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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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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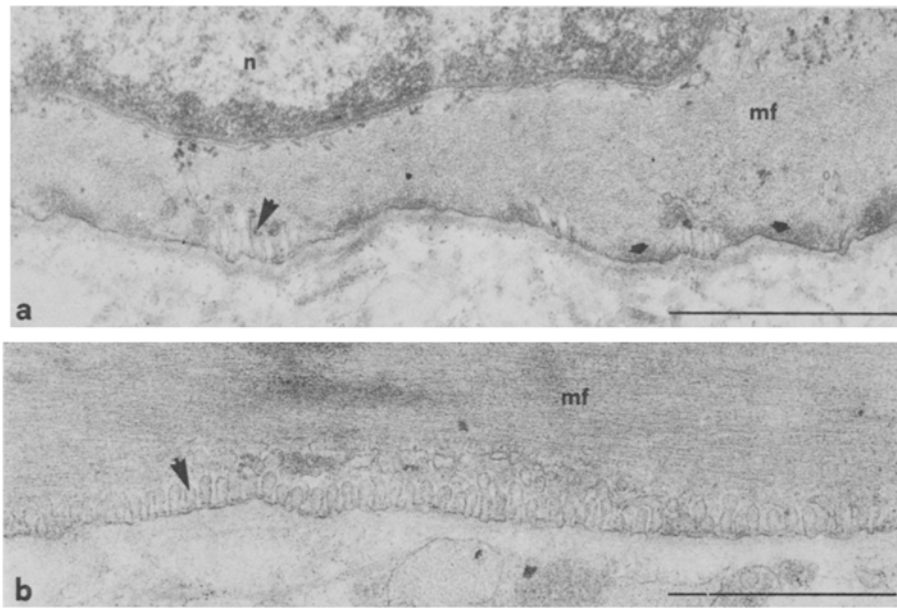

Fig. 1. Conventionally fixed and sectioned smooth muscle cell. a) Section perpendicular to the cell axis. Groups of membrane invaginations (↓) are seen with dense areas (▼) in between. The myofilaments (mf) in cross section are seen as dots. Nucleus (n). × 42,000. b) Longitudinal section. The membrane invaginations (↓) are regularly spaced along the entire length of the cell membrane. The myofilaments (mf) in longitudinal section are seen as fine threads. × 42,000.

preventing contraction of the vessels[11]. The cell surface, as viewed from the cytoplasmic side of the cell, displayed uniform crater-like structures (Figure 2) which are believed to be broken off necks of the vesicles attached to the membrane (Figure 1 a and b); the small orifice connecting the interstitial space with the lumen of the vesicles. The inner and outer diameter of these 'craters' were found to average 180 Å and 440 Å, respectively. The corresponding orifices in transverse and longitudinal sections were about 300 Å wide. Striking was, however, the aspect of bands composed of one to several rows of vesicles. These bands were of undetermined length and paralleled the longitudinal axis of the cell body. The membrane between bands of vesicles was of variable width and smooth, apart from small particles below the 50 Å level. Along well-developed rows, the inter-vesicle distance was fairly constant and was found to average 850 Å. The corresponding figure, as determined from longitudinal sections (Figure 1b), was somewhat smaller (700 Å), probably due to some shrinking during the dehydration and/or embedding process. This parallel arrangement of surface vesicles was conspicuous whenever large enough portions of the cell surface are exposed during cleaving. However, it should be pointed out that the vesicle bands are not always of such a uniform width as the one depicted on Figure 2. They were often less

