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3. Type B toner is used in the Rank Xerox 3600 copier, and type H in Rank Xerox 3100 copier.
4. In a normally operating xerographic copier or duplicator, 25 mg of toner are present on a typical copy of a typed sheet 21.6 by 27.4 cm.
5. G. Löfroth, personal communication.
6. The addition of polymer to a solution of a mutagenic chemical (for example, nitropyrene) resulted in decreased revertant counts and a leveling of the activity versus mutagen concentration slope. This reduction in mutagenic activity appears to be influenced by polymer solubility. Whether the dissolved polymer reduces the effective concentration of the mutagenic chemical in solution or is encapsulating or hindering the diffusion of the chemical into the bacterial cells remains to be determined. The modified procedure adopted consisted of preparing a slurry of toner, 5 mg/ml in DMSO, and agitating it for 30 seconds at 30-minute intervals for 5 hours. In the *Salmonella* assay we used 50-, 100-, and 200-μl amounts of the slurry.
7. Carbon black A is a medium color furnace black manufactured by Columbian Chemicals Division of Cities Service Company and is marketed under the name of Raven 5750. Carbon black B is a pelletized long flow furnace black manufactured by the Special Blacks Division of Cabot Corporation and is marketed under the name of Black Pearls L.
8. Mixing conditions: twin-shell blender without intensifier agitated at room temperature for 30 minutes.
9. Extraction conditions: 48-hour Soxhlet extraction using all glass equipment, coarse porosity glass thimble (Ace Glass, Inc., No. 68B-04) and shielded from the light.
10. Microsomes were prepared from the livers of Aroclor 1254–induced rats (F344N, male Fischer, 25 mg of protein per milliliter).
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12. Reverse-phase chromatography was chosen to minimize the possibility of irreversible adsorption on the column. The toluene in the extract solution was concentrated by vortex evaporation (50°C, 50 torr) and the liquid residue taken up in acetonitrile. This was eluted through a Waters C-18 SepPak with acetonitrile and the eluate was concentrated and chromatographed to furnish three fractions. The SepPak was backflushed with tetrahydrofuran. No mutagenicity was found in this solution so that in subsequent chromatography it was assumed that all mutagens were elutable with acetonitrile.
13. Mass spectra data were obtained on a Nuclide Corporation 12-90G instrument with a temperature-programmed solids inlet probe (source temperature 250°C, ionizing voltage 70 eV, ionizing current 100 μA, beam current 105 A/μg, and resolution 1:1000). The ^{13}C-NMR data were obtained at 20.1 MHz with complete proton broadband decoupling on a Bruker WP-80 pulsed Fourier-transform spectrometer (110°C with concentrations of approximately 1 percent by weight in 99.5 percent DMSO-d_6). The ^{1}H measurements were made at 250 MHz on a Bruker WM-250 instrument.
14. Nitric acid (2 ml, 70 percent) was added dropwise to about 10 mg of fluoranthene or pyrene in the absence of solvent. The reaction mixture was filtered using glass frit washed with water and cold methanol and dissolved in methylene chloride.
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29. The usual tester strains (TA1535, TA1537, TA1538, TA100, and TA98) have the *UvrB* genotype which is expressed in an enzyme deficiency (uv-nuclease). This deficiency, the product of the *UvrB* gene, renders the bacteria sensitive to ultraviolet light and to some chemical mutagens that form adducts with DNA.
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6 March 1980

Cells Isolated from the Embryonic Neural Retina Differ in Behavior in vitro and Membrane Structure

Abstract. *Several subpopulations of cells were isolated from trypsin-dissociated embryonic (14 days) chick retinas. The cells of each subpopulation differed in associative behavior measured by cell aggregation and stationary culture assays and in glycoproteins that contain glucosamine. Freeze-fracture analysis showed that these populations also differed in intramembrane particle content.*

During embryogenesis, the neural retina develops from a simple epithelium into a multilayered structure in which each cell layer is discrete and restricted to specific functions. The formation of the layers could be considered a result of associative differences between the different cell types of the retina. Several studies have shown that soluble factors promote the tissue-specific adhesion of retina cells to each other (*1*). However, none of these materials discriminates between cells in the tissue. Rutishauser *et al.* (*2*) demonstrated the presence of a material on cells of retina and other neural tissues that appears to affect fasciculation of axonal outgrowths. Although this is clearly relevant to an aspect of retina organization, it is not clear if it is related to the formation of the cellular layers, which are detectable before the formation of the plexiform layers. In order to examine differences among the cells directly, we have been attempting to develop techniques to isolate specific cell types from the neural retina of the embryonic chick. Of the procedures we considered (*3*), we found that a buoyancy equilibrium method allowed us to isolate four cell populations that differ in associative behavior and membrane structure.

