between the two cell types and the intensity with which their cytoplasm is stained (*11*).

In some regions of the epidermis, cells of the stratum corneum are organized into discrete columns (*12*). These columns extend downward through the granular and spinous cell layers. A cluster of 10 to 12 basal keratinocytes is positioned beneath each column. This arrangement has been termed an epidermal proliferative unit (*13*). It has been proposed that the basal cells in the center of each column are stem cells. However, radiobiological and other data (*14*) indicate that stem cells are more likely to reside in the tips of deep rete ridges or follicles than in the epidermal proliferative unit. Our findings strongly support this prediction.

The structural and kinetic observations presented here demonstrate the existence in primate epidermis of a discrete population of basal cells whose properties satisfy the general criteria established for stem cells. These presumptive stem cells have a specific distribution in the epidermis and are easily identified morphologically. The epidermis, in which sequential stages of cell maturation are well preserved in histological sections, is readily accessible to experimental manipulation, and thus provides an excellent model for studies on the biological properties of stem cells.

ROBERT M. LAVKER
Department of Dermatology, Duhring Laboratories, University of Pennsylvania School of Medicine, Philadelphia 19104

TUNG-TIEN SUN
Departments of Dermatology, Ophthalmology, and *Cell Biology and Anatomy, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

References and Notes

1. A. B. Cairnie, P. K. Lala, D. G. Osmond, *Stem Cells of Renewing Cell Populations* (Academic Press, New York, 1976); D. W. Van Bekkum, G. J. Van den Engh, G. Wagemaker, S. J. L. Bol, J. W. M. Visser, *Blood Cells* **5**, 143 (1979); P. Quesenberry and L. Levitt, *N. Engl. J. Med.* **301**, 819 (1979).
2. P. Parakkal, W. Montagna, R. A. Ellis, *Anat. Rec.* **143**, 169 (1962); T. Miraglia, J. W. daCosta Nery, M. L. C. Guedes, *Acta Anat.* **96**, 97 (1976).
3. R. M. Lavker, *J. Invest. Dermatol.* **73**, 59 (1979).
4. A. S. Breathnach, Ed., *An Atlas of the Ultrastructure of Human Skin* (Churchill, London, 1971), p. 50; K. A. Holbrook and G. F. Odland, *J. Invest. Dermatol.* **65**, 16 (1975).
5. G. F. Odland and T. H. Reed, in *Ultrastructure of Normal and Abnormal Skin*, A. S. Zelickson, Ed. (Lea & Febiger, Philadelphia, 1967), p. 61; W. Montagna and P. F. Parakkal, Eds., *The Structure and Function of Skin* (Academic Press, New York, 1974).
6. To confirm the suprabasal location of labeled nuclei, three-dimensional reconstructions were made from six to ten serial sections, each 7 μm thick.
7. Epidermal cell division has generally been thought to be restricted to cells resting on the basal lamina [H. Pinkus, *J. Invest. Dermatol.* **19**, 431 (1952); K. Fukuyama and I. A. Bernstein, *ibid.* **36**, 321 (1961); G. D. Weinstein and E. J. Van Scott, *ibid.* **45**, 257 (1965); C. P. Leblond, R. C. Greulich, J. P. M. Pereira, in *Symposium on Wound Healing*, W. Montagna, Ed. (Pergamon, New York, 1964)]. However, a significant degree of suprabasal labeling has been reported in human epidermis [T. Ashihara, T. Kitamura, O. Takeoka, S. Fujita, M. Kodama, M. Shinoda, I. Hashimoto, *Arch. Histol. Jpn.* **28**, 399 (1967); N. S. Penneys, J. E. Fulton, G. D. Weinstein, P. Frost, *Arch. Dermatol.* **101**, 323 (1970)] and in cervical and vaginal epithelium [H. E. Averette, G. D. Weinstein, P. Frost, *Am. J. Obstet. Gynecol.* **108**, 8 (1970)].
8. R. M. Lavker and T. T. Sun, unpublished observations.
9. B. M. Kopriwa and C. P. Leblond, *J. Histochem. Cytochem.* **10**, 269 (1962); J. M. Lachapelle and T. Gillman, *Br. J. Dermatol.* **81**, 603 (1969).
10. There is evidence that basal keratinocytes in human epidermis are kinetically heterogeneous. Recently, R. A. Briggerman (*J. Invest. Dermatol.*, in press) grafted normal human upper thigh skin to the backs of nude mice. Beginning 6 weeks later, [^{3}H]thymidine was injected every 6 hours for 16 days. Autoradiographs revealed incorporation of [^{3}H]thymidine in the rete ridges, with a high degree of suprabasal labeling. These findings suggest that cells at the tips of rete ridges, identified here as nonserrated, are probably stem cells.
11. A. S. Breathnach, in *An Atlas of the Ultrastructure of Human Skin*, A. S. Breathnach, Ed. (Churchill, London, 1971), p. 108; A. N. Raick, *Cancer Res.* **33**, 269 (1973); A. J. P. Klein-Szanto, *J. Cutaneous Pathol.* **4**, 275 (1977); ———, S. K. Makor, T. J. Slaga, *Carcinogenesis* **1**, 399 (1980); T. J. Slaga, A. J. P. Klein-Szanto, L. L. Triplett, L. P. Yotti, J. E. Trosko, *Science* **213**, 1023 (1981).
12. I. C. Mackenzie, *Nature (London)* **222**, 881 (1969); D. N. Menton and A. Z. Eisen, *J. Ultrastruct. Res.* **35**, 247 (1971); I. C. Mackenzie, K. Zimmerman, L. Peterson, *J. Invest. Dermatol.* **76**, 459 (1981).
13. T. D. Allen and C. S. Potten, *J. Cell Sci.* **15**, 291 (1974); C. S. Potten, *Cell Tissue Kinet.* **7**, 77 (1974); W. J. Hume and C. S. Potten, *J. Oral Pathol.* **8**, 3 (1979).
14. C. S. Potten, in *Stem Cells and Tissue Homeostasis*, B. D. Lord *et al.*, Eds. (Cambridge Univ. Press, Cambridge, 1979), pp. 317–334.
15. We thank M. Cullen, P. Bonitz, and H. Santana for technical assistance. These investigations were aided by grants EY 02472 and AM 25140 from the National Institutes of Health. T.-T.S. is the recipient of NIH research career development award EY 00125.

