treated females 15 minutes after application.

Our results indicate that exposure to male urine may produce measurable endocrine and neurohormonal changes in LHRH and NE and that these changes are measurable in posterior, but not anterior, olfactory bulb tissue. These changes implicate olfactory bulb LHRH and NE as possible mediators for LH secretion and may offer support for a possible role of the vomeronasal system in the activation of female reproduction in this species.

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11. The female prairie vole, an induced ovulator, will remain reproductively suppressed (with regard to the induction of ovarian function and onset of estrus) if housed with male or female family members or other females, and requires stimuli from an unfamiliar male to activate reproductive processes (*2*, *3*). Uteri from females in the present experiment were weighed to further verify reproductive suppression. Uterine weights of females in this experiment were less than 20 mg, which indicates an absence of prior activation.
12. For the radioimmunoassay for LH we used the NIAMDD rat LH system obtained from the Rat Pituitary Program of the NIAMDD, NIH. Sensitivity for the LH assay at 80 percent binding is 5 ng. Samples (100 μl) were run in duplicate. This LH assay for vole serum has been used and validated [V. D. Ramirez, J. E. Hauffe, L. L. Getz, V. D. Ramirez, *Trans. Ill. Acad. Sci.* **72**, 42 (1979)].
13. For the radioimmunoassay for LHRH we used a procedure previously described in which free and bound hormone are separated with ethanol. The antibody was provided by T. M. Nett and used at a final dilution of 1:200,000. The sensitivity of this assay at 80 percent binding is between 0.5 and 1.0 pg of a synthetic LHRH standard (Beckman) [D. E. Hartter and V. D. Ramirez, *Endocrinology* **107**, 375 (1980)].
14. Catecholamine concentrations were determined by a radioenzymatic assay previously described, with the exception that tetraphenylborate was added for the extraction of catecholamines. The sensitivity for both NE and dopamine is 32 pg (twice the blank value) [J. Becker and V. D. Ramirez, *Neuroendocrinology* **31**, 18 (1980); L. L. Zschaeck and V. D. Ramirez, *J. Neural Transm.* **39**, 29 (1976)].
15. The olfactory bulbs were placed on ice and perpendicularly bisected along the longitudinal axis into bilateral anterior and posterior halves. Each half was further divided into right and left halves. The tissue samples were weighed, and samples from anterior and posterior olfactory bulbs were placed in separate vials containing either 100 μl of 0.1*N* $HClO_4$ for catecholamine extraction, 100 μl of 0.1*N* HCl, for extraction of LHRH. Observations from our laboratory indicate no lateralization of catecholamines or LHRH in olfactory bulb tissue, and sample extracts from right and left olfactory bulbs were equally represented in the two assays. Tissue extracts were homogenized and either brought to 10 percent volume by weight with 0.1*N* $HClO_4$ for catecholamine determination or to a final volume of 1.0 ml with 0.1*N* HCl for LHRH assay. Both catecholamine and LHRH extracts were centrifuged for 20 minutes at 5000 rev/min (Beckman J-21B, J.S.7.5 head) and duplicate samples from the supernatant were taken for the respective assays.
16. In an experiment with unstimulated female voles ($N = 5$), a significant ($U = 1$, $P < .008$) LHRH concentration was localized in posterior (15.2 ± 0.94 pg/mg) compared to anterior (4.5 ± 0.99 pg/mg) olfactory bulb tissue. Varying volumes of olfactory bulb samples used in the assay resulted in a dose response parallel to the LHRH standard curve.
17. Nonparametric Mann-Whitney *U* tests were used for pairwise comparisons.
18. We measured LHRH concentrations from additional groups of females (five per group) stimulated with urine from either males castrated 21 days previously or intact males and decapitated at 30, 60, or 120 minutes later. The combined results (mean ± standard error) for each time interval for the two groups were: anterior tissue = 5.2 ± 1.3, 4.6 ± 0.5, 4.8 ± 0.5 pg/mg; posterior tissue = 17.0 ± 2.7, 21.3 ± 3.4, 25.6 ± 2.8 pg/mg). Urine from castrated males, although less effective than that of intact males in increasing uterine weight, does significantly increase uterine weight in comparison to nonstimulated females (*2*). The elevated LHRH concentrations at 60 minutes in urine-stimulated females are apparently maintained 120 minutes after urine stimulation.
19. In another experiment with unstimulated female voles ($N = 5$) a significant ($U = 4$, $P < .05$) localization of NE was obtained from posterior (250 ± 24 pg/mg) compared to anterior (192 ± 27 pg/mg) olfactory bulb extracts.
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23. An anatomical pathway for the olfactory-stimulated LH release in male rats exposed to odors from estrous females may involve accessory olfactory bulb projections to the medial preoptic area where LHRH neurons or other projections of the medial preoptic area to the medial basal hypothalamus may stimulate LH release [F. Scalia and S. S. Winans, *J. Comp. Neurol.* **161**, 31 (1975); L. A. C. Conrad and D. W. Pfaff, *Science* **190**, 1112 (1975)]. However, olfactory-dependent LH release also occurs after lesions are made in the medial preoptic area [F. Kamel and A. I. Frankel, *Horm. Behav.* **10**, 10 (1978); E. L. Ryan and A. I. Frankel, *Biol. Reprod.* **19**, 971 (1978)]. Thus there may be other pathways or mechanisms of action through which olfactory or gustatory stimuli mediate LH release.
24. We thank N. Ramirez for performing the catecholamine assays and G. Jackson and E. Roy for their comments on this manuscript. This work was supported by NSF BNS 79-25713 (C.S.C.), NSF PCM 77-04656 (V.D.R.) and NIH HDO9328 and NSF DFB 78-25864 (L.L.G.).

