paper chromatograms. The partially purified inhibitor reduced germination 50 percent at a concentration of 2 μg/ml. We report here the isolation and identification of two germination inhibitors from water extracts of bean rust uredospores which have the properties of the self-inhibitors described by Bell and Daly (*3*).

Inhibitors were extracted from uredospores of the bean rust fungus *Uromyces phaseoli* (Pers.) Wint. by stirring in water (1 liter for every 10 g of uredospores). After filtration through sintered glass to remove the spores, the inhibitors were partitioned into diethyl-ether and taken to dryness on a rotary evaporator. The residue was then taken up in a small volume of ether, spotted onto thin-layer plates of silica gel, and chromatographed in a solvent consisting of benzene and ether (80 : 20 by volume). After extraction from silica gel with ether, the preparation was dried and sublimed under vacuum in a short path (5 mm) apparatus onto a condenser cooled with Dry Ice in acetone.

The active zone of inhibitor occurred on the silica-gel plates as a single ultraviolet fluorescent band at R_F 0.6 and was located by testing water extracts of 1-cm zones for the capacity to inhibit uredospore germination. Potency of the inhibitor was assayed at each step in the purification process by similar procedures.

The mass spectrum of the inhibitor showed the following diagnostic peaks [mass to charge (*m/e*); parent peak (P)]: 222 (P, base peak); 207 (P-CH_3); 191 (P-OCH_3); and 163 (P-$COOCH_3$). In addition, there were fragments at *m/e* 119, 105, 91, 77, and 39, all indicative of aromaticity. The infrared spectrum had absorption peaks at 1709 and 1692 cm^{-1} (ester carbonyl); 1590 and 1512 cm^{-1} (aromatic); 1305 cm^{-1} (methoxy); and 985 cm^{-1} (*trans*). The ultraviolet absorption spectra of the inhibitor dissolved in methanol had absorption maximums at 320, 291, and 232 nm, but the small quantities available prevented determination of extinction coefficients. The spectral data suggested that the inhibitor was the methyl ester of a dimethoxycinnamic acid. Comparison of the spectra of each of the six dimethoxycinnamate isomers clearly showed that methyl 3,4-dimethoxycinnamate was the only possible candidate.

While the masses and relative abundance of the various fragments in the mass spectrum of synthetic methyl *trans*-3,4-dimethoxycinnamate were identical to those of the unknown, their infrared spectra were inconsistent at several points unless the synthetic compound was first dissolved in methanol and irradiated for 3 hours with ultraviolet light at 254 nm. After irradiation of the synthetic compound, the infrared spectrum was identical to that of the inhibitor. This phenomenon and the appearance of a double carbonyl peak in the infrared spectrum suggested that some of the pure synthetic compound was converted to a second form during irradiation. Consequently, a further study of the inhibitor was carried out by gas chromatography with columns of Convalex-10 (5 percent on Chromosorb W) and by thin-layer chromatography on plates of cellulose irrigated with water.

As originally demonstrated by Bell and Daly (*3*), it was found that the inhibitor was composed of two compounds. These compounds had gas-chromatographic retention times (3.2 and 6.0 minutes) and R_F values (0.3 and 0.6) on cellulose plates that were identical to the components in the mixture obtained by irradiation of the synthetic compound. Each of these compounds could be converted to the other by irradiation with ultraviolet light, and the mass spectra of the isolated compounds prepared by the gas chromatography from the native inhibitor were identical to each other and to the two isomers prepared from irradiated synthetic methyl 3,4-dimethoxycinnamate (*4*).

The infrared spectrum and the melting point of 68°C (*5*) showed that the original synthetic methyl 3,4-dimethoxycinnamate was the *trans* isomer. After irradiation of this compound in methanol with ultraviolet light, the new compound which was produced was resolved from the *trans* isomer by gas chromatography. This new compound had a melting point of 91°C, which was reported previously for the *cis* isomer to be 92° to 93°C (*5*). In addition, the infrared spectrum of this compound lacked the absorption peak at 985 cm^{-1} (*trans*) and had a single carbonyl peak at 1709 cm^{-1}. An equimolar mixture of these two isomers provided an infrared spectrum which was identical to that of the inhibitor, and it is concluded that the inhibitor from bean rust uredospores is a mixture of methyl *cis*- and *trans*-3,4-dimethoxycinnamate.

