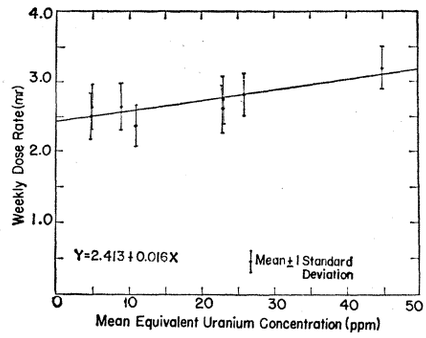

Fig. 2. Weekly dose rate from external radiation and local equivalent uranium concentration.

tooth specimen was developed (*6*). Prior bovine and human studies had suggested that teeth may be used to indicate the body burden of radium-226 if it is acquired by chronic exposure (*7*). Each sample included teeth from persons ranging in age from under 15 to over 65. No association between the concentration of either radium-226 or polonium-210 with age was noted.

The mean concentrations of radium-226 in teeth ranged from 0.009 to 0.025 pc/g of ash (Table 3). For analysis of variance, a log transformation was used in view of the marked differences in intracategory variability and the tendency of this variability to increase as the mean value for the category increases. Differences between geological categories were significant ($p < .01$). However, in contrast to the findings with respect to external radiation, the linear component of intercategory variability in the concentration of radium-226 in teeth which is related to equivalent uranium concentration is not significantly greater than the residual component which is associated with other factors of a local nature ($p > .05$). The present data would therefore suggest that if an effect of local bedrock radioactivity on the dose from radium-226 is present, it is too small to be detected in the number of teeth analyzed. Consistent with these findings is the observation that the concentration of radium in samples of tap water from the eight municipalities varies from 0.01 to 0.08 pc/lit. but shows no significant correlation with bedrock radioactivity ($r = -.51$; $p > .05$) (*8*).

Table 4. Estimated mean annual bone tissue dose (mrem/year) from natural internal emitters in northern New England.

Emitter	Relative biological effect	
	4	10
Radium-226	4.6	11.6
Thorium-228	3.3	8.3
Lead-210	14.7	36.6
Potassium-40*	15.0	15.0
Carbon-14*	1.6	1.6
Total	39.2	73.1

* Standard value.

Values for the concentration of polonium-210 in teeth range from 0.047 to 0.061 pc/g of ash. Intercategory variation is not statistically significant ($p > .05$). Differences between individuals with respect to this radioisotope are more marked than is the case for radium-226.

The concentration of radium-224 was measured in only 46 teeth with an unequal distribution between the eight geological categories. It was considered inadvisable, on the basis of this small sample, to evaluate the relationship between the tissue dose from this element and bedrock radioactivity. The mean value for teeth from all regions is 0.007 pc/g of ash.

The annual dose to bone tissue from radium-226, lead-210, and thorium-228 may be estimated from their concentration in teeth. Although polonium-210 and radium-224 were measured, the amounts of these daughter products may be assumed to be in equilibrium with their longer-lived parents lead-210 and thorium-228, since the teeth were extracted a year or more before analysis. Table 4 shows the estimated annual dose to bone tissue for a relative biological effect of 4 and 10. These values were calculated from the overall mean concentration of the relevant radioisotopes from all municipalities, since there was no evidence in the present data to suggest that the tissue dose from these natural internal emitters varies significantly in the study area as a function of bedrock radioactivity.

These results indicate some of the difficulties inherent in the use of bedrock radioactivity as an index of geographic variation in population exposure to background radiation. As far as external emitters are concerned, it was possible to demonstrate a significant linear relationship between population dose rate and equivalent uranium concentration. However, a ninefold difference in equivalent uranium concentration between the categories of highest and lowest bedrock radioactivity was found to correspond to a difference in dose rate in the order of only 43 mr/yr. No significant relationship between equivalent uranium concentration and the level of radium-226 and polonium-210 in teeth was observed. Thus, while the geological data suggested considerable intercategory variation in terrestrial sources of radioactivity, dosimetric evaluation of population exposure revealed relatively small differences only with respect to external gamma radiation (*9*).

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18 March 1963

Microdetermination of Calcium by Aequorin Luminescence

Abstract. *A bioluminescent protein, aequorin, isolated from the jellyfish* Aequorea *in dilute disodium ethylenediaminetetraacetate solution, emits light on addition specifically of Ca^{++} or Sr^{++}, thus providing the basis for a simple, quantitative micromethod for the determination of these cations, especially in biological fluids.*

Homogenates of photogenic tissues of the jellyfish *Aequorea* contain a bioluminescent protein, aequorin, which has recently been extracted in disodium ethylenediaminetetraacetate (EDTA-2Na) solution and purified chiefly by chromatography on diethylaminoethyl cellulose (DEAE) (*1*). Light emission at a rate that is first order with respect to the concentration of aequorin takes place on addition specifically of calcium (or to less extent strontium) salts in slight excess over the molar equivalent of EDTA-2Na in the solvent. No other factor, among many tried, replaced the Ca^{++} or Sr^{++} in this reaction. Unlike most other bioluminescent systems (*2*), neither the

Table 1. Calcium content of milk and horse serum as determined by EDTA-titration and the aequorin-luminescence method.

