formably overlies a "blue" clayey silt. Test excavations in the bone-bearing horizon yielded a number of specimens which were rather uniformly distributed along the 80-ft (24 m) profile. The faunal assemblage included the following: *Mammut americanus, Mammuthus* sp., *Bison antiquus, Odocoileus* sp., and *Equus* cf. *complicatus*. Their position in the deposits under the high terrace of post-Tazewell age and the lack of evidence of reworking or mixing with modern faunas indicates that the fossils at this site may be of approximately the same age as Zone C, or the clay layer immediately below it, at KEN-1.

No bones or teeth of small vertebrates were found during the preliminary excavations in 1962, although test screening was done.

In addition to the major excavations at Big Bone Lick in 1962, 15 test holes were drilled with a power auger furnished by the Commonwealth of Kentucky, and samples were taken for analysis and examination for microfauna and microflora. Wood was collected for carbon-14 analysis. A topographic map with a 1-ft (⅓ m) contour interval and a scale of 1 inch (2½ cm) to 100 ft (30 m) was made of the area. Test holes were plotted on this map. Temporary bench marks were established at the collecting localities. Excavations and fossil localities were recorded so that data will be readily available for future reference.

Present investigations indicate three terrace levels at Big Bone Lick above the present floodplain of the creek, the highest being of Tazewell age and the two lower terraces of post-Tazewell age. Distinct bone-bearing alluvial deposits appear to be related to the terrace sequence. Future investigations may reveal relationships of the alluvial deposits to each other and to the fauna; age determinations will be based on carbon-14 dating of woody material that accompanies the bones. The sequence of terraces appears to be similar to that of the adjacent Ohio Valley, indicating that the history of the site is a part of the regional sequence of events (*9*).

C. Bertrand Schultz
Lloyd G. Tanner
University of Nebraska State Museum, Lincoln 8
Frank C. Whitmore, Jr.
Louis L. Ray
U.S. Geological Survey, Washington 25, D.C.
Ellis C. Crawford
William P. Behringer Memorial Museum, Covington, Kentucky

References and Notes

1. H. F. Osborn, *Science* **82**, 533 (1935).
2. W. R. Jillson, *Big Bone Lick* (Standard Printing Co., Louisville, Ky., 1936), p. 164.
3. G. G. Simpson, *Proc. Am. Phil. Soc.* **86**, 130 (1943).
4. H. G. Rice, Jr., *ibid.* **95**, 608 (1951).
5. J. Leidy, *J. Acad. Natural Sci. Phila.* **7**, 372 (1869).
6. J. A. Allen, *Mem. Mus. Comp. Zool. Harvard* **4**, No. 10 (1876).
7. O. P. Hay, *Carnegie Inst. Wash. Publ.* **322** (**1923**), 401, 403 (1923).
8. Other members of the field party were: R. A. Cochran II, Donald Cox, B. N. Haynes, Gerald Petsche, W. H. Pile, Jr., G. G. Schaber, and D. C. Worstell.
9. C. B. Schultz received a grant from the American Philosophical Society; Schultz and L. G. Tanner were awarded a supplemental grant from the University of Nebraska Research Council; and the U.S. Geological Survey furnished funds to F. C. Whitmore and L. L. Ray. Many have contributed to making the preliminary excavations at Big Bone Lick State Park possible. Special thanks are due George Lyon, Kentucky State Dept. of Parks; Roger Arnold, superintendent of Big Bone Lick State Park; William Fitzgerald and Bruce Ferguson of the Big Bone Lick Historical Association; F. W. Johnson, deputy manager of exploration, Standard Oil Co. (New Jersey); and W. M. Lucas, Florence, Ky. Publication authorized by the director, U.S. Geological Survey.

1 July 1963

■

Bending Waves of the Posterior Flagellum of Ceratium

Abstract. *Flagellar bending waves, as shown by multiple-flash photomicrographs of the posterior flagellum of* Ceratium, *are not sine waves. The bent regions are circular arcs; these regions are separated by short segments without bending.*

Photomicrographs of sea urchin spermatozoa obtained by Gray (*1*) indicated that the bending waves which pass along the flagella and cause propulsion of the spermatozoa have the form of sine waves. This conclusion has been the starting point for many theoretical discussions of the movement of this type of flagellum. We have recently obtained photographs of the bending waves of flagella of a variety of protozoa and marine invertebrate spermatozoa which, typically, show a different type of wave form. In this report we discuss only the posterior (longitudinal) flagellum of the marine dinoflagellate *Ceratium*, which is particularly suitable for precise photography because it is larger (up to 200 μ long) and develops more bending waves per flagellum than the invertebrate sperm flagella we have also examined. Jahn, Harmon, and Landman (*2*) have recently described the quite different movements of the transverse flagellum of *Ceratium*.

