A Human Cell Line From a Pleural Effusion Derived From a Breast Carcinoma[1,2]

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SUMMARY—A primary culture, 734B, was grown from a pleural effusion of a patient with metastatic mammary carcinoma. This culture continually produced free-floating cells used to initiate serial passages that could be transferred at 25-day intervals. A stable cell line, MCF-7, was derived from the free-floating cells and has been maintained for over 90 weekly passages. MCF-7 and 734B retained several characteristics of differentiated mammary epithelium, including the cytoplasmic estrogen receptor and the capability of forming domes. Four lines of evidence attest to the human origin of the cells.—J Natl Cancer Inst 51: 1409–1416, 1973.

ATTEMPTS to cultivate long-term differentiated epithelial cell cultures from human breast tumors have met with only limited success (*1–6*). Three distinct difficulties in establishing such cultures are: 1) rapid overgrowth by connective tissue, 2) initial multiplication followed by death of tumor cells in primary cultures, and 3) failure of tumor cells to survive serial transfers (*2, 5–10*).

This report describes the cultivation of a differentiated, epithelial-like cell culture (*11*) derived from a pleural effusion of a patient with metastatic breast carcinoma. Free-floating cells of the primary culture were used to initiate a stable cell line. Both primary and stable cell lines have been maintained to date (3 yr).

MATERIALS AND METHODS

Nodules.—A 69-year-old female (Caucasian, blood type O, Rh positive) underwent a mastectomy of her right breast for a benign tumor and a radical mastectomy of her left breast for a malignant mammary adenocarcinoma 7 and 3 years, respectively, before initiation of the primary culture. Local recurrences appeared on the left chest wall immediately after postoperative radiotherapy. These recurrences were controlled for 3 years by radiotherapy and hormotherapy. In June 1970, widespread nodular and erythematous pattern of recurrences spread over the left anterior chest wall. Nodules were removed and placed in culture; the spilling technique (*1*) and the collagenase treatment (*12*) were used. In addition, 4 plasma clot cultures (*3*) were made.

Pleural effusion.—Two months after removal of the nodules from the chest wall, the patient developed a pleural effusion. A small clot in the effusion was fixed in neutral buffered formalin and embedded in paraffin; sections were stained with hematoxylin and eosin. The cells in the fluid were washed twice (800× *g*) and placed in culture at twofold dilutions of the packed cell volume (1:200–1:800). Two 4-ounce glass bottles and one T25 flask (Falcon) were seeded at each dilution.

Cell culture.—Cells were cultured in Eagle's minimal essential medium and nonessential amino acids with 20 μg insulin/ml prepared in either Hanks' or Earle's salt solution. All media contained 250 U penicillin and 250 μg streptomycin/ml and were supplemented with 20% calf serum; after 4 months, 10% calf serum was used for the primary cultures. Medium was replenished 3 times weekly (50–90%) in cultures not transferred weekly. For cells transferred weekly, medium was replaced on days 3 and 6. Trypsin (0.025% for 5 min) was used for transferring cultures. Free-floating passages were initiated by centrifuging the cells in the supernatant at 210×*g* and resuspending with medium. Coverslips in Leighton tubes were seeded with 250,000 cells for morphologic studies. Chromosome preparations were made of early free-floating passages 2–5 at 202–213 days, and trypsin passages 2 and 39 after 587 days in vitro. Cultures were incubated with colchicine at a final concentration of 0.004% for 4–5 hours. Metaphase plates were prepared according to the procedure of Moorehead et al. (*13*) and stained with dilute Giemsa.

The species of origin for MCF-7 was examined by 2 other methods: 1) cell-surface antigenicity evaluated by membrane immunofluorescence reactivity with species-specific antisera[5] (*14*) and 2) molecular weight determinations for the 28S ribosomal RNA (*15*). RNA was prepared from MCF-7 cell suspensions by treatment with 1% sodium dodecyl sulfate (SDS) in 0.01M Tris-HCl, 0.15M NaCl, 0.001M ethylenediaminetetraacetate (TNE) buffer, pH 7.4, to which 10 μg/ml dextran sulfate was added. Extraction was performed with a 1:1 phenol:cresol mixture. After ethanol precipitation, the RNA was electrophoresed in 3.6% acrylamide gels containing 0.5% agarose (*16*).

