ties directly. An important role can also be played here by radio emissions from spacecraft in interplanetary flight and in orbit about, or emplaced on, other planets.

> R. A. PRESTON, R. ERGAS H. F. HINTEREGGER C. A. KNIGHT D. S. ROBERTSON I. I. SHAPIRO A. R. WHITNEY

Massachusetts Institute of Technology, Cambridge 02139

A. E. E. ROGERS Haystack Observatory, Northeast Radio Observatory Corporation, Westford, Massachusetts 01886

T. A. CLARK

Goddard Space Flight Center, Greenbelt, Maryland 20771

## **References and Notes**

- 1. The received signal was so strong that very small microwave horns (less than 1 m in diameter) eter) could have been employed in place the large parabolic antennas that were of available at the sites
- 2. H. F. Hinteregger, I. I. Shapiro, D. S. Robert-A. F. Hinteregger, T. I. Shapiro, D. S. Kober-son, C. A. Knight, R. Ergas, A. R. Whitney, A. E. E. Rogers, J. M. Moran, T. A. Clark, B. F. Burke, *Science* 178, 396 (1972). Defini-tions given there for specialized terminology re not repeated here.
- 3. By differenced delays and differenced delay rates, we mean, respectively, the differences in group delay and in phase delay rate for the propagation of signals from the satellite to the different ground sites. The measurement totals follow since (i) a few observations at Green Bank were either unsuccessful or lacked phase calibrations, and (ii) the third base-line provides only redundant data. The constraint of closure insures that the sum of the three differenced delays or delay rates from each of the three baselines must vanish.
- 4. The Planetary Ephemeris Program of the Massachusetts Institute of Technology, de-veloped largely by M. E. Ash, was modified for this purpose
- 5. For a detailed discussion, see R. A. Preston, thesis, Massachusetts Institute of Technology (1972).
- 6. The orbital elements are not referred to the midpoints of the observations, but have been extrapolated backward in time through an interval somewhat greater than the total ex-tent of the tracking session.
- 7. We note that a 10-degree orbital inclination would have resulted in a thousandfold improvement in the epoch offset estimate.
- 8. The phase calibration signals (2) were only recorded at the beginning of each observa-tion. From a comparison of the calibration results from neighboring observations, we conclude that at times there may have been uncompensated drifts in the receiver phase characteristics large enough to account for the observed residuals
- 9. Of course, VLBI observations require substantial common visibility of the source from the participating ground sites; this visibility will decrease as the satellite's altitude de-creases. Also, atmospheric effects are harder to model accurately the lower the elevation angle of the source as seen from the ground site.
- 10. Some of the VLBI observations of the TACSAT signals were separated by only 9 minutes, and it may be possible to connect the values of the fringe phase unambiguously between these measurements and thus obtain the analog of the counted Doppler observable (5).
- Radar tracking of passive (metallic) satellites could also yield very high echo-delay ac-curacies, but large ground facilities are required to obtain a sufficient antenna gain. The

far shorter wavelengths at which lasers operate allow even small optical telescopes to have much larger gains.

- 12. This ideal situation is tempered by the fine structure and internal kinematics of distant natural sources of continuum radio radiation. But these usually are found to be of the order of  $10^{-3}$  arc second and are of little consequence for geodetic applications [C. A. Knight, D. S. Robertson, A. E. E. Rogers, I. Roge Clark, K. Van-Knight, D. S. Robertson, A. E. E. Rogers, I. I. Shapiro, A. R. Whitney, T. A. Clark, R. M. Goldstein, G. E. Marandino, N. R. Van-denberg, *Science* 172, 52 (1971); A. R. Whit-ney, I. I. Shapiro, A. E. E. Rogers, D. S. Robertson, C. A. Knight, T. A. Clark, R. M. Goldstein, G. E. Marandino, N. R. Van-denberg, *ibid.* 173, 225 (1971)].
- 13. Such ties to the earth's center of mass degrade with the decrease in parallax accompanying an increase in the altitude of the satellite relative to the length of the baseline.
- For satellites in orbit about other planets, VLBI observations from the earth of both the 14. satellites and extragalactic radio sources in neighboring parts of the sky can serve to orient the solar system with respect to the inertial frame formed by these sources.
- 15. A. R. Whitney et al., in preparation. 16. We thank the Tri-Service Test Directorate for

