Tetracycline Effects on Statolith and Nematocyst Differentiation in *Aurelia*

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The effect of tetracycline HCl on synthesis of calcium sulphate statoliths in *Aurelia* is reported. Tetracycline inhibits synthesis of statoliths and nematocysts when administered at an early stage of strobilation. The tetracycline, however, is not incorporated into the developing statoliths or nematocysts. As the tetracycline does not combine with the calcium of the calcium sulfate dihydrate statoliths of *Aurelia*, an explanation for its inhibitory effects on statoliths and nematocyst differentiation apparently does not rest with an incorporation-related factor.

In vitro studies of four inorganic calcium systems and tetracycline revealed that calcium sulfate dihydrate (gypsum) did not incorporate tetracycline nor did its isostructural equivalent, calcium hydrogen phosphate dihydrate (brushite). Calcium carbonate and calcium phosphate (apatite) did incorporate tetracycline. The explanation for these different behaviors of calcium can be found in the crystal structure of the respective compounds, namely, whether or not the Ca ion is readily available to react with tetracycline.

Key words: Tetracycline --- Development --- Calcification --- Statolith --- Nematocysts --- *Aurelia*.

L’effet de la tétracycline HCl sur la synthèse des statolithes de sulfate de calcium chez *Aurelia* a été étudié. La tétracycline inhibe la synthèse des statolithes et nématecystes à un stade précoce de strobilation. La tétracycline, cependant, n’est pas incorporée dans les statolithes ou nématecystes en formation. Comme la tétracycline ne se combine pas avec le calcium des statolithes de sulfate de calcium dihydraté d’*Aurelia*, l’explication des effets d’inhibition sur la différenciation de statolithes et nématecystes ne semble pas liée avec un facteur en rapport avec l’incorporation.

Des études in vitro de quatre systèmes inorganiques de calcium et de tétracycline montrent que le sulfate de calcium dihydraté (gypse) n’incorpore pas la tétracycline: il en est de même de son équivalent isostructuraux, le phosphate de calcium hydrogéné dihydraté (brushite). Le carbonate de calcium et le phosphate de calcium (apatite) incorpore la tétracycline. L’explication des différences de comportement du calcium peut être liée à la structure cristalline des composés respectifs, et, en particulier, au fait que l’ion Ca est prêt ou non à réagir avec la tétracycline.

Es wird über die Wirkung von Tetracyclinchlorhydrat auf die Synthese von Calciumsulfat-Statolithen bei *Aurelia* berichtet. Wird das Tetracyclin in einem Frühstadium der Strobilation verabreicht, so hemmt es die Synthese der Statolithen und der Nematocysten. Das Tetracyclin wird jedoch nicht in die sich bildenden Statolithen oder Nematocysten eingebaut. Da sich das Tetracyclin nicht mit dem Calcium der Calciumsulfatdihydrat-Statolithen der *Aurelia* verbindet, so kann dessen Hemmwirkung auf die Statolithen und die sich differenzierenden Nematocysten offenbar nicht mit einem einbaubedingten Faktor erklärt werden.

Untersuchungen, die in vitro mit vier verschiedenen anorganischen Calciumsalzen und Tetracyclin ausgeführt wurden, zeigten, daß weder Calciumsulfatdihydrat (Gips), noch dessen...
isostrukturelles Aequivalent Calciumhydrogenphosphatdihydrat (Bruschit) Tetracyclin ein- 
bauen. Dagegen inkorporieren Calciumcarbonat und Calciumphosphat (Apatit) das Tetra-
cyclin. Die Erklärung für dieses unterschiedliche Verhalten der Calciumsalze findet sich in 
der Kristallstruktur der betreffenden Verbindungen, d.h. es hängt davon ab, ob das Calciumion 
für die Reaktion mit Tetracyclin leicht verfügbar ist.

Introduction

The ability of tetracycline to incorporate into the bones and teeth of higher 
organisms is a well-documented phenomenon (Andre, 1956; Saxen, 1966; Bever-
lander et al., 1961; Owen, 1961; Wallman and Hilton, 1962). Less is known con-
cerning the incorporation of tetracycline into calcifying systems of lower animals.

Bevelander et al. (1960a, 1963) reported that tetracycline was readily in-
corporated into the developing sand dollar and into the shell of Pinna. Jensen 
and Cumming (1967) discovered that tetracycline was taken into the scales of 
the winter flounder and the otoliths of the cod. Tetracycline has also been found 
to affect development adversely in lower organisms as well as higher ones (see 
Bevelander, 1965, for review).

Jellyfish polyps which synthesize calcium sulfate statoliths during meta-
morphosis (Spangenberg and Beck, 1968) provide a model system lor the in-
vestigation of possible tetracycline uptake into a calcium sulfate system and for 
the study of potential developmental effects of tetracycline on the organism. This 
paper reports the results of an investigation, including inorganic incorporation 

Methods and Materials

Tetracycline Testing. Aurelia aurita polyps from cultures maintained under controlled 
conditions in this laboratory were used. Methods for culture of the organisms and induction 
of strobilation (metamorphosis) are described elsewhere (Spangenberg, 1965, 1967). Only 
organisms in a very early stage of strobilation (with one segment and tentacles) were used for 
testing (Fig. 1). The organisms were placed in small culture dishes containing tetracycline HCl 
solutions ranging from 1 to 12 mg per cent in concentration. The strobilae remained in the 
tetracycline throughout the experiment at 27°C in the dark and they were not fed during the 
testing period. The organisms were examined daily for ephyra development. After 72 to 96 h 
the ephyrae matured and were released from the strobilae (Fig. 1). Wet films were made of 
the ephyrae in a drop of the test solution and examined under a Leitz Ortholux phase micro-
scope. The number of statoliths in each rhopalin was recorded for each organism. The general 
size of the statoliths was also noted and the number of small statoliths (those less than one-
third the size of an average statolith) was recorded. Nematocyst number was estimated 
throughout the test organisms and controls, and the quantity of nematocysts, was noted 
particularly in the nematocyst patches at the base of the lappets of the ephyrae (Fig. 1).

The tetracycline HCl used for these studies was taken from fresh stock (Nutritional Bio-
chemical Corp.). Several batches of tetracycline with a shelf-life past six weeks (under re-
frigeration) did not evoke the developmental changes achieved with recently purchased 
compounds. The tetracycline solutions were prepared in buffered artificial sea water (Spangen-
berg, 1965) immediately before testing. Minor pH changes were equivalent in all concentrations 
of the tetracycline.

Fluorescence Examination of Organisms. Approximately 100 ephyrae from strobilae de-
vloping in a range of 1–12 mg per cent tetracycline were examined under a Leitz Laborlux 
microscope equipped for fluorescence microscopy. The ephyrae were from strobilae placed in 
the tetracycline solutions during early strobilation. A wet film was made of the ephyrae