THE VARIATIONS IN THE LIPIDS OF THE UTERINE MUCOSA IN THE PIG.*

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The structural changes in the uterine mucosa associated with the phenomena of estrus, menstruation, implantation of the embryo, pregnancy, and the puerperium have, for a long time, been of marked interest to the histologist. The staining reactions of the endometrium with Nile blue, Sudan III, Scharlach R, and osmic acid have indicated the possibility of a connection between the lipid content and the state of activity of the tissue. Most of the information we have on the subject is, however, based upon these staining reactions only, largely because of the technical difficulties involved in the study of such tissue by the chemical methods which have been available.

The work of many investigators, beginning with Ancel and Bouin (1) has indicated that the pregestational proliferation of the glandular tissue of the endometrium may be dependent upon the action of the hormone of the corpus luteum. Recently Corner and Allen (2) have prepared an active extract of corpus luteum and demonstrated a typical glandular proliferation of the endometrium following its injection into rabbits. This suggests that the cyclic changes in the chemical composition of the endometrium may be expected to run parallel to the various stages of growth and degeneration of the corpus luteum. It has seemed to us, therefore, that parallel chemical studies of the lipids of the

* It is our purpose here to consider the data presented primarily in relationship to the specific physiology of the sexual cycle. For the discussion of the data on endometrium in its relationship to the general metabolism of lipids the reader is referred to the previous paper.
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endometrium and the corpus luteum in the same animals should yield some information of value in the interpretation of the biochemical nature of the phenomena associated with estrus, menstruation, and the nutrition of the fetus.

The whole mechanism for the nutrition of the embryo from the stage of migration of the fertilized ovum to the uterus to the time of birth must function through the uterine mucosa. Moreover, in most animals this tissue undergoes, at more or less regular intervals, a cycle which involves growth and elaboration of special glandular structures, followed by retrogression when the ova are not fertilized. Certain stages of its cycle, i.e. estrus and menstruation, are of special interest to the biochemist because they are associated with a condition of congestion or hemorrhage, which in turn must be associated with or dependent upon recurrent changes in the chemical composition of tissue.

The phenomenon of menstruation in the primates, since it is the most spectacular example of this cyclic recurrence of congestion and hemorrhage, has been most frequently studied. The apparent increase in the fragility of the erythrocytes of menstrual blood has recently been ascribed by Hermstein (3) to the increase in the lecithin-cholesterol ratio which he has found in menstrual blood collected directly from the human uterus. His average value for the ratio phosphatide-cholesterol is 1.2 for circulating blood and 1.5 for menstrual blood. His average values for the lecithin-cholesterol ratios in circulating blood are exactly the same during the menstrual period as during the intermenstrual period, although the absolute values for lipid during menstruation are higher. But, since he has only two observations per case in twelve cases altogether, and blood cholesterol varies so much from day to day, it is difficult to see how his figures for circulating blood can have any significance. If, however, we assume that the function of cholesterol is primarily to place a check upon the hemolytic properties of lecithin, or those of the unsaturated fatty acids liberated on the destruction of lecithin, his reasoning seems logical.

Klaus (4) has shown a large increase in the choline content of circulating blood and perspiration during menstruation. But he found in the menstrual discharge itself, trimethylamine, instead of choline. He believes that the menstrual destruction of the uterine mucosa is associated with a breakdown of lecithin, and that the trimethylamine results from bacterial decomposition of choline. An otherwise unaccounted for rise in the total non-protein nitrogen content of circulating blood which has been noted by Okey and Erikson (5) during the first stages of menstruation may likewise possibly be taken as indicative of lecithin destruction.

Okey and Boyden (6) found a considerable increase in the lecithin-cholesterol ratio in circulating blood during the early stages of menstruation.
tion. But this increase in the ratio was due, not to an absolute increase in blood lecithin concentration, but rather to a very marked decrease in cholesterol concentration. These findings have very recently been confirmed by Kaufmann and Mühlebeck (7). Later work (Okey, Ehrhardt, and Steinmetz, unpublished results) has indicated that the free, rather than the esterified cholesterol is chiefly involved.

