of water or saliva applied to the joint $B$ keeps the air in the calibrator moist. The Brodie solution is brought to the desired mark, and the gas pressure in the calibrator and the manometer is equilibrated to air. In case the apparatus does not have a three-way stopcock, the pressure can be equalized by pulling out the plug of the stopcock or by opening the side arm of the vessel.

The plunger is pulled out from the lower to the upper position, the manometer fluid is adjusted back to the mark, and the pressure is read. As a check, the plunger is pushed down again, and the procedure is repeated. If there is no leak the readings should agree to within less than 1 mm.

The gas volume of the respirometer is calculated according to the following formula: $V(Pb-Pw) = (V + Vc)\ (Pb-Pw-h)$ which, solved for $V$, gives: $V = Vc\ (\frac{Pb-Pw}{h} - 1)$ where $V$ = volume of the respirometer, $Vc$ = volume withdrawn by the calibrator, $Pb$ = barometric pressure, $Pw$ = water vapor pressure, and $h$ = change in pressure in the manometer. All pressures are expressed in mm of Brodie solution. For accurate calibrations it is recommended to check the specific gravity of the Brodie solution.

The present method was checked on 10 respirometers that had been accurately calibrated by mercury. The results of both methods agreed within 1%. Actually, 7 out of the 10 were within ± 0.5%.

### Reference

1. MÜNZER, E., and NEUMANN, W. *Biochem. Z.*, 1917, **81**, 319.

## Anodic Decalcification of Mineralized Tissue[1, 2]

**Jack De Ment[3] and Ellis B. Jump[3]**

*School of Dentistry,*
*University of Oregon, Portland*

A large part of our knowledge and understanding of the nature of such mineralized tissues as teeth and bone rests directly upon decalcification methods that involve various acid solutions. The solubility of a mineralized tissue, such as enamel, in different acids differs greatly and appears to depend upon factors other than pH alone (*6*).

This is a preliminary report of studies on methods and means for the anodic decalcification of such normal mineralized tissues as teeth, bone, eggshell, coral, mollusk shell, and such pathologic calcified tissues as osteomata and ectopic calcifications.

[1] Read before the Northwest Section, International Association for Dental Research, at the University of Washington, Seattle, May 8, 1949.

[2] This study was made possible by the financial support of the Oregon State Dental Society.

[3] Respectively, research assistant and professor and head of the Department of Anatomy and Histology, School of Dentistry, University of Oregon, Portland.

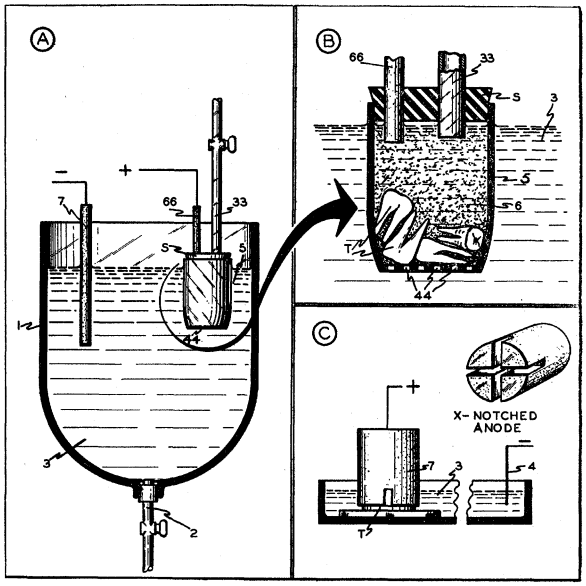


FIG. 1. Anodic decalcification apparatus, showing arrangement for gross specimens, *A*, and the tissue-anode assembly, *B*; thin-ground section apparatus, *C*.

The electrically forced migration of the mineral constituent of bone, with use of strong acid solutions, was first described by Richman *et al.* (*8*) and later by Friedland (*4*). These investigators relied upon the movement of $Ca^{++}$ to a cathode—somewhat the reverse of electrodeposition, a "deplating" of the calcium phosphates and carbonates from the bone. Tests upon tooth organ material were apparently not undertaken in the earlier studies.

