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—Research Note—

金魚鰾的初級細胞培養

Monolayer Culture of Goldfish (*Carassius auratus*) Swimming Bladder

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In the present study, an attempt was made to culture the cells of swimming bladder of goldfish (*Carassius auratus*). Prior to the experiment, the fish was dipped in 500–600 ppm chlorine water and wipped with cotton saturated with 70% ethyl alcohol three times over a period of thirty minutes. The swimming bladder was then removed from the fish by using sterile forceps and scissors. The tissue was then placed immediately into sterile Hank's balanced salt solution (HBSS). In the preparation of primary culture, the tissue explant technique was used. The swimming bladder obtained as describe above was finely minced and washed three times in HBSS. The small tissue fragments were transferred into a flask containing growth medium L-15 (GM L-15) and incubated in 32±1°C. GM L-15 is Lebovitz's L-15 supplemented with 10% Foetal calf serum and 400 μg/ml penicillin, 400 μg/ml streptomycin and 10 μg/ml fungizone. All the serum, medium and antiseptic compounds were obtained from M. A., Bioproducts, Walkersville, Maryland, USA. When the confluent monolayer had formed, cells were disloged using 0.1% trypsin in EDTA/PBS solution as described previously (Chen *et al.*, 1981) and then transferred into two flasks with fresh GM L-15. The cultured media were rountinely changed at a interval of 3–4 days.

The results showed that the cells had been subcultured 6 times in GM L-15 at 32±1°C since their initiation within approximately three months. In these primary cultures of goldfish swimming bladder only epitheloid cells with polygonal morphology were observed (Fig. 1). Multinucleated cells (Fig. 1 see arrows) were found in the cultures. In comparison, the cells obtained in the present study were larger than those obtained from eel kidney and ovary (Chen and Kou, 1981; Chen *et al.*, 1982). Although the cells were subcultured at a split ratio of 1:2 in GM L-15, it took approximately 15–20 days to become a confluent cells sheet at 32±1°C. The results demonstrated that the multiplication *in vitro* of the cells from goldfish swimming bladder was slower than those from EK-1 (Chen *et al.*, 1982), EO-2 (Chen and Kou, 1981), RTG-2 (Wolf and Quimby, 1962) and FHM (Gravell and Malsberger, 1965) lines which were rountinely used in our laboratory for the study of viruses from warm water fishes. Since the slow multiplication of goldfish swimming bladder cells resulted from the present study, the valuation for these cells on the study of fish viruses is limited.

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Fig. 1. Primary cell culture of goldfish (*Carassius aurats*) swimming bladder, Arrows showed multinucleated cells. ×143

中 文 摘 要

本研究乃在嘗試培養金魚的鰾細胞。實驗結果顯示，將金魚鰾細胞培養於含 10% 牛犢血清的 Leibovitz's L-15 培養液及 32±1°C 的情況下，細胞可以生長分裂但速率緩慢，當一個培養瓶 (Flask) 中的細胞長滿時，把其中的細胞分至二個培養瓶中做繼代培養，約十五至二十天細胞才會再度長滿，因而三個月內僅得到六個繼代培養。培養的鰾細胞皆爲多角形的表皮樣細胞，並可觀察出多核細胞的存在。這種鰾細胞比本實驗室所發展出來的鰻魚腎臟及卵巢細胞爲大。由於生長速率緩慢，所以可供病毒研究的應用價值可能相當有限。

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