ADHESION AMONG NEURAL CELLS OF THE CHICK EMBRYO

IV. Role of the Cell Surface Molecule CAM
in the Formation of Neurite Bundles in Cultures
of Spinal Ganglia

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ABSTRACT
The cell adhesion molecule (CAM) is involved in adhesion among embryonic retinal and brain cells and has been detected in a variety of neural tissues. This paper describes the use of spinal ganglion cultures and specific anti-CAM antibodies to determine the distribution of CAM on plasma membranes of nerve processes, and to assess the results of perturbation of its function during the growth of neurites from ganglia. The results indicate that CAM is distributed over the entire surface of nerve processes, and that specific anti-CAM Fab' fragments alter the morphology of neurite outgrowth. In particular, it was observed that anti-CAM inhibits formation of nerve bundles, so that the ganglion becomes surrounded by a tangled net of fine processes. Growth cone functions, such as neurite elongation, motility, and attachment to the substratum, did not appear to be affected by the antibody. These studies suggest that one of the major functions of CAM is to mediate side-to-side adhesion between neurites to form fascicles, and raise the possibility that this molecule serves a key role in embryogenesis of nerve tissues.

KEY WORDS spinal ganglion cultures antibodies neurite-neurite adhesion cell surface proteins

Interactions between neural cell membranes, as evidenced in cell aggregation, and in formation of nerve bundles and synapses, are considered to be central factors in nerve tissue development and function. In previous studies on the in vitro aggregation of chick embryo neural cells, we have used an immunohistochemical assay to detect and isolate a cell surface molecule from retina that is involved in the initial formation of cell-cell bonds (1, 19). The molecule, called cell adhesion molecule (CAM), has an apparent mol wt in sodium dodecyl sulfate of ~140,000, and can be detected on the surface of cells from a number of neural tissues. The amount or accessibility of CAM and related molecules is correlated with cell adhesiveness in vitro (14, 15), and Fab' fragments prepared from specific anti-CAM antibodies inhibit adhesion between both retinal and brain cells (14, 15).

A major goal of this research is to determine the relevance of CAM and cell aggregation to normal embryological development, in particular to examine whether membrane-membrane adhesions influence the arrangement and specification
of neurites during the formation of nerve tissue. In the present report, we have used anti-CAM antibodies and Fab' fragments in conjunction with methods for culture of spinal ganglia (7) to analyze the distribution of CAM on the surface of neurites, as well as to examine the function of CAM during formation of neurite bundles.

MATERIALS AND METHODS

Antibodies, Fab' Fragments, and Lectins

The procedures for purification of CAM from chick embryo retina, production of anti-CAM and anti-retinal cell antibodies in rabbits, and preparation of monovalent Fab' fragments have been described in detail elsewhere (1, 19). Antibodies to chick embryo fibroblasts were obtained after 10 weekly intraperitoneal injections of rabbits with cells from secondary cultures. Concanavalin A and the divalent succinyl-concanavalin A derivative were prepared as reported previously (4).

Spinal Ganglion Cultures

Dorsal root ganglia were excised from 10-day-old chick embryos and placed in 35-mm collagen-coated tissue culture dishes (BioQuest, BBL & Falcon Products, Cockeysville, Md.) containing 2 ml of Dulbecco's Modified Eagle Medium (DMEM; Microbiological Associates, Walkersville, Md.) supplemented with 1/10 volume of fetal calf serum (Microbiological Associates), 1 U/ml nerve growth factor (Burroughs Wellcome Co., Research Triangle Park, N. C.), and cytosine arabinoside (10^{-5} M) to inhibit mitosis of non-neuronal cells. When prepared for scanning electron microscopy, the ganglion cultures were grown on collagen-coated glass cover slips.

