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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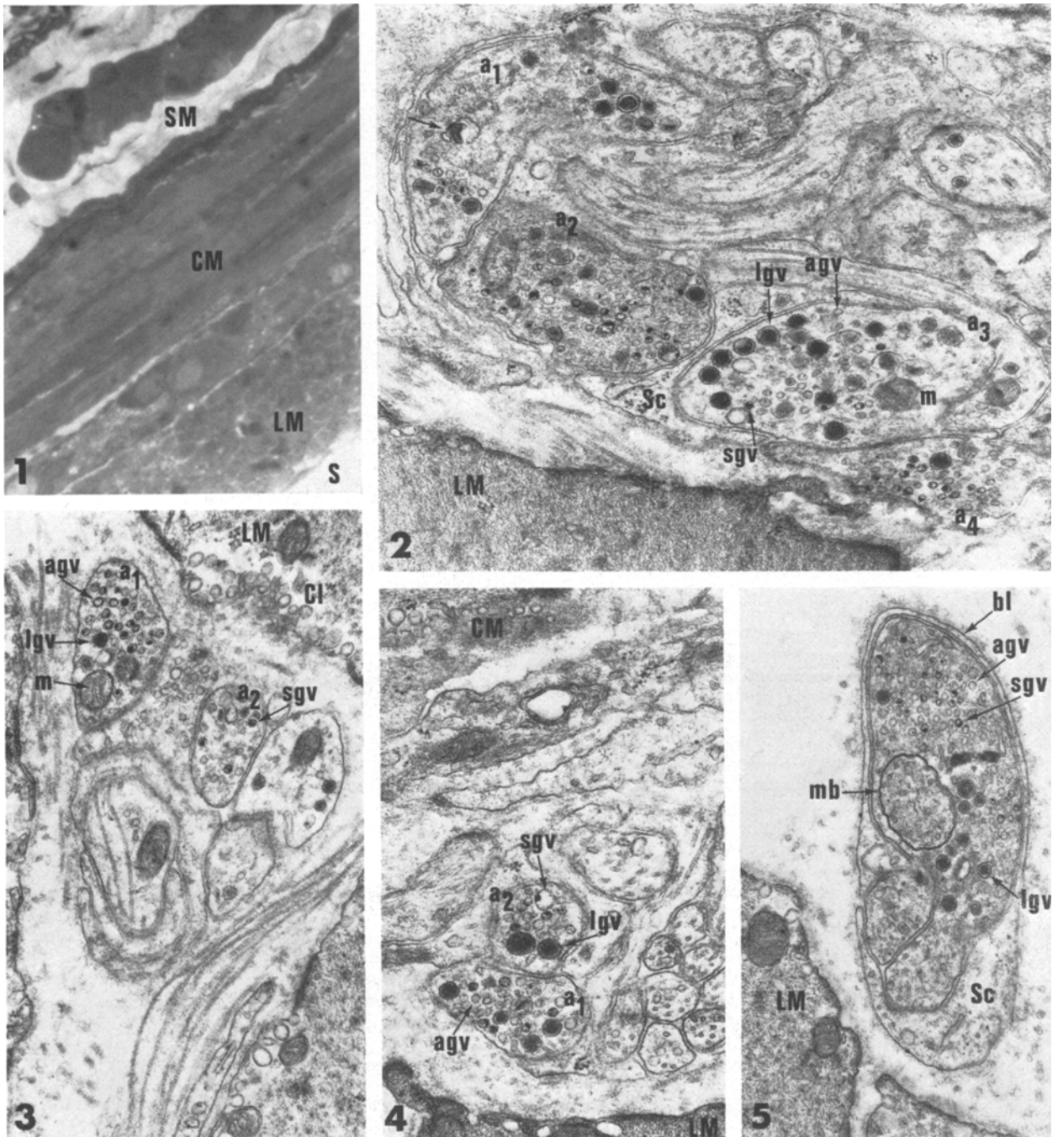

All illustrations are taken from transverse sections of the duodenum of treated rats (1 h, i.v. 6-OHDA 100 mg/kg).

Fig. 1. Light photomicrograph of a semi-thin section. S, serosa; LM, longitudinal coat; CM, circular coat; SM, submucosa. × 570.

Fig. 2. Part of a medium-sized nerve bundle in the core of the longitudinal coat. a_1–a_4, adrenergic axons; arrow in a_1 points to a degenerating mitochondrion; LM, smooth muscle; Sc, Schwann cell cytoplasm; sgv small granular vesicle; lgv, large granular vesicle; agv, agranular vesicle; m, degenerating mitochondrion. For futher discussion see text. × 27,500.

Fig. 3. A small nerve bundle in the core of the longitudinal coat. a_1 and a_2, adrenergic axons; agv, agranular vesicle; lgv, large granular vesicle; m, mitochondrion; sgv, small granular vesicle; LM, smooth muscle; CI, caveolae intracellulares. × 27,500.

Fig. 4. Part of a medium-sized nerve bundle in the myenteric plexus. a_1 and a_2, adrenergic axons; agv, agranular vesicle; lgv, large granular vesicle; sgv, small granular vesicle; LM, smooth muscle in longitudinal coat; CM, smooth muscle in circular coat. × 32,000.

Fig. 5. A small nerve bundle on the serosal aspect of the longitudinal coat containing a degenerating adrenergic axon. LM, smooth muscle; mb, membrane-bound body; bl, basal lamina; agv, agranular vesicle; sgv, small granular vesicle; lgv, large granular vesicle; Sc, Schwann cell cytoplasm. × 27,500.

