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# Oxygen isotope variations of phosphate in mammalian bone and tooth enamel

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**Abstract**—About eighty specimens from ten different species of mammals, collected from different areas under different climatic and environmental conditions, were measured for the oxygen isotopic composition of their bone and tooth phosphate. The equations relating these values to the mean oxygen isotopic composition of local meteoric water were also derived. The same equation can be used for goats, roe-bucks, and mouflons, despite the biological differences among these species. Measurements were made on about fourty different specimens of rabbit and hare from Europe, Africa, and Canada, but in this case the data obtained clearly show no direct relationship between the oxygen isotopic composition of local meteoric water and the isotopic composition of the skeletal phosphate. However, there seems to be an inverse relationship between the relative humidity of the studied areas and the $\delta^{18}O(PO_4^{3-})$ of the skeletal phosphate, thus suggesting the use of fossil bones of these mammal species as recorders of palaeoenvironmental relative humidity. Finally, a new equation was derived for the isotopic scale for horses, on the basis of all the previous data and of a few newly obtained results.

## 1. INTRODUCTION

Isotopic scales, calibrated on living organisms of the same species and relating $\delta^{18}O(PO_4^{3-})$ of their skeleton to $\delta^{18}O$ of the environmental water, are useful for quantitative interpretation of palaeoclimatological and palaeohydrological conditions in continental areas made on the basis of isotopic analyses of fossil bones and teeth. Previous papers (Longinelli, 1984; D'Angela and Longinelli, 1990; Ayliffe et al., 1992; Sanchez Chillon et al., 1994; Bryant et al., 1994) reported the experimental equations obtained for this relationship measuring bone and tooth phosphate on living or recent mammals. Isotopic scales were calibrated for humans, pigs, deer, mice, cattle, sheep, elephants, and horses. Some of these scales have been successfully used for palaeohydrological and palaeoclimatological studies (D'Angela and Longinelli, 1993; Bryant et al., 1994). Despite the problems arising from taphonomic processes which bones normally undergo after their burial, and which may affect their oxygen isotopic composition significantly even after a relatively short time, quantitative information on palaeoclimates may be obtained, at least for the last 30–40 thousand years or so. To extend as far as possible the use of this technique to the study of different climatic conditions and different environments of the past, it is important to make available as many isotopic scales as possible. We have for a long time attempted to calibrate more scales, but many problems arise from the difficulty of obtaining recent wild specimens which took in no manmade food at all. In fact, according to previous measurements, the specimens which took in manmade food even as a partial contribution to their usual diet showed variable but meaningful deviations of the $\delta^{18}O(PO_4^{3-})$ of their bones and of the $\delta^{18}O$ of their body water from the expected values calculated on the basis of the existing equations. Unreliable results were obtained in the case of cattle (D'Angela and Longinelli, 1990), deer, and wild boar from national parks (A. Longinelli, unpubl. data) and horses (Bryant et al., 1994; A. Longinelli, unpubl. data). We report here $\delta^{18}O(PO_4^{3-})$ values of bones and/or teeth of goats, roe-bucks, rabbits, hares, and of a few additional horses.

## 2. SAMPLE PREPARATION

The samples were prepared according to the procedure proposed by Tudge (1960) (see also Longinelli, 1965). The final product of the chemical purification procedure ($BiPO_4$) was heated in vacuo at 450°C for about two hours to dehydrate the bismuth phosphate and to avoid rehydration, according to the suggestions of Kahru and Epstein (1986). However, there is some confusion in regard to this procedure and the related effects. Ayliffe and Chivas (1990) conclude that drying the $BiPO_4$ at high temperature is not crucial for correct measurement of $\delta^{18}O(PO_4^{3-})$. Shemesh et al. (1988) state that heating the $BiPO_4$ at 420°C "dehydrates the sample and transforms the $BiPO_4$ from a monazite-type structure to a high-temperature structure (Mooney-Slater, 1962)." This seems incorrect since Mooney-Slater (1962) states that the conversion to the high-temperature form of monazite-type powders occurs between 600 and 700°C, while crystalline precipitation products from acidified solutions undergo the high-temperature modification by prolonged heating at temperatures of about 800°C. Luz et al. (1990) report that their "$BiPO_4$ is heated to 130°C in vacuum to remove the last traces of water (Shemesh et al., 1988)." Again, this seems incorrect since Mooney-Slater (1962) states that traces of the hexagonal type of $BiPO_4$ (slightly hydrated) persist to temperatures as high as 350°C. However, we agree with Shemesh et al. (1988) that, if the transfer of the sample from the drying vessel to the reaction vessel is carried out in a few seconds while both vessels are being purged with dry nitrogen, little or no atmospheric moisture may be added to the $BiPO_4$ samples. In our opinion, a very important point is the experimental proof of Kahru and Epstein (1986) that, after heating the $BiPO_4$ ($\alpha$-form) to 145°C, the sample is almost completely rehydrated in about 15 minutes. In contrast, dehydration occurs and rehydration of the sample is prevented by heating to 420°C or more ($\beta$-form) so that the isotopic effect of hydration water is negligible even when $BiPO_4$

