difficult, and gel interpretation is straightforward. A single technician with homogenates of as few as 10^7 cells can comfortably analyze 10 to 20 cell lines for the seven allozymes discussed in a single workday (*30*).

STEPHEN J. O'BRIEN
GAIL KLEINER
Laboratory of Viral Carcinogenesis, National Cancer Institute, Bethesda, Maryland 20014
RUSSELL OLSON
Flow Laboratories, Rockville, Maryland 20852
JOHN E. SHANNON
American Type Culture Collection, Rockville, 20852

References and Notes

1. W. Nelson-Rees and R. P. Flandermeyer, *Science* **191**, 96 (1976); *ibid.* **195**, 1343 (1977).
2. ———, P. K. Hawthorne, *ibid.* **184**, 1093 (1974).
3. K. S. Lavappa, M. L. Macy, J. E. Shannon, *Nature (London)* **259**, 211 (1976).
4. S. M. Gartler, *ibid.* **217**, 750 (1968).
5. ———, *Natl. Cancer Inst. Monogr.* **26**, 167 (1967).
6. S. H. Hsu, B. Z. Schacter, N. L. Delanez, T. B. Miller, V. A. McKusick, R. H. Kennett, J. G. Bodmer, D. Young, W. F. Bodmer, *Science* **191**, 392 (1976); J. H. Kersey, E. J. Yunis, G. J. Todaro, S. A. Aaronson, *Proc. Soc. Exp. Med.* **143**, 453 (1973).
7. T. C. Hsu and K. Benirschke, *An Atlas of Mammalian Chromosomes* (Springer-Verlag, New York, 1967).
8. F. Montes de Oca, M. L. Macy, J. E. Shannon, *Proc. Soc. Exp. Biol. Med.* **132**, 462 (1969); S. J. O'Brien, G. Kleiner, J. Kern, J. Reynolds, *Isozyme Bull.* **9**, 20 (1976).
9. M. Nei, *Am. Nat.* **106**, 283 (1972).
10. H. Harris and D. A. Hopkinson, *Ann. Hum. Genet.* **36**, 9 (1972); *Handbook of Enzyme Electrophoresis in Human Genetics* (North-Holland, Amsterdam, 1976).
11. H. Harris, in *Isozymes, Genetics and Evolution*, C. Markert, Ed. (Academic Press, New York, 1975), vol. 4, p. 131.
12. R. Lewontin, *Annu. Rev. Genet.* **7**, 1 (1973).
13. R. A. Fildes and H. Harris, *Nature (London)* **209**, 261 (1966); S. Raplez, E. B. Robson, H. Harris, *Ann. Hum. Genet.* **31**, 237 (1967).
14. R. E. Tashian, *Am. J. Hum. Genet.* **17**, 257 (1965); *Proc. Soc. Exp. Biol. Med.* **108**, 364 (1961).
15. W. H. P. Lewis, *Excerpta Med. Int. Congr. Ser.* **233**, 111 (1971).
16. D. J. Giard, S. A. Aaronson, G. J. Todaro, P. Arnstein, J. K. Kersey, H. Dosik, W. Parks, *J. Natl. Cancer Inst.* **51**, 1417 (1973).
17. W. H. P. Lewis and H. Harris, *Ann. Hum. Genet.* **32**, 317 (1969).
18. N. Spencer, D. A. Hopkinson, H. Harris, *ibid.* **32**, 9 (1968).
19. R. Hirschhorn, *J. Clin. Invest.* **55**, 661 (1975).
20. Y. Edwards, D. A. Hopkinson, H. Harris, *Ann. Hum. Genet.* **35**, 207 (1971).
21. S. J. O'Brien and R. J. MacIntyre, in *Genetics and Biology of* Drosophila, M. Ashburner and T. R. F. Wright, Eds. (Academic Press, New York, in press), vol. 2.
22. G. J. Brewer, *An Introduction to Isozyme Techniques* (Academic Press, New York, 1970).
23. M. W. Steele, W. J. Young, B. Childs, *Biochem. Genet.* **2**, 159 (1968).
24. M. F. Perutz and H. Lehman, *Nature (London)* **219**, 902 (1968).
25. R. G. Davidson, *Ann. Hum. Genet.* **30**, 355 (1967).
26. N. Auersperg and S. M. Gartler, *Exp. Cell Res.* **61**, 465 (1970).
27. S. Povey, S. E. Gardiner, B. Watson, S. Mowbray, H. Harris, E. Arthur, C. M. Steel, C. Blenkinsop, H. J. Evans, *Ann. Hum. Genet.* **36**, 247 (1973).
28. S. J. O'Brien, *Isozyme Bull* **9**, 47 (1976); S. J. O'Brien, G. F. Kleiner, N. Takeichi, J. Heller, C. W. Boone, in preparation.
29. W. F. Benedict, *J. Natl. Cancer Inst.* **9**, 435 (1972); N. Rucker, C. Mark, R. Kouri, *ibid.* **54**, 157 (1975).
30. Since this manuscript was submitted, a similar analysis was reported by S. Povey, D. A. Hopkinson, H. Harris, L. M. Franks [*Nature (London)* **264**, 60 (1976)], using basically the same gene-enzyme system.
31. C. R. Shaw and R. Prasad, *Biochem. Genet.* **4**, 297 (1970).
32. F. H. Ruddle and E. A. Nichols, *In Vitro* **7**, 120 (1971).
33. D. A. Hopkinson and H. Harris, *Ann. Hum. Genet.* **30**, 167 (1966).
34. ———, *ibid.* **31**, 359 (1968).
35. A. Yoshida, E. Beutler, A. G. Motulsky, *Bull. WHO* **45**, 243 (1971); H. N. Kirkman, *Adv. Hum. Genet.* **2**, 1 (1971).
36. D. A. Hopkinson, M. A. Mestriner, J. Cortner, H. Harris, *Ann. Hum. Genet.* **37**, 119 (1973).
37. We thank Dr. Harry Harris for discussions and population data on gene-enzyme systems that influenced our selection of enzymes; and M. Macy and J. Simonson for technical assistance.

