CALIBRATION OF DENTAL LAYERS IN SEVEN CAPTIVE HAWAIIAN SPINNER DOLPHINS, *STENELLA LONGIROSTRIS*, BASED ON TETRACYCLINE LABELING

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ABSTRACT

To calibrate dentinal and cemental growth layer groups (GLGs) with real time, a study was conducted on the teeth from seven captive Hawaiian spinner dolphins that had been treated clinically with tetracycline (TCL) at numerous times over multiple years at Sea Life Park, Hawaii. To monitor layer accumulation as it occurred for 1 year, we gave single injections to three animals every 3 months and pulled a tooth from each every 6 months. By comparing dental-layer patterns between TCL labels that had been introduced at 6-month and 1-year intervals, annual patterns were distinguished. In the dentine, a thin, light layer (the first being the neonatal line) was formed about every 6 months. Each annual GLG contained 13 lunar monthly layers (LMLs). Using LML or light-layer counts, age, month, and year of birth were estimated for each of the seven specimens. All seven deposited nearly the same dentinal GLG thickness in the same year of life. Estimates of birth months indicated that five of the animals were born in late summer or early autumn and two were born in spring. Comparisons of dentinal labels with clinical records for a captive-born animal showed that TCL given to its mother was imparted via milk to the nursing calf. Time calibration of cemental GLGs showed that usually one cemental GLG was deposited annually, but in some cases a GLG was formed every second year or twice a year.

The technique of "reading" layers or growth layer groups (GLGs, terminology of Perrin and Myrick 1980) in teeth, developed to determine ages for pinnipeds in the early 1950's by Scheffer (1950) and Laws (1952), is now used routinely in dolphin studies (see reviews by Klevezal' and Kleinenberg 1967; Jonsgård 1969; Scheffer and Myrick 1980). Early work on dolphins (e.g., Nishiwaki and Yagi 1953; Sergeant 1959), showing a correlation between apparent age and number of GLGs led to the working assumption that GLG-deposition cycles are constant, each GLG usually, but not always, interpreted as representing 1 yr. Critical analysis of this assumption has been impaired by a lack of suitable material.

Three approaches have been used in efforts to calibrate dental GLGs with time and to determine their deposition rate: 1) In vivo labeling of tooth layers, 2) multiple extractions of teeth over time, and 3) examination of teeth from animals of known age. Nishiwaki and Yagi (1953) labeled the layered dentine in four wild-caught striped dolphins, *Stenella coeruleoalba*, by intramuscular injection of lead acetate paste. None of the four survived long enough for the labels to provide useful data.

Nielsen (1972) treated a young wild-caught harbor porpoise, *Phocoena phocoena*, with tetracycline (TCL) three times over a 370-d period. Three fluorescent labels were found in thin sections of its teeth examined in ultraviolet (UV) light "... but the uniform [unlayered] dentine made it impossible to determine the number of growth-layers formed per year" (Nielsen 1972:72).

Best (1976) administered oral doses of TCL hydrochloride, "Mysteclin-V", on each day over an 8-d period to each of three wild-caught dusky dolphins, *Lagenorhynchus obscurus*. Labels were detected in teeth of two of the three specimens after their deaths. In one specimen, dentine accumulated for 703 d between treatment and death averaged 200 μm/yr and 0.56 μm/d. In the other (older) specimen, the average deposition rate in dentine between the treatment label and the pulp-cavity wall was 77 μm/yr and 0.21 μm/d. Best concluded that the thickness of GLGs decreases significantly with age in dusky dolphins.

Gurevich et al. (1980) successfully introduced a single TCL label into the teeth of three of four wild-caught adult common dolphins, *Delphinus delphis*. The three labeled animals died 328, 354, and 441 d, respectively, after the date of treatment. By estimating the dentinal pattern laid down in about 1 yr, the investigators characterized an annual GLG. They estimated the ages of the animals by assuming that the GLGs in the unlabeled regions of the teeth rep-

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resented the same amount of time as the single GLG interpreted from the labeled region of each tooth.

A study by Hui (1978) included two tooth extractions made 2.5 yr apart from a captive male bottlenose dolphin, *Tursiops truncatus* (No. 10, "Kona"). Comparisons of longitudinal thin sections of the two teeth led Hui to conclude that "... almost three dentin layers [GLGs] had been deposited during the intervening period..." (p. 11). Other than indicating GLG boundaries in figures of the two thin sections (his fig. 3), Hui did not describe the GLGs or their components.

Three published studies (Sergeant 1959; Sergeant et al. 1973; Hui 1978) have attempted to demonstrate time content in GLGs using teeth of known-age, i.e., captive-born dolphins. All three had access to only a small number of specimens, all of *Tursiops truncatus*. Apparently, the investigators knew the ages of the specimens before defining and counting dentinal GLGs in the teeth, and no assurance was provided that the GLGs counted corresponded to annual periods between birth and death. Hui's study demonstrated that GLGs may be defined in such a manner as to verify the age that is already known for a specimen (Myrick 1980a). The incorrect age data (3.3 yr) provided to Hui for one of two "known-age" specimens studied by him (Hui 1978) led to his subsequent division of its dentinal layering pattern into three GLGs and a small fraction (Hui[4]). The original clinical records for the specimen (Hui's No. 29, LACM 54698) show, however, that the dolphin was born on 28 August 1965 and died on 8 August 1969, at nearly 4 yr of age.

Used independently, teeth of known-age animals, single-labeled teeth, or teeth extracted on two dates do not provide reliable means by which to determine tissue accumulation rates fully or to define GLGs with precision. Each method yields only two dates bracketing a segment of layered tissue into which the known elapsed time is divided. Myrick (1980b) described approaches that combine the use of two or more labels and two or more tooth extractions over an extended period to monitor rates and calibrate GLGs. The present paper is an account of such a study which used TCL-labeled teeth from seven captive Hawaiian spinner dolphins, *Stenella longirostris*.

MATERIALS AND METHODS

The study consisted of two phases. The first was a retrospective examination of TCL labels in the dolphins' dental tissues produced incidentally by clinical treatments administered during their captivity at Sea Life Park, Hawaii. Teeth were used from four frozen carcasses (Nos. WFP 606, 669, 670, and 671[5]), including one specimen of known age, and three live animals (Nos. ACM 103, 104, and 106) from which teeth were extracted in early 1980.

The second phase was a 1-yr monitoring of tissue-accumulation rates in teeth of three live animals. Each animal was given intramuscular injections of TCL at about 3-mo intervals and underwent three tooth extractions during the monitored period.

To restrain the dolphins during injections and extractions, an elevated rigid litter was placed near the edge of the dolphin holding tank in which the water level had been lowered to a depth of 0.5 m. The sloped tank bottom inclined the litter at an angle of 20° relative to the water surface. Each dolphin in turn was guided on its belly onto the litter until the front half of its body was above the water surface. In this position the dolphin could be held firmly with little apparent discomfort to the animal.

The procedure used to extract teeth was adapted for the spinners from the method described by Ridgway et al. (1975) for bottlenose dolphins. The dolphin's mouth was held open by moistened rolled toweling placed around the upper and lower jaws. Carbocaine[6] (5-10 cc) was injected into the right or left interalveolar nerve immediately behind the anterior border of the mandibular foramen. After allowing about 10 min for the anesthetic to take effect, a tooth was removed from the middle of the corresponding mandibular tooth row using an elevator and an extractor. The vacated alveolus was packed with cotton soaked with a ferric solution to control bleeding and promote healing.

Liquamycin 100, a form of TCL, was injected into the dorsal musculature between the dorsal fin and the blow hole. To reduce the possibility of local inflammation of the tissue—a problem known to result from concentrations of TCL—each dose (25 mg/kg body weight) was distributed along the dorsum at three separate sites.

Untreated (cut or ground) thin sections and decalcified and haematoxylin-stained (D/S) thin sections are the two most widely used preparations for dolphin teeth in age determination studies (see Perrin and Myrick 1980: 21 ff.). D/S sections produce simpler, more uniform GLG patterns, but de-

[4]Clifford Hui, Naval Ocean Systems Center, San Diego, Calif., pers. commun. 1981.

[5]Skeletons are in the synoptic collection at Southwest Fisheries Center, NMFS, La Jolla, Calif.

[6]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

calcification removes TCL labels (Nielsen 1972). We prepared the spinner teeth using both methods.

Untreated, mid-longitudinal thin sections, 100 μm thick, were prepared by hand grinding and polishing teeth using 240 and 600 grit Al_2O_3 on a glass plate. Other teeth were decalcified in RDO[7] for 6-8 h, rinsed, and cut with a microtome in longitudinal plane to produce 30 μm thick sections that were stained in Mayer's haematoxylin for 15-30 min. Untreated and D/S preparations were mounted on slides in Permount or glycerin gel and covered with coverslips.

To determine the pattern components of GLGs, the D/S and untreated thin sections were examined in plain transmitted light 39X and 150X with a Zeiss photomicroscope. TCL labels were viewed at the same magnifications with UV reflected light using a Zeiss fluorescent vertical illuminator with a filter-reflector No. 44-75-05 combination attached to the same instrument.

Retrospective Calibration of Dentinal GLGs

Dates and durations of treatment, date of birth (for one specimen) or capture, and dates of death (for four carcasses) were taken from clinical records maintained for each dolphin during its captive life at Sea Life Park (for summaries see Myrick et al. in press). Data for each specimen were transcribed onto a calibration chart as the chronological series of event blocks, the relative width of a given block corresponding to the length of a given period of treatment.

