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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30 September 1965

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

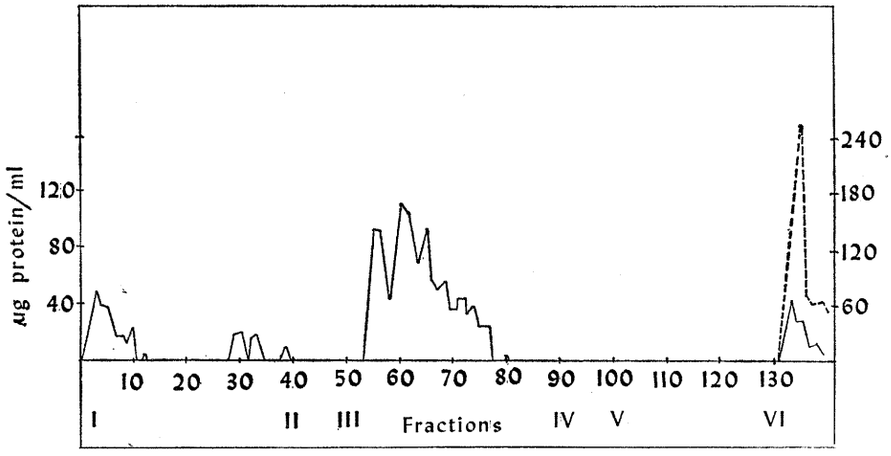

Fig. 1. Effluent diagram of 40 to 60 percent alcohol fraction of *D. discoideum*: Fraction III (43 ml) was applied on a column (17 by 230 mm) of DEAE-cellulose. The effluent was collected in 10-ml fractions. Buffers used: (*I*) Tris-maleate, 300 ml, 0.005*M*, *p*H 6.0, siphoned into mixing chamber containing 100 ml of 0.005*M* tris-maleate, *p*H 8.0, at the same rate that the mixture leaves the chamber and enters the column for elution. (*II*) Tris-maleate, 100 ml, 0.005*M*, *p*H 6.0, placed directly on the column. (*III*) Tris-maleate, 300 ml, 0.05*M*, *p*H 6.0, siphoned into 100 ml of 0.005*M* tris-maleate, *p*H 6.0. (*IV*) Tris-maleate, 100 ml, 0.05*M*, *p*H 6.0, placed directly on the column. (*V*) Tris-maleate, 200 ml, 0.05*M*, *p*H 6.0, containing 0.1*M* NaCl, siphoned into 100 ml of 0.05*M* tris-maleate, *p*H 6.0. (*VI*) Tris-maleate, 100 ml, 0.05*M*, *p*H 6.0, containing 0.2*M* NaCl, placed directly on the column. Purified trehalase was obtained from fractions 131 to 139 inclusive. Dotted lines refer to micrograms of glucose.

Table 1. Purification of trehalase.

Total units (No.)	Total protein (mg)	Specific activity	Total recovery (%)
Crude homogenate (fraction I)			
483	1550	0.311	
Supernatant (fraction II)			
494	1700	0.291	102
Alcohol (40 to 60 percent) precipitate (fraction III)			
248	46	5.39	51.2
Column eluate			
48.9	1.8	27.17	10.1

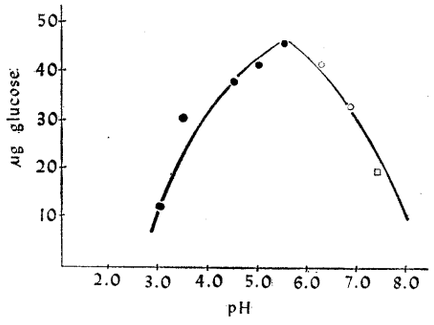

Fig. 2. The relation of *p*H to activity of trehalase. Buffer concentration is 0.1*M*. Conditions of assay are as described in text. ●, citrate; ○, phosphate; □, tris-HCl.

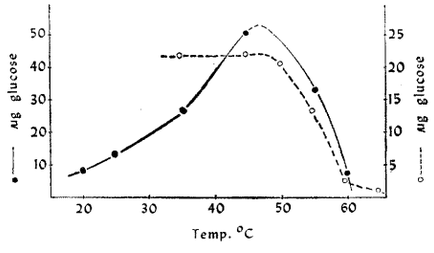

Fig. 3. Relation of temperature to activity of purified slime-mold trehalase. ●——, The incubation mixture was kept at the given temperatures for 30 minutes. ○——, The purified enzyme was kept at the above temperatures for 15 minutes; it was then assayed. The amount of glucose was determined by the Glucostat method.

