Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 1727–1731, March 1988
Geophysics

Oxygen and carbon isotopic compositions of gases respired by humans

(isotopic fractionation/hemoglobin levels/lung membrane diffusion/atmospheric oxygen)

SAMUEL EPSTEIN AND LEILA ZEIRI*

Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125

Contributed by Samuel Epstein, November 17, 1987

ABSTRACT **Oxygen-isotope fractionation associated with respiration in human individuals at rest is linearly related to the fraction of the O_2 utilized in the respiration process. The slope of this relationship is affected by a history of smoking, by vigorous exercise, and by the N_2/O_2 ratio of the inhaled gas. For patients who suffer anemia-related diseases, the slope of this relationship is directly proportional to their level of hemoglobin. These results introduce a new approach for studying the mechanisms of O_2 consumption in human respiration and how they are affected by related diseases.**

The preferential use of the ^{16}O isotope in respiration was known from the early work of Dole and Jenks (1), Lane and Dole (2), and Dole (10), from their analysis of gas samples involved in respiration by plants and by one sample from a human individual. However, this early work was primarily aimed at explaining why atmospheric O_2 is enriched in ^{18}O compared to the oxygen in the hydrosphere.

In this paper we present a study of the isotopic fractionation of O_2 associated with the respiration by humans. We analyzed breath samples from people of different sexes, ages, and weights, as well as samples from people who suffered anemia-related diseases. We determined the relative proportions of O_2, N_2, and CO_2 gases in the breath samples, as well as the isotopic composition of oxygen in both O_2 and CO_2 and carbon in the CO_2 gases. The isotopic analyses are reported as:

$$\delta^{18}O = \left(\frac{^{18}O/^{16}O \text{ (sample)}}{^{18}O/^{16}O \text{ (standard)}} - 1 \right) \times 1000.$$

For $\delta^{13}C$, the $^{18}O/^{16}O$ ratios are substituted by the $^{13}C/^{12}C$ ratio. The precision of measurement is $\pm 0.05‰$ (part per thousand).

Although in this paper we deal only with human respiration, the techniques we developed here as well as the results we obtained should be applicable to studies of the mechanisms of oxygen-isotope fractionation involved in the different members of the terrestrial and marine biota. One of the objectives of this work was to provide a basis for future clinical investigations of problems relating to consumption of O_2 by humans as well as to contribute to studies of the causes of the ^{18}O enrichment of atmospheric O_2.

EXPERIMENTAL

The experimental procedures are based on well-established techniques (3–5). Our subjects inhaled atmospheric air, whose O_2 concentration and isotopic composition are accurately known. They held their breath from between 10 and 60 sec and exhaled some of the air into a balloon. A fraction of the air in the balloon was quickly transferred to a glass volume sealed by two stopcocks. A measured aliquot of this sample was transferred into a vacuum line for separation into its different components for volumetric and isotopic analyses. In some cases the latter part of the exhaled air was selected for analyses to maximize the fraction of the O_2 used in the respiration process.