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Age Determination for the Shanidar 3 Neanderthal

Abstract. *Close agreement between the age at death estimated by macroscopic and microscopic methods was obtained for the Shanidar 3 Neanderthal. This suggests the possibility of obtaining age at death estimates by microscopic methods in other fossil hominids where the skeletal remains are highly fragmentary and macroscopic methods are not applicable.*

Age assessment for fossil hominids has relied primarily on macroscopic indicators. These include morphological changes of the pubic symphysis and sacroiliac joints, cranial suture closure, the degree of dental occlusal attrition, proximal femoral trabecular bone loss, and a variety of osteoarthritic changes (*1*). These indicators have been shown to provide ages of death from recent skeletal samples that are reliable primarily for individuals under 40 years of age. The age at death determinations for the fossils are based on the assumption that the rates of skeletal growth and degeneration among Pleistocene human populations were similar to those of preindustrial modern populations, although some processes, such as dental attrition, may have occurred at a faster rate.

Analysis of the degree of remodeling of cortical bone and the development of

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secondary osteons in a small core of bone from the femoral midshaft diaphysis (*2*) may provide accurate age at death estimates, especially for individuals over 40 years of age, for which traditional macroscopic techniques are imprecise. In order to investigate the applicability of this technique to Pleistocene fossil hominids, we estimated the age at death for the Shanidar 3 Neanderthal by both macroscopic and microscopic techniques. The Shanidar 3 Neanderthal, from the upper Mousterian levels of Shanidar Cave in northern Iraq, has a geological age of at least 45,000 to 50,000 years (*3*) and is morphologically similar to the other Neanderthals from the Near East and Europe (*4*).

The Shanidar 3 partial skeleton retains portions of the left pubic symphysis and auricular surface, both of which exhibit the characteristic age-related changes of recent humans (*5*, *6*). The pubic symphysis exhibits stage 5 for both the first and third components of the McKern and Stewart system (*5*) (the second component is indeterminate), suggesting an age at death of at least 38 years. The morphology of the auricular surface suggests an age of at least 40 years, probably between 40 and 50 years, according to the scale of Kobayashi (*6*). These age estimates are supported by the presence of relatively advanced osteophytosis on the lower thoracic and upper lumbar vertebrae and the occurrence of arthritic exostoses at the costovertebral, cubital, talocrural, and subtalar articulations. The preserved teeth suggest a more advanced age, since the crowns of the teeth were largely or completely removed by occlusal attrition. These data together suggest an age at death of at least 40 years of age.

Table 1. Quantification of secondary osteons in a femoral bone fragment from Shanidar 3. Values represent mean calculations from four adjacent periosteal fields.

