THE BONE MARROW IN THE ANAEMIA OF PREGNANCY

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We have already shown that the incidence of anaemia among pregnant women is high in Glasgow, namely 20% (Scott and Govan, 1949). The majority have an iron deficiency anaemia, but at least 4.3% of those patients with a microcytic hypochromic blood picture do not respond completely to iron therapy and only show further improvement once liver in some form is given.

It is now well recognized that the megaloblastic anaemias of pregnancy may present a completely atypical blood picture (Stevenson, 1938; Abramson, 1938; Segerdahl, 1941; Davidson, Davis, and Innes, 1942b; Callender, 1944; Wolff and Limarzi, 1946), and to-day haematologists tend more and more to diagnose this condition from the bone marrow picture. Thus within the last 10 years studies of larger numbers of cases have appeared, and a condition which at one time was considered rare (Beckman (1921) reported six in 60,000 normal deliveries) has been given greater prominence. Indeed, opinions would appear to have veered in the opposite direction, for in some of Segerdahl's (1941) cases a diagnosis of pernicious anaemia of pregnancy was made when megaloblasts were as low as 0.6%. It is doubtful whether this figure alone can be regarded as pathognomonic. Small percentages of cells resembling megaloblasts have been reported in the marrows of normal pregnant women (Daniachij, 1936; Russo, 1937), although the majority of authors do not hold this opinion (Hansen, 1938; Forssell, 1939; Markoff, 1939; Callender, 1946).

The problem of the megaloblast is a difficult one. Differentiation of the megaloblastic marrow may not be easy, and Mallarmé (1948) recognized intermediary forms in his "incipient" cases of pernicious anaemia, while Leitner (1948) has suggested that there may even be a "hypochromic prepernicious" stage. In pregnancy differentiation is even more difficult because the marrows are dimorphic and the percentage of the megaloblasts may not be high. In this hospital an initial sternal puncture is a routine procedure on all cases of anaemia with haemoglobin readings less than 8 g. %. Though the majority of marrows examined were normoblastic and a few megaloblastic, there were others containing intermediary forms which were very difficult to classify.

This paper is an attempt to relate the marrow morphology to the type of anaemia and to determine the essential features of each variety. It is, therefore, necessary to establish certain diagnostic criteria entailing a description of some of the abnormal precursors in the red cell series. Normoblasts, megaloblasts, plasma

FIG. 1.—A, film showing a type b intermediate cell ($\times 1,200$). B, film showing a type c smear cell ($\times 1,300$).

cells, and reticulum are very fully described in the standard textbooks of haematology, but there are three abnormal cells which occur in the anaemias of pregnancy and require description.

Abnormal Cells in Anaemias of Pregnancy

Type a : Macronormoblast.— This has been described already (Whitby and Britton, 1946), but, as we have found it in small numbers in marrows other than those essentially macronormoblastic, we would state that it was larger than normal with a larger nucleus and a chromatin network more open than one would expect in comparison with the degree of haemoglobinization of the cytoplasm.

Type b : Intermediate Cell.—In many of the marrows an early cell was found

which could not be placed in either the megaloblast or normoblast series, nor did it seem correct to call it an “early macronormoblast.” About $20\ \mu$ in diameter, it had a large, pale pink, almost homogeneous nucleus whose chromatin network was never very distinct. Occasionally small condensations of the chromatin could be seen, but these were unusual. The cytoplasm was never a deep blue, but all other shades could be seen even to a clear sky-blue, when it resembled rather closely the typical megaloblast. Nucleoli could not usually be demonstrated by ordinary Romanowski stains.

Type c : Smear Cell.—This was a very immature cell, which, owing to the tendency to disintegrate and lose its cytoplasm when the marrow smears were made, was called a smear cell. Usually the nucleus was seen without any surrounding cytoplasm, but careful searching of the fields revealed that where the cytoplasm was attached it was clear blue. The nuclei appeared to be structurally similar to those of the promegaloblast. Vital staining brought this out more clearly and stained the nucleoli a clear sky-blue. From their appearance and relation to other cells these seemed to be red cell precursors, but this was not definite, and possibly they were similar to the cells described by Rohr (1940) as “large proliferated reticulum cells” and by Callender (1944) as “haemocytoblasts.” We have included them with the plasma cells and reticulum cells in our differential counts.

