NATIONAL BUREAU OF STANDARDS REPORT

9078

Progress Report
on
Surface Studies of Natural and Synthetic Bone Mineral and Teeth

NBS

U.S. DEPARTMENT OF COMMERCE

NATIONAL BUREAU OF STANDARDS

THE NATIONAL BUREAU OF STANDARDS

The National Bureau of Standards is a principal focal point in the Federal Government for assuring maximum application of the physical and engineering sciences to the advancement of technology in industry and commerce. Its responsibilities include development and maintenance of the national standards of measurement, and the provisions of means for making measurements consistent with those standards; determination of physical constants and properties of materials; development of methods for testing materials, mechanisms, and structures, and making such tests as may be necessary, particularly for government agencies; cooperation in the establishment of standard practices for incorporation in codes and specifications; advisory service to government agencies on scientific and technical problems; invention and development of devices to serve special needs of the Government; assistance to industry, business, and consumers in the development and acceptance of commercial standards and simplified trade practice recommendations; administration of programs in cooperation with United States business groups and standards organizations for the development of international standards of practice; and maintenance of a clearinghouse for the collection and dissemination of scientific, technical, and engineering information. The scope of the Bureau's activities is suggested in the following listing of its four Institutes and their organizational units.

Institute for Basic Standards. Applied Mathematics. Electricity. Metrology. Mechanics. Heat. Atomic Physics. Physical Chemistry. Laboratory Astrophysics.* Radiation Physics. Radio Standards Laboratory:* Radio Standards Physics; Radio Standards Engineering. Office of Standard Reference Data.

Institute for Materials Research. Analytical Chemistry. Polymers. Metallurgy. Inorganic Materials. Reactor Radiations. Cryogenics.* Materials Evaluation Laboratory. Office of Standard Reference Materials.

Institute for Applied Technology. Building Research. Information Technology. Performance Test Development. Electronic Instrumentation. Textile and Apparel Technology Center. Technical Analysis. Office of Weights and Measures. Office of Engineering Standards. Office of Invention and Innovation. Office of Technical Resources. Clearinghouse for Federal Scientific and Technical Information.**

Central Radio Propagation Laboratory.* Ionospheric Telecommunications. Tropospheric Telecommunications. Space Environment Forecasting. Aeronomy.

---

* Located at Boulder, Colorado 80301.

** Located at 5285 Port Royal Road, Springfield, Virginia 22171.

NATIONAL BUREAU OF STANDARDS REPORT

NBS PROJECT
311.05-11-3110561

December 31, 1965

NBS REPORT
9078

Progress Report
on
Surface Studies of Natural and Synthetic Bone Mineral and Teeth

By

William V. Loebenstein

This investigation is part of the dental research program conducted by the National Bureau of Standards, in cooperation with the Council on Dental Research of the American Dental Association; the National Institute for Dental Research; the Army Dental Corps; the Aerospace Medical Division, USAF School of Aerospace Medicine; and the Veterans Administration.

IMPORTANT NOTICE

NATIONAL BUREAU OF STAND
for use within the Government. Befo
and review. For this reason, the pub
whole or in part, is not authorized
Bureau of Standards, Washington, D.
the Report has been specifically prep

Approved for public release by the
director of the National Institute of
Standards and Technology (NIST)
on October 9, 2015

counting documents intended
ected to additional evaluation
ng of this Report, either in
ice of the Director, National
Government agency for which
tor its own use.

NBS

U.S. DEPARTMENT OF COMMERCE

NATIONAL BUREAU OF STANDARDS

Surface Studies of Natural and Synthetic Bone Mineral and Teeth

Abstract

Low temperature nitrogen adsorption measurements have been performed using dentin and bone as well as synthetic calcium phosphate preparations. Surface areas ranging from about 1-$\frac{1}{2}$ to nearly 150 $m^2 \cdot g^{-1}$ have been found. Pore size distribution calculated from the isotherm data shows promise of being a valuable and sensitive tool for aiding in the comparison of crystallographic forms and for detection of subtle changes in structure not measurable by other means presently in use. The pore structure of bone mineral in the range 20 - 300 Å appears to be entirely independent of the collagen matrix.

1. Introduction

Some studies have been made on bone mineral principally by Beebe and coworkers [1,2] utilizing low temperature nitrogen adsorption techniques for measuring the surface properties. The present investigation utilizes a similar approach for studying bones and teeth as well as a few representative synthetic preparations. Some questions which have been resolved relate (a) to the effect of collagen on the surface area of dentin; (b) the effect of heat treatment on the pore structure of anorganic bone and (c) the similarities (and differences) in the differential pore volume distributions of natural bone mineral and synthetic preparations.

2. Techniques Employed

Conventional volumetric nitrogen adsorption measurements were made at 77°K for all experiments using a simplified surface area apparatus described in 1951 [3]. Where surface areas only were obtained, the measurements were confined within the relative pressure region where the "Free Surface" BET equation is valid; i.e., between 0.1 and 0.3. However, for pore size determinations, the complete isotherms were required.

