

Morphology and Growth, Tumorigenicity, and Cytogenetics of Human Neuroblastoma Cells in Continuous Culture¹

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SUMMARY

Continuous cell lines, SK-N-SH and SK-N-MC, were established in cell culture from human metastatic neuroblastoma tissue and maintained *in vitro* for 1 to 2 years. SK-N-SH comprises two morphologically distinctive cell types, a small spiny cell and a large epithelioid cell. SK-N-MC is composed of small fibroblast-like cells with scant cytoplasm. In monolayer culture both cell lines form disoriented growth patterns and reach high saturation densities. Population-doubling times were 44 and 32 hr for SK-N-SH and SK-N-MC, respectively. Inoculum levels of 10^7 cells of both lines produced tumors confirmed by histopathological examination, at frequencies of 30 to 40% in cheek pouches of conditioned Syrian hamsters. SK-N-SH cells are characterized by high dopamine- β -hydroxylase activity while SK-N-MC cells have no detectable activity. However, for SK-N-MC but not SK-N-SH, the presence of intracellular catecholamine was indicated by formaldehyde-induced fluorescence. The lines are near-diploid with several chromosomal markers; SK-N-MC cells contain double-*minute* chromosomes. Growth, biochemical, and cytogenetic properties confirmed that the lines comprise malignant cells of neurogenic origin.

INTRODUCTION

The potential usefulness in cancer research of continuous cell lines established *in vitro* from human tumors is widely accepted. However, there are various practical requirements for their development. A fundamental one is that of providing adequate conditions for cell survival and proliferation. Another is the identification of the cultured cells as being derived from the diagnosed cancer. In our program for establishment of long-term cultures of human neuroblastoma cells, we have been concerned with that aspect of cell identity as well as with delineation of cell properties, since neuroblastoma is unusually diversified in its clinical manifestations and since the cultured cells may differ considerably in morphological characteristics (5, 6, 14). To date, 16 biopsy specimens from 13 different patients have been placed into culture, and 4 continuous cell lines have been obtained. In the present report we describe 2 of these, SK-N-SH and SK-N-MC, which have been maintained for approximately 1 and 2 years, respectively.

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MATERIALS AND METHODS

Cells and Culture Methods

The bone marrow sample (Case 1) was cultured in Eagle's minimum essential medium supplemented with 20% fetal bovine serum, penicillin (100 i.u./ml), streptomycin (100 μ g/ml), and fungizone (2.5 μ g/ml) in plastic flasks. Erythrocytes were washed off after 2 days when attachment and some growth of cells were noted. Cultures were transferred 3 to 4 times at irregular intervals. After about 3 months SK-N-SH cells were routinely transferred every 2 weeks with replacement of medium on Days 5, 9, and 12.

The small piece of tumor tissue (Case 2) was minced in the medium described, and tumor pieces were treated with 0.125% trypsin and 0.02% EDTA in calcium- and magnesium-free phosphate-buffered salts solution to obtain a cell suspension. Clumps of cells were attached to plastic culture flasks after 2 to 3 days, and by about the 2nd month SK-N-MC cells could be transferred at weekly intervals with a medium replacement on Day 5. In plastic flasks cells attached after approximately 24 hr, while in glass vessels attachment occurred only after 2 days.

Both cell lines were routinely maintained in Eagle's medium containing 15% fetal bovine serum, penicillin, streptomycin, and nonessential amino acids (Eagle's formulation). For culture transfers, cell monolayers were exposed to the EDTA-trypsin solution for 3 to 5 min. The cell lines were tested for the presence of *Mycoplasma* through the courtesy of Dr. Jørgen Fogh, Memorial Sloan-Kettering Cancer Center. Organisms were not detected.

Several fibroblast-like cell lines, F-ECH, F-LSO, and F-TGL, were established in culture from apparently normal human biopsy material (mediastinal cyst, intestine, and ovary, respectively) and served as controls. F-HT, established from skin of a patient with Hodgkin's disease, was kindly provided by Dr. M. Eisinger, Memorial Sloan-Kettering Cancer Center. For experiments these cells were maintained and transferred similarly to the neuroblastoma lines.

Population-doubling Time and Saturation Density

Replicate 60-mm glass plates were seeded with 2×10^5 cells in 6 ml of growth medium per plate. For these determinations, Eagle's medium supplemented with 20% serum,

nonessential amino acids, penicillin, streptomycin, and fungizone was used. Medium was replaced 2 times per week. For estimates of population-doubling time, the cells in duplicate plates were counted 3 to 5 times per week. For saturation density determinations plates were counted during about a 10-day period after stationary growth phase was reached. A Coulter counter was used for all cell counts.

Plating Efficiency

For determinations of plating efficiency, 200 ± 20 cells were plated in 5 ml of medium supplemented with 15% fetal bovine serum and nonessential amino acids, penicillin, streptomycin, and fungizone at previously indicated concentrations in 60-mm plastic dishes. Colonies were fixed and counted at 14 days. Only colonies consisting of more than 5 cells were scored.

Heterotransplantation

For determination of tumor-producing capacity, cells were inoculated into each cheek pouch of 19- to 22-day-old female, weanling, golden Syrian hamsters. Animals received a s.c. injection of 2.5 mg of cortisone acetate at the time of inoculation and twice weekly thereafter. Cheek pouches were examined once a week for 4 to 6 weeks, tumors were measured, and samples were excised for histological preparation. Measurements were made in 3 dimensions and size was reported in cu mm. Only those tumors growing progressively during at least a 3-week period, attaining a minimum size of 100 cu mm before regression, and/or receiving histopathological confirmation were considered "positive." Because of the large inoculum size (10^7 cells/pouch), comparison was made with human fibroblast populations.

Chromosome Studies

Preparation of metaphase cells for chromosome observation was carried out with standard procedures of acetic alcohol fixation and acetic orcein staining of air-dried coverslip preparations previously subjected to Colcemid ($0.025 \mu\text{g/ml}$) for 1 hr and 0.56% KCl solution for 20 min.

