A RAPID METHOD FOR PURIFICATION AND QUANTITATIVE ESTIMATION OF PROGESTERONE FROM LUTEAL TISSUE*

BY ROBERT G. LOY,† W. H. McSHAN, AND L. E. CASIDA

(From the Departments of Genetics and Zoology, University of Wisconsin, Madison, Wisconsin)

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Investigations of certain problems in animal reproduction require that data be obtained on the progesterone content of corpora lutea in individual animals. A rapid and reliable method of chemical analysis is required, since a relatively large number of determinations are necessary in order to evaluate properly the variation among individual animals.

Methods reported in the literature include those of Pearlman and Cerceo (1) and Noall et al. (2) for the detection, estimation, and isolation of progesterone from the placenta, of Butt et al. (3) for the estimation of progesterone from blood, of Edgar (4) for the determination of progesterone from blood and tissue, and of Samuels (5), as modified by Wiswell and Samuels (6), for estimation of the steroid from liver tissue.

Attempts by us to apply a slight modification of the method of Wiswell and Samuels (6) to extracts of luteal tissue gave unsatisfactory results because of interfering substances such as the more polar pigments which were not removed by solvent partition. The method reported here, however, is based in part on that of Samuels (5) with the introduction of modifications which permit a satisfactory estimation of the progesterone content of corpora lutea of sows and cows.

Method

The corpora lutea1 (0.9 gm. to 4.7 gm. of tissue) which were stored in a freezer in a small amount of 95 per cent ethanol were chopped finely in a

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1 Sow corpora lutea were made available by H. L. Self, D. W. Waldorf, H. G. Spies, and D. R. Zimmerman from other investigations at the University of Wisconsin.
Waring blender. The ethanol in which the tissue was stored was used to make up to a volume of 150 ml. The tissue was transferred and rinsed with an additional 150 ml. of ethanol into a flask and refluxed for 1 hour on a steam bath. It was allowed to settle and the supernatant liquid was decanted through filter paper. The tissue was extracted twice more in the same manner with 100 ml. of 95 per cent ethanol each time. Additional extractions failed to yield any detectable progesterone.

The ethanol extracts were combined and evaporated under reduced pressure for column adsorption chromatography on aluminum oxide (Merck) suitable for chromatographic adsorption. A column was prepared by pouring a suspension of 5 gm. of adsorbent in Skellysolve B into a chromatography tube 10 mm. in diameter and 250 mm. long. Glass wool plugs were used at the bottom and top of the column. All solvents used were redistilled.

The extract was redissolved in 20 ml. of Skellysolve B and applied to the column, followed by two 15 ml. volumes used to rinse the flask. The column was then developed with 100 ml. of Skellysolve B, followed by 100 ml. of 5 per cent chloroform-Skellysolve B. A solution of 20 per cent chloroform-Skellysolve B eluted all material which had an absorption maximum near 240 μm.

The last fraction was evaporated to dryness under reduced pressure and transferred to a separatory funnel with two 5 ml. volumes of petroleum ether (b.p. 30-60°) and two 5 ml. volumes of 70 per cent methanol. The material was then subjected to an eight transfer countercurrent distribution system as described by Pearlman (7) with use of separatory funnels. The solvent system was petroleum ether (30 ml.)-70 per cent methanol (10 ml.).
The solutions contained in the center five funnels, which theoretically contain 93 per cent of the progesterone introduced into the first funnel, were combined, evaporated to dryness under reduced pressure, and redisolved in a suitable amount of absolute ethanol, and the absorbance at 230, 240, and 250 μm was determined with a Beckman model DU spectrophotometer. From the absorbance values obtained, corrected absorbances due to progesterone at 240 μm were calculated by use of the equation worked out by Allen (8): CDP_{240} = OD_{240} - (OD_{230} + OD_{250})/2, where CDP_{240} = calculated absorbance due to progesterone at 240 μm and OD_{230}, OD_{240}, and OD_{250} = observed absorbances at 230, 240, and 250 μm, respectively.

Similar CDP_{240} values were then calculated from absorbance readings for varying concentrations of pure progesterone in ethanol and used for preparation of a standard curve (Fig. 1). Quantitative estimation of progesterone was made by interpolation on the standard curve.

**Results**

When sufficient amounts of tissue from an ovary were available, the extract was divided into two aliquots and each was subjected to the remainder of the method independently. The results of these duplicate analyses, selected at random, are given in Table I.

The absorption curves from 220 to 260 μm for a typical pair of duplicate analyses are shown in Fig. 2. Inspection of the curves between 260 and 290 μm showed no absorption maximum in this range.