It would appear, therefore, logical to postulate that somewhere in the body there may be a tissue in which cholesterol is used up at an unusually rapid rate at the time of retrogression or destruction of the endometrial cells. This may be merely a mechanism for added defense against bacterial invasion during the period of congestion or hemorrhage. But if this need for cholesterol arises as a result of rapid decomposition of phospholipids with consequent liberation of highly unsaturated or toxic fatty acids which must be rendered harmless by combination with cholesterol, it is reasonable to expect an accumulation of cholesterol ester in this tissue. Corpus luteum, because of its high lipid content, and endometrium, because of the possible intimate connection between the variations in its lipid partition and the production of periodic congestion and hemorrhage seemed worth investigating in this connection. While the animal most readily available for the study, i.e. the pig, offered the advantage of a plentiful supply of corpus luteum for chemical analysis from a single animal, nevertheless the comparative simplicity of the cyclic histological changes in the endometrium of this species promised a correspondingly less marked variation in the lipid content. However, the possibility of correlating the findings on the two tissues in the same animal has been considered to outweigh this objection, and the data on endometrium are accordingly presented.

EXPERIMENTAL.

The general method used for collecting and sampling the tissue has been described in the previous paper. After the blocks of corpora lutea and uteri had been set aside for histological study, large pieces of the uteri were taken for the chemical study. Each uterine horn was split open and the mucosa removed as cleanly as possible by dissection with scissors. Except in one case,
each sample was taken from a single uterus. Sample 30–33 was a composite taken from four uteri of the same day.

The endometrial tissue for lipid determination was weighed, ground with sand, and extracted with alcohol and then with ether exactly as described for corpus luteum in the preceding paper. Because of the lower lipid content of the endometrium, it was, however, necessary to use large samples. Moreover, it was soon evident that there was a great variation in water content of the tissue at different stages of the sexual cycle. Hence, separate portions of the samples of uterine mucosa were weighed, dried on the water bath, and then in the desiccator over sulfuric acid, reweighed, and the moisture content calculated in the usual way.

Determinations of the different lipid constituents were made on aliquot portions of the alcohol-ether extracts. The method used for phospholipids was the chromate-sulfuric acid oxidation of Bloor (8) and that used for total fatty acids (9) was based upon the same principle. Cholesterol and cholesterol esters were estimated by micro oxidation of the digitonide according to a new method of which a preliminary report has been published (10). The value for neutral fat was obtained by subtracting from the value for total fatty acids, the amounts of fatty acid in combination in the phospholipid (assumed to be two-thirds of its weight) plus the amount of fatty acid in combination with cholesterol as cholesterol esters, or approximately three-fourths of the weight of the bound cholesterol. This value for residual fatty acids, may be multiplied by 1.05 to convert it to glyceride, but this calculation has not been carried out, because the value for residual fatty acid carries the errors of all the determinations, which may very well aggregate more than this correction.