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Ultrastructure of 40-Million-Year-Old Insect Tissue

Abstract. *Examination of the ultrastructure of preserved tissue in the abdomen of a fossil fly (Mycetophilidae: Diptera) entombed in Baltic amber revealed recognizable cell organelles. Structures that corresponded to muscle fibers, nuclei, ribosomes, lipid droplets, endoplasmic reticulum, and mitochondria were identified with the transmission electron microscope. Preservation was attributed to inert dehydration as well as the presence of compounds in the original sap which functioned as natural fixatives. This evidence of cell organelles in fossilized soft tissues represent an extreme form of mummification since Baltic amber is considered to have formed about 40 million years ago.*

Well-preserved fossilized soft tissues of animals are rare and have been examined with the light (*1*, *2*) or scanning electron microscope (*3*, *4*). Cell ultrastructure has apparently not been studied in soft tissues more than 1 million years old.

During routine examination of insect fossils in Baltic amber, a well-preserved female fly (Mycetophilidae: Diptera), which appeared to have internal soft tissues, was observed. Baltic amber is considered to have originated in the late Oligocene to early Eocene, or about 40 million years ago (*5*).

The piece of yellow transparent Baltic amber was broken through the abdomen of the fly. Some of the body cavity was filled with the original plant sap, which had since become amber. The rest of the body cavity was filled with Araldite 6005 and polymerized for 8 hours at 60°C. The blocks were trimmed and sectioned with glass knives (Porter-Blum MT2 ultramicrotome). Sections were treated with saturated aqueous uranyl acetate for 20 minutes and then with lead citrate for 5 minutes. The sections, mounted on slot grids, were observed in a Philips EM 300 electron microscope.

The amber specimen containing the fly was confirmed as being Baltic amber by infrared spectroscopy (*6*). Beck *et al.* (*7*) have shown that Baltic amber is distinguishable from related ambers by an absorption band of medium intensity at 1160 to 1150 cm^{-1} (8.6 to 8.7 μm) which is preceded by a more or less flat shoulder nearly 0.5 μm wide. From a 1.5-mg sample of the amber containing the fly specimen, a spectrum was obtained which matched that of well-preserved Baltic amber (*6*).

