on the degree of maleness. On the other hand, male fetuses form an alien factor in the maternal organism, and are thus more easily absorbed than female fetuses. This theory is in agreement with the one advanced by Hoelzel at the University of Chicago that in well-nourished male rats more *X*-chromosome–bearing sperms than *Y*-chromosome–bearing sperms are reabsorbed, whereas in well-nourished female rats more male than female fetuses are absorbed.

A Method for the Rapid Preparation of Histological Sections

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The preparation of tissue specimens for paraffin embedding is a tedious and time-consuming process. The method described here considerably shortens the time required and reduces the number of operations. Particular economies in this respect have been achieved in the dehydrating step, which has been reduced to a single, simple operation.

Thin pieces of fresh tissue about 4 mm thick, are placed in a modified Bouin's fluid consisting of: 80% ethyl alcohol, 150 ml; 40% formalin, 60 ml; glacial acetic acid, 15 ml; and picric acid crystals, 1 g. A minimum of 35–45 min immersion is required to fix the tissues. No harm is done by permitting them to remain in the fixative overnight. Washing in water is not necessary. Zenker's fluid, Helley's fluid, etc., also may be used, provided that the proper prescribed procedure, including washing, is followed for each fixative.

After fixing in the modified Bouin's fluid, the tissues are cut into slices 1–2 mm thick. This can be done with a razor blade or sharp-edged knife.

Dehydration is rapidly accomplished by placing 4–8 slices of the fixed tissue in an Allihn filter tube (porosity of disk "medium," height above disk about 110 mm, capacity about 45 ml). Excess fixative is removed by rinsing with 5 ml acetone and decanting. The filter is then filled with pure acetone, which is allowed to run freely by gravity. When the tube is about half full it is refilled to the top with additional acetone. This process is repeated once again. By the end of an hour the filter will have emptied and the dehydration process will have been completed.

As soon as the last of the acetone has disappeared through the disk, the tissues are cleared with xylene. This may be carried out conveniently by pouring xylene into the Allihn tube. When the tissues become translucent they are removed immediately, since too long an exposure to xylene will render them brittle. Kidney slices may become translucent in 15 min, whereas spleen slices may require 45 min.

The cleared slices are placed in small, labeled, galvanized screen baskets, 1 in. in diameter and 3 in. high. These are placed in 250-ml beakers containing melted paraffin (Tissuemat, mp, 54°–56° C) and maintained in a vacuum oven at 58° C and 560 mm Hg pressure. A desiccator, in an ordinary thermostatically controlled oven, connected to a Cartesian manostat with a vacuum filter pump, provides a satisfactory vacuum oven. After 30 min in the vacuum oven, the tissues are placed in a fresh beaker of pure paraffin and kept at 58° C for 30 min.

After the infiltration is completed, the slices are embedded in paraffin in the usual manner. It has been found useful on occasion to embed many tissues in the same block, using a cardboard box as a mold. If the box is large (3″ × 3″ or larger) care must be taken to use flexible cardboard sides to permit the paraffin to contract on cooling without splitting the block.

In the method described, dehydration is first favored by the presence of 80% alcohol in the fixing solution. It is greatly intensified by the technique which makes use of the sintered glass filters. This procedure tends to promote a high concentration gradient between the water in the tissue and the acetone outside by constantly allowing the partly diluted acetone to escape through the bottom of the filter while simultaneously replacing it with fresh fluid from above.

The use of a partial vacuum helps to remove the xylene and thus to favor the infiltration by a paraffin that is comparatively pure. Too high a vacuum tends to separate and disrupt the tissues.

The method has been used routinely in our laboratory for more than a year. By its use, it is possible to start with fresh tissues in the morning and to complete the preparation of sections for microscopic study before the end of the working day. Good results have been obtained with such difficult preparations as those showing clearly the cilia of the respiratory epithelium or the ciliated brush borders of the proximal convoluted tubules of the kidney.

An Apparatus for Determining Bone Density by Means of Radioactive Strontium (Sr^{90})[1]

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During investigations upon regional differences in the physical properties of the compacta of the leg bones of man and the dog the density was one of the properties studied. Since one of the investigators (CCC) is studying the effects, in the dog, of alterations in the blood supply of the femur, it was necessary to have a method for detecting very slight differences in density. It was therefore decided to determine the density by the percentage transmission of β-rays through the bone samples.

[1] This investigation was supported (in part) by a research grant from the National Institutes of Health, USPHS.

