MICROSCOPICAL EXAMINATION OF THE “BUFFY COAT” FROM THE HAEMATOCRIT IN THE INVESTIGATION OF ANAEMIA IN PREGNANCY

BY

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Microscopical examination of the “buffy coat” is an accepted but not widely adopted haematological procedure. It is particularly valuable in the diagnosis of pernicious anaemia, aleukaemic leukaemia, malaria, and disseminated lupus erythematosus. In the diagnosis of anaemia in pregnancy and the puerperium this simple technique has received only scant attention; thus a mere mention of its use is made by Lillie, Gatenby, and Moore (1954). The purpose of this paper is to show how useful it can be in the diagnosis particularly of pernicious anaemia of pregnancy.

Technique

A heparinized or oxalated sample of venous blood is spun in a Wintrobe tube in the usual way for the haematocrit reading. The “buffy coat” is sucked along with the immediately subjacent erythrocytes and a very small amount of plasma into a long, fine pipette. This material is divided between two or three or more slides, depending on the amount available. To obtain an even film, the pipette itself or the corner of a clean slide is used to mix the cells and plasma before spreading; the material is then spread as for an ordinary blood film, and stained with Leishman’s solution. The stained and now dry slide is prepared for microscopy by rolling a thin film of microfilm over it with a glass rod. This allows a 4-mm. objective to be used for scanning and the immersion lens to be swung in at once for the exact identification of doubtful cells.

The blood, whether in heparin or in oxalate, retains a satisfactory morphology for six to eight hours at room temperature; the structure of the leucocytes is largely destroyed in 24 hours, although megalocytes may still be recognizable. At 4° C. the diagnostic criteria for megaloblastic anaemia are adequately preserved for 24 hours.

The Cytology of the Buffy Coat

Certain blood cells are concentrated in the buffy coat (Rodan, 1949). A detailed account of these is now given with comments on their significance in pregnancy and the puerperium.

Nucleated Red Cells.—In normal pregnant women only a very occasional late normoblast is seen, but in cases of anaemia in pregnancy the nucleated red cells are often numerous and may be of specifically diagnostic type.

Megaloblasts or transitional megaloblasts afford the main criterion for the diagnosis of “pernicious anaemia of pregnancy.” It must be stressed that in evidence for this diagnosis only polychromatic or well-haemoglobinized cells are reliable, as the earlier, basophilic megaloblasts are not sufficiently distinctive to allow of their being differentiated from early basophilic macronormoblasts or from the large, basophilic irritation lymphocytes which are often quite numerous in various types of anaemia. It is also emphasized that even in a severe megaloblastic case the nucleated red cells show a wide range of size and nuclear structure, and that only a variable minority are definitely diagnostic.

In cases of dimorphic anaemia the megaloblasts may be relatively small, though they show characteristic delay in nuclear maturation with precocious, yet inadequate haemoglobinization. Such a megaloblast might well be given the paradoxical title of micromegaloblast. If the iron deficiency in such cases be treated first, the megaloblasts attain the more usual large size.

Small, pyknotic, hypochromic normoblasts are seen in hypochromic microcytic anaemia. Thus the finding of only this type of erythroblast is a useful confirmation of uncomplicated iron deficiency. It is of interest, however, that such cells may be found in the maternal buffy coat not only in the common iron deficiency anaemia of pregnancy, but also, very rarely, in a woman who is not anaemic, but into whose circulation there has been massive, occult, transplacental haemorrhage from the foetus. In such circumstances the pyknotic normoblasts in the maternal buffy coat are undoubtedly of foetal
origin. This subject is presented in detail in another paper (Goodall, Graham, and Cameron, 1956).

Normochromic normoblasts may be found in anaemia secondary to pre-eclamptic toxemia, but it should be borne in mind also that the signs of the pre-eclampsia are often secondary to anaemia, especially megaloblastic anaemia. Thus it is particularly important that the buffy coat be examined in any patient with anaemia and pre-eclamptic toxemia.

Macronormoblasts may be found in the buffy coat after severe ante- or post-partum haemorrhage, during therapeutic response in iron deficiency anaemia, in leukaemia, in haemolytic anaemia and in pernicious anaemia of pregnancy. Thus these cells are not of any specifically diagnostic value. What is important is that they should be differentiated from transitional megaloblasts. Macronormoblasts show a uniformly coarse condensation of nuclear chromatin, whereas in transitional megaloblasts there is a dual type of chromatin arrangement, in which coarser condensations are set in a background of finer stippling or reticulation.