Observations of the ultrastructure showed a strip of tissue adjacent to the cuticle and approximately 12 μm wide in the abdomen of the fly; it showed a high degree of preservation. From the location, we concluded that this tissue represented epidermis and associated muscle. The degree of preservation varied considerably and a few areas contained cellular remains with morphologically identifiable organelles. Nuclei were identified on the basis of their size, location within the cell, structure, and clearly defined boundaries (Fig. 1A). The nuclei were generally flattened and elongate, with a slightly undulate border as is normally observed in insect epidermal

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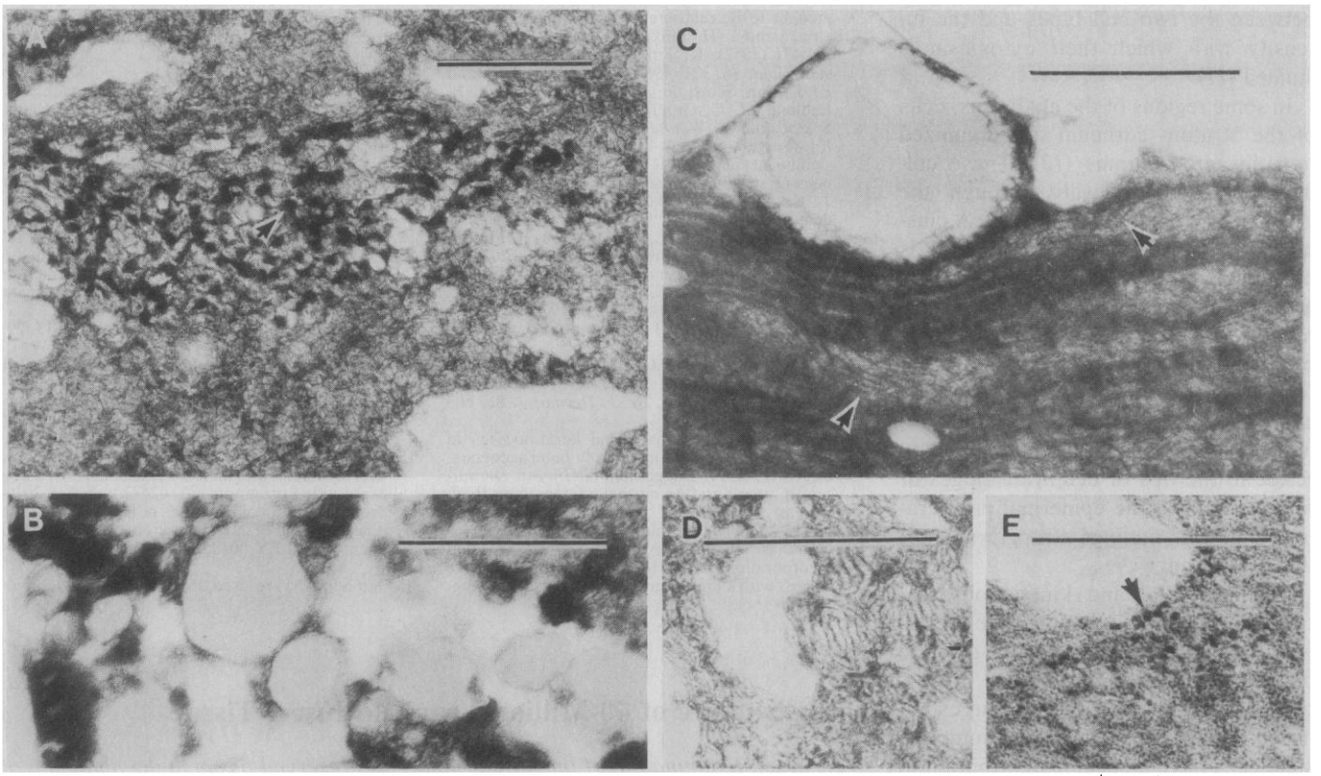