In each thin section showing distinct fluorescent labels under UV light, label thicknesses and interlabel distances were measured. Label-measurement data for each dolphin were entered on its chart as a series of blocks below the event blocks, with spacing and thickness scaled to the corresponding measurements. The treatment and label blocks were compared for spacing and thickness to identify the date each label was introduced. Connecting lines were drawn from the beginning and the end of each matched pair of blocks (Fig. 1C).

A UV photograph of each thin section was used to identify and letter key labels that enclosed 6- or 12-mo segments of dentine. Labels and structural landmarks in the UV photograph were traced with a china marker on an overlay of transparent plastic. Using the landmarks, the tracing was lined up on the corresponding plain-light photograph onto which the labels were reproduced to delineate layering patterns within the time segments. Each marked photograph was then inspected for repeating layer components to define GLGs and their subunits in the untreated thin section. GLGs defined in the labeled dentine of each thin section were used as a basis for identifying similar GLGs in the unlabeled regions of the dentine and permitted a complete series of GLG-thickness measurements and an estimate of dentinal age in years to be made for each animal.

Dentinal GLGs in dolphin teeth are most easily discerned in the region of the "shoulder", i.e., along a transect from near the base of the neonatal line (the first layer of the postnatal dentine), downward and inward at about a 30°-40° angle to the margin of the pulp cavity (for examples see Perrin and Myrick 1980: fig. 2; Hui 1978: figs. 1, 2, 3). For consistency, measurements of GLG and label thickness, taken perpendicular to the long axis of the teeth of the Hawaiian spinner dolphins, were made along transects at a similar position and angle (Figs. 1A, B). However, a GLG or label may vary in thickness in localized regions of the dentine and may not be the same on both sides of a tooth because of tooth asymmetry. For these reasons, measurements were made on the most symmetrical side of a tooth and in regions where GLGs and labels were clearest and least variable in thickness; departing slightly from a uniform angle of transect. GLGs in the dentine of the corresponding D/S thin sections were defined and counted with the aid of GLG-thickness measurements obtained from the untreated section.

Retrospective Calibration of Cemental GLGs

Because fewer labels were observed in the cementum than in the dentine of the same untreated thin section, it was assumed that those visible represented condensed forms of only the brightest, thickest, or closely spaced groups of dentinal labels. This has been verified in bottlenose dolphins (Myrick 1980b) and recently in the present sample of Hawaiian spinners by observations that bright dentinal labels at the tooth base are continuous with cemental labels. Hence, cemental labels were lettered to correspond to the brightest dentinal labels, and the cemental layers between labels were calibrated using the time segments represented between the dentinal labels.

The annual GLG pattern was defined as precisely as possible using the calibrated segments of the tissue, and the cemental GLG definition was tested by comparing the dentinal GLG count with the cemental GLG count in untreated thin sections. In D/S thin

[7]A commercial rapid decalcifying agent available through Dupage Kinetic Laboratories, Inc., Plainfield, Ill.

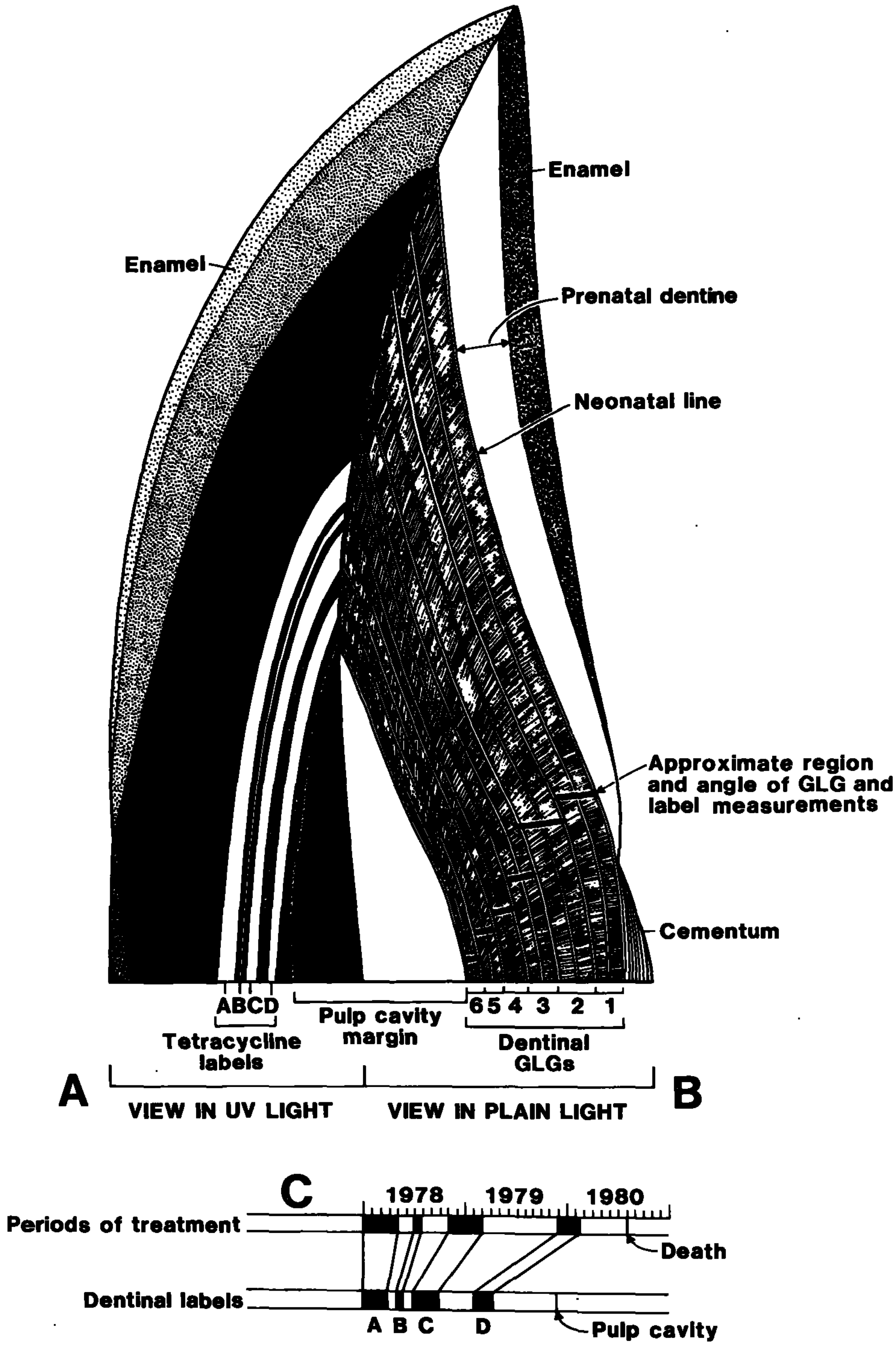

FIGURE 1.—Line drawing of hypothetical dolphin tooth in thin section showing appearance of TCL labels, A, B, C, D, under ultraviolet light (1A, left-hand side) and dentinal growth layer group (GLG) layering patterns under plain transmitted light (1B, right-hand side), and standard positions in tooth where label and GLG thickness are measured. 1C illustrates method of identifying labels in tooth section with TCL treatment dates by comparing relative thickness and spacing of labels with treatment periods.

sections, cemental GLGs were defined indirectly by comparing them with the pattern and number of cemental GLGs determined in untreated sections.

Direct Monitoring

Calculation of depositional rates and calibration and definition of GLGs in dentine and cementum were achieved by comparing tooth specimens containing successively introduced labels and/or additional tissue accumulated over the 1-yr period of monitoring. To make determinations, for cases in which labels were not distinct or not successfully produced, the additional tissue was measured from structural landmarks or labels in the extracted series of thin sections.

RESULTS

Dentinal labels.—The untreated thin sections for all seven specimens contained multiple labels. Most attempts to match labels with treatments were successful (Figs. 2-6). However, in four specimens more labels occurred than could be accounted for from clinical records. In the only captive-born specimen, WFP 670, numerous TCL labels were observed (Fig. 7A, B), but only three were found to have been caused by intentional therapeutic treatments (Fig. 7D, labels C, F, and G). Labels A and B apparently were a result of TCL impaired to the then-calf through the milk of its mother, who was treated with the drug for two periods while the calf nursed. The other labels appear to have resulted from frequent ingestion of stolen TCL-dosed smelt intended for other dolphins being treated at various times while sharing a common tank with this animal.

No treatment was recorded for label A found in the dentine of dolphin carcass WFP 669 (Fig. 4A, C) and live dolphin ACM 104 (Fig. 6A, C). Judging from the relative positions of the "A" labels to the other labels for which matches were found with recorded treatments, "A" labels were introduced into both specimens at or about their respective dates of capture. It is a fairly common practice in commercial aquaria to give medication (often tetracycline) to newly captured dolphins recovering from stress of capture and adjusting to the captive environment[8].

Labels B and G in the dentine of dolphin carcass WFP 671 could not be identified from clinical records (Fig. 5A, C), although the numerous other labels match well in relative thickness and spacing with the treatment dates for this specimen.

