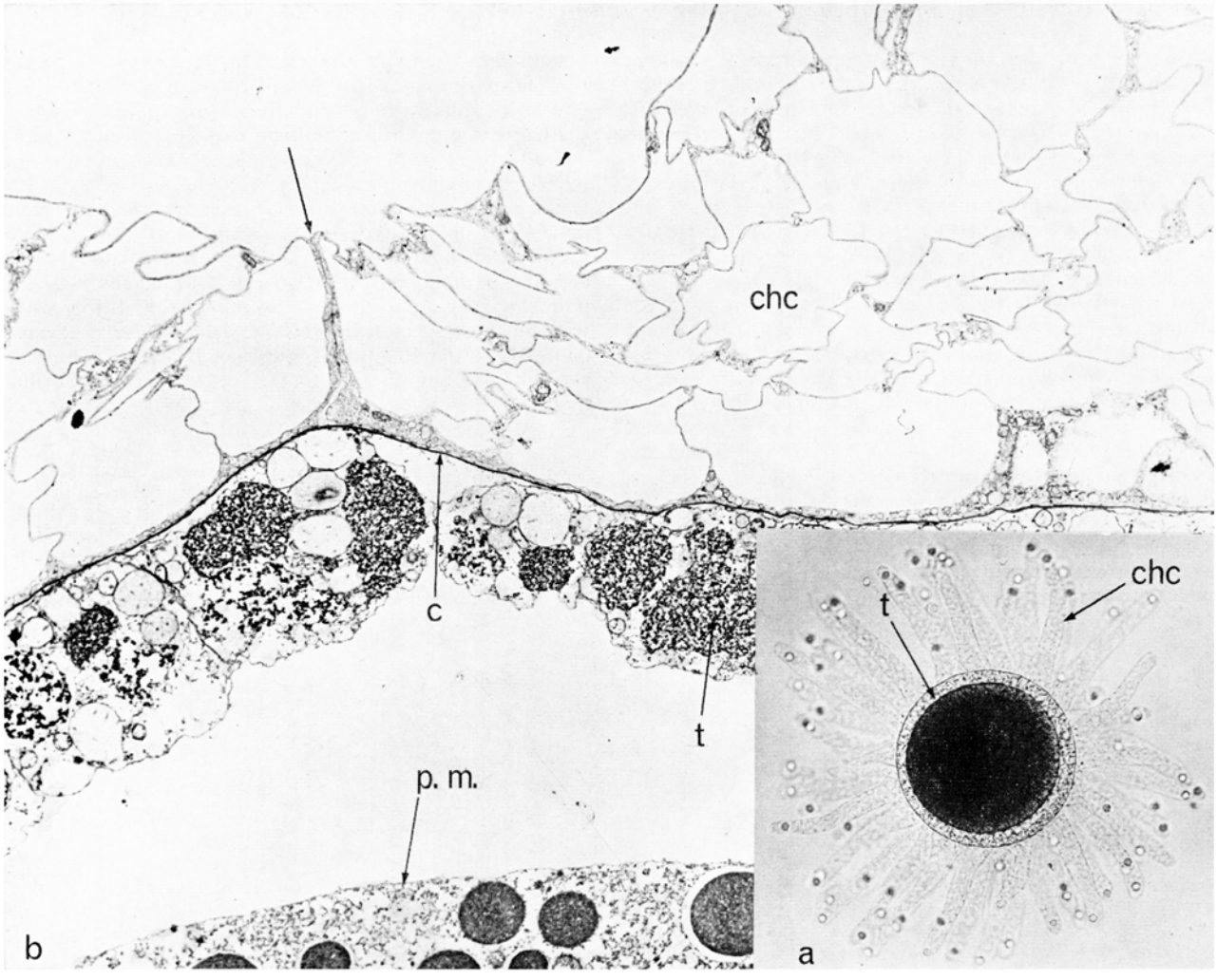

Fig. 2. Egg of *Ciona intestinalis*. (a) × 145; (b) × 4900. c, dense core of chorion; chc, chorion cell; p.m., plasma membrane; t, test cell. Arrow: area of contact between 2 chorion cells.

material. In *Ciona*, the test cells form a continuous sheet around the perivitelline space, whereas in *Ascidia* the test cells are widely spaced. In both egg types, the innermost envelope is a plasma membrane.

Basically then the 2 eggs do not differ in their envelopes. Both have chorion cells, chorion, test cells, and plasma membrane. Whether or not the merely quantitative differences that we observed are causally related to the difference in fertilization behavior was not studied in this analysis.

Zusammenfassung. Unbefruchtete Eier der Tunikaten *Ascidia nigra* (selbstfertil) und *Ciona intestinalis* (selbststeril) wurden elektronenmikroskopisch untersucht. Quantitative, nicht aber qualitative Unterschiede wurden in der Feinstruktur der Eizellen der beiden Arten gefunden.

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The Occurrence of Nerve Growth Factor in Teleost Fishes

BUEKER[1] investigating the effects of implanted tumors on the development of the nervous system of the chick embryo found that sarcoma 180 caused enlargement (hypertrophy and hyperplasia) of the spinal ganglia in the segments adjacent to it. LEVI-MONTALCINI and HAMBURGER[2] found that the sarcoma stimulated growth of the sympathetic ganglia to a greater extent than the spinal ganglia. Subsequently these workers isolated a 'nerve growth stimulating factor' from the sarcoma and devised an in vitro bioassay in which a spinal ganglion from a 7- or 8-day chick embryo was placed in hanging drop culture containing tissue culture medium, rooster plasma and the fraction to be assayed[3]. At optimum concentrations of the nerve growth factor (NGF) a dense

halo of outgrowing nerve fibers ('4-plus growth') is seen around the ganglion after 18–24 h of incubation at 37 °C. The assay is semiquantitative and growth is described as 1-plus, 2-plus, 3-plus and 4-plus as determined by the density and length of the nerve fibers.