12 November 1976

Regional Specialization of Reptilian Scale Surfaces: Relation of Texture and Biologic Role

Abstract. *The iridescent body scales of the fossorial uropeltid snakes produce these interference colors by keratinous ridges spaced at 2500 Å. The pattern inhibits wetting of the surface and adhesion of soil particles and thus reduces friction between the snake's trunk and walls of its tunnel. The epidermal scales of the blunt tail show a sharply defined pattern of spines and ridges with convergent flutings. Dirt caught here forms a plug that protects the snake's caudal end. The sharp transition of surface textures suggests (i) that selection for each of the two roles is great, and (ii) that the interference colors of many fossorial snakes indicate that friction as well as dirt adhesion are being reduced.*

Recent studies (*1*) have resurrected the hypothesis (*2*) that the keratinized surface ornamentation of reptilian skin reduces wear and friction. This hypothesis may explain why certain reptiles have regular microstructure that produces interference colors of unknown function (*3*). Although this explanation may be plausible, it lacks proof and does not explain the diversity of structural patterns seen (*4*). We here describe a system in which the biological role of these microstructures changes along a well-defined line so that the associated structural differences may be analyzed.

The rough-tailed (or shield-tailed) snakes (family Uropeltidae) comprise some 35 relict species of specialized (*5*) fossorial animals restricted to the southwest Indian hill country and Sri Lanka. The cylindrical trunk of these elongate cone-headed animals is always extremely smooth and shows iridescence along bands parallel to the long axis (Fig. 1). The name of the group refers to a spinose patch of variable size on the truncate caudal tip, the rough area of which is always restricted to part or all of the distally visible zone. The transition between the two zones is sharp, and structural colors extend to the immediate edge of the rough tip.

Scanning electron micrographs (*6*) show the surface texture of the two regions. The widely overlapping scales along the trunk (Fig. 2E) of all species examined show an ornamentation of regular ridges on a spacing of 2500 Å (Fig. 2H). The grooves between the ridges contain round to oval pits at an apparently random spacing. The pits are sometimes filled with irregular matrix of uncertain origin (Fig. 2, G and I). The ridges are aligned parallel to the long axis of the body, even on scales, such as the lateroventral ones, that are asymmetrical. In general, the exposures of the topmost squamous cells are transversely subelliptical, and each ellipse has a pointed lateral tip. The sutures cross the ridges at near right angles, interdigitating for 2000 to 5000 Å on a 4000 to 5000 Å spacing, so that the ridge ends of successive scales do not align. When the dorsal surface of these snakes is examined in bright light (sunlight) with the visual axis just parallel to the path of the incident light, one sees a band of interference colors on each side of the midline with the colors ranging from blue (medially) to orange-yellow (laterally). The transition of colors corresponds to the changing spacing of ridges normal to the incident light around the curvature of the body.

