Reports

Oxygen Isotope Fractionation in Reactions Catalyzed by Enzymes

Abstract. A study has been made of some of the enzymatic mechanisms responsible for the previously reported fractionation of oxygen isotopes by whole organisms. The data indicate that the fractionation occurs through the activity of metalloenzymes.

In their respiratory processes aerobic organisms have been reported to fractionate the oxygen isotopes O^{16} and O^{18} (*1*, *2*). The fractionation factor, which is a measure of the relative rates of consumption of the two isotopes, varies from organism to organism (*1*). In an attempt to study the metabolic mechanisms involved we have found that several enzymatic reactions prevalent in aerobic organisms are among the sources of the fractionation.

The cytochrome oxidase and tyrosinase enzyme systems were chosen because of their widespread occurrence in living organisms. Oxidations were carried out in a Warburg reaction vessel. The essential measurements were the number of moles of oxygen consumed and the change in the O^{18}/O^{16} ratio in the closed system. From this information a fractionation factor, α, characteristic of each reaction was calculated from the equation:

$$\alpha = \frac{\ln n/n_0}{\ln y/y_0 + \ln n/n_0}$$

where n_0 is the number of moles of oxygen in the container at time t_0 when the reaction starts, n is the number of moles of oxygen at time t, y_0 is the percentage of O^{18} in the oxygen at time t_0, and y is the percentage of O^{18} in the oxygen at time t; α is defined as the ratio of the percentage of O^{18} in the n moles of oxygen in contact with the respiring mechanism at time t to the percentage of O^{18} in the dn moles of oxygen consumed between t and $(t + dt)$. The equation above is based on the assumption that α is constant.

Rat liver mitochondria were used as the source of cytochrome oxidase. The standard Warburg assay for cytochrome oxidase was followed; succinate was used as substrate (*3*). For the two tyrosinase reactions the Warburg assay procedures recommended by Mallette and Dawson (*4*) were used. Using both *p*-cresol and catechol as substrates made it possible to compare the fractionation involved in the two types of tyrosinase activity. The tyrosinase solutions used were types Ty5483 and Ty547B of the Worthington Biochemical Corp.

The mass spectrometric analyses were carried out on a mass spectrometer similar in many respects to that described by McKinney *et al.* (*5*). A vacuum manifold, with a mercury Toepler pump and a liquid air trap for water vapor, was constructed to transfer gas, without contamination, from the Warburg manometer to spectrometer sample tubes. The Warburg manometer was modified slightly to prevent loss of manometer fluid during extraction of the gas sample. For convenience, atmospheric air was used, since the spectrometer was sensitive enough to obviate the need for pure oxygen.

The individual measurements and average values of α are listed in Table 1. All of these values lie within the range of measurements reported in earlier papers for whole organisms (*1*, *2*). The measurements with cytochrome oxidase were made at two different temperatures, 37° and 25°C. Originally it was decided to run these reactions at the higher temperature in order to increase reaction rate. Later it was found that reaction rate depended more on the quality of the rat liver than on the temperature, and that an adequate reaction rate was obtained at 25°C with good liver preparations. The lower temperature was used in the remainder of the measurements, since less time was required to bring the reaction vessel to the temperature of the bath.

It might be worth while to measure α as a function of temperature, as it is known that the relative reaction rates of O^{16} and O^{18} vary slightly with temperature (*6*); however, the enzyme preparations used are active only in a narrow temperature range (10° to 40°C), and in this range variations in α are probably insignificant (± 0.001). The data indicate that α for each type of reaction is independent of average reaction rate, time of reaction, and total oxygen consumption. Control runs were made by keeping the enzyme preparations in the Warburg vessels separated from their substrates. In the control runs no fractionation was found.

The variations in α from system to

Table 1. Measurements of fractionation factor (α) for the cytochrome oxidase and tyrosinase systems.

	Sample	Temp. (°C)	Total O_2 consumption (ml)	Total reaction time (min)	α
			Cytochrome oxidase		
	1	37	0.115	80	1.015
	2	37	0.160	90	1.014
	3	37	0.198	28	1.011
	4	25	0.194	30	1.012
	5	25	0.153	32	1.011
Av. α					1.013 ± 0.002
			Cresolase		
	1	25	0.320	130	1.011
	2	25	0.173	45	1.011
	3	25	0.183	30	1.007
	4	25	0.199	40	1.015
	5	25	0.190	38	1.008
Av. α					1.010 ± 0.002
			Catecholase		
	1	25	0.186	60	1.007
	2	25	0.199	70	1.014
	3	25	0.222	70	1.011
Av. α					1.011 ± 0.004

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

system are not significant. This might be expected for the two tyrosinase reactions, because the monohydric oxidation involves conversion to *o*-dihydric form as a first step (*7*). For this reason the α measured for the complete monohydric oxidation is an average for the two steps in the reaction, and this average is weighted toward the *o*-dihydric step, since two molecules of oxygen are involved in the *o*-dihydric step for each molecule of oxygen in the monohydric step. For other enzymatically controlled processes a significant difference in α for two different reactions catalyzed by the same enzyme would indicate the presence of more than one active site on the enzyme.

