Discussion

Cell block techniques employed in parallel or instead of smear preparations are used in many laboratories to examine cytologic material and to bridge the gap between cytology and histology.⁸ The advantages of this approach, either when agar^{4,6} or when plasma-thrombin^{2,3} is used for entrapping cells are that, without loss of the fine cytologic characteristics, the histological arrangement of cell clusters can be appreciated. Additional advantages include lack of overlapping or obscuring by blood or other debris which makes reading of smears difficult.

The celloidin film, which forms a semipermeable membrane around the sediment, allows dehydration and embedding in paraffin but not the escape of cells nor, even more dangerous for the diagnosis, the entry of "floating" cells. We found the "cell-bag" technique faster and easier with definite advantages over the agar or plasma-thrombin methods. In addition, there was no dispersion of the sediment and the cells remained grouped. This technique was applied with success on cytologic material from various sources and may be applicable in other fields such as endometrium.

A definite diagnosis with histological typing was reached in 74% of carcinoma of the lung. False-negative diagnoses (26%) were related to extension of necrosis and to lack of viable cells. No false-positive cases were observed. These results compare favourably with those obtained by other procedures.⁸ The number of the present cases is, however, too small to allow a definite evaluation of the relative advantages of the "cell-bag" technique over the conventional smear procedure, and is a matter for future studies.

The results obtained on cytologic material from various sources indicate that embedding of the sediment in a celloidin film is a simple and reliable procedure, and can be helpful in the investigation of cell clusters and tissue fragments.

The skilful technical assistance of Domenico Romeo and Ermanno Occhiali is gratefully acknowledged. This work was partly supported by CNR (Rome) grant No 80 01493 96.

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An improved method for sequential light and scanning electron microscopy of the same cell using localising microcoverslips

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Over the last few years, scanning electron microscopy (SEM) has proved a promising tool for cytological research with possible future uses in service diagnosis.¹⁻³ A fundamental limitation lies in a lack of experience with the SEM images of various normal and abnormal cells. It follows that a prerequisite for the effective use of SEM is the development of effective techniques for correlative studies of the same cell by both light and SEM. Although many technical proposals have been advanced to allow the sequential examination of individual cells,³⁻⁵ the procedures have certain limitations. This paper presents a modification of an inexpensive method,⁶ using localising microcoverslips. This method minimises the inconvenience of correlative cytological work and ensures the precise identification of individual cells by both light and SEM.

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Accepted for publication 19 October 1981

Material and methods

SPECIAL PREPARATION

Preparation of localising microcoverslips

Copper finder grids (3 mm diam) (Maxtaform H2, 200 mesh) are fixed, shiny surface down, to round 6 mm diameter grease-free microcoverslips, using a minute amount of epoxy resin final monomer (EMIX). Two methods of attachment can be employed. For *complete* grid-glass attachment, the resin is spread on the coverslip using a small camel hair brush. The amount of resin needed is exceedingly small and is spread into the thinnest possible film on the glass surface. The finder grid is then placed in the centre of the resin-coated area and pressed gently against the glass with the tip of a clean wooden lab stick. For *partial* grid-glass attachment, the grid is placed on the microcoverslips with part of its edge in contact with a tiny resin droplet. Most of the grid thus lies flat on the glass, but is not bonded to it with resin. With the tip of a clean wooden lab stick, the area of the grid edge in contact with the resin is pressed gently against the glass to ensure firm adhesion. The microcoverslips with bonded grids are then placed in an oven at 60°C overnight for polymerisation and are subsequently stored in a dust-free environment. Examination by light microscopy allows any grid with excessive resin deposits to be discarded.

Preparation of carrier slides

A specially prepared slide was introduced by a modification of the method of Domagala *et al.*⁶ The carrier slide is designed to support the localising microcoverslip during the examination of the wet preparation by light microscopy. A square shaped shallow trough is formed in the middle of a 3" × 1" (7.6 cm × 2.5 cm) standard glass slide by applying streaks of clear nail polish to form an elevated enclosure with walls of about 1 mm in height. The nail polish walls of this enclosure are designed to support the edges of a standard square coverslip.

Preparation of grid map

A simple sketch of the finder grid is drawn on graph paper, with letters and numbers fixed in their appropriate sites (Fig. 1). Photocopies of this map serve as a record of cell localisation for each specimen examined.

SPECIMEN PREPARATION FOR LIGHT MICROSCOPY

Fixation

Cells from body cavity fluids submitted for diagnos-

tic purposes are fixed in suspension using 2% glutaraldehyde in 0.1 M phosphate buffer with 0.1 M sucrose (pH 7.4, 300 mosmol), at room temperature for one hour and are then stored at 4°C until required.