Case Reports

Case 1 (L. S.). In May 1970, this 4-year-old girl developed an upper respiratory infection that was unresponsive to antibiotics. Progressive dyspnea led to chest radiographic examination which revealed opacification of the left chest cavity. Thoracentesis revealed a bloody effusion and repeat radiographs demonstrated tumor masses containing stippled calcifications in the left upper lobe. Progressive dyspnea continued and was not ameliorated by repeat thoracentesis and administration of fibrinolysin. An emergency left thoracotomy revealed a large neuroblastoma in the upper chest, extending into both upper and lower lobes. The major portion of the primary tumor was excised. Urinary catecholamines and VMA² levels were

abnormally elevated. Following surgery she was treated with radiation therapy (1800 rads ^{60}Co over a 17-day period) applied to the left lung and mediastinum. This was followed by treatment with a sequence of chemotherapeutic agents (vincristine, cyclophosphamide, daunomycin). While under treatment the patient developed metastatic disease in her femur, bone marrow, liver, and epidural space. Increasing amounts of catecholamines and VMA were found in her urine. Her urinary cystathionine was 416 mg/g creatinine, which is well over 70 times the upper normal value. Additional radiation to the involved sites and chemotherapy with other agents (trifluoromethyl-2'-deoxyuridine and adriamycin) were given with little response. A bone marrow aspiration was obtained in December 1970, from which the SK-N-SH cell line was established. After progressive debilitation and continued growth of tumor, the patient died in January 1971.

Case 2 (M. M.). A 12-year-old girl developed a nodule on her left chest wall in February 1968. This was removed and diagnosed as neuroblastoma. She received radiation therapy to the left thorax (4430 rads; 2 MeV) over a 4-week period. She received an additional 2000 rads to the same region in March 1969 for a benign lung density. In March 1970, proptosis and left orbital mass occurred which was biopsied; the histological diagnosis was neuroblastoma. The proptosis and mass decreased in size following radiotherapy to the involved left orbit (3500 rads ^{60}Co) and systemic chemotherapy (alternating courses of vincristine and cyclophosphamide for 8 months). In November 1970, proptosis and the orbital tumor mass recurred. An additional course of radiotherapy was given to the left orbit (4000 rads ^{60}Co) with temporary clinical improvement. This was followed with additional chemotherapy (vincristine and cyclophosphamide) until April 1971, when a 2nd orbital tumor recurrence was noted. Temporary control of further tumor growth with adriamycin was achieved for 4 months and with actinomycin D for 2 weeks. At this point the patient was transferred to Memorial Hospital where an enucleation of the globe was performed. A biopsy specimen of the metastatic neuroblastoma tissue situated behind the globe was placed into culture, giving rise to the SK-N-MC cell line. Repeated urinary determinations for catecholamines, VMA, and cystathionine were normal. The histology of all the slides was reviewed and was considered to be consistent with that of neuroblastoma.

RESULTS

Morphology and Growth Characteristics in Culture. Cell line SK-N-SH comprised 2 distinctly different cell types. One was a small, dense cell with scant cytoplasm forming focal aggregates (Fig. 1). These cells had delicate processes, usually short, but sometimes exceeding $100 \mu\text{m}$ in length. The other type was a comparatively large epithelioid cell. In newly transferred cultures these cells were the first to attach and extensively proliferate. As the culture aged, the small "spiny" cells accumulated until an old culture consisted predominantly of dense mounds of the small cell type.

² The abbreviation used is: VMA, vanillylmandelic acid.

This cell line was plated many times on either glass or plastic. The predominating colony type consisted of a mixture of epithelioid and small, spiny cells (Fig. 2). Occasionally, depending on the age of the culture used for plating, it was possible to obtain an apparently pure colonial population as illustrated by Figs. 3 and 4.

The SK-N-MC cell line was composed of fibroblast-like cells with little cytoplasm (Fig. 5). However, these cells did not resemble normal human fibroblasts which are larger, more flattened and stretched out, and more oriented when grown under similar cultural conditions. When plated, there were generally 2 types of colonies, both with dense mounding centers but one with more radiating, fibroblast-like cells at the periphery (Fig. 6). That this small difference between the 2 kinds of colonies is probably real is supported by observations of the subcloned line SK-N-MC-IXC (see Chart 3 legend) which consisted entirely of the more radiating type of colony (Fig. 7).

Six apparently morphologically homogeneous SK-N-SH clones were established. Cloning was carried out by isolation of colonies since other more rigorous methods were not successful with these neuroblastoma populations. Of the 6 lines, 2 were epithelioid (clones 13 and 31) and 4 consisted of small cells with processes (clones 21, 22, 30, and 32). Three lines (clones 22, 30, and 32) initially consisting of small, densely mounding cells were observed to have both cell types in similar proportion to the parental line after 3 to 4 months. Clonal line 13 appeared entirely epithelioid. When it could be replated, after about 1 month, nearly all colonies were large and epithelioid with clearing centers consisting of the small, spiny cells. These cells overgrew and the line died out. Subcloning of epithelioid cells was not successful. Line 21 seemingly comprised the small cell type with long cell processes (Fig. 8). Flattened, epithelioid cells were seen after 4 to 5 months. Finally, clonal line 31 appeared to contain only epithelioid cells both when plated and when observed during the course of 10 culture transfers over a 4-month period. Thereafter, several aggregates of small cells with processes were noted.

Rate of Growth, Saturation Density, and Plating Efficiency. The average population-doubling time of SK-N-SH cells was 44 hr, and that of SK-N-MC cells was 32 hr. As indicated in Chart 1, the doubling times of each cell line measured several times between the 10th and 24th culture transfers were consistent. Both neuroblastoma lines attained high cell densities at stationary growth phase (Chart 1; Table 1). In contrast, 3 human fibroblastic lines, F-ECH, F-LSO, and F-TGL, attained maximum cell densities that were approximately 13-fold lower (Table 1).

Plating efficiencies were determined in 2 experiments for each cell line, with values of $29.1 \pm 1.8\%$ for SK-N-SH and $13.5 \pm 2.6\%$ for SK-N-MC.

Heterotransplantation. When SK-N-SH cells were inoculated into cheek pouches of cortisonized hamsters, no tumors were produced at an inoculum size of 10^6 cells, whereas the average frequency was 32% with 10^7 cells (Table 2). The tumors were small, appearing late in the course of the experiment, but grew progressively (Chart 2). The SK-N-MC line produced tumors with an average frequency of 38% with an inoculum size of 10^7 cells (Table 2).

