

Tetracycline-Labeled Human Bone from Ancient Sudanese Nubia (A.D. 350)

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Abstract. *Nubian bone recovered from an X-group cemetery (A.D. 350 to 550) exhibits a pattern of fluorescence identical to that of modern tetracycline-labeled bone. When it is viewed under ultraviolet light at 490 angstroms, fluorophors are visible as a characteristic yellow-green fluorescence on surfaces that were actively mineralizing at the time of exposure. Contamination of stored grains provided the proper environment for cultivation of tetracycline-producing Streptomyces. Evidence for exposure to antibiotics in an archeological population is relevant to studies of the evolution of R factors and to the interpretation of health and disease within the population.*

When thin sections of compact bone from an archeological population of Sudanese Nubians were viewed under a fluorescence microscope, a pattern of fluorescence identical to that of modern tetracycline-labeled bone was observed (1). This bone predates the antibiotic era by at least 1400 years.

The prescribing of tetracyclines as broad-spectrum antibiotics in the 1950's led to the discovery that their use causes staining and fluorescence in calcifying tissues (2). In compact bone, tetracycline is bound to the surfaces of Haversian systems (osteons) actively mineralizing at the time of the administration of the drug; mature osteons remain unaffected. The tetracyclines are active ion chelators, forming complex calcium and protein compounds (3) that produce intense yellow-green fluorophors (2). This fluorescence persists through time (4) and has been useful in the analysis of bone dynamics (5).

The fluorophor-labeled bone was recovered from an X-group (6) cemetery on the west bank of the Nile River opposite the town of Wadi Halfa in the Sudan. The X-group (Ballana) period (A.D. 350 to 550) represents a phase of independent rule after the decline of the Meroitic Kingdom (350 B.C. to A.D. 350) and preceding the rise of Christianity (A.D. 550

to 1300) in Nubia. The X-group people were intensive agriculturalists, cultivating the floodplains of the Nile.

The X-group population has been extensively studied. Samples of both compact (7) and trabecular (8) bone were examined macroscopically. Microscopic examination of compact bone was carried out (9), and there was gross examination of dentition (10) and changes in cranial morphology (11). Patterns of pathology were related to the adaptation of the group (12).

The material was excavated in a state of excellent preservation—in most cases with hair and naturally mummified tissue adhering to the skeletons. Thin sections were taken from the area immediately distal to the lesser trochanter on 15 left femurs. The undecalcified, unembedded sections were mounted with Hillquist epoxy on petrographic slides (13), ground by hand to a thickness of 90 μm with carborundum paper of 320, 400, and 600 grit, and polished. The sections were viewed at 490 \AA with a fluorescence microscope (Reichert NR340101) with a darkfield VG-1 filter. Fluorescence was observed in all samples.

Serial extraction of collagen indicates little postmortem degradation. Analyses of hydroxyproline, proline, and mucopolysaccharides suggest that collagen

was present in levels comparable to that extracted from biopsy or autopsy material (14).

Several observations support the hypothesis that tetracycline caused the bone fluorescence. The fluorophors were visible at the same wavelength (490 \AA) and had the same color (yellow-green) as oxytetracycline-labeled bone. In addition, the pattern of fluorescence occurred in a manner histologically identical to that of labeling by tetracycline.

In vivo, tetracycline can only be deposited on actively mineralizing surfaces, forming a discernible pattern in osteons and across the cortical surface. Intense fluorescence corresponds to areas that were actively mineralizing at the time of exposure to the tetracyclines; low concentrations of fluorophors correspond to the areas that were mineralized later (15). The patterns of fluorescence in the X-group bone are directly comparable to those observed in modern tetracycline-labeled bone.

About 30 percent of all the osteons we observed contained fluorophors; some were completely fluorescent even though they were adjacent to nonfluorescing osteons (Fig. 1A). Most of the fluorescing osteons were located in the endosteal third of the compact bone. Femurs from older individuals showed some fluorescence in the subperiosteal area. No other age (or gender) pattern was discernible.

In most instances, fluorescence appeared as wide bands corresponding to the lamellar bone in the osteons (Fig. 1). In most of the labeled osteons, the brightest fluorescence was present in the inner one-third, followed by the outer and middle thirds. Areas of high mineral density surrounding Haversian canals fluoresced brightly (Fig. 1B); they correspond to the edge sclerosis seen in microradiographs.

