

Since fish noises predominate in other regions, it seems strange that none were identified here, except for those caused by their biting and pulling at the hydrophone. It is possible that some of the sounds might be of fish origin, but it is significant that only the bites were detected in the absence of *Delphinapterus*.

Particularly striking is the great variety of *Delphinapterus* sounds and their rapid and apparently continuous succession. This loquaciousness contrasts markedly with most terrestrial herd mammals and compares with such chatterboxes as monkeys and men. Among the Cetacea it would appear not to be limited to *Delphinapterus*, as is indicated by the continuity of related but less varied sounds heard by one of us in the presence of different porpoises in the open Atlantic, as well as those on phonograph records at the Woods Hole Oceanographic Institution which are believed to be of porpoises. The considerable difference between the sounds we heard in the presence of *Delphinapterus* and the less spectacular ones associated with *Delphinus* (Kullenberg, 7; Fraser, 5), *Tursiops* (McBride, 8, p. 19; 9, pp. 112-113), and other pelagic forms encourages us to hope that these underwater calls may be sufficiently characteristic to be helpful in distinguishing cetaceans at sea. Such listening probably will have to be carried into the supersonic range.

Only toothed whales (Odontoceti) have figured in the reports so far encountered. It would be of interest to learn of any authenticated instances of hearing underwater sounds from baleen whales. Among these, our experience with listening apparatus is limited to the solitary individuals of *Balaenoptera acuto-rostrata* mentioned earlier; we distinguished no underwater sounds even when a whale was within 300 yards.

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Colorimetric Estimation of Succinic Dehydrogenase by Triphenyltetrazolium Chloride¹

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Methylene blue or other redox dyes are extensively used as indicators of dehydrogenases. Enzyme activities are frequently defined by the anaerobic decolorization time of a redox dye, but photometric estimation of the decolorization can be carried out in special Thunberg tubes only if the reaction mixture is not very turbid (3). It was found in our laboratory that triphenyltetrazolium chloride is a suitable indicator of the succinic dehydrogenase activity of tissue homogenates. The advantages of this technique are that the system does not require anaerobic conditions and that the enzyme activity can be followed quantitatively by colorimetric measurements.

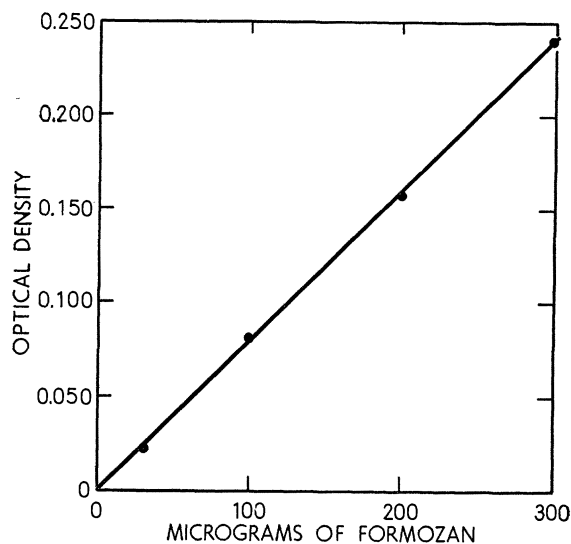


FIG. 1. Straight line relationship between the amount of reduced tetrazolium salt (formozan) and optical density ($\log I_0/I_x$).

Tissue homogenates in the presence of succinate in a buffered (pH 7.4) medium reduce the colorless tetrazolium salt to a red water-insoluble formozan. The formozan (2) can be easily dissolved in acetone, which, by precipitating tissue proteins, leaves a clear supernatant ready for colorimetric measurement. Under given conditions, in the absence of succinate the tissue homogenates tested do not reduce the tetrazolium salt except under strongly alkaline conditions.

If this principle is applied, the colorimetric determination of succinic dehydrogenase can be carried out according to the following procedure: Into 15-ml calibrated centrifuge tubes are pipetted 0.5 ml of 0.1 M phosphate buffer (pH 7.4), 0.5 ml of 0.2 M sodium succinate, 1.0 ml of 10% tissue homogenate (i.e. 0.1 to 1.0 ml of homogen-

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ate, depending on the enzyme content, made to 1.0 ml with distilled water), and lastly 1.0 ml of freshly prepared 0.1% triphenyltetrazolium chloride solution. After shaking, the tubes are placed in a constant temperature bath at 38° C for a period of 15 to 30 min. Immediately after removal of the tubes from the bath, 7 ml of acetone are added and the tubes are stoppered and shaken vigorously. The precipitate is then centrifuged, and the clear supernatants are drawn off for the determination of optical density. The color is stable for several hours and can be measured by the Klett photometer using a 420 filter. There is a linear correlation between the amount of dye and optical density ($\log I_0/I_x$) in the range between 20 and 300 μ g. To obtain a calibration curve, varying amounts (20 to 300 μ g) of tetrazolium salt are reduced by a few crystals of sodium hydrosulfite in the system as described (Fig. 1). Furthermore, the amount of dye measured by optical density is a linear function of the amount of enzyme present (Fig. 2). The

