SHORT COMMUNICATION

LEUKOCYTE CULTURE OF THE EEL WITHOUT AUTOLOGOUS SERUM

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Though the application of the leukocyte culture technique with phytohemagglutinin (PHA) to the chromosome study of vertebrates can be traced back more than 14 years, this technique was successful mainly with non-fish species. Ojima *et al.* (1970), and Heckman and Brubaker (1970), however, successfully cultivated leukocytes of fresh-water cyprinid fishes, gold fish (*Carassius auratus*) and carp (*Cyprinus carpio*). Paying attention to some difference in species-specific growth factors between cells of cyprinid and salmonid species, a reproducible method of culturing leukocytes from rainbow trout (*Salmo gairdneri*) was developed by using a culture medium saturated with oxygen (Heckman *et al.* 1971).

In the above two kinds of culture methods for fish leukocytes and in usual methods for other vertebrates, the autologous serum is simultaneously introduced into the culture medium when cells are inoculated. However, no significant toxic and inhibitory effects on the leukocyte growth are caused by the addition of autologous serum.

We found that for successful leukocyte culture of the eel removal of autologous serum was an essential factor. A new technique of culturing leukocytes from Japanese eel (*Anguilla japonica*) for chromosome study is described in this paper with particular interest in removal of autologous serum.

Technique: Six adult females of Japanese eel (*Anguilla japonica*) with body weight of 128.0-355.0 g and body length of 47.0-60.0 cm were collected in Korean fresh-water. The specimens were transported alive to our laboratory aquarium and were kept at 28°C until they were used.

All fishes were thoroughly rinsed with 70% alcohol before sacrifice. Anticoagulant (0.2 ml, Difco blood separation vial) was drawn into a sterile 5-ml syringe through a needle of a size commensurate with the size of the fishes. Usually, a 23-gauge (20 mm) needle was suitable. For convenience of blood aspiration, the pericardium was removed before blood collection, since it was rather rigid comparing with that of other fish species.

About four milliliter of whole blood was then drawn by puncture of ventriculus cordis. The blood was then centrifuged at 350 rpm for 5 minutes to separate leukocytes in a refrigerated system. The supernatant of leukocyte-rich plasma was collected by 3 to 4 repeated sedimentations at 5-minute intervals. By this procedures

about two milliliter of leukocyte suspension was obtained in each specimen. The leukocyte suspension thus prepared was again centrifuged at 800 rpm for 10 minutes to remove the serum. The cell pellet was washed with a balanced salt solution for fish, and the washing solution was removed through centrifugation.

Cultures were set up with and without autologous serum, the latter being tested for a possible important factor. Culture medium consisted of 10 ml GIBCO MEM Spinner medium (10 $\times$), 80 ml of sterile triple distilled water, 1 ml of L-glutamine (200 mM, Merck) and 5 ml of streptomycin-penicillin mixture (Flow Lab., 5,000 u/ml). Finally, the medium was supplemented with 10 ml of fetal calf serum (Difco). Difco Chromosome Microtest Kit was also used as an alternative test medium. Initial pH of the culture medium was adjusted at 7.2 with 10% sodium bicarbonate. One ml of culture medium was added to the leukocytes collected in a measuring conical tube. Cells were gently dispersed to make a homogeneous cell suspension. Four ml of culture medium was placed in culture bottle (17 mm in diameter, 50 mm deep), to which freshly prepared Difco PHA-M (0.1 ml) was added. Half milliliter of the leukocyte suspension was seeded in each bottle containing 4 ml of medium (approximately 1.0×10^6 cells/ml). Cultures were incubated in thermostatically controlled water bath at 25°-28°C for 5 days. During this culture period, bottles were not agitated. Two hours before termination of the culture, colcemid (Ciba) was added at a final concentration of 0.5 μ g/ml. Cells were harvested as a usual manner: Hypotonic treatment was conducted with 0.075 M KCl for 15 minutes at room temperature. Cells were fixed with chilled fresh 1:3 acetic-alcohol at 4°C for 15 minutes. Following two changes of the fixative, the cells were finally resuspended in one milliliter of fixative, and air dried on slides. The slides were stained with basic fuchsin.

Results and Discussion: The effect of the removal of autologous serum from cultures was obvious; about 100 to 500 mitotic figures were obtained per culture in four repeated experiments, and a large number of excellent mitotic figures were available for chromosome analysis (Fig. 1). On the contrary, the leukocyte cultures containing autologous serum were coagulated after one day of incubation. And they eventually failed to grow with very rare mitotic figures. In only one of four cultures containing autologous serum we obtained less than ten metaphases.

The serum was yellowish brown in the carp, funa and rainbow trout, while it

Fig. 1. Metaphases from cultures of leukocytes of *A. japonica*. Bars indicate 5 μ .

it showed a bright or slightly greenish coloration in the eel. This phenomenon may be an interesting problem from the hematological point of view. Due to the lack of definite hematological information about this problem in the eel (Sano 1957; Johansson *et al.* 1974), it is very difficult to elucidate the effect of autologous serum upon leukocyte cultures.

It should also be noted that the present technique may not always promise a satisfactory result in other fish species. In homoiothermic vertebrates, different leukocyte culture methods have been developed for different taxonomic groups. The same problem may exist in dealing with fishes as pointed by Heckman and Brubaker (1970) and Denton (1973).

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