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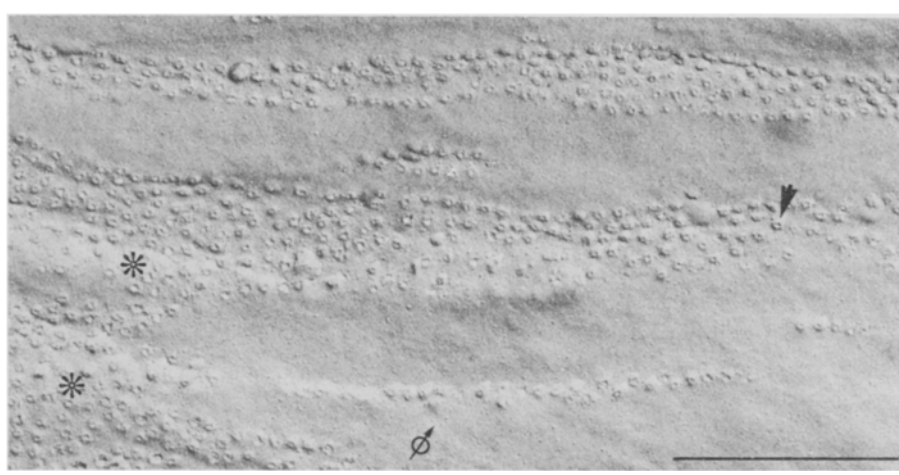

Fig. 2. Surface view of a freeze-etched cell membrane of a smooth muscle cell (as seen from the cytoplasmic side). Vesicles fractured at the necks, leaving 'craters' (↓), lie in longitudinal bands. Fusion of bands leads to accumulation of vesicle 'craters' (✻). The longitudinal cell axis is horizontal. Shadowing direction (↑). × 42,000.

regularly shaped and tended to fuse with adjacent bands of vesicles (Figure 2), forming patches with a density of about 170 vesicles per μm^2.

The vesicles have often been termed pinocytotic implying a physiologic function upon which there is no general agreement at the present time. As DEVINE et al.[5] pointed out, one very rarely observes vesicles away from the cell membrane, and the question whether they are involved in any transport process is still open, although the vesicles could disintegrate very rapidly after detachment from the plasma membrane. In recent studies, the possibility was discussed that the invaginations are in some way analogous to the T-system of striated muscle[5,6,12]. At present, the significance of the arrangement of membrane areas into bands with and without vesicles is even less understood. PROSSER et al.[2] made a first reference to a striate pattern of vesicles in smooth muscle of the intestinal and urogenital tract, and recently longitudinal rows of membrane invaginations have been reported on smooth muscle cells from mesenteric arteries and vas deferens of guinea-pigs[5] as well as from avian arteries[6]. It has been demonstrated that the dense areas which are likely to represent attachment sites for the myofilaments[1,6] are only found in the longitudinal surface regions in which no vesicles are present[5-7]. This finding has prompted DEVINE et al.[5] to regard the vesicle-free areas of the cell membrane as the attachment areas of the myofilaments. Whatever the interpretation, there is increasing evidence that the arrangement of membrane invaginations and areas of membrane devoid of invaginations into bands, is a common feature of smooth muscle cells.

Zusammenfassung. Mit Hilfe der Gefrierätzung wurde die Oberfläche glatter Muskelzellen von Kaninchenarterien (A. ilica) dargestellt. Die Aufsicht zeigt ein charakteristisches Muster von parallel zur Zellachse verlaufenden Streifen, welche aus einer bis mehreren Reihen einheitlich grosser Membraneinstülpungen (Caveolae intracellulares) bestehen.

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Zum Schlüpfprozess bei Fischen: II. Gewinnung und Charakterisierung des Schlüpfsekretes bei der Regenbogenforelle (*Salmo gairdneri* Rich.)

Das abgelegte Forellenei ist während seiner gesamten Embryonalentwicklung von einer derben Schale (Chorion) umgeben, die vordringlich einen schützenden Charakter hat und nach eigenen Beobachtungen aus drei Schichten aufgebaut ist[1]. Diese Schichten bilden zusammen eine Hülle, die das Forellenei befähigt, einen Druck von 2,5 bis 3,5 kg auszuhalten, womit es den natürlichen Bedingungen bestens angepasst ist[2]. Zum Zeitpunkt des Schlüpfens ist deshalb ein besonderer Prozess zu erwarten, der es dem herangewachsenen Embryo gestattet, diese Hülle zu sprengen, um in das freie Wasser zu gelangen. Im einzelnen setzt sich dieser Vorgang aus den folgenden Teilschritten zusammen: der Bildung eines Schlüpfsekretes, seiner Ausschüttung und seiner Wirkung auf die Eihülle.

