In vitro Cultivation of Cells from Ovotestis Tissue of Pigmented Biomphalaria glabrata

Author: Iwanaga, Yuzuru

Source: Zoological Science, 19(3) : 271-274

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.19.271

ZOOLOGICAL SCIENCE **19**: 271–274 (2002)

[SHORT COMMUNICATION]

In vitro Cultivation of Cells from Ovotestis Tissue of Pigmented *Biomphalaria glabrata*

Yuzuru Iwanaga*

Department of Immunology and Parasitology, Hiroshima University School of Medicine, Kasumi 1-2-3, Minami-ku, Hiroshima 734-8551, Japan

ABSTRACT—Cells derived from ovotestis tissue of pigmented *Biomphalaria glabrata*, Puerto Rican strain were cultured in double diluted GIT medium supplemented with modification of amino acids components of pigmented *B. glabrata*, ovotestis and mid-gut region and 3% inactivated fetal calf serum. As a result, two types of cells, epithelial and fibroblastic like cells increased in number during the cultivation. It seem that the medium used in this study is a suitable medium for cultivation of cells from ovotestis of pigemeted *B. glabrata* . These two types of cells have been maintained by successive transplantation for over 3 passages.

Key words: *Schistosoma mansoni, Biomphalaria glabrata,* ovotestis, cell culture, GIT medium

INTRODUCTION

Cell and organ culture of invertebrates have been used to study the physiology proper of those species. Although primary tissue and cell cultures of mollusks have been frequently reported (Perkings and Manzel, 1963; Burch and Cuadros, 1965; Manaka, *et al*., 1980; Iwanaga *et al*., 1985), these reports have not maintained cells and tissues over 2 months, except reports for cell lines designated HA (Vago and Chastang, 1958) and Bge (Hansen, 1974).

It is well known that different geographic strains of *Schistosoma mansoni* have different rates of infection within different subspecies of *Biomphalaria* snails (Files and Cram, 1949; Paraense and Correa, 1963; Kagan and Geiger,1965; Iwanaga *et al*., 1992). This suggests that physiological and biochemical differences exist between *Biomphalaria* snails and that these differences affect the growth of *Schistosoma mansoni* larvae. In the course of development of *S. mansoni* larvae in the snail, sporocysts almost grow in near the reproductive organs especially, digestive gland and ovotestis region of the snail, and there start mature cercarial production (Iwanaga *et al* ., 2000). Establishment of cell cultures from *Biomphalaria* snails will facilitate study of the interaction between the parasite and the intermediate hosts and permit characterization of the metabolic pathways of these cells. Cell cultures from embryo of *B. glabrata* have been reported to had been established successfully (Hansen, 1974; Hansen, 1976 a,b; Yoshino *et al*., 1999). However, that from the ovotestis have not yet to be grown in vitro.

In the present study, we have established long-term cell cultures from the ovotestis of pigmented *B. glabrata*, Puerto Rican strain.

* Corresponding author: Tel. +81-82-257-5176;
FAX. +81-82-257-5179.
E-mail: yiwanaga@hiroshima-u.ac.jp

MATERIALS AND METHODS

Juvenile snails (5–7mm in shell diameter) of pigmented *Biomphalaria glabrata* used in the present study originated from the Puerto Rican strain. The snail has been maintained in our laboratory for many generations.

A basal culture medium was based on GIT medium (Nihon Parmaceutical Co., Ltd.,). GIT is a cell culture medium the development of which was enabled by the successful separation of the cell growth factor GFS from animal sera, and can be used in any mode of adherent or non-adherent cell cultivation. And so, it has been demonstrated that GIT is useful for culturing a wide variety of cells (Murakami *et al*., 1982; Sasai *et al*., 1985). In this study, 100ml of GIT was diluted 1 : 1 with double distilled water and supplemented with 10ml of amino acids components on empirical modification, as shown in Table 1, to 200ml of diluted GIT medium (medium A), and final concentration of amino acids in medium A was adjusted to the concentration of four times amount (400mg) of amino acids components (percentage by weight) of pigmented *B. glabrata*, ovotestis and mid-gut region as shown in Table 2. Two different media were prepared for this study; 1. Basal culture medium (medium A). 2. Basal culture medium supplemented with 3% fetal calf serum inactivated at 56°C for 30 minutes (medium B). These two media were prepared with penicillin G, 100 IU and streptomycine sulfate, 100 μg

Table1. Basal culture medium for cultivation of tissues and cells from pigmented *Biomphalaria glabrata*

Components of basal culture medium (Medium A)

1. Diluted (x2) GIT medium----------200ml
2. Amino acids solution*----------10ml

*Amino acids solution (mg/10ml)