The extinction coefficient ($E_{320}^{1\%}$) of methyl *trans*-3,4-dimethoxycinnamate was 790 in absolute methanol. With this value, it was determined that approximately 4 μg of native inhibitor can be extracted from each gram of spores by our procedures. Each stereoisomer is equally toxic, and germination of 50 percent of the spores (ED_{50}) is inhibited by 5×10^{-3} μg/ml. The ED_{50} values were determined from a plot of the probit of percent inhibition against log concentration. The native inhibitor and the synthetic esters were equally toxic to spore germination, since their ED_{50} values were identical.

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3 August 1970; revised 10 September 1970

Spontaneous in vitro Neoplastic Transformation of Adult Human Prostatic Epithelium

Abstract. *The possible significance of spontaneous transformation of epithelium from a benign prostatic adenoma containing glandular hyperplasia is discussed.*

A variety of normal human tissues have been propagated in tissue culture, but most, with the exception of hematopoietic tissue, have a limited life span in vitro. Hayflick (*1*) postulates that this finite life span reflects a rapid "aging" process that culminates in cell death. However, it is well known that both animal and human cells will survive indefinitely in culture if they become "transformed." Transformation is a rather loosely defined, irreversible cellular alteration mainly characterized by rapid cell growth, neoplastic morphol-

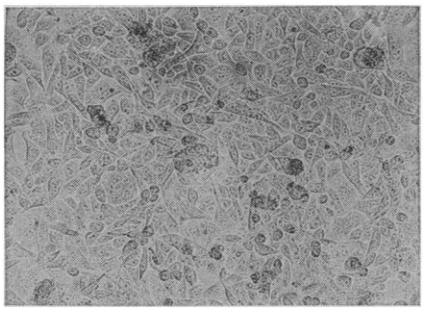

Fig. 1. MA 160 cells in tissue culture. MA 160, passage 49, appears epithelial. Individual cells have a bizarre morphology with a large nucleus or nuclei. Loss of contact inhibition and piling up of cells is shown (×80).

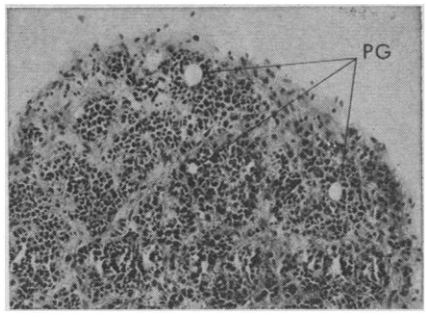

Fig. 2. Section through hamster cheek pouch tumor. Tumor was produced by injection of cell suspensions of MA 160 as detailed herein. Anaplastic nature of tumor and pseudo-gland (*PG*) formation is seen (×40).

ogy, heteroploidy, and the ability of the transformed cells to survive and produce tumors in autologous hosts. Transformation can be caused by specific agents or it may occur spontaneously. Although a variety of human cells have been transformed by viruses and chemicals, spontaneous in vitro transformation of normal adult tissues has occurred infrequently (for review see *2*).

In our investigation, a portion of benign prostatic adenoma removed at surgery was minced into fragments of approximately 2 mm on edge and explanted on glass in Eagle's minimum essential medium supplemented with 0.1 mmole of nonessential amino acids, 1 mmole of sodium pyruvate (*3*), 10 percent fetal bovine serum, and antibiotics (100 units of penicillin and 100 μg of streptomycin per milliliter). While both fibroblast-like and epithelial-like cells migrated from explants, the epithelial-like cells predominated and the fibroblasts were not apparent after the third passage. When the primary cultures developed monolayer confluency, they were washed with disodium versene (0.02 percent) and the cell sheet was dissociated with a mixture of equal parts of versene (0.02 percent) and pancreatin (0.25 percent). From the date of explanation to passage 3 the elapsed time was 7 weeks; the elapsed time for the next 3 passages was 3 weeks. By passage 35 the interval between passages was 4 days. While the reference line has been continuously cultured on Eagle's minimum essential medium (with supplements named above), cultures have also been maintained with RPMI 1640 medium supplemented with 10 percent fetal calf serum (*4*). The cells adapt easily to suspension cultures with population levels of 1×10^6 cell/ml in Eagle's minimum essential medium for suspension cultures supplemented with 5 percent fetal calf serum. This cell line has been carried through more than 200 passages during the past 4 years and has been designated MA 160 (*5*).