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samples are not fluorinated immediately after thermal treatment. In regard to the small enrichment in $^{18}O$ shown by the samples heated to 420°C, when compared to the results yielded by the same samples after heating to 130°C, we believe that this is related to the presence of traces of water persisting in the hexagonal $BiPO_4$ after heating to 130°C.

The standard deviation of our measurements, calculated on sets of 5–10 fluorinations on the same batch of $BiPO_4$ is about ±0.15 permil ($1\sigma$). When measuring $BiPO_4$ heated in vacuo at 130°C the standard deviation is slightly worse being close to ±0.2 permil ($1\sigma$). However, the chemical treatment of the sample is the critical part of the procedure: impure $BiPO_4$ samples (slightly yellowish to brownish) are normally discarded since their oxygen yield is generally low and their isotopic reproducibility quite bad.

## 3. RESULTS

### 3.1. Goats and Roe-bucks

Bones from living or recently deceased goats and mouflons were obtained from different areas and measured for the oxygen isotopic composition of their phosphate (Table 1). The mean oxygen isotopic composition of local meteoric water was either measured directly in the studied area or nearby (Table 1) or else extrapolated from data published by the I.A.E.A. in Vienna (I.A.E.A. reports on environmental isotope data: 1969, 1970, 1971, 1973, 1975, 1979, 1983, 1986, and 1990). In the latter case, the uncertainty of the reported value is obviously larger than in the case of direct measurements. This is particularly true in the case of specimens from Oman (S. Arabia). Representative values for the atmospheric precipitation in this area are difficult to obtain both because of the difficulty in obtaining statistically meaningful samples and the interference of marked evaporation processes, which precipitations may undergo in this very dry area.

Despite the relatively large biological difference between goats (*Capra ibex*) and mouflons (*Ovis ammon musimon*) and the fact that previous measurements often showed different behaviours for different species living under the same environmental conditions (Longinelli, 1984; D'Angela and Longinelli, 1990), we used the results from both groups of mammals to build the same isotopic scale. It appears that this procedure was reasonably justified. In fact, according to the results reported in Fig. 1, all the data taken into account seem to obey the same law and to fit the same equation relating the mean $\delta^{18}O(PO_4^{3-})$ values to the mean $\delta^{18}O$ of environmental water. The least-squares fit of the goat and mouflon data yields the following equation:

$$\delta^{18}O(PO_4^{3-}) = 0.91\ (\delta^{18}O(H_2O)) + 24.39 \qquad (R^2 = 0.99). \quad (1)$$

The results obtained from mouflons are somehow surprising. In fact, from the biological point of view, a mouflon should be considered a sheep (*Ovis*) rather than a goat (*Capra*). According to the equation previously established for sheep (D'Angela and Longinelli, 1990), a $\delta^{18}O(PO_4^{3-})$ of about

Table 1 - Oxygen isotopic composition of bone phosphate from recent goat, mouflon, roe-buck and horse skeletons from different locations and mean $\delta^{18}O$ value of local meteoric water.

