CHAPTER II

EXCRETION OF PROGESTATIONAL STEROIDS IN THE MILK DURING LACTATION
INTRODUCTION

After administration, the progestational steroids are taken up in the tissues from the blood, metabolized in the body and excreted in the urine and faeces. During lactation these may be excreted in the milk of lactating mothers. The question of excretion of progestational steroids and their metabolites in the milk and the possibility of any biological activity of the excreted products in the milk assumes great significance if these drugs are used for fertility control during lactation. Furthermore, lactational amenorrhea is quite a prolonged and indefinite period. It is estimated that the mean lactation time in some women is of the order of 13.5 months (Malkani and Mirchandani, 1960). It thus becomes imperative to provide lactating women with a safe and effective method for conception control. The use of progestational steroids is an effective method for fertility control during lactation, however the problem of excretion of these steroids in the milk and the possibility of transmission of these to the infant
being nursed remained to be investigated. This chapter deals with the excretion of norethynodrel and chlormadinone acetate in the milk of lactating women and goats. The estrogenic activity of excreted compounds in the milk has been estimated by bioassay procedures.

MATERIALS AND METHODS

Subjects—The women used in this study were indoor patients from the Obstetrics Ward of the All India Institute of Medical Sciences Hospital. The women had been delivered of still births. The experiments were carried out after day 4 or 5 of parturition.

Goats—The female goats were of a local breed. They were housed in air-conditioned quarters. They were fed soaked grams, green vegetables and had free access to water.

Radio-inert steroids—The radio-inert steroids used in this study were:

1) Enovid (Norethynodrel 5.0 mg + Mestranol 0.075 mg).
2) Norethynodrel 5.0 mg (17α-ethyl 17B-hydroxy 5 (10)-estren-3-one) batch No. MA-Q-924. Estrogen free norethynodrel was provided by Dr. Victor Drinn of G.D. Searle & Co., Chicago.

3) Chlormadinone acetate 0.5 mg (6-Chloro-17α-hydroxy preg-4-6-diene 3,20 dione acetate).

4) Megestrol acetate 5.0 mg (6-methyl-17α-hydroxy preg-4, 6-diene-3,20 dione acetate).

3H-norethynodrel: 3H-norethynodrel was obtained as mentioned on page 41-43 of this thesis. The 3H-norethynodrel was mixed with radio-inert norethynodrel whenever necessary. It was filled in gelatin capsules and administered orally at 8 a.m. Prior to the administration of 3H-norethynodrel women received Enovid (5.0 mg tablet) for two days.

3H-chlormadinone acetate: 1α-3H-CAP was supplied by Dr. Heckt Lukari, E. Merck, Darmstadt, W. Germany in the form of tablets containing 50 μCi chlormadinone acetate per 0.5 mg tablet. Women were orally administered tablets containing 3H-CAP, while in the case of goats, each tablet was suspended and dissolved in
0.5 ml olive oil and fed to them through a stomach tube. Both women and goats were pretreated with radio-inert steroid (0.5 mg daily) for 3-4 days and on day five \(^3\)H-CAP was administered.

**Collection of milk samples:** Milk samples from women were collected 4 hourly during the day for a period of 5-7 days. In the case of goats the animals were milked in the morning and the evening.

In studies on the detection of estrogenic activity in human and goat milk after the administration of oral progestins, the human or goat milk was expressed twice daily at 8.00 a.m. and 8.00 p.m. for 3 days. The milk collected for three days before the administration of the drug was labelled "Control milk". For the following three days oral gestagens were administered and the breast milk was again expressed twice daily. This milk was labelled as "Post gestagen Milk". Estrogenic activity was assayed in 12 specimens collected from each subject (human or goat).

**Estimation of radioactivity in the milk:** In the earlier experiments, radioactivity in the milk was
estimated by adding an equal volume of 10% trichloroacetic acid to precipitate the proteins. The supernatant was then added to diotol scintillation liquid (10 ml) and counted in a Liquid Scintillation Spectrometer (Packard, Model 3314). The precipitate was solubilized in hyamine hydroxide and counted as usual. Quenching corrections were carried out with an internal standard.

