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NATIONAL MARINE
FISHERIES SERVICE
Panama City Laboratory
Panama City, Florida

Age Determination Studies
in Marine Turtles

Preliminary Report (II)

to

National Marine Fisheries Service
(Southeast Fisheries Center)

and

Fish and Wildlife Service
Endangered Species Program
(Region 2)

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I. Literature review.

In addition to new papers, two bibliographic reviews produced by the U.S. Department of the Interior, were discovered. As usual, they show a heavy bias towards mammals and birds with few studies of reptiles or amphibians.

II. Acquisition of known-age and other critical specimens.

At present three critical collections are available for study (known-age material in Thailand; rare ear and eye bones in Peru and Ecuador). Funding has just been received to cover transportation, and these materials will be shipped as soon as possible to the Smithsonian Institution. In addition, Dr. J Wood, Cayman Turtle Farm, has agreed to send more known-age material.

III. Preparation.

A. Bones.

Most effort has gone into thin sectioning decalcified material. It was recently learned that the best samples for decalcifying should be 2 to 3 mm thick, not 6 or 7 mm, as we have been preparing. At present, decalcification in the commercial preparation (RDO) takes up to two days; the mixture of Hydrofloric-Hydrochloric acids may take 10 days. With thinner samples, decalcification will take less than half this time. It is unclear if there is any advantage in RDO over the acid mixture, other than time.

There seem to be no major differences between sections cut on the freezer microtome or embedded and cut in parafin, but this will be investigated further. Frozen sections can be cut with less processing, but the material is less durable. We are still experimenting with different stains, although hematoxylin alone seems to be best.

B. Keratinous epidermal scales.

No further investigation has been done on this topic.

C. Eye lenses.

The available information deals especially with mammals and birds; there seems to be no published information on turtles. Furthermore, the published accounts often do not explain the complete procedure needed. In general the technique has not been widely applied, although numerous preliminary studies have been published; with birds it is useless, except for the first few months of life.

Lenses of Mexican Ridley Turtles (*L. olivacea*) have been cleaned and weighed. A draft of these results is now in preparation.

Studies of the insoluble protein fraction in eye lenses reveal that this measure is a more precise indicator of age than is gross weight. The technique is relatively simple but, unfortunately, requires fresh (or frozen?) material, which is not available.

IV. Results.

Bone sections cut by a variety of techniques are illustrated in Figure 1 through 17. These include decalcified pieces cut on a freezing microtome, decalcified pieces embedded in parafin and cut on a standard microtome, and thin undecalcified sections cut on a diamond bladed saw and exposed to low level X-rays (microradiograph). Three species are represented: Chelonia mydas, Eretmochelys imbricata, and Caretta caretta.

These preparations show clear growth layers and incremental lines. However, the interpretations of these phenomena is not simple. Known-age animals of 14 and 18 months show more incremental lines in femurs than would occur if they were laid down annually. Some humeri, however, show a single line, but the amounts of bone either side do not seem to correspond to the

growth periods available if the line represented one year.

In one preparation (Figures 6, 7 and 8) several complicating factors are illustrated. "Bundle bone" occurs in large areas and completely disrupts the banding pattern: on the ventral surface there are no obvious striations, on the dorsal surface, there are well over five lines. The banding pattern in other parts of the bone show many layers of variable widths.

Another section of the same bone (Figure 9) without large areas of bundle bone shows more contiguous layers but they still do not have an obvious relationship to its age of 18 months.

Preparations of a humerus of a 42-month old animal show three clear layers. As a fourth layer could previously have been in the area of spongy bone, and hence have been destroyed by remodeling, this seems to show an annual relationship of growth layers. The femur, however, shows only a single layer. In addition to these differences between bones and parts of bones, there are remarkable differences in countable lines depending on exactly where a section is examined. An adult C. caretta shows distinct lines that diverge or fuse from a single source. Lines running in compact bone may disappear into spongy bone (Figures 14 and 15). Some incremental lines, rather than running straight have loops and bends.

In summary, this indicates that incremental lines are not simply laid down annually. Deciphering just how to read these lines, and relate them to time, will be necessary before a technique can be usable.

Figure 1

Femur of 14 month old captive-reared Chelonia mydas (LC 199) showing two concentric lines (1 and 3) with a partial line between (2)



Fig. 1

Figure 2

Humerus of 14-month old captive reared Chelonia mydas (LC 199) showing one concentric line

