

# PREPARATION OF SEPARATE ASTROGLIAL AND OLIGODENDROGLIAL CELL CULTURES FROM RAT CEREBRAL TISSUE

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## ABSTRACT

A novel method has been developed for the preparation of nearly pure separate cultures of astrocytes and oligodendrocytes. The method is based on (a) the absence of viable neurons in cultures prepared from postnatal rat cerebra, (b) the stratification of astrocytes and oligodendrocytes in culture, and (c) the selective detachment of the overlying oligodendrocytes when exposed to sheer forces generated by shaking the cultures on an orbital shaker for 15–18 h at 37°C. Preparations appear >98% pure and contain  $\sim 1-2 \times 10^7$  viable cells (20–40 mg of cell protein). Three methods were used to characterize these two culture types. First, electron microscopic examination was used to identify the cells in each preparation (mixed and separated cultures of astrocytes and oligodendrocytes) and to assess the purity of each preparation. Second, two oligodendroglial cell markers, 2',3'-cyclic nucleotide 3'-phosphohydrolase (EC 3.1.4.37) and glycerol phosphate dehydrogenase (EC 1.1.1.8) were monitored. Third, the regulation of cyclic AMP accumulation in each culture type was examined. In addition to these studies, we examined the influence of brain extract and dibutyryl cAMP on the gross morphology and ultrastructure of each preparation. These agents induced astroglial process formation without any apparent morphological effect on oligodendrocytes. Collectively, the results indicate that essentially pure cultures of astrocytes and of oligodendrocytes can be prepared and maintained. These preparations should significantly aid in efforts to examine the biochemistry, physiology, and pharmacology of these two major classes of central nervous system cells.

The cellular heterogeneity of the central nervous system has largely prevented investigators from describing the biochemical characteristics of well-defined cell populations within this system. To circumvent this problem, we and others have directed our efforts toward developing new tech-

niques that could be used to prepare homogeneous populations of neurons, oligodendrocytes, or astrocytes (1, 4, 7, 13, 15, 20, 25). The separation of cells in primary culture has a number of advantages over other methods often used to purify brain cells (20). As a result, we have concentrated

our efforts on studying the growth of brain cells in culture in the hope of using differences in the properties of these cells to develop a method of cell separation. Such differences include (a) cellular adhesiveness, (b) medium requirements, (c) developmental time-courses, and (d) growth patterns. These parameters have been used by a number of investigators to separate neuronal and non-neuronal cells in the peripheral nervous system (20).

The methods described in this paper enabled us to prepare essentially pure cultures of oligodendrocytes and astrocytes. These cultures have been characterized by (a) ultrastructural analyses, (b) enzymatic marker studies, and (c) pharmacological responsiveness. Each of these approaches suggests two distinct nonoverlapping (<2%) cell populations. The separate astroglial and oligodendroglial cultures can be maintained for several weeks and should significantly extend our ability to study these two major cell classes.

## MATERIALS AND METHODS

### *Preparation of Source Cultures*

The preparation of mixed glial cell cultures (source cultures) has been described elsewhere (18). However, with respect to cell separation, several important points should be emphasized. First, the use of 1- to 2-d-old rat pups (not younger) ensures the absence of viable neurons in the cell suspension obtained from dissociating the cerebral cortex (17). Second, the length of the initial culture period should be held between 7 and 9 d. Periods shorter than this are not sufficient to obtain the stratification of astrocytes and oligodendrocytes. Periods longer than this often result in the clustering of astrocytes above the normal bed layer. Because of the nature of the subsequent separation procedure, such astroglial clusters break up and contaminate the oligodendroglial preparation. Third, it is imperative to use culture flasks having screw-on caps that can be completely tightened to prevent medium spill during the shaking period. Finally, with respect to cell separation, cell cultures prepared by mechanical sieving techniques have been found to be similar to cultures prepared via trypsinization. The culture medium used in these experiments was Basal Medium Eagle's with Earle's balanced salts containing 15% fetal calf serum, 0.1% glutamine, 0.6% glucose, and no added antibiotics. The culture flasks are incubated at 37°C in a water saturated 6% CO<sub>2</sub>:94% air atmosphere. The medium was changed after the first 3 d and three times a week thereafter.

### *Enzyme Induction*

Hydrocortisone was prepared as a 1 mg/ml stock solution in absolute ethanol. The stock solution was diluted into fresh medium at the time of each experiment to give a final concentration of 1.38  $\mu$ M (2). Control cultures received an equivalent amount of absolute ethanol. N<sup>6</sup>,O<sup>2'</sup>-dibutyryl cyclic AMP (Bt<sub>2</sub>cAMP) was dissolved in culture medium the day of the experiment and sterilized by filtration. The final concentration of Bt<sub>2</sub>cAMP was 1 mM. Fresh treatment medium was added after 24 h, and the treatments were terminated at 48 h.

### *Enzyme Assays*

Methods used to prepare culture homogenates and assay glycerol phosphate dehydrogenase (GPDH; EC 1.1.1.8) and lactate dehydrogenase (LDH; EC 1.1.1.27) activity have been described previously (2). Cell cultures used to determine cyclic nucleotide phosphohydrolase (CNPase; EC 3.1.4.37) activity were rinsed five times with 0.9% NaCl and then harvested by scraping with a rubber policeman into 0.4 ml of H<sub>2</sub>O. The harvested samples were held at -20°C until assayed. Just before assay, the samples were sonicated for 20 s with a Sonipen (Technic International) and monitored microscopically for complete cellular disruption. Aliquots were removed and assayed for protein content. CNPase activity was determined by the method of Prohaska et al. (26). GPDH and LDH assays were performed in duplicate and CNPase activity in quadruplicate. We defined 1 U of enzyme activity as the amount that will catalyze the transformation of 1 nmol of substrate per minute at 30°C. Specific activity is expressed as units of enzyme activity per milligram of total tissue protein. Unless otherwise indicated, the specific activities represent the average of the activities of four cultures.

### *Cyclic AMP*

The methods used to measure changes in intracellular cyclic AMP levels were described previously (18).

### *Protein Assay*

Protein content was measured by the method of Lowry et al. (14) using crystallized bovine serum albumin as a standard.

### *Electron Microscopy*

Cell cultures were rinsed three times with phosphate-balanced saline (PBS) and then fixed in PBS containing 4% glutaraldehyde for 2 h at room temperature and overnight at 4°C. They were then postfixed in osmium tetroxide for 30 min and dehydrated in a graded series of ethanol. The cells were then embedded *in situ* and processed (3).

