SHORT COMMUNICATION

CHROMOSOME PREPARATION FROM CULTURED SCALE EPITHELIUM OF TELEOST FISH

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In 1969, Denton and Howell described a technique for the observation of chromosomes from the epithelium that overlies the scales of teleost fishes. However, the number of metaphase cells in the scale epithelium is usually small. In this report, a technique for the observation of chromosomes from the cultured scale epithelium in a teleost fish, the carp (*Cyprinus carpio*), is described.

Cultivation of scale epithelium: Several scales were scraped from a carp and the tissues were immersed in PBS (phosphate-buffered saline, Dulbecco and Vogt, 1954). The scales were then washed in three changes, 10 min each, of PBS containing penicillin (100 IU/ml), streptomycin (100 μ g/ml) and kanamycin (60 μ g/ml). The scales were then placed in a Petri dish (6 cm in diameter) and attached to the surface of the dish with a perforated cellophane. They were cultivated at 28-30°C in a humidified incuba-

Fig. 1. The scale epithelium extended to the glass wall after 24 hours of cultivation.

Fig. 2. Metaphase chromosomes in scale epithelium cultivated 24 hours.

tor for one day. The culture medium consisted of a mixture of 85 ml of NaHCO_3 -free TC-199 containing penicillin (100 IU/ml), streptomycin (100 $\mu\text{g}/\text{ml}$), kanamycin (60 $\mu\text{g}/\text{ml}$), mycostatin (50 U/ml) and HEPES (15 mM), and 15 ml of fetal calf serum. The initial pH of the culture medium was adjusted to about 7.4 by NaOH.

Harvesting: After 24 hours of cultivation and growth, the scale epithelium extended to the glass wall of the Petri dish as is shown in Fig. 1. Two or three hours before harvesting, colchicine solution was added to the Petri dish for a final concentration of 1 μg per ml. At the termination of culture, the cellophane paper and scales were removed from the Petri dish and the sheets of epithelial cells were washed by Ca^{++} - and Mg^{++} -free PBS followed by 0.1% trypsin for 10 minutes at 37°C. After trypsinization, the cells were treated according to the usual air-drying technique for chromosome preparation.

Results: Scale epithelium cultivated twenty-four hours *in vitro* contains sufficient numbers of metaphase cells for chromosome study (Fig. 2).

LITERATURE CITED

- Denton, T. E., and W. M. Howell, 1969 A technique for obtaining chromosomes from the scale epithelium of teleost fishes. *Copeia* 1969, No. 2: 392-393.
- Dulbecco, R., and M. Vogt, 1954 One-step growth-curve of western equine encephalomyelitis virus on chicken embryo grown *in vitro* and analysis of virus yields from single cells. *J. Exptl. Med.* 99: 183-199.