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A Cell Line Derived from the Fin of the Goldfish, *Carassius auratus*

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ABSTRACT A new fibroblastic cell line was established from the caudal fin of the goldfish, *Carassius auratus*. The cells, designated CAF have been subcultured over 80 times since their initiation in August 1977. There was no difference in the growth rate between 10 and 20% fetal calf serum. At 5% fetal calf serum and 20% calf serum, the growth curves fell slightly lower than the 10 and 20% levels of fetal calf serum. CAF cells were cultured at various temperatures. The rate of cell growth increased with increasing temperature over the range of 16 to 33°C, whereas no cell growth was observed at 6°C. Plating efficiency of CAF cells was estimated to be 10%, when 2,000 or more cells per dish were inoculated at 26°C. (*Zool. Mag. 88: 321-324, 1979*)

A number of fish cell lines have been established in the field of fish virology (Wolf and Quimby, 1969). SJU-1, the only cell line derived from somatic cells of the goldfish, was developed by G. J. Rio *et al.* (1973) in search of an assay system for interferon studies at the *in vivo* and *in vitro* levels. In describing all these cell lines, reference has hardly or not at all been made to their plating efficiencies. In this report a new cell line, designated CAF, derived from the caudal fin of the goldfish, *Carassius auratus*, was dealt with. It has a relatively high plating efficiency of about 10%, which is much lower than those of various mammalian cell lines, but which still can be used for studies of the effects of radiation and other environmental agents on fish cells *in vitro*.

Establishment of a new fish cell line

A goldfish (one-year old, 10 cm in total length) was sacrificed and the caudal fin was removed. The fin was sterilized in 1% sodium hypochlorite and 70% alcohol for one minute each, and then washed once with sterilized distilled water and twice with TC-199. Thereafter, the fin was minced with a pair of scissors as finely as possible, and was planted in a 30-ml plastic flask (Falcon 3012) containing 5-ml HEPES-buffered culture medium (pH, 7.2), which consists of TC-199, 80%, supplemented with fetal calf serum, 20%, streptomycin sulfate, 100 μg/ml, penicillin, 100 IU/ml and Amphotericin B, 1.3 μg/ml. Cells were incubated in a closed system at 26°C. Half of the culture medium was renewed twice a week. Cells were

Table 1. Successive cultivation of the cells, designated as CAF, derived from the fin of the goldfish, *Carassius auratus*.

Passage	Days for confluent cell sheet formation (26°C)	Culture media
Primary-5th	3-5	TC-199* with 20% FCS, Streptomycin 100 μg/ml, Penincillin 100 IU/ml, Fungizone 1.3 μg/ml
6th-17th	6-16	
18th-33rd	4-11	
34th-43rd	4-11	TC-199* with 10% FCS, Streptomycin 100 μg/ml, Penicillin 100 IU/ml, Fungizone 1.3 μg/ml
44th-84th	7	

*pH 7.2 (HEPES buffer)

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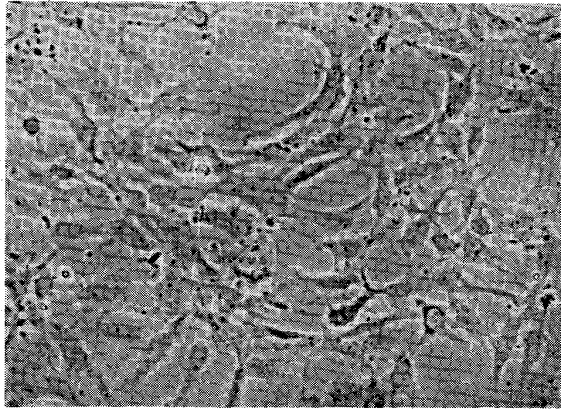

Fig. 1. Phase contrast microphotograph of CAF cells. (×260)