### *Brain Extract*

Brain extract was prepared by the method of Lim et al. (13). Briefly, brains were removed from 10 adult rats, homogenized in Tyrode's balanced saline, and dialyzed against sterile water for 4 d at 4°C. The dialysate was lyophilized and stored at -20°C until used. On the day of the experiment, brain extract was reconstituted in complete culture medium (5 mg brain extract/ml), filtered through a 0.45- $\mu$ m filter (Millipore Corp., Bedford, Mass.) and applied to cultures for 20 h.

## RESULTS

### *Initial Observations*

While characterizing the effect of the age of the "donor" tissue on the cell types found in culture, we made two important observations. First, we noted that neurons did not survive tissue dissociation when cultures were prepared from pups older than 2 d postnatal (17). Second, oligodendrocytes always grew on the top of the astroglial cell layer. The stratification of oligodendrocytes and astro-

cytes (see Figs. 1 and 2) developed rapidly after the first few days in culture and appears to correspond to the period of astroglial cell proliferation. The fact that oligodendrocytes could be seen growing on the culture dish surface shortly after culture preparation (before the formation of the confluent astroglial bed) indicates that (a) oligodendrocytes do not require contact with astrocytes for growth and (b) oligodendrocytes are present in the initial cell suspension and do not necessarily result from the differentiation of a precursor cell within the bed layer. It should be noted, however, that when cultures were prepared from prenatal nervous tissue, oligodendrocytes could not be identified until after 7–10 d of culture growth (17). These results suggest that the original cell suspension contains oligodendroglial precursors that have not yet morphologically differentiated.

#### *Cell Separation*

Several different approaches were used in an attempt to separate astrocytes (bed-layer cells) and oligodendrocytes (surface cells). Initial experiments indicated that both (a) mild trypsinization and (b) chelation of divalent cations resulted in the detachment of both cell types. Lowering the incubation temperature to 4°C had similar results. Partial separation was achieved by vigorously squirting the surface of mixed cultures (source culture) with culture medium. A few astrocytes and most of the oligodendrocytes were removed by this method. When this cell suspension was replated, nearly all of the cells attached within 60 min; such cultures contained both oligodendrocytes and astrocytes at a ratio of ~8:1 respectively. However, because astrocytes proliferate much more rapidly than oligodendrocytes, this ratio rapidly decreased. Following this partial separation, the source culture contained an enriched population of astrocytes ( $\geq 90\%$ ) that could either be used immediately or cultured for a few days to allow the replenishment of lost astroglial cells.

The partial success of this technique suggested that, under the appropriate conditions, oligodendrocytes might be physically removed without significantly affecting the attachment of the astrocytes in the bed layer. In an attempt to standardize this technique, primary cultures (in 75 cm<sup>2</sup> plastic flasks (Falcon Labware, Div. Becton, Dickinson Co., Oxnard, Calif.)) were firmly fixed onto the surface of an orbital shaker (model G76, New Brunswick Scientific Co., Inc., Edison, N. J.) and

agitated for various times and velocities. It was immediately apparent that this method could be used to prepare both astroglial and oligodendroglial cultures.

#### *Optimal Procedure for Cell Separation*

The preparation of nearly pure cultures of oligodendrocytes and astrocytes from rat cerebral cortical tissue is diagrammatically presented in Fig. 3. Cultures were prepared from 1- to 2-d-old rat cerebral cortical tissue and grown for 9 d in Falcon flasks (75 cm<sup>2</sup>) as described in Materials and Methods. On the 10th d (24 h after the last medium change) the cell cultures were gently rinsed three times with complete medium to remove floating cells. Each culture flask (with 10 ml of fresh medium) was placed into a culture chamber and allowed to equilibrate with the CO<sub>2</sub>-air atmosphere for 2 h. We then removed the flasks from the chamber, completely tightened the caps, and securely fixed the flasks onto the surface of an orbital shaker. Once secured, the flasks were shaken for 15–18 h (37°C, 250 rpm, stroke diameter of 1.5 in). This procedure usually began in the afternoon so that the cultures were shaken overnight. Following the shaking period, the suspended cells from all flasks were collected, pooled, and filtered through Nitex 33. The cultures were rinsed three times and the rinses were filtered and pooled with the cells already collected. At this point, the filtrate contained a single cell suspension of oligodendrocytes and a few small clumps of astrocytes. The culture flasks contained a nearly intact layer of astrocytes and a few oligodendrocytes scattered across the surface. The purity of each fraction at this point was assessed by light microscopy to be ~95%. Because contaminating astrocytes are present almost exclusively as cell clumps, the purity of the oligodendroglial cell fraction was greatly increased by filtering the suspension through Nitex 17. The cells in the filtrate were then pelleted by centrifugation (40 g 5 min) and plated into culture flasks. These oligodendroglial cell cultures (Fig. 4) were ~98% enriched. The purity of these cultures could be increased to >99% by allowing 24 h for the cells to attach, reshaking the cultures (for a few minutes by hand), and plating the detached oligodendrocytes. The purity of the astroglial cell fraction was increased beyond 95% by two successive steps. First, the flasks were vigorously shaken by hand. When all oligodendrocytes had detached (determined by