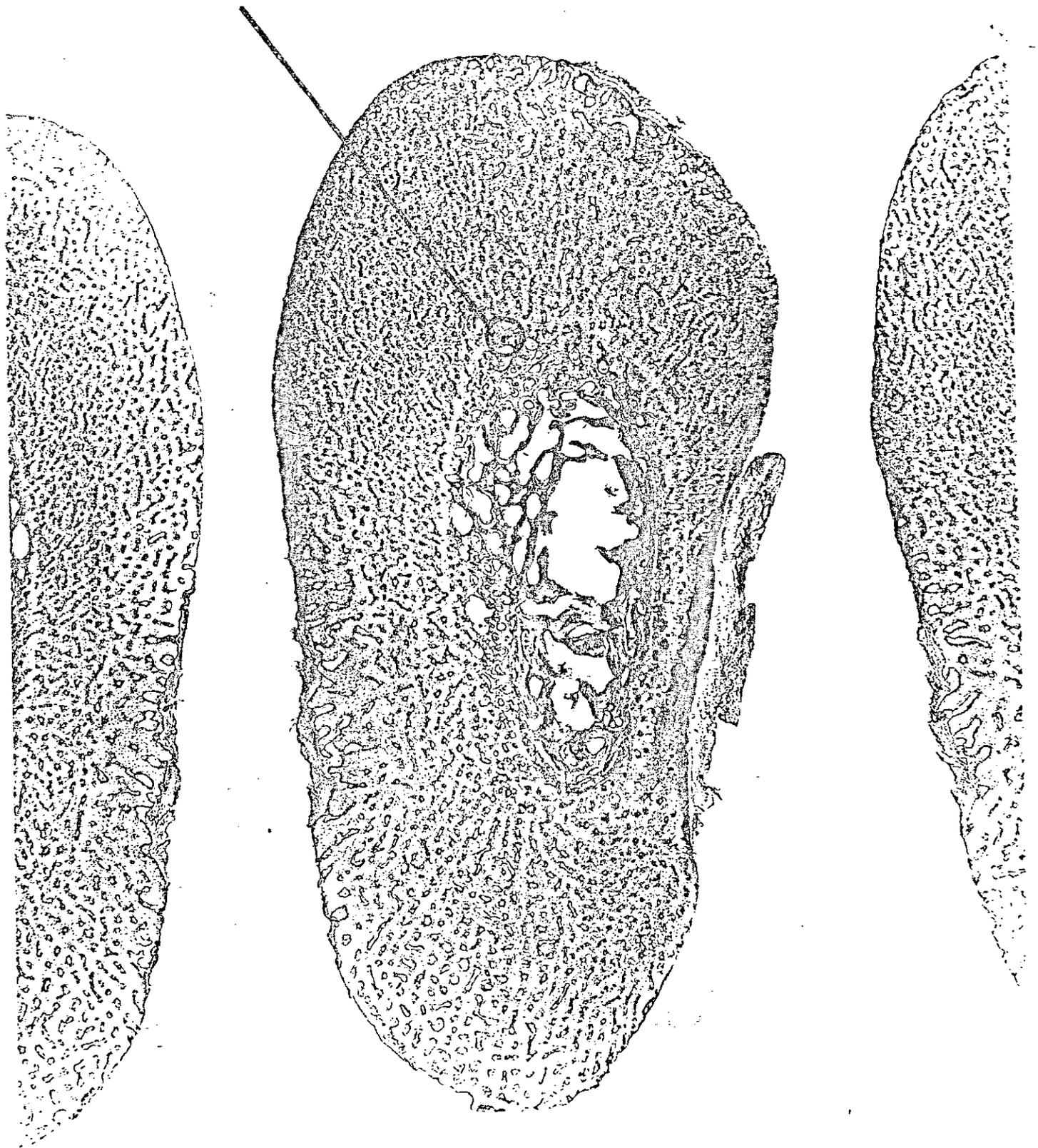


Fig 1

Figure 3

Femur of 18-month old captive-reared Chelonia mydas (LC 200) showing three clear bands separated by two concentric lines (1 and 2), in an area of bundle bone line 2 becomes at least 3 clear lines.

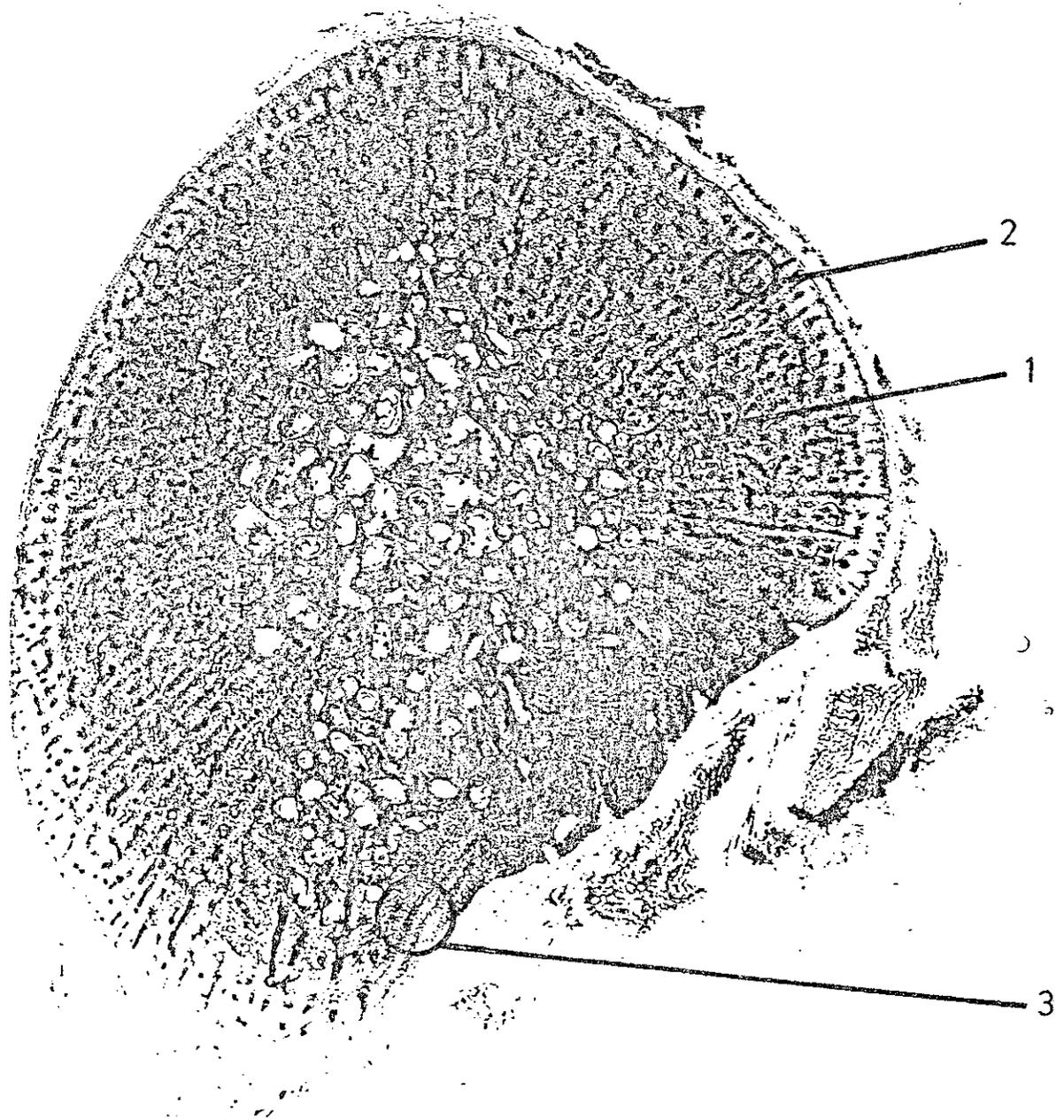


Fig. 2

Figure 4

Two sections of humerus of 18-month old captive-reared Chelonia mydas
(LC 200) showing a contiguous incremental line outside of medulary bone.



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Figure 5

Femur of 18-month old captive-reared Chelonia mydas (LC 201) showing evidence of three bands, (a, b, c) but only one clear incremental line (1).