cooperation in scheduling; R. Levinson, Aerospace Corp., and W. Snyder and L. Riley, Hughes Aircraft Co., for providing TACSAT orbit determinations based on Air Force data; and J. Klobuchar, Air Force Cambridge Re-search Laboratory, and M. J. Davis, Stanford Electronics Laboratories, for providing esti-mates of the electron content of the ionosphere mates of the electron content of the ionosphere. We also thank W. E. Howard, III, National Radio Astronomy Observatory, and D. L. Jauncey, Cornell University, for the use of the Mark I VLBI recording systems; and H. Peters, Goddard Space Flight Center, for aid with the hydrogen-maser frequency standards. The experimenters at Massachusetts Institute of Technology were supported in part by the National Science Foundation and in part by the Advanced Research Projects Agency. Research at the Haystack Observatory is sup-ported by NSF grant GP-25865 and NASA grant NGR22-174-003, contract NAS9-7830. The National Radio Astronomy Observatory is operated by Associated Universities, Inc., under contract with the National Science Foundation. The Owens Valley Radio Observatory is operated by the California Institute of Technology with support from the National Science Foundation.

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## **Incised River Meanders: Evolution in Simulated Bedrock**

Abstract. A flume 60 feet (18.28 meters) long was employed to study the controls of lateral and vertical incision of a sinuous stream in simulated bedrock. When 100 percent of the available sediment load was entrained the flow incised vertically at bends. When less than 100 percent of the load was entrained the flow downcut laterally outward at bends. The effects of helicoidal currents and shear stress localization explain the loci of erosion and deposition.

Meandering rivers that have incised in bedrock and yet have maintained a sinuous pattern may be of two basic types: (i) those which have slip-off convex spurs and undercut concave banks at bends (lateral incision), and (ii) those which have vertical concave and convex banks at bends (vertical incision). Nearly 80 years ago Science published a classic debate between Davis (1) and Winslow (2) concerning the incised Osage River of Missouri and how its incised meander pattern was related to the geologic history of the surrounding region. A major question was whether the existing meander pattern was closely related to a previous pattern (inheritance through superposition), or whether lateral incision had markedly altered a previous pattern. The field studies of Davis and Winslow depended largely on interpretations of valley morphology and alluvial deposits; thus, only inferences could be drawn concerning the crux of the problem-the mechanics and controls of vertical and lateral incision. Through the years, the reasons for the differences between the two types of meanders and their significance have remained an unsolved problem (3, 4).

In the work reported here, erosion

at meander bends was studied by using simulated bedrock consisting of 70 percent sand, 19 percent original silt clay, and 11 percent added kaolinite clay. This mixture was poured as a slurry into a tilting, recirculating flume 60 feet (18.28 m) long by 4 feet (1.22 m)wide (Fig. 1). The slurry was leveled and allowed to dry and harden for 2 weeks. After drying, the material was cohesive and capable of maintaining vertical banks 1.5 feet (45.75 cm) high. Under the imposed flow conditions erosional grooves, scour channels, potholes, and erosional ripples developed in a remarkable simulation of features in natural bedrock channels.

A sinuous channel was manually excavated in the simulated bedrock (Fig. 1), and incision was induced through an increase of flume slope. The channel morphology at bends and crossings shown in Fig. 2 evolved during 73 hours at a discharge rate of 0.10 cubic foot per second (0.0028  $m^3$  sec<sup>-1</sup>), sand feed rates from 30 to 50 g/min, and a maximum slope of 0.0167. Initial erosion was a maximum at the inside of the bends, and the channel was incised vertically (Fig. 2, a and c). The maximum erosion continued at the inside of the bends (convex bank) until scour had so decreased





Fig. 1 (left). Downstream view of a sinuous channel in simulated bedrock in the flume at the Hydraulics Laboratory, Engineering Research Center, Colorado State University. Gravel was placed at local low places on the bedrock surface along the channel to prevent overflow at initial bank-full stage. The gravels had no discernible effect on the localization of erosion in the channel. Fig. 2 (right). Transverse profiles of incision through time at two bends (a and c) and the crossing between (b) for the channel shown in Fig. 1. Arrows point to the outside (concave bank) of the bends; distances along the flume are given at each sequence. Stipled areas represent sand deposition; elapsed time is given for each profile in hours.

the gradient and velocity that deposition of the sediment load began. Sand deposition began on the inside of the bends, the flow began to cut laterally, and the site of maximum erosion shifted to the outside (concave bank). In contrast, incision at crossings was nearly vertical (Fig. 2b).

In many studies of flow in meandering alluvial rivers the highest velocity in a meander has been found to be localized near the concave bank (3). However, at flood stages alluvial rivers have been observed to straighten out that is, to erode most at the convex bank at the apex of a bend. Since similar hydraulic mechanisms undoubtedly operate in both alluvial and bedrock channels, the different channel boundary effects must act to influence the final channel morphology.

