HAEMATOLOGICAL CHANGES IN PREGNANCY FOLLOWING OVULATION-INDUCTION THERAPY

BY

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Summary

An examination has been made of the changes in the major haematological indices that occurred in five women who became pregnant after ovulation-induction therapy. It would appear that, given successful ovulation-induction, the ensuing pregnancy presents an essentially normal haematological picture.

There is a substantial literature dealing with the haematological changes that occur in pregnancy, and many of these changes are reasonably well established (Hytten and Leitch, 1971). However, the advent of laboratory automation brings greater scope to studies in this field, for it provides simultaneous and highly precise measurements of the relevant variables; because of this precision statistically significant trends can be elucidated with fewer experimental observations. The opportunity was taken to investigate haematological changes that occurred in five women who became pregnant after induction of ovulation by gonadotrophins as they moved from the non-pregnant state through pregnancy into the puerperium. The clinical details of these women and their pregnancies are given by Macdonald et al. (1973). Iron supplements were not given to any of these subjects.

METHODS

The sampling procedure has been described (Macdonald et al., 1973). Sequestrenated whole blood specimens were obtained for haematology and heparinized samples were used for determining erythrocyte osmotic fragility.

The haematology was carried out on a Coulter Counter Model S, which measures the following variables within maker's specified limits. White cell count (+3 per cent), red cell count (+1 per cent), haemoglobin (+0.1 g./100 ml. blood), mean cell volume (+1 per cent), haematocrit (+1 per cent), mean cell haemoglobin (+2 per cent) and mean cell haemoglobin concentration (+3 per cent). The first four quantities are direct measurements, while the remaining three are obtained by computation from appropriate combinations of the first four values. For each variable, the readings of the five individuals were grouped at 30-day intervals from 13 ± 3 days pre-ovulation onwards, and the group means and standard errors were recorded. The post-partum measurement was made on the 6th postnatal day.

Erythrocyte osmotic fragility was measured at 20 °C. on fully oxygenated whole blood at 1/21 dilution in serially reducing concentrations of aqueous NaCl. The serial values of per cent haemolysis thus obtained were converted to
probits, and the best straight line of probit vs NaCl concentration was fitted by the method of least squares. The mean cell fragility, that is the concentration of NaCl at which 50 per cent haemolysis occurs, was then calculated from the regression equation. The individual mean cell fragilities for each of the five subjects were grouped as before, and the group means and standard errors were recorded.

RESULTS

The serial group mean values and standard errors of the eight haematological variables examined here are presented in Table I. Although the numbers are small they accurately represent the changes that occurred in a group of women who had successful pregnancies, and as such reflect in a general way the overall pattern of change from conception to parturition. Because this work is concerned mainly with trends, the data are presented graphically in Figures 1 and 2.

Figure 1(a) shows how the blood haemoglobin concentration changes in moving from the non-pregnant state, through pregnancy into the puerperium. In very early pregnancy the haemoglobin concentration begins to fall and continues to do so until about 30 days post-ovulation; it remains virtually constant until term. By the 6th day of the puerperium the red cell count is restored to the non-pregnant level. The decrease in this phase amounts to about 2 g./100 ml. From mid-pregnancy onwards the haemoglobin concentration rises again and regains approximately 1 g./100 ml. near term; a further 1 g./100 ml. increase occurs in the puerperium, where the non-pregnant level is fully restored by the sixth day.

The haematocrit exhibits a similar response (Fig. 1(b)), declining by about 5 per cent from the earliest point to mid-pregnancy. In the second half of pregnancy the haematocrit rises, regaining about 2 per cent by term and a further 4 per cent by the sixth day of the puerperium.

Somewhat surprisingly, the trend in the red cell count, which is depicted in Fig. 1(c), differs appreciably from that exhibited by the haematocrit. The red cell count falls by about 0.40 x 10^6/mm. between the first pregnancy reading and the end of the 1st trimester; it then rises by about 0.10 x 10^6/mm. at mid-pregnancy, and remains virtually constant until term. By the 6th puerperal day the red cell count is restored to the non-pregnant level.

The trend of mean cell volume (Fig. 1(d)) shows a decrease of about 10 µm. between the first pregnancy reading and mid trimester. This is followed by a steady increase to non-pregnant MCV near term, with a drop of about 6 µm. by the sixth puerperal day.

