FINE STRUCTURE OF MILLIPORE FILTERS

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Millipore filters and membranes are being used with increasing frequency in biological and chemical investigation. However, the structure of these filters, as well as some of the problems associated with histological techniques involved in their use, do not appear to be widely known. Early electron microscope studies (5) of Millipore filters were restricted to replicas of surface texture and to the measurement of average pore size. In a work on transfilter mesonephrogenic tubule induction, Grobstein and Dalton (2) suggested that morphological and dimensional changes might occur in the filters during specimen embedding and sectioning. Recently McCombs et al. (3) deliberately swelled or dissolved the filter in acetone to facilitate sample preparation.

In this note, we wish to comment on the fine structure of and some properties of the filters in order to facilitate the work of subsequent users of these materials. The observations reported here were made during a study of bone induction across Millipore filters, the details of which are described elsewhere (1). To summarize, filters composed of mixed esters of cellulose, bearing the manufacturer's designation HA, VC, or VF, with respectively, 0.45, 0.1, and 0.01 μ pores, formed the walls of diffusion chambers which enclosed fragments of tumor tissue (osteosarcoma). It was found that new bone was induced to grow along the outer surfaces of the chamber walls under certain in vivo conditions. This growth resulted in a layer of tumor tissue separated from a layer of induced bone by the filter. Thin sectioning and electron microscopy were then carried out in order to study the structures which grew into the filter pores. From the observations made, insight into the mechanism of bone induction was obtained.

Initial problems encountered in the preparation for Araldite embedding of the composite specimens were overcome by introducing minor modifications into the commonly employed embedding procedure. These were the substitution of isopropyl alcohol for ethanol in the dehydration step and the use of toluene in place of propylene oxide as the transitional solvent between isopropyl alcohol and the resin mixture. These steps were necessary since the usual procedure swelled or even dissolved the filters. Furthermore, methacrylate embedding proved less desirable than Araldite embedding.

The first specimens examined in the electron microscope proved to be confusing. These specimens were sections of tissue and filter and were confusing in that the filter matrix appeared electron-lucent, producing the illusion that cytoplasmic extensions were growing into the filter material rather than into the pores (Fig. 1). Therefore, it became necessary to examine the filter without tissue growth. Further samples were prepared to ascertain the effect of fixatives and embedding techniques on filter structure. Thus, some segments of filters were fixed in 6% buffered glutaraldehyde, postfixed in 1% buffered osmium tetroxide, and dehydrated in graded dilutions of isopropyl alcohol, while others received no treatment prior to Araldite embedding. Sectioning was carried out on a Reichert ultramicrotome fitted

