

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 $31.5\,^{\circ}\text{C}$ to $4\,^{\circ}\text{C}$ and maintained for 2 days, slowly spread out over the surface of the culture bottle. They resumed normal growth (initially accelerated) when returned to $31.5\,^{\circ}\text{C}$.

When analyzed at the 17th passage, the stemline karyotype ⁸ of the testis cell line was composed of 116 chromosomes ⁹. The diploid chromosome number for *Carassius auratus* has been variously recorded as 96–104 ¹⁰, 100 ¹¹ and 104 ¹². 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 classes ¹⁸.

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

jusqu'à $39\,^{\circ}$ C, les cellules du testicule en séries de 28 sous-cultures à $31.5\,^{\circ}$ C et 6 sous-cultures à $37.5\,^{\circ}$ C.

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- ⁸ A. LEVAN, W. W. NICHOLS, M. PELUSE and L. L. CORIELL, Chromosoma 18, 343 (1966).
- ⁹ L. S. McKenzie, N. G. Stephenson and E. M. Stephenson, unpublished.
- 10 S. Ohno and N. B. Atkin, Chromosoma 18, 455 (1966).
- ¹¹ Y. OJIMA, S. HITOTSUMACHI and S. MAKINO, Proc. Jap. Acad. 42, 62 (1966).
- ¹² B. CHARELLI, O. FERRANTELLI and C. Cucchi, Experientia 25, 426 (1969).
- ¹⁸ 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

Specimens		Colour	Arthro- podin	Sclerotin	Total extractable protein	Chitin	Minerals	Reference
Scorpions								
Palamnaeus bengalensis	SC AM	Black White	10.2 31.3	53.6 32.3	63.8 63.6	31.9 31.9	4.3 4.5	5 5
Buthus tamulus gangeticus	SC AM	Brown White	21.1 29.5	43.0 34.7	64.1 64.2	31.8 31.7	4.0 4.0	5 5
Buthus	SC				68.1	31.9		2
Buthus	SC				67.4	32.6		6
Pandinus	SC				69.8	30.2		6
Spider Mygale	SC				61.8	38.2		6
Mite Trombidium grandissimum	SC	Red			63.4	34.6	2.0	5
Phalangid Damon	sc					38.2		6
King crab Limulus	SC				high	24.5		6
Limulus	SC					28.0		7
Millipedes Thyroglutus malayus Julus	SC SC	Light brown	8.7	2.5	10.1	33.8	55.0 56.3	5 8
Centipedes								
Scolopendra morsitans	SC	Orange brown	15.0	53.1	68.1	31.4	0.2	5
	AM	White	39.1	23.5	62.6	37.2	0.2	6
Scolopendra cingulata	SC				high	31.0		6
Lithobius	SC				high	31.0		o

SC, sclerite cuticle; AM, arthrodial membrane.

contents of the cuticle get reduced if the cuticle is cleaned in water or alcohol below 96% grade², the cuticle in these experiments was cleaned in 96% alcohol and dried in the oven at 95°C for 24 h. The cuticle was completely decolorized after 14 days in 15% potassium hydroxide. It was, therefore, not necessary to treat it with potassium permanganate and sodium bisulphite in the estimation of chitin. It was, however, washed thoroughly with distilled water followed by weak acid water. The calcareous diploped cuticle was analyzed in undemineralized condition since the values of chitin and arthropodin were found lower after 2% HCl³ or 30% aqueous soln. of sodium hexametaphosphate⁴.

The percentage values of the components of dry weight of cuticle are summarized in the Table. Data available for other arachnids and myriapods is also given for the sake of comparison.

From a review of the percentage of each cuticular component of the different types of cuticles shown in the Table giving almost the same values for chitin, it is clear that chitinization is independent of the extent of sclerotization. The observation for the arachnid and myriapod cuticle confirms that for the cockroach *Periplaneta americana*⁹. Considerably higher values for chitin are reported for the calcareous cuticle of diplopods⁶. Using a different method, chitin values in the calcareous cuticle of a diplopod were obtained which, however, tally with those of the other arthropods. This shows that chitinization is also independent of mineralization.

It is also evident that quite a significant amount of arthropodin is present even in the sclerite cuticle of these arachnids and myriapods. Its value in the sclerite cuticle of these arthropods decreases with the increasing degree of sclerotization, i.e. from cuticles of various shades of brown to the black cuticle.

The amount of extractable protein in the diplopod cuticle is, however, very little as compared to that of the other arthropods. It appears that it stands in inverse relationship to the amount of mineralization. The mineralization therefore seems to have taken the place of a large proportion of the protein that would have otherwise been needed for hardening. A very low value of sclerotin shows that out of the total protein only a very little undergoes sclerotization in diplopods.

Zusammenfassung. Es werden Angaben über den prozentualen Gehalt der cuticularen Bestandteile einiger indischer Spinnen und Myriapoden gemacht. Die Chitinisierung ist unabhängig von der Sklerotisierung und bei Diplopoden unabhängig von der Verkalkung. Bei Diplopoden wurde ein sehr geringer Gehalt an extrahierbarem Protein festgestellt, wohl bedingt durch die starke Verkalkung.

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- A. G. RICHARDS, The Integument of Arthropods (University of Minnesota Press 1951).
- ² G. FRANKEL and K. M. RUDALL, Proc. R. Soc. London, Ser. B 134, 111 (1947).
- ³ M. Lafon, Bull. Inst. océanogr. Monaco 45, 1 (1948).
- ⁴ R. A. C. Wilks, Nature 142, 958 (1938).
- 5 Present author.
- ⁶ M. Lafon, Annls Sci. nat., sér. Bot. Zool. 11, 113 (1943).
- ⁷ A. G. Richards, Science 109, 591 (1949).
- 8 A. Kelly, Jena Z. Naturwiss. 35, 429 (1901).
- ⁹ F. L. Campbell, Ann. ent. Soc. Am. 22, 401 (1929).