Subsequent investigations in the development of a rapid and an accurate procedure for the estimation of radioactivity in the milk led to the use of a method in which 0.3 ml of the milk was dissolved in an equal volume of 10% KOH. 15 ml of diotol scintillation liquid was added to each sample and the samples were counted as usual. Both the methods gave comparable results.

Counting radioactivity: Diotol scintillation liquid or simple scintillation liquid was used for counting of samples as described in Chapter I.

Preparation of milk extracts: For studies on estrogenic activity in milk, the total quantity of milk in each specimen was refluxed with an equal volume of alcohol for 30 minutes and was centrifuged at 2000 r.p.m. for
15 minutes. The residue was discarded and the supernatant was concentrated under vacuum to half its volume. Milk proteins were precipitated with acetone and centrifuged. This was repeated 4 to 6 times until the supernatant was clear and free of proteins. The supernatant was then evaporated to a volume of about 2 ml. It was transferred to a vial and dried under nitrogen. The residue was dissolved in 0.6 ml olive oil.

Bioassay for estrogenic activity: - Both the "control milk" and "post-gestagen milk" specimens were assayed for estrogenic activity by the uterine weight response in the immature mouse (Edgren and Calhoun, 1955). For the study of control and post-gestagen specimens, 6 mice weighing 6 - 8 g were used per group. The milk extract of each control or post-gestagen milk specimen was dissolved in 0.6 ml olive oil and was administered at a dose of 0.2 ml/mouse/day for 3 days either by injection or by gavage. Autopsies were performed on the fourth day; uteri were weighed on a Roller Smith torsion balance. The body weight of the mice was determined. A statistically significant increase in the uterine weight in the post-gestagen group as compared to the control group was considered as an index of estrogenic activity.
RESULTS

Excretion of $^3$H-norethynodrel and its metabolites in the milk of lactating women.

The excretion of radioactivity in the milk due to norethynodrel and its metabolites after the administration of $^3$H-norethynodrel to lactating women is presented in Table I. It may be seen that after the administration of different amounts of radioactivity to norethynodrel containing varying amounts of the inert steroid, the percent radioactivity coming out in the milk ranged from 0.45 to 1.52 per cent. On an average 1.1% of the administered $^3$H-norethynodrel was excreted in the milk over a period of five days.

The daily pattern of excretion of radioactivity in the milk of patients B.K., S.B., U.D. and L.S. are presented in Figures 1, 2, 3 and 4 respectively.

Excretion of $^3$H-CAP and its metabolites in the milk of lactating women.

Table II gives the percent excretion of radioactivity over a period of 6-7 days in the milk of 10 lactating women after the oral administration of 50 μc
| Case | Days | Activity in Milk | Patient | Age at Death | % of Total Dose of H-38
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.75</td>
<td>3.0 mc/5.0 mc²</td>
<td>5</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.4</td>
<td>4.8 mc/0.79 mc²</td>
<td>5</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1.75</td>
<td>3.0 mc/5.0 mc²</td>
<td>4</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1.75</td>
<td>3.0 mc/5.0 mc²</td>
<td>6</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>

Table I

| 6 days in milk | H-38 not absorbed | % absorbed | H-38 absorbed | % absorbed | Patient | Case | % of total dose of H-38
|----------------|-------------------|------------|----------------|------------|---------|------|------------------|

Women after the administration of H-38 not absorbed.

Excretion of radioactivity in breast milk of lactating...

