Period.

Major Field: Nutrition and Biochemistry

Biographical Information:

Personal Data: Born at Taiwan, Republic of China, December 8, 1939, daughter of Ta-Kue and Pi-yu Liao.

Education: Attended elementary school in Taipei, Taiwan; graduated from Taiwan Provincial Taipei First Girls' High School in 1958; received the Bachelor of Science Degree from the Department of Pharmacy, National Taiwan University in 1962; Received Master of Science in Nutrition and Biochemistry at Utah State University in 1968.

Professional Experience: From March, 1963 to August, 1965, worked as an analyst in the Department of Quality Control, Pfizer Taiwan Ltd., Taiwan, China.