Lysine-HCl	4.1	Histidine	4.1
Threonine	20.3	Aspartic acid	59.0
Gultamic acid	53.1	Serine	27.4
Glycine	32.5	Proline	20.7
Cystine-HCl	2.2	Alanine	29.5
Methionine	4.7	Valine	14.7
Leucine	25.7	Isoleucine	8.2
Phenylalanine	13.3	Tyrosine	4.5

Table 2. Contents of amino acids of pigmented *Biomphalaria glabrata,* ovotestis and mid-gut region

Lysine	123.0+	(3.3)≠	Cystine	31.2	(1.0)
Aspartic acid	567.0	(15.3)	Isoleucine	127.0	(3.4)
Glutamic acid	533.0	(14.4)	Phenylalanine	156.0	(4.2)
Alanine	287.0	(7.8)	Arginine	117.0	(3.2)
Methionine	57.6	(1.6)	Serine	279.0	(7.5)
Tyrosine	91.2	(2.5)	Glycine	320.0	(8.6)
Histidine	67.5	(1.8)	Valine	186.0	(5.0)
Threonine	238.0	(6.4)	Leucine	293.0	(7.9)
Proline	227.0	(6.1)			

+ nmo/mg ≠ Percentage by weight

per ml. The pH of the media was adjusted to 7.2 to 7.4 with 1N-NaOH prior to use. For amino acids analysis of pigmented *B. glabrata*, ovotestis and mid-gut region, the sample that extracted according to the technique of Iwanaga and Tsuji (1985) were hydrolyzed in 6N-HCl at 110°C for 24 hr in evacuated tubes, and analyzed with a amino acid analyzer (Hitachi KLA-5). Contents of amino acids of the snail showed in Table 2.

For preparation and maintenance of culture of ovotestis tissues, prior to removing the tissues, snail's shell was wiped with 70% ethanol and air-dried. The shell was then cracked by gently applying pressure with tweezers. A cluster of tissues were removed with sterile micro-knives so as to form a piece, 2–4 mm wide. This tissue was then washed twice in balanced salt solution (BSS) (Chernin, 1963). The pieces were placed onto petridishes containing 0.25% trypsin solution in BSS prepared without calcium and magnesium (CMF) for 10 min. Petridishes were gently agitated every 2 to 3 minutes. After trypsin treatment for 10 minutes, the pieces were rinsed with BSS and placed in T-flask (25p, Slim Type, Iwaki Glass Co., Ltd) containing CMF to attach the pieces to the bottom. The CMF was then removed and replaced with 5 ml of medium A. T-flask were maintained at 24 to 26°C. The tissues were cultivated for approximately 60 days. About half of the medium was withdrawn and renewed at 2 times a week during cultivation. BSS and CMF used in this study contained 500IU/ and 300 μg/ml penicilline and streptomycine, respectively.

For maintenance of cells derived from tissues, three types of cells from the tissue culture, namely, amebocytic, epithelial like and fibroblastic like cells appeared on the surface of T- flask after 1 to 10 days of culture. After 2 to 3 weeks initiation, the epithelial and fibroblastic like cells were mechanically dislodged from the surface with Pasteur pipet. The suspension of cells was centrifuged at 1,000 rpm for 10 minutes, and the cells suspension was transferred to T-flask containing 5 ml of fresh medium B. Primary culture was started with approximatelly 2×10^2 cells per ml. Cell counts for growth rate were determined by hemocyto-meter after10, 20 and 30 days. T-flask were maintained at 24 to 26°C, and about half of the medium renewed every 3 to 4 days. Cells grown in the primary culture were trypsinized according to the technique of Hansen (1974) 35 days after the start of a cell culture, and the suspension of cells, about 2×10^2 cells per ml, was resuspended in T-flask containing 5 ml of medium B. When cells populations reached about 3×10^2 cells per T- flask, part of the cells were transferred to a new T- flask.

RESULTS AND DISCUSSION

Amebocytic cells migrated out from explanted tissue 20 to 24 hr after the culture was started, and they formed a colony of cells near the tissue after 5 to 7 days (Fig. 1). The cells were variable in shape and size. Peak cell movement from the explants was observed at the end of the first two weeks, but the cells continued to migrate from the explants moved slowly during the end of culture period. The stage of proliferation of cells could not observed. At 10 to14 days after initiation, the tissue culture yielded outgrowths of epithelial and/or fibroblastic like cells reported by Kitamura (1970) and Hansen(1976a). The growth rates of epithelial and fibroblastic like cells were faster in number than that of amebocytic cells until 7 weeks after culture (Fig. 2), but the number of epithelial like, fibroblastic like and amebocytic cells declined slowly and some cells failed to continue to develop and died and disappeared during the succeeding week, and so, it was exactly difficult to count the lived cells during cultivation.