S.G. 30
Fig. 2

Daily excretion of radioactivity ($^3$H-norethynodrel and its metabolites) in the milk of lactating patient S.B. after the oral administration of 8.0 mc of $^3$H-norethynodrel.
Fig. 3

Daily excretion of radioactivity (3H-norethynodrel and its metabolites) in the milk of lactating patient U.D. after the oral administration of 3.0 μc of 3H-norethynodrel.
Daily excretion of radioactivity ($^{3}$H-norethynodrel and its metabolites) in the milk of lactating patient S.B. after the oral administration of 3.0 μc of $^{3}$H-norethynodrel.
$^{13}H$-CAP. The volume of milk of different patients is also shown in the Table and presented in the descending order. It may be seen that women yielding higher milk volume showed higher excretion of steroids in the milk while low excretion of radioactivity in the milk was found in women having low yield of milk. Patient R.A. had a total milk volume of 1,546.0 ml and the radioactivity excreted was 1.05 per cent of the administered oral dose. On the other hand the lowest amount of radioactivity excreted in the milk of patient A.S. was 0.164 per cent with a total milk volume of 180.0 ml.

Details of the daily excretion of radioactivity and the daily milk volumes of the 10 women are shown in Figs. 5, 6 and 7. It may be seen that in general, the volume of milk had a profound effect on the amount of radioactivity excreted in the milk. The amount of radioactivity was directly proportional to the volume of milk obtained. Wherever the milk volume was low or high the excretion of radioactivity followed the same pattern.

Since some of the women in the group did not have an established lactation, repeated expression of
Table II

EXCRETION OF RADIOACTIVITY IN THE MILK OF LACTATING WOMEN AFTER THE ORAL ADMINISTRATION OF $1\alpha\text{-}^3\text{H} \text{CHLORMADINONE ACETATE}.$

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Patient's Name</th>
<th>Total Milk Vol. (ml)</th>
<th>Radioactivity % Oral Dose Exc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>R.A.</td>
<td>1546.0</td>
<td>1.05</td>
</tr>
<tr>
<td>2.</td>
<td>R.D.</td>
<td>610.5</td>
<td>0.596</td>
</tr>
<tr>
<td>3.</td>
<td>B.L.</td>
<td>504.0</td>
<td>0.435</td>
</tr>
<tr>
<td>4.</td>
<td>S.A.</td>
<td>517.0</td>
<td>0.45</td>
</tr>
<tr>
<td>5.</td>
<td>S.L.</td>
<td>370.0</td>
<td>0.331</td>
</tr>
<tr>
<td>6.</td>
<td>J.W.</td>
<td>360.0</td>
<td>0.25</td>
</tr>
<tr>
<td>7.</td>
<td>S.S.</td>
<td>292.4</td>
<td>0.24</td>
</tr>
<tr>
<td>8.</td>
<td>A.D.</td>
<td>213.0</td>
<td>0.185</td>
</tr>
<tr>
<td>9.</td>
<td>M.J.</td>
<td>134.0</td>
<td>0.124</td>
</tr>
<tr>
<td>10.</td>
<td>A.S.</td>
<td>130.0</td>
<td>0.164</td>
</tr>
<tr>
<td></td>
<td><strong>Average</strong></td>
<td></td>
<td><strong>0.38</strong></td>
</tr>
</tbody>
</table>
Fig. 5

Pattern of excretion of radioactivity in the milk of four lactating women, S.L., B.L., A.D. and M.J. after the oral administration of tablet containing 50.0 μc of 1α-3H-chlormadinone acetate. The results are expressed as percent excretion of the total dose administered.
Fig. 6

Pattern of excretion of radioactivity in the milk of three lactating women A.S., S.A. and J.W. after the oral administration of a tablet containing 50.0 µc of 1α-3H-chlormadinone acetate. The results are expressed as percent excretion of the total dose administered.
Fig. 7

Pattern of excretion of radioactivity in the milk of three lactating women, R.A., R.D. and S.S. after the oral administration of a tablet containing 50.0 μc of 1α-3H-chlormadinone acetate. The results are expressed as percent excretion of the total dose administered.
milk with the help of a breast pump resulted in slight increase in the volume of milk on day 4 and 5 after the administration of $^3$H-chlormadinone acetate. This resulted in a corresponding increase in the excretion of radioactivity in the milk.