Ceratium divaricatum (?) was collected in plankton samples from Newport Bay, California, near the Kerckhoff Marine Laboratory. Specimens were photographed within a few hours of collection, in sea water on a microscope slide under a standard cover glass, at a temperature of about 19°C. Illumination for multiple-flash photography was provided by a General Electric FT-230 flash tube operated at approximately 10 watt-seconds per flash, as described in a previous paper (*3*). An initial magnification of 50× on Kodak Tri-X film was used for most of the photographs.

Two multiple-flash, darkfield photomicrographs of the posterior flagellum of a specimen of *Ceratium* are shown in Fig. 1. The cell is stationary and the flagellum is bending in a plane approximately parallel to the surface of the slide. The beat frequency of this specimen was determined by stroboscopic observations and photographs at several flash rates to be 30 per second. Other specimens had similar beat frequencies. Most specimens showed bending waves with wavelengths similar to those of the specimen in Fig. 1, but often the amplitude of the waves was less and decreased towards the tip of the flagellum.

In Fig. 2, *A* and *B*, a wave pattern traced from another photograph taken at slightly higher magnification is compared with a sine wave of equal amplitude and wavelength. Although the patterns are very similar, the resolution in the photographs of this large flagellum is sufficient to show that the flagellar waveform does not correspond exactly to a sine wave. This can be seen clearly if sine waves of proper dimensions are drawn on paper and compared directly with the images of the flagellar waveforms projected by a photographic enlarger. A more objective comparison was made by measuring the ratio between the chord length *a-a* (Fig. 2*A*) at half-amplitude and the half-wavelength *b-b* on a series of photographs of two specimens of *Ceratium*. The mean of 30 measurements of this ratio was 0.743, compared with a theoretical value of 0.667 for a sine wave. The standard deviation of the measure-

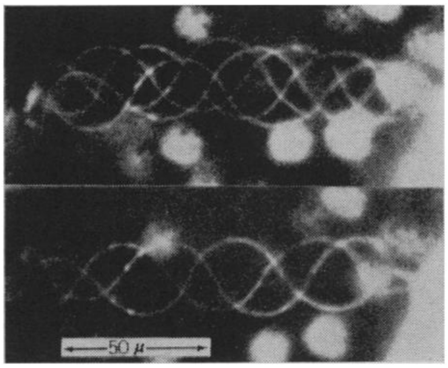

Fig. 1. Multiple-flash photomicrographs of the posterior flagellum of *Ceratium*. The posterior end of the cell body is visible at the right edge of the photographs. Flash rate, 25 per second.

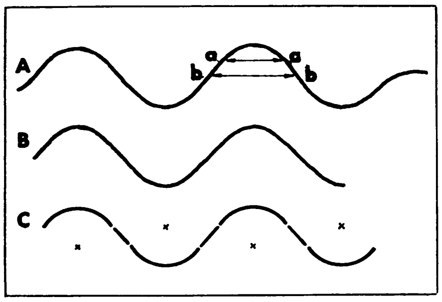

Fig. 2. *A*, Curves traced from a photograph of a flagellar waveform; *B*, curve constructed from the function $y = \sin x$, with wavelength and amplitude matched to the waveform in *A*; *C*, curve constructed of circular arcs and straight lines to match the waveform in *A* as closely as possible.

ments was 0.025; the difference is significant well beyond the 0.001 level of probability. The deviations from a sine wave are not therefore simply random irregularities, but indicate that the regular form of the wave is not sinusoidal.

The photographs also reveal that the bent regions of a flagellum are usually circular arcs in which the curvature is constant throughout the bend. Wave patterns which fit the flagellar waveforms almost exactly can be constructed from circular arcs and straight lines as shown in Fig. 2*C*. In this example, all the arcs were drawn with the same radius, indicating that the amount of bending in the bent regions was constant over most of the length of the flagellum. Periodic active bending must occur all along the flagellum to maintain the constant amplitude of the bending waves (*1*, *4*).

The difference between the sine wave and the wave constructed with circular arcs and straight lines is small and does not seriously affect the results of integration obtained for estimating the propulsive effect and energy expenditure, so that previous conclusions based on sine wave calculations (*5*) are not invalidated. The significance of the difference lies in its implications about the mechanism of wave generation.

