Counts it is possible to calculate the number of each species present in the undiluted specimen.

In order to ensure that no organisms were being lost with this technique, endocervical swabs of five subjects were taken through a sterile Cusco's speculum at the same time as the aspirate. Each swab was broken into a bijou containing 2 ml of glucose broth and mixed vigorously by vortex mixer. The resultant suspension was diluted and plated out in an identical manner to the aspirate. After 48 h incubation the plates were examined and the isolates recovered by the two techniques were compared. In all five cases there was no qualitative difference in the organisms isolated. However, while the cervical flora obtained by aspiration can be accurately enumerated, that recovered from the swabs cannot since the volume of mucus on each swab is unknown.

Dithiothreitol is widely used in the liquefaction and homogenisation of sputum and has not been shown as inhibitory to the microbial flora of this secretion. Similarly, no antimicrobial activity was detectable following the treatment of cervical mucus. However, to exclude this possibility, a suspension of Neisseria gonorrhoeae, probably the most labile organism potentially isolated from the cervix, was prepared and diluted 1:1 with either dithiothreitol or glucose broth. The number of viable organisms in each suspension was estimated according to the method of Miles and Misra. No difference was noted. It is likely that since dithiothreitol does not inhibit the growth of the gonococcus it should not do so to any other more rigorous constituent of the cervical flora.

The method described is rapid, technically easy and precise. We recommend its use when quantitative assessment of the microbial flora of the cervix is required.

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References


Ring sideroblasts and myelodysplastic syndromes

In the May 1983 issue, Juneja et al1 conclude that ring sideroblasts are not a distinctive feature of sideroblastic anaemia but “also seem to occur frequently in refractory anaemia with excess of blasts.” Moreover, they state that their findings are in marked contrast to those reported recently by myself and colleagues,2 since we found that none of the cases of refractory anaemia with excess of blasts had more than 5% ring sideroblasts. I believe that the conclusions by Juneja et al1 are confusing. For example, faced with a patient having anaemia and 40% ring sideroblasts with 10% myeloblasts in the bone marrow, which diagnosis should one make? Refractory anaemia with excess of blasts and ring sideroblasts, or primary acquired sideroblastic anaemia? I will try to clarify this point.

In counting sideroblasts, it is of fundamental importance to distinguish between ferritin (or intermediate) sideroblasts and ring sideroblasts. High numbers of ferritin sideroblasts are usually found in patients with iron loading anaemias. Such patients have high transferrin saturation, increased proportion of diferric transferrin, and an iron supply to the erythroid marrow which is in excess of that required for haemoglobin synthesis. This excess iron is stored as ferritin in the cytoplasm of developing red cells, which therefore may contain two to 10 iron-staining particles.3 Many patients with myelodysplastic syndromes have iron overload and increased numbers of ferritin sideroblasts. On the other hand, ring sideroblasts are found in patients with impaired porphyrin synthesis, in whom the mitochondria become entrusted with iron. Precise criteria for the distinction between ferritin sideroblasts and ring sideroblasts have been defined by Hillman and Finch.4 The term sideroblastic anaemia should be reserved for anaemia with a significant number of ring sideroblasts in the bone marrow,5 usually over 30%. Nevertheless, some haematologists call sideroblastic anaemia cases of refractory anaemia with ferritin sideroblasts. Apparently, Juneja et al1 and we2 adopted the same criteria for defining ring sideroblasts. Thus, the discrepancy between the two studies cannot be explained by different criteria in counting ring sideroblasts. Such discrepancy is due to different criteria in defining myelodysplastic syndromes.

In the Department of Internal Medicine, University of Pavia, Pavia, Italy, we have studied more than 70 cases of myelodysplastic syndromes. In 20 such patients we found more than 30% ring sideroblasts, and therefore we made a diagnosis of primary acquired sideroblastic anaemia. Three of these patients had also an excess of blasts in the bone marrow, and two of them eventually developed acute leukaemia. Juneja et al1 would have called these cases refractory anaemia with excess blasts and ring sideroblasts.

In the other patients studied in Pavia, ring sideroblasts were almost absent. Due to difficulties in distinguishing between ferritin sideroblasts and ring sideroblasts in subjects with marked iron overload, in some cases 1 to 4% ring sideroblasts were recorded. Since these subjects had increased numbers of ferritin sideroblasts, I believe that also those recorded as ring forms were indeed of the ferritin type. Anyhow, all patients with refractory anaemia with excess of blasts had less than 5% ring sideroblasts. It should be considered that Dreyfus,4 revising 29 cases of the disorder he defined, found that morphological abnormalities in erythroblasts were not conspicuous, and ring sideroblasts could only sometimes be seen.