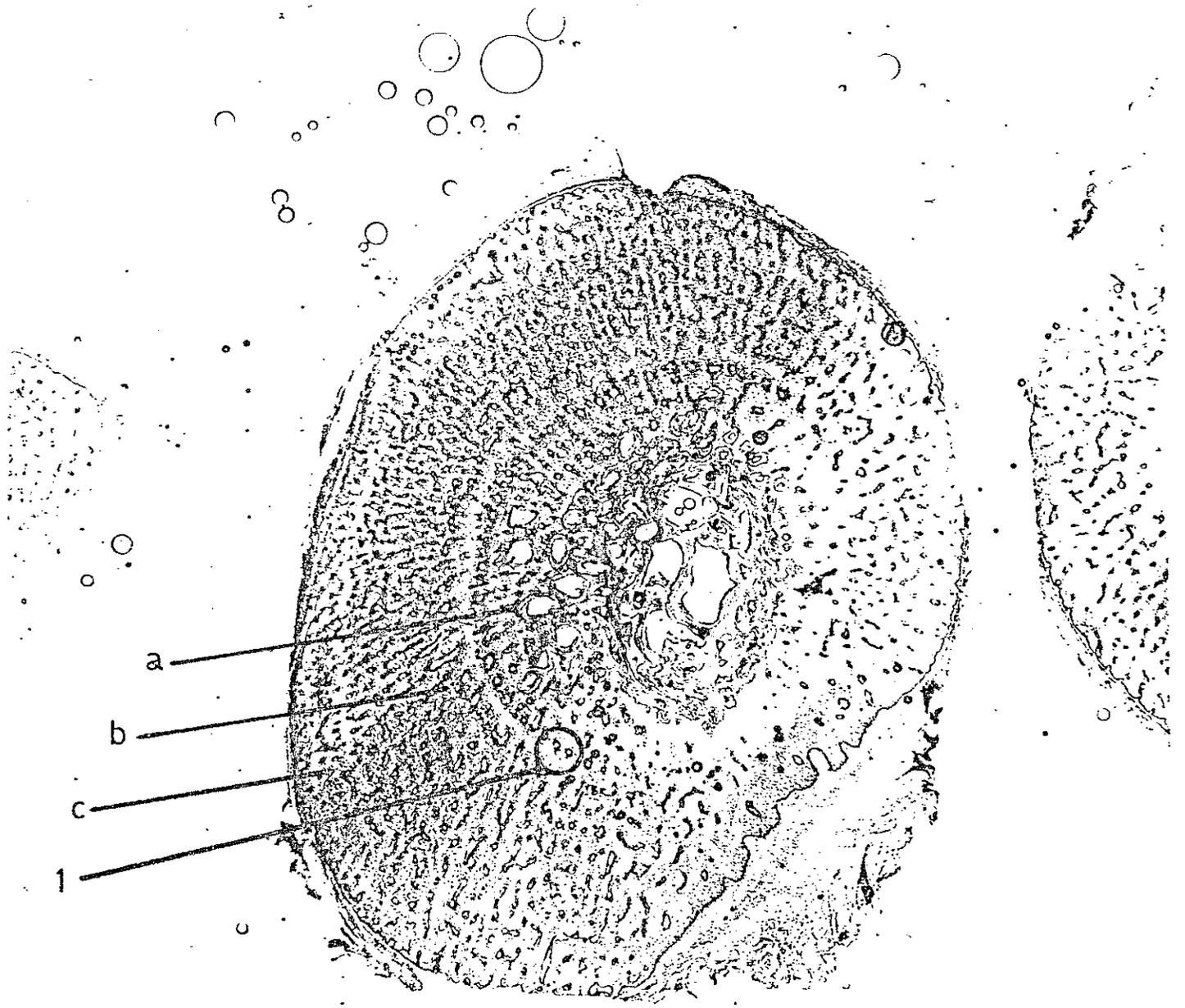


Fig 5

Figure 6

Humerus of 18-month old captive-reared Chelonia mydas (LC 201) showing large (ventral) area of bundle bone (bb) two areas amplified in Figures 7 and 8 (F7 and F8).



Figure 6

Figure 7

Humerus of 18-month old captive-reared Chelonia mydas (LC 201) showing ramification of lines in patch of dorsal bundle bone (1), and possible natal bone (2).

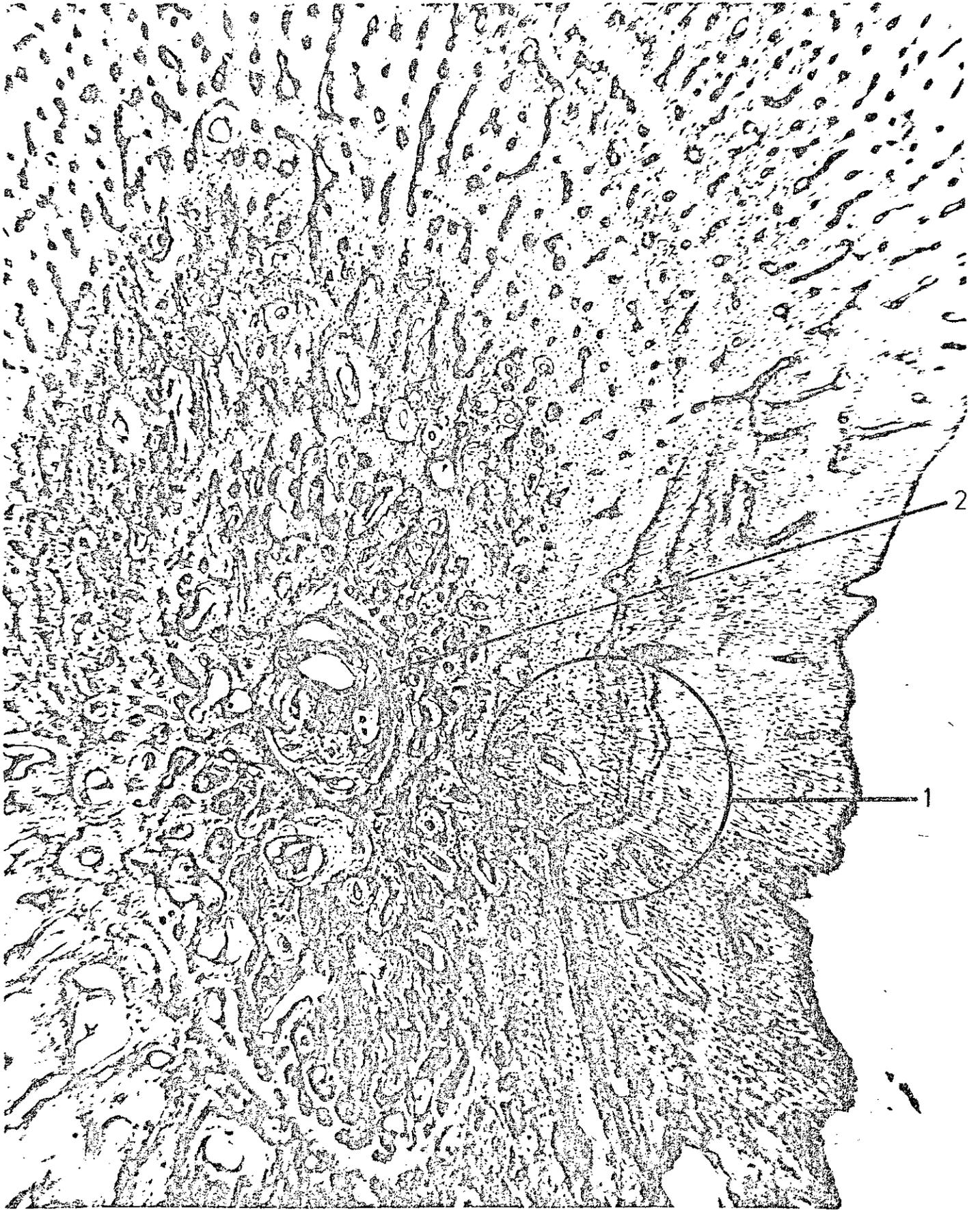


Fig 7

Figure 8

Humerus of 18-month old captive-reared Chelonia mydas (LC 201) showing many clear bands.



