

include compaction of soil, replacement of forest herbs with grasses of alpine meadows, and consequent increase in root growth, impeding tree seedlings to establish¹⁰. They may combine (synergism) to cause an irreversible decline in regeneration of brown oak.

The ban on tree cutting clamped about two decades ago has, in part also contributed to failure of regeneration of brown oak, as well as of most tree species, except the shade-enduring (*Quercus floribunda* Lindl.⁴) by restricting formation of gaps of an adequate size.

The failure of regeneration would not come to public notice as long as old trees persist. Reports of failure of regeneration of brown oak in three separate areas (viz., Naini Tal, Pindari and the present area)^{3,10} located more than 300 km apart, need to be taken as a serious warning, warranting action before the problem assumes an element of surprise.

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Primary cell culture from explants of heart tissue of Indian major carps

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Heart tissue of Indian major carps was explanted in minimum essential medium supplemented with 15% fetal bovine serum. Fibroblast-like cells emerged from the explant and formed a monolayer within a week. The cell monolayer could be harvested for passage by either trypsinization or scraping.

FISH cell lines are indispensable in fish virology besides their importance in toxicology, biomedical research, biotechnology and many other basic studies¹. Most of the established fish cell lines are derived from cold water fish^{2,3}. Very few cell lines have been developed from the tissues of warm water fish cultured in Asia.

In India, a cell line from the gill tissue of mrigal, *Cirrhinus mrigala* has been developed⁴. Primary cell cultures from kidney of stinging catfish, *Heteropneustes fossilis*⁵ and from caudal fin of rohu, *Labeo rohita*⁶ have also been developed.

Indian major carp culture has been established on a commercial scale in India. The growing carp culture industry has suffered due to several incidences of mass mortality of carps in culture systems suspected due to microbial diseases, particularly of viral etiology⁷. Besides, mortality due to increased aquatic pollution is also on the rise. Hence, there is a need to develop cell

lines for the major culturable varieties of fish in India. This paper describes the development of primary cell culture from the heart tissue of the Indian major carps, catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*).

Major carp fry (5-8 g) were obtained from the State Government Fish Farm, Gajanur, Shimoga and reared to fingerlings in 25 m² cement tanks at the fish farm of College of Fisheries, Mangalore. The fish were fed daily with artificial feed⁸. The fingerlings (12-15 g) were transferred to laboratory and maintained for 5 days in aquaria containing tap water treated with KMnO₄ (5 ppm). These fish were then transferred to sterile water overnight. They were killed instantly by a hard blow on the head, disinfected in calcium hypochlorite (500 ppm) for 5 min, washed in sterile water and swabbed with 70% ethyl alcohol. The heart was removed aseptically from several fingerlings using sterile scissors and forceps and pooled in a vial containing cold phosphate buffer saline (PBS). Pooled heart washed twice in PBS were squeezed to remove blood cells and washed again with PBS. The organ was cut into small fragments of approximately 1 mm³ and washed twice at 1000 rpm in PBS.

The pellet consisting of small pieces was resuspended in 2-3 ml of PBS containing antibiotic-antimycotic solution (penicillin, 250 IU/ml; streptomycin, 0.25 mg/ml and amphotericin B, 0.625 µg/ml Sigma, USA) and further washed twice in a sterile petri dish. The tissue pieces were transferred to a 25 cm² tissue culture flask (Tarsons, India) and then distributed uniformly by flooding with 1 ml PBS supplemented with antibiotic-antimycotic solution as above. Excess PBS was then pipetted out to the last drop and the tissue

