are young and the small gill appears inefficient for suspension feeding.

It appears that N_2 fixation could be a significant supplementary source of combined-N compounds for some shipworms. For shipworms from the Sargasso Sea, the time required to double cellular N (T_{DN}) (*10*) through N_2 fixation alone can be as short as 1.4 days. The T_{DN} for the three smallest *L. pedicellatus* (average weight 370 μg) assayed averaged 32 days. Carbon and nitrogen contents of these shipworms were measured with a Perkin-Elmer 240 elemental analyzer, and for 52 shipworms the average C : N ratio was 4.29 (range 3.52 to 9.33), and N constituted an average of 5.74 percent (range 3.78 to 8.27) of shipworm dry weight. The five smallest shipworms assayed had an average N content of 6.08 percent (range 4.60 to 8.21), whereas the five largest averaged 5.67 percent (range 5.16 to 6.37). This suggests that the juvenile shipworms had an adequate supply of combined-N compounds.

A slightly curved, rod-shaped (0.3 by 1.5 μm) bacterium capable of fixing N_2 was isolated from the cecum of *L. pedicellatus*. The isolate is gram-negative and appears to be of the family Spirillaceae. No N_2 fixing bacteria were obtained from the gill or the gland of Deshays (*11, 12*). The N_2 fixing isolate will grow either anaerobically in Bray dishes or Pankhurst tubes (*13*) or aerobically in petri dishes. It will only reduce acetylene under anaerobic conditions. The isolate is capable of liquefying cellulose (Sigma MN-300).

Nitrogen fixation occurs in another wood ingester, the termite (*14*). However, this is apparently the first report showing a potentially significant contribution of combined N to the nutrition of an animal (*15*). This discovery explains in part how shipworms are able to grow on a low-nitrogen diet. In the deep sea there are other cellulose-ingesting mollusks (subfamily Xylophagainae), as well as echinoderms and crustaceans that eat plant material of terrestrial origin, and wood entering the deep sea is rapidly decomposed (*16*). Nitrogen fixation could be an important source of nitrogen for these and possibly other deep-sea organisms.

EDWARD J. CARPENTER
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543
JOHN L. CULLINEY
Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138

References and Notes

1. E. B. Cowling and W. Merrill, *Can. J. Bot.* **44**, 1539 (1966).
2. J. D. H. Strickland, *Fish. Res. Board Can. Bull. 122* (1966).
3. L. J. Greenfield and C. E. Lane, *J. Biol. Chem.* **204**, 669 (1953).
4. R. D. Turner, *A Survey and Illustrated Catalogue of the Teredinidae* (Museum of Comparative Zoology, Cambridge, Mass., 1966).
5. K. J. Eckelbarger and D. J. Reish, *Bull. South. Calif. Acad. Sci.* **71**, 48 (1972).
6. J. L. Culliney, unpublished observations of *Teredo navalis* at Beaufort, N. C., June and July 1973; the water temperature was 26° to 28°C.
7. W. D. P. Stewart, G. P. Fitzgerald, R. H. Burris, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 2071 (1967).
8. For the assay, high-purity acetylene gas (1 or 5 cm^3) was injected through a septum into serum bottles (5 or 20 ml, respectively) half-filled with seawater filtered through glass fiber. Blanks with dead shipworms were done with every experiment, and contaminating ethylene in the acetylene was subtracted for each shipworm sample. Shipworms were incubated in the dark at 25°C on ship or 15°C in the laboratory. After incubation for about 6 to 24 hours [shipworms are capable of living anaerobically; see C. E. Lane, R. W. Sims, E. J. Clancy, *Am. J. Physiol.* **183**, 3 (1955)] the worms were killed with 50 percent TCA, the bottles were shook, and 100 μl of the gas phase was withdrawn with a gas-tight syringe. Ethylene production was measured by injection of the gas into a Packard model 417 gas chromatograph with a stainless steel column (1.8 m by 0.3 cm) containing Porapak R and operated at 50°C. To convert moles of ethylene produced to moles of ammonia fixed, the factor 1.5 was used.
9. E. M. Hulburt, *Bull. Mar. Sci.* **14**, 33 (1964); *ibid.* **17**, 330 (1967).
10. $T_{DN} = (\ln 2)/[(N_2$ fixed per day)/(N content)], where nitrogen concentrations are per milligram dry weight.
11. J. D. Popham and M. R. Dickson, *Mar. Biol.* **19**, 338 (1973).
12. Bacteria were initially isolated by aseptically streaking onto Difco Marine Agar in petri dishes. Isolated colonies were transferred to axenic culture and then into modified Hino and Wilson medium [R. J. Seidler, P. E. Aho, P. N. Raju, M. J. Evans, *J. Gen. Microbiol.* **73**, 413 (1972)] with the addition of NaCl (23 g/liter), $MgSO_4$ (5 g/liter), and sucrose (2 percent), but without mannitol. A small piece (0.5 cm^2) of Whatman No. 1 filter paper was added to the medium.
13. N. E. R. Campbell and H. J. Evans, *Can. J. Microbiol.* **15**, 1342 (1969).
14. J. R. Beneman, *Science* **181**, 164 (1973); J. A. Breznak, W. J. Brill, J. W. Mertins, J. C. Coppell, *Nature (Lond.)* **244**, 577 (1973).
15. J. R. Postgate, *J. Appl. Bacteriol.* **37**, 185 (1974); see p. 195.
16. R. D. Turner, *Science* **180**, 1377 (1973).
17. Supported by NSF grant GA 37993 and ONR contract N0014-67-A-0298-0027 with Harvard University. We thank K. L. Smith, Jr., for stimulating advice and Barbara Culliney, Junell Sayles, Charleen Van Raalte, and Charles Price for technical assistance. Charles Remsen and Brian Schroeder advised on microbiological methods, and Ruth Turner kindly confirmed identifications of shipworm species and critically reviewed the manuscript. Contribution No. 3461 from the Woods Hole Oceanographic Institution.