In teeth of live dolphin ACM 103 the labels were indistinct. The presence of TCL, introduced clinically during three periods of treatment over 2 yr and experimentally at 3-mo intervals in 1980, was indicated only by several areas of hazy fluorescence in the dentine near the pulp cavity.

Dentinal GLG pattern.—The use of plastic overlays of key labels enclosing 6-mo or 1-yr segments of dentine on plain-light photographs of the dentine for each specimen permitted repeated calibrations of the annual dentinal layering pattern for six of the seven specimens (the seventh specimen, ACM 103, had no discrete labels). In untreated thin sections, a dentinal GLG contained four major components deposited in the following sequence: 1) A thin, light (GLG-boundary) layer, 2) a thicker dark layer, 3) another thin, light (mid-GLG) layer, and 4) a second thick, dark layer (Figs. 3A, 4B, 5B, 6B).

In addition to the four components, many of the earliest deposited GLGs had an infrastructure composed of finer alternating dark and light layers. Counts made at 150× under low transmitted light showed that each of these annual GLGs contained 13 pairs of fine layers (Figs. 3A, 4B, 6D, 7C). Where layers were sufficiently distinct to be counted between labels (e.g., between label B and M, Fig. 4A, B), counts indicated that each pair ["LML," (lunar monthly layer) Myrick 1980b] represented about 1 lunar month. The full complement of LMLs was visible throughout the dentine in the captive-born specimen, WFP 670, i.e., 13 LMLs in each of the first three complete GLGs and 9 in the incomplete fourth GLG (Fig. 7C). In specimen ACM 103, 13 LMLs were observed in the first 12 of the 14.5 GLGs present (Fig. 8). But in other specimens, LMLs were clear enough to be counted only in the first five or six GLGs.

In D/S thin sections, the annual GLG pattern consisted of two lightly stained and two darkly stained layers. The thin, light, GLG-boundary layers and mid-GLG layers in untreated thin sections corresponded to the lightly stained layers in D/S thin-sections (Fig. 9A, B). LMLs were indistinct in almost all GLGs in D/S preparations.

Age-specific GLG thickness.—Table 1, showing dentinal GLG thickness measurements made from the most symmetrical side of the tooth of each of the seven dolphins, indicates that for each animal a GLG of a specific thickness was produced that appears to be related to the year of life in which the GLG was formed, i.e., an age-specific GLG thickness.

[8]William A. Walker, Los Angeles County Museum of Natural History, Los Angeles, Calif., pers. commun. 1982.

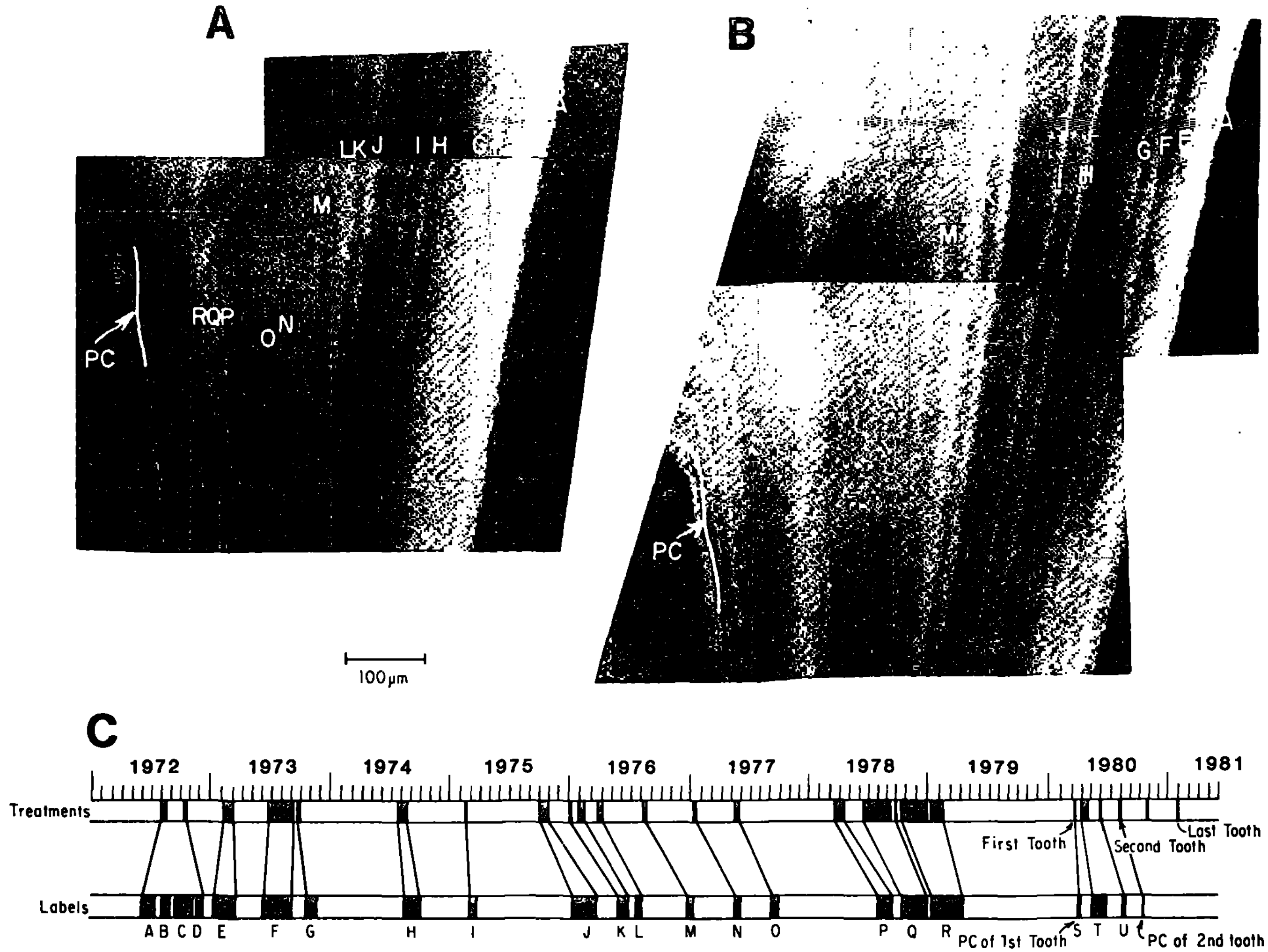

FIGURE 2.—Labeled teeth from live dolphin ACM 106. A. Untreated thin section of tooth extracted in March 1980, showing labels (lettered) introduced through TCL treatments from 1972 through 1979 (UV, 150×). B. Tooth extracted 4 mo after tooth in 2A showing three labels introduced during first part of the experimental labeling period. Label T was produced from a clinical treatment administered over 1-mo period (UV, 150×). C. Chart showing treatments and corresponding dentinal labels as interpreted from relative label positions and thicknesses.

