ABSTRACT

Portions of an extensive literature were reviewed to evaluate the status of our knowledge relative to progesterone in the bovine. Studies related to the secretion and metabolism of progesterone have been hampered by the lack of chemical methods sufficiently sensitive and specific to quantitate the hormone in small samples. Certain bioassays are extremely sensitive but lack specificity and have undefined limits of error. Spectrophotometric determination of progesterone or progesterone derivatives appears specific but is sensitive to only 0.1-0.5 μg. Fluorometric, gas chromatographic, and double-isotope derivative methods promise sensitivity to 0.01 μg with adequate specificity and precision.

Progesterone and 20 α-hydroxy-Δ 4 -pregnene-3-one appear to be the principal progestins in the corpus luteum (CL) of cycling and pregnant cows. Progestins have also been reported in bovine ovaries, adrenals, and blood. The CL appears to be the most important source of progesterone throughout bovine pregnancy. The CL from a previous cycle contains measurable progesterone for two or three days after estrus. Progestin in the CL and ovarian vein blood declines rapidly during proestrus from maximum levels observed 14-16 days post-estrus. In the pregnant cow, CL progestin levels decline about 40% from mid-cycle levels by 42-56 days of pregnancy, further decline slowly until 200 days, then rise again to the 28-day level; after 250 days, a decline to parturition begins. Similar changes are seen in blood levels of the hormones during pregnancy. Progesterone appears important in pregnancy maintenance, expression of estrus, normal cyclic function, and in hypophyseal-gonad interrelationships.

Thirty years have elapsed since the first isolation of progesterone as a crystalline compound. Since that time, most attempts to use the hormone therapeutically to correct faulty reproduction in the bovine have generally proven unsuccessful. Basic mechanisms involved in the different phases of reproduction are only generally understood, and many details are almost completely unknown. Specific sites or qualitative and quantitative aspects of progesterone action and the precise role of the hormone in the hypothalamo-hypophyseal-ovarian axis (5, 60, 64, 127) remain obscure.

For many years progesterone (Δ 4 -pregnene-3,20-dione) was thought to be the only naturally occurring progestational agent. In more recent years, however, several other compounds have been isolated. The most important of these, quantitatively, appear to be 20 α-hydroxy-Δ 4 -pregnene-3-one (20 α-ol), 20 β-hydroxy-Δ 4 -pregnene-3-one (20 β-ol) and 17 α-hydroxy-Δ 4 -pregnene-3,20-dione (17-hydroxyprogesterone). In 1936, the A.I.M.A. Council on Pharmacy and Chemistry (3) adopted the term progestin to indicate progestational agents without regard to the state of chemical purity. Progestin will be used in this paper to refer to this group of hormones in biological media or in pure form.

The purpose of this paper is to review the methodology in progestin research and the levels and importance of progestin in the bovine, using comparative data where they appear applicable. No hormonal action is completely independent of the effects of other hormones, and progesterone is no exception; nonetheless, the effects of progestin on reproduction will be isolated here, insofar as is possible. For a more complete presentation of hormonal interrelationships, the reader is referred elsewhere (5, 10, 38, 40, 41, 114, 140, 157).

BIOLOGICAL ACTIONS OF PROGESTERONE

The physiological and pharmacological actions of progestins have been recently listed (98); therefore, no attempt will be made here to present an all-inclusive discussion of the properties. A general listing of these actions, however, should be valuable for a better understanding of the complexity of the hormonal aspects of reproduction.

First of all, progestins are essential for the uterine development necessary for implantation, blastocyst development, and maintenance of the fetus and of uterine tone during pregnancy.
Moreover, progestin may be important as a conditioning agent for normal bovine estrus (92) and may have an effect on tubal and uterine transport of sperm and ova (98). These hormones may inhibit fertilization at certain dose levels in vivo (98) and in vitro (27). Profound effects are also noted on vaginal epithelium (108, 157), ovarian function (31, 49, 106, 142), gonadotropin elaboration (45, 84), and oxytoxin (50) and relaxin (98, 157) actions. Pharmacological doses may antagonize the actions of estrogens or corticoids (32, 98, 157).

Many of the above actions are mimicked to one degree or another by progestins other than progesterone, but no compound has yet been found which duplicates exactly all of the effects of the parent hormone, progesterone. The biological significance of the 20-hydroxy compounds, for example, is not understood; they may well be naturally occurring metabolites of progesterone. Both isomers have twice the activity of progesterone in the Hooker-Forbes intruterine assay in mice (155). In the Clauberg rabbit bioassay, 20 β-ol has 10-20% of the activity of progesterone (80, 155), and 20 α-ol is 30-50% as active (155). After studying its effects on the endometrium, delay of menstruation, production of withdrawal bleeding, cessation of dysfunctional bleeding, cervical mucus, vaginal smear, and several other factors in the human, Lauritzen (80) reported that 20 β-ol is a true progestational hormone with nearly half the activity of progesterone.

Conversely, 20 β-ol did not support deciduomata formation in the ovarioectomized rat when 3-mg injections were used (0.5 mg progesterone is effective) (147). In addition, both 20-hydroxylated compounds failed to maintain pregnancy in the ovarioectomized mouse, even with large doses (146).

It seems likely that the biological effectiveness of progestins other than progesterone will remain obscure until considerably more is known about the mechanism of action of these hormones (25, 53, 79, 106, 141). It is disappointing to note that few results have been forthcoming in this area, although rapid advances are being made toward the elucidation of the primary actions of other steroid classes (59, 74, 104, 141). It is probable that the advent of better methodology will facilitate such studies with the progestins.