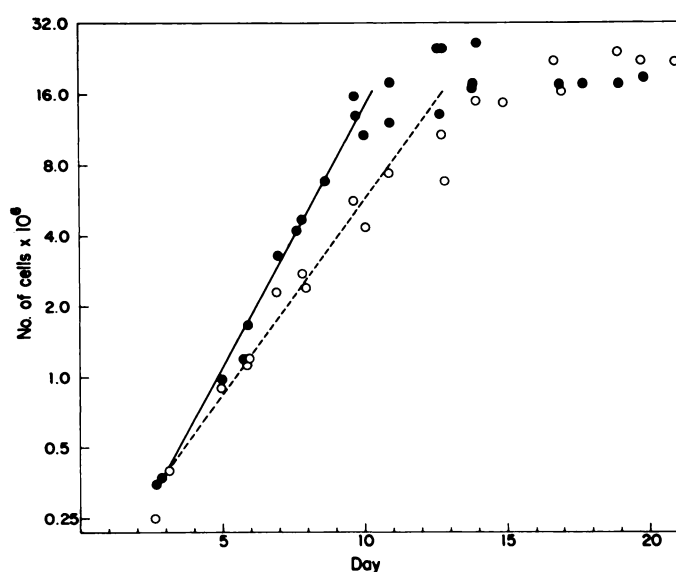


Chart 1. Determinations of cell number during exponential growth phase (2 experiments per cell line) and during stationary growth phase (3 experiments per line). Each point represents the mean of 2 values. O, SK-N-SH; ●, SK-N-MC.

Table 1
Saturation density of neuroblastoma and fibroblast-like cell lines
Estimates of saturation density are expressed as the mean of 2 to 4 cell counts from duplicate 60-mm plates during a 10-day period following onset of stationary growth phase.

Cell lines	No. of days in culture	Saturation density (10 ⁴ × no. of cells/sq cm)
SK-N-SH	94	113
	169	104
	351	114
SK-N-MC	132	125
	149	128
	225	89
F-ECH	82	9
F-LSO	70	7
	94	5
F-TGL	86	13

A few tumors occurred with the smaller cell inoculum. SK-N-MC tumors appeared early, grew rapidly, and attained a large size (greater than 1400 cu mm) by the end of the experimental period (Chart 2). All of the nodules appearing after inoculation of 10^7 fibroblast-like cells regressed by the 3rd week, reaching their maximum size (30 to 120 cu mm) at 7 days (Table 2).

Microscopic sections of the masses growing in the cheek pouch had the appearance of malignant tumors. In a tumor produced by the SK-N-SH line (Fig. 9), the cells are small and are loosely arranged in an irregular manner. The nuclei are round or oval and the cytoplasm is moderately abundant and pale. There is little or no necrosis; mitotic figures are numerous. In a tumor formed by SK-N-MC cells (Fig. 10), the cells are small and compactly arranged. The nuclei

Table 2

Frequency of tumors produced in Syrian hamster cheek pouches in individual experiments

Cells were inoculated into the cheek pouch of 19- to 22-day-old weanling hamsters receiving 2.5 mg of cortisone acetate s.c. at time of inoculation and 2 times per week thereafter. Pouches were examined once a week for 4 to 6 weeks.

Cell line	No. of days in culture	Tumor incidence ^a	
		10 ⁶ cells/pouch	10 ⁷ cells/pouch
SK-N-SH	88	0/12	
	95	0/10	
	109	0/4	
	347		2/10
	372	0/4	2/8
	490		4/8
	497		4/12
		0/30	12/38 (32%)
SK-N-MC	114	1/11	11/12
	128	0/5	0/5
	222		0/10
	226	3/11	3/11
		4/27 (15%)	14/38 (38%)
F-ECH	73	0/6	0/5
F-HT	102	0/5	0/4
	130		0/12

^a Number of tumors per number of pouches inoculated.

are round or oval with moderate amounts of pale cytoplasm. There are a few areas of necrosis; mitotic figures are numerous.

Attempts to enhance tumorigenic potential of both lines by several sequences of passage from cheek pouch to culture back to cheek pouch were not effective.

Karyotype Analysis. Metaphase preparations of SK-N-SH were analyzed and counted after approximately 2 and 7 months in continuous culture (Table 3). There were sharp modes at 47 chromosomes. As seen in Fig. 11, the additional chromosome, *M1*, is a long, structurally abnormal submetacentric marker of presently unknown origin. A second marker chromosome, *M2*, is probably a translocated No. 21-22 since a chromosome was consistently missing from this group and since the *M2* marker was occasionally seen to have satellites.

The SK-N-MC cell line was examined at approximately the 1st and 8th months (Table 3). Initially, the modal chromosome number was 47. At later examination it was 46, and there was a proportion of cells with only 45 chromosomes. The frequency of metaphases of higher ploidy was less than 5% in both cell lines. The karyotypes were somewhat less homogeneous than those of the SK-N-SH line and there was greater karyotypic deviation (Fig. 12). A No. 2 chromosome was consistently missing. In approximately one-half of the karyotyped cells a No. 3 chromosome was also missing and it appeared somewhat abnormal in the remainder. In 50 to 70% of the metaphases examined, a B-group and a D-group chromosome were absent. One or 2 additional F-group chromosomes were present in all cells. There were 4 consistent markers; *M1* and

M2 are probably translocated and deleted G-group chromosomes, respectively; *M3* is a very long submetacentric chromosome in part comprising a No. 3 chromosome as ascertained by quinacrine fluorescence techniques (investigations in progress); *M4* is a long subtelocentric chromosome possibly assignable to the D-group.

In the clonal subline, SK-N-MC-IX, all cells examined lacked a Chromosome 2, 3, 4-5, and 21-22. The 4 markers were present as well as an abnormal metacentric chromosome sometimes resembling a No. 3. There were 2 additional F-group chromosomes. The modal chromosome number was 47.

