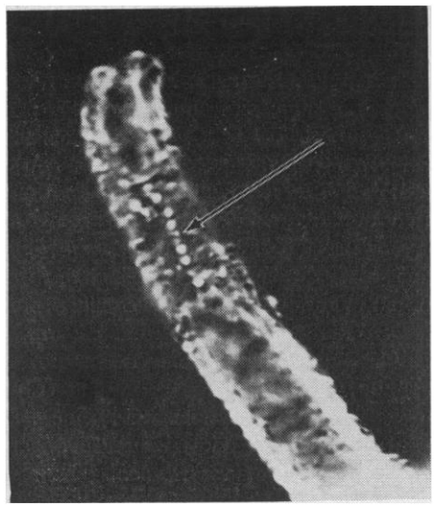

Fig. 1. *Rhizobium* infection thread progressing through the interior of a living, unstained clover root hair; photographed with a yellow Tiffon No. 12 filter on Kodak Ektachrome Infrared Aero Film, type 8443; × 410 at the film plane. The color of the background and of the root-hair protoplasm is light blue; the root-hair wall is yellow, and the "string" of *Rhizobium* cells (arrow) is red. Because of the difficulty in converting color transparencies to black-and-white photographs, certain artifacts in other areas of the picture appear white, but were present as colors other than red in the original.

materials of the habitat appear in contrasting false colors. Both dead (heat killed) and living bacteria are revealed in this manner, regardless of whether they occur on soil or root-hair surfaces, or are embedded within soil materials or plant tissue in the soil. Variation in the film, from one lot number to another, in regard to film speed and color shifts has been experienced, but the bacteria photograph red in spite of these problems. Moreover, the bacteria appear red regardless of whether a yellow or a red filter is inserted in the light path, while all other colors undergo a change in relation to the particular light filtration being employed.

In addition to samples of soil, activated sludge, tissue of plant-root hair, and cell-culture lines, a series of pure cultures of microorganisms were photographed by this technique. All bacteria photographed a red false color, provided that the objective was focused on the lower portion of the cell—the side nearest to the condenser. Species of *Nocardia, Streptomyces,* and fungi either did not photograph a red false color or were sporadic in their response. Plant-root tissue did not respond with a red color; accordingly, one could photograph bacterial cells on the surface of a legume root hair, or photograph *Rhizobium* cells as they penetrated as an infection thread down the center of the root hair (Fig. 1).

It is not known why this technique permits differentiation of bacterial cells from other materials, but one assumes that the physical-chemical conditions existing at the surface of the microbial cell, possibly including its curvature, are involved. Another consideration is that the cells are not photographed solely by infrared illumination, since long-wavelength visible light is still present after filtration, and the film is sensitive to this light. In this regard, one should also note that the focal point of the microscope is not recalibrated for the use of infrared film—a common procedure for infrared photography—and that the resolution at the film plane approaches that for conventional color-transparency film instead of presenting the low-resolution level that one might expect if only infrared were involved. Thus long-wavelength visible light may be a factor in the results observed by me.

As I have said, heat treatment of soil does not interfere with the infrared visualization of bacteria in this habitat; the same is true for soil treated overnight at refrigeration temperature with chloroform or carbon tetrachloride. However, treatment with toluene, which lyses bacterial cells, at times destroys the cells in soil so that they cannot be observed by this technique.

This technique presents interesting possibilities beyond its use for ecological studies of soils and comparable environments. The ability to see bacteria within plant tissue, without changing or harming the tissue, could find use in plant-disease relations. The same may be true for animal tissue, but this possibility has not been investigated. Lastly this technique may provide a means for detecting the presence of microbial life, whether living or dead, in extraterrestrial samples, either *in situ* or after they have reached Earth; in the latter instance the samples could first be autoclaved as a precaution before observation.

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References and Notes

1. H. J. Conn, *N.Y. State Agr. Exp. Sta. Tech. Bull. 64* (1918); Y. Seifert, *Pochvovedenie* **2**, 50 (1958); S. Strugger, *Can. J. Res. B* **26**, 188 (1948); S. Winogradsky, *Ann. Inst. Pasteur* **39**, 299 (1925).
2. Paper 3313 in the journal series of the Pennsylvania Agricultural Experiment Station.

