Résumé. Après stimulation électrique de la gencive chez le singe et du nerf lingual chez le chat, une réponse réflexe de courte latence à été enregistrée au niveau du muscle temporal et du masseter. La latence de cette réponse est plus rapide que celle de la réponse digastrique induite par le même stimulus. La réponse temporale de courte latence peut également être observée en l'absence tant d'une réponse digastrique que du réflexe d'ouverture de la gueule.

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The Effect of *Lophozozymus pictor* Toxin on HeLa Cells

A crude extract of the coral reef crab, *Lophozozymus pictor*, has recently been reported to be toxic to rats and mice. Large doses rapidly depress the respiration and blood pressure and death follows within a few minutes; with low doses the action is more insidious and death only occurs after 20 or 30 h[1]. These findings suggested that the toxin may have a general cytotoxic action; HeLa cells have been used to study this possibility.

The toxic extract was prepared as described earlier[1]. 1 ml of extract contained 300 mouse units (MU)[2]. HeLa cells (Strain E, Commonwealth Serum Laboratories, Australia) were grown in monolayers in medium 199 (Burroughs Wellcome & Co.) containing 10% foetal calf serum (DIFCO), penicillin 100 units/ml and streptomycin 100 µg/ml in non-corrosive borosilicate culture tubes with non-toxic silicone rubber stoppers. The tubes were incubated in a Model T26 Rollertherm incubator at 37°C and rotated at $^1/_5$ g/min Trypsin 0.25% (Type III, Sigma Chemical Co.) was used for trypsinization of the cells and cell counts were made with a Neubauer haemocytometer.

The effect of the extract on HeLa cell attachment to glass was tested. Freshly introduced HeLa cells will normally attach themselves to the inside surface of the tube after about 2 h. Aliquots of approximately 1×10^5 cells in 1 ml medium were distributed in culture tubes. The extract (300 MU/ml) was added to the tubes to give concentrations from 0.006 to 60 MU/ml. Tubes with no toxin added were used as controls. All tubes were incubated for 48 h and observed with an inverted microscope at regular intervals for cell attachment to glass. The results show that at concentrations higher than 0.006 MU/ml, HeLa cells failed to adhere to glass (Table I).

Table I. Effect of *Lophozozymus pictor* toxin on HeLa cell attachment to glass

Toxin mouse units/ml	HeLa cell attachment to glass after 48 h incubation
0 (Control)	yes
0.006	yes
0.06	no
0.6	no
6.0	no
60.0	no

The effect of the toxin on cellular morphology was next investigated. Similar aliquots of HeLa cells as above were first distributed in tubes and allowed to adhere and grow for 48 h. The medium was then replaced with 1 ml aliquots of medium containing concentrations of toxin ranging from 0.003 to 3.0 MU/ml. Control tubes contained no toxin. The tubes were observed for cell toxicity over a 96 h period. Toxicity was correlated to the rounding of the polygonic cells, the appearance of granules and refractile bodies in the cytoplasm and cell lysis.

The cytoplasm of many of the cells stained less prominently with alcoholic eosin than the controls. The cells detached from the glass tubes within 20 h after cytotoxicity was first noted. The minimal concentration required to give a toxic effect was 0.006 MU/ml. The period before the appearance of cytotoxicity in 50% of HeLa cells varied with the dose of toxin and ranged from 10 min to 4 days (Table II). These results are of interest since in the whole animal the death times also varied from a few min to 30 h depending on the dose[1].

Table II. Effect of *Lophozozymus pictor* toxin concentration on the time of appearance of cytotoxicity in HeLa cells

Toxin mouse units/ml	Time at which cytotoxicity was observed in 50% of HeLa cells
0 (Control)	no toxicity after 4 days
0.003	no toxicity after 4 days
0.006	4 days
0.03	3–5 h
0.06	$1^1/_2$–2 h
0.3	35–60 min
0.6	25 min
3.0	10–15 min

The results of this study show that HeLa cells provide a more sensitive assay for the toxin than the mouse toxicity test. This cell culture method is therefore useful in following the purification of the toxin.

Zusammenfassung. Ein wässeriger Extrakt einer besonderen Krabbenart (*Lophozozymus pictor*) erweist sich als toxisch gegenüber HeLa-Zellen. Es kommt zu Umwandlungen der Zellform sowie des Cytoplasmas. Die Reaktion scheint leicht erfassbar und ist für einen Toxizitätstest besser geeignet als der bis jetzt verwendete Mäusetest.

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