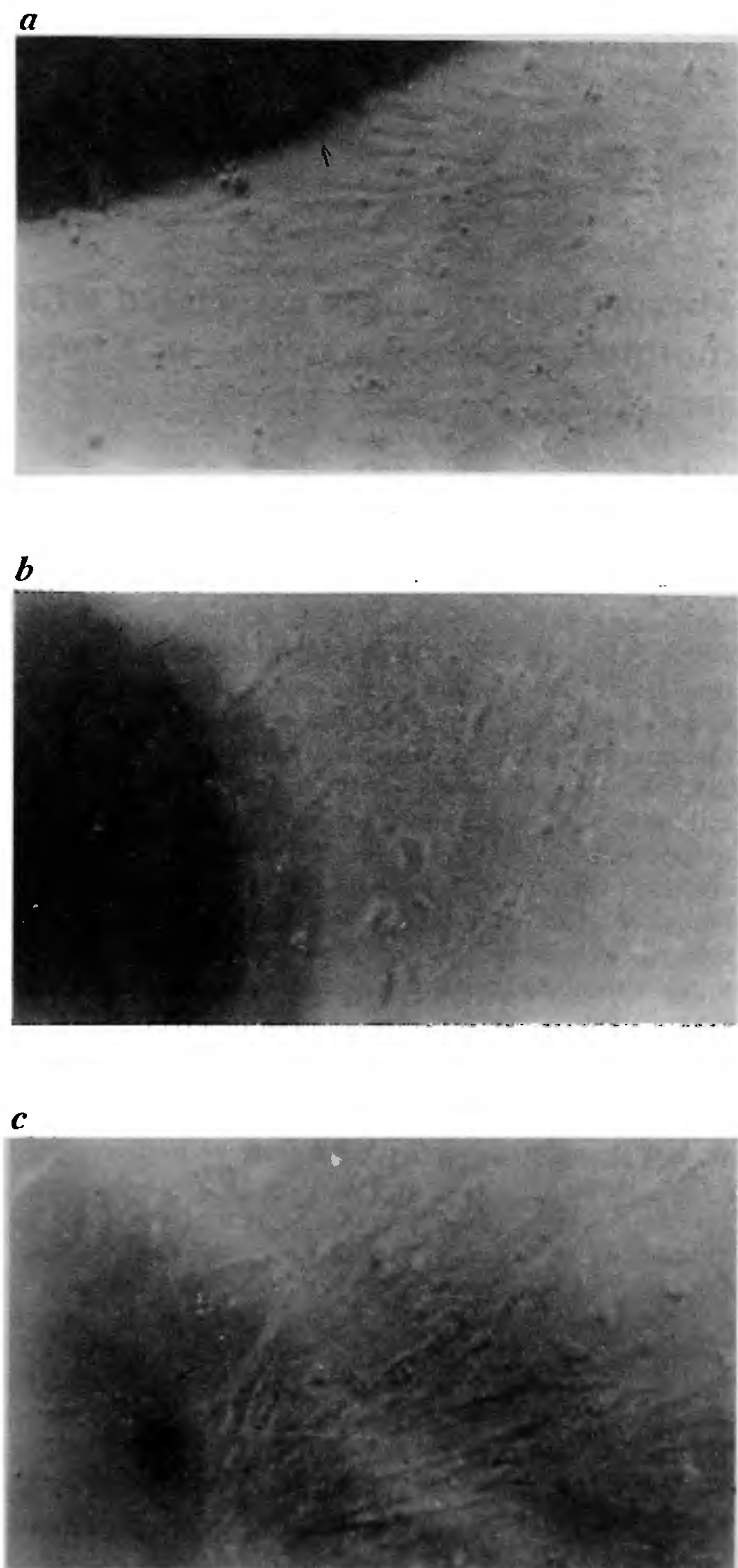


Figure 1. Primary culture (three-day-old) from the heart tissue explant (arrow) of (a) rohu (*Labeo rohita*); (b) catla (*Catla catla*); (c) mrigal (*Cirrhinus mrigala*), (100 ×).

