were quantitatively reproducible. Lower concentrations of VCN are ineffective, perhaps because of their inability to release the optimum amount of sialic acid to bring about cellular deformabiliy. Thus it appears that the observed effect as depicted in the Figure is due to the action of VCN on the cell membrane structure of amoebae.

The exact mechanism as to how VCN leads to the disintegration of the membrane structure followed by the lysis of the cell body of amoeba is not known. Removal of sialic acid might be associated with the loss of the rigidity of the cell membrane structure[5] leading to deformation[6] of the surface structure of the cell. These phenomena might change the modes of normal transport across cell membranes[13–15], thus creating an imbalance in the osmotic potential inside and outside the membrane. In this situation, at some points, any two parts of the membrane structure may fall apart leading to the consequence detailed above.

It might be relevant to point out that this could be an isolated example of the observed effects because of the unique surface configuration of amoeba. In the case of mammalian cells, this kind of situation is not normally observed in vitro, even if incubation is extended for longer periods and higher concentrations of VCN are used[10, 11].

Summary. Removal of cell surface sialic acid with neuraminidase brings about cell deformation in amoeba. The membranes of these deformed cells are eventually ruptured leading to the liberation of the cell mass.

P. K. Ray and S. Chatterjee[16]

Biomedical Group, Modular Laboratories,
Bhabha Atomic Research Centre,
Trombay, Bombay-400 085 (India), 3 May 1974.

[13] L. Weiss and C. J. Levinson, J. Cell Physiol. *73*, 31 (1969).
[14] D. H. Brown and A. F. Michael, Proc. Soc. exp. Biol. Med. *131*, 568 (1969).
[15] A. Vaheri, E. Ruoshatri and S. Nordling, Nature New Biol. *238*, 211 (1972).
[16] We thank Dr. E. W. Rajasekhar for his valuable help in preparing the manuscript.

Two Types of Bipolar Cells in the Chick Retinal Development[1]

Bipolar cells comprise the bulk of the inner nuclear layer of the retina. These cells are important intermediates of the retinal pathways because they form synaptic contacts with both photoreceptor terminals, processes of amacrine cells and ganglion cells[2–5]. Extensive studies by Ramón y Cajal[6–8] indicated that, for example in the chicken, there are 2 types of bipolar cells. However, there appears to be little existing data on when these two types of bipolar cells arise and what their morphological characteristics are. This study is an attempt to shed some insight on this problem.

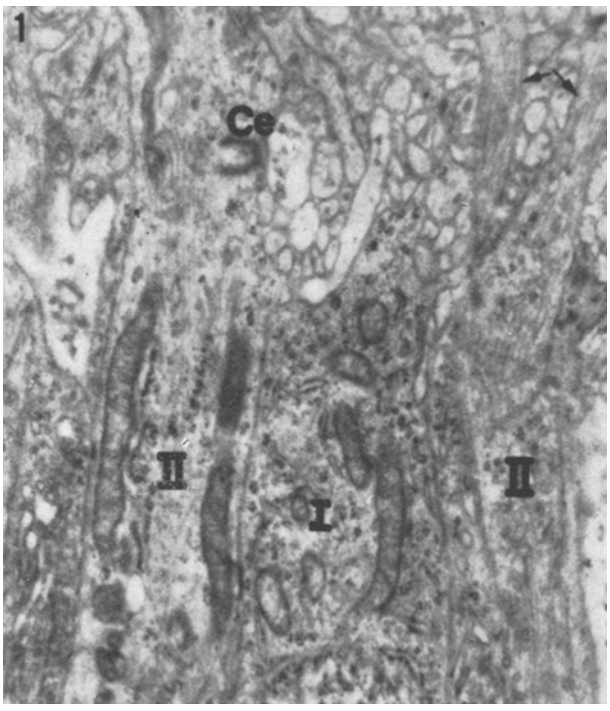

Fig. 1. Type I and II bipolar cells are distinguished at stage 40 of chick embryonic retina. Note a centriole (Ce) in one of the type II cells and external projections (arrows) in the other. ×28,800.