Figure 9

Humerus of 18-month old captive-reared Chelonia mydas (LC 201) showing
less area of bundle bone and 5 distinct bands.

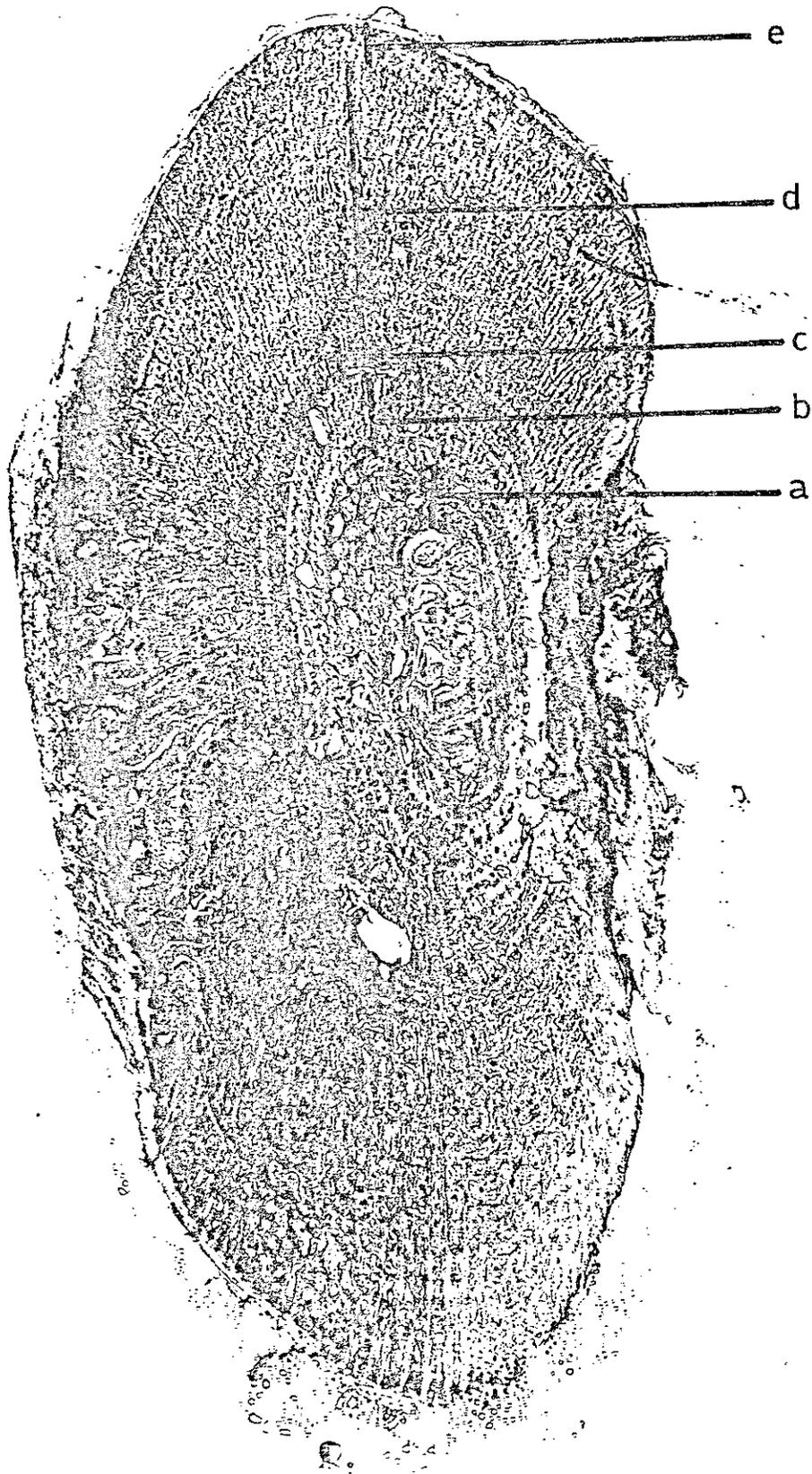


Figure 10

Humerus of 42-month old captive reared Chelonia mydas (LC 204) showing three bands separated by two concentric lines (1 and 2)

