Hormonal Substances in Human Milk, Cow’s Milk and Dairy Products

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ABSTRACT

Hormone-like substances are normal constituents of cow’s and human milk. Progesterone has been quantified in cow’s milk and dairy products with butter containing a high value of 300 ng/g. Estrogenic substances have been detected in cow’s and human milk but literature values are sparse or tend to vary widely possibly because quantitative methods available lacked specificity and sensitivity. Pregnanediol is rather unique to human milk and its presence in elevated levels has been associated with prolonged neonatal jaundice of nursing infants. The concentration of this steroid in affected mother’s milk appears to be in the range of 150-450 ng/ml. 17-Ketosteroids may be present in cow’s and human milk but here again limited literature values vary widely. Corticoids have been reported in cow’s milk at a concentration of 3.1-3.7 ng/ml. Thyroxine and triiodothyronine are present in human milk but practically undetectable in cow’s milk. Progesterone has been quantified in cow’s milk and dairy products with butter containing a high value of

The steroid hormones as elaborated by the endocrine system occur in relatively small concentrations in body fluids. In fact, concentrations of these substances are so low in body fluids that only recently has it been possible to routinely quantify them. Use of microtechniques employing thin-layer chromatography, column chromatography, gas-liquid chromatography, radioisotopes, competitive binding assays, and radioimmunoassays has made it possible to estimate nanogram and picogram quantities of steroid hormones in one milliliter of milk. The primary impetus behind measuring various steroid hormones in milk resides in their diagnostic value to veterinarians and in aiding the husbandryman to manage reproduction. For example, maintenance of elevated concentrations of progesterone in milk 19 or more days after breeding is being recommended as early evidence of pregnancy. Since the dairy cow is usually pregnant 180 to 220 days at the end of her lactation period, market milk contains mixtures of milk from pregnant and nonpregnant cows. Thus, it is only natural that small amounts of steroid hormones associated with the reproductive cycle are present. By the same token, human milk also contains many of the same steroid hormones. It has been only within the past few years that the steroid hormone content of dairy products has been measured with a view toward the public health implications. Most analyses indicate that these hormones are present in the nanogram or picogram per milliliter level and, as such, should not constitute a public health threat. Thus development of ever increasing sensitive assays for these substances demonstrates their presence in natural foods such as cow’s milk and mother’s milk and, in effect negates the argument for zero tolerance.

This report is not an exhaustive review of the literature but is intended to give an overview of the subject. It’s likely that peptide hormones would be digested in the alimentary tract, at least in adults. Thus, for the most part, peptide hormones are not included in this review. However, prolactin in milk has been studied in some detail (11) and has been quantified using radioimmunoassay techniques (29). Individual samples of cow’s milk vary widely in prolactin concentration but usually range from 5 to 200 ng/ml with an average prolactin concentration of about 50 ng/ml (29). Quantities of steroid hormones present in human milk, cow’s milk, and dairy products will be discussed. Hormones which have been measured in these foods include progesterone, estrogens, pregnanediols, and 17-ketosteroids. In addition, miscellaneous hormones such as the corticoids and thyroxine will be covered briefly.

PROGESTERONE

Presence of progesterone in cow’s milk during pregnancy has been proven by isolation and characterization using gas-liquid chromatography coupled with mass spectrometry (12). There has been considerable disagreement and confusion, however, on the concentration of progesterone in cow’s milk. Reasons for the disparate analyses have been attributed to possible existence of progesterone metabolites which interfere with assay procedures, differences in assay techniques among laboratories, sample deterioration with storage, nonspecificity of antisera used in radioimmunoassay procedures, and time at which milk samples were taken. A major reason for differences in progesterone concentrations in milk appears to be the sampling procedure. It is well known that the amount of fat in milk samples is dependent on the time during the
milking process at which a sample is obtained (49). Also there is a positive correlation between the fat content of the milk sample and the progesterone concentration (21, 23, 37). Thus, from a consumer's point of view, only pooled milk samples from complete milkings are relevant with regard to progesterone concentration. Samples from partial milkings would give a biased concentration of progesterone. Consequently, only those papers in the literature that clearly indicated the analysis for progesterone represented whole milk samples or pooled whole milk samples will be considered in this report.