8 November 1967

Viral Neoplastic Transformation of Hamster Prostate Tissue in vitro

Abstract. *Cultures of hamster prostatic tissues infected with simian virus 40 undergo transformation in vitro, and the transformed cells produce malignant tumors when injected into homologous hosts. Tartrate-inhibited acid phosphastase is found in the cultures of transformed cells and in the tumors they produce. Tartrate-inhibited acid phosphatase activity is elevated in the serum of tumor-bearing animals.*

The growth characteristics of certain mammalian tissues may be altered in vitro by a process referred to as "transformation," which is identified by increased acidity of the growth media, with accelerated growth of cells to form multiple layers and clumps. The transformation may be spontaneous (*1*), chemically induced (*2, 3*), or produced by infection with a variety of oncogenic viruses (*4, 5*). Transformation in vitro of murine prostatic tissues by methylcholanthrene was recently reported; the transformed cells produced a variety of histological types of tumors when implanted in syngeneic hosts (*3*). We now report transformation of hamster prostatic tissue by the oncogenic DNA simian virus 40 (SV40).

Prostatic tissue was obtained from 20 neonatal Syrian (golden) hamsters and separated into dorsal-, lateral-, and ventral-lobe portions. It was minced into 1-mm explants and then used to establish two 2-ounce (57-g) prescription-bottle cultures from each lobe by methods described (*5*). When the cells began to migrate from the explants on the 2nd and 3rd days, one culture bottle each of dorsal-, lateral-, and ventral-lobe tissue was infected with a $10^{7.9}$ $TCID_{50}$ (tissue-culture infective dose, 50-percent effective) of SV40. The second bottle of each tissue was kept as a virus-free control.

All cultures were incubated at 37°C and fed three times weekly with medium No. 199 containing 20 percent of fetal bovine serum. Cultures were observed daily for evidence of transformation characterized by accelerated growth of cells to form multiple layers, and by increased acidity of the growth medium. Evidence of transformation in the infected cultures was noted 5 to 8 days after infection. The control cultures were observed for 30 days without evidence of transformation. Infective SV40 could not be recovered from the transformed cells after they were grown

in direct contact with monolayer cultures of primary kidney cells of African-green monkey for 21 days. Immunofluorescence studies demonstrated the viral T antigen in the transformed cells.

Twenty-two days after infection, all bottles were subcultured by trypsinization and transferred to 32-ounce bottles. After 18 days of growth in the large bottles, the transformed cells were scraped from the bottles and injected subcutaneously into adult male hamsters that had received 400 r of whole-body irradiation 24 hours earlier. Each hamster received approximately 40 $\times 10^6$ cells.

All animals developed palpable tumors within 15 to 40 days. The tumors produced by cells derived from dorsal- and ventral-lobe tissue were palpable after 15 to 20 days, respectively, while the tumors derived from lateral-lobe tissue did not become palpable until the 37th day. Subsequently all tumors have been easily transplantable to unirradiated male hamsters and have been carried through 15 generations so far.

The tumors produced by the transformed ventral- or dorsal-lobe tissue were soft and hemorrhagic; they grew rapidly and killed 100 percent of the animals within 25 days. Histologic examination showed tumors to be composed of sheets of pleomorphic polygonal cells, with very little fibrous stroma. There was no evidence of epithelial differentiation, such as epithelial-type cells or gland formation (Fig. 1). The tumors produced by the transformed lateral-lobe tissue were firm and grew slowly; histologically they were fibrosarcomas composed of elongated cells and abundant collagen (Fig. 2). The tumors derived from each of the three lobes produced metastases which were usually found in the periaortic lymph nodes, although some animals also had pulmonary metastases.

Table 1. Determinations of acid phosphatase (AP) in SV40-transformed cell cultures and in tumors. Sigma-104 phosphate substrate (*p*-nitrophenol phosphate) was used for determinations of specific activity, the conditions of assay according with *Sigma Tech. Bull. 104.* SV40 HK, tissue of hamster kidney transformed in cell culture with SV40 virus.