Mycoplasma contamination of MCF-7 cells was tested by standard culture methods[6] as well as autoradiographically, with silver grain distribution over cells evaluated after a 4-hour ^{3}H-thymidine labeling interval (*17*).

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[3] Michigan Cancer Foundation, 110 East Warren Avenue, Detroit, Mich. 48201.

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[5] Tests graciously performed by Dr. C. S. Stulburg, The Child Research Center of Michigan, Detroit, Mich.

[6] Tests kindly performed by Dr. L. Hayflick, Department of Medical Microbiology, Stanford University, Stanford, Calif.

Specimen number 734B was assigned to the pleural effusion and this designation has been maintained for the primary and the first 25 passages derived from this effusion. After a year of serial trypsin passages, MCF-7 denoted the stable cell line.

RESULTS

Nodules

Fibroblasts overgrew the tumor cells obtained from the spilling procedure and the collagenase treatment. One culture was maintained for a year; however, only fibroblasts were observed. Two plasma clot cultures exhibited epithelial growth; after transfer, only fibroblasts were present and these cultures were discarded at 3 months.

Pleural Effusion

A few mature erythrocytes, granulocytes, and lymphocytes were observed in sections of the clot. The predominant cell was epithelial and most of these were in small clumps with considerable pleomorphism. The only mitotic figures found were in the epithelial plaques. Some epithelial cells formed ductlike structures (fig. 1). Macrophages and "signet ring" cells were also evident.

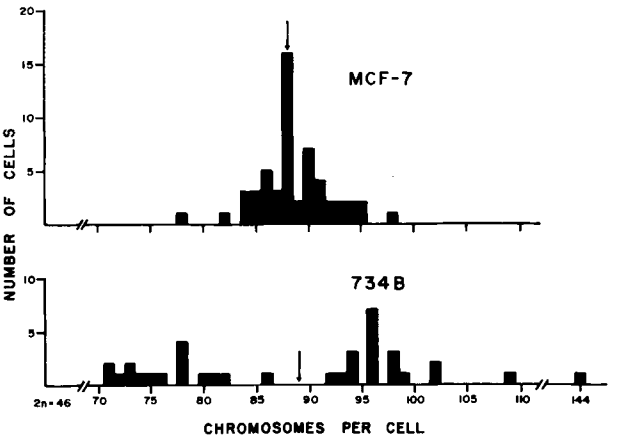
Primary cultures, at the high dilutions, grew more rapidly and produced confluent monolayers; yet free-floating cells were continually shed into the supernatant. Monolayers were eventually reestablished with grossly visible plaques of epithelial-like cells in all cultures after 5 months. Initially, stained smears of the free-floating cells contained fibroblasts, large elongated cells with vacuolated and striated cytoplasm, macrophages, and an occasional multinucleated cell. After 6 months, smears exhibited small epithelial clumps, individual cells in mitosis, and macrophages.

Free-Floating Passages

Secondary passages were made from the free-floating cells of the primary cultures. Epithelial plaques (fig. 2) were observed among large immature cells (fig. 3). In early secondary passages, small clumps and individual epithelial-like cells (fig. 4) were noticed between the immature cells. After 6 months, plaques of epithelial-like cells became the dominant feature. These cells frequently formed a rosette (fig. 5).

Cytogenetic characterization of passage 2 indicated a mean chromosome number of 89 with considerable variation (text-fig. 1). This passage typifies a mixoploid culture with a high percentage of the cells being near tetraploid.

Free-floating cells were serially passaged from the primary culture after 28 days. Eight serial transfers were made at approximately 25-day intervals. These free-floating cells readily reattached in new culture flasks and multiplied, as shown by the large number of mitotic figures after the addition of colchicine. As in the primary cultures, complete monolayers were not produced and detachment was common. Since the cell types present in passages 3–9 were essentially the same (fig. 6) as the late secondary passages, passage 8 was frozen with dimethyl sulfoxide after 256 days in vitro. Recovery of cells after over a year in liquid nitrogen storage was demonstrated by a 69% viability as determined by the trypan blue exclusion test and growth of the cells from the frozen stock.



