0073-5655/81/0619-0631 $01.50/0
IN VITRO Vol. 17, No. 7, July 1981

THE FINE STRUCTURE OF CONTINUOUS HUMAN NEUROBLASTOMA LINES SK-N-SH, SK-N-BE(2), AND SK-N-MC

ELIZABETH N. BARNES, JUNE L. BIEDLER, BARBARA A. SPENGLER, AND KATHERINE M. LYSER[1]

Department of Pathology, Columbia University College of Physicians and Surgeons, New York, New York 10032 (E. N. B.); Laboratory of Cellular and Biochemical Genetics, Memorial Sloan-Kettering Cancer Center, Walker Laboratory, Rye, New York 10580 (J. L. B., B. A. S.); and Department of Biological Sciences, Hunter College of the City University, New York, New York 10021 (K. M. L.)

(Received July 7, 1980; accepted December 5, 1980)

SUMMARY

Cell cultures of the continuous human neuroblastoma lines SK-N-SH, SK-N-BE(2), and SK-N-MC at exponential and stationary growth phase have been examined by electron microscopy. At the level of fine structure these cells did not show typical neuronal differentiation such as extensive granular endoplasmic reticulum or neurites with microtubules and neurofilaments. Instead they were characterized by abundant free ribosomes, moderate Golgi complexes, and usually scant granular endoplasmic reticulum, features similar to the fine structure of early normal embryonic autonomic neurons. However, in several respects appearance of differentiated features of the neuroblastoma cells did not follow the pattern observed for normal neurons, suggesting noncoordinate expression of neuronal phenotypic properties. First, an occasional neuroblastoma cell had as extensive granular endoplasmic reticulum as would be found at later stages in normal developing neurons. Second, the cellular processes of these neuroblastoma cells did not have the fine structure of developing or mature axons in vivo. Third, few dense core vesicles were found in SK-N-SH and SK-N-BE(2), though these organelles are numerous in early normal adrenergic neurons and the adrenergic character of these two lines is apparent from other studies that have demonstrated expression of neurotransmitter synthesizing enzymes (SK-N-MC is cholinergic). The fine structural characterization of these continuous human neuroblastoma cell lines will allow this parameter to be utilized with other approaches in future experimental studies.

Key words: human neuroblastoma; electron microscopy; continuous cell lines.

INTRODUCTION

Establishment of continuous cell lines of human neuroblastoma has made possible a variety of experiments. As part of continuing studies of the basic biology and control of neuronal differentiation in such cells, we have examined in detail the fine structure of the continuous cell lines SK-N-SH, SK-N-BE(2), and SK-N-MC (1-6) under standard culture conditions. Various features of these cells and of other similar lines of malignant neuronal cells have been described, but their morphology at the level of electron microscopy has been considered only briefly (1-10). In order to monitor as many parameters as possible in assessing experimental results and in evaluating potential therapeutic agents, we have investigated the overall pattern of ultrastructure and fully characterized this aspect of these continuous human neuroblastoma lines. (Studies of other human neuroblastoma lines generally have given an incomplete picture.) This approach elucidates the level of differentiation expressed, for the fine structure of normal developing neurons changes markedly from early to mature stages (11).

Peripheral neuroblastomas are derived from neural crest cells and may be compared with their

[1]To whom all correspondence should be addressed at Box 1030, Hunter College CUNY, 695 Park Avenue, New York, New York 10021.

normal counterparts, developing autonomic neurons. Development of sympathetic neurons in human fetuses and laboratory mammals has been described (12,13). Just before neural crest cells aggregate to form sympathetic ganglia, one can identify two kinds of cells, early sympathetic neurons and those that appear to be neuronal stem cells. The probable neuronal precursor cells are stellate and have abundant free ribosomes, scant granular endoplasmic reticulum, moderately developed Golgi apparatuses, and some large (about 100 nm) dense core vesicles. Early sympathetic neurons have more extensive cytoplasm that is similar in fine structure to that of the precursor cells. Even at this early stage, axons contain microtubules, agranular reticulum, and a few dense core vesicles. As the neuron matures the perikaryon increases in size, the Golgi complex becomes more prominent, and numerous longer cisternae of granular endoplasmic reticulum appear. The cytoplasm of the mature neuronal perikaryon contains extensive rough-surfaced endoplasmic reticulum (RER) and Golgi membrane systems as well as other organelles such as free ribosomes, mitochondria, microtubules, and neurofilaments. The RER, which is the fine structural basis of a classical attribute of the neuron, the Nissl substance, is a particularly useful criterion in assessing the extent of cytological differentiation because it undergoes conspicuous change from early to late stages.

Many continuous neuroblastoma cell lines, among them the SK-N-SH, SK-N-BE(2), and SK-N-MC cell lines described in this report, express some neuronal features under standard culture conditions. The SK-N-SH and SK-N-BE(2) lines are predominantly adrenergic whereas SK-N-MC appears exclusively cholinergic (4,6). The near-diploid karyotype of many human neuroblastoma lines makes them useful for combining cell genetics with other approaches to the study of control of differentiation. In addition, an interesting set of anomalies of metaphase chromosomes, a long, nonbanding homogeneously staining region (HSR), and small, paired chromatin bodies known as double minutes (DMs) have been described. The prevalence of one or the other of these two types of chromosome abnormalities in human neuroblastoma is notable (14,15). SK-N-BE(2) cells have HSRs; SK-N-MC cells have DMs and, occasionally, HSRs (2,3,5).

We find that, for the most part, these cells in culture do not display characteristic neuronal differentiation as determined by fine structure analysis. Considered together with other features, such as neurotransmitter synthesizing enzyme expression, our results suggest that SK-N-SH, SK-N-BE(2), and SK-N-MC cells are comparable in general to normal autonomic neurons at early stages of their differentiation. However, neuronal characteristics are expressed, at least to some extent, in a noncoordinate fashion.

Materials and Methods

Neuroblastoma cell lines and culture methods. Cell line SK-N-SH was established from an aspiration biopsy of a bone marrow metastasis 8 months after excision of a large thoracic primary from a 4-yr-old female with continuing elevated levels of urinary catecholamines and vanillylmandelic acid (1,4). SK-N-BE(2) was from a bone marrow biopsy from a 2-yr-old male (2,4); SK-N-MC was from an orbital metastasis 3 years after presentation with a small thoracic neuroblastoma in a 15-yr-old female with normal levels of urinary catecholamines (1,2,4). All three patients had undergone repeated courses of chemotherapy and radiotherapy (3). Cells of all three lines were demonstrated to be tumorigenic in cheek pouches of immunosuppressed hamsters (1,4).