The scales of the tail immediately fringing the sudden blunt termination show the same kinds of ridges and pores, except that the ridges are not aligned and often appear to be more shallow. More posteriorly, the surface undulates on a spacing greater than and independent of the surface exposures of the squamous cells of the keratinized Oberhäutchen. The actual architecture of the system is species specific (*7*) (in Fig. 2, compare A–C with F). The undulant surface may show one or more ridges per scale, and the terminal scale covering the distal tip tends to become enlarged and modified into a keratinized shield (possibly underlain by an enlarged terminal ossification of the vertebral column) (*8*), that bears sharp ridges and spines of different sizes in a regular pattern. Such spines and

ridges are always deeply fluted with the grooves convergent rather than parallel (Fig. 2D).

Uropeltids are found in various types of cohesive soils, mostly under a forest canopy. Even when excavated, they always show a clean and shiny trunk except for the caudal patch, which bears a plug of soil that is often as long as the animal's diameter. When the snakes crawl through semiliquid mud, this material is shed from the surface as the animal twists among fingers or grass stems. In contrast, the caudal plug sticks to the shield; even when the tip is rubbed, there remains a layer of adherent dirt.

Microscopy shows that the surface of the body scales is poorly wetted by drops of water, which form sharp edges and do not spread. However, the caudal surface is difficult to examine, as sand grains and other particles wedge among the convergent flutings ascending the spine. Other particles adhere to these mechanically captured particles, and the mud plug remains patent by internal cohesion.

The dirt-shedding mechanism clearly reduces the coefficient of friction between the snake and the wall of its tunnel. It permits the animal to remain clean even in the sticky muds in some of these areas with high rainfall. This is particularly critical because some of the soils become very hard when dry and could entrap or damage scales to which they adhere, as well as imposing very significant wear to a tunnel dweller. Reduction of "surface free energy" (*9*) with consequent inhibition of interaction of soils with the skin will then be advantageous.

The blunt uropeltid tail is not used for digging, and the animals have only a limited capacity to reverse and none to turn about in the tunnel. The dirt plug protects the snake against predators such as other snakes, ants, and beetles that may enter the tunnel behind it (*10*).

In some "primitive" uropeltids in which the caudal boss is smaller than the distal tip, the ridges and modification of adjacent scales extend outward to cover the posteriorly facing portion of the caudal boss. In contrast, the smooth zone extends just up to and around the widest diameter anterior to the blunt termination. The sharp transition from the ridges spaced at 2500 Å to the rougher zone, which generally occupies only one or two scales documents the selective advantage of keeping adhesion (as well as friction) to a minimum along the length of the trunk. The sharp transition also reflects the advantage of having the entire distal surface modified and mud-covered, thus keeping small predators from

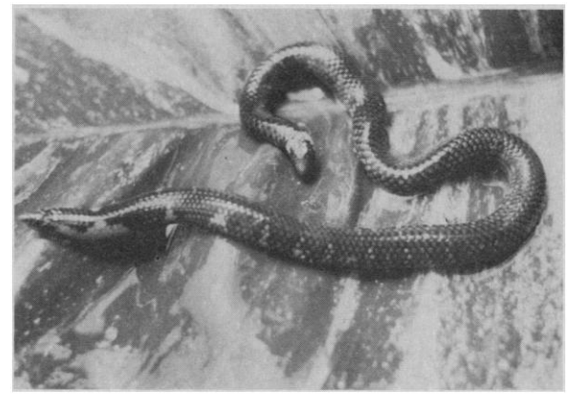

Fig. 1. Adult specimen of the uropeltid snake *Rhinophis blythi* from Sri Lanka. The pointed head (left) appears superficially like a tail, and the blunt, spotted tail (center) with its caudal shield gives the impression that it is the snake's head. In *R. blythi* the caudal shield is smaller than the distal portion of the tail, and the surrounding scales bear ridges and other irregularities up to the full diameter of the body. The length of the snake is approximately 30 cm.

