trol than with IAA-treated sections. Also, there was no difference in the isozymal composition of the extracellular enzyme from control and IAA-treated tissue. Therefore, the differences in the tissue cannot be accounted for by differential leakage. Although high concentrations of IAA inhibit peroxidase action (*8*), the concentration used is too low to produce this effect.

Gibberellic acid (GA_3), which induces growth and whose effects on the peroxidase isozymes of plant tissue may be measured quantitatively (*9*), appears to act like IAA in this system, but much less effectively and rapidly. When added with IAA, it enhances the effect of the latter.

Therefore, it appears that peroxidase B develops as a consequence of aging, perhaps in response to a diminution in the supply of endogenous auxin. The application of exogenous IAA to stem sections prevents the depletion of growth hormone and concomitantly represses the formation of this specific peroxidase.

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Radium Isotope Accumulation in Animal Thyroids

Abstract. *Uranium and thorium daughters are ten times more concentrated in thyroids from some bovine animals than in the teeth of the same animals. These radioactive isotopes are believed to be from natural sources, but their resulting annual dosage of thyroid radiation has exceeded that from iodine-131 fallout.*

During continuous observation of animal thyroids for I^{131} from radioactive fallout, a small percentage of the glands contained long-lived radioactivity, which was interpreted to indicate the presence of radium (*1*). Within the past 2 years we have observed, in 28 of 275 bovine thyroids from Cali, Colombia, measurable quantities of gamma radiation at 0.24, 0.29, 0.35, and 0.61 Mev; these energies are the energies of radium daughters. The possibility that the radiation was due to surface contamination was disproved because we found a slightly greater concentration of radioactivity in the center of three glands than in their peripheries. Two of the thyroids were radioactive in 1961, and they showed no measurable loss of radioactivity by 1965. It may be relevant that 97 percent of 1700 supposedly unselected cattle thyroids sent from Colombia have been abnormally large, and the country has extensive areas of endemic goiter. We found radium daughters in only 8 of 750 cattle thyroids from the United States; however, the common presence of I^{131} in North American thyroids may have masked the presence of radium-daughter radiation in some samples. Teeth of 12 Colombian animals with the largest concentrations of radioactivity in their thyroids were also tested; the long-lived radioactivity per gram (wet weight) of thyroid was more than four to ten times greater than the radioactivity per gram (wet weight) of teeth. In four cases, however, the teeth from a Colombian animal contained more long-lived radioactivity than the thyroids, but in these animals both tissues contained minimally detectable quantities of radium.

Five typically radioactive thyroids were selected for detailed quantitative study to identify the long-lived radiation as originating from short-lived daughters of the uranium-radium and thorium series. Table 1 summarizes the results of the analyses.

Method A utilized coincidence-counting techniques (*2*) to identify and quantitate Ra^{226} and Th^{228}, while method B used gas-emanation techniques in which radon and thoron were separated and quantitated. In method A, the coincident 0.609 and 1.12-Mev gamma rays of Bi^{214} served to identify the Ra^{226} daughters. The samples, which had been preserved for more than 2 months, were sealed in plastic containers to prevent loss of Rn^{222} (half-life 3.82 days) and to allow the Bi^{214} daughter to build up to equilibrium with its parent Ra^{226}. Because the daughters of Ra^{226} (half-life 1620 years) which occur between its first daughter Rn^{222} (3.82 days) and Bi^{214} (19.7 minutes) are very short-lived, the measurement of Bi^{214} provided a precise measurement of the Ra^{226} in the preserved samples.

Studies of the uptake of natural radionuclides by plants in areas of high natural radioactivity have been interpreted to indicate that Ra^{226} and Ra^{228} are taken up directly from the soil (*3*). These isotopes do not appear to be produced in the plants in significant amounts by decay of their precursors; however, the major source of Th^{228} in plants appears to be from the decay of Ra^{228}, which is taken up directly. The uptake of these radionuclides by animals may be similar to that in plants. The Ra^{226} was probably concentrated by the thyroid as Ra^{226}. Even though Ra^{226} could be formed in the thyroid

Table 1. Radium-226 and Th^{228} in thyroid and teeth of cattle. Analyses were made by nondestructive coincidence gamma spectroscopy (A) and performed by Perkins (*2*), except on the sample for which the slaughter date was 14 November 1964: this sample was analyzed by radon and thoron emission (B), with the assay performed on the ashed tissue by A. T. Keane. The weights used as a basis for the figures were wet weights of trimmed tissues preserved with paraformaldehyde powder.

Slaughter date	In thyroid			In teeth	
	Ra^{226} (pc/g)	Th^{228} (pc/g)	Calculated dose (mrem/day)*	Ra^{226} (pc/g)	Th^{228} (pc/g)
United States					
14 Oct. 64	2.6	2.3	53		
28 Apr. 65	1.6	0.84	24		
Colombia					
26 May 65	2.0	3.0	61	0.17	0.32
26 May 65	1.8	2.9	58	0.09	0.37
14 Nov. 64	3.0	4.0	83		

* See reference *4*: μrad/day = 51 [(pc Ra^{226}/g) $\times$ (weighted average Mev/disintegration) + (pc Th^{228}/g) $\times$ (weighted average Mev/disintegration)] or mrem/day = $10^{-3} \times (10 \times 51)$ [(pc Ra^{226}/g) $\times$ (4.8 + 30 percent of 24.4) + (pc Th^{228}/g) $\times$ 31.9].

from its parent Th^{230} (half-life 8×10^4 years), examination of the gamma-ray spectra of the thyroids in Table 1 showed that any Th^{230} that may have been present was insignificant compared with that which would be required to produce the observed Ra^{226}.