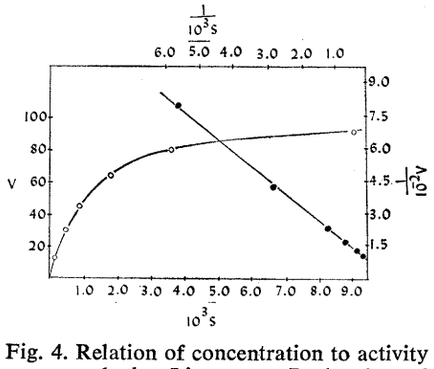

Fig. 4. Relation of concentration to activity curve and the Lineweaver-Burk plot of purified slime-mold trehalase. A K_m of $1.2 \times 10^{-3}M$ was calculated from the above curves.

cold distilled water. The amoebae were broken by passing them once through a French press. Unless otherwise stated, trehalase activity was assayed in the following reaction mixture: 0.6 ml of citrate buffer, *p*H 5.5 (0.1*M*), 0.5 ml of trehalose (25 or 50 μmole/ml), and 0.1 ml of enzyme extract. Once the enzyme purification was beyond the diethylaminoethylcellulose (DEAE-cellulose) step, 0.3 ml of the enzyme preparation was used in the incubation mixture. The reaction mixture was incubated for 30 minutes at 35°C. The reaction was stopped by boiling for 10 minutes, cooling, and bringing to *p*H 7 by addition of NaOH. The glucose in 1.0 ml of the clear supernatant was assayed by the glucose oxidase method (*9*).

Glucostat Special (Worthington Biochemical) was used throughout our experiments, since the lower grade of glucose oxidase contains large quantities of trehalase. The absorbancy of colored end product was read at 401 mμ. Proteins were assayed (*10*) and the final color was read at 600 mμ. Enzyme activity is the number of micrograms of glucose produced under the conditions of enzyme incubation. In all experiments one unit of enzyme activity is equivalent to 1 μmole of glucose released in 30 minutes at 35°C.

After 48 hours of incubation, amoebae from several flasks were collected, washed, and broken with the French press. This crude fraction was numbered I. It was then centrifuged at 8200*g* for 10 minutes to give fraction II. Fraction II was first brought to 40 percent alcohol by addition of 100 percent ethanol at 0°C. The precipitate was discarded and the 40 percent alcohol supernatant was then brought to 60 percent alcohol. This precipitate was kept and dissolved in 40 ml of tris-maleate buffer (*p*H 8.0, 0.005*M*) to give fraction III. Fraction III was then dialyzed overnight against 2 liters of the same buffer. The solution was then applied on top of a DEAE-cellulose column which was prepared according to the method of Friedman (*7*). The elution was carried out at a rate of about 30 ml/hr. Table 1 summarizes the various steps in the purification procedures.

The elution profile of trehalase as it comes off the DEAE-cellulose column (Fig. 1) is a homogeneous, sharp peak very late in the elution.

The purified enzyme was tested for activity at various *p*H's with the appropriate buffer systems, and it was found to have maximum activity at *p*H 5.5 (Fig. 2). This value is identical to that for trehalase of both purified *Neurospora* and insects.

The purified enzyme was tested against five other substrates similar to trehalose (melibiose, lactose, raffinose,

sucrose, and trehaloseamine). Trehalase did not utilize these substrates. Furthermore, 280 μmole of glucose added per milliliter to the assay system did not inhibit the enzyme.

The maximal activity of the enzyme occurs at about 45°C (Fig. 3). If the enzyme is kept at 55°C for 15 minutes, then assayed at 35°C, it loses only 36 percent of its original activity, but when it is kept for 15 minutes at 60°C it loses 75 percent of its original activity. Slime-mold trehalase is more resistant to heat than purified insect trehalase (*7*). Purified trehalase from *Neurospora* is, on the other hand, even more resistant to heat; it loses negligible activity if kept at 60°C for 15 minutes (*7*). The purified slime-mold enzyme was stable for 6 months when kept at −20°C.

A Lineweaver-Burk plot of concentration against activity gives a value of $1.2 \times 10^{-3}M$ for the Michaelis constant (K_m) of the enzyme-substrate complex and a maximum activity of 27.2 units/mg of protein.

In general the properties of purified slime-mold trehalase resemble those of the trehalases from other sources. Its behavior on a DEAE-cellulose column is almost identical to blow-fly trehalase. Where purified trehalases have been used, trehalose is the only substrate that is utilized. The K_m of *D. discoideum* trehalase of $1.2 \times 10^{-3}M$ is higher than the value obtained from other purified trehalases (*7*).