ASSAY METHODS FOR PROGESTERONE

Studies related to the secretion and metabolism of progesterone have been hampered to date by the lack of accurate chemical techniques sufficiently sensitive and specific to facilitate the quantitation of the hormone in small plasma samples. Until quite recently it was necessary to collect reproductive tissues at surgery (55, 89) or slaughter (42, 158-161) for the estimation of progesterone. Present methods require the use of large volumes of blood (118).

Certain biological assays are extremely sensitive, but lack specificity and have undefined limits of error (36, 98).

BIOASSAY OF PROGESTINS

Several excellent reviews of progestin bioassay methods are available (98, 108, 157). In general, the assays based on end points such as complete progestational proliferation, uterine granulosa/mucosa areas, carbonic anhydrase activity, deciduomata formation, pregnancy maintenance, or parturition delay [for specific assays and references, see (98, 157)] lack sensitivity and are often difficult and time-consuming. The local progestational tests by McGinty and Hooker and Forbes (see 108, 157) are the most sensitive progesterone assays known, but these tests utilize a subjective end point and lack specificity. Furthermore, these methods have consistently indicated progesterone levels in biological fluids that cannot be verified by chemical assay (36). In fairness to the bioassay, it should be pointed out that animal responses are essential for the assessment of activity of crude extracts and of individual compounds and are valuable in the isolation of unknown substances. The bioassay has played a vital role in progestin research to date and will be equally important in mechanism of action studies and in the evaluation of the newer synthetic progestins (45, 61, 62).

CHEMICAL METHODS

Chemical methodology applicable to progesterone research has been reviewed in recent years (101, 114, 154). However, an abundance of new methods has appeared which may be of special significance, depending on the objectives of various research programs dealing with large animal production.

Extraction and purification. Extraction of steroids from tissues and fluids has generally been accomplished by the use of organic solvents or mixtures of solvents (54, 154). Zander (154) has shown that pretreatment of plasma samples with sodium hydroxide as proposed by Short (118) improves the precision of the final progesterone quantitation. In addition, NaOH decreases the formation of solvent-plasma emulsions during ether extraction.
One of the difficulties involved in solvent extraction is the great quantity of lipid extracted. Several methods have been utilized to remove this fatty material, including centrifugation or filtration in the cold (57, 134) or solvent partitioning (39, 118). A further suggestion has been the use of an acetylation step following extraction. In this procedure (16), the crude extract is subjected to acetylation following preliminary chromatography. Since progesterone cannot be acetylated, the other impurities may be converted to derivatives which will not remain with progesterone during subsequent chromatography.

Zander (154) has reviewed the use of Girard's Reagent T for the separation of ketones from crude extracts, but points out that this procedure has not been applied to routine microanalytical assays for progesterone.

To obtain a more nearly pure extract from biological samples, Zaffaroni et al. (153) applied dialysis for the extraction of steroids from blood, but recovery of added progesterone was too low for this method to be valuable. DeVenuto et al. (28) modified the dialysis procedure by extracting continuously in a Soxhlet extractor. Referring to this procedure as perextraction, these workers reported 100% recovery of progesterone-C\(^{14}\) added to the blood. Furthermore, no discernible lipid, protein, or pigment was noted in the extract. The method is limited by the relatively small volume of fluid that can be extracted, but the lack of lipids or chromagens in the extract might further the use of perextraction as quantitative measurement of progestins becomes more sensitive.

Separation of individual steroids. Countercurrent distribution has been used for the separation and purification of steroids (83, 100, 101, 108, 114), but has been utilized in only one routine method (83). Application of this excellent method appears to have been limited by the relatively long time required to separate and purify a single sample.

Wilson et al. (149) devised a method using column chromatography for the separation of steroids on synthetic aluminum silicate with aqueous ethanol. Other workers have used similar systems with silica gel, celite, aluminum oxide (105, 130), or other supporting media in the columns. Column chromatography is advantageous for the removal of large amounts of impurities, but packing of columns and analysis of individual fractions is very time-consuming unless automation is applied (4, 48).

The widespread use of paper chromatographic techniques in the separation and identification of progestins is evidence of the advantage of this procedure (15, 16, 102). Since the early use of these systems for the separation of adrenal steroids (15, 16), paper chromatography systems have been devised and studied for the separation of virtually all steroids. Extensive reviews and books on the paper chromatographic separation of steroids are available (16, 102). In substance, steroid chromatographic systems utilize biphasic organic solvents for the separation of compounds on a paper strip or sheet. Resolution, sharp separation, and simplicity are distinct advantages of this tool.

A new method of adsorption and partition chromatography—an open column—has been developed in recent years. Thin-layer chromatography (TLC), though in its infancy in steroid research, has already been heralded as a micro-chemical technique that will supplement or even replace paper chromatographic methods. The speed (20-40 min) compared to that of paper chromatography (4-24 hr), the wide range of distinct resolution (5-500 \(\mu g\)), and the noticeably sharp separation of compounds are distinct advantages of TLC (63). Furthermore, the organic layer on a glass support allows the use of corrosive spray reagents and can be prepared with luminous indicators (115). The method has already been tested for a great number of steroids in various solvent systems and complete separation data are available for progestins (81).

Enzymatic methods have become more valuable in recent years for the separation and identification of steroids, although this has not been their primary role in progestin research. Henning and Zander (67) probably offered the best example of this application of enzymatic methods when they described the separation of 20 \(\alpha\)-ol and 20 \(\beta\)-ol by 20 \(\beta\)-hydroxysteroid dehydrogenase. The specific enzyme quantitatively converts 20 \(\beta\)-ol to progesterone but leaves 20 \(\alpha\)-ol unchanged. The separation can then be accomplished by chromatography, and the 20 \(\beta\)-ol can be regenerated by the same enzyme and different cofactors (67).