Embryonic (14 days) chick retinas, which contained all of the cell layers, were dissociated with 1 percent trypsin in calcium- and magnesium-free Tyrode's solution (*4*). The single cells were then resuspended in Eagles minimal essential medium containing 10 percent fetal calf serum (*5*). The suspension was divided among three solutions of Percoll (*6*) in medium and then adjusted to final densities of 1.019, 1.034, and 1.060 g/ml. These suspensions were incorporated into a step gradient with a top layer of medium and a cushion of 1.090 g/ml. The total volume of the gradient was 190 ml, and it contained up to 10^9 cells. The gradient was placed in a HS-4 rotor and centrifuged at 770*g* for 1 hour in a Sorvall RC-2B centrifuge equipped with a slow acceleration modification. Cells were collected from the interfaces, and the populations were labeled 1, 2, 3, and 4 in order of increasing buoyant density. The cells in each fraction were counted and classified by size. The four fractions contained 35 percent of the original cells; the rest were between the interfaces. Phase-contrast micrographs of the populations are given in Fig. 1, a to d, and the relative proportions of the cells are summarized in Table 1.

The cells in fraction 1 are large—78

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Table 1. Distribution by cell diameter of starting suspension of dissociated embryonic chick retinas and relative proportions in four cell populations after fractionation.

Suspension	Percent of cells		
	> 10 μm	5 to 10 μm	< 5 μm
Whole	5	29	66
Fraction 1	78	22	0
Fraction 2	19	54	27
Fraction 3	0	36	64
Fraction 4	0	12	88

percent have diameters greater than 10 μm. They are spherical with large, pale nuclei containing one or two nucleoli and are similar in general appearance to ganglion cells in the intact retina. Fractions 2 and 3 contain mixed populations in which cells of an intermediate size are found. In fraction 4, 88 percent of the cells have diameters less than 4 μm. The small cells resemble the cells of the outer portion of the inner nuclear layer of the retina, and the intermediate cells are similar to those of the inner portion of the same layer. We have not yet determined the precise origin of the different cells in the populations.

The populations differ in associative behavior. When the cells of each population were allowed to reassociate in a rotation-mediated aggregation system (*7*), the aggregates formed after 24 hours were different in size and shape. Figure 1, e to h, presents low-magnification dark-field micrographs of aggregates of the different fractions. Cells of fractions 1 and 4 produced small aggregates; those of fractions 2 and 3 were larger. Cultures of fraction 1 contained small spherical aggregates and unaggregated single cells. Fraction 3 aggregates had a roughly ovoid shape. Fractions 2 and 4 formed lobular aggregates.

The cell populations behaved differently in stationary culture as well. After 3 days in stationary culture, the cells developed a common configuration in which a layer of flat, mitotic cells was found closely apposed to the substrate, with neural cells adhering only to the flat cells. Although this general pattern was observed in all cultures of the different cell populations, variation was considerable depending on the proportions of cells in each fraction. Fractions 2 and 3 had a large proportion of flat cells, and these cells rapidly became confluent on the surface. The other cells formed small colonies on the flat cells (Fig. 1, j and k). Fractions 1 and 4 had very low proportions of flat cells, and most of the surface of the dish was bare. The neural-like cells attached only to the flat cells and formed characteristic structures on this cellular substrate. The cells of fraction 1 formed clusters of fewer than 50 cells that in turn sent out fascicles of fibers that contacted other clusters (Fig. 1i) and appeared to be similar to cultures of neural tissue (*8*). The cells of fraction 4 formed dense, spherical aggregates containing about 400 cells attached to the flat cells.