Two previous studies have yielded hydraulic data on flow at bends in rigid open channels. Ippen et al. (5) determined experimentally that the highest velocity gradient and highest shear stress gradient were both located at the convex bank at the apex of a bend in a curved trapezoidal channel with a rigid boundary. Immediately downstream from the apex the highest shear and velocity contours crossed the channel and approached the concave bank. Sediment was not used in their experiments, but Yen (6) studied flow in bends in which he first allowed a cohesionless sand to form a stable alternate bar bed topography, and then

stabilized the bottom by replacing it with concrete. He, too, found that the highest shear stresses were near or just before the apex at the inside of the bend and that the contours crossed the channel downstream. By including the bar bed topography effect in the analysis, however, Yen determined that secondary helicoidal currents were more important than shear stress in determining the pattern of bed topography in a movable-bed channel. Thus, even though shear stresses were higher at the convex bank of a bend, the secondary currents forced sediment there and a bar formed.

These results explain the seemingly anomalous localization of scour shown in Fig. 2. Before sediment deposition began, all the sediment load was entrained and was transported around the bend at the inside in response to helicoidal secondary currents. The highest shear stress was also at the inside of the bend, and the maximum scour occurred there. The scour was aided by corrosion as the sand load was concentrated in the area of highest shear stress. However, when incision had decreased the gradient and velocity sufficiently to permit deposition, the secondary currents, more important than shear stress, transported the sand to the inside of the bend, where it was deposited. Then the site of maximum erosion shifted to the outer bank, and the flow began to incise laterally.

This explanation for the modes of

lateral and vertical incision is supported by information from natural rivers on the Colorado Plateau. The San Juan River in the Goosenecks near Bluff, Utah, is an example of a river that is vertically incised and appears to be superimposed from a previous pattern. The Escalante River, also in southeast Utah, exhibits lateral incision; the sinuosity has apparently increased during downcutting (7). The experimental results suggest that the vertically incised meanders of the San Juan may have resulted from downcutting during low-frequency discharges of large magnitude which entrained all of the alluvium in the channel. Even if lateral incision was effective at lower discharges, higher discharges resulting in vertical incision would have controlled the morphology. The large drainage area of the San Juan is probably sufficient to have contributed such high discharges in the past. The Escalante, however, is a small river and probably did not receive discharges of such magnitude that all of the available sediment load was entrained. Moore (7) observed that the smaller rivers on the Colorado Plateau were dominantly laterally incised. This conforms with the present conclusions, as larger rivers with larger drainage areas would be more likely to have flood discharges of sufficient magnitude to permit vertical incision.

The simulated bedrock used in this study was isotropous, but in nature

variances in lithology, fracture patterns, regional structure, and other geologic factors have significant effects on incision. Moreover, the sinuous experimental channel was incised as a result of a slope increase, while incision in nature may occur from other causes as well. Nevertheless, the experimental evidence explains the morphology of some incised meanders on the Colorado Plateau, and the results may be applicable to other incised meanders.

**R.** G. Shepherd\* Geology Department, Colorado State

University, Fort Collins 80521

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  \* Present address: Bureau of Economic Geology, University of Toxyon Avuita 787116.
- University of Texas, Austin 78712.

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## Survival of Mouse Embryos Frozen to -196° and -269°C

Abstract. Mouse embryos survived freezing to  $-196^{\circ}C$ . Survival required slow cooling (0.3° to  $2^{\circ}C$  per minute) and slow warming (4° to  $25^{\circ}C$  per minute). Depending on the specific rates used, 50 to 70 percent of more than 2500 frozen and thawed early embryos developed into blastocysts in culture after storage at  $-196^{\circ}C$  for up to 8 days. When approximately 1000 of the survivors, including some frozen to  $-269^{\circ}C$  (4°K), were transferred into foster mothers, 65 percent of the recipients became pregnant. More than 40 percent of the embryos in these pregnant mice gave rise to normal, living full-term fetuses or newborn mice.

Most attempts to preserve multicellular mammalian systems such as tissues and organs by freezing have failed, partly because the approaches have been empirical. However, an understanding of the mechanisms of freezing injury in single cells is emerging, and we applied that understanding to the freezing of one such multicellular system-early mouse embryos.