Materials and method. Fertile white leghorn chicken eggs were incubated in standard conditions and staged according to Hamburger and Hamilton[9]. Chick embryonic eyes from stages 36 to hatching were taken out and bisected into halves. Then the posterior halves were prefixed with 6% buffered glutaraldehyde and postfixed with 1% buffered osmium tetroxide, dehydrated in ethanol, cleared in propylene oxide and embedded in Epon. Sections were cut with a Porter-Blum MT 2 microtome and stained with uranyl acetate and lead citrate, and then examined with a Zeiss EM 9S2 electronmicroscope.

Results. Bipolar cells cannot be distinguished until stage 36 when both plexiform layers are formed and the presumptive bipolar nuclei become distinguishable by their ellipsoidal shape. The cytoplasm of these cells at stage 36 have a few mitochondria, some rough endoplasmic reticulum and few ribosomes. Some bipolar cells often lie close together resembling a telophase phenomenon, indicating that they have just completed a mitotic division. At stage 40, dendritic projections can be seen arizing from the bipolar cell bodies towards the outer plexiform layer (Figure 1) and at this time 2 types of bipolar cells can be distinguished. The ultrastructural details of these 2 types of bipolars are outlined in the

[1] Supported by NIH grant No. EY00477 to DBM.
[2] J. E. Dowling, Proc. R. Soc., Lond. *170*, 205 (1968).
[3] J. E. Dowling, Invest. Ophthal. *9*, 655 (1970).
[4] J. E. Dowling and B. B. Boycott, Proc. R. Soc. Lond. *166*, 80 (1966).
[5] J. E. Dowling and B. B. Boycott, Cold Spring Habor Symp. quant. Biol. *30*, 392 (1965).
[6] S. Ramón y Cajal, Anat. Anz. *4*, 111 (1889).
[7] S. Ramón y Cajal, Cellule *9*, 119 (1892).
[8] S. Ramón y Cajal, translated by S. A. Thorpe and M. Glickstein, *The Structure of the Retina: A Complete Work* (Charles C. Thomas, Springfield 1972).
[9] U. Hamburger and H. L. Hamilton, J. Morph. *88*, 47 (1951).

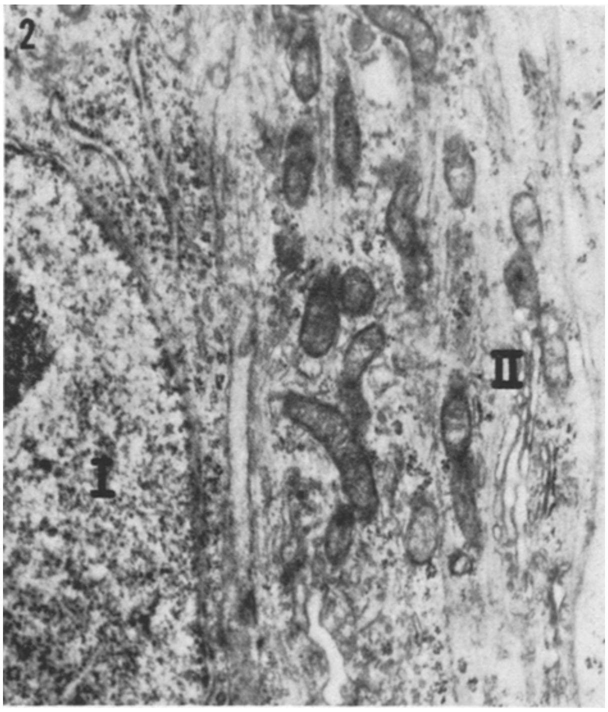

Fig. 2. Type I and II bipolar cells at the same stage. The nucleus of the type I bipolar is large and located more externally. Note the presence of some rough endoplasmic reticulum near the nucleus. On the other hand, the long dendritic trunk in the type II bipolar contains a golgi apparatus, mitochondria and neurotubules. × 28,800.

