PLASMA KININ AND KININOGEN LEVELS IN WOMEN DURING PREGNANCY AND IN LABOUR

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Summary. Kininogen and kinin levels in venous plasma were determined in groups of normal adult men and women and in pregnant women at different periods of gestation and in labour.

Kininogen levels were found to be significantly higher in the pregnant women than in the male and female controls. A significant positive linear correlation was found between period of gestation and plasma kininogen level.

Differences between the mean plasma kinin levels in groups of control adults and pregnant women were in most cases not significant.

No significant linear correlation was found between kinin and kininogen levels in plasma in any of the groups studied and no definite trends were found in the kinin and kininogen levels at delivery.

INTRODUCTION

During labour there is an increase in the oxytocic activity of maternal blood which cannot be attributed to oxytocin (Armstrong & Stewart, 1960; Centaro, Periti & DeLaurentis, 1961). It now seems likely that the activity is due, at least in part, to the prostaglandins (Karim, 1969), but the belief that polypeptides may contribute to it has led several workers to study the precursors of the plasma kinins (kininogens) in the blood in order to determine the extent to which they are mobilized in pregnancy and labour. Kininogen rather than kinin levels have been estimated because, until recently, it has not been possible to determine the free kinin levels in plasma with any degree of accuracy, since rapid enzyme action gives an extremely short half-life to the kinins, both in vivo and in vitro (Saameli & Eskes, 1962).

Martinez, Carvalho & Diniz (1962) found that the kininogen content of plasma fell during labour but returned to prepuratum values in the puerperium. Approximately 25% of the kinin stores were found to be mobilized during labour and the authors calculated that 2 to 5 mg of kinin (as bradykinin) would be released in parturient blood during expulsion—enough to confer oxytocic activity on the plasma. However, no kinin activity as such could be detected in the plasma and it was suggested that this might have been due to rapid

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destruction \textit{in vivo} of the kinins. Periti & Gasparri (1966) repeated and extended the work of Martinez et al. (1962). From data on forty-four pregnant and 138 non-pregnant women, they concluded that plasma kininogen during the last 2 months of pregnancy is higher than normal, with a significant positive arterio-venous difference, and that venous plasma kininogen during labour diminishes greatly but returns to \textit{prepartum} levels during the puerperium.

In this study, we have repeated the work of Periti & Gasparri with respect to kininogen estimations and have, in addition, estimated kinin levels, using a slightly modified version of the method of Brocklehurst & Zeitlin (1967) which ‘fixes’ the kinins at the moment of withdrawal of the blood specimen. This method relies on the estimation of whole blood kinin and kininogen levels, followed by calculation of the plasma levels using the haematocrit figure.

\section*{MATERIALS AND METHODS}

\textbf{Subjects studied}

A control group of eighteen normal adult men and women and a group of fifty-four pregnant women, some of whom were tested several times during their pregnancy, were studied. At the time of entry into the study, all these women had uncomplicated pregnancies. The control group, whose ages ranged from 22 to 46 years, was divided into two sub-groups according to sex, with nine subjects in each. The results for the pregnant women were divided into groups according to the gestational age of the fetus (in completed weeks) as follows:

- Group 1: gestational ages 1 to 20 weeks, results from six women aged between 18 and 29 years.
- Group 2: gestational ages 21 to 28 weeks, results from fifteen women aged between 18 and 43 years.
- Group 3: gestational ages 29 to 33 weeks, results from twenty-four women aged between 17 and 43 years.
- Group 4: gestational ages 34 to 37 weeks, results from ten women aged between 19 and 34 years.
- Group 5: gestational ages 38+ weeks, results from ten women aged between 19 and 43 years.

Specimens were also obtained from ten women at delivery. These were taken (1) in early labour (approximately 5 hr before delivery); (2) at full dilatation; (3) at expulsion of the fetus; and (4) 5 min \textit{post partum}. Puerperal specimens were also obtained from three women.