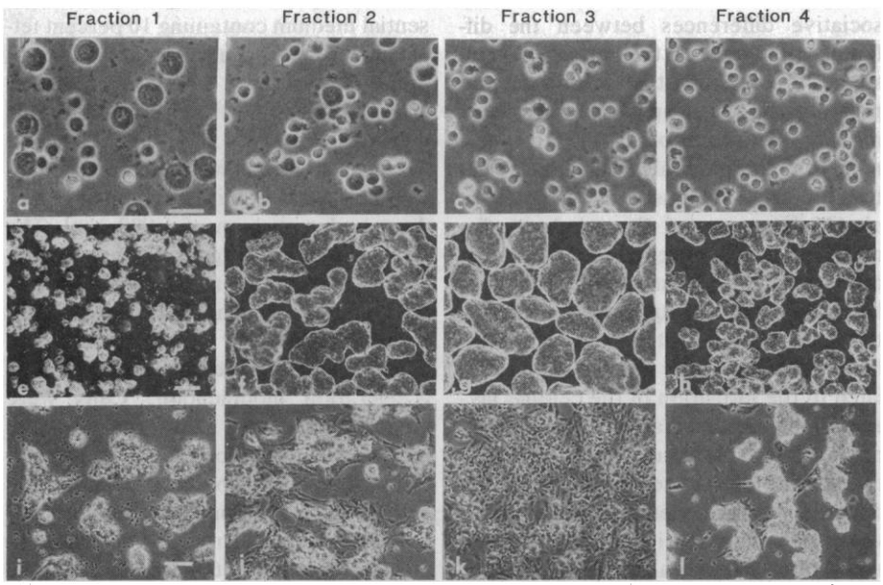

Fig. 1. (a to d) Phase-contrast micrographs of cells of four fractions. Scale bar, 20 μm. (e to h) Dark-field micrographs of cell aggregates from the four fractions. Cells (15×10^6) in 3 ml of medium were placed in a 25-ml Erlenmeyer flask on a gyratory shaker at 70 rev/min (3/4 inch center of rotation) for 24 hours. Scale bar, 0.1 mm. (i to l) Phase-contrast micrographs of stationary cultures of cells from the four fractions. Cells were seeded on plastic tissue culture dishes at a concentration of 2×10^5 cells per square centimeter. The cultures were fed every day, and these micrographs were taken on day 3.

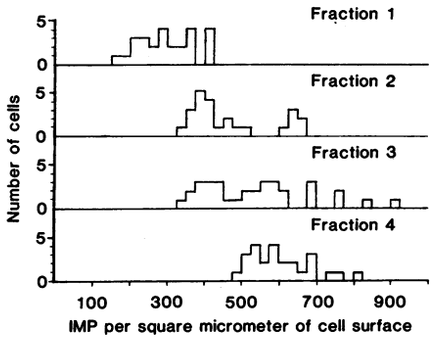

Fig. 2. Histogram of the intramembrane particle (*IMP*) distribution counted on the P-face of cells in the four populations.

The third characteristic we examined was the distribution of intramembrane particles (IMP) after freeze-fracture replication. [In another study (*9*), we found that different layers within the intact, developing retina have characteristic numbers of IMP.] Cells were fixed for 1 hour with 2.5 percent glutaraldehyde in 0.08*M* phosphate buffer, *p*H 7.4 (*10*), rinsed with buffer, and transferred to 25 percent glycerol 1/2 hour before use. Freeze-fracture replicas were prepared in a Denton DFE-3 unit with the use of apposed specimen holders (*11*). Particles were counted on the P-face of cells, and the micrographs were identified under code. The results are summarized in Fig. 2. Although there is some overlap between the fractions, the cells of fraction 1 have fewer IMP's per square micrometer of cell surface than do cells in fraction 4, and fractions 2 and 3 are intermediate between these extremes. The IMP density of fraction 1 cells is of the same magnitude as that of ganglion cells in the intact retina (*10*), an indication that the cells of fraction 1 are ganglion cells.

Cells of the four fractions (5×10^6 cells per milliliter; 3 ml) were grown in the presence of 25 μCi of tritiated glucosamine. Cells were lysed and the cell extract was subjected to sodium dodecyl sulfate gel electrophoresis (*12*). The gels were analyzed by autoradiography (*13*). Nineteen bands were common to all fractions, and five glycopolypeptides—260, 92, 87, 57, and 54 kd, respectively—were distributed unevenly between the fractions. The 260- and 92-kd materials were found primarily in fraction 1, with a small amount in fraction 2 (Table 1). We assume that these materials in fraction 2 arise from the almost 20 percent of the cells in this fraction that are of the ganglion cell type. The 87- and 57-kd materials were found in fraction 2, 3, and 4; the 54-kd material was primarily in fraction 4.

The IMP analysis demonstrated a gradient of increasing IMP density with in-

creasing cell buoyant density. This apparent gradient was not matched by the associative behavior of the cells as monitored by the size of the aggregates. The largest aggregates formed from cells of fraction 3; the aggregates of fraction 1 and 4 were considerably smaller. It appears then that IMP density is a characteristic that is not directly related to the size of aggregates of the cells. The size of the 24-hour aggregates, however, does appear to correlate with the number of flat cells that grow out in stationary cultures. Since the flat cells are able to adhere not only to artificial substrates but also to dissimilar cell types, they could form a bridge between cells in the aggregate. This would allow a large aggregate to form by alternately adding flat cells and other cells.

