we detect or foretell disasters to our salmon, and protect them accordingly? The answer is not in speculation, but in accurate records and their analysis by use of such equipment as we can devise and have here described. It lies in multiplication of such points and times of observation as have been indicated in our graphs, a multiplication which can only take place if equipment is devised which is accurate, cheap to make and saving of labor, and if it is diversified in form to meet the varied conditions under which it must be used.

To create such instruments requires time, money and scientific knowledge of the fish themselves.

*The Accuracy of Human Bone Composition Determination from Roentgenograms**

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ABSTRACT: *Several techniques have been developed to determine bone mineral content in living subjects. This paper describes and compares three common techniques, two of which involve photogrammetric methods.*

THERE has been a recent and rapid rise of interest in roentgenogrammetric techniques for determining bone mineral content in living subjects. Documentation is so recent that when queried two years ago about the feasibility of a film technique for estimating bone mineral in man, the chief of the medical research division for a large x-ray film producer replied that he seriously doubted such a technique could be developed.

Despite numerous publications, none have demonstrated by direct comparison the relationship between the roentgenological answers and bone compositions for human material.

The most common techniques break down into three basic categories:

1. the first method is the comparison of the passage of light through bone images on x-ray film with a standard index image appearing on the same film;
2. the second is quantitative determination of the silver salt of an image on an exposed x-ray film; and finally
3. a direct measurement of the radiation passing through the exposed anatomical site, using detectors other than films such as tubes and scintillation counters.

Of these three techniques the first is the oldest and most widely used. In its simplest form a light beam is passed through the film at a predetermined location, and the light intensity is compared to a step wedge of some material which is x-rayed on the same film. Efforts to validate the results obtained with such a technique have been meager. Mainland ('57) found that the results obtained on an os calcis film could be reproduced on the same film, but that results from a densitometer analysis of a second os calcis film introduced larger errors. Most of the between-film error appears to be a location problem. Slight differences in positioning from one exposure to the next produce significant differences in the film opacity.

More recent investigations have solved the location problem by using a tracing densitometer (Balz, *et al.* '57, Lackman '55, Omnell

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'57, Williams, *et al.* '57). This permits the determination of film opacity across a linear section of bone image, and greatly reduces between-film error.

Despite the number of publications on bone mass and density using the film opacity technique, there has been little verification of the technique on human bone. Error identification has been limited to theoretical considerations and investigation of machine and film differences.

The technique in which the film is chemically analyzed and compared to the bone composition has been accomplished by Virtama ('57). This technique is basically a quantitative determination of the metallic silver produced on the section of film which represents a given bone. Virtama checked the results of this unique method against the bone ash of 86 cadaver phalanges. He eliminated many cases by a selection of the films, and analyzed his material using variance analysis. It is therefore impossible to make a direct comparison to the usual coefficient of reliability. However, it appears that the correspondence between his silver determinations and bone ash represents a correlation coefficient of .7 to .8.

Recently described techniques for the determination of bone mineral content *in vivo* involve the use of a scintillation counter which directly measures the x-radiation as it passes through the body segment under study (Mayer '56, Gershon-Cohen, *et al.* '58). While this technique holds much theoretical promise, no verification or reproducibility studies have, as yet, been published.

PENN STATE TECHNIQUE

The bone density determination technique at The Pennsylvania State University has a long history. It started some 20 years ago under Pauline B. Mack. In this period of time the method evolved from a spot technique using an ivory step wedge (Mack, *et al.* '39 and Mack, *et al.* '49), to the present bone density computer which employs an aluminum-zinc alloy wedge.

This machine was fully described in an article by Brown and Birtley in 1951, and its use will be only briefly described here. The wedge image is traced in a photodensitometer and a light opacity curve is obtained on a recorder. This curve is then assigned to a function transformer. The bone and soft tissue image appearing on the same film is then traced at a preselected site called a trace path. An electromechanical integrating device sums the signals from the bone and soft tissue trace, and a total mass count is obtained by the summing process in terms of the mass of aluminum alloy. This value has been termed the x-ray mass coefficient. Values reported in this paper have not been multiplied by the constant frequently used in this laboratory to obtain ivory equivalent readings. An aluminum-zinc wedge was chosen because of its mechanical properties and the similarity between its x-ray absorption and that of bone ash (Schraer '58). The translation of these readings into estimates of density requires a determination of the volume of bone or soft tissue traced. By limiting the trace path width to one millimeter, the volume estimates have been reduced to area estimates. At present, area estimates based on film measurements can be calculated only for the finger and os calcis of man. However, formulae are being developed, for a number of skeletal locations, based on the empirically derived relationship between diameter and area.