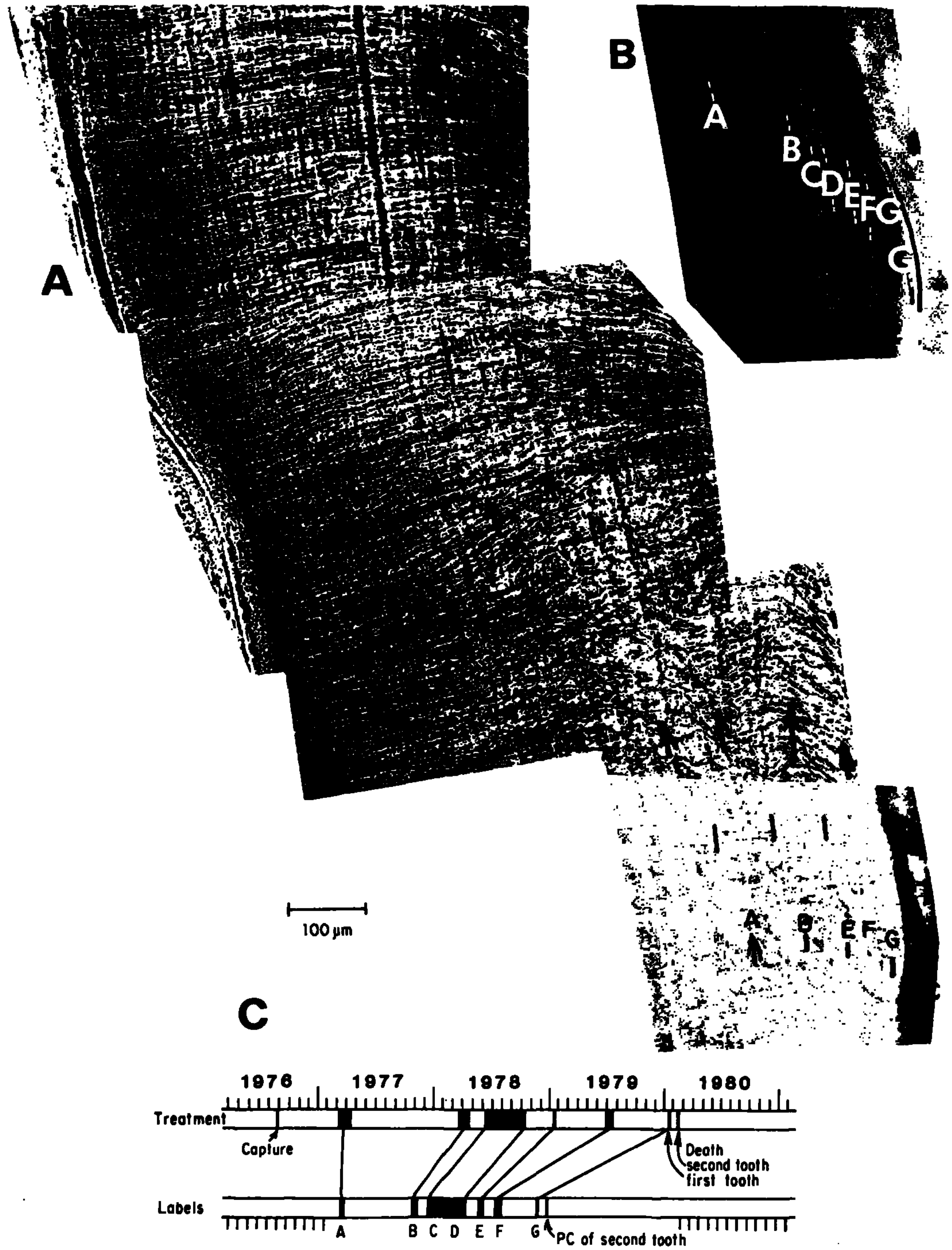

FIGURE 3.—Labeled tooth taken from dolphin carcass WFP 606. A. Untreated thin section in plain light showing about eight annual GLGs in dentine (separated by arrows). GLGs divided approximately in half by thin, light mid-GLG layers (heavy dark marks). GLGs 6, 7, and 8 were interpreted from positions of tetracycline labels (lettered). Finer dark layers represent lunar monthly layers (150×). B. Dentine labels in UV light (150×). C. Chart showing dates labels were introduced.

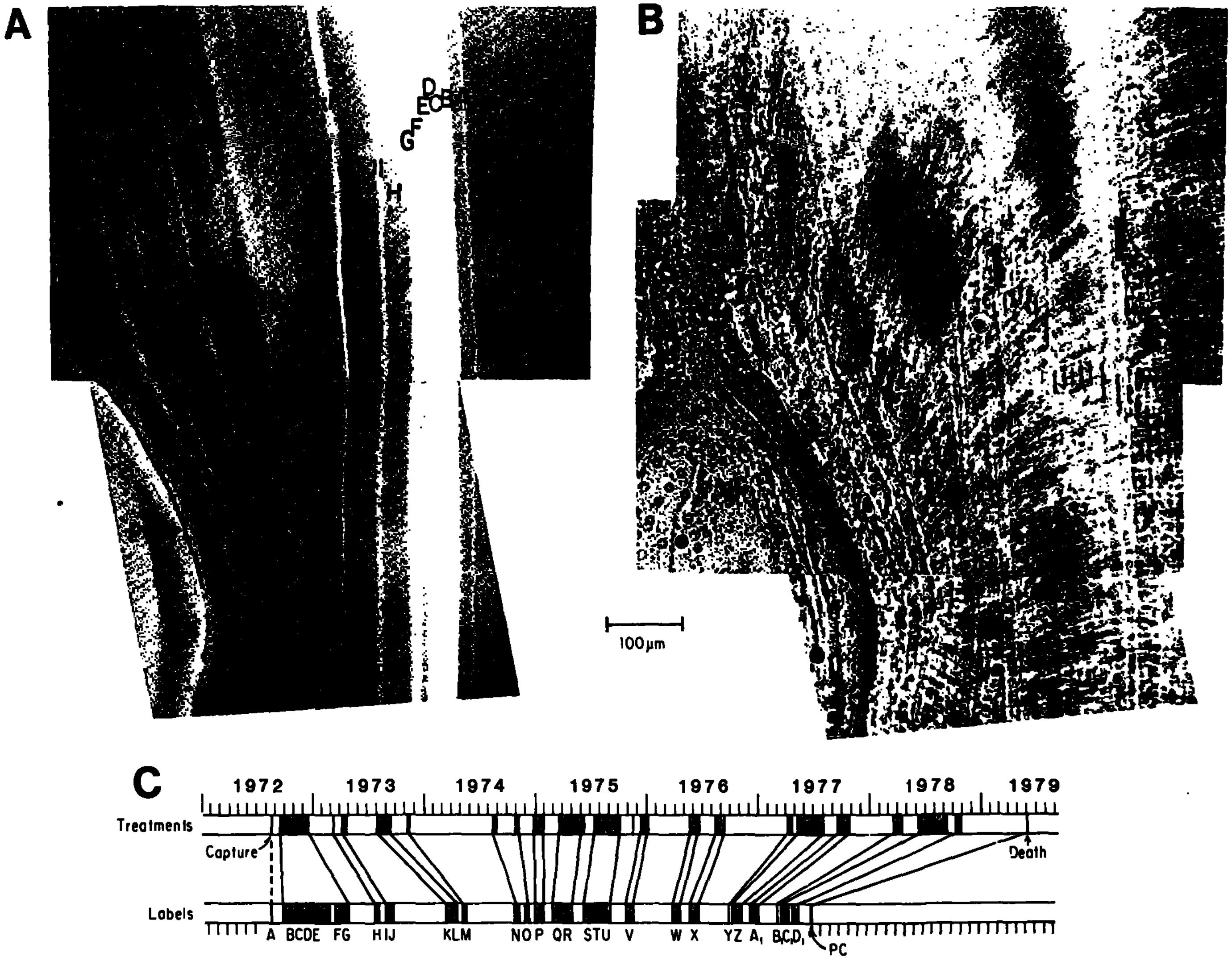

FIGURE 4.—Labeled tooth from dolphin carcass WFP 669. A. TCL labels (lettered) in region of dentine pulp cavity (PC) (UV, 150×). B. Plain-light view of dentine as in 3A showing annual GLGs bordered by light boundary layers (heavy dark marks) as interpreted from key TCL labels. Fine dark lines represent LMLs. (Heavy lines delineating GLGs are not placed at the standard positions used to measure GLG and label thickness.) C. Chart showing TCL treatments and corresponding dentinal labels identified by comparing relative positions and thicknesses of labels with spacing of treatment blocks.

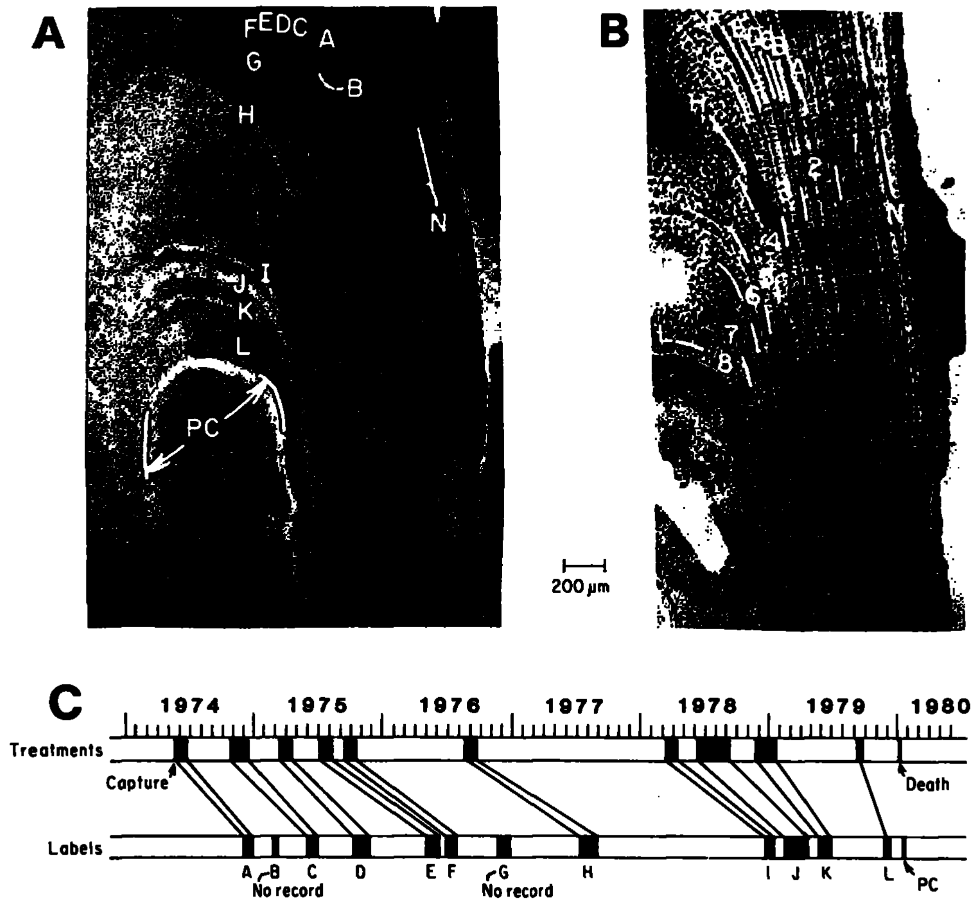

FIGURE 5.—Labeled tooth of dolphin carcass WFP 671. A. Untreated thin section in UV light showing TCL labels (39×). B. Thin section in plain light showing almost eight complete GLGs as interpreted from labels. Light GLG boundary layers appear to have been deposited in or about March (39×). C. Chart showing match between labels and treatments.

TABLE 1.—Mean age-specific thicknesses (µm) of completed dentinal growth layer groups (GLGs) in teeth of seven Hawaiian spinner dolphins, *Stenella longirostris*. Values are averages of at least three measurements per specimen, taken perpendicular to the long axis of the tooth in a stairstep fashion downward and inward from the base of the neonatal line to the pulp-cavity wall.

