A further analysis of this model has cast light on the important and much disputed question of whether a fully differentiated cell such as a smooth muscle cell (containing specific structural proteins) can undergo mitotic division without first dedifferentiating. It is held that dedifferentiation is associated with mitosis in specifically committed cells[3].

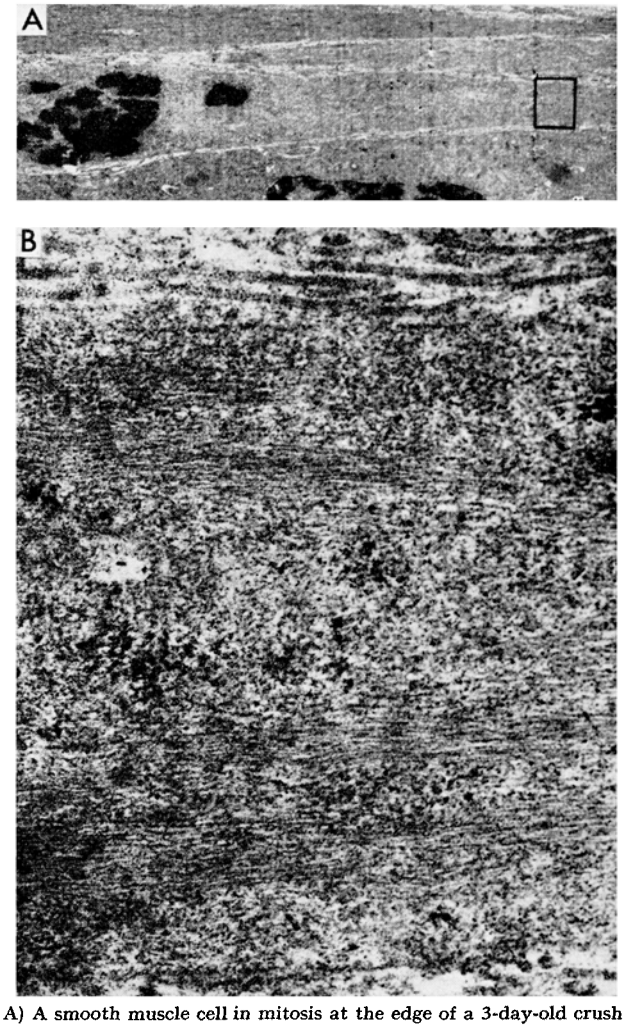
Electron microscopic examination of cells within and surrounding the crush lesion after 3, 5, 7 and 10 days shows significant ultrastructural features of the regeneration process in smooth muscle cells.

At 3 and 5 days, the cytoplasm of uncrushed smooth muscle cells adjacent to the lesion shows a relative increase in rough endoplasmic reticulum, particularly in the perinuclear zone where large ribosome-lined cisternae filled with amorphous material are common. Myofilaments occupy the bulk of the cytoplasm. In similar cells which contain fewer cisternae, free ribosomes are abundant and microtubules run longitudinally throughout the cytoplasm. Myofilaments occupy a well defined layer in the peripheral cytoplasm. Autoradiographic studies with $H_3$ thymidine[2] indicate that these are premitotic cells in late S or G2 phase[4]. Smooth muscle cells in mitosis have similar cytoplasmic features (to the above), including myofilaments (Figure 1). Myoblasts which appear to arise from this mitotic division dominate the lesion. They have large open-faced nuclei with dispersed chromatin and prominent nucleoli. Their cytoplasm contains many polyribosomes, and a thin peripheral layer of myofilaments.

From 5–10 days, increasing numbers of smooth muscle cells with a large concentration of cytoplasmic myofilaments are found in the lesion. By 14 days, only relatively mature smooth muscle cells are seen.

HOLTZER et al.[5–7] used chondrocytes as a model to study the relationship between DNA synthesis and the synthesis of cell specific substances (in this case chondroitin sulphate). They found that not only are these 2 synthetic processes mutually exclusive, but that chondrocytes in vitro progressively lose their ability to produce chondroitin sulphate following mitosis. They concluded that mitosis in a committed or differentiated cell is associated with dedifferentiation.

Such is not the case with smooth muscle regenerating in vivo. The presence of cytoplasmic myofilaments within the smooth muscle cells at all myogenic stages (including mitosis) demonstrates the specific nature of this cell.




A) A smooth muscle cell in mitosis at the edge of a 3-day-old crush lesion. ×4000.
B) An enlargement of inset in A), showing myofilaments. ×58,000.

*Zusammenfassung.* Elektronenmikroskopisch wird erstmals die Regeneration von glatten Muskelzellen in vivo beschrieben und festgestellt, dass diese Zellen entgegen der herrschenden Auffassung keine Differenzierung durchmachen, bevor sie sich teilen.

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## Demonstration of Virus Particles in Ovine Pulmonary Adenomata

Several spontaneous and transmissible avian[1], rodent[2–6] and, as recently reported, feline[7–9] neoplasms, have been found to be associated with and probably caused by C type viral particles. The present electron-microscopic study of ovine pulmonary adenomata indicates that this neoplasm is also associated with C type viral particles. This disease of sheep is characterized by papilliform proliferations of the epithelium of the alveoli and bronchioli and by frequent metastatic involvment of different organs or tissues[10,11]. Thus the term carci-

noma of the lung, rather than lung adenomatosis, was recently proposed[11]. Electron microscopical studies show that most of the tumor cells appear to be derived from the B alveolar cell, one of the two known types of alveolar epithelial cells[12].