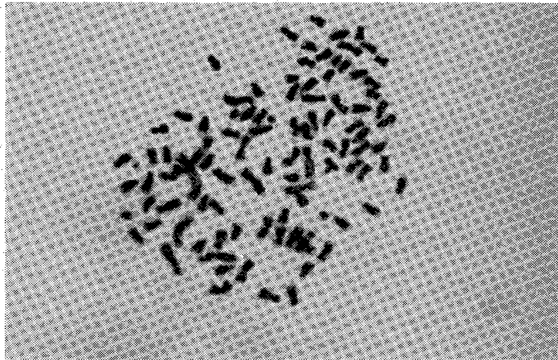

Fig. 2. A typical metaphase figure. (×1,700)

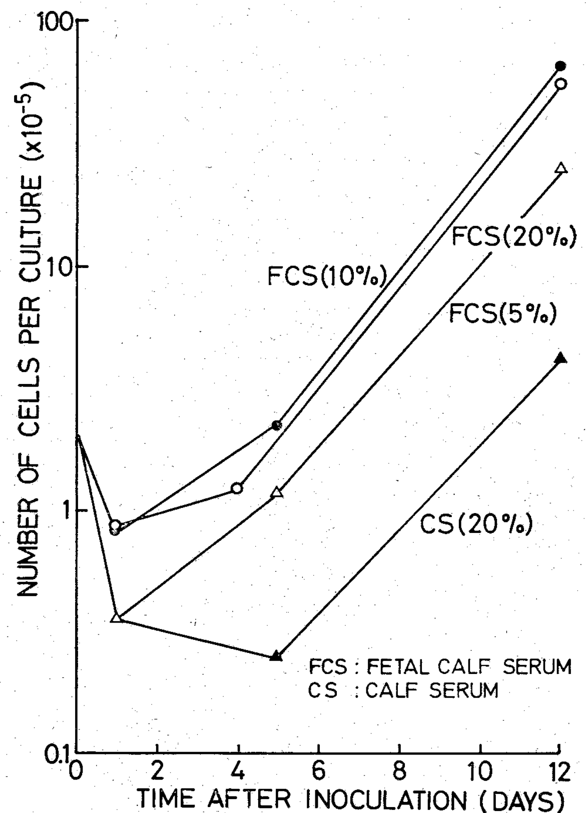

Fig. 3. Effect of concentration of fetal calf serum on cell growth at 26°C.

divided into two new flasks at confluency using 0.1% trypsin solution. Time to confluency varied in early passages, from 3–16 days, but at the 44th passage and later, the time settled down to 7 days (Table 1). The new cell line derived from this primary culture was designated as CAF. The cells were fibroblast-like (Fig. 1). Chromosome preparations were made at passage 61 by means of routine air-drying techniques. Counting of 67 well-spread metaphases showed a wide variation of chromosome numbers. The mode was 100 with 19 metaphases (28%). A typical metaphase having 100 chromosomes is shown in Fig. 2. Cells at passage 56 were tested using Bacto-thyoglycollate medium which verified that they were free of microbial contamination.

Characteristics of CAF cells

Cell growth was tested at 26°C at three different concentrations of fetal calf serum (FCS) and at one level of calf serum (CS) by inoculating 2×10^5 cells per dish (5 cm in diameter). Results obtained are shown in Fig. 3. At 20% CS, cell growth was delayed considerably and at 5% FCS, the growth curve fell slightly lower than the 10 and 20% levels of FCS. There was practically no difference between 10 and 20% of FCS. Although the kinds and concentrations of serum affected the lag phase of cell growth, it should be noted that no differences in population doubling time were observed among the four serum conditions tested. Taking this result into consideration, FCS content for culture medium was reduced from 20% to 10% from the 34th passage and beyond.

To find the optimal temperature, the culture dishes containing $2\text{–}5\times10^5$ cells per dish were incubated at 6, 16, 26 and 33°C immediately after inoculation (Fig. 4). At 6°C, about 10% of the cells inoculated adhered to

