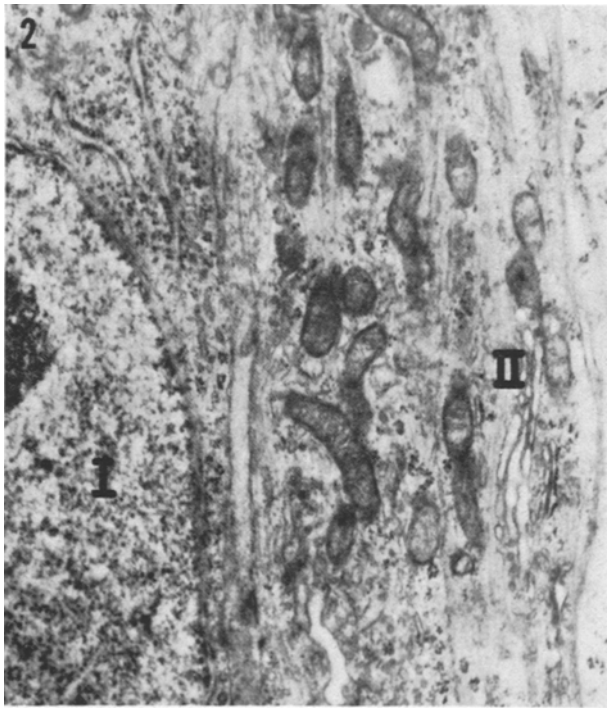

Fig. 2. Type I and II bipolar cells at the same stage. The nucleus of the type I bipolar is large and located more externally. Note the presence of some rough endoplasmic reticulum near the nucleus. On the other hand, the long dendritic trunk in the type II bipolar contains a golgi apparatus, mitochondria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

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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.

Transmission electron microscopy showed that the plasma membrane of the oocyte was separated from the plasma membranes of the somatic cells by an electron lucent, intercellular space several hundred Ångstroms wide. Except at the foregoing cell-to-cell association zone, the oocyte was enveloped by a thin chorion, which was actually a continuation of the basal lamina underlying the inner layer of the ovary.

By the sample of 11.45 h, the breakdown of the germinal vesicle and the disappearance of the nucleolus signaled the start of oocyte maturation. Ovulation had also started, as each oocyte was just beginning to enter the ovarian lumen by passing through the epithelium lining the ovary. By subsequent samples (12.00 h noon, 12.15 h and 12.30 h), an ever increasing portion of each oocyte was projecting into the ovarian lumen (Figures 1a and 2). The oocytes were constricted by passage through the epithelium, but