Quantitation of progesterone. Zarrow et al. (156) confirmed that the \(\Delta^1\)-ene-3-one grouping on the steroid nucleus must be present for progestational activity. This same group may be quantitated by its strong absorbance at 240 \(\text{nm}\) in alcoholic solutions (16, 34). Although the \(\Delta^1\)-ene-3-one grouping is not specific for progestins, ultraviolet absorption can be a reliable method for their detection and quantitation if suitable separation and purification is ac-
accomplished. Wiest (145) measured the absorption of progesterone at 240 nm by direct spectrophotometric scanning of the paper chromatogram.

Other spectrophotometric assays have been reviewed by Zander (154) and are based on the absorbance of ultraviolet light at wavelengths other than 240 nm by progesterone derivatives. These include the progesterone bis-thiosemicarbazone at 300 nm, the progesterone bisdinitrophenylhydrazone at 380 nm, the bisisonicotinic acid hydrazone at 380 nm, and the sulfuric acid–ethanol chromagen at 290 nm. As pointed out by Zander (154), these methods have the advantage of shifting the quantitation wavelength away from that of interfering materials, but lack specificity and make it difficult to identify the isolated material as progesterone.

The limit of sensitivity of the spectrophotometric methods ranges from 0.5 to 1.0 µg if micro-cells are used in the spectrophotometer. If not, five times as much hormone is needed (Table 1). Recently, Sommerville et al. (130) reported a modification of the thiosemicarbazide reaction and reported sensitivity to 0.1 µg using micro-cells. This is the most sensitive spectrophotometric method reported for progesterone to date, but the specificity problems mentioned above still apply. The fact that other (nonspectrophotometric) assays yield lower results for progesterone levels in non-pregnant women (130, 150, 152) suggests that this method may measure thiosemicarbazone derivatives of other compounds in addition to progesterone, since the progesterone derivative is not isolated prior to spectrophotometric evaluation (130).

Fluorometric procedures have been used since 1948 for the quantitation of steroid estrogens (71); however, progesterone exhibits less than 1% of the fluorescence shown by estradiol in sulfuric acid (2, 143). For this reason, this procedure was of little value for the determination of progesterone until it was shown that pretreatment of the hormone with methanolic potassium hydroxide would increase the peak one hundred-fold (143). Using a micro-cell adaptation of this method, Short and Levitt (123) were able to detect 0.05 µg of progesterone standard. However, when samples assayed by this method and the spectrophotometric method of Short (118) were compared, the fluorescence results tended to be higher at low concentrations of progesterone and lower at high concentrations. This prompted these workers (123) to question the specificity of the fluorescence reaction, especially because of high and variable fluorescence of paper blanks.

Although the theory of gas chromatography was recognized as early as 1941, it was not until 1952 that this procedure was first successfully used (72). By 1960, the technique was only beginning to be applied to steroid separation and qualitative identification (107). As recently as 1963, the first quantitative assays of progesterone by gas chromatography appeared. These were difficult, time-consuming assays with less sensitivity than available methods (51). In 1964, the first highly sensitive gas chromatographic assay for progesterone was reported (152). The method is reported sensitive to less than 0.02 µg of progesterone with excellent precision. Gas chromatography also appears to offer specificity that cannot be equalled by spectrophotometric or fluorometric methods. The procedure can also be adapted to isotope dilution methods for recovery estimates or quantitative measurements (22, 75, 77).

The introduction of isotopic reagents for the estimation of steroid hormones has overcome the problem of inadequate sensitivity of conventional chemical procedures. Keston et al. (76) first applied the isotope derivative principle to the estimation of amino acids in 1946,

<table>
<thead>
<tr>
<th>Quantitation method</th>
<th>Standard cells</th>
<th>Micro-cells</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrophotometry *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In alcohol</td>
<td>2.5</td>
<td>0.5</td>
<td>13, 34, 82, 118, 154</td>
</tr>
<tr>
<td>In sulfuric acid: ethanol</td>
<td>2.5</td>
<td>0.5</td>
<td>34, 105, 154</td>
</tr>
<tr>
<td>Derivatives</td>
<td>2.5</td>
<td>0.1–0.5</td>
<td>34, 130, 154</td>
</tr>
<tr>
<td>Fluorometry</td>
<td>0.2</td>
<td>0.05</td>
<td>2, 123, 143</td>
</tr>
<tr>
<td>Gas chromatography</td>
<td>0.005–0.02</td>
<td>17, 75, 77, 152</td>
<td></td>
</tr>
<tr>
<td>Double-isotope derivative</td>
<td>0.005–0.02</td>
<td>111, 150, 151</td>
<td></td>
</tr>
</tbody>
</table>

* For a more complete listing and comments on precision and recovery, see Reference 154.
but it was not until recently that the principle was used for steroid hormones (11, 14, 70). The general method entails addition of an isotopically labeled steroid (e.g., C\(^{14}\)-labeled) to the unknown sample. The endogenous steroid is extracted and purified in a mixture with the labeled compound, and both are converted to a derivative, using a reagent labeled with a second isotope (e.g., H\(^{3}\)). The amount of H\(^{3}\) in the final isolated derivative quantitates the amount of derivative formed and the C\(^{14}\) measures procedural losses. This procedure appears to be limited in sensitivity only by the specific activities of the derivative-forming reagent and the labeled steroid.