Another interesting feature of the results was the fact that the levels of radioactivity in the milk due to CAP and its metabolites did not decline completely even by 6th or 7th day.

Excretion of $^3$H-CAP and its metabolites in the milk of lactating goats.

Three goats were administered $^3$H-CAP and milk samples were collected for a period of 5-6 days. The amount of radioactivity excreted in the milk over a period of 5-6 days was 0.68%, 0.319 and 2.80% for goat No. I, II and III respectively as shown in Table III.

Testing of estrogenic activity in the milk of lactating women after the administration of contraceptive steroids.

Table IV gives the analysis of estrogenic activity in the milk of eleven women after the oral administration of Enovid, norethynodrel and chlormadinone
<table>
<thead>
<tr>
<th>%</th>
<th>0.31%</th>
<th>3.5%</th>
<th>0.68%</th>
<th>1.231%</th>
<th>1.0%</th>
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<tbody>
<tr>
<td></td>
<td>1.75</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>0.65</td>
<td>0.041</td>
<td>1.66</td>
<td>0.076</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>0.98</td>
<td>0.045</td>
<td>3.05</td>
<td>0.100</td>
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<td></td>
<td>1.18</td>
<td>0.097</td>
<td>0.087</td>
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<td></td>
<td>0.68</td>
<td>0.104</td>
<td>0.75</td>
<td>0.277</td>
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<tr>
<td></td>
<td>0.97</td>
<td>0.203</td>
<td>2.00</td>
<td>0.881</td>
<td>0.2</td>
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</table>

<table>
<thead>
<tr>
<th>Dose (ml)</th>
<th>Radioactivity</th>
<th>Dose (ml)</th>
<th>Radioactivity</th>
<th>Dose (ml)</th>
<th>Radioactivity</th>
<th>Dose (ml)</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.14</td>
<td>500</td>
<td>0.202</td>
<td>200</td>
<td>0.305</td>
<td>600</td>
<td>0.145</td>
<td>180</td>
</tr>
<tr>
<td>0.21</td>
<td>500</td>
<td>0.202</td>
<td>200</td>
<td>0.305</td>
<td>600</td>
<td>0.145</td>
<td>180</td>
</tr>
<tr>
<td>0.28</td>
<td>500</td>
<td>0.202</td>
<td>200</td>
<td>0.305</td>
<td>600</td>
<td>0.145</td>
<td>180</td>
</tr>
</tbody>
</table>

(Note: Table III)

AFTER THE ORAL ADMINISTRATION OF 14C-CH3CORMINONE CAROTATE
EXCRETION OF RADIOACTIVITY IN THE MILK OF LACTATING COWS
| S.H.O. Subject | No. of Subject | Mean Protein Content of Milk Extract | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight |
|----------------|----------------|--------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|
| S.H.O. Subject | No. of Subject | Mean Protein Content of Milk Extract | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight |
| S.H.O. Subject | No. of Subject | Mean Protein Content of Milk Extract | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight |
| S.H.O. Subject | No. of Subject | Mean Protein Content of Milk Extract | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight | Mean Protein Content of Post-Cesarean Uterus Weights mg/100 Body Weight |

The Varlee Response of Immature Female Mice to Post-Cesarean Human Milk.

Table 4.
acetate. When Enovid was administered to 3 women estrogenic activity was found in 2 women R.K. and R.B. as shown by a significant increase ($P<0.01$) in the weight of immature mouse uterus.

After the administration of norethynodrel to six women, milk extract of two women (A.N. and P.K.) caused a significant increase ($P<0.01$ and $P<0.05$ respectively) in the uterine weight of immature mouse indicating thereby the presence of estrogenic activity in the milk of these two women. No estrogenic activity could be detected in the milk of other four patients, S.I., M.J., V.W. and S.S.

The milk extracts of two women H.R. and S.Y. processed after the administration of CAP did not show any estrogenic activity.