Use of localising microcoverslips

A gridded microcoverslip is fixed, grid uppermost, to the middle of the trough formed on the carrier slide using a small amount of nail polish. A drop of poly-L-lysine solution^{7,8} is placed over the area of the grid, left for one hour and then rinsed with distilled water and air dried. A drop of the fixed cell suspension in buffer, concentrated as necessary by centrifugation, is placed over the grid. The preparation is then maintained in a moist chamber for one hour, rinsed gently with buffer, stained with diluted Giemsa stain⁵ and rinsed again with buffer. Drying must be carefully avoided. The stained preparation should be examined and photographed within 15 min, since the stain fades rapidly.

Light microscopy

The shallow trough in the carrier slide, containing the localising microcoverslip with wet, Giemsa-stained cells, is filled with buffer and covered with a square glass coverslip. The preparation is then ready for examination by light microscopy. Cells of interest are identified in various locations in the grid mesh and photographed at low and high magnification to record their location on the grid and their light microscope morphology (Figs 2 and 3). The locations of cells of interest are marked by dots or small circles on the grid map.

SPECIMEN PREPARATION FOR SEM

After light microscopy, the localising microcoverslip is detached from the carrier slide and postfixed with 1% osmium tetroxide, in the same buffer. The coverslip is then dehydrated in a series of alcohols and dried by the critical point method, using the Polaron E3000 CPD apparatus. Great care must be taken to keep the cell preparation wet throughout the intermediate stages of this procedure. The microcoverslip is subsequently mounted with conductive silver paint, grid side uppermost, on an aluminium stub and coated with gold using the Polaron E5100 series 2 cool sputter coater. The cell preparations used in these studies were examined on the Cambridge Scientific Instrument Company S600 scanning electron microscope, and on the ISI 3A scanning electron microscope.

Results

The relocation by SEM of cells identified and

Fig.1

Fig.2

Fig.3

Fig.4

Fig.5

Fig. 1 A finder grid photographed under the light microscope (LM).

Fig. 2 LM. Low magnification micrograph to show the location of the square in which the cells of interest are found. A group of tumour cells is located in the square below and to the right of the number nine.

Fig. 3 LM. High magnification micrograph to show the group of cells identified in Fig. 2.

Fig. 4 SEM. Low magnification micrograph to show the location of the cells of interest in the grid. Compare with Fig. 2.

Fig. 5 SEM. Cell cluster at higher magnification, to show the cellular surface detail. Numerous microvilli characterise the tumour cells. Adjacent red cells and a lymphocyte can be identified. Compare with Fig. 3.

Technical methods

mapped using the light microscope was found to be simple, quick and accurate. The finder grid, shown without cells in Fig. 1, provided immediate and precise cell localisation. With the light microscope, a cluster of malignant cells surrounded by numerous lymphocytes, red cells and histiocytes can be identified in Fig. 2 in the square immediately below and to the right of the number 9. At higher magnification (Fig. 3) details of the cell cluster can be distinguished, although the wet preparation imposes certain limitations. With SEM, the same square is easily located (Fig. 4) showing the same cells at low magnification in relation to the landmarks of the finder grid. Higher magnification (Fig. 5) shows cellular details such as surface microvilli on the tumour cells. Different cell types, such as tumour cells, red cells and lymphocytes can be confidently identified and related to their light microscopic counterparts in Fig. 3. Cellular morphology is well preserved and artefacts are not a problem. Charging effects are not encountered. Using this technique, we have processed over 100 cytological specimens with satisfactory results. In no case has the grid become detached from the coverslip during any of the stages of processing or examination. The numbers of available cells retained within the area of the grid have always been sufficient for extensive morphological study.

Discussion

Most of the currently used correlative procedures for sequential light and scanning microscopy depend either on the use of certain asymmetric marks^{5 9 10} and circles, etched on glass or other substrates,^{3 6 11} or on the use of graticuled glass^{4 12-14} for the relocation by SEM of preidentified cells. For various reasons, none of these methods is fully acceptable in practice as a convenient correlative technique. In the first category, the marked area is inconveniently large, even when subdivided into smaller units.⁶ These methods, therefore, are time-consuming and imprecise, especially with highly cellular specimens. In the second category, the graticuled area is even larger than in the first, increasing the problems of localisation. Moreover, this type of glass is not readily available from laboratory suppliers. It can be made in the laboratory, but its manufacture calls for highly sophisticated equipment and for special skill not routinely available in many hospital departments.