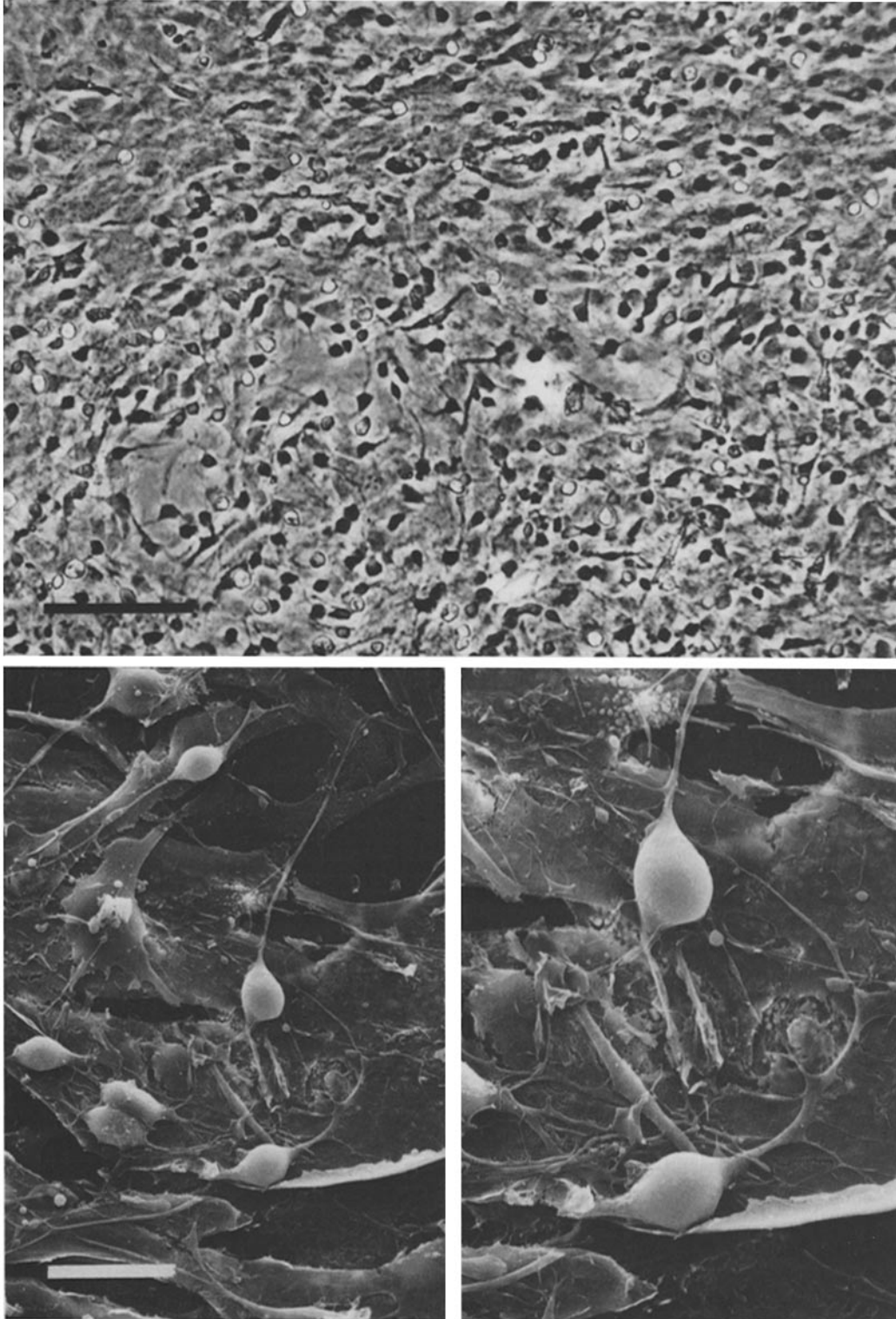


FIGURE 1 Top, dissociated cerebral cells from 2-d-old rats after 9 d in culture. The phase-dark cells have been identified in this study as oligodendrocytes. They are resting on top of a bed of flat cells, ultrastructurally identified as astrocytes (phase contrast,  $\times 230$ ). Bar,  $100 \mu\text{m}$ . Bottom, scanning electron micrographs of this culture clearly reveal the stratification of oligodendrocytes and astrocytes. Bar,  $20 \mu\text{m}$ . Left,  $\times 970$ ; right,  $\times 1,950$ .