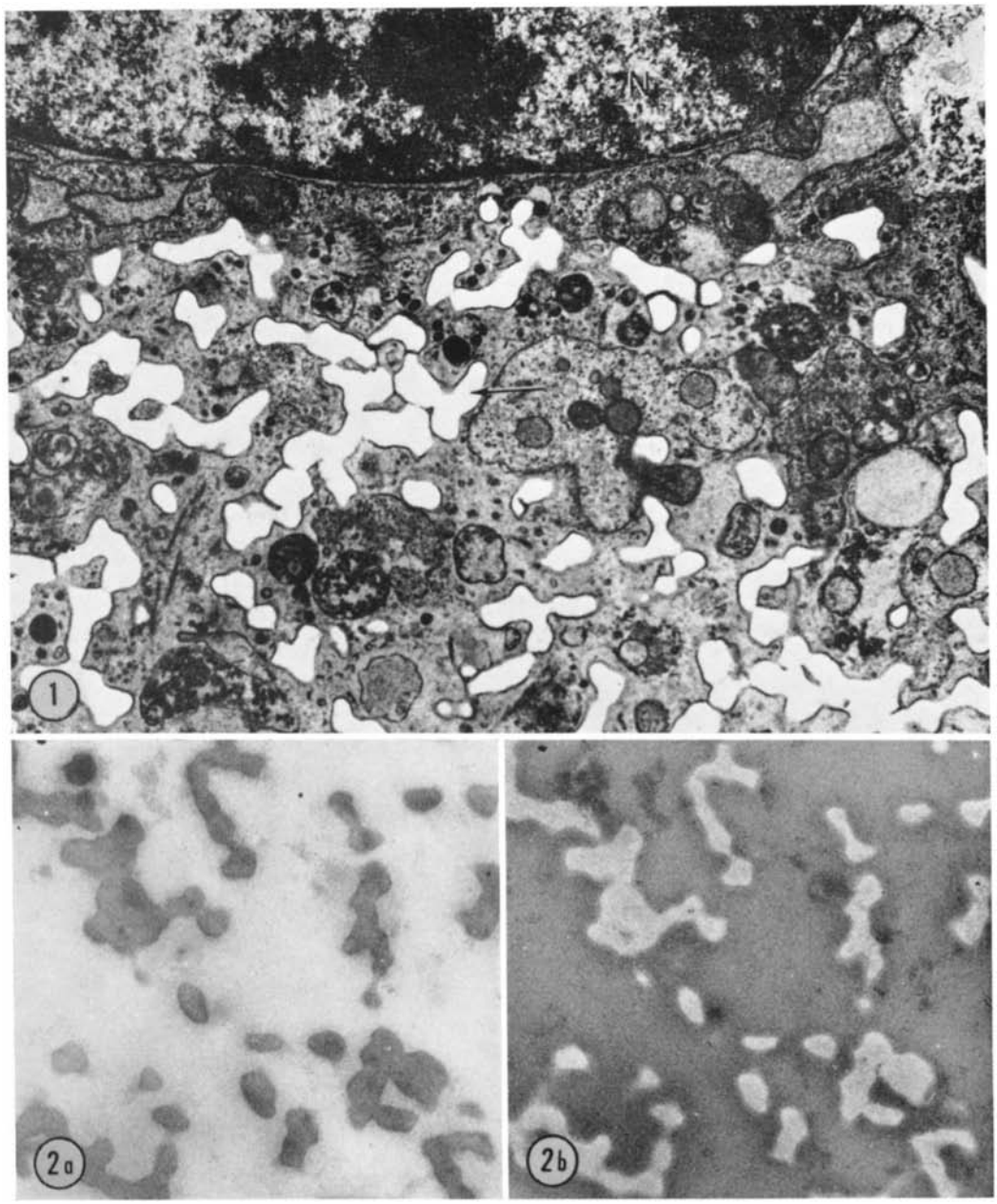

FIGURE 1 Tissue-filter junction showing nucleus (*N*) of an osteosarcoma cell above and cytoplasmic extension into filter pores below. While most of the structures are plasma membrane-bounded, a few free subcellular components are seen. Irregular, white areas (arrow) represent sites where filter matrix was present before its sublimation in the electron beam. Uranyl acetate, lead citrate staining. × 12,000.

FIGURE 2 *a* and *b* 2 *a*, Micrograph showing 0.45 μ Millipore filter embedded in Araldite and thin sectioned. Filter matrix is surrounded by embedding medium which fills the filter pores. 2 *b*, Same area as that seen in Fig. 2 *a*, 30 sec after exposure to the electron beam. Filter matrix has been sublimated, leaving electron-lucent holes in the embedding medium. Uranyl acetate and lead citrate straining. × 12,000.