Specimen	GLG number														
no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
WFP 670	240	240	180	—	—	—	—	—	—	—	—	—	—	—	—
WFP 669[1]	240	240	172.5	150	127.5	127.5	92.5	85	65	57.5	—	—	—	—	—
WFP 671	240	240	165	140	120	95	80	—	—	—	—	—	—	—	—
ACM 104	240	230	170	140	130	90	90	70	70	60	55	—	—	—	—
ACM 103	240	240	180	160	110	80	80	70	60	60	60	65	55	40	40
ACM 106	240	240	150	130	110	90	90	60	60	60	55	—	—	—	—
WFP 606	240	240	180	150	120	90	90	—	—	—	—	—	—	—	—
N	7	7	7	6	6	6	6	4	4	4	3	1	1	1	1
$\bar{x}$	—	238.6	171.0	145.0	119.6	95.4	87.0	71.3	61.3	58.1	55.0	—	—	—	—
SD	—	3.8	11.0	10.5	8.4	16.5	5.6	10.3	2.5	2.4	5.0	—	—	—	—
SE	—	1.4	4.2	4.3	3.4	6.7	2.3	5.2	1.3	1.2	2.9	—	—	—	—

[1]Mean values of measurements in untreated and D/S (decalcified and haematoxylin-stained) sections.

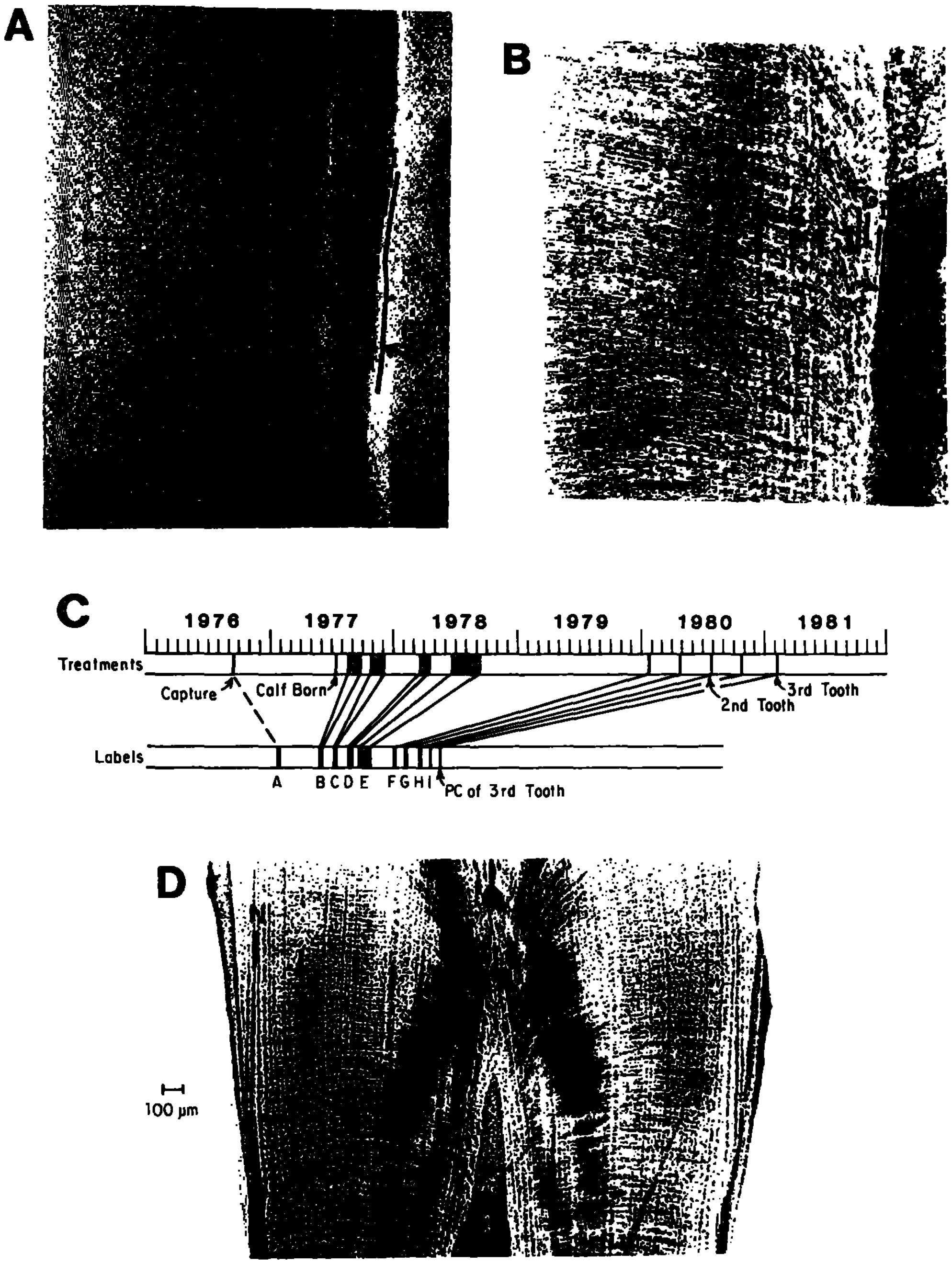

FIGURE 6.—Tooth of live dolphin ACM 104 extracted 2 February 1981. A. Untreated thin section in UV light showing location of TCL labels (150×). B. Same section as in 6A in plain light showing position of key labels bracketing last 4 yr of deposition. Light GLG boundary layers appear to have been deposited in or about August. C. Chart showing match of labels and treatments. D. Thin section showing 11 complete annual GLGs (separated by dark marks) as interpreted from labels (39×).

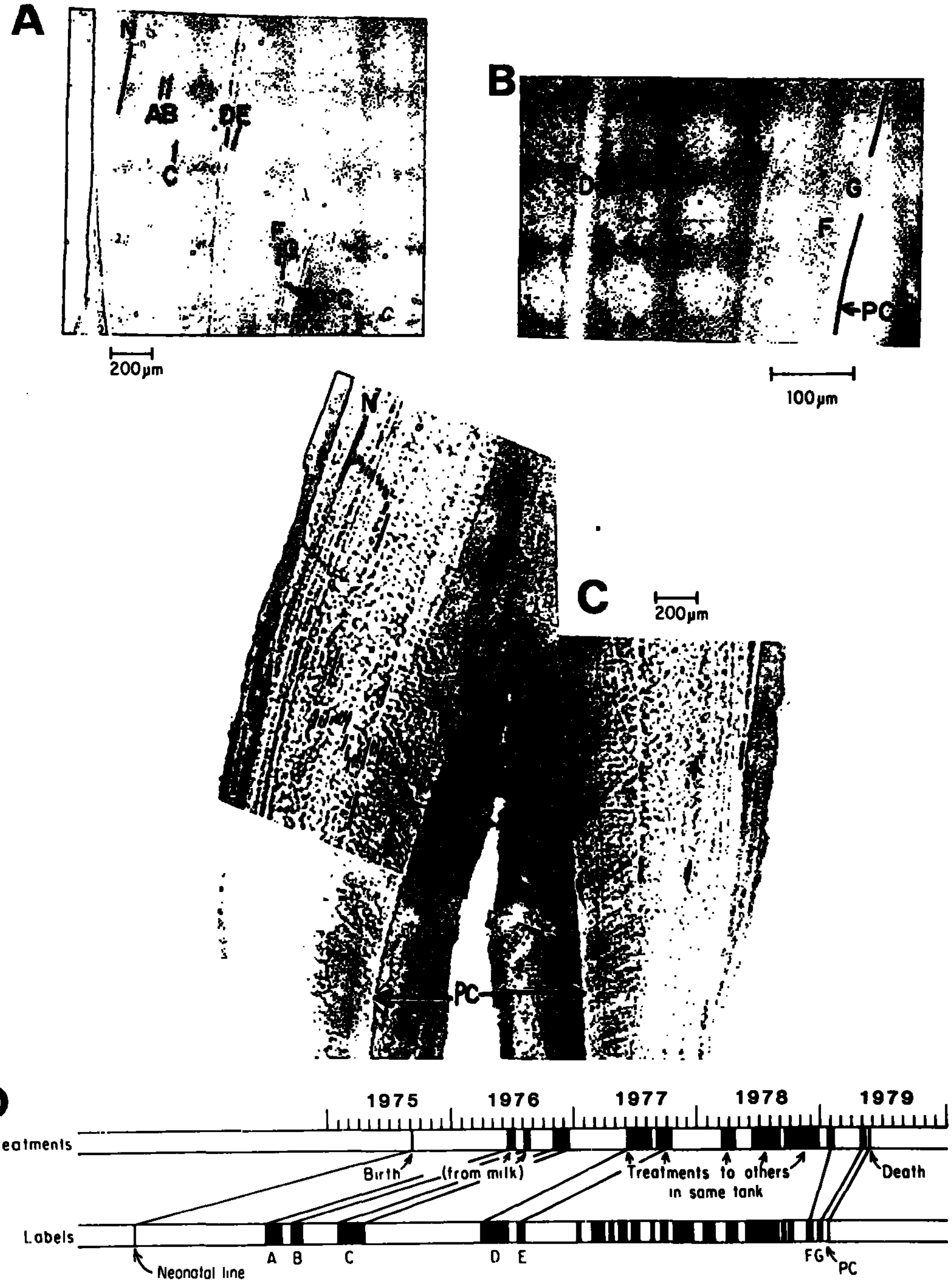

FIGURE 7.—Teeth from dolphin carcass WFP 670 (captive-born animal). A. Untreated thin section showing TCL labels in dentine. Labels A-B apparently represent TCL imparted to this animal through its mother's milk (UV, 39×). N = neonatal line; PC = pulp cavity margin. B. Portion as shown in 1A showing numerous labels from TCL-dosed smelt stolen from other dolphins occupying the same tank. Labels F and G represent direct treatments administered shortly before death (UV, 150×). C. Thin-sectioned tooth showing three entire and one partial GLGs (indicated by heavy dark marks) in the postnatal dentine as interpreted from TCL labels. LMLs are indicated by fine dark markers (plain transmitted light, 150×). D. Chart showing dates of direct and presumed incidental introduction of TCL and corresponding labels identified in the dentine by relative label position and thickness.

