

Diagramme des absorbances à 230, 260 et 280 nm des fractions du ler pic de la colonne C2. Les absorbances des fractions du 2ème pic, qui commence ici au tube no 24, n'ont pas été mesurées. (Le foie est broyé dans LiCl 2 M, puis l'homogénat est centrifugé. Le surnageant est déposé sur une colonne C1 d'Ultrogel A2, équilibrée avec un tampon de force ionique élevée. L'absorbance à 260 nm de l'éluat de C1 est enregistrée. Sur une 2ème colonne C2 d'Ultrogel A 2, équilibrée avec un tampon de faible force ionique, on fait passer successivement de la protéase et le 1er pic de C1.)

Débit: 3 ml/cm² · h. Volume des fractions: 7,5 ml.

trent de plus l'absence d'ARN et de nucléotides. Une limite supérieure de contamination par l'ARN peut être estimée à 0,1%. Les «gros» ARN (ARN nucléaires géants, ARN ribosomiques) sont insolubles dans LiCl 2 M et sont déjà éliminés totalement ou presque dans la première étape de broyage suivi de centrifugation⁷. Si même il en restait dans le surnageant, ils seraient adsorbés sur C18. Les ARNt et les oligonucléotides par contre sont solubles dans LiCl 2 M et ne sont pas adsorbés sur l'agarose en milieu de force ionique élevée. Mais leur poids moléculaire est faible (26000 environ pour les ARNt), et ils sont totalement séparés des ADN par filtration sur Ultrogel A 2 dont la limite d'exclusion est de 50 · 106. Le taux d'ADN obtenu, déterminé par spectrophotométrie, est de 2,33±0,13 mg/g de tissu frais (il est de 1,3 pour Ganguli et al.3 et de 2 pour Kirby⁹). Le coefficient de sédimentation S_{20,w}, déterminé à l'aide d'une ultracentrifugeuse analytique M.S.E., est de 26,2 (les valeurs trouvées dans la littérature vont de 23 S^{1,9} à $24,4 S^3$).

- 1 J.E. Loeb et J. Chauveau, Biochim. biophys. Acta 182, 225 (1969).
- 2 J.P. Savitsky et F. Stand, Biochim. biophys. Acta 114, 419 (1966).
- 3 P.K. Ganguli, R. Brasseur et L. Gyenes, Prep. Biochem. 3, 313 (1973).
- 4 O.H. Lowry, N.J. Rosebrough, A.L. Farr et R.J. Randall, J. biol. Chem. 193, 265 (1951).
- 5 W.C. Hutchison et H.N. Munro, Analyst 86, 768 (1961)
- J.D. Mandell et A.D. Hershey, Analyt. Biochem. 1, 66 (1960).
 J.E. Besson, J. Champier, A.M. Chambosse, J. Radisson et M.
- J.E. Besson, J. Champier, A.M. Chambosse, J. Radisson et M. Berger, Can. J. Biochem. 56, 129 (1978).
- 8 S.L. Petrovic, J.S. Petrovic, R.A. Markovic et Z.A. Knezevic, Prep. Biochem. 4, 509 (1974).
- 9 K.S. Kirby, Biochem. J. 104, 254 (1967).

Cornea regeneration in the Pacific giant octopus, Octopus dofleini, and the common octopus, O. vulgaris

G. Dingerkus and E. D. Santoro¹

Department of Ichthyology, American Museum of Natural History, New York (New York 10024, USA), and Biology Department, City College of New York, New York (New York 10031, USA), 27 August 1980

Summary. Cornea regeneration in a Pacific giant octopus, Octopus dofleini, occurred within 10 days after the injury was observed. Surgical removal of the cornea in 2 common octopi, O. vulgaris experimentally duplicated this cornea regeneration within a 10-day period. It is, therefore, concluded that besides sucking discs, arms, and nerve fibres, octopi can also regenerate corneal tissue.

The regeneration of sucking discs, arms, and nerve fibres has been well documented in octopi²⁻³. However, there have been no reports of cornea regeneration in octopi. Past studies have revealed that certain gastropods have the capacity to regenerate eye tissues, however, their eyes are dissimilar to those of cephalopods, especially octopi⁴. Numerous similarities exist between the eyes of octopi and vertebrates⁵⁻⁷. However, the vertebrate cornea is derived from ectodermal and mesodermal components, whereas the cephalopod cornea is only ectodermal in origin. Among vertebrates, lens regeneration occurs in salamanders, and cornea repair, but not cornea regeneration, has been observed in various species⁸⁻¹⁰.

On 9 February 1979, a female Pacific giant octopus, Octopus dofleini, arrived at the New York Aquarium. The specimen, New York Aquarium No.02-415, was captured on 3 January 1979 by Blakely Rocks, Elliott Bay, off Seattle, Washington, at a depth of 20-25 m, and weighed approximately 18 kg with a 2.5-m arm-span. Upon arrival aquarist Peter Fenimore and the senior author noted that the left eye of the animal had been damaged and that the cornea was missing. A rough jagged edge of cornea, about 1 mm in width, was observed around the circumference of the eye (figure 1). The cause and exact date of injury could not be determined. The animal was placed into and maintained in a 200-gallon semi-open natural seawater aquarium at 4-7 °C, with a daily diet of 2.5 kg herring, mackerel, clam, or crabs.