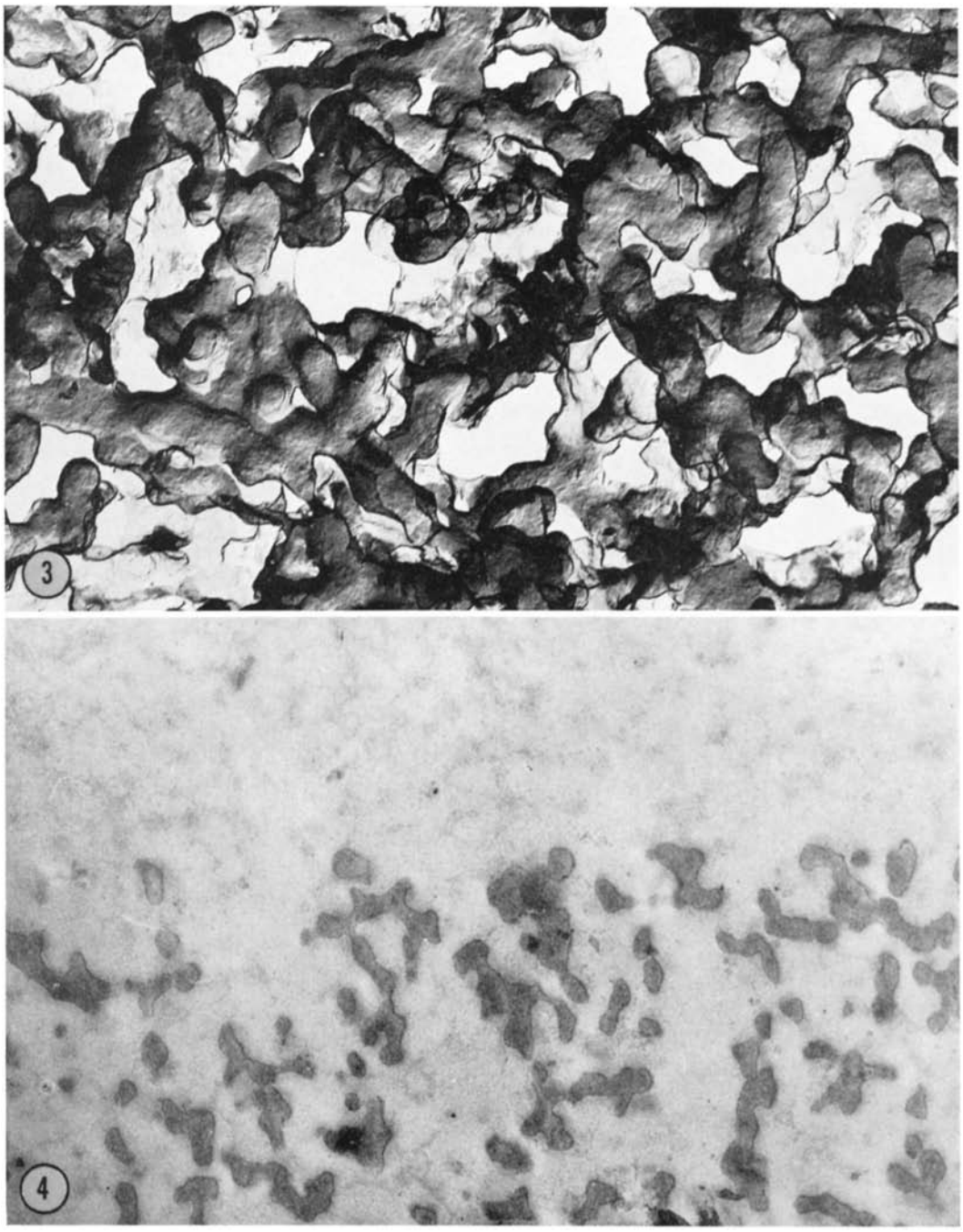

FIGURE 3 Carbon platinum–shadowed replica of 0.45 μ filter surface to show irregular size, shape, and distribution of filter pores (white areas). Shadowed at tan $^{-1}0.5$ from upper left hand corner. × 12,000.

FIGURE 4 Micrograph of thin-sectioned, Araldite-embedded, 0.45 μ filter showing the filter surface and cross-sectional view of the subsurface. Lead citrate strain. × 12,000.

with a diamond knife. In addition to thin sections of filters, carbon-platinum shadowed replicas were examined in order to study the surface texture of the filters.

All sectioned specimens presented a similar appearance in the electron microscope, and none of the preembedding treatments modified the structure of the filters. The pores of VF filters were, generally, the narrowest; those of VC filters were intermediate in width; while those of HA filters were the widest of the three. Comparison of the filters which had been used in the bone induction experiment with the filters which had not been exposed to tissue growth established that little if any deformation took place in the pores as a consequence of penetration by cytoplasmic extensions and collagen fibrils.

An unexpected property of the filter material, irrespective of rated pore size, was its rapid sublimation even to mild exposure in the electron beam. This effect is illustrated in Fig. 2 where the initial appearance of the filter may be compared with the appearance of the same area 30 sec after exposure to the electron beam under normal operating conditions. Virtually all of the filter material has disappeared, leaving holes in the Araldite matrix. This feature complicates the interpretation of sections, such as the section seen in Fig. 1.

The internal structure of the filters may now be considered in the light of these observations. While it has frequently been assumed that the filters are membranes with parallel, surface-to-surface perforations, our observations indicate that the pores are tortuous channels. If a replica of the surface (Fig. 3) is compared with a section cut perpendicular to the surface (Fig. 4), a three-dimensional representation of the filter structure becomes possible. Channels showing a wide size distribution appear to course at random and to have numerous blind pockets. This structural feature may possibly lead to entrapment of some of the particles which are smaller than the rated pore size, an effect reported by Moore and Peck (4).

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