**Analysis of goat milk for estrogenic activity.**

Four goats were administered norethynodrel (5.0 mg) daily. The control and post-gestagen milk samples were assayed for estrogenic activity. The results (Table V) showed the presence of estrogenic activity in the milk of 3 out of 4 goats. One goat
<table>
<thead>
<tr>
<th>Value</th>
<th>Mean + S.E.</th>
<th>Mean + S.E.</th>
<th>Mean + S.E.</th>
<th>Mean + S.E.</th>
<th>Mean + S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.4</td>
<td>68.4 + 3.56</td>
<td>81.1 + 7.36</td>
<td>64.4 + 5.95</td>
<td>83.5 + 7.6</td>
<td>50.1 + 6.1</td>
</tr>
<tr>
<td>&gt; 0.01</td>
<td>32.4 + 1.7</td>
<td>36.6 + 2.1</td>
<td>36.1 + 4.87</td>
<td>38.7 + 2.1</td>
<td>44.4 + 2.4</td>
</tr>
<tr>
<td>&gt; 0.005</td>
<td>32.4 + 1.7</td>
<td>36.6 + 2.1</td>
<td>36.1 + 4.87</td>
<td>38.7 + 2.1</td>
<td>44.4 + 2.4</td>
</tr>
<tr>
<td>&gt; 0.001</td>
<td>32.4 + 1.7</td>
<td>36.6 + 2.1</td>
<td>36.1 + 4.87</td>
<td>38.7 + 2.1</td>
<td>44.4 + 2.4</td>
</tr>
<tr>
<td>&gt; 0.0001</td>
<td>32.4 + 1.7</td>
<td>36.6 + 2.1</td>
<td>36.1 + 4.87</td>
<td>38.7 + 2.1</td>
<td>44.4 + 2.4</td>
</tr>
</tbody>
</table>

The table shows the response of immature female mice to post-gestational cast Mila.

Table 5.
was administered 5.0 mg megestrol acetate. The post-gestagen milk extract of this goat did not significantly increase the weight of immature mouse uterus, thereby indicating the absence of any estrogenic activity in the milk.

**DISCUSSION**

The results revealed that progestational steroids and their metabolites are excreted in the milk when administered during lactation. It was found that after the administration of $^3$H-norethynodrel on an average 1.1 per cent (range 0.45 to 1.52%) of the administered dose was excreted in the milk as norethynodrel and its metabolites. Thus 1.1% excretion of the steroid, when calculated on the basis of 5.0 mg of norethynodrel in $^3$H-novid tablet would mean that 55.0 ug of norethynodrel and its
metabolites (assuming the molecular weight of the metabolites is approximately the same as that of norethynodrel) are excreted in the breast milk. This is quite a significant amount of the steroid and its metabolites but it is not known whether the latter represent biologically active or inactive compounds.

The studies on the excretion of chlormadinone acetate and its metabolites in the milk of 10 lactating women showed that on an average 0.38 per cent of the administered dose (range 0.124 to 1.05%) was excreted in the milk after the administration of a tablet containing 500 µg of $^{3}$H-CAP. A direct relationship between the volume of the milk and the amount of the steroid excreted in the milk became evident. The women yielding higher milk volume excreted higher amounts of steroids, while the women having lower volumes of milk excreted lower amounts of steroids. Such a conclusion was further supported by the data on the excretion of $^{3}$H-chlormadinone acetate and its metabolites in the milk of goats where the high milk yielding goats excreted higher amounts of $^{3}$H-CAP and its metabolites. This suggestion was also obvious when daily milk samples of
women and the amount of steroids excreted in it were compared. These results, therefore, lead to the suggestion that a woman yielding higher amounts of milk may run the risk of excreting higher amounts of steroids in the milk. And such a high milk volume may have higher proportion of biologically active substances.