Material and Methods

Forty-six patients were investigated. All had attended the antenatal clinic and were admitted to hospital for investigation. Sternal puncture was performed and smears made according to the method of Davidson, Davis, and Innes (1942b). The films were stained with May-Grunwald and cresyl blue and a differential count on 400 cells made in each case. Haemoglobin levels were estimated with a photoelectric colorimeter.

In the statistical analysis the standard error was calculated for each group and for the figures noted in Table I. In comparing the percentages Fisher's *t* test was applied, and the value of *t* checked by means of the angular transformation. (Only the significant points of the statistical analysis are mentioned here.)

Results

Of the 46 patients examined, 39 had a microcytic hypochromic blood picture. The remaining seven had macrocytic anaemias. Examination of the bone marrows showed that these cases could be divided into four groups.

Group 1.—This consisted of 11 patients with an average haemoglobin of 7.85 g.% and a red cell count of 3.63 m./c.mm. Haemopoiesis was normoblastic in every case and the cells of this series numbered 15.39%, the majority being polychromatic normoblasts. Macronormoblasts were rare and mitosis infrequent. Plasma cells and reticulum cells were not increased. Marrow reticulocyte counts were made, but in no case was the percentage higher than 1.0. All these patients were given iron, and they showed a very satisfactory response.

Group 2.—The second group of 17 patients included four who had already been treated with oral iron and had shown no improvement. Though their results are given separately in the table, they show such a marked similarity to the remaining 13 patients that we considered they should be included. The average haemoglobin was 5.2 g. % and the red cell count 3.16 m./c.mm. Both these readings are significantly lower than those in Group 1, but the drop in haemoglobin is the more striking. When the marrows were examined the striking feature was the marked degree of hyperplasia of the red cell series. Haemopoiesis was essentially normoblastic, but there was a significantly larger number of macronormoblasts. The normoblast count (including the macronormoblasts) was increased to 29.22%, and though this in itself was significant an analysis of the cells at each stage of maturation showed that the increase was most marked in the polychromatic normoblasts and less so in the basophilic cells. The early pronormoblasts and late orthochromatic normoblasts showed no significant change. Mitoses had increased to 1.4%, and the marrow reticulocyte count of 2.7% was also significantly increased. Plasma and reticulum cells showed no significant change. In almost every smear examined a few type b abnormal early cells were seen, yet every patient responded to iron, and the four patients who failed to improve on oral iron recovered completely on intravenous iron.

Group 3.—Seven patients formed this group and all had very severe anaemia. The average haemoglobin was 4.43 g.% and the red cell count 3.27 m./c.mm. The haemoglobin reading was significantly lower than that of Group 1 anaemias, but, due to wider variations in the red cell counts of the patients within this group (S.E. ± 0.42), the drop in the mean red cell count was not significant. The blood picture was very similar to that of Group 2 anaemias. Indeed, it was practically impossible to recognize these anaemias from their peripheral blood pictures, though occasionally a more marked anisocytosis was suggestive of the third group. In the bone marrow, however, distinguishing characteristics were apparent. Although haemopoiesis was still essentially normoblastic, typical megaloblasts were present to the extent of 2.4%. The total normoblast count was lower than in Group 2,

