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Thirteen New Mammary Tumor Cell Lines from Different Mouse Strains*

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Abstract—*Seventeen mammary tumors transplantable in various strains of mice were cultured to obtain established cell lines. All but four tumors have been successfully established as monolayers, have been subcultured regularly, and produced tumors on inoculation into syngeneic mice. Histological examination showed that seven lines (SHF and SHK in C3H mice, S6C in ACA mice, S3W in ASW mice, S2Y in ABY mice, SBfnHA and SBfnHB in CBA mice) are adenocarcinomas, three (TA3Ha, TA3St in A mice and SBfnHD in CBA mice) are poorly differentiated carcinomas, and three (SHG in C3H mice, S40C in ACA mice and SBfnHC in CBA mice) are spindle celled, 'sarcoma-like' tumors associated with much reticulin and collagen. Electron-microscopy demonstrated that all lines, including the 'sarcoma-like' SHG, S40C and SBfnHC are carcinomatous. Eleven lines showed secretion vacuoles consistent with the presence of milk protein. SHG, S40C and SBfnHC are thus to be regarded as spindle-celled carcinomas. All cell lines, except SHG and SBfnHC, were sensitive to antimammary tumor virus serum. SHK, S2Y and SBfnHB showed extremely high reactivity to this antiserum. These thirteen established cultured lines promise to be useful in the further study of mouse mammary tumors.*

INTRODUCTION

DESPITE numerous attempts to establish long-term cultures of mammary tumor cells, few such lines are available. This is claimed to be due to the frequent spontaneous degeneration of tumor cells in primary and serial cultures, and overgrowth by fibroblastic stromal cells and possibly sarcomatus transformation of such cells [1, 2]. The availability of such cell lines remains highly desirable because they would provide a fundamental tool for the study of mammary tumor cells. In order to analyse cell-surface antigens of mouse mammary tumors, we have attempted to establish lines from 17 spontaneous and virus-induced transplantable mammary tumors from seven different strains of mice.

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MATERIALS AND METHODS

Tumors

Thirteen *spontaneous* mammary tumors (SHF, SHG, SHJ and SHK from C3H mice, SlD from DBA/2 mice, TA3Ha and TA3St from A mice, S6C, S40C, S41C and S42C from ACA mice, S3W from ASW and S2Y from ABY mice) and four mammary tumors originating in female CBA mice foster nursed by C3H mothers (SBfnHA, SBfnHB, SBfnHC and SBfnHD) were used. The histology of Ta3Ha and TA3St and details of the maintenance and transfer of these tumors have been described [3]. The transplantable generations of all tumors at the time of explantations are shown in Table 1.

Cell cultures

Tumors were minced, suspended in Dulbecco's modified Eagle's minimal essential medium (MEM) (powder, Flow Laboratories, U.K.) with Earle's salts, supplemented with 10% fetal calf serum (GIBCO BIO-CULT, Glasgow, Scotland), penicillin (250 i.u./ml), and streptomycin (100 μg/ml), and pipetted to separate the cells. A suspension of free cells

Table 1. In vivo *transplantation generation at explantation and* in vitro *passage generation of established mammary tumor cultured lines*

Tumors	Mouse strains	*In vivo* transplant generation at explantation	*In vitro* passage generation on October 20, 1978
SHF	C3H	13	154
SHG	C3H	8	72*
SHJ	C3H	2	Failed to maintain
SHK	C3H	2	78
TA3Ha	A	708	91
TA3St	A	253	87
S6C	ACA	17	94
S40C	ACA	13	84
S41C	ACA	7	Failed to maintain
S42C	ACA	2	Failed to maintain
S3W	ASW	4	100
S2Y	ABY	6	97
S1D	DBA/2	2	Failed to maintain
SBfnHA	CBA	28	148
SBfnHB	CBA	21	147
SBfnHC	CBA	10	100
SBfnHD	CBA	20	96

*December 20, 1977.

and several small tumor fragments was placed in Falcon (250 cm^2) flasks in 20 ml medium. Undisturbed 2-day incubation at 37°C and 5% CO_2 atmosphere in a CO_2 incubator (ASSAB, Medicine AB, Stockholm) allowed most of the fragments to attach. Growth medium was subsequently renewed twice a week and subcultures made by dissociating the cells of the monolayers with a trypsin–EDTA solution (GIBCO, Glasgow, Scotland). Additional tumor fragments were frozen in a viable state in complete medium with 10% dimethylsulfoxide (DMSO) and stored at −90°C to keep early generation of tumor cells.

Overgrowing fibroblastic cells, if any, were removed from primary cultures by two methods. The first is modified from Owens and Hackett [4] (selective detachment of fibroblasts with trypsin–EDTA treatment). The mixed cell sheet was rinsed once with CA^{2+} and Mg^{2+}-free phosphate buffered saline (PBS), fresh trypsin–EDTA (2 ml) was added, and the cell sheet incubated at 37°C for a few minutes. The trypsin–EDTA was agitated horizontally across the cell sheet until inspection showed more than 70% of the cells to be detached. The less rapidly detached cells, mostly islands of epithelium-like cells, were then rinsed, supplied with fresh medium and returned to the incubator. This enzymatic weeding was repeated several times at 3–4 days intervals until patches of epithelium-like cells covered at least 50% of the culture surface. Only then could a successful subculture be made in SHF, SHG Ta3St, S6C, S40C, S3W, S2Y, SBfnHB and SBfnHC lines. Another technique employed is that of free-floating passages [5]. In the case of SBfnHD, epithelial cells were continually shed into the supernatant. The free cells were serially passaged from the primary culture, and readily reattached in fresh culture flasks.

