obtained. In our hands a maximal response corresponds to about 50 γ of our standard ACTH protein. Assuming, therefore, a minimum of an equivalent of 50 γ/0.5 ml, the original 28 mg contained a minimum of 400 γ of activity. Thus, a minimum of an activity equivalent to 170 mg of an ACTH protein can be obtained from 1 kg of fresh sheep glands.

It is of interest to note that the only other anterior pituitary hormone that could be demonstrated in these extracts was the follicle-stimulating hormone (FSH), which was apparently present in large quantity. However, it could be separated from the ACTH activity by dialysis, since the FSH did not pass through a dialysis membrane.

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Simple Calibrator for Warburg Respirometers[1]

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The standard method of calibrating Warburg respirometers is to determine the volume by weighing with mercury. Less laborious methods have been devised, but most of them lack sufficient accuracy or simplicity. With the present simple calibrator a Warburg respirometer can be calibrated in a few minutes, accurately within 1%.

An accurately known volume of gas is withdrawn from the respirometer with a thermostabilized syringe device. The volume of the apparatus is calculated from the resulting change in manometer reading. The principle is well known and was applied in the Münzer-Neumann method (*1*).

The calibrator (Fig. 1) is essentially a syringe surrounded by a water jacket. The metal or plastic barrel is about 5 cm long and is drilled and reamed for a ⅛-in. drill rod, which serves as the plunger. The upper end of the barrel is provided with a short piece of tygon tubing, which provides an airtight seal for the plunger. A metal spring clip is fastened to the upper end of the barrel and presses lightly against the plunger. Two fine grooves (*C* and *D*) 3 cm apart are machined around the plunger. These serve to arrest the plunger accurately when they engage the metal clip. The apparatus is calibrated by measuring the diameter of the drill rod with a micrometer. The distance between the grooves is measured to within 0.1 mm. A slight film of grease travels with the plunger through the tygon seal, and adds to the volume displaced by the plunger. This amount was found to be negligible ($< 0.1\%$) when a light grease such as vaseline was used. The volume extracted by our calibrator is 234 mm³, known to within ± 0.2 mm³.

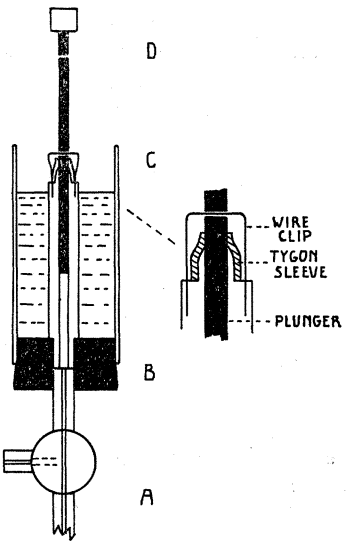

FIG. 1.

The volume of gas determined by the present procedure includes not only the volume of the Warburg apparatus up to the lower opening of the stopcock (Fig. 1, *A*) but, in addition, all air spaces included between *A* and *C*.

The volume *AB* can be determined by filling it with water from a tuberculin syringe. This volume in 10 of our apparatus averaged 80 mm³. The gas volume *B* to *C* can be kept negligible by having a flat connection at *B*, and the plunger in the low position, ending flush with *B*.

Each Warburg vessel is charged with a volume of water from a tuberculin syringe equal to *AB* (in our case 80 mm³). The respirometers are placed in the water bath at room temperature, and the water jacket of the calibrator is filled with water from the water bath.

After a period of thermoequilibration, the calibrator is attached to the respirometer (Fig. 1). A small amount

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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%.

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Anodic Decalcification of Mineralized Tissue[1, 2]

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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.

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

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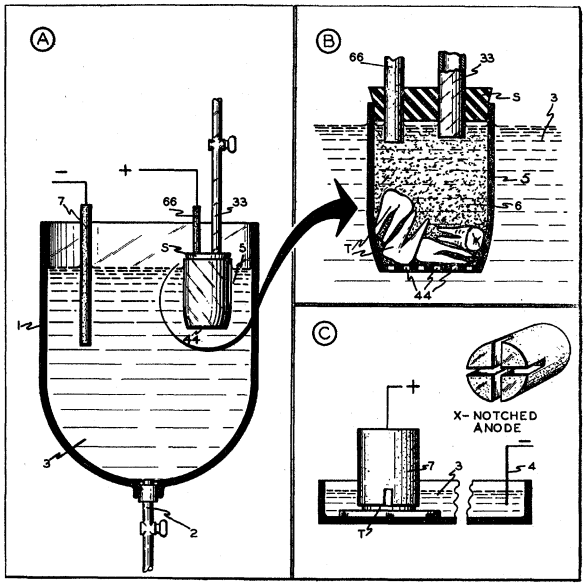

FIG. 1. Anodic decalcification apparatus, showing arrangement for gross specimens, *A*, and the tissue-anode assembly, *B*; thin-ground section apparatus, *C*.

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 6×10^9 ohms (*3*) downward for dry dentin to between 17.5×10^4 and 36×10^5 ohms for human enamel (*1, 2*). Moist dentin, however, is a conductor, showing values between 84×10^4 and 1.7×10^6 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,