The respired CO_2 and H_2O was extracted by cycling a 20- to 30-cm^3 aliquot of the exhaled air for about 15 min through a liquid nitrogen-cooled trap. This process isolated the condensible CO_2 and H_2O from the noncondensible gas in the transpired sample. The CO_2 was released by warming the trap in a dry-ice bath and was transferred into a sample tube for manometric and isotopic measurements. The H_2O was pumped away. The air O_2 was converted to CO_2 by cycling the remaining CO_2/H_2O-free aliquot of the exhaled air over a carbon rod that was heated to red heat by passing current through it (see Fig. 3) (5). Upon formation, the CO_2 was frozen out in liquid nitrogen-cooled traps and isolated for manometric and oxygen-isotope analyses. The kinetics of the O_2-to-CO_2 reaction was tested on air samples, whose concentration and $\delta^{18}O$ content of O_2 is well known, to determine the best conditions for complete and rapid conversion of O_2 to CO_2. Incomplete conversion can cause serious errors in the yields and in the isotopic data. An incomplete conversion of exhaled air O_2 to CO_2 is usually due to the formation of noncondensible CO near the end of the reaction. Consequently the residual noncondensible air fraction, which should consist almost entirely of N_2 and be free of any oxygen-containing compounds, was tested for the presence of CO by circulating it over cupric oxide at 850°C. When CO was present, it was converted to condensible CO_2, which could be isolated and measured precisely. Its presence in the N_2 fraction indicates that the original conversion of the O_2 to CO_2 in the exhaled sample was incomplete. Such samples were discarded, and the experiment was repeated. Actually, only upon rare occasions was it necessary to discard such a sample. In summary, the O_2 and the expired CO_2 present in the aliquots of the exhaled breath sample were both analyzed separately for their volume and $\delta^{18}O$ values. The $\delta^{13}C$ was also determined for the expired CO_2.

The oxygen-isotope fractionation associated with the respiration processes was determined by plotting the fraction (X) of the inhaled O_2 used in respiration against the $\delta^{18}O$ of the unreacted O_2. The value of X was calculated in the following way.

For the aliquot of an exhaled sample: $X = 1 - A/A°$, where A is the volume of O_2 in the aliquot of the expired air, and $A°$ is the volume of the initial O_2 in the aliquot of inhaled air. Since the ratio of N_2/O_2 in the atmosphere is 3.76, $A° = B/3.76$, where B is the volume of N_2 in the aliquot of the respired sample.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*Permanent address: Department of Chemistry, Ben-Gurion University of Negev, P. O. Box 653, Beer-Sheba 84105, Israel.

Now $B = V - (A + C)$, where V is the total volume of the aliquot of expired air from which the H_2O was removed, and C is the volume of CO_2 in the exhaled sample. Thus $A^o = [V - (A + C)]/3.76$.

Therefore, $X = 1 - [A(3.76)]/[V - (A + C)]$. The quantities C, A, and V are measured. Consequently X is calculable. The value of X also may be calculated as X_1, where: $X_1 = 1 - A/(A + C)$. However, the value of X_1 is lower and less accurate because some of the O_2 is used in the production of H_2O, and the volume of CO_2 in an expired sample is not equivalent to the volume of O_2 taken up in the blood. The difference between X and X_1 is actually small but variable, depending upon the experiment. We used X_1 only in the case when air samples enriched in O_2 were used in the respiration experiments.

RESULTS AND DISCUSSION

The Relationship Between $\delta^{18}O$ of O_2 in Respired Air and X; the z Value. The change in the isotopic composition of O_2 as a function of the amount used during respiration was determined for 14 normal, healthy volunteers (Table 1), who varied in age between 6 and 64 years. Straight-line relationships with characteristic slopes were obtained for each of the people involved (Fig. 1). This relationship for a specific person was obtained by using exhaled breath samples taken at random times, each exhaled sample representing a single point on the line. The longer the subject held his breath after inhaling, the larger the fraction of the inhaled O_2 that was used and the higher the $\delta^{18}O$ of the O_2 in the expired sample.

The data points are not included in Fig. 1 because it would complicate the graphs too much by some overlapping points. However, a representative curve, which includes the data points taken as described above for subject 5, is shown in Fig. 2. Included in this figure are the data obtained for six consecutive samples (1–5, 10) from a single exhalation. As expected, the first aliquot was the least depleted in O_2; the last sample was the most depleted in its O_2 and had the highest $\delta^{18}O$ value. These graphs (Figs. 1 and 2) show that it is almost irrelevant how the exhaled breath is sampled to preserve the characteristic line for a person, as long as the subject is not performing strenuous exercise (see below).