Reimplantation of cultured cells

The tumorigenic potential of the cultured cells was determined by s.c. inoculation into syngeneic untreated weanling mice or irradiated (400 rad) mice to suppress non-specific immunity of the hosts [6]. Various viable cell numbers (10^3–10^6/0.2 ml) of trypsin–EDTA dispersed cells were inoculated into syngeneic mice which were examined weekly for progressively growing tumors at the inoculation site. Only tumors at least 1 cm in diameter were scored as positive. Tumors reaching this size invariably progressed to kill the animal. Some tumors (SHF, SHG, S3W, SBfnHA, SBfnHB and SBfnHC) were re-explanted in culture flasks to check whether the epithelial morphology was still maintained.

Light-microscopy and electron-microscopy (EM)

Monolayer cultures were fixed in methanol and stained with Giemsa for photography. Tissues from each original transplanted tumor and from each tumor arising after inoculation of cell cultures were fixed in 10% formalin, processed routinely and sections were stained with haematoxylin and eosin (HE), with Gomori's stain for reticulin, with Masson's trichrome stain for collagen, with Southgate's mucicarmine for mucin and with periodic acid–Schiff (PAS) for mucopolysaccharides. The low-speed centrifugation pellet of trypsin–EDTA dispersed culture cells and tissues from tumors developing after inoculation of cell cultures were fixed with 2% glutaraldehyde in Sorensen's buffer, and embedded in araldite (ARA) for EM. Sections were cut with an LKB ultratome II using freshly cut glass knives, double stained with uranyl acetate and lead citrate and viewed in Philips EM 301G.

Micrototoxicity assays

Rabbit anti-mouse MTV serum (Lot No. 666) absorbed *in vivo* [7] was kindly provided

by Dr. J. Hilgers (The Netherlands Cancer Institute, Amsterdam). In addition to the mammary tumor cultured lines, four ascitic tumors: YAC (a Moloney lymphoma in A mice), ,RBL-5 (a Rauscher lymphoma in C57BL/6 mice), P815 (a methylcholanthrene-induced mastocytoma in DBA/2 mice) and SESO (a polyoma virus-induced sarcoma in A mice) and two cultured lines: Ha2 (a MSV-induced sarcoma in CBA mice) and MC57M (a methylcholanthrene-induced sarcoma in C57BL mice) were used as target cells. The microcytotoxicity assay was performed as follows: 2 μl of serial dilutions of antiserum from 1:10 in BSS (balanced salt solution) with 1% gelatin (BSS/gel) were injected under paraffin oil into the rings of microtrays (Møller–Coats A/S Moss, Norway) with an automatic Hamilton syringe. One thousand target cells in 1 μl were added to each droplet and the plates were incubated for 20 min at 37°C. Subsequently 1 μl rabbit complement was added at a dilution of 1:3. Plates were incubated for a further 45 min when 0.5 μl trypan blue was added to each drop and the results were read microscopically. The cytotoxic index (CI) was calculated as follows: $CI = 1 - t/c$ (t: percentage of unstained cells in the test sample, c: the percentage of unstained cells in the control sample). CI exceeding 0.2 being regarded as positive.

RESULTS

Morphology in vitro

The primary outgrowth from all tumors consisted of epithelial cells in monolayer. Initially the cells tended to grow as small islets, from which sheets of epithelial cells subsequently migrated as the culture aged. Of the 17 tumors only SHJ, S41C, S42C and S1D failed to survive serial transfer despite three attempts at establishment in culture. SBfnHA showed pleomorphic structure, consisting of clear epithelial cells (Fig. 1). The nuclei of these cells were large, and lobulated with multiple nucleoli. Aberrant mitotic figures, binucleate and multinucleate cells were common and the nuclear-cytoplasmic ratio was high. Some of the cells of this line contained vesicular structures. There were also degenerating cells with breakdown of nuclei and hydropic vacuolization of the cytoplasm. SBfnHA also showed rosette formation, suggesting acinar organization and has maintained epithelial morphology.

SHK and TA3Ha consisted of epithelial cells without marked polymorphism. SHK showed the so called 'dome formation' described by McGrath [8] consisting of a continuous layer of epithelial cells raised above the level of the adjacent monolayer; the area under the raised cells containing only occasional cells when confluence was reached. Other cell lines, SHF, SHG, TA3St, S6C, S40C, S3W, S2Y, SBfnHB, SBfnHC and SBfnHD initially formed a mosaic pattern on plastic surfaces, with islands of densely packed polygonal and cubiodal epithelial cells separated by networks of cells intermediate in morphology between the epithelial and fibroblastic types. At subconfluence, the intermediate cells looked spindle-shaped. However, these cells also had features which were not characteristic of fibroblasts: the nuclei were round to ovoid rather than elongated, as would be expected in fibroblasts. These nuclei were sometimes displaced peripherally rather than centrally located. The cells had features characteristic of malignant cells: high nuclear-cytoplasmic ratio, irregular nuclear margins, large nucleoli and basophilic cytoplasm. After repeated passage *in vitro* with selective detachment of fibroblasts or free-floating passage, islands of polymorphic epithelial cells have become dominant in S3W and S6C. SHF, TA3St, S40C, SBfnHB, SBfnHC and SBfnHD have maintained a mosaic pattern in which epithelial cells predominate. TA3St and SBfnHD were characterized by aggregating floating cells, when confluence was reached. In SHG and S2Y, the epithelial cells typical of the early passages were lost during later passage. S2Y consisted of cells intermediate between epithelial and fibroblastic type, but which were never oriented in parallel rows and never formed a whorled pattern. SHG has developed fibroblastic cytology and morphologic characteristics after repeated *in vitro* passage.

