plored further such discrepancies remain unexplained.

Summary. Linoleic-1-C^{14} acid was rapidly incorporated into the liver lipids of both control and carbon tetrachloride treated rats. Twenty minutes after the injection, a pronounced fall in incorporation into phosphatidyl ethanolamine with a concurrent rise in incorporation into cardiolipin and phosphatidyl choline fractions was observed in the degenerating liver. The increased incorporation of radioactivity into cardiolipin and phosphatidyl choline was reflected in an increased content of linoleic acid in these phospholipids but not in phosphatidyl ethanolamine. These results suggested the existence of separate pools of linoleic acid for incorporation into individual phospholipids.

1. Sgoutas, D. S., Metabolism, in press.
2. Folch, J., Lees, M., Sloane-Stanley, G. H., J. Biol. Chem., 1957, v226, 497.
3. Bartlett, G. R., ibid., 1959, v234, 466.
4. Skipsky, V. P., Peterson, R. F., Barclay, M., Biochem. J., 1964, v90, 374.
5. Maling, H. M., Frank, A., Horning, M. G., Biochim. Biophys. Acta, 1962, v64, 540.

Received July 5, 1966. P.S.E.B.M., 1966, v123.

Measurement of Bone Mass from Ultrasonic Transmission Time.* (31467)

CLAYTON RICH, ELI KLINIK, RAY SMITH, AND BEN GRAHAM†
Radioisotope Service, Veterans Administration Hospital and Departments of Medicine and Radiology, University of Washington School of Medicine, Seattle

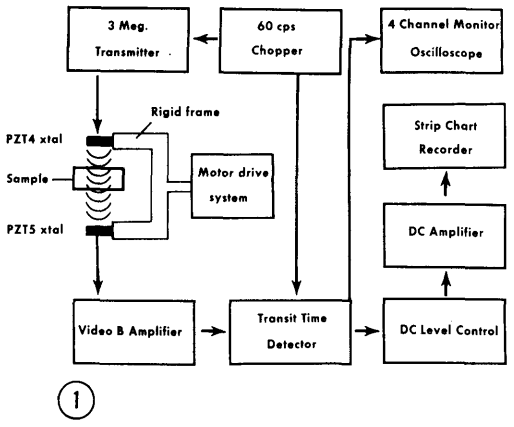
The major emphasis in the medical diagnostic applications of ultrasound has been on measurement of the reflections of sound from interphases within the tissue examined(1,2). Some early work was based upon absorption of sound and measurements of transit time through tissue have been made(3), but no systematic attempt to evaluate the accuracy of this last principle for measurements of body structure has previously been carried out.

The measurement we describe here is based upon transmission time and depends on the fact that velocity of sound is greater in solids than in liquids. Therefore, if a pulse of sound is passed through a limb which contains bone and soft tissues, the velocity will be greater in the bone than in the soft tissues and the time necessary for sound to traverse the limb will depend upon the distance of the sonic path in each.

Experimental. We have modified the cardiometer and sonodistometer, instruments that provide a continuous measurement of the distance between structures, such as the walls of the heart or blood vessels(4,5), to develop a system which can be used to measure the velocity of sound in any given medium. Ceramic barium titanate transducers with a natural thickness mode of vibration of 3 megacycles are fixed to each end of a rigid "U" shaped frame (Fig. 1). A 900v step of 50 nsec rise time is applied 60 times a second to one transducer to generate a series of abrupt sonic pulses. These pass through the water or other medium separating the transducers and are converted by the other transducer to a variable and often much smaller voltage, depending upon the amount of absorption or reflection of sound in the tissue examined. A linear amplifier circuit is used to amplify the signal from the receiver transducer to about 2v, within input limits between 100 μv and 10v. A voltage ramp is started at the time the transmitter transducer is pulsed and cut off by the signal from the receiver output. Because the voltage rises at a constant rate during the time between these two signals, the maximum voltage attained is directly proportional to the time between transmission and reception of the pulse. The highest volt-

* These studies were supported in part by a grant from USPHS (A-4701).

† Dr. Graham was an Advanced Fellow in Academic Radiology of the James Picker Foundation.



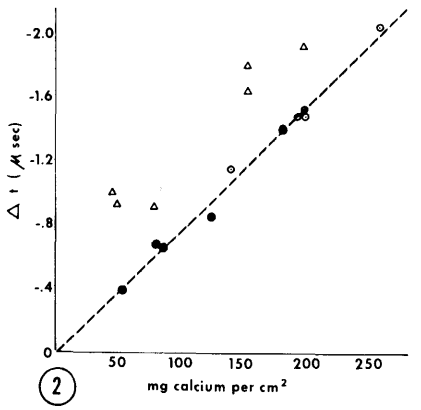


FIG. 1. Block diagram.

