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Fibril Formation in Stationary Cultures of Mouse Lung Cells. (24279)

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It has recently been reported(1) that a strain 929 of L fibroblasts agitated in a serum-free medium gave rise to the formation of fibers. This fiber material was shown to be digested by collagenase, partially degraded by trypsin, and resistant to alpha amylase and hyaluronidase. The observations reported here are cited to bear out this report using a different cell line in a non-agitated medium, to present new information regarding the period of appearance and disappearance of the fibrils, and to present photographic evidence of the cell fibril complex.

Materials and methods. The line of cells used in this report was isolated from lung tissue of new-born Swiss mice, NIH strain. The original isolation was made using trypsin dispersed cultures prepared by a procedure similar to that of Youngner(2). Prior to their growth on a serum-free medium the cells were in their 110th passage on a medium consisting of 10% horse serum and 90% medium #199 (3). Penicillin and streptomycin were added in a final concentration of 50 units each per ml. Morphologically the cells appeared fibroblast-like, although small numbers of epithelioid cells were in evidence. The cells were routinely passed every 4 days by trypsinizing, centrifuging, resuspending, and inoculating, (hemocytometer count) 3 x 10^5 cells per ml in T-30 flasks, the total volume being 5 ml.

During the course of adapting these cells to a serum-free medium consisting of 99% medium #199, 1% Difco Bactopeptone, and 100 mg% glucose (BPD) it was noticed that a floating layer of cells was formed. These cells were separate from the cell layer which had attached to the glass surface, and appeared in the early passages after 5 to 8 days of incubation at 37°C. The floating cells were seen as discrete patches often 3-4 mm in diameter. Photomicrographs of this material were obtained by inverting and reinverting the T-30 flasks so that the unattached cells adhered temporarily to the glass surface opposite the normal cell layer. In this way the natural configuration of the material could be studied. Portions of the material were then removed and tested with several enzymatic preparations. Results of these tests showed that the material was resistant to digestion by hyaluronidase and trypsin but susceptible to digestion with collagenase.

Fig. 1 shows a typical growth of mouse lung cells on a glass surface using a 10% horse serum medium. The cells form an even sheet which is extremely difficult to dislodge physically. Fig. 2 illustrates the tangled mass of