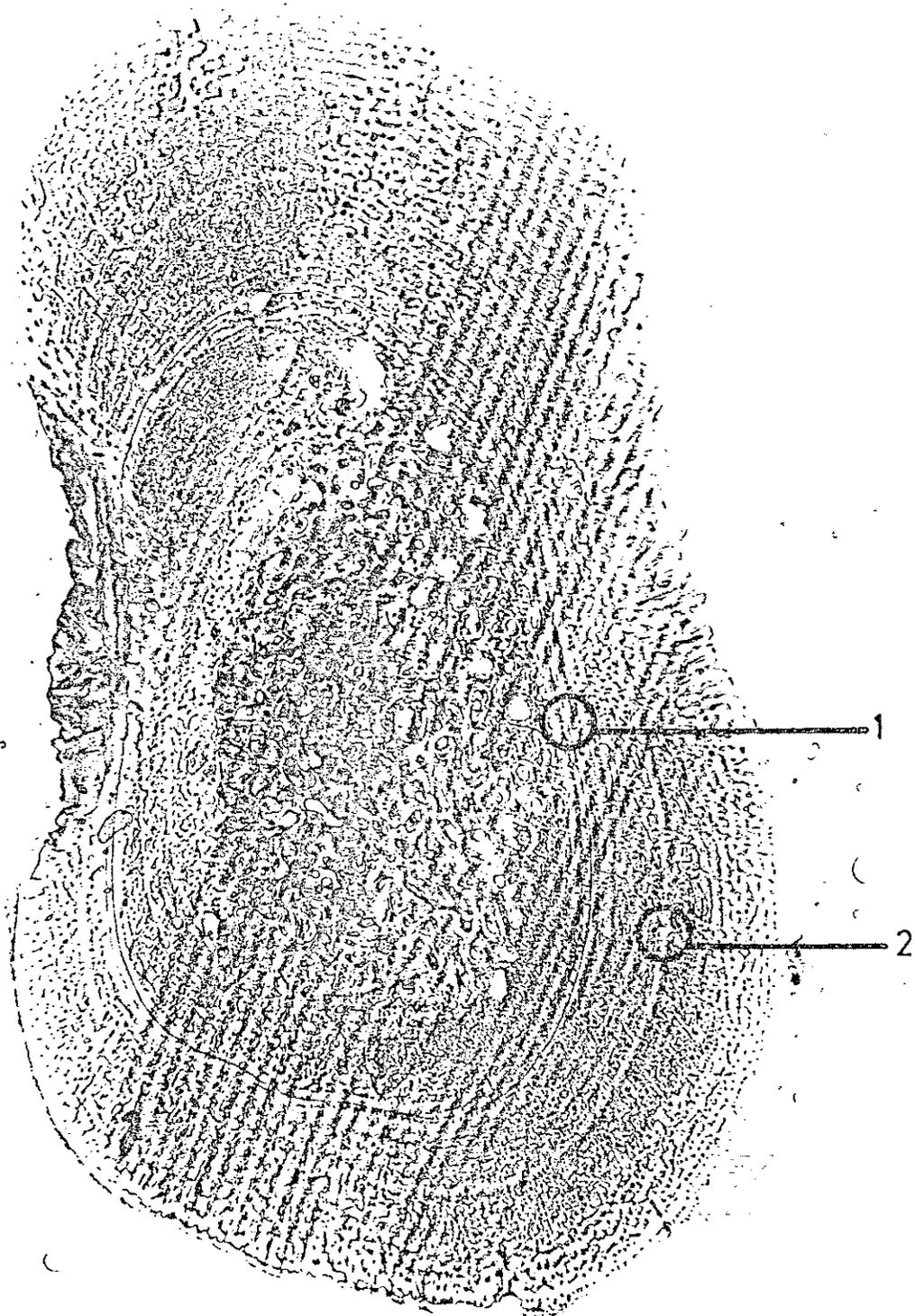


Figure 10

Figure 11

Femur of 42-month old captive-reared Chelonia mydas (LC 209) showing poorly defined bands and lines.



Figure 11

Figure 12

Femur of 42-month old captive-reared Chelonia mydas (LC 209) showing well defined lines (1).

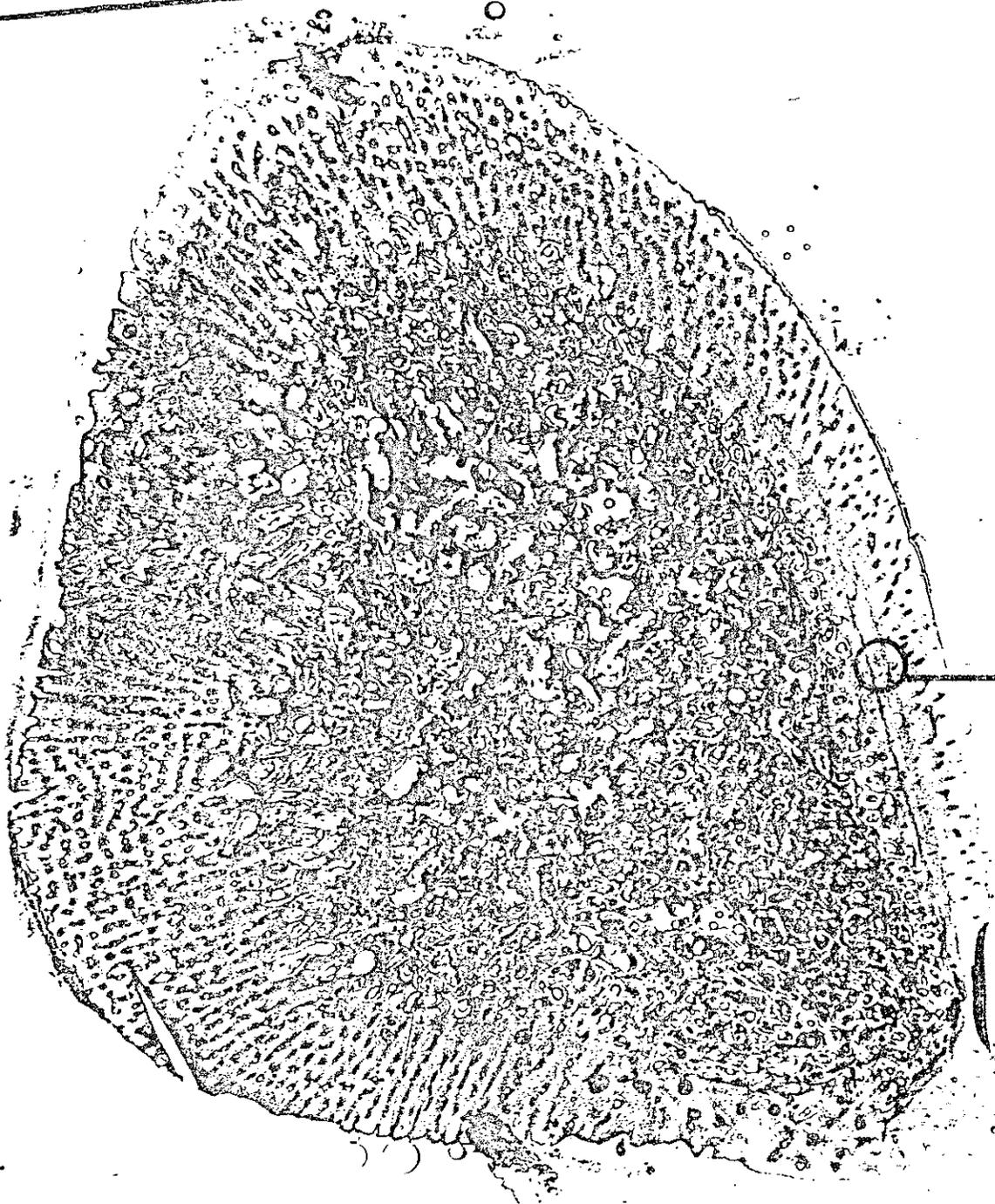


Fig 12

Figure 13

Femur of 42-month old captive-reared Chelonia mydas (LC 209) showing one distinct incremental line (1).