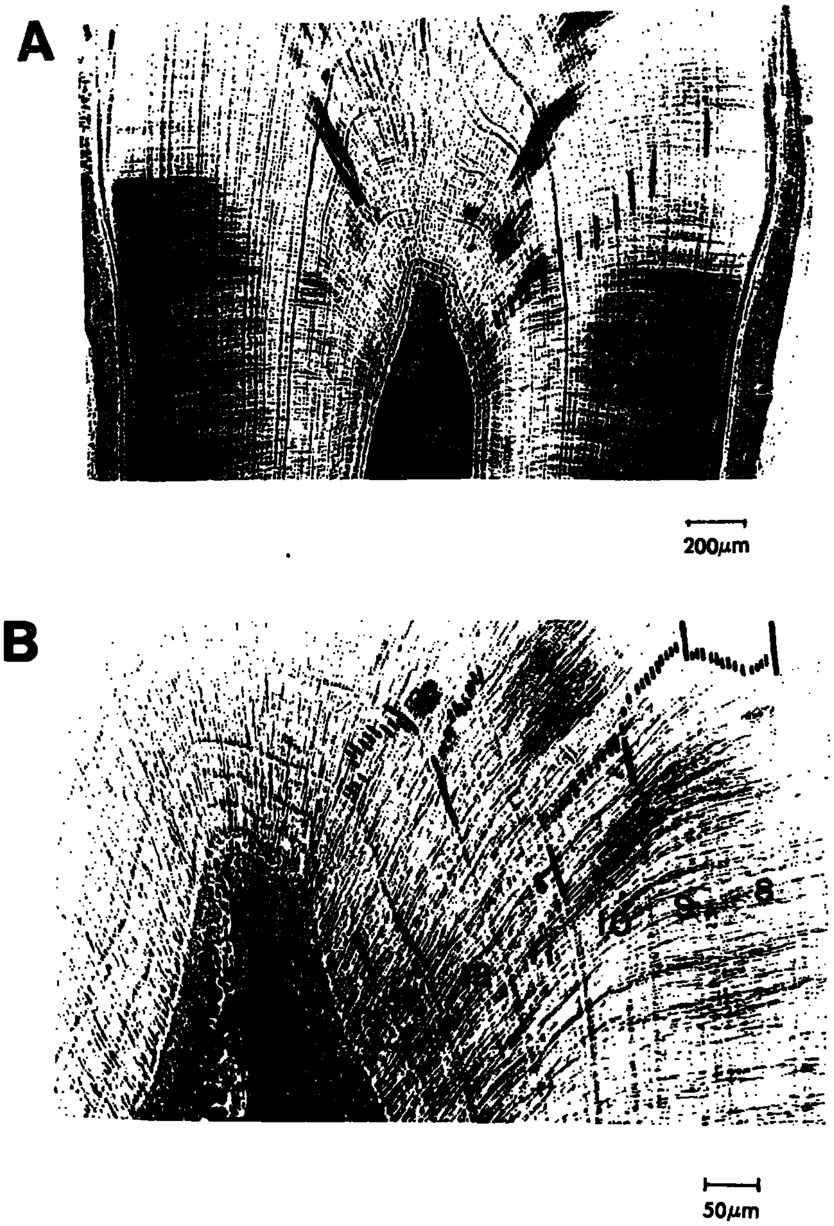

FIGURE 8.—Untreated tooth of live dolphin ACM 103 extracted 25 January 1980. A. About 14½GLGs indicated (heavy dark marks) (39×). B. GLGs 8-14½ showing thin, light boundary layers with dark margins. Thirteen LMLs indicated in each GLG 8-12 are particularly well developed (150×).

Comparisons of age-specific GLG thickness among the specimens suggest that the animals deposited a GLG of similar thickness in the same year of life. In the first and second year, 240 μm thick GLGs were deposited. In the third, fourth, fifth, sixth, and seventh years, thickness of GLGs averaged 171, 145, 119, 95, and 87 μm respectively. From the 8th to the 11th year, GLGs were between 71 and 55 μm thick. The data in Table 1 represent averages of at least three measurements per GLG per specimen.

Cemental labels.—Relatively few TCL labels were

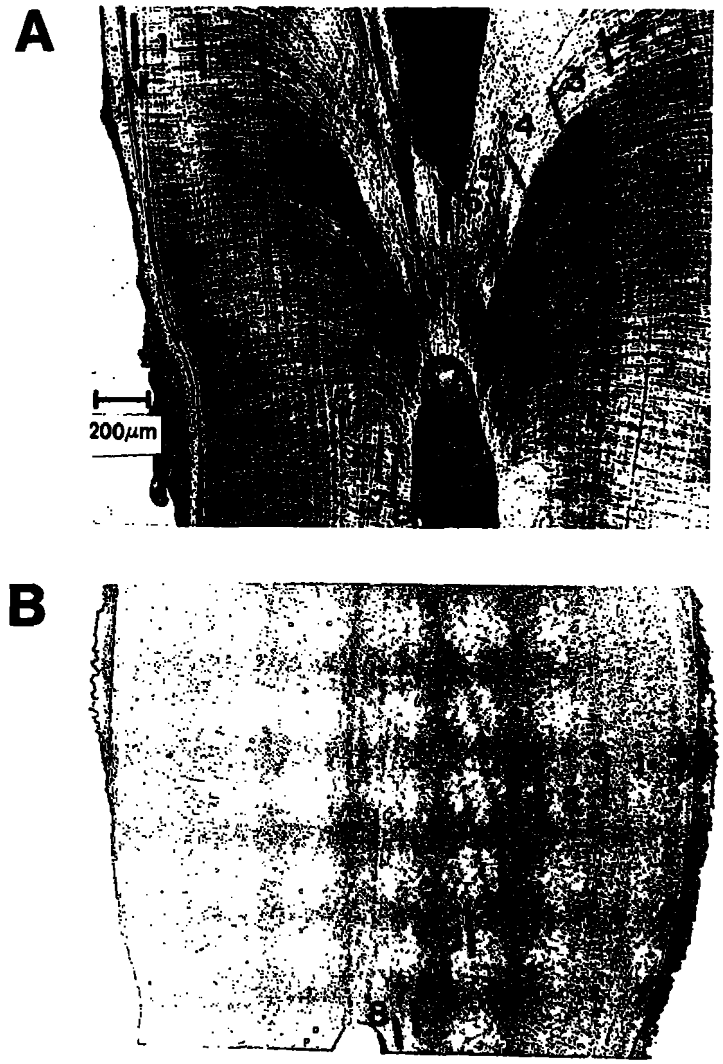

FIGURE 9.—Comparison of GLG patterns in teeth from dolphin carcass WFP 606 prepared by two methods: A. Untreated thin section (39X). B. Decalcified and stained thin section (39X).

found in the cementum compared with those in the dentine of the specimens. In the captive-born specimen, WFP 670, with about 25 dentinal labels, the cementum contained only three labels. In specimen WFP 669, only four cemental labels were observed (Fig. 10A) compared with 30 dentinal labels (Fig. 4A). The cementum in the other specimens had either zero or 1 label, despite the numerous dentinal labels observed for each.

Cemental GLG pattern.—In untreated thin sections, a cemental GLG consisted of a dark layer and a light layer (Fig. 10B). In D/S sections it was composed of a dark-stained layer, corresponding to the dark layer in untreated sections, and a lightly stained layer (Fig. 11). In both types of preparations, the dark layers contained larger concentrations of cementocytes than did the light layers.

Calibration of cemental GLGs.—Calibrations of cemental GLGs with those in the dentine were carried out using the assumption that cementum is a less sensitive recording structure than dentine (Klevezal' 1980) and that labels occurring in the cementum corresponded only to the brightest and thickest labels or label groups in the dentine. Thus, for example, the four labels detected in the cementum of specimen WFP 669 (Fig. 10A) were flagged with the same letters used to identify multiple label concentrations in the dentine (Fig. 4A).

