as we have mentioned) in gaseous NH_3. [Some free sugars probably react to form the corresponding amino sugars (*12*).]

Other more complex biochemicals, such as proteins and higher polysaccharides, did not migrate. Glucose migrates rapidly; galactose, only slowly—a result much like that in liquid NH_3. Nucleosides such as adenosine and guanosine migrate readily, but the corresponding purines (adenine and guanine) give no indication of chromatographic movement. It is generally accepted that the notorious insolubility of these purines in liquids is due to strong intermolecular forces, mainly hydrogen bonding, between solute molecules (*13*); this idea is again confirmed by our observation that caffeine (1,3,7-trimethylxanthine) migrates in dense gaseous NH_3 but xanthine does not. In addition to these examples, high-molecular-weight polyethylene glycols, such as the gas-chromatography stationary phases Carbowax-4000 and Carbowax-20M, migrate readily in dense NH_3 gas.

Solution in liquid CO_2 is believed to involve primarily dispersion, quadrupole and acid-base interactions (*14*). The CO_2 molecule can form an acceptor-donor complex with the highly polarizable π-electrons of multiple bonds. The order of solubility is roughly the order of unsaturation. Thus we observe that the terpenes, corticol steroids, and sterols migrate in dense CO_2 gas with progressively less solubility; the highly conjugated carotenoids migrate readily at 170 atm, while the above steroids barely migrate at 1300 atm. Amino acids do not migrate at all.

The necessity for careful choice of the gaseous solvent to be used in dense gas chromatography is illustrated by the migration of nucleosides and related compounds in NH_3 and CO_2. We find, as expected, that NH_3 is the better of the two solvents for carbohydrates, although CO_2 provides some migration. Conversely, CO_2 is the better for the difficult purines, causing a slight but promising migration. The nucleosides migrate slightly in CO_2 just like their constituent carbohydrate and purine fragments. As we have mentioned, NH_3 readily promotes migration of the nucleosides.

Such examples stress the unique migrational characteristics of dense gases, especially for biomolecules. Migration has been emphasized because it is an essential prerequisite to separation. The fact that useful separations of moderately large biomolecules can be achieved has been demonstrated by the direct separation in CO_2 at 40°C of ergosterol, cholesterol, and lanosterol; of squalene and squalane; and of α- and β-carotene. In Fig. 3 we show the last separation on a 1.5-m, 15-percent Ucon column at 500 atm (Ucon begins to come off at about 700 atm). Because of the instability of carotenoids, this separation is impractical by ordinary gas chromatography. With continued improvement of system parameters and further inquiry into the nature of dense-gas solubility and transport phenomena, dense-gas chromatography will probably be able to effect increasingly difficult separations.

LILIAN MCLAREN
MARCUS N. MYERS
J. CALVIN GIDDINGS
Department of Chemistry,
University of Utah,
Salt Lake City 84112

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Infrared Color Photography: Selective Demonstration of Bacteria

Abstract. *A microscopic procedure employs infrared color photography to enable visualization of unstained microorganisms in natural habitats. The procedure can be used for either living or dead microorganisms; it enables visualization of microbial cells embedded within component materials of the habitat.*

Visualization *in situ* of indigenous microorganisms in natural habitats, such as soil or marine muds, has proved difficult because of the inability of the microscopist to distinguish between microorganisms and colloidal humic materials and clay particles of similar size and shape; and stains often accumulate on habitat surfaces so as to preclude visualization of embedded microorganisms. These statements apply regardless of whether unstained soil is observed by phase microscopy, or stained soil is observed by light microscopy (*1*). These difficulties can now be overcome by use of Kodak Ektachrome Infrared Aero film, type 8443, to photograph the unstained organisms as wet mounts in their natural habitats.

The material to be examined is either suspended in water on a microscope slide and covered with a cover slip, or first allowed to dry on a microscope slide before resuspension and addition of the cover slip. The latter procedure can be used to decrease microbial motility. The slide is placed on the stage of a microscope having apochromatic objectives and a 12-volt conventional microscope light source; a green photographic filter is placed in the light path beneath the condenser. The condenser is lowered and the light intensity is decreased so that focusing can be accomplished on particulate matter on the microscope slide, although individual bacterial cells often cannot be seen because of the absence of a stain. An area of interest on the slide is brought into focus, the condenser is raised to a position just beneath the slide, and, in place of the green filter, either a yellow Tiffon No. 12 or a medium-red 25A Ednalite filter is placed in the light path below the condenser. The light output is then increased to 5 amp, and photographs are taken at 0.01 second, a speed fast enough for photography of moving microbial cells. The viewing magnification is 1560, while magnification at the film plane is slightly greater than 500. The film is processed with Kodak Ektachrome E-3 or E-4 chemicals without infrared inspection.

Bacteria in the resultant color transparencies appear in red "false" color, while all other inorganic and organic

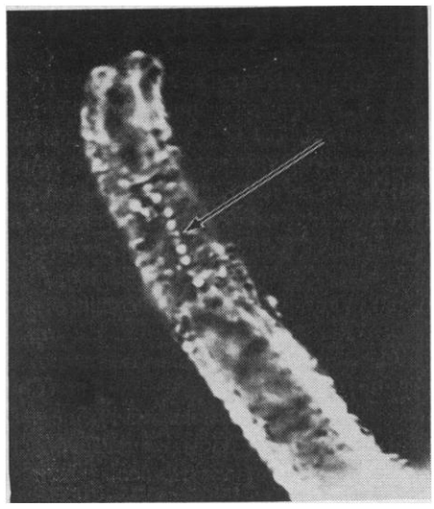

Fig. 1. *Rhizobium* infection thread progressing through the interior of a living, unstained clover root hair; photographed with a yellow Tiffon No. 12 filter on Kodak Ektachrome Infrared Aero Film, type 8443; × 410 at the film plane. The color of the background and of the root-hair protoplasm is light 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.

L. E. CASIDA, JR.

Department of Microbiology, Pennsylvania State University, University Park 16802

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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