To assess the effect of Fab' fragments or lectins on growth of neurites, the proteins were dissolved (10 mg/ml) in phosphate-buffered saline, pH 7.4 (PBS), dialyzed against DMEM, and added (10-400 μl) to the medium at the beginning of the culture period except where otherwise noted. Growth of neurites was recorded photographically after 16, 24, and 32 h by phase-contrast microscopy or scanning electron microscopy, or continuously by cinematography (details given below). The extent of outgrowth from each ganglion was measured in terms of its diameter. Neurite morphology was scored by visual estimation of the percentage (to the nearest 20%) of total neurite outgrowth present in thick (4-15 μm diam), intermediate (1.2-4 μm), and thin (0.4-1.2 μm) fascicles. Results from 10-20 ganglia were averaged for each experimental condition.

Scanning Electron Microscopy

Ganglia cultured on cover slips were washed with PBS and fixed with glutaraldehyde (2% in PBS, 2-4 h, 25°C). After sequential incubations with water, ethanol (50, 70, and 100%), and acetone, the samples were dried using a Denton liquid-CO₂ critical point dryer (Denton Vacuum Inc., Cherry Hill, N. J.). An Edwards 150 Sputter Coater (Edwards High Vacuum, Inc., Grand Island, N. Y.) was used to coat the ganglia with gold and observations were made in an ETEC Autoscan microscope (ETEC Corp., Hayward, Calif.). Each micrograph is a representative field from 20 to 50 micrographs of 10 to 20 ganglia.

Electron Microscopy

Ganglia grown on culture dishes were treated at 25°C with glutaraldehyde (2.5%, 20 min), osmium tetroxide (1%, 1 h), and uranyl acetate (saturated aqueous solution, 1 h); dehydrated through 50, 70, and 100% ethanol, and embedded by adding Epon directly to the dish. Regions of the hardened Epon that contained a ganglion were cut out and sectioned in the plane of neurite outgrowth.

Localization of CAM

CAM was detected on the surface of neurites by use of anti-CAM antibodies and either fluorescein-labeled goat anti-rabbit immunoglobulin (9), or complexes of anti-immunoglobulin with rabbit anti-horseradish peroxidase and horseradish peroxidase that can be localized by oxidation of diaminobenzidine in the presence of H₂O₂ (18). For studies using the electron microscope, the peroxidase procedure was incorporated between the glutaraldehyde and osmium tetroxide treatments.

Cinematography

 Cultures containing 1 mg/ml anti-CAM Fab' or Fab' from unimmunized rabbits were photographed at 15-s intervals using phase-contrast optics × 125.

RESULTS

When spinal root ganglia from chick embryos are cultured in the presence of nerve growth factor, a halo of processes and fascicles (bundles of processes) appears around each ganglion over a 24-h period (7). The major result reported here is that addition to these cultures of monovalent Fab' fragments directed against a molecule involved in neural cell adhesion causes a dramatic change in the morphology but not the diameter of the neurite halo (Fig. 1). The overall change is observed as a decrease in the number of fascicles with a large diameter, accompanied by an increase in the number of processes consisting of one or a few neurites. The following experiments were designed to investigate the nature, specificity, and mechanism of this effect.
FIGURE 1 Representative outgrowth of neurites from thoracic ganglia (G) cultured for 24 h in medium containing (a) 1 mg/ml Fab' from unimmunized rabbits and (b) 1 mg/ml anti-CAM Fab'. Ganglia cultured in medium containing 1-5 mg/ml anti-fibroblast Fab' were identical to Fig. 1a. The presence of anti-CAM Fab' has resulted in a tangled outgrowth of fine processes rather than the thick and relatively straight fascicles formed in cultures without this antibody. × 82.

Presence of CAM on the Surface of Neurites

Using anti-CAM antibodies in conjunction with fluorescence microscopy and the peroxidase-antiperoxidase staining procedure, we have demonstrated that most of the CAM in a chick embryo is associated with neural tissue, and that it is nearly uniform in its distribution on the surface of both retinal cell bodies and their neurites (14). In this report, these observations are extended to processes that grow from spinal ganglia. As shown in Fig. 2, CAM is present on all ganglion neurites and does not appear to be preferentially located on the shafts or tips of the processes. In electron micrographs of sections through a fascicle (Fig. 2c), CAM was again nearly uniform in its distribution on surface membranes, similar amounts being present in regions of contact between two neurites and on isolated membranes.