| TABLE I
Haematological changes in pregnancy |
<table>
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<tr>
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<tr>
<td>Time interval (days)</td>
<td>Blood haemoglobin (g./100 ml. blood)</td>
<td>Blood haematocrit (% PCV)</td>
<td>Erythrocyte count (x 10^6/mm.³ blood)</td>
<td>Mean cell volume (µm.³)</td>
<td>Mean cell haemoglobin (µg./cell) cells</td>
<td>Mean cell Hb concentration (g./100 ml. cells)</td>
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<tr>
<td>Non-pregnant</td>
<td>13.0 ± 0.2</td>
<td>38.3 ± 0.5</td>
<td>4.06 ± 0.10</td>
<td>96.8 ± 2.2</td>
<td>33.5 ± 1.0</td>
<td>34.0 ± 0.4</td>
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<td>0-29</td>
<td>13.2 ± 0.2</td>
<td>38.4 ± 0.7</td>
<td>4.03 ± 0.12</td>
<td>97.0 ± 1.7</td>
<td>33.3 ± 0.7</td>
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<td>30-59</td>
<td>12.6 ± 0.3</td>
<td>36.4 ± 0.9</td>
<td>3.89 ± 0.15</td>
<td>92.0 ± 2.4</td>
<td>31.4 ± 1.0</td>
<td>34.4 ± 0.4</td>
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<td>60-89</td>
<td>11.6 ± 0.2</td>
<td>34.1 ± 0.7</td>
<td>3.61 ± 0.11</td>
<td>88.8 ± 2.4</td>
<td>30.0 ± 0.9</td>
<td>33.9 ± 0.3</td>
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<td>90-119</td>
<td>11.3 ± 0.2</td>
<td>33.1 ± 0.3</td>
<td>3.69 ± 0.17</td>
<td>89.3 ± 2.9</td>
<td>30.7 ± 1.2</td>
<td>34.4 ± 0.4</td>
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<td>120-149</td>
<td>11.1 ± 0.5</td>
<td>32.6 ± 1.2</td>
<td>3.74 ± 0.16</td>
<td>88.0 ± 2.0</td>
<td>29.6 ± 1.1</td>
<td>33.7 ± 0.7</td>
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<td>150-179</td>
<td>11.7 ± 0.1</td>
<td>34.7 ± 0.3</td>
<td>3.77 ± 0.15</td>
<td>91.2 ± 2.4</td>
<td>31.3 ± 1.7</td>
<td>33.6 ± 0.5</td>
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<tr>
<td>180-209</td>
<td>11.0 ± 0.3</td>
<td>33.1 ± 0.8</td>
<td>3.69 ± 0.16</td>
<td>88.5 ± 2.7</td>
<td>29.9 ± 1.2</td>
<td>33.2 ± 0.3</td>
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<tr>
<td>210-239</td>
<td>12.0 ± 0.4</td>
<td>35.0 ± 1.2</td>
<td>3.70 ± 0.18</td>
<td>93.5 ± 1.8</td>
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<td>240-269</td>
<td>11.8 ± 0.5</td>
<td>34.8 ± 1.3</td>
<td>3.67 ± 0.15</td>
<td>97.8 ± 1.8</td>
<td>33.3 ± 1.2</td>
<td>33.8 ± 0.4</td>
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<tr>
<td>Postpartum</td>
<td>13.4 ± 0.1</td>
<td>38.9 ± 0.4</td>
<td>4.18 ± 0.16</td>
<td>91.8 ± 2.6</td>
<td>32.0 ± 1.7</td>
<td>34.1 ± 0.5</td>
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Each value represents the group mean ±S.E.M. of 2-3 determinations of a given variable on each of the 5 subjects within the corresponding time interval.
Serial changes in blood haemoglobin (a), haematocrit (b), erythrocyte count (c) and mean cell volume (d) in moving from the non-pregnant state, through pregnancy into the puerperium. The points denote group mean values and the vertical lines indicate ±1 standard error of the mean. Each group mean is derived from 2-3 determinations on each of the 5 individuals.

With the mean cell haemoglobin, shown in Figure 2(a), there is a decrease of about 4 μg. cell by mid-pregnancy, with a slight rise near term and no further change on the sixth day of the puerperium.

Changes in mean cell haemoglobin concentration (Fig. 2(b)) are very small indeed; there is the suggestion of a decrease from early pregnancy and a slight increase towards term. This variable is derived from haemoglobin and haematocrit; it therefore compounds the error in each and thus reflects greater uncertainty in its measurement.

Unlike the automatically measured variables, the determination of red cell osmotic fragility is a manual operation which is rather less precise. With this in mind it would appear (Fig. 2(c)) that there is probably little change in osmotic fragility during the first half of pregnancy. After mid-pregnancy there is a marked increase in osmotic fragility, which rises by about 17 mg. NaCl and flattens out near term. By the sixth puerperal day erythrocyte osmotic fragility has returned to the non-pregnant level.

The trend in leucocyte count, shown in Figure 2(d), is quite different from the others, for there is an immediate and considerable increase of 2·3×10^3/mm. This increase appears to attain its maximum by the 15th day post-ovulation; the white cell count then falls precipitately by about 3·5×10^3/mm. on the 60th day post-ovulation. From this point onwards the leucocyte count rises steadily by 2·0×10^3/mm. to a plateau near term. The sixth day puerperal level is almost identical to the non-pregnant value.

