Green Bone

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Objective: To describe the unusual finding of yellow-green–colored bone during routine orbital surgery, to detail its investigation, and to demonstrate its benign nature.

Methods: When green bone was found, specimens were sent for light and fluorescent microscopy, ultraviolet photography, and spectrophotometry.

Results: Yellow-green bone was encountered in 3 patients during orbital tumor excision or orbital fracture repair procedures. The only common cause was prior use of tetracycline during adolescence. All patients had healthy white dentition. In all cases, absence of neoplasia was demonstrated histologically. The bone fluoresced with a bright yellow-green color when exposed to 365-nm ultraviolet light. Histologic analysis demonstrated fluorescence located near the haversian canals. Spectrophotometry revealed absorption at 4 wavelengths specific to tetracycline: 230, 275, 380, and 440 nm.

Conclusions: Fixation of tetracycline and ensuing fluorescence occurs mostly in areas of new bone growth and mineralization. This happens during childhood but also with bone remodeling associated with tumors or fractures. Once mineralized, teeth should therefore not be affected if tetracycline exposure occurs after ages 8 to 10 years. This paucity of external clues can lead to the surprising but innocuous surgical finding of green bone. Careful history and proper investigation can confirm its origin.

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Proper surgical planning and preparation are key to a successful operation. Nevertheless, surgeons should always expect the unexpected, as cases are often fraught with unusual findings that may require further investigation or management.

Three cases of relatively routine surgery in an oculofacial and orbital surgery service are described herein because of the surprising intraoperative finding of unusual yellow-green–colored bone. Because this hue was associated with a tumoral process in 2 of the cases, there was concern about possible invasion of the adjacent bone. Upon postoperative questioning, all patients reported at least 1 year of tetracycline use during adolescence. This study reports the detailed investigation that followed this finding in all 3 patients and demonstrates the benign nature of the bone discoloration.

Methods

The patients were seen between January 2003 and October 2004. When yellow-green-brown bone (very similar to a dark khaki color; CMYK=0, 3, 43, 26; RGB=189 183 107) was found during surgery, specimens were sent for light and fluorescent histologic analysis, ultraviolet photography, and spectrophotometric analysis. This dark khaki color will be referred to as khaki in the body of the text. Charts were reviewed retrospectively.

Fluorescence and Light Microscopy

After overnight fixation in a 10% phosphate-buffered formalin (pH 7.0), 1.0-mm sections of bone were placed into Karnovsky’s KII solution (2.5% glutaraldehyde, 2.0% paraformaldehyde, and 0.025% calcium chloride, in a 0.1–mol/L sodium cacodylate buffer; pH 7.4), fixed overnight at 4°C, and stored in a cold buffer. The bone sections were then postfixed in osmium tetroxide, stained en bloc with uranyl acetate, dehydrated in graded ethanol solutions, infiltrated with propylene oxide and Epon mixtures, flat embedded in pure Epon, and polymerized overnight at 60°C. Two- and 3-µm sections were cut with a tungsten knife (Delaware Diamond Knives, Wilmington, Del), stained with toluidine blue, and examined by light microscopy using a Zeiss Axioshot microscope (Carl Zeiss, Göttingen, Germany). Representative unstained areas were chosen for fluorescence microscopy using a 4’6-diamidino-2-phenylindole excitation wavelength (maxi-
mum emission, 377 nm) on an Olympus BX60 fluorescence microscope (Olympus Optical Co, Hamburg, Germany). Digital images were captured for both fluorescence and light microscopy using a Spot RT-SE6 digital camera (Diagnostic Instruments, Sterling Heights, Mich).

GROSS ULTRAVIOLET PHOTOGRAPHY

Representative sections of bone were photographed after formalin fixation under a 365-nm UV-A lamp (Spectroline EA-240; Spectroline Corp, Westbury, NY). Photographs were taken with 400 ASA film (Kodak, Rochester, NY) using a 35-mm reflex camera with a 60-mm lens (Nikon, Melville, NY).

SPECTROPHOTOMETRY

To quantify the tetracycline content of the bone specimen in patient 3, the bone was first milled to a powder using a mortar and pestle. Three milliliters of 0.25 N sodium hydroxide was added to the crushed bone and the solution was transferred to a test tube. The solution was incubated at 40°C for 1 hour and then centrifuged. The supernatant was transferred to a cuvette (quartz path length, 10 mm) (Agilent, Boeblingen, Germany) for scanning.