Several methods for determining pore size were considered [4,5,6] before the method of Cranston and Inkley [7] was adopted. This preference was based on the fact that the method was capable of yielding a surface area that was nearly completely independent of the BET value and could, therefore, be used as a cross check. It was also preferred because a pore-size calculation could be based on the adsorption isotherm for the calcium phosphate materials used in this study. This lessened the experimental error that would have resulted from the use of the desorption isotherm required by other methods.[1]

The Cranston and Inkley method (like other pore-determination procedures based on desorption isotherms) considers the amount of nitrogen that is retained at each relative pressure value to be partitioned into two quantities: (1) that used to fill completely those pores the diameters of which are

---

[1] Before the first point below saturation on a desorption isotherm can be determined, it is generally necessary to remove several small increments of gas, each contributing to a cumulative error. The alternative of removing a single large increment might prove to be too large resulting in the point being too far down on the isotherm.

equal to or smaller than d and (2) that which adsorbs on the surfaces of all pores the diameters of which are greater than d. The quantity d is, of course, a function of relative pressure and increases in a non-linear manner. The thickness of the adsorbed layer for a large number of different non-porous materials as a function of relative pressure was found by Cranston and Inkley to be a reasonably well fitted by a single curve thus making it possible to calculate the partitioning described above.

3. Surface Areas of Anorganic As Distinguished From Crude Dentin and Bone

Samples of crude dentin (325-400 mesh) which had been mechanically separated from the enamel, dried and stored at temperatures of -20° for a year were re-examined. The surface areas of the dentins, as determined by nitrogen adsorption, were found to be 10.5, 10.7 and 11.1 $m^2 \cdot g^{-1}$, respectively. These values agreed with measurements of the same samples determined a year earlier (prior to storage).[2]

Yet another sample of dried crude dentin with particles which passed through a sieve of 400 mesh yielded a surface area of only 6.8 $m^2 \cdot g^{-1}$, indicating a rather large degree of variability.

In another experiment, a sample of bovine femur shaft (10-20) mesh was obtained from Armour and Co. Its label indicated that it had been boiled and defatted, only. A good BET plot (based on seven points) revealed a surface area of only 1.47 $m^2 \cdot g^{-1}$.

Similar samples of dentin and of bone were rendered anorganic by extraction with ethylene diamine according to the method of Williams and Irvine [8] and of Peckham, et al [9]. The resultant samples were free of all but a trace of organic matter as determined by fluorescence emission through the courtesy of S. Mancewicz (of the ADA staff at the National Naval Medical Center). The samples of anorganic dentin so prepared were dried at a temperature of 60°C, but their weights were corrected for 5.15 percent water subsequently removed. They possessed a surface area of 130 $m^2 \cdot g^{-1}$ (dry basis) or nearly an acre an ounce! After its initial determination, a sample was exposed to 200°C and to 300°C preheating for prolonged periods of time in a stream of helium followed, in each case, by a redetermination of surface area. The results (see Fig. 1) are in qualitative agreement with Beebe's findings [1] for bone mineral showing a maximum in surface area at about 200°C.

Samples of anorganic bovine femur head and bovine femur shaft were each pre-treated in the same way by purging in a stream of helium for four hours at 200°C. The resultant surface areas were 114 $m^2 \cdot g^{-1}$ and 117 $m^2 \cdot g^{-1}$, respectively. These values are in excellent agreement with one another, as well as with the results for bone mineral reported by Beebe. There seems little doubt that the ten-fold (or greater) increase in surface area resulted from removal of the dried collagen the presence of which denied access even of nitrogen molecules to virtually all of the micro-porous structure of bone or of dentin. That this clogging of pores was not attributable to the presence of fat was confirmed by the low surface area of the de-fatted sample of bone.

---

[2] Unpublished results obtained in these laboratories on the surface areas of a dozen samples of crude dentin ranging in sieve fractions from (<200->325) to (<400) mesh had been determined by G. M. Brauer and W. H. Wiedeman. The results showed no statistically significant difference in surface area among the sieve sizes and averaged 10.0 $m^2 \cdot g^{-1}$.

The collagen molecule is known to be made up of a large number of basic "building blocks" each containing three polypeptide chains which coil around a common axis. These basic units have a diameter of about 15 Å and are 3000 Å in length. If allowed to denature, the spiraled polypeptide chains collapse irreversibly to a rather impervious mass. It is conjectured that the samples of crude dentin used in the early part of this work contained denatured collagen which denied access of nitrogen to all but 10 percent of the existing pore area which was available in the anorganic bone or tooth mineral.

4. Surface Area of Synthetic Hydroxyapatite Converted from Octacalcium Phosphate

It can be shown that when octacalcium phosphate (OCP) is converted to form hydroxyapatite (HA) according to the reaction $2Ca_8H_2(PO_4)_6 \cdot 5H_2O = Ca_{10}(OH)_2(PO_4)_6 + 6CaHPO_4 + 8H_2O$, a shift takes place in the angle between the a and b axes from about 108° in OCP to 120° in HA. This is accompanied by measurable changes in the corresponding lengths of the unit cell axes. Whether these shifts in dimensions might cause corresponding changes in surface area was a matter which might readily be resolved at this time. Care had to be taken to insure that a representative sample from the same batch of OCP was used in the reaction because different preparations of HA may vary in their surface area over one or more orders of magnitude as will be seen later. (Both samples were prepared and made available through the courtesy of the NBS dental research staff).