The data on the excretion of chlormadinone acetate in the milk showed that the radioactivity due to CAP and its metabolites, did not decline by fifth and sixth day after the oral administration of one tablet containing 0.5 mg $^3$H-CAP. It thus indicated that $^3$H-CAP was possibly stored in the body and was getting slowly released into the plasma and thus excreted in the milk. Other work carried out in this laboratory showed that chlormadinone acetate has a long half-life of the order of 80.1 hours in the plasma of women (Dugwekar, Narula and Laumas, 1973a) and of the order of 35.1 hours in the plasma of monkey (Chapter III). It was seen that CAP showed a high uptake in the fat (Chapter I) of the rat. Similar results on the high uptake of chlormadinone acetate in the fat have been reported in the women (Gallegos et al., 1970, Dugwekar
It thus led to the conclusion that the CAP was stored in the fat and was slowly released into the blood and from the blood it was continuously excreted in small amounts in the milk over a number of days. This explained the reasons for the observation that the levels of chlormadinone acetate in the milk did not decline by five to six days after the oral administration of $^3$H-chlormadinone acetate. It has been shown that the mammary gland takes up hormones, antibodies, minerals and other chemical constituents from the blood (Linzel, 1969). The prolonged half-life of norethynodrel (Laumas, Murugesan and Hingorani, 1971 and CAP (Chapter III) in the plasma showed that these steroids were available in the blood to be taken up by the mammary gland and excreted in the milk. The progestational steroids and their metabolites may not be just lying absorbed in the mammary gland but may be bound to receptor proteins. Since retention of CAP in the mammary gland has been found in the other studies (Chapter I), it is possible that the milk proteins may have a higher affinity for the progestational steroids and its metabolites, and thus higher volumes of milk having more amounts of milk proteins may be able to
remove more of the steroids from the mammary glands into the milk. The women yielding higher amounts of milk excreted higher amounts of steroids in the milk. It suggests that the higher amounts of milk or the milk proteins may be able to remove the correspondingly higher amounts of steroids bound to the receptor-proteins in the mammary gland and thus excrete them in the milk of women yielding high amounts of milk.

The question of the biological activity of the excreted steroid and its metabolites in the milk is of considerable practical importance when women taking progestational steroids for fertility control breast feed the baby at the same time because it is possible that the active metabolites of the administered steroid excreted in milk may exert biological effects on the infant; it is known that extremely small amount of estrogens (Jensen and Jacobson, 1962; Stone and Martin, 1967; Laumas, 1967; 1969) and progesterone (Lawson and Pearlman, 1964; Laumas and Farooq, 1966b; 1967) are taken up by the target tissue when physiologic doses of these hormones are administered. These findings are corroborated by the reports in the literature about the effects of steroids and their metabolites which are transmitted to the infant through milk. (Curtis (1964) reported gynaecomastia in a male infant whose mother was taking Enovid and at the same time feeding the infant. Vaginal changes
resembling those after the administration of estrogen were seen in children whose mothers were breast feeding and at the same time taking oral contraceptives (Lauritzen, 1967). Breibart, Bongiovani and Eberlein (1963) have observed advanced skeletal development in 5-6 breast-fed infants whose mothers were taking Pranone (17α-ethynyl testosterone) or Norlutin (17α-ethynyl-17 hydroxy-4-estren-3-one) as oral contraceptive.