Influence of Anti-CAM Fab' on Ganglion Cultures: Kinetics, Substrate Effects, Dose Response, Specificity, and Reversibility

To provide a quantitative estimate of the anti-CAM effect, a simple scoring system based on direct observation was employed. This included the average extent of radial growth and the fraction of outgrowth associated with thick (4-15 μm), intermediate (1.2-4 μm), and thin (0.4-1.2 μm) processes (see Fig. 1). As shown in Table I, an effect of anti-CAM on ganglia could be observed as soon as neurites appeared (after about 12 h of culture). Cultures were routinely scored after 24 h when substantial outgrowth had occurred. Growth after 35 h mainly consisted of straight, well-spaced fascicles whose morphology was less dramatically altered by anti-CAM Fab'.

The pattern of neurite growth was also affected by the substratum on which the ganglia were placed. In the presence of Fab' from unimmunized rabbits, equally large fascicles formed when the plastic culture dishes were used directly or coated with collagen. When the anti-CAM Fab' was present, however, thinner processes were observed with the collagen substratum. When the dishes were coated with a more adhesive substrate such as the lectin from wax beans, thin fascicles appeared even in the absence of anti-CAM Fab', and it was more difficult to observe the effect of the antibody. Consequently, all experiments have been carried out with collagen-coated surfaces. Because the collagen coating is not uniform across the entire surface, at least 10 ganglia in each dish were scored, and results were compared from dishes that had been coated at the same time. Similar precautions were taken with respect to the number of flat non-neural cells in the cultures, but differences in the density of these cells did not appear to have a large effect on the extent or morphology of neurite outgrowth.

The effect of anti-CAM Fab' on neurites was
As controls for the specificity of the anti-CAM Fab' effect, Fab' from unimmunized rabbits or from rabbits immunized with chick embryo fibroblasts were also tested (Table I). Neither reagent caused any change in neurite growth or appearance, including concentrations of anti-fibroblast antibodies that bound to neurite plasma membranes in amounts similar to or greater than anti-CAM (as assessed in antibody dilution studies with the peroxidase-anti-peroxidase procedure). Concanavalin A and its dimeric derivative succinyl-concanavalin A, which also bind to cell surface components but do not inhibit neural cell adhesion (1), caused a drastic retraction and thickening of neurites that was easily distinguished from the alterations induced by anti-CAM. Finally, to demonstrate that the decrease in fascicle diameter was caused by anti-CAM, purified CAM was shown to neutralize the ability of the Fab' to affect neurites (Table I).

To examine the possibility that anti-CAM interferes with nerve growth factor or some other component present in the medium, the Fab' was also added to cultures of mouse embryo spinal ganglia. There was no change in the outgrowth of mouse neurites, from which we conclude that anti-CAM acts directly on the chick ganglia. This result is consistent with the fact that anti-chick CAM Fab' does not affect adhesion between mouse neural cells (our unpublished observation).

The maximal effect of anti-CAM Fab' was observed only when it was present continuously during the culture (Table II). Either a delay in its addition or its removal before the experiments were scored caused a shift from smaller to larger fascicles. It appeared, however, that both the formation of thick fascicles and the anti-CAM Fab' effect were reversible in that some preexisting thick fascicles disappeared upon addition of the Fab', and conversely, some thin processes coalesced into larger ones upon its removal.

Cinematographic Studies

Because outgrowth of neurites and formation of fascicles are dynamic processes which appear to be reversible (Table II), cinematography was used to evaluate the sequence of events that leads to the results shown in Fig. 1. The following general observations were made from movies of a number of ganglia:

(a) In the absence of anti-CAM, large optically refractile fascicles were gradually built up as more
### TABLE I