The original sample of OCP had a specific surface of $4.3\ m^2 \cdot g^{-1}$. The treated sample, after reaction, was estimated to be 48 percent converted to HA as determined principally from the change in Ca:P ratio and qualitatively confirmed by its modified infrared spectrum. Its surface area was found to be $4.0\ m^2 \cdot g^{-1}$. These two values are in sufficient agreement that it is of no consequence that the conversion was incomplete.

The surface areas of different preparations of synthetic hydroxyapatite were found to range from one or two square meters per gram to nearly 100. The resultant area appears to be extremely dependent upon the conditions of preparation. Commercial preparations for use in chromatography were, in general, fluffy, extremely finely divided, and had surfaces of about $50\ m^2 \cdot g^{-1}$, or more.

5. Results of Pore-Size Experiments

Changes in the overall surface area of anorganic bone brought about by deliberate modification of the environmental conditions may be insignificant despite the fact that profound irreversible changes in the microporous structure might very well have taken place. This is possible when the loss of surface area resulting from the destruction of small pores is nearly compensated by the increase in area on the walls of newly created large pores. The above example is dramatically exhibited by the behavior of anorganic bovine femur shaft as a result of successivly higher pre-heating temperatures which is shown in Figure 2. The curve designated by "160°C" was heated no higher than that temperature during its drying and purging step and this was only to be sure that bulk water, CO_2 and other impurities were expelled. This curve was found to be in rather good agreement with the natural bone char curve[3] reported by Barrett [10] who used a different method [4] for measuring pore

---

[3] Unpublished results for the pore-size distribution of charcoals alone suggest that most of the volume attributable to the carbonaceous component of bone char would appear in the smallest pores (less than about 50 Å) so that most of the detailed pore structure of the bone substrate would be preserved intact.

sizes. The 160°C sample had a surface area of 101/2 $m^2 \cdot g^{-1}$ and a mean[4] pore diameter of 88.4 Å. When this same sample was subjected to a prolonged heat treatment at 300°C, the change in surface area was barely significant -- it had increased to to 107.9 $m^2 \cdot g^{-1}$ (in qualitative agreement with Beebe's results [1] and with the behavior of dentin as reported earlier in this paper). In its pore structure, however, several profound effects are discernible from Figure 2:

a. The largest peak, i.e., (36-60 Å) was modified to the greatest extent.

b. The second largest peak (100-110 Å) was only slightly attenuated.

c. Smaller peaks and troughs (for the most part) retained their identities, exhibiting only slight relative changes.

d. The decrease in small pores as result of heating was, to a considerable extent, compensated by the increase in large pores, i.e., greater than 120 Å. The mean pore diameter had, in fact, increased to 93.9 Å as a result of the 300°C pre-treatment.

When the sample had been pre-treated at 450°C, the above mentioned effects became more pronounced. In some instances, new peaks and troughs were formed or became more clearly defined, while elsewhere they diminished in amplitude. It is interesting that the peak between 100 and 110 Å was virtually unchanged on heating from 300° to 450°C. This was also found for a lesser peak at 195 Å although the former peak had diminished from its 160° value, while the latter peak increased. The surface area had dropped to 91.9 $m^2 \cdot g^{-1}$ as the result of the 450° heating, while the mean pore diameter had increased to 108.4 Å.

The pore size distribution of two preparations of synthetic hydroxyapatite are shown in Figure 3. In overall appearance, the curves had very little in common and could hardly be identified as the same compound based on their respective pore volume profiles. The lower curve shows a more uniform distribution and a considerably greater surface area (the ordinate scale of the upper curve is magnified ten fold that of the lower.). The uniform distribution is characteristic of the "amorphous" state. The "amorphous" sample[5] had been precipitated rapidly and seemed characteristic of most commercial preparations designed with high surface areas to function as adsorbents in packing of columns for use in chromatography. This sample had a surface area of 67.7 $m^2 \cdot g^{-1}$. The upper curve of Figure 3 shows a much more distinctive pattern which typifies the specificity or higher degree of order exhibited by crystalline substances. This particular batch had been prepared by the dental research staff at NBS where the crystals had been constrained to grow very slowly over a period of five days. The surface area was 11.4 $m^2 \cdot g^{-1}$. Its detailed pore volume profile discloses principal peaks at about 42 Å and 104 Å, corresponding precisely with that of natural bone mineral. In fact, when its pore volume profile is compared with that of

---

[4] A surface-averaged mean pore diameter is used throughout this work. It is defined as $\bar{d}_s$ in the equation $\bar{d}_s = \frac{\Sigma d' \Delta S}{\Sigma \Delta S}$ where d′ is the diameter corresponding to each increment of pore area ΔS used in the summation approximating the integral.

