Most of the material sediments directly on to the grid, and large clumps of virus are usually easily seen in the first grid square. Almeida and Waterson[4] stated that polio antigen-antibody aggregates require approximately 1 hour of spinning at 15 000 rpm. However, in our study, a large number of virus aggregates were seen after a spinning time of only 5 minutes at 2100 *g* in a clinical type bench centrifuge, but it is likely that spinning for a full half-hour would be necessary to sediment small clumps of virus into the grids. These aggregates consisted of both empty and core particles, free of cell debris, showing typical electron microscopic antigen-antibody reaction.

A further feature of the technique described is that a quite low concentration of antibody may be used; around four times the neutralising titre is optimal. In addition the positive results obtained from diluted virus suspensions (Table 2) suggest that virus may be detected in cultures of low infective titre. It is important to avoid taking too much sample, otherwise an unacceptable quantity of debris, mostly from tissue culture cells, is deposited on the grid. It is likely that this method could be used satisfactorily for the typing of other viruses.

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A technique avoiding carcinogens for the demonstration of myeloperoxidase in blood and bone marrow smears

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For the demonstration of myeloperoxidase in leucocytes in haematological smears of blood or bone marrow a widely used current technique is a modification of that of Kaplow[1] where the substrate 3,3 di-amino-benzidine (DAB) is substituted for the original benzidine dihydrochloride. As the recognised carcinogenic properties of benzidine and its derivatives result in their production and distribution becoming increasingly limited, 2,7-fluorenediamine has been used as an alternative substrate[2,3] which, while apparently satisfactory in demonstrating the enzyme, is still not free of potential carcinogenicity.[4]

We have adapted a histochemical method which employs pre-mixed *p*-phenylenediamine and pyrocatechol[5] and which totally avoids the use of known or suspected carcinogens. This technique has been tried before without success,[3] but when modified as we suggest it seems to give perfectly satisfactory results.

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Material and methods

Peripheral blood films were obtained from healthy controls, and bone marrow smears were examined from patients with acute leukaemia at diagnosis and in remission. Slides were fixed for 1 minute in 10% formaldehyde in 95% ethanol and washed under running tap water for 2 minutes. They were then

rack-stained for 10 minutes with freshly prepared Hanker-Yates reagent (described by Hanker *et al.*[5] and available from Polysciences Inc, Paul Valley, Industrial Park, Warrington, PA, USA), 0·1% in 0·1 M Tris HCl buffer at pH 7·6, hydrogen peroxide being added to a final concentration of 0·0004% immediately before use. Films were then washed in tap water and counterstained for 40 seconds with Giemsa solution diluted 1:10 in buffered distilled water pH 6·8. The substrate was stored undissolved at 4°C between applications.

Smears so stained were compared for the proportion of peroxidase positive cells with duplicate samples stained using DAB as a substrate in the established method of Kaplow,[1] the duplicate studies being applied to five normal blood films and to six bone marrows from patients with acute myeloid leukaemia.

Results

QUALITATIVE

Myeloperoxidase activity was demonstrated by brownish-black granules in the cytoplasm of cells of the myeloid series. Eosinophils were stained strongly and some monocytes showed a distinct, fine granulation. Lymphocytes and lymphoblasts were uniformly negative.

Staining was comparable with simultaneously stained smears where the DAB substrate was used and Auer rods were clearly demonstrated (see Figure). Results were also comparable on fresh or sequestrene samples, and on smears that had been frozen unfixed at —20°C for up to three years.