A United States Pharmacopeia tetracycline hydrochloride solution was used as a reference sample. Its concentration was 20 µg/mL. The medium was 0.25 N sodium hydroxide. It was also incubated at 40°C for 1 hour.

Both solutions, as well as the blank solution (0.25 N sodium hydroxide), incubated at 40°C for 1 hour, were scanned from 220 to 500 nm using a ultraviolet-visible spectrophotometer (Hewlett-Packard, Palo Alto, Calif). All 3 solutions were tested for absorption at peaks of 230, 275, 380, and 440 nm. These peaks were chosen because they were the most prominent on spectroscopic analysis of the tetracycline reference sample (Figure 1).

REPORT OF CASES

PATIENT 1

A 49-year-old white man had episodes of burning and tingling in his left superolateral orbit. He noted a tender non-inflammatory mass in the outer corner of his upper eyelid that gradually enlarged in size during a period of 3 years. He had been treated with oral tetracycline for 3 years from ages 14 to 17 years for severe acne problems and for cysts at the back of his neck. His past medical and ocular histo-

ries were otherwise unremarkable. He smoked 1 pack of cigarettes per day for 18 years. His daily medications included multivitamins, zinc, and nicotine patches.

The patient has worked in the field of the research and development of industrial textile colors for 25 years. During the first 2 years of his employment, some of the dyes used contained cadmium. Although these dyes were present in the building, the patient has never been in direct contact with these products. The patient also worked as a metal grinder for about 6 months at the beginning of his career. The patient's general physical examination results were unremarkable. Of note, even though he has never had crowning, veneering, or teeth-whitening procedures, he had healthy white dentition with no discoloration (Figure 2) and no bony or articular anomalies.

The patient’s corrected visual acuity was 20/20 OU. Examination confirmed the presence of a mass involving the anterior superior orbit associated with left temporal eyelid ptosis. Although the results of the rest of the ocular and orbital examination were unremarkable, a computed tomography scan demonstrated a 15 × 7-mm uniform low-density tumor of the left lacrimal gland with no evidence of bony erosion. On magnetic resonance imaging, there was no perineural, intracranial, or nodal extension. The tumor seemed encapsulated and limited to the palpebral lobe of the lacrimal gland, which was excised. The lesion abut-

Figure 1. Spectroscopic analysis of a tetracycline reference sample tested for typical absorption peaks at 230, 275, 380, and 440 nm.

Figure 2. Patient 1 had healthy white dentition with no discoloration even though his bones were green-brown because of tetracycline use during adolescence.
The bone was described as green-brown but showed no evidence of neoplastic invasion of bone on histologic analysis. The patient underwent an orbitotomy to excise the remaining lacrimal gland and surrounding tissues when histologic analysis demonstrated an adenoid cystic carcinoma. The bone taken from the superolateral orbit was described as green-brown but showed no evidence of malignancy (Figure 3). The patient's total bilirubin serum concentration was tested and found to be normal at 0.4 mg/dL. He has had no recurrence 18 months after completion of adjuvant treatments.

PATIENT 2

A 36-year-old white man had an 18-month history of progressive proptosis and decreased vision in his left eye with no associated pain. His medical history was significant for severe acne during adolescence, which was treated with tetracycline from ages 15 to 17 years. He has been smoking 1 to 2 packs of cigarettes per day for the last 20 years. He was also taking lexofenadine, celecoxib, and bupropion. He has worked as a musician his whole life.

On examination, his corrected visual acuity was 20/20 OD and 20/25 OS. Frank hypoglobus and 6 mm of proptosis were measured in the left eye. He had limited up-gaze and abduction of the left eye but no associated optic neuropathy. A painless palpable mass was found in the left lacrimal gland. A dilated fundus examination revealed choroidal folds in the macular area, but the results of the rest of the ocular examination were unremarkable. His teeth also had a normal white color. The results of the rest of his general physical examination were unremarkable.

An orbital computed tomography revealed a 22 × 14-mm, bilobed, well-circumscribed mass centered on the orbital lobe of the left lacrimal gland, with evidence of bone remodeling of the orbital roof and lateral wall (Figure 4). A lateral orbitotomy with bone flap was performed to excise the tumor. The encapsulated tumor was completely removed. Interestingly, the orbital rim had an intense yellow color (Figure 5). The bone also seemed softer in this area and was therefore sent to our pathology department. Histopathologic results were consistent with a pleomorphic adenoma of the lacrimal gland. There was no evidence of malignancy in the bone that was removed.

His latest follow-up was 6 months after the operation. Proptosis, hypoglobus, and motility restriction had completely disappeared and visual acuity was improving.