TEXT-FIGURE 1.—Numbers of chromosomes per metaphase plate of the MCF-7 (39th passage) and of 734B (passage 2) are plotted as histograms. The mean chromosome number of each passage is marked by *arrow*.

MCF-7

A combination of free-floating passages 2–4 were used to initiate a serial trypsin passage after 7 months. From the 25th transfer onward, an approximate threefold increase in cell number was observed every 7 days. This increase does not represent the total cell multiplication, for single cells and small clumps of cells were seen in the supernatant removed in feeding the cultures. The cells observed in the trypsin passage were primarily epithelial-like, exhibiting a polygonal morphology (fig. 7); however, macrophages and "signet rings" (fig. 8) were also observed. Before confluence was reached, rosettes were formed, and these organized cells usually stained more intensely than the surrounding cells.

Cytogenetic studies of the 39th passage indicated a distinct stem line of 88 chromosomes. The variation in chromosome number per cell was considerably less than in passage 2. The chromosome pattern was typical of human cultures with the increase in chromosomes being primarily more metacentrics and subtelocentrics (table 1).

The species of origin was shown to be human when tested against human- and mouse-specific antisera (table 2). Coelectrophoresis of MCF-7 cell RNA

TABLE 1.—*Karyotype of MCF mammary carcinoma cell line*

Chromosome No.	85	(Subtetraploid.)
Long metacentrics	15	26 metacentrics.
Short metacentrics	11	
Long subtelocentrics	28	35 subtelocentrics.
Short subtelocentrics	7	
Long acrocentrics	2	18 acrocentrics.
Short acrocentrics	16	
Minutes	6	

(85th trypsin passage cells) with human and mouse cell RNA verified the humanness of MCF-7 cells (text-fig. 2). With a molecular weight (mol wt) of 1.67 × 10^6 daltons for mouse cell 28S ribosomal RNA (*15*), the mol wt of MCF-7 cell RNA, which migrated identically with other human cell 28S RNA, was calculated to be 1.75×10^6 daltons. This mol wt is characteristic of human and higher primate ribosomal RNA to the preclusion of ungulate and rodent as well as other groups (*15*).

Culture tests for mycoplasm at the 75th and 85th trypsin passage by culture methods were reportedly negative. Moreover, 85th passage cells did not exhibit extranuclear DNA synthesis; this confirmed freedom from mycoplasma by an independent test.

Domes

Domes consisted of a continuous layer of epithelial cells raised above a monolayer with the surface area under the raised cells having only an occasional cell (*18*). Unequivocal 3-dimensional "dome" formation was observed in a secondary passage after a confluent monolayer was obtained. This occurred at 54 days in passage with 500 days in vitro and was reproduced in later secondary passages.

The 29th trypsin passage was seeded into 4 Leighton tubes. With the observation of dome formation in the secondary passage, this passage was allowed to grow to confluence and domes were observed in 3 of the tubes after 31 days in passage (522 days in vitro). To determine the reproducibility of dome formation, cells from subsequent serial passages were inoculated into T25 flasks. Again, confluence was essential for dome formation and domes were produced in all flasks (table 3) (figs. 9, 10).