Double-minute Chromosomes. Cells of the SK-N-MC line, but not SK-N-SH cells, were observed to contain a variable number of double-minute chromosomes. Their frequency declined during the course of *in vitro* cultivation (Table 3). After approximately 1 year, cells with double-minute chromosomes were only occasionally found. However, when 3 clonal sublines were surveyed for the presence and number of small chromatin bodies, they were observed in about 90% of the cells (Chart 3). The distributions of the number of double-minute chromosomes per cell were simi-

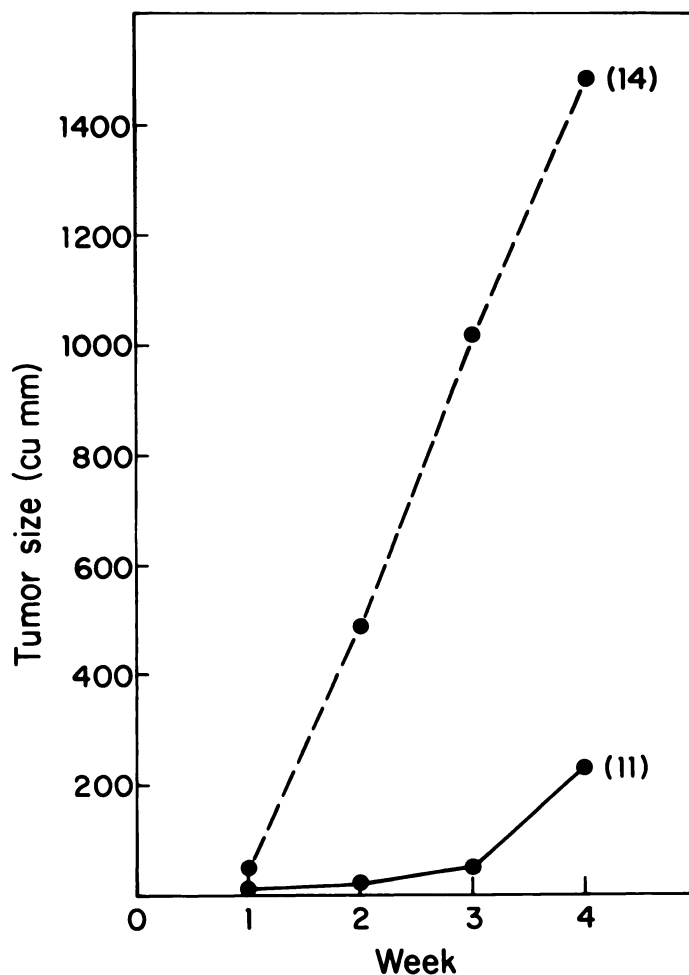


Chart 2. Mean size of tumors produced by the neuroblastoma lines in cheek pouches of conditioned Syrian hamsters. Numbers in parentheses, number of tumors. —, SK-N-SH; ---, SK-N-MC.

Table 3

Distribution of chromosome numbers, breakage frequency, and frequency of double-minute chromosomes

Metaphase cells were prepared with a standard sequence of Colcemid (0.025 μ g/ml) and hypotonic (0.56% KCl) treatment followed by acetic alcohol fixation and acetic orcein staining.

Cell line	No. of days in culture	No. of chromosomes/cell ^a								Cells with breaks (%)	Cells with double- <i>minute</i> chromosomes (%)
		44	45	46	47	48	49	50			
SK-N-SH	51			2	42	6			16	0	
	228		1	2	42	5			4	0	
SK-N-MC	42	2	3	9	22	11	2	1	20	70	
	244	3	16	25	5	1			0	14	

^a Fifty cells were counted for each group.

lar for the clone SK-N-MC-IX and its subclone, SK-N-MC-IXC. Approximately 75% of cells contained more than 10 double-minute chromosomes per cell with modes in the 21 to 30 range. A small proportion contained 100 to 200. In the subclonal SK-N-MC-IIE population about 60% of cells had 10 or fewer double-minute chromosomes per cell; however, there was a small component of cells with quite high numbers. The size of the double-minute chromosomes varied. They could be categorized as "barely visible," "faint," as most commonly observed (Fig. 13), "small," and "medium" (Fig. 14). Although the double-minute chromosomes were classified by size for each cell in the survey, there were no discernible patterns of difference between the 3 clonal lines.

Metaphases were also scored for chromosome breaks and exchanges. Only chromatid breaks were observed, 1 or occasionally 2 per cell. The frequency was 7% for SK-N-MC-IX, 3% for SK-N-MC-IXC, and 15% for SK-N-MC-IIE. Therefore, the overall breakage frequency for SK-N-MC cells including that reported in Table 3 was not higher than can be expected for cultured cells not having double-minute chromosomes.

Catecholamine and Dopamine- β -hydroxylase. In a recent study (7) of catecholamines in neuroblastoma cells, as detected by the paraformaldehyde-induced fluorescence method, fluorescing cells were observed in the twice-cloned SK-N-MC-IIE subline. Fluorescing cells were observed in the parental SK-N-MC but not the SK-N-SH populations (unpublished results). SK-N-MC-IIE showed a greater extent and intensity of apple-green fluorescence, exceeding that of C-1300 (neuro-2A) mouse neuroblastoma cells in culture.

The parent lines and several clonal sublines were also tested for the presence of dopamine- β -hydroxylase in a preliminary study (L. S. Freedman and M. Goldstein, personal communication). There was no detectable activity in either parental SK-N-MC cells or in the twice-cloned SK-N-MC-IXC population. SK-N-SH cells, however, had high dopamine- β -hydroxylase activity. Similarly high values were obtained with an adrenergic clonal line (N-115-G) of C-1300, as described in a recent report (3). Lower activity was found for parent C-1300 mouse neuroblastoma cells in culture (1). In each of 2 clonal lines of SK-N-SH composed of small, spiny cells there was moderate enzyme activity while no activity was detected in 2 cloned, epithelioid populations.

DISCUSSION

By their growth behavior *in vitro* and *in vivo* the 2 cell lines, SK-N-SH and SK-N-MC, appeared to fulfill certain criteria of malignancy. Saturation densities were high (greater than 10^6 cells/sq cm). Plating efficiencies were moderately high despite slow attachment. Growth patterns in monolayer culture were those generally associated with cancer; disoriented cell arrays as well as rounding and mounding, appear to result from low cellular adhesiveness. Finally, the cell populations were tumorigenic at a high inoculum level. This was corroborated by frequent histopathological examinations.