An alternative way of obtaining $\bar{d}_s$ is to assume that the total volume V and area S are contained within a single long pore of diameter $\bar{d}_s$ and length l such that $V = \frac{\pi \bar{d}_s^2 l}{4}$ and $S = \pi d_s l$. An equation for $\bar{d}_s$ is then obtained in terms of V and S by eliminating l between these two equations; thus $\bar{d}_s = \frac{4V}{S}$.

[5] This sample was prepared and made available through the courtesy of Dr. Yoram Avnimelech.

anorganic bovine femur shaft, nearly every peak and trough has its corresponding counterpart at the same pore diameter values, as can be seen from Figure 4.

A sample of synthetic octacalcium phosphate prepared by the NBS dental research staff had a surface area of only 1.41 $m^2 \cdot g^{-1}$, yet its detailed pore volume profile (when magnified 100 times) disclosed considerable detail and can be compared with anorganic bovine femur shaft in Figure 5. While there are points of marked divergence as would be expected (such as that found between 32 Å and 76 Å, and between 180 Å and 210 Å), the remarkable thing is the degree of similarity in most of the remaining portion of the curves. Of course, a certain degree of resemblance should naturally exist between the profile of OCP and HA because of the closeness of their crystallographic relationship. It does seem rather strange that anorganic natural bone (generally believed to consist, more or less, of HA) bears a closer overall resemblance in its pore volume profile to crystalline OCP than it does to an "amorphous" preparation of HA.

The significance of the location of the peaks and troughs in the mineral component of bone and teeth and in synthetic calcium phosphates has not been determined. The possibility that they might be artifacts peculiar to the Cranston and Inkley method must be examined:

(a) No corespondence of peaks and troughs was found when the pore volume profile of porous glass (determined by the same method) was compared with any of the curves of the calcium phosphates studied here.

(b) A reasonably good correspondence was found with bone char by Barrett and Brown [10] (as mentioned earlier) where the Cranston and Inkley method was not used.

Further study will be required before the problem can be completely resolved regarding not only the detailed location of the various peaks and throughs, but the reason for their very existence. It is clear, however, that the collagen or organic component of the animal skeleton can in no way be involved in this phenomenon. The synthetic crystalline preparations studied here, which corresponded so closely in profile with the mineral component of natural bone, had had no exposure to organic material during any part of their preparation.

6. Summary

It has been shown that much can be learned about the detailed structure of the surface of teeth, bones, and synthetic calcium phosphates by use of gas adsorption techniques. The following points are highlighted:

6.1 The removal of dried collagen from bone or dentin increases the surface accessible to nitrogen by some ten-fold.

6.2 The surface area of anorganic dentin at first increases as a result of heat treatment up to about 200°C and then decreases.

6.3 The surface area of synthetic octacalcium phosphate remains essentially unchanged upon conversion to hydroxyapatite.

6.4 One of the effects of heating anorganic bone is the building up of larger pores at the expense of smaller ones.

6.5 A scrutiny of the fine structure of the pore volume profile uncovers some very interesting facts:

(a) So-called "amorphous" preparations of hydroxyapatite possess a more-or-less uniform distribution of spaces between particles of the solid matter with no recognizable pattern despite their large surface areas.

(b) Crystalline samples, even down to the lowest measurable surface area, are characterized by distinctive patterns of peaks and troughs which, in turn, vary in the degrees to which their respective amplitudes are modified by heat treatment.

(c) Some of the detailed patterns for anorganic bone are shared by both HA and OCP, while others are more closely aligned with HA.

(d) The relative amplitudes of peaks and troughs for the mineral component of bone and teeth as well as their location along the pore diameter axis are dependent not upon their organic matrix, but rather, upon their crystallographic make-up.

7. References

1. Beebe, R. A. "Adhesive Restorative Dental Materials", Indiana University Medical Center Workshop, sponsored by National Institutes of Health and U. S. Public Health Service of H.E.W., Edited by Ralph W. Phillips p-75 (Sept. 1961).
2. Beebe, R. A. and Emmett, P. H., J. Phys. Chem. 65 184 (1961).
3. Loebenstein, W. V. and Deitz, V. R., J. of Research NBS 46 51 (1951).
4. Barrett, E. P.; Joyner, L. G. and Halenda, P. P. J. Amer. Chem. Soc. 73 373 (1951).
5. Innes, W. B., Analytical Chemistry 29 1069 (1957).
6. Pierce, C., J. Phys. Chem. 57 149 (1953).
7. Cranston, R. W. and Inkley, F. A., Advances in Catalysis Vol. IX (1956).
8. Williams, J. B. and Irvine, J. W., Jr., Science 119 771 (1954).
9. Peckham, S. C.; Losee, F. L. and Ettleman, I., J. Den. Res. 35 947 (1956).
10. Barrett, E. P. and Brown, J. M., Proc. of the 1st Tech. Sess. on Bone Char (NBS) 198 (1949).