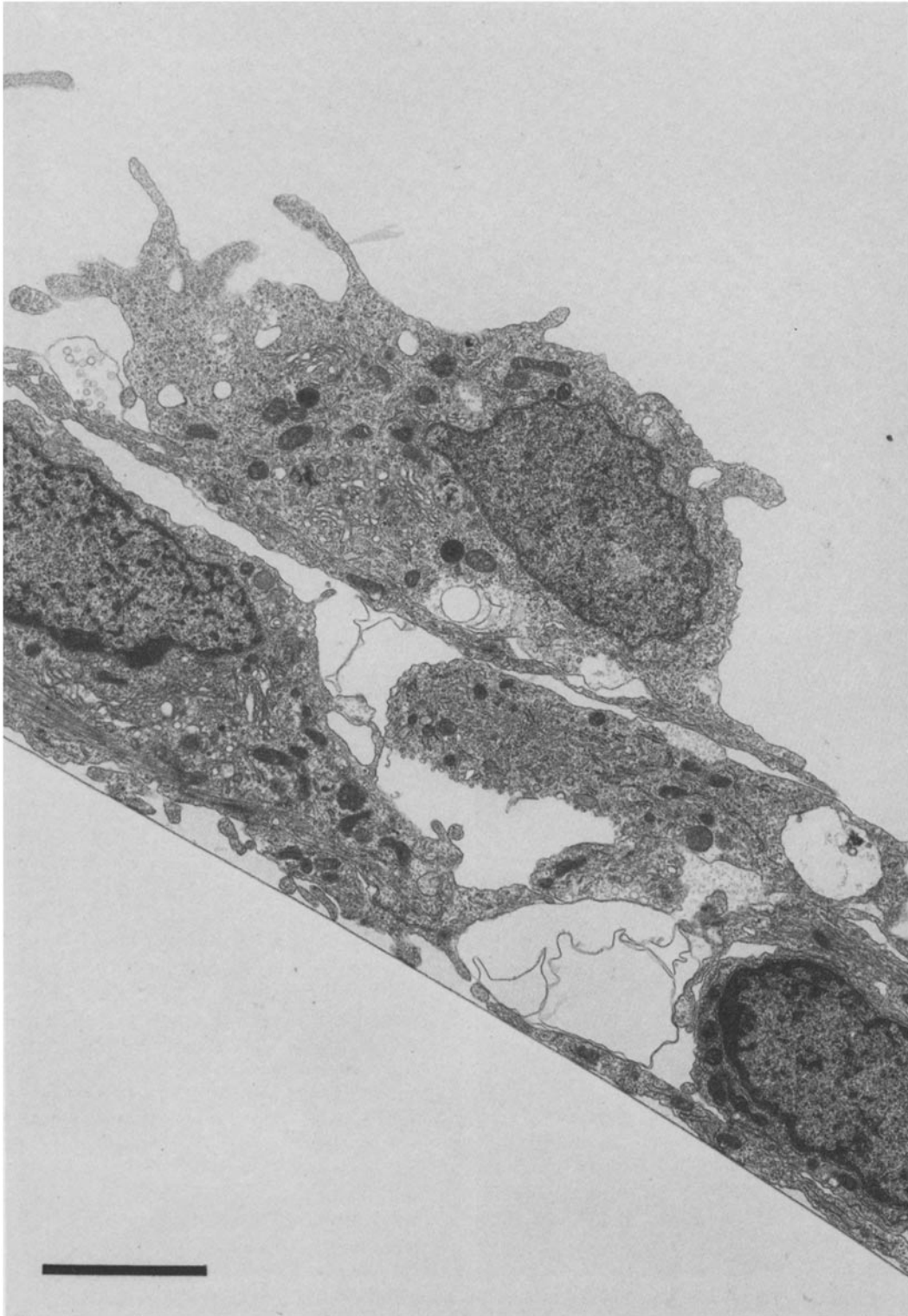


FIGURE 2 Vertical thin section through a primary mixed glial culture shows the ultrastructure of the two cell types. The astrocytes under the oligodendrocytes display characteristic bundles of filaments. Bar, 2  $\mu\text{m}$ .  $\times 12,750$ .

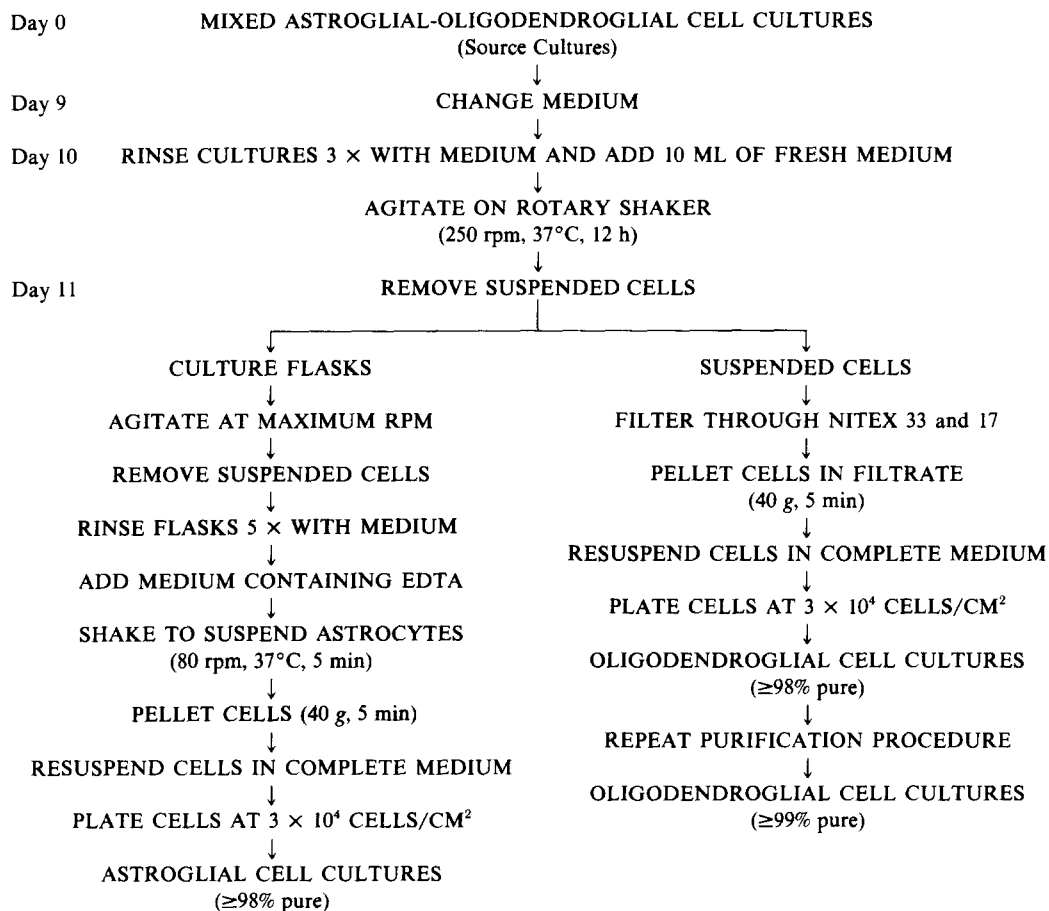


FIGURE 3 A flow chart for the preparation of separate astrocytes and oligodendrocytes. Details are given in the text.

light microscopy) from the surface, the medium was removed and the culture flasks were rinsed five times with fresh medium (the cell suspension removed during this procedure was usually discarded because it contained both oligodendrocytes and astrocytes). Second, the astrocytes were replated at about one-third of their confluent density. We suspended the astrocytes by adding culture medium containing 5 mM EDTA, waiting 5 min, and then gently shaking the flasks. The cells were collected, pelleted by centrifugation (40 g, 5 min), resuspended in fresh medium, and then plated at  $\sim 30,000$  cells/cm<sup>2</sup>. Because astrocytes proliferate very rapidly relative to oligodendrocytes, the degree of oligodendrocyte contamination rapidly declined. The resulting astroglial cell cultures (Fig. 4) appeared to be >98% pure by all criteria used (see below).

### Morphological Characterization

The nature of the cells present in these cultures were studied by transmission electron microscopy under two different conditions: (a) mixed astroglial-oligodendroglial cell cultures and (b) purified surface cultures. The criteria used to identify oligodendrocytes and astrocytes were essentially those described by Peters et al. (24). Figs. 1 and 2 illustrate the typical growth pattern of a mixed glial cell population after 9 d in vitro. In each of these micrographs, two distinct cell types can be seen. In Fig. 1, cells identified as oligodendrocytes can be seen resting on top of a bed layer of cells that have been characterized as astrocytes. At this level of magnification, oligodendrocytes are distinguished from astrocytes by their (a) relatively small size, (b) very fine cell processes, (c) dark cell