**DISCUSSION**

Published work on the haematological changes in pregnancy has recently been reviewed by
Hytten and Leitch (1971), and the observations made here are evaluated largely in the light of their comment.

The pattern of change shown by blood haemoglobin concentration is in good general agreement with other studies. But, with the advantage of frequent and precise serial observations from before conception, the initial fall is discernible by 45 days post-ovulation. The lowest concentration also appears to be reached earlier than generally accepted, about mid-pregnancy, and this is followed by the slow rise that others have reported. The further rise in the puerperium seems to be an upward step rather than simply a continuation of the process initiated at mid-pregnancy and probably reflects the return to normal non-pregnant plasma volume and red cell volume.

The haematocrit values reported here are lower than is generally accepted but, since they are computed from the directly measured cell volume and the independently measured cell count, the usually troublesome factors like applied centrifugal force and trapped plasma are irrelevant. The trend found here is again in good agreement with other work but, as with haemoglobin, the initial drop is apparent at 45 days post-ovulation and the lowest value occurs at mid-pregnancy. This is followed by the accepted slow rise to near term, with a stepwise rise into the puerperium.

There is little information about the red cell count in pregnancy, but what there is suggests a decrease similar to that of haemoglobin concentration. The precision of this direct measurement is high, so the comparatively uniform standard error found here is due almost entirely to variation between the individuals of the group. The red cell count decreases steadily from early pregnancy and reaches a statistically significant minimum about the 90th day post-ovulation; that is, rather earlier than haemoglobin or the haematocrit. There may then be a slight rise that flattens out around mid-pregnancy to a level which is significantly lower than that of early pregnancy, and remains virtually constant to near term; which implies that neither the rise in haemoglobin nor the rise in the haematocrit that occur in this period can be accounted for by increasing red cell numbers.

Although there is some consensus that mean cell volume probably does not change in pregnancy, there remains uncertainty about this quantity. Our observations indicate an absolute decrease in the independently measured mean cell volume that is evident by the 45th day post-ovulation. This decrease continues and reaches a minimum about mid-pregnancy, thereafter increasing to the non-pregnant volume near term. The sixth day puerperal MCV, however, drops sharply and significantly to below the non-pregnant level. We cannot, at present, offer an explanation of these changes, but it is noteworthy that while the increasing haematocrit of the second half of pregnancy cannot be explained by red cell count, it could be at least partly due to an absolute increase in cell volume during this period.

Little is apparently known of mean cell haemoglobin in pregnancy, although a slight decrease towards term has been noted. Our observations show a trend that is, as expected, broadly similar to that exhibited by blood haemoglobin concentration. There is a decrease by the 45th day post-ovulation and a minimum about mid-pregnancy; after this point the cell haemoglobin increases steadily to term and on into the puerperium. In this instance, and again because of the unchanged red cell count, the net increase in mean cell haemoglobin from mid-pregnancy to term could explain the rise in blood haemoglobin concentration.

It is well known that the mean cell haemoglobin concentration varies little in pregnancy, and that is our observation also. At best it can be said only that there is a barely discernible trend similar in shape to those of blood haemoglobin concentration and mean cell haemoglobin.

Within the limits of the experimental observations, erythrocyte osmotic fragility is apparently unchanged from the non-pregnant level through to mid-pregnancy. After this point osmotic fragility increases rapidly and then flattens out at a significantly higher level towards term. By the sixth puerperal day osmotic fragility has returned to the non-pregnant level. The only other study of this kind (Robertson and Cheyne, 1972) employed a different method of determining erythrocyte osmotic fragility which gives uniformly higher results. Nevertheless, these authors found much the same overall trend, although they reported that osmotic fragility
dropped slightly just before term. Robertson and Cheyne (1972) suggested that the increased osmotic fragility of late pregnancy was due to the reduced colloid osmotic pressure of plasma, but since the change in plasma albumin is already well-developed before the osmotic fragility change can be detected, this explanation is questionable.

The white cell count is remarkable in its demonstration of a highly significant rise by the 15th day post-ovulation; the more so because it appears that this rise could be initiated before ovulation. There is, moreover, support for this observation in the literature. Cruickshank et al. (1970) report that in two women who became pregnant after similar treatment, the neutrophil count increased. In their words this increase, "appeared to be a continuation of events that were happening before conception". In our view this transient increase in leucocyte count is distinct from the well-established leucocytosis of pregnancy that is apparent in the slow rise between the 45th day and term.

There are two main conclusions to be drawn from this study. The first is that once ovulation has been successfully induced in women with secondary amenorrhoea due to hypothalamic-pituitary failure, conception leads to an essentially normal haematological picture in the ensuing pregnancy. The second conclusion is that the initial expansion of blood volume appears to be a major factor in these changes. The fall in blood haemoglobin concentration, haematocrit and red cell count in early pregnancy, and the relatively constant red cell count in the second half of pregnancy—when plasma volume change is less marked—supports that view.

References