

gations of serum neutralization (1) and the large amount of data available on the natural resistance of *C. adamanteus* to a wide variety of crotalid venoms (14) suggest that at least partial neutralization of other venoms by serum albumin may be expected as well.

WILLIAM C. CLARK

Yale College,
New Haven, Connecticut 06520

HAROLD K. VORIS

Department of Biology, Yale
University, New Haven, Connecticut

References and Notes

1. H. Peterson and T. Koivastik, *Z. Immunitätsforsch. Allergie Klin. Immunol.* **102**, 324 (1942); V. B. Philpot and R. G. Smith, *Proc. Soc. Exp. Biol. Med.* **74**(3), 521 (1950); V. B. Philpot, *Herpetologica* **10**, 158 (1954); P. J. Deoras and V. B. Mhasalkar, *Toxicon* **1**, 89 (1963).
2. B. R. Criley, in *Venoms*, E. E. Buckley and N. Porges, Eds. (AAAS, Washington, D.C. 1956).
3. F. E. Russell and R. Eventov, *Toxicon* **2**, 81 (1964).
4. J. T. Litchfield and F. Wilcoxon, *J. Pharmacol.* **96**(2), 99 (1949).
5. M. Phisalix, *Bull. Soc. Pathol. Exot.* **20**, 986 (1927).
6. S. Rosenfeld and S. Glass, *Amer. J. Med. Sci.* **199**, 482 (1940).
7. An EC470 vertical gel electrophoresis apparatus (E. C. Apparatus Corp.), 5 percent cyanogum gels, and a 0.1M tris-borate buffer system [tris(hydroxymethyl)aminomethane] at pH 8.9 was used. Samples were diluted one-to-one in a 5 percent glucose buffer prior to the application of 5 μ l to each pocket. Runs were made at 4°C, 300 volts, and 40 to 70 ma for 120 minutes.
8. M. G. Delaville and J. Delaville, *Ann. Pharm. Fr.* **12**, 109 (1954).
9. Add 0.5 ml of 10 percent TCA solution to each 1.0 ml of serum; centrifuge at 10,000 rev/min for 10 minutes; discard supernatant; add 8 ml of 95 percent ethanol to each 1.0 ml original serum sample; shake 2 minutes; centrifuge at 10,000 rev/min for 20 minutes; discard precipitate; dialyze supernatant against three changes of 0.005M pyridine solution for 72 hours; lyophilize.
10. J. Poortmans, J. Van Fraechem, M. Segers, *Biochim. Biophys. Acta* **127**, 380 (1966).
11. H. A. Sober, F. J. Gutter, M. M. Wyckoff, E. A. Peterson, *J. Amer. Chem. Soc.* **78**, 756 (1956). In this case a glass column (40 by 1.5 cm) was four-fifths filled with coarse DEAE-cellulose (Sigma Chemical Co.). The starting buffer was 0.02M KH_2PO_4 and 0.01M Na_2HPO_4 at pH 6.6; the second and third buffer were 0.17M KH_2PO_4 and 0.01M Na_2HPO_4 at pH 5.6 and 3.5, respectively. Column runs were made at 22°C, and elution buffers changed at 30-minute intervals.
12. B. W. Grunbaum, P. L. Kirk, W. A. Atchley, *Anal. Chem.* **32**, 1361 (1960). The buffer was 3.18M sodium 5,5-diethylbarbituric acid and 0.51M 5,5-diethylbarbituric acid at pH 8.6. The runs were made at 22°C, 150 volts, and 2.5 ma for 30 minutes.
13. P. F. Crosby, *Diss. Abstr.* **17**(10), 2137 (1957).
14. H. L. Keegan and T. F. Andrews, *Copeia* No. 4, 251 (1942); P. L. Swanson, *ibid.* No. 4, 242 (1946); A. A. Nichol, V. Douglas, L. Peck, *ibid.* No. 4, 211 (1933); H. K. Gloyd, *Science* **78**, 13 (1933); W. P. Allyn, *Proc. Indiana Acad. Sci.* **46**, 224 (1937).
15. Supported in part by NSF undergraduate research participation grant GY-4193. We thank Drs. C. G. Sibley and T. M. Uzzell for use of equipment, and Drs. C. I. Bliss and K. Corbin for their advice and assistance.

