C/V-values of 8 fragments in the intestines of mouse, rat and golden hamster

Segment	Duodenum				Jejunum		Ileum		
Numbers	1	2	3	4	5	6	7	8	Means
Mouse	7.6[a]	7.3	5.8	4.9	4.5	4.2	4.3	4.6	5.4
	1.18[b]	1.33	1.04	0.57	0.59	0.68	0.53	0.70	
Rat	18.2	16.7	14.3	13.2	12.2	10.4	10.5	10.2	13.2
	3.15	3.22	1.82	1.67	1.29	0.88	0.123	0.83	
Golden	5.3	5.3	4.1	4.5	4.0	4.2	4.1	3.3	4.4
hamster	1.44	0.68	0.76	0.71	0.68	0.79	0.57	0.49	

[a] The average value of 9 measurements on 3 animals [b] S.D.

b). In Figure a), the edge of crypt was brought into focus, so that villi were visualized as dim spindle-like images.

The small intestine was cut up evenly into 8 segments and the number of crypt and villus was mesaured, respectively, in the phrotographs taken at 3 different regions in each segment. In the Table are shown the ratios of crypts to villus of 3 animals tested; hereafter the ratio will be referred to as 'C/V-value'. The Table reveals that there are apparent gradual decreases of the C/V-values along the intestine from upper duodenum to lower ileum, and that the averages of all 8 segments coincide with the value at the 4th segment in all animals tested. At the 4th segment, therefore, the mean C/V-values are comparable with each other. It is noticeable that the mean C/V-value is different in each species and that of rat is markedly higher than those of the others. These facts may imply the probable variance in static or dynamic aspects of epithelial cell population of various animals, and furthermore the difference of mean survival times in the gastrointesinal death induced by radiation.

In fact, the author has gained a few noticeable features on dynamic aspects of epithelial population through reconsidering the problem by introducing the C/V-values into the 2-dimensional pattern known from the studies of conventional histological sections. They will be reported elsewhere.

Zusammenfassung. Quantitative Bestimmung der Krypten- und Zottenzahl im Darm der Nagetiere. Die Zahl der Zotten ist bei verschiedenen Tieren eine unterschiedliche und nimmt zudem vom Duodenum gegen das Coecum zu ab.

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Myoneural Junctions in Larval Ascidian Tail

Myoneural junctions are typically formed at the surfaces of muscle cells by peripheral nerve fibers which extend from a centrally located spinal cord or nerve cord. Two instances are known, however, in which this pattern is reversed. In the nematode worm *Ascaris*[1] and in the primitive chordate *Amphioxus*[2] it is the muscle cells that send processes toward the nerve cord, and myoneural junctions are established at the surface of the nerve cord rather than within the muscle.

The tail of the larval ascidian *Amaroucium constellatum* is known to contain acetylcholinesterase[3]; however, no myoneural junctions have been found previously in the tail muscle[4]. This report presents evidence for a unique type of muscle innervation in this animal which bears some resemblance to that reported previously in *Ascaris* and *Amphioxus*: muscle fibers run right alongside the dorsal nerve cord in close apposition to it throughout the entire length of the tail and it is at this interface that the structural specializations characteristic of myoneural junctions occur.

Methods. Free-swimming larvae were collected from parent colonies of *Amaroucium constellatum*. Colonies which have been kept in running water in the dark for several hours will readily produce hundreds of larvae within 15 min after their exposure to light. The rapidly-swimming larvae were collected with a Pasteur pipette and immediately placed in a freshly-prepared solution of 5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.4). Specimens were fixed for approximately 3 h at room temperature, followed by a rinse in buffer, and were post-fixed with 1% OsO_4 in phosphate buffer. The tissue was then dehydrated in a graded series of methanol solutions followed by propylene oxide, and embedded in Araldite. Thick sections for light microscopy were stained with toluidine blue. Thin sections were stained with uranyl acetate und lead hydroxide, and then examined by electron microscopy. Numerous animals were surveyed and in two cases serial sections of the tail were made as follows: a survey series of 5 to 10 sections 1–2 μm thick was made, followed by a series of thin sections. This pattern was repeated for the length of the tail. In 4 other animals, random thick and thin sections at various levels representing the entire tail were studied. In one experiment, tails were amputated from free-swimming larvae at various distances from the trunk and at various time intervals after emergence from the parent organism until the beginning of tail resorption. The amputated tails were then observed for signs of motility.