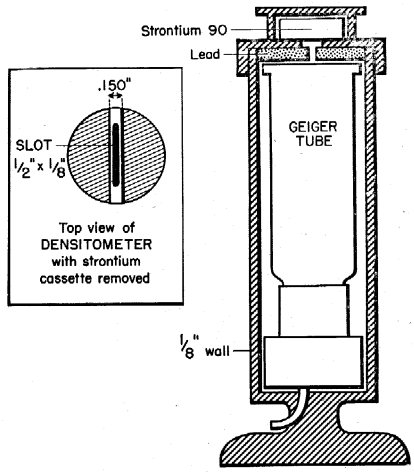

FIG. 1. Diagram of apparatus for measuring bone density by means of radioactive strontium (Sr^{90}).

β-rays were selected since nuclear theory shows that the percentage of their transmission by any material is proportional to the logarithm of the density of the material. The curve obtained by plotting the log of the intensity (cpm) against the weight per unit area (density × thickness) is a straight line. Within the maximum and minimum limits of the weight per unit area all materials will fall upon this curve.

Radioactive strontium (Sr^{90}), with a half-life of 25 years, was used as a source of the β-rays, and the percentage of them transmitted through a bone sample was recorded with a Geiger counter. The concentration of the β-ray source was equivalent to approximately 1/100 μc, which is far below the danger threshold for human tissue. Thus the operator could use the apparatus for 8 hr a day continuously without harmful effects. The main part of the apparatus is illustrated diagrammatically in Fig. 1.

The apparatus consists of a closed brass cylinder for the Geiger tube (TGC 2) and a brass cassette for the radioactive material. The head of the cylinder is lined with lead and has a groove for the sample, which is held in place by small clips. In the bottom of the groove is a small slit, so that the β-rays penetrating the portion of the sample overlying the slit can reach the Geiger tube. The cassette fits tightly to the head of the cylinder but can be removed so that the head can be changed to accommodate a sample of a different size. Reference marks on the head and the cassette insure that the geometry of the radioactive material and the sample is always the same. Four aluminum strips of known density but of varying thickness were used to calibrate the densitometer.

Each day, before testing the bone samples, the random (background) cpm was obtained without the strontium. Then, with the strontium in place, the cpm was taken for each aluminum strip. Three readings were taken for each strip and the final cpm was based on the average. In all cases the net cpm, obtained by subtracting the background cpm from the average, was used in computations. The curve for the aluminum strips was then plotted on semilogarithmic single-cycle paper. This curve (Fig. 2) was then used to determine the weight per unit area (g/cm²) of the bone samples.

The net cpm for each bone sample was located on the calibration curve of the aluminum strips, and the weight per unit area of the sample read directly from the curve. The weight per unit area of the sample divided by its thickness gives its density in g/cm³. The thickness of the samples was measured with a micrometer to the nearest five 10/1000 of an inch. A minimum of 4,000 counts/sample was taken. With this method density differences of less than 1% could be detected.

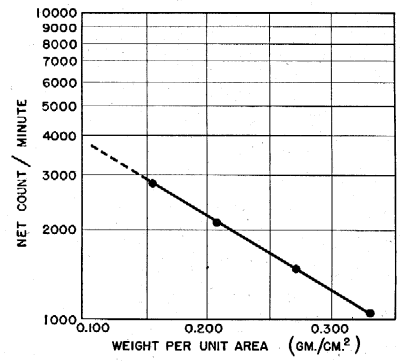

FIG. 2. Calibration curve of aluminum strips.

The samples of human bone were obtained from the compacta of the anterior, posterior, medial, and lateral quadrants of the proximal, middle, and distal thirds of the femur and tibia. Because of their smaller size the human fibulas were simply divided into thirds and the dog femurs into quadrants. The samples were then reduced to a standardized size in a milling machine and by use of emery cloth. The specimens were air-dried at room temperature before testing. In current studies on the dog femur, the samples are placed in a desiccator for 24 hr before testing. This provides a greater standardization of conditions.

As an example of the results obtained by use of the apparatus, the density values obtained by two of the authors (FGE and ML) in a preliminary study of samples of human bone may be cited. The samples were obtained from the bones of a relatively young man, a middle-aged man, and an old man. In all, 137 samples from the femurs, 135 samples from the tibias, and 26 samples from the fibulas were tested. In each bone (Fig. 3) the average density decreased with the increased age of the individual whose bones were studied. The decrease in density was greatest in the fibulas and least in the femurs. Some regional differences in the density within a single bone (Fig. 4) were also noted. The density of the proximal third of all three bones was practically identical, but the middle third of the tibia and the distal third of the femur were the most dense regions of the respective bones. The average density of all the samples from each bone

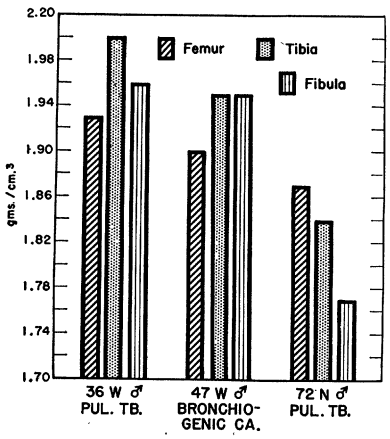

FIG. 3. Average density values (g/cm³) for 298 samples of compact human bone with respect to the age of the individual from whom the samples were taken.