All cell lines showed EM features consistent with their being comprised of malignant epithelial cells, large irregular nuclei, clumped chromatin and prominent multiple nucleoli. The cell lines, including SHG, also maintained EM features characteristic of epithelium, such as tight junctions and desmosomes (especially in SHF, TA3St, S6C, S40C and SBfnHD) and abundant tonofilaments (especially in S6C, S3W, SBfnHA, SBfnHB and SBfnHD) (Table 2). The presence of lipid droplets, well-developed Golgi apparatus, dilated rough endoplasmic reticulum (RER), vesiculated smooth endoplasmic reticulum

Table 2. Ultrastructural characteristics of established mammary tumor cultured lines and tumors which followed their in vivo *inoculation*

Tumors	*In vitro* generation	Tight junctions Desmosomes	Tono filaments	Lipid droplets	Well-developed Golgi	Dilated RER	Vesicularized SER	Numerous polyribosomes	Casein, secretion vacuoles	Mini acini
SHF (C*)	59	++†	+	−	+	+	+	++	+	−
SHF (T‡)	70	+	+	−	+	+	+	+	+	−
SHG (C)	30	+	+	−	−	+	+	+	−	−
SHG (T)	52	+	++	+	+	+	−	++	−	−
SHK (C)	10	+	+	−	+	+	−	++	+	−
SHK (T)	2	++	+	−	++	+	+	++	+	+
TA3Ha (C)	2	+	+	+	+	+	+	++	++	−
TA3Ha (T)	14	++	++	−	+	+	+	+	+	−
TA3St (C)	5	++	+	−	−	−	−	+	−	−
TA3St (T)	9	++	+	+	−	−	−	+	+	−
S6C (C)	12	++	++	−	−	+	+	+	++	+
S6C (T)	10	++	+	+	++	+	+	+	+	+
S40C (C)	26	++	+	+	+	−	+	+	+	−
S40C (T)	12	+	++	+	+	−	−	+	+	−
S3W (C)	14	+	++	−	+	+	+	++	+	−
S3W (T)	10	+	+	−	+	+	+	++	++	+
S2Y (C)	10	+	+	−	++	++	+	+	++	−
S2Y (T)	3	++	+	−	+	−	+	++	+	+
SBfnHA (C)	60	+	++	−	−	−	+	−	+	+
SBfnHA (T)	27	+	++	+	++	+	+	−	+	+
SBfnHB (C)	59	+	++	+	++	−	−	++	+	−
SBfnHB (T)	28	+	+	+	+	−	−	++	+	−
SBfnHC (C)	36	+	+	−	+	+	+	+	−	−
SBfnHC (T)	27	+	+	−	+	+	+	+	−	−
SBfnHD (C)	13	++	++	−	+	++	−	+	+	−
SBfnHD (T)	2	+	+	+	++	+	−	++	+	+

*Cultured cells.
†(++) Prominent, (+) Present, (−) Absent.
‡Tumor tissue.

(SER) and numerous polyribosomes indicated secretory activity such as is characteristic of mammary epithelial cells. These features were seen in most of the lines. The mammary origin of the cells was further supported by EM evidence of the presence of secretory droplets consistent with those containing milk protein, casein, which were prominently seen in TA3Ha, S6C and S2Y (Fig. 2).

Tumorigenicity of cultured lines

After different periods of growth *in vitro*, we implanted cultured cells into irradiated young adult or untreated weanling mice to determine whether after prolonged culture, the cells could still induce tumors. The results of these inoculations are summarized in Table 3. When cultured cells were first inoculated into syngeneic mice, the inocula, except for those from the long-passaged lines, TA3Ha and TA3St which grow to 1 cm diameter within 2 weeks, needed on average 2–3 months to grow to tumors of 1 cm diameter. On subsequent reimplantation in the case of SBfnHA, SBfnHB and SBfnHC, the frequency of takes and the speed of growth of the tumors increased quickly, so that within 1 month, tumors greater than 2 cm diameter developed. Three other tumors, S2Y, S6C and SBfnHD which were not repeatedly implanted, also showed an increased growth rate after repeated passage *in vitro*.