Cell lines were grown in Eagle's minimum essential medium (MEM) supplemented with nonessential amino acids (Eagle's formulation), 15% fetal bovine serum, penicillin (100 IU/ml), and streptomycin (100 μg/ml) in 25-cm^2 culture flasks (Falcon Plastics, Los Angeles, CA) or on plastic cover slips in 35- and 60-mm petri-style dishes. The medium was partially replaced three times a week. Material for this study included two stationary phase cultures of each line [SK-N-SH, Passages 35 and 36; SK-N-BE(2), Passages 34 and 35; and SK-N-MC, Passages 51 and 52], and two exponential phase cultures of each line [SK-N-SH, Passages 9 and 35; SK-N-BE(2), Passages 12 and 35; and SK-N-MC, Passages 16 and 52]. Cell densities at stationary phase were 0.98×10^6 cells/cm^2 for SK-N-SH, 1.3×10^6 cells/cm^2 for SK-N-BE(2), and 1.5×10^6 cells/cm^2 for SK-N-MC. SK-N-SH and SK-N-MC were tested for *Mycoplasma* by the direct microscopical demonstration method with orcein staining after hypotonic treatment (16) by Dr. Jørgen Fogh, Memorial Sloan-Kettering Cancer Center, New York, and SK-N-BE(2) was tested by the uridine phosphorylase assay (17) by Dr. Harvey Ozer, Hunter College, New York. Organisms were not

detected in these tests. In addition, in approximately 800 electron micrographs examined, no evidence of *Mycoplasma* was found.

Electron microscopy. Cultures were rinsed briefly with buffered saline and fixed in the flask or on cover slips in 3% glutaraldehyde in Millonig's phosphate buffer (0.1 *M*, pH 7.4) with 1 m*M* $CaCl_2$ for 1 h at room temperature. They were postfixed 1 h in 1% OsO_4 in the same buffer, rapidly dehydrated in ethanol, and embedded in Epon 812 (18) or Spurr's medium (19). Sections were routinely cut perpendicular to the substrate; electron micrographs illustrating this report are from such sections, with exceptions noted that are from sections cut parallel to the substrate. One to three-micrometer sections were stained with toluidine blue for light microscopy or examined unstained by phase microscopy. Sections for electron microscopy were stained with 25% uranyl acetate in methanol (20), and some were counterstained with lead citrate (21) or bismuth subnitrate (22).

For comparison of neuroblastoma cells and normal developing neurons, paravertebral sympathetic ganglia from three second trimester human fetuses were partially dissociated by teasing and cultured in petri-style culture dishes in MEM with FBS and nerve growth factor (2 U/ml, Wellcome Laboratories, Greenville, NC) for 8 d. Cultures were processed for electron microscopy as described above, except that cacodylate buffer was used, and uranyl acetate staining was carried out during dehydration.

Results

General features. When SK-N-SH and SK-N-BE(2) cells are viewed by light microscopy as they grow in cell culture, two morphological types are usually observed. There are neuroblastlike cells that are rounded and sometimes have processes, whereas the substrate-adherent pleomorphic cells are large, flat cells. (In the past the latter have been referred to as "epithelial-like" cells, but there is no evidence of an epithelial origin.) The flat cells are particularly apparent in SK-N-SH cultures, but are seen also in SK-N-BE(2) cultures. The SK-N-MC cells are somewhat fibroblastlike but less flattened and less oriented than human fibroblasts under similar culture conditions (1,6).

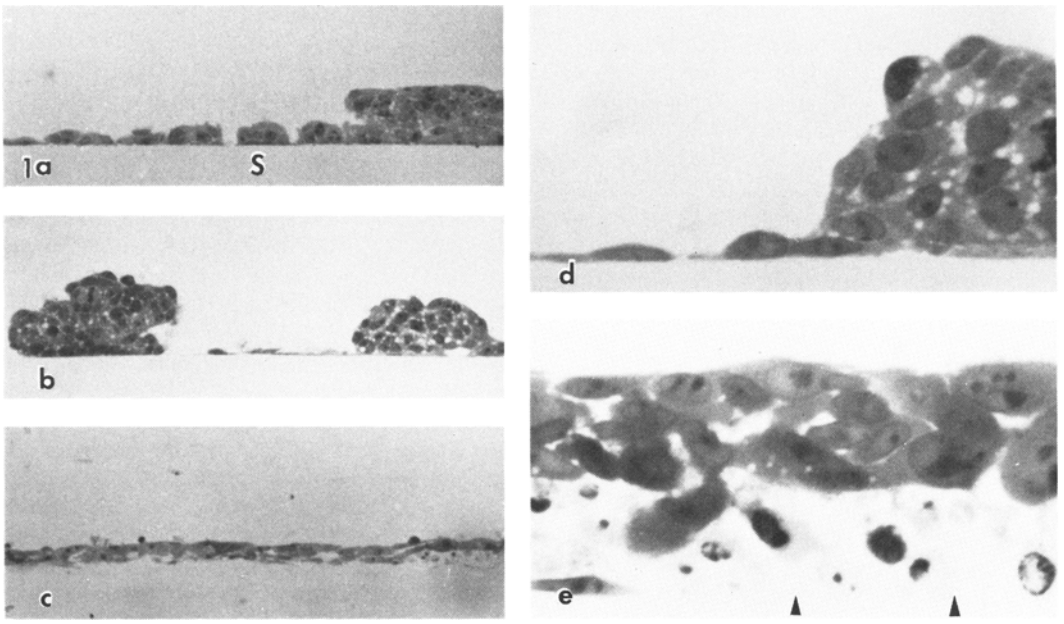


FIG. 1. Light micrographs of 1 to 2 μm sections of stationary phase cultures, cut perpendicular to the substrate (*S* in *a*, *arrow heads* in *e*) and stained with toluidine blue. In SK-N-SH(*a*) and SK-N-BE(2) (*b,d*) cultures, note aggregates of round or polygonal cells and apparent monolayers of cells flattened against the substrate. Compare to the continuous layer of varying thickness in SK-N-MC (*c,e*). At the right edge of *c* and in *e*, a necrotic area underlying the upper layers of healthy cells is visible. *a,b,c* ×220; *d,e* ×800.

Observations of 1 to 3-μm sections cut perpendicular to the substrate and of low magnification electron micrographs confirmed these descriptions and provided the means to distinguish various areas of the cultures in higher magnification electron micrographs. In stationary phase SK-N-SH and SK-N-BE(2) cultures, some cells formed aggregates or clumps up to 12 cells deep, as seen in Figs. 1*a*, *b*, *d*, and 2. Such clumps comprised rounded or irregular neuroblastlike cells. Between the clumps the cells formed a monolayer as observed with the light microscope. However, electron micrographs showed that in such areas there was extensive overlapping of lateral cytoplasmic extensions of cells and many nuclei were sandwiched between