TABLE I
BONE MARROW FINDINGS IN FOUR GROUPS OF CASES OF ANAEMIA OF PREGNANCY

Group	No. of Cases	Hb (g. %)	R.B.C. (m./mm. ³)	Reticulocytes (%)	Normoblasts				Megablasts				Macro-normoblasts			Abnormal Early Erythroblasts (%)	Normoblast Total (%)	Megablast Total (%)	Mitosis	
					Pronormo-blast (%)	Basophilic (%)	Polychro-matic (%)	Orthochro-matic (%)	Promegalo-blast (%)	Basophilic (%)	Polychro-matic (%)	Orthochro-matic (%)	Basophilic (%)	Polychro-matic (%)	Orthochro-matic (%)				Red Cell Series	White Cell Series
1	11	7.85 (0.27)*	3.63 (0.12)	—	0.52 (0.13)	1.52 (0.34)	7.44 (1.13)	5.22 (1.16)	—	—	—	—	0.05 (0.05)	0.49 (0.8)	0.15 (0.11)	—	15.39 (1.34)	—	0.19 (0.069)	0.14 (0.069)
2	17	5.22 (0.24)	3.16 (0.12)	2.72 (0.38)	0.90 (0.29)	3.46 (0.48)	13.63 (1.0)	6.69 (0.78)	—	—	—	—	0.67 (0.11)	2.52 (0.43)	1.35 (0.46)	0.9	29.22 (1.29)	—	1.43 (0.15)	0.2 (0.07)
3	7	4.43 (0.49)	3.27 (0.42)	2.57 (1.1)	0.50 (0.23)	2.26 (0.45)	5.46 (0.65)	5.71 (1.33)	0.1	0.6	0.9	0.8	0.89 (0.3)	3.40 (0.33)	2.18 (0.58)	0.6	20.40 (2.11)	2.44 (0.48)	0.75 (0.13)	0.21 (0.4)
4	6 1	5.41 (0.64) 7.29	2.13 (0.24) 3.19	0.67 (0.44) 3.0	1.08 (0.17) 1.5	2.13 (0.46) 2.25	2.04 (1.06) 5.5	2.17 (0.78) 11.0	1.42 0.25	1.04 1.0	1.29 0.75	0.79 1.0	1.04 (0.40) 2.25	1.79 (0.38) 7.25	1.38 (0.15) 6.0	1.0 0.5	11.63 (2.6) 37.25	4.54 (1.14) 3.0	0.54 (0.15) 1.8	0.21 (0.16) 2.0
Post-haemorrhagic	4	4.8	20.2	3.8	0.43	1.3	7.5	9.8	—	—	—	—	0.9	1.9	1.6	—	23.4	—		

* Figures in brackets represent the standard error.

but it was significantly increased compared with Group 1 anaemias. Analysis of the individual cell-types showed a significant increase in the number of macro-normoblasts, most marked at the basophilic stage and progressively less as development proceeded through the polychromatic to the orthochromatic stage. A slight decrease occurred in the ordinary normoblast series at every stage of development, but this was not statistically significant.

Despite the fact that these megaloblastic marrows did not give the impression of being specially active, nevertheless the number of cells in mitosis had increased to 0.75%, and the marrow reticulocyte count of 2.57% was also significantly raised. There was also an insignificant increase in the plasma and reticulum cells.

The patients of this group showed only a partial response to iron therapy and ultimately proved resistant even to intravenous iron. A change to liver therapy produced an immediate improvement with the appearance of a reticulocytosis in the peripheral blood.

Group 4.—This group consisted of seven patients with a macrocytic blood picture. The findings in six were fairly uniform. The seventh will be considered separately. Of the six patients, five were seen antenatally and one in the puerperium.

The average haemoglobin of these six patients was 5.41 g. % and the red count 2.13 m./c.mm.; i.e., compared with the Group 1 anaemias there was a significant drop in both haemoglobin and red cell levels, and, as would be expected, the fall in the red cells was the more striking.

The marrow was dimorphic in every case. Megaloblasts averaged 4.54% and normoblasts 11.63%. Although this was the lowest percentage of normoblasts of any group, the diminution was not statistically significant when compared with Group 1 anaemias. This was surprising, but detailed analysis of the normoblastic series showed that there was a marked diminution in the orthochromatic normoblasts and this was of definite statistical significance. The greater proportion of the normoblast series was composed of earlier forms of normoblasts and of macro-normoblasts, a feature not seen in Group 1.

Reticulo-endothelial cells showed a very significant increase in the marrows of the macrocytic anaemias. This was mainly due to the presence of large immature smear cells, though the plasma cells themselves averaged as much as 3.2%. There was no increase in the marrow reticulocytes, but mitotic figures in the red cell series were more frequent and this was significant.

All six patients were given liver principle and showed a satisfactory response to treatment.

The remaining member of the group had a haemoglobin of 7.29 g. % and a red cell count of 3.19 million. Though the red cells were macrocytic the patient had also a marked hypochromia. The marrow contained 3% megaloblasts and 37.25% normoblasts, of which 15.5% were macronormoblasts. The marrow was very cellular and hyperplasia of the red cell series obvious. As can be seen from Table I, there were a considerably greater number of mature normoblasts compared with the other cases of macrocytic anaemia. In addition mitotic figures were frequent, 1.8%, and marrow reticulocytes, 3%. Plasma and reticulum cells were not exceptionally high. All these findings tended to suggest that the marrow hyperplasia in this case was producing more mature forms, and we decided to withhold liver

and review the case. Spontaneous recovery occurred, but the rate of improvement was slower than other cases treated with iron and liver.