The ability to preserve viable embryos by freezing them to low temperatures would have applications in genetics and developmental biology and could facilitate the upgrading of domestic animals. Before 1971, attempts to freeze mammalian embryos below  $-20^{\circ}$ C had, with rare exceptions, failed (1). Then it was reported that 55 to 65 percent of mouse eight-cell embryos and early blastocysts survived freezing to -79°C for 30 minutes if they were suspended in a 7.5 percent solution of polyvinylpyrrolidone in a modification of Dulbecco's phosphate-buffered salt solution (PBS medium) and cooled at 60°C  $min^{-1}$  (2). However, the embryos would not survive longer than 30 minutes at that temperature, and two-cell embryos did not survive at all.

Because of these limitations, we explored the cryobiological factors-chiefly suspending medium, cooling rate, final temperature, and warming ratethat might influence survival of preimplantation stages of mouse embryos (one-, two-, and eight-cell embryo, and blastocyst). The criteria of survival (2) were development to expanded blastocysts in culture and development to living mice in the uterus of a foster mother.

Embryos were obtained from randombred Swiss-Webster albino (specific pathogen-free, National Laboratories) or  $F_1$  hybrid [(C57BL  $\Im$ ) × (C3H/ AN  $\delta$ )F<sub>1</sub> Cum] virgin female mice (6 to 12 weeks old) mated with  $F_1$  hybrid males. (Parental source did not appear to affect survival after freezing and thawing.) The females were induced to superovulate by the intraperitoneal injection of 5 international units (I.U.) of serum gonadotropin from pregnant mares and 5 I.U. of human chorionic gonadotropin given approximately 48 hours apart, and were then mated individually. One-, two-, and eight-cell embryos and early blastocysts were recovered from the reproductive tracts at 22, 44 to 46, 66 to 70, and 90 to 96 hours, respectively, after the injection of human chorionic gonadotropin (3). Embryos of a given age were pooled and washed twice in 2 ml of PBS medium at room temperature (2, 4). Cumulus cells were removed from one-cell embryos by exposure to hyaluronidase (150 U.S. Pharmacopeia units per milliliter) in PBS medium for 3 to 5 minutes before washing.

Ten to 40 embryos in  $\sim 0.001$  ml of

medium were pipetted into tubes (10 by 100 mm) containing 0.1 ml of PBS medium. Except where noted, the tubes were cooled to  $0^{\circ}$ C, and 0.1 ml of 2M dimethyl sulfoxide (DMSO) or glycerol at 0°C was added. After 15 minutes the samples were transferred to a bath at  $-3.5^{\circ}$  to  $-4.5^{\circ}$ C, and seeded 2 minutes later with a minute ice crystal. After another 5 minutes, they were transferred to baths cooling at 0.3° to  $40^{\circ}$ C min<sup>-1</sup> (5). Samples were cooled to  $-78^{\circ}$ ,  $-196^{\circ}$ , or  $-269^{\circ}$ C, kept at these temperatures for 1 minute to 192 hours, and thawed in four ways: (i) a 35°C water bath for 35 seconds (rate 450°C min<sup>-1</sup>); (ii) an ice bath (rate 215°C min<sup>-1</sup>); (iii) air at room temperature (rate 25°C min<sup>-1</sup>); or (iv) 20 ml of ethanol in tubes (38 by 200 mm), the ethanol, initially at  $-110^{\circ}$ C, warming by contact with room temperature air (rate  $4^{\circ}C \text{ min}^{-1}$ ). (Cooling and warming rates were measured between  $-65^{\circ}$  and  $-10^{\circ}$ C.)

Thawed samples were immediately diluted at  $0^{\circ}$ C by the addition of 0.2, 0.2, and 0.4 ml of medium at 45second intervals. Each sample tube was emptied into an embryological watch glass containing 1 ml of medium and rinsed with 1 ml of medium. The embryos were washed by transfer through two changes of fresh medium at room temperature. They were then examined at  $\times$  50 magnification to determine the number recovered, the number exhibiting normal or abnormal morphology, and the number that had degenerated. All embryos except those that had totally degenerated were transferred to a modified Krebs-Ringer bicarbonate medium (6) and cultured by Brinster's method (7) in small droplets of culture medium under mineral oil at 37°C in a mixture of 5 percent  $CO_2$  in air. Except for one-cell embryos (8), they were cultured until they developed into blastocysts or expanded blastocysts (24 to 90 hours, depending on the developmental stage at the time of freezing). The number of embryos recovered after thawing was 90.3 percent of the total frozen. Survival is defined as the percentage of recovered embryos that developed into blastocysts. Of the surviving embryos, 9.7 percent exhibited one or more damaged blastomeres after thawing, but were also capable of developing into fetuses (first footnote, Table 1) and into viable young (9).

Dimethyl sulfoxide, one of the more effective protective agents, received the chief emphasis (9a). The reported toxicity for embryos (2, 4) was nearly