We measured kininogens of low and high molecular mass along with prokallikrein activity in plasma of women with a normal menstrual cycle. We observed no difference between results for the follicular and luteal phases. We assayed the same constituents in women who were taking oral contraceptives (combined estroprogestative) and found that activity of prokallikrein and concentrations of low- and high-molecular-mass kininogens were significantly increased. Lastly, we studied the components of the kallikrein–kininogen system during pregnancy. We also observed a marked increase in their concentrations in plasma, despite a decrease in total proteins. Specifically, prokallikrein and kininogens increase continuously with gestational age, reaching their maxima around the eighth month of pregnancy. At that time, more than 50% of observed results fall outside the normal reference interval. Our observations are even more striking when prokallikrein and kininogens are expressed in units per gram of total proteins, to account for the hemodilution. After delivery, the concentrations of prokallikrein and low- and high-molecular-mass kininogens decline promptly, returning to normal within three days.

Additional Keyphrases: prokallikrein • low- and high-M, kininogens • menstrual cycle • contraceptives • pregnancy • labor and delivery

Prokallikrein and kininogens of high and low relative molecular mass (HMWK and LMWK, respectively) are glycoproteins in plasma that are synthesized by hepatic cells. The first two constituents are essential for activating Hageman factor (Factor XII), which in turn transforms prokallikrein into kallikrein (EC 3.4.21.34). Kallikrein liberates bradykinin from HMWK. Similarly, the glandular kallikreins (tissue kallekrein, EC 3.4.21.35) can liberate kallidin or lysyl-bradykinin from LMWK. These kinins stimulate the smooth-muscle fibers (1) and are powerful autacoids (endogenous mediators having pro-inflammatory properties).

Until recently, only biological methods have been available for the exploration of the kallikrein–kininogen system (2). Now, however, prokallikrein activity can be determined by amidolytic methods involving chromogenic or fluorogenic substrates (3) or by immunological methods (4–6). We previously described an automated method for the enzymic determination of plasma prokallikrein (7) and proposed specific radioimmunoassays for both kininogens (8). Such rapid techniques, applicable to large series of plasma specimens, may further our knowledge of the kallikrein–kininogen system.

Our previous studies establishing reference values for kininogen compounds in healthy individuals showed that they were not influenced by sex or age (8). Here, we report our investigation of the influence of estroprogestative contraceptives and pregnancy on concentrations of prokallikrein and kininogens in plasma. Pregnancy is known to affect many plasma proteins (e.g., acute-phase proteins and coagulation factors), but little has been reported concerning kininogens; the few relevant studies performed have involved only a limited number of pregnant or parturient women (9–11). In our comprehensive investigation of the kallikrein–kininogen system in women, we compared the results for women having normal, unperturbed menstrual cycles with those for pregnant women and women who were taking oral contraceptives. We studied the evolution of prokallikrein, HMWK, and LMWK concentrations during gestation, taking into account hemodilution, through the time of membrane rupture and on to the seventh postpartum day.

Materials and Methods

Patient populations. Our study subjects were 56 healthy women, ages 29 ± 6 years (mean ± SD), with normal menstrual cycles. Nineteen were in the estrogen phase and 37 in the luteal phase, as determined by anamnesis and a progesterone quantification to confirm the luteal phase. Moreover, we were able to study 19 other women both before and after ovulation. We also studied the influence of oral contraceptives in 46 women (ages 23 ± 5 years) who were taking a combination of estrogen/progesterone medication. These women were all in good health and undergoing no other treatment.

We also measured the components of the kallikrein–kininogen system during pregnancy. Data were obtained from 195 pregnant women during prenatal consultation, one sampling per woman; for practical reasons, we could not perform a longitudinal study. Figure 1 shows the distribution of gestational age, expressed as weeks after the last menstruation, at the time of blood sampling. All pregnancies evolved normally until birth. In 13 of these normal parturients, we monitored the concentrations of prokallikrein, HMWK, and LMWK from the time of membrane rupture, through labor and delivery, and then daily until

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After puncture, "endogeneous" zymes were observed in the HMWK.