Results. In transverse sections of the proximal part of the tail, 8 muscle cells can be seen surrounding the notochord. The dorsal nerve cord, or neural tube, is situated at the dorsal edge of the notochord, wedged be-

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tween the medial aspects of opposing muscle cells (Figure 1). Myofibrils are located peripherally in each muscle cell around a sarcoplasmic core containing pigment granules, glycogen, and mitochondria (Figure 2). In longitudinal sections, the myofibrils exhibit typical cross striations. The neural tube in cross sections has a central lumen lined by cells having large, granular nuclei surrounded by a thin rim of cytoplasm. Toward the periphery of the neural tube, processes ranging in diameter from 0.5–2.0 μm and containing vesicles and occasional mitochondria can be seen (Figures 2 and 3). Clusters of 25 or more vesicles may be found in these processes where they face the muscle cells and in some instances the vesicles form several closely packed rows just beneath the axolemma (Figure 4). The majority of vesicles are clear and have an average diameter of approximately 500 Å. The cleft between the axonal and muscle cell membranes varies in width from approximately 300–1000 Å (average width 500–600 Å) and contains a prominent basal lamina. Occasionally 1 axon faces 2 muscle cells. Slight infolding of the sarcolemma occurs occasionally (Figure 3) but the long, narrow junctional folds typically seen in vertebrate neuromuscular junctions[5] are absent. No other types of membrane specializations are found at the sarcolemmal surface; the prejunctional (axonal) membrane, however, sometimes has dense patches associated with it (Figure 3). This relationship between the neural tube and the muscle cells is repeated at all levels of the tadpole tail from trunk to distal end.

Tail amputation experiments were carried out either in very early rapidly-swimming larvae, or slightly older larvae just beginning to undergo tail resorption. In older larvae, excised tails failed to twitch, whether cut proximally or distally. In younger, rapidly-swimming animals, the excised tails twitched once or twice before becoming completely immobile and unresponsive when the amputation was done at the junction of trunk and tail or immediately distal to this junction. If the tail was amputated more caudally it did not twitch once severed.

Discussion. Several possible means of tail innervation may be hypothesized in *Amaroucium*. First, only the muscle cells in the most rostral part of the tail may be innervated by nerve processes whose cell bodies are located in the visceral ganglion of the trunk. Impulses may then travel from one muscle cell to the next along the length of the tail by means of specialized electrically conducting junctions between cells. The second possibility is that the dorsal neutral tube carries with it processes which innervate the tail muscle all along its length and that the parent nerve cell bodies are again located in the visceral ganglion. The third possibility is that motor nerve cell bodies are located in the neural tube itself all along its length and that they give rise to segmental axons.

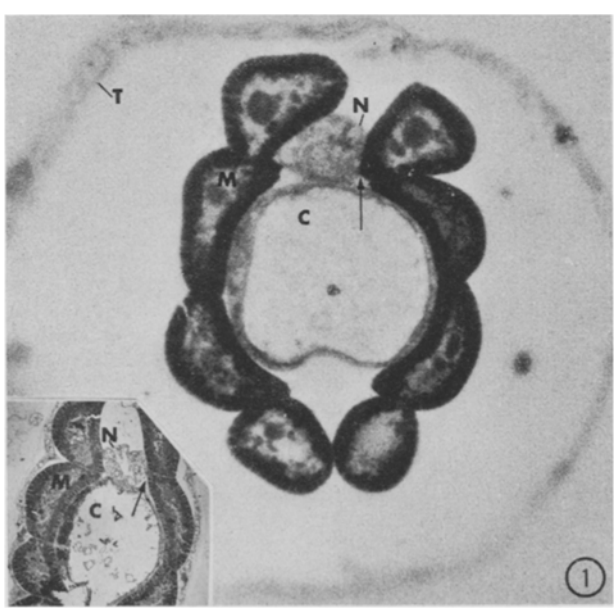

Fig. 1. Photomicrograph of tail (cross-section) showing surrounding tunic (T), notochord (C), neural tube (N)and 8 muscle cells, one of which is labeled (M). Arrow indicates junction between the neural tube and a muscle cell. Figures 2–4 were taken from this region (× 750). Inset: Low power electron micrograph showing equivalent features. The neural tube here appears hollow.