The animal was inspected daily and on the 10th day after noting the injured eye, a new cornea was observed. At this

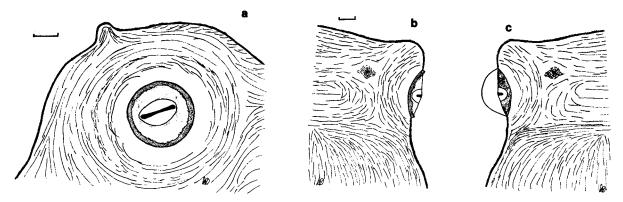


Fig. 1. Drawings of the Pacific giant octopus, O. dofleini, NYA 02-415, showing the extent of cornea damage to the left eye in a lateral and b frontal views; c the normal right eye in frontal view. Scale bars equal 1 cm.

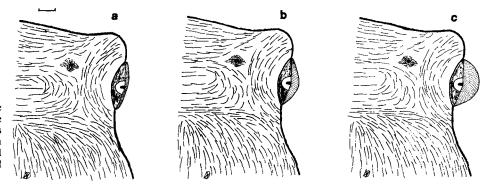


Fig. 2. Drawings showing the regenerated cornea on: a the 1 day it was observed, b 5 days later and c 10 days later, when the regenerated cornea had bulged outwards to its normal position. Scale bars equal 1 cm.

time the new cornea was initially in close proximity to the iris and gradually began to bulge outwards until 10 days after it was first seen it assumed the shape of the normal right cornea (figure 2). During this same period the regenerating cornea was at first semi-opaque in color. It gradually cleared to become transparent and indistinguishable from the unaffected eye.

The Pacific giant octopus, a display specimen, was not used to experimentally confirm the above observations. Instead, 2 female common octopi, *Octopus vulgaris*, were obtained. These were both captured off Key West, Florida, on 13 March 1979 and arrived at the New York Aquarium the following day. Each had approximately a 40-cm arm-span. They were maintained in separate 10-gallon open natural seawater aquaria at 22 °C. They were each fed 2-3 shrimps daily. After acclimation periods of 12 and 22 days, respectively on 3 March and 5 April 1979, a cornea from each octopus was surgically removed under cryoanasthesia. The cryoanasthesia was performed by placing the animal and approximately 3 1 of its tank water into an ice bath. The water temperature was dropped from 22 °C to 9 °C in

approximately 30 min. During induction of the cryoanasthesia respirations decreased until at 9°C they totally ceased and both animals ejected ink. At this stage they were totally immobile. Surgery was performed out of water and lasted about 15 min. Upon completion of the cornea removal the octopi were placed into clean cold seawater, which was slowly allowed to return to the original tank temperature. During this period the normal respiratory mode was re-established. Both octopi resumed feeding on the first postoperative day. Both specimens completely regenerated their corneas; one in 10 and the other in 9 days. The octopi were observed to hold the surgically altered eye withdrawn into their heads during their recuperative periods.

This evidence clearly shows that in addition to sucking discs, arms, and nerve fibres, octopi can also regenerate corneal tissue. The rapidity of cornea regeneration in octopi suggests that more research into the developmental and biochemical processes of this phenomenon may be useful to understanding the mechanisms underlying cornea regeneration.

- 1 The authors would like to thank U.E. Friese, B. Borowski, P. Burn, J.W. Atz, G.J. Nelson, H. Feinberg, P. Fenimore, C. Roper, M. Nixon, M. Segalla, G. Clark, D.O. Calligaro, J.M. Anderson and R.J. Koestler for assistance and advice. Illustrations were drawn by G.D. This work was supported by the New York Aquarium of the New York Zoological Society, while G.D. was at the New York Aquarium.
- 2 M. M. Lange, J. exp. Zool. 31, 1 (1920).
- 3 M.J. Wells, in: Physiology and Behavior of an Advanced Invertebrate: Octopus, p.280. Chapman and Hall, London 1978.
- F. Nonne, Roux Arch. 105, 430 (1925).
- 5 M.J. Wells, in: Physiology and Behavior of an Advanced Invertebrate: Octopus, p.143. Chapman and Hall, London 1978.
- 6 R.D. Barnes, in: Invertebrate Zoology, 3rd edn, p. 425. Saunders, Philadelphia 1974.
- 7 C.P. Hickman, in: Biology of the Invertebrates, 2nd edn, p. 402. Mosby, St. Louis 1973.
- 8 R.W. Reyer, Devl Biol. 14, 214 (1966).
- 9 R.W. Reyer, Q. Rev. Biol. 29, 1 (1954).
- 10 F.R. Johnson and K.M.H. McMinn, Biol. Rev. 35, 364 (1960).