\textbf{Withdrawal of blood specimens}

Blood was obtained from the antecubital vein through a wide bore needle which was attached to a two-way tap, one outlet of which was connected to a 20-ml capacity polythene syringe containing 15 ml of chilled redistilled ethanol and the other to a 2-ml capacity syringe. Five ml of blood were taken up directly into the ethanol which temporarily inactivated plasma enzymes and precipitated the proteins. The tap was then turned and 2 ml of blood were taken into the smaller syringe. As soon as possible after the venepuncture, the blood–ethanol mixture was shaken in the syringe and forced through another wide-bore
needle into a siliconed Pyrex glass centrifuge tube, thus dispersing the precipitated proteins. These tubes were then stoppered and stored for up to 20 hr at 4°C.

The blood in the 2 ml syringe was added to an EDTA-coated tube and stored at 4°C until needed for the haematocrit estimation.

Estimation of kinin and kininogen in blood

This was carried out using a slightly modified version of the method of Brocklehurst & Zeitlin (1967) which entails the initial ethanol precipitation described above, in which the kininogen is precipitated with the protein and the kinin is localized in the supernatant. This is followed by centrifugation at 4°C and further purification procedures.

Extraction of kinin

The supernatant and washings were heated at 100°C for 10 min to inactivate the enzymes and then evaporated to dryness under reduced pressure. The residue was dissolved in water and acidified with HCl to pH 1.0.

A slight modification was made to the method in that the solution was heated at 100°C for 20 min. Without the modification, the addition of oxytocin to blood specimens gave falsely high values for kinin but, after the heating stage, the addition of 2 mU oxytocin (Synthetic Oxytocin, A grade, Calbiochem) per ml of blood produced no effect on the assay of kinin or on the recovery of added bradykinin.

Next, 5 g NaCl was added to the hot acidified solutions which were then cooled to produce a saturated solution. After centrifugation, the supernatants were removed and extracted with redistilled butanol. This extraction has the effect of excluding potassium ions from the organic phase and Brocklehurst & Zeitlin (1967) found that it also effectively excluded bases such as histamine, 5-hydroxytryptamine and adrenaline.

The extracts were then dried under reduced pressure and finally heated to 80°C and subjected to a vacuum of 0.1 Torr for 10 min to drive off the last trace of butanol, as it has been found that even small amounts of butanol can interfere with the assay. The dried extracts were then stored at −20°C for periods up to 1 month: tests have shown that such storage does not result in any loss of activity.

Treatment of kininogen-containing precipitate

The precipitate was first heated at 100°C for 10 min with 80% ethanol to give complete denaturation and then centrifuged. After two water washes, the precipitate was suspended in 15 ml 2.5 M-NaCl and homogenized. Aliquots (0.2 ml) of the homogenate were incubated with trypsin in phosphate buffer (pH 8) for 30 min at 37°C to convert the kininogen to kinin. The incubates were then heated at 100°C for 10 min to destroy the trypsin, quickly cooled and deep-frozen in acetone/solid CO₂ mixture. These were also stored at −20°C for up to 1 month.

Bioassay of kinins

This was performed on the isolated superfused oestrous rat uterus preparation
at 30°C (Gaddum, 1953) using DeJalon's solution as the nutrient medium. The contractions of the uterus were recorded isotonically and the superfusion rate was kept constant at 5 ml/min.

A semi-automatic apparatus similar to that described by Colquhoun & Tattersall (1970) was used. The preparation was supplied with doses of test and standard solutions at fixed intervals from an 'AutoAnalyzer' sampling unit (Technicon Ltd) slightly modified to give a cycle in which the dose was in contact with the preparation every 3 min for 20 sec.

The assay was a simple bracketing design in which the contractions produced by the unknown kinin-containing solutions were compared with those produced by standard bradykinin solutions (Koch-Light Chemicals Ltd) in the range of 50 to 250 pg/ml. Within this concentration range, no tachyphylaxis was found to occur and the discrimination ratio ranged from 12.5 to 25%. The kinin specimens were usually diluted 25 times for assay. The 'kininogen' specimens, on the other hand, contained much more kinin and these were usually diluted up to 200 times. Owing to these high dilutions it was not found necessary to compensate the diluting solution for ions in the extracts and incubates and unmodified DeJalon's solution was used.