The double-isotope derivative principle was first applied to the estimation of aldosterone (11) and testosterone (70) by forming the acetate of these hormones using acetic anhydride-H\(^{3}\) and C\(^{14}\)-labeled steroids. This method could not be applied per se to the estimation of progesterone, since the hormone cannot be acetylated; therefore, it was necessary to investigate other derivatives. Riondel et al. (111) first applied the double-isotope derivative principle to progesterone estimation using progesterone-H\(^{3}\) with thiosemicarbazide-S\(^{35}\) as the derivative-forming reagent. A later report by Woolever and Goldfein (151) advocated the conversion of progesterone to 20 \(\beta\)-ol, using tritiated sodium borohydride and progesterone-C\(^{14}\) as the isotope sources. This method is reported sensitive to 0.01 \(\mu\)g of progesterone or less (150), and appears highly specific and adequately precise (151).

EVALUATION OF METHODS AND POSSIBILITIES FOR FUTURE DEVELOPMENT

A major problem in progesterone research has been to develop methods with greater sensitivity without losing reliability. As shown in Table 1, methods employing spectrophotometric end points are sensitive only to 2.5 \(\mu\)g of the hormone unless micro-cells are used. This adaptation increases sensitivity fivefold, but also magnifies the problem of interference from impurities. Barring new developments in ultraviolet instrumentation, or discovery of a new color-forming reagent, it appears that the spectrophotometric methods have nearly achieved their peak sensitivity. These methods, however, should continue to be of great value where extreme sensitivity is not the primary criterion.

The fluorometric procedure used by Short and Levitt (123) is quite sensitive (Table 1) but requires adequate purification and extreme care with reagents and paper to insure the necessary specificity. Further advances might be made by the conversion of progesterone to a more highly fluorescent compound such as Finkelstein et al. (44) reported for estimation of testosterone.

Judging from the very rapid advances in gas chromatography, one must expect the excellent method of Yannone et al. (152) to be superseded soon by even more sensitive (22), equally specific methods. The developmental possibilities for gas chromatography in qualitative and quantitative steroid assays are extensive.

Although the double-isotope derivative methods currently available for progesterone research are extremely sensitive (Table 1), it is not unreasonable to believe that sensitivity can be further increased tenfold or more. Use of borohydride of a higher specific activity (50 mC/mm was used in the original study—200 mC/mm and higher is now available) could, in itself, increase sensitivity remarkably. Other developments might include the chemical (103) or enzymatic (67) conversion of progesterone to a hydroxylated derivative (e.g., 20 \(\beta\)-ol), followed by acetylation with labeled acetic anhydride. The analysis could then be carried out with higher recovery, using a conversion reagent available with a very high specific activity. A less likely possibility is the conversion of progesterone to 20 \(\beta\)-ol by 20 \(\beta\)-hydroxysteroid dehydrogenase (67), using tritium-labeled reduced nicotinamide adenine dinucleotide (NADH\(^{3}\)) as a tritium donor. This conversion should be quantitative, but one would likely have to prepare NADH\(^{3}\) in the laboratory (1) and the compound would probably fail to meet the specific activity requirements.

PROGESTINS IN THE BOVINE SOURCES AND ENDOGENOUS COMPOUNDS

Edgar (35), in 1953, appears to have been the first to identify progesterone in the bovine corpus luteum (CL). Following gonadotrophin treatment of a calf, he isolated 20 \(\mu\)g of progesterone from 60 g of luteal tissue. A year later, Hayano et al. (66) demonstrated the conversion of progesterone to 20 \(\beta\)-ol by bovine CL in vitro; and, in 1957, Zander et al. (155) isolated 20 \(\alpha\)-ol and 20 \(\beta\)-ol from human ovaries.

In 1958, Gorski et al. (57) reported the isolation of progesterone and 20 \(\beta\)-ol from ovaries of nonpregnant cows. A third ultraviolet absorbing area, corresponding to 20 \(\alpha\)-ol or \(\Delta^4\)-androstene-3, 17-dione, was noted, but insufficient amounts were present for identification. In a subsequent publication, Gorski et al. (58) demonstrated the presence of progesterone and 20 \(\beta\)-ol in corpora lutea, ovaries, and adrenals
of a pregnant cow, but were unable to detect either progesterin in 1,400 g of placental tissue, one liter of uterine venous blood, 305 ml of uterine venous plasma, or blood or tissues of the 258-day male fetus. Others have supported the report of progesterone and 20 β-ol in ovarian and adrenal tissue in the bovine (9, 40, 41). Short (117) was unable to detect progesterone in 1 kg of bovine placental tissue, but Melampy et al. (93) reported high levels of a compound thought to be progesterone in bovine placenta. Later, Bowerman and Melampy (13), using a different chemical method, could detect progesterone in only one sample of eight analyzed.

Progesterone and 20 β-ol have been isolated from mare placentae (116) and progesterone and 20 α-ol from ewe placenta (125). The 20 α-ol compound is very high in the rabbit (128), rat (17, 145), and ewe (99, 125).

SYNTHESIS AND METABOLISM OF PROGESTERONE IN THE BOVINE

Rapid developments in steroid biochemistry, especially the use of radioactive tracers, have helped to elucidate the biosynthetic and metabolic pathways of progestins. Use of acetate-C\(^{14}\) in vitro incubations with bovine CL has shown that simple carbon compounds may be precursors of the progestin nucleus (136). The intermediate compounds probably include acetoacetate, hydroxymethylglutarate, and squalene, which is a precursor of the cholestane series of compounds (96). Cholesterol is probably hydroxylated at the C-20 position and cleaved at the 21-carbon by a bovine side-chain desmolase (137). The resulting pregnenolone is converted by two enzymes to progesterone. Duncan et al. (33) demonstrated in vitro synthesis of progesterone in swine CL and commented on several factors affecting this synthesis.