PATIENT 3

A 26-year-old white man came to us after he was allegedly struck by a brick to the right side of the face. He had taken systemic tetracycline for a period of 1 year, starting at age 14 years, for acne problems. He had been smoking about half of a pack of cigarettes per day for the last 6 months. He was taking methylphenidate, sertraline, and divalproex sodium. He is currently a student and has had no prior employment.

On examination, his uncorrected visual acuity was 20/200 OD and 20/25 OS. His right eye was proptotic by 1 mm and there was hypoglobus. His ocular motility in the right eye was limited, especially in upgaze. He had malar flattening and lateral canthal dystopia. Open fractures were found in the frontozygomatic area and along the inferior orbital rim. The ocular examination re-
vealed a hyphema, an intraocular pressure of 26 mm Hg, and a macular scar in the right eye. His teeth had a normal white color. His general examination results were unremarkable.

A computed tomography scan revealed a highly comminuted zygomaticomaxillary complex fracture with large orbital floor and medial wall deficits. A zygomaticomaxillary fracture repair with open reduction and internal fixation was performed 2 weeks after the trauma. During the surgery, the exposed bones were noted to be generally khaki in color. Some of the fragments were sent to our pathology department. Postoperatively, the patient was left with only minor residual upgaze restriction in the right eye.

The histology of the bone fragments did not show any evidence of malignancy, but the pathologist described the bone as green-yellow. Additional tests were done to elucidate the origin of this discoloration. Bone photographed under ultraviolet light clearly demonstrated a yellow-green fluorescence as compared with a control (Figure 6). Histologic sections examined under ultraviolet light showed fluorescence located near the haversian canal surfaces and embedded throughout lamellar bone (Figure 7).

Finally, the piece of bone was submitted to spectrophotometric analysis. Absorbance was measured at 230, 275, 380, and 440 nm, peaks typical for tetracycline. Compared with background absorbance, ratios of 51:1, 125:1, 71:1, and 44:1, respectively, were found. The presumed concentration of tetracycline in bone was estimated to range from 6.1 ng/g to 760 ng/g (Table).

### Table. Spectrophotometric Analysis of Bone in Patient 3

<table>
<thead>
<tr>
<th>Wavelength, nm</th>
<th>S/N Absorbance Ratios Above Background</th>
<th>Tetracycline Concentration in Bone, ng/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>230</td>
<td>51:1</td>
<td>760</td>
</tr>
<tr>
<td>275</td>
<td>125:1</td>
<td>376</td>
</tr>
<tr>
<td>380</td>
<td>71:1</td>
<td>94.9</td>
</tr>
<tr>
<td>440</td>
<td>44:1</td>
<td>6.1</td>
</tr>
</tbody>
</table>

In the patients described here, there was no real osteolysis but only bone remodeling. Patients also had no history of hematoma or spontaneous bleeding in the vicinity of the tumor that could cause bilirubin staining. Patient 3 may have had some bleeding following facial fractures, but his bone was extensively tested, and the pattern of fluorescence as well as the spectrophotometry findings were compatible with the presence of tetracycline rather than unbound bilirubin, bilirubin bound to albumin, or lumirubin.

There are several other possible causes of khaki-colored bone, but none of the following were applicable.

#### COMMENT

Three patients requiring relatively routine surgery in an oculofacial and orbital surgery service were seen. To the surgeon’s surprise, the bone encountered was unusually yellow-green-brown (khaki) in place of the typical ivory-cream color usually seen. In the first 2 cases, there was concern about possible neoplastic involvement of the bone, but pathologic evidence proved that this was not the case.

In the setting of neoplasia, yellow osteoid has been described in association with tumors caused by osteolysis and lysis of red cells with increased bilirubin. The changes were found in demineralized trabecular bone.
to the patients described here. Although serum bilirubin was only tested in the first patient, none of the patients had clinical jaundice. Long-term exposure to high levels of cadmium can cause yellow discoloration of teeth and osteoporosis and/or osteomalacia. This occurs mostly by inhalation of cadmium by pottery and ceramic workers, manufacturers of nickel-cadmium batteries, and metal or plastic workers. Cadmium is also found in dyes used for printing and in semiconductors. Moreover, cigarette smoking is a major source of nonindustrial cadmium exposure. Apart from patient 1, who may have had minimal exposure to cadmium or metal dust, the patients described here did not work in any of these industries. On the other hand, they were all cigarette smokers. The khaki bone color found during surgery is unlikely due to the cigarette smoking; we have performed bone surgery on hundreds of patients who smoke, and this finding has never been encountered. Thalidomide also reportedly causes yellow-green discoloration of bones in dogs. None of our patients were exposed to thalidomide. Skulls from patients with diabetes have been found to be yellow-brown or darker than normal, but this has been disputed. None of our patients had diabetes. Dietary carotenoids have also been suspected but never proven to cause yellow discoloration of bone. Finally, it is important to note that the human skull has a light yellow color that becomes more saturated with age. In this series, the oldest patient was 49 years, and the khaki discoloration found during the surgeries was definitely outside of the normal range.