In 1956, Pigato and Guzzonato (41) attempted to quantify progesterone metabolites in the morning milk of five cows pregnant from 3 to 7 months. Whole milk samples were hydrolyzed with acid and the conjugated and non-conjugated hormones were extracted into benzene. Estrogens were separated from androgenic 17-ketosteroids and 20-ketosteroids (progesterone metabolites) by solvent fractionation. The 17-ketosteroids were separated from the 20-ketosteroids using the Girard T reagent. The 20-ketosteroids were quantified using the colorimetric reaction of Zimmermann (57). Levels of 20-ketosteroids ranged from 40 ng/ml for the cows pregnant 3 months to 96 ng/ml for the cows pregnant 7 months. As we shall see, these values for 20-ketosteroids are approximately one thousand-fold greater than those reported subsequently for progesterone in milk. It seems likely that the nonspecific solvent fractionation and colorimetric procedures could include adventitious ketonic materials thus yielding high values.

Laing and Heap (27) determined the progesterone levels in samples taken from the whole volume of milk obtained at afternoon milking of a herd of British Friesian cows. Milk samples were analyzed for progesterone by a competitive protein binding assay. The reliability of the method was comparable with that obtained for the assay of plasma progesterone. Of 16 cows in the herd known not to be pregnant but still lactating the average progesterone concentration was 5.10 ng/ml with a range of 1.30 to 15.89 ng/ml. In 17 animals known from later clinical examination to be pregnant the average progesterone concentration was 19.69 ng/ml with a range of 7.1 to 35.6 ng/ml. Samples were analyzed over the 19th to 215th day of pregnancy and there was a tendency for the progesterone concentration to decrease in the latter stages of pregnancy. However, subsequent research from the English group has consistently indicated lower levels of progesterone in the milk of pregnant cows (10,20). A partial explanation might lie in the sampling techniques used in these later studies whereby initial portions of the milking appeared to be taken resulting in lower fat and progesterone concentrations. On the other hand, Heap et al. (20) reported a serious interference in the radioimmunoassay technique used to quantify the progesterone. They observed that milk samples analyzed by a highly specific antiserum gave results consistently lower than those measured with a less specific antiserum. They suggested that the competitive protein binding assay and the radioimmunoassay using the less specific antiserum detected another and, as yet, unidentified compound(s) present in milk at an average concentration of about 1-2 ng/ml.

Nuti et al. (37) analyzed the pooled milk of 4 cows in mid-diestrus (days 11-14) using 2 radioimmunoassay procedures employing two different antisera. The mean concentrations of progesterone varied from 7.43 ± 0.95 to 12.30 ± 2.42 ng/ml depending upon the method.

Subsequently, this same research group (15) analyzed the progesterone concentrations in pooled milk from the bulk tank of a dairy farm over a 12-month period and a variety of dairy products from four markets. A radioimmunoassay procedure was used with minimal cross-reaction with other steroids. The antigen used for immunization and as a standard was a pure, synthetic progesterone. Validity of the assay was proven (16) as indicated by parallelism among unknowns, whether milk or plasma, and between unknowns and the standard. Furthermore, validity of the assay procedure was confirmed using gas-liquid chromatography (37).

The results of these analyses are listed in Table 1. The mean progesterone concentrations varied from 2.1 ng/ml in skim milk to 133 ng/ml in butter. The progesterone concentrations tended to correlate with the fat content of the dairy product with butter being the highest in progesterone.

Hoffman et al. (22) also determined the concentration of progesterone in milk products using a radioimmunoassay procedure. The samples were obtained directly from the dairy or from the market. The results of these analyses are listed in Table 2. In general, there seems to be fairly good agreement between the results in Tables 1 and 2. However, the concentration of progesterone in the butter sample in Table 2 is substantially higher than in butter in Table 1.

Progesterone given by mouth to humans is much less effective compared to that administered by injection (17). This would imply that the progesterone is poorly absorbed from the gastrointestinal tract or that it is substantially modified biochemically after absorption.