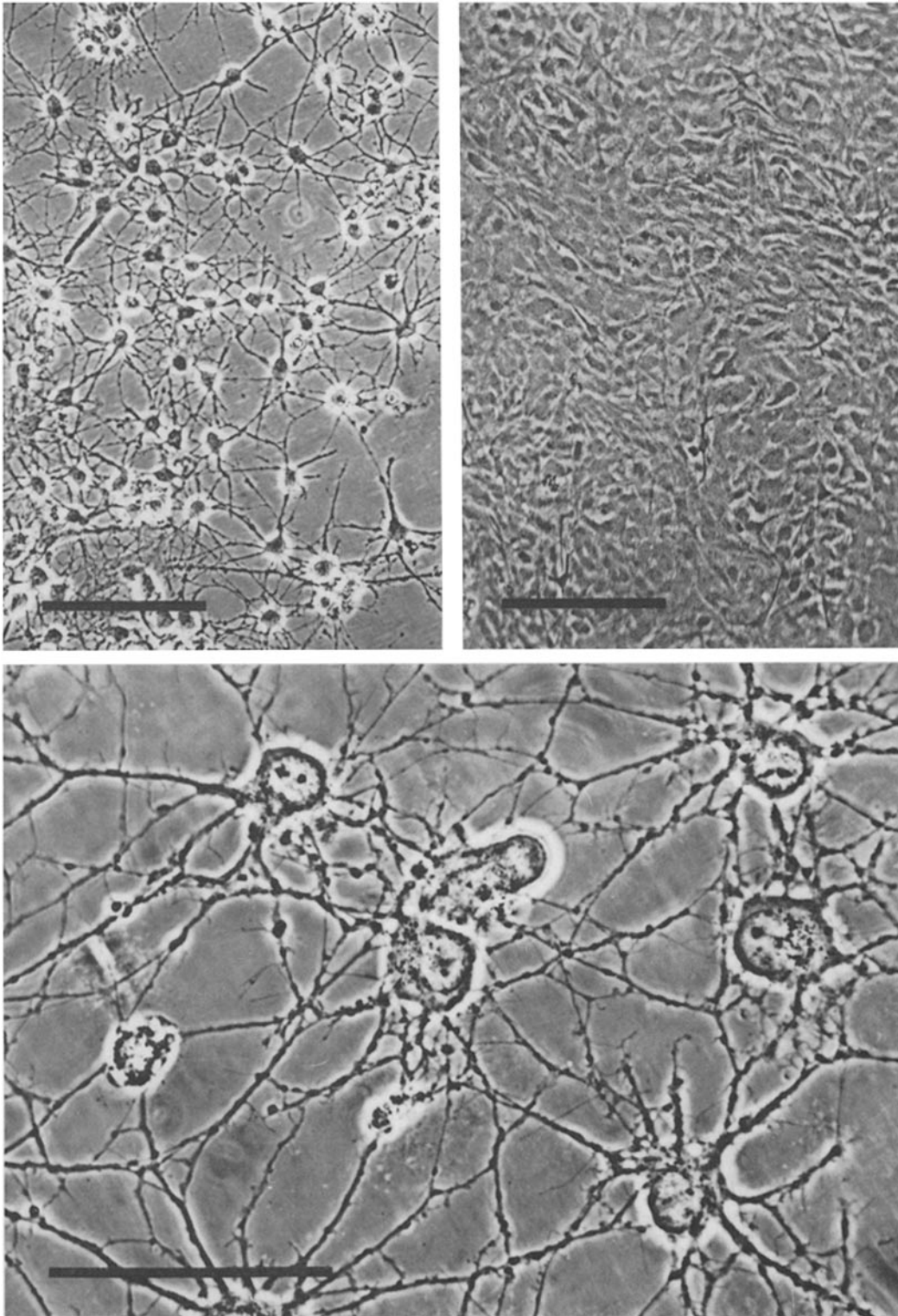


FIGURE 4 Top, phase contrast of oligodendroglial cell culture (left) and astroglial cell culture (right). Bar,  $100\ \mu\text{m}$ .  $\times 300$ . Bottom, higher magnification of an oligodendroglial cell culture showing ramification of processes. Bar,  $50\ \mu\text{m}$ .

bodies, and (d) inability to proliferate to cell soma contact (marginal contact between oligodendroglial cell processes seem to halt the proliferation of these cells). In contrast, astrocytes are relatively large cells that usually do not possess visible cell processes (at this magnification) in confluent cultures. Astrocytes always proliferated rapidly to form a confluent bed layer of cells. In Figs. 1 and 4, the astrocytes appear as the phase-grey cells upon which the phase-dark oligodendrocytes can be seen. Cell cultures prepared from the meningeal tissue that normally surrounds the cerebral cortex presented a very different picture at all levels of characterization (morphological, enzymatic, and pharmacological; data not shown).

Both the stratification of the two cell types and the ultrastructural features that were used to identify these cells positively as astrocytes and oligodendrocytes can be seen in the electron micrograph shown in Fig. 2. The cells in the basal layer (astrocytes) possess a large oval nucleus with perinuclear clumped chromatin. The Golgi apparatus and rough endoplasmic reticulum are poorly developed, lysosomes are numerous, and a thick cell process arises from the cell body that contains bundles of filaments. The cell outline is irregular and, most importantly, numerous astroglial filaments oriented in different directions can be seen in the cytoplasm. Microtubules were rarely seen in these cells. Cells with these features were always found in the basal layer. Fig. 5 further illustrates the ultrastructure of astrocytes. The typical positioning and ultrastructural features of cells characterized as oligodendrocytes are shown in Figs. 1, 2, 4, and 6. These cells have an eccentric nucleus with finely clumped chromatin and dilated perinuclear and cytoplasmic endoplasmic reticulum cisternae. In the cytoplasm, numerous microtubules (Fig. 6) were present and the Golgi apparatus was always well developed. These cells contained relatively few lysosomes compared to astrocytes. Bundles of astroglial filaments were never observed in these overlying cells. The oligodendrocytes present show a similar ultrastructure whether grown on top of astrocytes or as isolated cells on plastic culture dishes. In addition to these studies, we have examined the effect of (a) brain extract (13) and (b)  $Bt_2cAMP$  (27) on the morphology of these two cell populations. Although these agents appear to have little, if any, effect on the morphology of oligodendrocytes, they cause extensive changes in astrocytes (data not shown). However, even in the presence of these agents, astrocytes do

not resemble the cells identified as oligodendrocytes. In summary, we have carefully monitored the ultrastructural and gross cellular morphology of these two cell populations in at least 20 preparations, with similar results. On the basis of these studies, we estimate that the purity of both the astroglial and oligodendroglial cell cultures is in >98%.