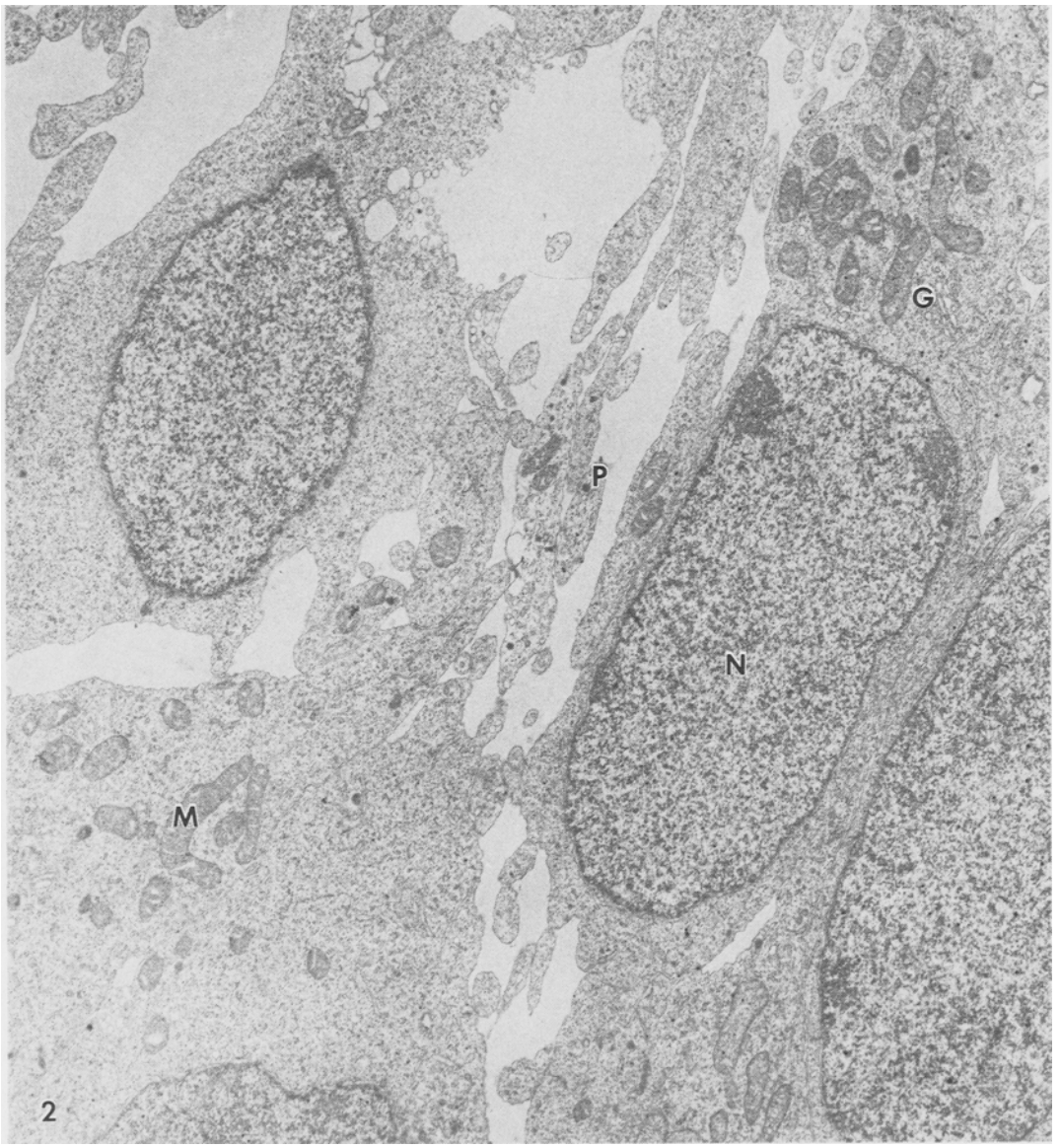


FIG. 2. Low power electron micrograph of stationary phase SK-N-SH cells near the center of a clump. Note extensive areas of cytoplasm filled only with free ribosomes. As is typical, mitochondria (*M*), Golgi complexes (*G*), and other organelles, when present, are not randomly distributed but tend to be clustered. Cell processes (*P*) are seen in spaces between the cells. *N*, nucleus. The substrate is to the right of the field shown. ×15,000.

flat extensions of several other cells; such areas are referred to as "pseudomonolayers." Individual cells in the pseudomonolayer were not identified before embedding and sectioning, and areas between clumps included both neuroblastlike and flat cells. However, those that were flat, with flat nuclei, and with extensive cytoplasm lateral to the nucleus (Fig. 3), were assumed to be substrate-adherent pleomorphic cells, because neuroblastlike cells, even when not aggregated, appear rounded and refractile in living cultures.

In SK-N-MC stationary phase cultures (Fig. 1*c*, *e*) the thickness varied from one or two nuclei in depth to six nuclei, but there were no dense cellular aggregates, and the upper surface of the culture was continuous. Extensive areas of necrosis often were present under the topmost cell layers. Compared with cells of the other two lines, stationary phase SK-N-MC cells displayed less variation in shape. All of them exhibited some degree of elongation or flattening, but the most elongated SK-N-MC cells were distinctly less flattened than the flattest cells in the other lines.

Exponential phase cultures of all three cell lines comprised small clumps of cells as well as monolayers and isolated cells. As in denser cultures, there were some flat cells in SK-N-SH and SK-N-BE(2) cultures during exponential growth phase.

Fine structure. Fine structure analysis showed little evidence of neuronal differentiation in these cells (Figs. 2–5). In particular, neurites, extensive granular endoplasmic reticulum, and arrangement of Golgi apparatus typical of a differentiated neuron were not observed. Rather, cytological features typical of neuronal cells at early stages of differentiation were prominent; these included numerous free ribosomes, sparse granular endoplasmic reticulum, and a moderately de-

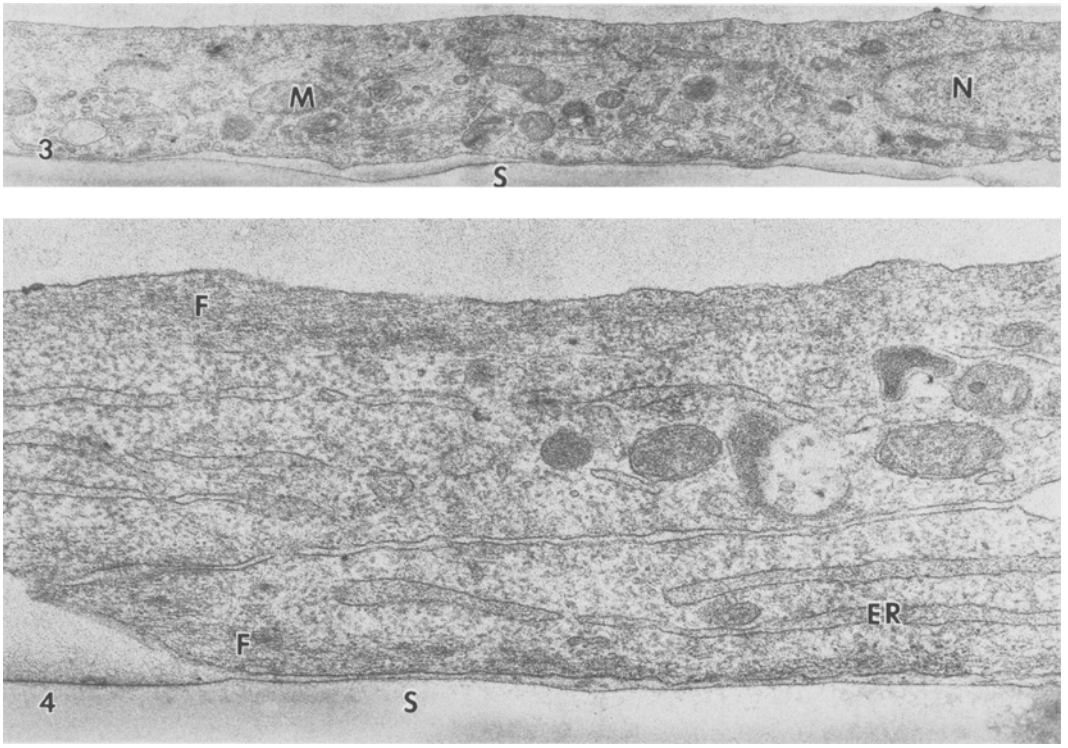


FIG. 3. Low power electron micrograph of a flat cell from an exponential phase SK-N-SH culture. The cell is less than 1.5 μm thick, even in the nuclear region, which extends about 5 μm to the right of this field with no increase in the height of the cell. The culture appears as a monolayer here, but in a nearby area flat cells overlap (*see* Fig. 4). *N*, nucleus; *M*, mitochondrion; *S*, substrate. ×18,000.

