Upsala J Med Sci 78: 153–159, 1973

Electron Microscopy Studies on Cytologic Material Acquired by Fine Needle Biopsy

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ABSTRACT

Tumour material, collected by fine-needle aspiration according to Franzén (4) has been fixed and embedded for study with the transmission electron microscope (TEM). Different kinds of tumours, mainly from the head and neck region as well as experimental animal material (liver and salivary gland tissue from guinea-pigs) have been studied. It is possible to get material of good quality by careful processing of the specimens and the paper contains a number of micrographs showing such specimens. There is every reason to regard the results presented as an indication that electron microscopy can be used for more detailed studies in connection with fine-needle biopsy. It should be tried especially in those cases where it is of interest to follow a tumour during radiotherapy or treatment with antibiotics or cytotoxic drugs.

INTRODUCTION

Fine needle biopsy has become an important aid in the diagnosis of tumour material in oncologic clinics (7). For more than 10 years the first author has carried out cytologic work of this kind, and fine needle biopsy according to Franzén has become a routine procedure at the University Hospital of Uppsala. A combination of different techniques is of great importance for a fast and reliable histo-pathologic diagnosis of tumours. In many countries, approaches using tissue culture, fluorescence techniques and ultraviolet absorption methods are being developed. Electron microscopy on operation and exfoliated material (3, 5) has also been used and much work has been carried out in these fields during recent years. This technique has been used in our group especially for a study of salivary gland tumours (2).

Electron microscopy of material acquired by fine needle biopsy seems, however, not to be used to any mentionable extent (1, 6). This is surprising as in this way it should be possible to collect material well suited for an ultrastructural analysis. By aspiration, a small group of cells can be well fixed and well suited for electron microscopy. In this paper we intend to show that it is possible to use aspirated material for a detailed study of the inner structure of tumour cells and that it is possible to get a high quality preparation. Our studies show that important information can be obtained with this technique. We have therefore made a systematic study of factors of importance for good conservation and preparation of such material. One portion of the study has been carried out on human tumour material and the other on material obtained from experimental animals.

Questions of major concern regarding a fine needle biopsy technique can be summarized thus:

1. Can tumour material acquired by fine needle biopsy be used for electron microscopic studies?
2. Can such studies contribute in a meaningful way to increased information of diagnostic or scientific value?
3. What factors are of major importance for the quality of the material to be studied?

The present paper will deal with some of the more important factors in relation to the questions listed above. It has the nature of a preliminary publication.

MATERIAL AND METHODS

Initially this study was started with a pilot series of head and neck tumour specimens collected from patients in the ENT-clinic at the University Hospital. At present this material consists of 40 cases of different kinds of tumours. Some of the specimens were collected by sternal puncture. In those cases a rather coarse needle was used.

Some factors which appeared to affect the quality of the final results were:

Needle diameter and length
Type of aspiration
Fixative used

Fixation time
Material handling
Ways to recognize important portions in embedded material.

In an attempt to elucidate the importance of these variables we have started a series of animal experiments. We have chosen aspirated material from the liver and the submandibular gland in guinea pigs for this study. The liver cells were chosen as they have a well established structure of rather uniform type and the submandibular gland because its cells correspond in structure to parts of our clinical material.

The material was collected by aspiration with a modified Franzén-syringe. The needles were of regular, disposable type. To evaluate the importance of the needle diameter we have systematically used needles with a diameter ranging from 0.6–1.2 mm. It was found that in most cases sufficient material could be collected using 0.6–0.7 mm thick needles. When the material was hard to acquire cells from, as was the case in the normal submandibular glands, a short 0.7 mm needle was necessary. In those cases where osmic acid was used as the only fixative a specially made platinum needle was used to prevent reaction with the needle material.

The cells were gently immersed into a siliconized glass tube (diameter 6 mm) containing the fixative in its lower half. The aspirated material consisted of single cells diffusely distributed or of groups of cells often in the shape of thin threads.

When the material had dropped into the fixative, some more of the fixative was added. We have used 2.5% glutaraldehyde in 0.4 M phosphate buffer with pH 7.2–7.4 and the fixation time was varied from 1 to 48 hours. The time used at present is 4 hours. The specimens were then washed in several changes of sucrose buffer and post-fixed in 1.5% osmic acid in veronal buffer for 1–4 hours. We have found 1.5 hours to give a good result.

