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Relation between D/H ratios and $^{18}O/^{16}O$ ratios in cellulose from linen and maize—Implications for paleoclimatology and for sindonology

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Abstract—The $^{18}O/^{16}O$ ratios of cellulose and the D/H ratios of cellulose nitrate were determined for linen, a textile produced from the fibers of the flax plant *Linum usitatissimum*, and for maize (*Zea mays*) from a variety of geographic locations in Europe, the Middle East, and North and South America. The regression lines of δD values on $\delta^{18}O$ values had slopes of 5.4 and 5.8 for the two species. Statistical analysis of results reported in the only other study in which samples of a single species (the silver fir *Abies pindrow*) that grew under a variety of climatic conditions were analyzed yielded slopes of ~6 when δD values of cellulose nitrate were regressed on $\delta^{18}O$ values of cellulose. The occurrence of this previously unrecognized relationship in three species suggests it may obtain in other plants as well. Determining the basis for this relationship, which is not possible given current understanding of fractionation of the isotopes of oxygen and hydrogen by plants, should lead to increased understanding of how D/H and $^{18}O/^{16}O$ ratios in cellulose isolated from fossil plants are related to paleoclimates. The separation of most linen samples from Europe from those originating in the Middle East when δD values are plotted against $\delta^{18}O$ values suggests it may be possible to use the isotope ratios of cellulose prepared from the Shroud of Turin to resolve the controversy concerning its geographic origin.

INTRODUCTION

THE SHROUD OF TURIN has been the subject of continuing controversy since it surfaced in France over six hundred years ago. A major issue concerns its origin. No one knows how old the Shroud is or where it came from, but opinions differ sharply nonetheless. Some think the Shroud was the burial cloth of Jesus. Proponents of authenticity believe the linen cloth was manufactured in or near Palestine during the early first century A.D. Opponents of this position refer to a letter written in 1389 A.D. by Pierre d'Arcis, Bishop of Troyes (WILSON, 1978). In it the bishop states that his predecessor had conducted an investigation into the matter and had turned up an artist who confessed to the forgery. In the sceptics' view, the Shroud is thus a product of European technology of the 14th century. While these opposing positions in no way exhaust all possibilities, they have nonetheless remained predominant. It therefore seems appropriate that some experiments be done to test one hypothesis against the other.

The available scientific information regarding the origin of the Shroud is largely inconclusive. RAES (1976) observed that neither thread-twist nor weave type of the Shroud is sufficiently distinctive to identify the origin of the linen fabric. He did, however, find traces of cotton (*Gossypium herbaceum*) fibers included in the linen threads. This observation suggested that the linen may have been spun with the same equipment used previously for cotton. The cotton inclusions support a Middle Eastern manufacture, but do not prove it. A second line of evidence supporting a Middle Eastern origin comes from the pollen studies of FREI (1979). This work is inconclusive, however, because of lack of control studies and detailed descriptions of experimental procedure. None of the observations made during the most recent scientific study of the Shroud in 1978 (SCHWALBE and ROGERS, 1982) bear directly on age or geographic origin.

In 1984 Larry Schwalbe from Los Alamos National Laboratories invited us to become associated with STURP, the Shroud of Turin Research Project, a consortium of researchers interested in investigating various aspects relating to the origin and subsequent history of the Shroud and in persuading the Roman Catholic Church authorities in charge of the Shroud to permit such investigations. Our contribution to the program was to involve stable isotopic analysis of cellulose purified from a piece of the Shroud in order to limit its possible geographic origin, thereby adding some evidence to the question of its authenticity.

The rationale behind our approach is as follows. If the Shroud is the burial cloth of Jesus, the flax (*Linum usitatissimum*) plants that were processed to produce the linen probably were grown in the Middle East in the 1st century A.D. On the other hand, if it is not authentic, the plants probably were grown in Europe in the 14th century A.D. We expected that stable isotopic analysis of cellulose from the Shroud would allow us to decide between these two geographic possibilities because (1) δD and $\delta^{18}O$ values of meteoric water are influenced by climatic factors, with temperature being the most important determinant (DANSGAARD, 1964; FRIEDMAN *et al.*, 1964); (2) D/H and $^{18}O/^{16}O$ ratios of cellulose reflect the corresponding isotope ratios of the meteoric water a plant used and the humidity of the environment in which it grew (EPSTEIN *et al.*, 1976, 1977); so that (3) cellulose

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synthesized by flax plants in the hot, dry climate of the Middle East should have higher deuterium and ^{18}O concentrations than that from flax plants that grew in colder, wetter Europe (DANSGAARD, 1964; FRIEDMAN *et al.*, 1964; YURTSEVER, 1975; GAT, 1980). It should be noted that because trade has occurred between the two regions for more than two millennia, radiocarbon dating of the Shroud will provide a more rigorous test of its authenticity than stable isotope analysis, but, in any case, determining the geographic origin of the Shroud is not without interest.