The lines shown in Fig. 1 are the least squares-calculated curves of the data using Eq. **1**:

$$z = (y - b)/X, \qquad [1]$$

in which y is the $\delta^{18}O$ of the O_2 in the exhaled sample, X is the fraction of the inhaled O_2 that is used in respiration, and b is the $\delta^{18}O$ of the atmospheric O_2 inhaled. The value of z indicates the magnitude of the oxygen-isotope fractionation associated with the respiration of any particular person. The values for z and σ, the standard deviation, are given in Table 2.

Table 1. The vital statistics of participants in respiration experiment

Participant	Years smoked	Age	Sex	Height, cm	Weight, kg
1	13	43	M	176	90
2	22	40	M	185	93
3	9	27	F	168	52
4	12	33	M	176	82
5	32*	63	M	173	82
6	5	22	F	172	55
7	25	45	F	168	58
8	0	6	M	112	19
9	0	52	F	155	57
10	0	35	M	189	86
11	0	26	M	191	91
12	0	30	F	166	52
13	0	28	F	170	61
14	0	30	F	167	59

*Smoked for 32 years; stopped 10 years ago.

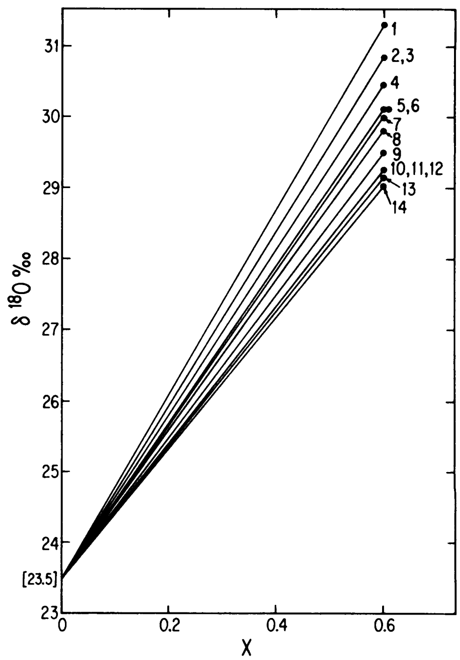

FIG. 1. Relationship between $\delta^{18}O$ of O_2 in the exhaled breath samples and the fraction of the inhaled O_2 utilized (X). The curves represent the least-squares line for the data for each subject (see Table 2). The $\delta^{18}O$ of the atmospheric oxygen is 23.5‰. The relationship was measured for 14 subjects, but the curves for some of the subjects were the same within experimental error. Consequently, only one curve was drawn to represent these subjects (e.g., 2 and 3, 5 and 6, and 10, 11, and 12).

Three interesting aspects of the oxygen-isotope fractionation in human respiration are: (*i*) humans preferentially use ^{16}O in respiration and affect the $^{18}O/^{16}O$ ratio of atmospheric O_2, confirming the initial, but less accurate, preliminary work of Lane and Dole (2); (*ii*) the relationship between the $\delta^{18}O$ of the O_2 in the respired sample of air and the fraction of O_2 used (X) is linear; and (*iii*) the isotopic fractionation associated with respiration in humans varies significantly between individuals.

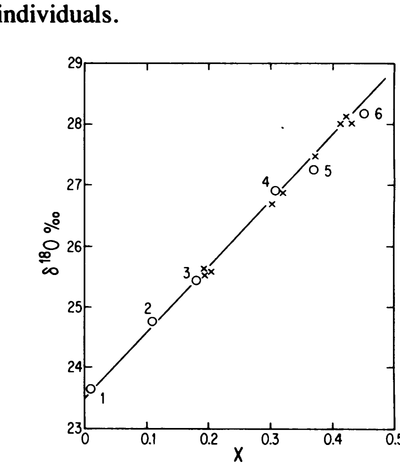

FIG. 2. Relationship as in Fig. 1 for subject 5. The x points represent different exhaled samples; the longer the time the breath was held, the higher were the X and $\delta^{18}O$ values. The data represented by circles numbered 1–6 are samples from a single exhalation. They are numbered in the succession they were taken.