The 2 cell lines established from metastatic tumor tissue of patients with clearly diagnosed disease were dissimilar in their morphological aspects. However, each was found to possess certain attributes which may be representative of neuroblastoma cells. Morphologically, the predominating cell population of the SK-N-SH line was composed of small densely aggregating cells similar, from description, to the IMR-32 neuroblastoma line isolated by Tumilowicz *et al.* (14). Like IMR-32, SK-N-SH was composed of 2 distinctly different cell types, the less predominating type being epithelioid in this instance. However, as with the IMR-32 cell line, results of plating and cloning experiments with SK-N-SH suggested to us that the spiny and epithelioid cells are part of a spectrum of phenotypic expressions. There was further indication of morphological differentiation with the occasional appearance of long, delicate cell processes such as previously described for human neuroblastoma cells *in vitro* (5). The formation of cell processes was most pronounced in a cloned population (Fig. 8) derived from SK-N-SH. Acquisition of conclusive evidence for possible interconversion between the different morphological types (spiny and epithelioid) requires sequences of cloning and subcloning, now in progress.

The most convincing evidence at present that SK-N-SH cells are indeed of neuronal origin is the finding of high levels of activity of dopamine- β -hydroxylase, an enzyme distributed only in sympathetic nervous tissue. In serum of 10 out of 22 neuroblastoma patients, extremely high activity values were found by Goldstein *et al.* (4). Elevated activity was found also in mouse neuroblastoma C-1300 tumors and in derived clonal cell lines established *in vitro* (1), although to a lesser degree. In SK-N-MC cell dopamine- β -hydroxylase was not detectable. Paradoxically,

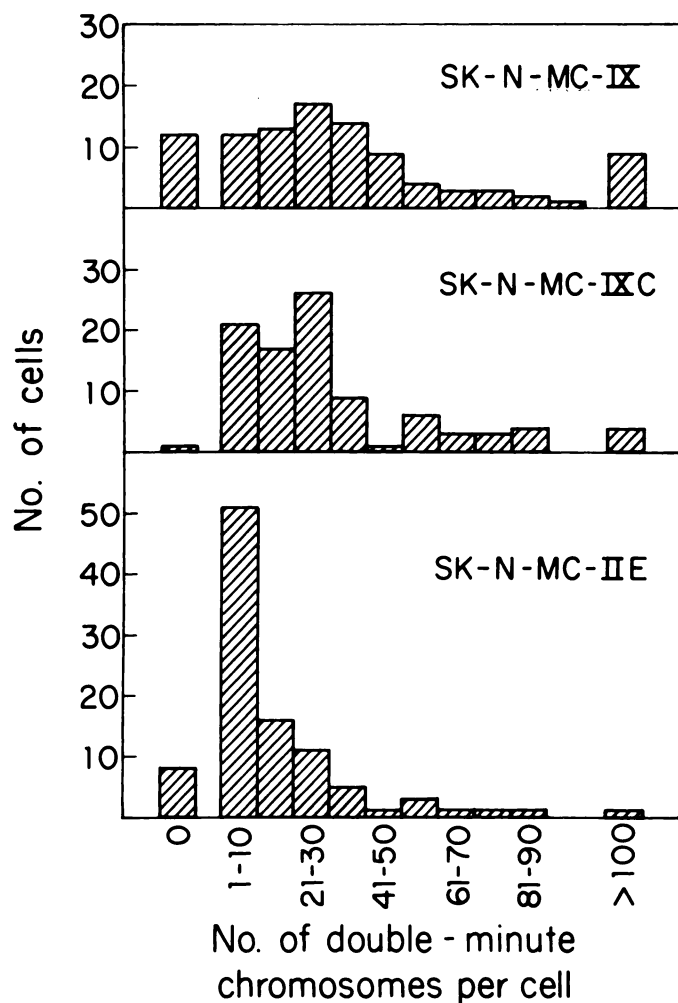


Chart 3. Distribution of the numbers of double-minute chromosomes per cell for 100 metaphases each of 3 clonal sublines derived from SK-N-MC. SK-N-MC-IX was cloned 49 days after establishment of SK-N-MC *in vitro* and was maintained an additional 100 days before preparation of metaphases for survey of double-minute chromosomes. SK-N-MC-IXC was subcloned from SK-N-MC-IX after 105 days of growth as a clone. Preparations for survey were made after 51 days of growth as a subclone and a total of 205 days *in vitro*. SK-N-MC-IIIE was derived from clonal line SK-N-MC-II following a growth period of 105 days after cloning. Metaphase preparations for survey were obtained after 44 days as a subclonal line and a total of 198 days *in vitro*.

the presence of intracellular catecholamine(s) was indicated by histochemical fluorescence techniques (7).

Further evidence of a different nature for the neurogenic origin of SK-N-MC cells is provided by the finding of double-minute chromosomes first described in a human tumor by Spriggs *et al.* (13). The occurrence and possible derivation of these very small paired chromatin bodies have recently been discussed in detail (11, 12). Although found most frequently in malignant gliomas and neuroblastomas, they have been reported also for several other human tumor types as well as for murine sarcomas. There are at least 2 other published accounts of the persistence of double-minute chromosomes in human tumor cells established in culture. White and Cox (18) observed chromatin bodies in 5 to 10% of metaphases of a rhabdomyo-

sarcoma up to 8 months *in vitro*. They were also present in thyroid carcinoma cells when examined after about 10 months, as described by Jones *et al.* (8). These chromosome preparations were recently reviewed in consideration of the present findings. Approximately one-half of the thyroid carcinoma cells contained double-minute chromosomes within a size range similar to that for SK-N-MC cells. Our observation concerning the distribution of double-minute chromosomes in 3 clonal sublines concurs with those of Levan *et al.* (9) for a human neuroblastoma tumor. It is apparent that the distribution is inexact, leading to either loss or accumulation. Even in these clonal lines there was considerable size variation. The range of both sizes and numbers was remarkably similar to that described for 6 human tumors by Cox *et al.* (2), suggesting a common basis for the formation of double-minute chromosomes.