Because of the reluctance of the ecclesiastical authorities who control the Shroud to permit destruction of even a small piece of it without evidence that the analysis in question would prove useful in resolving the issues at hand, we embarked on a program of analyzing linen samples that came from the two areas of most likely geographic origin. In doing so, we inadvertently produced the first set of isotope ratios of cellulose from samples of a single species that grew over a wide geographic range and hence were subject to a variety of climatic conditions. Analysis of these data produced evidence for a previously unrecognized relationship between the δD and $\delta^{18}O$ values of cellulose. Once we realized the significance of this observation, we assembled samples of another plant, maize (*Zea mays*), grown over a wide geographic range, and observed that a similar relationship existed between the δD and $\delta^{18}O$ values of cellulose for this species. Ironically, our results for linen samples indicate that stable isotopic analysis of the Shroud possibly will not necessarily permit discrimination between a Middle Eastern and a European origin, but the results for linen and maize reported here have fundamental implications for interpreting stable isotope ratios of cellulose in modern and fossil plants.

MATERIALS AND METHODS

The geographic origins and ages of the linen and maize samples we analyzed are given in Table 1. A more detailed description of the provenance of each sample is presented in the Appendix.

Linen samples were examined with a binocular microscope at 50× magnification, which permitted visualization of the characteristic structure of individual linen fibers (MCCRONE, 1980) to insure that no other fibers used in textiles (*e.g.* cotton, silk, wool) were present. Prior to cellulose extraction, linen samples were cut into small strips, while maize samples, all of which consisted of the stem that connects the cob to the stalk of the plant, were ground to less than 40 mesh in a Wiley mill. Cellulose was extracted from ~100 mg samples of linen and ~500 mg samples of maize by the method of WISE (1944). An aliquot of each sample was then nitrated using the acetic anhydride method as in DENIRO (1981), producing cellulose nitrate that contained only non-exchangeable carbon-bound hydrogen (EPSTEIN *et al.*, 1976). Oxygen isotope ratios of cellulose were determined by the method of RITTENBERG and PONTICORVO (1956) as modified by BURK (1979), except h-benzoquinoline was used instead of quinoline to separate HCl from CO_2. Hydrogen isotope ratios of cellulose nitrate were determined as described by NORTHFELT *et al.* (1981). All isotope ratios are reported in the δ notation, where

$$\delta = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000‰$$

and R represents $^{18}O/^{16}O$ for $\delta^{18}O$ values and D/H for δD values. The standard was standard mean ocean water (SMOW). The precisions of the isotopic analyses were ±2‰ for δD values and ±0.5‰ for $\delta^{18}O$ values.

Table 1. δD values of cellulose nitrate and $\delta^{18}O$ values of cellulose (given in ‰) from linen (designated JD) and maize stems (designated BM) of the indicated provenance and age. Ages are A.D. unless otherwise indicated.