The principal advantages of the present method are its simplicity and its economy. Finder grids in various sizes and patterns are readily available from laboratory suppliers. The compact surface area provided by the finder grid and the subdivision

of this area into uniquely identified microsquares makes the task of relocation of the preidentified cell by SEM remarkably easy and reliable. No special skills or apparatus are needed. The carrier slide is easy to prepare and can be reused. The cell yield is high compared with some procedures, perhaps because only a minimum of specimen manipulation is needed and perhaps because the relatively prominent partitions formed by the grid bars offer a certain degree of protection to the cells lying between.

A simple procedure used by Shay and Walker 1980,¹⁵ has some of the merits of our method, but has serious drawbacks for correlative studies. This procedure involves making a negative print of a finder grid on a glass microcoverslip, by metal evaporation in a sputter coater. The finder grid is then removed and the "printed" coverslip is used for cell localisation.

When we compared this method with the attached-grid procedure described above, various difficulties were encountered. In particular, the gold coating used to outline the grid pattern interfered significantly with the recording of the light microscopic appearances of the cells. When a thinner coating layer was used to improve visibility, the grid pattern could barely be seen by SEM, making cell localisation difficult. Moreover, if the cell preparation was not metal-coated after light microscopy, serious charging effects were encountered on SEM, but after adequate coating the grid pattern was again obscured to a degree that made cell localisation difficult. Backscattered electron imaging might help to overcome the problems described above.

For routine practical purposes, the attached finder grid method which we propose at present offers to the cytologist a reliable and inexpensive means for the correlative light microscopic and SEM study of individually identified cells.

The work of Dr KM Saleh was supported by a grant from the University of Basrah, Iraq.

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Letters to the Editor

Decontamination of automated laboratory equipment

In the Howie Code of Practice (1978)¹ paragraph 25(a) lays down procedures for the routine decontamination of the parts of automated equipment used in chemical pathology laboratories that are in direct contact with samples. These procedures, involving the washing through of liquid lines with either water or the manufacturer's wash fluid are considered adequate for dealing with infectious agents in normal routine work. When dialyser membranes have to be changed the system should be washed with a solution of strong hypochlorite followed by a water wash.

Recently we have been asked to advise on a suitable procedure for dealing with microbial contamination that might occur in a laboratory housing large expensive automated equipment such as that found in many chemical pathology laboratories. Although this is an unlikely event, it might, depending on the nature of the micro-organism, or the level of contamination, call for the decontamination of the exterior of the equipment as well as decontamination of the liquid lines.

Liquid lines can be dealt with by the use of a suitable disinfectant such as strong hypochlorite. The exterior of the equipment can be decontaminated by sealing the laboratory and then decontaminating the room and its contents with formaldehyde. This is a well established procedure for space decontamination.² More limited decontamination could be carried out by *ad hoc* arrangements such as enclosing the equipment within a polythene film tent fabricated on site using polythene sheeting and adhesive tape and generating formaldehyde within the tent.

The point of interest here is whether exposure to formaldehyde would have any detrimental effect on components of the equipment such as electrodes, electric circuits etc necessitating expensive refurbishing after decontamination. Experience in the Microbiological Safety Reference Laboratory in which many pieces of equipment such as an automatic image analyser, cameras, microscopes and electronic control boxes have been repeatedly exposed to formaldehyde have not shown any detrimental effects from this treatment. An example of an item of equipment that might be considered

vulnerable to treatment with formaldehyde, a cartridge, including dialyser assembly, from a Technicon SMAC (Sequential Multiple Analyzer with Computer) has been subjected to tests.

The SMAC cartridge was placed inside a sealed chamber (16 cubic feet) and 10 ml of formalin solution (40% wt/vol formaldehyde) plus 10 ml of water vaporised by heat. The chamber was opened after about 15 h and aired for 3 h to remove residual formaldehyde. This procedure was carried out on four occasions. On the first occasion, two flat surfaces of the cartridge were heavily contaminated with about 2×10^7 spores of *Bacillus globigii* suspended in either water or neat horse serum. These inocula were allowed to dry before exposure to the formaldehyde. The surfaces were sampled after one cycle of decontamination. After fumigation no viable spores were recovered from either of the deliberately contaminated surfaces demonstrating that the exposure to formaldehyde was effective for sterilising surfaces heavily contaminated with a resistant micro-organism.

Visual inspection of the SMAC cartridge after four cycles of decontamination showed no obvious changes, discolora-