That the 2 continuous lines maintained chromosome numbers in the diploid range, as did the neuroblastoma cells described by Tumilowicz *et al.*, is not surprising since diploid and near-diploid tumor cells in patients with metastatic neuroblastoma who have had extensive drug and/or radiation therapy is not an uncommon finding (10, 12, 16). However, heteroploid populations in patients studied before or after therapy have also been found (2, 15-17). Karyotype analysis of "diploid" tumor cells has revealed the presence of abnormal marker chromosomes in several instances (9, 12, 16). It is probable that the marker chromosomes characterizing SK-N-SH and SK-N-MC cells represent a component at least of the *in vivo* tumor population.

The development of successful methods of culturing human neuroblastoma cells over long durations permits the luxury of time to explore a variety of biological phenomena. Preliminary study of their cytogenetic, biochemical, and tumorigenic properties indicates that such cells retain many of their original characteristics over repeated transfers in cell culture and further supports their use as a tumor model system.

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Figs. 1 to 7. May-Grunwald-Giemsa preparations. $\times 75$.

Fig. 1. SK-N-SH cell line in monolayer culture showing 2 cell types.

Fig. 2. Prevalent mixed colony type of SK-N-SH.

Fig. 3. Colony of small, spiny cells of SK-N-SH.

Fig. 4. Epithelioid colony of SK-N-SH.

Fig. 5. SK-N-MC cell line in monolayer culture.

Fig. 6. Colonies of SK-N-MC showing the 2 colony types characteristic of the line.

Fig. 7. Colonies with a loose peripheral network of cells, the colony type characterizing the clonal line SK-N-MC-IXC.

Fig. 8. Unfixed monolayer of clonal line 21 derived from SK-N-SH showing long intercellular processes. $\times 219$.

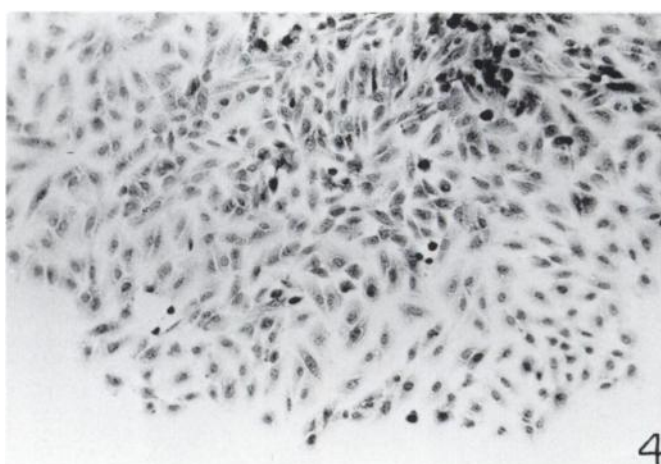
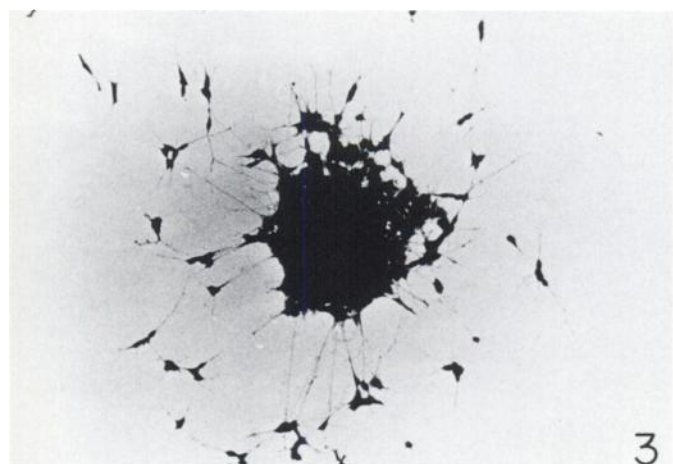
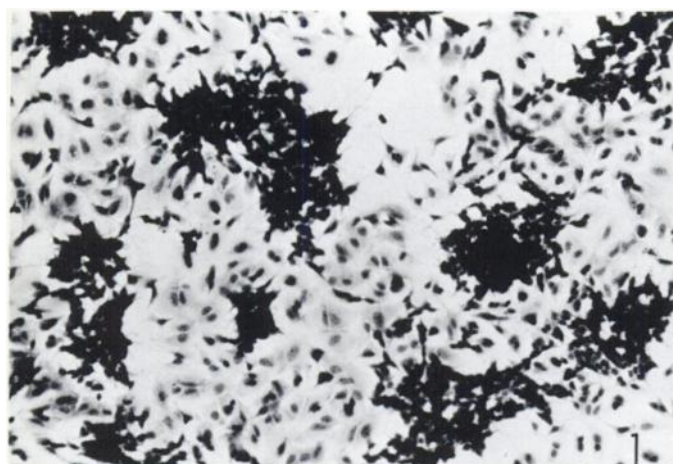
Figs. 9 and 10. Sections of tumors produced in hamster cheek pouches by SK-N-SH and SK-N-MC cells, respectively. H & E, $\times 300$.