The anodic decalcification of hard tissue is contrary to the general belief that, for a substance to undergo anodic attack or anodic dissolution, it must be a conductor of electrical energy (*5*). Decalcification of tooth substance occurs despite the relatively great electrical resistances involved, which vary from $6 \times 10^9$ ohms (*3*) downward for dry dentin to between $17.5 \times 10^4$ and $36 \times 10^5$ ohms for human enamel (*1, 2*). Moist dentin, however, is a conductor, showing values between $84 \times 10^4$ and $1.7 \times 10^6$ ohms (*7*). Factors modifying the electrical conductivity in tooth substances are the amount of moisture, degree and type of mineralization, available supply of soluble salts, and the conditions of the determination.

As might be expected, the nonmineral parts of the tooth appear to carry the current best, and most of the current seems to be conducted along the dentinal tubules. Our studies have indicated that, in customary acid decalcification of teeth, the enamel is most readily attacked, the dentin and cementum much less so; in anodic decalcification the opposite is usually observed.

*Gross specimens.* For the decalcification of whole teeth and masses of bone, the apparatus shown in Fig. 1 *A* and *B*, was developed: The teeth, *T*, are embedded in 10–20 mesh 50% ferrosilicon, within a sieved Gooch crucible, 5, provided with a 2-hole rubber stopper, *S*. Through one hole of the stopper a hard carbon rod, 66,

is inserted, to make contact with the FeSi. Inserted through the other hole is a glass tube, 33, in communication with a supply of electrolyte, 3.

The FeSi-embedded tissue specimen is loosely packed into the sieved crucible (e.g., Coors No. 3), with space allowed at the top. This space facilitates free flow of the electrolyte through the system. The anode member, *B*, is suspended within a 4-liter open-neck bell jar, 1, supported in an inverted position. A cathode, 7, is also supported within the open jar, several cm from the anode assembly. The lower neck of the bell jar is fitted with a stopper and glass cock, 2, for regulating the level of the electrolyte and for draining the system.

An electrolyte is then placed in the bell jar, its level being brought within about 0.5 cm of the crucible edge. An additional supply of electrolyte is placed in a reservoir unit, situated above the main assembly, *A*, for gravity or gas-bubble feed through the anode crucible and out its sieved bottom, 44, into the bath.

With the apparatus shown in *A* and *B* of Fig. 1, the resistance is generally between 200 and 400 ohms, depending upon the nature and size of the specimen(s), the packing of the FeSi, the conductivity of the electrolyte, and the temperature. Between 100 and 400 ma is usually the current drawn by one such unit.

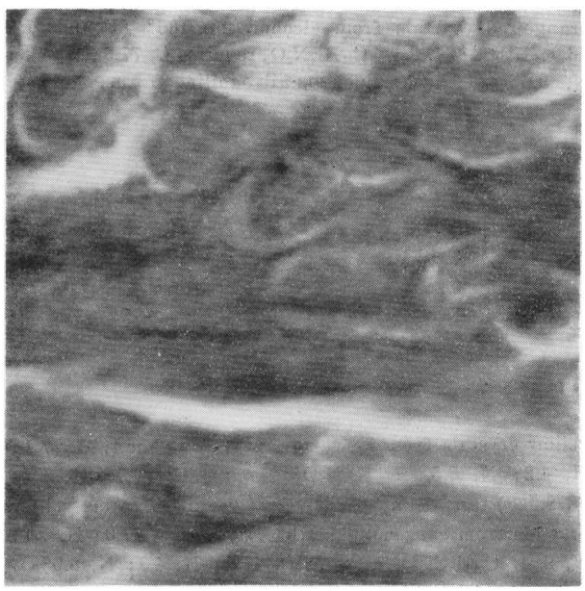

FIG. 2. Human tooth enamel anodically decalcified (2% NaCl; 30 hr), showing in transverse section the organic matrix (hematoxylin; × 500).

