PROGRESS REPORT

Microradiography of Thin Bone Sections**MASTER**

Contact microradiography of thin sections of bone offers a refined method for the quantitative determination of bone mineral. Since there is no distortion of actual specimen size to X-ray image, the resulting analysis is highly accurate and evaluation of the bone density distribution over a given volume of bone can be determined.

A method of microradiography has been developed using bone slices of 50-100 micron thickness. These bone slices and an aluminum step wedge standard are irradiated with soft X-rays, i.e., copper K_{α} , using an instrument ⁽¹⁾ which is capable of recording four unit X-ray images and an aluminum standard per frame of 35 mm spectroscopic film (Kodak 649-0). After X-raying, the film is subject to the usual careful development and fixing. It is then placed in the FIDAC (Film Input to Digital Automatic Computer) densitometer where the film frame is scanned giving about 350,000 sampled points and their respective densities distinguished as one of sixteen grey levels. The grey levels corresponding to each point on the film are fed directly into a high-speed core memory of an IBM Model 44 Computer. The FIDAC instrument is equipped with an automatic film mover which transports the roll of film to the next frame after the previous one has been scanned and digitalized. The multi-specimens per frame, the automatic and high speed scanning and conveyance of the film, the direct input to the computer all are factors making feasible large-scale studies.⁽²⁾

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Computer programs were written to process the digitized bone images contained in the computer's memory. The bone mineral distribution is calculated on a per unit volume basis. The 50-100 micron bone slices made by the microtome are wedge-shaped. Thickness measurements on eight typical locations of the bone specimen are made by a micrometer (.001 mm sensitivity). These values along with their respective coordinates relative to the bone position as scanned by FIDAC are input to the computer where a least square program calculates constants for the equation of the best fitting surface plane of the wedge. The thickness of any point on the bone slice may then be automatically calculated from the constants. This value will be used with the area of a scan spot to give the amount of bone mineral on a per unit volume basis.

The density of the aluminum standard is known and the thickness of the respective steps in the aluminum standard have been measured with a 0.001 mm sensitivity micrometer. From the FIDAC scan, each aluminum thickness corresponds to a definite grey level value. The aluminum weight of each thickness was calculated by

$$\begin{aligned} \text{Weight} &= \text{Volume} \times \text{Density} \\ &= \text{Thickness in cm,} \times (\text{Area of one FIDAC spot, in cm}^2) \times 2.699 \text{ g/cm}^3 \end{aligned}$$

Here, the aluminum weight in grams is given for the area covered by one FIDAC Spot.

The bone weight for each grey level spot made by the FIDAC scan was calculated from the aluminum weight determined above for that grey level and the ratio of the mass attenuation coefficient

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ents of aluminum and hydroxyapatite (the mineral component of bone) as follows:

$$\text{Bone Weight} = \text{Aluminum Weight} \times ([\mu/\rho]_{\text{Al}} / [\mu/\rho]_{\text{HA}})$$

where $(\mu/\rho)_{\text{Al}}$ and $(\mu/\rho)_{\text{HA}}$ are the mass attenuation coefficients of aluminum and hydroxyapatite respectively. The total amount of bone mineral in the specimen is the summation of all the grey levels and their equivalent weights. Since the area of each scan spot and the thickness at that point is a fixed value, the amount of bone mineral per unit volume can be calculated.

The corroboration between computer calculated and actual bone weight can be made by ashing the bone specimen.

Photomicrographs made on 35 mm film from the microradiographs (in which the bone mineral was determined as outlined above), were also scanned by the FIDAC instrument to determine mineral micro-density, i.e., on a histological basis. In taking the photomicrographs, care was exercised in relating the location of the area seen in the microscope field to the same area of bone in the microradiograph. This is necessary in order to correlate the bone mineral found on the histological scale to that found on the macro scale i.e., on the microradiograph. A photomicrograph of the microradiograph of the aluminum step wedge standard was taken under the same light intensity and magnification as that for the bone microradiograph. The method of calculation for equivalent aluminum and bone weights was the same as given above. The quantitative values of bone mineral found in the photomicrographs have been correlated to those found in the microradiographs in terms of the same area.

Microdensities of themselves can be of great aid in elucidating the histological structure of bone. Moreover, subtle variations in bone density which can be a key to incipient disease, i.e., osteoporotic or osteomalactic, would be detected more readily in the automatic scanning of photomicrographs, whereas these conditions may not be observed from the X-rays until the diseases are in advanced states.

In addition to microdensities, the FIDAC scan of the photomicrograph was analyzed by computer programs to obtain parameters relating to the microstructure of bone. These parameters include the count of lacunae and Haversian systems found per unit area; the perimeters and surface areas of these microstructures; and the bone growth and resorption rate ratio. All of these parameters can be automatically determined, and hence, this method offers a tool for quick and accurate analysis which would be long and tedious if performed manually.

Therefore, the use of the FIDAC densitometric scanner and automatic computer programmed analysis provides a feasible means for large scale screening and processing of X-rays of bone specimens. The large scale screening and analyses are vital to establishing statistically sound norms to characterize both normal and diseased bone. This should eventually be of immense aid in medical diagnosis.

REFERENCES

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