Zur Gewinnung des Schlüpfsekretes öffneten wir Eier, die unmittelbar vor dem Schlüpfen standen, weil erst zu diesem Zeitpunkt die Gewähr besteht, dass die perivitelline Flüssigkeit auch aktives Schlüpfsekret enthält[1]. Während die perivitelline Flüssigkeit in den frühen und mittleren Entwicklungsstadien dünnflüssig ist, nimmt ihre Viskosität zum Schlüpfen hin immer mehr zu, so dass maximal nur 5 µl gewonnen werden können. Diese Menge ist für biochemische Untersuchungen aber zu gering. Wir entwickelten deshalb eine Methode, die von der Beobachtung ausging, dass Sauerstoffentzug bei kurz vor dem Schlüpfen stehenden Forellen zu einer Beschleunigung des Schlüpfvorganges führt. Die Eier, die am besten wenige Stunden vor dem natürlichen Schlüpfen stehen sollen, wurden zu 100 Stück in eine Petrischale gegeben, auf Eihöhe mit verschiedenen Puffern überschichtet und vorsichtig mit Stickstoff begast. Der günstigste Wirkungsgrad der Begasung liegt im richtigen Verhältnis des noch vorhandenen zum schon verdrängten Sauerstoff, erkennbar an den heftigen Bewegungen der Embryonen, die dabei sehr wahrscheinlich die das Schlüpfsekret enthaltenden Drüsen an der Eischale aufreiben und damit den Schlüpfvorgang einleiten. Das überschüssige Sekret gelangt nach dem Aufbrechen der Schalen in den Puffer und kann zusammen mit der perivitellinen Flüssigkeit abgetrennt werden (im folgenden als Rohextrakt bezeichnet). Ammoniumcarbonat-Puffer (0,1 *M*; pH 8,0) und Veronal/HCl-Puffer (0,05 *M*; pH 8,0) erwiesen sich am günstigsten und ergaben eine Schlüpfquote von wenigstens 95%, während Phosphat-Puffer (0,1 *M*; pH 8,0) den ungünstigsten Wirkungsgrad erzielte. Die Schlüpfquote betrug hier nur um 35%, weil die übrigen Embryonen ihre Bewegungen vorzeitig einstellten und noch im Ei abstarben. Der so gewonnene Extrakt wurde bei −20 °C eingefroren.

Die Aktivität des so gewonnenen Schlüpfsekretes konnte durch Verdauungsversuche mit Eierschalen gesichert werden, die im Augenpunktstadium präpariert waren und in dest. Wasser im Kühlschrank aufbewahrt wurden. Mit Hilfe solcher Eihüllen als natürlichem Substrat konnte für das bei −20 °C eingefrorene Sekret eine Mindesthaltbarkeit von einem Jahr festgestellt werden. Dass diesem Sekret ein enzymatischer Charakter zugebilligt werden kann, liess sich durch Erhitzung vor der Zugabe zum Substrat zeigen. Verschiedene Temperaturen zwischen 5 bis 60 °C ergaben eine völlige Inaktivierung ab 40 °C.

Für Untersuchungen zur Zusammensetzung des Schlüpfsekretes kamen elektrophoretische Methoden und enzymatische Tests zur Anwendung. Mit Hilfe von Cellulose-Acetat-Folien gelang es, den Rohextrakt in vier Banden aufzutrennen. Unterzieht man den Extrakt jedoch der besser auflösenden Acrylamid-Gel-Elektrophorese (7%iges Gel, *Tris*-Glycin-Puffer, pH 8,3), so ergeben sich bei progressiver Anfärbung mit Coomassie Brillant Blue R 250[3] sieben Banden, wovon sich in anodischer Laufrichtung betrachtet die erste Bande von den übrigen deutlich absetzt.

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