The suspension of two types of cells, migrated out from explanted epithelial and fibroblastic like cells was placed in a T-flask containing 5 ml of medium B. In preparations of cells stained with Giemsa, one type, i.e., epithelial like cells were characterized by polygonal cell with a central, granular nucleus and appeared patch-like in the monolayer. The fibroblastic like cells exhibited different sizes, and contained large nucleus (Fig. 3). The growth rate of cells showed in Fig. 4. The cells increased in number to 3 to 4 fold at 30 days after initiation. It seemed that fibroblastic like cells relatively increased in numbers than that of the epithelial like

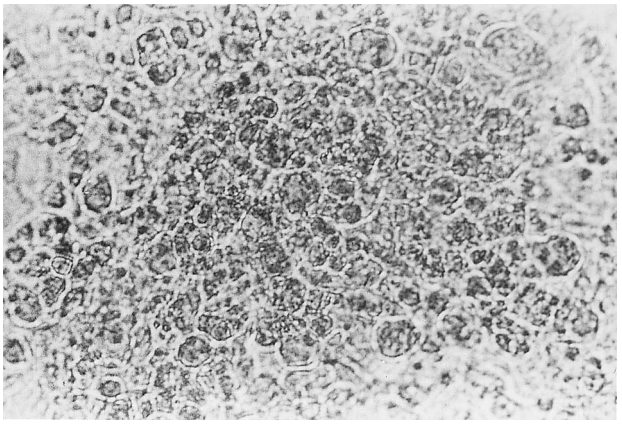

Fig. 1. A colony consisting of amebocytic cells, 7 days after cultivation, x200

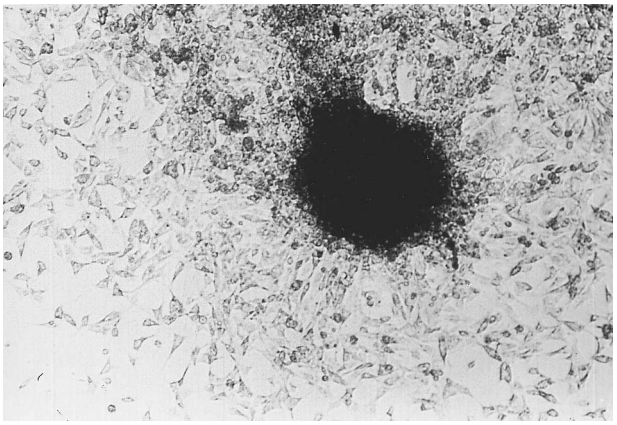

Fig. 2. Epithelial and fibroblastic like cells migrated from the ovotestis gland tissue, 7 weeks after cultivation, x100

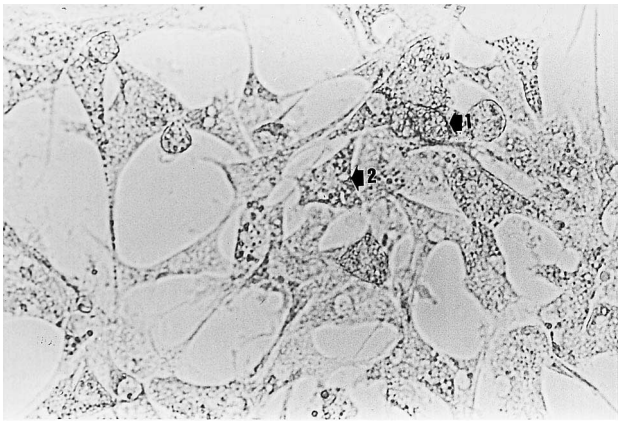

Fig. 3. Epithelial (arrow 1) and fibroblastic like (arrow 2) cells at 2 nd subculture, 10 days after seeding, x400

cells, but they then slowly decreased in number and became granular at 50 days after inoculation(data not shown). Hansen(1976a) reported that primary cultures of cells from embryos of *B. glabrata* started with about 1×10^4 cells, and cells usually were well grown by 10 days, but they then slowly decreased in numbers and died, so the cells rapidly needed to transfer to new flak. In present study, inoculation started with 2×10^2 cells, and they activity lived by next inoculation. It seem that inoculation of cells number in primary culture is a debatable point.