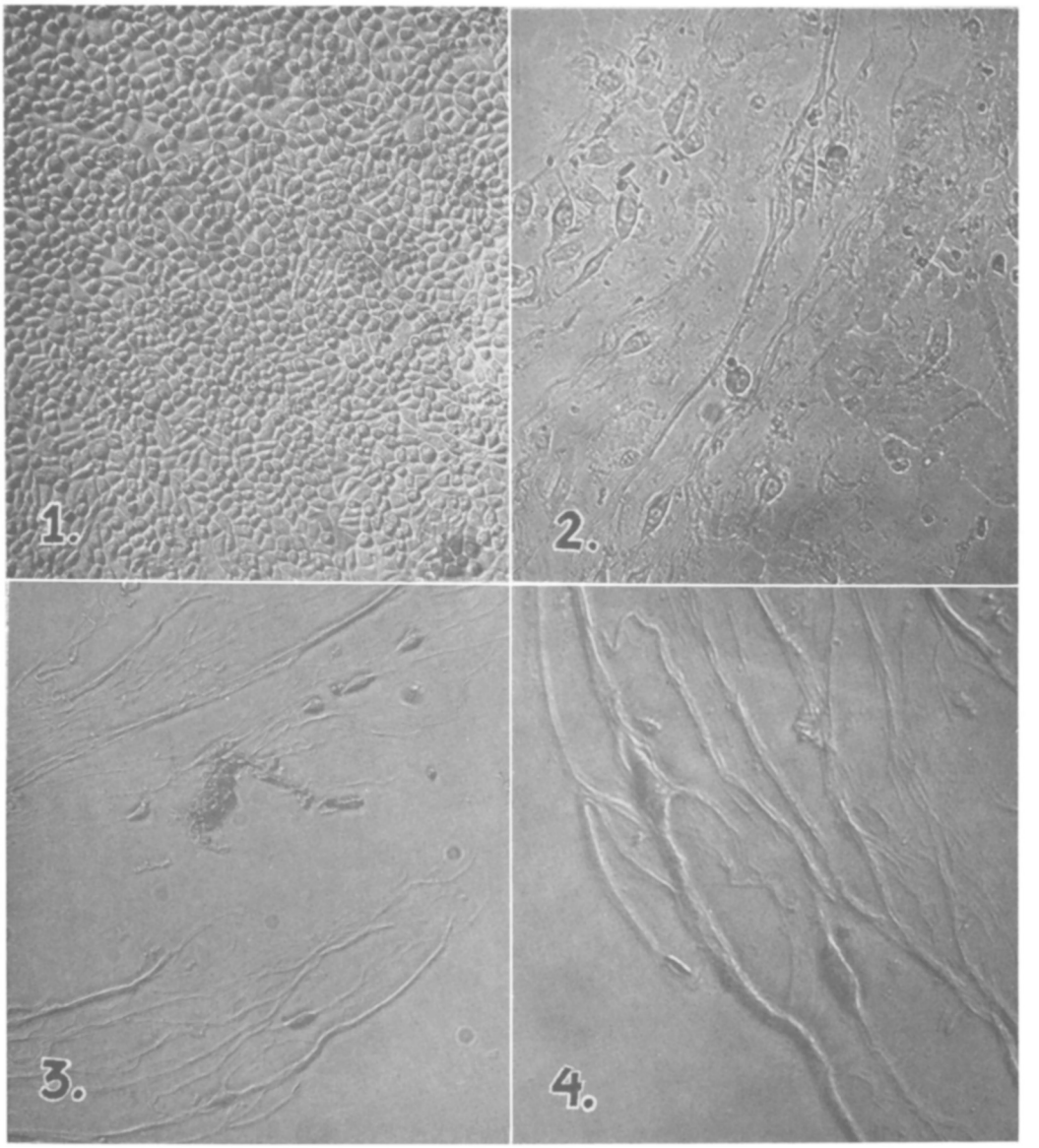

FIG. 1. Normal culture of mouse lung cells grown on 10% horse serum. × 180.
FIG. 2. Floating sheet of cells and fibers seen on 1st passage of mouse lung cells on Bactopeptone-dextrose medium. × 180.
FIG. 3. Discrete fiber material shown in association with mouse lung cells, 5th passage on Bactopeptone-dextrose medium. × 180.
FIG. 4. Higher magnification of the material shown in Fig. 3. × 400.

material which made up the floating layer in the first passage on BPD medium. This response gave way to the more discrete fiber formation seen in Fig. 3. Notice that the fibril material seems to arise from single cells and crosses the entire field of vision. This type of response did not occur until the 4th to 6th passage, Fig. 4 is an enlargement of the material shown in Fig. 3 and affords a better view of the origin of the fibers. The transition from large masses of floating material to better defined strands was not an immediate but a gradual one.

These cells are now in their 21st passage on

BPD and no longer give rise to fibril formation upon routine passage. It is possible to induce a surface type growth by scraping the cells from the glass surface and transplanting them into new BPD media with a minimum of agitation. Under these conditions the floating cells never produce the long strands shown in Fig. 3, but grow and expand as cell sheets with a morphology similar to, but not as compact as, the attached cell sheet.

Early passages of the L cell line(4) in the BPD medium produce floating cell sheets similar to those seen with the mouse lung line. The discrete fibrils seen in the case of the mouse lung cells have not been seen with the L cells.

Discussion. As a result of the above investigation certain facts concerning fibril formation can be stated. It is certain that fibrils can arise without agitation of the medium, and are therefore not the result of entwining of single strands of material. From the photomicrographs it appears that the fibrils arise from the cell body *per se* and are not the result of entrapment of cells in fibrous material released by the degenerated cells. The fact that the fibrils are no longer seen once the cells become adapted to a rapid growth in the BPD medium points up the fact that they may be the result of unfavorable growth conditions as previously hypothesized(1). Attempts to stimulate a similar response by other sub-optimal conditions in the absence of BPD have failed. It is probable therefore that the Bactopeptone itself is at least partly responsible for formation of this material.

Summary. The presence of fibril-like material in a strain of mouse lung cells grown in a serum-free medium is described. This material was resistant to digestion by hyaluronidase and trypsin and susceptible to digestion with collagenase. The period of appearance and disappearance is discussed and photographic evidence of the cell body-fibril association is presented.

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Elastin Content of the Human Lung.*†‡ (24280)

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Elastic tissue is readily demonstrated histologically in the parenchymal tissue of the human lung but no quantitative chemical studies of it have been reported in this tissue. Such investigations are now in progress in this laboratory. Chemical estimation of elastic tissue at present must be based on its content of the fibrous protein, elastin. The purpose of this communication is to report the amount of elastin in the human lung, and the correlation between pulmonary elastin concentration and age. In addition, the normal concentrations of elastin have been compared with those obtained in lung parenchyma subjacent to areas of bulla formation. It has been suggested that elastic tissue plays a role in the pathogenesis of such lesions.

Of several methods of elastin determination which have been reported(1,2,3), the method of Lowry *et al.* was chosen for study of the

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