Thorium-228 was identified and measured by observing the coincidence gamma rays at 0.583 and 2.614 Mev emitted by its daughters Tl^{208} in sealed samples. In the thorium series there are no daughters between Th^{228} (half-life 1.9 years) and the measured product Tl^{208} that have half-lives longer than that of Ra^{224} (3.62 days). If the thyroid concentrated thorium, then Th^{228} could have been the source of the thorium daughters; however, it is likely that the precursor of Th^{228}, Ra^{228} (half-life 5.7 years), was first concentrated and that it decayed through its short-lived Ac^{228} daughter (half-life 6.1 hours), which produced Th^{228}. The parent of Ra^{228} is Th^{232}, with a half-life of 10^{10} years, but no specific tests were applied for this original member of the thorium series.

It is interesting that the thyroids from the United States contained more Ra^{226} than Th^{228}, while those from Colombia contained more Th^{228} than Ra^{226}; however, the sources of thyroid radium and thorium have not been identified.

The estimated rates of radiation dosage (see Table 1) were calculated by the methods used for bone (*4*); it was assumed that 70 percent of the Rn^{222} (half-life 3.8 days) with its daughters was completely lost through the circulation. No loss of thoron (Rn^{220}, half-life 51.5 sec) was assumed, and the concentration of Ra^{228} was assumed to equal the observed Th^{228} concentration. The relative biological effectiveness of alpha particles was assumed to be 10. Two groups of 30 Colombian thyroids were examined to relate histological evidence of hyperplasia with radium content; 8 of these contained radium and 16 were severely hyperplastic, but there was no simple or consistent relation between the two variables. Previous investigators (*5*) have shown that calcium concentrates much more in the thyroid than in other soft tissues; therefore, it might be reasonable to expect radium to be concentrated with calcium in thyroid glands, but concentration of radium in thyroids would not be expected to exceed greatly that in teeth. No information is available, for any animal species, regarding thyroid metabolism of radium or the biological turnover of thyroid radium.

It is believed that these radioactive isotopes are daughters of the natural uranium-radium and thorium series, but the sources and routes of intake are unknown. The radiation doses (Table 1) can be compared with radiation doses received by animal thyroids during the periods of greatest worldwide fallout of I^{131}. Colombian animals were not studied during the year of maximum I^{131} fallout. The animal populations with the greatest reported amounts of I^{131} were sheep from Tennessee (*6*) in 1957. There was a brief period during that year when I^{131} concentrations were 10^4 times greater than the Ra^{226} concentrations (Table 1), and during the succeeding 12 months these sheep thyroids were exposed to an estimated accumulation of 23 rem (*6*) from I^{131}. These values can be compared to the 30 rem per year attributable to natural uranium and thorium daughters in the thyroid sample taken in Colombia 14 November 1964 (Table 1).

It appears coincidental that 97 percent of the bovine thyroids from Colombian animals are abnormal and that 10 percent have unexplained concentrations of natural radioisotopes; at present, there is no evidence of any relation between the two observations.

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Trehalase from Dictyostelium discoideum: Purification and Properties

Abstract. *The hydrolytic enzyme, trehalase, was isolated and purified approximately 90-fold from the cellular slime mold* Dictyostelium discoideum. *The purified trehalase has an optimal temperature of 45°C and shows maximum activity at* p*H 5.5 in citrate buffer. Its Michaelis constant is* 1.2×10^{-3}M.

Dictyostelium discoideum, a cellular slime mold, is an organism whose life cycle provides excellent material for the study of differentiation (*1*). The life cycle of the cellular slime molds has been reviewed by Bonner (*2*).

The nonreducing disaccharide trehalose, α-glucosido glucose, is present in many lower fungi (*3*); it is also the characteristic blood sugar of insect hemolymph (*4*). Clegg and Filosa (*5*) have reported the presence of trehalose in spores of *D. mucoroides*. The sugar constituted 7 percent of the total dry weight. Ceccarini and Filosa (*6*) found trehalose in another species of the cellular slime molds, *D. discoideum*; trehalose was present at each development stage (less than 0.5 percent of the total dry weight), but it increased dramatically at a stage which was arbitrarily called "later culmination," in which the sorus (mature structure containing spores) is not yet formed but is very near completion. Trehalose was found in spores at more than 5 percent of the total dry weight.

I now report that trehalase, a hydrolytic enzyme that splits trehalose into two glucose molecules, is present in *D. discoideum*. It has been isolated and purified from a variety of organisms (*7*).

Amoebae were grown in liquid culture (*8*). Trehalase-negative *Escherichia coli* were first grown on rich nutrient media for approximately 24 hours at 37°C, washed twice by centrifugation with 0.016*M* Sorensen buffer, *p*H 6.0, and suspended in the same buffer at a concentration of 10^{10} cells per milliliter. Spores of *D. discoideum* were then added to give a final concentration of 3×10^3 to 10^4 spores per milliliter. After about 48 hours of incubation at 21° ±2°C, when most of the bacteria had been eaten and the growth of the amoebae had reached a stationary state, the amoebae were washed free of the remaining bacteria with cold buffer or