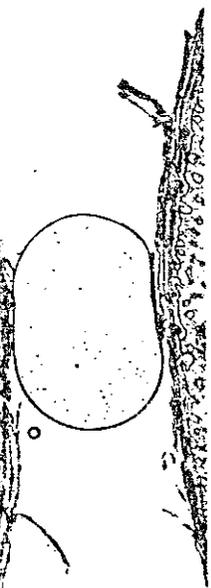
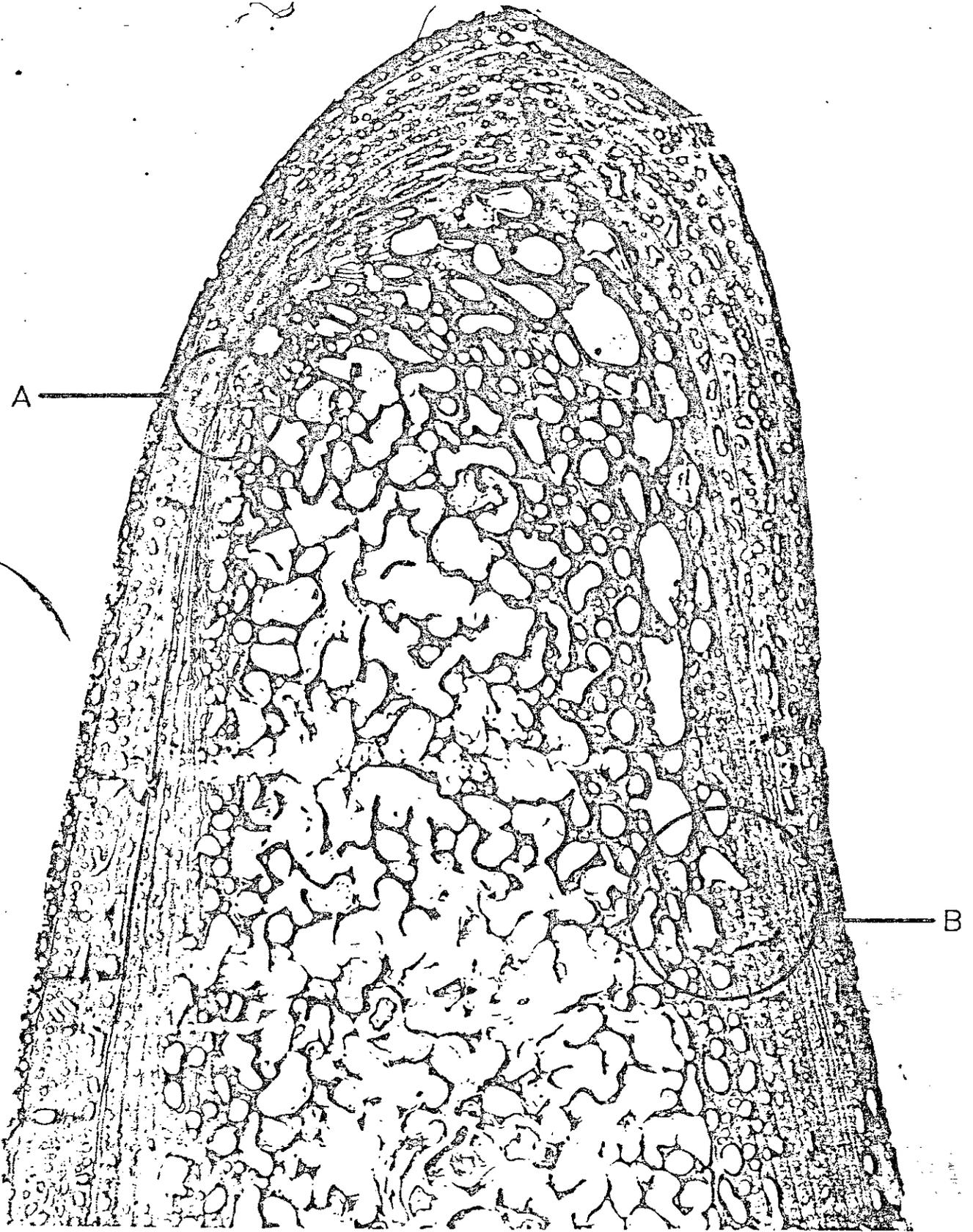
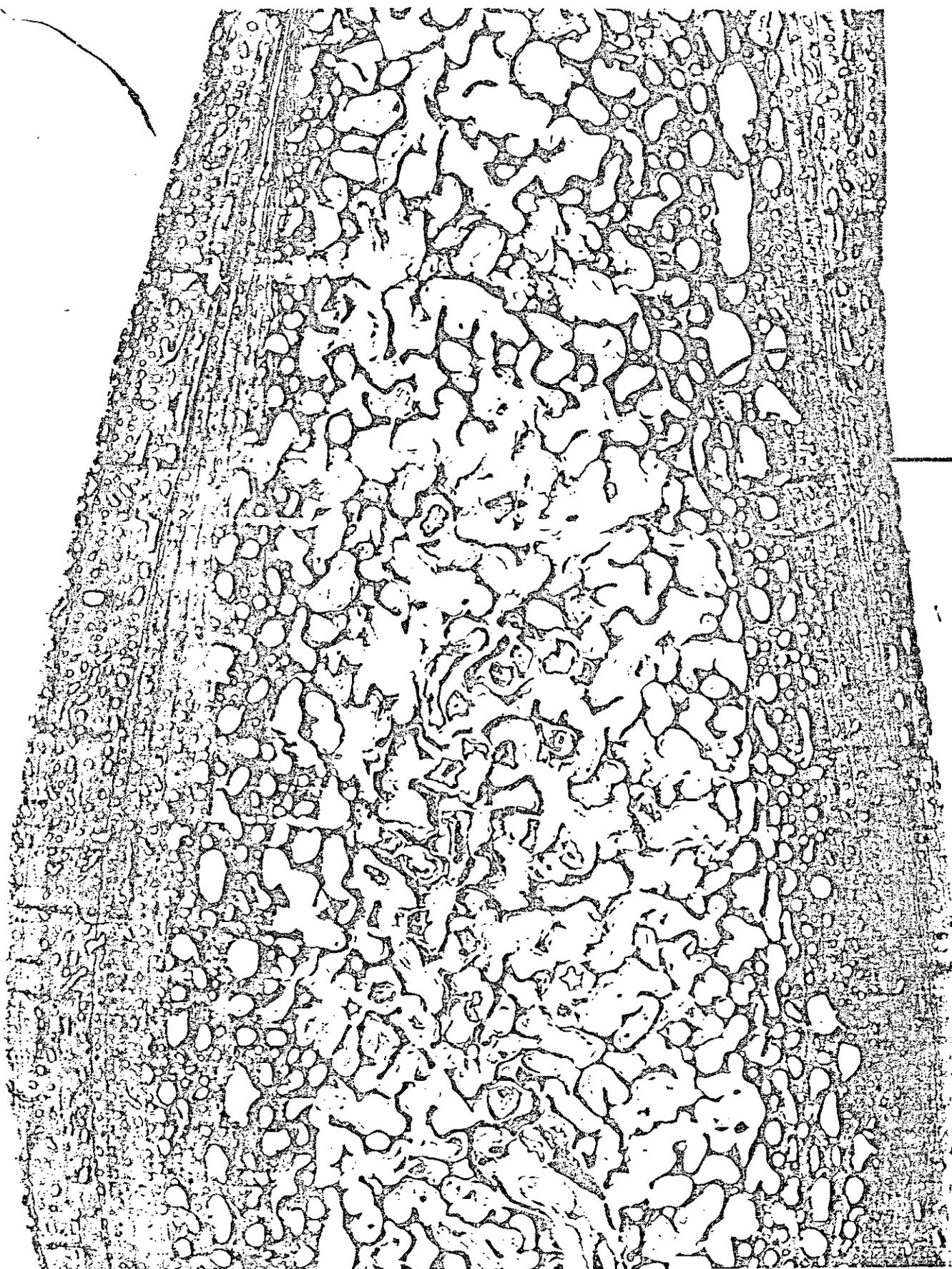