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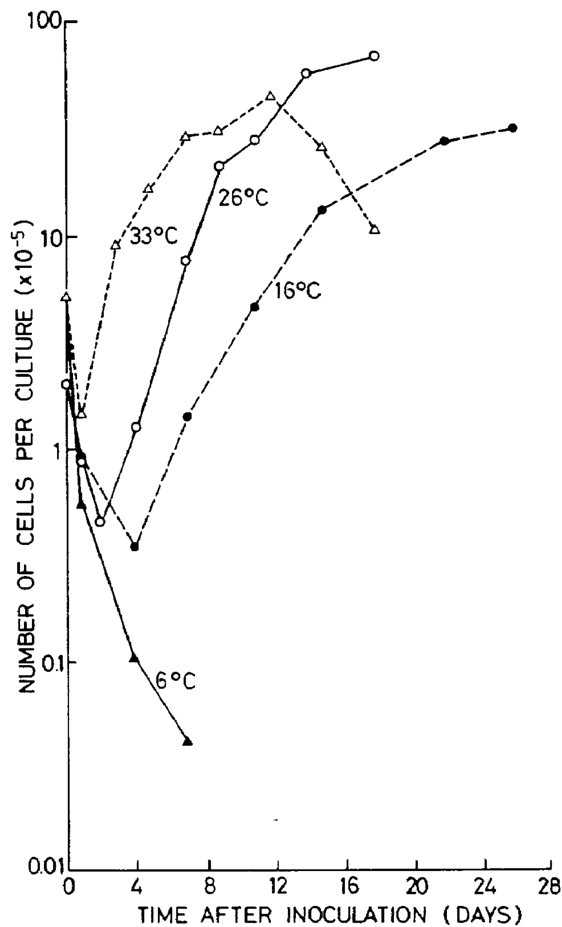

Fig. 4. Growth curves of CAF cells kept at 6, 16, 26 and 33°C.

the dish wall, but rapidly came off. No growth was observed at this low temperature. At 16°C, cell growth was rather slow and the cell number was still increasing on the 26th day after inoculation. Cells grew well at both 26 and 33°C with a higher growth rate obtained in the latter. From the growth curves, population doubling times at 16, 26 and 33°C were calculated at about 1.6, 1.2 and 0.8 days, respectively. According to data in Rio *et al.* (1973), the optimal temperature at pH 7.2 was 20°C, though cell growth was followed for only five days in their experiments. Our data apparently are not in agreement with theirs.

To test colony forming ability, plastic dishes containing 700, 1,400 and 2,800 cells in the same medium except that the concentra-

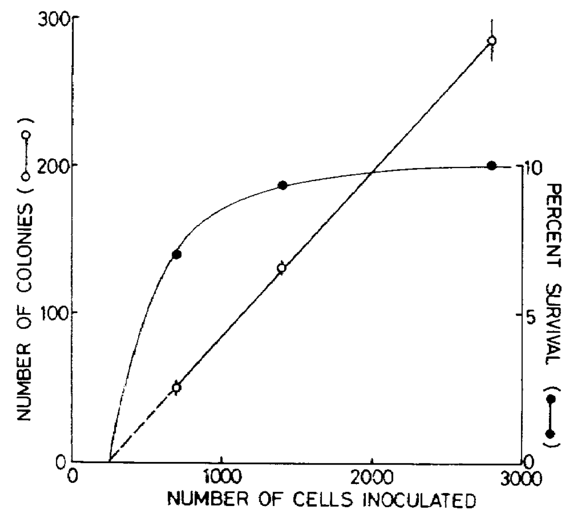

Fig. 5. Relationship between number of cells inoculated and colonies formed.

tion of fetal calf serum was increased to 20%, were kept at 26°C for 10 to 14 days. Cells were fixed in 10% formalin and stained with 0.2% Gentian violet. Colonies, which consisted of more than fifty cells each, were counted under a dissecting microscope. Plating efficiencies were calculated at 7.0, 9.3 and 10.1%, respectively (Fig. 5). It is known from Fig. 5 that when 2,000 or more cells per dish were inoculated, the plating efficiency was around 10%. The plating efficiencies of the established fish cell lines described in ATCC (The American Type Culeture Collection) are less than 1% except for the one derived from the goldfish (ATCC CCL 71, CAR), the efficiency of which is approximately 6%. Compared to those values, CAF cells have a higher plating efficiency, which makes it possible to use this cell line in *in vitro* studies of the effects of radiation and other environmental agents on fish cells.

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Culture.

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