Table 2. The fractionation of oxygen isotopes per fraction of O_2 used and the $\delta^{18}O$ and $\delta^{13}C$ of the respired CO_2

	Respired O_2		Respired CO_2	
Participant	z‰	σ‰	$\delta^{18}O$‰	$\delta^{13}C$‰
1	13.0	0.04		
2	12.2	0.05	−5.4	−19.6 to −22.6
3	12.2	0.09	−6.8	−21.9 to −22.0
4	11.6	0.09	−5.6	−20.0 to −23.0
5	11.0	0.14	−5.4	−22.4 to −23.0
6	11.0	0.06		
7	10.8	0.04		
8	10.5	0.23	−4.3	−20.0 to −22.5
9	10.0	0.04	−7.4	−21.8 to −23.2
10	9.6	0.12	−6.1	−21.6 to −22.6
11	9.6	0.07	−6.4	−18.7 to −19.8
12	9.6	0.05	−4.9	−22.0 to −23.0
13	9.4	0.05	−4.9	−22.0 to −23.5
14	9.2	0.12	−5.7	−21.0 to −23.5

σ is the standard deviation. The number of data, per participant, ranged between 5 and 17. z = ($\delta^{18}O$ of O_2 in the exhaled gas − 23.5)/X, where X is the fraction of the atmospheric O_2 used and 23.5 is the $\delta^{18}O$ of atmospheric O_2.

A number of experiments were performed to determine some of the nonbiological factors affecting the degree of oxygen-isotope fractionation during respiration.

Isotopic Fractionation in Combustion of Graphite. Basically O_2 is consumed by humans for oxidation of organic matter. It was of interest to ascertain if some very simple experiments, such as the oxidation of carbon, could provide some information useful for evaluation of the factors that govern the isotopic effects observed in Fig. 1. By using the apparatus shown in part in Fig. 3, samples of pure O_2 of known $\delta^{18}O$ values and atmospheric O_2 samples were converted into CO_2 to various degrees of completion. The resulting CO_2 was analyzed for CO_2 yields and $\delta^{18}O$ values. With these data and a simple material balance, the yield and $\delta^{18}O$ of the unreacted oxygen were calculated, and plots similar to those for the respired samples shown in Fig. 1 were constructed.

Two types of experiments were performed. In one case the oxygen or air samples were circulated over a hot graphite rod by means of a Toepler pump shown in Fig. 3. The heated graphite rod was in a glass trap that represented about one-fourth of the total volume of the system. It was immersed in liquid N_2 to freeze out the CO_2 as it formed. In the other case, the total reacting gas was confined in the trap containing the heated graphite. Under these circumstances, the gas circulated because of the strong temperature gradient established between the heated graphite in the center of the trap and the cold walls. This rapid circulation decreases the effect of diffusion of the O_2 through the N_2 to the location of the hot carbon, and the collision rate of the O_2 on the surface of the hot graphite probably controls the initial isotopic fractionation.

The relation betweeen the $\delta^{18}O$ of the unreacted O_2 and X, the fraction of the O_2 reacted, for pure O_2 and for air is shown in Fig. 4. For the purpose of comparison, the $\delta^{18}O$ of the initial O_2 in all experiments was normalized to a value of 23.5‰.

The results in Fig. 4 show that the $\delta^{18}O$ of the pure O_2 varies most strongly with X (curve 1). This reaction, in the confined volume of the trap, shows the most simple relationship described by the Rayleigh equation:

$$\frac{(^{18}O/^{16}O)_s}{(^{18}O/^{16}O)_o} = \frac{1000 + \delta^{18}O_s}{1000 + \delta^{18}O_o} = f^{(\alpha-1)}.$$

The $\delta^{18}O$ of the residual O_2 (s), which has not reacted as compared with the $\delta^{18}O$ of the initial O_2 (o), is equal to the fraction of the O_2 unreacted (f) to the power of $\alpha - 1$, where α is the fractionation factor for this reaction. Note that in our terminology, $X = 1 - f$.