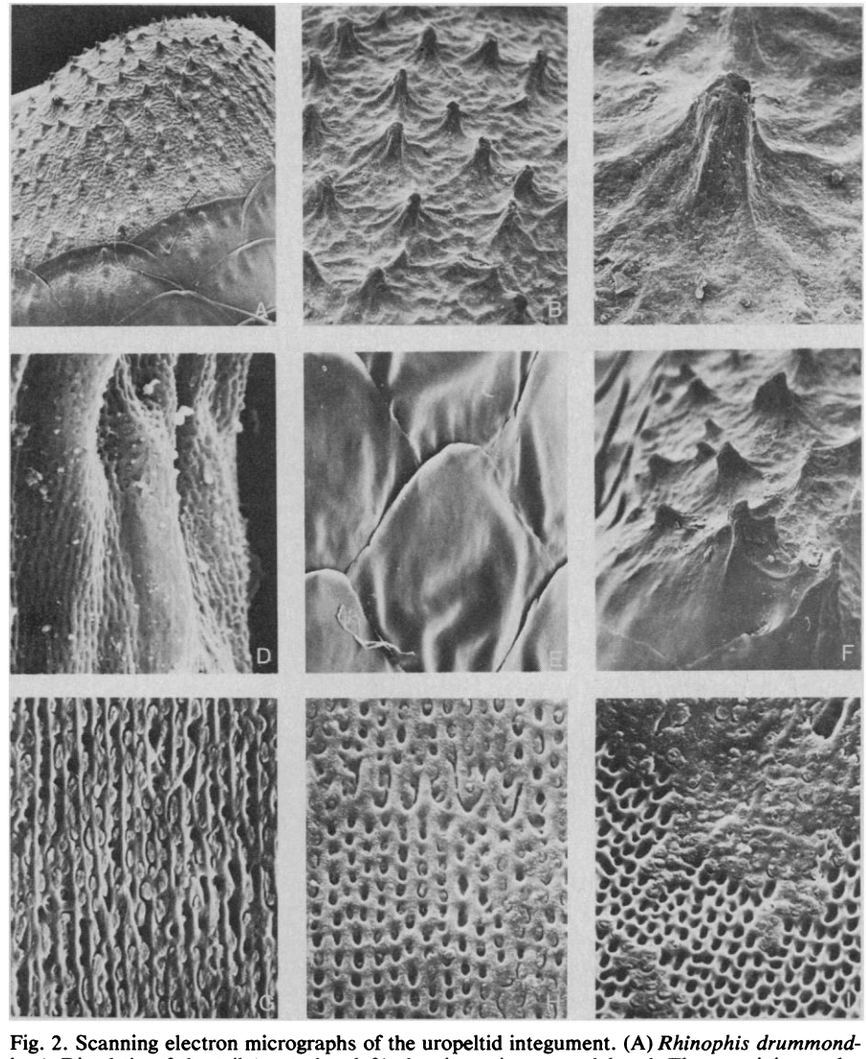

Fig. 2. Scanning electron micrographs of the uropeltid integument. (A) *Rhinophis drummond-hayi*. Distal tip of the tail (ventral to left) showing spinous caudal pad. The margining scales show some undulations at the sites of the underlying spines but no particular roughening of the surface (×10). (B) Same specimen showing the relative placement of the spines and their connecting ridges (×33). (C) Same specimen showing a single cone. Note the irregular fluting along the sides (×106). (D) *Uropeltis phillipsi*. Lateral view of a single projecting spine to show the irregular fluting among which the sand grains tend to be caught (×2600). (E) *Rhinophis philippinus*. Detail of dorsal scales to show their generally overlapping arrangement at midbody. The undulations represent an artifactual drying deformation (×33). (F) Same object as (D), lower left is ventral, to show the much more irregularly placed and relatively longer distal spines on the caudal pad of this species (×26). (G) *Rhinophis philippinus*. Detail of middorsal scales to show the striations and the intermediate pits, here filled with coating. View represents geometric center of (E) (×8000). (H) *Rhinophis drummond-hayi*. Detail of dorsal skin to show the interlocking ridges along the surface borders between two squamous cells (×8000). (I) *Rhinophis philippinus*. Detail of dorsal surface showing a patch of keratinous grid partially shielded by coating (×6700).

bypassing the shield to attack the body (*11*).