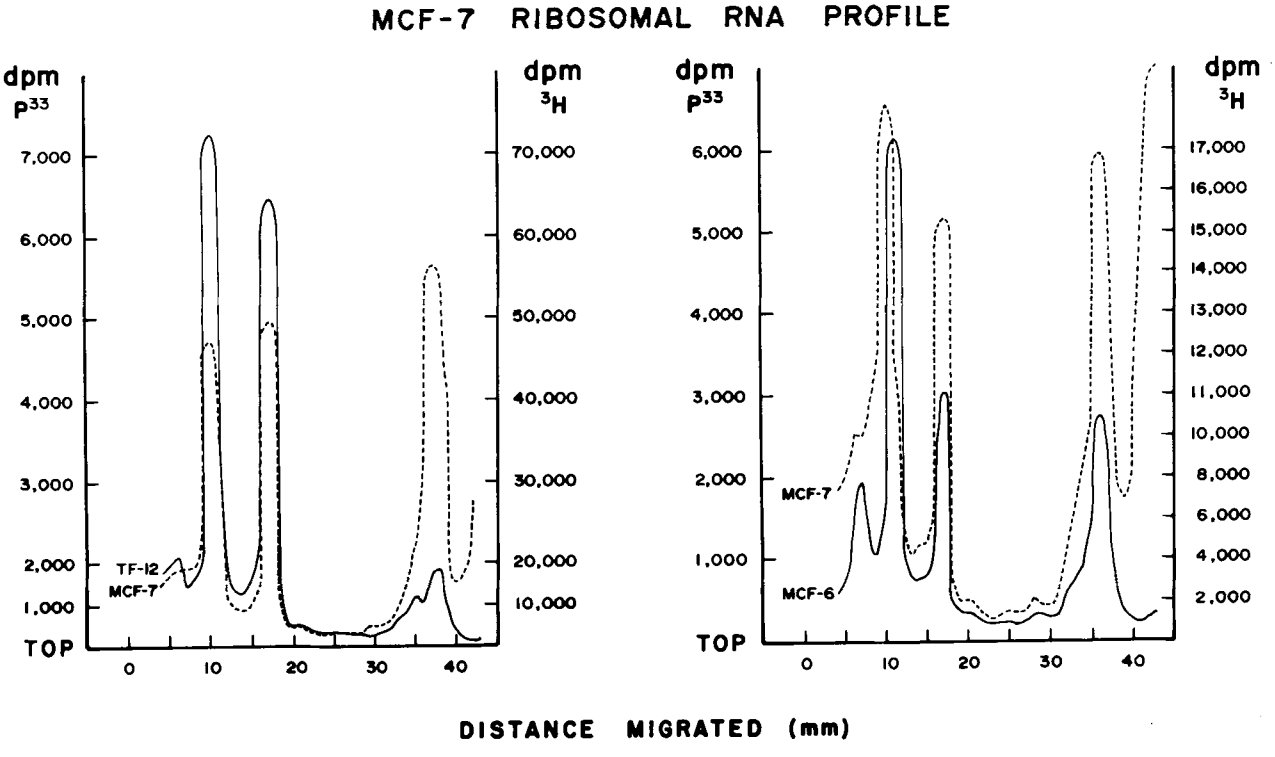
TABLE 2.—*Cell surface reactivity of MCF-7 cells to species-specific antisera (14)*

	MCF-7 cells	Mouse cells
Antimouse:		
1:8	−	4+
1:32	−	4+
Antihuman:		
1:8	4+	−
1:32	4+	−

TABLE 3.—*Effect of age on dome formation in MCF-7*

Passage	Days in passage at confluency	Days in passage at dome formation	Number positive
			No. tested
29	20	31	3/4*
38	20	38	4/4†
39	20	41	6/6†
40	27	34	3/3†
45	13	22	4/4†
46	13	24	7/7†

*Leighton tubes.
†T25 Falcon flasks.



TEXT-FIGURE 2.—MCF-7 cells (17×10^6) were labeled 18 hours to 100 μCi ^{33}P (New England Nuclear, carrier-free). Cultured mouse mammary tumor cells (MCF-6) and human foreskin fibroblasts (TF-12) were labeled 18 hours with 200 μCi ^3H uridine (Nuclear Dynamics, sp act=20 Ci/mmole). RNA was extracted with SDS-phenol-cresol and ethanol precipitated. Redissolved RNA's were combined for coelectrophoresis of MCF-7 and MCF-6 as well as MCF-7 and TF-12. RNA was sized in 3.6% acrylamide–0.5% agarose gels (*16*). Gels were cut in 1-mm slices, dissolved in H_2O_2, and counted in aquasol.

Eventually, the primary cultures did produce a confluent monolayer; however, with the continual spotty detachment, confluence was not maintained for extended periods. The initial domes in the primary culture were observed at 571 days in a confluent monolayer.

Since that time, domes were seen routinely but only when a contiguous area of cells was present. Typical domes had a 0.45 mm diameter; some had a 0.8 mm diameter and some were as small as 0.05 mm. There appeared to be an organization of a basement layer of cells from which the domes arose (fig. 11).