In cultivation of embryonic cells from *B. glabrata* reported by Basch and Diconza (1973) and Hansen (1976 a), they observed at least three types cells, that is, amebocytic, epithelial like and fibroblastic like cells, and a cell line designated Bge (*Biomphalaria* embryo) that has been maintained through many subcultures of colonies of epithelial and/or fibroblastic like cells. In our observations of the tissues culture in the present study, three types of cells, i.e., amebocytic, epithelial like and fibroblastic like cells were observed near margins of explanted tissue. In previous experiments, subculture of these three types of cells was made repeatedly at about 2 weeks intervals with mediun A

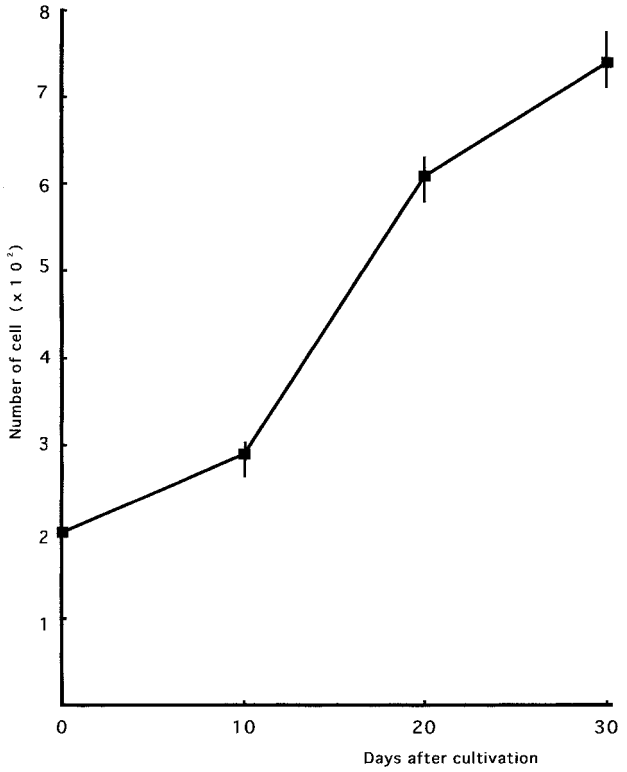

Fig. 4. The growth curve of cells

for 2 months, but the cells were not increased noticeably, especially amebocytic cells were not growth (not published).

Serial cultures were made using the same medium that was used initially for cell culture. There was at least 3-fold increase in number over the primary culture in the third passages of culture. Cells suspension obtained from every serial culture always contained two types of cells, and no morphological change were observed. Mitotic figures were usually seen, but chromosome number and karyotype of cells could not count. For long-lived culture of cell and/or tissues of invertebrate, Grace(1966) established cell line from the *Aedes* mosquitos using 900mg amino acid components. and also Hansen(1976a) maintained the cells form embryos of *B. glabrata* by Schneiders Drosophila medium diluted to 22%(included about 240mg amino acids). It seem that amount of amino acids components in medium is the important factor for long-term culture. Basch and Diconza (1973) maintained embryonic cells from *B. glabrata* with medium 199 and components from analyses of hemolymph from adult *B. glabrata*, but the cell could not maintained long-term. It was considerable that amount of total amino acid components of the hemolymph in medium was not enough during the culture period. This problem is need to reexamine. In the present study, we have been able to culture cells derived from ovotestis of *B. glabrata* by using GIT medium supplemented with 400mg of amino acids components of the snail and 3% inactivated fetal calf serum. These cells showed active growth, and the cells have been subculturing over 3

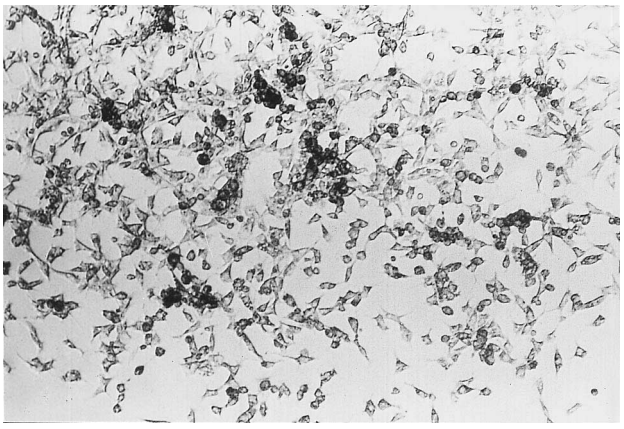

Fig. 5. Living cells at 3 rd subculture, 7 days after seeding, x100

passages at the present culture, it seem to be possible to establish the cell line from ovotestis of pigmented *B. glabrata*. Although the medium used in the present paper was a suitable medium for cultivation of ovotestis, the significance nutrients of the medium for culture of ovotestis were not evaluated. Two types of cells have been subculturing over 3 passages at the present study using medium B (Fig. 5).