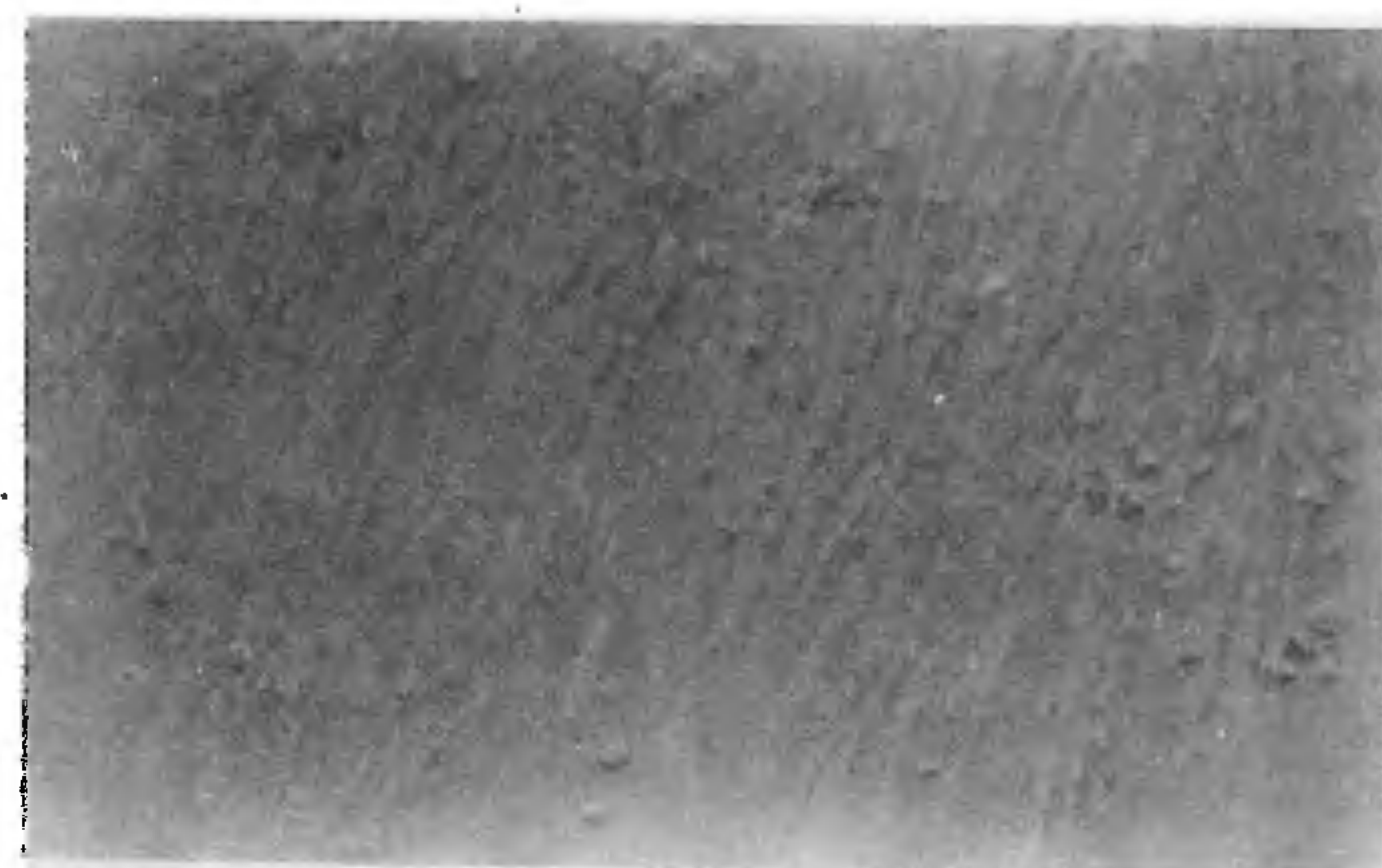


Figure 2. Five-day-old monolayer of cells from the heart tissue explant of catla (*Catla catla*), (100 ×).

antimycotic solution, at dosage already mentioned above, was added to the flask and incubated at room temperature ($28 \pm 2^\circ\text{C}$). After 24 h the medium was replaced with 5 ml of fresh MEM supplemented with FBS and antibiotic–antimycotic solution and incubated at room temperature ($28 \pm 2^\circ\text{C}$). The tissue explants were observed for growth and formation of monolayer of cells using an inverted microscope (Olympus, Japan).

Within 12–15 h, several types of cells were found emerging from the tissue fragments and attached to the flask. After 48 h, large, elongated, fibroblast-like cells appeared around several explants. The cells were found to migrate away from the explant, attach and multiply. On the third day, spindle-shaped fibroblast-like cells were seen spreading over an area of 1 mm^2 around the tissue (Figure 1 a–c). These cells multiplied and a good monolayer, comprising mostly of fibroblast-like cells were obtained within 5 days of tissue explanting (Figure 2). On the sixth day, growing cells close to the explants were found to be rounding off, but the cells away from explant were found to be in good shape. It is interesting to find that several heart pieces were still twitching in the flask. The twitching rate was 60 times/min in the beginning which decreased gradually and lasted for 6–7 days after the explant.