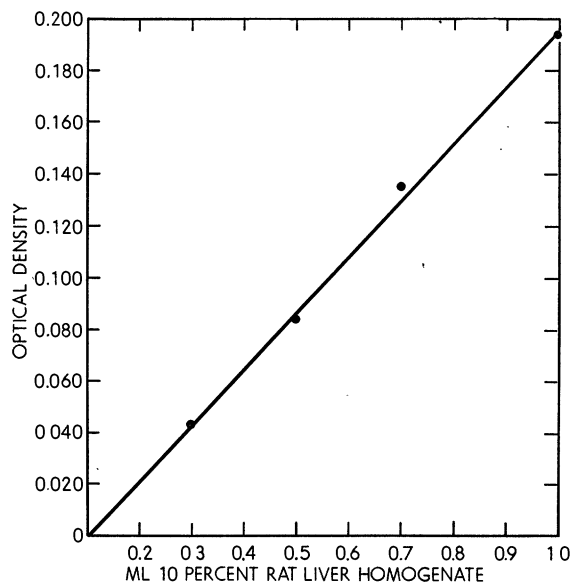


FIG. 2. Straight line correlation between the amount of enzyme (rat liver homogenate) and the amount of reduced tetrazolium salt, as measured by optical density. Time of incubation, 10 min; temperature, 38° C.

rate of reduction of the dye gives a curve resembling that of most enzymatic reactions. During the first 30 to 40 minutes the curve is approximately linear. Under anaerobic conditions (nitrogen) the rate of reaction is initially greater, while tending to decrease more rapidly (Fig. 3).

In view of these findings it is quite simple to determine quantitatively the succinic dehydrogenase activity of tissue homogenates. The enzyme activity of various tissues may be expressed in terms of micrograms of dye reduced in 10 minutes by 1 milligram of tissue; however, it is necessary to take into account the unequal adsorption of the dye by equivalent amounts of different tissues. Consequently, two controls are run with the unknown; the

first contains no succinate and serves as a tissue blank, while the second also contains no succinate but a

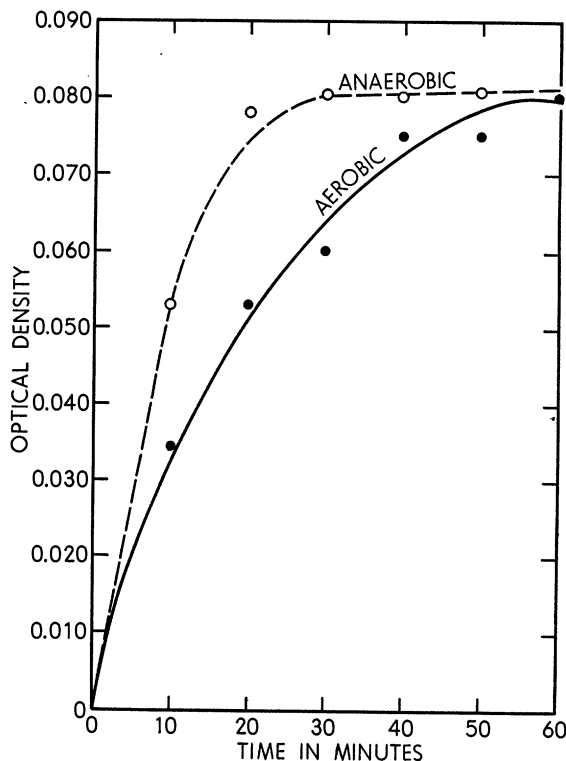


FIG. 3. Comparison between the rate of reduction of the tetrazolium salt by 0.25 ml of 10% rat liver homogenate under aerobic and anaerobic (N_2) conditions.

given amount, viz., 100 μ g, of the tetrazolium chloride which is reduced by sodium hydrosulfite. The latter is the reference standard for a specific tissue. The experimental tube contains all the constituents. Typical experimental data obtained with 10% homogenates of rat kidney, liver, brain, heart, and testis are presented in Table 1.

This method can be used in the study of enzyme inhibitors. Sodium malonate (0.5 ml of 0.1 M solution)

TABLE 1

Tissue	μ g of dye reduced/ mg of tissue*/10 min
Kidney	1.72
Liver	1.56
Brain	1.00
Heart muscle	0.75
Testis	0.43

* Wet weight.

produces a 75% inhibition of the succinic dehydrogenase activity of rat liver homogenate (0.5 ml of 10% homogenate); ethyl urethane (0.5 ml of 2% solution), 46% inhibition. Cyanide (1%) on the other hand causes no inhibition. Triphenyltetrazolium chloride does not interfere with succinate oxidation by tissue homogenates either

in the absence or in the presence of added cytochrome C, as measured in the Warburg apparatus (1).

