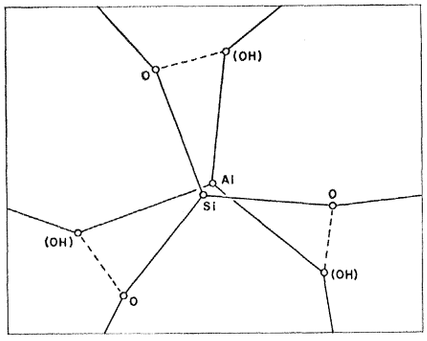

Fig. 1. Relation of the surface hydroxyl groups of one layer to the surface oxygens of an adjacent layer in dickite. A similar relation exists in kaolinite (*3*).

Their results show two features which require further discussion. First, weak sharp bands persist at 3695, 3670, and 3650 cm^{-1} in the deuterium-substituted kaolinite. These can reasonably be ascribed to a small proportion of kaolinite layers which were not expanded by hydrazine treatment, and so were not available for deuterium exchange. Although x-ray diffraction measurements on hydrazine-treated kaolinite showed only a weak 7.1 Å line (*6*), it must be remembered that an x-ray reflection requires a number of adjacent 7.1 Å spacings; a small number of isolated 7.1 Å spacings would not be detectable. Second, although the 3670 cm^{-1} band of well-crystallized kaolinite is the weakest of the four and has its dipole change nearly parallel to the sheets (*1*, *7*), the residual absorption in the deuterium-exchanged kaolinite is as strong as that at 3695 cm^{-1} and has a component perpendicular to the kaolinite layers (*8*). This can be ascribed to residual hydroxyl groups which have deuteroxyl groups as neighbors. The sharp absorption bands of well-crystallized kaolinite arise from dipole-dipole coupling between the hydroxyl groups in the surface layer and probably require a well-ordered region of hydroxyl groups covering several primitive cells in any one sheet. In the deuterium-substituted kaolinite, some residual hydroxyl groups would be entirely surrounded by deuteroxyl groups; others would have one, two, or more hydroxyl groups as first and second neighbors, giving a range of coupling forces, which would be expected to give rise to broad hydroxyl absorption in the 3640 to 3700 cm^{-1} region, showing a marked degree of polarization perpendicular to the kaolinite sheets. The spectra of Ledoux and White (*8*) do in fact show this feature, of which the maximum appears to be near 3670 cm^{-1}. Similar arguments apply to the deuteroxyl absorption at 2698 cm^{-1} (*8*). The effects of disorder are also evident in halloysite, which, because of its poorer crystallinity, does not show the fine details of the kaolinite spectrum (*8*). The diffuse absorption of kaolinite in the 3650 to 3690 cm^{-1} region (*1*), which has a component perpendicular to the kaolinite layers, probably arises from less highly crystalline regions such as crystal surfaces and slip planes within the crystals.

It is noteworthy that the position at which the inner hydroxyl group absorbs, 3620 cm^{-1}, is unaffected by replacing the surface sheet of hydroxyl groups in kaolinite by deuteroxyl groups. This indicates that there is little coupling between the inner hydroxyl group and the surface groups, presumably because they are oriented nearly perpendicular to each other.

Although the argument presented here shows that the results of infrared studies need not be taken to conflict with those of x-ray investigations on kaolinite, it must be noted that the related minerals, dickite and nacrite (*7*), give hydroxyl absorption patterns surprisingly different from those of kaolinite. Application of the selective deuteration technique of Ledoux and White (*8*) should prove valuable in interpreting these differences.

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5 June 1964

Adenovirus-like Particles from Cancers Induced by Adenovirus-12 but Free of Infectious Virus

Abstract. *Particles resembling adenovirus were recovered from tumors induced by adenovirus-12. The particles were similar to incomplete adenovirus in size, subunit morphology, and density. The presence of such incomplete viral units helps to explain why attempts to isolate infectious virus from adenovirus-induced tumors have failed despite the serological evidence that viral elements are present.*

There are three human adenoviruses (types 12, 18, and 7) that induce malignant tumors in laboratory animals (*1*). Such tumors appear to be free of infectious virus, although they may contain high concentrations of viral complement-fixing antigens (*2*). These tumor antigens found by Heubner *et al.* (*2*) are similar to the type-specific antigens produced by adenoviruses during their normal growth in tissue culture cells. In the present study adenovirus-induced tumors were extracted, and the extracts were examined by quantitative electron microscopy (*3*) to determine whether they contained organized viral structures.