### Enzymatic Characterization

To further characterize the cells present in these cultures, we monitored the activity of (a) CNPase (11, 22), (b) LDH (5), and (c) GPDH (2, 5, 6, 21) in the presence and absence of treatments that have previously been reported to alter the activity of these enzymes (Table I).  $Bt_2cAMP$  induced LDH activity in both astroglial and oligodendroglial cell cultures. However, the magnitude of induction was much greater in oligodendroglial cell cultures. In contrast to LDH activity, CNPase activity was present only in the oligodendroglial cell cultures and was not significantly altered by the inclusion of 1 mM  $Bt_2cAMP$  in growth media for 48 h. Increasing the amount of astroglial protein to three times that used in oligodendroglial assays did not result in the detection of CNPase activity in astroglial culture homogenates. Because CNPase appears to be localized to myelin and oligodendrocytes, these results support our contention that the astroglial cell cultures are essentially free of oligodendrocytes.

Within the rat nervous system, the hydrocortisone induction of GPDH appears to be an excellent marker for oligodendrocytes (5, 12). The results presented in Table I indicate that hydrocortisone treatment increased GPDH activity by nearly threefold in astroglial cell cultures and 16-fold in oligodendroglial cell cultures (relative to uninduced cultures). If all of the GPDH activity in hydrocortisone-treated cultures was attributable to oligodendrocytes, the results suggest that <2% of the protein in the astroglial homogenate was derived from oligodendrocytes. The difference in the magnitude of GPDH induction between the two culture types indirectly suggests that a small pool of GPDH was present in the astroglial cell cultures that was not induced by hydrocortisone treatment. This experiment has been repeated several times and the results presented represent the highest level of GPDH activity observed in astroglial cell cultures. GPDH activity and inducibility remained low even after prolonged periods of *in vitro* growth after cell separation (data not shown).



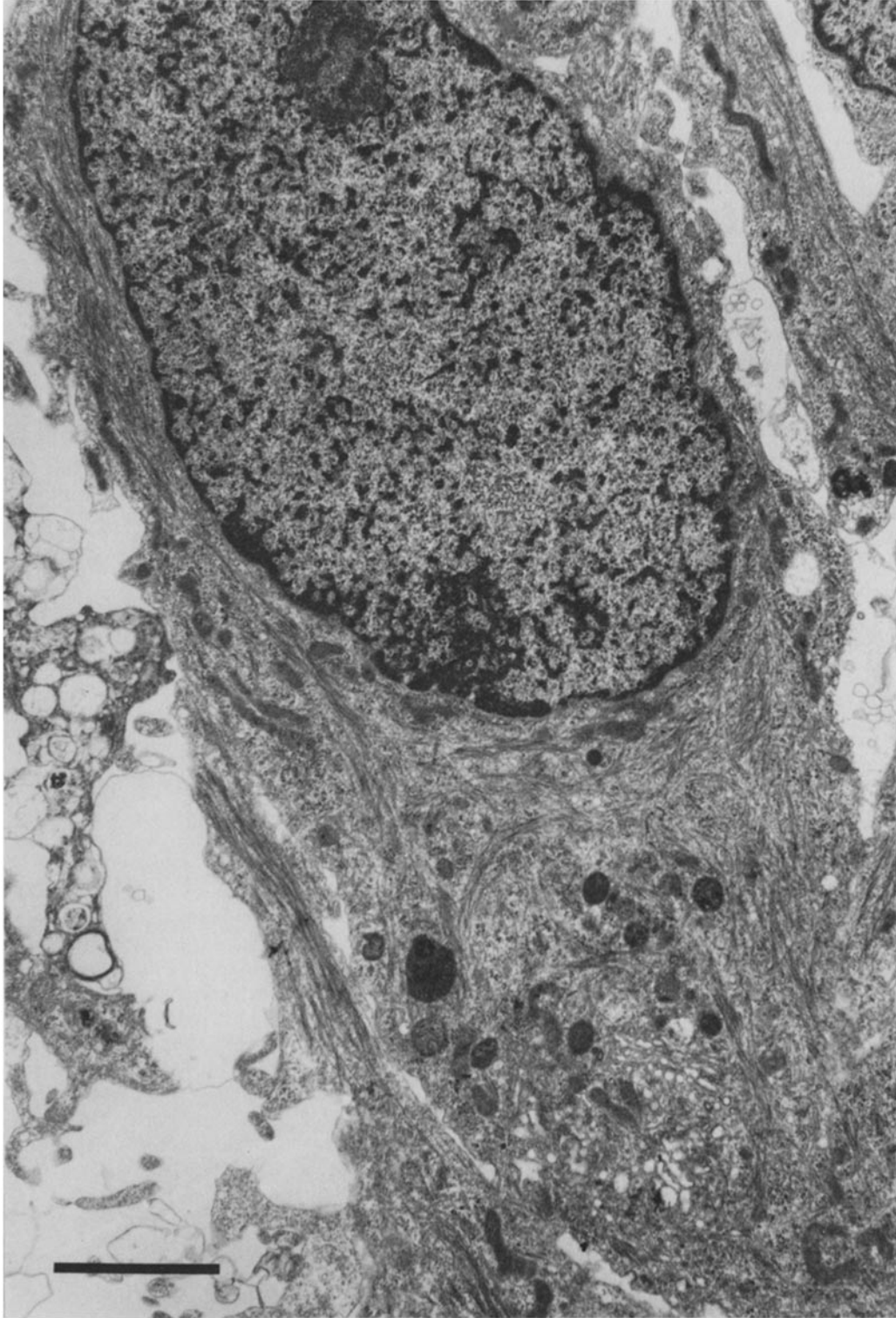


FIGURE 5 The typical ultrastructural features of astrocytes can be seen in this electron micrograph. Note that the cell is much larger than oligodendrocytes (Fig. 6) and that bundles of filaments occupy much of the cytoplasm throughout the cell body and processes. Bar, 2  $\mu\text{m}$ .  $\times 14,600$ .