After fixation the specimens were dehydrated and embedded in Epon 812. Sometimes the cells were stirred up during one of the different steps in the manipulation and in those cases the specimen was gently centrifugated.

To reduce the possibilities of contamination, and as it is often impossible to move the material from one jar to another, the whole fixation and embedding procedure of each specimen has been carried out in the same siliconized glass tube. In this way contamination is practically excluded, the material is little damaged and no material lost if carefully handled. The material aspirated often has the form of a minute thread and it can be well recognized if a glass tube is used. When the epon had polymerized and cooled, the glass tube was put into a vice with flat faces and crushed carefully. Usually the glass just snapped off the plastic core which contained the cells to be studied. In the case when the sample contained few blood-cells the tumour material was easily recognized and could be trimmed directly for ultra-thin sectioning. If there was some blood mixed with the sample, which should be avoided as much as possible, the specimen had to be sectioned and studied in phase contrast microscopy. After orientation in this way the trimming for TEM took place. On many occasions the blood-free samples were also sectioned for light microscopy and stained with 1% paraphenylenediamine in water for examination. The sections for TEM were stained in 20% uranylacetate in methanol and post-stained in lead citrate. A Siemens Elmiskop I A was used for all TEM.

RESULTS

It was found that in most cases sufficient material could be collected using 0.6–0.7 mm thick needles. When the material was hard, fibrotic and difficult to acquire cells from, a short 0.7 mm needle was needed. To reduce the possibilities of contamination and as it often was impossible to move the specimens, we found it important to use the same container for the whole process. We found a siliconized glass tube to work well. The best fixation was obtained by using 2.5% glutaraldehyde in phosphate buffer for 4 hours and post fixing in 1.5% osmic acid for 1.5 hours.

In Fig. 1, a bone marrow cell from a myeloma case has been prepared by the method described below. The micrograph shows that it is possible to obtain a good quality preparation and that it is possible to discern many of the intracellular components of the cell. The endoplasmic reticulum and the mitochondria are well preserved and the nucleus and nucleolus also. Figure 2 from the same case shows that it is possible to study many cells in close relation to each other. These micrographs are from our more recent preparation and they are the result of improvements reached from our experimental studies.

In Figures 3 (A–D) and 4 (A–D) several different tumour cells are presented. These were prepared according to the technique described. In Fig. 3 the material has been collected from two different tumours and it can be seen that it is possible to get good fixation not only in single cells but also in rather large groups of cells. It is also possible to obtain good information in large cell groups regarding nuclear structure, mitochondria, and different components of the endoplasmic reticulum as well as the interrelations between the cells.

In Fig. 4 A–D we have chosen four different portions from a malignant tumour of low grade differentiation from the left maxilla in a woman aged 73. The material was collected by direct puncture through the wall of the maxillary sinus in fossa canina. In this case single cells or a considerable number of cells in groups were found in the aspirated material. The material contained

