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A celloidin bag for the histological preparation of cytologic material

G BUSSOLATI *Institute of Pathological Anatomy and Histology, University of Turin, Italy*

Cytologic material may contain cell clusters and tissue debris, which are often missed on smears. The processing in parallel of both cytologic smears and sediment when available has been recommended,¹⁻⁵ for tissue fragments present in the latter increase diagnostic precision. Paraffin embedding of the sediment for histological examination presents obvious technical difficulties and it has been proposed that entrapping cytologic material in agar⁴ or in plasma-thrombin^{2,3} makes the sediment compact and easier to handle.

We have devised a technique in which the sediment is folded in a celloidin film which, being permeable but insoluble in water, alcohol, and xylene, keeps the sediment compact during the process of paraffin-embedding. This method bears some resemblance to that proposed in 1936 by Wihman⁷ who employed a sausage-skin to collect and process the sediment of exudates and transudates. Such a procedure, however, has been neglected in recent times.

Material and methods

Celloidin (Chroma Ges, Stuttgart) flocks were soaked in absolute alcohol and then dissolved in ether to obtain a 10% solution in absolute alcohol-ether (1:1) which was kept in a well stoppered jar at room temperature. Neocolloidine, commercially available in solution ("Neocolidine", BDH) gave less satisfactory results being more soluble in alcohol.

The cytologic material (serous fluids; washings, brushings and needle aspirates arriving at the laboratory suspended in saline) were quickly centrifuged and routine smears prepared. The sediment was then suspended in fixative (usually 95% alcohol or 10% formalin) for histological processing.

Celloidin bags ("cell-bags") were prepared (Fig. 1) by pouring the celloidin solution to the top of polypropylene plastic centrifuge tubes. The celloidin was then recovered and the tubes left upside down for a short time. A thin film of celloidin about 20-50 μ m thick remained over the inner surface of the tubes,

Fig. 1 Scheme of celloidin bag preparation for embedding of sediments. (a) Centrifuge tube is filled with celloidin solution. (b) Celloidin is recovered, but a thin film remains over the walls of the tube, which is then partly filled with chloroform. This hardens celloidin and is then discarded before the next step. (c) Cytologic material (dispersed in fixative) is poured in the tube. (d) After centrifugation (3000 rpm for 10 min) the clear supernatant is discarded. (e) The celloidin bag with the sediment at its bottom is extracted from the tube. (f) The "cell-bag" is processed for paraffin embedding by conventional histological means.

which were then partly filled with chloroform and stoppered. This hardens the celloidin and prevents excessive drying of the film. Immediately before use, the chloroform was discarded and the cytologic material suspended in fixative was poured inside the tubes, which were then stoppered.

After centrifugation, the clear supernatant was discarded. The celloidin bag, with the sediment at its bottom, was gently extracted with a finger or forceps. The "cell-bag" was then "closed over" the sediment by simple pressure and the excess of celloidin discarded. The "cell-bag" and its contents was passed quickly through a 1% alcoholic solution of eosin which made it easier to trace the sediment in the paraffin blocks. It was then processed routinely through alcohols, xylene and paraffin and embedded in paraffin. Histological sections were cut and routinely stained with haematoxylin and eosin, the Papanicolaou technique or other conventional staining procedures. The presence of celloidin did not affect either the cutting or the staining.

The present method has been in use in this laboratory for the last seven months: a total of 627 cases including pleural, peritoneal and pericardial fluids, bronchial washings and scrapings, needle aspirates from the lung, the breast, the lymph-nodes and bone

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marrow have been processed with the "cell-bag" technique.

After the initial experiments some specimens—for example, needle aspirates of the lung, were processed only with the "cell-bag" techniques since the diagnostic material arrived already fixed in 10% formalin and smears were not made.

Results

The celloidin bag facilitated collection of whole sediment, including isolated cells, and prevented loss of cytologic material during the process of dehydration and embedding. The celloidin was cut together with the sediment and stained faintly with eosin.

On histological sections the cells retained their diagnostic nuclear and cytoplasmic characteristics. In addition, neoplastic cells in serosal fluids (Fig. 2) often appeared grouped in nests and gland-like structures. In other cytologic material, especially in

Fig. 2 Pleural fluid from a breast cancer patient. The neoplastic cells are arranged in nests and gland-like structures. Haematoxylin and eosin $\times 100$.

needle aspirates (Figs. 3, 4), cell clusters or small tissue blocks were often observed allowing a histological diagnosis.

In 50 consecutive cases of needle aspirates of the lung where all the material was processed histologically and smears not made the value of this technique was assessed by comparing the diagnosis with that ascertained in subsequent surgical biopsies

Fig. 3 Bone marrow aspirate. Areas of haematopoietic bone marrow among blood cells. Haematoxylin and eosin $\times 25$.

Fig. 4 Needle aspirates in a case of lung carcinoma. Small tissue blocks allow a histological diagnosis. Haematoxylin and eosin $\times 100$.

or by clinical evidence of response to treatment (in 36 and 14 cases respectively). A diagnosis of neoplasia with tentative histological typing was reached in 31 (74%) of 42 cases of cancer. No false-positive cases were observed.

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.

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Requests for reprints to: Prof G Bussolati, Istituto di Anatomia e Istologia Patologica, Università di Torino, Via Santena 7, 10126 Turin, Italy.

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

KM SALEH,** PG TONER, KE CARR,* HE HUGHES† *University Department of Pathology, Glasgow Royal Infirmary, the *Department of Anatomy, Glasgow University and the †Department of Cytology, Glasgow Royal Infirmary Glasgow G4 0SF*

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.

**Present address: Department of Pathology, Basrah Medical College, Iraq.

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