Subsequently much richer sources of NGF were found in snake venom[4] and in mouse submaxillary salivary glands[5]. NGF has been detected in the serum and variety of organs, especially the axial region of mouse and chick embryos[6]. Moderate amounts have been detected in the axial region of young tadpoles, the sympathetic chain of new-born mice, and the axial region of goldfish[7]. The present research was undertaken to investigate the extent to which NGF could be detected in teleost fishes.

Materials and methods. A total of 12 families of fishes comprising 22 species was sampled for the presence of NGF. Most specimens were adults, with the exception of *Brachydanio rerio* and *Rhinichthys atratulus* which were young. In testing the various fishes for the presence of NGF, spinal axes were homogenized, centrifuged at 500 rpm for 10 min, and the supernatant mixed with tissue culture medium 199 in 5-, 25- and 125-fold dilutions. The bioassay was performed with 0.04 cm^3 of the fraction to be assayed and 0.02 cm^3 rooster plasma, using lumbosacral spinal ganglia dissected out from 7- or 8-day chick embryos. Control ganglia received only tissue culture medium. For small fish the entire spinal axis was homogenized. However, for large specimens with hard bones this was impractical, so the spinal cord and ganglia were dissected out and homogenized.

Results. The results of the bioassays performed on spinal axes of fishes from different families are summarized in the Table, and it can be observed that a variety of different species gave positive results. Many of the ganglia, including controls, showed extensive outgrowth of fibroblasts, which can be distinguished from nerve fibers. The strongest fraction from most of the fishes proved lethal to the ganglion, and various lipid droplets and cell debris were seen in these cultures. The 5- and 25-fold dilutions most frequently provided the best substrate for growth. Control ganglia showed, in general, no nerve outgrowth.

Results of bioassays of spinal axes of teleost fishes for NGF

Family	Species	Best growth
Cyprinidae	*Carassius auratus*	2-plus
	Brachydanio rerio	2-plus
	Pimephales promelas	0
	Rhinichthys atratulus	1-plus
	Notemigonus chrysoleucas	trace
Centrarchidae	*Ambloplites rupestris*	1-plus
	Lepomis macrochirus	1-plus
	Lepomis gibbosus	1-plus
	Pomoxis nigromaculatus	trace
Poeciliidae	*Xiphophorus helleri*	trace
	Gambusia manni	0
	Lebistes reticulatus	0
Cyprinodontidae	*Rivulus strigatus*	0
	Fundulus heteroclitus	trace
Pleuronectidae	*Pseudopleuronectes americanus*	0
Serranidae	*Centropristes striatus*	1-plus
Labridae	*Tautogolabrus adspersus*	1-plus
	Tautoga onitis	trace
Batrachoididae	*Opsanus tau*	trace
Tetraodontidae	*Spheroides maculatus*	0
Percidae	*Perca flavescens*	0
Anguillidae	*Anguilla rostrata*	trace

Discussion. Many species of fishes gave positive results when tested on embryonic chick ganglia indicating that their spinal axes have a nerve growth promoting substance. One should not conclude that the species which gave negative results lack NGF or a similar substance, since it may be that only young fish have enough to be detected by these methods, or that there was insufficient material in the preparations. Also, in a crude homogenate, the presence of proteolytic enzymes which could destroy the nerve growth promoting protein is likely. In this type of assay only a positive result is conclusive. A negative result is not since there are many factors which could alter the response. In many of the fish which gave positive results the growth obtained was comparable to that obtained from crude homogenates of spinal axes of other animals. Bueker, Schenkein and Bane[6] used microsomal, nucleoprotein, and protein fractions of the tissues assayed, and so obtained much better results than could be obtainable from a crude homogenate. Their use of embryonic material in large quantities is also favorable to good growth. With the majority of fishes tested only adults were available, and in very limited supply. Despite these drawbacks many species gave 1-plus and 2-plus activity. From the limited amount of data obtained it might appear as if fish in the family Cyprinidae have, in general, higher amounts of NGF in their axial regions than other families of teleosts. But this may be only an artifact due to the use of younger fish or the availability of larger amounts of material from larger numbers of specimens assayed together.

Conclusion. It has been shown that a number of species in a variety of families of teleost fishes possess, in their spinal axes, a nerve growth promoting substance which can cause outgrowth of nerve fibers from spinal ganglia explanted from 7- and 8-day chick embryos[8].

Résumé. Le «nerve growth factor» a été découvert dans les axes spinaux de quelques poissons lors d'un essai biologique, in vitro, provoquant la croissance de fibres nerveuses des ganglia spinaux des embryons de poulet.

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[8] Acknowledgments. I wish to express my sincere appreciation to Dr. A. Perlmutter for his interest and helpful suggestions. I wish to thank Dr. E. Bueker for his advice and the use of his equipment. This research was part of a dissertation in partial fulfillment of the requirements for the Doctor of Philosophy degree at New York University.
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