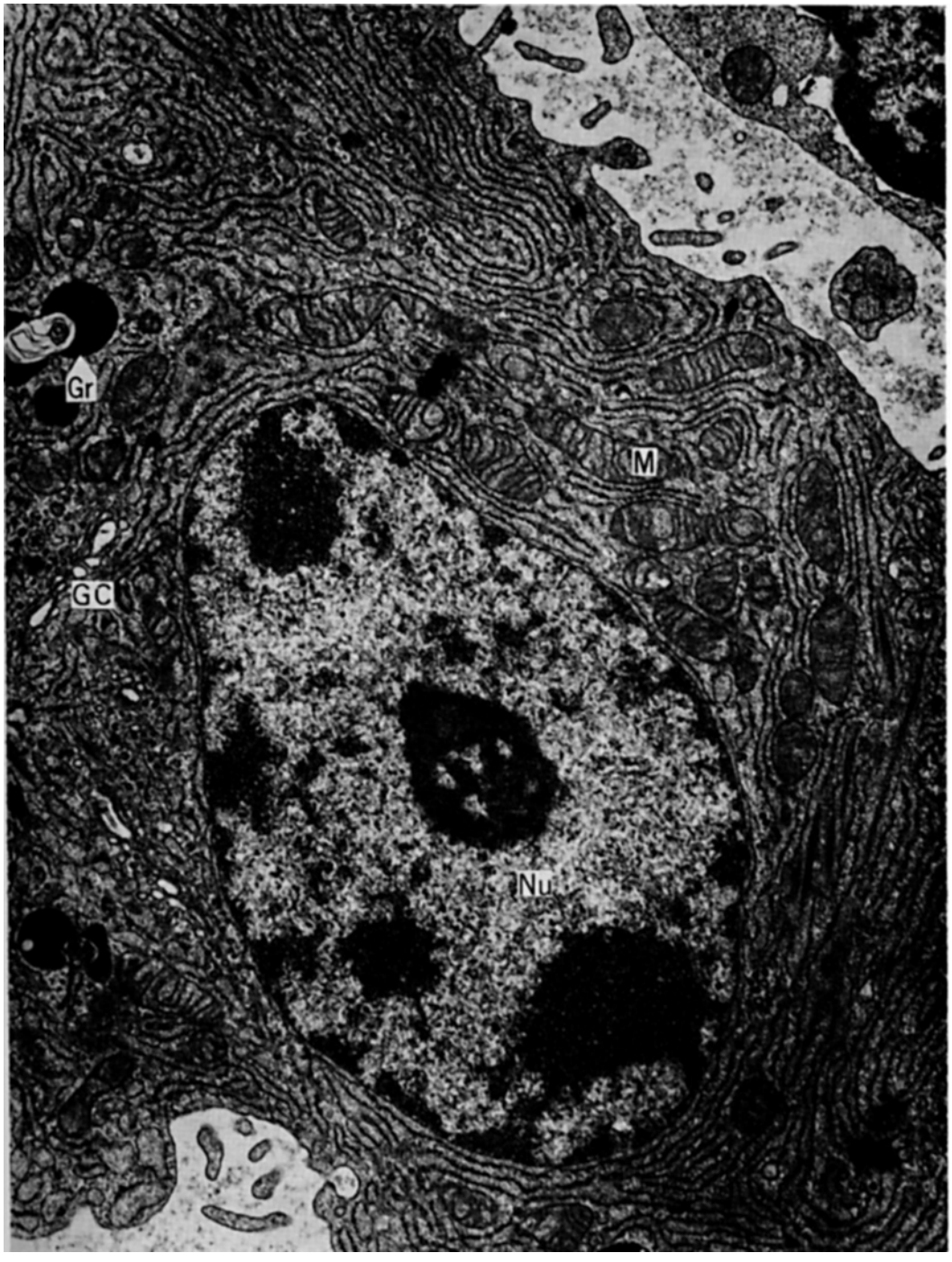

Fig. 1. Bone marrow cell from a myeloma case. *Gr* = granules, *M* = mitochondria, *N* = nucleus, *GC* = Golgi complex. × 20 000.