Histological examination of the prostate gland from which the cell line was derived showed marked glandular hyperplasia throughout. There were some areas of squamous metaplasia but multiple sections failed to show malignancy. The patient has not developed any clinical signs of cancer 4 years postoperatively.

Cells of the MA 160 line have an epithelial appearance in tissue culture (Fig. 1). The fine structure of the MA 160 cell is characterized by a well-developed rough endoplasmic reticulum, many microvilli, and desmosomes. Thus, the electron microscopic findings are consistent with a secretory cell or prostatic epithelium (*6*).

The karyotype of MA 160, passage 24, had a modal number of 64, which is triploid for human cells, whose normal diploid number is 46. There is a second peak in the polypoloid range of 123 to 137. Extra chromosomes appeared to be random additions. Cytogenetic studies from passage 10 revealed a Y chromosome.

Suspensions containing between 10^6 and 10^7 cells derived from passages 45, 49, 82, and 85 were injected both into the cheek pouch and subcutaneously in neonatal hamsters that previously had received 500 r of whole-body radiation. Solid tumors usually appeared within 5 days in both sites and continued to grow for approximately 2 weeks, after which time they were rejected if the animals received no additonal immunesuppression. The tumors were composed of anaplastic, epithelioid cells; pseudo-gland formation frequently was seen within the tumors (Fig. 2). Invasion of surrounding connective tissues and nerves was observed, although distant metastases did not occur.

Cultures for mycoplasma have been negative and no mycoplasma have been seen with the electron microscope in cells carried through over 100 subcultures. Furthermore, multiple attempts to demonstrate viruses with the electron microscope, by cocultivation experiments, and by inoculating a variety of human cell lines with cell-free filtrates from MA 160 have been unsuccessful.

The MA 160 cell does not contain more tartrate-inhibitable acid phosphatase (the so-called "prostatic" fraction) than other cells in tissue culture (*7*). Furthermore, when these cells are grown in high concentrations of zinc alone or with zinc and testosterone they do not increase their intracellular zinc more than other cell lines such as WI 38 (*7*). Therefore, we have been unable to show that these cells have any specific biochemical markers that would help to identify them as prostatic epithelium.

Gartler (*8*) recently has reported that many heteroploid human cell lines are contaminated with HeLa cells. He bases this argument on finding a rapidly migrating (A) component on electrophoresis of intracellular glucose-6-diphosphatase (G6DP) from a wide variety of human cells in culture. This fast or A component of G6DP is supposedly found only in cells from Negroes. The MA 160 cell also contains rapidly migrating G6DP (*8*) even though it was derived from the prostate of an adult caucasian male. There are at least three explanations for this finding. First, it is possible that the patient has Negro ancestry. Second, the A component of G6DP may eventually be found to be present in all cells carried long-term in vitro. In other words, the presence of this enzyme may not be as specific a marker as has been thought. Third, of course, it is possible that this cell line became contaminated with HeLa cells.

However, there are additional findings which argue against these cells being HeLa. First, it is possible to distinguish fixed preparations of MA 160 from several strains of HeLa cells with the light microscope. MA 160 cells are larger and show more bizarre morphology, including a tendency to form multinucleated giant cells. Second, the karyotype of MA 160 is different than that previously reported for several different strains of HeLa (*9*); that is, although MA 160 is also heteroploid there is, as previously mentioned, a sharp distribution in two modes. Also,

the presence of a Y chromosome suggests that these cells were derived from a male. Third, the ultrastructure of MA 160 differs from that of HeLa cells. The well-developed rough endoplasmic reticulum and the presence of desmosomes are findings that generally have not been found in HeLa cells (*10*). Fourth, Robertson (*11*) has found differences in the alkaline phosphatase content between MA 160 and HeLa cells. Fifth, the fact that these cells form glandlike structure in the cheek pouch and subcutaneous tissues of immune-suppressed hamsters supports their being derived from prostatic epithelium. HeLa cells also produce solid tumors under similar conditions but pseudo-gland formation has not been reported. Attempts by us to demonstrate pseudo-gland formation in tumors produced by the injection of suspensions of HeLa cells into the cheek pouch of immune-suppressed hamsters were unsuccessful.

