Fractionation of Oxygen Isotopes during Respiration

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The stable isotopes of oxygen have been separated by a number of physical processes such as thermal diffusion and distillation. In addition to the physical methods of separation, the oxygen isotopes (*1*) are known to fractionate in the following types of chemical reactions: (i) equilibrium isotopic exchange reactions such as those between carbonate ions and water or carbon dioxide gas and water, (ii) decomposition reactions such as the decomposition of hydrogen peroxide (*2*) or the decomposition of ammonium nitrate (*3*), and (iii) oxidation reactions such as the formation of oxide films on metals (*4*).

Isotopic fractionation of oxygen in living systems was investigated for the photosynthesis reaction by Dole and Jenks (*5*), who found that the liberated oxygen had the isotopic composition calculated for isotopic exchange equilibrium between oxygen and liquid water. Because the equilibrium constant for this reaction, as calculated by Urey (*6*), is very close to unity, photosynthetic oxygen has an isotopic composition close to that of the oxygen in water with definitely less O^{18}, speaking relatively, than the oxygen already present in the atmosphere (*1*). Dole, Hawkings, and Barker (*7*) found only a very small fractionation factor, about 1.003, for the consumption of oxygen in bacterial respiration, but Rakestraw, Rudd, and Dole (*8*) discovered that sea life consumed the O^{16} isotope in the oxygen of air dissolved in the ocean at a slightly more rapid rate than the O^{18} isotope, the fractionation factor being 1.009 (*9*).

Barker (*10*) and Rabinowitch (*11*) suggested independently that the cause of the Dole effect (*12*), which is the greater ratio of O^{18}/O^{16} in atmospheric oxygen than in the oxygen of water, might be the result of fractionation during the back thermal reaction in the oxygen cycle, or in other words in the respiration of oxygen. Barker (*7*) thought that the bacteria living in the soil would be mainly responsible.

To calculate the fractionation factor for the relatively greater consumption of O^{16} as compared with O^{18} by respiration required to substantiate the back thermal reaction theory of the Dole effect, it is necessary to know the average O^{18} content of photosynthetic oxygen, fresh water, and ocean water and to make an estimate of the relative contribution of photosynthesis from fresh and ocean waters. From the estimates given by Rabinowitch (*11*, p. 7), we have assumed that 85 percent of photosynthesis occurs in the oceans and 15 percent in fresh water. Using the percentage of O^{18} values given in Table 1, we can calculate that photosynthetic oxygen contains on the average 0.2003 percent of O^{18}. The ratio of the percentage of O^{18} in atmospheric oxygen to that in photosynthetic oxygen, 0.2039/0.2003, gives what we might call the oxygen isotope 16 production factor, or 1.018. For a steady-state condition, this must also equal the fractionation factor during respiration. It was the purpose of the research described here to study oxygen isotope fractionation factors during the respiration of typical biological systems and to test the back thermal reaction theory of the Dole effect. We felt that the earlier work of Dole, Hawkings, and Barker (*7*) needed to be repeated, using different biological systems and more accurate isotope abundance measuring equipment.

Plan of the Experiment

Using an apparatus similar to that described by Brown (*13*), we allowed various organisms to grow in air with the carbon dioxide removed by absorption in KOH solution and with the respired oxygen being continually replaced by pure oxygen from a Saran balloon. After a length of time, the oxygen of the air in the flask containing the organism was analyzed for its percentage of oxygen and for the percentage of O^{18} in the oxygen. The mass spectrometer used for these measurements has already been described (*9*).

