Bull. Inst. Zool., Academia Sinica **11**(1): 33-36 (1972)

AN IMPROVED TECHNIQUE FOR THE CULTIVATION OF HOUSE FLY EMBRYONIC CELLS

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Received for publication, June 28, 1972

ABSTRACT

K. Y. Jan, C. C. Hu and C. M. Hou (1972) *An Improved Technique for the Cultivation of House Fly Embryonic Cells.* Bull. Inst. Zool., Academia Sinica **11**(1): 33-36. The eggs of house fly from 4 to 11 hours old in 25±1 C may be used for cell culture, but 7-hour-old eggs give best cell growth. A continuous cell line was obtained when embryonic cells were cultivated in D20 medium plus 10% fetal calf serum and some antibiotics. This line has been maintained for 10 months and a dozen of metaphase cells obtained were all with normal diploid chromosomes.

Cell lines of 12 species of insects[1] have been established. With some improvements these cell lines will undoubtedly provide a significant experimental device for many fields of reserch. The house fly is one of the most popular laboratory animals and also of medical importance. However, the progress of house fly cell culture is relatively sluggish. Primary cultures have been obtained from eggs, larvae and pupae of house fly and various growth patterns have also been noted[8,10,23]. But on the whole, the results have not been particularly good. These cultures were only maintained for brief periods and from which no metaphase chromosomes have been obtained. In this commnuication we report some improvements on the culture condition, in particular, the stage of eggs and the medium used.

MATERIALS AND METHODS

The culture of house fly (*Musca domestica* L.) was maintained as previously described[15]. Eggs were collected at every 30 minutes and incubated at 25±1 C. Eggs taken out at 1, 2, 3,··· up to 12 hours after incubation were used for cell culture to determine which give better growth.

The eggs were shaken with detergent and washed in tap water. They were then put in a petri dish with some distilled water. Clean eggs were picked up with a pasteur pipette. Three hundred eggs were put into a finger shape nylon cloth bag and the opening of the bag was tightened by a paper clip. Hereafter, the preparation was carried out in a germ free chamber and the tools and solutions used were aseptic. The bag was dipped in 0.05% mercuric chloride in 70% ethyl alcohol for 15 minutes, 3% sodium hypochlorite for 2 minutes, washed in distilled water for three times, washed once in balanced salts solution[8] and once in culture medium. During these procedures, a pasteur pipette was used to suck and blow the solutions passing through the bag. This gives a thorough contact of the surface of the eggs with the solutions.

The bag containing eggs was squashed by a

glass rod in a watch glass with 2 ml of medium. The suspension was spun in a clinic centrifuge at 300 rpm for 2 minutes. Pellets were resuspended in 2 ml of medium. Grace medium[9], Schneider medium[21], H5 medium[18], H6 medium[14], X2 medium[8] and D20 medium[7] supplemented with various proportions of lobster hemolymph, fetal calf serum, whole chicken egg ultrafiltrate and bovine plasma albumin were tested for cell growth. The inoculum was cultured in a Leighton tube with tight rubber stopper and incubated in 26±1 C. The cultures were observed under a Nikon inverted microscope. Chromosome preparation was proceeded as that described by Dolfini[5].

RESULTS

Some of the eggs start to hatch at 12 hours after incubation. Eggs of 4 to 11 hours are found to be suitable for setting a primary culture, but 7-hour eggs give best cell growth. This is judged by the comparison of increasing cell density from daily observation. A primary culture can be maintained in H6 medium or X2 medium supplemented with 20% fetal calf serum, 10% whole chicken egg ultrafiltrate and 1% bovine plasma albumin. Nevertheless, D20 medium plus 10% fetal calf serum gives best growth and the procedures are simpler.

Appreciable cell growth was observed around 7 days after inoculation. There are at least three kinds of cells i. e., round, fibrous and star-shape. Cells grow and/or aggreate in various types. The first type is the formation of half floating multiple vesicles. These vesicles are round, hollow balls and in various sizes. The second type is the formation of monolayer cell sheets adhering to the bottom of the culture tube. They may be composed of round or fiber-shape cells. The third type is the axial forming cells. They are round shape and compactly aligned. The fourth type is a star-shape arrangement. Rhythmic contractile movement was observed often at the axial arranged tissues and occasionally at the star-shape tissues. The contractile movement at the axial tissues may be initiated as early as the second day of incoulation and lasted for three weeks. From the daily observation, it is apparent that some degrees of cell aggregation and differentiation is initiated during the beginning two or three weeks of cultivation; and these cell organizations gradually disintegrate thereafter.

In general the cell growth rate declines after two to three weeks. However, in a total of fifty primary cultures prepared, one line has been maintained for ten months up to the moment. This cell line has been subcultured for five times (this was done only whenever the cells became over-crowed in a tube), and once a week, half of the medium in each tube was replaced by a new medium. A dozen of diploid metaphase figures from this line were obtained recently.