Primary validation of the present technique was based on theoretical considerations and a comparison of film values to packets of $Ca_3(PO_4)_2$. McFarland ('54) published an article demonstrating the high reliability of bone density estimates for the human os calcis. Schraer, H. and Schraer, R. ('56) later demonstrated that accurate determinations of bone ash, calcium and phosphate of femurs could be made from roentgenograms of living rat thighs. In a study recently published by the present authors (Baker and Schraer '58) it was shown that the dry weight of the human femur could be estimated from a roentgenogram of the dry femur. However, the present report represents the first effort, using a large sample, to determine human bone composition quantitatively using the Penn State bone density computer.

METHODS

For this study an embalmed cadaver population was used. It consisted of 35 males of mixed racial background who averaged 68 years of age at time of death. These cadavers were preserved with embalming fluid. Despite the presence of x-ray opaque substances, such as arsenic, in the embalming fluid the quantity of such substances was so small as to cause no appreciable effect on the roentgenograms. The phalanges 5-2, humeri and femorae of this sample were x-rayed intact, after defleshing and after defatting and drying. On each set of films the mass was determined for three trace paths on the phalanx and across the mid-sections of the humerus and femur. The entire phalanx and six milli-

TABLE I

THE RELATIONSHIP BETWEEN X-RAY MASS COEFFICIENTS MADE ON INTACT CADAVER BONES AND THE SAME BONES DRIED AND DEFATTED

Bone	*Sample size* N	*Intact bone vs. dry bone correlation coefficient*	*Intact soft tissue vs. dry bone correlation coefficient*	*Intact bone and soft tissue vs. dry bone correlation coefficient*
Humerus mid section	34	.76 (r)	.54 (r)	.82 (R)
Femur mid section	35	.32	.52	.70 (R)
Phalanx 5-2 proximal epiphysis	16	.63	.47	.92 (r)
Phalanx 5-2 mid shaft	16	.61	.55	.94
Phalanx 5-2 distal epiphysis	16	.70	.78	.84

meter slices from the mid-shaft of the long bones were used to determine dry weights, ash content and calcium content. Defatting was accomplished in a soxhlet apparatus with an ether alcohol miture (1:1), drying in and oven at 105°C., and ashing in a muffle furnace at 600°C. Calcium determinations were made by means of a back titration method using EDTA (Yalman et al. In press.)

RESULTS

Statistical relationships between bone mass values of the intact bone and excised bone films are shown in Table 1. The first order correlations (r) (Table 1) are not high enough to provide satisfactory predictions. In some cases the soft tissue mass coefficient is more highly correlated to the excised bone mass coefficient than the intact bone mass estimation. This prompted the use of a multiple correlation (R) for the femur and humerus. For the finger the total mass including soft tissue was summed and correlated to the excised bone value (Table 1). These results were much more promising and indicated that in areas where there are small quantities of soft tissue, the major error lies in the soft tissue correction and is not inherent in the technique.

There seems to be a very direct relationship between the error and the thickness of soft tissue. Referring again to Table 1 it will be noticed that the correlation values which include both soft tissue and bone, progress from a satisfactory .94 on the finger to a poorer .82 on the upper arm and descend to an almost useless .70 on the thigh. These results indicate that a film technique which uses a wedge and a broad spectrum x-ray source is probably of use only on the areas of the human body where there is a small amount of soft tissue surrounding the bone. It is certainly clear that the present technique cannot be applied without modification for the much desired determination of lumbar vertebrae mineral content.