Microstructure feature	Value
Secondary osteon lamellae area	0.287 mm^2
Haversian canal area	0.046 mm^2
Secondary osteon lamellae and Haversian canal area	0.333 mm^2
Secondary osteons	19.0 mm^{-2}
Haversian canal and primary osteons	20.25 mm^{-2}
Secondary osteon perimeter	0.394 mm
Haversian canal perimeter	0.127 mm

Extensive postmortem changes in the Shanidar 3 femoral fragment made it fracture easily when sectioning was attempted without embedding. A two-stage embedding process was thus employed to obtain a sufficiently thin section of bone for microscopic examination of the secondary osteons. The sample was immersed in 10 percent polyvinyl acetate for 60 minutes and then air dried for 48 hours. A 250-μm thin section, dehydrated successively for 20 minutes each in solutions of 50, 65, 85 and 100 percent ethanol, was immersed in propylene oxide for 1 hour, then in a diluted mixture of epoxy embedding medium, and finally in the embedding medium alone. An 80-μm thin section was cut and examined with a phase-contrast microscope with a 10 by 10 μm eyepiece grid. On the basis of stereological principles of morphometry (*7*), seven variables were quantified (Table 1).

The secondary osteons are well preserved in the femoral section of Shanidar 3 (Fig. 1) in spite of extensive postmortem replacement of the organic and mineral phase of the bone. The cracking that is apparent in the interstitial lamellae and to a lesser extent in the osteon lamellae is due to postmortem changes. The results obtained from the stereological quantification of the secondary osteons (Table 1) are consistent with those obtained from forensic skeletons (*8*), cadavers (*9*), and archeological skeletons (*10*).

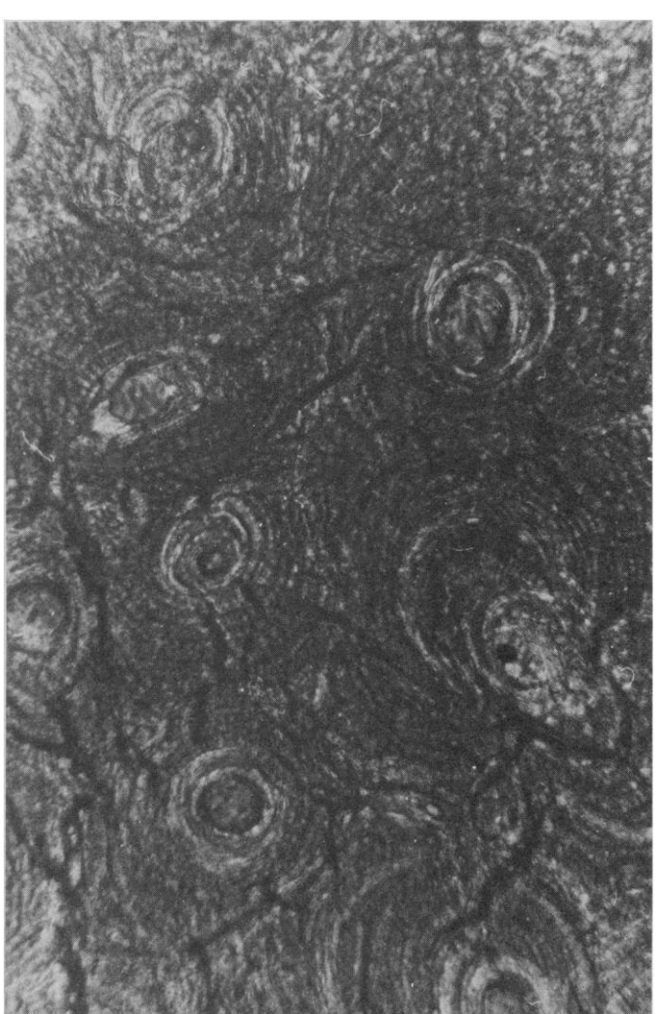

Fig. 1. Photomicrograph of the femoral midshaft section from Shanidar 3. Secondary osteons are apparent throughout the section in spite of extensive postmortem mineral replacement (×173).

Our estimation of the age at death of the Shanidar 3 Neanderthal is 42 ± 8.2 years. For this estimate we used the regression equation for age $y = 8.387 + 100.133x$, where x is the osteon lamellae plus Haversian canal area (*2*). This equation is standardized from known ages at death of U.S. whites. Our estimated age at death (42 years) agrees well with the estimated age derived by the macroscopic method (40 to 50 years). The absolute accuracy of these age-estimating techniques cannot be determined in extinct Neanderthal populations. However, where the fossil material is sufficient to allow the application of both techniques, a basic agreement in the age assignments improves the confidence of a reasonably accurate age at death.

It is often the case in fossil finds that the features on the skeleton required for macroscopic age determination are lack-

ing, whereas fragments of long-bone midshafts are often recovered, thus making the microscopic technique the only reliable aging technique. Since the amount of material needed for analysis by the microscopic technique is small (0.4-cm section), it may be applied to highly fragmentary and poorly preserved fossil finds.

