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实验研究

湖北钉螺肝脏细胞原代培养的初步研究

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摘要
目的 研究湖北钉螺肝脏细胞的原代培养方法，观察肝脏细胞琥珀酸脱氢酶（SDH）和乳酸脱氢酶（LDH） 的活性分布。 方法 解剖湖北钉螺取肝脏，用0.2%（体积比）苯扎溴铵（新洁尔灭）浸泡及含抗生素的生理盐水无菌洗涤、磨碎、过滤，收集肝脏细胞。采用联合法和静置悬浮法接种培养。细胞培养液配方: 50 ml M199溶液、3 mg/ml 水解乳蛋白溶液30 ml(平衡盐溶液配制)及胎牛血清20 ml, 混匀，附加常量抗生素（青霉素100 IU/ml、链霉素100 μg/ml、卡那霉素50 μg/ml），混匀，调节pH值为7.2～7.4。于26.5 ℃ 培养。联合法培养的肝脏细胞分别进行Giemsa、SDH和LDH染色，显微镜观察活细胞与染色细胞形态、SDH和LDH的分布，以及观察静置悬浮法培养的肝脏细胞形态。 结果 联合法培养的肝脏细胞贴壁后形态各异，以圆形、椭圆形为主, 也有三角形和不规则形等。细胞大小约（4～16） μm×（6～20） μm。较小细胞形成细胞簇，细胞核不明显，细胞质丰富、透亮。散在分布的单个细胞稍大，细胞核较大而明显，细胞质较少。培养5～7 d 的肝脏细胞逐渐退化。Giemsa染色将细胞分为两种，一种为细胞质呈蓝色，细胞核紫红色；另一种是细胞质呈紫红色，细胞核呈蓝色。SDH和LDH染色，细胞质出现大小不同、深浅不一的蓝色颗粒。静置悬浮法培养的肝脏细胞不易贴壁，多为圆形，细胞核不明显。细胞较小，约为（4～6） μm×（6～8） μm。培养3 d 均被细菌污染。 结论 联合法更适合湖北钉螺肝脏细胞原代培养，SDH和LDH活性位于肝脏细胞质中。

关键词 湖北钉螺 肝脏细胞 细胞培养 细胞大小 琥珀酸脱氢酶 乳酸脱氢酶
分类号

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本刊中 包含“湖北钉螺”的 相关文章
本文作者相关文章
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Primary Culture of the Cells from *Oncomelania hupensis* Liver

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Abstract
Objective To develop a method for primary culture of cells from *Oncomelania hupensis* liver， and to observe the distribution of succinate dehydrogenase （SDH） and lactate dehydrogenase （LDH） in the cultured cells. Methods *O.hupensis* was anatomized to separate the liver. Livers were soaked in 0.2% benzalkonium bromide and washed by physiological saline containing antibiotics in turns. Cells from the liver were harvested by mechanical mulling and filtering. The isolated cells were then incubated with methods of the combination culture and standing suspension culture， respectively. The culture medium for the cells was a mixture of Medium 199 （50 ml）， 0.3% lactoalbumin hydrolysate dissolved in a balanced salt solution （BBS，30 ml）， and fetal calf serum （FCS，20 ml） containing a moderate amount of antibiotics （100 IU/ml penicillin， 100 μg/ml streptomycin and 50 μg/ml kanamycin） at pH 7.2-7.4 under the temperature of 26.5 ℃. The cells were stained by using Giemsa and Pearson methods （for SDH and LDH respectively） to observe the shape of cultured cells and enzyme distribution in cells. The living and stained cells were microscopically observed. Results Under microscope， the attached cells incubated with method of the combination culture showed round， elliptic， triangular and irregular shapes, with more round and elliptic cells. The size was approximately （4-16）μm×（6-20）μm in average. The clustered cells with an unclear nucleus and abundant and lucid cytoplasm were smaller than diffused cells with a large， obvious nuclei and less cytoplasm. Degeneration was observed after culturing for 5-7 days. The cultured cells could be divided into two types based on the color shown after Giemsa staining. The first type cells showed blue cytoplasm and mauve nuclei while the second type cells were opposite. There were blue granules in different sizes and shade in the cytoplasm after SDH and LDH staining. It was difficult for the cells to attach the wall of the culture flask using method of the standing suspension culture. The shape of the cultured cells were almost round with unclear nuclei， and the size was about （4-6）μm×（6-8）μm in average. The cells incubated with the standing suspension method were found to be contaminated after culturing for 3 days. Conclusion The combination culture method is suitable for primary

culture of the cells from *O.hupensis* liver and the cells show activities of both SDH and LDH in cytoplasm.
Key words *Oncomelania hupensis* Liver Cell culture Cell size Succinate dehydrogenase （SDH） Lactate dehydrogenase （LDH）

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