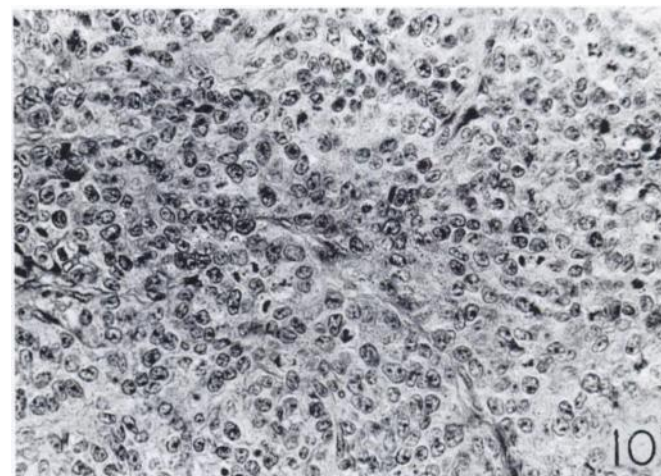
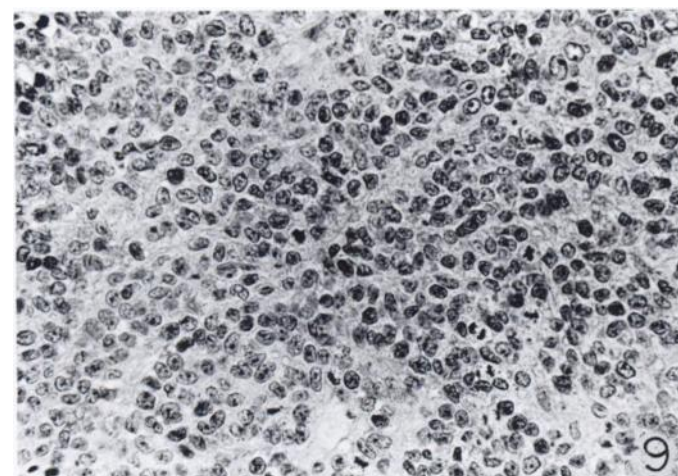
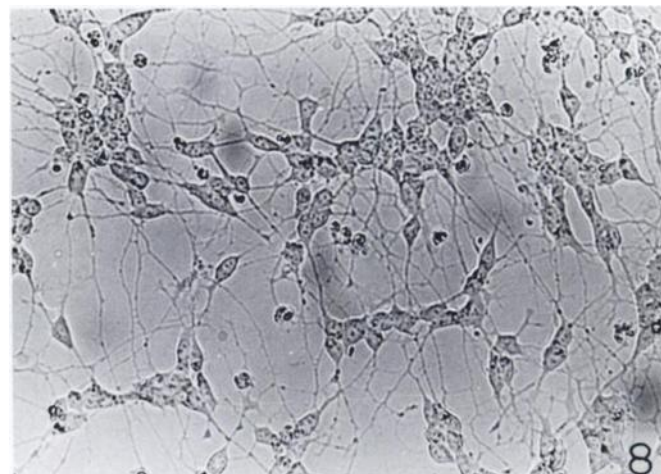
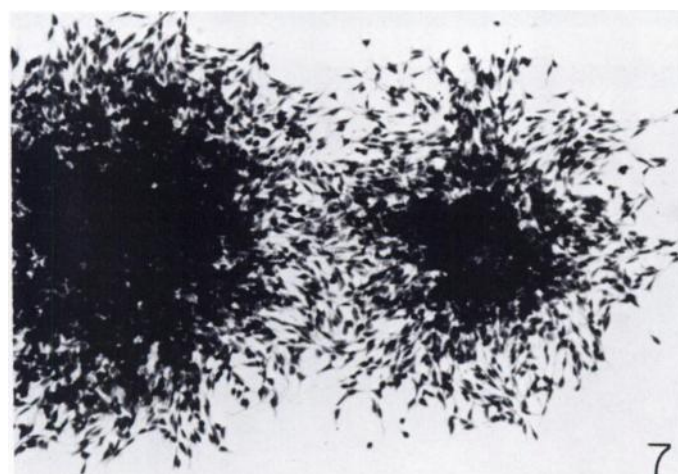
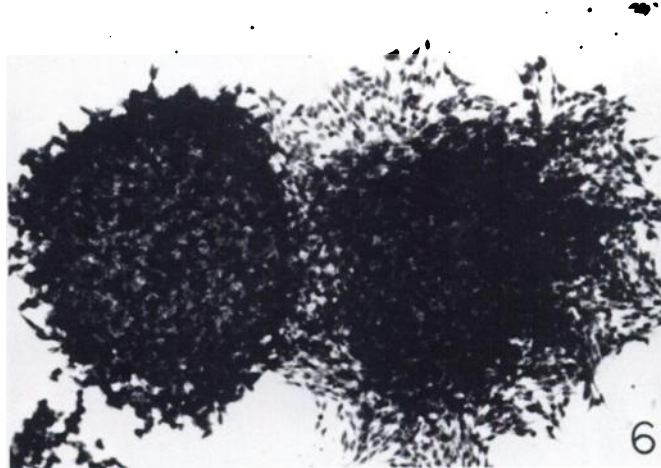
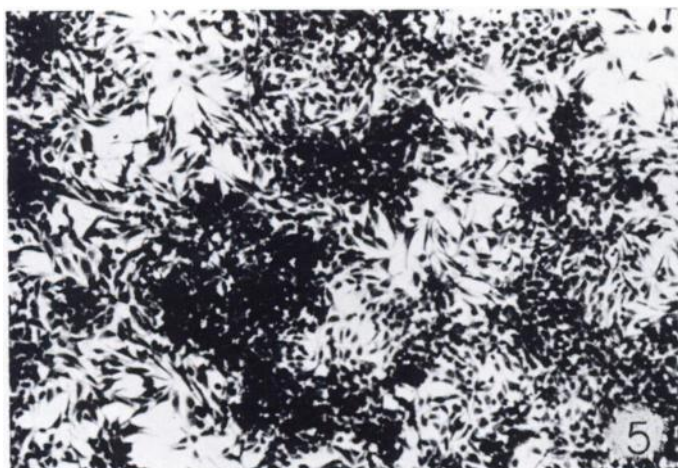
Fig. 11. Representative karyotype of SK-N-SH. $\times 1800$.

