

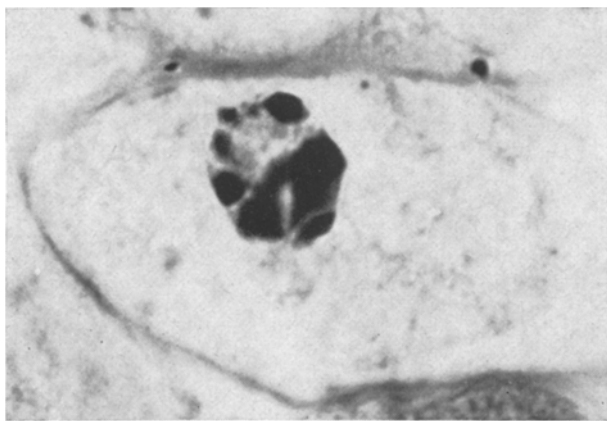


Fig. 5. Karyorhexis induced by 48 h treatment with vernoflexuoside solution (10^{-3} M/l).

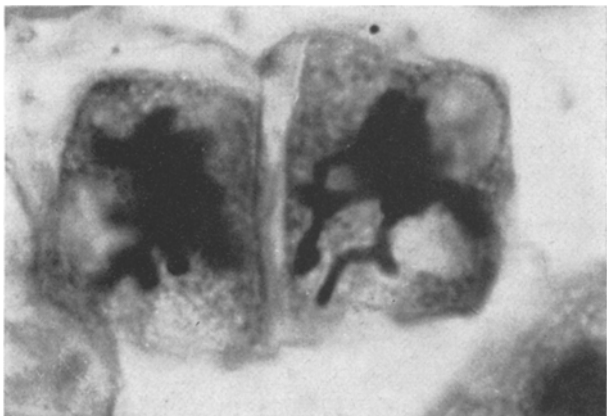


Fig. 6. Irregular congregation and partial agglutination of chromosomes in metaphase induced by 24 h treatment with vernoflexuoside solution (10^{-5} M/l).

The onion roots treated for 48 h with Vf solutions retained the ability to continue their growth and normal development when they were washed and transferred into tap water again. This may be proof that Vf does not show cytotoxic effect in the concentrations investigated.

On the basis of microscopical analysis of the squashes, the mitotic index values for each concentration of Vf solutions were calculated (Figure 2).

The following concentrations of Vf aqueous solutions produced mitotic disturbances or nucleus structure degeneration symptoms: 1. chromatine margination (Figure 3), at 10^{-3} – 10^{-4} M/l concentration of Vf after 24 and 48 h; 2. pycnosis (Figure 4) at 10^{-4} – 10^{-5} M/l concentration after 4, 24 and 48 h; 3. karyorhexis (Figure 5) at 10^{-3} – 10^{-4} M/l concentration after 48 h; 4. irregular chromosomes congregation and partial agglutination in metaphase (Figure 6) at 10^{-4} – 10^{-6} M/l concentration after 24 and 48 h.

Among these mitotic disturbances and symptoms of nucleus structure degeneration, pycnosis was the most frequent phenomenon. According to GULJAJEV⁶, pycnosis occurs after chromatin margination and its final step is karyorhexis. These three phenomena were very distinct in the material investigated when treated with Vf in 10^{-3} – 10^{-5} M/l concentrations. These observations lead to the conclusion that Vf shows some of the chromatoclassical properties⁷.

The calculated mitotic indexes confirm the very significant cytostatic activity of Vf in concentrations of 10^{-3} and 10^{-4} M/l (Figure 2). This activity may be attributed to the conjugated α -methylene- γ -lactone grouping, which occurs in Vf structure. KUPCHAN⁸ underlined the fact that these groupings may inhibit phosphofructokinase in cells by binding the active SH-group of the enzymes. The interaction of sesquiterpene α -methylene- γ -lactones with thiol groups is well established and it may explain biological activity of these compounds.

⁶ W. A. GULJAJEV, in *Kljetočnoje jadra i jeno ultrastruktury* (Nauka, Moskva 1970).