FIG. 4. Near the area shown in Fig. 3, overlapping portions of two flat cells constitute a "pseudomonolayer" as defined in the text. In each cell several elongated profiles of granular endoplasmic reticulum (*ER*) as well as numerous free ribosomes are visible. Microfilaments (*F*), which are about 7 nm d, are evident in association with cell surfaces. *S*, substrate. ×60,000.

veloped Golgi apparatus. There were relatively few differences among cells of the three lines.

Nucleus. In sections cut perpendicular to the substrate, nuclei of flattened cells appeared as elongated ovals. Nuclei of other cells were oval or polygonal. In many SK-N-MC cells, both growth phases, and in some exponential phase SK-N-BE(2) cells, there were finger-shaped nuclear invaginations about 300 to 500 nm d (Fig. 5). Chromatin was largely dispersed. However, the nuclei were less "vesicular" than in mature neurons, and heterochromatin was sometimes present as a narrow peripheral rim, most pronounced in the SK-N-MC cells.

Ribosomes and rough surfaced endoplasmic reticulum. The cytoplasm of early neurons includes abundant free ribosomes and scant RER; mature neurons are characterized by extensive

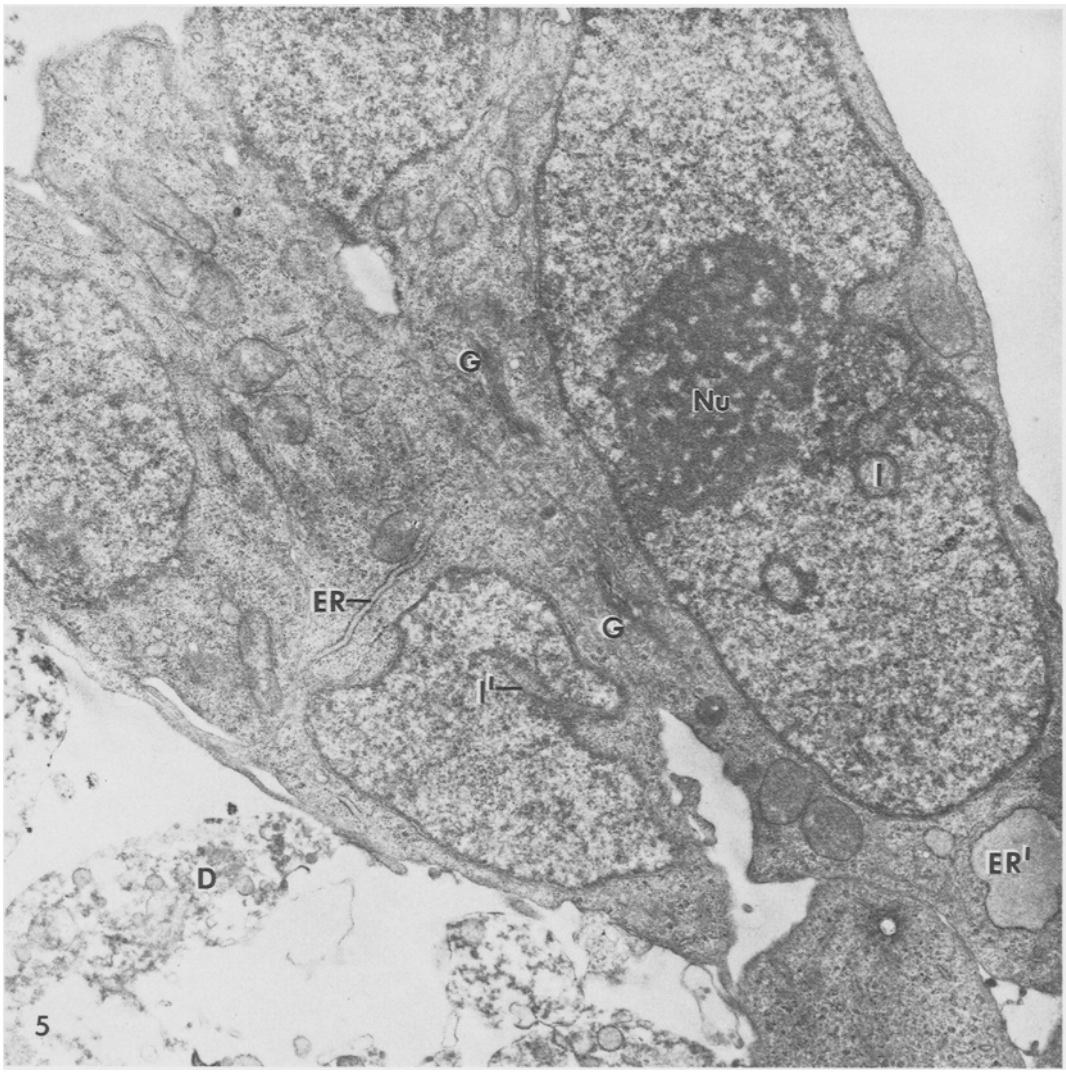


FIG. 5. Stationary phase SK-N-MC culture, from an area 3 to 4 nuclei deep, showing the upper layer of cells with cellular debris (*D*) between it and the lower layer of the culture that lies against the substrate out of the field to the lower left. Fingerlike cytoplasmic invaginations of nuclei are seen in transverse (*I*) and longitudinal (*I'*) section. Both flat (*ER*) and distended (*ER'*) granular endoplasmic reticulum cisternae are present. *Nu*, nucleolus; *G*, Golgi complex. ×11,000.

RER, which, in many neuron types, including sympathetic cells, is in parallel arrays with interspersed free ribosomes (Nissl bodies). In all SK-N-SH, SK-N-BE(2), and SK-N-MC neuroblastoma cells examined, the most prominent cytoplasmic organelles were free ribosomes that were virtually ubiquitous (Figs. 2, 5). In most cells RER was sparse and appeared as scattered short, single cisternae. In cells of all three lines in both growth phases, there were only occasional parallel arrays of cisternae, and it was rare indeed to observe an array of more than four cisternae. Single cisternae tended to be somewhat longer in SK-N-MC cells than in cells of the other two lines. Extensive dilatation of the RER was encountered in most SK-N-MC cells (Fig. 5) and in some flattened exponential phase cells of the other two lines.

Golgi apparatus. Moderately extensive Golgi complexes were present in cells of all three neuroblastoma lines. They were almost invariably near the nucleus (Figs. 5, 6). However, there was never a suggestion that such complexes surrounded the nucleus as they generally do in mature neurons.