<table>
<thead>
<tr>
<th>Product</th>
<th>No. of samples</th>
<th>Fat %</th>
<th>Progesterone concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole milk</td>
<td>12</td>
<td>11.3</td>
<td>±0.6</td>
</tr>
<tr>
<td>Skim milk</td>
<td>12</td>
<td>4.6</td>
<td>±0.4</td>
</tr>
<tr>
<td>Cream</td>
<td>12</td>
<td>58.7</td>
<td>±5.3</td>
</tr>
<tr>
<td>Unprocessed products</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole milk</td>
<td>4</td>
<td>3.5</td>
<td>±0.5</td>
</tr>
<tr>
<td>Skim milk</td>
<td>4</td>
<td>0.01</td>
<td>±2.1</td>
</tr>
<tr>
<td>2% Milk</td>
<td>4</td>
<td>2.0</td>
<td>±0.4</td>
</tr>
<tr>
<td>Buttermilk</td>
<td>4</td>
<td>2.0</td>
<td>±2.0</td>
</tr>
<tr>
<td>Half-and-half</td>
<td>4</td>
<td>13.0</td>
<td>±2.5</td>
</tr>
<tr>
<td>Cream</td>
<td>4</td>
<td>35.0</td>
<td>±5.8</td>
</tr>
<tr>
<td>Butter</td>
<td>4</td>
<td>79.6</td>
<td>±5.1</td>
</tr>
</tbody>
</table>

*From Ginther et al. (15).*
Relatively large oral doses of progesterone are essential to evoke a contraceptive response in human females. Pincus (42) administered 300 mg/day by mouth over the menstrual cycle and observed an inhibition of ovulation in 30 of 50 women. Analysis of urine samples collected on days 19 to 21 of the cycle indicated that approximately 5% of the ingested progesterone was excreted in the urine as pregnanediol. This would suggest that the oral progesterone was poorly absorbed. However, one must realize that absorbed progesterone could also have been excreted as metabolites other than pregnanediol.

Stone and Kupperman (50) gave 1,000 mg of progesterone daily to each of 13 patients for 10 to 12 days, starting with the 8th day of the cycle. Of 16 cycles studied, inhibition of ovulation was evident in only six cycles. Thus, even when massive doses of progesterone are administered orally, the biological response is far from universal. In view of the large oral doses of progesterone necessary to effect a contraceptive response in human females, it is clear that the nanogram levels of progesterone found in dairy products would not have a significant biological effect.

**ESTROGENS**

Bioassay procedures that detect total estrogenic activity have been used to derive a semiquantitative indication of the presence of estrogenic materials in milk and milk-products (36, 55). Munch (36) was able to detect only estrogenic activity but no androgenic activity in milk. However, Vogt et al. (55) observed an increase in uterus weights of juvenile mice fed low levels of dried whole milk powder but a reversed increase in the weights of uteri from juvenile mice fed high levels of whole milk powder. They could not confirm the uterotrophic activity of whole milk powder by feeding skim milk powder or butter.

Turner (54) made an early attempt to quantify the estrogenic activity in dried whole milk and colostrum using a bioassay procedure. In each assay, 10 ovariectomized mice were fed dried whole milk for 10 days and the uterus wt/body wt % was determined. A standard curve for estrogenic activity was prepared by feeding graded amounts of diethyl stilbestrol added to a mixture of commercial dried skim milk and butter. This diet would, of course, include any endogenous estrogens in the skim milk-butter feed. From the standard curve, estrogenic activity was reported as diethyl stilbestrol equivalents.

The uterus wt/body wt % values for mice fed mouse chow compared to those fed dried whole milk were 0.04 and 0.05%, respectively. Using diethyl stilbestrol as the standard, Turner was unable to detect 90 ng or less of estrogenic activity per gram of dried whole milk. However, there is a discrepancy in the standard curve calculation which resulted in an overestimation of diethyl stilbestrol equivalents by a factor of 10. Thus, the above value should be 9 ng or less of estrogenic activity per gram of dried whole milk. All values reported subsequently will be corrected.

Estrogenic activity was not detectable in milk of 11 nonpregnant cows. Subsequently, Turner assayed the milk of 32 pregnant cows. Of 10 cows pregnant from 27 to 97 days, only a single cow produced milk which stimulated uterine weight in excess of the control range. Of 11 cows pregnant from 104 to 193 days, three cows produced milk estimated to contain 14 to 18 ng of diethyl stilbestrol equivalent/g of dried milk. Since milk is 88% water, this would amount to about 1.7 to 2.2 ng/ml of fluid milk. Of 11 cows pregnant 200 to 268 days the average uterine wt/body wt % was 0.09. Milk from one cow contained approximately 35 ng/g dried milk (4.2 ng/ml); whereas, the milk of three cows in this group gave a response equivalent to about 18 ng/g dried milk (2.2 ng/ml). Thus the milk sample with the highest activity in all of Turner's studies had a diethyl stilbestrol equivalent of 4.2 ng/ml.