Almost nothing is known about the etiology and natural history of carcinoma of the prostate in man. For example, there is considerable debate as to whether cancer of the prostate arises de nova from normal prostatic cells or whether these tumors develop gradually with benign hyperplastic epithelium as an intermediate stage. Previously it was not possible to study the biology of single human prostatic cells since this tissue had not been grown as a monolayer culture. Recently, however, not only MA 160 but also other benign and malignant human prostatic tissue has been propagated as cell cultures and cell suspensions (*7*). If, when these techniques are used, epithelium from benign prostatic adenomas consistently undergoes spontaneous in vitro neoplastic transformation it would suggest that these cells are premalignant. Further work is needed to resolve this question as a basis for further research in this area.

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30 July 1970

Sex Pheromones of the Southern Armyworm Moth: Isolation, Identification, and Synthesis

Abstract. *Two sex pheromones have been isolated from the female southern armyworm moth,* Prodenia eridania *(Cramer), and identified as* cis-*9-tetradecen-1-ol acetate, identical with the sex pheromone of the fall armyworm moth,* Spodoptera frugiperda *(J. E. Smith), and* cis-*9,*trans-*12-tetradecadien-1-ol acetate.*

The larval stage of the southern armyworm moth, *Prodenia eridania* (Cramer), is a climbing cutworm which is an occasional pest of vegetable crops in the South (*1*). In 1962, Butt (*2*) showed that the female southern armyworm moth possesses a sex pheromone for males which is apparently produced in the tips of the abdomen; extraction of the tips with ethanol or methylene chloride removed the attractant and gave an active extract. He also reported that the adult males respond to the attractant only during night hours.

Southern armyworms reared in the laboratory at Brownsville, Texas, are sexed in the pupal stage and placed in separate cages. Virgin females are used as a source of the sex pheromone and males are used in bioassay (*3–5*). Techniques were developed to assay for the presence of the pheromone (*6*). The most satisfactory method of bioassay is to subject caged male moths, under photographic darkroom conditions, to the vapors expelled from a glass pipette treated with the test material. The males readily respond with a characteristic dance when the test material is active. The isolation procedure described herein was monitored by means of this test.

Abdomens (132,000) of virgin female moths were originally extracted with ethanol, but ethyl esters, apparently formed during extraction, made subsequent purification steps difficult. Subsequently 177,000 more abdomens were extracted with methylene chloride or hexane. The extract was dissolved in 20 volumes of acetone, and the solution was kept overnight at −10°C. The acetone, containing a large amount of inactive, precipitated white solid was filtered rapidly through a cold Büchner funnel. After being washed with cold acetone, the solid was dissolved, precipitated, filtered, and washed twice more. The combined mother liquors and washings were freed of solvent at 20 mm-Hg (bath below 40°C). At this point, the material originally extracted with ethanol, and contaminated with ethyl esters, was saponified with alcoholic KOH, and the inactive neutral material obtained therefrom was acetylated with acetyl chloride to give additional active material that was handled separately.

All active material was subjected to the sweep codistillation technique developed by Storherr *et al.* (*7*) for the cleanup of oils in the determination of pesticides. The procedure consisted essentially of subjecting a heated sample, coated on glass beads, to a flow of nitrogen and an intermittent flow of hexane, which removed the active material from the bulk of the sample. Active material thus obtained was chromatographed on a column of Florisil (*8*) and eluted successively with hexane and then with 3 percent ethyl ether in hexane. The latter solvent removed the active material, which was then chromatographed on a column of silica gel impregnated with silver nitrate (*9*) and eluted successively with hexane and 5, 10, and 25 percent ethyl ether in hexane. The latter two eluates, which contained all the activity, were combined and subjected to preparative gas chromatography on an OV-1 column (*10*). The active material emerged from the column as a single peak with a retention time of 5 minutes. It was subjected to preparative gas chromatography on a diethylene glycol succinate (DEGS) column (*11*), giving two active components with retention times of 9.4 minutes (compound **I**) and 12.4 minutes (compound **II**).

In approximately 20 percent of the