Morphology in vivo

To determine whether cells after culture could reproduce *in vivo* the morphology characteristic of the tumor from which they originated, tumors were examined by light microscopy and EM. The histology of the original tumors: SHF, SHK, S6C, S40C, S3W, S2Y, SBfnHA, SBfnHB, SBfnHC and SBfnHD maintained by serial passage without interpolation of cell cultures showed them to be carcinomas, the morphology of which varied from that of well differentiated papillary adenocarcinoma, through less differentiated

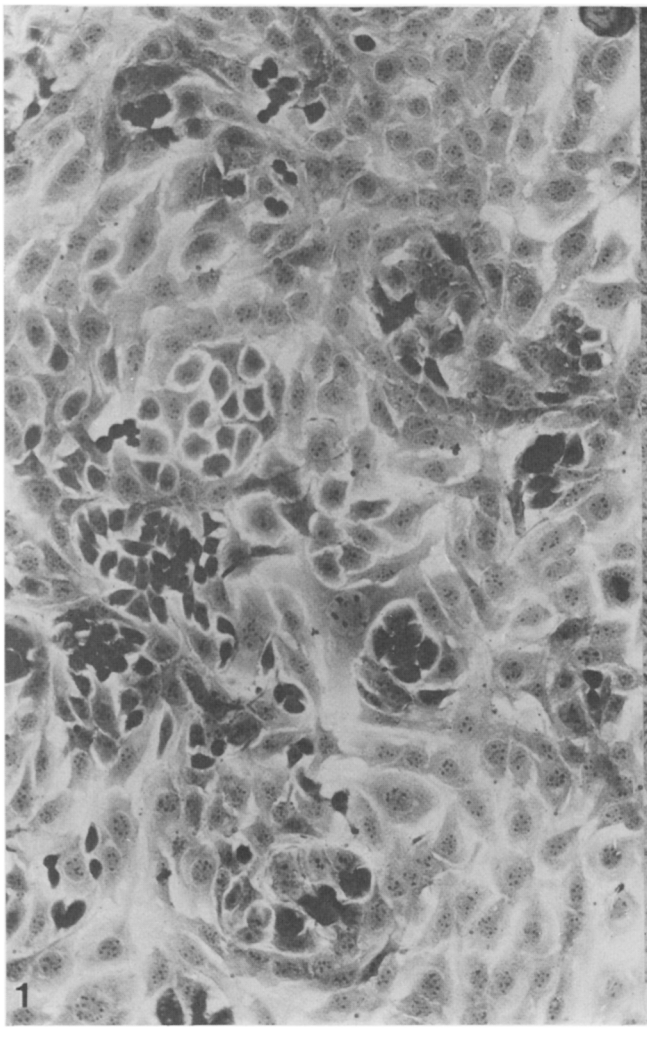

Fig. 1. *Bright-field photomicrograph of a* 2-day-*old culture of SBfnHA cells in passage* 148 *stained with Giemsa which demonstrates polymorphorous epithelial morphology* (× 125).

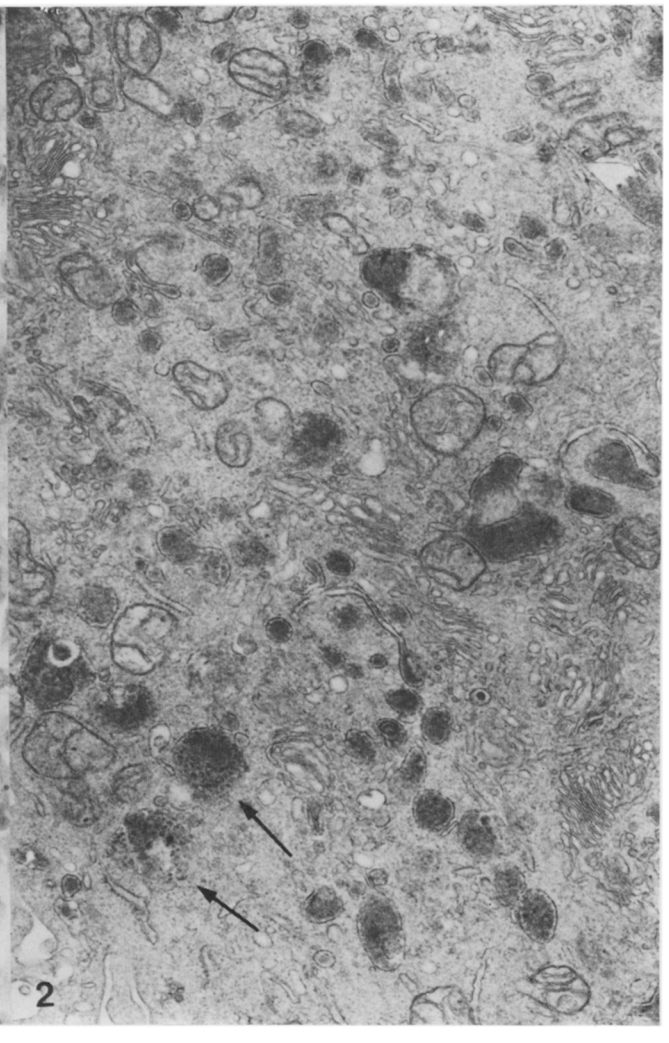

Fig. 2. *Electron micrograph showing milk protein droplets* (*arrows*), *dilated Golgi apparatus and numerous polyribsomes* (*S2Y*, × 26,000).