FIG. 2. Changes from transmission time in water when different thicknesses of bovine bone are placed between the transducers. Decrease in transmission time is shown on the ordinate and the mass of calcium through which the sound passes (mg Ca per cm^2 normal to the sonic path) on the abscissa. Circles represent values for cortical and triangles for trabecular bone.

age attained by the ramp is held on the storage capacitor of a peak voltage detector until the next time the transmitter fires; the storage capacitor is then discharged and a new charge is stored on it by the ramp. The series of peak voltages so obtained is fed into an amplifier which integrates the waveforms and amplifies them to drive a strip chart recorder. Because the storage time per cycle is about 1.67×10^4 μsec while the off time is only 10 to 15 μsec the voltage so obtained is within 0.01% of the true peak voltage. Thus, a voltage is established which is directly proportional to the transit time.

The distance between the transducers is calculated from the observed transit time when sound passes through water at 20°C, according to the assumption that c, the velocity, is 0.143 cm per μsec(1). Thereafter, the velocity in any other substance is calculated from the relationship $c = d/t$, where $t =$ μsec and the distance, d, is known. Using this method, we found the velocity of sound in corn oil, bovine fat tissue and muscle to be 0.146, 0.154 and 0.163 cm per μsec, respectively; in agreement with published values(1,3). The velocities in human plasma protein (6 g per 100 ml) and blood (40% erythrocytes) were 0.151 and 0.156 cm per μsec,‡ all in contrast to that in cortical bone, of 0.288 cm per μsec. Thus, it is evident that the velocities in soft tissue are similar and sufficiently different from in bone so that it is practical to use this system for estimation of the bone mass.

When rectangles of fresh bovine cortical bone were interposed between the transducers, a reduction in transmission time over that through water alone was measured. After the sonic measurement was recorded, the dimensions and weight of the samples of bone were determined. The bone was then extracted in acetone for 16 hours, dried at 110°C for 2 hours, reweighed, ashed at 500°C and the amount of calcium present determined by titration with versine(6). The observed reductions in transit time from those in water and the thickness, wet and dry weights, and calcium content found on one set of measurements are given in Table I. In every case, the arbitrary assumption is made that the sonic beam is of unit width and the mass is given for a rectangular section one cm^2 normal to the dimension through which the sonic beam was passed. The precision of the individual measurements was high, and in these measurements of compact bone, the correlation between change in transit time and length, wet and dry weight all seemed equally good. The slightly greater variation when the

‡ The results of measurements on complex tissues such as fat and muscle vary somewhat, depending upon the composition of the sample. However, the precision in repeated measurements of the the same sample or of homogenous samples such as oil, water or plasma is the same as shown in Table I for bone.

TABLE I. Change in Transit Time per cm Sonic Path in Bone and per g Wet Bone, Dry Bone or Calcium in a Rectangle of the Dimensions 1 cm × 1 cm × the Length of Sonic Path in Bone.

Bone sample				Reduction from transit time in H_2O (μsec)		Change in transit time			
	Wt (g)						Per g per cm² normal to sonic path (—μsec/g/cm²)		
Length of sonic path (cm)	Wet	Dry	Calcium	Readings	Mean ± S.D.	Per cm path in bone (—μsec/cm)	Wet	Dry	Calcium
.304	.560	.537	.140	1.14, 1.10, 1.11	1.117 ±.019	3.68	2.00	2.09	8.00
.399	.740	.721	.198	1.53, 1.52, 1.51	1.520 ±.010	3.81	2.05	2.11	7.67
.400	.741	.718	.199	1.47, 1.47, 1.49	1.477 ±.010	3.70	2.00	2.06	7.44
.400	.745	.720	.196	1.48, 1.50	1.490 ±.014	3.73	2.00	2.07	7.60
.562	1.043	.994	.260	2.03, 2.03	2.030	3.61	1.95	2.04	7.81
				Mean		3.706	2.000	2.074	7.704
				S.D.		.074	.036	.027	.213
				S.D. (% of mean)		2.0	1.8	1.3	2.8

change in transit time was correlated with mass of calcium was probably because of variations introduced by the chemical analysis.

The correlation between mass of calcium per cm² section and reduction of transmission time is shown in Fig. 2. The data from Table I are represented by open circles and those from measurements made a year ago by closed circles. The regression line was obtained by the method of least squares and the correlation coefficient was 0.99.

When rectangles of trabecular bone were used instead of cortical bone (open triangles) there was not a good correlation; the change in transit time varying by 20% or more of what would have been predicted from the mass of bone present. This was true no matter what parameter of bone measurement was compared to transit time, but the correlation was better for mass of calcium than for wet or dry weight or thickness of the sample.