Comparison of the two bipolar cell types at stage 40

	Type I	Type II
Nuclear position	Close to outer plexiform layer	Close to inner layers
Nuclear morphology	Large (2 µm wide differ from horizontal cell nuclei which are spherical)	Smaller (1.5 µm wide)
Cytoplasm	Scanty	Scanty
Cytoplasmic organelles	Mitochondria, more rough endoplasmic reticulum, free ribosomes, some neurotubules	Long mitochondria, less rough endoplasmic reticulum free ribosomes, prominent long golgi, more neurotubules
Morphology of dendritic processes	Short branches of dendritic processes from cell body	Long main dendrite leading from body, then giving out branches

Table (Figures 1 and 2). In rare instances, a centriole may be present in the main dendrite of the type II bipolar cells (Figure 2) at this stage.

Discussion. Bipolar cells are derived embryologically from the outer neuroblastic layer[10,11] and initially they appear spindle-shaped and cannot be easily differentiated from the other retinal cells. By stage 36, when the outer plexiform layer appears and separates the photoreceptor cells from the inner nuclear layer, bipolar cells can be clearly identified. Our electronmicroscopic data on these 2 types of chick bipolar cells appear to confirm RAMÓN Y CAJAL's studies. Type I bipolar cells (RAMÓN Y CAJAL's outer bipolars) with large nuclei and short dendritic branches can be distinguished from the type II bipolar cells (RAMÓN Y CAJAL's inner bipolars) each of them has a smaller nucleus and a single long main dendritic trunk. Furthermore, our results also indicate that there are differences between cytoplasmic organelles in the 2 types of bipolar cells.

It is hoped that, based on the ultrastructural characteristics of these 2 types of chick bipolar cells, additional understanding of the complex relationships between the bipolars and the different retinal cell types can then be obtained.

Summary. Two types of bipolar cells are identified in the chick embryonic retina. They can be distinguished by their cytoplasmic organelles.

D. T. YEW and D. B. MEYER

Department of Biology, University Science Center, Chinese University of Hong Kong, Shatin, N. T. (Hong Kong), and Department of Anatomy, Wayne State University, Detrot (Michigan, USA), 2 April 1975.

[10] I. MANN, Br. J. Ophthal. *12*, 449 (1928).
[11] I. MANN, *The Development of the Human Eye* (Grune and Stratton Inc., New York 1950).

Ovulation in an Echinoderm (*Comanthus japonica*)

Ovulation in several invertebrate groups may be defined as the passage of oocytes through a layer of epithelial cells. This phenomenon has been observed directly for ctenophores[1] and has been inferred from comparisons of ovarian histology before and after ovulation for coelenterates[2], priapulids[3], pentastomids[4] and cephalochordates[5]. The present report describes the foregoing type of ovulation[6] for the first time in an echinoderm; moreover, the oocytes are illustrated during their transepithelial passage.

The echinoderm studied was *Comanthus japonica*, a crinoid for which the spawning date is predictable from the lunar calendar[7]. On the predicted day, almost all the oocytes in all the females begin maturation with germinal vesicle breakdown shortly before noon, and the mature ova are spawned later that afternoon[8,9]. To demonstrate ovulation in *Comanthus*, we removed ovaries every 15 min throughout the morning and afternoon of the spawning day. These ovaries were fixed by previously published methods[10] for light- and electron microscopy.

Up through 11.30 h, every oocyte had a germinal vesicle containing a single nucleolus. Each oocyte lay mainly in the intermediate layer of the ovary[11]; however, the end of the oocyte nearest the ovarian lumen was closely associated with a plaque of cuboidal somatic cells belonging to the otherwise squamous epithelium lining the ovary.