⁷ G. DEYSSON, Soc. bot. Fr., *Mémoires* 117, 95 (1970).

⁸ S. M. KUPCHAN, D. C. FESSLER, M. A. EAKIN, T. J. GIACOBBE, *Science* 168, 376 (1970).

Bakterien im Ovar aquariengehalteter Meeresfische

Bacteria in the Ovary of Marine Fishes Held in Aquaria

K. J. GÖTTING¹

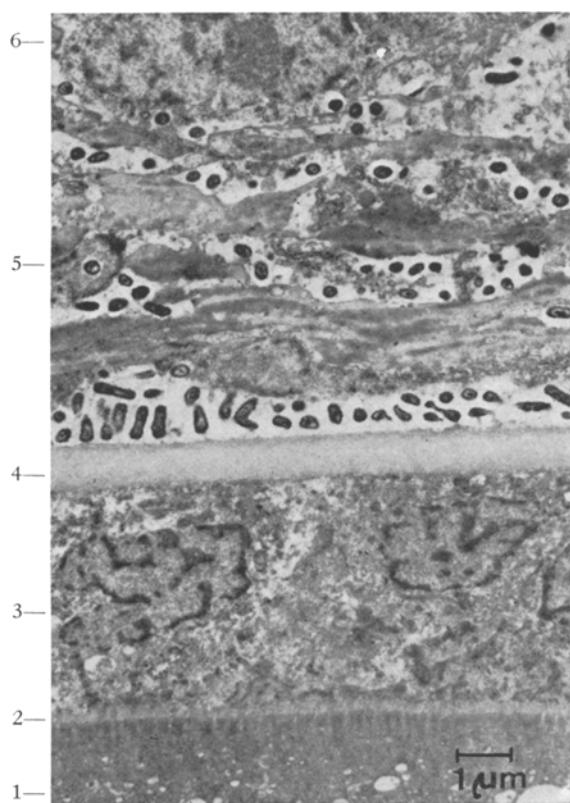
I. Zoologisches Institut der Justus-Liebig-Universität, Stephanstrasse 24, D-63 Giessen (Bundesrepublik Deutschland, BRD), 28. August 1975.

Summary. In the ovary of the ovoviparous teleost *Zoarces viviparus* L. kept in aquaria, gram-negative bacteria are found. These penetrate the tissue up to the basement membrane which separates the follicle epithelium from the theca.

In Aquarien gehaltene Meeresfische sind auch dann zahlreichen negativen physischen und physiologischen Einflüssen ausgesetzt, wenn der derzeit übliche technische Standardaufwand getrieben wird (Kühlung, Belüftung, Abschäumung usw.). Es ist seit langem bekannt, dass die ♀♀ vieler mariner Fische in Gefangenschaft Schwierigkeiten haben, ihre Eier abzusetzen und dann an Legenot zugrundegehen können. Dieses Problem wird

sich auch bei künftigen «fish farming» in grösserem Ausmass stellen.

Bei den ♀♀ lebendgebärender (ovoviviparer) Fische wie der Aalmutter (*Zoarces viviparus* L.) verzögern sich im Aquarium die Oogenese und die Embryonalentwicklung. Die meisten Embryonen sterben vor der Geburt. Früh absterbende Embryonen können offensichtlich – wie degenerierende Oocyten – resorbiert werden, während in



Dünnschnitt durch die peripheren Teile der Eizelle und die umgebenden Follikelschichten. *Zoarces viviparus*, ♀, 13 cm (Nr. 114; fixiert am 14.11.68. Glutaraldehyd, Vestopal W). 1. Oocytoplasma; 2. Cortex; 3. Follikel epithel; 4. Basallamelle; 5. Theca folliculi mit zahlreichen Bakterien in den Interzellularräumen; 6. Bindegewebe.