7 October 1974

Pregnancy in Cactus Mice: Effects of Prolonged Copulation

Abstract. *The postejaculatory copulations of cactus mice are demonstrated to function in facilitating neuroendocrine responses necessary for pregnancy. Whereas estrous cycles were altered after just 10 percent of the tests terminated after one ejaculatory series, females became either pregnant or pseudopregnant after 80 percent of tests continued at least to sexual satiety (30 minutes with no copulations).*

While a few mammalian species cease copulating upon attaining their first ejaculation of an episode, most continue and attain multiple ejaculations (*1*). The function of this continued copulatory activity, particularly in species with spontaneous ovulation, is not clear. Copulation is known to function not only in the transfer of sperm from male to female, but also in triggering neuroendocrine reflexes in the female. These neuroendocrine responses, ovulation in some species and the initiation of a functional luteal phase (pseudopregnancy) in others, are as critical to pregnancy as is sperm transfer. Available data on laboratory rats and mice suggest that a single ejaculatory series is sufficient for initiation of the neuroendocrine reflexes producing pregnancy and pseudopregnancy in these species (*2, 3*). In this report, we show that in cactus mice copulation after ejaculation is critical to such responses.

Cactus mice ejaculate after several discrete insertions (intromissions) with intravaginal thrusting (*4*). An "ejaculatory series" is defined as a group of intromissions leading up to and including an ejaculation. A pause of about 7 minutes follows the first ejaculatory series, after which copulation is resumed. However, a second ejaculation rarely is attained (*4*). Indeed, we have never observed more than one ejaculation in a test when the female was in naturally occurring estrus. Ejaculations are readily discriminated from intromissions without ejaculation, using behavioral criteria (*4*). Vaginal smears taken from females receiving many intromissions, but no behaviorally identified ejaculations, reveal an absence of sperm in the female's genital tract. Thus cactus mice are ideal for investigation of stimulatory effects of copulation, since such effects can be separated from possible effects of additional sperm transfer.

The subjects were ten adult, laboratory-reared cactus mice, *Peromyscus eremicus*, from the colony maintained at the University of Florida (*4*). Stock originally were trapped near the Rillito River in Arizona. The females were mated with 12 males from the same colony.

An attempt was made to complete six tests with each female—two tests in each of three conditions. In the first condition (1E), the female was introduced into the male's home cage and the pair was permitted to copulate until the completion of one ejaculatory series. The female was then returned to her home cage. In the second condition (SAT), copulation was permitted until attainment of a satiety criterion of 30 minutes without copulation. The 30-minute criterion is the standard, if somewhat arbitrary, criterion for regarding a male as sexually satiated. The female was then removed. Tests in the third and final condition (SAT + ON) were identical to those in the SAT condition, except that after attaining the satiety criterion, the female was permitted to remain unobserved in the male's home cage overnight. (One of the scheduled 60 tests could not be conducted because of the death of the female.)

Animals were housed individually and maintained on a reversed light-dark cycle. Vaginal smears were taken each morning with a thin wire loop. Tests were conducted on the first day of disappearance of leukocytes from the vaginal smear, provided the female had shown a stable baseline of at least two successive cycles of 7 days or less. Following copulation, a female could show any of three possible responses: (i) continue to cycle (display a proestrous or estrous vaginal smear in 7 days or less), (ii) pseudopregnancy (a 10- to 15-day diestrus), or (iii) pregnancy. All females classed as pregnant delivered litters after 28 to 32 days, except one which suffered an apparent spontaneous abortion on day 19.