If active bending is generated by short, mechanically independent elements distributed along the flagellum (*1*, *4*), the shape of the waveform is determined by, and therefore provides information about, the control mechanism which coordinates the activity of the bending elements. If the control mechanism involves transmission of information along the flagellum to trigger active bending, the wave pattern composed of circular arcs and straight lines could be generated by a simple on-or-off control of bending. This would appear to require much less information than would have to be transmitted to generate a sine wave by controlling the amount of bending or the bending moment.

Machin has proposed (see *4*) that active bending is triggered by bending induced by the activity of other regions of the flagellum. This proposal was originally formulated in terms of a linear system generating sinusoidal waves. In a recent paper (*6*), Machin has demonstrated that this model requires non-linear behavior of the active bending elements in order to generate the propagated, unidirectional waves which appear on flagella. The on-or-off character of the waveform composed of circular arcs and straight lines clearly implies non-linear activity of the bending elements, and increases the plausibility of Machin's model (*7*).

C. J. Brokaw
Leigh Wright

Kerckhoff Marine Laboratory,
Corona del Mar, California, and
Division of Biology, California Institute of Technology, Pasadena

References and Notes

1. J. Gray, *J. Exptl. Biol.* **32**, 775 (1955).
2. T. L. Jahn, W. M. Harmon, M. Landman, *J. Protozool.* **10**, 358 (1963).
3. C. J. Brokaw, *J. Exptl. Biol.* **40**, 149 (1963).
4. K. E. Machin, *ibid.* **35**, 796 (1958).
5. J. Gray and G. J. Hancock, *ibid.* **32**, 802 (1955); F. D. Carlson, *Proc. Natl. Biophys. Conf., 1st, 1957* (Yale Univ. Press, New Haven, 1959), p. 443; G. I. Taylor, *Proc. Roy. Soc. London, Ser. A* **211**, 225 (1952).
6. K. E. Machin, *Proc. Roy. Soc. London, Ser. B* **158**, 88 (1963).
7. Supported in part by grant GM-06965 from the National Institutes of Health.

4 October 1963

Contamination of Commercial Rabbit Albumin Preparations by Bovine Albumin

Abstract. *Seven commercial rabbit serum albumin preparations obtained from two different sources were found to contain bovine serum albumin. The extent of contamination in three representative samples varied from 0.35 to 0.54 percent.*

During preliminary studies in which rabbit serum albumin (RSA) was employed as a hapten carrier for rabbit immunizations, certain findings suggested that the commercial preparations in use contained bovine serum albumin (BSA). This prompted the detailed examination of one such preparation, and a survey of other lots for similar contamination. In all, seven commercial RSA samples prepared by Cohn fractionation, and one made in this laboratory (RSA La Jolla) were the method of Schwert (*1*), were tested. All except the one prepared in this laboratory (RSA La Jolla) were contaminated, as determined by quantitative precipitation, agar-gel diffusion, and inhibition of the reaction between I^{131}-labeled BSA and rabbit antiserum to BSA.

One-half milliliter of a pooled hyperimmune rabbit antiserum to BSA was absorbed with 234 mg of RSA No. 51

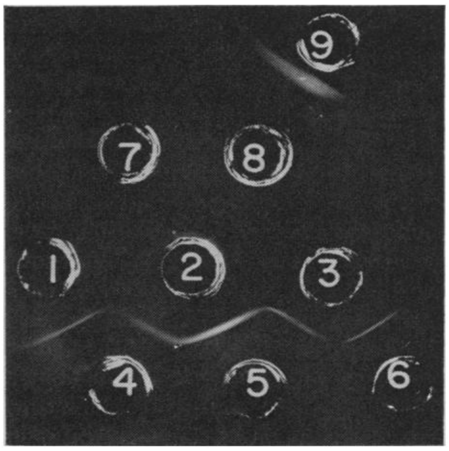

Fig. 1. Reactions in agar gel of BSA and commercial RSA No. R51 with antiserums to bovine serum albumin and commercial rabbit serum albumin. The continuity of the wavy line between wells 1 and 4, 2 and 5, and so forth, denotes a reaction of identity. Well 1 and 3: rabbit "anti-commercial RSA"; well 2: hyperimmune rabbit anti-BSA; well 4 and 6: commercial RSA No. R51 (160 μg N); well 5: BSA (2 μg N); well 7: RSA (La Jolla) (160 μg N); well 8: RSA (La Jolla) (10 μg N); well 9: bovine anti-RSA.