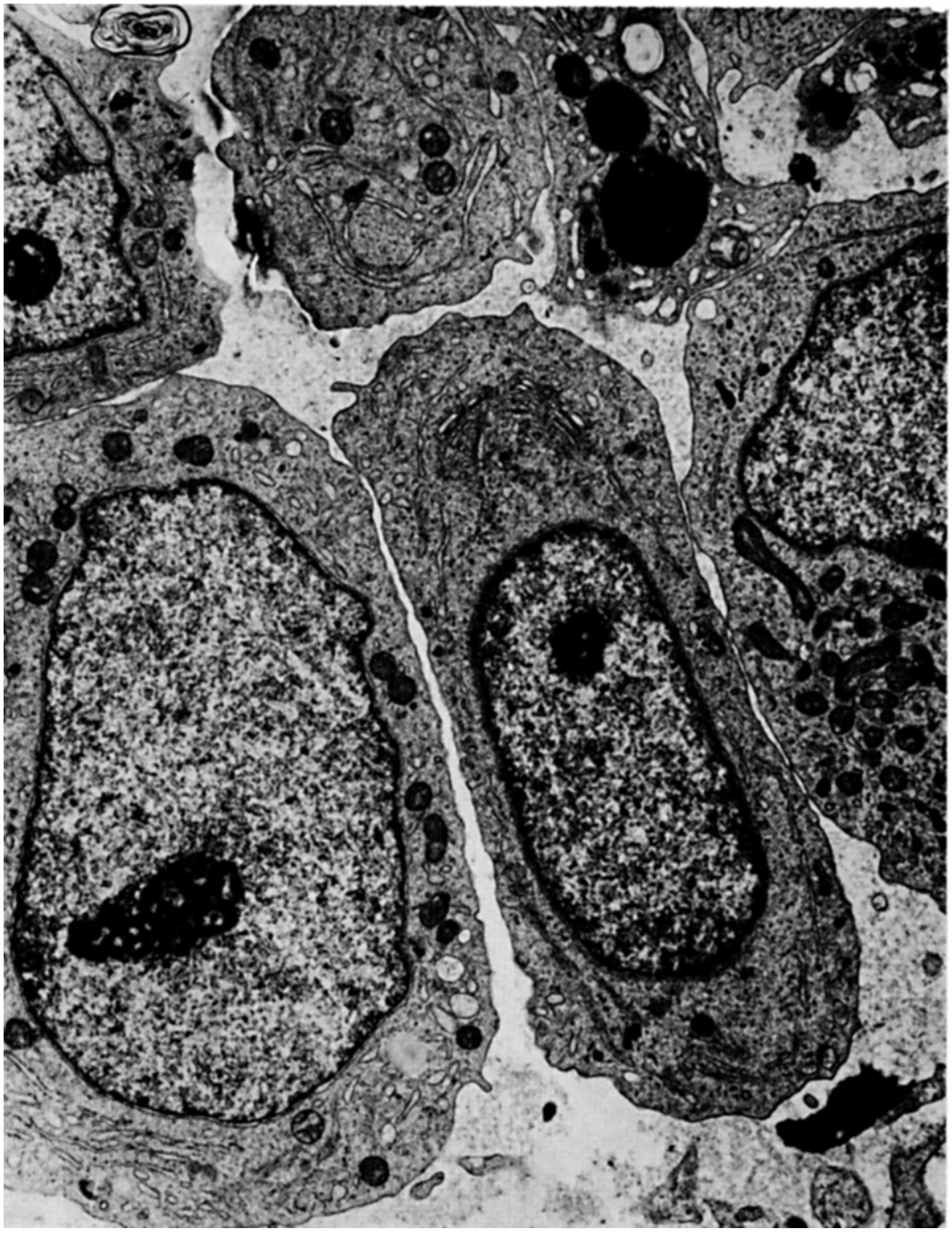

Fig. 2. Several cells from the same myeloma cases as in *Fig. 1.* × 12 000.