Turner also assayed 18 samples of milk collected at monthly intervals. The average uterine wt/body wt % over the 18 months was 0.06. However, when cows were on pasture there was evidence of estrogenic activity in the milk. For example, in August and September this amounted to about 13.5 ng/g dried milk (1.6 ng/ml of milk). It is known that uterotrophic substances are produced by plants (5).

Pope and Roy (43) were unable to detect estrogenic activity in normal milk using a mice bioassay. They could detect <1 ng of 17β-estradiol per ml of milk.

Monval-Gerondeau et al. (35) estimated the estrogens in cow's milk using a double isotopic labeling procedure. Only those estrogens occurring in the milk fat were quantified. The sensitivity of the method was about 12 pg/ml of milk for estrone, 10 pg/ml for 17β-estradiol and 16 pg/ml for estriol. The reproducibility of the method was very good. Table 3 lists the concentration of estrogens found in samples of commercial milk products. The highest total level of estrogens occurred in the September milk samples containing 114 pg/ml.

Lunaas (28) analyzed milk from one nonpregnant cow over 4 days for conjugated and non-conjugated estrone and estradiol. Selective extraction procedures were used to separate conjugated from non-conjugated estrogens. Hormones were quantified using a spectrofluorometric procedure which is both highly sensitive and nonspecific. The free estrone concentration in milk was 20-40 pg/ml;

### TABLE 2. Concentration of progesterone in various dairy products

<table>
<thead>
<tr>
<th>Product</th>
<th>Fat (%)</th>
<th>Progesterone concentration (ng/ml or g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole milk</td>
<td>3.5</td>
<td>12.50</td>
</tr>
<tr>
<td>Skim milk</td>
<td>0.1</td>
<td>1.40</td>
</tr>
<tr>
<td>Cream</td>
<td>32.0</td>
<td>43.00</td>
</tr>
<tr>
<td>Buttermilk</td>
<td>1.0</td>
<td>6.50</td>
</tr>
<tr>
<td>UHT whole milk</td>
<td>3.5</td>
<td>11.75</td>
</tr>
<tr>
<td>Low fat UHT milk</td>
<td>1.5</td>
<td>6.00</td>
</tr>
<tr>
<td>Low fat sour milk</td>
<td>1.5</td>
<td>4.20</td>
</tr>
<tr>
<td>Unsweetened condensed milk</td>
<td>10.0</td>
<td>12.25</td>
</tr>
<tr>
<td>Butter</td>
<td>82.0</td>
<td>300.00</td>
</tr>
<tr>
<td>Nonfat dry milk</td>
<td>1.5</td>
<td>17.10</td>
</tr>
<tr>
<td>Whole milk powder</td>
<td>25.0</td>
<td>98.40</td>
</tr>
</tbody>
</table>

*aFrom Hoffman et al. (22).*
whereas the conjugated estrone ranged from <20-40 pg/ml. The free estradiol ranged from 60 to 200 pg/ml and the conjugated estradiol varied from 120 to 160 pg/ml. Thus the total estrogen in milk from a nonpregnant cow was <220-440 pg/ml.

Recently, Monk et al. (33) estimated the free estrone and estradiol in the total milk from one quarter using radioimmunoassay techniques. The whole milk was extracted with ether and the extract was used for quantification of the steroids. Recovery of added free estrogens to the milk was very good when corrected for procedural losses. Table 4 lists the concentrations of free estrogens found in the milk of cows at various stages of pregnancy. The total free estrogens in milk ranged from 87-146 pg/ml which is about the same level found by Minval-Gerondeau et al. (35).

Subsequently, Chicchini (7) determined 17β-estradiol and estrone in the milk of six nymphomaniac and six normal cows during the estrus cycle. He apparently sampled the entire milking in each instance. However, the method of Itrich (24) was used to quantify the estrogens. In this method, the fat phase is discarded and the conjugates in the aqueous phase are hydrolyzed by hydrochloric acid with possible destruction of some estrogens. This is followed by various partitioning steps between organic solvent and alkaline aqueous phases. The estrogen mixture is then subjected to chromatography on A185 by using organic solvents to develop the column. This procedure separates 17β-estradiol, estrone, and estriol which are then quantified using a modification of the Kober (25) reaction utilizing strong sulfuric acid and a substituted phenol to form the final color complex which is estimated colorimetrically. It's doubtful this reaction would be very specific, and the specificity of the method would depend in part on the fractionation procedure. The ranges of estrogens found in the milk of six normal cows by Chicchini (7) were as follows: at estrus, 0.25-0.80 µg/100 ml; 17β-estradiol and 1.85-4.63 µg/100 ml of estrone; at mid-estrus 0.27-0.48 µg/100 ml 17β-estradiol and 0.92-1.08 µg/100 ml of estrone. Thus the estrogen values varied between 2.3 ± 0.9 µg/100 ml for 17β-estradiol and 9.2 and 46.3 ng/ml for estrone. These values are substantially higher than those of previous investigators.