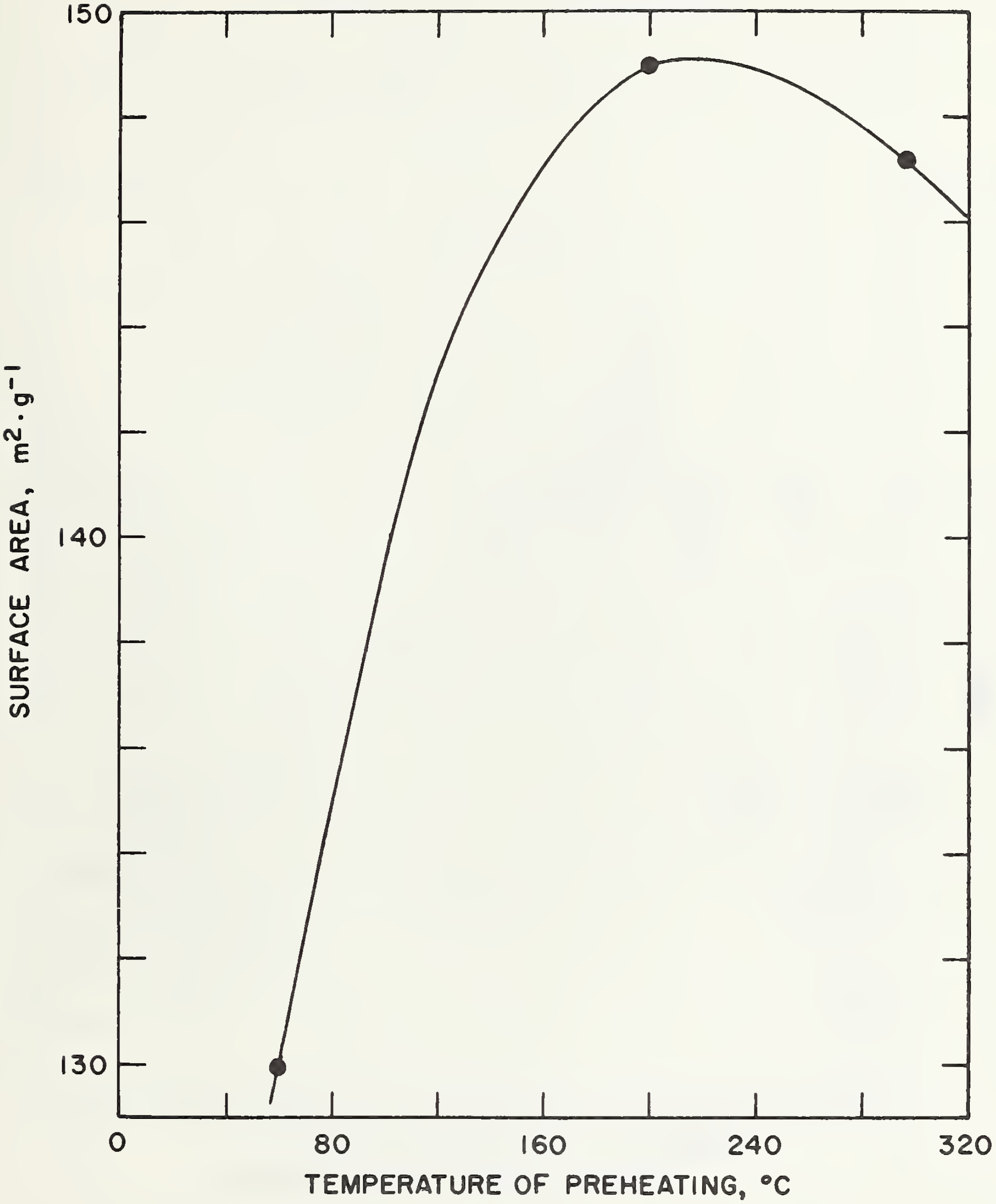

Figure 1. Surface Area of Dentin as a Function of the Temperature of Pre-heating.