It should, perhaps, be stated that the analytical determination of lipids in the endometrium presented more difficulties than that of the lipids in the corpus luteum, because of the interference of some non-lipid material, possibly glycogen, soluble in hot alcohol and precipitated from the alcohol-ether solutions on standing. This necessitated refiltration of the extracts of the cooling solution. The analytical results are summarized in Table I.
<table>
<thead>
<tr>
<th>Approximate date</th>
<th>Sample No.</th>
<th>Free cholesterol (moist weight)</th>
<th>Cholesterol esters (moist weight)</th>
<th>Total cholesterol (moist weight)</th>
<th>Leucithin (moist weight)</th>
<th>Dry weight</th>
<th>Total cholesterol (dry weight)</th>
<th>Leucithin (dry weight)</th>
<th>Free cholesterol (dry weight)</th>
<th>Lecithin</th>
<th>Lecithin (dry weight)</th>
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<td>Day after previous estrus.*</td>
<td>21</td>
<td>34</td>
<td>0.10</td>
<td>0.00</td>
<td>0.10</td>
<td>0.5</td>
<td>11.8</td>
<td>0.856</td>
<td>0</td>
<td>4.4</td>
<td>4.9</td>
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<tr>
<td>21</td>
<td>13</td>
<td>0.115</td>
<td>0.016</td>
<td>0.13</td>
<td>0.74</td>
<td>14.5</td>
<td>1.01</td>
<td>0.22</td>
<td>0.79</td>
<td>4.9</td>
<td>4.8</td>
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<td>Day after estrus.</td>
<td>2</td>
<td>30-33</td>
<td>0.114</td>
<td>0.033</td>
<td>0.147</td>
<td>0.7</td>
<td>19.0</td>
<td>0.82</td>
<td>0</td>
<td>0.82</td>
<td>4.9</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>0.173</td>
<td>0.025</td>
<td>0.198</td>
<td>1.0</td>
<td>15.0</td>
<td>1.32</td>
<td>0</td>
<td>1.32</td>
<td>7.6</td>
<td>5.8</td>
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<tr>
<td>6</td>
<td>35</td>
<td>0.162</td>
<td>0.0</td>
<td>0.162</td>
<td>0.7</td>
<td>13.6</td>
<td>0.96</td>
<td>0</td>
<td>0.96</td>
<td>6.05</td>
<td>5.8</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>0.149</td>
<td>0.0</td>
<td>0.198</td>
<td>1.09</td>
<td>11.2</td>
<td>1.04</td>
<td>0.33</td>
<td>0.69</td>
<td>4.95</td>
<td>4.7</td>
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<tr>
<td>10</td>
<td>15</td>
<td>0.139</td>
<td>0.032</td>
<td>0.171</td>
<td>1.00</td>
<td>13.4</td>
<td>1.23</td>
<td>0.29</td>
<td>0.94</td>
<td>4.48</td>
<td>3.6</td>
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<td>11</td>
<td>26</td>
<td>0.077</td>
<td>0.040</td>
<td>0.117</td>
<td>0.55</td>
<td>13.2</td>
<td>0.78</td>
<td>0.04</td>
<td>0.74</td>
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<td>5.6</td>
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<td>14</td>
<td>36</td>
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<td>0.0</td>
<td>0.131</td>
<td>0.76</td>
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<td>0.95</td>
<td>0.23</td>
<td>0.72</td>
<td>4.86</td>
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<td>25</td>
<td>0.082</td>
<td>0.027</td>
<td>0.100</td>
<td>0.54</td>
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<td>15</td>
<td>16</td>
<td>0.126</td>
<td>0.039</td>
<td>0.165</td>
<td>0.6</td>
<td>11.2</td>
<td>0.95</td>
<td>0.23</td>
<td>0.72</td>
<td>4.86</td>
<td>4.8</td>
</tr>
<tr>
<td>16-17</td>
<td>22</td>
<td>0.001</td>
<td>0.004</td>
<td>0.095</td>
<td>0.54</td>
<td>13.2</td>
<td>0.78</td>
<td>0.04</td>
<td>0.74</td>
<td>4.32</td>
<td>5.6</td>
</tr>
<tr>
<td>19-20</td>
<td>28</td>
<td>0.079</td>
<td>0.006</td>
<td>0.085</td>
<td>0.4</td>
<td>11.6</td>
<td>0.73</td>
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<td>0.68</td>
<td>3.6</td>
<td>4.7</td>
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<tr>
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<td>0.106</td>
<td>0.015</td>
<td>0.121</td>
<td>0.6</td>
<td>14.8</td>
<td>0.91</td>
<td>0.10</td>
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<td>4.27</td>
<td>4.9</td>
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<tr>
<td>19</td>
<td>24</td>
<td>0.101</td>
<td>0.037</td>
<td>0.138</td>
<td>0.57</td>
<td>10.8</td>
<td>1.28</td>
<td>0.35</td>
<td>0.93</td>
<td>5.13</td>
<td>4.1</td>
</tr>
<tr>
<td>Pregnancy.</td>
<td>10</td>
<td>0.172</td>
<td>0.199</td>
<td>0.371</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>

* Time of estrus.
The cyclic changes in the uterine mucosa of the pig have been described by Corner (11). 3 or 4 days after estrus, proliferation of the glandular cells begins and attains its height at the 8th to 10th day; i.e., when the corpus luteum has become well developed. After the 15th day, i.e. at the time of the retrogression of the corpus luteum, there is a slow reversion to the resting type of structure. The changes which take place during the first 15 days are the same whether or not pregnancy follows ovulation. There is considerable congestion during the period just preceding the retrogression of the corpus luteum but never hemorrhage.