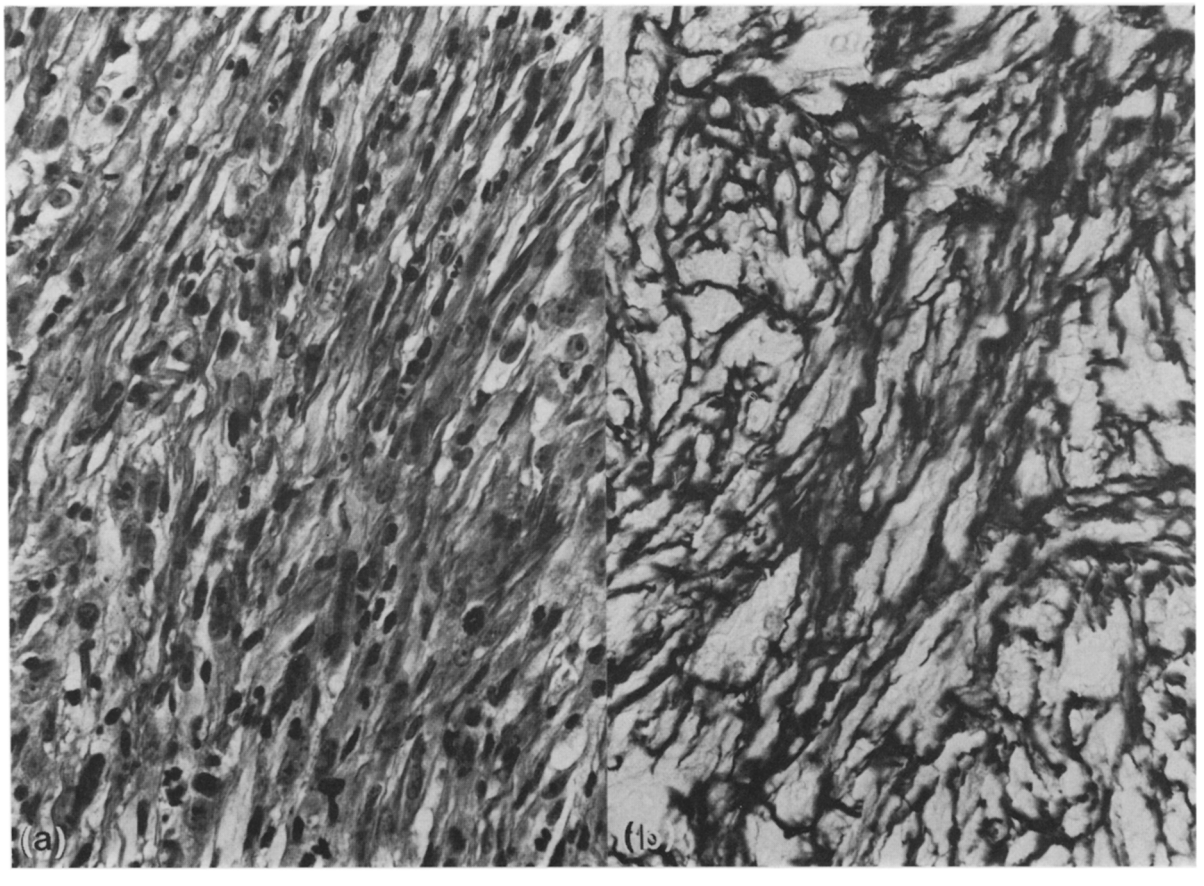

Fig. 3. Spindle celled carcinoma. (*a*) (*SHG-TC, H.E.* × 500). (*b*) (*SHG-TC, reticulin stain* × 500).