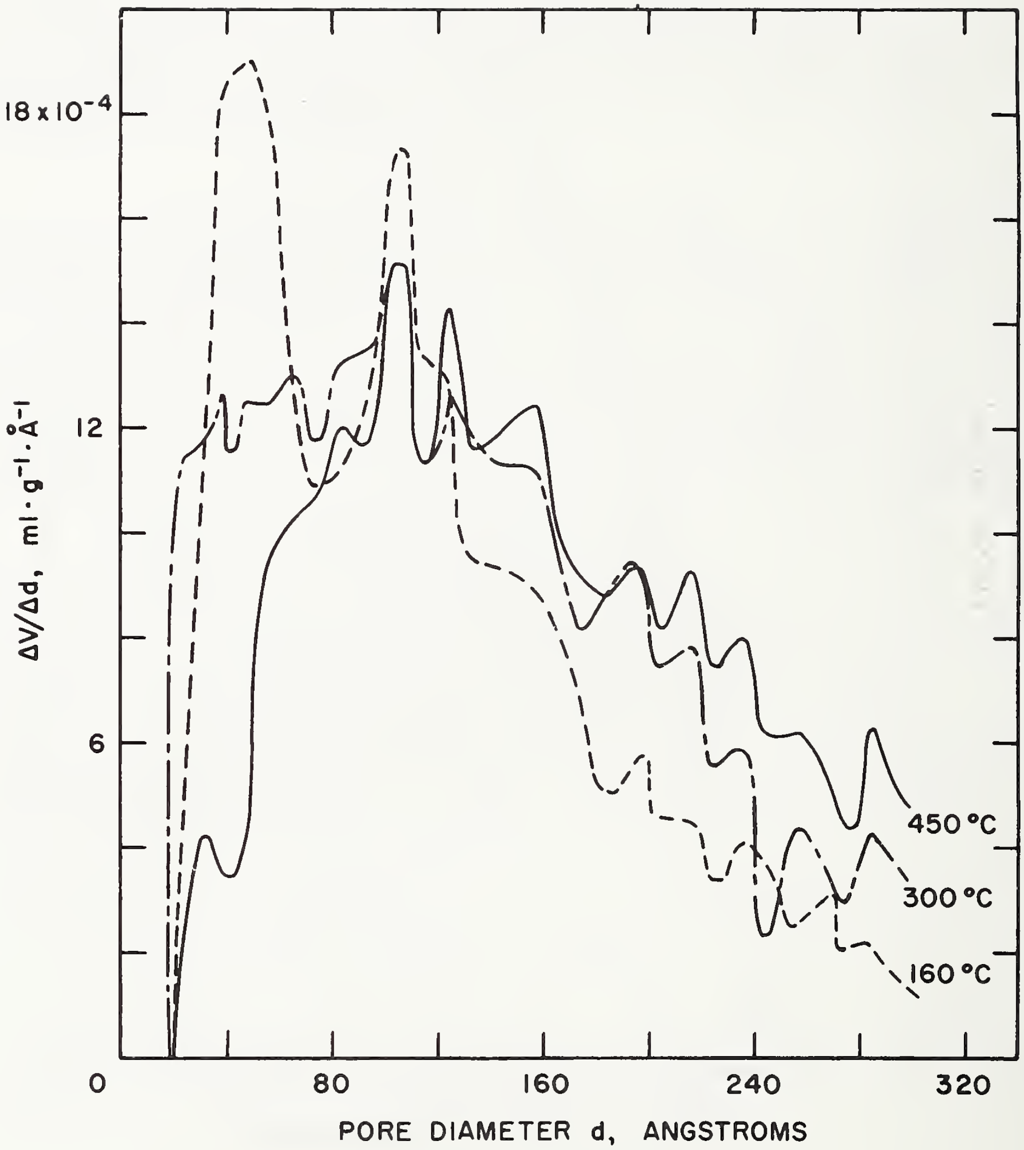

Figure 2. Changes in the Differential Pore Volume Profile of Anorganic Bovine Femur Shaft brought about by Preheating at 300°C and at 400°C.