Let a fractionation factor α be defined as the ratio of the percentage of O^{18} in the oxygen of the air in contact with the organism to the percentage of O^{18} in the oxygen being consumed by the organism at any moment:

$$\alpha = y/y_r \qquad (1)$$

where y is the percentage of O^{18} in the air of the flask at any selected time and y_r is the percentage of O^{18} in the oxygen being consumed by respiration at that same selected moment. This definition of α makes α greater than unity if O^{16} is consumed at a relatively more rapid rate than O^{18}. The differential material balance equation for the O^{18} isotope on respiration of dn moles of oxygen then becomes

$$n_0(y + dy) = n_0 y - y_r dn + x_0 dn \qquad (2)$$

where n_0 is the initial number of moles of oxygen in the respiration chamber (should be constant throughout the experiment) and x_0 is the percentage of O^{18} in the oxygen entering the respiration chamber from the Saran balloon. Eliminating y_r by means of Eq. 1 and integrating, we obtain

$$\alpha = \frac{y - y_0 e^{-m/\alpha}}{x_0 - x_0 e^{-m/\alpha}} \qquad (3)$$

where m equals n/n_0, the ratio of oxygen consumed to the amount of oxygen initially in the respiration flask. Inasmuch as α is close to unity, α can be calculated from Eq. 3, first assuming α in the exponential terms to be equal to y/x_0. The calculation can then be repeated using the new value of α. Or, if the organism respires enough so that m is 3 or 4, the exponential terms become insignificant and α is then equal to y/x_0. If an excess of pure oxygen leaks into the flask, the analysis of the air will indicate such an effect and a correction to the data can be readily applied.

A special apparatus was used for the *Homo sapiens* experiment in which the human being under observation breathed through one tube from a reservoir of air whose oxygen supply was continually replenished, and breathed out through another tube. His exhaled breath passed first through a large KOH tube, then through a sampling flask before it was rebreathed. Two rubber balloons at-

Table 1. Oxygen-18 content of oxygen from various sources.

Source	O^{18} (%)	Reference
Air	0.2039	(*16*)
Fresh water	0.1981	(*17, 18*)
Ocean water	0.1995	(*18*)
Photosynthetic oxygen from fresh water	0.1991	(*5*)
Photosynthetic oxygen from ocean water	0.2005	(*5*)
Average photosynthetic oxygen	0.2003	

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tached to the KOH reservoir enabled the gas volume of the system to expand and contract with each breathing cycle and permitted quick estimates to be made of the amount of additional oxygen required.

Results and Conclusions

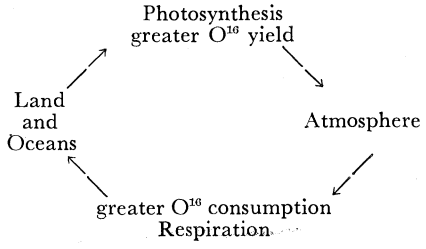
Figure 1 illustrates the data that are also collected in Table 2, where the order of agreement between successive experiments can be seen. The dotted vertical line of Fig. 1 represents the fractionation factor required to account quantitatively for the Dole effect. If we assume that the consumption of oxygen is entirely by respiration with allocation to various organisms according to the schedule, 75 percent to bacteria, 10 percent to other fungi (5 percent to molds and 5 percent to mushrooms), and 15 percent to higher plants (leaves and roots), a composite α equal to 1.016 is calculated. This division of the respired oxygen among the various species has no quantitative basis; however, if the allocation is changed to 50 percent bacteria, 10 percent molds, 10 percent mushrooms, 15 percent leaves, and 15 percent vegetables, the composite α is scarcely changed; it is 1.017. The forest litter experiments were performed on material (leaves, sod, and so forth) that was collected during October when biological activity was declining; nevertheless, the fractionation factors obtained were close to the average values estimated. Within the limit of uncertainties of the calculations and of the experimental results, the composite fractionation factors are in good agreement with the value of α equal to 1.018 that is required to explain the Dole effect.

It is interesting to note that α for the fiddler crab, 1.010_5, a marine animal, agrees well with the value 1.009 found for α by Dole, Lane, Rudd, and Zaukelies (*9*) in the consumption of oxygen by marine organisms living in the ocean.

There was a considerable fluctuation in the results for the bacterial experiments for reasons unknown to us.