While the finding of macronormoblasts is evidence of response to treatment in cases of proved iron deficiency, the buffy coat may also give useful information in assessing treatment in the megaloblastic cases. Thus, as early as 12 hours after folic acid has been given, the nucleated red cells may show reversion to normoblastic type, though this change usually takes about two days. Yet even these more delayed responses precede the rise in reticulocytes. It should be noted, however, that, in the presence of infection, this normoblastic change is not always a sure sign that response will be early and adequate; one severe megaloblastic case, complicated by pneumonia, had an entirely normoblastic buffy coat within 24 hours of the start of folic acid therapy, yet did not show a reticulocyte response till the infection was overcome.

Mature Erythrocytes.—As was found by Rodan, macrocytes and microcytes are concentrated with the nucleated red cells just below the leucocyte layer; indeed macrocytes can be found in every case in which megaloblasts are present, even though the mean corpuscular volume is low or normal. Thus, when examining a buffy coat as a screening test one can assess quite quickly whether a further search for megaloblasts is likely to be successful. It has, however, been found that in patients with dimorphic anaemia receiving initial iron therapy before folic acid the macrocytes are no more numerous, and may even be fewer in the buffy coat than in the ordinary film, though, as mentioned above, the now larger megaloblasts are still concentrated. In such cases the juxta-leucocytic erythrocytes are mainly hypochromic and thus less dense microcytes, while the better haemoglobinized macrocytes are at a lower level in the haematocrit.

Leucocytes.—The leucocytes can also be studied in films from the buffy coat, though it must be remembered that their distribution is such that differential counts are quite unreliable. Macropolycytes and giant metamyelocytes were seen in many of the cases of megaloblastic anaemia, but in the cases studied these changes in the granular leucocytes, characteristic as they are, were less sensitive indicators of folic acid deficiency than the alterations in the erythroblasts. Myelocytes, pro-myelocytes, and myeloblasts were found in pernicious anaemia of pregnancy, but they were observed also in other severe anaemias.

Erythropagocytosis.—Numerous erythrophages, both polymorphonuclear and mononuclear, were found in most of the cases of megaloblastic anaemia, but, although this finding should properly raise suspicion in the mind of the observer, it occurs, unfortunately, in other conditions. Its occurrence in pernicious anaemia of pregnancy is probably a manifestation of a lytic process affecting the abnormal maternal erythrocytes. The other conditions in which the phenomenon is seen are all related to uterine activity, either normal labour or abnormal labour, or false alarms, and it may well indicate the presence of foetal erythrocytes within the maternal circulation.

Present Investigation

The investigation took place over a period of 18 months (from September, 1954, to March, 1956). The clinical material consisted of 198 pregnant or puerperal patients who had a haemoglobin of less than 11 g. per 100 ml. (venous sample). The total number of antenatal cases was 2,252, but among patients delivered in hospital there was an additional smaller number of selected cases from other clinics; thus a true incidence of anaemia cannot be deduced from these crude figures. The anaemic cases were divided into two main groups; first, those in whom megaloblastic anaemia was suspected; and, second, those obviously due to iron deficiency alone or to haemorrhage, and corrected by appropriate therapy.

Group 1.—Group 1 consisted of 50 cases.

Selection.—In 43 there was definite suspicion of megaloblastic anaemia on the grounds of initial
severity, or failure to respond to iron therapy, or association with pre-eclamptic toxemia. The other seven patients had haemorrhage at term, but not quite enough to account for the degree of anaemia.

Ordinary Blood Examination.—The haemoglobin was estimated as oxyhaemoglobin on an E.E.L. photo-electric colorimeter. The P.C.V. was obtained in all cases, and the M.C.H.C. calculated. The size of the red cells was assessed on the Leishman-stained ordinary blood film during a thorough search, particularly for nucleated red cells. For the purpose of this investigation the red cell count is being ignored, as in some of the cases time was not available for a reliable type of red cell estimation.

Examination of Buffy Coat.—Theuffy coat was examined in all cases, as described under technique.

Marrow Biopsy.—Sternal puncture was made on 41 patients, including all the post-haemorrhagic cases. The marrow was not examined in eight, either because the patient could not come into hospital or because there was severe pre-eclampsia, but the buffy coats from these cases showed either megaloblasts or transitional megaloblasts, and response to folic acid confirmed the obvious diagnosis of megaloblastic anaemia. The case of acute myeloblastic leukaemia did not have a marrow biopsy, but the diagnosis, which was quite obvious from the blood film and buffy coat, was confirmed by post-mortem examination, including microscopic examination of the marrow.

Group 2.—Group 2 contained 148 cases.

The examination of the peripheral blood was similar to that of Group 1. The marrow was not examined.

Findings

Group 1.—In Table I the 42 cases in which the marrow was examined are assessed according to the nucleated red cell picture in the ordinary blood film.

