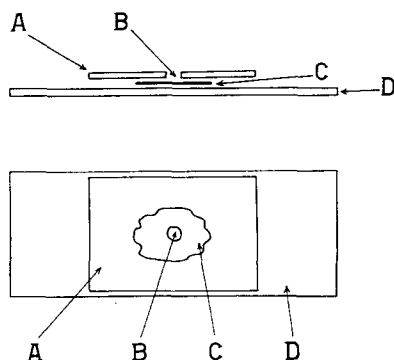


**Technique for Dissection and Measurement of Refractive Index of Osteones.\*** BY ANTONIO ASCENZI AND CLAUDINE FABRY. (*From Istituto di Anatomia e Istologia Patologica, Università di Roma, Italy.*)†

It is known that a continuous process takes place in bone whereby mature osteones (Haversian systems) are broken down and new osteones are formed. Historadiographic study has shown that the mineral salts content of osteones increases gradually toward a maximum (1, 2, 8). In order to obtain information on the chemical and physical properties of the growing osteone, we have developed a dissection technique for isolating single osteone units.



TEXT-FIG. 1. Diagrams showing the section (C) in line with the coverslip (A) and the slide (D); B marks the central hole of the coverslip.

Cross- and longitudinal sections of the femoral diaphysis of ox were ground on glass plates to a thickness of about 30 microns, and the specimens examined by microradiography (the apparatus is described elsewhere (6)), to permit recognition of varying degrees of calcification in osteones. The microradiographs were taken on Eastman Kodak spectroscopic plates 648.

In order to obtain a more sharply defined image of the bone structure, the dissection was performed under a polarizing light microscope. Since steadiness of the specimen is of fundamental importance, the section was mounted on the microscope stage by pressing it between a slide and coverslip with clips. The coverslip was a plexiglas rectangle in which had been cut a 2 mm. circular hole through

which to perform the dissection. Adhesion of the section to both slide and coverslip was increased, also as a means of steadying the specimen, by frosting their surfaces, leaving polished and clear only a central area of about 4 mm. diameter, to allow passage of the light beam. Dissection was performed with a very sharp needle at about 80 to 100 magnification. (See Text-fig. 1).

Isolating a single osteone is a delicate operation that may take as much as half an hour's time. The operator may adapt the physical arrangement of the experiment to his own individual idiosyncrasies, but we have found the following to be a most effective

TABLE I  
*Refractive Index of 35 Dissected Osteones*

No. of osteones	Refractive index
9	1.555
4	1.556
4	1.557
2	1.558
6	1.559
4	1.563
6	1.564

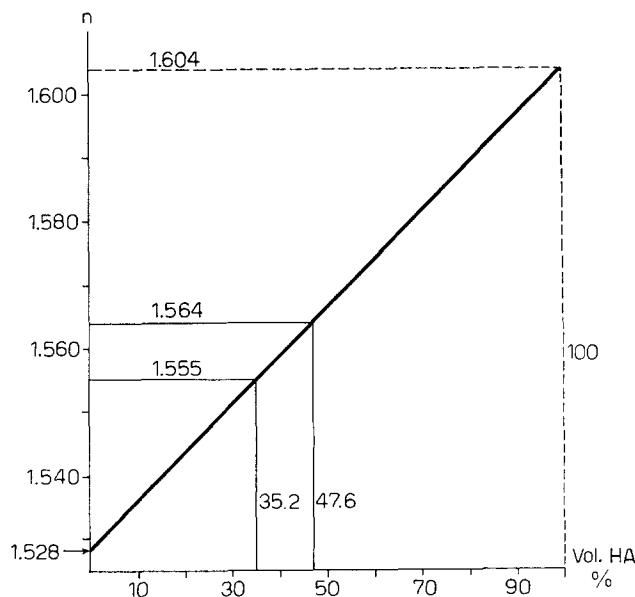
means of achieving the necessary stability: The right arm rests on the work bench, and the heel of the right hand rests firmly on the microscope stage. The needle is manipulated from this position, by using the body of the objective lens as a fulcrum. The fingers of the left hand press the coverslip, thus helping further to steady the section.

When the operator attains skill in this type of dissection, differences in degree of calcification become readily recognizable. The least calcified units are relatively soft and become detached by "tearing," while the oldest osteones are of very compact structure, and can only be removed by "breaking." The rough "tearing" outline of a slightly calcified osteone is shown in Fig. 1, and the sharp "breaking" outline of a highly calcified unit is shown in Fig. 2.

Once the technique is mastered, one can isolate fragments of transverse-sectioned osteones ranging from  $\frac{1}{2}$  down to  $\frac{1}{4}$  the total area. Fig. 3 shows a

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TEXT-FIG. 2. Graph plotting osteone refractive indexes against per cent volume of hydroxyapatite.

slightly calcified osteone, and Fig. 4 shows a highly calcified unit.

To measure the refractive index ( $n$ ) of isolated osteones, monochromatic light (5891 Å) was used, and both the Becke, and Schroeder van der Kolke methods were applied.<sup>1</sup> A series of mixtures of oil of lavender ( $n = 1.473$ ) and essence of cinnamon ( $n = 1.612$ ) was used as reference fluids, and prepared so as to give  $n$ -gradations of 0.002. It is essential that particular attention be paid to dissecting the osteone within its boundaries so that fragments from contiguous osteones do not contaminate the field and falsify measurements.