| Sample No. | Species | Location | No. of specimens | $\delta^{18}O$ (V-SMOW) | |
|---|---|---|---|---|---|
| | | | | ($PO_4^{3-}$) | local rain |
| 1 | Capra ibex | Tallin, Estonia | 1 | +14.7 | -11.0 |
| 2 | Capra sp. | Scotland (700 m a.s.l.) | 2 | +16.2 | -8.5 |
| 3 | Capra sp. | Turkey | 2 | +16.8 | -8.0° |
| 4 | Capra ibex | Sardinia, Italy (850 m.a.s.l.) | 1 | +17.4 | -7.5 |
| 5 | Capra ibex | Montecristo Island, Italy | 7 | +19.3* | -5.7 |
| 6 | Ovis ammon musimon (mouflon) | Circeo Nat. Park, Italy | 6 | +19.4** | -5.8 |
| 7 | Capra ibex (?) | Oman, S. Arabia | 1 | +22.3 | -2.0° |
| 8 | Capra ibex (?) | Oman, S.Arabia | 1 | +23.7 | -1.0° |
| 9 | Capreolus capreolus | Gröningen (The Netherlands) | 1 | +16.2 | -8.0° |
| 10 | Capreolus capreolus | Mt. Bondone (Trento, Italy) | 2 | +16.0 | -9.0 |
| 11 | Capreolus capreolus | Trento, Italy | 1 | +17.4 | -8.0 |
| 12 | Capreolus capreolus | Regensburg (Germany) | 3 | +14.3 | -11.0° |
| 13 | Capreolus capreolus | Bayerische Wald (Germany) | 2 | +14.0 | -12.0° |
| 14 | Capreolus capreolus | Mt. Stelvio Nat. Park (Italy) | 1 | +13.1 | -13.0 |
| 15 | Equus caballus | Colhué huapi (Argentine) | 1 | +17.3 | -8.5 |
| 16 | Equus caballus | A. Chasicó (Argentine) | 1 | +18.2 | -6.1 |
| 17 | Equus caballus | A. Chasicó (Argentine) | 1 | +18.8 | -6.1 |
| 18 | Equus caballus | Sarmiento (Argentine) | 1 | +16.1 | -8.5 |
| 19 | Equus caballus | A. Chasicó (Argentine) | 1 | +19.2 | -6.1 |
| 20 | Equus caballus | P. Valdés (Argentine) | 1 | +16.6 | -7.5 |
| 21 | Equus caballus | Mendoza (Argentine) | 1 | +16.2 | -7.2 |

The standard deviation of our phosphate isotope measurements is about ± 0.15 per mil ($1\sigma$)

* Mean value of seven consistent results from seven different specimens.

** Mean value of six consistent results from six different specimens.

° Extrapolated isotopic values from IAEA data (see references).

The local rain values for samples 1 and 2 were suggested by Dr. Ferronsky (IAEA) and by colleagues of the Museum of Natural History (London) respectively; the values of samples 4, 5 and 6 were evaluated according to monthly measurement carried out by the authors at Genova, Pisa, Palermo and the outskirts of Roma for several years; the values of samples 10, 11 and 14 were evaluated from monthly measurements carried out for 3 years at 3 different stations in nearby areas; all the isotopic results for Argentina are extrapolated from IAEA data, from a few measurements carried out by the authors and according to discussions with Dr. H. Panarello (CONICET-Buenos Aires).

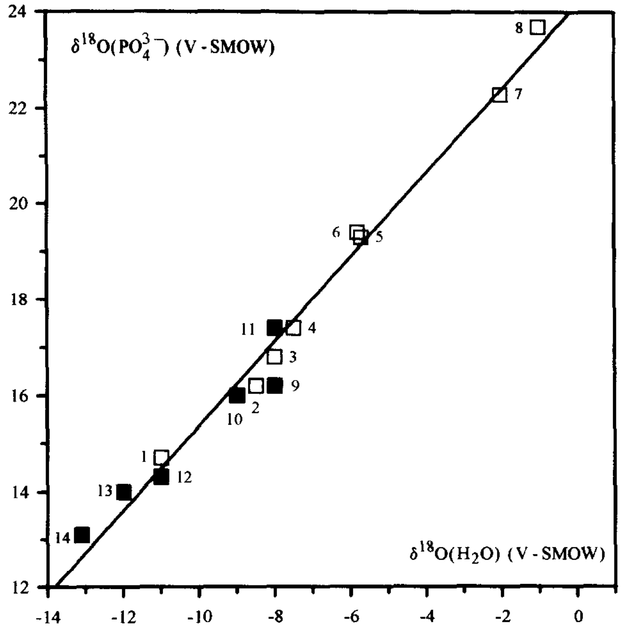

FIG. 1. Relationship between the oxygen isotopic composition of local meteoric water (abscissa) and the mean oxygen isotopic composition of bone phosphate in goats (*Capra ibex*, open squares No. 1 to 4 and 6 to 8), mouflons (*Ovis ammon musimon*, open square No. 5) and roe-bucks (*Capreolus capreolus*, black squares) (Table 1) from different locations (ordinate). The uncertainty of the $\delta^{18}O(H_2O)$ values is between ±0.5 permil and ±1.0 permil. The least-squares fit yields the following equation: $y = 0.88x + 24.1$.

+18.6 should be obtained from sheep for a mean annual value of −5.8 for the oxygen isotopic composition of local meteoric water. For the case of mouflons from Central Italy, the measured value is +19.4 (+19.1 the correct value calculated from the goat equation), rather far from the sheep value.

