SHORT COMMUNICATION

A PHA-CULTURE METHOD FOR CELLS FROM THE RENAL TISSUE OF TELEOSTS

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In direct methods for the study of chromosomes of fishes, kidney, spleen and gill are usually used as tissue sources. On the other hands, various types of tissue culture methods were developed by Chiarelli et al. (1969), Chen (1970) and Ojima et al. (1972) for the study of teleost chromosomes. The blood culture technique, originally developed for human chromosomes, was advantageously modified for fish chromosomes by Ojima et al. (1970) and Heckman et al. (1970). Following the discovery of phytohaemagglutinin (PHA) as an initiator of mitosis in human lymphocytes by Nowell (1960), the application of this agent to blood cultures has resulted in outstanding contributions to human cytogenetics. A new technique for the study of fish chromosomes is described in this paper, with particular interest in the mitotic frequency of the PHA-stimulated culture-cells from the renal intertubular tissue which has been known to contain immature blood cells (Catton, 1951).

Procedures: Cyprinus carpio, and some other species of teleosts were sterilized by rinsing body with 70% ethanol. The kidney was aseptically removed and minced with scissors. The tissue fragments thus obtained were transferred into a test tube with Eagle’s MEM. The cells were liberated from the renal intertubular tissue by a gentle pipetting. Then, the test tube was allowed to stand for several minutes at room temperature to obtain cell suspension. The upper part of the cell suspension, free of coarse tissue fragments, was collected in another test tube. The cell density (except erythrocytes) was determined with a hemocytometer. Duplicate cultures were prepared by seeding the cells at the final cell density of 2×10⁶ cells/ml. The medium for culture consisted of 85% of HEPES-buffered Eagle’s MEM (HEPES: 15 mM, antibiotics: penicillin: 100 IU/ml, streptomycin: 100 μg/ml and kanamycin: 60 μg/ml) and 15% of fetal calf serum. Two sets of cultures were set up: One was without PHA and the other contained PHA-M (Difco) at a concentration of 10 μl/ml. Three milliliters of cultures were placed in TD-15 flasks which were stoppered with aluminium foils. The cultures were kept in a humidified incubator for 3 days at 29±1°C.

In order to examine the effect of PHA, four hours prior to harvest, ³H-thymidine and colchicine were added to the cultures at a final concentration of 1 μCi/ml and 1 μg/ml respectively. At the termination of incubation period, each set of cultures was transferred in centrifuge bottles and centrifugation was made at 800 rpm for 5 min. After
removal of supernatant, cells were exposed to one milliliter of hypotonic solution (0.075 M KCl) for 10 min at room temperature. One milliliter of methanol-glacial acetic acid (3:1) were added to fix the cell suspension. The cells were centrifuged, and the supernatant was replaced with fresh fixative. Following three changes of fixative, 15 min each, the cells were finally resuspended in a small volume of fixative, one drop of the cell suspension was transferred on a clean slide and air-dried. Autoradiographic slides were made according to the dipping method. After 10 day's exposure at 4°C, the slides were developed and stained with Giemsa's solution. A total of 300 nuclei (except erythrocyte) was checked in each culture for labelled cells showing more than

Fig. 1. Percentages of labelled cells in two independent cultures (I and II) from renal intertubular tissue of *Cyprinus carpio*. The vertical bars indicate standard errors. PHA+: Cultures with PHA. PHA−: Cultures without PHA.

Fig. 2. a. PHA-added culture of cells from *C. carpio*. b. Non-PHA culture. (Labelled slides). lc: Labelled cells. m. Mitoses. The scales indicate 20 μ.

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Findings: The percentages of labelled cells observed in Cyprinus carpio are shown in Fig. 1. In the PHA-added culture, a large number of mitotic and labelled cells were observed. On the contrary, such figures were rare in the non-PHA culture (Fig. 2). From the above data it is very possible to see that there are at least two different cell-populations, in one of which the cells proliferate in vitro under the stimulus of PHA, while in the other the cells can reproduce without PHA.

Based on the autoradiographic sequence, erythrocytes were well-identified by their outstandingly small-sized nuclei and intense affinity to Giemsa's stain.

Thus it appears that there is a cell-population in the renal intertubular tissue of teleosts which proliferates under the effect of PHA. The culture technique is proved advantageous for the chromosomal study of fishes, particularly in those of small-size in which blood supply is not sufficient for culture (Fig. 3).

LITERATURE CITED