In some cases, such as in WFP 669, plastic overlays were used to determine that a cemental GLG rep-

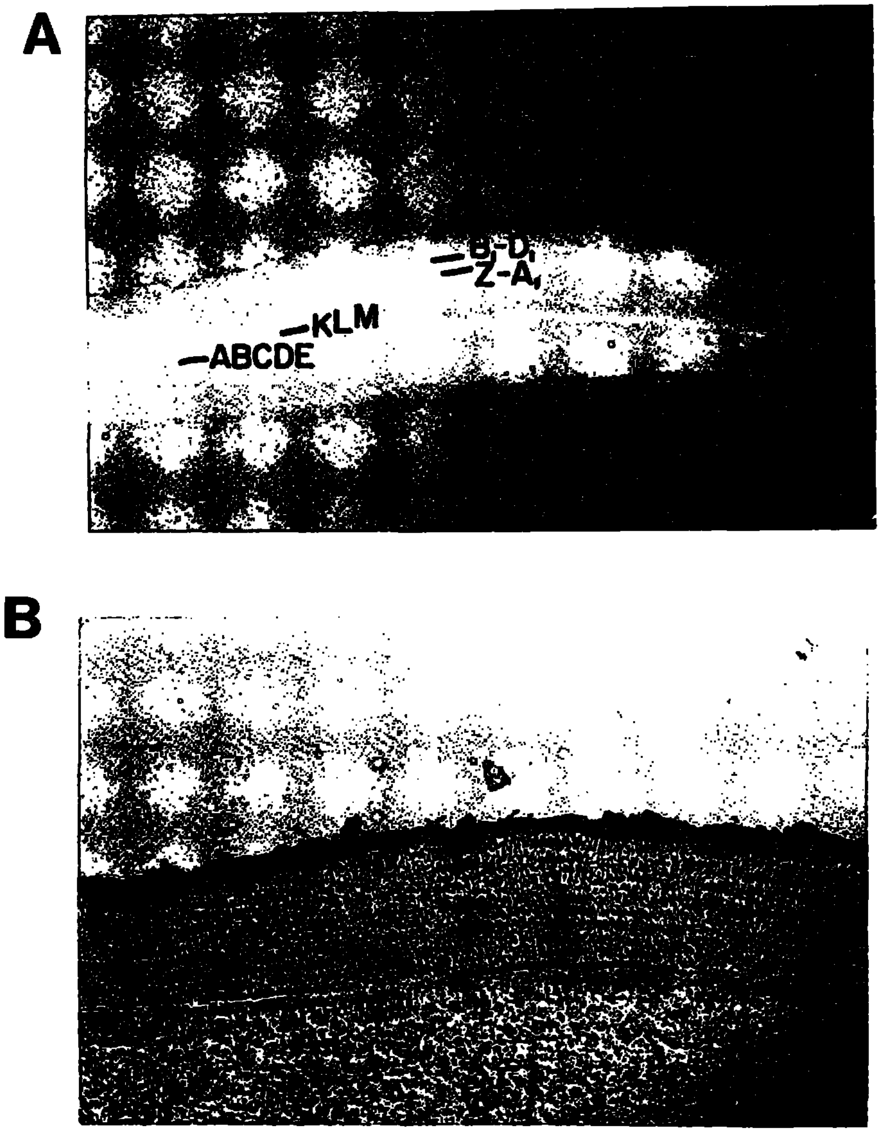

FIGURE 10.—Tooth cementum of dolphin carcass WFP 669. A. TCL labels interpreted as corresponding to lettered dentinal labels (150×). B. Positions of TCL labels (arrows) in layered cement. About 10 GLGs are indicated (150×).

resented the same amount of time as a dentinal GLG, i.e., 1 yr (e.g., Fig. 10B). In other cases, where labels were absent or where only one label occurred, calibration of cemental GLGs with dentinal GLGs was made indirectly by comparing GLG counts from both tissues. This method usually demonstrated a one-to-one relationship of GLGs in dentine and cementum, but in a few regions of the cementum of the captive-born specimen, WFP 670, there were twice as many GLGs as in the dentine (Fig. 11), indicating that a GLG may have been deposited twice a year in the cementum. In expanded regions of the cementum in another specimen (ACM 104; see Table 2), the cemental count was equal to the dentinal count; but in thinner regions the cemental count was only half that of the dentine, suggesting a cemental GLG being deposited every 2 yr in some cases.

Direct monitoring.—The results of examinations of thin sections of the series of three teeth from each of three live animals, taken at the beginning, at midpoint, and at the end of a 1-yr monitored period are

FIGURE 11.—Cementum of dolphin carcass WFP 670 tooth in decalcified and stained thin section. The number of dark layers is eight, about double the age in years of this captive-born specimen (150×, plain transmitted light).

presented in Table 2. Although distinct labels were not always successfully introduced, dentinal and cemental GLGs continued to be accumulated at a uniform rate of one per year. A comparison of accumulated dentine and labels in the first two extracted teeth of specimen ACM 106 (Fig. 12) showed two experimental treatments and one (unscheduled) clinical treatment accounted for in the second tooth (Fig. 2A-C). Specimen ACM 103, in which premonitor labels were indistinct, showed no experimental labels but clearly showed continued accumulation of dentine, the third extracted tooth having added about one GLG over the 1-yr period. No experimentally introduced labels were observed in the (less sensitive) cementum in any teeth of the three animals, but cemental deposition of about one complete GLG occurred in each animal for the period.

Seasons of birth.—By determining the dates of key dentinal labels introduced at or near the thin, light component layers of GLGs and by noting the approximate time of formulation of component layers in the teeth extracted during the monitor period, it was found that GLG-boundary and mid-GLG layers were formed at about 6 mo intervals. In five specimens, GLG-boundary layers were deposited in or about August and the mid-GLG layers were deposited in or about March. In the two other specimens the timing was reversed, i.e., GLG-boundary layers formed in March, mid-GLG layers in or near August. Proceeding on the assumption that the timing of layer formation (determined from the labeled or monitored

TABLE 2.—Results of examinations of teeth extracted from three live Hawaiian spinner dolphins, *Stenella longirostris*, over a 1-yr period monitoring accumulation of layers and labels. GLGs = growth layer groups.

Specimen no. and tooth	Date of tooth extraction	Date label introduced	Dentine		Cementum	
			Additional labels	No. GLGs	Additional labels	No. GLGs
ACM 103						
First	25 Jan. 1980	25 Jan. 1980 30 Apr. 1980	—	14.5	—	14.5
Second	30 July 1980	30 July 1980 30 Nov. 1980	indistinct	15.0	None	15.0
Third	2 Feb. 1981	—	indistinct	15.5	None	15.5
ACM 106						
First	19 Mar. 1980	19 Mar. 1980 11-28 Apr. 1980[1] 5 June 1980	—	10.3	—	10
Second	30 July 1980	30 July 1980 30 Nov. 1980	3	10.7	None	10+
Third	2 Feb. 1981	—	indistinct	11.2	None	11
ACM 104						
First	25 Jan. 1980	25 Jan. 1980 30 Apr. 1980	—	([2])	—	([2])
Second	30 July 1980	30 July 1980 30 Nov. 1980	2	10.7	None	[3]5/10
Third	2 Feb. 1981	—	4	11.3	None	[3]6/11

[1]Unscheduled medical treatment 18 d in duration.
[2]Not examined because of poor preparation of section.
[3]Cementum showed a number of GLGs equal to that of the dentine as well as half that of the dentine.