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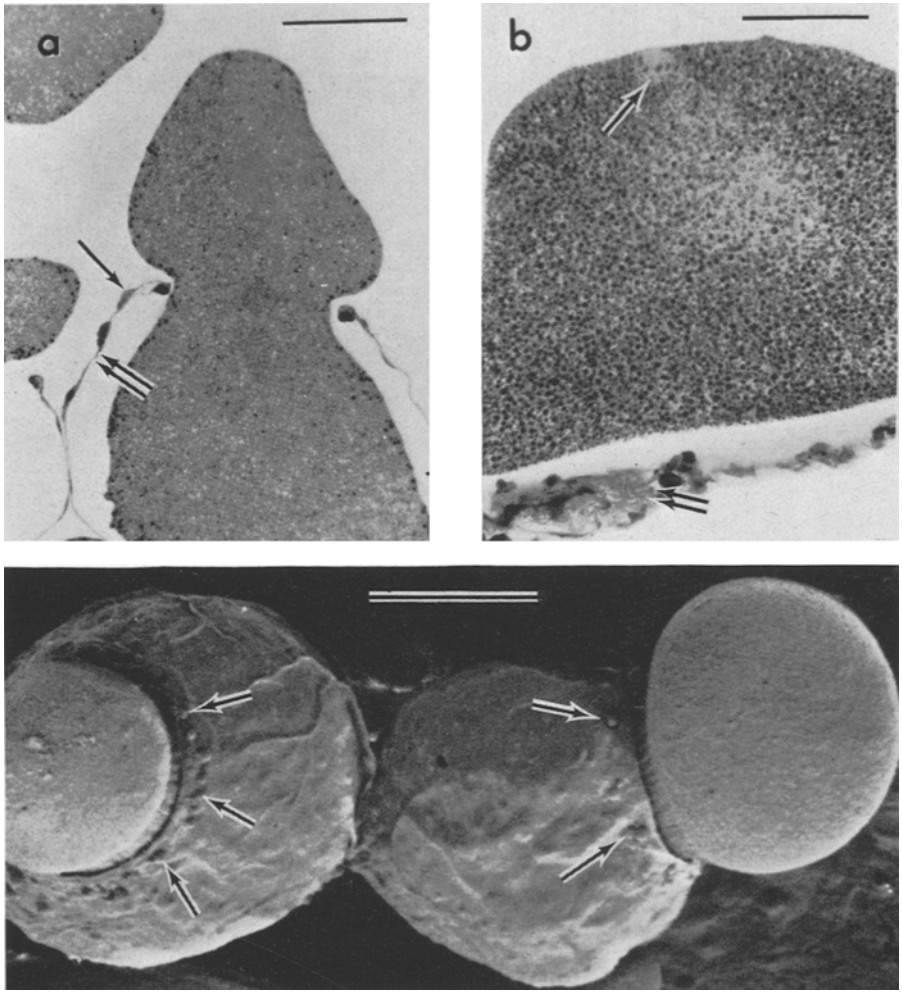

Fig. 1. (top). Light micrographs of *Comanthus* ovaries fixed on the day of spawning. A) Oocyte fixed at 12.15 h during ovulation. The oocyte is passing through the inner ovarian epithelium (single arrow) and is emerging into the ovarian lumen towards the top of the figure. In this figure, the chorion (twin arrows) cannot be distinguished from the epithelium lining the ovary. B) Oocyte fixed at 13.00 h, just after the end of ovulation. The oocyte, containing the metaphase plate (single arrow) of the first maturation division, lies free in the ovarian lumen. The stripped off chorion (double arrow), now much collapsed and thickened, lies beneath the inner epithelium of the ovary. The scale lines are 50 μm long.

Fig. 2. (bottom). Scanning electron micrograph of the luminal surface of a *Comanthus* ovary fixed during ovulation (12.15 h on the day of spawning). 2 oocytes are passing into the lumen through circular openings in the epithelium lining the ovary. The cuboidal cells (arrows) of the epithelium are distributed around the periphery of each opening. Elsewhere the epithelium consists of squamous cells not individually distinct at this magnification. The scale line is 50 μm.

expanded again as they entered the lumen. Scanning electron microscopy demonstrated that each oocyte emerged through a circular opening located in the midst of the plaque of cuboidal somatic cells; during the passage of the oocyte, the cuboidal cells were distributed around the perhiphery of the opening (Figure 2, arrows). Nothing is yet known about the dynamic cause(s) of ovulation in *Comanthus*; more work is needed to assess the possible roles of oocyte motility, somatic cell motility, chorion contractility, ovarian proteolytic enzymes, and pressure changes in the subchorionic space.

By 12.45 h ovulation was over, having taken approximately 1 h from start to finish. All the oocytes were now lying free in the ovarian lumen, and most of them had reached metaphase of the first maturation division (Figure 1b). The chorions had been stripped off the oocytes during ovulation and remained, much thickened and folded, beneath the lining epithelium of the ovary (Figure 1b). In this epithelium, no conspicuous openings remained after ovulation. For at least several hours after ovulation, the epithelium lining the ovary was still differentiated into plaques of cuboidal cells and squamous cells. Oocyte maturation, which had started simultaneously with ovulation, continued for several more hours after the end of ovulation. Maturation proceeded in the ovarian lumen through the two maturation divisions and ended with the formation of an egg pronucleus in each ovum. At spawning, which took place shortly before 16.00 h, the ova were expelled from the ovarian lumen into the sea water by simultaneous rupture of the ovarian and body walls.