**Effect of Anti-CAM Fab' on Neurite Growth from Dorsal Root Ganglia**

| Type     | mg/ml | Culture time (h) | Growth (μm) | 15-4 μm | 4-1.2 μm | 1.2-0.4 μm |
|----------|-------|------------------|-------------|--------|---------|-----------|
| UR       | 0.5   | 12               | 0-0.2       | 50     | 35      | 15        |
| aCAM     | 0.5   | 12               | 0-0.2       | 10     | 35      | 55        |
| UR       | 0.5   | 24               | 0.6-1.1     | 55     | 35      | 10        |
| aCAM     | 0.5   | 24               | 0.6-1.1     | 20     | 30      | 50        |
| UR       | 0.5   | 40               | 1.2-1.6     | 60     | 30      | 10        |
| aCAM     | 0.5   | 40               | 1.3-1.6     | 20     | 40      | 40        |
| UR       | 0-4   | 24               | 0.7-1.0     | 50     | 35      | 15        |
| aCAM     | 0.1   | 24               | 0.7-1.2     | 30     | 35      | 35        |
| aCAM     | 0.25  | 24               | 0.7-1.0     | 20     | 35      | 45        |
| aCAM     | 0.5   | 24               | 0.6-1.2     | 20     | 25      | 55        |
| aCAM     | 1     | 24               | 0.7-1.1     | 15     | 25      | 60        |
| aCAM     | 2     | 24               | 0.6-1.1     | 25     | 25      | 50        |
| aFib     | 0.25-5| 24               | 0.7-1.2     | 55     | 35      | 10        |
| aCAM + CAM| 0.5  | 24               | 0.6-1.3     | 50     | 35      | 15        |

* UR = unimmunized rabbit; aCAM = anti-CAM; aFib = rabbit antibodies against chick fibroblasts; aCAM + CAM = anti-CAM preincubated with 100 U of CAM.

\[ \text{Range of radial outgrowth at end of culture period.} \]

\[ \text{Average percentage of outgrowth contained in nerve bundles of the indicated diameter; 10-20 ganglia were scored in each experiment.} \]

### TABLE II

**Addition and Removal of Anti-CAM Fab' at Different Times during Ganglion Cultures**

| Fab' (0.5 mg/ml) | Type* | Time added (h) | Time removed (h) | Growth (μm) | 15-4 μm | 4-1.2 μm | 1.2-0.4 μm |
|-----------------|-------|----------------|------------------|-------------|--------|---------|-----------|
|                 | UR    | 0              | 30               | 0.8-1.3     | 80     | 20      | 0         |
|                 | aCAM  | 0              | 30               | 0.9-1.2     | 30     | 0       | 70        |
|                 | aCAM  | 6              | 30               | 0.8-1.5     | 30     | 10      | 60        |
|                 | aCAM  | 16             | 30               | 1.1-1.3     | 35     | 15      | 50        |
|                 | aCAM  | 24             | 30               | 0.9-1.4     | 50     | 10      | 40        |
|                 | UR    | 0              | 24               | 1.2-1.5     | 50     | 35      | 15        |
|                 | aCAM  | 0              | 24               | 0.9-1.4     | 20     | 20      | 60        |
|                 | aCAM  | 0              | 16               | 1.1-1.4     | 30     | 30      | 40        |
|                 | aCAM  | 0              | 6                | 1.0-1.2     | 35     | 40      | 25        |

* UR = unimmunized rabbits; aCAM = anti-CAM.

\[ \text{Range of radial outgrowth after 30 h of culture.} \]

\[ \text{Average percentage of outgrowth contained in nerve bundles of the indicated diameter; 10-20 ganglia were scored in each experiment after 30 h of culture.} \]

and more neurites became attached to them; the splitting of a thick fascicle was only occasionally observed except at its initial point of contact with the substratum.

(b) When anti-CAM was present, there were many more isolated growth cones, and thick fascicles could be formed transiently but rapidly split into smaller bundles which grow in different directions. The end result of these events was that the thick and radially directed processes that formed without anti-CAM were replaced by a tangled net of relatively fine neurites (Fig. 1).

(c) The rate of growth and the filopodial activity of neurites and their growth cones were not...
obviously affected by anti-CAM.

(d) The number and movement of non-neural cells were not altered by the antibody.