The only etiology that was common to all 3 patients was prior use of tetracycline. For all patients, absence of neoplasia was demonstrated histologically. The bone was shown to fluoresce with a bright yellow-green color when exposed to ultraviolet light with a wavelength of 365 nm (Figure 6). In fact, histologic sections demonstrated the same type of fluorescence (Figure 7). In addition, spectrophotometry of the bone specimen from patient 3 revealed absorption at 4 wavelengths specific to tetracycline.

**TETRACYCLINE**

Tetracyclines were first introduced for clinical use in 1947. These antibiotics inhibit protein synthesis by binding to the 30S subunit of bacterial ribosomes. Tetracyclines are therefore active against gram-positive and gram-negative bacilli, enterobacteriaceae, chlamydia, spirchetes, mycoplasma, rickettsia, actinomycyes, atypical mycobacteria, and amoebae. They are often used for prolonged periods in the treatment of acne. Some of the most frequent side effects encountered with tetracyclines include gastrointestinal irritation with abdominal discomfort, nausea, and vomiting. They can produce hepatotoxicity, vestibular toxicity, and azotemia. Skin photosensitization is also possible with long-term use. Additionally, they can cause superinfection with fungi, staphylococci, and Clostridium difficile. Hypersensitivity reactions and even anaphylaxis sometimes occur. Drug fever and pseudotumor cerebri are rarer.

Another notable side effect is the yellow-to-brown tooth discoloration caused by tetracycline exposure during the second or third trimesters of gestation or during early childhood. Odontogenesis of primary and permanent dentition occurs during this period. Both can be affected. Children are at risk of discoloration of permanent teeth up to the age of 8 to 12 years. Tetracycline is therefore generally contraindicated during these time periods. Notably, there have been reports that staining can occur when tetracyclines are given during long periods of time in adulthood because of continued demineralization and remineralization of the enamel or dentine. If affected, initially fluorescent green teeth eventually become brown and nonfluorescent owing to a process of photo-oxidation. The same phenomenon can occur in bone that is unprotected from light, but in teeth, the exposed enamel and dentine, where tetracycline is deposited, are affected. Whether or not enamel and dentine hypoplasia or hypomineralization can result from tetracycline exposure is debatable. Interestingly, fingernails and toenails are also at risk of blackish discoloration and onycholysis from the use of tetracycline, especially after exposure to the sun. Unlike teeth that are fully calcified at the end of childhood, nails can be affected into adulthood because they maintain an active state of growth.

Because of its strong propensity for chelating cations like magnesium and calcium, tetracycline can also become incorporated into the inorganic phase of bone. Calcium binds to the 4-ringed nucleus common to all cyclines, rather than to its attached radicals, possibly forming a tetracycline-hydrochloride-calcium orthophosphate complex. This attachment is very stable and after parenteral administration, tetracycline localizes to the skeleton very rapidly. Bone then becomes an excellent reservoir for the antibiotic for prolonged periods of time. In this study, tetracyclines have been found in bones from an ancient Sudanese Nubian population, dating from 350 AD. It was also found in skeletons and dental enamel in a population from Dakleh Oasis, Egypt, dating back to the late Roman period. In both of these cases, ingested stored grains may have been contaminated by streptomycetes that could produce the antibiotic.

Tetracycline can thus emit a yellow-green fluorescence under high-intensity ultraviolet illumination. Appropriate excitation wavelengths vary in the literature from 365 to 490 nm. Variations in these values may depend on hydration of the specimen, use of incident vs transmitted light, and whether tetracycline is chelated to calcium. In this study, an excitation peak of 365 nm was used for macroscopic fluorescence, and transmitted light at a wavelength of 377 nm was used for fluorescence microscopy on bone samples chelated by tetracycline.

After absorption, tetracycline emits fluorescence between 480 and 660 nm, with a maximal peak between 515 and 525 nm. Although not precisely measured in this study, the emission spectra for yellow-green light was 377 to 597 nm for yellow and 492 to 577 nm for green. This phenomenon is known to disappear in decalcified bone, indicating again that tetracycline is firmly attached to its calcium.