Subsequently, Chicchini (6) determined the estrogen content of milk from nine Friesian cows on each of the first 7 months of pregnancy. Here again the methods of Itrich (24) and Kober (25) were used to quantify the hormones. The average total estrogen contents were 12, 16, 24, 55, 101, 236, and 494 ng/ml. These values are an order of magnitude higher than those previously reported (7) and considerably higher than the results of other investigators.

Pascoli (40) reported even higher values for the total estrogens in milk from several cows. Estrogens were measured colorimetrically using the Kober (25) reaction. Values for total estrogen ranged from 2.95 to 3.70 µg/ml of milk. Since these levels are several orders of magnitude higher than values generally reported by other workers, they are of doubtful validity. In view of the wide variation in estrogenic activity reported in cow's milk, a careful, detailed study of estrogen levels in cow's milk and dairy products is called for.

To put the estrogen content of cow's milk into a better perspective, we might consider the estrogens evident in human milk. In 1953, Rossi (45) used a rather nonspecific solvent partitioning fractionation technique to isolate the total estrogens in postpartum human milk. Total estrogenic content of the morning and evening milk as estimated by the Kober (25) reaction averaged 1.8

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### TABLE 3. Estrogens in commercial milk samples

<table>
<thead>
<tr>
<th>Product</th>
<th>Fat % (g/100 ml)</th>
<th>No. of determinations</th>
<th>Estrone (pg/ml)</th>
<th>Estradiol (pg/ml)</th>
<th>Estriol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasteurized commercial milk</td>
<td>3.4</td>
<td>10</td>
<td>31</td>
<td>10</td>
<td>37</td>
</tr>
<tr>
<td>Pasteurized commercial milk</td>
<td>3.4</td>
<td>4</td>
<td>78</td>
<td>30</td>
<td>6.3</td>
</tr>
<tr>
<td>Partially skimmed powder</td>
<td>1.8</td>
<td>4</td>
<td>28</td>
<td>&lt; 5</td>
<td>7</td>
</tr>
</tbody>
</table>

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### TABLE 4. Average quantities of estrone and estradiol in milk during pregnancy

<table>
<thead>
<tr>
<th>Stage of pregnancy (days)</th>
<th>No. of cows</th>
<th>Estrone (Average ± SE)</th>
<th>Estradiol (Average ± SE)</th>
<th>Sum (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>55-81</td>
<td>5</td>
<td>57 ± 20</td>
<td>85 ± 9</td>
<td>142 ± 20</td>
</tr>
<tr>
<td>107-145</td>
<td>4</td>
<td>35 ± 13</td>
<td>52 ± 14</td>
<td>87 ± 22</td>
</tr>
<tr>
<td>205-209</td>
<td>4</td>
<td>97 ± 21</td>
<td>49 ± 21</td>
<td>146 ± 27</td>
</tr>
</tbody>
</table>

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*From Monk et al. (33).*
and 0.9 µg/ml in 11 women with a male fetus and 1.1 and 0.6 µg/ml in nine women with a female fetus, respectively. Sas et al. (46) determined the steroid content of pooled samples of milk from 10 healthy women on the 2nd to 10th day and on the 15th, 20th, and 25th day postpartum. Estrogens were determined by the colorimetric method of Ittrich (24). Total estrogen (estrone, estradiol, estranol) values corrected for procedural losses are given in Table 5. The estrogen content of human milk over the first few weeks postpartum is substantially higher than those values for cow's milk reported by most other investigators. Sas et al. (46) estimated the daily amounts of estrogen consumed in the mother's milk by the nursing infants. These values are also in Table 5. As indicated in Table 5, the concentration of estrogens in milk postpartum goes through a maximum at about 5 days then declines to about the levels found in cow's milk at 25 days. Comparison of the levels of estrogens in human milk to those in cow's milk suggests that a nursing infant would receive more estrogens over the first month from human milk than cow's milk. However, much more detailed and extensive analyses will be necessary to firmly establish these values. The reported values for estrogens in milk that were derived using nonspecific fractionation and colorimetric procedures. Furthermore, the chemistry of the Kober reaction is exceedingly complex, and Oliver et al. (38) have emphasized the need for strict controls over the purity of the reagents, the reaction vessels, the heating temperature, and the necessity for a knowledge of the specificity of the reaction mixtures. Since the specificity of these colorimetric techniques depends, in part, on prior fractionation procedures, it is not unreasonable to suspect that plant phenolics derived from the cow's diet, for example, may be contributing to the high estrogenic values. In any event, further carefull, detailed studies on quantifying estrogenic activity in human and cow's milk are needed.