In the previously reported data on living systems average α values ranged from 1.007 for frogs to 1.025 for spinach leaves (*1*). It is probable, therefore, that fractionation processes other than the three enzyme reactions occur in living systems, and further research is indicated. The fractionation factors obtained in the present work would be expected to be closest to the values for unicellular organisms, because in these organisms there are no complex oxygen transport systems which might cause significantly different fractionation. At present the only data available on unicellular organisms are for bacteria, for which Lane and Dole (*1*) reported an average value of 1.015. The significance of this average value is questionable, however, since it is the result of a simple average of seven values of α ranging from 1.008 to 1.029 for two different types of bacteria. Therefore, the fact that 1.015 is close to the values for the enzymes reported here is not necessarily significant.

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20 August 1958

Condylostoma—an Enemy of Bivalve Larvae

Abstract. In laboratory cultures, where larvae of such bivalves as oysters and clams were kept together with large ciliates of the family Condylostomidae, the latter were seen ingesting the larvae. A single *Condylostoma* could contain as many as six larvae. Related species may destroy many bivalve larvae in nature.

Because of their small size and long pelagic existence, larvae of such bivalves as oysters, clams, and mussels are eaten by many holoplanktonic animals ranging from protozoans to fishes. Thorson (*1*), in his excellent account of reproduction and larval development of Danish marine invertebrates, offers a brief review of the enemies of pelagic larvae. His review, however, shows that, with the exception of the dinoflagellate *Noctiluca,* few protozoa subsist, in part, on lamellibranch larvae. My co-workers and I think, therefore, that our observations of *Condylostoma* sp., a heterotrich of the family Condylostomidae, feeding on larvae of the hard clam, *Venus mercenaria,* and the American oyster, *Crassostrea virginica,* are of biological interest and, perhaps, practical significance.

The discovery that *Condylostoma* feeds on lamellibranch larvae was made last winter when populations of an unidentified species of *Condylostoma* established themselves in several large glass vessels serving as intermediate reservoirs for mixed phytoplankton cultures which were fed routinely to the oysters and clams maintained in special trays during the winter (*2*). The temperature in these vessels usually ranged between 16.0° and 20.0°C, and the salinity of the water was normally near 27.0 parts per thousand. Individual *Condylostoma* varied in size from approximately 400 to 900 μ when expanded. In some instances their peristomes reached a length of 225 to 250 μ. They were apparently well adapted to the conditions under which they existed, because small groups, when placed in 500-cm^3 beakers to which a considerable quantity of mixed phytoplankton was added, lived for 2 to 3 months.

The first observation that *Condylostoma* ingests lamellibranch larvae was made when some of the plankton containing these ciliates was fed to cultures of young larvae of the common clam, *Venus mercenaria.* While examining the larvae, we noticed that some were guided into the gullet by the undulating membrane in the peristome of *Condylostoma* and that they were finally engulfed by the ciliates.

The number of larvae that can be ingested by a single *Condylostoma* depends upon the size of the predator and the size of the larvae. In one individual, measuring 661 μ and having a peristome approximately 178 μ long, six larvae could be seen. In smaller individuals, such as the one shown in Fig. 1, only one or two larvae were found. Progressive digestion of the soft parts of the ingested larvae was indicated by the changes occurring in their appearance. Usually the ones ingested first were distinctly paler in color than those ingested later, probably because of the disappearance of the fleshy part. Regardless of the frequent and prolonged observations on feeding *Condylostoma,* ejection of the shells of the larvae has never been observed. It is possible, therefore, that the shells also are eventually entirely digested within the body of the predator.

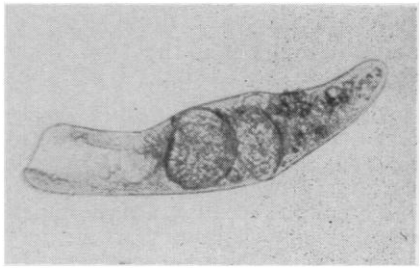

Fig. 1. *Condylostoma* with two ingested larvae of *Venus mercenaria* (*3*). (About × 100)

Perhaps what we saw taking place under laboratory conditions occurred because of the crowding of *Condylostoma* and lamellibranch larvae. It would probably seldom take place in nature, largely because *Condylostoma* is not very common on oyster bottoms or in the masses of water over the oyster beds where larvae are found during the summer. Our observations suggest, however, that members of another closely related family, Folliculinidae, may present a danger. These ciliates are at times extremely numerous in oyster-producing areas such as Chesapeake Bay, where, on occasion, they virtually cover the oyster shells planted as cultch to obtain a new generation of oysters. Some of these may be capable of engulfing oyster larvae, especially when the latter descend to the bottom to undergo metamorphosis into juvenile oysters. The same may be true of other members of the suborder Heterotrichina in which the peristomes and gullets may expand to become large enough for the passage of lamellibranch larvae. Such studies suggest many interesting possibilities.

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3. I wish to express my thanks to Mrs. Matoira Chanley for taking the photograph.

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