These observations confirm the advantage of a regularly ridged keratinized shield in reducing adhesion and friction. They may be extrapolated to explain the function of the smooth ventral shields of most snakes (as well as many lizards), and they indirectly document the advantages both of regular ecdysis and of more frequent shedding when the skin is damaged (*12*). The reduced friction promotes the effectiveness of lateral undulation, but the shedding of foreign objects is more important to elongate limbless animals that lack alternate methods of grooming. Consequently, the structural interference colors of reptiles lack specific function but are, instead, side effects (*13*).

CARL GANS
DUSAN BAIC

Division of Biological Sciences, University of Michigan, Ann Arbor

References and Notes

1. W. H. Fahrenbach and D. D. Knutson, *J. Invest. Dermatol.* **65** (No. 1), 39 (1975); C. Gans and D. Baic, in *Recherches Biologiques Contemporaires*. L. Arvy, Ed. (Vagner, Nancy, 1974), p. 265; M. K. Jackson and H. W. Reno, *Herpetologica* **31**, 350 (1975).
2. C. Gans, *Am. Zool.* **2** (No. 2), 167 (1962); *Nat. Hist.* **75** (No. 2), 10 (1966); *ibid.* (No. 3), 36; *Biomechanics* (Lippincott, Philadelphia, 1974).
3. M. Silveira, *Sci. Cult. Notas Technica* **13**, 79 (1961); E. A. Monroe and S. E. Monroe, *Science* **159**, 97 (1968).
4. S. Aotta, *J. Sci. Hiroshima Univ. Ser. B. Div. 1*, **30**, 109 (1940); A. R. Hoge and P. de Souza Santos, *Science* **118**, 110 (1953).
5. C. Gans, *Endeavour* **32**, 60 (1971); M. J. Rajendran, thesis, Madurai University, India (1973).
6. Excised specimens were prepared for scanning electron microscopy by critical-point drying and gold coating after treatment with alcohol and a detergent solution.
7. C. Gans, *Das Tierreich (Berlin)* **84**, 1 (1966); M. A. Smith, *The Fauna of British India. Reptilia and Amphibia*, vol. 3, *Serpentes* (Taylor and Francis, London, 1943); F. Wall, *Ophidia Taprobanica or the Snakes of Ceylon* (Colombo, Ceylon, 1921).
8. L. Baumeister, *Zool. Jahrb., Abt. Anat. Ontog. Tiere*, **26**, 1 (1908); R. Hoffstetter and J. P. Gasc, in *Biol. Reptilia* **1** (No. 5), 201 (1969).
9. R. E. Baier *et al.*, *Science* **162**, 1360 (1968).
10. The caudal pad also enhances the function of head mimicry, probably directed to jungle fowl and other common gallinaceous birds sharing the forest floor [C. Gans, in *Morphology and Biology of Reptiles*, A. d'A. Bellairs and C. B. Cox, Eds. (Linnean Society of London, London, 1976)]. The tail is stuck out and the pointed head is hidden when the snake is exposed on the surface. Eye spots alongside the cloaca, and lines more posteriorly, divert pecks to the caudal cap, which is protected by mud and bone.
11. Observations of snakes in artificial tunnels confirm that these snakes do try to block the opening with the tail when attempts are made to insert straws or similar small objects.
12. Disease states may often be detected by changes in the surface of a snake's skin, which then becomes undulant and dull and may show breaks in the free edges of the scales even before obvious lesions appear. Apparently shedding maintains a "low-energy surface" (*9*).
13. Examination of uropeltid color by itself in ignorance of its origin might well result in the assumption that the characteristics were selectively "neutral." However, the concept of neutrality should be applied to the totality of a character state rather than to a single way of viewing it.
14. We are grateful to many for helping us collect animals in the field as well as for cooperation from the Smithsonian Institution Entomological Research Project in Sri Lanka. Photographs were taken on the scanning electron microscopes (Jeol JFM-U3) of the Royal Ontario Museum (Division of Systematics) and at the Laboratory of Scanning Electronmicroscopy of The University of Michigan (Dr. W. C. Bigelow, director) and printed by D. Bay. S. P. Hollingsworth, R. Hewson, and E. Linn helped with the microscopy. Supported by NSF grant BMS 71 01380.