Sweat et al. (136) have shown that a wide spectrum of hormones is produced from progesterone by ovarian tissue in vitro. Using C\(^{14}\)-labeled compounds, these workers reported the conversion of progesterone to both 20-hydroxy derivatives, 17-hydroxyprogesterone, 6 β-hydroxyprogesterone, and pregnanediol. It seems likely that more sensitive methods will demonstrate this entire spectrum and probably still more progestins in samples from endogenous sources.

After progesterone enters the circulation, it is subject to metabolism in the blood. Coyle and Romanoff (24) have demonstrated the in vitro conversion of progesterone-C\(^{14}\) to 20 β-ol and three more polar compounds by bovine blood. Miller et al. (97) estimated the half-life of free progesterone to be between 18 and 59 min, with an average for 11 cows of 33.8 min. These values must be considered maximal because progesterone-C\(^{14}\) was not isolated, but rather total radioactivity per sample was measured and reported as progesterone. Short and Rowell (126) estimated the half-life of progesterone in the blood of the ewe to be 7-8 min, using values of isolated progesterone-C\(^{14}\).

Studies with progesterone-C\(^{14}\) in women indicate that hydroxylated, saturated metabolites of progesterone are excreted mostly in the urine, whereas the corresponding ketonic compounds are excreted via the bile (20). In both cases, most of the hormone is conjugated as the glucuronide. Mayer et al. (91) reported pregnanediol and two nonconjugated progesterone metabolites in sow and gilt urine, and rabbit urine has been shown to contain progesterone metabolites (19). In contrast to these animals, progesterone metabolites have only occasionally been reported in the urine of ruminants (10). Williams (148) administered progesterone-4-C\(^{14}\) to dairy cows and recovered only 3% of the radioactivity in urine, whereas nearly 50% was found in the feces. Boscott (12), after injecting 100 mg progesterone daily into goats, found no progesterin metabolites in the urine. Other workers failed to find pregnanediol in the urine of cows. The studies of Miller and Turner (95, 96) indicate that progesterone is converted to androgenic substances in the liver and excreted via the bile into the feces. In addition, progesterone metabolites may be excreted in the milk (148) or as carbon dioxide (54).

QUANTITATIVE ESTIMATES OF PROGESTINS

Nonpregnant cows. One hesitates to summarize reported quantitative levels of progestins, because of the uncertainty of how efficiently the hormones were extracted and purified, especially in earlier studies. However, enough evidence exists to warrant doing this to show generalized trends.

As shown in Table 2, the progestin levels in corpora lutea of cycling cows agree remarkably well for three of the more complete studies (42, 55, 89). Data from other studies support these three (47, 132, 133), but there are some conflicting data (23). In the majority of studies using bovine corpora lutea, progesterone concentration (µg/g) showed an initial rise during the early proliferative stages of the CL (Days 1-4 of the cycle), followed by a more or less constant level (25-35 µg/g) until Day 9 or 10.
<table>
<thead>
<tr>
<th>Days since estrus</th>
<th>Mares et al. (89)</th>
<th>Gomes et al. (55)</th>
<th>Gomes et al. (55)</th>
<th>Edgar &amp; Ronaldson (37)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. CL</td>
<td>CL Wt (g)</td>
<td>Prog. (μg/g)</td>
<td>20 β-ol</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>2</td>
<td>1.54 c</td>
<td>6.8</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>3</td>
<td>1.22 c</td>
<td>6.5</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>3</td>
<td>0.74 d</td>
<td>6.3</td>
</tr>
<tr>
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<td>4</td>
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<td>25.8</td>
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<td>8</td>
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<td>4</td>
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</tr>
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<tr>
<td>21</td>
<td></td>
<td>1</td>
<td>2.92 d</td>
<td>6.3</td>
</tr>
</tbody>
</table>

a For additional data and other species, see References 13, 23, 33, 40, 41, 57, 58, 78, Figure 1.

b For additional data and other species, see Table 3.

c Corpora lutea of previous cycle.

d No progestin detected.
Thereafter, the concentration of the hormone again increased to a peak on Days 14 and 15, followed by a decline to the next estrus (Table 2). Detectable progesterone is present in the regressing CL for two or three days after the next estrus period. The levels of 20 β-ol in the CL contributed 5-20% of the total progestins, apparently depending on the method of CL collection. Since progesterone can be converted to 20 β-ol by the incubating CL, it seems likely that collection of tissues at slaughter (42) might be expected to show higher levels of 20 β-ol than collection from the living animal (55, 89). The data indicate that such is the case. Progesterone concentrations in the CL of the bovine (Table 2) appear to be paralleled by concentrations in the ovarian venous effluent (55). Table 2 shows also the concentration of progesterone in the ovarian vein blood of the cycling cow (55) and ewe (37).

Armstrong et al. (7) have shown that bovine CL exhibits maximum in vitro progesterone synthesis (270-300 μg/g/2 hr) when the gland is removed surgically 4-13 days post-estrus. A gradual decline in synthetic capacity was noted between Days 14 and 18 post-estrus (80 μg/g/2 hr). No detectable synthesis occurred with 19-day-old CL. Luteinizing hormone (LH) stimulated increased progesterone synthesis by all CL collected prior to 19 days post-estrus, but had no discernible effect on luteal tissue obtained on Day 19 or later. Progesterone synthesis in these latter CL's could be only partially restored by addition of pregnenolone or glucose-6-phosphate plus NADP+ to the incubation medium (7). Duncan et al. (33) reported the in vitro synthesis of progesterone by slices of porcine CL. As with the bovine CL (7), glands collected 4-16 days post-estrus were able to synthesize progesterone, but those obtained on Day 18 contained no detectable progesterone before or after incubation.