DISCUSSION

Since human malignant mammary tumor cells are less capable of withstanding isolation procedures than cells of benign lesions (*1*, *2*), they may require a period of adaptation to in vitro life before they can withstand transfers by enzymatic or mechanical procedures (*1*, *9*, *10*). The continued availability of free-floating aggregates of epithelial cells for serial transfers may have been responsible for the successful establishment of MCF-7. Multiplying cells in the aggregates of the pleural effusion, with morphologic features typical of tumor cells (*19*) as opposed to normal cells (*20*), could have permitted the eventual selection of tumor cells in the cultures.

The rapid production of a pleural effusion after the widespread local recurrence of mammary tumor cells in the chest wall suggested infiltration by way of the parietal pleura (*21*) which would explain the numerous aggregates of tumor cells with the presence of minimal hematopoietic cells.

The spilling technique and collagenase treatment, which liberate a high percentage of epithelial cells from tumors (*1*), did not yield enough cells from the nodules in the chest wall to permit growth of visible epithelial plaques in culture. Colonies of epithelial cells in the plasma clot cultures indicated that these cells could divide, and their rapid multiplication was typical of mammary adenocarcinomas before overgrowth by fibroblasts (*2*).

The primary cultures exhibited the typical rapid proliferation of most metastatic tumors in vitro (*8*). The initial dominant cell—large, immature, and striated—did not appear to be epithelial in origin. The cell type of the striated cells was not determined; they could have been mesothelial in origin or abnormal fibroblasts. The presence of fibroblasts was evident by typical fibroblastic morphology and by the usual collagenization of the cultures, with complete removal of the cells from some tissue culture flasks.

The human origin of MCF-7 was demonstrated cytogenetically (*22*); the most chromosomes were metacentrics and subtelocentrics with only 21% acrocentrics.

Surface antigenicity and ribosomal RNA molecular weight determinations on cells of subsequent passage confirm the human origin of MCF-7 and contraindicate nonhuman cell contamination during serial subculture. In addition, studies on isozyme synthesis in MCF-7 show synthesis of human glucose-6-phosphatase isozyme B (C. Stulburg, personal communication).

The genesis of domes from MCF-7 monolayers which are analogous in structure and hormonal requirements to the "domes" generated from monolayers of mouse mammary epithelium (*18*, *23*, *24*) strongly suggest the conservation of some differentiated mammary epithelial cell function. This suggestion is reinforced by the recent finding of specific estrogen receptor in MCF-7 (*25*) and preliminary indications of α-lactolbumin synthesis (unpublished observation).

The biologic properties of MCF-7 suggest this line to be an excellent substrate for attempting to isolate human breast cancer viruses. Such studies are in progress.

