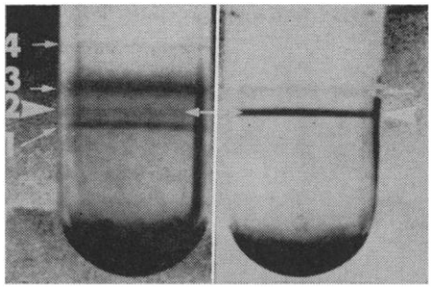

Fig. 3. Bands in a preparation of infective adenovirus-12 from tissue culture (left), and in an extract of adenovirus-induced tumor (right). Band No. 2 is a minor component of the virus preparation but a major component of the tumor extract.

and No. 3 are common to both preparations.

The composition of the gradient of adenovirus-12 is shown in Fig. 4, in which results of particle counts (*3*) and density determinations (*6*) for each of the fractions is plotted. Estimates of the general locations of the bands are shown on the chart. Most of the virus (88 percent) was contained in the fractions 12, 13, and 14. The densities of these three fractions ranged from 1.325 to 1.270. Band No. 2, present in both gradients, had a density of 1.27 to 1.30. Thus, band No. 2 in the virus gradient represents the less dense portion of the crude virus population and is probably that fraction of particles containing lesser amounts of DNA. Band No. 2 in the tumor extract gradient had a density exactly equal to that in the virus gradient and contained a high concentration of structures similar to those in Fig. 2.

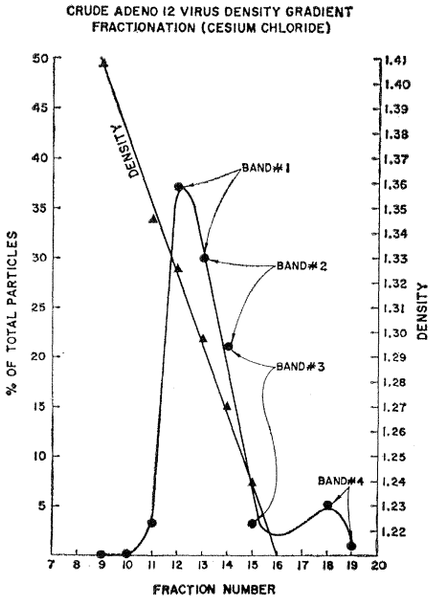

Fig. 4. Density-gradient fractionation of a crude, unpurified preparation of adenovirus-12 in cesium chloride.

The conclusion that the structures seen in the adenovirus-induced tumor extracts may be imperfectly constructed adenovirus particles is based upon their similarities in (i) size, (ii) morphology of their structural subunits, and (iii) density in cesium chloride gradients, and also upon their absence in hamster tumors induced by SV40. It appears that adenovirus-induced tumor cells are capable of producing substantial quantities of viral material, as indicated by the intensity of this band in cesium chloride and the large number of particles it contained. The density of these particles (1.27 to 1.30) is less than that of the most dense adenovirus particles (1.32 to 1.34), but they are more dense than about 11 percent of the particles in the adenovirus preparation.

These findings indicate that the attempts to isolate infectious adenovirus from tumors induced by this agent have failed because of (i) a lack of sufficient amounts of DNA (genetic information) within the particles to confer infectivity (indicated by low density of the particles), or (ii) a lack of sufficient organization of the protein subunits to provide a transfer of viral DNA from the tumor cells to susceptible cells in test cultures. Tumor cells appear incapable of producing particles with well-ordered subunits. This imperfect construction or assembling of protein subunits may be a reflection of the incompleteness of the viral genome, which is suggested by the low density of the particles. Until now viral material from these tumors was recognized only by serological methods. The present study furnishes information concerning the degree of organization characteristic of these viral elements.

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Pulmonary Alveolar Cell Inclusions: Their Development in the Rat

Abstract. *Cytoplasmic inclusions in the special pulmonary alveolar cells of adult, newborn, and fetal rats were investigated by electron microscopy and histochemistry, and lung extracts were analyzed for surface-tension activity. The inclusions were positive in the periodic acid-Schiff test after digestion with diastase and were not stained with toluidine blue; they formed myelin figures and possessed alkaline phosphatase activity. Both the typical surface tension activity and the inclusions developed 2 days before birth. These properties differentiated the inclusions from mitochondria.*

Considerable circumstantial evidence, recently summarized (*1*), links a cytoplasmic inclusion in the special pulmonary alveolar "corner" cell with the production of lung surfactant, a highly surface-active lipoprotein (*2*) that lines the terminal airspaces and stabilizes them against collapse (*3*). The large corner cells project into the airspaces with characteristic microvilli on the surface exposed to air (*4*); the inclusions are osmiophilic, appear lamellated, and are in close proximity to mitochondria.