Blood sampling. Discarding the first milliliter to remove "endogeneous" tissue activator, we collected blood by venipuncture, after overnight fasting, into a polystyrene tube containing sodium citrate, 0.1 mol/L, as anticoagulant. After centrifugation (10 min, 15 °C, 1500 × g), we decanted the plasma with a plastic pipette and stored it at −30 °C. Quantification was performed within a month.

Plasma assay. Plasma total proteins were measured by the biuret method. Prokallikrein enzyme activity was determined at 37 °C by an automated method with an Hitachi 705 automated analyzer (Naka Works, Katsuda, Japan) (7); the activator was ellagic acid (Cephosett; Nyegaard, Oslo, Norway) and the chromogenic substrate was "PK chromozyme" from Boehringer, Mannheim, F.R.G.

Total kininogens and HMWK were radioimmunoassayed (8). The antigens were purified in our laboratory and antisera were raised in rabbits. Because of the complete and equimolar cross reaction of HMWK in the LMWK radioimmunoassay, we quantify total kininogens with this assay. The anti-HMWK antiserum is immuno-adsorbed with LMWK before use, therefore making it highly specific for HMWK. The concentration of LMWK is determined as simply the difference between total kininogens and HMWK. For more detail, see Adam et al. (8).

Statistical analysis. For each woman, we calculated the ratio of LMWK to HMWK. We also expressed prokallikrein and kininogens in units per gram of total proteins, so as to take into account possible hemodilution. The mean, standard deviation, and cumulative frequency distribution were determined for each variable in the three groups of women investigated: normal, those taking contraceptives, and pregnant. We used Student's t-test to compare the mean values for the different groups.

Results

Normal menstrual cycle. The mean ± 1 SD values for all the variables we investigated in women with normal cycles (see Table 1) agreed with the values we reported in previous studies (7, 8) of a much larger population of healthy volunteers (n = 450): 265 ± 41 mg/L for total kininogens, 90 ± 12 mg/L for HMWK, 178 ± 42 mg/L for LMWK, and 713 ± 110 U/L for prokallikrein. For the 19 women for whom results were available in both phases of the menstrual cycle, we observed no significant differences in plasma concentrations of these analytes. For example, the values for HMWK was 92 ± 10 mg/L in the follicular phase and 91 ± 12 mg/L in the luteal phase, and LMWK was 174 ± 40 and 170 ± 37 mg/L, respectively.

Oral contraceptives and pregnancy. As Table 1 shows, concentrations of prokallikrein, total kininogens, HMWK, and LMWK are significantly greater (p < 0.05) in women who are taking oral contraceptives, whereas total proteins remain unchanged. During pregnancy there is also a marked increase in the constituents of the kallikrein−kininogen system, despite a significant drop in total protein concentrations owing to the concomitant hemodilution. Inspection of the cumulative frequency distributions for the three groups (Figure 2) reveals that prokallikrein and kininogens concentrations in normal, untreated women are notably lower than those measured during pregnancy or in women taking contraceptives. Note that the distribution curves of prokallikrein and LMWK for the last two groups are practically superimposable, whereas for HMWK the values observed in the oral contraceptive group are significantly higher than during pregnancy (118 vs 108 mg/L; p < 0.05).

Evolution of the kallikrein−kininogen system during pregnancy. To study the evolution of the components of the kallikrein−kininogen system during pregnancy, we plotted the results obtained from the 195 pregnant women vs gestational age (Figure 3); the shaded zones correspond to the reference intervals determined for healthy women (7, 8). From the eighth week onwards there is a marked decrease in total protein concentration in plasma, whereas prokallikrein activity and kininogens concentrations increase continuously until the sixth month of pregnancy, when the mean values stabilize towards the upper limit of the reference interval.