28 June 1976; revised 8 October 1976

Reconstitution of Chromatin Subunits

Abstract. *The recovery of the subunit structure of chromatin after dissociation and reconstitution is markedly affected by the procedure used. Some procedures give complete regeneration of subunits, but the procedure most commonly used for reconstitution gives poor yields of subunit-containing chromatin.*

Reconstitution of chromatin from its three main constituents, DNA, histones, and nonhistone chromosomal proteins, has been widely used to investigate transcriptional specificity (*1–3*) and chromatin structure (*4–10*). These studies have shown that the reconstituted chromatin may regain many of the properties of native chromatin, including specifically restricted transcriptional potential, thermal denaturation profile, circular dichroism spectrum, x-ray diffraction pattern, nuclease limit digest profile, and ultrastructure. In the case of transcriptional specificity, the presence of endogenous messenger RNA (mRNA) on the chromatin complicates the results (*11*). Nevertheless, when the presence of such contaminating RNA was taken into account, transcriptional specificity was still demonstrable in the reconstituted chromatin (*11*). In this report, the fidelity of various reconstitution techniques in regenerating the subunit (ν body) structure of chromatin (*12, 13*) is analyzed. It is shown that one of the methods commonly used for reconstitution gives poor yields of subunit-containing chromatin. Other regimes, however, give an excellent recovery of chromatin which is indistinguishable from the native starting material with respect to ultrastructure, sedimentation velocity, and nuclease sensitivity.

Chromatin subunit dimer fractions were collected from preparative sucrose gradients of micrococcal nuclease-treated chicken erythrocyte nuclei as previously described (*13*). Erythrocyte chromatin is uniformly heterochromatic, and contains no appreciable protease activity (*14*). After complete dissociation by dialysis into 2.5*M* NaCl, 6.0*M* urea, and $2.5 \times 10^{-4}M$ EDTA at *p*H 8.0, chromatin dimers were allowed to reassociate by gradient dialysis through various reconstitution procedures, the final buffer solution in each treatment being $2.5 \times 10^{-4}M$ EDTA (Table 1). Samples were removed at each stage in the treatment and prepared for electron microscopy by staining with aqueous uranyl acetate (*13*). The final reconstituted products were also analyzed on sucrose gradients both before and after a brief digestion with micrococcal nuclease, and peak fractions were examined with the electron microscope (Figs. 1 and 2). By this method, the proportion of reassociated fragments which regained the sedimentation properties of native dimers could be determined. Nuclease digestion of reconstituted dimers gave two further measures of the fidelity of the reassociation. First, the yield of monomers derived from the splitting of the dimers could be compared to that obtained by digestion of native dimers; and second, the amount of free DNA in the reconstituted chromatin could be monitored, because the conditions were such that naked DNA would be digested, and the breakdown products would appear at the top of the gradient.

Treatment of chromatin with 2.5*M* NaCl and 6.0*M* urea at *p*H 8.0, which is widely employed in reconstitution studies (*1–3*), dissociates all the histones (*10*) and most of the nonhistone proteins. In experiments with rat liver chromatin, it has been estimated (*15*) that 3 to 5 percent of the protein remained bound to DNA under similar conditions. In this study, the dissociated chromatin cosedimented with purified DNA on sucrose gradients, and appeared as linear fibers (2.5 nm in diameter) in the electron microscope (*16*). The DNA-histone complexes which formed as the dissociated chromatin was dialyzed to EDTA could be classified according to their ultrastructure as follows: native-like dimers consisting of two 8- to 10-nm spherical subunits interconnected by a 2.5-nm diameter fiber of DNA; particles with one subunit plus a 40- to 50-nm "tail"; particles consisting of one subunit but little or no tail; particles which had formed no subunits; and overlapping or aggregated material. Table 1 shows the distribution of particles obtained from the dimer regions of sucrose gradients when the various reconstitution procedures were used. Examination of the material ap-