The nature of the cell inclusions is disputed. Schulz considered them to be transformed mitochondria (*5*), and described various stages of degenerated cristae leading to lamellar forms. Klaus *et al.* (*6*) also found the mitochondrial fraction of lung homogenate to contain surface-active components. Other electron microscopists, however, have found no definite transitional forms (*1*, *7*, *8*). Karrer pointed to the ammonium sulfide affinity of the inclusions (*7*), which is not shared by mitochondria. Campiche (*1*) found that $KMnO_4$-fixation completely altered

the lamellar appearance after osmium fixation. The structure which Macklin (*9*) referred to variously as a "secretory granule" or osmiophilic body or vacuole, in his extensive studies by light microscopy, was clearly distinguishable from alveolar cell mitochondria.

In our investigations, we first sought to determine in the rat whether the inclusion body of the special alveolar cell corresponds to Macklin's secretory granule, and whether or not it is a mitochondrion in transition. When sections of araldite-embedded lung were infiltrated with alkaline toluidine blue (*p*H 11.5), the special inclusions resembled what Macklin called "intracytoplasmic fluid pools" (*9*) and were completely unstained. Inclusions within the macrophages of the lung, which morphologically may resemble those in the corner cells, were uniformly stained with toluidine blue. "Overflow" granules free in the airspace of the lung of the newborn rat formed myelin figures, resembling those formed by phospholipid-protein complexes. In the alveolar cells, material which gave a positive reaction in the periodic acid–Schiff (PAS) test after digestion with diastase was localized by alternate thick sections for histochemistry and thin sections for electron microscopy. A large-scale map identified the portions of the corner cells that contained the PAS-positive material. Such material was limited to cytoplasmic inclusions that still contained material which appeared solid by electron microscopy. Mitochondria were unstained. This may indicate that the granule contains mucoid, although sphingomyelins which are highly surface-active (*10*) are also resistant to diastase and PAS-positive. The extreme density of inclusions found in the alveolar cells of a child with mucoviscidosis (*11*) also favors a mucoid composition. With Mallory's phosphotungstic acid-hematoxylin stain, the corner cell mitochondria were colored purple whereas the surrounding special inclusions appeared as clear, unstained "halos."

No acid phosphatase enzyme activity was demonstrated in cell inclusions of the alveolar wall, even though it was present in lung macrophage inclusions. The inclusions of corner cells showed abundant alkaline phosphatase activity in their membranes (Fig. 1), while the mitochondria of the cell had no such activity.

To our knowledge, alkaline phosphatase has not been described in association with mitochondria in any cell, whether studied by analysis of fractions obtained by differential centrifugation or by histochemical methods. These data suggest, therefore, that the inclusion body and the Macklin granule are probably identical, and unrelated to mitochondria.

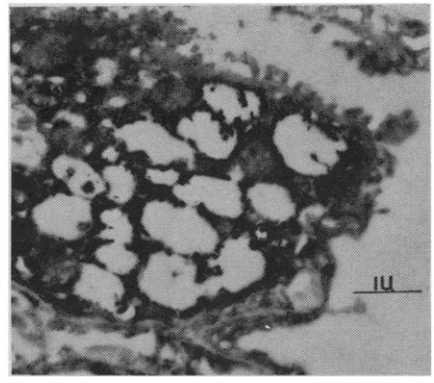

Fig. 1. Alveolar corner cell stained to demonstrate alkaline phosphatase activity. The tissue was fixed in cold formalin-calcium for 1 hour prior to the enzyme reaction, and afterward was fixed in buffered osmic acid and embedded in araldite. The lead phosphate is localized about the periphery of the vacuoles which represent the inclusion bodies. Control sections failed to demonstrate this localization. (× 11,200)

Normal surface activity develops in the lung of fetal mice at the same time during gestation (*12*) that alveolar cell inclusions first appear (*8*). In our study we extended these functional-morphological observations to the fetal rat. Pooled lungs from fetuses in each of the last 5 days of gestation were studied on a Wilhelmy surface-tension balance. The results showed that normal surface activity develops in this species 2 days before birth. Two out of four pooled samples of lung extracts from fetuses at this gestational age (body weight approximately 3 g) showed surface activity; lung extracts from all more mature fetuses and newborn animals showed similar, normal surface activity, whereas extracts from all the younger fetuses showed deficient activity. Normal activity was defined as achievement of a minimum surface tension of 20 dyne/cm or a stability ratio of 0.85 or greater, according to Clements' formula (*13*).

Micrographs prepared from lungs of littermates were studied to determine the first appearance of alveolar cell inclusions. These also developed in fetuses 2 days before birth. The alveolar cells of younger fetuses bulged with cytoplasmic glycogen and contained well-formed mitochondria and endoplasmic reticulum, but not inclusions. Glycogen had almost disappeared from the alveolar cells by the time inclusions were present.

The coincident development of alveolar cell inclusions and onset of what may be a mature function of these cells (that is, the production of surfactant) in fetal lungs do not prove that these events are related. No data exist to show that these inclusions manufacture surfactant, and their identity remains uncertain. If the production of surfactant is a secretory process as Macklin suggested, one may argue that secretory units would be expected to appear as part of a general differentiating process in the development of alveolar epithelium. The localization of alkaline phosphatase in these structures may even suggest an analogy to maturing duodenum (*14*), where this enzyme heralds morphological and functional differentiation of the epithelial cells.

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