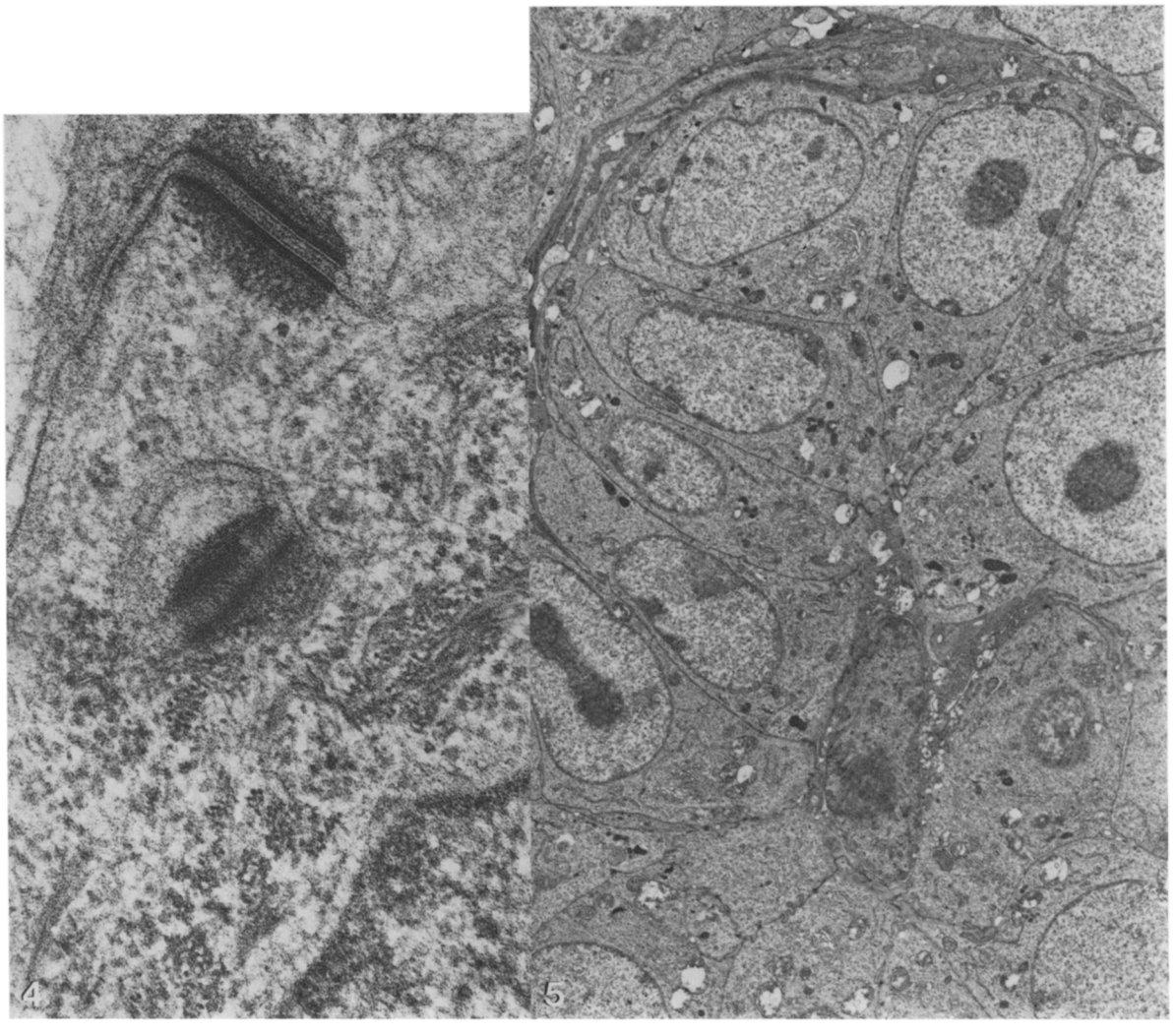

Fig. 4. *Electron micrograph showing the formation of typical desmosomes between adjacent tumor cells* (*SHF*, ×66,000).

Fig. 5. *Electron micrograph showing formation of a 'mini acinus'. Note the close packing of the cells and the long straight areas of membrane to membrane contact. Basement membrane material is evident and a small lumen is discernible* (*S3W*, ×26,000).

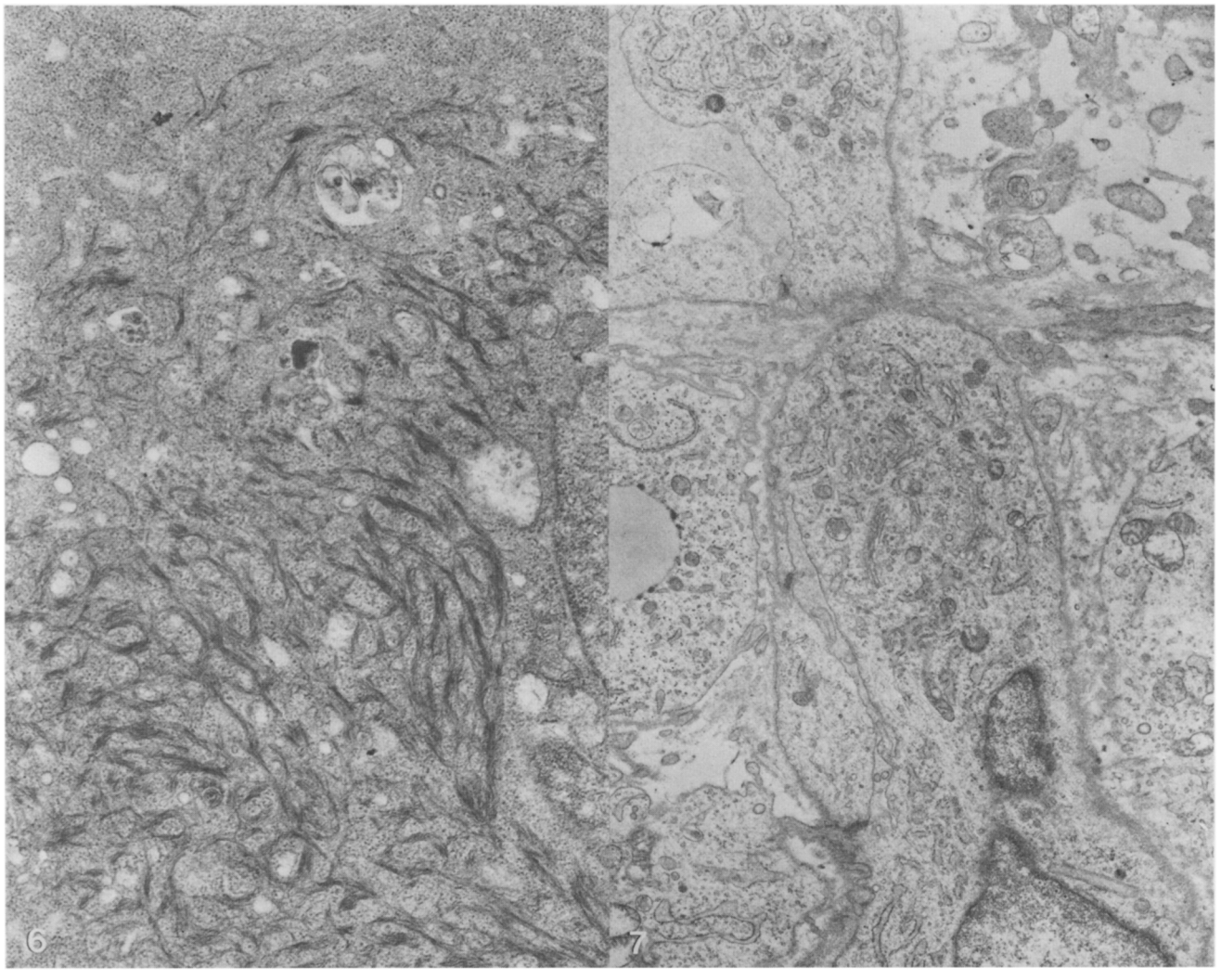

Fig. 6. Electron micrograph showing carcinomatous features of the line cultured from a S40C-induced tumor. Note the presence of a very large number of tonofilaments (× 26,000).