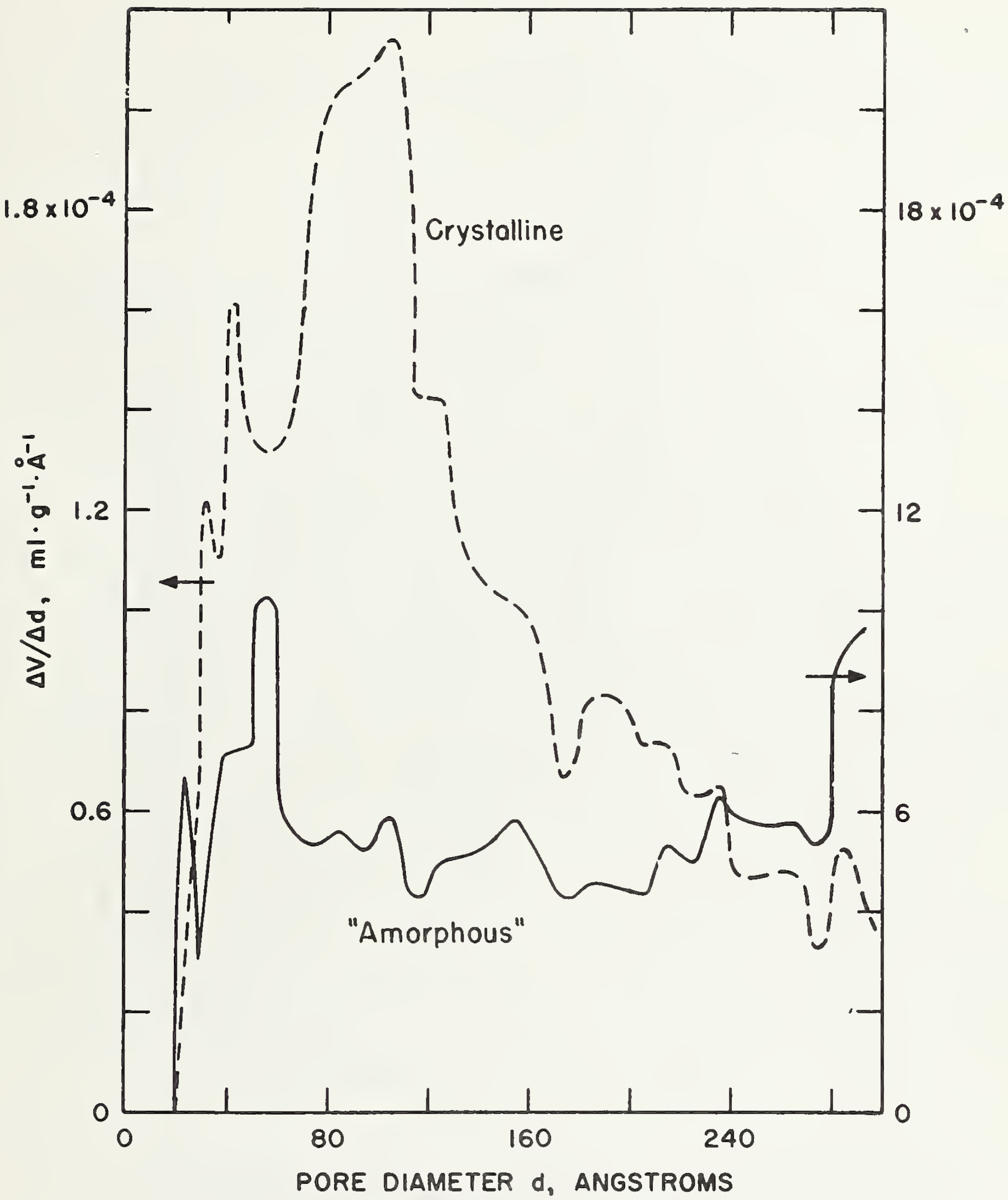

Figure 3. Comparison of the Differential Pore Volume Profiles of Amorphous and of Crystalline Synthetic Hydroxyapatites.