Fractions from the column, collected before and after the progesterone-containing fraction, were run through the countercurrent distribution system. Their absorption spectra were determined and are also shown in Fig. 2.

A study of the curves for duplicate samples has shown that they may differ one from another in absolute value over part or the entire range studied, but their conformation is very similar in the range 230 to 250 μm. Use of Allen's calculation provided estimates of progesterone concentration which were generally well within the limits of the error of the method. Statistical treatment of all results of duplicate analyses gave a repeatability figure of 0.79 (P < 0.01).

The use of Allen's method of calculation presumes that the absorption spectrum of impurities in the wave length range involved approaches a straight line. An indication that this is true may be taken from the fact that the curves of the two discard fractions from the column approach linearity in this region. This is only suggestive, since the impurities of the fractions preceding and following do not necessarily have the same
absorption spectra as those in the one presumed to contain progesterone. In addition, however, the high repeatability of the results seems to justify the use of this correction.

**Table I**

*Results of Duplicate Estimates of Progesterone Content and Concentration of Sow Corpora Lutea*

<table>
<thead>
<tr>
<th>Ovary</th>
<th>Tissue equivalents per estimate</th>
<th>Progesterone estimate</th>
<th>Progesterone concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm.</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>A</td>
<td>2.35</td>
<td>84.0</td>
<td>91.5</td>
</tr>
<tr>
<td>B</td>
<td>1.99</td>
<td>66.0</td>
<td>76.5</td>
</tr>
<tr>
<td>C</td>
<td>0.85</td>
<td>25.4</td>
<td>25.4</td>
</tr>
<tr>
<td>D</td>
<td>1.43</td>
<td>108.0</td>
<td>108.0</td>
</tr>
<tr>
<td>E</td>
<td>2.05</td>
<td>213.0</td>
<td>207.0</td>
</tr>
<tr>
<td>F</td>
<td>1.29</td>
<td>53.1</td>
<td>53.1</td>
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<tr>
<td>G</td>
<td>1.14</td>
<td>43.5</td>
<td>45.0</td>
</tr>
</tbody>
</table>

![Figure 2](http://www.jbc.org/)

**FIG. 2.** Ultraviolet absorption curves for analyses of aliquots of a single luteal extract (X, O) and for discard fractions from the chromatography column (●, 5 per cent chloroform in Skellysolve B; □, 100 per cent chloroform). The discard fractions correspond to the curve (X).

Recovery of samples of pure progesterone added in varying amounts to aliquots of a luteal extract indicates recoveries of 77 to 92 per cent.

Work with paper partition chromatography, in which several of the systems described by Reineke (9) are employed, failed to result in resolution of the ultraviolet-absorbing material of the extract into more than one
component. Furthermore, a single ultraviolet-absorbing spot was obtained when pure progesterone and extract were mixed and run on paper.

The results of reestimation of the progesterone content of the extract after paper partition chromatography were in good agreement with original estimations.

The biological activity of the extract was tested by injecting an amount estimated to contain 1 mg. of progesterone into a spayed estrual rabbit. On autopsy the uterus showed a progestational proliferation of ++ to +++ as described by Allen (10).

DISCUSSION

The use of an adsorption column as the first step in the purification of progesterone is in keeping with the requirement for a rapid method. Attempts at solvent fractionation have proved inefficient. It was found that, when an initial solvent partition was performed, followed by the column chromatography step, the spectral curve of the resulting material was very erratic in the range 220 to 260 mp. Similar findings were reported by Samuels (5). The aluminum oxide column seems to remove substances which interfere with subsequent countercurrent distribution.

The countercurrent distribution was used rather than a simple solvent partition owing to the presence of pigment impurities which have a wide range of polarities. These pigments, along with many non-pigment impurities, are distributed largely in tubes 1 and 2 and 8 and 9 which are discarded. The total time required to perform all operations of the method is 7 to 8 hours.

In a practical application of the method to the study of the progesterone content of swine corpora lutea, results of analyses show a repeatability between ovaries from the same sow of 0.78 ($P < 0.01$). This seems to indicate a satisfactory degree of reproducibility of results for the method including extraction. Some work has been done to test the usefulness of the method for analyzing individual cow corpora lutea. The results of this preliminary work have been comparable to those obtained with swine corpora lutea.

It seems probable that, with appropriate modification of the extraction procedure, the method can be applied satisfactorily to the analysis of certain body fluids for progesterone.

SUMMARY

A method for the purification and quantitative estimation of progesterone from corpora lutea of sows and cows is presented. The method is based

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upon column adsorption chromatography, countercurrent distribution, and estimation of progesterone by ultraviolet absorption at 240 μm.

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