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11. We thank V. Frederich and M. Goss for assistance in preparing the sections.

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Terminal Cretaceous Extinctions and the Arctic Spillover Model

Gartner and McGuirk (*1*) have outlined an Arctic spillover model to account for the observed abrupt marine and terrestrial extinctions at the Cretaceous-Paleocene boundary. Although they devote nearly all of their attention to discussion of climatic effects that might ensue subsequent to the hypothetical spillover event, we would like to redirect attention toward the Arctic Ocean, even though Watts *et al.* (*2*) have concluded that the sedimentary section that forms the basis for the model is slumped allochthonous sediment.

In chronological order, Gartner and McGuirk's model requires first, a Late Cretaceous cessation of exchange of marine water between the Arctic Ocean and the world ocean. Although available data (*3*) support a closed connection between the North Atlantic and the Arctic Ocean throughout the Cretaceous, paleocontinental maps (*4*) and paleoceanic reconstructions (*5*) depict a wider connection than exists at present between the North Pacific and Arctic oceans during Late Cretaceous time. Patton and Tailleur (*6*) presented evidence that the compressional tectonics between North America and Eurasia occurred sometime between the middle Late Cretaceous and middle Tertiary but were most probably related to opening of the North Atlantic. Pitman and Talwani (*7*) stated, on the basis of tectonic studies, that the size of the gap between Alaska and Siberia was significant prior to 60 to 63 million years ago.

Second, the model requires that normal marine organisms within the pre-isolated Arctic Ocean be ecologically replaced by species tolerant of fresh or brackish conditions during the isolation period. Although no deep-sea core from the Arctic Ocean has yet recovered a continuous sequence across the boundary, two cores that bracket the Cretaceous-Paleocene boundary have been recovered from the central Arctic Ocean. Core 437 contains a flora that correlates with the Late Cretaceous *Lyramula furcula* silicoflagellate zone, and core 422 bears the Early Paleocene (Danian) *Corbisema hastata* silicoflagellate zone which has been correlated with the *Cruciplacolithus tenuis* coccolith zone. All silicoflagellates are marine. In an earlier exchange (*8*), Gartner and Keany erroneously cited the reported presence (*9*) of the Late Cretaceous silicoflagellate species *Vallacerta siderea* at DSDP Site 275 to represent abnormal salinity. Their error was admitted by personal communication on 1 May 1979. In support of their model, Gartner and McGuirk cite Tourtelot and Rye's (*10*) mollusk isotope data which refer to Campanian and early Maestrichtian mollusks whose most northerly sites are at latitudes 70°; they also conclude that if the isotopic data reflect dilution, the salinity difference was not substantial.

Third, the model requires a trigger mechanism of rifting between Greenland and Norway that is simultaneous with the extinction event. The extinction event has been placed at the base of or just below anomaly 29 (*11*). Talwani and Eldholm (*3*), in a study of the evolution of the Norwegian-Greenland Sea, place the initiation of rifting between anomaly 24 and anomaly 25 time: anomaly 24 is present whereas anomaly 25 is missing. Hence, opening of the Norwegian-Greenland Sea postdated the extinction event by millions of years. If causes and effects are not nearly simultaneous, logic requires that the cause precede the effect.

Finally, the proposed model based on the present volume of the Arctic Ocean utilizes a volume calculation of total water available for the spillover event. The Eurasian Basin, which approximates one-half the present Arctic Ocean (*7*), originated well after the extinction event. Vogt *et al.* (*13*) place the time of initial rifting of the Lomonosov Ridge from Eurasia at anomaly 24 time, nearly 10 million years after the extinction event. Further, the Canada Basin, during Late Cretaceous time, was only 1500 m deep (*14*) as compared to its present depth of 3800 m. Consequently, since volume is a function of both area and depth, the model's estimate of water available to blanket the world ocean with a layer of low salinity water is inaccurate.

To summarize, the hypothetical solution to the problem of the massive terminal Mesozoic extinction event is not useful because it does not account for the boundary limits imposed by available data.

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Clark and Kitchell (*1*) do not take into account that the conclusion of Watts *et al.* (*2*) is based on cores not from Ekofisk but from the nearby Abuskjel structure and that Perch-Nielsen *et al.* (*3*) were unable to demonstrate the presence of

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