A Culture Strain (LAC) of Human Epithelial-Like Cells from an Adenocarcinoma of the Lung. (23524)

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A number of tissue culture cell lines derived from human tissues have been reported (1,4). Only two of these cell lines have been isolated from patients with carcinoma of the lung and in neither case was the isolation made directly from the primary tumor. One, the "Maben" cell, was derived from pleural fluid of a patient with an adenocarcinoma of the lung(3), and the other, the Detroit 6 cell, originated from the bone marrow of a patient with lung cancer(5). The purpose of this report is to describe the isolation, maintenance, morphological characteristics and some of the viral susceptibilities of a cell line (strain LAC) derived from a human adenocarcinoma of the lung.

History of cell strain. A right pneumonectomy was performed on a 55-year-old white man with an adenocarcinoma of upper lobe of the right lung on Oct. 19, 1955. Histologically the tumor was composed of large areas of undifferentiated carcinoma (Fig. 1) as well as areas of well differentiated adenocarcinoma (Fig. 2). Mucin secretion by tumor cells was demonstrable with Mucicarmine, the Rhinehart stain, and the periodic-acid Schiff reaction after diastase digestion.

Some of the tumor was treated with trypsin solution according to the method of Youngner(6). Another portion was finely minced and suspended in Eagle's basal medium without trypsin digestion. Both cell preparations were then centrifuged and resuspended in a medium consisting of 40% human serum and 60% Eagle's basal medium. Aliquots were placed in 2-oz. prescription bottles.

Results. Within 2 days, groups of cells were evident on the glass surfaces in all cultures. The cells were elongated and pleomorphic and were fibroblast-like in appearance. They had elongated nuclei with prominent nucleoli (Fig. 3). Scattered through the cultures were groups of larger irregularly shaped cells with nuclei containing coarse chromatin granules and large nucleoli which frequently had a peri-nucleolar halo (Fig. 4). Cell growth was not rapid and only 6 subcultures were made over an 11-week period. Small colonies of epithelial-like cells were first noted in the 6th subculture. These cells rapidly outgrew the slowly multiplying fibroblast-like cells. In Fig. 5, a culture of these epithelial-like cells is seen with a large atypical mitotic figure in center of field. Nuclear pleomorphism and hyperchromatism are evident with several small irregularly shaped nucleoli present. Phagocytosis of degenerating cells by other cells may be seen.

Four cultures of the epithelial-like cells were preserved by freezing so that they would be available if the cell line were lost. Preservation of cell cultures was done using a modification of established technics(7-9). Glycerol, USP, (sterilized by autoclaving at 10 lb pressure for 15 minutes) was added to make a 15% final concentration of glycerol. The stopper was secured to the 2-oz. culture bottle with masking tape and the cells under the glycerol-containing medium were allowed to freeze slowly in a solid carbon dioxide storage chest at approximately −70°C. For use, the culture was rapidly thawed in a 37°C water bath with resulting loss of attachment of the cells to the glass surface. The cells were then sedimented at 500 rpm for 10 minutes in the International, size 2, centrifuge, the supernatant decanted, the cells resuspended in fresh growth medium in a 2-oz. culture bottle and incubated at 36°C. Cell multiplication was evident within 24 hours. It was estimated that ¼ to ½ of the cells survived such prolonged storage.* Shortly thereafter all active cultures of the LAC cell were lost due to a combination of poor cul-

* Other tissue culture cell lines have been preserved in this manner (HeLa (Gey), D189 (Leighton), J96 (Osgood), monkey kidney) for as long as 18 months.

tural conditions and bacterial contamination.

A culture which had been stored frozen for 3 months was thawed and cultivation was resumed. Subsequently the cell line has maintained continuous rapid proliferation for over 14 months. 0.25% trypsin solution was used to suspend cells for passage. The human serum content of the growth medium has been