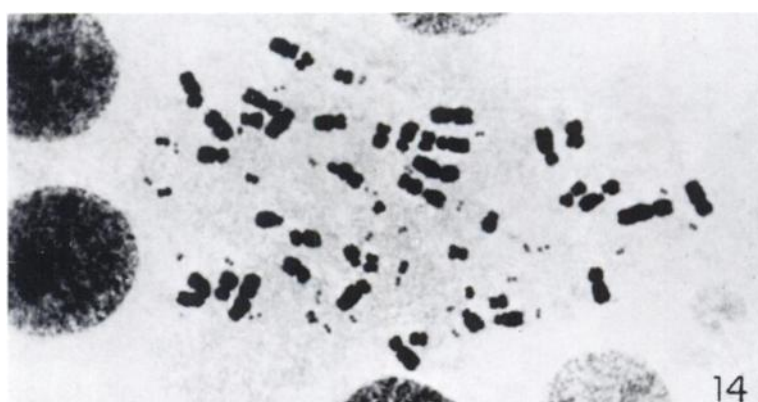
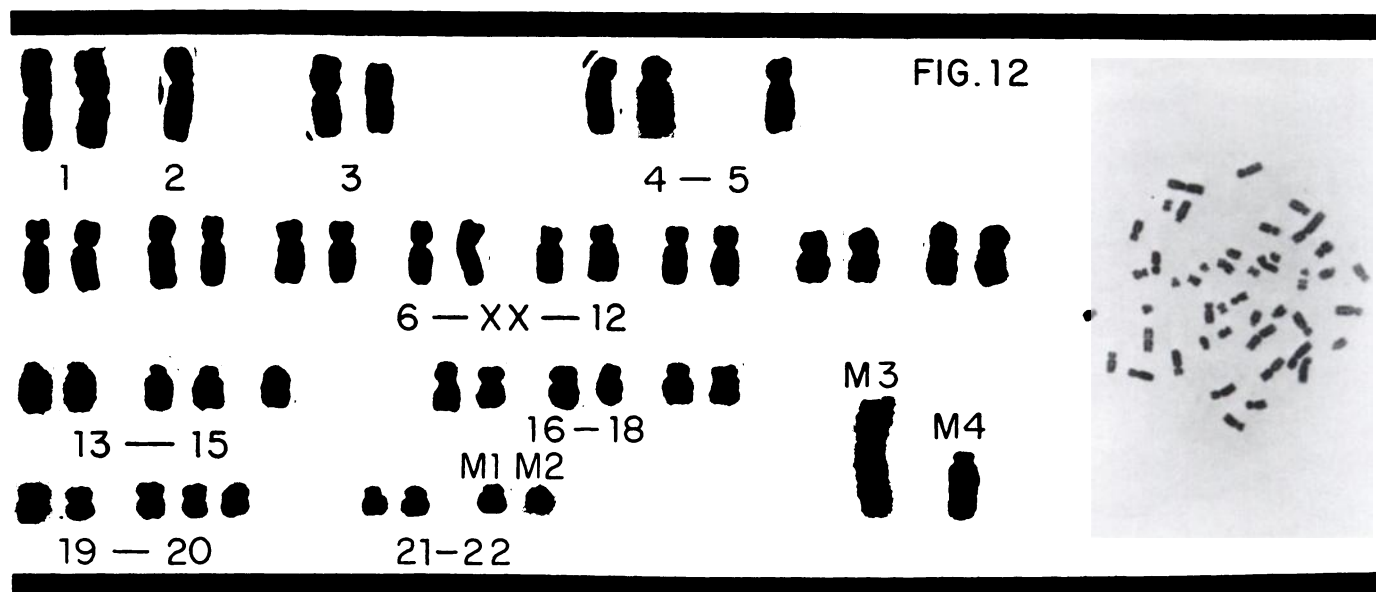
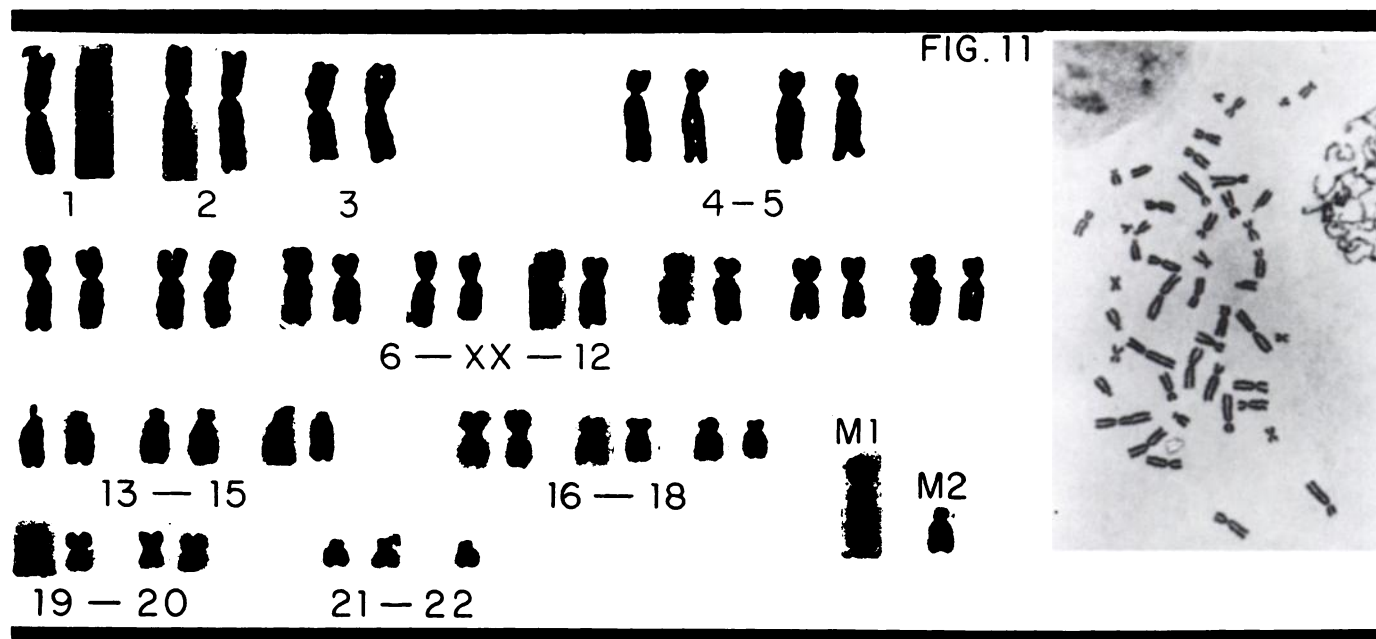
Fig. 12. Representative karyotype of SK-N-MC. $\times 2200$.

Fig. 13. Metaphase cell of SK-N-MC-IX with more than 100 "faint" and "small" double-*minute* chromosomes.

Fig. 14. Metaphase cell of SK-N-MC-IXC with "small" and "medium" double-*minute* chromosomes. The medium-sized chromatin bodies are the largest observed in these cells.







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