When prokallikrein and kininogen are expressed in units per gram of total protein concentration, obviating effects of hemodilution, their increase is even more pronounced (Figure 4). From the 20th week of gestation until delivery, the mean values are noticeably outside the reference interval, especially prokallikrein and LMWK (p < 0.001).

To confirm our findings, we determined for each analyte and for each month of gestation the proportion of values falling outside the reference limits (Table 2). We found that these proportions increase throughout pregnancy. Prokallikrein and HMWK follow a parallel evolution: before the 12th week all values are within the 95% reference interval, but the percentage of values outside rapidly increases, being more than 40% of observed values for prokallikrein and 30% for HMWK by the 24th week of gestation. In contrast, LMWK increases more rapidly; in fact, between the fourth and eighth weeks, 11% of the values already exceed the upper reference limit. By the seventh month of pregnancy, as much as 71% of LMWK values exceed the upper limit. Finally, all three analytes decrease slightly in the last month of pregnancy.

Kallikrein−kininogen system behavior before and after delivery. In the 13 women who were monitored from delivery until hospital discharge, the prokallikrein activity and the concentrations of HMWK and LMWK decrease promptly after membrane rupture (see Figure 5). The values measured 12 h after delivery are already markedly lower than those measured beforehand, particularly for prokallikrein and LMWK. By three days after delivery, the concentrations of all three analytes had returned to normal.

Discussion

The increase in estrogen resulting from pregnancy or ingestion of oral contraceptives stimulates the hepatic synthesis of glycoproteins, especially the coagulation factors. For instance, a recent study (14) has shown an increase of
Table 1. Mean (and SD) for Some Variables in Women with Normal Menstrual Cycles, and Women Taking Oral Contraceptives or during Pregnancy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal menstrual cycle (n = 56)</th>
<th>Oral contraceptives (n = 46)</th>
<th>Pregnancy (n = 195)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>29 (6)</td>
<td>23 (5)</td>
<td>27 (5)</td>
</tr>
<tr>
<td>Total proteins, g/L</td>
<td>63.5 (5.8)</td>
<td>63.4 (4.5)</td>
<td>58.9 (4.3)*</td>
</tr>
<tr>
<td>Prokallikrein, U/L</td>
<td>695 (112)</td>
<td>893 (155)*</td>
<td>861 (166)*</td>
</tr>
<tr>
<td>Total kininogens, mg/L</td>
<td>269 (45)</td>
<td>363 (44)*</td>
<td>374 (75)*</td>
</tr>
<tr>
<td>HMWK, mg/L</td>
<td>91 (11)</td>
<td>118 (14)*</td>
<td>106 (12)*</td>
</tr>
<tr>
<td>LMWK, mg/L</td>
<td>178 (44)</td>
<td>245 (39)*</td>
<td>266 (67)*</td>
</tr>
</tbody>
</table>

*Significantly different from normals at p < 0.05 (Student's t-test).

Fig. 2. Cumulative frequency distribution for prokallikrein, LMWK, and HMWK in normal women (O), in women taking contraceptives (X), and in pregnant women (Δ)

Fig. 3. Changes in concentrations of total proteins, prokallikrein, LMWK, and HMWK during pregnancy.

Each point represents the mean ± SE for each analyte. Shaded areas: normal reference intervals for nonpregnant women.

Factors VII and X and fibrinogen during pregnancy. Prokallikrein and kininogens, also glycoproteins, could be similarly affected. Although the physiological role of LMWK remains uncertain, prokallikrein and HMWK accelerate the activation of the Hageman factor in contact with a negatively charged surface.

Our current knowledge of the behavior of the kallikrein–kininogen system in women taking oral contraceptives or in pregnancy is limited. More recent immunological methods (4–6, 8) for quantifying kininogens and enzymatic methods (3) for measuring prokallikrein activity have made it possible to investigate in more detail the pathophysiology of the kallikrein–kininogen system. For instance, in two recent papers (7, 8) we established 95% limits for reference values for prokallikrein, total kininogens, HMWK, and LMWK.