Sample	Location	Age/Year Collected	$\delta^{18}O$	δD
JD-1	Belgium	16th century	+26.8	-113
JD-2	Egypt	1st-3rd centuries	+32.5	-96
JD-3	Portugal	14th century	+24.0	-145
JD-4	Egypt	4000 years B.P.	+31.2	-101
JD-5	Egypt	5th century	+31.8	-88
JD-7	Egypt	10th century	+31.1	-90
JD-8	Egypt	12th century	+30.2	-98
JD-10	Egypt	20th century	+32.4	-88
JD-11	Belgium	post-7th century	+24.0	-132
JD-12	Belgium	20th century	+25.1	-138
JD-13	Belgium	20th century	+25.0	-131
JD-14	Poland	20th century	+22.8	-154
JD-15	Poland	20th century	+22.5	-146
JD-21	Italy	19th century	+23.6	-139
JD-22	Spain	17th-18th centuries	+22.8	-139
JD-23	France	18th century	+26.7	-114
JD-24	Greece/Turkey	17th-18th centuries	+24.1	-136
JD-25	France	20th century	+26.4	-107
JD-31	Austria	20th century	+22.6	-148
JD-41	Germany	20th century	+24.8	-133
JD-48	Germany	19th century	+24.2	-130
JD-50	Germany	19th century	+23.6	-134
JD-56	Israel	~135 A.D.	+26.1	-103
JD-57	Israel	9000 years B.P.	+29.8	-103
JD-58	Israel	9000 years B.P.	+25.4	-112
JD-64	Israel	1st century B.C.	+26.6	-112
JD-65	Israel	515 B.C.-70 A.D.	+28.5	-100
JD-66a	Israel	73 B.C.-73 A.D.	+27.4	-111
JD-66b	Israel	73 B.C.-73 A.D.	+27.3	-111
BM-101	Phillipines	1915	+27.2	-33
BM-104	Phillipines	1953	+21.8	-74
BM-106	China	1937	+18.1	-72
BM-110	El Salvador	1922	+26.6	-71
BM-111	Brazil	1899	+26.4	-63
BM-114	Peru	1942-44	+21.6	-51
BM-116	Peru	1942-44	+23.5	-72
BM-120	Bolivia	1942-44	+30.8	-3
BM-121	Bolivia	1942-44	+28.5	-10
BM-132	Brazil	1942-44	+22.1	-66
BM-134	Paraguay	1942	+27.7	-12
BM-155	Massachusetts	1881	+26.2	-44
BM-159	North Carolina	1984	+27.3	-8
BM-178	Massachusetts	1942	+28.2	-18
BM-1000	Finland	1986	+17.3	-69

RESULTS

The isotope ratios of linen and maize samples are given in Table 1. In Fig. 1 a plot of δD *versus* $\delta^{18}O$ values for each species is given.

For linen samples, the least squares regression fit of the δD *versus* $\delta^{18}O$ values is

$$\delta D = 5.8\delta^{18}O - 274‰$$

with an r^2 value of 0.86, which is significant at the $P = 0.005$ level (Table 2). The least squares line for the δD *versus* $\delta^{18}O$ values of maize has the form

$$\delta D = 5.4\delta^{18}O - 178‰$$

and an r^2 value of 0.59, which is also significant at the $P = 0.005$ level (Table 2).

DISCUSSION

The observation that the slopes of the least squares lines for δD values of cellulose nitrate *versus* $\delta^{18}O$ values of cellulose for linen and for maize are similar suggested that this is a fundamental relationship that might occur in other terrestrial plant species. Accordingly, we surveyed the literature in order to determine if the relationship had been observed previously. Although the $\delta^{18}O$ values of cellulose and the δD values of cellulose nitrate prepared from terrestrial plants have been

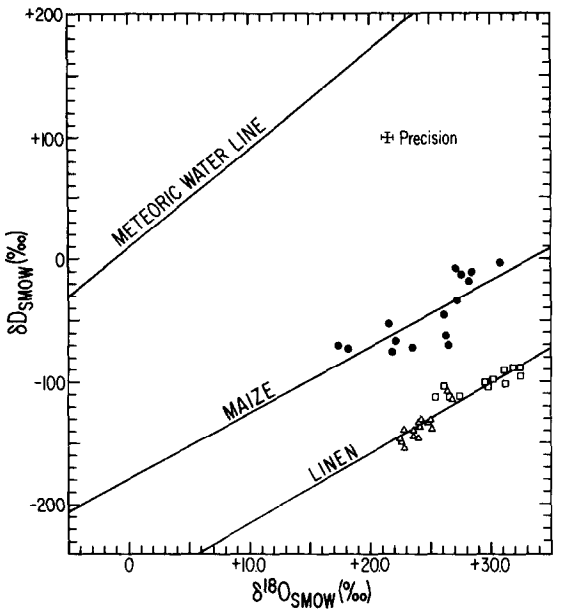

FIG. 1. δD values of cellulose nitrate plotted against $\delta^{18}O$ values of cellulose for the maize (indicated by circles) and linen (indicated by triangles for European samples and by squares for Middle Eastern samples) specimens listed in Table 1. The meteoric water line is from CRAIG (1961). The regression lines for maize and linen are given by the equations $\delta D = 5.4\delta^{18}O - 178‰$ and $\delta D = 5.8\delta^{18}O - 274‰$, respectively.