Dense core vesicles. Dense core vesicles (about 100 nm d, with electron opaque cores separated from the membrane by a light halo) appear in de-

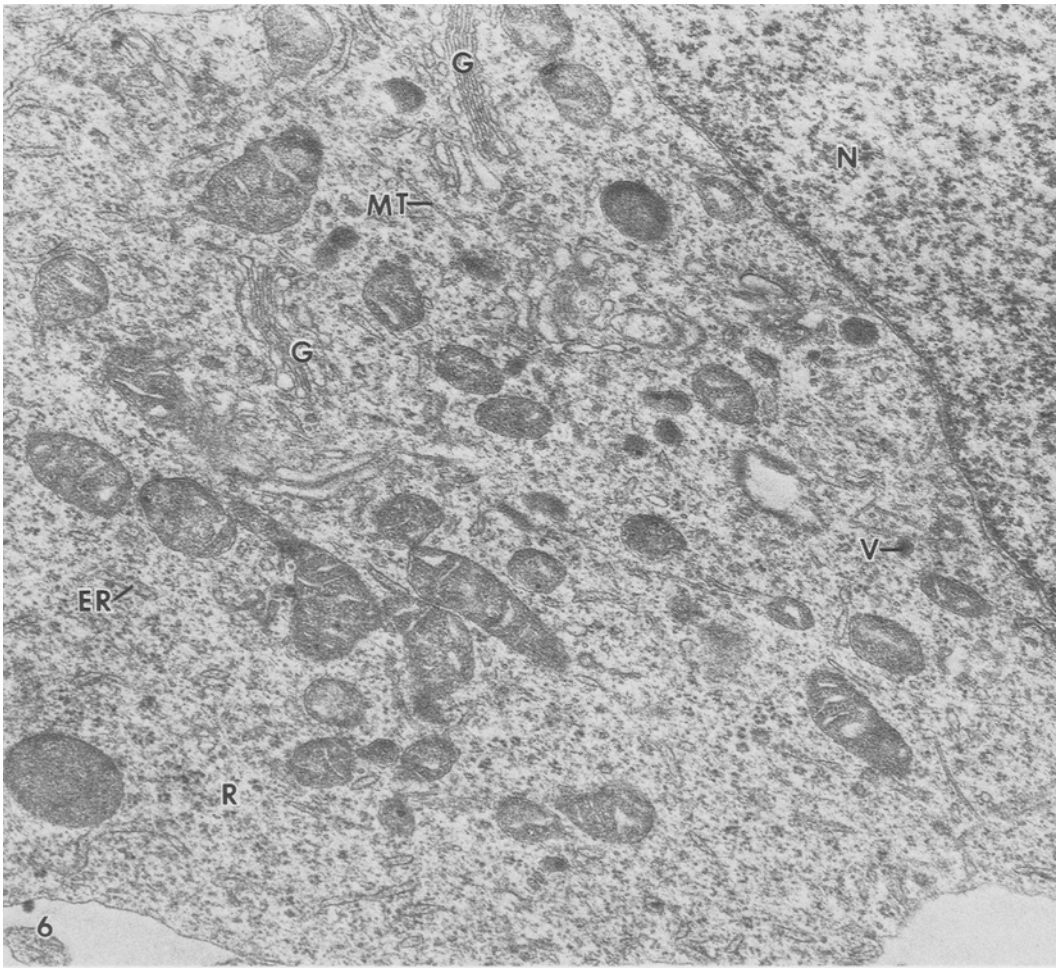
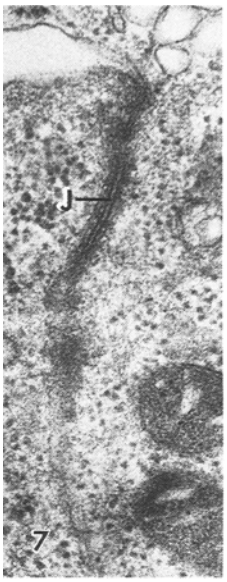
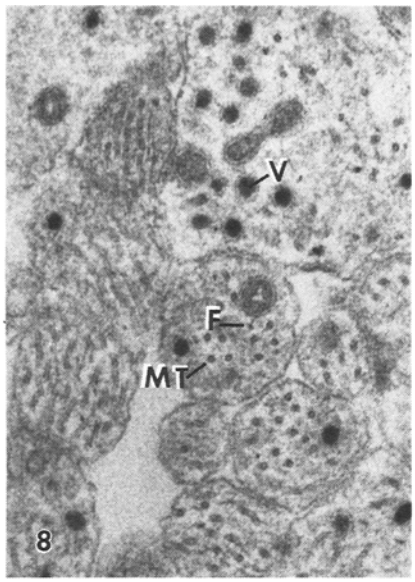
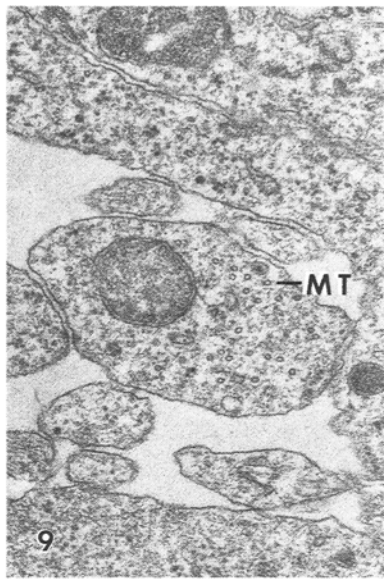


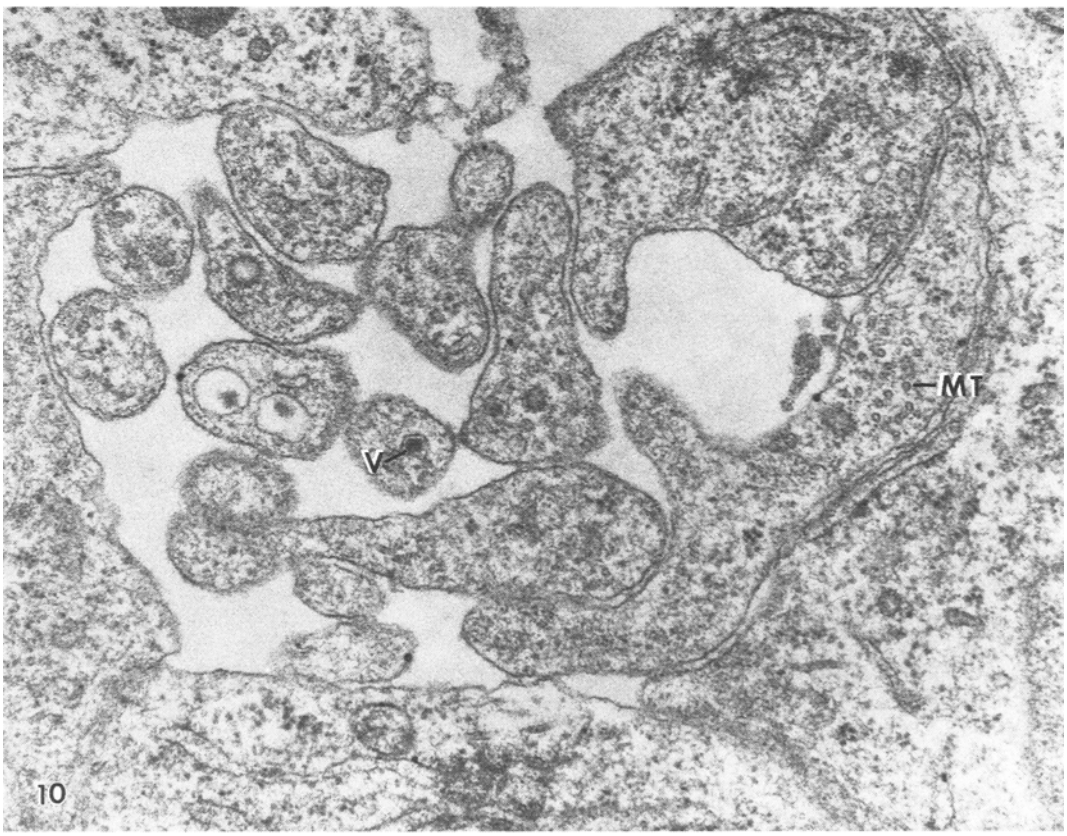
FIG. 6. The edge of the nucleus (*N*) and adjacent cytoplasm of a cell from a clump. The cytoplasm contains the typical clustered organelles in addition to the usual numerous free ribosomes (*R*). The Golgi complex is relatively well developed (G). Cisternae of granular endoplasmic reticulum (*ER*) are short and scattered. A number of microtubules (*MT*) and a single dense core vesicle (*C*) are evident. SK-N-SH, stationary phase culture. ×31,000.

