

terials from the atmosphere would account for the nitrogen that is essential to the growth of green algae on snow. Nival plants which are capable of fixing atmospheric nitrogen would generally rely upon the mineral content of wind-transported dust. Thus, some autotrophic organisms may be regarded as representatives of the aeolian zone. Wind-blown organic materials explain the presence of enchytraeid worms, collembola, fungi, and bacteria, and possibly other organisms which live in the snow of high mountainous regions.

Another category of the nival division of the aeolian zone is composed of scavengers, chiefly insects and arachnids, which feed on the many wind-transported, dead insects of mountain snow fields. Mani (4) described these animals at length and found that truly phytophagous species decrease as the snow line is approached and that omnivorous and scavenging species increase.

In the Himalayas, populations of insects such as the stone fly *Rhabdopteryx* may be enormous at elevations above 4900 m where they live in torrents emerging from glaciers. The water and the rocks of the stream bed are nearly devoid of algae, and it appears that the insects in these streams subsist on organic debris released from the ice. The aquatic fauna near the glacier furnishes the summer food supply of the white capped redstarts (*Phoenicurus leucocephalus*), which nest and rear young in the vicinity of the stream. Other birds, and perhaps some aquatic mammals, may also be dependent upon insects of the community.

In the eastern Himalayas, at elevations up to 5550 m, stagnant, silted, glacial pools, without noticeable algal growths, support enormous populations of phyllopod crustaceans or chironomid midge larvae. On the surface of pools which have bacterial slime, there is generally a blanketing population of Collembola. Occasionally the pools are visited by birds which migrate across this otherwise inhospitable portion of the Himalayas, but contamination of the pools by excreta is rare. There appears to be no alternative to the assumption that animals of these aquatic habitats survive primarily on organic debris, initially of aeolian origin, which has been released from the glacier.

These considerations of the aeolian zone extend to other mountain ranges

and to the polar regions. Wide areas which are devoid of green plants, owing to edaphic and climatic factors, are characteristic of many high mountain ranges. Aeolian life may well exist in these locations if only in the form of bacteria and fungi. The communities of snow, glacial torrents, and glacial pools are commonly represented in many mountain ranges of the world, and temporary aeolian communities may be present in winter snows over wide areas of the earth.

The scavenging insects of snow and cryophytic algal growths are the more obvious polar representatives of the aeolian zone. Kol (5) draws attention to wind-blown dust particles which act as sources of minerals for cryovegetation in Alaska. Different species of algae grow in response to the chemical nature of this debris. Savile (6) has suggested the possibility that a polar terrestrial aeolian community might exist in areas of shattered limestone surrounded by more fertile terrain. He has, however, clearly emphasized the near sterility of the Beaufort formation in the Canadian arctic (7). His observations suggest that organic debris and microorganisms are virtually absent. Coniferous wood, possibly of late Tertiary origin imbedded in the ground, is still intact and undecayed. Similar conditions have been described in many parts of Antarctica. It would appear that terrestrial aeolian animals are not widespread in polar regions.

Where surface water is available, mosses and lichens are frequently present. This flora is generally more abundant in polar environments than it is in the high regions of the Himalayas and, hence, polar terrestrial communities at the highest latitudes are more likely to be based upon these photosynthetic plants. The work of Smith (8) indicates that the lichens themselves are dependent upon organic and inorganic materials in precipitation. The nutritional status of plants in extreme environments is unclear, but it is conceivable that lichens, and possibly other photosynthetic terrestrial plants, may be representatives of the aeolian zone. Llano (9) supports this view by pointing out the possible role of wind-blown guano as a source of nutrients for lichens living far within the Antarctic interior.

Nutritional materials of aeolian origin are ubiquitous and the organisms that utilize these various substances are nearly so. Debris feeders are rather

inconspicuous in most situations, but at high altitudes and latitudes, where climatic or edaphic conditions are very unfavorable for photosynthetic plants, some saprophytic and scavenging organisms are able to survive. On snow or other surfaces where small quantities of water permit the growth of photosynthetic plants, the aeolian materials directly or indirectly supply essential nutrients. The release of aeolian materials by the melting of snow and glacial ice supplies nutrients to aquatic organisms. The terrestrial, nival, and aquatic divisions of the aeolian zone can thus be distinguished from alpine or tundra communities and conceived as the constituent parts of a discrete ecosystem, the limits and detailed structure of which are not adequately known (10).