*Thin-ground specimens.* For the decalcification of thin-ground sections of teeth a relatively simple arrangement was developed (Fig. 1 *C*): The tissue section, *T*, is placed upon a plane microscope slide, within a Petri dish, in contact with either a lump of 50% FeSi or the end of a hard carbon rod, 7. When a carbon rod is employed, the contact end is preferably notched or slotted longitudinally, to permit escape of anodic oxygen; the same function is served by the irregular surface of the

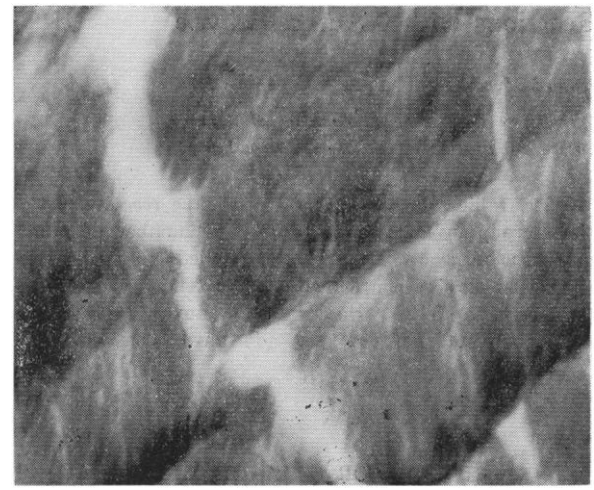

FIG. 3. Human tooth enamel anodically decalcified (2% NaCl; 30 hr), showing longitudinal view of the organic matrix replica of perikymata (hematoxylin; × 390).

FeSi. Complementing the tissue-anode assembly is a cathode, 4. An electrolyte, 3, is then added to cover the specimen. This system should draw approximately 20–60 ma, depending upon its size.

By means of anodic decalcification the rate of demineralization for a given electrolyte is accelerated. A wide choice of materials is available which otherwise do not qualify as decalcifying agents. Whole teeth may be decalcified in as little as 10–15 hr, as contrasted to the several weeks often required in nonelectric methods. Using 2% nitric acid (with or without such antioxidants as di-tert-butyl-*p*-cresol) or 5% lactic acid, the tooth matrices are obtained as firm yellow or white products, respectively. With alkali chlorides the mineral matter of teeth, bone, and eggshell is thrown into the bath as a white, flocculent precipitate which, in the case of teeth and bone, is accompanied by a yellowish or brownish oil-like material. Other compounds, such as sodium formate (3.3%), cause a green substance to appear in the roots of human teeth. The enamel cuticle and plaques are dislodged from the tooth surface and, in many instances, can be recovered intact as a cast of the crown. Metal sols may be formed, usually during the early stages of processing in electrolytes of low conductivity, and these are dispersed in the hard tissue; e.g., we have produced purple of Cassius in human tooth enamel. The anodic decalcification of thin-ground sections (Fig. 1 *C*) permits visual or photographic study of the sequence of changes that occur.

### References


1. BJORN, H. *Svensk Tandlak. Tid.*, 1946, **39**, 48.
2. EHRENFELD, H. *Z. Stomatol.*, 1927, **25**, 1039.
3. FARMER, F. M. *Int. critical Tables*, 1927, **2**, 310.
4. FRIEDLAND, L. M. *Tech. Bull.*, 1948, **18**, 4.
5. KRONSBEIN, J. *J. Electrochem. Soc.*, 1948, **94**, 353.
6. LEICESTER, H. M. *Biochemistry of the teeth.* St. Louis Mosby, 1949. Pp. 93–94.
7. MATHIS, H., and ADLER, P. *J. Dental Research*, 1937, **16**, 338; *Z. Stomatol.*, 1937, **35**, 760.
8. RICHMAN, I. M., GELFAND, M., and HILL, J. M. *Arch. Path.*, 1947, **44**, 92.