veloping sympathetic neuron precursors before the terminal mitosis (23, 24). They tend to decrease in number in the cell soma as the neuron matures (13); however, they are prominent in partially mature cultured sympathetic neurons of human fetuses in the perikaryon as well as in









neurites (Fig. 8). Similar dense core vesicles were seen only in very small numbers in exponential and stationary phase SK-N-SH and SK-N-BE(2) cells. They were found singly or in small groups, in random locations in the cells (Figs. 6, 10). Cholinergic neurons do not have dense core vesicles, and none were found in SK-N-MC cells.

Cytoskeleton and cell processes. Microtubules, intermediate filaments (10 nm), and microfilaments (7 nm) are found in neuronal precursors as well as in mature neurons. The most striking feature associated with normal neuronal cell differentiation is a characteristic distribution of tubules and filaments in the axon. The axoplasm contains microtubules and intermediate filaments (also mitochondria and smooth surfaced vesicles, but not numerous ribosomes, granular endoplasmic reticulum, or Golgi complexes). Microfilaments are found particularly in apposition to the plasma membrane and in the growth cone microvilli.

In cells of all three neuroblastoma lines, microtubules, intermediate filaments, and microfilaments were present, but the processes observed were not axonal in their fine structure (Figs. 8–10). Membrane-limited round or oval profiles (cross or oblique sections of processes), about 1 μm or less in diameter, were numerous among SK-N-SH and SK-N-BE(2) cells in clumps in some regions; they were noted infrequently on top of or among flattened cells. Distinctly fewer were found in SK-N-MC cells than in the other lines. In most cases, the cytoplasm of processes was similar to that of cell bodies, with many free ribosomes, some mitochondria, and occasionally a dense core vesicle. In one area of a stationary phase SK-N-SH culture there were 60 cross or oblique sections of processes, all including numerous ribosomes, only 6 including any microtubules.

Cell to cell connections. Infrequent intercellular junctions were seen in SK-N-SH and SK-N-BE(2) cultures (Fig. 7). They appeared as densities of fine filamentous material immediately underlying apposed plasma membranes of cell bodies. In a few instances similar densities were associated with the plasma membrane where it was in contact with the substrate. Although they were specifically sought, no other junctions and no synapses were observed.

Other organelles. Other organelles typically found in neurons as well as in many other cell types were present but not remarkable in these neuroblastoma cells. These included numerous, widely distributed mitochondria; pairs of centrioles; single multivesicular bodies; dense bodies with the appearance of lysosomes; bristle coated vesicles; apparent pinocytotic invaginations; glycogen deposits; isolated, randomly located cilia; small arrays of annulate lamellae; and large arrays of clear vesicles surrounded by a protrusion of the cell membrane ["mounds" (25)].

Discussion

These studies show that human neuroblastoma cells of the SK-N-SH, SK-N-BE(2), and SK-N-MC lines maintained under standard culture conditions are generally comparable in their fine structure to early developmental stages of sympathetic and other nerve cells that have large nuclei with prominent nucleoli, cytoplasm with numerous free ribosomes, scanty rough endoplasmic reticulum, and moderately developed Golgi complexes (12,13,26–29). The presence of transmitter synthesizing enzymes in neuroblastoma cells also is consistent with an early stage of differentiation in autonomic neurons (23,24). Brief descriptions of other continuous human neuroblastoma lines suggest that they are similarly immature in level of differentiation (7–10).

Our fine structure observations, however, combine with other data to indicate that the tumor cells do not correspond exactly to their normal counterparts in pattern of development. Most cell processes observed in SK-N-SH, SK-N-BE(2),

FIG. 7. Edges of two SK-N-SH stationary phase cells with a junctional area (*J*) between them. From material sectioned parallel to the substrate. ×47,000.

FIG. 8. Cross and oblique sections of axons in a primary culture of a sympathetic ganglion from a 16 wk human fetus, 8 d in vitro. Note 10-nm filaments (*F*), numerous microtubules (*MT*), clusters or single dense vesicles (*V*) in some axons. ×40,000.

FIG. 9. Section of a neuroblastoma cell process with microtubules (*MT*) but not typical axonal morphology. Compare with Fig. 8. SK-N-SH stationary phase culture. ×40,000.

FIG. 10. Cluster of cell processes in a space between SK-N-BE(2) cells in a clump. None of the processes has the typical fine structure of an axon. A few microtubules can be found, including a group (*MT*) in what seems to be an irregular sheetlike cell extension. *V*, dense core vesicle. Exponential phase culture, sectioned parallel to the substrate. ×40,000.

and SK-N-MC cultures maintained in serum-supplemented medium do not resemble axons of normal sympathetic neurons. Typical neurites with microtubules, smooth-surfaced reticulum, and a few dense core vesicles are seen in sympathetic ganglia at relatively early stages of development (13). Human fetal ganglion cells in culture have been observed to form numerous typical axons (Fig. 8) (30; Lyser, unpublished observations). In clinical specimens, as well as in organ cultures, processes with the fine structure of axons have been seen in some "undifferentiated" neuroblastomas but not others (31-35). Either because cells were removed from the culture vessels before fixation or because this aspect was not considered in the available reports (7-10), no comparisons can be made with IMR-32, nor with the CHP and LA-N lines. With appropriate manipulation of some lines and clones, it is possible to enhance greatly formation of processes that are more like axons of normal neurons (36,37; Lyser et al., unpublished observations). Although light microscopic observations indicate that there may be some fluctuations over time in the extent of process formation in continuous lines of neuroblast-like cells, occurrence of large proportions of neuroblastoma cell processes that are not "neuritelike" in the cultures examined in this study suggests that a more rigorous definition of a process as a neuronal characteristic must be applied.

Abundance of dense core vesicles does not seem to be correlated in human neuroblastoma lines with levels of activity of enzymes for transmitter synthesis nor with the developmental pattern in normal sympathetic ganglion cells. As indicated in Table 1, several predominantly adrenergic lines [SK-N-BE(2), LA-N-1, IMR-32, and SK-N-SH] have dense core vesicles, but there is no apparent quantitative correlation within the adrenergic group, and dense core vesicles are most abundant in LA-N-1, a line which demonstrates no activity of the enzymes necessary for the synthesis of dopamine and norepinephrine (6,10). Although neither the present study nor previous reports of the fine structure of human neuroblastoma lines include material processed by specific histochemical procedures, there are many reports of sympathetic neurons that are fixed by routine glutaraldehyde osmium methods and that have conspicuous dense core vesicles, the catecholamine content of which is indicated by correlated formaldehyde-induced fluorescence studies (12,13,27,38).