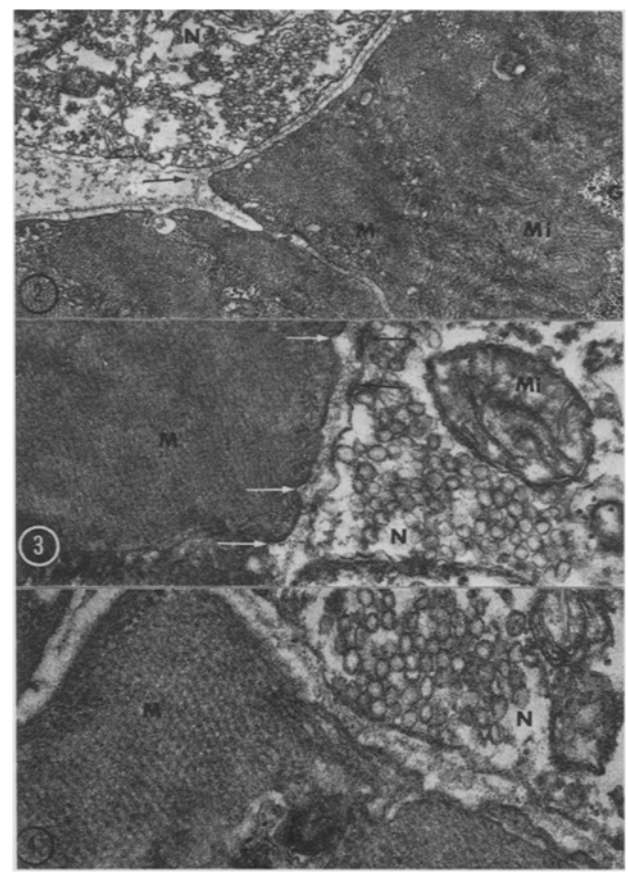

Fig. 2–4. Electron micrographs showing junction between neural tube (N) and muscle cells (M).

Fig. 2. The position of the arrow corresponds to that in Figure 1. Part of the nucleus of a neural tube cell is visible at the upper left(*). The adjacent process, which contains innumerable vesicles, is separated from a muscle cell by a narrow cleft containing a basal lamina. Mi, mitochondrion; G, glycogen. ×15,000.

Fig. 3. White arrows show shallow infoldings in post-junctional muscle cell membrane. Black arrows indicate dense patches associated with prejunctional axonal membrane. Mi, mitochondrion. × 43,000.

Fig. 4. Axonal vesicles are lined up in rows immediately adjacent to the pre-junctional plasma membrane. × 43,000.

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The evidence presented here indicates that structures resembling the neuromuscular junctions of vertebrate skeletal muscle occur all along the length of the tail at the surface of the neural tube. The origin of the axons that form these junctions is, however, uncertain. Cell bodies located in the neural tube itself are obvious candidates, but cytologically they resemble ependymal or glial elements as much as motor neurons. The cell bodies giving rise to the axons seen in the neural tube might be located in the visceral ganglion – an impression which is suggested although not proved by the tail amputation experiments. If nerve cells occurred segmentally all along the neural tube, the severed tail might be expected to continue twitching for many minutes after its separation from the trunk. Other factors, such as change in ionic concentrations in the region of the tail stump or a state comparable to spinal shock might also be responsible for the inability of the amputated tail muscle to contract, however. In *Boltemia* larvae, CLONEY[6] has shown that excised tails twitch for many minutes before finally degenerating, suggesting that in this animal the cell bodies of the motor neurons probably are located in the tail.