**Effect of Anti-CAM Fab’ on Cultures of Brachial, Thoracic, Lumbar, and Sacral Ganglia**

To investigate the possibility that ganglia from different parts of the spinal column vary in their response to anti-CAM Fab’, sacral, lumbar, thoracic, and brachial ganglia from 10-day embryos were cultured in medium containing either Fab’ from unimmunized rabbits or anti-CAM Fab’ (Table III). These groups differed in three respects: the diameter of outgrowth (with either Fab’) after 24 h, the thickness of fascicles formed in the absence of anti-CAM Fab’, and the degree to which thin processes were produced by the addition of anti-CAM Fab’. The extent of outgrowth, which may have been influenced by differences in the time required for the ganglia to attach to the substratum, was not affected by anti-CAM Fab’. With respect to fascicle diameter, it was observed that for each type of ganglion anti-CAM Fab’ caused a similar degree of change from thick to thin fascicles. Thus, thoracic ganglia, which, without the Fab’ produced a larger proportion of thick processes than the other ganglia, also had fewer thin neurites when cultured with anti-CAM; conversely, brachial and lumbar ganglia, which form relatively few thick processes without anti-CAM, grew a dense net of fine neurites when the antibody was present.

**Scanning Electron Microscopy of Ganglion Neurites**

Although it was necessary to use light microscopy in observing large numbers of ganglia under different experimental conditions, the greater resolution and relief of scanning electron microscopy make it a superior method for visualizing neurites and fascicles.

In Fig. 3 are shown scanning electron micrographs of ganglia from different segments of the spine that have been cultured in the presence and absence of anti-CAM. The pictures represent the same experiment shown in Table III except that the procedures used to prepare samples for scanning electron microscopy required the use of collagen-coated glass cover slips rather than collagen-coated petri dishes. The glass was less uniformly covered by the collagen, so that some ganglia produced only a few neurites. Nevertheless, the overall effect of the antibody, a conversion of thick fascicles to a tangled net of fine processes, was similar in the two experiments. Although it is difficult to count neurites in cultures containing fascicles, it also appeared that the presence of anti-CAM in some cases increased the total number of individual neurites emerging from

### Table III

**Effect of Anti-CAM Fab’ on Different Classes of Spinal Ganglia from 10-Day Chick Embryos**

| Spinal ganglia | Fab* (1 mg/ml) | Growth? (mm) | Fascicles$| |
|---------------|----------------|-------------|-----------|
|               |                | 15-4 µm     | 4-1.2 µm  | 1.2-0.4 µm|
| Brachial      | UR             | 0.6-1.1     | 20        | 50        | 30        |
|               | aCAM           | 0.7-1.0     | 10        | 20        | 70        |
| Thoracic      | UR             | 0.6-1.0     | 50        | 35        | 15        |
|               | aCAM           | 0.7-1.1     | 25        | 40        | 35        |
| Lumbar        | UR             | 0.5-1.0     | 30        | 50        | 20        |
|               | aCAM           | 0.6-0.9     | 20        | 25        | 55        |
| Sacral        | UR             | 0.8-1.2     | 35        | 50        | 15        |
|               | aCAM           | 0.8-1.3     | 25        | 35        | 40        |

* UR = unimmunized rabbits; aCAM = anti-CAM.

† Range of radial outgrowth after 30 h of culture.

§ Average percentage of outgrowth contained in nerve bundles of the indicated diameter; 10-20 ganglia were scored in each experiment after 30 h of culture.
FIGURE 3 Typical outgrowth of neurites from spinal ganglia after 24 h of culture in medium containing 1 mg/ml Fab' from unimmunized rabbits (a, c, e, and g), or 1 mg/ml anti-CAM Fab' (b, d, f, and h). Ganglia were taken from the brachial (a and b), thoracic (c and d), lumbar (e and f), and sacral (g and h) regions of spinal columns from 10-day-old embryos. In all cases, the region shown represents an area centered midway between the edge of the ganglion and the edge of the outgrowth (see Fig. 1). The different classes of ganglia formed fascicles of varying thicknesses, but within each class the anti-CAM Fab' consistently caused a marked decrease in the diameter of the processes. A comparison of Fig. 3a and b suggests that anti-CAM Fab' may also increase the number of neurites emerging from a ganglion. × 670.
a ganglion (compare Fig. 3a and b).

