extracts. The tubes were incubated at 37 °C and examined periodically. Cell numbers were obtained by trypsinizing, diluting, and counting cells in an electronic cell counter (Coulter Electronics, Model B).

The cell lines tested were: hamster kidney (BHK-21), human embryonic lung (WI-38), primary human embryonic kidney (HEK), diploid human embryonic kidney, diploid human embryonic lung, primary human embryonic skin and muscle, and diploid human skin (Microbiological Associates).

The most marked effects on cell growth were seen with the usually slow-growing primary HEK cultures, as shown in Figure 1. In this instance, enhanced proliferation was noted with the EDTA tumor extract. Figure 2 depicts typical growth curves obtained with the test fractions on HEK cells. The tumor precipitate (TP) and serum precipitate (SP) both enhanced cellular proliferation, but the curves for TP showed a lag phase with long period of continuous multiplication, whereas SP produced an immediate sharp rise in cell number followed by a rapid decline of the population. Neither the serum nor tumor supernatants (SS, TS) showed any effects on proliferation beyond those of controls. Non-precipitated tumor serum gave growth curves like those of the SP additive, and whole EDTA tumor extract was similar to TP in growth effect. Thus, the growth regulating agent(s) are those precipitable with 3% acetic acid. Varying degrees of enhanced proliferation were also seen when the TP and SP fractions were added to the BHK-21 hamster kidney line, both primary and continuous diploid human embryonic kidney, and lung lines, but not with the skin, or skin and muscle cells tested.

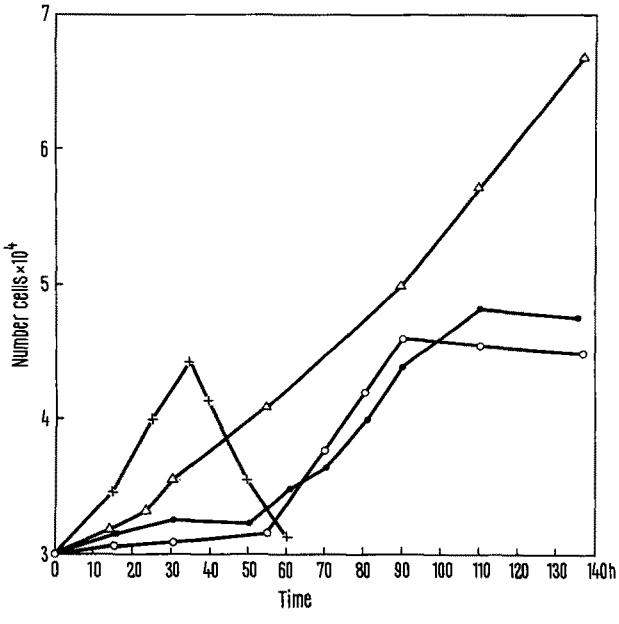

Fig. 2. Growth curves of primary human embryonic kidney cells after the addition of tumor or serum extracts. ×–×, acetic acid precipitate from serum containing the abnormal component (SP); △–△, acetic acid precipitate from the EDTA tumor extract (TP); ●–●, serum supernatant or serum with the abnormal component removed by acetic acid treatment (SS); ○–○, control cells containing an additional 0.1 ml media. Samples of normal human serum or EDTA solution could be substituted as controls with the same growth pattern resulting.

These results indicate marked enhancement of cell proliferation by addition of either the EDTA Wilms' tumor extract or the isolated abnormal serum component. The difference in growth kinetics between TP and SP is not yet accounted for. In view of the fact that lung is a primary target for metastasis of Wilms' tumor, it is of interest that the acid-precipitable fractions from tumor and serum promote growth of both kidney and lung cells, but do not affect proliferation of skin cells or mixed skin and muscle cells in vitro.

In view of evidence relating protein-polysaccharides on the cell surface to control mechanisms of cell growth[3,4], it may be reasonable to conjecture that the mucoprotein in Wilms' tumor serum is involved in metastasis. This viscous material may act as a carrier for malignant cells and enlodge them at metastatic loci by a specific adhesive mechanism. The substance may then aid in proliferation of the malignant cell once it reaches such a site[5].

Zusammenfassung. Anomale Komponenten, die aus Blutserum und Tumoren von Patienten mit Wilmschen Tumoren isoliert wurden, fördern das Wachstum der Nieren- und Lungenzellen-Kulturen.

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Ultra-Structural Specificity in Regenerating Smooth Muscle

In an experimental crush injury to the taenia of the guinea-pig cacum in which approximately 10,000 cells are crushed, damaged smooth muscle cells are rapidly replaced. By 14 days following the crush there is little microscopic evidence of the lesion[1].

From 3 days postoperatively onwards, high levels of DNA synthesis (shown autoradiographically by H_3 thymidine uptake) and mitotic figures in the surrounding uncrushed smooth muscle cells indicate that new cells in the lesion arise by a process of myoblastic regeneration[2]. Small spindle-shaped myoblasts invade the site of the lesion and mature into smooth muscle cells within 7–10 days. A few myoblasts undergo subsequent mitosis before maturation.

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].

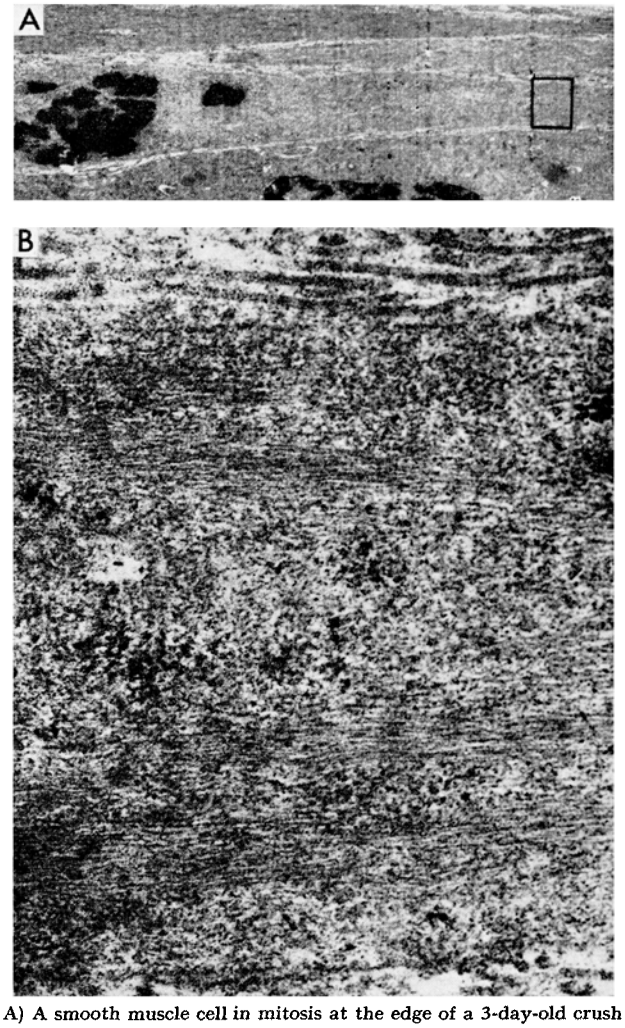
Electron microscopic examination of cells within and surrounding the crush lesion after 3, 5, 7 and 10 days shows significant ultrastructural features of the regeneration process in smooth muscle cells.

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-