Hamsters bearing tumors induced by adenovirus-12 (*4*) were killed by decapitation and exsanguinated as completely as possible. Tumors were removed aseptically, cut into small pieces, and rinsed three times in Eagle's serum-free medium. Small weighed pieces of individual tumors were ground into a thick paste with a mortar and pestle, and measured volumes of Eagle's medium were added slowly during grinding until the concentration of tumor (in suspension) was 20 percent. These homogenates were then frozen and stored at −20°C. When needed they were thawed and allowed to stand for a few minutes until large debris had settled. The turbid supernatant fluids were filtered through thin layers of Celite held by filter paper in Buchner funnels. The resulting filtrates, which showed no visible turbidity, were used for electron microscopy and density-gradient fractionation studies.

For electron microscopy appropriate dilutions were made of Celite-clarified suspensions of adenovirus-12 (grown in tissue culture) and tumor extracts, small droplets of each being allowed to dialyze into 2 percent agar (in saline). The particulate material was transferred from the agar surface to a collodion membrane and negatively stained in 0.5 percent potassium phosphotungstate or in 1 percent uranyl acetate (*3*).

Figure 1*A* is an electron photomicrograph of crude adenovirus-12 (grown in KB cells and harvested at 48 hours) prepared as described. There is a regular arrangement of the subunits (capsomeres) constituting the protein shell (capsid) of these particles. Figure 1*B* shows two typical fields of adenovirus-12 which had been purified by cesium chloride density-gradient fractionation and then stored at 4°C for about 6 months. The large number of free capsomeres have resulted from the partial degradation of capsids. The large pointers indicate two partially degraded particles, and the arrows indicate subunits on the ragged periphery of these particles.

Figure 2 shows the morphology of structures seen in several extracts of different tumors induced by adenovirus-12 (*4*). Some of these structures (large pointers) are almost exactly the same in diameter as the particles observed in suspensions of adenovirus-12 produced in tissue culture, and show a strong resemblance to the partially degraded virus particles in Fig. 1*B*. The subunits of the purified particles in Fig. 1*B* and those of the tumor particles in Fig. 2 are practically indistinguishable. These somewhat disorganized particles were found in fairly high concentrations (10^8 to 10^9 particles per milliliter of 20 percent tumor suspension) in three of the four tumors examined, and in low concentration in the fourth. A large amount of irregularly shaped material with virus-like subunits was also present (Fig. 2, right). Virus-free tumors induced by SV40 (*5*) were examined and were free of the adenovirus-like particles or virus subunits described above.

Because of the morphological similarities between the particles extracted from adenovirus-induced tumors and the partially degraded adenovirus-12, we compared their behavior in equilibrium density gradients. Celite-clarified filtrates of both preparations were carefully layered over cesium chloride density gradients (16 ml) contained in lusteroid cellulose tubes (2.5 by 7.5 cm). The tubes were centrifuged for 3 hours at 25,000 rev/min in the Spinco SW-25.1 rotor. Deceleration was carried out without braking and required about 45 minutes. Tubes were removed carefully, photographed with oblique lighting, and the fractions were separated by the bottom-drip method. Figure 3 shows a gradient containing adenovirus-12 (left) and a gradient containing tumor extract (right). The tube containing virus (left) shows four distinct bands, and the tube containing tumor extract (right) shows two bands. Bands No. 2

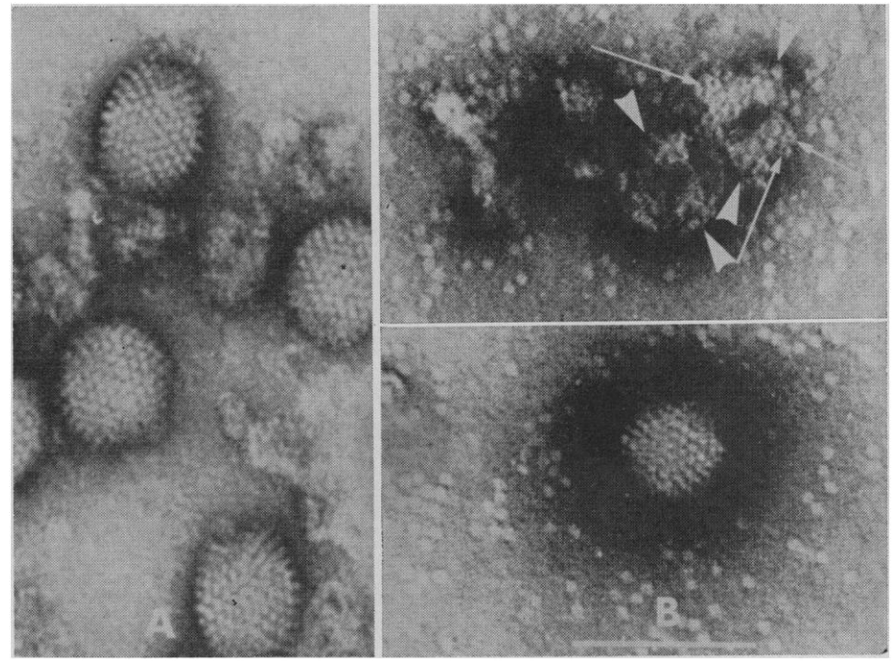

Fig. 1. Adenovirus-12. (*A*) Fresh preparation of crude, unpurified material harvested from tissue culture. (*B*) Top and bottom. The purified preparation after storage at 4°C for 6 months. Free capsomeres are abundant. Scale, 100 mμ.