ACKNOWLEGMENT

The author is grateful to Dr. Satoru Oka, Professor emeritus of Hiroshima University for amino acids analysis of *B. glabrata* and to Mr. Yosei Kajiwara, Miss Junko Arai and their staffs, Nihon Parmaceutical Co., LTD for partly supplying medium and to Prof. Masamoto Kanno, Hiroshima University for his cooperation throughout this research.

REFERENCES

Basch PF, Diconza JJ (1973) Primary cultures of embryonic cells from the snail *Biomphalaria glabrata*. Am J Trop Med Hyg 22: 805–815

Burch JB, Cuadros C (1965) A culture medium for snail cells and tissues. Nature 206: 637–638

Chernin E (1963) Observations on hearts explanted in vitro from the snail, *Australorbis glabratus*. J Parasitol 49: 353–364

Files VS, Cram EB (1949) A study of the comparative susceptibility of snail vectors to strain of *Schistosoma mansoni*. J Parasitol 35: 555–560

Grace TDC(1966) Establishment of a line of mosquito (Aedes aegypti L.) cells grown in vitro. Nature 21: 366–367

Hansen EL (1974) A cell line from the fresh water snail *Biomphalaria glabrata*. Int Res Commum Systems 2**:** 1703

Hansen EL (1976 a) A cell line from embryos of *Biomphalaria glabrata* (Pulmonata): Establishment and characteristics. Invertebrate Tissue Culture, Research Appricatıons, Acadmic Press, London and New York, pp75–99

Hansen EL (1976 b) Application of tissue culture of a pulmonate snail to culture of larval *Schistosoma mansoni*. Invertebrate Tissue Culture, Applications in Medicine, Biology and Agriculture, Academic Press, London and New York, pp 87–97

Iwanaga Y, Tsuji M (1985) Studies on host-parasite relationship between *Schistosoma japonicum* and *Oncomelania* snails (1) Antigenic communities between the Chinese srain of *Schistosoma japonicum* adult worm and *Oncomelania* snails. Jpn J Parasitol 34(1): 1–6

Iwanaga Y, Tsuji M, Oka S (1985) Primary cultivation of tissues from *Oncomelania hupensis hupensis*. Jpn J Malacol 44: 103–109

Iwanaga Y, Santana J V, Goncalves F (1992) Studies on common antigenicities between the Belo Horizonte strain, Brazil of *Schistosoma mansoni* eggs and *Biomphalaria* snails by immunoelectrophoresis. Southeast Asian J Trop Med Pub Hlth 23: 98–102

Iwanaga Y, Tanabe M, Takeuchi T (2000) Comparative studies for development of *Schistosoma mansoni* sporocysts in Puerto Rican and Brazilian *Biomphalaria glabrata*. Hiroshima J Med Sci 49: 125–128

Kagan IG, Geiger S (1965) The susceptibility of three strains of *Australorbis glabtatus* to *Schistosoma mansoni* from Brazil and Puerto Rico. J Parasitol 51: 622–627

Kitamura S (1970)Establishment of cell line from *Culex* mosquito. Kobe J Med Sci 16: 41–50

Manaka K, Furuta E, Shimozaki A (1980) Primary tissue culture of cells in the slug, *Incilaria bilineata*. Zool Magazine 89: 252–263

Murakami H, Masui H, Sato GH, Sueoka N, Chow TP, Kano-Sueoka T(1982) Growth of hybridoma cells in serum-free medium: Ethanolamine is an essential component. Proc Natl Acad Sci USA 79: 1158–1162

Paraense WL, Correa LR (1963) Susceptibility of *Australorbis tenagophilus* to infection with *Schistosoma mansoni*. Rev Inst Med Trop Sao Paulo 5: 23–29

Perkings FO, Manzel RW (1963) Maintenance of oyster cells in vitro. Nature 204: 1106–1107

Sasai S, Umemoto S, Fujimoto T (1985) Establishment of a medium for mass culture of mammalian cells. Abst. 3rd International cell culture congress (Sendai, Japan): 46

Vago C, Chastang S (1958) Obtention de lignees cellulaires en culture de tissus d invertebres. Experientia 14: 110–111

Yoshino TP, Coustan C, Modat S, Castillo MG (1999) The *Biomphalaria glabrata* embryonic (BGE) molluscan cellline: Establishment of an in vitro cellular model for the study of snail host-parasite interactions. Malacologia 41: 331–343

(Received August 15, 2001 / Accepted December 3, 2001)