Considering the large difference between the x-ray absorption capacity of soft tissue elements and calcium it would seem, a priori, that the fat and water contained in even large human bones should not contribute a significant error when the bare bone is x-rayed. However, as shown in Figures 1 and 2, a significant difference was found between the mass coefficients made on films of excised untreated bones and the same bones when dried and defatted. Thus, a small error was contributed by the fat and water content of the bone. Some of the difference might be attributed to between-film error, but most of this error is due to the fat and water as indicated by the relationships between film readings and actual bone composition (see Figure 2).

When the bone has been defatted and dried, the film evaluation method has all the accuracy that is desirable in a biological technique. Table 2 demonstrates that bone x-ray mass coefficients can be translated into dry bone, bone ash, or bone calcium equivalents in the case of humeri and femorae, where a direct comparison between film value and bone composition was made.

For the phalanx, selected trace paths were compared to the total bone weight and composition. The resulting correlations are given in Table 3. The correlations are lower and, while the use of a single trace path (mid-shaft) for estimating total bone mineral content provides reasonable accuracy, the use of all three in a multiple correlation provides slightly better estimate of dry bone weight or even total bone calcium.

In Figures 1 and 2 the regression lines obtained for the femur x-ray mass coefficient and humerus x-ray mass coefficient to bone

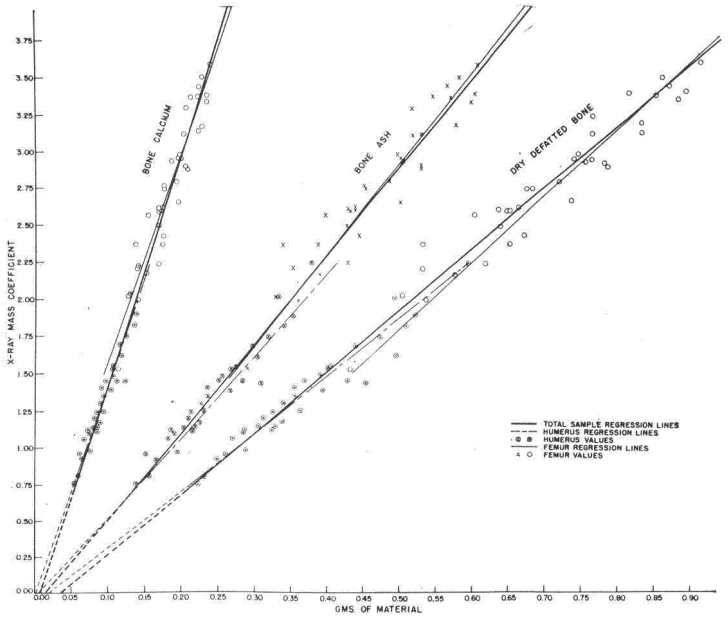

FIG. 1. X-ray mass coefficient of excised bone against underlying components.

composition do not vary significantly from each other. It was, therefore, possible to draw a regression line based on the combined data from both bones. If extrapolated, these lines intercept very closely to the zero ordinate. This combination of facts indicate that the present regressions can be used for translating any bone x-ray mass coefficient in this range into dry bone weight, bone ash or even bone calcium. Since the mid-section of the femur probably contains the greatest amount of mineral in any human long bone

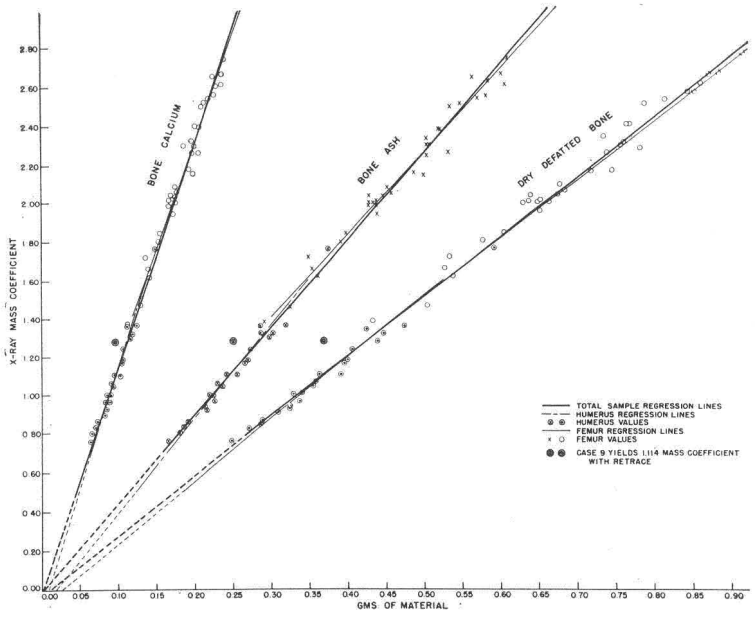

FIG. 2. X-ray mass coefficient of dry defatted bone against underlying components.