Several details of neurite bundling and branching could be detected in the higher magnification studies. First, it was clear that many of the thick fascicles that were formed in the presence of anti-CAM, in particular those near the periphery of the outgrowth, consisted of loose clumps of individual neurites as opposed to the smoother and
FIGURE 4 Thick fascicles in the periphery of the outgrowth that had formed in the presence of (a) Fab' from unimmunized rabbits and (b) anti-CAM Fab'. Although such fascicles appeared similar in phase-contrast micrographs and both were scored as 15- to 4-μm processes in Tables I-III, the neurites were less densely packed in the presence of anti-CAM Fab'. × 5,000.

FIGURE 5 Fascicles passing from the ganglion (G) to the substratum (S) that had formed in the presence of (a) Fab' from unimmunized rabbits, and (b) anti-CAM Fab'. In contrast to outgrowth on the substratum (Fig. 3), this region of outgrowth, which is only in contact with the medium, was not dramatically altered by the presence of anti-CAM Fab'. × 500.

FIGURE 6 Branching of fascicles (arrows) in the presence of anti-CAM Fab' near the point of attachment to the substratum (S). × 500.

FIGURE 7 Neurites emerging from a brachial ganglion (G) through medium containing anti-CAM Fab'. With these ganglia, the point of branching (Fig. 6) often reached the ganglion itself so that thick fascicles (Fig. 5b) were not observed. × 500.
tightly packed fascicles seen in cultures without the Fab' (Fig. 4).

Furthermore, thick fascicles that were not affected by anti-CAM were observed largely in regions near the ganglion where the processes were not in contact with collagen or cells (Fig. 5). At the point where such fascicles attached to the substratum, there was a tendency for the bundle to split, and this fragmentation was accentuated by the presence of anti-CAM (Fig. 6). In the case of brachial ganglia, which have less tendency to form thick fascicles, even the processes leaving a ganglion were reduced in diameter by anti-CAM (Fig. 7).

**DISCUSSION**

When cultured in vitro, a variety of nerve tissues produce neuronal processes that can stick to each other to form bundles, called fascicles (10, 16, 19). In the studies reported here, we have found that monovalent Fab' fragments that bind to the cell surface molecule CAM affect the formation of these bundles. Because antibodies against CAM have also been shown specifically to prevent the initial adhesion of neural cells to each other (14, 19), the simplest interpretation of this result is that CAM participates in the formation of side-to-side adhesions between individual neurites. The following discussion will focus on the consistency of this interpretation with results from our own and other laboratories, the possibility of alternative hypotheses, and the significance of these adhesions in developing neural tissue.

In terms of gross morphology, a neurite consists of two parts, the shaft and the growth cone. The growth cone can adhere to and move along a solid substratum by means of highly motile filopodia, and linear growth of a neurite occurs by addition of new membrane components at or near this structure (2, 3).

Neurite shafts are by comparison static structures. Although they can stick to each other to form fascicles (11, 20), they do not adhere to most types of substratum (2, 13). As shown previously (11) and confirmed by our observations, these side-to-side adhesions are actively formed through neurite-neurite contacts during their outgrowth from ganglia. Our studies suggest that the pattern of neurite fasciculation and branching in vitro reflects a continuous competition between side-to-side adhesions involving CAM and forces which tend to pull neurite bundles apart, such as movement of growth cones in different directions along the substratum. When side-to-side adhesions are not perturbed, thick fascicles are observed. When these adhesions are reduced by addition of anti-CAM Fab', the other forces on individual growing neurites appear sufficient to prevent fasciculation and even to disrupt preexisting bundles of processes; conversely, removal of the antibody restores adhesiveness and thick fascicles reappear. In contrast, growth cone functions do not appear to be affected by anti-CAM, in that the rate and extent of neurite growth was not changed by the antibody.