In the Phylum Echinodermata, the type of ovulation described above for *Comanthus* probably occurs in many crinoids, holothurians and ophiuroids[12]. In most echinoids, by contrast, maturing oocytes pass through a stratified epithelium of non-germinal cells[13], and there are no chorions to be stripped off and left behind in the inner layer of the ovary. In asteroids, the retraction of the follicle cells from the maturing oocytes[14] is undoubtedly a part of ovulation; unfortunately, an exact description of asteroid ovulation is not yet possible, since the structure of the inner layer of the ovary is imperfectly understood at present.

Summary. An invertebrate type of ovulation is described for the first time for an echinoderm. In this echinoderm, which is a crinoid, ovulation is the passage of maturing oocytes through temporary openings in the epithelium lining the ovary. After ovulation, which takes about 1 h, the oocytes lie free in the ovarian lumen; there they quickly finish maturing into ova which are spawned into the sea water.

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Degeneration of Adrenergic Axons in the Longitudinal Muscle Coat of the Rat Duodenum Following Treatment with 6-Hydroxydopamine

Several authors[1–3] have recently alluded to the virtual absence of an adrenergic innervation of the longitudinal coat of the muscularis externa of mammalian gut. This contrasts with the circular coat which is said to receive a direct adrenergic innervation. During the course of a study on the submucous ganglia of the rat duodenum following treatment with 6-hydroxydopamine[4] (6-OHDA), degenerating adrenergic axons, unrelated to blood vessels, were observed in passing, in both coats of the muscularis externa. In view of the paucity of information on the adrenergic innervation of the longitudinal coat of mammalian gut, it was decided to study the problem further.

Adult albino rats weighing 250–300 g were given a single i.v. injection through the saphenous vein of 100 mg/kg 6-OHDA (25 mg/ml 6-OHDA HCl dissolved in a solution containing 1 mg/ml ascorbic acid). Control rats were injected i.v. with an equivalent volume of ascorbic acid. Subcutaneous heparin (1,000 U) was administered to all rats at the time of the i.v. injection. All rats were sacrificed about 1 h after the experiments. The duodenum was fixed by intracardiac perfusion for 15 min with a solution containing 4.0% glutaraldehyde in 0.10 *M* cacodylate buffer at pH 7.2[5]. Following perfusion, a short segment of duodenum was distended slightly with fresh fixative and tied at both ends to maintain the distension. The whole segment was removed and immersed in fresh ice-cold fixative for a further 2–3 h, after which it was sliced into thin rings. The material was post-fixed in 1% osmium tetroxide, dehydrated with acetone and embedded in araldite. Semi-thin transverse sections of the duodenum were stained with methylene blue and prepared for light microscopy. Ultra-thin transverse sections were stained with aqueous saturated uranyl acetate and lead citrate and examined in a Hitachi HS-8 electron microscope.

The longitudinal coat of the rat duodenum was 30–35 µm thick and, though clearly thinner than the circular coat, was about 4/5 the latter's thickness (Figure 1).

Electron microscopy of the 6-OHDA treated rats showed, in the core of the longitudinal coat, small to medium-sized nerve bundles whose axons were sometimes seen to approach individual muscle fibres (Figures 2 and 3). Adrenergic axons within nerve bundles could be recognized by their content of small granulated vesicles as well as agranular vesicles and large granular vesicles[6]. Figure 2 shows part of a nerve bundle partially enveloped by Schwann cell cytoplasm and containing 4 adrenergic axons, 3 of which (a_1–a_3) were showing early degeneration (general darkening, swollen vesicles and degenerating

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