Calculation of results

The haematocrit estimation was performed to determine the relative amounts of plasma and cells in the blood. It is upon the premise that all the kinin and its precursor were in the plasma (Brocklehurst & Zeitlin, 1967) that the calculation of plasma levels from the whole blood values using the haematocrit factor was carried out.

RESULTS

Recovery experiments

When bradykinin, 5, 10 and 25 ng, was added to specimens of blood (5·0 ml), the mean recoveries were respectively 85% \((n = 2)\), 76% \((\text{S.E.M.} \ 7.4; \ n = 5)\) and 88% \((\text{S.E.M.} \ 7.0; \ n = 8)\).

Control group

The results for this group are shown in Table 1. When Student's \(t\) test was applied to the mean plasma kinin levels for the two groups (male and female), no significant difference was found \((P>0.7)\). Similarly, no significant difference was found between the mean plasma kininogen levels \((P>0.9)\). No significant linear correlation was found between the kinin and kininogen levels in the combined group \((r = 0.074; \ P>0.1)\).

Groups of pregnant women

The results for these groups are shown in Table 2. The kinin levels appear similar throughout pregnancy and when Student's \(t\) test was applied to the means of the various groups, no significant differences were found. The mean kinin levels for the five groups were also compared with the mean level for the female control group and the differences were found to be not significant, except in the case of Group 1 where \(0.025 > P>0.02\).
The mean kininogen levels on the other hand were raised above that for the female control group and this was found to be significant for each group (0·005 > P > 0·001 for Group 1 and P < 0·001 for the other four groups). Although a slight rise was found in the mean kininogen levels as gestation increased, none of these changes was statistically significant, e.g. when the means for Groups 1 and 5 were compared, 0·1 > P > 0·05. Also, in none of these groups was there a significant linear correlation between the kinin and kininogen levels.

Table 1
PLASMA KININ AND KININOGEN LEVELS IN CONTROL GROUPS OF NORMAL HUMAN ADULTS

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
<th>Combined group</th>
</tr>
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<tbody>
<tr>
<td>Mean plasma kinin level</td>
<td>1·17 ± 0·20</td>
<td>1·08 ± 0·14</td>
<td>1·12 ± 0·12</td>
</tr>
<tr>
<td>(as bradykinin)</td>
<td>n = 9</td>
<td>n = 9</td>
<td>n = 18</td>
</tr>
<tr>
<td>Mean plasma kininogen</td>
<td>2·78 ± 0·28</td>
<td>2·76 ± 0·12</td>
<td>2·77 ± 0·15</td>
</tr>
<tr>
<td>level in µg/ml ± S.E.M.</td>
<td>n = 9</td>
<td>n = 9</td>
<td>n = 18</td>
</tr>
<tr>
<td>(as bradykinin equiv.)</td>
<td></td>
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</table>

Table 2
PLASMA KININ AND KININOGEN LEVELS IN GROUPS OF PREGNANT WOMEN AT DIFFERENT PERIODS OF GESTATION

<table>
<thead>
<tr>
<th>Gestation (weeks)</th>
<th>Group 1 (1 to 20)</th>
<th>Group 2 (21 to 28)</th>
<th>Group 3 (29 to 33)</th>
<th>Group 4 (34 to 37)</th>
<th>Group 5 (38+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean plasma kinin</td>
<td>0·60 ± 0·09</td>
<td>1·08 ± 0·14</td>
<td>1·01 ± 0·11</td>
<td>1·18 ± 0·14</td>
<td>0·83 ± 0·19</td>
</tr>
<tr>
<td>level in ng/ml ± S.E.M.</td>
<td>n = 6</td>
<td>n = 15</td>
<td>n = 24</td>
<td>n = 26</td>
<td>n = 10</td>
</tr>
<tr>
<td>Mean plasma kininogen</td>
<td>4·02 ± 0·40</td>
<td>4·35 ± 0·24</td>
<td>4·94 ± 0·24</td>
<td>5·05 ± 0·25</td>
<td>5·24 ± 0·39</td>
</tr>
<tr>
<td>level in µg/ml ± S.E.M.</td>
<td>n = 6</td>
<td>n = 15</td>
<td>n = 23</td>
<td>n = 26</td>
<td>n = 10</td>
</tr>
<tr>
<td>(as bradykinin equiv.)</td>
<td></td>
<td></td>
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</table>