Fig. 1. Electron micrographs of fossilized fly tissue. (A) Epidermal cell illustrating typical ultrastructure. The nucleus in the center of the figure is distinguished by its electron-opaque clumps of chromatin-like material (arrow). Cytoplasmic vacuoles and cytomembranes are apparent in vesicular areas similar to agranular reticulum. (B) Lipid inclusions are apparent within vacuolated cytoplasm. (C) Muscle fibrils appear as electron-opaque bundles among mitochondria containing cristae (arrows). An associated tracheole with its separated lining (considered to be a lipoprotein) and small tubercules on the inner surface is present. The outer plasma membrane is also visible. (D) Membranous arrays characteristic of endoplasmic reticulum cisternae. (E) Electron-opaque granules of uniform size (arrow) are similar to ribosomes. All scale bars are 0.5 μm.

tissue (*8*). Electron-opaque clumps, similar in appearance to and probably representing chromatin, were throughout the nucleus. Nucleoli were not observed. The cytoplasm contained vesicular membrane arrays similar to agranular reticulum (Fig. 1D). Many vacuoles were present, representing poor preservation or an artifact of shrinkage.

Other organelles were identified on the basis of their morphology and homology with present-day insect cells (*8*). These included lipid droplets (Fig. 1B), elongate bodies with membraneous cristae representing mitochondria (Fig. 1C), electron-opaque granules approximately 18 nm in diameter corresponding in shape and size to ribosomes (Fig. 1E), and several types of membranous arrays, some of which appeared similar to myelin arrays on the basis of spacing and configuration. The cytoplasm also contained electron-opaque granules which were lysosomal in nature. Muscle bands were clearly defined (Fig. 1C). The muscle fibers appeared as bundles of compressed electron-opaque fibrils. Interspersed between the fibrils were large mitochondria with a less opaque matrix that contained paired membranes corresponding to cristae. Also associated with the muscle bands were tracheoles. In most cases, the tracheole lining was still present, although it had separated from the inner sheath. This lining is reportedly composed of lipoprotein (*9*). Tubercles on the interior surface of the tracheole were evident as was a thin outer membrane, possibly representing the plasma membrane.

Thick sections of the fly tissue made on the ultramicrotome were placed on glass microscope slides, stained with aqueous methylene blue, and examined under a light microscope. The stain was absorbed by the tissues.

The ultrastructural remains of fossilized insect tissues in Baltic amber corresponded to what one would expect to find in a routine examination of present-day insects. The character of the tissues in the fossil fly resembled present-day tissues that had been dehydrated with ethylene glycol (*10*). The use of this compound results in a type of physical preservation known as inert dehydration. This could be one of the main factors in the preservation process of amber inclusions. Sugars and terpenes, both present in tree sap, might combine with water in the tissues to aid in the dehydration process. In addition to the physical method of tissue preservation, the sap might also have contained chemical components that assisted preservation. This is probable since lipid inclusions were observed, and they normally are not preserved when inert dehydration occurs along (*10*).

In essence, what we are describing is an extreme case of mummification, involving the preservation of insect tissue by drying and natural embalming (that is, entrance of plant sap into the body cavity). Finding cellular organelles in tissue of this age is an indication of the stability of cellular structure preserved by natural processes.

GEORGE O. POINAR, JR.
ROBERTA HESS
Division of Entomology and Parasitology, University of California, Berkeley 94720

References and Notes

1. H. Brenner, *Nova Acta Leopold.* **7**, 95 (1939).
2. E. Voigt, *Palaeontol. Z.* **31**, 35 (1957).
3. P. Mierzejewski, *Ann. Med. Sect. Pol. Acad. Sci.* **21**, 81 (1976A).
4. ———, *Ann. Soc. Geol. Pologne* **46**, 291 (1976B).
5. S. G. Larsson, *Entomonograph* **1**, 1 (1978).
6. C. W. Beck, personal communication.
7. ———, E. Wilbur, S. Meret, *Nature (London)* **201**, 256 (1964).
8. D. L. Smith, *Insect Cells* (Oliver & Boyd, Edinburgh, 1968).
9. M. J. Locke, in *The Physiology of Insecta*, M. Rockstein, Ed. (Academic Press, New York, 1964), vol. 3, pp. 379–470.
10. D. C. Pease, *J. Ultrastruct. Res.* **14**, 356 (1966).

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