Table 2. Percentage of Results Exceeding the Reference Interval for Prokallikrein, LMWK, and HMWK in Relation to Gestational Age

<table>
<thead>
<tr>
<th>Gestational age, weeks</th>
<th>Prokallikrein</th>
<th>LMWK</th>
<th>HMWK</th>
<th>Out-of-range results, %*</th>
</tr>
</thead>
<tbody>
<tr>
<td>4–8</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>11 (11)</td>
</tr>
<tr>
<td>8–12</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>17 (36)</td>
</tr>
<tr>
<td>12–16</td>
<td>6 (12)</td>
<td>8 (30)</td>
<td>16 (29)</td>
<td></td>
</tr>
<tr>
<td>16–20</td>
<td>17 (21)</td>
<td>8 (30)</td>
<td>25 (42)</td>
<td></td>
</tr>
<tr>
<td>20–24</td>
<td>46 (51)</td>
<td>30 (42)</td>
<td>51 (66)</td>
<td></td>
</tr>
<tr>
<td>24–28</td>
<td>50 (54)</td>
<td>30 (48)</td>
<td>48 (60)</td>
<td></td>
</tr>
<tr>
<td>28–32</td>
<td>55 (50)</td>
<td>32 (57)</td>
<td>71 (86)</td>
<td></td>
</tr>
<tr>
<td>32–36</td>
<td>48 (48)</td>
<td>36 (56)</td>
<td>57 (75)</td>
<td></td>
</tr>
<tr>
<td>36–40</td>
<td>44 (44)</td>
<td>33 (52)</td>
<td>48 (63)</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parentheses represent the percentage of results falling outside the standardized reference interval (see ref. 8) after correction for total protein concentration.
and we found that they were not sex- or age-related. Here, we used standardized reference intervals for kininogens in terms of total protein concentrations, to improve data interpretation in case of hemodilution as observed in pregnancy. As expected from our first study (8), we did not find any significant difference in kininogen concentrations and pro-
kallikrein activities between the follicular and luteal phases of the menstrual cycle, which suggests that the human kallikrein–kininogen system is not related to the ratio of estrogen to progesterone at physiological concentrations.

The influence of estrogens and progesterones on the kalli-
krlein–kininogen system has been studied in animals, with biological techniques (14, 15), but the results are contradictory. Thus, daily injection of estrogens or progesterones to female rats reportedly increased the concentration of plasma kininogens (14), whereas another study (16) showed that high doses of progesterone decreased plasma kininogens in rats. One could argue, however, that the changes affecting kininogens in rat were related to the ratio of estrogen and progesterone, and our study in fact confirms that the constituents of the kallikrein–kininogen system are significantly increased by combined estrogen/progesterone contraceptives.

Several authors have studied the behavior of prokalli-
krlein, HMWK, or total kininogens in small groups of pregnant women (10-11, 14, 16, 17). All demonstrated a definite increase in these protein concentrations during pregnancy, although the hemodilution was not taken into account. In normal pregnancy, plasma volume increases by 1.5 L. This hypervolemia necessarily causes hematocrit and plasma protein concentrations to decrease, particularly the albumin fraction. We found that concentrations of prokallikrein and both kininogens are statistically higher in pregnancy than in menstruating women who are taking no oral contraceptive. All constituents of the kallikrein–kininogen system show a progressive increase with gestational age, and this increase becomes even more apparent when concentrations are corrected for hemodilution. As have others (9, 18-22), we studied the behavior of the kallikrein–kininogen system during puerperium and delivery. We observed that concentrations of both kininogens and prokallikrein decrease immediately after the membrane rupture, but return to normal as soon as the third postpartum day, in agreement with the findings of others (9, 14).

We suggest that the increase of the kallikrein–kininogen system constituents during estrogenic increases depends on an increased hepatic synthesis; indeed, prokallikrein is not activated, as indicated by its own increase. Further research is needed to support our assumption and to verify whether the constituents of the kallikrein–kininogen system are useful for detecting abnormalities in pregnancy.

References