Tissue	Specific activity in protein (Σ units mg^{-1} hr^{-1})		Percentage of total activity	
	Total AP	"Prostatic" AP	"Prostatic" AP	Nonspecific AP
	Tumors in vivo			
Dorsal lobe	2.0	1.553	77	23
Ventral lobe	0.862	0.564	66	34
Lateral lobe	2.6	2.32	89	11
SV40 HK	1.255	0.777	61	39
	Cultures in vitro			
Dorsal lobe	1.975	0.573	29	71
Ventral lobe	1.684	.496	29	71
Lateral lobe	2.245	1.429	64	36
SV40 HK	1.616	0.647	40	60

Ammonium sulfate precipitation techniques were used to isolate acid phosphatase. A considerable fraction of the phosphatase was inhibited by tartrate, an inhibition supposedly specific for acid phosphatase of prostatic origin (*6*) (Table 1). Tartrate-inhibited acid phosphatase (TIAP) fraction of the acid phosphatase activity was from 66 to 90 percent of the total for tumors of prostatic origin. Furthermore, the TIAP activity in serum of animals bearing the tumors was higher by from 65 to 500 percent than the norm in hamsters. However, acid phosphatase with a TIAP fraction was not found only in the tumors produced by the SV40-transformed prostatic tissue: tumors produced by SV40-transformed hamster kidney tissue also had acid phosphatase activity with a considerable TIAP fraction, but animals bearing such tumors had normal TIAP activity in the serum.

Freeze-thaw lysis of cells grown in vitro produced a protein-rich slurry which, when cleared by centrifugation, also had acid phosphatase activity (Table 1). The smaller percentage of TIAP activity found in the in vitro system may reflect the absence of endogenous hormone stimulation.

The presence of TIAP in the SV40-transformed cells derived from hamster prostate gland, in the tumors produced by these cells, and in the serum of tumor-bearing animals suggests the possible value of the system as a model for human prostatic carcinoma. The finding of TIAP in cells derived from nonprostatic tissues, such as the hamster-kidney tissue, indicates the need for further study of the relation of TIAP to the prostate gland and to the tumors derived from it and other tissues.

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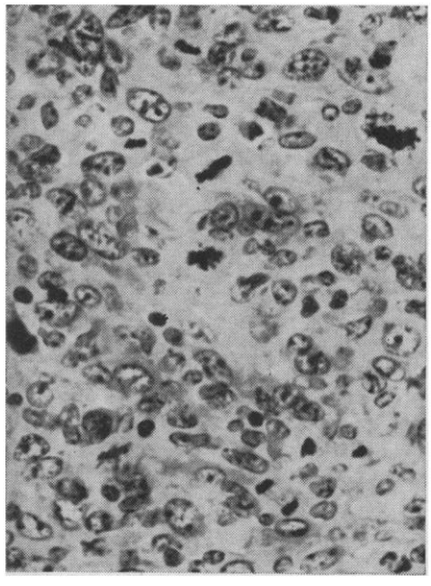

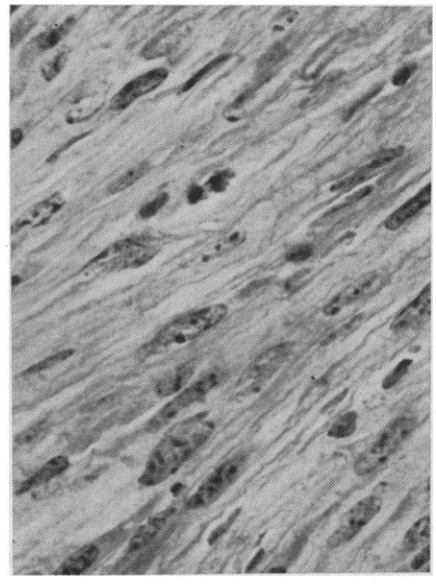

Fig. 1 (left). Tumor derived from transformed dorsal-lobe tissue; microscopically identical with tumor arising from transformed ventral-lobe tissue (× 460). Fig. 2 (right). Tumor derived from transformed lateral-lobe tissue (× 460).

References and Notes

1. K. K. Sanford, *Intern. Rev. Cytol.* **18**, 249 (1965).
2. I. Lasnitzki, *Brit. J. Cancer* **5**, 345 (1951).
3. C. Heidelberger and P. T. Iype, *Science* **155**, 214 (1966).
4. M. Vogt and R. Dulbecco, *Proc. Nat. Acad. Sci. U. S.* **46**, 365 (1960).
5. S. A. Wells, Jr., A. S. Rabson, R. A. Malmgren, A. S. Ketcham, *Cancer* **19**, 1411 (1966).
6. K. Jacobsson, *Scand. J. Clin. Lab. Invest.* **12**, 367 (1960).
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