Similar considerations may be applied to the results obtained from roe-bucks. Again, both the genus and the species of roe-bucks are different from goats but, despite this difference, the results obtained from the measured specimens of *Capreolus capreolus* (Table 1, Fig. 1) apparently fit the set of data obtained for goats very well. In fact, the equation calculated from all the reported results (goats plus roe-bucks) is almost identical to that derived only from the goat results. The new equation obtained is the following:

$$\delta^{18}O(PO_4^{3-}) = 0.88\,(\delta^{18}O(H_2O)) + 24.1 \qquad (R^2 = 0.98). \quad (2)$$

The difference between Eqns. 1 and 2 is only −0.03 in the slope value and −0.25 in the intercept. It would be extremely interesting to obtain at least a few results from roe-bucks living in areas with relatively heavy isotopic composition of local meteoric water, but for reasons mainly related to the biological characteristics of this species, this would be very difficult. Taking into account the very small difference between Eqns. 1 and 2, it seems reasonable to suggest the use of the latter equation for the three different species of mammals which seem to behave similarly: goats, mouflons, and roe-bucks.

### 3.2. Rabbits

Despite the worldwide presence of this mammal, we encountered quite a number of problems in obtaining suitable material for isotopic measurements. Nevertheless, we were able to run a number of measurements on specimens from several climatically-different areas. The species studied and the location of specimens are reported in Table 2 along with the relevant isotopic data. It was important to obtain specimens from climatically different areas since, in the case of rabbits, difficulties were anticipated in obtaining a good relationship between the two variables which are normally taken into account in deriving these equations. This is mainly due to the small size of this mammal and to the relatively large amount of grass eaten. Since rabbits normally do not drink or drink only small amounts of water, the ratio between the amount of water intaken daily with food and the total amount of body water is relatively large. It is well known that the process of evapotranspiration is able to enrich the water in grass and plant leaves in $^{18}O$ (Dongman et al., 1974; Förster, 1978; Burk and Stuiver, 1981). It follows that different areas with the same mean annual $\delta^{18}O(H_2O)$ but with very different rates of evapotranspiration affect the mean isotopic composition of grass water and may cause similar differences in the mean $\delta^{18}O$ of rabbit body water and, consequently, of rabbit bone phosphate.

According to Ayliffe and Chivas (1990), who experienced the same problem in the case of Australian Macropods, assuming as a first approximation the $\delta^{18}O$ of soil water to equal the $\delta^{18}O$ of local meteoric water and the isotopic composition of atmospheric water vapour to be closely approximated by the expression ($\delta_{mw} - \epsilon_{eq}$), the following equation can be used when the atmospheric humidity plays an important role,

$$\delta_{lw} = \delta_{mw} + (1 - h)(\epsilon_{eq} + \epsilon_k), \qquad (3)$$

where $\delta_{lw}$ is the isotopic composition of leaf water, $\delta_{mw}$ is the isotopic composition of meteoric water, $h$ is the relative humidity, and $\epsilon_{eq}$ and $\epsilon_k$ are the equilibrium and kinetic isotope enrichment factors in permil. Measuring bones of white tailed deer from N. America, Luz et al. (1990) proved that, in this case too, the $\delta^{18}O(PO_4^{3-})$ values were directly related to both isotopic composition of precipitation and relative humidity.

Under these conditions, the reliability of the proposed equations from the point of view of quantitative palaeoclimatological reconstructions becomes quite poor, the relationship between the two main variables being directly related to environmental conditions which cannot be correctly evaluated. The results obtained from rabbits confirm these hypotheses as may easily be seen in Fig. 2. In addition to the overall dispersion of the data it is important to note that all the samples from numbers 28 to 39 (right upper side of Fig. 2) come from Somaliland and Ethiopia where the overall variability of the $\delta^{18}O$ of rain water is definitely small (I.A.E.A. reports on environmental data). In contrast, a huge range of $\delta^{18}O(PO_4^{3-})$ values (about 8 permil) was measured in rabbit and hare bones. The most positive values come from specimens collected in the extremely dry area of northernmost Somaliland, while the less positive samples relate to relatively humid areas (sample no. 28 from Afgoi and sample no. 31 from Giohar, both on the banks of the Webi Shebele river, not far from Mogadishu).

Table 2 - Oxygen isotopic composition of bone phosphate from recent specimens of rabbit and hare from different locations and mean $\delta^{18}O$ value of local meteoric water and mean summer relative humidity for a group of rabbits from Spain.