Specimen	EDTA			Aequorin luminescence		
	Volume tested (undiluted)	Ca^{++} found		Dilution tested (2 ml)	Ca^{++} found	
		mg	%		mg/ml	%
Milk brand X	0.5	0.589	0.118	1:1000	0.00120	0.120
Milk brand Y				1:1000	0.00116	0.116
Horse serum	1.0	0.170	0.0170	1:100	0.00181	0.0181
(commercial ampoule)	2.0*	0.332*	0.0166*	1:200	0.00094	0.0188

* Same specimen tested independently by the Schwartzkopf Microanalytical Laboratory.

luminescence rate nor the total light emitted is influenced by molecular oxygen, from virtually no dissolved oxygen to complete saturation. Moreover, although the system is heat labile and is susceptible to inactivation by heavy metal ions, such as Hg^{++}, or other protein denaturants, it is not affected by various substances which might be reasonably encountered in natural sources. With a suitably low concentration of EDTA, 0.01 μg of Ca^{++} in 2 ml of solution (about 10^{-7} molar) can be easily detected, and by appropriate refinements of the apparatus for measuring light in extremely small (micro) volumes, it should be possible to extend this sensitivity to detect 0.0001 μg of Ca^{++}, or less.

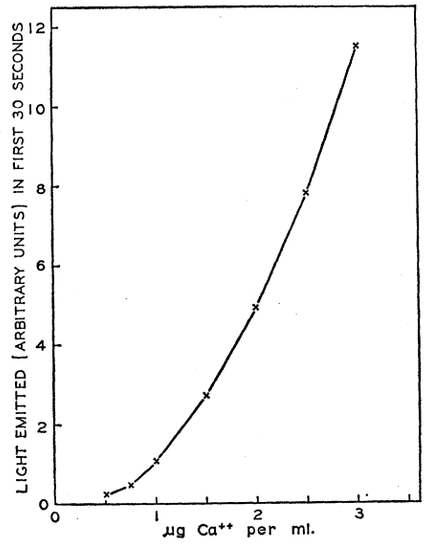

Fig. 1. Calibration curve showing the relation between the amount of Ca^{++} per ml in 2 ml of various dilutions of calcium acetate in 0.005*M* magnesium acetate (abscissa), and amount of light emitted in the first 30 sec after adding the respective dilutions to 1.5 μg of aequorin contained in 0.1 ml of 0.005*M* EDTA-2Na solution in the reaction vessel (ordinate). Under the conditions of the test, the relation is linear on a log-log scale, with a slope of 2.25. The maximum light emitted with an excess of Ca^{++} amounted to 22.5 on our scale; the quantum efficiency (photons/mole aequorin) has been computed to be 0.14 on the basis of quantitative, though indirect, evidence of molecular weight (*1*, *3*).

The specificity and sensitivity of aequorin luminescence to Ca^{++} or Sr^{++} provide the basis for a simple, quantitative micromethod for determining these cations as illustrated by the following data, where horse serum and cow's milk were used as convenient unknowns. A calibration curve (Fig. 1) was first established with dilutions of a known concentration of calcium acetate in 0.005*M* magnesium acetate made up in deionized distilled water. The magnesium salt was inert in the luminescence reaction but served to retard the rate by competing with Ca^{++} for chelation with the EDTA-2Na in the solution; otherwise, the rate tends to be "all-or-none" at concentrations of Ca^{++} and EDTA-2Na approaching molar equivalence. Scrupulously clean pipettes and vessels, previously washed with 10 percent HCl, were used throughout. The procedure consisted of adding 0.1 ml of 0.005*M* EDTA-2Na solution containing approximately 15 μg of practically pure aequorin per milliliter to the reaction cuvette and initiating the luminescence reaction by adding 2 ml of a specified dilution of the calcium solution. The light emission, in arbitrary units, was measured by means of a photomultiplier with output-integrating and amplifying circuit connected to a Sanborn automatic recorder (*3*). The same procedure was used with 2 ml aliquots of the unknowns at appropriate dilutions in 0.005*M* magnesium acetate. The results are summarized in Table 1 which includes, for comparison, data obtained by an ordinary EDTA titration method (*4*) with murexide used as an indicator.

For microanalyses of Ca^{++} in biological fluids in particular, the aequorin test requires smaller volumes (as little as 2 μl) of specimens; it is simpler, faster, and more sensitive than polarographic (*5*), flame photometric (*6*), or EDTA titrimetric methods (*7*), and it is not subject to complication by the presence of other ions such as sodium, potassium, magnesium, or phosphate, or by the presence of protein. Although it is difficult to distinguish between Ca^{++} and Sr^{++} at low concentrations, the latter cation would not be significant in tests on most biological fluids.

Pure aequorin is fairly stable in saturated ammonium sulfate solution containing EDTA-2Na at dry-ice temperatures for long periods of time; the ammonium sulfate does not interfere with the test. Since aequorin retains its activity after drying in a vacuum at room temperature, it is reasonable to expect that it can be preserved in a lyophilized state for an indefinite period. With a suitable quantity of starting material, a maximum yield of 10 percent of the amount present in the organisms can possibly be achieved. We estimate that an average of 100 μg of aequorin is contained in a mature, living specimen of *Aequorea*. The minimum amount of aequorin needed for a calibration curve together with one test for Ca^{++} in an unknown would thus be equivalent to the amount that could be obtained, as an average with a 10-percent yield, from one specimen of the jellyfish *Aequorea* (*8*).

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