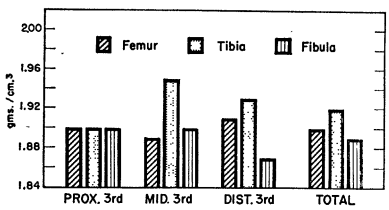

FIG. 4. Average density values (g/cm³) of 298 samples of compact human bone with respect to the region of the intact bone from which the samples were taken.

was greatest in the tibia and least in the fibula. The variation between the most and the least dense third of each bone was 1.0% in the femurs, 2.6% in the tibias, and 1.6% in the fibulas. The average density of the tibias was 1.0% greater than that of the femurs and 1.6% greater than that of the fibulas. The average density of the femurs was 0.5% greater than that of the fibulas.

The sensitivity of the apparatus is also illustrated by the initial results obtained by one of the authors (CCC) in an investigation of the effects of a reduction in the blood supply on the density and other physical properties of bone. This work is being done on the dog, the only other species so far studied. The blood supply to one femur was reduced by operative procedures, the opposite femur serving as the control or normal bone. The density values for 48 samples of normal bone taken from the femurs of 6 dogs are presented in Table 1.

The density changes present 48 hr after the blood supply to the right femur had been reduced by ligation of the nutrient artery plus a periosteal decapsulation of the entire femoral shaft are shown in Table 2.

In density studies the accuracy with which the thickness of the specimens can be controlled is an important factor. Among the 298 samples of human bone whose density was determined, the thickness of 87% of them was within the standardized range (.08 ± .01 in.); 12% were .06–.07 in. thick; 6% were .05–.06 in. thick; and 5% were .04–.05 in. thick. The thickness of the samples from the dog femur was controlled with a similar degree of accuracy.

The average values for the specific gravity of human compact bone found by some of the earlier investigators are: 1.934, Wertheim (*1*); 1.9304, Krause and Fisher (cited by Hulsen, [*2*]); 1.936, Aeby (cited by Rauber [*3*]); 1.901 for the fresh femur and tibia of a man 30 years of age and 1.825 for a woman 56 years of age, Rauber (*3*); and 1.933 for the femur, Hulsen (*2*). Mack, cited by Keys, *et al.* (*4*), measured the density of bone, by means of teleroentgenograms, in 32 living subjects at 24 weeks of starvation and after 6 and 12 weeks of rehabilitation. The bones studied were the left little finger, the left femur, and the left calcaneum. The average values, in terms of equivalent ivory thickness (cm), for the region of the femur about 5 cm proximal to its most distal tip (i.e., its distal third), for the semistarvation and normal subjects were 2.571 and 2.243, respectively, for one group and 2.462 and 2.300, respectively, for a second group. Our average density values (g/cm³) for the human bones are 1.90 (femur), 1.92 (tibia), and 1.89 (fibula). The values given by Mack, *et al.* (*4*) for human bone are not directly comparable to those obtained by the authors, since the former were taken from living individuals. Consequently, the overlying tissues, fat, blood, and other constituents of the marrow, as well as superimposition of bone, would be involved. These factors may account for the higher density values obtained by Mack. The previous investigators mentioned did not study regional differences in the density of individual bones or the influence of blood supply upon the density of bone.

The final results of these studies of the density of human bone and how the density of the dog femur is influenced by a reduction in the blood supply to the bone will be published separately and more extensively elsewhere.

TABLE 1

DENSITY (G/CM³)—NORMAL

Quadrant	Right femur	Left femur	Difference	% Difference
Anterior	1.790	1.810	0.02	1.10
Posterior	1.835	1.800	0.035	1.90
Medial	1.810	1.830	0.02	1.09
Lateral	1.840	1.830	0.01	0.50

TABLE 2

DENSITY (G/CM³)—PARTIALLY AVASCULAR AND NORMAL—48 HR AFTER OPERATION

Quadrant	Right femur (operated)	Left femur (normal)	Difference	% Difference
Anterior	1.94	1.98	0.04	2.02
Posterior	1.84	1.89	0.05	2.64
Medial	1.80	1.93	0.13	6.73
Lateral	1.86	1.94	0.08	4.12

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Some Effects of High-Intensity Ultrasound on Tobacco Mosaic Virus[1]

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Several investigators have reported the effect of sonic and ultrasonic frequencies on tobacco mosaic virus. Early reports of the exposure of tobacco mosaic virus to sonic frequencies were made by Stanley (*1*) and by Takahashi and Christensen (*2*). Exposure of tobacco mosaic virus to sonic and ultrasonic frequencies was reported by Kausche, Pfankuch, and Ruska (*3*), who showed that the virus could be broken into shorter particles by suitable exposure, with the infectivity reduced. Oster (*4*) exposed tobacco mosaic virus to a frequency of 9,000 c. He found that, as the time of exposure was increased, the basic virus unit, 280 mμ long, was broken into fragments one half and one fourth the original length.