TABLE 2

RELATIONSHIP BETWEEN EXCISED BONE MASS COEFFICIENTS AND THE COMPOSITION OF THE UNDERLYING BONE

	Sample	*Dry defatted wt. r*	*Ash wt. r*	*Calcium wt. r*
Roentgenograms of wet macerated bone				
humerus	32	.97	.96	.96
femur	35	.96	.94	.94
Roentgenograms of dry defatted bone				
humerus	24	.98	.98	.96
femur	35	.99	.98	.98

cross section, it should now be possible to apply these results to any human long bone cross section.

DISCUSSION AND CONCLUSIONS

The results of this study indicate that the major barrier to accurate bone mineral content determinations from roentgenograms of the living man is the error introduced by the soft tissues. Schraer in his work with rats did not find a serious error due to soft tissue, even though the ratio of soft tissue to bone in a rat thigh is not greatly different from that in man. If anything the rat thigh has a higher ratio of soft tissue to bone (Newmann '53). Thus, it seems reasonable to suggest that it is the absolute depth of soft tissue which is significant and not its ratio to bone. This is further supported by the fact that as broad band x-radiation passes through progressively greater depths of soft tissue, there is progressive absorption of the longer wave portions of the x-ray spectrum.

Several suggestions have been made for means of eliminating the soft tissue error. Jackson ('51) was among the first to suggest that immersing the body part and wedge standard under comparable water depths would equalize the absorption over the bone and wedge, thereby making the wedge and bone film opacities similar. While this is feasible when dealing with small depths of soft tissue, preliminary experiments in which thighs were x-rayed under water to correct for soft tissue have been unsuccessful. Required x-ray exposures are high, and the resulting films are too diffuse and fuzzy, for accurate x-ray mass coefficient determinations of bone.

The use of a scintillation counter technique would reduce the radiation load on the individual. This is desirable in light of the recent emphasis on reducing human x-ray exposure. However, it is not certain that this technique would reduce soft tissue error unless a selective band receiver or a monochromatic radiation source was used.

It has also been suggested that the use of a narrow band radiation source which was not absorbed to any great extent by soft tissue would improve the results with film. The narrow band radiation could be obtained from either an emitting isotope source or from a standard x-ray tube fitted with special fil-

TABLE 3

RELATIONSHIP BETWEEN EXCISED BONE X-RAY MASS COEFFICIENTS AND THE COMPOSITION OF THE UNDERLYING BONE

		Phalanx 5-2		
Trace path location	*Sample*	*Total bone dry wt. correlation coeff.*	*Total bone ash wt. correlation coeff.*	*Total bone calcium wt. correlation coeff.*
Proximal epiphysis	30	.90 (r)	.90 (r)	.90 (r)
Midshaft	30	.94	.94	.93
Distal epiphysis	30	.86	.86	.86
Using all three trace paths	30	.95 (R)	.95 (R)	.95 (R)

ters. (Ackerman personal communication). This suggestion sounds promising but will require considerable research before its value can be ascertained.

In conclusion it can be stated that the measurement of x-ray film opacity of bone images can provide accurate information on the bone mineral content in many cases. The application of the technique is limited to those anatomical sites where the bone is covered by a shallow layer of soft tissue. Techniques such as the one described have been profitably applied in medical and biological research. However, to the best of our knowledge, the problem of accurately assessing the mineral content of bone surrounded by large masses of soft tissue is still to be solved.

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ERRATA: Yearbook Issue, page 262. Change Rosenau, Henry G. to Rosenau Milton D. and Rosenbaum, Milton D. to Rosenbaum, Henry G. Also on page 240 omit Brooks, Ernest G. etc. and on page 245 add Earnest, G. Brooks, President, Fenn College, Cleveland, Ohio.