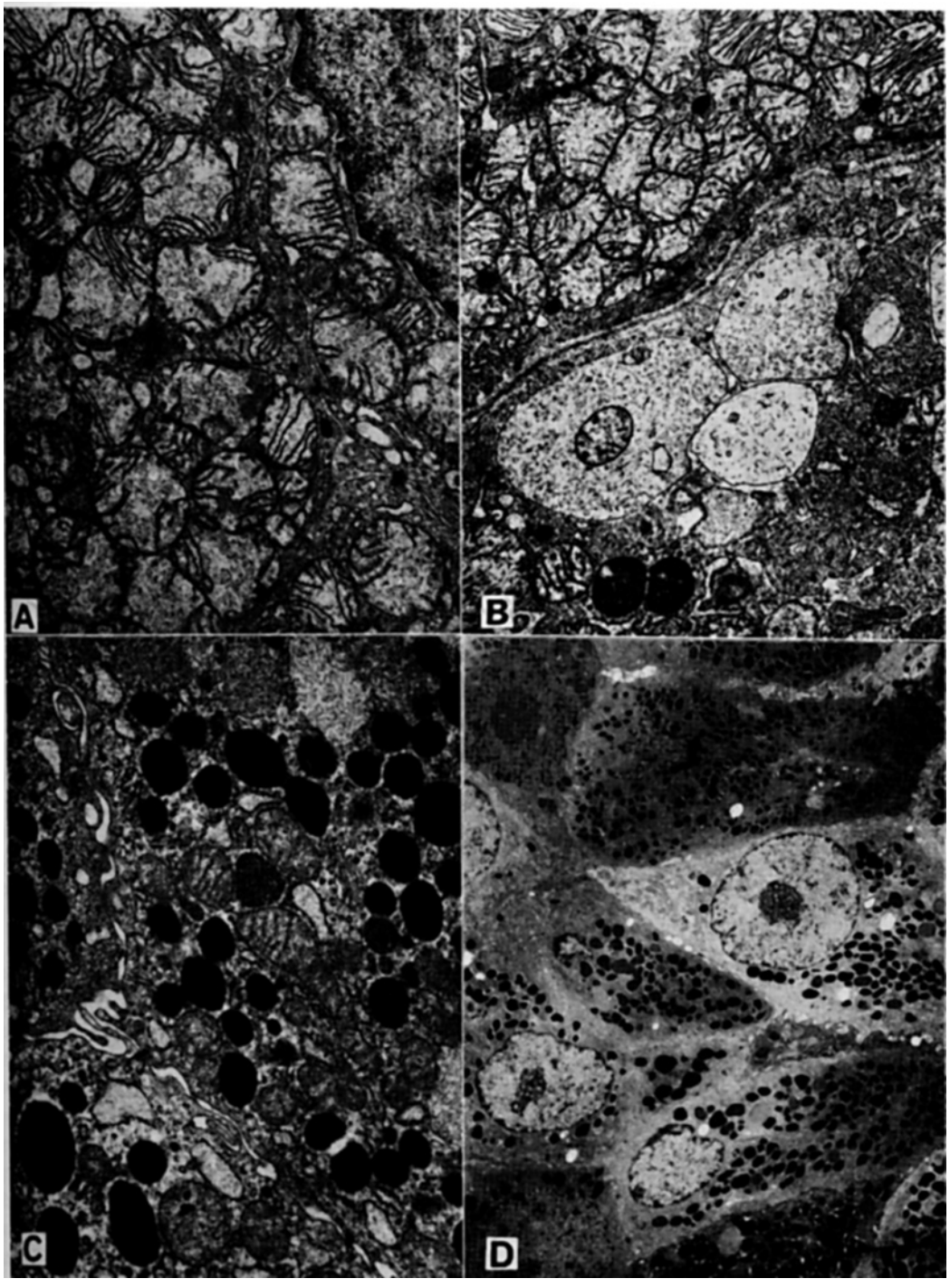

Fig. 3. (*A, B*) Adenolymphoma of the parotid. The cytoplasm is packed with mitochondria, a typical finding in oncocytes. (*C*) Acinic cell tumour of the parotid. A cluster of tumour cells, where the cytoplasm is filled with dark granules. The cell borders are irregular and nearby cells are joined by intercellular foldings. (*D*) Lower magnification from the same case as in (*C*). (*A*) × 25 000, (*B*) × 18 500, (*C*) × 16 500, (*D*) × 5 000.