The recent effort by the FAB Group6 to formulate new diagnostic criteria for the myelodysplastic syndromes should be useful to remove at least part of the confusion presently existing in this field. I suggest that, as a further refinement, the category of primary acquired sideroblastic anaemia should be divided into two subcategories: sideroblastic anaemia with only erythroid abnormalities, and sideroblastic anaemia with abnormalities in other cell lines.

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Dr Juneja and colleagues reply as follows:

We are surprised that Dr Cazzola should have found the conclusions confusing, despite our attempt to be as objective as possible. As mentioned, the cases in our study were classified according to the FAB proposals. Accordingly, any case of a myelodysplastic syndrome with \( \geq 5\% \) blasts in the marrow would be classified as refractory anaemia with excess of blasts. It is also mentioned in the FAB proposals that ringed sideroblasts may be seen in refractory anaemia with excess of blasts and no limit on their percentage has been laid down unlike that for acquired idiopathic sideroblastic anaemia. Moreover, it has been shown that the percentage of marrow blasts and not the ringed sideroblasts is the more important factor that correlates with prognosis in the myelodysplastic syndrome. It is, therefore, important that the cases of myelodysplastic syndrome with excess \( \geq 5\% \) marrow blasts irrespective of the percentage of ringed sideroblasts be classified separately and not included in the group acquired idiopathic sideroblastic anaemia, the cases of which according to the FAB proposals, have a normal \(<5\%\) marrow blast count. Likewise it may not be justified to classify a case of myelodysplastic syndrome with 20–30\% marrow blasts and 30\% ringed sideroblasts as acquired idiopathic sideroblastic anaemia. All these points are discussed in greater detail in our second article which will be published shortly.

We also wish to point out that Cazzola et al defined the cases of refractory anaemia with excess of blasts on the basis of the percentage of marrow blasts and promyelocytes (10–30\%), whereas we defined these on the basis of the percentage of blasts only, in the marrow (5–20\%) and in the peripheral blood (<5\%), as in the FAB proposals. Therefore, the diagnostic criteria in the two studies were not identical. Even if we take the criteria of the blasts and promyelocyte together for defining refractory anaemia with excess of blasts, as in the study by Cazzola et al, we still find eight cases which have \( >30\% \) ringed sideroblasts and 19 cases which have \( >5\% \) ringed sideroblasts.

Like Cazzola et al, we appreciate the difference between the intermediate (or ferri- tin) and the ringed sideroblasts and only cells meeting the criteria of five or more Prussian blue positive granules covering one-third or more of the circumference of the nucleus were included in our study.

We agree that the recent FAB proposals are a step forward, but we feel that they should not be modified until there is sufficient evidence to the contrary.

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Evidence for polymorphism of transferrin receptors in human skin

It was with considerable interest that we read the paper on transferrin receptors in human tissues by Dr Gatter and colleagues in the May 1983 issue. In light of the possible importance of these receptors we feel it is important to communicate that our findings in normal skin with the use of monoclonal anti-transferrin receptor sera and immunofluorescence methods are at variance with those of Gatter et al. They reported transferrin receptor-positive cells in the basal layer of the epidermis using four monoclonal antibodies to transferrin receptors studied with the aid of the immunoperoxidase technique.

Using cryostat sections of normal full-term human placentae as the control tissue for normal human skin, we have carried out experiments which fulfil the necessary criteria for the demonstration of transferrin receptors. These are: (a) direct immunofluorescence with the use of tetramethyl rhodamine isothiocyanate conjugated goat anti-human transferrin; (b) direct immunofluorescence, as above, following preincubation with 50 \( \mu\)l of 2.5 mg/ml transferrin in phosphate-buffered saline (PBS); and (c) indirect immunofluorescence by using the immunoglobulin (Ig) fraction of rabbit antihuman transferrin and fluorescein conjugated sheep antirabbit IgG. These reactions were consistently negative in normal skin.

The monoclonal antibody OKT9 has been well characterised as recognising transferrin receptor. In none of our experiments did this antibody react with cryostat sections of skin when used with fluorescein isothiocyanate conjugated goat anti-mouse IgG. However linear positivity was found uniformly on the syncytiotrophoblastic plasma membranes of chorionic villi (Figure).

It is apparent that several monoclonal antibodies which recognise transferrin receptors differ in their patterns of reactivity. For example 83/25 reacts with T, B and non-T, non-B cell lines and OKT9 preferentially recognises acute lymphoblastic leukemia T cells. In addition it has been shown that L5-1 primarily reacts with erythroid precursor cells. It is possible that the transferrin receptor is a family of structurally related but antigenically and functionally distinct molecules much as was suspected as a result of studies of breast adenocarcinoma cells. This is borne out by the present observations which show that monoclonal OKT-9 antibody does not recognise basal cells in human skin, but the monoclonal antibodies to transferrin receptors used by Dr Gatter and colleagues gave a quite different result. Barring some type of unforeseen technical artefact, we interpret these results as being another manifes-
Ring sideroblasts and myelodysplastic syndromes.

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