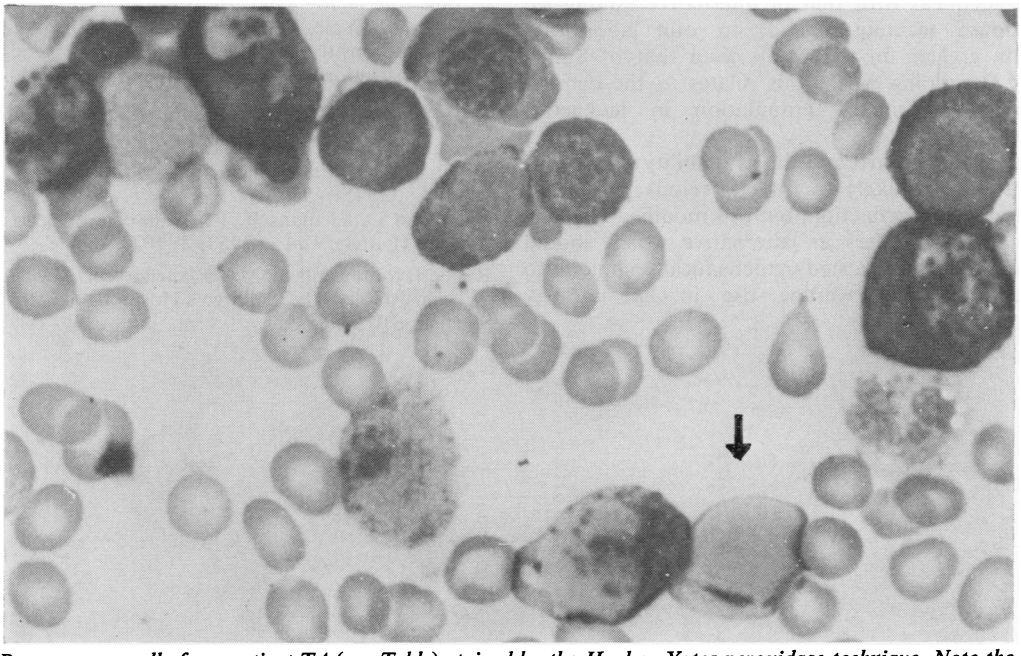
QUANTITATIVE

Using the Hanker-Yates and DAB substrates, five healthy peripheral blood films were compared for the proportion of peroxidase positive cells out of a 200-cell differential count. In no case did the proportion differ by more than 4·5%.

Six cases of acute myeloid leukaemia were also examined, and the mean proportion of peroxidase positive cells evaluated by two observers each counting 500 blast cells on slides stained by each technique are shown in the Table. From this it will be seen that the technique described is no less sensitive than that using DAB as a substrate.

Discussion

As benzidine is a proven carcinogen, a number of myeloperoxidase methods have been published which offer alternative substrates.[1-3 7] Unfortunately, all of these alternatives are also potentially carcinogenic, albeit perhaps less so, but the method we describe



Bone marrow cells from patient TA (see Table) stained by the Hanker-Yates peroxidase technique. Note the prominent Auer rods (arrowed). Original magnification × 1400.

Comparison of primary granule stains in different acute myeloid leukaemias

Patient	Type of leukaemia*	% Blasts positive		
		Sudan Black B	DAB peroxidase	Hanker-Yates peroxidase
TA	M6	56	59	67
RJ	M1	4	5	8
RM	M3	99	99	100
RT	M3	100	99	99
CW	M2	44	47	54
BF	M1	7	7	8

*FAB classification[6]

uses reagents considered totally free of hazard in this respect. The technique employs the oxidative coupling reaction of *p*-phenylenediamine in the presence of pyrocatechol to form an insoluble copolymer, and while it was developed for use in histological sections to demonstrate horseradish peroxidase,[5] we believe that it can satisfactorily demonstrate myeloperoxidase in blood and bone marrow smears, despite previously being considered of no value for this purpose.[3]

We found that it was important to pay attention to the concentration of substrate and incubation time, both of which are less than those found necessary in histological preparations. Also the amount of hydrogen peroxide we used is less than that recommended in the original method.[5] With such modifications the sensitivity achieved appears to be identical with that of alternative established peroxidase techniques and, in our laboratory, slightly greater on the whole than that of Sudan Black B staining where this relates to the demonstration of primary granulation in leukaemic myeloblasts.

The health hazard and the availability of benzene derivatives are likely to force previous peroxidase substrates into extinction, but this modified Hanker-Yates method offers an alternative which should allow a tried and trusted cytochemical technique to be continued in routine use in haematology laboratories.

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