

premature to draw conclusions regarding relationships between the 2 lines of evidence.

The differences in the distribution of activity during activity periods probably correlate most closely with hunting and feeding habits in the field. One might expect a tendency toward continuous activity by animals subsisting on very small prey and plant materials, and toward bout-like activity by animals that take fairly large prey. The behavior of our animals fits roughly into this pattern. The tendency of the tropical diurnal species to nap during midday lends weight to the applicability of some aspects of laboratory findings to field behavior. The grison, in particular, has been reported to take shelter during midday heat⁹. The fact that a siesta also is taken in constant moderate temperatures in the laboratory suggests that the field habit is not solely a response to high temperatures.

The avid treading of wheels by cat- and dog-sized mammals suggests a new approach to their care and exhibition in zoos. Firstly, a suitably large wheel may offer a more desirable or efficient outlet for activity than the conventional zoo enclosure. Secondly, exhibits of mammals walking, trotting, galloping, performing acrobatics, and competing for the use of wheels probably would interest observers more than ones of their lying

prone, sleeping, or occasionally pacing to and fro. The same approach also might succeed with larger mammals. A detailed report of these studies will appear elsewhere^{6, 12}.

Zusammenfassung. Die Lokomotion sowie die phasische Aktivität sechs mittelgrosser Carnivoren und eines Makaken wurde am Laufrad untersucht. Die Laufaktivität nachtaktiver Tiere war stark durch die Lichtintensität beeinflusst, nicht aber die der diurnalen Tiere. Bei künstlichem Zwielicht waren erstere sehr aktiv, letztere wenig oder überhaupt nicht. Die Bedeutung dieser Ergebnisse für die phasische Aktivität im Freiland und den damit zusammenhängenden Grad der Adaptation des Auges wird diskutiert.

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The Culture of Goldfish Cells (*Carassius auratus*) at High Temperatures

It is known that if amphibian and reptilian tissues are selected from donor species ranging into tropical latitudes, growth and differentiation are possible in vitro at and sometimes above 37°C^{1, 2}. Less is known regarding the upper limits for fish cell cultivation, although successful, short-term, primary cultures of goldfish cells at 37°C have been recorded³. The growth rate of the FHM (fathead minnow) cell line⁴ is maximal at 34°C, cell death occurring at 38°C. As the highest incipient lethal temperature of the goldfish, *Carassius auratus*, is regarded as being approximately 40°C⁵, the probability of culturing goldfish cells at 37°C seemed high.

Material and methods: Primary cultures from a variety of adult organs of *Carassius* were prepared from aseptically minced tissues treated with 0.25% trypsin for 15 min at approximately 25°C. After washing with serum-enriched medium, the explants were incubated at 31.5°C on collagen films inside small Petri dishes maintained in an atmosphere of 5% CO₂ in air. The most effective medium used was Eagle's minimum essential medium supplemented with 20% young (3–6 month) calf serum and 0.1% lactalbumin hydrolysate. Antibiotics routinely used included sodium penicillin G (40 IU/ml), streptomycin sulphate (50 µg/ml) and amphotericin B (Fungizone) (2.5 µg/ml). Successive subcultures were carried out in flat McCartney bottles. Cell samples were transferred to other temperatures as required.

Results and discussion: Good primary outgrowths were obtained within 3–6 days from heart, spleen, ovary, testis and kidney. The most successful line⁶ has been one derived from testis cells. At present this has been serially cultivated for 28 passages, occupying 140 days, with a period of 177 days' storage in liquid nitrogen at –196°C⁷ between passages 17 and 18.

Cells have been maintained for 6 passages and 31 days at 37.5°C without obvious morphological changes and with no apparent mitotic inhibition. Only preliminary experiments involving quantitative comparisons of growth rates have so far been carried out. Over a 3-day period, cells plated in similar numbers and densities showed overall population increases of 47% at 31.5°C, 105% at 35°C and 92% at 37.5°C. Standardized coverslip cultures incubated for 24 h at 31.5°C, 35°C, 37.5°C and 39°C showed normal cells and mitoses (Figures 1–3). The incidence of fragmented nuclei was possibly higher at 37.5°C and above but the frequency of this condition has not yet been analyzed. In cultures incubated for 24 h at 41.5°C, although a few relatively normal cells were still present (Figure 4), changes associated with cell death were generally apparent. Only a few mitoses, all obviously abnormal, could be found. The limit of heat tolerance of *Carassius* cells in vitro appears to be quite closely correlated with that of the fish in vivo⁸.