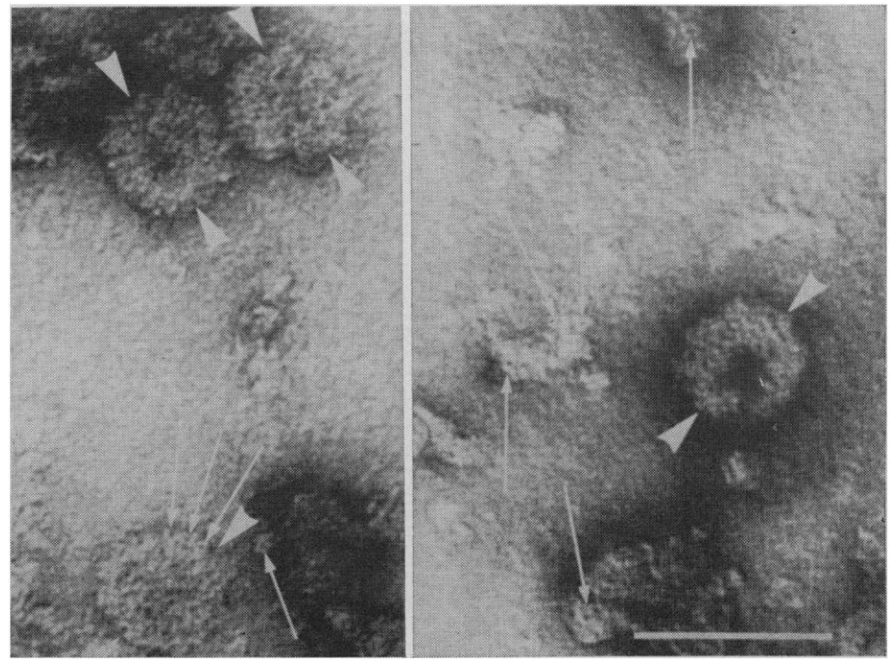

Fig. 2. Particles resembling partially degraded adenovirus, as found in crude, unpurified extracts of adenovirus tumors. Scale, 100 mμ.

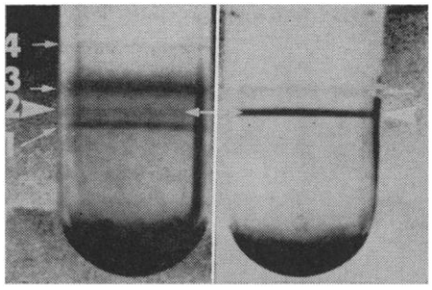

Fig. 3. Bands in a preparation of infective adenovirus-12 from tissue culture (left), and in an extract of adenovirus-induced tumor (right). Band No. 2 is a minor component of the virus preparation but a major component of the tumor extract.

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.

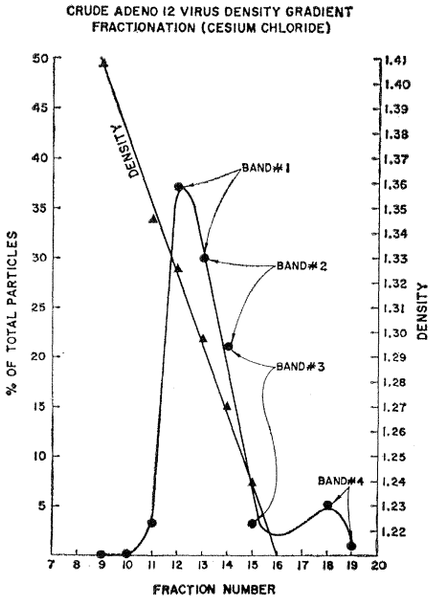

Fig. 4. Density-gradient fractionation of a crude, unpurified preparation of adenovirus-12 in cesium chloride.

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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7. We thank M. Trousdale for technical assistance. Supported in part by PHS research grants CA-04600 and AI-05382 and by NIH research career development award 1-K3-CA-13,120 to K. O. Smith.

15 June 1964

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