It can be generalized from several studies that growth in weight of the CL and progesterone content, synthesis, and secretion are closely related to the degree of luteinization as determined by histological studies (26, 89). During the first five days of the cycle, the granulosa cells of the CL are gradually luteinized (26), the CL grows in weight (55, 89), and progesterone content and synthesis become detectable (7, 55, 89). After Day 6, the theca interna cells become luteinized (26), the CL grows larger, and progesterone concentration is higher (55, 89). Degeneration of the luteal cells begins on Day 16 or 17 in the nonpregnant bovine (26, 89), corresponding to a decrease in weight and progesterone concentration (55, 89), rate of release (55), and a lowered capacity to synthesize (7). Similar changes in RNA/DNA ratios and per cent Type I and Type II cells (46) were shown by Mares et al. (89).

In a study designed to compare luteal and blood plasma progestins in the nonpregnant bovine, Gomes et al. (55) showed a significant correlation between CL progestins (μg/g or μg/CL) and ovarian vein progestin concentration (μg/ml of plasma). However, they reported that the peripheral blood progesterone levels found in their study did not reflect the stage of the estrous cycle, quantity of concentration of progesterin in the CL, or concentration of progesterin in ovarian vein blood plasma.

Ovarian vein progestin concentrations in the cow and ewe show similar trends (Table 2) and indicate that CL levels of the hormone may adequately reflect secretion rates. The work of Stormshak et al. (135) in the cycling ewe supports the hypothesis that luteal progestin content is indicative of the amount of hormone released into the blood, but Clegg et al. (23), in a study involving only seven cows, suggest that CL levels of progesterone do not reflect secretory rate.

It is discouraging to note that Gomes et al. (55) found no relationship between tissue or ovarian vein plasma progesterone and peripheral blood plasma levels of the hormone. It should be pointed out, however, that micro-cell attachments were not used for the spectrophotometric quantitative step (55). Thus, readings were often made near the limit of sensitivity (Table 1), increasing the possibility of error. McCracken (86) has shown that peripheral blood plasma progesterone levels begin to decrease within 15 min after CL removal and fall to nondetectable levels within 24 hr, indicating that a positive relationship may exist between ovarian and peripheral levels of the hormone. This would suggest that a highly sensitive assay allowing analysis of multiple samples from one animal throughout the cycle might yield results which would lead to a better understanding of ovarian-peripheral progesterone relationships.

Pregnant cows. Figure 1 was drawn in an attempt to generalize available data on progesterin levels in corpora lutea and peripheral blood plasma of pregnant cows. CL data are combined from the studies of Zimbelman et al. (158-161, control data), Stormshak and Erb (134), Estergreen et al. (43), and Gomes et al. (56). The data on peripheral plasma hormone levels are taken from the reports of Short
Fig. 1. Progestin (progesterone and 20-α-ol) in corpora lutea and peripheral blood plasma of pregnant cows. a References 40, 41, 43, 56, 134, 158-161. b References 13, 56, 87, 117.

CL levels of progesterone, both in total (μg/CL) and in concentration (μg/g), are in excellent agreement for several studies (56, 134, 158-161). Others (13, 93) tend to report lower quantitative values; therefore, these are not shown on the curve in Figure 1. Of the combined data in the CL curve in Figure 1, the values of control cows reported by Zimbelman et al. (158-161) tend to be lower; but the differences are not large when the other data are restricted to cows under 10 yr of age. Since earlier studies have shown very close parallelism between total progesterone in the CL and progesterone concentration (40, 41), only the latter has been shown in Figure 1.

In several studies, essentially no difference was found in CL progestins 14-16 days following estrus in pregnant or nonpregnant cows (40, 47, 134). Pregnant cows show a slight decline by Day 19, but the most pronounced decline occurs after Day 42. A leveling off is seen after Day 56 and a further decline at about 120 days of pregnancy. A later rise reaches a peak at about 250 days, followed by a decline to the time of parturition (Figure 1). Similar changes are seen in peripheral blood progesterone levels (Figure 1). Gomes et al. (56) have shown that ovarian vein plasma progestin concentration also declines after 250 days in the bovine. This concentration reaches undetectable levels after parturition (43). Progestin was also undetectable in a CL obtained eight days post-partum (134). Edgar and Ronaldson (37) reported that ovarian vein blood progesterone remained at about 1.5 μg/ml during Days 7-28 of pregnancy in the ewe, then increased to over 2 μg/ml on Day 35. Thereafter, the concentration remained near 1.5 μg/ml until Day 111, followed by a gradual decline to nondetectable levels at 140 days. These workers (37) suggested, from their data, that uterine contents in the pregnant ewe are not an important source of progesterone. However, other work has shown that the ewe does not abort following ovariectomy after Day 60 of pregnancy (18), peripheral blood progesterone levels in the pregnant ewe do not fall after ovariectomy, and the placenta of the ewe has been found to be a source of progesterone and 20-α-ol (125).

Other species. Although this paper is primarily concerned with bovine progestins, and it is often difficult to extrapolate between species, a certain amount of reference to comparative data may be helpful in elucidation of the role(s) of progesterone in reproductive processes. For this reason, a summary of progesterone levels in the blood of a number of species...
is shown in Table 3. These data have previously been summarized in part (35, 41, 54). For tissue and follicular fluid levels of the hormone, the reader is referred elsewhere (35, 40, 41, 113).

