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IMPROVED METHOD OF CELL-ISOLATION AND CELL CULTURE FOR THE STUDY OF MECHANISM ON PHYSIOLOGICAL MODULATION IN THE MUSCLE. S.Nagaki and N.Ai. Dept.of Biol.,Tokyo Gakugei Univ.,Koganei,Tokyo.

Muscle contraction produced by intrinsic chemical modulator, as PGs, makes an intentional move as the typical and essential behavior for animal. But the action mechanism of modulator is not clear. PGs seem to have ability for changing excitability in the muscle. So, it is attacked to prepare excitable cell membrane for the study.

In this study, it is used the cardiac muscle with spontaneous and repetitive action from adult crab, *Sesarma haematochier*, and attempted to make cultured cardiac muscle by improved method.

At first, adult crab was carefully washed and disinfected by chlorination, and then cardiac muscle was taken out from the isolated heart with fine scissors. The muscle was rinsed with Ca-Mg free salts solution repeatedly. Then, the muscle was cut to fine pieces. In the next, they were centrifuged at 4°C with 3000 rpm for 5 min. Precipitate after these procedure was added to 0.005% trypsin and managed with fine pipette for cutting more pieces. In the last, materials were slowly filtrated. On determination of culture medium, cell-size and its frequency were calculated, and also osmotic pressure were adjusted. Cell-culture was performed under condition for 24hr at 15±1°C. Single cell or cluster were used for experiments of modulator and measurement of membrane potentials.

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FEEDING AND RESPIRATION IN ADULT LAMPREYS. R.Kawasaki¹ and C.Rovainen². ¹Col. Biomed. Technol. Niigata Univ., Niigata and ²Dept. Cell Biol. and Physiol., Sch. Med. Washington Univ. St. Louis, MO, USA.

In lampreys, intake of food and oxygen are performed (together in the larva, or separately in the adult) by rhythmic activities of "branchial (or oropharyngeal) muscles". American lampreys (*Ichthyomyzon unicuspis*, juveniles and adults), were tested in lake water of 10 C. After pithing behind the last gill pores, the head was held in an apparatus and the sucker vacuum was monitored with a pressure transducer. EMGs were recorded from the sucker, buccal region (muscles for sucking) and branchial muscles. Minced goldfish meat and skin were extracted. This extract plus food dye was used as test solution.

Feeding behavior was initiated by infusion of the test solution into the sucker cavity; sucker vacuum and EMG recordings showed characteristic profiles of this behavior. Feeding and swallowing was evidenced by the food dye observed in the gut in the dissected lamprey. Infusion of dyed lake-water failed to initiate this behavior, and no colour was observed in the gut.

From our results, "some criteria" for feeding behavior in the lamprey could be originally proposed. Feeding and branchial respiratory movement respectively lasted with their own rhythms, suggesting division of central pattern generator function of "branchial nervous system" for feeding and respiration in adult lampreys.

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COMPARATIVE STUDIES OF AIRBREATHING FUNCTIONS IN GOBID FISHES-II. CHARACTER IN EPIDERMAL STRUCTURE OF AIRBREATHERS K.IWATA¹ and I.KAKUTA². ¹Biol.Labo. Fac.of Edu. Wakayama Univ., Wakayama and ²Fac.Of Appl. Biol. Hiroshima Univ., Hiroshima

Amphibious fish such as *Periophthalmus cantonensis* (Tobihaze) is known to have a unique skin: blood capillaries penetrating into epidermis and very large cells locating in the middle layer of epidermis. In the present study, We found another three types of epidermal structures from the dorso-lateral part of skin of other gobid airbreathers as follows: (1) In *Tridentiger obscurus* (Chichibu) with developed scales, the epidermis covering the upperside of intruding scales composes of only 2-3 layers, and its lowest layer has large sized cells, while a very thin epidermal layer covers over the under-surface of intruding scales. (2) In *Chasmichthys doli-cognathus* (Agohaze) with small scales, the cavities of scale pockets lined with the vascularised dermal layer enlarge. (3) In *Luciogobius guttatus* (Mimizuhaze) without scales, the epidermis lined with vascularised dermis composes of the mixed cells: small cells and large cells with vacuoles.

The large cells in the epidermis and the large scale pockets seem to serve as a retention of water in the skin during out of water.

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VARIATION IN AND OXYGEN-BINDING PROPERTIES OF *DAPHNIA MAGNA* HEMOGLOBIN

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The oxygen-binding and molecular properties of the purified hemoglobin from *Daphnia magna* were investigated. There were inverse correlation between P 50 value (oxygen affinity) and hemoglobin concentration in hemolymph. The P 50 values ranged from 1.1 to 5.3 Torr and the n values (Hill's coefficient) from 1.6 to 1.2 in 0.1 M phosphate buffer of pH 7.2 at 20 C. The purified hemoglobin was separated into at least six components in isoelectric focusing. Although hemoglobin-rich and hemoglobin-poor animals possess the same hemoglobin components, the hemoglobin of the former with high pI values possesses these components in greater amounts. These results indicate that *Daphnia magna* may become acclimated to various oxygen environments through adjustment of the proportions of hemoglobin components differing in affinity and subunit cooperation.