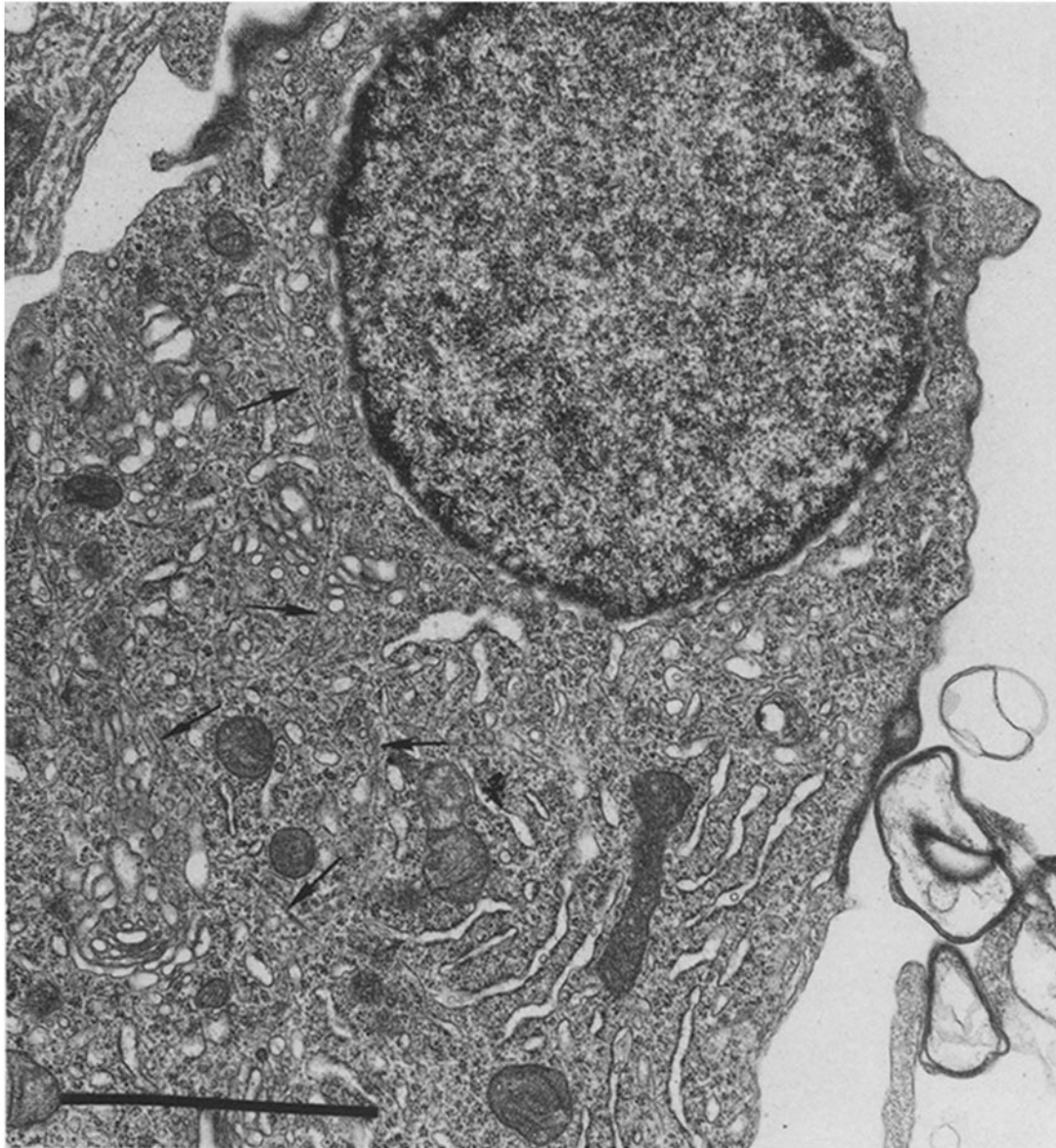


FIGURE 6 The typical ultrastructural features of oligodendrocytes can be seen in this electron micrograph. Note the enlarged cisternae, well-developed Golgi apparatus, microtubules (arrows), darkly stained cytoplasm, and small eccentric nucleus. Bar, 2  $\mu$ m.  $\times$  25,400.

These studies indicate that biochemically the two culture preparations were quite different and support our morphological identification of oligodendrocytes.

#### *Pharmacology*

Table II shows the effect of several pharmacological agents on cyclic AMP accumulation in

astroglial and oligodendroglial cell cultures. In these cultures the ability of  $\alpha$ -adrenergic receptors to reduce cyclic AMP accumulation appears to be restricted to astrocytes. The results also indicate that astrocytes are primarily responsible for the marked accumulation of cyclic AMP that occurs in mixed glial cell cultures in response to adenosine. In contrast, the effect of prostaglandin  $E_1$

TABLE I  
Regulation of Marker Enzymes in Astrocytes and Oligodendrocytes

Enzyme/treatment	Amount of Marker Enzyme	
	Astrocytes	Oligodendrocytes
	<i>U/mg protein</i>	
CNPase		
(-) Bt <sub>2</sub> cAMP	N.D.	441 ± 11
(+) Bt <sub>2</sub> cAMP	N.D.	507 ± 13
GPDH		
(-) Hydrocortisone	4.0 ± 0.3	43.0 ± 1.8
(+) Hydrocortisone	11.9 ± 0.9	688.5 ± 5.1
LDH		
(-) Bt <sub>2</sub> cAMP	2022 ± 51	1147 ± 30
(+) Bt <sub>2</sub> cAMP	2700 ± 132	2910 ± 112

Hydrocortisone was added to a final concentration of 1.38 μM in fresh media. N<sup>6</sup>,O<sup>2</sup>-dibutyryl cyclic AMP (Bt<sub>2</sub>cAMP) was dissolved in fresh media to a final concentration of 1 mM. Fresh treatment medium was added after 24 h and the treatment was terminated at 48 h. Units of enzyme activity are defined as nanomoles of substrates used per minute at 30°C. Each value represents the mean of three cultures ± SEM. N.D., nondetectable.

TABLE II  
Regulation of Cyclic AMP

Treatment	Amount of cyclic AMP	
	Astrocytes	Oligodendrocytes
	<i>pmol/mg Protein</i>	
Control	8.7 ± 0.2	8.9 ± 0.4
Norepinephrine	28.7 ± 2.9	50.1 ± 9.0
Norepinephrine plus phentolamine*	180.6 ± 10.1	62.0 ± 4.4
Adenosine	44.3 ± 1.6	14.3 ± 0.7
Prostaglandin E <sub>1</sub>	67.6 ± 8.8	187.4 ± 31.2

The concentration of all drugs, except for adenosine, was 3 μM. Adenosine was used at 100 μM. All incubations were carried out for 5 min. Each value represents the mean of three cultures ± SEM.