Discussion

There is still considerable disagreement on the subject of "the bone marrow of pregnancy." Most authors admit that there is a slight tendency to moderate hyperplasia with the appearance of macronormoblasts, and report a shift to the left of the granular series as the pregnancy advances, but they have not found these results significant in comparison with non-pregnant women (Forssell, 1939; Pitts and Packham, 1939; Callender, 1946). Others, like Daniachij (1936), Hansen (1938), and Markoff (1939), disagree with this conclusion and feel justified in speaking of a bone marrow of pregnancy. Daniachij also reported the presence of megaloblasts in his cases. Callender (1946) and Montoya Gómez (1947) disagreed with Daniachij, and we too have never found any megaloblasts in the normal cases we have examined. Anaemia in pregnancy does not usually become apparent till the third trimester, and none of our cases was seen earlier than this. In order to establish criteria we have selected a few of the reports on the marrows of normal pregnant women seen in the last three months of pregnancy (Table II). Pitts and Packham's (1939)

TABLE II
BONE MARROW FINDINGS IN NORMAL PREGNANT WOMEN

Author	Normoblasts				Megaloblasts				Normoblast (total %)	Megaloblast (total %)	Myeloid (total %)	R.E.S. Cells (%)	Monocytes	Lymphocytes	Megakaryocytes
	Pronormoblast	Basophilic	Poly-chromatic	Ortho-chromatic	Pronormoblast	Basophilic	Poly-chromatic	Ortho-chromatic							
Daniachij (1936)		0.6	15.0			0.6			15.6	0.6	72.9	0.6	1.0	9.3	—
Hansen (1938) ..		23.4			—	—	—	—	26.7	—	62.0	0.5	1.8	7.7	0.6
Pitts and Packham (1939) ..	0.2	2.8	5.3	2.4	—	—	—	—	10.7	—	63.2	—	0.7	12.0	—
Callender (1946)	0.6	2.4	12.8	7.0	—	—	—	—	22.8	—	68.7	2.2	1.0	4.3	—
Gómez (1947) ..									25.05						
Mild anaemias, present series ..	0.5	1.5	7.4	5.2	—	—	—	—	15.4	—	73.0	3.0	1.0	7.4	—

figures show the lowest percentage of normoblasts, but when they made their sternal punctures they withdrew 10 ml. and probably diluted their specimens with blood. They also included some patients with anaemia in their series, since they reported minimum haemoglobin values of 8.97 g. %.

Apart from the finding of megaloblasts our figures for the mild normoblastic anaemias are probably nearer Daniachij's (1936). Compared with Callender's (1946) nine cases examined during the third trimester and those of Montoya Gómez (1947) and Hansen (1938), there appears to be less hyperplasia of the erythroid elements.

The degree of hyperplasia and the stage of red cell development at which it occurs are the most striking features in these cases of anaemia. As a control the

figures may be compared with the values found in four cases of post-haemorrhagic anaemia. These four patients were essentially healthy and their anaemia was entirely due to blood loss. The marrow counts are given at the foot of Table I. The presence of mitotic activity in the marrow and of reticulocytes both in the marrow and in the peripheral blood may be taken as evidence of hyperplasia. In addition the reticulocytosis indicates that there is no interference with maturation, and, as one would expect, the highest percentage of nucleated red cells occurs in the orthochromatic normoblast type. When, however, primary or dyshaemopoietic anaemia occurs the picture is altered. In Group 1 there is a reduction in the percentage of normoblasts, most marked in the orthochromatic type, with a tendency to increase in the earlier forms. As the anaemia increases in severity the changes and tendencies become more marked. A marked increase in the percentage of normoblasts occurs in Group 2, but this increase is entirely confined to the phases before the orthochromatic, especially the basophilic and polychromatic. At this stage in the anaemic process there was also an increase in the number of macronormoblasts, particularly the more mature forms. A new type of cell, the megaloblast, makes its appearance in Group 3, and with it the percentage of normoblasts becomes diminished although less markedly in the earlier forms. Macronormoblasts, on the other hand, have undergone a further increase and for the first time plasma cells and reticulum cells showed a tendency to increase. An even greater reduction in the normoblastic series occurred in Group 4. Macronormoblasts were also diminished although the earlier forms were increased. Megaloblasts were greatly increased and a similar marked increase occurred in the plasma and reticulum cell series.