Concern for the infant-nursing mothers taking contraceptive steroids has been expressed. Molen et al. (32) have calculated that a child consuming 600 ml of breast milk from a mother receiving 5 mg 17 a-ethynyl-4-estren-17-β-01 and 150 µg 17 a-ethynyl-1,3.5(10)-estra- diene-3, 17β-diol 3 methyl ether; 17 a-ethyl estradiol 3-methyl ether would get about 8 µg of steroid metabolites per day.

TABLE 5. Estrogen content of mother's milk and daily estrogen dose to the nursing infant.

<table>
<thead>
<tr>
<th>Days post-partum</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total estrogen (corrected) (ng/ml)</td>
<td>241</td>
<td>403</td>
<td>465</td>
<td>843</td>
<td>336</td>
<td>595</td>
<td>312</td>
<td>351</td>
<td>186</td>
<td>140</td>
<td>57</td>
<td>15</td>
</tr>
<tr>
<td>Daily consumption of milk (ml)</td>
<td>50</td>
<td>160</td>
<td>320</td>
<td>350</td>
<td>450</td>
<td>480</td>
<td>500</td>
<td>530</td>
<td>560</td>
<td>600</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Daily dose of estrogen (µg/day)</td>
<td>12</td>
<td>65</td>
<td>149</td>
<td>249</td>
<td>151</td>
<td>250</td>
<td>156</td>
<td>136</td>
<td>104</td>
<td>84</td>
<td>34</td>
<td>9</td>
</tr>
</tbody>
</table>

From Sas et al. (46).

PREGNANEDIOL

Pregnane-3α, 20β-diol represents a rather unique situation with regard to infants nursing on mother's milk. Apparently, this steroid does not exist to any appreciable extent in cow's milk but does appear in mother's milk. The presence of this steroid in human milk has been associated with the occurrence of neonatal unconjugated hyperbilirubinemia or neonatal jaundice.

In 1963, Arias et al. (3) first reported that breast milk of mothers nursing newborns with prolonged unconjugated hyperbilirubinemia strongly inhibited the formation of o-amino-phenol glucuronide and of bilirubin glucuronide in vitro. Thus the steroid interfered with normal excretion of bilirubin. The inhibitory activity was negligible in milk of randomly examined mothers. These same investigators (2, 4, 14) stated subsequently that this inhibitory activity was not present in cow's milk and that pregnane 3α, 20β-diol was present in inhibitory but not in noninhibitory human milk. Pregnane-3α, 20β-diol competitively inhibits glucuronon transferase activity in vitro. Furthermore the unconjugated hyperbilirubinemia could be produced in very young, full term infants by feeding pregnane 3α, 20β-diol at levels of approximately 1 mg/day, which is the quantity the above authors estimated to be secreted per day in breast milk. This type of neonatal jaundice is rapidly reversible and immediately disappears when the affected infant is placed on a formula of cow's milk (56).

The incidence of breast milk jaundice has been estimated to vary between 1 in 200 to 1 in 500 breast fed infants (56). Severe hyperbilirubinemia in the neonatal period can result in neurotoxicity known as kernicterus which results in irreversible brain damage (56).

Severi et al. (48) estimated the concentration of pregnane-3α, 20β-diol using thin layer techniques for separation followed by colorimetric analysis. Krauer-Mayer et al. (26) claimed this method of analysis had a sensitivity of 0.05 µg and practically absolute specificity. Concentrations of pregnane-3α, 20β-diol in human milk samples implicated in neonatal jaundice were estimated at 150 to 450 ng/ml by Severi et al. (48) and at 200 to 420 ng/ml by Krauer-Mayer et al. (26). In the latter analyses, the 420-ng/ml value was observed in breast milk 42 days postpartum.

