As reported in the Table, the cells were oncogenic in isogeneic hosts until 180th day, and the concomitant occurrence of intra and extra cellular Bittner-like particles was ascertained. After the 233th day the cells were no more able to induce tumors in C3H/He Ar/IRE although their inoculum size was 20 times larger. By electron microscopy it has always been possible to correlate the lost of oncogenicity to the absence of viral particles.

Relationship between the occurrence of virus particles and the oncogenicity observed for salivary adenocarcinoma cell cultured in vitro

Age of the culture	No. of cells inoculated	Site of inoculation	Tumor takes in C3H/He strain	Intracellular particles/cells[a]
20 days	40,000	s.c.	100% (30/30)	116
45 days	40,000	s.c.	100% (30/30)	128
100 days	40,000	s.c.	80% (24/30)	102
121 days	80,000	s.c.	70% (21/30)	73
180 days	80,000	s.c.	70% (21/30)	60
233 days	800,000	s.c.	0% (0/30)	0
253 days	800,000	s.c.	0% (0/30)	0
253 days	800,000	i.p.	0% (0/30)	0

[a] Each value represents an averaged figure obtained counting 10 different cells.

On the basis of these results we can conclude that the virus is the causative agent of the salivary gland adenocarcinoma. Experiments are now in progress on the relations of the virus and on its characteristics, in order to know its most important properties and any possible differential character toward typical Bittner-virus.

So far as the observations concerned the mammary tumor, it has been reported[1,2,5] that the virus-free cells when injected in virus-carrier animals are able to assume again the host's virus particles. In contrast to this from the data above reported, it seems that the cells isolated from the salivary gland tumor are unable to utilize viral particles occurring in the host in order to replace their original oncogenicity.

Riassunto. Cellule portatrici di virus derivate da adenocarcinoma della ghiandola salivare coltivate in vitro sono oncogeniche per l'ospite isogenico per circa sei mesi di coltura. Dopo tale periodo perdono l'oncogenicità e non è più possibile ritrovare particelle virali. Sulla base di questi risultati si conclude che il virus è l'agente causale dell'adenocarcinoma della ghiandola salivare del topo C3H/He.

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[5] K. K. SANFORD, *Cancer Cell in Culture* (Ed. H. KATSUTA; University of Tokyo Press, Tokyo 1968), p. 281.

Growth Promotion by Extracts from Wilms' Tumor in vitro

We have recently isolated a relatively homogeneous protein-polysaccharide complex from Wilms' tumor which resembles an abnormal serum component detected in Wilms' tumor-bearing children[1]. The tumor component was isolated by a procedure previously used to obtain acid mucopolysaccharides (AMPS) from the cell surface of normal and polyoma-virus transformed fibroblasts in culture[2]. The fibroblast AMPS extracts promote or inhibit cell proliferation depending on whether they are derived from viral-transformed or normal cells, respectively. We explored, therefore, the possibility of a similar activity in AMPS cell surface materials derived from Wilms' tumor, and in serum from Wilms' patients.

The abnormal component was isolated from sera of Wilms' patients by adding 1 volume of 6% acetic acid per volume of serum and separating the supernate by centrifugation at $5000 \times g$. The precipitate was washed in 3% acetic acid and redissolved in 3 volumes of pH 8.5 buffered isotonic saline. Both serum precipitate (SP) and serum supernate (SS) were assayed for growth effects on cells in culture.

A portion of fresh Wilms' tumor was washed with saline, minced, and extracted with 0.02% disodium EDTA in calcium- and magnesium-free phosphate buffered saline. About 400 g of minced tumor was stirred with 300 ml EDTA solution in a spinner culture flask. After 1 h, this suspension was centrifuged ($5000 \times g$, 15 min), the viscous supernate was diluted 1:2 in saline, and made up to 3% acetic acid by volume, as with the serum samples. The resulting tumor precipitate (TP) and its supernate were also tested in growth studies.