### Table I

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<th>Cases in which Marrow was Examined</th>
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<td>Ordinary Film</td>
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<tr>
<td>Megaloblasts or transitional megaloblasts</td>
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<td>Macronormoblasts</td>
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<td>Normoblasts</td>
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in the buffy coat and in the marrow. In some of the cases nucleated red cells were not actually seen in the ordinary blood film, though present in the buffy coat in small numbers, but for practical purposes these have been assessed as normoblastic on the blood film.

In Table II the same 42 cases are presented in more detail, as diagnosed after sternal puncture and

### Table II

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<th>DETAILS OF CASES IN TABLE I AS ASSESSED AFTER MARROW BIOPSY</th>
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<tr>
<td>Megaloblastic or transitional megaloblastic erythropoiesis</td>
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<td>Macronormoblastic erythropoiesis</td>
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<td>Normoblastic erythropoiesis</td>
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<tr>
<td>The normoblastic cases include: Hypochromic microcytic anaemia</td>
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<tr>
<td>Post-haemorrhagic anaemia in puerperium</td>
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<tr>
<td>Slightly hypoplastic marrow</td>
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<tr>
<td>Acute myeloblastic leukaemia</td>
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<tr>
<td>Folic acid deficiency</td>
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<td>Total</td>
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* Though morphologically dissimilar these cases are all biologically related in that they required folic acid before making an adequate response.

response to therapy. It is convenient here to comment on certain cases. The case with a macronormoblastic marrow showed intense leucoblastic activity, an almost pseudo-leukaemic picture, but responded to folic acid. Similarly three normoblastic cases (tabulated as “folic acid deficiency”) which were refractory to iron therapy (intramuscular in two) improved after receiving folic acid by mouth. These cases, though not classified on morphological grounds as “pernicious anaemia of pregnancy,” are obviously closely akin biologically to the megaloblastic group.

Table III shows the reliability of the various diagnostic methods based on the need for folic acid. The total number of cases requiring folic acid was
Group 2.—Normoblasts were the only nucleated red cells found in the ordinary blood film and buffy coat from 148 cases of simple iron deficiency or post-haemorrhagic anaemia, the majority of which were of mild degree. Similarly, in over 100 normal pregnant women only an occasional normoblast was seen in the buffy coat.

Discussion

It is clear from Table I that in the diagnosis of megaloblastic anaemia the buffy coat method is very much more sensitive than the ordinary blood film, though not quite so reliable as marrow biopsy. The practical value of the test is emphasized by the fact that all the cases in which the marrow showed a fully-developed megaloblastic picture had diagnostic cells in the buffy coat, while in all cases with a haemoglobin of less than 8.5 g. per 100 ml. the buffy coat gave a true reflection of the marrow. Even among the less severe cases, there were two with haemoglobin of more than 10 g. per 100 ml. in which a few transitional megaloblasts were found in the buffy coat.

In Table III also it can be seen that from the point of view of assessing on morphological grounds whether a patient requires folic acid the buffy coat is much more reliable than the ordinary blood film and only a little less than marrow biopsy. Thus the buffy coat was diagnostic in 24 out of 30 cases in which the marrow was diagnostic. In only one case in which the marrow was diagnostic did the buffy coat fail to raise any suspicion of folic acid deficiency. The patient had a urinary infection, and it is tempting to suggest that this complication may have prevented release of abnormal cells from the marrow, just as the reticulocytes failed to escape from the marrow in the case of pneumonia described above (page 249).

From these facts and figures it is obvious that the microscopical examination of the buffy coat is a useful aid to the diagnosis of pernicious anaemia of pregnancy. It is fully diagnostic for severe cases of folic acid deficiency, will detect most of the moderate cases, and provides suggestive clues in many of the mild cases. Its use should obviate the need for sternal puncture in nearly all cases of anaemia in pregnancy, even refractory cases. Although sternal puncture in skilled hands is a minor operative procedure and not greatly upsetting to the patient, it appears to be particularly troublesome to pregnant women, in whom it should be used only if the morphology of the buffy coat, the other haematological data, and the clinical findings have failed to establish the exact nature of the anaemia. It is surely good practice that the justice of accurate diagnosis should be tempered with the mercy of avoiding a sternal puncture.

Summary

Microscopic examination of the buffy coat, controlled by marrow biopsy, has been applied to the investigation of anaemia of pregnancy, and, by giving a remarkably true reflection of the type of erythropoiesis in the marrow, has enabled the diagnosis of megaloblastic anaemia to be made even when the rest of the peripheral blood picture was not characteristic. Although a very occasional mild case of pernicious anaemia of pregnancy may be missed by examination of the buffy coat, the use of this simple method as a screening test for cases of anaemia in pregnancy and the puerperium allows one to obtain a diagnosis more easily and with a welcome reduction in the discomfort of the patient.

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References