The appearance of the stationary cultures of fractions 2 and 3 suggests that the smaller cells adhere more readily to the flat cells than to each other. The cells of fraction 4, however, tended to adhere more closely to each other than to the flat cells, but not at all to the substrate. This led, in stationary cultures, to the formation of compact clusters on top of flat cells and a significant population of unattached aggregates.

The associative interactions between the cells in the different fractions are extremely complex, but it is now possible to use our separation procedure to isolate and analyze components of the mixture. This analysis is currently under way.

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Influence of Siphonophore Behavior upon Their Natural Diets: Evidence for Aggressive Mimicry

Abstract. *Collection by divers permitted determination of the natural diets of siphonophore species within 11 genera. Siphonophores that swim rapidly to spread their tentacles capture small prey, whereas those that swim very weakly capture much larger prey. Nematocyst batteries of two species of weak swimmers closely resemble copepods and fish larvae. Morphology, behavior, and diet suggest that these two species attract large prey by mimicking other zooplankton.*

Siphonophores, pelagic cnidarians of the class Hydrozoa, have been likened to spiders in capturing prey that bump into a nearly invisible sticky network (*1*). The tentacles of siphonophores form a three-dimensional web, armed with millions of nematocysts that inject toxin into the prey on contact. The diets of these nonvisual predators depend in part on prey behavior, including avoidance of the predators and the ability to escape if contacted (*2*). The diets of siphonophores must also depend on predator behaviors that counteract prey avoidance and escape. The types and sizes of prey captured by various siphonophores differ according to the swimming activity and gastrozooid size of the siphonophore species. In addition, two of the siphonophore species examined may attract large prey by the movements of tentacular structures that resemble small zooplankton, an indication of aggressive mimicry among zooplankton.

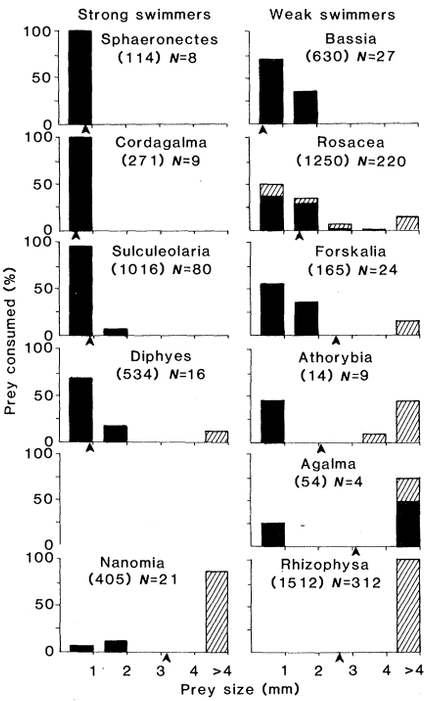

Fig. 1. Comparison of the sizes (measure of the greatest dimension, usually length) of natural prey captured by strongly and weakly swimming siphonophores. Gastrozooid length is indicated by an arrowhead under each horizontal axis. Copepod prey are indicated by black bars, noncopepod prey by diagonally shaded bars. Numbers of gastrozooids examined are in parentheses. Percentages are based on *N*, the number of measurable prey items.

While scuba diving at depths of 0 to 25 m, I collected siphonophores in jars and immediately killed them in situ with formaldehyde solution. I examined ingested prey in the following numbers of colonies of these species: 60 *Bassia bassensis*, 53 *Nanomia bijuga*, 47 *Rosacea cymbiformis*, 14 *Agalma okeni*, 6 *Sulculeolaria quadrivalvis*, 3 *S. turgida*, and 11 *Diphyes dispar* from the Gulf of California (*3*); 6 *Athorybia rosacea*, 10 *S. chuni*, 3 *S. monoica*, 15 *Forskalia edwardsii*, and *F. tholoides* from both the Sargasso Sea (*4*) and the Gulf of California; 11 *Cordagalma cordiformis* and 2 *S. biloba* from the Sargasso Sea; and 13 *Sphaeronectes gracilis*, 7 *Diphyes dispar*, 5 *S. quadrivalvis*, and 2 *S. chuni* from the California current (*5*). Since species of *Sulculeolaria* and of *Forskalia* consumed similar prey, data are presented by genus. All gastrozooids ("stomachs") were dissected or mounted whole on microscope slides with cover slips for prey identification and measurement at magnifications of ×25 to ×100.

Differences in the diets of several siphonophore species corresponded to differences in their swimming patterns. Some species swim rapidly (strong swimmers), often in an arc or in spirals (*1*, *6*). These siphonophores drift be-

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