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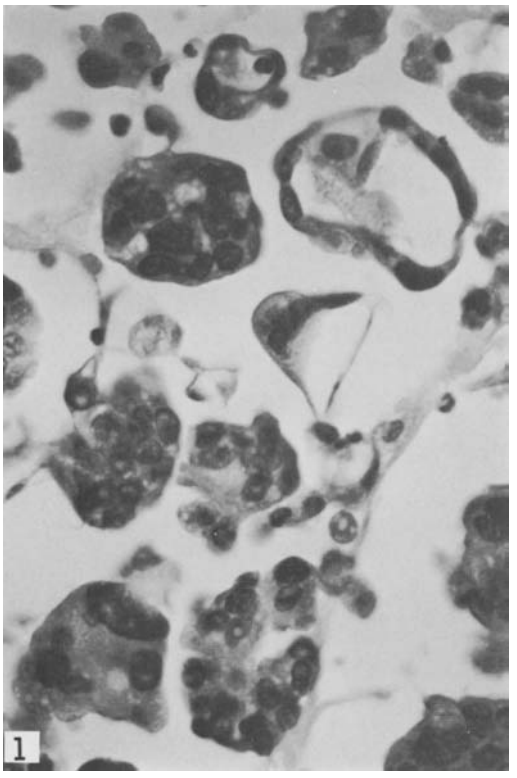
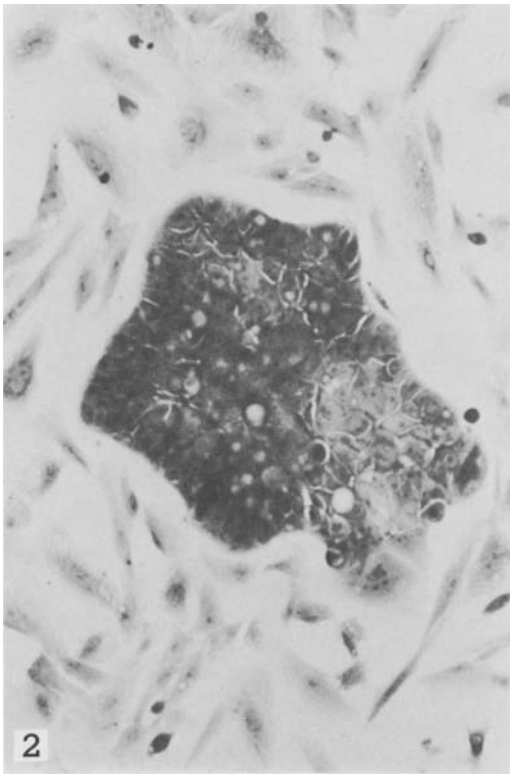
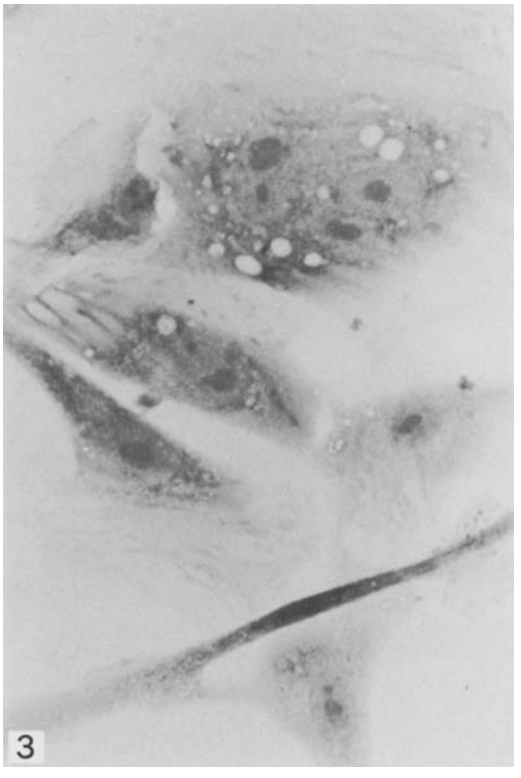






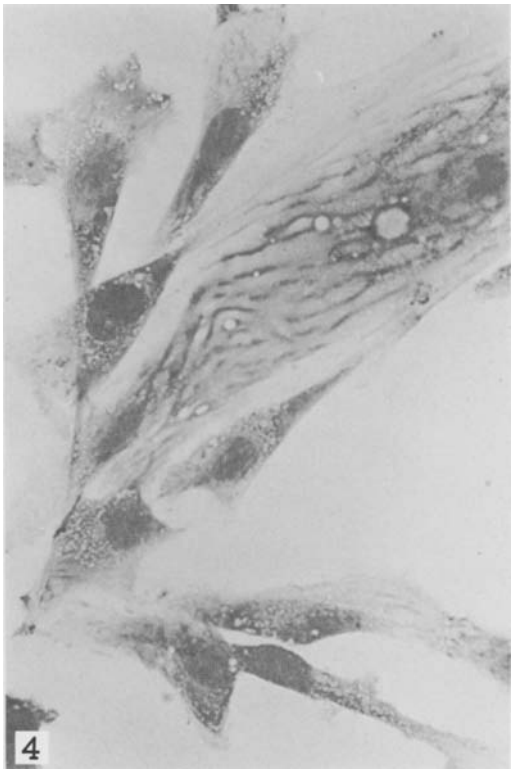
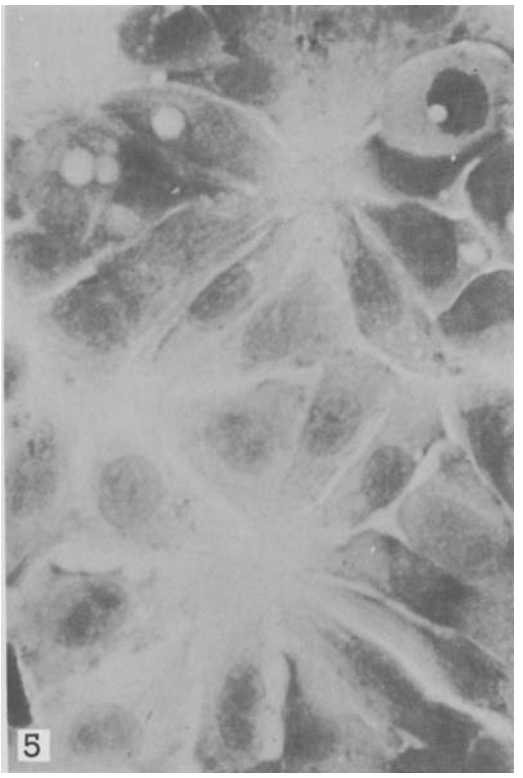