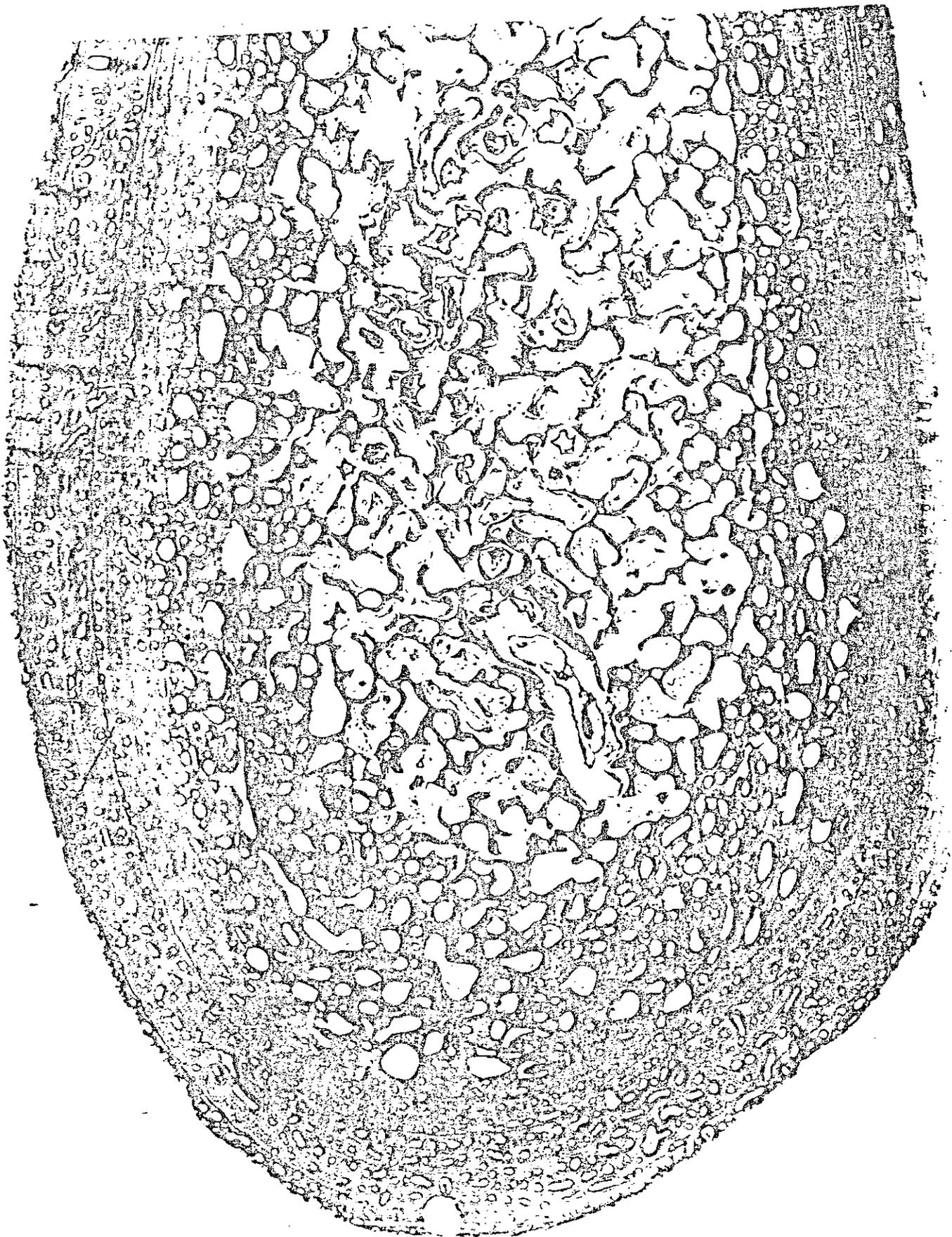
Figure 14 (a, b and c)

Humerus of adult Caretta caretta (USNM 881536) showing distinct incremental lines which fuse and ramify (A), disappear into spongy bone (B).





B



1
r. 11.

Figure 15

Humerus of adult Caretta caretta (USNM 881536) showing close up of area where lines fuse.