Fontaine et al. (13) reported on four cases of breast milk jaundice resulting from milk containing 75 to 385 ng of pregnane-3α, 202-diol per ml. Using a gas-liquid chromatographic method, Tanaka (53) determined
concentrations of this steroid to be 2.38 to 0.4 μg/ml of breast milk depending upon the time postpartum. Sevelli and Battista (47) reported on 11 cases of neonatal jaundice caused by pregnanediol in mother’s milk. However, the levels of pregnanediol (0.4-2.2 mg/ml) in the breast milk reported by these workers is orders of magnitude higher than that reported by other workers in the field.

Apparently breast milk implicated in neonatal jaundice contains elevated levels of pregnane-3α, 20β-diol for extended periods, whereas the steroid level of milk from normal women decreases rapidly from 350 ng/ml on the second day postpartum to undetectable levels on the third day postpartum (36). The view that pregnane-3α, 20β-diol in breast milk is responsible for breast milk jaundice is not universally held, however (46).

17-KETOSTEROIDS

In an early paper on 17-ketosteroids in postpartum human milk, Rossi (44) used solvent fractionation to obtain the androgens which were quantified with the colorimetric reaction of Zimmermann (57). By this method the average 17-ketosteroid content of the morning and the evening milk was 103 and 66 μg/ml in 11 women with male fetuses and 146 and 97 μg/ml in nine women with female fetuses, respectively. Sas et al. (46) found lower levels of 17-ketosteroids in pooled milk from normal, healthy lactating women over the first 25 days postpartum. The 17-ketosteroids studied included dehydroepiandrosterone, androsterone, androstenedione, androstanediol, and chloro-dehydroepiandrosterone. Extremely high levels of these steroids were reported in human milk. Uncorrected total 17-ketosteroids ranged from 12.52 μg/ml at day 2 postpartum to a high of 35.60 μg/ml at day 4 postpartum to a low of 0.4 μg/ml at day 25 postpartum. However, Darling and Harkness (11) suggest that contaminants might be responsible for the high values of Sas et al. (46).

Pascoli (40), using a colorimetric technique, reported extremely high values for the 17-ketosteroids of cow’s milk. The concentration of these steroids varied from 66.4 to 83.4 μg/ml of milk. Subsequently, Pigato and Guzzonato (41), using relatively nonspecific procedures of fractionation and colorimetric analysis (41), estimated the 17-ketosteroids in milk of five cows pregnant from 3 to 7 months. Values varied from a high of 45 μg/ml to a low of 36 μg/ml and decreased as pregnancy progresses. These enormous amounts of 17-ketosteroids in cow’s milk were not confirmed by Darling et al. (10) using more sensitive and sophisticated techniques. Darling et al. (10) surveyed the steroids in cow’s milk by oxidizing the steroid mixture to steroid ketones which were separated and estimated using gas-liquid chromatography. The 5α-androstane 3,17 dione obtained from the milk of four pregnant cows varied from 0.7 ± 0.4 ng/ml to 5.0 ± 2.2 ng/ml over 136 days of pregnancy. Darling et al. (10) suggested that the precursors for the above oxidized steroid were in part androsterone and epiandrosterone. Thus the concentrations of 17-ketosteroids in cow’s milk appear to be relatively low.

MISCELLANEOUS HORMONES

Paape et al. (39) reported that the levels of corticoid in cow’s milk ranged from 3.1-3.7 ng/ml.

Strbak et al. (51) determined the amount of thyroxine, which was concentrated in the lipid phase, in human milk using a protein displacement method. For five determinations on human milk the levels were 46 ± 8 ng/ml. In a second series of analyses, the values ranged from 50 to 72 ng/ml of milk. There was no evidence of thyroxine in cow’s milk. Subsequently, Strbak et al. (52) used a competitive binding assay with a high specificity for thyroxine to analyze human milk. These workers examined 94 samples of human milk from 45 lactating mothers from the 3rd day up to the 9th week after delivery. Immediately after initiation of lactation the thyroxine content of the milk was only 13 ± 3 ng/ml. During subsequent days of lactation the levels of thyroxine in milk increased. Analysis of cow’s milk formulas showed very small or a doubtful content of thyroxine. Montalvo et al. (34) examined human breast milk andcolostrum from four healthy euthyroid mothers for thyroxine and triiodothyronine. Milk was obtained on day one, 1 week and 1 month postpartum. Colostrum contained a mean of 6.5 ± 2 ng/ml of thyroxine and a mean of 0.38 ± 0.09 ng/ml of triiodothyronine (T₃). At 1 week postpartum the mean thyroxine level was 96 ± 4 ng/ml whereas the mean T₃ value was 1.13 ± 0.25 ng/ml. One month postpartum the levels of thyroxine had decreased to 20.7 ± 6 ng/ml and those of T₃ to 0.99 ± 0.06 ng/ml. Thus thyroxine and T₃ are secreted in significant amounts in human milk but hardly at all in cow’s milk.