Several other observations are consistent with this conclusion. A very adhesive substratum such as plastic coated with wax bean agglutinin, which should be a good surface for attachment and migration of growth cones (6, 17), caused a decrease in the thickness of fascicles both in the presence and absence of anti-CAM. Similarly, a fascicle that traversed from the ganglion through the medium to the substratum began to branch at a point proximal to its initial contact with the substratum, presumably because the growth cones migrated in different directions along the surface and therefore pulled the neurite bundle apart. As would be predicted, the presence of anti-CAM caused these branch points to occur closer to the ganglion.

Another mechanism that would explain most of our results is that anti-CAM increases the adhesion of neurites to the substratum. This possibility would appear less attractive in view of the fact that anti-CAM Fab' is known to inhibit cell-cell adhesion. Moreover, we have not detected any effect of the antibody on the rate of neurite growth, or the rate of cell-substrate and ganglion-substrate attachment. In some cases, the presence of anti-CAM resulted in an increase in the total number of neurites emerging from a ganglion. Such effects can be induced by using a more adherent substratum such as polylysine (6), but it would seem more likely that anti-CAM promotes growth onto the substratum by decreasing neurite-neurite and neurite-cell interactions within or on the ganglion.

Although the effect of anti-CAM on fascicles is clear, it is not yet possible to conclude that CAM is directly responsible for the appearance of neurite bundles. In particular, it has not been proven, either with neurites or in cell aggregation, that CAM is a direct ligand in the formation of bonds between two plasma membranes. Our experiments suggest that the anti-CAM effect does not
involves inactivation of nerve growth factor, although indirect mechanisms, such as the binding of anti-CAM to a receptor for the factor, cannot be excluded. Other substances that bind to the cell surface, such as lectins, also cause a change in neurite morphology, but in general these agents produced a drastic condensation and retraction of processes that was clearly different from the anti-CAM effect. Furthermore, when an Fab' preparation was used that binds to neural processes but does not contain antibodies which react with CAM, neither the growth or appearance of the neurites was altered.

CAM is present in virtually every nervous tissue (14) and is found on the membranes of cell bodies, neurite shafts, and growth cones. Specificity in its action could be achieved through control of its concentration or behavior at the cell surface as a function of time or position. In addition, the presumed competition of CAM-mediated binding with the in vivo interaction between a growth cone and its local environment could produce a variety of morphological configurations (8). We have observed that the amount of cell adhesion molecule that reappears on retinal cells after trypsinization of tissue varies with developmental age and is correlated with cell adhesiveness (14). In this regard, it would be interesting to test whether ganglia from different parts of the spinal column, which produce fascicles of varying thicknesses, also differ in their CAM content.

An obvious role for CAM-mediated adhesion between neurite shafts is that this is the formation of nerve trunks that occur in many parts of the nervous system. The orderly gathering of individual neurites together, their collective migration along a specific nerve tract, and the branching of the trunk are important aspects of neuronal architecture and specification (11, 12, 17, 20, 21). Our observations on CAM would be consistent with a role in the gathering and branching steps, provided that a mechanism exists to modulate its function at a given place or time. Although the present in vitro studies cannot provide detailed information about directed growth of nerve trunks in an embryo, the neurites observed in cultures with anti-CAM appeared to follow a more tortuous path than the nerve bundles that grew out in the absence of this antibody. It has been reported that the processes formed in culture by pieces of goldfish retinal tissue tend to curve in a clockwise direction (5). This phenomenon appears to be an inherent property of the neurites, and it would be pertinent to investigate whether anti-CAM can affect such patterns in a suitable system.

The arrangement of nerve cell bodies and neurites within a tissue is another aspect of development that could be influenced by CAM function. In time-lapse movies of spinal ganglion or retinal tissue cultures, cell bodies occasionally translocated along the processes. Because the cell bodies also have CAM on their surface (14), it is possible that such movements, and similar phenomena during normal neuronal development (10, 16), are influenced by adhesions involving this molecule. In studies on the formation of histotypic structures in aggregates of retinal cells, we have observed (14) that the presence of anti-CAM inhibits sorting out of cell bodies and neurites into regions similar to the layers formed during development of retinal tissue. In view of the results obtained here, it may be illuminating to carry out similar experiments with cells from other highly stratified nervous tissues, such as the cerebellum.

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