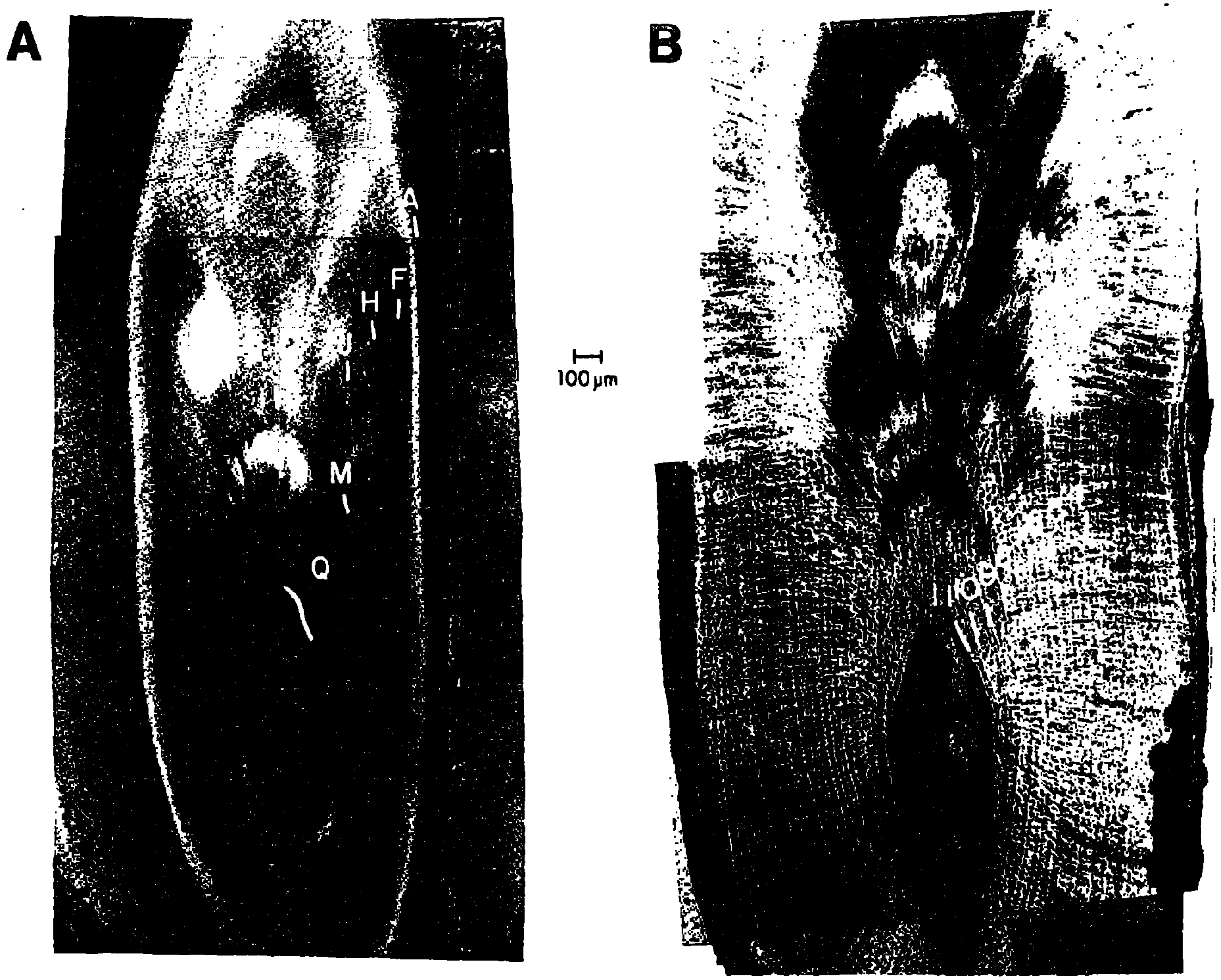

FIGURE 12.—Tooth of live dolphin ACM 106 extracted 30 July 1980. A. Untreated thin section in UV light showing key labels A-M each introduced 1 yr apart, M and Q 2 yr apart, and almost 1 yr of dentine accumulation represented between Q and the pulp-cavity margin (39×). B. Thin section as in 12A (plain light) showing GLGs as interpreted from labels. Positions of labels A and F are indicated. Horizontal white lines indicate positions and angle of transect. GLG thickness measurements were taken for GLGs 1-3.

regions of the dentine) was uniform throughout all of the dentine for a given specimen, GLG-boundary and mid-GLG layers were counted in reverse order of deposition up to the first boundary layer, the neonatal line, to estimate month and year of birth.

Table 3 summarizes the month- and year-of-birth estimates made from boundary-layer counts in six specimens and birth dates taken from park records of two captive-born specimens, WFP 670 and the calf of ACM 104. Six were born in late summer/early autumn and two in March.

TABLE 3.—Estimated birthdates of eight captive Hawaiian spinner dolphins, *Stenella longirostris*..

Specimen no.	Month and year of birth
ACM 103	August 1964
ACM 106	August 1969
WFP 669	August 1969
ACM 104	September 1969
WFP 606	March 1972
WFP 671	March 1973
WFP 670[1]	8 September 1975
Calf of ACM 104[2]	21 July 1977

[1]Born in captivity.
[2]Born in captivity, survived 3 d.

DISCUSSION

Age-Specific GLG Thickness

Dentinal GLG thickness appears to be age-specific for the Hawaiian spinner dolphin teeth examined. There was little variability from tooth to tooth or from animal to animal in the sequence of GLG thickness through the 11th GLG, despite deposition of a specific GLG in some specimens while still in the wild and in other specimens during their captive lives. This suggests that, to some extent at least, the amount of dentine deposited by animals at a given age may be predetermined and that animals of a given stock, species, or higher common phylogenetic affinity may follow the same or similar pattern of age-specific GLG deposition unaffected by environment.

Used in conjunction with the GLG component-layer pattern, the regularity in thickness of age-specific GLGs may be useful as an aid in locating GLG boundaries and counting GLGs in teeth of wild Hawaiian spinner dolphins and dolphins of related species in which GLG thickness and component-layer patterns are found to be similar. When measurements are taken at standard positions in the teeth of such dolphins, one may make fairly rapid age estimates without having to examine each GLG in detail (see Myrick et al. 1983).

Lunar Monthly Layers (LMLs)

Laws (1962) was the first to suggest that the system of fine layers within dentinal GLGs of pinniped teeth corresponded to lunar monthly cycles. Putative LMLs have been reported in dentine of dugongs (Kasuya and Nishiwaki 1978; Marsh 1980), in dentine of beaked whales ("short cycles," Kasuya 1977; "accessory layers," Perrin and Myrick 1980:3, 5), in fossil dolphin teeth (Myrick 1979), and in the mandibular bone (Myrick 1980b) and dentine of modern dolphins (Myrick 1980b; Hohn 1980a, b). Hui (1978) reported finding no relationship between the fine layers that he counted in a tooth from a known-age bottlenose dolphin and its age in lunar months; but with no prior knowledge of its age, Myrick (1980b) made dentinal LML counts in the same specimen that closely agreed with its known age.

The present study has furnished verification that LMLs are deposited with lunar-monthly regularity in the animals studied. In the 3.7-yr-old captive-born spinner dolphin (WFP 670), 13 LMLs were counted in each of the three complete annual dentinal GLGs and 9 were counted in the partial fourth GLG. Where LMLs were visible between TCL labels in the dentine in this and other specimens, they were found to correspond consistently in number to the time in months represented between labeling dates.

Where LMLs could be seen clearly, no departure from the 13 LML/GLG pattern was detected in the teeth used in the present study. Variability has been reported in studies of other marine mammals. Marsh (1980:197) found only "about 12 [LMLs] per GLG" in the dentine of the deciduous incisor of a dugong. Ten to 15 LMLs/GLG were observed in dugong tusks by Kasuya and Nishiwaki (1978). Kasuya (1977) found between 11 and 13.4 LMLs ("short cycles")/GLG in teeth of Baird's beaked whales, *Berardius bairdii*. Hohn (1980b) counted 10-13 LMLs/dentinal GLG in Atlantic bottlenose dolphin teeth. Presumably, LML variability will be found to occur also in Hawaiian spinner dolphins when larger samples are examined.

Relationship of Cemental GLGs to Dentinal GLGs

None of the teeth of the studied specimens had reached the stage of pulp-cavity occlusion or dentinal irregularity that necessitated age estimation solely from cemental GLG counts (Kasuya 1976; Myrick et al. 1983). Although the pulp cavities were small in some specimens and some later-administered TCL failed to produce distinct labels, none showed evidence of cessation of dentine deposition.

All cemental GLG counts corresponded in number to dentinal annual GLG counts except in the case of specimen WFP 670, where some regions of the cementum showed double the number, and in specimen ACM 104, where in some places the cemental count was half that of the dentine. The finding that in some cases cemental GLGs may form at half or twice the rate of dentinal GLG deposition points up the problem of using cemental GLGs to estimate ages without reference to the dentine (Myrick et al. 1983).

Evidence for an Internal Clock

In the dentine of the animals studied, a thin GLG boundary layer, beginning with the neonatal line, was formed in the month of birth and on anniversaries of the month of birth. Mid-GLG layers were formed about 6 mo after formation of boundary layers. Where LMLs could be calibrated, one was found to form about every (lunar) month with high uniformity in relative spacing. Such a cycle of deposition is indicative of an internal clock, or clocks. The pattern commences at birth and apparently is reset with solar and/or lunar regularity without perceptible alteration by fluctuation in the dolphins' natural or captive environment or in calendric season of birth. That it may not be a totally free-running system, i.e., not without external cues, is suggested by the precisely synchorized deposition of the fine and coarse patterns of the dentine repeated over many years.

Age at Sexual Maturity

Perrin et al. (1977) indicated that sexual maturity may be reached in females of *Stenella longirostris* at an average 5.5 yr (range of 5-9 yr) and the average period of gestation may be about 11 mo. From the study of dentinal GLGs and TCL labels of specimen ACM 104, it was possible to determine that this animal was about 8-yr-old when she gave birth to her calf. Assuming an 11-mo gestation, we estimate that she would have been 7-yr-old when she conceived. It is not known whether the pregnancy resulted from fertilization at her first or subsequent ovulations. ACM 104 remains alive. This precludes examination of her ovaries for ovulation scars.

Reproductive Seasonality

Based on the birth records of specimen WFP 670 and the calf of ACM 104 and the deductions made from dentinal layers, six animals were born in late summer/early fall, and two were born in March. Since all animals in the study represented the same population off Kona, Hawaii, the early-spring and early-fall birth patterns might indicate a corresponding two-cycle pattern of reproductive peaks for the wild population generally. Such a seasonal pattern has been suggested by Norris and Dohl (1980, fig. 16), but Wells (in press), who has studied the population in considerable detail, concluded that the breeding season occurs from spring to fall, with most births in the fall. Our sample was too small to verify Wells' findings.

Tetracycline Exposure to the Calf Through the Milk

The first two labels found in the dentine of specimen WFP 670, the captive-born animal, were interpreted as having been introduced through milk received by the calf while the mother was being treated with TCL. This recommends a possible practical application in indirectly treating newborns in ill health. Excessive handling of such animals frequently results in a worsening of their condition, making the treatment more dangerous than the malady. (Nursing calves not on solid food cannot be treated with TCL-dosed fish and must be force-fed or injected with drugs.) Separating the young calf from its mother may produce additional complications.[9] If treatments for the calf could be administered through the milk by treating the mother with TCL-dosed food, it seems likely that most of the problem could be minimized. The question invites further study.

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