Fig. 7. Electron micrograph showing carcinomatous (epithelial) features of the line cultured from a SBfnHC-induced tumor. Note the presence of tight junctions (desmosomes), tonofilaments, Golgi apparatus and abundant endoplasmic reticulum (× 26,000).

Table 3. Tumorigenicity of established mammary tumor cultured lines

Tumors	*In vitro* generation	Viable cell No.	Tumor take in Irradiated mice*	Tumor take in Untreated mice
SHF	40	10^6	3/20	
SHG	15	10^6	4/18	
SHK	3	10^6	6/6	
SHK	5	10^5		4/10
TA3Ha	5	10^5	5/5	
TA3St	6	10^5	5/5	
S6C	4	10^6	3/3	
S6C	8	10^5		8/8
S40C	5	10^6	4/6	4/13
S3W	4	10^6	10/10	
S3W	15	10^5		7/7
S2Y	12	10^6	5/5	
S2Y	15	10^5		9/9
SBfnHA	13	10^4	6/6	
SBfnHA	22	10^5		6/15
SBfnHB	20	10^5	10/12	
SBfnHB	37	10^5		3/5
SBfnHC	25	10^4	12/12	
SBfnHC	26	10^4		10/10
SbfnHD	5	10^5	4/4	
SBfnHD	18	10^5		13/13

*Received 400 rad whole-body irradiation 24 hr previously.

but still recognisable adenocarcinomas to solid tumors in which the cells include polygonal, cuboidal and oval cells. In many tumors the appearance varied from area to area. Tumors SHG, TA3Ha and TA3St were poorly differentiated solid carcinomas without any evidence of attempts at acinous formation (Table 4). All tumors fell within the broad group of B-type adenocarcinomas according to Dunn's classification [9].

The histology of the tumors developing after inoculation of cultured cells showed a wider range of appearances. Tumors derived from cultures of SHF, SHK, S6C, S3W, S2Y, SBfnHA, SBfnHB and SBfnHD fell within the broad range of carcinomas described above but each tumor was less well differentiated than its equivalent maintained by serial passage *in vivo*. The tumors derived from cultures of TA3Ha and TASt were solid poorly differentiated tumors similar to those developing during serial passage *in vivo*. Cultures of S40C, SHG and SBfnHC yielded strikingly spindle celled tumors of moderate pleomorphism in which there was abundant reticulin and collagen fibers, arranged in a pericellular manner in some areas (Fig. 3). The light microscopic appearance of tumors from these last three lines were those of fibrosarcomas. Mitotic activity was prominent in all tumors but those from lines SBfnHB and SBfnHD showed exceptionally frequent mitoses (up to 80 per high power field—×400). Lymphoid cell infiltration was prominent in tumors from lines TA3St, S40C, S3W, S2Y and SBfnHC.

EM examinations revealed that the cells of tumors induced by all lines had sparse to prominent tight junctions or desmosomes (Fig. 4) (Table 2). Tumors induced by lines SHG, TA3Ha and SBfnHA had abundant tonofilaments (5–8 nm). Lipid droplets, well-developed Golgi apparatus, dilated RER, vesiculated SER or numerous polyribosomes were seen in most of the tumors induced by cultured lines. Tumors from lines of SHF, SHK, TA3Ha, TA3St, S6C, S40C, S3W, S2Y, SBfnHA, SBfnHB and SBfnHD showed milk protein formation, and those from lines SHK, S6C, S3W, S2Y, SBfnHA and SBfnHD demonstrated acinus formation (mini acini) (Fig. 5). Although SHG, S40C and SBfnHC resembled fibrosarcomas on light microscopic examination and on staining for reticulin and collagen distribution, they showed definite epithelial characteristics (Figs. 6, 7).

The results showed that the 13 lines were mammary tumor cell lines; SHF, SHK, S6C, S3W, S2Y, SBfnHA and SBfnHB being adenocarcinomas, TA3Ha, TA3St and SBfnHA poorly differentiated carcinomas, and SHG, S40C and SBfnHC spindle celled carcinomas.

Sensitivity to anti-MTV serum

The cultured lines were tested for sensitivity to antimouse MTV serum in a complement-dependent microcytotoxicity assays (Table 5). With the exception of SHG and SBfnHC all were sensitive to this serum. Lines SHK, S2Y and SBfnHB showed especially high sensitivity with titers of 20480, 20480 and 10240, respectively. Leukemia cell lines: YAC, RBL-5 and P815 and sarcoma cell lines: Ha2, SESO and MC57M did not show any reactivity with this serum.