In the present study, 35 cross-sectioned and microradiographed osteones were selected to determine the range of refractive indexes. The results are presented in Table I.

The refractive index of osteones with the least amount of mineral salts is 1.555. The value increases progressively with the increase of calcium salts that occurs with age, reaching a maximum of 1.564 in oldest bone. The maximum value corresponds with figures obtained in previous measurements in human and ox femoral diaphyses (3). The techniques used here cannot detect non-uniform distribution of calcium salts in a single

osteone (2). The refractive index of decalcified, air-dried bone, at room temperature, is 1.530.

Some data recently furnished by Rowland, Jowsey, and Marshall (7) bear on the subject at hand. These investigators have determined the content of mineral substance both in osteones in which calcification is minimal, and in osteones in which the process has gone to its maximum. They express the amount of calcification in terms of grams of calcium per cubic centimeter, and grams of hydroxyapatite (HA) per cubic centimeter.

Regarding ox bone, used by us in the present study, they report the following data:

Young, slightly calcified osteones: 0.444 gm. Ca/cm.<sup>3</sup> and 1.11 gm. HA/cm.<sup>3</sup>

Osteones at maximum calcification: 0.600 gm. Ca/cm.<sup>3</sup> and 1.50 gm. HA/cm.<sup>3</sup>

Having found, on the basis of the weight and volume of the elementary crystal cell, that the specific gravity of HA is 3.15 gm./cm.<sup>3</sup>, one can calculate from the data of Rowland *et al.* that the per cent volume of HA grows from 35.2 in the young osteone to 47.6 in the maximally calcified osteone.

If the increment of the refractive index is plotted as a linear function of the per cent volumetric increment of HA, the graph in Text-fig. 2 is obtained. If an extrapolation is performed, the graph shows that at the limit values of 0 and 100 per cent volume of HA, the refractive indexes are 1.528 and 1.604 respectively. These figures agree surprisingly

<sup>1</sup> For details on the latter method, see A. N. Winchell, *Elements of Optical Mineralogy*, New York, J. Wiley & Sons, 1947.

well with those found by us for the decalcified bone (1.530) and by Ascenzi (4-6) for bone deprived of its organic substance (1.600).

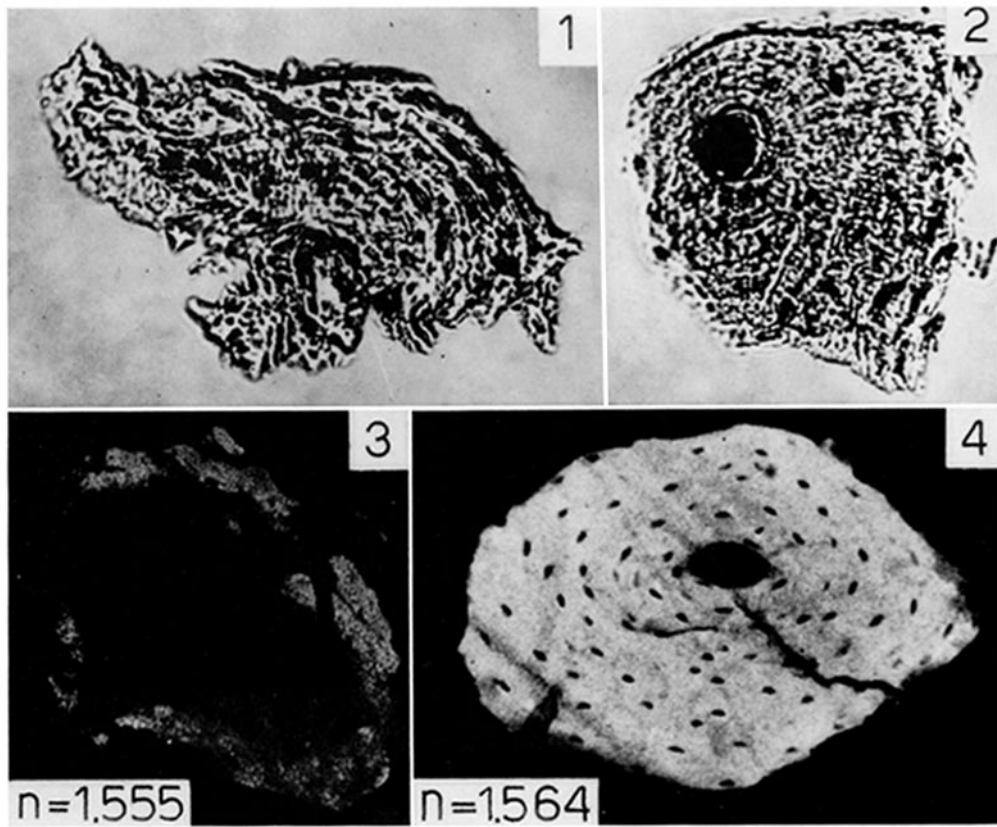
Further work is in progress.

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## EXPLANATION OF PLATE 90

- FIG. 1. Microphotograph of a slightly calcified osteone removed by tearing (cross-section).  $\times 210$ .  
FIG. 2. Microphotograph of a highly calcified osteone removed by breaking (cross-section).  $\times 290$ .  
FIG. 3. Microradiograph of a slightly calcified, dissected osteone (cross-section).  $\times 190$ .  
FIG. 4. Microradiograph of a highly calcified, dissected osteone (cross-section).  $n$  = refractive index.  $\times 170$ .



(Ascenzi and Fabry: Refractive index of osteones)