| Sample No. | Species | Location | Summer R.H. (%) | $\delta^{18}O$ (V-SMOW) | |
|---|---|---|---|---|---|
| | | | | ($PO_4^{3-}$) | local rain |
| 1 | Sylvilagus cfr. floridans | Ontario, Canada | | +19.3 | -10.5 |
| 2 | Sylvilagus cfr. floridans | Ontario, Canada | | +18.9 | -10.5 |
| 3 | Sylvilagus cfr. floridans | Ontario, Canada | | +18.3 | -10.5 |
| 4 | Oryctolagus cuniculus | Alicante (Confrides), Spain (1200 m.a.s.l.) | 60 | +20.1 | -8.0 |
| 5 | Oryctolagus cuniculus | The Netherlands | | +19.0 | -8.0 |
| 6 | Oryctolagus cuniculus | Segovia (S. Tomé), Spain (1000 m.a.s.l.) | 55 | +20.4 | -8.0 |
| 7 | Oryctolagus cuniculus | Castellón (Atienza), Spain | 50 | +23.4 | -7.0 |
| 8 | Oryctolagus cuniculus | Valencia (Algar de Valencia), Spain | 45 | +23.5 | -6.0 |
| 9 | Oryctolagus cuniculus | Granada (Illora), Spain (700 m.a.s.l.) | 50 | +21.6 | -7.5 |
| 10 | Oryctolagus cuniculus | Zamora (Bajo Guareña), Spain | 55 | +20.9 | -7.3 |
| 11 | Oryctolagus cuniculus | Granada (Albolote), Spain | | +20.4 | -7.5 |
| 12 | Oryctolagus cuniculus | Crema, Italy | | +17.7 | -7.5 |
| 13 | Oryctolagus cuniculus | Crema, Italy | | +17.4 | -7.5 |
| 14 | Oryctolagus cuniculus | Brioni, Croatia | | +16.6 | -6.7 |
| 15 | Oryctolagus cuniculus | Wiltshire, U.K. | | +15.4 | -6.5 |
| 16 | Oryctolagus cuniculus | Wiltshire, U.K. | | +14.4 | -6.5 |
| 17 | Oryctolagus cuniculus | S. Sardinia, Italy | | +16.0 | -5.5 |
| 18 | Oryctolagus sp. | Kerguelen Isl. | | +16.5 | -5.5 |
| 19 | Oryctolagus sp. | Kerguelen Isl. | | +16.7 | -5.5 |
| 20 | Oryctolagus sp. | Kerguelen Isl. | | +17.4 | -5.5 |
| 21 | Oryctolagus cuniculus | Lugo, NW Spain | 70 | +17.5 | -6.0 |
| 22 | Oryctolagus sp. | Badajoz , Spain | 55 | +19.6 | -6.3 |
| 23 | Oryctolagus sp. | Badajoz (Olivenza), Spain | 50 | +21.2 | -6.3 |
| 24 | Oryctolagus sp. | Cáceres (Valle de Alcántara), Spain | 40 | +24.5 | -6.3 |
| 25 | Oryctolagus cuniculus | S. Sardinia, Italy | | +21.2 | -5.5 |
| 26 | Oryctolagus sp. | Cádiz (Puerto S. María), Spain | 50 | +21.7 | -5.0 |
| 27 | Oryctolagus sp. | Cádiz (S. L. Barrameda), Spain | 50 | +22.5 | -5.0 |
| 28 | Lepus crawshai | Afgoi, Somaliland (2°07'N; 45°03'E) | | +18.5 | -1.0 |
| 29 | Lepus habessinicus | Addis Ab., Ethiopia (9°02'N; 38°42'E) | | +19.5 | -1.5 |
| 30 | Lepus habessinicus | Addis Ab., Ethiopia (9°02'N; 38°42'E) | | +20.0 | -1.5 |
| 31 | Lepus crawshai | Giohar, Somaliland (2°46'N; 45°30'E) | | +20.5 | -1.0 |
| 32 | Lepus crawshai | Uanle Uen, Somaliland (2°37'N; 44°54'E) | | +20.8 | -1.0 |
| 33 | Lepus crawshai | Afmedù, Somaliland (0°28'N; 42°6'E) | | +22.0 | -1.0 |
| 34 | Lepus crawshai | Bur Akaba, Somaliland (2°48'N; 44°05'E) | | +23.0 | -1.0 |
| 35 | Lepus crawshai | Bur Akaba, Somaliland (2°48'N; 44°05'E) | | +22.7 | -1.0 |
| 36 | Lepus capensis | El Bur, Somaliland (4°40'N; 46°37'E) | | +24.5 | -1.0 |
| 37 | Lepus habessinicus | Awash Nat. Park, Ethiopia (8°54'N; 40°00') | | +25.6 | -1.5 |
| 38 | Lepus habessinicus | Berbera, Somaliland (10°30'N; 45°03'E) | | +25.3 | -1.0 |
| 39 | Lepus habessinicus | Garoe-Scimbir., Somaliland (8°24'N; 48°28'E) | | +26.5 | -1.0 |
| 40 | Oryctolagus cuniculus | Granada (Baza), Spain | 45 | +22.3 | -7.5 |
| 41 | Oryctolagus cuniculus | Tallin, Estonia | | +14.3 | -11.0 |

The standard deviation of our phosphate isotope measurements is about ± 0.15 per mil ($1\sigma$).