For pure O_2 gas, we calculate $\alpha = 1.031$, which is equal to $(34/32)^{1/2}$, indicating that the fractionation could be due to the collision frequency of the oxygen with the graphite rod.

In the case of the atmospheric gas (21% O_2), where the gas involved in the reaction is in the confined volume of the trap (Fig. 4, curve 2), the initial oxygen-isotope fractionation of 1.031 is similar to that for pure O_2 but decreases as the O_2 is removed from the air and the N_2/O_2 ratio increases. The value of z (Eq. 1) is 31‰. The reaction that gives the lowest fractionation factor associated with the combustion of carbon involves the circulation of the air through the Toepler pump and trap system (Fig. 4, curve 3). In this case, the value of z (Eq. 1) is 18.5‰ and is governed by the presence of a high N_2/O_2 ratio. The initial oxygen-isotope-fractionation factor for the human respiration is only ≈1.013, and the z value is 13‰ (Fig. 4, curve 4).

A comparison of the $\delta^{18}O$ vs. X plot for a respired sample with those obtained from the combustion of carbon provides some useful hints on the factors that govern the isotopic

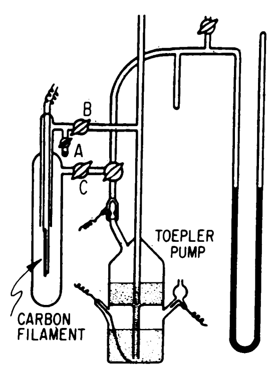

FIG. 3. Toepler pump apparatus, when connected to the rest of the vacuum line, circulates a sample of air over the hot carbon filament to convert the O_2 in the air quantitatively to CO_2. A is a short tube that contains a molecular sieve that allows the transfer by cooling of the original CO_2/H_2O-free aliquot of air sample or pure O_2 to the trap volume within stopcocks A, B, and C.

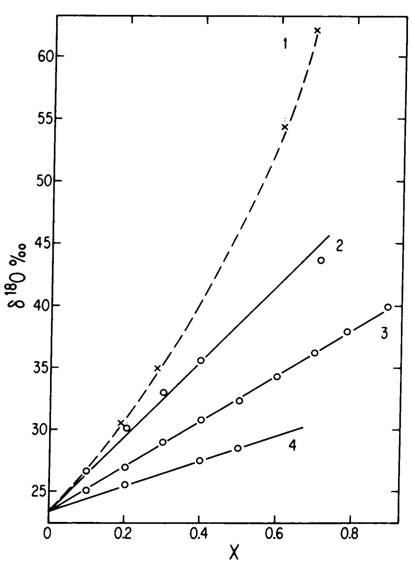

FIG. 4. Relationship between $\delta^{18}O$ and X curves: 1, the conversion of pure O_2 to CO_2 in the confined volume of the trap (the x points are the data, and the broken line is the calculated relationship with the Rayleigh equation); 2, the same experiment as 1, using air as the starting material; 3, circulation of air through the vacuum line and over the graphite; 4, subject 5 from Fig. 2 for comparison.

fractionation found in the respiration process. The condition most similar to O_2 uptake in the lung is probably represented by curve 2 in Fig. 4, where the oxidation was carried out in a confined volume containing the total air sample and the hot graphite rod. Like O_2 in the lung, the O_2 in experiment 2 of Fig. 4 is in continuous contact with the site of O_2 uptake (the graphite rod). However, the oxygen-isotope fractionation observed for the respired samples (Fig. 4, curve 4) is lower than any of those observed for the graphite oxidation. This fact alerts us to the possibility that, in the respiratory process, factors other than simple oxidation of organic matter affects oxygen-isotope fractionation.