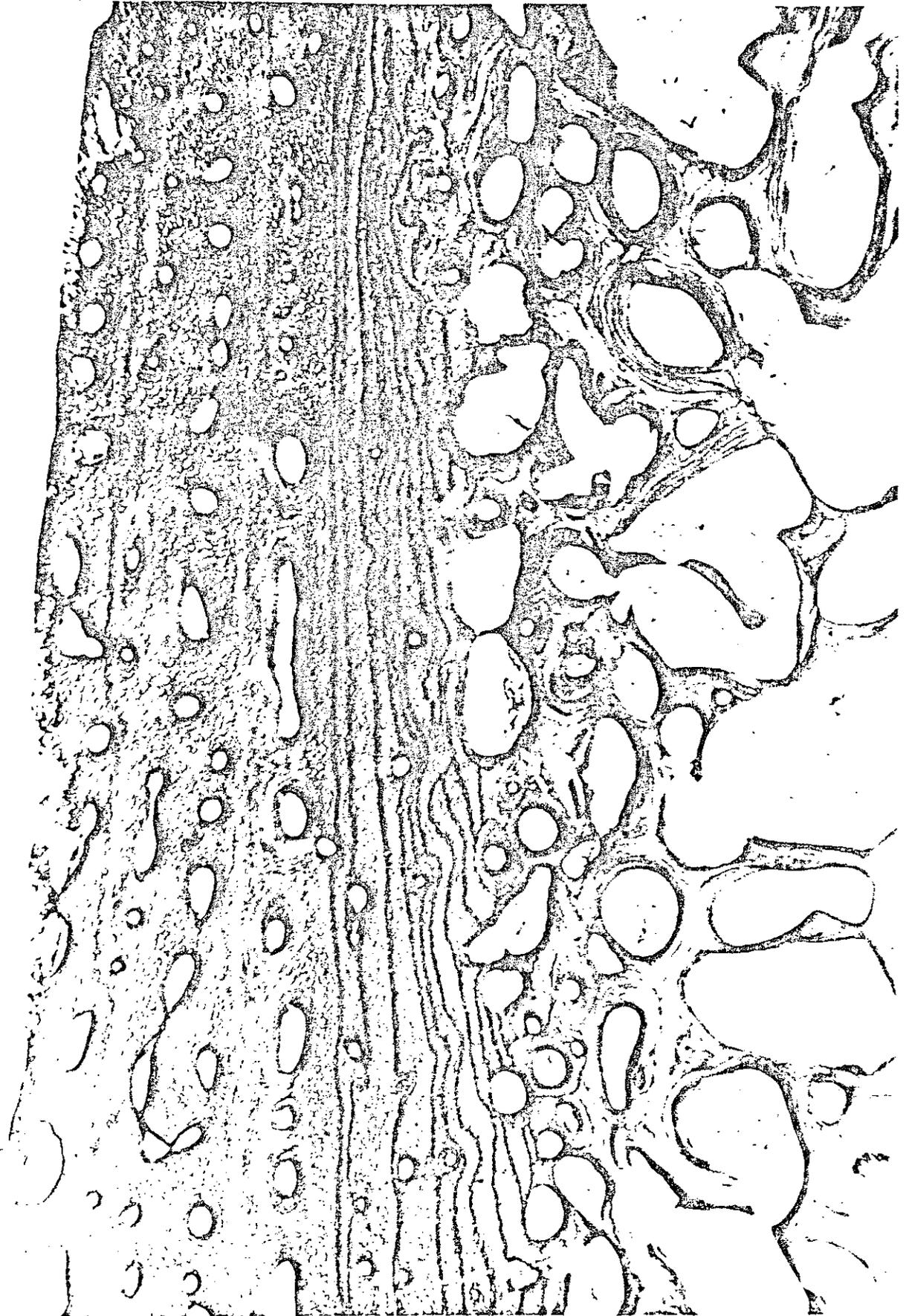


Figure 16

Humerus of adult Caretta caretta (USNM 881536) showing distinct incremental lines, some of which have an erratic, wandering form (10).

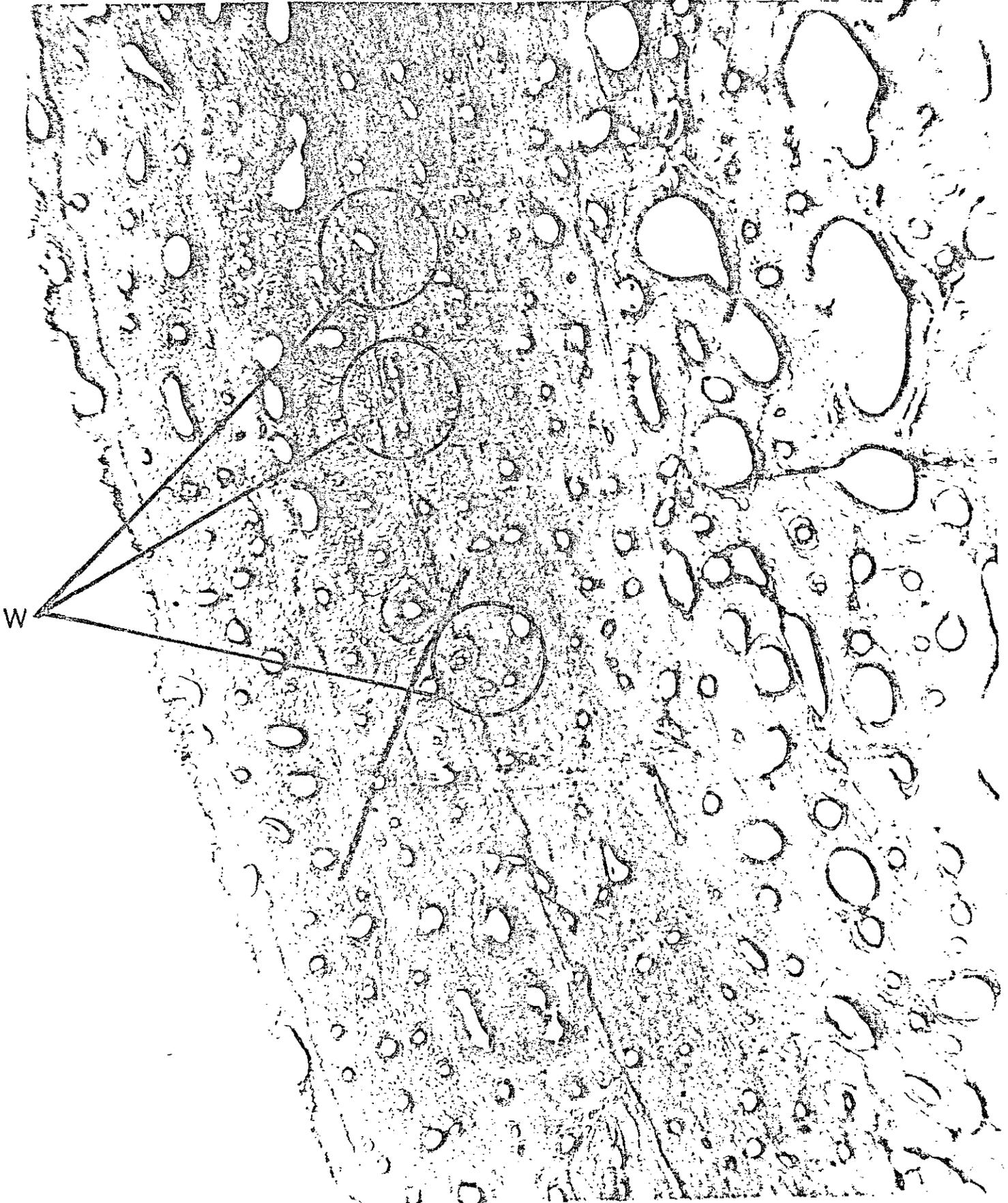


Figure 17

Microradiograph of an adult Eretmochelys imbricata (JFEi 151), showing banded pattern of mineral densities.