Because dense core vesicles are observed at early stages in normally developing neurons, we expected to find appreciable numbers in the adrenergic neuroblastoma lines. Studies combining [^{3}H]thymidine labeling with formaldehyde-induced fluorescence, electron microscopy, or immunohistochemistry show catecholamines, dense core vesicles, and transmitter enzymes to be present in sympathetic ganglion cells before the terminal mitosis in laboratory animals (23,24). Human fetal sympathetic ganglion cells in vivo and in culture contain numerous dense core vesicles after glutaraldehyde osmium fixation (Fig. 8, and unpublished observations) and show formaldehyde-induced fluorescence (30). Thus, it appears that normal human sympathetic neurons at early stages of development do have catecholamine(s) in the dense core vesicles, although there

TABLE 1

COMPARISON OF PRESENCE OF DENSE CORE VESICLES WITH NEUROTRANSMITTER BIOSYNTHETIC ENZYME ACTIVITIES IN HUMAN NEUROBLASTOMA CELL LINES

Neuroblastoma Cell Line	Dense Core Vesicles[b]	Enzyme Activity[a]			
		Choline Acetyl-Transferase	Tyrosine Hydroxylase	Aromatic L-Amino Acid Decarboxylase	Dopamine-Beta-Hydroxylase
LA-N-1	++[c]	0	3.05	0	0
SK-N-BE(2)	+[d]	0	54.1	22.9	5.79
SK-N-SH	+[c,d]	0	0.017	5.7	14.66
IMR-32	+[c]	0	1.47	1.26	0
LA-N-2	0[c]	9.40	0.23	0.16	3.69
SK-N-MC	0[d]	3.52	0	0	0

[a] Enzyme activities expressed as nanomoles of product per hour per milligram protein, data from (6), where statistical analysis may be found.

[b] Estimate of abundance of dense core vesicles in electron micrographs, ++, many; +, few; 0, none observed.

[c] Data from (10).

[d] Data from this report.

is some question as to which catecholamines are present (39).

The paucity of dense core vesicles in the SK-N-SH and SK-N-BE(2) neuroblastoma cells may reflect a disturbance of developmental controls resulting from malignant transformation, and may be related to the failure of these cells to produce detectable norepinephrine from radioactive precursor (4). Perhaps there is a deficiency in the mechanism of forming vesicles and incorporating into them dopamine-β-hydroxylase, the enzyme which catalyzes the conversion of dopamine to norepinephrine and seems to be located in dense core vesicles (40,41). Presence of dense core vesicles in generally morphologically undifferentiated neuroblastomas has been correlated with a better prognosis than that for patients with similar (evidently adrenergic) tumors in which dense core vesicles are not found (42). Such correlation might reflect the degree of disruption of developmental controls. There is no apparent correlation between the presence of dense core vesicles and that of neurites in these tumors.

Differences in fine structure among the three lines were few. The SK-N-MC cells' lack of dense core vesicles supports other evidence of their cholinergic character. Other differences in fine structure among the lines do not seem related to neuronal differentiation. In addition, no significant ultrastructural differences were found between exponential and stationary phase cultures in any line, although specific activities of some neurotransmitter synthesizing enzymes are higher during stationary phase than during exponential growth phase (4,43).

In the SK-N-SH and SK-N-BE(2) lines, the pattern of cell to cell relationships, with clumps of rounded cells and "pseudomonolayers" containing flat cells, somewhat resembles that in primary cultures of dissociated neural cells, where nonneuronal cells form a background layer with neurons on top. Neurons tend to be rounded in such cultures, and in some instances aggregate in clusters (44,45). However, although substrate-adherent pleomorphic clones, including those arising from conversion of neuronlike cells, lose the transmitter synthesizing enzyme activities of the parental line or clone (6), they do not have the typical spindle shape of Schwann cells in primary cell cultures (46). It is not known whether the flat neuroblastoma cells express any glial "markers." The lack of conspicuous differences between the flat and neuroblastlike cells with respect to cytoplasmic organelles may reflect the immature level of differentiation of all these neuroblastoma cells and corresponds to the general similarity in fine structure of many immature cell types in normal embryos.

With respect to two prominent features, general fine structure characterized by abundant free ribosomes along with sparse granular endoplasmic reticulum and the expression of neurotransmitter-synthesizing enzyme activities, the continuous human neuroblastoma cell lines SK-N-SH, SK-N-BE(2), and SK-N-MC are similar to early autonomic neurons. However, the presence of a few cells with more extensive granular endoplasmic reticulum than is found in early neurons, the paucity of dense core vesicles in lines expressing enzyme activities for catecholamine synthesis, and the nonneuritelike morphology of most of the cell processes suggest that expression of various differentiated features may be independent to some degree. We anticipate that experiments designed to test the capacity of the human neuroblastoma cells to undergo further differentiation, for example, coculture with embryonic tissue, culture in conditioned media, or culture with agents such as cyclic AMP and nerve growth factor, will yield some answers to questions raised by these fine structural studies.

REFERENCES

1. Biedler, J. L.; Helson, L.; Spengler, B. A. Morphology and growth, tumorigenicity, and cytogenetics of human neuroblastoma cells in continuous culture. Cancer Res. 33: 2643-2652; 1973.
2. Biedler, J. L.; Spengler, B. A. Metaphase chromosome anomaly: Association with drug resistance and cell-specific products. Science 191: 185-187; 1976.
3. Biedler, J. L.; Spengler, B. A. A novel chromosome abnormality in human neuroblastoma and antifolate-resistant Chinese hamster cell lines in culture. J. Natl. Cancer Inst. 57: 683-694; 1976.
4. Biedler, J. L.; Roffler-Tarlov, S.; Schachner, M.; Freedman, L. Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. Cancer Res. 38: 3751-3757; 1978.
5. Biedler, J. L.; Ross, R. A.; Shanske, S.; Spengler, B. A. Human neuroblastoma cytogenetics: Search for significance of homogeneously staining regions and double minute chromosomes. Evans, A. E. ed. Advances in neuroblastoma research. New York: Raven Press; 1980; 81-96.
6. Ross, R. A.; Joh, T. H.; Reis, D. J.; Spengler, B. A.; Biedler, J. L. Neurotransmitter-synthesizing enzymes in human neuroblastoma cells: Relationship to morphological diversity. Evans, A. E. ed. Advances in neuroblastoma research. New York: Raven Press; 1980: 151-160.