A monolayer comprising mainly of fibroblast-like cells with a sharp and clear outline developed in a week's time. These cells were of both dendritic and spindle shape with visible cytoplasmic granules. A confluent monolayer was formed in the flask within 8 days in the case of catla, 10 days in case of rohu and 20 days in case of mrigal. This variation in attaining confluency could be due to the difference in explant density and the growth rate of the species. In nature, catla is the fastest-growing species followed by rohu and mrigal. As such there was no difference in cell type from the heart tissue explant of the three species.

pieces were incubated in semi-dried condition for 6–7 h at room temperature ($28 \pm 2^\circ\text{C}$). Minimum essential medium (MEM) (Sigma, USA), supplemented with 15% fetal bovine serum (FBS) (Sigma, USA) and antibiotic–

After obtaining the monolayer, cells were passaged to new flasks by trypsinization. The old medium was removed and replaced with 1 ml of 0.025% trypsin-EDTA. The enzyme activity was arrested by adding 1 ml of FBS after a minute. The detached cells along with FBS were centrifuged at 1000 rpm for 10 min. The pellet was then suspended in MEM by gentle titration and the contents transferred to a tissue culture flask for incubation. Alternatively, the monolayers were also harvested by scraping, employing a sterile rubber policeman and passaged to a new flask in MEM.

The trypsinized cells attached to flasks in clumps and individually, retaining the original fibroblast-like shape. Cells were in good condition for about a week, after which they died. The cells that were detached using a sterile rubber policeman appeared in clumps and attached well in the new flask. These clumps of cells showed growth along the periphery. Attachment of cells and monolayer formation was better with harvesting by scraping than with trypsinization. After harvesting the cells (either by scraping or trypsinizing), the few pieces of tissue and undetached monolayer in the original flask resumed normal cell growth and formed monolayer. Some tissue pieces in the original flask showed emerging cells which did not spread away from the explant; rather they stayed close to the tissue and formed a network.

In this study, a successful primary culture could be obtained using only the synthetic medium (MEM) supplemented with 15% FBS without using muscle extract. Homologous fish muscle extract has been used for successful development of primary cultures^{4,6}.

In general, growth and development of cell monolayer from heart tissue explants were good and easy to maintain. Besides, heart tissue of Indian major carp was found ideal for cell culture, as it is easy to collect aseptically and also it is contamination-free compared to other visceral organs. Hence, there is scope and prospect for development of cell line from heart tissue of carps. Work on the establishment of heart cell line from the primary cultures and its characterization is under progress.

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Microgravity changes associated with continuing seismic activities in Koyna area, India

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High-precision gravity measurements were carried out during June–August, 1995 in Koyna area, India, which is known for its high intraplate seismic activities. Daily observations were recorded at four stations with respect to a reference station to monitor the possible gravity changes arising due to crustal deformations associated with seismic activities. Significant relative gravity changes (15–30 μgal) of almost same pattern were observed at different stations which is 2–3 times of the observational error. A good correlation was noticed between the relative change in the gravity field from one station to the other and with the occurrences of tremors of magnitude 2–3 which are approximately twenty in number during this period. In most of the cases (18), the relative gravity field first increases and then decreases when the seismic events take place. The observed changes in gravity field seem to be associated with seismic activities, and may be attributed to mass redistribution or seismogenic deformations such as opening of the cracks, etc. in processes taking place during an earthquake. However, the phenomenon is not fully understood and needs more such experiments over longer duration to be conducted in different geological/tectonic settings to understand its actual nature and the mechanism.

TEMPORAL variations in local gravity field over a small time period can occur due to deformation of crustal rocks in the form of mass re-distribution and change in elevation¹. The study of variation in the gravity field due to such deformations has been made possible with the advent of high precision gravimeters which measure the variations in gravity field up to microgal levels. Hence, repeat gravity observations using such instruments help to study both aseismic and co-seismic deformations and mass redistribution. For example, the measure of vertical aseismic deformation in response to loading due to La-Grande-2 reservoir, Quebec has been

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