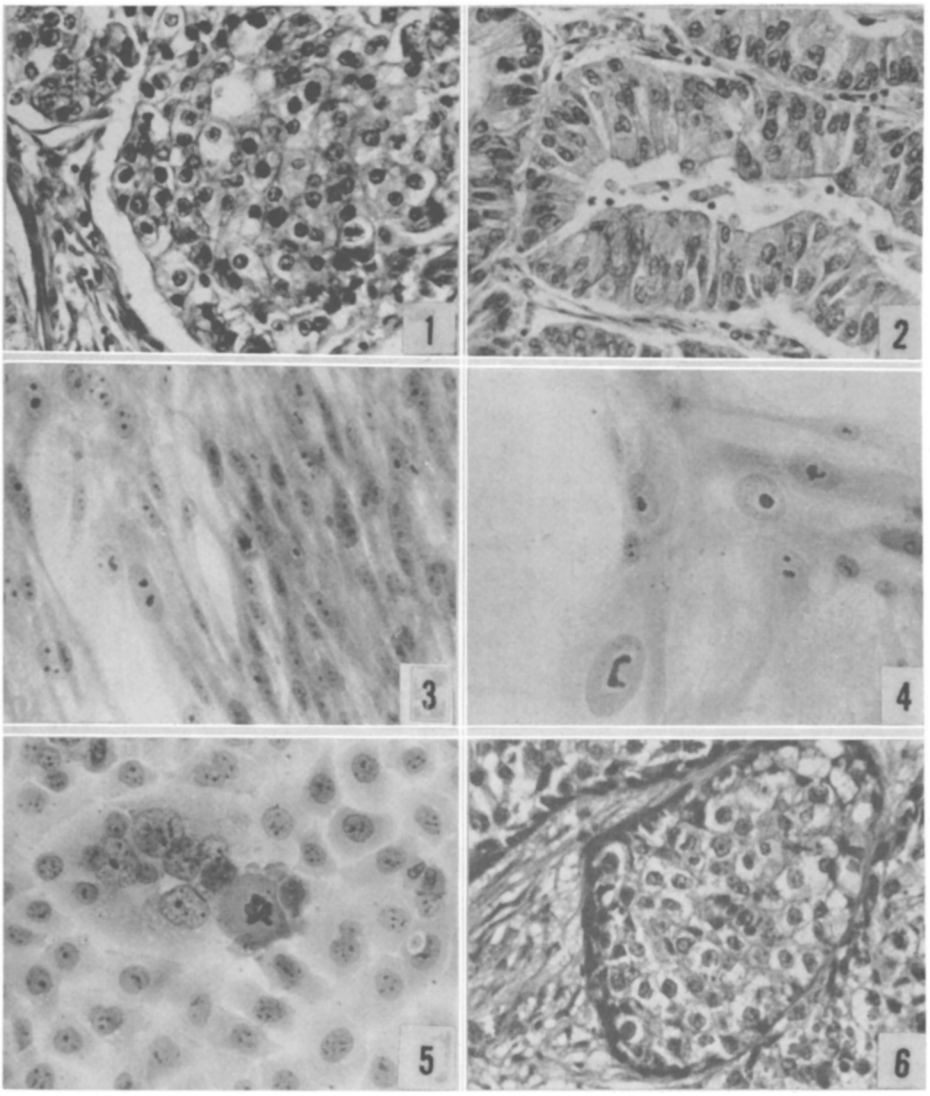

PLATE 1. All photomicrographs are taken at 420 × magnification.

FIG. 1. Section of primary carcinoma of lung in an undifferentiated area. (Hematoxylin & Eosin).

FIG. 2. Section of primary carcinoma of lung with mucin-secreting columnar cells resembling bronchial type epithelium. (H & E).

FIG. 3. Fibroblast-like cells in early tissue culture passages of carcinoma of lung. (Papanicolau stain).

FIG. 4. Large irregularly shaped cells in early tissue culture passages of carcinoma of lung. (Papanicolau stain).

FIG. 5. Epithelial-like cells in later tissue culture passages of carcinoma of lung. Note large atypical mitotic figure near center of field. (Papanicolau stain).

FIG. 6. Tumor in subcutaneous tissue of cortisone-treated irradiated rat after injection of LAC cells. Note resemblance to primary tumor in upper left. (H & E).

TABLE I. Virus Susceptibility of LAC Cell.

Virus	Maintenance medium*	Passages	Cytopathogenesis§	Remarks
Poliovirus—Type I	BME† + 10% ES‡	5	+	
Coxsackie B2	*Idem*	5	+	
" A9	"	1	0	
Vaccinia	"	5	+	
Herpes simplex	"	3	+	Isolated directly from human "cold sore"
Sendai	"	5	0	Cytopathogenesis on 1st passage only
Yellow fever	BME + 4% ES		0	

* Before maintenance medium was added, tubes were rinsed 3 times with serum-free BME.
† Basal Medium Eagle.
‡ Horse serum.
§ Titrations of infectivity were not performed.

decreased to 10% with undiminished rapidity of growth.

Transplantation to rats. It has been possible to transplant the cell line to cortisone-treated irradiated rats both subcutaneously and intraperitoneally with production of tumor nodules(11,12). In the sections of these nodules in the rat, collections of epithelial cells with morphologic features of an undifferentiated carcinoma are seen (Fig. 6). These are similar to the undifferentiated areas in the primary tumor (Fig. 1). In this regard it is of interest that intracellular mucin has been found in these tumor nodules in the rat, and the mucin has the same staining reactions as mucin found in the well-differentiated portions of the original tumor from which the cell line has been derived. It should be noted that tumor nodules with a similar histologic appearance and with intracellular mucin-like material have been produced in rats after injection of HeLa cells.

Susceptibility to viruses. Cultivation of a number of viruses on the LAC cells was attempted. The viruses used included Mahoney strain of Type I poliovirus, Coxsackie A9 and B2†, monkey kidney adapted vaccinia virus, Sendai virus, and the yellow fever virus (17D vaccine strain). Since this part of the study is continuing, a preliminary summary is presented in Table I.