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References and Notes

1. L. W. Swan, *Sci. American* **205**, No. 4, 68 (1961); *Natural Hist.*, in press.
2. R. W. G. Hingston, in *The Fight for Everest*, by E. F. Norton (Edward Arnold, London, 1925), p. 287.
3. A. T. Wilson, *Nature* **183**, 318 (1959); **184**, 99 (1959).
4. M. S. Mani, *Introduction to High Altitude Entomology* (Methuen, London, 1962).
5. E. Kol, *Smithsonian Inst. Misc. Collections* **101**, No. 16, 4 (1942).
6. D. B. O. Savile, personal communication.
7. ———, *Can. J. Botany* **39**, 909 (1961).
8. D. C. Smith, *Ann. Botany* **24**, 184 (1960).
9. G. A. Llano, *Sci. American* **207**, No. 3, 220 (1962).
10. Himalayan expeditions yielding pertinent information in this report were supported by the Sierra Club and the National Science Foundation (1954) and by the World Book Encyclopedia (1960-1961).

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Microradiography of Fossilized Teeth

Abstract. *Microradiographic studies of fossil reptilian and amphibian teeth reveal an excellently preserved microstructure with little if any apparent mineralization except in the region of obvious fractures. X-ray diffraction has shown that the invading mineral near fractures is hematite, and x-ray spectroscopy has shown that relatively large amounts of yttrium are present.*

Microradiography in which characteristic x-rays from a target appropriate to the sample are used as the radiation source makes it possible to recognize much microscopic detail in thin sections



Fig. 1. Photomicrograph through a transverse section of a tooth of *Machaeroprostopus* showing the enamel (below), the dentinoenamel junction, and (above) the dentinal tubules terminating in this region (about $\times 165$).

through hard tissues such as tooth, bone, and shell and to identify some of the chemical elements responsible for such detail. We are using these methods to examine a variety of hard tissues, both fresh (1) and fossilized. We have obtained preliminary results on fossilized reptilian and amphibian teeth.

The specimens were collected from the Lower Red member of the Triassic Chinle formation in northeastern Arizona at a location north of U.S. Highway 66 and west of the paved road through Petrified Forest National Monument. The reptilian teeth are probably from a species of the genus *Machaeroprostopus* (2), and the amphibian teeth are probably from *Eupelor fraasi fraasi* (Lucas) (3). Thick sections were cut with a thin diamond or silicon carbide saw, and their composition was determined by vacuum x-ray spectroscopy as well as by x-ray diffraction of powdered fragments. For optical microscopy and microradiography the sections were reduced to a thickness of 10 to 25 μ by hand-grinding and polishing with diamond abrasives. Contact microradiographs were prepared; a demountable x-ray tube made in the laboratory and equipped for our purposes with a target of either metallic titanium or calcium carbonate, was used as a radiation source. The titanium was used because the calcium and phosphorus of a tooth are particularly opaque to its K radiation; calcium is, on the other hand, especially transparent to its own, not much longer, x-rays issuing from the CaCO_3 target. For radiography a thin section was maintained in direct contact with Eastman 649-0 ultrafine-grained film or plate during irradiation;

helium filled the space between the sample and the tube window. Titanium foil served as light shield and filter for the titanium radiation; when a CaCO_3 anode was employed, the light shield was aluminized Mylar plastic. When the apparatus was operated at a low voltage (15 to 20 kv) to minimize the production of white radiation, exposure times for the insensitive fine-grained emulsion were of the order of 5 minutes. The micrographs were examined visually under the microscope and photographed at magnifications of between 200 and 800. By changing the tube target between exposures, micrographs of the same specimen could be taken with x-rays of different wavelengths. No attempt was made to photograph identical fields of the sections with visible light and with x-rays, but many pairs of photographs of the same regions were prepared.

These photographs reveal a familiar microstructure. The enamel shown at the bottom of Fig. 1 is well preserved, and the dentine above it is filled with readily recognizable tubules. A study of this ancient dentine has been particularly interesting. Similar microradiographs of human teeth (1) show that in some regions the tubules are more or less completely filled with calcium, while in others they are transparent to the titanium radiation so strongly absorbed by calcium. Such tubules devoid of calcium are found in the fossil teeth. In Fig. 2 they appear as a sequence of black vertical rods especially evident in the dark band (middle); in other microradiographs, they are seen end-on as packets of black circles. It had been expected that any empty spaces originally present in these teeth would, during prolonged fossilization, have become filled with silica or another mineralizing substance. Actually our radiographs show mineralization only in regions adjacent to a fracture (Fig. 3). The horizontal crack is partly filled with radio-opaque material which extends out from it, but only into tubules cut by it. In this case the mineral introduced during fossilization could be identified by dissolving away the apatite of the section. Opaque rodlets the dimensions of the tubules remained, and these were shown by x-ray diffraction to consist of hematite (Fe_2O_3). It is clear that the iron-rich invading solutions did not penetrate beyond the damaged area.