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7 October 1965

Blood Oxygen and Ecology of Porpoises of Three Genera

Abstract. *Blood volumes, hemoglobin concentrations, packed-cell volumes, and heart weights were determined in three genera of propoises which differ from one another in behavior and ecology. The estimate for the total blood-oxygen content of the highly active, deep-diving, pelagic species* Phocoenoides dalli *was almost three times greater than that for the coastal-dwelling species* Tursiops truncatus, *and about 70 percent greater than for the less active pelagic species,* Lagenorhynchus obliquidens. *Heart weights of* Phocoenoides dalli *were about 140 percent greater than in* Tursiops truncatus *and 55 percent greater than in* Logenorhynchus obliquidens.

Members of the order Cetacea appear to be the best divers among the mammals. The bottlenose whale (*Hyperoodon rostratus*) can remain below the surface for as long as 2 hours (*1*), and the sperm whale (*Physeter catodon*) is evidently able to dive to a depth of at least 1000 meters (*2*). Some cetaceans are among the fastest swimming creatures in the sea (*3*). Deep dives, dives of long duration, fast swimming, and thermoregulation in cold waters may place unusual physiological requirements for oxygen transport upon these mammals.

We studied the blood volumes, heart weights, hemoglobin concentrations, and packed-cell volumes (hematocrit) of three genera of small cetaceans: *Tursiops truncatus* (Montagu), *Lagenorhynchus obliquidens* (Gill), and *Phocoenoides dalli* (True). The data were gathered over 2 years from five *P. dalli* (80 to 124 kg in body weight), nine *L. obliquidens* (60 to 110 kg), and 12 *T. truncatus* (62 to 155 kg). The Dall porpoises (*P. dalli*) that we studied are to our knowledge the only members of this species ever to survive in captivity for an extended period. Measurements of blood volume in living cetaceans had not previously been made.

Venipunctures were made in small veins on the ventral surface of the flukes. These veins are quite prominent in *L. obliquidens* and *P. dalli* but are palpable only in certain individuals of *T. truncatus*. The blood-volume measurements were made by the I^{131} technique (*4*) with a volemetron (*5*). The test dose, 2 ml (10 μc), of serum albumin tagged with I^{131} was injected into a vein on the ventral surface of the flukes. After 10 minutes an 8-ml blood sample was drawn from a vein on the opposite side of the flukes. Packed-cell volumes (*6*) and hemoglobin concentrations (*7*) were measured each time blood volumes were measured, as well as during numerous physical examinations.

Hemoglobin concentration in *P. dalli* ranged from 17.8 to 23.7 g/100 ml of whole blood. In *L. obliquidens* the hemoglobin concentration ranged from 16.0 to 19.6 g with a mean of 17.0 g, and in *T. truncatus* from 13.2 to 15.3 g with a mean of 14.4 g (Table 1). Concurrently measured packed-cell volumes ranged from 52 to 63 percent in *P. dalli* with a mean of 57 percent. In *L. obliquidens* the packed-cell volume ranged from 50 to 59 percent and the mean was 53 percent. *Tursiops truncatus* had a mean packed-cell volume of 45 percent with a range of 40 to 48 percent (Table 1).

Striking differences are found in blood volumes of the three species (Fig. 1 and Table 1): 143 ml/kg of body weight for *P. dalli*, 108 ml/kg for *L. obliquidens*, and 71 ml/kg for *T. truncatus*. Estimated differences in total blood-oxygen content are perhaps better indicators of the physio-

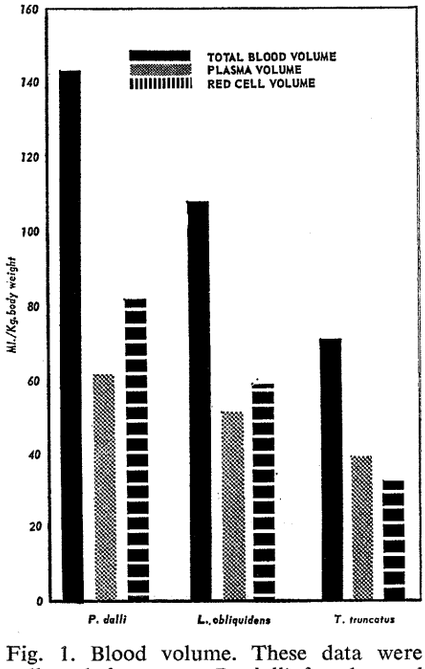

Fig. 1. Blood volume. These data were collected from two *P. dalli* females and one male, two *L. obliquidens* males and two females, and one *T. truncatus* male and three females.