7 March 1969; revised 24 April 1969

Cartilaginous Dermal Scales in Cephalopods

Abstract. *Epidermal scales of the squid Cranchia scabra are composed of tissue which stains metachromatically with toluidine blue, and which is similar in appearance to hyaline cartilage.*

The origin of cartilage tissues in the vertebrates is a problem whose solution would contribute significantly to our knowledge and understanding of the earliest stages of vertebrate evolution. Because of the lack of information concerning the existence of cartilage tissues in early vertebrates, there is current disagreement on whether cartilage preceded bone in vertebrate evolution or vice versa and on whether the earliest fishlike vertebrate ancestors had bony or cartilage skeletons, or both. Romer (1) first proposed, and recently (2) reemphasized, that cartilage probably arose as an embryonic adaptation of early vertebrates, which permitted them to successfully cope with the problem of a rigid endoskeleton in a rapidly growing and transforming embryo. In Romer's view, bone preceded cartilage in the evolution of vertebrates. Denison (3) on the other hand, has supported the opposite view. Recently, Moss (4) attempted to reconcile these opposing views, as follows—"Romer (1964) forcefully states his position that cartilage did not precede the first appearance of bone (but rather is neotenus); others (Denison, 1963) adopt a contrary point of view. In evaluating this argument, it seems to me that an eclectic position may be held.

It seems to be reasonably certain that no one seriously contends that any ancestral form possessed a cartilaginous dermal skeleton, and similarly, no one doubts that the earliest Agnatha, as well as their presumed ancestors, possessed a cartilaginous endoskeleton. If these two skeletal sites are differentiated, it appears that both points of view may be reconciled." Moss's suggestion has been accepted by many workers as reasonable.

Until recently most biologists believed that cartilage, like bone, was a uniquely vertebrate tissue and was not to be found in the invertebrates (5). However, true endoskeletal cartilage does indeed occur in the invertebrates (6). Dermal cartilage tissues also exist in the invertebrates. In a study of cephalopods, Clarke (7) reported a squid *Lepidoteuthis grimaldii* with dermal scales similar in many ways to the scales of fish. We now report the existence of cartilaginous dermal scales in a squid *Cranchia scabra* from Florida coastal waters (8).

The transparent dermal scales of *C. scabra* are easily seen as individual projections (Fig. 1). The scales are made of rounded and ovoid cells (some apparently recently divided) suspended in a relatively homogeneous matrix (Fig. 2). Scale components stain metachromatically with the cationic dye toluidine blue, indicating the probable presence of acid mucopolysaccharides. The histological and histochemical appearances of this tissue are typical for a variety of hyaline cartilages, both invertebrate and vertebrate.

Although this finding does not bear

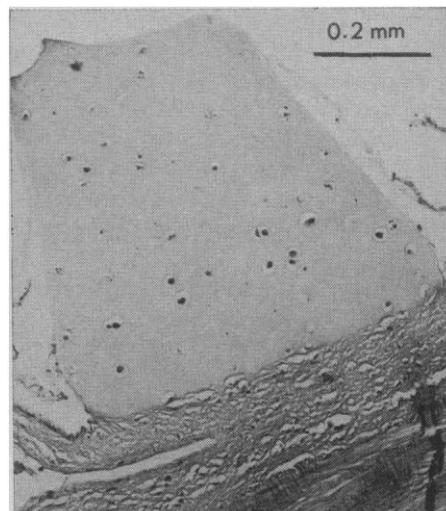
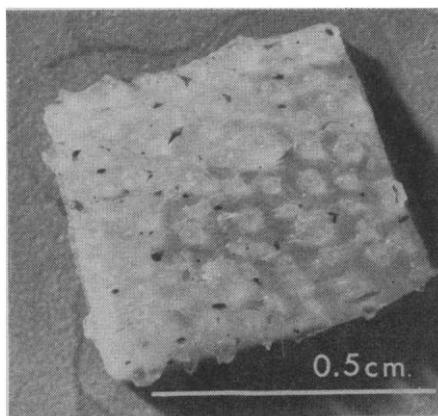


Fig. 1 (left). An excised square of skin of *Cranchia scabra* showing gross appearance of cartilage scales. Fig. 2 (right). Hematoxylin-eosin stained section through a cartilage scale and the connective and muscle tissues beneath. Histologic appearance of the scale is that of typical hyaline cartilage.

directly upon problems of vertebrate cartilage evolution, it is significant for study of such problems. By analogy, although cephalopod eye and brain have no direct evolutionary relationships with vertebrate eye and brain, the cephalopod organs have evolved remarkable similarities to the analogous vertebrate organs (9). In both classes of animals, these organs have also responded similarly to ecologic change and challenge. For this reason, study of cephalopod eyes and brains has contributed to our understanding of their vertebrate counterparts (10). I therefore believe that the existence of dermal cartilaginous scales in cephalopods should motivate us to open our minds to the possible existence of similar structures in the vertebrates. A critical reexamination of fossil and living vertebrates in the above perspective would surely be worth the effort (11).