† Coxsackie A9 and B2 viruses were obtained from Dr. Alexis I. Shelokov, monkey kidney adapted vaccinia virus was obtained from Dr. Joel Warren, and Sendai virus was obtained from Dr. Kenneth K. Takemoto.

Discussion. The histogenetic concepts of the derivation of epithelial-like cell strains and transformations of fibroblast-like cells to epithelial-like cells in tissue culture have been discussed in considerable detail recently(1,10, 13) and will not be considered in this report.

A new tissue culture cell line is in some ways analogous to a new bacteriologic medium used for the study of previously uncultured microorganisms. Such cell strains might support and indicate the growth of as yet undetected viruses. The use of a tissue culture cell derived from bronchial type epithelium would seem to be of special value in attempts to isolate agents from respiratory diseases. The viral spectrum of the LAC cell (Table I) is as yet not sufficiently complete to allow final evaluation of the usefulness of the cell; however, further viral studies are now in progress.

Summary. 1) Isolation, maintenance, and morphological features of a cell strain (LAC) derived from a human adenocarcinoma of the lung are described. 2) Preliminary viral studies indicate that the LAC cell is susceptible to poliovirus, Coxsackie B2 virus, vaccinia virus and herpes simplex virus.

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1. Berman, L., and Stulberg, C. S., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 730.
2. Eagle, H., *ibid.*, 1955, v89, 362.
3. Frisch, A. W., Jentoft, V., Barger, R., and Losli, E. J., *Am. J. Clin. Path.*, 1955, v25, 1107.
4. Jordan, W. S., PROC. SOC. EXP. BIOL. AND MED.,

1956, v92, 867.

5. Berman, L., Stulberg, C. S., and Ruddle, F. H., *Blood,* 1955, v10, 896.

6. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.

7. Scherer, W. F., and Hoogasian, A. C., *ibid.,* 1954, v87, 480.

8. Merryman, H. J., *Science,* 1956, v124, 515.

9. Polge, C., Smith, A. U., and Parkes, A. S., *Nature,* 1949, v164, 666.

10. Leighton, J., Kline, I., and Orr, H. C., *Science,* 1956, v123, 502.

11. Toolan, H. W., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 572.

12. Suskind, R. G., Huebner, R. J., Rowe, W. P., and Love, R., *ibid.,* 1957, v94, 309.

13. Westwood, J. C. N., Macpherson, I. A., and Titmus, D. H. J., *Brit. J. Exp. Path.,* 1957, v38, 138.

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Effect of Glucagon on Gastric Secretion. (23525)

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Zollinger and Ellison(1) recently have described a group of patients with progressive recurrent benign peptic ulcerations of the duodenum and upper jejunum associated with non-beta cell (presumably alpha cell) adenomas of the pancreas. These patients characteristically have a very high rate of gastric secretion and resistance to medical and surgical therapy. Although some of these patients have coexisting multiple adenomatosis of other endocrine glands(2) there is no clinical or laboratory evidence of hyperinsulinism. Since glucagon is secreted by pancreatic islet alpha cells which resemble the tumor cell type, Zollinger and Ellison suggested that glucagon might be the ulcerogenic factor. This study was designed to determine the effect of glucagon on gastric motility and secretion and thus to investigate(3) the likelihood of this substance being of pathogenic significance in the peptic ulceration associated with these bizarre pancreatic tumors.

Materials and methods. Gastric secretion: Nine adult males (age 29 to 59 years) were used; 4 of these patients had proven duodenal ulcer; 3 were diagnosed as psychophysiologic gastrointestinal reactions; and the remaining 2 patients were hospitalized for minor surgical problems unrelated to the gastrointestinal tract. Soon after awakening in the morning after a 12-hour fast, the patient was placed at bed rest for approximately 30 minutes before the experiment was begun. A No. 16 Levine tube was placed in the stomach and connected to a constant source of suction (100 mm Hg) with an intervening trap for collection of gastric secretions. The stomach was emptied and the initial specimen discarded. Gastric aspiration specimens were collected at 15-minute intervals during a one-hour control period, at the conclusion of which 1.5 mg of glucagon* in 10 cc of saline was rapidly injected by way of a previously established intravenous saline infusion in the forearm, and collections continued as before. The test period was continued for a maximum of 2 hours longer, or until the post glucagon infusion secretory rate approached the pre-infusion control. Venous blood samples were drawn without stasis from an indwelling needle in the antecubital vein and analyzed in duplicate. Capillary blood was drawn from a finger puncture. Glucose concentrations were determined by the Nelson-Somogyi method(4). The volume and free hydrochloric acid content was determined on each 15-minute sample(5). Gastric motility studies were performed on a series of 8 patients, 4 of whom had proven duodenal ulcers, the other 4 serving as normal controls. Motility was measured by an intragastric balloon attached to a recording device as designed by Hlad. As with the secretory studies, a one-hour pre-glucagon injection period was

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