The seemingly perfect preservation

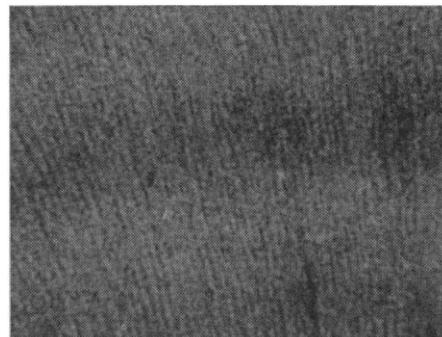


Fig. 2. Microradiograph made with titanium K x-rays of a section through the dentine of a tooth of *Eupelor fraasi fraasi*. The tubules, lying in this region almost parallel to the plane of the section, are dark (radiotransparent). Horizontally crossing the micrograph is a segment of one of the several concentric zones of hypocalcification commonly seen in the dentine of these teeth. Part of the next ring is seen at the bottom (about $\times 325$).

of the microscopic fine structure of these fossil teeth without evident mineral replacement suggests that they have been remarkably little altered during more than 100 million years. The tubules are, on the average, somewhat narrower than those in the recent teeth we have examined, and x-ray diffraction of the fossil dentine indicates that the microcrystals of its calcium phosphate may be larger than they are in present-day dentine. These observations could imply a slow crystal growth over the years (4), though there is no way to be sure that this has occurred since there are no living species of these genera.

Another aspect of our results which bears on the question of mineral replacement has been the demonstration, by x-ray spectroscopy, that there are relatively large amounts of yttrium in the fossil teeth. Smaller amounts of neodymium and gadolinium are also

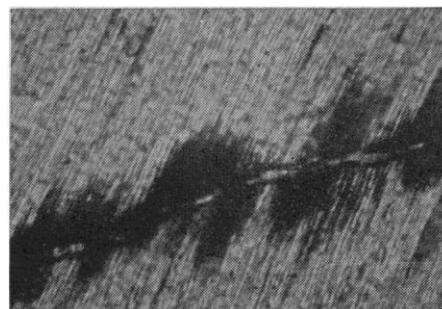


Fig. 3. Photomicrograph of a section through a fractured tooth of *Machaeroprostopus* showing partial filling of the crack and intercepted dentinal tubules with (black-appearing) hematite (about $\times 325$).

present, but no more than a trace of any other rare earth has been found, and neither yttrium nor any other rare earth has been found in the matrix embedding the teeth. In all samples the yttrium and strontium contents have been nearly the same (around 0.2 percent). The enamel has not been especially rich in them, and they bear no relation to the amount of iron oxide present as a replacing mineral.

These experiments (5) indicate that combined radiographic and spectroscopic methods using characteristic soft x-rays offer a profitable new way to study the fine structure of fossilized hard tissues.

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References and Notes

1. R. W. G. Wyckoff and O. Croissant, *Biochim. Biophys. Acta* **66**, 137 (1963).
2. C. L. Camp, "A study of the phytosaurs," *Mem. Univ. Calif. No. 10* (1930).
3. E. H. Colbert and J. Imbrie, "Triassic metoposaurid amphibians," *Bull. Am. Mus. Nat. Hist.* **110**, art. 6 (1956).
4. G. P. Brophy and T. M. Hatch, *Am. Mineralogist* **47**, 1174 (1962).
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l- α -Tocopheryl Acetate:

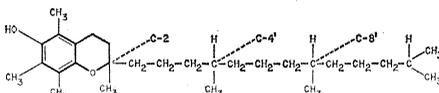
Biological Activity

Abstract. *l*- α -Tocopheryl acetate has 42 percent of the activity of *d*- α -tocopheryl acetate when compared in the rat hemolysis test. Based on this activity a potency ratio of 1.4 : 1.0 for *d*- α -tocopheryl acetate compared to *dl*- α -tocopheryl acetate was established; this confirms the currently accepted biological ratio. Preparations of *dl*- α -tocopheryl acetate from phytol and from isophytol were equally active in the rat hemolysis test.

l- α -Tocopherol, an epimer of the naturally occurring *d*- α -tocopherol, has recently been synthesized (1). This epimer has at C-2 the inverse configuration of natural *d*- α -tocopherol; at the two other centers of asymmetry C-4' and C-8', however, it is the same as the natural product. A mixture of 50

percent of these *d*- and *l*-epimers is identical with synthetic *dl*- α -tocopherol derived from phytol. Synthetic *dl*- α -tocopherol prepared from isophytol (instead of from phytol) has racemic carbon atoms at C-4' and C-8'.