Oxygen Isotope Cycle

Similar to an oxygen cycle in nature, we can now write down an oxygen isotope cycle as follows:



Photosynthesis yields oxygen containing a higher O^{16}/O^{18} ratio than the oxygen of the atmosphere, while respiration consumes oxygen containing a higher O^{16}/O^{18} ratio than the oxygen of the atmosphere and the same ratio as that of photosynthetic oxygen, thus leading to the nonequilibrium steady-state value of the O^{16}/O^{18} ratio in the atmosphere. In other words, the O^{16}/O^{18} ratio of atmospheric oxygen has risen to a point such that the ratios for photosynthetic oxygen delivered to the atmosphere and the oxygen extracted from the atmosphere by respiration are equal (*14, 15*).

Table 2. Oxygen isotope fractionation factors during respiration. The average fractionation factor for all vegetables was 1.009; for all bacteria 1.015.

Organism	Fractionation factor	
	Expt.	Av.
Homo sapiens	1.015	1.018
	1.019	
	1.019	
Spinach leaves	1.029	1.025
	1.024	
	1.023	
Crab (*Uca pugilator*)	1.011	1.010_5
	1.011	
	1.009	
	1.011	
Frog (*Acris crepitans*)	1.006	1.007
	1.009	
Carrot	1.010	
	1.008	
Potato	1.009	
Mushrooms (*Agaricus campestris*)	1.025	1.023
	1.023	
	1.022	
Molds (*Penicillium*)	1.019	1.018
	1.018	
	1.017	
Bacteria (*Aerobacter aerogenes*)	1.012	
	1.029	
Bacteria (*Achromobacter fischeri*)	1.008	
	1.013	
	1.017	
	1.021	
	1.008	
Forest litter (upland oak and hickory forest)	1.014	
Forest litter (subclimax oak and maple floodplain forest)	1.016	

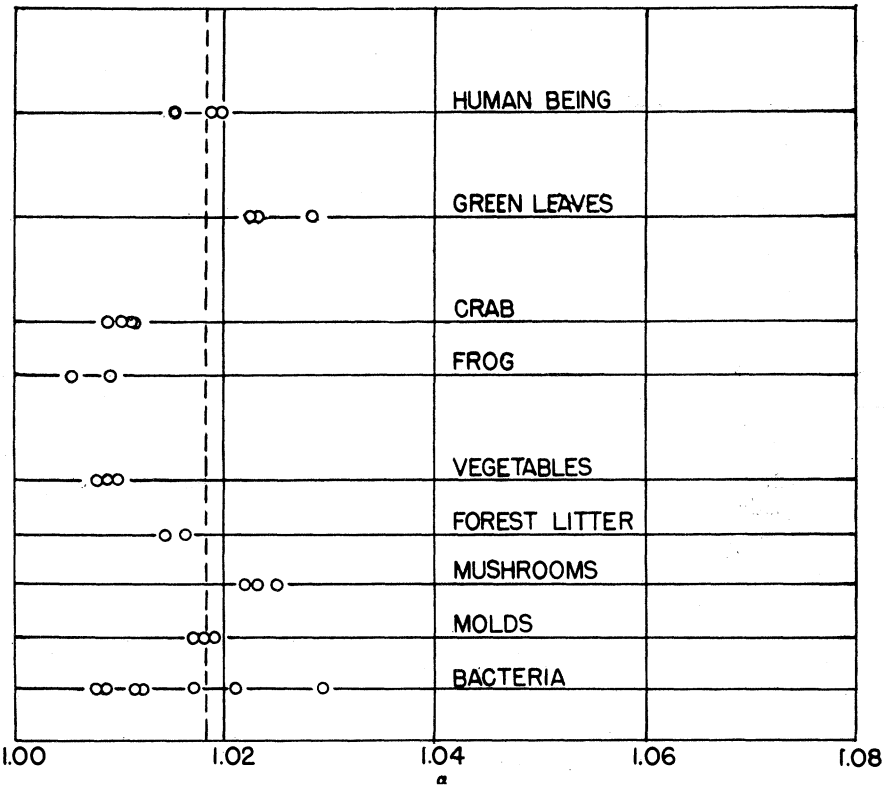


Fig. 1. Oxygen isotope fractionation factors for various biological systems.