Mineralization occurs on the subperiosteal and endosteal surfaces (16) as well as within osteons (17). Fluorescence was observed in the lacunae (Fig. 1), in the lamellae on the subperiosteal surface, and on the endosteal surface. In the diaphysis, fluorophors appeared as bands 5 to 10 μm in thickness on the subperiosteal and endosteal surfaces. In cross section, they formed concentric rings (18).

A small percentage of osteons exhibited "feathering," a phenomenon described by Frost (19) as incompletely mineralized bone matrix in humans. In vivo, this bone is relatively impermeable to tetracycline (19), the feathered portion not being labeled. Figure 1C depicts a

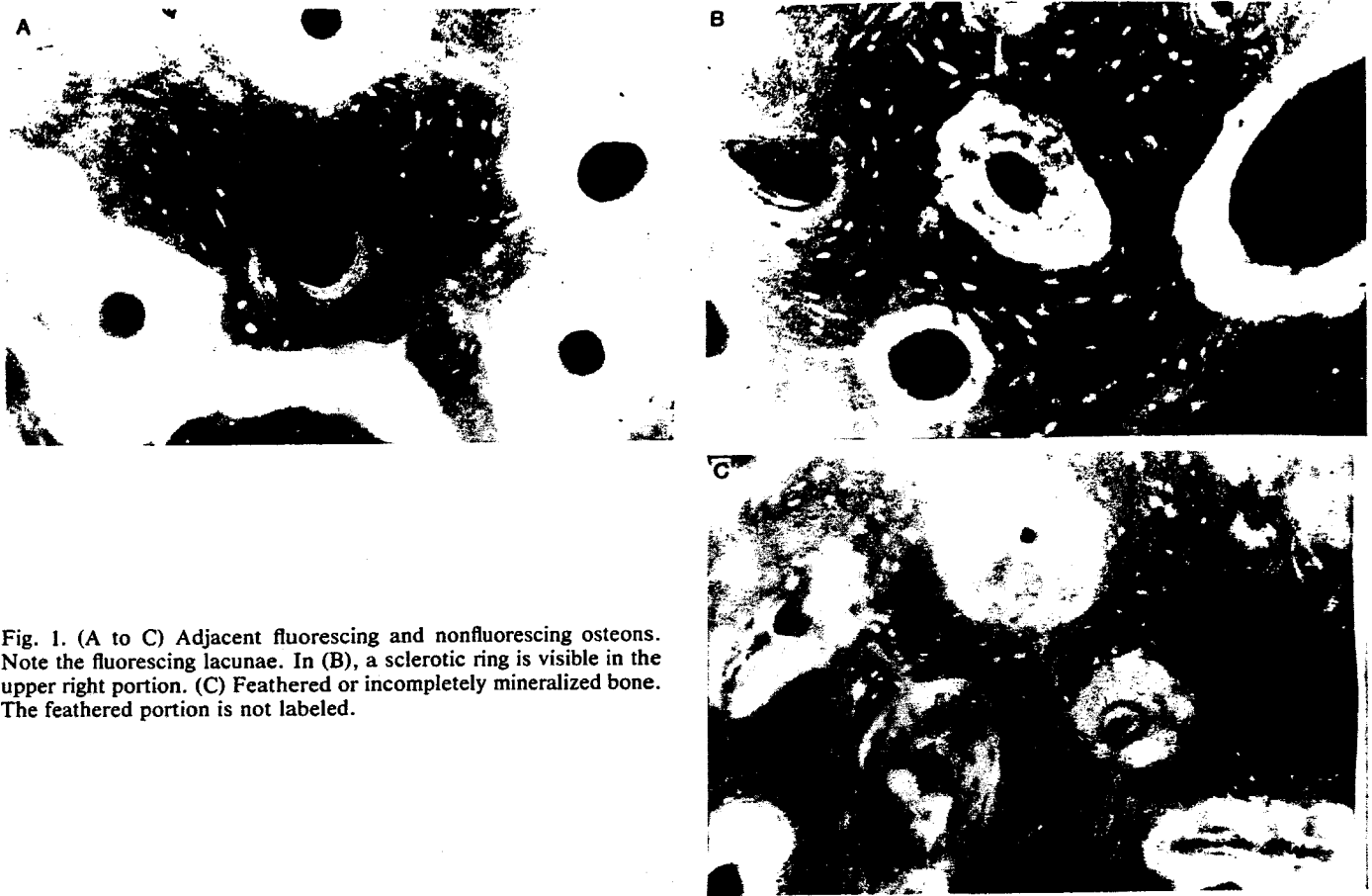


Fig. 1. (A to C) Adjacent fluorescing and nonfluorescing osteons. Note the fluorescing lacunae. In (B), a sclerotic ring is visible in the upper right portion. (C) Feathered or incompletely mineralized bone. The feathered portion is not labeled.

feathered osteon; the labeled, normally mineralized portion indicates that the osteon was in the formative process at the time of its exposure to tetracycline.