DISCUSSION

In order to study the immunology, virology and genetics of murine mammary tumor cells, it is necessary to have available single cell suspensions from tumors arising in different strains. Ascitic cells from tumors effusion are potentially a good source of such suspensions but are not often or regularly available. The obvious alternative, the establishment of tumor cell lines from solid tumors, has proved difficult, mainly because of the lack of a

Table 4. Histology of original and cultured line-induced tumors

Tumors	Passage generation*	Acinous formation	Collagen	Reticulin	Mitotic rate†	Lymphoid infil tration	Diagnosis‡
SHF (O)§	4	+‖	−	−	H	−	Ad
SHF (C) ¶	13–70	++	−	−	VII	−	Ad
SHG (O)	25	−	−	+	H	−	Ca
SHG (C)	8–52	−	++	++	H	−	SCCa
SHK (O)	1	+	−	−	VH	−	Ad
SHK (C)	1–2	+	+	+	VH	−	Ad
TA3Ha (O)	736	−	−	++	H	−	Ca
TA3Ha (C)	708–14	−	−	++	L	−	Ca
TA3St (O)	268	−	−	++	H	−	Ca
TA3St (C)	253–9	−	+	++	H	++	Ca
S6C (O)	4	+	−	−	L	−	Ad
S6C (C)	17–10	+	−	−	L	−	Ad
S40C (O)	17	++	−	+	H	−	Ad
S40C (C)	13–12	+	+	++	L	++	SCCA
S3W (O)	8	+	−	+	L	+	Ad
S3W (C)	4–10	+	−	+	L	++	Ad
S2Y (O)	6	+	−	−	H	+	Ad
S2Y (C)	6–3	+	−	−	L	++	Ad
SBfnHA (O)	43	+	−	+	H	−	Ad
SBfnHA (C)	28–27	+	+	+	H	+	Ad
SBfnHB (O)	31	++	−	+	H	−	Ad
SBfnHB (C)	21–28	++	+	+	EH	−	Ad
SBfnHC (O)	5	++	−	+	H	−	Ad
SBfnHC (C)	10–28	−	++	++	L	++	SCCa
SBfnHD (O)	15	++	−	+	H	−	Ad
SBfnHD (C)	20–2	−	−	++	EH	−	Ca

**In vivo* transplantation generation–*in vitro* passage generation.
†(EH) extremely high; (VH) very high; (H) high; (L) low.
‡(Ad) adenocarcinoma; (Ca) carcinoma; (SCCa) spindle celled carcinoma.
§Original tumours.
‖(++) prominent; (+) present; (−) absent.
¶Cultured line-induced tumours.

routine method for the selective cultivation of tumor cells free from overgrowth by fibroblastic stromal cells. Recent technical advances such as selective detachment of fibroblasts [4], free floating passage [5], enzymatic dispersion [10] and selective plating [11] permit the isolation of epithelial cells from mixed cell population. In the present study, using these techniques it has proved possible to establish long-term culture lines from 13 of 17 mammary tumors, all of which show evidence that they consist of malignant epithelial cells. Morphology remained most stable when the tumors were passaged serially *in vivo*, and was less maintained when the tumors were subjected to serial subculture.

Three of the tumors (S40C, SHG and SbfnHC) arising from inoculation of cultured cells showed features of special interest. On light microscopic examination they resembled fibrosarcomas and yet they had ultrastructural characteristics indicating their epithelial nature. They are thus to be regarded as spindle celled carcinomas. According to reviews by Sanford *et al.* [1] and Fasske *et al.* [2], the spindle cell appearance on light microscopy of mouse mammary carcinomas during transplantation *in vivo*, culture *in vitro* and serial reimplantation may be due to dedifferentiation of the carcinoma cells into more primitive forms or malignant transformation of host connective tissue cells by infection with tumor viruses from the carcinoma cells. Em studies of the three spindle celled tumor lines in this study indicate clearly that the cells are epithelial and are best regarded as spindle celled carcinomas.

The term carcinosarcoma does not seem appropriate or helpful in this situation. It is therefore mandatory to examine spindle celled cultures from mammary tumors by electron microscopy before considering them as due to overgrowth of stromal cells. Previously published accounts of this situation which do not

Table 5. Sensitivity of cultured lines derived from mouse mammary tumors to anti-mouse MTV serum

Tumors	Cytotoxic titer* with anti-MTV serum
Mammary tumors	
SHF	160
SHG	—†
SHK	20480
TA3Ha	320
TA3St	640
S6C	10
S40C	80
S3W	10
S2Y	20480
SBfnHA	80
SBfnHB	10240
SBfnHC	—
SBfnHD	1280
Leukemias	
YAC	—
RBL-5	—
P815	—
Sarcomas	
Ha2	—
SESO	—
MC57M	—

*Reciprocal of serum dilution producing more than 0.2 of cytotoxic index.
†No positive reaction at 1:10 serum dilution.

include ultrastructural analysis will require to be reviewed and their conclusions reassessed.

All lines except for the spindle celled carcinomas, SHG and SBfnHC, expressed MTV-associated antigens, and SHK, S2Y and SbfnHB showed especially high sensitivity to anti-MTV serum. These findings also supported the view that the lines derived from mammary carcinoma cells. In addition, we have recently demonstrated that most of the cultured lines expressed a MTV-strucural component, gp52 [12]. The S3W cultured line also expresses an individually specific, unique antigen probably unrelated to MTV [13]. Similar investigations of the other cultured lines have been done to identify and characterize unique and shared antigens on them [12].

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