References and Notes

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R. C. Osburn, Connoisseur of Living

When I first met Raymond C. Osburn in 1936, he was 64 years old and chairman of the department of zoology and entomology at Ohio State University. I came to him as a new member of the staff on the entomological side of his department. A tall, slim gentleman with the lines of his face emphasizing his happy nature, he received me graciously in his office. Looking at me through his horn-rimmed glasses with a lighted stogie in his hand, he talked easily about the department and his own interests. He was thoroughly at home in this book-filled, specimen-cluttered room, which contained a work table, a roll-top desk, and a highboy desk, at which he sometimes stood to do his work.

Osburn had worked in that room since 1917 and in the opinion of some members of his staff had become too comfortable there to exert himself competitively for the benefit of his department. I believe, however, that he was too gentle and honest and too absorbed in his professional work to apply pressure or to indulge in campus politics to obtain what was needed. He tacitly encouraged the senior members of his staff to help themselves, if they could; and they did. He was not without accomplishment, however, in the expansion of facilities for zoological research and teaching at O.S.U., for it was generally acknowledged that he was responsible for the establishment of the Franz Theodore Stone Laboratory at Put-in-Bay, Ohio, through the generosity of Julius F. Stone.

The only instrument I remember in Osburn's office was a binocular dissecting microscope, for he was essentially a direct observer of nature and a natural philosopher who did not resort to experimentation and instrumentation—an old-fashioned naturalist.

Osburn's broad professional interests are indicated by his membership in 22 societies, national and local; by the places in which he chose to spend his summers, usually hydrobiological stations; and by his numerous publications of amazing variety. He worked on Bryozoa, oysters, fish, dragonflies, and two-winged flies, particularly the beneficial flies of the family Syrphidae. He promoted the care and use of natural resources in Ohio, gave generous service to *Biological Abstracts,* and held office in many of his societies. Although no scientific law or well-known hypothesis is associated with his name, he added to zoological knowledge all along the line, and his advice and help were in demand in the aquatic side of his work.

Surprisingly, Osburn's most comprehensive and important work was done after his retirement at age 70 in 1942. Then he was called to Southern California to study the collections of Bryozoa made by the Hancock Foundation expeditions in Pacific waters from Alaska to Peru. He described and directed the illustration of many new species and produced three volumes on the taxonomy and distribution of these marine organisms. No one was more surprised than Osburn when, in recognition of the excellence of his work as represented by the first volume, he was named to receive the Daniel Giraud Elliot medal of the National Academy of Sciences for 1950. I was present in the auditorium of the National Academy on the evening of 26 April 1954 when Alexander Wetmore presented Osburn to the audience, and the award was conferred upon him by Detlev W. Bronk. At 82 he stood as straight and responded as easily and gracefully as ever. Then he and Mrs. Osburn departed for a visit to one of their favorite regions, the Great Smokies. What an inspiration to young biologists was this grand climax to Osburn's scientific career! He died on 6 August 1955.

To me Osburn was more important as a connoisseur of living than as an impersonal scientist. He loved literature and was noted among his students and friends for his ability to read poetry aloud. I once requested him to read *Tam O'Shanter* at a Hallowe'en party—he knew it by heart and declaimed it with relish in his rolling bass voice. He was noted also for his skill in composing light verse for any occasion. It sparkled with his quips and always had the light touch without barbs.

Thanks to modern electronics, Osburn seemed to be with us on the morning of 29 November 1955 in Cincinnati when Ohio State University alumni of his department assembled at breakfast in his memory. A recent tape-recording of his recitation of some of his most humorous verse was heard, and in our imagination we saw again that tall, gallant figure, stogie alight, eyes twinkling, head mobile, performing as of old.

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Everyone who enjoys thinks that the principal thing to the tree is the fruit, but in point of fact the principal thing to it is the seed. Herein lies the difference between them that create and them that enjoy.—FRIEDRICH WILHELM NIETZSCHE, *Maxims.*