The data in Fig. 4 point out the importance of the N_2/O_2 ratio in affecting oxygen-isotope fractionation—namely, the higher the N_2/O_2 ratio is, the lower the isotopic fractionation. This effect was found in human respiration as well (Fig. 5). The slope of the curve of the $\delta^{18}O$ vs. X relationship increases by a factor of 2, reminiscent of the fractionation factor observed for the combustion of graphite with pure O_2.

A Mechanism for Isotopic Fractionation in Air–Hemoglobin Interaction. It is well known that respiration in humans is a multistep process. O_2 in the alveoli diffuses through two membranes into the pulmonary capillaries and then into the blood cells where it reacts with the hemoglobin. There are various diffusion steps, as well as chemical steps, that may be responsible for the fractionation of the oxygen isotopes. Our experiments may be useful to identify the steps in the respiration process that are critical in determining the magnitude of the oxygen-isotope fractionation.

Let us consider the simplest type of multistep mechanism, whereby the oxygen goes through a two-step process in the production of CO_2. The two-step process could involve oxygen diffusing through the pulmonary membranes with a rate constant k_1, followed by the reaction of O_2 with the hemoglobin with a rate constant k_2, followed by other reactions to form CO_2.

$$O_2 \underset{k_1}{\overset{\alpha_1}{\rightarrow}} [O_2] + \mathrm{Hb} \underset{k_2}{\overset{\alpha_2}{\rightarrow}} \mathrm{Hb}\,[O_2], \qquad [2]$$

in which α is the fractionation factor and k represents relative rates. Let us suppose that these two steps have different kinetic oxygen-isotope fractionations (α_1 and α_2, respectively). The oxygen-isotope fractionation of the total process depends on the relative rates of steps k_1 and k_2. If k_1 is the slow step and, thus, the rate-controlling one for the total respiration process, the oxygen-isotope-fractionation factor associated with step 1 (α_1) will be primarily responsible for the overall oxygen-isotope-fractionation factor in the respiration process. Thus, this step will determine the total oxygen-isotope fractionation that we observe. Similarly, if step k_2 is the rate-controlling step, then the fractionation factor α_2 will determine the overall fractionation for the O_2 utilization. In the first case, once the O_2 passes through the membranes, the O_2 will be used rapidly and completely; thus, no isotopic fractionation will take place in the second step. In the second case, O_2 will simply diffuse back and forth through the membranes destroying the diffusion–fractionation effect. If the rates for step k_1 and step k_2 are comparable, then the total fractionation factor will be determined by a combination of α_1 and α_2. If this simple model is useful, then it should account for the magnitude of the oxygen-isotope-fractionation factors of respiration as well as suggest experiments whose results could be predicted based on this simple model.

Our data in Tables 1 and 2 show that those people who smoke have a significantly higher fractionation factor than the nonsmokers have. Of the 14 subjects tested, those who smoke have z values greater than 10.5 ‰ and those who do not smoke have z values less than 10.5‰. This observed effect is well beyond experimental error. The higher fractionation factor could be due to damage of membranes and, presumably because of this, greater difficulty for O_2 to diffuse through these membranes (6). This should result in a slower k_1 step (Eq. 2). The slower the rate, the larger the effect of step k_1 in determining the total oxygen-isotope fractionation. If α_1 is greater than α_2, then the effect of smoking on the oxygen-isotope fractionation we observed is expected. Obviously, this important isotope effect can be tested further by examining a larger number of subjects.

The Relationship Between the Degree of Oxygen-Isotope Fractionation (z Value) and the Level of Hemoglobin. The simple model proposed above could be tested another way. The rate of uptake of O_2 from the lung to the blood should depend on the hemoglobin concentration. The lower the concentration of hemoglobin, the smaller is the k_2 value and the more important is α_2 in determining the overall oxygen fractionation (z value) for the respiration process.