PROGESTIN AND NORMAL CYCLIC FUNCTION

Observations from several experiments indicate that progesterone is associated with the availability of LH during late proestrus or early diestrus (40). McCann (84) found that progesterone or estradiol benzoate alone had little effect on LH release in the ovariectomized rat, but progesterone injections into estrogen-primed females reduced plasma LH to non-detectable levels. As reviewed earlier by Erb (40), ratios of progesterone to estradiol of 12.5:1 to 75:1 appear to be optimal for the induction of estrus in the ovariectomized cow, but ratios higher than 75:1 will block this

### TABLE 3

A summary: Levels of progesterone in blood or plasma as determined by chemical assay

<table>
<thead>
<tr>
<th>Species</th>
<th>Reproductive status</th>
<th>Fraction of blood</th>
<th>Progesterone (µg/100 ml)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow *</td>
<td>Preovulatory−1 day</td>
<td>Peripheral plasma</td>
<td>0.20</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>Cycle−12 days</td>
<td>Peripheral plasma</td>
<td>0.19</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>−12 days</td>
<td>Peripheral plasma</td>
<td>0.92</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>−1 day</td>
<td>Peripheral plasma</td>
<td>Not detected</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>−13 days</td>
<td>Peripheral plasma</td>
<td>1.06</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>−2−5 days</td>
<td>Peripheral plasma</td>
<td>0.71</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>−6−8 days</td>
<td>Peripheral plasma</td>
<td>0.36</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>−9−15 days</td>
<td>Peripheral plasma</td>
<td>0.46</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>−18−20 days</td>
<td>Peripheral plasma</td>
<td>1.68</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Pregnant−90 days</td>
<td>Peripheral plasma</td>
<td>0.09</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>−10−49 days</td>
<td>Peripheral plasma</td>
<td>1.18</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>−50−89 days</td>
<td>Peripheral plasma</td>
<td>0.9</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>−90−129 days</td>
<td>Peripheral plasma</td>
<td>1.4</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>−130−169 days</td>
<td>Peripheral plasma</td>
<td>0.4</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>−170−209 days</td>
<td>Peripheral plasma</td>
<td>3.1</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>−210−249 days</td>
<td>Peripheral plasma</td>
<td>3.4</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>−250−280 days</td>
<td>Peripheral plasma</td>
<td>4.0</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>−111−117 days</td>
<td>Ovarian plasma</td>
<td>264</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>−250−254 days</td>
<td>Ovarian plasma</td>
<td>259</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>−281−282 days</td>
<td>Ovarian plasma</td>
<td>140</td>
<td>56</td>
</tr>
<tr>
<td>Ewe *</td>
<td>Cycle</td>
<td>Ovarian plasma</td>
<td>95</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>Mid-luteal</td>
<td>Ovarian plasma</td>
<td>104</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>Late luteal</td>
<td>Ovarian plasma</td>
<td>125</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Pregnant−6 days</td>
<td>Ovarian plasma</td>
<td>130</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>−1−4 wk</td>
<td>Ovarian plasma</td>
<td>215</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>−5−6 wk</td>
<td>Ovarian plasma</td>
<td>160</td>
<td>35, 37</td>
</tr>
<tr>
<td></td>
<td>−7−16 wk</td>
<td>Ovarian plasma</td>
<td>125</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>−17−18 wk</td>
<td>Ovarian plasma</td>
<td>40</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>−19 wk</td>
<td>Not detected</td>
<td>0.43</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>−20 wk</td>
<td>Not detected</td>
<td>0.68</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>−25 days</td>
<td>Not detected</td>
<td>0.91</td>
<td>37</td>
</tr>
<tr>
<td>Sow</td>
<td>Cycle−Mid-luteal</td>
<td>Peripheral plasma</td>
<td>0.75</td>
<td>117, 120</td>
</tr>
<tr>
<td></td>
<td>Pregnant−40 days</td>
<td>Peripheral plasma</td>
<td>1.70</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>−48 days</td>
<td>Peripheral plasma</td>
<td>3.12</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>−49 days</td>
<td>Peripheral plasma</td>
<td>2.92</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>−53 days</td>
<td>Peripheral plasma</td>
<td>2.04</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>−55 days</td>
<td>Peripheral plasma</td>
<td>0.91</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>−99 days</td>
<td>Peripheral plasma</td>
<td>1.04</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>−112 days</td>
<td>Peripheral plasma</td>
<td>1.20</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>−113 days</td>
<td>Peripheral plasma</td>
<td>0.34</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>−114 days</td>
<td>Peripheral plasma</td>
<td>0.67</td>
<td>120</td>
</tr>
<tr>
<td>Goat</td>
<td>Pregnant−134 days</td>
<td>Ovarian plasma</td>
<td>230</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>−112 days</td>
<td>Peripheral plasma</td>
<td>0.71</td>
<td>117</td>
</tr>
</tbody>
</table>

* For additional data, see Table 2 and Figure 1.
condition (92). In intact cows, 10 mg of progesterone administered during estrus reduces the time to next ovulation by about 10 hr (62). Work with the bovine and the rat indicates that progesterone accomplishes this end by enhancing the hypothalamo-hypophyseal release of ovulating hormone (likely LH).

Foote et al. (47) observed a high incidence of cystic ovaries following supravaginal removal of the CL on Day 14 of the cycle. Staples et al. (132) produced cystic ovaries in a significant proportion of heifers when CL inhibition and precocious ovulation were induced with oxytocin. Conversely, 50 mg of progesterone daily will inhibit ovulation and estrus effectively, but large follicles still appear on the ovary (144). This suggests that a minimal level of progesterone is necessary to enhance release of gonadotrophin, but a higher level may be inhibitory.