Although each segment of the tail muscle is innervated in *Amaroucium*, there is no obvious explanation as to how muscle cells which are not directly adjacent to the neural tube in cross-section are activated. Perhaps within each segment the 4 members of each row of cells are mechanically or electrically interconnected by specialized junctions, as suggested by BERRILL and SHELDON in *Styelopsis*[7].

The innervation of the tail muscle in larval *Amaroucium* thus resembles that reported previously in *Ascaris* and *Amphioxus* in both of which myoneural junctions are established at the surface of the nerve cord, rather than within the depths of the muscle. *Amaroucium* differs, however, in that the muscle cells do not give rise to elongated innervation processes.

Zusammenfassung. Elektronenmikroskopische Untersuchungen der Neuriten in der Peripherie des dorsalen Nervenstranges im Schwanz der Aszidien-Larve *Amaroucium constellatum* zeigen, dass die in der Nähe liegenden Schwanzmuskelzellen Verbindungen mit den Neuriten eingehen, die den neuromuskulären Kontaktstellen im Skelettmuskel der Wirbeltiere ähnlich sind. Diese neuromuskulären Verbindungen verteilen sich über die Gesamtlänge des Schwanzes und sind offenbar die Innervation der Schwanzmuskulatur.

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[7] N. J. BERRILL and H. SHELDON, J. Cell Biol. *23*, 664 (1964).
[8] Supported by grants No. NS-07495 and No. NS-07197 from the NIH, U.S. Public Health Service.

Pattern of Membrane Invaginations at the Surface of Smooth Muscle Cells of Rabbit Arteries

Conventionally fixed sections of smooth muscle of blood vessels show numerous membrane invaginations[1–6] which have been termed 'caveolae intracellulares' or 'pinocytotic vesicles'[7]. From transverse sections it is apparent that these vesicles are not distributed at random, but rather lie in groups of usually less than 10 vesicles separated by dense cytoplasmic regions termed dense areas (Figure 1a). Longitudinal sections of smooth muscle cells can be found where dozens of vesicles are arranged at regular intervals (Figure 1b) indicating a grouping of the vesicles predominantly in the direction of the longitudinal axis of the cell. The actual arrangement, however, on the cell surface is not evident from single sections. Such information can be obtained by a laborious three-dimensional analysis from serial sections or by the more convenient freeze-etching technique which is capable of exposing a good portion of the cell surface[8,9]. The latter technique was used to shed some light on the surface pattern of membrane invaginations.

Burgunder rabbits of either sex and of different commercial sources were anesthetized with Numal® supplemented with ether as necessary. Their abdominal aorta and arteries more distally were perfused via a Silastic® cannula with 25 ml Krebs-Ringer solution (pH 7.4) immediately followed by approximately 400 ml 2.5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.0) at 22 °C with a pressure of 100 cm water for 20 min[10]. The iliac arteries were then dissected, cut into segments of 3 to 6 mm and immersed in the same fixative for another hour at 4 °C. The specimens were stored in 0.1 *M* phosphate buffer (pH 7.0) containing 7% sucrose. A) Segments for ultramicrotomy were postfixed with 2% osmiumtetroxide in 0.1 *M* phosphate buffer (pH 7.0) for 1 h, dehydrated in graded alcohols and embedded in Epon 812. Ultrathin transverse and longitudinal sections of oriented specimens were contrasted with uranyl acetate and lead citrate and examined with a Philips EM 300. B) Segments to be freeze-etched were washed 3 times in water for 20 min, immersed in 30% glycerol for 3 h and freeze-etched in a Balzers BA 510 A/M apparatus as described by MOOR[8,9]. The replicas were cleaned with 40% chromic acid and washed 3 times with distilled water.

A view of the overall aspect of the cell surface in freeze-etched preparations showed a membrane without folds (Figure 2), quite in contrast to a preparation in a similar study by DEVINE et al.[5]. This demonstrates the importance of an adequate perfusion pressure during fixation for

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