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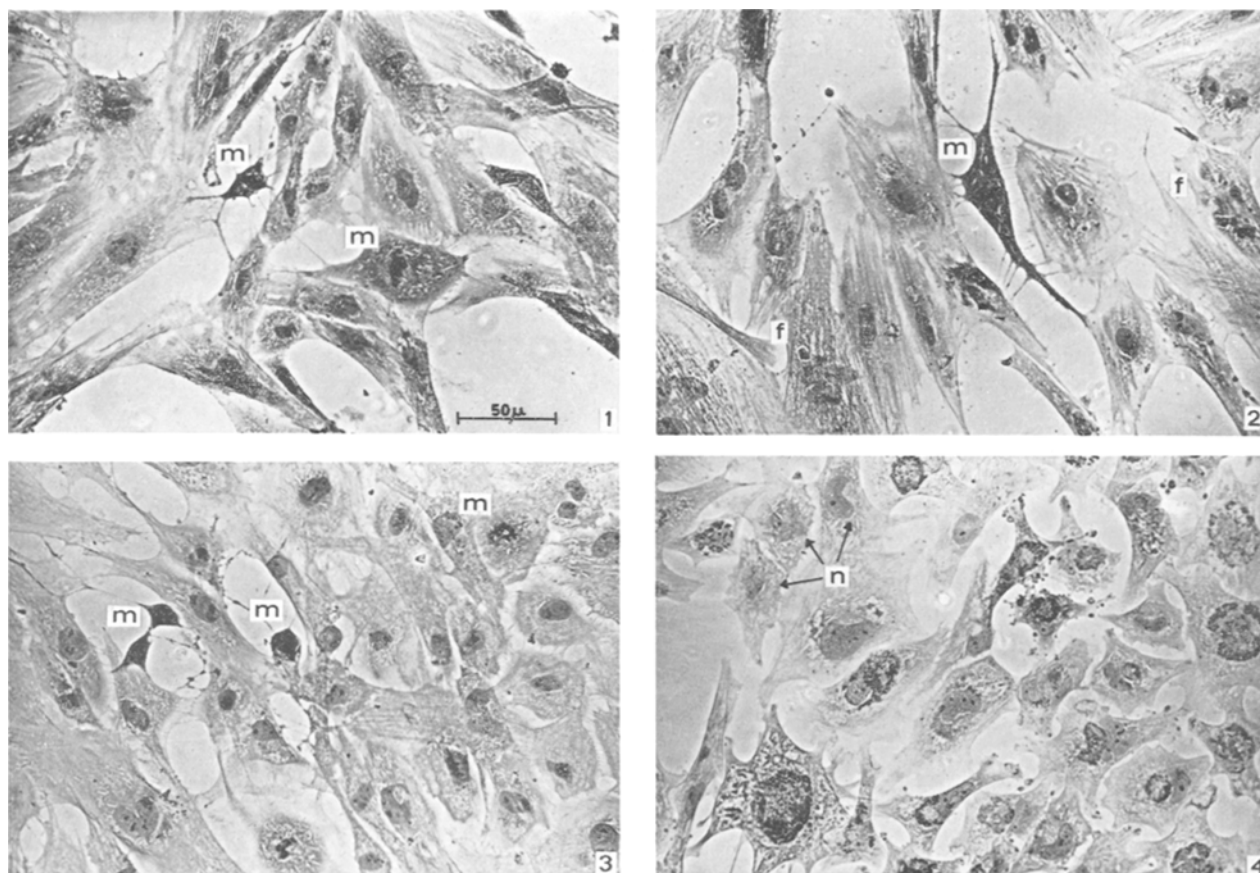


Fig. 1.-4. Phase contrast photographs of fixed and stained cultures grown for 24 h at 31.5°C, 37.5°C, 39°C and 41.5°C, respectively; m, mitoses; f, fragmented nuclei; n, normal cells (arrows). All magnifications as for Figure 1.

Cells transferred from 31.5°C to 4°C and maintained for 2 days, slowly spread out over the surface of the culture bottle. They resumed normal growth (initially accelerated) when returned to 31.5°C.

When analyzed at the 17th passage, the stemline karyotype⁸ of the testis cell line was composed of 116 chromosomes⁹. The diploid chromosome number for *Carassius auratus* has been variously recorded as 96-104¹⁰, 100¹¹ and 104¹². We have found a modal diploid number of 100 in primary cultures.

These preliminary results indicate that selection of a fish species with high heat tolerance as a tissue donor allows cells to be successfully cultured in vitro at temperatures at least up to 37.5°C and under conditions otherwise identical for the cultivation of mammalian cells. This opens the way for studies of behaviour and interaction of cells of two very diverse vertebrate classes¹³.

Résumé. Les cellules de *Carassius auratus* ont été cultivées in vitro avec succès à des températures allant

jusqu'à 39°C, les cellules du testicule en séries de 28 sous-cultures à 31.5°C et 6 sous-cultures à 37.5°C.

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¹³ We thank Dr. E. M. STEPHENSON for advice on technique and for assistance with photography. This work was supported by an University of Sydney Research Grant.

Cuticular Components of Common Indian Arachnids and Myriapods

The data on the cuticular components of the arachnid cuticle being scanty and relating only to fewer constituents of the cuticle as compared to that available for the insect cuticle¹, the present quantitative analysis was made of the cuticles of arachnids and myriapods having

various shades and degree of pigmentation and sclerotization, and also of their soft arthrodial membranes.

Standard routine methods¹ were employed for the biochemical estimations of arthropodin, sclerotin, chitin and mineral constituents of cuticle. Since the protein