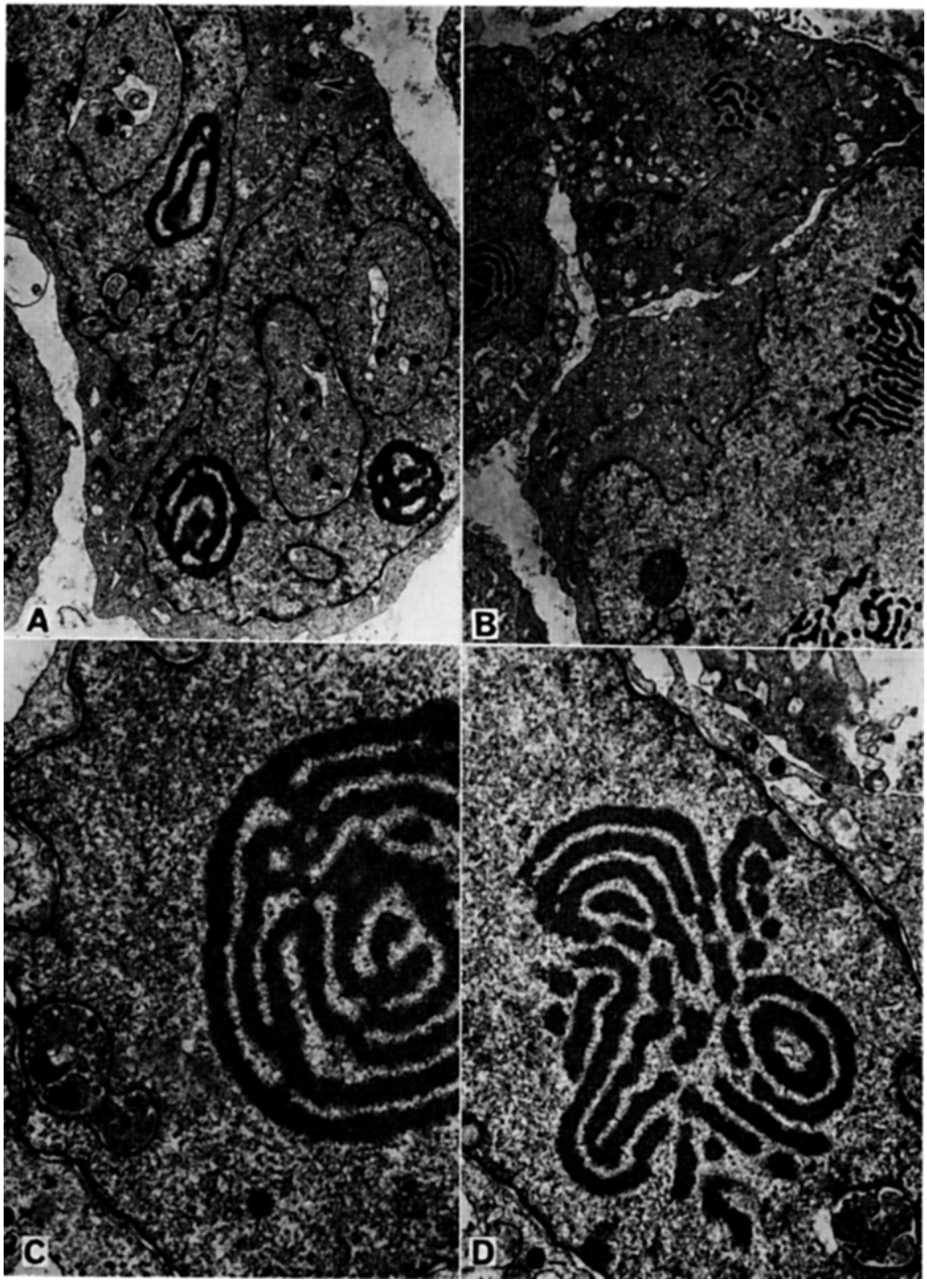

Fig. 4. (*A*) Malignant tumour of low grade differentiation from the maxilla. A tumour cell with two nuclei containing dense formations, evidently large nucleoli. The arrow indicates two centrioles. (*B*) Same tumour as (*A*), showing very irregular nucleus and nucleoli. (*C, D*) Higher magnifications from the same tumour as in (*A*) and (*B*). (*A*) × 11 600, (*B*) × 6 000, (*C*) × 22 000, (*D*) × 17 000.

several different forms of cells varying from cells with single nuclei to giant cells containing many nuclei. Most nuclei were very large and contained conspicuous dense formations not seen by conventional smear staining. These formations are evidently very large nucleoli (8). In many cells the cytoplasm contained dark granules (Fig. 4 A). Further, it was very common to see very irregular indentations in the nuclear membrane and in Fig. 4 C one such indentation contains a considerable number of virus-like particles.

DISCUSSION

Electron microscopy is becoming widely used in studies of biopsy material from human tumours. The present paper has shown that it is possible to collect material for TEM by fine-needle biopsy and to get good quality in such material.

It is shown that this technique widens the knowledge about the structure of the tumour cells. This is of interest in all tumours but especially in those cases where it is important to get repeated information regarding a special tumour during some form of treatment. The quality of our material has been considerably improved by animal experiments where different parameters such as needle diameter, fixative, specimen treatment have been studied. These studies are still continued and will be reported on later. Glutaraldehyde-osmium tetroxide fixation has been preferred in the present material, but we have also tried osmic acid fixation only.

There seems to be a considerable difference in the way in which different materials can be fixed by the technique used. Good results have been obtained from some specimens while others have shown a tendency of mitochondria to rupture as well as distortion of the endoplasmic reticulum. A well standardized technique is therefore very important if artefacts are to be distinguished from pathologic findings. Several factors of importance have been clarified through the animal experiments but systematic studies are necessary and are being carried out.

It seems from the above results that TEM can become an important addition in tumour diagnosis by fine-needle biopsy, and there is clear evidence that good fixation can be obtained with the technique used.

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Received February 20, 1973

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