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Determination of Radioactive Content of Rocks by Means of Geiger-Müller Counters

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Geiger-Müller counters are widely used in the search for radioactive substances, but their use for immediate quantitative determination has not been elaborated. During a survey of the Velence Mountains in Hungary we have developed a direct approximating method.

Brass G-M counters of the self-quenching type (45 mm in diameter, 88 mm in length and 1.8 mm in wall thickness), filled with 100 mm argon and 10 mm alcohol vapor, were used in connection with all battery amplifiers. The soft components of the γ -radiation were absorbed by a 2 mm-lead shield. In this way only the known penetrating γ -components of the known radioactive bodies (uranium, thorium, their decay products, and potassium) could reach the counter.

In most cases the counter was placed touching the rock wall to be investigated, but in one instance, we drove a bore, 5 cm in diameter and 50 cm deep, into the granite wall and placed the counter within. The counting rate (number of impulses/min) was determined and compared with the counting rate of the cosmic radiation, the last amounting to 40/min. We subtracted this value from the observed total counting rate, and divided the remaining rock-activity counting rate by this factor. We observed that the counting rate was about 6 times higher in the bore and 2-3 times higher immediately at the wall than the cosmic radiation. The activity of the rocks can be expressed approximately by this factor, and this kind of expression is, within some limits, independent of the counter dimensions. It can be used for comparison of measurements made by various brass counters. However, it is not independent of the material of the counter, because the γ -sensitivity of a G-M counter depends upon the atomic weight of its substance. As is known, the intensity of the cosmic radiation depends upon the geographical latitude. In this case it amounted to about 1 impulse/min $\cdot$ cm² of maximum square area of the counter (length $\times$ diameter).

The observed γ -radiation of rocks may originate from K, U, and Th, and their decay products in equilibrium.

ThC'', RaB + C and K are the sources of the hard γ -radiations, which affect the counter under such conditions. Softer components may have a minor part only, for they are absorbed by the lead shield, and the sensitivity of a brass counter is small for soft γ -rays.

Now it must be realized, that the direct determination of the proportion of the existing radioactive substances to each other in the rock is hardly possible with a G-M counter without any chemical separation. It is possible, however, to determine the total radioactivity of the rocks expressing it in Th- γ -equivalents, as measured by a brass G-M counter behind a 2-mm lead shield. We achieved it in the following way: It is possible to calculate the total amount of the hard γ -radiation that reaches the counter from the surrounding rock substance. In Fig. 1 we have

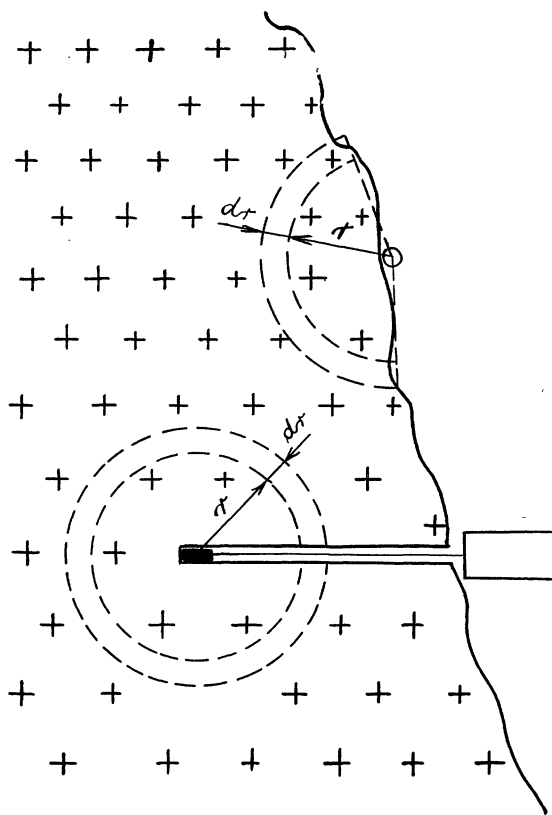


FIG. 1. Determination of Th and U concentration in rocks by a G-M counter tube.

taken the center of our coordinate system as the middle of the counter in the bore. (The counter may be regarded as small in comparison with the surrounding rock masses.) A small volume, dv , of the rock containing c g-Th-equivalents of radioactive substance/cm³ rock, will send a penetrating γ -radiation from r distance to the counter, giving the counting rate, dJ . It is obvious that $dJ = cAdv/r^2$, where A is the sensitivity of the given counter, that is, the rate of counting when 1 g Th (in radioactive equilibrium) is placed 1 cm from the counter.

Now we must take into account the self-absorption of the γ -radiation within the rock substance itself. The