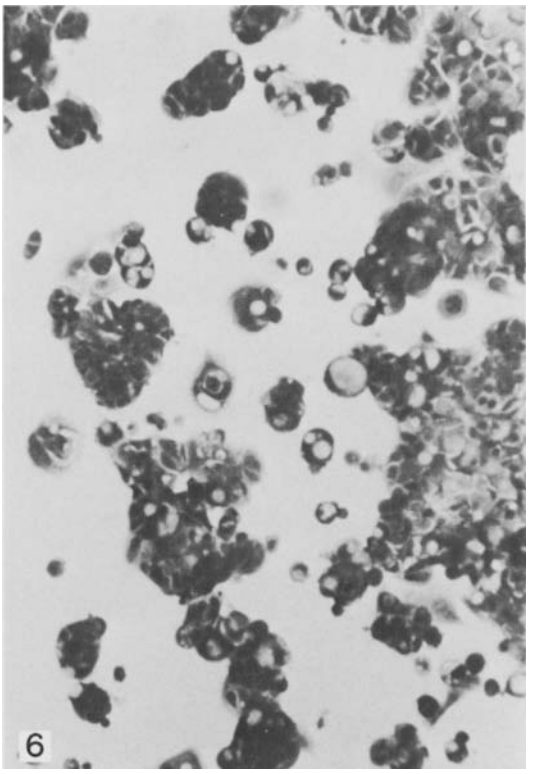
FIGURE 1.—Ductlike structures from epithelial cells.

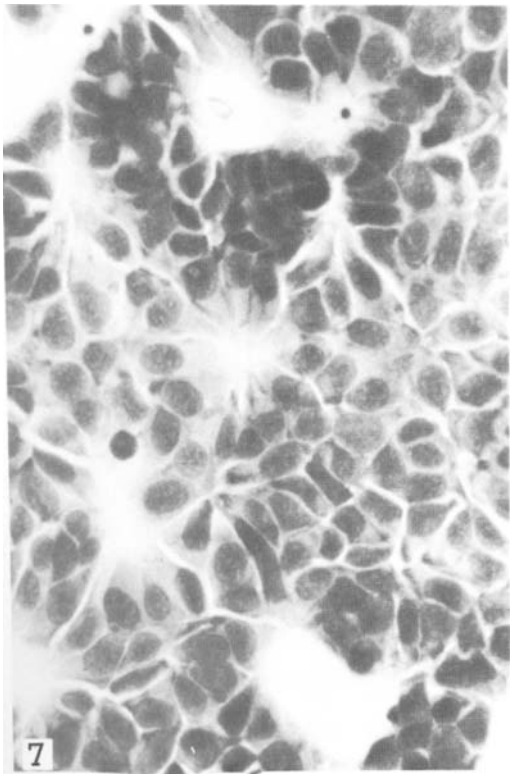
FIGURE 2.—Epithelial plaques.

FIGURE 3.—Large immature cells.

FIGURE 4.—Small clumps and epithelial-like cells.