The present results showed that the presence of estrogenic activity can be demonstrated in the milk after the oral administration of some oral contraceptives. Enovid which is a combination of a progestagen (norethynodrel) and an estrogen (mestranol) was administered to three women and biological activity was demonstrated in post-gestagen milk of 2 women. Whether the estrogenic activity was due to both the components of the Enovid tablet viz. norethynodrel and mestranol needed further evaluation. It has been reported that mestranol has low metabolic clearance rate and a prolonged half life of the order of 40-60 hours (Wijmenza and van der Molen, 1969) and it is excreted in the milk (Abdel-Aziz, Bialy, Keith and Williams, 1969). It is thus possible that the observed estrogenic activity in the milk after the administration of Enovid may either be
due to mestranol or norethynodrel and its metabolites or due to both. In order to test this point experiments were carried out where norethynodrel alone was administered to women and estrogenic activity of the milk was tested. In these experiments estrogenic activity was detected in the milk of two out of a group of six women. The variations in estrogenic activity found in the milk of women administered norethynodrel may be due to varying amounts of the steroid and its metabolites excreted into the milk. However, no correlation of the estrogenic activity with the amount of milk could be established. On the other hand estrogenic activity could be detected in the milk of 3 out of 4 goats, who had been administered norethynodrel. It thus appeared that the volume of daily secretion of milk which is much larger in the case of goats may have an effect on the excretion of norethynodrel and its metabolites in the milk. Norethynodrel per se has some estrogenic activity (Drill, 1966). 17α-ethynyl 19 norandrost-4-ene 3β, 10β, 17β-triol, which is a metabolite of norethynodrel has also been shown to possess estrogenic activity (Bialy et al., 1965). It is possible that unchanged norethynodrel or any of its estrogenic metabolites may contribute to the estrogenic
activity found in the milk. The estrogenic activity
detected in the milk of the two women after the
administration of norethynodrel is however significant.

The estrogenic activity was estimated by
three day immature mouse uterine weight assay. The
milk extracts were injected into the mice in some
experiments while in other experiments these were
orally administered to the mice simulating a situation
as it prevails in practice of a breast fed infant.
Bialy et al. (1965) have shown that the metabolite of
norethynodrel possessing estrogenic activity viz.
17α-ethynyl-19-nor androst-4-ene-3β-10β, 17β-triol
is active by both injection and oral route.

Pincus, Bialy, Layne, Paniagua and Williams
(1966) did not find any estrogenic activity in the
milk of lactating women after the oral administration
of norethynodrel and ethynodiol diacetate. However,
the plan of assay of estrogenic activity used by
these authors differed from the present investigation.
Pincus et al. (1966) injected only half the milk
extract to a mouse while in the present investigation
the total extract from 1 milk sample was administered
to 1 mouse. Abdel-Aziz et al. (1969) have shown that
mestranol and van der Molen et al. (1969) have shown that lynestrenol are also excreted in the milk of lactating women.

While the data discussed above related to the analysis of estrogenic activity after the administration of progestational steroids which were derivatives of 19-nor steroids, it remained to be seen whether progestational steroids which are derivatives of 17α-acetoxy progesterone gave rise to any estrogenic activity in the milk. Chlormadinone acetate and megestrol acetate have no estrogenic activity and an evaluation of the estrogenic activity of their metabolites is not yet available. Administration of chlormadinone acetate and megestrol acetate to lactating women and goats did not give rise to any estrogenic activity as assayed by immature mouse uterine weight method.

A number of factors may contribute to the passage of contraceptive steroids into the milk and the significance of the observed estrogenic activity. The amount of the steroid and its metabolites passing into milk depends upon the quantity of the milk and
the half-life of the steroid in the plasma, which will indicate the duration of time for which the steroid is available in the plasma for excretion into the milk. Furthermore the rates at which the steroid and its metabolites with progestational or estrogenic activities, present in the blood are excreted into the milk may together contribute to the final ratio of progestational and estrogenic activity in the milk. It is also possible that progestational effects may be antagonized by estrogens and estrogenic effects inhibited by progestins and at times there may be certain potentiations (Edgren, Jones, Peterson and Gillen, 1967). Other pharmacological activities like the progestational or androgenic activities of the steroids excreted in milk also need evaluation.

The present findings thus lead to the conclusion that there is some possibility of transmission of estrogenic activity to the breast fed infant of a mother taking those progestational steroids which possess estrogenic activity or are used in combination with an estrogen. On the other hand, the administration of oral progestins which are derivatives of 17α-
acetoxy progesterone may not result in the transmission of estrogenic activity to the breast fed infant. The latter category of progestational steroids could thus be considered for fertility control during lactation. Further studies need to be carried out to search for a progestational steroid which will not result in the transmission of any progestational and estrogenic activity to the breast fed infant.