To measure changes of transmission time in an intact limb, the sensing device was passed across the limb at a constant rate and a continuous record of signal transmission time made by an appropriate recording apparatus. This technique is analogous to that used in radioisotope scanning, and transcribes on paper an area formed by height, which is proportional to the change in transit time and the length which is proportional to the distance the transducers have moved. The fore limb of a rabbit was scanned in this manner each cm for 8 cm, starting 0.5 cm from the distal end of the humerus. The first 2 scans through the irregular and substantially trabecular bone near the joint were too irregular to interpret. In the other scans, it was easy to distinguish the area on the record due to bone from that due to soft tissue, and a representative scan is shown in Fig. 3. The fore limb was stripped of soft tissues and then scanned again at the

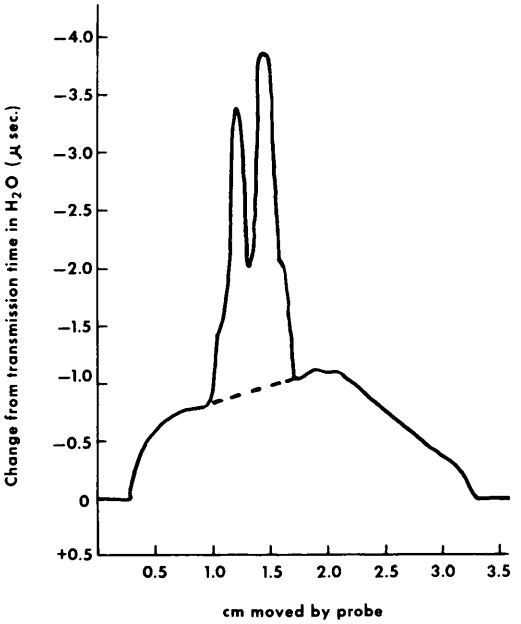


FIG. 3. Sonic scan of an intact rabbit fore limb. The intial gradual deflection indicates a reduction of transit time as the sonic beam passes through skin and muscle. The sharp upward deflection occurs at the edge of bone. The deflection is less in the middle of the bone trace where the beam passes perpendicularly through the cortex than laterally where it passes tangentially through a greater distance of bone. The area of the scan due to bone alone is that above dashed line, and it is this area which is used for calculation of bone mass.

TABLE II. Comparison of Ultrasonic and Chemical Measurements of Fore Limb of a Rabbit.

Position of scan*	Area of scan (cm^2) Intact limb	Bone only	mg calcium/cm bone Predicted	Found
2.5	26.8	26.5	174	187
3.5	26.8	27.5	181	177
4.5	28.5	26.5	174	187
5.5	24.5	24.0	156	167
6.5	23.0	22.0	145	143
7.5		18.0	118	125
Standard		39.5		260

* cm from distal end of humerus.

same places, as was a rectangle of bovine cortical bone of known composition which served as a standard. The areas due to bone were measured by planimetry and are given in Table II. The predicted amount of calcium in the bone at the level of each scan was calculated from the relationship, $M_u = A_u M_s / A_s$, where M_u and M_s are the mass of calcium per cm^2 normal to the sonic path of the unknown and the standard, respectively and A_u and A_s the areas from bone in the sonic scans. In Table II, the results are compared to the amounts of calcium subsequently found when the corresponding 1 cm segments of bone were analyzed chemically. The difference between the estimations of bone calcium made by the sonic method and the results of chemical analysis of these six samples was -6.33 ± 7.58 mg.

Discussion. The potential advantages of this system, which we can now use for scanning the human forearm, are its precision, safety, speed of operation and, in respect to bone, its accuracy. The principal difficulty at present is that the correlation between mass and transmission time found for cortical bone does not hold for trabecular bone. This may be because of structural differences between compact and cancellous bone. It is probable also that it is in part a technical problem which can be approached through changes in design of the instrument. There is much greater absorption of sound in trabecular bone than in other tissues, presumably because of reflections of sound as it passes through the many irregularly arranged interphases. When this occurs, the energy transmitted by the first oscillation may be so reduced that the signal from the receiver transducer is not perceptibly greater than background. When this is the case, the voltage ramp is cut off by the current produced when the more powerful second or third oscillation is received, thus indicating a longer transit time than would otherwise have been found.

The inaccuracy when trabecular bone is measured and the fact that absorption of power may be too great if the beam is passed through a thick part of the body make it seem unlikely that measurement of vertebral mass will be practical.

Summary. When a beam of pulsed sound is passed between two transducers a fixed distance apart, the transit time depends upon the velocity of sound in the medium separating them. Because the velocities of sound in soft tissues are similar and much less than in bone, an instrument which records changes in transmission time can be used to determine the mass of bone present in an intact limb. When measurements were made on samples of cortical bone and of the humerus of a rabbit (either intact or stripped of the soft tissues) there was good agreement between the predicted and subsequently chemically determined mass of calcium present.

1. Carlin, B., Ultrasonics, McGraw-Hill, New York, 1960.

2. Newell, J. A., Phys. Med. Biol., 1963, v8, 241.

3. Ludwig, J., Acoust. Soc. Am. 1950, v22, 862.

4. Rushmer, R. F., Franklin, D. E., Ellis, R. E. Circ. Res., 1956, v4, 684.

5. Mullins, G. L., Digest of 4th Internat. Conf. on Med. Electronics, New York, 1960, p70.

6. Jones, J. D., McGuskin, W. F., Clin. Chem., 1964, v10, 767.

Received June 1, 1965. P.S.E.B.M., 1966, v123.