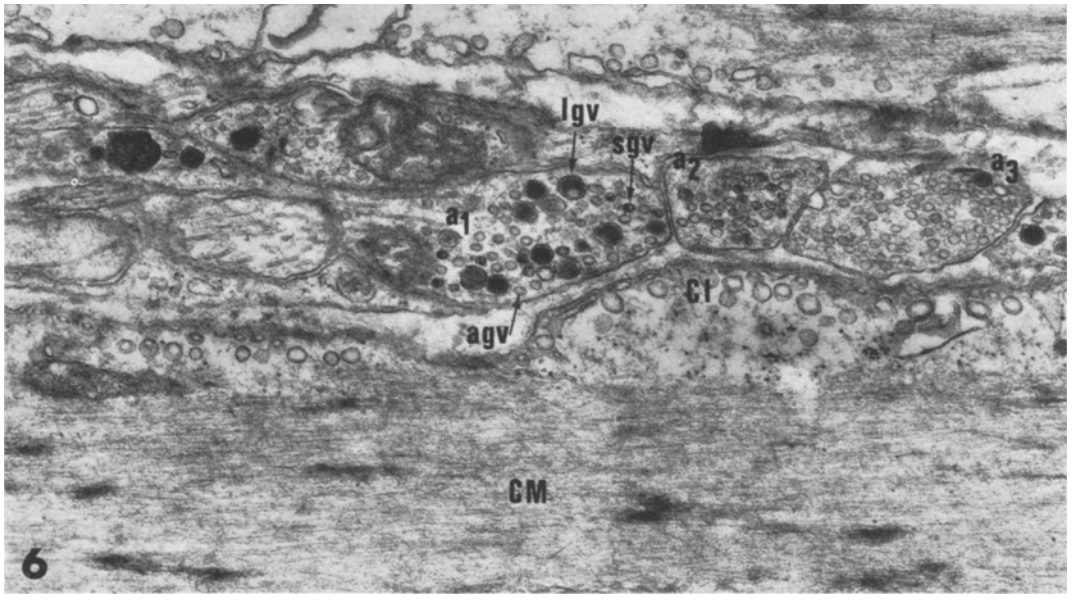

Fig. 6. Part of a medium-sized nerve bundle in the circular coat. a_1, adrenergic axon; agv, agranular vesicle; lgv large granular vesicle; sgv, small granular vesicle; a_2 and a_3, nonadrenergic axons with many agranular vesicles; CM, smooth muscle in circular coat; CI, caveolae intracellulares. × 27,500.

mitochondria) while the other axon (a_4) could not be distinguished from adrenergic axons present in controls. Where axon a_4 faced the tongue-like process of the muscle fibre, Schwann cell cytoplasm was absent and here the neuromuscular gap was about 55 nm. In Figure 3 two adrenergic axons (a_1 and a_2) were seen close to a muscle fibre with neuromuscular gaps of 150 nm or more. Axon a_1 was about $^2/_3$ 'naked' and, where it faced the muscle, the latter contained many caveolae intracellulares[7]. Figure 4 was taken from a medium-sized nerve bundle at the junctional region of the myenteric plexus and shows 2 adrenergic axons (a_1 and a_2) one of which (a_1) was partially 'naked' and facing the muscle fibre in the longitudinal coat with neuromuscular gap of about 315 nm.

Nerve bundles were rarely encountered on the serosal aspect of the longitudinal coat and definite nerve muscle relationships have so far not been seen here. However, on the occasions when small nerve bundles were present they sometimes contained within them degenerating adrenergic axons (Figure 5).

The results show that 6-OHDA may be used in combination with electron microscopy to demonstrate the presence as well as the distribution of adrenergic axons in the muscularis externa of mammalian gut (cf. THOENEN[8] and THOENEN and TRANZER[9]). The finding of the adrenergic axons within the nerve bundles that course in the longitudinal coat of the rat duodenum, and the report of their virtual absence in the guinea-pig ileum[1], support the view that there are regional differences in the adrenergic innervation of the smooth muscle of mammalian gut[2,3]. The ratio of the thickness between the longitudinal and circular muscle coats in the rat duodenum and the guinea-pig ileum appears different; 4:5 in the former and 1:2 in the latter[1]. Whether the thickness of the longitudinal coat, absolute or relative, may be reflected in its pattern of innervation is not clear, but it has been reported that the thick taenia coli of the guinea-pig receives an adrenergic innervation[10].

The pattern of innervation of the longitudinal coat of the rat duodenum resembled the multi-axonal neuromuscular relationships described in the smooth muscle of anuran gut[11,12]. Most of the junctions had wide gaps, 150 nm or more. Gaps that were about 50 nm or less were rarely encountered. This contrasts with the situation in the circular coat where narrower junctions were much more commonly found[1] (Figure 6).

BENNETT and ROGERS[10], in their study of the guinea-pig taenia coli, estimated that for nerve varicosities to produce a detectable permeability change they need to be within 300 nm of a smooth muscle. If similar cirteria are also applicable in the longitudinal muscle coat of the rat duodenum, then a proportion at least of the adrenergic neuromuscular relationships described in the present study may be considered to be functionally effective.

Summary. Following systemic treatment with 6-OHDA, electron microscopy of adult rat duodenum showed degenerating adrenergic axons in the longitudinal muscle coat making neuromuscular relationships with gaps of varied widths.

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