The mean values reported for the oxygen isotope composition of local rain were evaluated as follows: Italy and Croatia, according to unpublished measurements carried out in Trieste on monthly samples from several years and different locations; Spain, according to measurements carried out from a few years at CSIC Granada and to IAEA measurements; Tallin, personal communication from Dr. Ferronsky; Kerguelen Isl. according to some spring-water and isolated rain water measurements (Longinelli, unpublished); other locations, extrapolation from IAEA measurements (see references).

Lacking quantitative and detailed meteorological information on the relative humidity in this section of Africa, we attempted to report the oxygen isotopic composition of bone phosphate vs. a merely qualitative scale of relative humidity evaluated on the basis of a general knowledge of environmental conditions (Fig. 3). It is apparent that there is an inverse relationship between the two variables even though we cannot describe this relationship in quantitative terms.

Despite the difference in environmental conditions, similar considerations hold in the case of specimens from Spain. For example, samples 21–24 show a progressive drastic increase in $\delta^{18}O$ (+17.5 to +24.5) while the mean $\delta^{18}O$ values of local meteoric water are nearly identical. However, for the case of Spain, data on the relative humidity are available from the Ministry of Public Works and Transport. Using these data we have reported $\delta^{18}O(PO_4^{3-})$ vs. the mean relative humidity during summer in the area of origin of the specimens measured (Table 2, Fig. 4). We have chosen to use the summer values since the mean annual values differ little from each other, the marked variations being related to the long and mainly dry summer time which brings almost desert environmental conditions in some areas of the country. It is apparent that, again, there is an inverse relationship between the reported variables. The least-squares regression of the data yields the following equation:

$$\text{Summer R.H.} = -3.74\,(\delta^{18}O(PO_4^{3-})) + 133 \qquad (R^2 = 0.86). \quad (4)$$

The Ayliffe and Chivas (1990) equation calculated for australian Macropods plotting $\delta^{18}O(PO_4^{3-})$ vs. environmental relative humidities (averaged on a yearly basis) was the following: $H = -2.9\,(\delta^{18}O(PO_4^{3-})) + 128$. The minor differences observed between these two equations may perhaps be related to the use of summer relative humidities (this paper) instead of relative humidities averaged on a yearly basis. According to the above considerations it may be concluded that, as in the case of deer from N. America (Luz et al., 1990) and

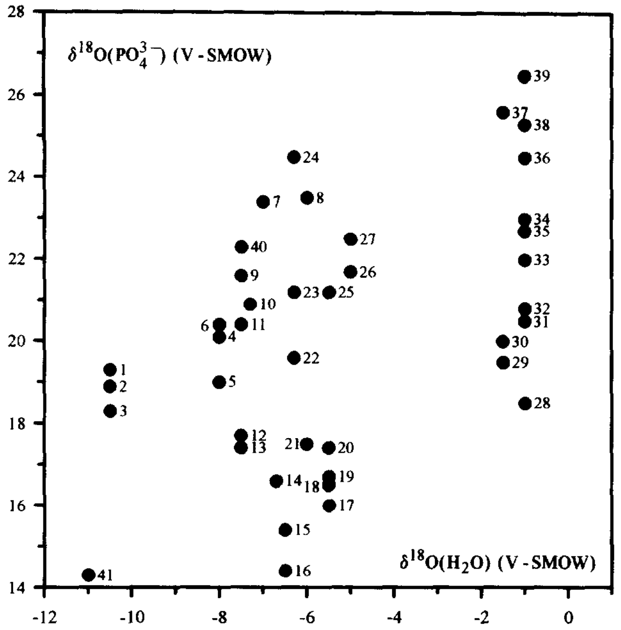

FIG. 2. Oxygen isotopic composition of rabbit bone phosphate (*Oryctolagus cuniculus, Sylvilagus* cfr. *floridans* and *Oryctolagus sp.*) and hare bone phosphate (*Lepus*, various species) from different areas (Table 2) vs. mean oxygen isotopic composition of local meteoric water. The uncertainty of the $\delta^{18}O(H_2O)$ is between ±0.5 and ±1.0 permil.

kangaroos from Australia (Ayliffe and Chivas, 1990), fossil bones of rabbit cannot be used for quantitative palaeoclimatological studies even though they may suggest, at least qualitatively, palaeoenvironmental (palaeohumidity) conditions.