The inhibition and synchronization of estrus and ovulation in cattle by progesterone and other progestins have been widely used for both practical and experimental reasons. The practical aspects have been adequately reviewed elsewhere (49, 61), but the value of such compounds in the experimental manipulation of cyclic functions is worthy of note here.

The injection of repositol progesterone (76 mg/e wt) into heifers on the day of heat or on Day 8 or 16 of the cycle significantly decreased pituitary levels of follicle-stimulating hormone (FSH) and LH and reduced the diameter of the CL. When progesterone (100 mg/e wt) was injected on Day 1 or Day 4 of the cycle, CL weight, progesterone content, and functional cells in the CL were reduced when inspected on Day 14; but estradiol had no apparent effect on the total amount of progesterone in the CL (83). This apparent luteolytic—or anti-luteotrophic—effect of progesterone offers a basis for the study of CL growth and maintenance. If one were to determine which gonadotrophin(s) were decreased in bovine pituitary effluent (30) following progesterone administration, it might be possible to determine the nature and normal level of the pituitary luteotrophin in the bovine and to add to our meager knowledge of progesterone-(uterine)-hypothalamo-hypophyseal-ovarian relationships. Such studies might also be helpful in determining whether other progestins act in the same manner as progesterone itself (106).

Corpus Luteum Maintenance

The most important factor in the onset of pregnancy, other than the presence of a zygote, is maintenance of the CL. The mechanism of this maintenance or, conversely, the mechanism of luteolysis in the nonpregnant bovine, is poorly understood. Our knowledge of these processes is most advanced for the rat and mouse; but it appears that prolactin, the luteotropic hormone (LTH) in these species, is ineffective for CL maintenance in other species (122). As mentioned earlier, Hansel and Wagner (62) reported the anti-luteotropic nature of oxytocin in the bovine. Simmons and Hansel (129), in an ingenious experiment using the prevention of this effect as an end point, reported that a luteotrophic factor is present in crude anterior pituitary extracts of the bovine.

The oxytocin-induced effect was also overcome by human chorionic gonadotrophin (HCG), but was unaffected by bovine growth hormone, ovine prolactin, or equine LH. It is disappointing that bovine LH was not available for this study (129), since HCG exhibits predominantly LH action (157), and ovine LH increased the in vitro synthesis of progesterone synthesis in human CL, as does HCG, but ovine LH is ineffective (110).

Any theory of CL maintenance or luteolysis must necessarily include a uterine effect, since hysterectomy will result in CL maintenance (5), oxytocin repression of the CL fails in the hysterec tomized cow (133), and insertion of foreign bodies into the uterus can cause precocious estrus and ovulation (62). These results suggest that a certain normal uterine condition is necessary during the early cycle for continued secretion of LTH in the bovine. Oxytocin, hysterectomy, or foreign objects disrupt this necessary condition. If a zygote does not enter the uterus, a substance from that organ may induce luteolysis, either by interrupting pituitary LTH release or by a direct LTH-over-riding effect on the ovary (122).

Progestins and Pregnancy

Essentiality of the CL. As reviewed earlier (41), the CL appears essential throughout pregnancy in the rabbit, rat, sow, goat, and bitch, but for less than full term in primates, mares, ewes, and guinea pigs. In all of the species studied to date, however, progesterone or its equivalent activity is required for the normal span of pregnancy. In the cow, the necessity of the CL during pregnancy remains controversial. It is generally concluded that the CL is required for about 200 days in this animal, but in one study removal of the CL after 200 days did not result in abortion prior to 250 days (88). In another study, when cows were ovariotomized after 200 days, eight of nine aborted between 238 and 274 days of
pregnancy (43). Furthermore, peripheral blood progesterone concentrations dropped to undetectable levels after ovariectomy (56), whereas this operation does not lower the hormone levels in peripheral plasma of the pregnant ewe. As pointed out earlier in this paper, attempts to isolate progesterone from bovine placenta have generally been unsuccessful (58, 117), but occasional positive results have been noted (13, 43, 93).

From the above evidence, one might propose the following hypothesis to explain the sources of progesterone in pregnancy maintenance in the bovine:

The major source of progesterone throughout pregnancy in the cow is the CL (58), but ovaries and adrenals might contribute a significant amount (58, 134) and body fat may store the hormone to be used if needed (87). The placenta in the cow produces a very low level of progesterone. In most cases, this level is insufficient to maintain pregnancy following ovariectomy but may be adequate when only the CL is removed. Because of individual variation in placental production of the hormone, an occasional cow may fall to abort following ovariectomy.

This postulated low level of progesterone synthesis is not unique among animals. If fetuses are removed from the rat in such a way that the placentae remain intact, 12 placentae will maintain one fetus in the ovariec-tomized rat (65). The general failure to find progesterone in bovine placenta cannot be considered proof of an absence of the hormone, since the levels may be so low as to have escaped detection by the methods used but high enough to exert an important local uterine effect (25, 50). Use of more sensitive assays (Table 1) should enable one to better resolve this question.

REFERENCES


(79) Kumar, D., and Barnes, A. C. 1963. Studies on the Mechanism of Action of


(139) TELEGDY, I. 1963. The Ovarian Secretion of Progesterone and 20α-Hydroxyprogren-4-en-3-one in Rats During the Estrous Cycle. Steroids, 2: 119.

(140) TEPPERMAN, J. 1962. Metabolic and End.