DISCUSSION

In the early stages of insect tissue culture, serious efforts were made to establish cell line from specific types of tissues. These efforts usually ended in abortive primary cultures, with the notable exception of the lines obtained from pupae or adult ovaries and larval hemocytes[1]. A current practice is to use whole embryos as inocula. However, certain ages of embryos may give rise to cell outgrowths or replications from dissociated cells much more readily than other ages do. Hirumi and Maramorosch[12], in experimenting with leafhopper embryos (*Macrosteles fescifrons*) in an 11-day incubation period, found that growing cells were only obtained from the embryos of 7th-8th day which is the time of blastokinesis. Chiu and Black[3] used only 7-day-old embryos of their leafhoppers (*Agallia constrica*) which have a total embryonic time of 11–12 days. Similarly, Landurau[16] found that the only satisfactory stage of embryos of *Blabera fusca* were those which already had a functional dorsal vesel–a relatively late stage in embryogenesis, past active mitosis. Horikawa and Fox[13] found that *Drosophila* eggs taken during the first 2 hours following fertilization did not yield cells capable of multiplying *in vitro*, but 8-hour eggs yielded a type of cell which entered logarithmic growth. Reflecting on *Drosophila* embryogenesis,

we recall that the first 5 or 6 hours (at 25 C) is the period of rapid mitotic activity and by the 8th hour the embryo is in a less active stage mitotically, while the emphasis is on differentiation[4]. Echalier and Ohanessian[7] found *Drosophila* eggs between 6–12 hours most suitable, and Lesseps[17] used *Drosophila* embryos about 11 hours old. In *Musca domestica* L., Greenberg and Archetti[10] used 4-hour-old eggs for culture; Eide and Chang[8] used 6-hour-old eggs from a total embryonic period of 10 hours. The present results show that house fly eggs from 4 to 11 hours old can be used for cell culture. These data indicate a fact that the *Drosophila* as well as the *Musca* have less critical embryonic period for cell culture. Although we feel that the 7-hour-old eggs somehow give best growth. In spite of all these, we as well as previous workers all have a common difficulty to assure that insect eggs collected at the same interval are at the same embryonic stage. But in practice, it is necessary to know that how long in incubation the post-oviposited eggs are good to be used for cell culture.

Trypsin has been successfully used for cell dissociation in house fly embryonic cell culture[23], but this procedure is unsatisfactory to us. Echalier and Ohanessian[7] cultivated *Drosophila* embryonic cells at 25–27 C. It also seems to us that the temperature variation of ± 2 C around 26 C does not appreciably affect the house fly cell growth. The only critical paper on temperature effects on the growing cells is that of Mitsuhashi[19] who determined 25 C to be optimal with both 20 C and 30 C causing a decrease in cell number. Trypsin[2] and Phytohemoglutanine[20] have been used for accelerating cell growth *in vitro*, but this desirable effect is not obtained by us. Sohi and Smith[22] have shown that maximum growth of *Adeds aegyti* cells was obtained with 10% fetal calf serum and maximum growth of *Antheraea eucalypti* with 5% of fetal calf serum. We have observed no appreciable effect in varying the concentration of fetal calf serum in 5, 10, 15 and 20% but the cell density was not measured. Perhaps insect hormones may have some effects on cell growth but we have not yet come to the position. In the present experiments penicillium G, streptomycin sulphate and mycostatin were added into the media in the concentration of 60 mg/l, 200 mg/l and 5 mg/l respectively. These antibiotics are found to be very satisfactory in preventing microbial contamination. Fungizone was not used because of its toxic to mosquito cells[18].

The culture conditions for insect cells are not as properly defined as that for mammalian cells. Factors such as cell development at the time of dispersion, culture procedures, cell density, medium, temperature etc. may affect cell growth. So far, for culturing house fly embryonic cells, perhaps the temperature is proper at 26 ± 1 C, the developmental stage is best at 7 hours old in 25 ± 1 C. We also know that the house fly embryonic cells grow better in D20 medium plus 10% fetal calf serum and some antibiotics, than those other media previously used[8,10,23], but this is by no means of the best. We have yet no data on the proper culture procedures and cell density.

According to Hayflick[11] the mammalian cells have a finite life-time *in vitro* as they do *in vivo*, unless they are transformed. We do not know whether the continuous growing cells of above mentioned are transformed cells. Loss of contact inhibition is one of the most distinct characters of the transformed mammalian cells *in vitro*[6]. Whereas, with the exception of those monolayer cells, the majority of insect cells *in vitro* show no contact inhibition even in those very young cultures. Karyotype polymorphism was detected in the continuous *Drosophila* cell line[5]. We have obtained a dozen of metaphases from our continuous cell line. All of them were with 12 chromosomes. Of course, the possibility of that some aneuploid cells exist in the culture still can not be precluded unless a larger sample of cells being checked.

ACKNOWLEDGEMENT

We are indebted to Drs. C. C. Huang and Y. S. Chow and Mr. F. J. Lin for helpful suggestions in the preparation of this manuscript.

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家蠅胚胎細胞培養方法之改進

詹崑源　胡鏡秋　何兆美

將家蠅剛產下之卵置 25±1 C 之環境下則 4 至11小時之間者皆可用以作細胞培養，但以 7 小時者生長最佳。用 D20 培養液加上百分之十的牛胚血清及少許之抗生素，有一系細胞培養至今已維持了十個月。其中作者曾見過12個分裂中期細胞，其染色體並不見有變異情形。