We have analyzed the isotopic composition of O_2 in breath samples taken from patients who suffer from various degrees of anemia of a variety of etiologies. This allows analysis of the effect of hemoglobin count on oxygen-isotope fractionation during respiration. There is a dramatic decrease in the z value for oxygen-isotope fractionation of patients with reduced hemoglobin counts (Fig. 6). The z value varies between 12‰ and 3‰ (Table 2). These data suggest that, at a low concentration of hemoglobin, the incorporation of O_2 by the hemoglobin becomes more influential in determining the overall fractionation factor, as if the reaction with hemoglobin is at least in part the rate-controlling step. The oxygen-isotope-fractionation factor associated with this step would be much lower than that associated with the diffusion

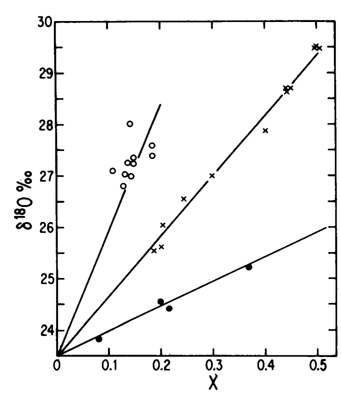

FIG. 5. Comparison of the $\delta^{18}O$ versus X plot for the breath samples of subject 4 (Fig. 1) at rest (×), with air enriched in O_2 (○), and during vigorous exercise (●). Note the high z values for the enriched-O_2 case and the low z values for the vigorous exercise case.

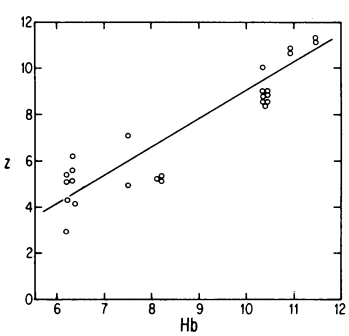

FIG. 6. Relationship between the z value and the hemoglobin count of the subjects examined. The diameter of the data points approximately equals the experimental error of the $\delta^{18}O$ values. The spread in z values is probably partially due to individual differences other than those due to hemoglobin concentration.

of oxygen through the pulmonary membranes. However, it remains to be determined whether the isotopic effect is due to the uptake of O_2 by hemoglobin *per se* or the actual oxidation process in the tissues.

The Effect of Exercise on the z Values. A series of runs were made to determine the effect of vigorous exercise on the relationship between $\delta^{18}O$ and X. Fig. 5 includes a plot of the isotope data for subject 4 at rest and during exercise. The fractionation of the oxygen isotopes drops drastically when the samples are taken during exercise. An exercising subject behaves as if the hemoglobin in the lungs is decreased, resulting in a decreased k_2 and a greater importance of α_2 in the overall fractionation. Alternatively, the greater expansion of the alveoli associated with exercise might increase the diffusibility of O_2 (perhaps by altering the membrane characteristics), resulting in a larger k_1 value and increasing the role of α_2 in determining the overall fractionation. Both of these alternatives would result in the relationship observed in Fig. 5. Clearly, the actual physical processes resulting in our observations await further study.

The $\delta^{18}O$ and $\delta^{13}C$ of the CO_2 Formed During Respiration. We also measured the $\delta^{13}C$ and the $\delta^{18}O$ of the CO_2 formed in the respiration process. These are shown in Table 2. The oxygen atoms of the CO_2 and those of the body H_2O are rapidly exchanged, catalyzed by carbonic anhydrase. Therefore, the $\delta^{18}O$ of the CO_2 should reflect the $\delta^{18}O$ of the body H_2O. The $\delta^{18}O$ of the body H_2O is the steady-state balance of the intake and output of oxygen atoms in various forms: for example, the $\delta^{18}O$ of air O_2, drinking H_2O, oxygen of food, etc., balanced by the $\delta^{18}O$ loss by evaporation, CO_2 and H_2O loss by respiration, and loss of oxygen by other natural processes. Luz *et al.* (7) have presented a discussion of this issue. For our subjects the $\delta^{18}O$ of the body H_2O has a range of about 3.1‰, which probably reflects each individual's diet, drinking habits, and oxygen-isotope fractionation during respiration. For members of the same family, subjects 4, 8, and 14, the $\delta^{18}O$ range is only 1.3‰.