A further test was carried out to determine whether there was a significant linear correlation between kininogen levels and period of gestation, using all the individual results from the fifty-four women (a total of eighty-seven results). The correlation coefficient (r) was 0·335 which was significant at the 99% level. The regression line was calculated for gestational age in weeks (x) against kininogen level in µg/ml of plasma (y) and this was y = 0·064x + 2·85. The slope of the line (0·064) had 95% confidence limits between 0·057 and 0·070.

Women in labour
Specimens were obtained from ten women during labour. These results are shown in Text-fig. 1, together with the levels found earlier in their pregnancy. When the mean kininogen level at the expulsion of the fetus for the ten
women (4·00 µg/ml ± 0·35 S.E.M. as bradykinin equiv.) was compared with the mean level in the group of pregnant women at 38+ weeks gestation (Group 5 in Table 2), a significant difference was found 0·05 > P > 0·025. This may indicate a lower kininogen level at delivery, but reference to Text-fig. 1 will show that there are no obvious trends in the kininogen and kinin levels during labour and delivery for the individual cases.
One abnormally high kinin result was obtained (Subject[e]) where the level of the 5-min post-partum specimen was greater than 50 times the other values for that patient, with no concomitant fall in the kininogen level. Again, in these ten women, no direct relationship between kinin and kininogen levels has been found.

DISCUSSION

The method used in this study was basically that of Brocklehurst & Zeitlin (1967) but an extra step was incorporated to remove oxytocin. Oxytocin levels in plasma during late pregnancy are high; Hawker, Walmsley, Roberts, Blackshaw & Downes (1961) found a mean level of 1·1 mU/ml ± 0·4 S.E.M. and it was found that these levels interfered with the assay of kinin. The simple modification outlined in the ‘Materials and Methods’ section was completely effective in removing this artefact without affecting the recovery of added bradykinin.

The plasma kinin levels for the normal adults are in general agreement with those reported by Brocklehurst & Zeitlin (1967). There are large differences in the reported kininogen levels for normal individuals; those in this study are in agreement with the findings of Periti & Gasparri (1966) but somewhat lower than those of Brocklehurst & Zeitlin (1967).

The rise in kininogen levels during pregnancy reported by Periti & Gasparri (1966) has also been shown to occur in this study. The other findings of these workers and of Martinez et al. (1962) of a significant decrease in kininogen at delivery were borne out statistically in our results for the ten women in labour. This could have been fortuitous, for on close inspection of the results in individual subjects, no obvious trends were found.

No kinin/kininogen relationship was found in this study and the postulate of Martinez et al. (1962) regarding the production of a large amount of bradykinin during expulsion has not been verified in nine out of ten women. In the case of one woman [e], there was a massive rise of kinin in the specimen taken 5 min post partum but, since there was insufficient specimen available to test this further (e.g. chymotrypsin incubation), this result could have been an artefact. It seems likely that, should a massive release of kinin occur at expulsion, this would be completely dissipated after 5 min, as the half-life of bradykinin in the blood is less than 30 sec (Saameli & Eskes, 1962).

It could be argued that increased kinin at expulsion would not be detectable in venous plasma owing to inactivation in the lungs (Ferreira & Vane, 1967). Even so, with a sensitive method such as that used here, some detectable residual kinin activity would be expected if there had been a massive release.

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