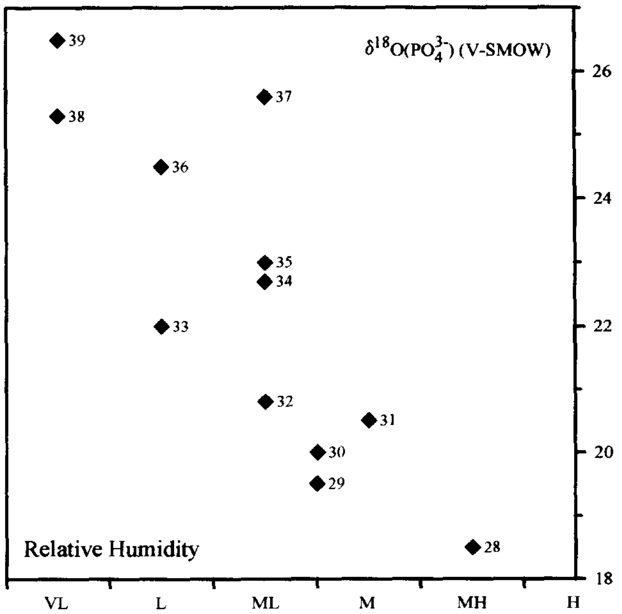

FIG. 3. Oxygen isotopic composition of hare bone phosphate from Somaliland and Ethiopia vs. the relative humidity of the area of origin. VL = very low; L = low; ML = Middle-low; M = middle; MH = Middle-high. Sample numbers refer to Table 2.

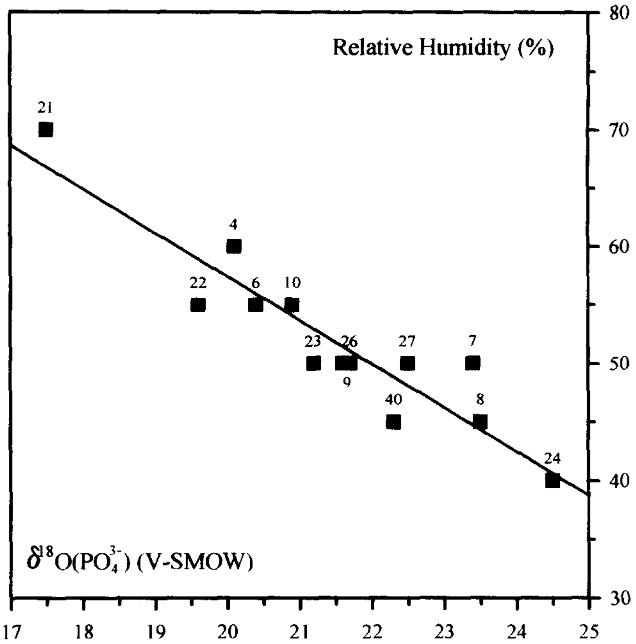

FIG. 4. Oxygen isotopic composition of rabbit bone phosphate (Spain—Table 2) vs. the mean local relative humidity during summer (data from the Ministry of Public Works and Transport). The analytical error in $\delta^{18}O(PO_4^{3-})$ is about ±0.15‰ ($1\sigma$); the error in the estimate of the relative summer humidity is probably ±5% ($1\sigma$) or slightly higher. The least-squares fit yields the following equation: $y = -3.74x + 133$. Ayliffe and Chivas (1990) equation for Macropods ($\delta^{18}O(PO_4^{3-})$ vs. relative humidity averaged on a yearly basis) yielded a similar equation (see text), the minor differences being perhaps related to the fact that we report summer relative humidities instead of averaged yearly values.

### 3.3. Horses

As mentioned previously, Sánchez Chillón et al. (1994) and Bryant et al. (1994) reported two tentative equations for the isotopic scale for horses which are very similar to each other even though the locations from which the specimens were collected were different, as were the species used: *Equus przewalskii, E. caballus,* and *E. asinus* in the former case and *Equus burchelli, E. caballus,* and *E. zebra* in the latter case. In this case the results are quite scattered probably because of the use of zebra samples along with N. America horse samples. Zebras migrate latitudinally and altitudinally and $\delta^{18}O(H_2O)$ estimates are very difficult. The reported equations were the following:

$$\delta^{18}O(PO_4^{3-}) = 0.73\,(\delta^{18}O(H_2O)) + 22.04$$

$$(R^2 = 0.94;\ \text{Sánchez Chillón et al., 1994}), \quad (5)$$

$$\delta^{18}O(PO_4^{3-}) = 0.69\,(\delta^{18}O(H_2O)) + 22.90$$

$$(R^2 = 0.69;\ \text{Bryant et al., 1994}). \quad (6)$$

During the fall of 1993, a few more samples of wild horses were collected in Argentina at different latitudes and elevations by one of the authors (B.S.C.). These specimens were measured for their $\delta^{18}O(PO_4^{3-})$ values using bones and one tooth. The results obtained are reported in Table 1 along with the suggested mean local $\delta^{18}O(H_2O)$ values extrapolated