The biological activity of *l*- α -tocopheryl acetate is



compared with *d*- α -tocopheryl acetate and *dl*- α -tocopheryl acetate derived from isophytol in Table 1, from data obtained in the rat hemolysis test (2, 3).

The results were evaluated statistically after arc sine transformation by standard procedures (4).

By the same method, *dl*- α -tocopheryl acetate from phytol was compared with *dl*- α -tocopheryl acetate from isophytol. *dl*- α -Tocopheryl acetate from isophytol showed an activity of 99.5 percent of that of the phytol derivative. The confidence limits for $P = 0.05$ were 89 to 113 percent.

All the investigated α -tocopheryl acetate preparations were examined for purity by gas chromatography. The degree of purity ranged between 92 and 99 percent. The biological results were corrected accordingly.

Based on the observations of Evans *et al.* (5) that vitamin E deficiency causes sterility in female rats, this symptom was originally used for determining the biological activity of vitamin E preparations. Later on, a number of other methods were developed corresponding to the various deficiency symptoms observed in rats and other animals. At present, the hemolysis test proposed by Rose and György (2) with the modifications of Friedman *et al.* (3) is mainly used. In comparative trials, there was good correspondence between the antisterility and the hemolysis test (6). Thus, with both methods the relative activity of *dl*- α -tocopheryl acetate against *d*- α -tocopheryl acetate was found to be 1 to 1.36 (7). Our results on the activity of *l*- α -tocopheryl acetate compared to *d*- and *dl*- α -tocopheryl acetate obtained with the hemolysis test are in good agreement with the established conversion factor of *dl*- to *d*- α -tocopheryl acetate (Table 1). Ames and Ludwig (8), however, found

Table 1. Activity of *l*- α -tocopheryl acetate compared with *d*- and *dl*- α -tocopheryl acetate.

Dose hemolysis (mg)	Av. ysis (%)	Potency*	
		Relative†	Calculated
<i>d</i> - α -Tocopheryl acetate (15 rats per dose)			
0.64	80.1		
0.90	57.3	100	
1.28	20.2		
<i>l</i> - α -Tocopheryl acetate (15 rats per dose)			
1.4	84.0	44 (38.1-50.0)	1.40:1 (1.33-1.44)
1.97	53.8		
2.8	29.8		
<i>d</i> - α -Tocopheryl acetate (25 rats per dose)			
0.64	70.3		
0.90	52.2	100	
1.28	14.6		
<i>l</i> - α -Tocopheryl acetate (25 rats per dose)			
1.4	83.3		
1.97	53.3	41.5 (37.2-46.2)	1.41:1 (1.36-1.45)
2.8	26.7		
<i>dl</i> - α -Tocopheryl acetate (from isophytol) (25 rats per dose)			
0.9	85.1		
1.27	59.0	100	
1.8	23.7		
<i>l</i> - α -Tocopheryl acetate (25 rats per dose)			
1.4	83.3		
1.97	53.3	62.5 (56.7-68.6)	1.38:1 (1.31-1.43)
2.8	26.7		

* Calculated potency is the ratio *d*- α - to *dl*- α -tocopherol acetate. † Within the confidence limits of 95 percent, $X_L = C^2M \pm CtsM$.

with the antisterility test for *l*- α -tocopheryl acetate only about half of the expected activity. Their results are also not in agreement with the direct comparison of *dl*- and *d*- α -tocopheryl acetate in the antisterility test by Harris and Ludwig (7).

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References

1. H. Mayer, P. Schudel, R. Rüegg, O. Isler, *Chimia Aarav* **16**, 367 (1962); P. Schudel, H. Mayer, R. Rüegg, O. Isler, *ibid.* **16**, 368 (1962).
2. C. S. Rose and P. György, *Federation Proc.* **8**, 1 (1949).
3. L. Friedman, W. Weiss, F. Wherry, O. L. Kline, *J. Nutrition* **65**, 143 (1958).
4. C. I. Bliss, in *Vitamin Methods*, P. György, Ed. (Academic Press, New York, 1951), vol. 2, p. 448.
5. H. M. Evans, E. A. Murphy, R. C. Archibald, R. E. Cornish, *J. Biol. Chem.* **108**, 515 (1934).
6. M. W. Dicks and L. D. Matterson, *Univ. Conn., Coll. Agr. Expt. Sta. Bull. No. 362* (1961).
7. P. L. Harris and M. I. Ludwig, *J. Biol. Chem.* **179**, 1111 (1949).
8. S. R. Ames and M. I. Ludwig, American Chemical Society Meeting, Atlantic City, Sept. 1962.

31 January 1963