Note added in proof: M. Moss has brought to my attention that some workers now believe that the first recognizable mineralized tissue in the vertebrates was a calcified cartilage in the dermal armor of ostracoderms (12). Thus, the critical reexamination suggested in my closing sentence is already under way, apparently with positive results.

PHILIP PERSON

Veterans Administration Hospital,
Brooklyn, New York 11209, and
Marine Biological Laboratory,
Woods Hole, Massachusetts 02543

References and Notes

1. A. S. Romer, *Amer. Natur.* 394 (1942).
2. ———, in *Bone Biodynamics*, H. M. Frost, Ed. (Little, Brown, Boston, 1964), p. 13.
3. R. H. Denison, *Clin. Orthop. Related Res.* 31, 141 (1963).
4. M. L. Moss, in *Biology of the Mouth*, P. Person, Ed. (AAAS, Washington, 1968), p. 37.
5. J. Schaffer, in *Handbuch der Mikroskopischen Anatomie des Menschen*, W. von Mollendorf, Ed. (Springer-Verlag, Berlin, 1930), Band 2, Teil 2, p. 1; L. Hyman, *The Invertebrates*, (McGraw-Hill, New York, 1940), vol. 1, p. 281; J. J. Pritchard, in *Biochemistry and Physiology of Bone*, G. H. Bourne, Ed. (Academic Press, New York, 1956), p. 1.
6. P. Person and D. E. Philpott, *Biol. Rev. (Cambridge)* 43, 1 (1969).
7. M. R. Clarke and G. E. Maul, *J. Zool. Proc. Zool. Soc. London* 139, 97 (1962).
8. I thank G. Voss and R. Young (Institute of Marine Sciences, Miami) for providing squid specimens and for informative discussions.
9. G. Wald, *Sci. Amer.* 201, 92 (1959).
10. J. Z. Young, *A Model of the Brain* (Oxford Press, Oxford, 1964).
11. It has recently been brought to my attention that S. Mirow and J. Rhodin (Dept. of Anatomy, New York Medical College) have found microscopic dermal cartilage bars in the skin of the common squid *Loligo pealii*.
12. T. Ørving, in *Current Problems of Lower Vertebrate Phylogeny*, T. Ørving, Ed. (Almqvist & Wiksell, Stockholm, 1968); R. H. Denison, *Fieldiana Geol.* 16, 131 (1967).

7 March 1969; revised 18 April 1969

20 JUNE 1969

Synaptic Vesicles in Electron Micrographs of Freeze-Etched Nerve Terminals

Abstract. Freeze-etched neuropil of the cat subformal organ was examined with the electron microscope for synaptic vesicles. Round vesicles were found exclusively in both unfixed and aldehyde-fixed specimens. Range of diameters and histograms failed to differ significantly between freeze-etched and conventionally prepared material. The mode of distribution of diameters was approximately 500 angstroms. Round stomata (approximately 350 angstroms in diameter) were found at the outer surface of the plasmalemma of nerve terminals; they are interpreted as pinocytotic vesicles.

The freeze-etching method, combined with electron microscopy (1), provides highly accurate views of profiles and surfaces of organelles and cells in the unfixed frozen state. Its application to nerve tissue has been particularly successful in the study of myelin (2), but no electron micro-

graphs of freeze-etched synapses have thus far been published. We have obtained suitable pictures from the cat subformal organ (SFO) which contains a large number of synapses in the neuropil (3). Twenty-eight subformal organs were carefully dissected and half were fixed with 3 percent buffered



Fig. 1. Freeze-etched neuropil of the cat subformal organ. Profiles of axon (ax) extending into presynaptic nerve terminal (pr). The bouton terminal contains many synaptic vesicles (sv) of various sizes. Two large concave spheric reliefs represent dark-cored vesicles (dv). Possible synaptic sites are blurred with shadows (sh). External surfaces (es) of axonal plasmalemma are characterized by granules and by pinocytotic stomata (arrows). One pinocytotic stoma is visible in the profile (double arrow). Glutaraldehyde fixation; primary magnification, $\times 20,000$.