Fluorescence also appeared on Volkman's canals. In these nongrowing surfaces, the mineral content was still increasing at the time of labeling, reflecting either the process of secondary mineralization or the final stage of primary growth (4).

Fluorophors other than tetracycline have been observed to bind to mineralizing bone. Alizarin (a derivative of madder) and quercetin (a flavine derivative) fluoresce in new bone under ultraviolet light, but are observed in the red and gold ranges, respectively (20). Naturally derived tetracyclines are observed in the green to yellow-green range (18). Porphyrins, carotenoids, lipofuscins, and oxidized cytochromes bind to bone and soft tissue, but are only visible in the red to orange range (18).

The possibility that the fluorescence in the bone was due to postmortem infestation by mold was also considered. Fungi of the genera *Mucor*, *Cladosporium*, *Candida*, and *Dematiaceae* all attack bone, some species even causing fluorescence (21). However, the fluorescence is diffuse (Fig. 2), not patterned as is labeling

by tetracycline. Also, the Nubian bones were undamaged, making mold infestation unlikely.

The occurrence of labeling by tetracycline in a preantibiotic population raises the question of the means by which they were exposed. It is hypothesized that grain stored in mud bins provided the medium for cultivation of *Streptomyces* (22), the moldlike bacteria from which nonsynthetic tetracyclines are produced. *Streptomyces*, which require a very dry, warm, alkaline environment, comprise 60 to 70 percent of the bacteria in the desert soils of Sudanese

Nubia (22). Subsisting through the breakdown of organic soil particles, the *Streptomyces* hold a selective advantage, in Nubian soils, over faster growing bacteria that are more dependent on moisture, acidity, and moderate temperatures (22).

Wheat, barley, and millet were staple crops of the X group, providing bread and beer as part of their diet. Storage of these grains in mud bins provided the environment and nutrients necessary for the cultivation of tetracycline-producing *Streptomyces*. We have isolated *Streptomyces* from grain and barley cul-

Fig. 2. Diffuse fluorescence caused by mold infestation. The sample is from a prehistoric Native American.



tured at 55°C on soil samples collected from the Sudan; some cultures exhibit obvious antibiotic behavior.

Tetracycline is effective against both Gram-negative and Gram-positive bacteria, Rickettsiae, spirochetes, and some viruses (23, 24). It also has an anti-malarial effect on humans (25). While it may not have been intended as a therapeutic substance, its consumption by the Nubians would have had broad implications for their health, disease, and demographic patterns and for the evolution of R factors (26) within that population. It may explain the extremely low rates of infectious disease found among the X group.

The side effects of tetracycline include temporary inhibition of bone growth in infants (25), vitamin B depletion (24), and interference with phagocytic activity (24) and with protein synthesis (24, 27). Extended exposure to therapeutic dosages of tetracycline inhibits spermatogenesis; smaller dosages slow sperm mobility (28). The bones of the X group have not yet been studied with a view toward determining dosages, but the amount of fluorescence suggests therapeutic levels.

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References and Notes

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13. All mounting and grinding materials were examined for fluorescence. A minute amount of fluorescence was observed with epoxy alone. This might add artifactual, background fluorescence to a section, but it would be diffuse, not patterned like labeling by tetracycline.
14. R. G. Brown, University of Massachusetts, performed the chemical analyses.
15. The rate of mineralization is higher at the beginning of the process than toward its completion. Seventy percent of the mineralization occurs within 1 to 2 days after deposition of the matrix; the remainder of the process may take up to 6 months.
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29. The original excavation and analyses were supported by grants GS7, GS286, and GS557 from the National Science Foundation. Subsequent analyses were supported by NIH research grant NIH-1-R01-02771-01. The analysis of tetracycline was partially funded by University of Massachusetts biomedical research support grant RR07048. We thank P. Hepler, B. R. Levin, M. J. Saunders, A. Swedlund, W. Putschar, B. Deres, and E. F. Vlach for their assistance.

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