ATTEMPT TO CULTURE *WUCHERERIA BANCROFTI IN VITRO*

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INTRODUCTION

The *in vitro* cultivation of filarial parasites has been a goal of parasitologists for many years (Weinstein 1986 ; Mak *et al.*, 1983), but it has only been recently that some success has been achieved. Early attempts were made on the *in vitro* culture of microfilariae and third-stage larvae of animal filarids and although growth of the parasites were reported, molting to the next stage was unusual. In later studies, Chen and Howells (1979) and Tanner (1981) reported development of *Brugia pahangi* and *Dipetalonema viteae* and more recently Franke and Weinstein (1984) cultured third-stage larvae of *D. viteae* to young adults and demonstrated the importance of a low oxygen tension for molting and differentiation to occur.

Efforts were also made to experimentally culture the human filarids, but success has only lately been reported by Mak and his coworkers (1983) who successfully cultivated third-stage larvae to fourth and fifth stages of *B. malayi*. Since little has been reported on the *in vitro* culture of *Wuchereria bancrofti*, we attempted the cultivation of third-stage larvae of this human filarid *in vitro* and this report presents our preliminary findings.

MATERIALS AND METHODS

The methods used were essentially those of Mak *et al.*, (1983). *Wuchereria bancrofti* third-stage larvae were from laboratory raised *Aedes togoi* or *Anopheles maculatus* that had been permitted to feed two weeks earlier on a human volunteer from Sorsogon Province in Southern Luzon, Philippine Islands.

The infected mosquitoes contained in paper cups were subjected to cold, placed onto a glass plate, and several drops of RPMI-1640, buffered with sodium bicarbonate and Hepes, were added to the plate. The mosquitoes were crushed by rolling a glass tube over them (Ash and Riley, 1970). The debris was then washed onto a fine mesh stainless steel screen fitted into a funnel (Baerman apparatus), RPMI containing penicillin (100 µg ml) and streptomycin (100 µg/ml) was added to the funnel, and following baermanization, the larvae were recovered, washed three times by individually placing them into sterile Petri dishes containing medium with a fine-tipped microdissection needle. Twenty-five to 45 larvae were then placed into 30 ml screw-capped tissue culture flasks containing RPMI-1640, antibiotics, 10% inactivated human AB serum and LLC-MK_2 rhesus monkey kidney cell-line as a feeder layer. Sterile techniques were used throughout and all manipulation carried out under a lamina flow hood. The cultures were incubated at 37°C and the medium changed daily. The larvae were examined under a phase

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contrast microscope for motility, and dead larvae removed. Some larvae were mounted onto a microscope slide, measured and examined.

RESULTS

In the first experiment, 45 third-stage larvae were introduced into the culture system and by 10 days, molting fluid could be observed in some of the larvae. By 12 days many of the larvae had molted to the fourth larval stage and shed cuticles could be seen in the medium. At this time 10 of the remaining 20 living larvae were placed into medium containing SP_2 mouse hybridoma cells and fetal calf serum and 10 retained in the original culture system. At 24 days, 6 of the 10 larvae in the former culture had died and the 4 remaining living larvae were removed from the medium and measured. The third-stage larvae were an average length of 1444 μm and 19 μm in width and the fourth-stage larvae at 24 days measured an average of 1656 μm by 37 μm. Only 5 fourth-stage larvae survived in the original cultures for 33 days and measured an average of 1848 μm by 36 μm.

In the second experiment, 25 third-stage larvae were introduced into cultures containing LLC-MK_2 feeder cells, human serum and medium. By 10 days, molting fluid was observed in 10 of 14 motile larvae, but by 16 days, all larvae had molted or were in the molting process. On day 19, only 10 larvae were alive and these were transferred to new cultures. By day 31, only 7 worms were still present and by 40 days, only 2 were alive. Several of the worms were lost during washing and the changing of medium. Two fourth-stage larvae recovered at day 40 were sluggish and when fixed and mounted measured an average of 1640 × 52 μm. The worms suffered some collapse when mounted, and when examined, very little structure could be observed. One worm was probably a male. There were no indications of female structures developing in the anterior end and premodial male reproductive cells were suggestive in the rectal region. The cuticle at the posterior end appeared to be separating. The larva was fourth-stage, but could have been on its way to the fifth-stage.

With each experiment, third-stage larvae from mosquitoes were also inoculated intraperitoneally into Mongolian gerbils. The animals were killed and examined periodically, but developing worms for comparison with cultured worms could not be found.

DISCUSSION

The *in vitro* cultivation of filarial parasites from third-stage larvae to young adults has been accomplished in recent years by Mak *et al.*, (1983) with *B. malayi* and *B. pahangi* using a feeder cell-line in the culture system. More recently, Franke and Weinstein (1984) reported success in the cultivation of *D. viteae* without feeder cell-lines but in an atmosphere of low oxygen tension. Both groups showed that the respective third-stage parasites could molt twice in the culture system and reach the adult stages. The development of *W. bancrofti* third-stage larvae to the adult stage has yet to be reported, but in the present study the parasite was able to molt once and reach a late fourth-stage or possibly an early young adult stage. Because of laboratory constraints, these studies were discontinued; however we feel that by continued use of the methods outlined, successful development of the parasite eventually could be achieved.

The development of *W. bancrofti* in culture was similar to that for *B. malayi* and *B. pahangi*. Molting of third-stage larvae of *W. bancrofti* occurred as early as 12 days and

all surviving larvae had molted to the fourth-stage by the 16th day in culture. In previous laboratory animal studies, Ash and Schacher (1971) reported the third molt of *W. bancrofti* larvae in 8 to 11 days in gerbils, while in monkeys, Cross *et al.*, (1979) reported fourth-stage larvae as early as 12 days. Young adult worms in monkeys were first found at 42 days. Worms from culture were smaller than those reported from animal experiments for comparable time periods. The largest fourth-stage larvae in cultures ranged in length from 1.6 to 1.8 mm in approximately 5 weeks while those in gerbils were over 3 mm (Ash and Schacher, 1976) and in monkeys (Cross *et al.*, 1979), at the same time the worms range in size from 4 to 5 mm.

We also attempted the cultivation of *B. malayi* and *B. pahangi* according to the methods of Mak *et al.*, (1983). Molting from the third to fourth-stage was found to occur as early as 11 days, and we were able to recover fourth-stage larvae after 4 to 5 weeks.

If efforts along these lines are continued, the *in vitro* cultivation of *W. bancrofti* should become a reality. In future studies it would be interesting to attempt to culture the parasite in the system reported by Franke and Weinstein (1984).

SUMMARY

Third-stage larvae of *Wuchereria bancrofti* recovered from laboratory raised *Aedes togoi* and *Anopheles maculatus* fed on a human volunteer were recovered by mass dissection methods and introduced into *in vitro* culture. LLC-MK_2 cells were used as feeder cells, and the culture medium consisted of RPMI-1640 buffered with Hepes and sodium bicarbonate and supplemented with human AB serum. The third-stage larvae molted as early as 12 days and those surviving had all molted by 16 days. The fourth-stage parasites averaged in length from 1.4 mm to a maximum of 1.8 mm. Some larvae remained alive in culture as long as 40 days and while the worms were distorted in fixation, possible primodial cells of a spicule could be visualized in the rectal region. The cuticle also appeared to be separating in the posterior end. Although complete development was not achieved, it seems that with a continuing effort, success could be obtained using this culture system with feeder cells.

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