\* Phentolamine is an α-adrenergic antagonist that blocks the α-adrenergic component of norepinephrine, leaving only the β-adrenergic activity. We have previously demonstrated that stimulation of alpha receptors always leads to decreased response to other agonists that increase cyclic AMP in glia (18, 19).

(PGE<sub>1</sub>) on cyclic AMP accumulation is significantly greater in oligodendrocytes than in astrocytes. These results indicate that a number of neurohumoral agents can influence cyclic AMP levels in both astrocytes and oligodendrocytes via membrane receptors and that these two cell pop-

ulations are pharmacologically distinct from one another.

## DISCUSSION

The results presented in this paper indicate that a mixed population of glial cells can be separated into astroglial and oligodendroglial cell fractions. The method used to separate the two cell populations is based on (a) the preparation of mixed glial cell cultures, (b) the stratification of astrocytes and oligodendrocytes in mixed glial cell cultures, and (c) the ability to remove oligodendrocytes selectively by vigorous shaking. Cultures have been characterized by light and EM morphological studies, cell marker enzymes, and pharmacological responsiveness. The results obtained with these glial cell cultures have been compared to the results obtained from similar studies examining meningeal cell cultures. Such experiments indicate that meningeal cells can easily be distinguished from either astrocytes or oligodendrocytes and that meningeal cells did not contribute significantly to the glial cell cultures.

Morphologically, astrocytes always reside in the bed layer of cells where they appear as rather diffuse phase-grey cells growing in a random fashion. Astrocytes are present in cultures prepared from cerebra ranging in age from 5-d prenatal to 5-d postnatal and always form a confluent multilayer upon which other cell types grow (neurons or oligodendrocytes, depending on the age of the donor tissue). Although astroglial processes cannot be seen with a light microscope, they are evident in EM preparations. Astroglial processes are relatively thick and possess bundles of filaments. Treatment of astroglial cells with either brain extract or Bt<sub>2</sub>cAMP results in marked morphological transformation of these cells. The perikarya tend to become spherical and extend long processes. However, even under these conditions, astrocytes do not resemble oligodendrocytes.

The major morphological feature of oligodendrocytes that allowed us to identify these cells at the light microscopic level was their small spherical perikarya, which always possessed several very fine, short cell processes. Oligodendrocytes never contained bundles of filaments typical of astrocytes but did possess an abundance of microtubules and extensive Golgi complexes and ER. The gross morphology and ultrastructural features of oligodendrocytes remained the same whether they were growing on the astroglial bed layer or in direct contact with the culture dish. Under present

culture conditions, oligodendrocytes proliferate only slowly and never reach confluence. Once completely removed from source cultures, oligodendrocytes do not reappear, indicating the absence of an oligodendroglial cell precursor population hidden within the astroglial bed layer. Examination of purified astrocytes and oligodendrocytes in rotation reaggregate cultures at the EM level enabled us to study the ultrastructure of a large number of cells growing under identical conditions. The results of these morphological studies indicate that two essentially nonoverlapping cell populations (<2%) can be prepared from 1- to 2-d-old rat cerebra. By classical morphological criteria, these two populations have been identified as astrocytes and oligodendrocytes.

Because the morphology of cells in culture may not accurately reflect the morphological features of those same cells *in vivo*, we decided to characterize these two cell populations further by monitoring the activity of two marker enzymes that have been shown *in vivo* to be localized in oligodendrocytes. The first marker, CNPase, has been reported by many investigators to be associated with myelin (9, 10, 16, 23). Although this enzyme is usually thought of as a myelin marker, the results presented here indicate that oligodendrocytes contain CNPase in the absence of visible myelin. CNPase activity was not detectable in homogenates prepared from astroglial cell cultures. Interestingly, Matthieu et al. (16) recently reported that CNPase activity continued to increase throughout the culture period of reaggregate cultures. Although we did not monitor CNPase activity as a function of the days in culture, we have done so with GPDH activity and find that it increases with time in culture (2). Elsewhere we have reported that within the nervous system, the hydrocortisone induction of GPDH activity appears to take place exclusively in oligodendrocytes (6, 12). As a result, we have used the presence of GPDH and its induction by hydrocortisone as an oligodendroglial cell marker. The fact that CNPase activity and the hydrocortisone induction of GPDH activity reside within the cell population identified as oligodendrocytes further substantiates the localization of the hydrocortisone effect.

Unfortunately, well-defined astroglial marker enzymes have not been described. Consequently, we have relied heavily upon the morphological criterion in the identification of these cells. However, the pharmacological studies presented in this

paper indicate that astrocytes possess  $\alpha$ -adrenergic receptors that, when stimulated, can modulate the amount of cyclic AMP that normally accumulates in response to  $\beta$ -adrenergic agonists. Interestingly, the  $\alpha$ -adrenergic modulation was absent from oligodendroglial cell cultures. Furthermore, the effect of adenosine and PGE<sub>1</sub> on astroglial and oligodendroglial cell cyclic AMP levels was also quite different. Thus, these pharmacological studies also suggest that two distinct cell populations have been isolated.

Because astrocytes proliferate much more rapidly than oligodendrocytes, purified astroglial cell cultures become progressively less heterogeneous and oligodendroglial cell cultures more heterogeneous during the first week immediately after cell separation. After the first week, this relationship does not persist, because astrocytes usually become confluent and stop dividing, whereas oligodendrocytes continue to proliferate. As a result, purified cultures of oligodendrocytes and astrocytes are usually used within a few days of preparation. However, unacceptable contamination can easily be overcome by repeating a simplified version of the original separation procedure. Under normal conditions, the separation procedure and subsequent growth period yield ~15–20 mg of oligodendroglial cell protein and ~35–45 mg of astroglial cell protein. Hence, this procedure yields ample protein to complete many different types of biochemical analyses.

A number of techniques have been developed for separating neuronal and glial cells in culture (8, 20). However, to our knowledge, this is the first method to allow the simultaneous preparation of essentially pure separate astroglial and oligodendroglial cell cultures from the same tissue. The power of such a method is illustrated in Table II, in which the pharmacology of these two cell classes is compared. By use of this system, it should be possible to address such questions as (a) what hormones are required for cell survival and proliferation, (b) whether intercellular interactions occur between astrocytes and oligodendrocytes, (c) whether either of these cell populations is a pharmacological target for CNS drugs, and (d) whether the formation of myelin by oligodendrocytes can be regulated.

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