A 0.1 ml aliquot of each test solution was added to a series of Leighton tubes containing known concentrations of trypsinized test cells in 1.5 ml of Eagle's minimum essential medium (MEM) supplemented with 10% newborn calf serum, and penicillin and streptomycin (Grand Island Biologicals). Control tubes were prepared from the same pools of cells, but contained 0.1 ml of media or normal human serum in lieu of serum or tumor

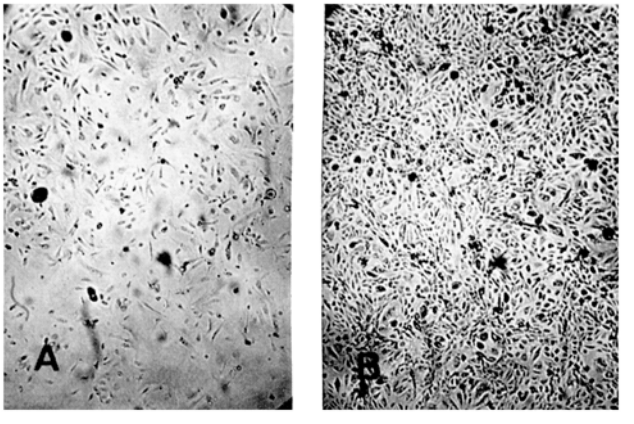

Fig. 1. Primary human embryonic kidney cell cultures in Leighton tubes after 105 h in culture (× 50). A) Control cells; with 0.1 ml normal human serum added. B) Test cells with 0.1 ml of an EDTA extracted Wilms' tumor fraction added (AMPS content; 27 µg determined as hexosamines plus uronic acids).

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.

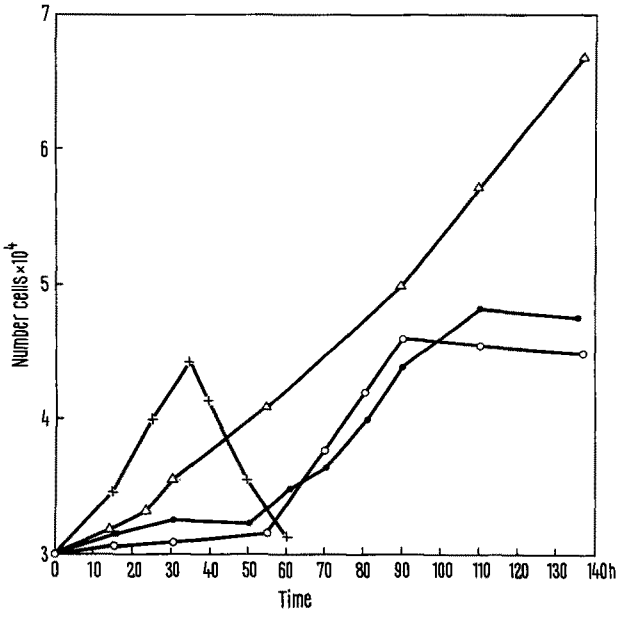

Fig. 2. Growth curves of primary human embryonic kidney cells after the addition of tumor or serum extracts. ×–×, acetic acid precipitate from serum containing the abnormal component (SP); △–△, acetic acid precipitate from the EDTA tumor extract (TP); ●–●, serum supernatant or serum with the abnormal 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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[1] S. ALLERTON, J. BEIERLE, D. POWARS and L. BAVETTA, Cancer Res. *30*, 679 (1970).
[2] J. W. BEIERLE, Science *161*, 798 (1968).
[3] V. DEFENDI and M. STOKER, *Growth Regulating Substance for Animal Cells in Culture* (Wistar Institute Symposium Monograph Number 7, 1967).
[4] L. WEISS, *The Cell Periphery, Metastasis, and Other Contact Phenomena* (North Holland Publishing Co., Amsterdam 1967).
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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.