Estrous cyclicity was altered in only 10 percent of the cases in which just a single ejaculatory series was permitted (see Table 1). Thus in cactus mice, in contrast to laboratory rats and mice, a single ejaculatory series with sperm transfer does not usually suffice to initiate neuroendocrine responses essential for pregnancy. By contrast, after 80 percent of the tests in the SAT and SAT + ON conditions females became pseudopregnant or pregnant.

Table 1. Number of mice that continued to cycle, became pseudopregnant, or became pregnant as a function of condition, expressed as the number positive/the number of tests, as well as in percentages.

Category	Condition		
	1E*	SAT†	SAT + ON‡
Continue cycle (3 to 7 days)	18/20 (90%)	4/19 (21%)	4/20 (20%)
Pseudopregnant (diestrus, 10 to 15 days)	1/20 (5%)	6/19 (32%)	4/20 (20%)
Pregnant (gestation, 28 to 32 days)	1/20 (5%)	9/19 (47%)	12/20 (60%)

* Completion of one ejaculatory series. † Satiety criterion attained and then the female was removed from the cage. ‡ SAT condition, except the female remained in male's cage overnight. (See text for details of these three conditions.)

There were no substantial differences between the latter two groups.

Measures of copulatory behavior in the first series were quite similar across groups and resembled the values reported earlier for this population (*4*). Copulation beyond the first ejaculation occurred in all tests in the SAT and SAT + ON conditions with a mean of 28.2 postejaculatory intromissions in the former condition and 22.0 in the latter. This is somewhat higher than the value reported for females in hormone-induced estrus (*4*). However, on no test was a second ejaculation observed to occur. Thus the copulations that were critical in initiating neuroendocrine responses of pregnancy were not accompanied by additional sperm transfer.

In both the SAT and SAT + ON conditions, the females becoming pregnant received significantly more postejaculatory intromissions than the females becoming only pseudopregnant (16.0 versus 36.8 in the SAT condition and 5.5 versus 29.5 during observation in the SAT + ON condition) (*5*). This suggests that in addition to a copulation-sensitive mechanism for the initiation of a functional luteal phase, a second mechanism, critical for true pregnancy, may exist. This mechanism might resemble that through which preejaculatory intromissions facilitate sperm transport in rats (*2*).

Many species continue to copulate after attaining ejaculation and have short estrous cycles in which pseudopregnancy is copulation-dependent (*1*, *6*). We believe this to be the first demonstration that postejaculatory copulations without sperm transfer can be critical to the initiation of the neuroendocrine responses that halt the estrous cycles and trigger a functional luteal phase.

DONALD A. DEWSBURY
DANIEL Q. ESTEP
Department of Psychology, University of Florida, Gainesville 32611

References and Notes

1. D. A. Dewsbury, *Q. Rev. Biol.* **47**, 1 (1972).
2. N. T. Adler, *J. Comp. Physiol. Psychol.* **69**, 613 (1969).
3. R. B. Land and T. E. McGill, *J. Reprod. Fertil.* **13**, 121 (1967).
4. D. A. Dewsbury, *Behav. Biol.* **11**, 315 (1974).
5. Mann-Whitney U tests: $U = 10$; $P < .05$ (two-tailed) in SAT condition and $U = 2$; $P < .02$ (two-tailed) in SAT + ON condition.
6. C. H. Conaway, *Biol. Reprod.* **4**, 239 (1971).
7. Supported by grant GB-33837X from the National Science Foundation.

3 September 1974; revised 18 November 1974

Sleep and Cardiac Rhythm in the Gray Seal

Abstract. *Telemetric studies of electroencephalograms, electrocardiograms, and electrooculograms and concurrent observations of behavior revealed that seals can sleep underwater, on the surface, or while hauled out. Rapid eye movement preceded slow wave sleep and was accompanied by increased respiratory rate and rhythmic tachycardia. While slow wave sleep occurred under all sleep conditions, rapid eye movement occurred only when a seal was hanging at the water surface or hauled out, never underwater.*

Although many investigators (*1*) have observed seals sleeping in various ways, there have been no neurophysiological investigations of sleep in pinnipeds. We were interested to learn if seals do sleep in or under the water and, if so, whether sleep in the water differs from sleep observed in terrestrial mammals (*2*).

Sleeping pinnipeds have been observed hauled out on land (*3*), on the sea surface (*1*, *4*), and underwater (*4*, *5*). Waking cardiac arrhythmia (bradycardia during apnea and tachycardia during breathing) in pinnipeds is eliminated by anesthesia (*6*). Since pinnipeds have been observed underwater, apparently asleep, for up to 23 minutes (*7*), we wanted to know if bradycardia oc-