7. Tumilowicz, J. J.; Nichols, W. W.; Cholon, J. J.; Greene, A. E. Definition of a continuous human cell line derived from neuroblastoma. Cancer Res. 30: 2110–2118; 1970.
8. Schlesinger, H. R.; Gerson, J. M.; Moorhead, P. S.; Maguire, H.; Hummeler, K. Establishment and characterization of human neuroblastoma cell lines. Cancer Res. 36: 3094–3100; 1976.
9. Gerson, J. M.; Schlesinger, H. R.; Sereni, P.; Moorhead, P. S.; Hummeler, K. Isolation and characterization of a neuroblastoma cell line from peripheral blood in a patient with disseminated disease. Cancer 39: 2508–2512; 1977.
10. Seeger, R. C.; Rayner, S. A.; Banerjee, A.; Chung, H.; Laug, W. E.; Neustein, H. B.; Benedict, W. F. Morphology, growth, chromosomal pattern, and fibrolytic activity of two new human neuroblastoma cell lines. Cancer Res. 37: 1364–1371; 1977.
11. Tennyson, V. M. The fine structure of the developing nervous system. Himwich, W. A. ed. Developmental neurobiology. Springfield, IL: Charles C. Thomas; 1970: 47–116.
12. Kanerva, L.; Hervonen, A.; Hervonen, H. Morphological characteristics of the ontogenesis of the mammalian peripheral adrenergic nervous system with special remarks on the human fetus. Med. Biol. 52: 144–153; 1974.
13. Tennyson, V. M.; Mytilineou, C. Fluorescence and electron microscopic studies of sympathoblasts in the fetal rabbit. Eränkö, O. ed. SIF cells: Structure and function of the small, intensely fluorescent sympathetic cells. Washington, D.C.: U.S. Govt. Print. Off.; 1976: 35–53.
14. Balaban-Malenbaum, G.; Gilbert, F. Double minute chromosomes and the homogeneously staining regions in chromosomes of a human neuroblastoma cell line. Science 198: 739–741; 1977.
15. Spengler, B. A.; Biedler, J. L. Prevalence of homogeneously staining regions (HSRs) and double-minute chromosomes (DMs) in human neuroblastoma cell lines. J. Cell Biol. 83: 159a; 1979.
16. Fogh, J.; Fogh, H. Procedures for control of mycoplasma contamination of tissue cultures. Ann. N.Y. Acad. Sci. 172: 15–30; 1969.
17. Levine, E. M. Mycoplasma contamination of animal cell cultures: a simple, rapid detection method. Exp. Cell Res. 74: 99–109; 1972.
18. Luft, J. H. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9: 409–414; 1961.
19. Spurr, A. R. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26: 31–43; 1969.
20. Stempak, J.; Ward, R. T. An improved staining method for electron microscopy. J. Cell Biol. 22: 697–701; 1964.
21. Reynolds, E. S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208–212; 1963.
22. Riva, A. A simple and rapid staining method for enhancing the contrast of tissues previously treated with uranyl acetate. J. Microscopie 19: 105–108; 1977.
23. Rothman, T. P.; Gershon, M. D.; Holtzer, H. The relationship of cell division to the acquisition of adrenergic characteristics by developing sympathetic ganglion cell precursors. Dev. Biol. 65: 322–341; 1978.
24. Rothman, T. P.; Specht, L. A.; Gershon, M. D.; Joh, T. H.; Reis, D. Replication of catecholaminergic neurons: occurrence in neural crest but not neural tube derivatives. Anat. Rec. 193: 669; 1979.
25. Pfenninger, K. H.; Bunge, M. B. Observations of plasmalemmal growth zones in developing neural tissue. J. Cell Biol. 59 (2, Pt. 2): 264a; 1973.
26. Pick, J.; Gerdin, C.; DeLemos, C. An electron microscopical study of developing sympathetic neurons in man. Z. Zellforsch. 62: 402–415; 1964.
27. Hervonen, A. Development of catecholamine-storing cells in human fetal paraganglia and adrenal medulla. Acta Physiol. Scand. (Suppl.) 368: 3–94; 1971.
28. Lyser, K. M. Early differentiation of motor neuroblasts in the chick embryo as studied by electron microscopy. II. Microtubules and neurofilaments. Dev. Biol. 17: 117–142; 1968.
29. Wentworth, L. E.; Hinds, J. W. Early motoneuron formation in the cervical spinal cord of the mouse: An electron microscopic, serial section analysis. J. Comp. Neurol. 177: 611–634; 1978.
30. Hervonen, H.; Hervonen, A.; Kanerva, L. Light and electron microscopic histochemistry of monoamines in human foetal sympathetic ganglion in culture. Histochem. J. 10: 271–286; 1978.
31. Goldstein, M. N.; Burdman, J. A.; Journey, L. J. Long-term tissue culture of neuroblastomas. II. Morphologic evidence for differentiation and maturation. J. Natl. Cancer Inst. 32: 165–199; 1964.
32. Misugi, K.; Misugi, N.; Newton, W. A. Fine structural study of neuroblastoma, ganglioneuroblastoma and pheochromocytoma. Arch. Pathol. 86: 160–170; 1968.
33. Greenberg, R.; Rosenthal, I.; Falk, G. S. Electron microscopy of human tumors secreting catecholamines: Correlation with biochemical data. J. Neuropathol. Exp. Neurol. 28: 475–500; 1969.
34. Tannenbaum, M. Ultrastructural pathology of adrenal medullary tumors. Pathol. Annu. 5: 145–171; 1970.
35. Lyser, K. M. Low- and high-voltage electron microscopy of a human neuroblastoma in long-term organ culture. Cancer Res. 34: 594–602; 1974.
36. Perez-Polo, J. R.; Werrbach-Perez, K.; Tiffany-Castiglioni, E. A human clonal cell line model of differentiating neurons. Dev. Biol. 71: 341–355; 1979.
37. Lyser, K. M.; Burmeister, D. W.; Carroll, J. M.; Zeevalk, G. K. Culture of human neuroblastoma cells in serum-free supplemented medium. Anat. Rec. 196: 240A; 1980.
38. Hervonen, A.; Kanerva, L. Cell types of human fetal superior cervical ganglion. Z. Anat. Entwicklungsgesch. 137: 257–269; 1972.

39. Hervonen, A.; Kanerva, L.; Hervonen, H. Ultrastructure of human fetal sympathetic ganglia after permanganate fixation—absence of granular vesicles. Neurosci. Lett. 5: 303–308; 1977.

40. Lundberg, J.; Bylock, A.; Goldstein, M.; Hansson, H.-A.; Dahlström, A. Ultrastructural localization of dopamine-β-hydroxylase in nerve terminals of the rat brain. Brain Res. 120: 549–552; 1977.

41. Winkler, H. The biogenesis of adrenal chromaffin granules. Neuroscience 2: 657–683; 1977.

42. Romansky, S. G.; Crocker, D. W.; Shaw, K. N. F. Ultrastructural study of neuroblastoma—evaluation of cytodifferentiation and correlation of morphology and biochemical and survival data. Cancer 42: 2392–2398; 1978.

43. Ross, R. A.; Joh, T. H.; Biedler, J. L.; Reis, D. J. Sequential expression of catecholamine enzymes in human neuroblastoma. Trans. Am. Soc. Neurochem. 9: 129; 1978.

44. Varon, S. S. In vitro study of developing neural tissue and cells: Past and prospective contributions. Schmitt, F. O. ed. The neurosciences: Second study program. New York: The Rockefeller University Press; 1970: 83–99.

45. Varon, S.; Raiborn, C. Excitability and conduction in neurons of dissociated ganglionic cell cultures. Brain Res. 30: 83–98; 1971.

46. Askanas, V.; Engel, W. K.; Dalakas, M. C.; Lawrence, J. V.; Carter, L. S. Human Schwann cells in tissue culture: Histochemical and ultrastructural studies. Arch. Neurol. 37: 329–337; 1980.

This work was supported by PSC-BHE Research Award 11612 from the City University of New York and in part by the National Cancer Institute Core Grant CA-08748 to the Sloan-Kettering Institute. E. N. B. was the recipient of a predoctoral fellowship under USPHS Training Grant GM02050.