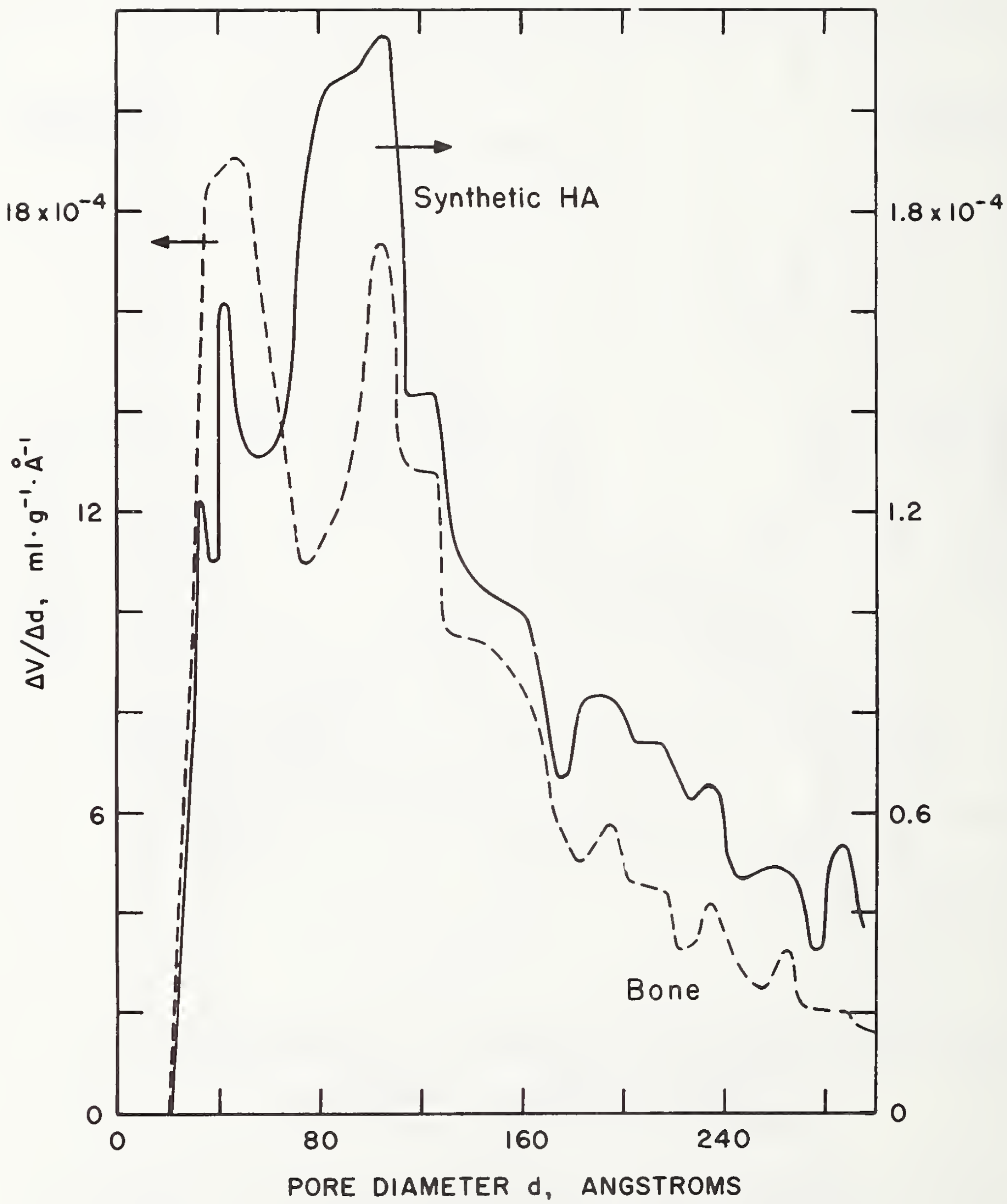

Figure 4. Comparison of the Differential Pore Volume Profiles of Anorganic Bouvine Femur Shaft and Crystalline Synthetic Hydroxyapatite.

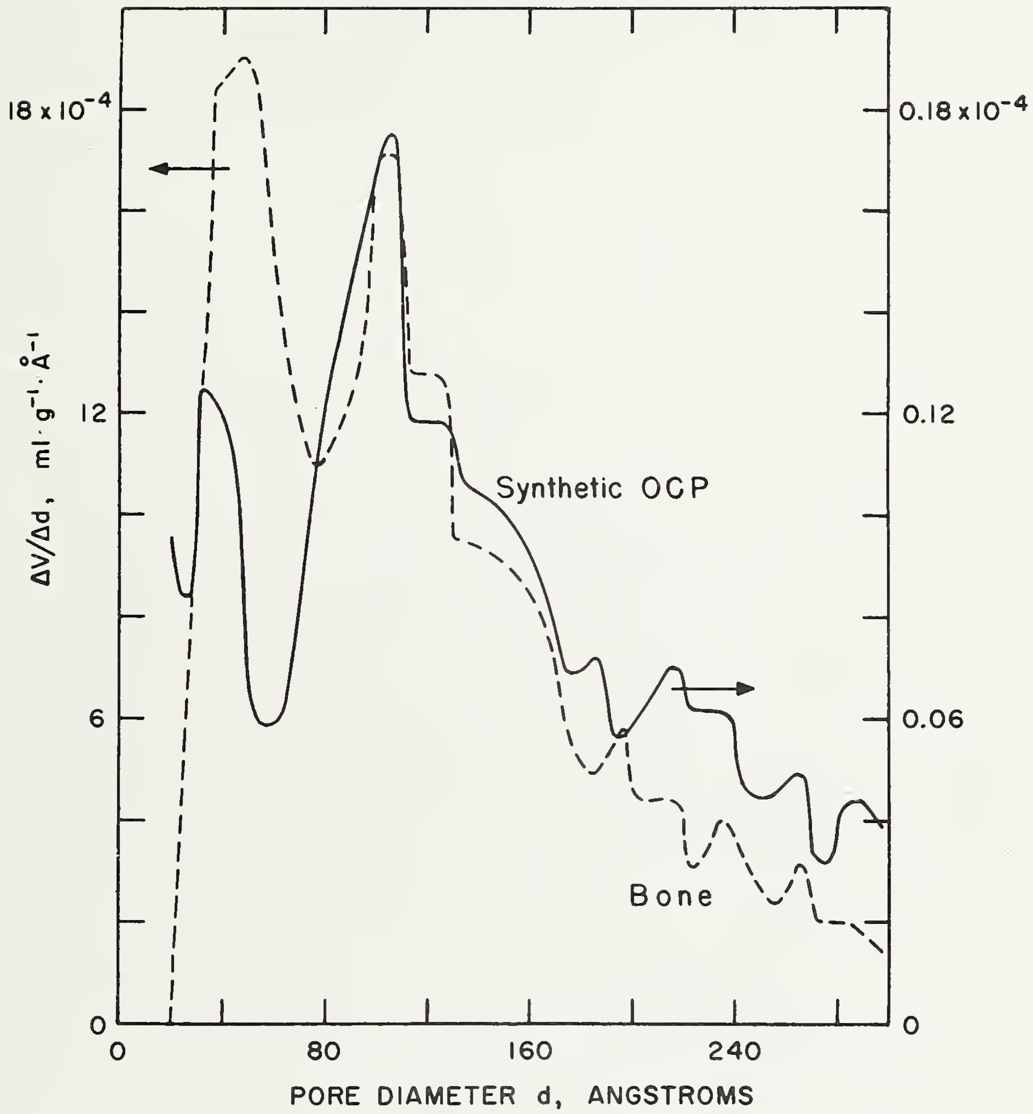

Figure 5. Comparison of the Differential Pore Volume Profiles of Anorganic Bovine Femur Shaft and Crystalline Synthetic Octacalcium Phosphate.

DEPARTMENT OF COMMERCE ★ NATIONAL BUREAU OF STANDARDS ★