In the present study, pulmonary tumor material from 8 adult Awassi sheep and from 1 lamb, all having a clinical and histopathological diagnosis of lung adenomata, were taken for electron microscopy. Lung tissue from 9 clinically healthy Awassi ewes, obtained from an abattoir, served as control.

Electron microscopic examination of thin sections of the ovine lung tumor revealed the frequent occurrence of virus particles adjacent to the free surface of the tumor cells. Virus budding from the tumor cell microvilli was also observed. Also, groups of virus particles were noted within the tumor cell cytoplasmic matrix; in extremely rare instances, particles were found to form at the granular endoplasmic reticulum membrane and also free within the cisternae. Viruses were not observed in the normal, control lung cells.

The extracellular viruses had the appearance of C type murine or feline virus particles[3]. The particles averaged 100 μm in diameter. Some possessed the outer coat, intermediate layer, and relatively electron-lucent nucleoid, approximately 50 μm in diameter, characteristic of 'immature' murine C type particles[3,4] (Figure 1). Others had a central located electron-dense nucleoid, characteristic of 'mature' C type virus particles[3,4] (Figure 2). The intracytoplasmic virus particles had the appearance of type A murine particles[2,3] ($A_2$, also called naked A) and were approximately 70–80 μm in diameter (Figure 3). In this particle the outer shell is thinner than the inner and its electron-lucent centre has a greater diameter than those type A particles[3] (type $A_1$) found within the cisternae (Figure 3, insert).

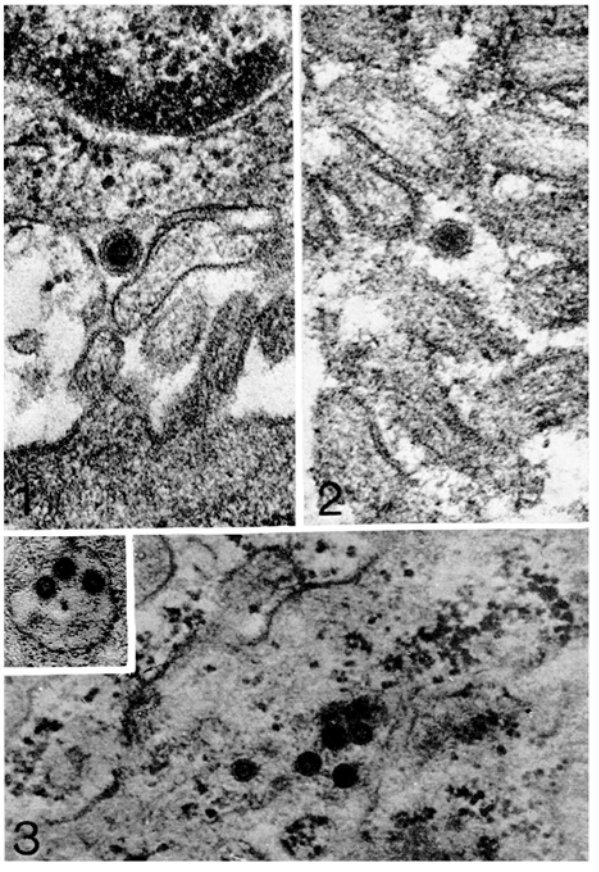

Fig. 1. An immature C type virus particle near the microvilli of a tumor cell. An intermediate membrane and nucleoid with a relative electron-lucent central area are present. ×60,000.

Fig. 2. A mature particle having an electron-dense nucleoid near the border of the tumor cell. ×70,000.

Fig. 3. A group of viral particles (A type) are seen within the cytoplasmic matrix of a tumor cell. ×38,000. Insert, virus particles within the cisternae of the granular endoplasmic reticulum. ×24,000.

The aetiology of sheep pulmonary adenomatosis is still the subject of much speculation, although positive transmission experiments with extract of affected lungs have been reported[13,14]. The aetiological significance of the virus particles described here is now being studied. To the best of our knowledge, no electron-microscopic observations have been reported on virus association in vivo in sheep having pulmonary adenomatosis; however, *Herpes*-like particles were found in cultivated macrophages from lungs of sheep suffering from this disease[15]. In the present study, nuclear particles of the *Herpes* type group were not observed. It is of interest that recently A and C type particles indistinguishable from those found in naturally-occurring ovine lung tumors were observed in two 16- and 24-month-old mice, having coincidently lung adenomatosis and leukemia. However, an etiological relation between these virus particles and the lung adenomatosis was considered unlikely[16]. The detection and demonstration of C type virus particles associated with this naturally occurring tumor extends the range of viral-tumor host systems from the avian, murine and feline to the ovine species, and from leukemia-sarcoma to epithelial tumors. Thus additional vistas to the cause of human neoplasia have become open.

*Zusammenfassung.* Elektronenmikroskopischer Nachweis bei Lungen-Adenokarzinomen von Schafen, dass diese jeweils zusammen mit Viruspartikeln auftreten, die morphologisch solchen aus verschiedenen spontanen und übertragbaren Neoplasmen von Geflügel, Nagetieren und Katzen ähnlich sind.

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