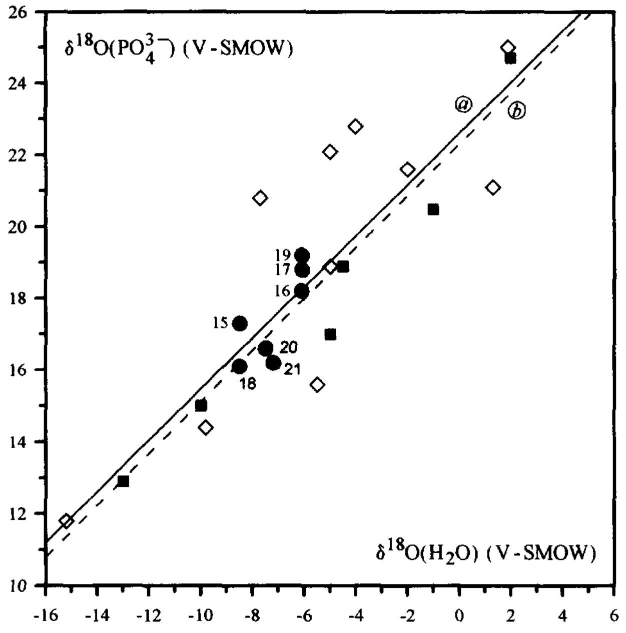

FIG. 5. Relationship between the oxygen isotopic composition of the local environmental water (abscissa) and the oxygen isotopic composition of bone and tooth phosphate of living equids (ordinate). The black dots refer to the samples reported in Table 1, the black squares refer to data from a previous paper by Sánchez Chillón et al. (1994) and the open diamonds refer to data from a paper by Bryant et al. (1994). Equation 6 (b in the Fig.) refers to black dots and black squares, Eqn. 7 (a in the Fig.) was calculated from all the data reported in this Fig.

from the I.A.E.A. data, from a few measurements carried out by the authors and some measurements carried out at the University of Buenos Aires (unpubl. data; H. Panarello, pers. commun.). The representative points are reported graphically in Fig. 5 along with previous results used by us and by Bryant et al. (1994) to derive Eqns. 5 and 6. The new equation which may be calculated taking into account our new data plus the Sánchez Chillón et al. (1994) results is the following:

$$\delta^{18}O(PO_4^{3-}) = 0.72\,(\delta^{18}O(H_2O)) + 22.29 \qquad (R^2 = 0.90). \quad (7)$$

If we consider all the results obtained so far on wild horses (Fig. 5), the least-squares fit yields the following equation:

$$\delta^{18}O(PO_4^{3-}) = 0.71(\delta^{18}O(H_2O)) + 22.60 \qquad (R^2 = 0.77). \quad (8)$$

The difference between Eqns. 7 and 8 is quite small so that we propose to use Eqn. 8 for palaeoclimatological studies on horse fossil bones and teeth in accordance with the fact that several different species living under completely different environmental conditions obey this equation reasonably well.

## 4. CONCLUSIONS

About 80 specimens from ten different species of mammals have been studied for the $\delta^{18}O(PO_4^{3-})$ of their skeleton. The results obtained allow the following conclusions to be drawn, which are of interest in view of the application of these measurements to quantitative studies of continental palaeoclimates:

1) fossil skeletal parts of goats, mouflons, and roe-bucks seem to be suitable material for this study and the same equation might be used to calculate the mean oxygen isotopic composition of palaeo-meteoric waters;
2) skeletal parts of rabbits and hares are not suitable for quantitative palaeoclimatological and/or palaeohydrological studies. In fact, as already proved in the case of other living organisms (Macropods in Australia and white tailed deer in N. America), their body water and, consequently, the $\delta^{18}O$ of their skeletal phosphate, seem to be strongly affected by evapotranspiration processes which deeply modify the oxygen isotopic composition of their primary food, i.e., grass and plant leaves. Fossil material from these mammal species may perhaps be used as a palaeoenvironmental recorder to obtain qualitative information on local relative humidity;
3) a new equation is proposed as an oxygen isotope scale for horses, replacing the two equations previously published. The new equation was recalculated on the basis of a relatively large number of different specimens belonging to several different species of the genus *Equus* and, consequently, it may be considered more representative and statistically meaningful than the previous ones. In fact, data for all five species of living horses used in this case obey this equation fairly well (at least for the case of wild specimens) despite the relatively large biological and environmental differences existing among the studied specimens and their provenances.

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