This is a report of some effects of high-intensity ultrasonic waves, oscillating at a frequency of 7 Mc, on the physical structure and infectivity of tobacco mosaic virus. This is a much higher frequency than has previously been used in ultrasonic exposure of viruses.

The juice from Turkish tobacco plants infected with tobacco mosaic virus was purified by the usual technique, involving alternate low- and high-speed centrifugation, and was finally suspended in a phosphate buffer at pH 7. A suspension consisting largely of the basic virus unit 280 mμ long was obtained in this manner.

The ultrasonic vibrations were produced at the face of a quartz crystal ground to vibrate at a frequency of 7 Mc. The crystal controls the oscillator circuit which drives it. Exposure of the virus suspension was made inside a thick-walled lucite cylinder of 5-cc capacity suspended with its lower end about 10 mm above the horizontally mounted crystal. Both ends of the exposure tube were sealed by a 0.003-in.-thick acetate membrane. The entire transducer and exposure tube assembly was mounted under transformer oil having a high dielectric constant. By this arrangement, the ultrasonic waves, originating at the crystal, pass upward through a layer of rapidly circulated ice-cooled oil into the exposure tube, out its upper end, and back into the oil reservoir, where they are deflected and dispersed by screen baffles. Although the outside of the exposure tube was cooled to approximately 10° C, some internal heating did occur, as indicated by a mercury thermometer inserted into the tube following treatment.

To quantitatively evaluate the effect of ultrasonic irradiation upon the infectivity of the virus, Scotia bean plants were infected at the time secondary leaves began to appear. Infection was produced by rubbing one of each pair of primary leaves with an aluminum spatula dipped in ultrasonically treated virus suspension and the other with untreated virus suspension. To aid infection, all leaves were first dusted with No. 600 Carborundum. Infectivity comparisons were made on the basis of 10 replications.

After exposure to ultrasonic energy, microdrop samples of the virus suspensions were deposited upon a thin film formed by evaporating a 0.2% solution of Formvar in ethylene dichloride. The microdrop sample was not allowed to evaporate to dryness, but was removed with a micropipette after standing several minutes. This deposit was then lightly platinum-shadowed *in vacuo* before being photographed in the Universal Model RCA electron microscope.

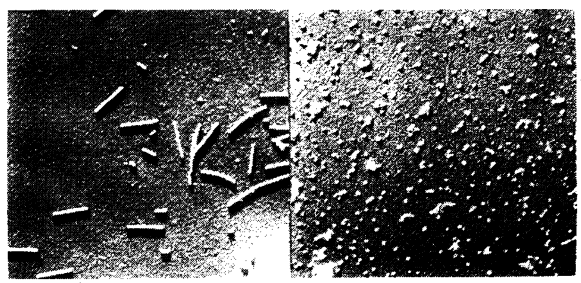

FIG. 1. Tobacco mosaic virus, × 20,000. Electron micrographs show effects of high-intensity ultrasound. Unexposed virus, consisting primarily of units 280 mμ long, is shown at left; highly fragmented virus (fragments 20 mμ–40 mμ long) shown at right.

Fig. 1 shows the results obtained by treatment at the maximum energy output of the oscillator. This high degree of fragmentation of the virus was accompanied by cavitation in the liquid. Experience has shown that the degree of fragmentation revealed by the electron micrographs was directly proportionate to the amount of cavitation as indicated by the number and volume of released gas bubbles. Heating of the virus suspension by ultrasonic energy was not in itself sufficient to inactivate the virus.

Lesion counts made on Scotia bean plants after inoculation of the leaves with unexposed virus and virus exposed to ultrasound for 3.3 min showed a reduction of approximately 95% in the infectivity of the virus exposed to ultrasound.

Using lower intensity levels, between approximately 140 w and 180 w power input, and varying the time of exposure, the following results have been demonstrated:

a) Ultrasonic irradiation of "aged" virus suspension produces an increase in virus infectivity through dispersion of aggregated clusters of virus rods.

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[2] The author wishes to acknowledge the capable technical assistance of John Kissel.