Tetracycline can also be detected in bone by using spectrophotometry. As previously described, the technique used in this study revealed peaks that were consistent with.
the presence of tetracycline. Peaks at 230, 275, and 440 nm are the same as those reported by Oklund et al. The peak at 380 nm is also the one recommended for identification by the United States Pharmacopeia.

Fixation of tetracycline to long and flat bones, and ensuing fluorescence, can be more or less generalized but occurs mostly in areas of new bone growth and mineralization, such as in the periosteal and endosteal surfaces (which are in close relation to blood transport), in new haversian canals, in a callus postfracture, or in malignant bone tumors. Accordingly, fluorescence in patient 3 was partly concentrated near the haversian canal surfaces. Because of this predilection for new bone, tetracycline labeling is a common experimental tool to assess bone growth and metabolism. As part of these tests, 2 administrations spaced in time led to the formation of 2 fluorescent bony zones. The distance between both allows measurement of growth. Fixation of tetracycline is less important in areas where bone is not growing, such as established cortical bone, but it can chelate calcium ions that have been exposed in microcracks.

In adults, deposition of tetracycline in bone seems to have no deleterious effect. Its effect may be more problematic in the young. In fact, tetracycline has been shown to inhibit mineralization and development of the chick embryo skeleton. It was also shown to slow bone maturation in monkeys. In humans, it has caused a reversible 40% reduction of fibular growth in premature children.

Because tetracycline seems to be problematic mostly in growing bones and teeth, tissues already mineralized should not be affected and should not become fluorescent green when exposed to tetracycline, unless new mineralization occurs. Because significantly discolored teeth were not noted in any of our patients, tetracycline exposure must have occurred when they were older than 8 to 10 years (after mineralization of teeth). In fact, all of the patients were exposed only during adolescence.

**BONE REMODELING**

Following skeletal maturity, bone continues to remodel throughout life and adapts to the mechanical demands placed on it. Throughout life, both cortical and trabecular bone undergo a balance of formation or resorption, with no net change in bone mass.

Based on the fact that tetracycline will stain areas of new bone formation or microcracks where calcium ions are exposed, the staining could possibly be more significant in the presence of a tumor. Neoplasia that remodels bone induces its absorption and redeposition. Neoplasia that erodes bone exposes calcium ions and also leads to new bone formation. In fact, Milch et al described a case of lytic metastases associated with tetracycline discoloration. Similarly, callus formation at fracture sites should also be expected to be stained with the antibiotic. In patient 3, no callus was yet seen at 2 weeks after the injury.

The generalized khaki color observed in the patients described results from tetracycline deposited in bones everywhere in the body during acne treatments. During adolescence, there is active bone growth and mineralization that favor generalized tetracycline deposition. Healthy adults remodel about 10% of their skeleton per year, releasing stored tetracycline into circulation. It is possible that tetracycline bone reserves from youth could thus be redeposited in areas of bone remodeling. In addition, the 10% bone remodeling that occurs in adults may allow any tetracycline ingested during that time to be deposited in those areas, as was seen in patients’ teeth. It is not yet known whether the prolonged course of doxycycline, often given to treat blepharitis and chalazia, is sufficient to affect bone.

In summary, tetracycline ingested during gestation and up to age 8 to 10 years will be deposited in mineralizing teeth in addition to bone. In other words, if teeth are brown, bone is likely discolored as well. As a general rule, if the antibiotic is administered during adolescence, while bones are still growing but permanent teeth are completely mineralized, bones may become discolored but teeth should remain unaffected, though there are exceptions. If tetracycline is taken as an adult, after all growth is completed, bones and teeth should remain generally unaffected, though areas of bone remodeling—which occur in the presence of tumors, fractures, or as part of the normal 10% turnover—may be subject to some tetracycline deposition. This tetracycline could then originate either from recent ingestion or from tetracycline reserves in the bone since youth and released during turnover.

**CONCLUSION**

The purpose of this article is to reassure the oculofacial surgeon who encounters khaki-colored bone during dissections, tumor excision, or fracture repairs. A patient’s history of prolonged tetracycline use can usually solve the mystery. Nevertheless, this should not dissuade the surgeon from performing necessary biopsies if tumoral bone invasion is suspected. When in doubt, the presence of tetracycline can be examined with macroscopic and microscopic fluorescence and spectrophotometry. Once satisfied with the results, tetracycline staining becomes nothing more than a marker of the patient’s prior antibiotic history.

**REFERENCES**