From the above it will be apparent that as the anaemic process increases in severity the percentage values for each cell series follow a curve. The peak in each curve, however, occurs at a different level of haemoglobin, the normoblast preceding the macronormoblast, which in turn occurs earlier than the megaloblast. This process can be rendered in graphic form as in Fig. 2. It may be said that with increasing anaemia successive waves of hyper-

plasia occur in the marrow affecting the normoblast first, then the macronormoblast, and finally the megaloblast. More detailed examination of the results indicates that changes of a similar nature occur within each cell series. As the anaemia increases in severity the hyperplasia occurs at a progressively earlier stage. This may be stated in another and possibly more accurate way by saying that increase in severity of the anaemia is associated with a slowing of maturation at an earlier stage of differentiation. Reference to the figures given for the total percentage of nucleated red cells indicates that, while there is some evidence of a true hyperplasia occurring in Group 2 anaemias, this is not apparent in the other groups. In this respect calcu-

FIG. 2

lation of percentages is misleading. An increase in the number of fat cells is a common finding in marrow smears from cases of very severe anaemia, and would tend to indicate a decrease in the number of haemopoietic foci. Apart from this it seems probable that there is some relationship between these groups of anaemias and that the megaloblastic type is merely one phase in the anaemic process.

Certain points still remain for discussion although few conclusions can be drawn from them. It will be noted that we found reticulocytes frequently in the marrow smears from cases of severe anaemia although none were present in the peripheral blood. Two possible explanations may be given. These reticulocytes may be abnormal cells. Alternatively their release into the peripheral blood may be conditioned by the rate of maturation of the nucleated red cells.

In any hyperplastic process one might expect information from an estimation of mitotic activity, but in marrow smears it is almost impossible to determine which type of red cell is undergoing mitosis. Changes were noted in the incidence of mitosis, but little significance can be attached to them.

So far we have not mentioned the abnormal erythroblast, type b. In appearance abnormal erythroblasts seemed to be an intermediate form between the normoblast and megaloblast. They were found in almost all marrow smears, but were most common in Group 4. It is possible that this cell has been mistaken for a megaloblast and this may account for communications reporting the presence of the latter cell in normal pregnancy marrows.

From this survey it would appear that, coincidental with the appearance of anaemia, there is a slowing of red cell maturation, and, as the severity of the process increases, maturation is slowed at progressively earlier phases. Apparent proliferation occurs in the stages before that at which maturation is halted. Cases showing hyperplasia even with a "shift to the left" as in Group 2 generally respond to iron. Where, however, the "shift to the left" is accompanied by relative inactivity in the erythroblast series the condition is likely to prove resistant to iron therapy. The apparent relationship between these various types of anaemia would suggest an upset in metabolism rather than a primary defect in the haemopoietic system.

Summary

The bone marrow has been studied in 46 cases of anaemia of pregnancy. In 28 the anaemia was of iron-deficiency type. The remaining cases were of pernicious variety.

In iron-deficiency anaemias marrows are entirely normoblastic. Main features are slowing of maturation, directly related to the severity of the anaemia, and hyperplasia at progressively earlier phases, that is a "shift to the left." As the anaemia increases in severity abnormal early erythroblasts, macronormoblasts, and reticulum cells become more common.

In pernicious anaemia of pregnancy the marrow is dimorphic and the peripheral red cells may be either microcytic or macrocytic.

Where the peripheral blood is microcytic there are few megaloblasts in the marrow. Normoblasts show a greater "shift to the left" and there is less evidence of hyperplasia in this cell-series. Macronormoblasts are much increased and both reticulum and plasma cells are common.

The microcytic megaloblastic anaemias show a partial response to iron, but normal blood values are only obtained if folic acid or liver is given.

In the macrocytic variety megaloblasts are common in the marrow. There is a diminution in the total number of normoblasts, although the "shift to the left" in this series is even more marked. A "shift to the left" is also apparent in the macronormoblasts. Reticulum and plasma cells are greatly increased. Patients with this variety show no response to iron.

It is suggested that these various types of anaemia are merely phases of the same pathological process and that there is an aetiological relationship between them.

The results would indicate that hyperplasia occurs in the red cell series as a result of slowing of maturation and is first apparent in the normoblasts, subsequently affects macronormoblasts, and finally megaloblasts as the anaemic process increases in severity.

Abnormal early erythroblasts may cause difficulty in interpretation and these cells may be mistaken for megaloblasts.

A statistical analysis of our results has been made and a copy of our protocols giving the details has been lodged with the Librarian of the British Museum. We would like to thank Dr. Robb, of the Mathematics Department, Glasgow University, for his help and guidance in the analysis.

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