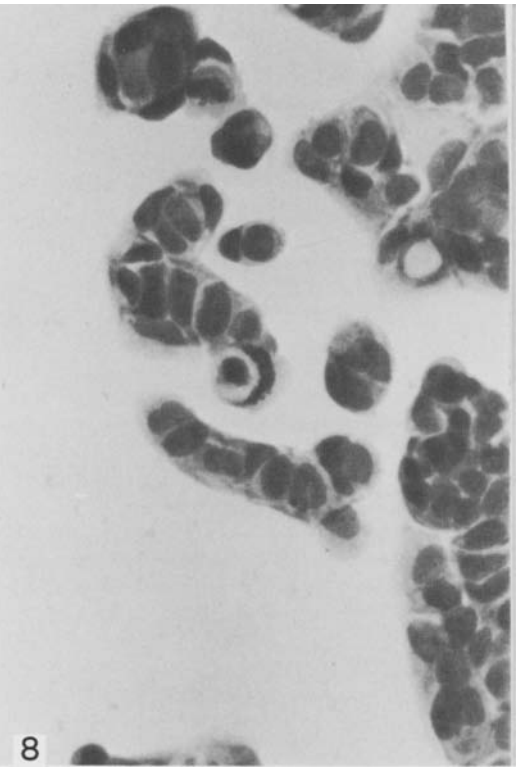


FIGURE 5.—A rosette.

FIGURE 6.—Cell types in passages 3–9.

FIGURE 7.—Epithelial-like cells exhibiting polygonal morphology.

FIGURE 8.—Macrophages and "signet rings."

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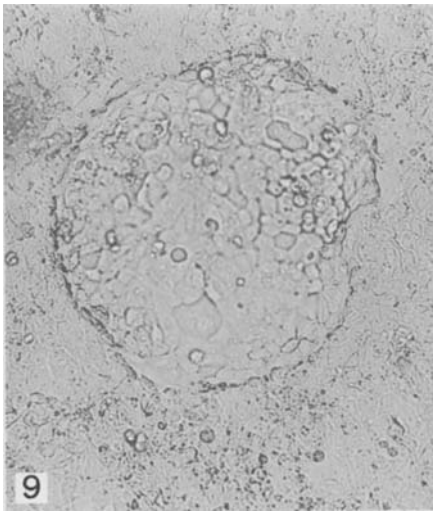

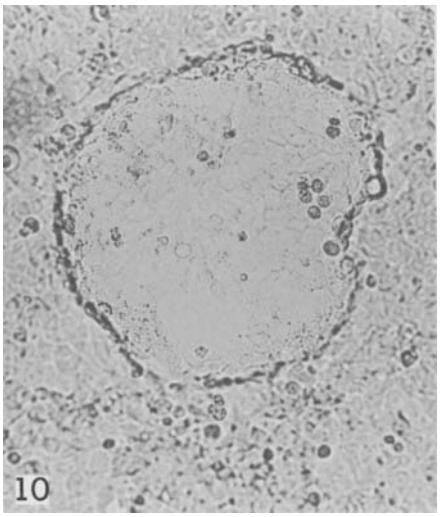

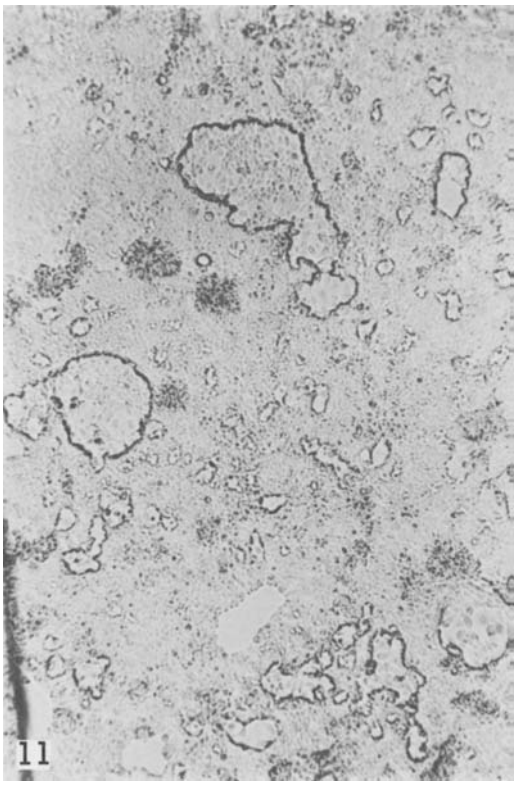

FIGURE 9, 10.—Trypsin passage; *note* domes.

FIGURE 11.—Basement layer of cells; *note* domes.