Because of the possibility that certain prostaglandins (PGF₂α) might be used to regulate the estrous cycle of cattle and sheep to allow insemination at preset times, considerable interest has arisen in measuring prostaglandin F (PGF) in milk before and after administration of these compounds. Manns (30), using a radioimmunoassay procedure, measured PGF in milk and blood of four cows for periods of 30 min before and 7 h after intramuscular injection of 30 mg of PGF₂α. Six PGF₂α and four control (vehicle only) injections were performed on the four cows. Maximal concentration of PGF in milk (.91 ± .12 ng/ml) occurred at 1 h after injection and declined to preinjection concentrations of .2 to .4 ng/ml at 7 h after injection. Hansel et al. (19) have carried out an experiment with six Holstein cows in which PGF₂α was measured in milk obtained at two regular milkings just before PGF₂α administration and in milk obtained at eight successive milkings after PGF₂α administration. Twice daily milkings were made at 0800 and 1800. The PGF₂α was administered into the uterus at 5- or 10-mg doses. The concentration of PGF before administration of the PGF₂α was approximately .14 - .15 ng/ml milk. After administration of the PGF₂α, concentrations of PGF varied between .17 to .23 ng/ml milk over the next seven milkings. On the eighth milking
after administration, the PGF concentration in milk had fallen to pre-administration levels to .12 ng/ml. The concentrations of the metabolites of PGF$_2$α in milk have not been measured yet.

If one assumes an average concentration of PGF in milk of 0.2 ng/ml and that it is concentrated in the fat phase, then one can readily calculate an approximate concentration in butter as 5 ng/g. To put this level in perspective, intraaortico instillation of 40 mg of PGF$_2$α is used to induce abortion in humans (17). Manns (30) points out that the quantities of PGF$_2$α in milk from treated or untreated cows is insignificant relative to those required orally to cause abortion in humans.

Apparently, exogenous PGF$_2$α can affect steroid metabolism. However, Hansel et al. (19a) found no evidence for elevated concentrations of testosterone in milk of PGF$_2$α-treated cows. Mean concentrations of testosterone in milk for three cows with active corpora lutea for the 10 milkings ranged from .45 to .71 ng/ml. On the other hand, in the work of Hansel et al. (19), milk progesterone concentrations for the three cows with functional corpora lutea were high (7.6 to 22.5 ng/ml) in the samples collected before treatment and declined to a concentration of less than 3.0 ng/ml by 72 h after treatment. Progesterone concentrations in milk in the remaining three cows rose from a mean of 4.3 ng/ml before treatment to a mean of 13.1 ng/ml. These animals were at days 2 to 5 of their estrous cycles when treated, and development of corpora lutea proceeds in a normal way when animals are treated with PGF$_2$α at this stage of the cycle.

Brewington et al. (6) failed to detect the conjugates of acne-causing hormones (glucuronides of testosterone, androsterone, or estradiol) in cow's milk at concentrations of <10-15 µg per liter.

CONCLUSIONS

The analytical procedures for progesterone in milk based on radioimmunoassay appear to yield reliable results. The levels of progesterone found in dairy products are biologically insignificant when compared to the massive oral doses of progesterone needed to exert a biological effect in humans. There is a great need to obtain reliable, quantitative data on estrogens in cow's and human milk. Older values based on colorimetric techniques are extremely high when compared to recent data predicated on more specific techniques. Pregnanediol in human milk has been associated with the occurrence of neonatal jaundice of nursing infants. Since this steroid is essentially absent in cow's milk, the jaundiced condition can be alleviated by substituting cow's milk for human milk. Disparate analytical results for 17-ketosteroids in milk point out the need for further, careful measurement of those steroids in human and cow's milk. Limited analyses indicate very low levels of corticoids, thyroxine, triiodothyronine, and prostandandins (PGF$_2$α) in cow's milk. Since milk is a very complex biological fluid reflecting metabolic processes, it is likely that other hormones and hormone metabolites will be found there.

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