The $\delta^{13}C$ should reflect the source of organic matter that is being used to provide the body energy. Although it will be generally true that the $\delta^{13}C$ content of the body reflects the $\delta^{13}C$ of the food used (8), the $\delta^{13}C$ of the CO_2 is probably less representative of the $\delta^{13}C$ of the total body carbon but more of the food intake at the time prior to sampling (9).

SUMMARY AND CONCLUSIONS

We have shown conclusively that the oxygen-isotope fractionation associated with respiration by humans is large compared to our precision of measurement. This fractionation varies from individual to individual. In addition, the fractionation of oxygen isotopes during respiration is affected by the hemoglobin count in the blood, by smoking, and by vigorous exercise. Our data suggests a variety of experiments that can be done to elucidate the mechanisms involved in causing these large oxygen-isotope fractionations. We can foresee that measuring the $\delta^{18}O$ of expired O_2 and CO_2 may provide a useful way of monitoring certain types of respiratory and blood diseases.

From a geochemical point of view, it would be useful to study the $\delta^{18}O$ of O_2 from a variety of sources, including ancient iron oxides and atmospheric O_2 in various localities. In particular, it would be interesting to ascertain the isotopic composition of O_2 in the early history of the Earth and to get some ideas of the magnitude of the respiration processes in the biota at that time. This kind of data may provide information about the N_2/O_2 ratio in the early Earth's atmosphere.

We acknowledge the cooperation of the volunteers who provided breath samples used in this research. We are grateful to Dr. Dan Cooper, Chief, Division of Respiratory and Critical Care, Department of Pediatrics, Harbor-University of California, Los Angeles Medical Center, who provided some of the samples and whose active interest was important to the completion of this work. We thank Dr. Charles Mittman, former executive Medical Director, and Mr. Kirk McClelland from the Pulmonary Department, both at the City of Hope, Duarte, CA, for providing some of the samples for our analyses. We thank Joseph Ruth and Eleanor Dent for their technical assistance and R. V. Krishnamurthy and M. J. DeNiro for fruitful discussions. We thank Professor John D. Roberts at Caltech for critically reading the manuscript. The research was supported by a National Science Foundation Grant EAR-8504096 and by auxiliary funds from the Weingart Foundation to the California Institute of Technology. This paper is contribution no. 4486, Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125.

1. Dole, M. & Jenks, J. (1944) *Science* **100**, 409.
2. Lane, G. A. & Dole, M. (1956) *Science* **123**, 574–576.
3. Epstein, S. & Mayeda, T. (1953) *Geochim. Cosmochim. Acta* **4**, 213–224.
4. Kroopnick, P. M. & Craig, H. C. (1972) *Science* **175**, 54–55.
5. Taylor, H. P., Jr., & Epstein, S. (1962) *Bull. Geol. Soc. Am.* **73**, 461–480.
6. Effros, R. M. & Mason, G. R. (1983) *Am. Rev. Respir. Dis.* **127**, 859–865.
7. Luz, B., Kolodny, Y. & Horowitz, M. (1984) *Geochim. Cosmochim. Acta* **48**, 1689–1693.
8. DeNiro, M. J. & Epstein, S. (1977) *Geochim. Cosmochim. Acta* **42**, **5**, 495–506.
9. Barstow, T. J., Cooper, D. M., Epstein, S. & Wasserman, K. (1988), in press.
10. Dole, M. (1955) in *Nuclear Processes in Geologic Settings: Proceedings of the Second Conference,* Nuclear Science Series (Natl. Acad. Sci.-Natl. Res. Counc., Washington, DC), Publ. 400, Rep. 19, pp. 13–19.