der Endphase absterbende Embryonen den Tod des Muttertieres bedingen. Ausserdem entwickeln sich im Ovar zahlreiche gramnegative Bakterien, für die die schleimige, mucopolysaccharidhaltige Ovarialflüssigkeit² ein günstiger Nährboden zu sein scheint. Interessant ist, wie weit die Bakterien zu den Eizellen vordringen. Jede Oocyte ist umgeben von einer Rindenschicht (Cortex; Figur). Darauf folgen nach aussen^{3,4}: perioocytärer Interzellularraum, Follikel epithel, Basalmembran, Theca folliculi und weiteres Bindegewebe. Das Bindegewebe ist stark vaskularisiert und von erweiterten Interzellularräumen durchsetzt. In diesen flüssigkeitserfüllten Räumen dringen die Bakterien vor, und zwar bis an die Basalmembran. Diese fungiert also nicht nur als Ultrafilter, sondern auch als Bakterien-Barriere. Bakterien erzeugen zahlreiche Fischkrankheiten^{5,6}. Ihr Einfluss auf den Verlauf der Oogenese bei *Zoarces* ist unbekannt. Bei freilebenden Tieren wurden sie bisher nicht beobachtet.

¹ Der Deutschen Forschungsgemeinschaft danke ich für die Förderung der Arbeiten, Herrn Prof. Dr. P. GIESBRECHT, Berlin, für Beratung.

² I. WEGMANN und K. J. GÖTTING, Z. Zellforsch. 119, 405 (1971).

³ K. J. GÖTTING, Micron, Lond. 1, 356 (1970).

⁴ K. J. GÖTTING, VIII. Int. Congr. Electron Microsc. (Canberra) 2, 668 (1974).

⁵ W. SCHÄPERCLAUS, *Fischkrankheiten* (Akademie-Verlag, Berlin 1954).

⁶ C. J. SINDERMAN, Adv. mar. Biol. 4, 1 (1966).

Evidence of a Pollen Esterase Capable of Hydrolyzing Sporopollenin

H. AHOKAS¹

Department of Genetics, University of Helsinki, P. Rautatiekatu 13, SF-00100 Helsinki 10 (Finland), 4 September 1975.

Summary. The activity of an esterase is associated with the germinal pore formation of the pollen grains of barley. Cytochemical evidence is given that the enzyme is capable of hydrolyzing sporopollenin. Some staining methods new to palynological studies were introduced.

Normally, the pollen grains of the graminaceous species carry one round germinal aperture, pore (Figure 1), the development of which was recently described². The male sterile barley mutant, *msg6cf*, was shown to produce inaperturate pollen³. The cytochemical studies of the mutant and non-mutant microspores revealed different sites of esterase activity. One of them, surface esterase, appeared in the primexine and then continued in the exine almost through the entire period of exine growth. Another was localized on the underside of the aperture region. This enzyme has been characterized as late poral esterase. These enzymes will be discussed below in connexion with some new staining methods developed during the study.

Methods. Napthol esters were mostly used as substrates for unfixed, frozen sections (Table). The cytoenzymological method took advantage of the simultaneous coupling method with freshly made HPR, hexazotate of pararosaniline (p,p',p"-tri-amino phenylmethylene HCl) as the

coupler^{4,5}. Cytological stainings were performed with unfixed frozen sections. Fast blue B salt (tetrazotized o-dianisidine) was applied as a filtered solution, concentration below 0.5% in 0.2 M phosphate buffer, at the final pH 8.0. Freshly made diazotate of p-nitroaniline was applied with the same method as HPR, but at a concentration 3 times higher, viz. 15 mM.

¹ Dr. J. R. ROWLEY is thanked for suggestions to the MS. The work was supported by the Finnish Academy and by a grant from the Eemil Aaltosen Säätiö Foundation, and from the Finnish Academy of Science.

² J. E. CHRISTENSEN and H. T. HORNER JR., Am. J. Bot. 61, 604 (1974).

³ H. AHOKAS, Ann. Bot. Fenn. 12, 17 (1975).

⁴ H. AHOKAS, Hereditas, 87, 33 (1975).

⁵ A. G. E. PEARSE, *Theoretical and Applied Histochemistry*, 3rd edn. (Churchill Livingston, Edinburgh, London 1972), vol. 2.