Since we find the greatest histological elaboration of the endometrium not at the time of ovulation, but rather at the time when the uterus is ready to receive and prepare for the nutrition of the fertilized ovum, we might expect to find the greatest chemical indication of activity in the endometrium at this time (i.e. the 8th to the 12th days). It will be seen from Table I that, in so far as the lipids are concerned, this is the case to a limited extent only.

**Total cholesterol** on the basis of moist weight is highest from the 3rd to the 10th day, which corresponds to the period of most rapid growth. The highest values for lecithin are to be found in the figures for the 7th to the 10th days. The diminution in percentage of lecithin after the 15th day is, however, not very striking. The values for the ratio lecithin to cholesterol are greatest at the period which corresponds to the greatest uterine activity.

**Cholesterol esters** are present throughout the entire cycle in quantities so small as to indicate no accumulation at any time, save perhaps in the case of very early pregnancy. Hence these data are in accordance with the hypothesis expressed in the previous paper; i.e., that the phospholipid content of a tissue is a function of its activity and that only a small amount of cholesterol esters are found in active tissues.

But, from the point of view of a possible explanation of differences in circulating lipid at different stages of the cycle, our findings are somewhat disappointing. It may be that we are dealing with a tissue in which the blood supply is so great that we can expect no accumulation of lipid material. The fact remains,
Okey, Bloor, and Corner

however, that the percentage of total lipid present in the endometrium of the pig is approximately that which Bloor (12) has found to be characteristic of lung, kidney, and pancreas of beef, rather than that of the specially differentiated, lipid-rich tissue to be expected from the discussions of Hermstein (3) and Watrin (13) of the human endometrium.

There is a possibility which must be considered, however; i.e., that the lipid content of the endometrial tissue in the pig may differ from that in other species of animals. There is no estrual or menstrual bleeding in the pig. The type of placentation is unusually simple. There is no burrowing of the chorionic villi into the endometrium but only a superficial contact between the membranes of the embryonic sac and the uterine lining. At birth of the young the membranes surrounding the fetus shell out without the extensive tearing of uterine tissue characteristic of most other species. Moreover, the amount of glandular proliferation in the endometrium at the time of implantation is less in the pig than in species with more elaborate placentation. Hence it is possible that lipid changes in the glandular tissue of our samples may have been masked by the high proportion of comparatively inert connective tissue: For this reason, we hope to make similar investigations of the lipids of this tissue in other species. But because this will have to be done in separate laboratories and because the work herein reported constitutes an integral part of our original investigation we are presenting the lipid figures for the endometrium of the pig together with those for the corpora lutea of the same animals.

The relative amount of corpus luteum tissue which may be present in the ovaries of a single animal is, on the other hand, greater in the pig than in most other species. But the total mass of corpus luteum tissue which we were able to obtain from any one animal at the stage of greatest development (15th day) averaged approximately 6 gm. and contained 0.4 to 0.6 per cent = 0.024 gm. of total cholesterol and approximately 4 per cent = 0.24 gm. of lecithin. Retrogression of the corpus luteum results in an increase in the percentage of cholesterol but in a decrease in total mass which more than compensates for it (see previous paper). Consequently we are justified in concluding that in the pig there is not at any stage of the sexual cycle an
accumulation of cholesterol sufficient to account for any consider-
able change in its concentration in the blood stream.

SUMMARY.

The total lipid content of the uterine mucosa of the pig on the
basis of dry weight, is approximately the same as that of lung,
kidney, and pancreas of beef. The water content of this tissue
is variable and high, especially during the stages of congestion.

There is a definite but not large increase in the percentage of
lecithin at the time of greatest elaboration of the endometrium.
This is, however, accompanied by an increase in free cholesterol.
Hence, the lecithin-cholesterol ratios are not as high as those in
the corpus luteum at the same time.

The amount of cholesterol ester present in the endometrium is
always very low. There is never any indication of accumulation
of this substance in the endometrial tissue. There is, moreover,
too little accumulation of cholesterol in the corpus luteum of this
species to account for any considerable change in the cholesterol
concentration of the blood.

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