presented by many workers (EPSTEIN *et al.*, 1977; STERNBERG and DENIRO, 1983a; STERNBERG *et al.*, 1984a,b; RAMESH *et al.*, 1985; STERNBERG *et al.*, 1985; TING *et al.*, 1985; EDWARDS and FRITZ, 1986; RAMESH *et al.*, 1986; STERNBERG *et al.*, 1986b) there is only one report involving many samples from a single species that grew under a variety of climatological conditions. In that study, RAMESH *et al.* (1985) analyzed cellulose from 30 annual rings along two radii of the trunk of a silver fir (*Abies pindrow*) tree from Kashmir, India. Our least squares analysis of their data for the two radii yielded lines with slopes of 5.8 and 6.0 (Table 2), similar to the slopes of 5.8 and 5.4 we observed for linen and maize. We note that RAMESH *et al.* (1986) reported a least squares line with a slope of 7.9 for these data. However, RAMESH *et al.* (1986) averaged the isotope ratios for the two radii before performing linear regression analysis, thereby obscuring the relation that existed in the values for each radius. We also believe the least squares analysis performed by RAMESH *et al.* (1986) may have been faulty, since we obtained a slope of 6.4 when we regressed the averaged data.

One complication in interpreting the data for linen involves possible isotope effects during the processing of flax plants to produce linen textiles. A variety of physical, chemical and biological processes are used to separate the long linen fibers from the woody portions of the stalks of the plants and to process the fibers into textiles (KLEINERT, 1972; GOLOVA and NOSOVA, 1973; CHESSON, 1978). Some of them (*e.g.* retting, a process in which the stalks undergo microbial fermentation or chemical oxidation) are possible causes of isotopic fractionation of cellulose. The evidence relating even remotely to isotope effects during such processes on δD and $\delta^{18}O$ values of cellulose is rather limited. YAPP and EPSTEIN (1977) presented data that indicate D/H ratios of cellulose nitrate are not affected by decomposition of plant materials occurring in the depositional environment. MARINO and DENIRO (1987) have shown that short-term (10–20 days) fermentation or molding and rotting do not substantially alter the δD values of cellulose nitrate and the $\delta^{18}O$ values of cellulose. Direct testing of the effects of the processes used to produce linen on the isotope ratios of cellulose in the fibers is not possible, because the fibers cannot be separated from other cellulose-containing components in the flax plant stalks except by the processes whose potential isotope effects are in question. Thus, in interpreting our linen data we have had to assume that either there were no isotope effects associated with the processes involved in producing linen, or, if there were fractionations, they were the same regardless of differences in techniques used to produce the linen samples we analyzed. If these assumptions are not valid, some of the variability in δD and $\delta^{18}O$ values we observed for linen may not reflect factors that determined these isotope ratios in cellulose as it existed in the flax plants.

The occurrence of similar relationships between the D/H and $^{18}O/^{16}O$ ratios of cellulose nitrate and cellulose for three species (*Linum usitatissimum, Zea mays* and *Abies pindrow*) suggests that δD and $\delta^{18}O$ values for other species may plot along lines with slopes between 5 and 6, with intercepts that might differ from species to species. Our observation of this previously unrecognized relationship should lead to increased reliability in paleoclimatic reconstructions based on isotopic analysis of cellulose from fossil plants provided its significance in living plants can be interpreted. Unfortunately, current understanding of how plants fractionate oxygen and hydrogen isotopes is inadequate to permit us to advance an explanation for the basis of the observations reported in this paper. We believe that the inadequacy of our understanding of these fractionation processes is also a major impediment preventing wider application of isotopic analysis of fossil cellulose as a method of climatic reconstruction.

To illustrate how little is known about isotope fractionation in plants, we present a simplistic climatic reconstruction from the data for two of our linen samples, JD-10 and JD-15, which are from Egypt and Poland respectively. The δD values of cellulose nitrate and the $\delta^{18}O$ values of cellulose for these two samples are shown in Fig. 2 as points E (Egypt) and P (Poland). The climatic information recorded in these cellulose data can be extracted by translating them back to the isotope ratios of the leaf water used in photosynthesis and of the

Table 2. Relation between δD values of cellulose nitrate and $\delta^{18}O$ values of cellulose for sample sets involving specimens of a single species that grew under a range of climatic conditions. We performed linear regression analysis to determine the line $\delta D = m\ \delta^{18}O + b‰$ for the data on linen and maize reported in Table 1 and for the data on annual rings along two radii of a single silver fir tree from Kashmir, India reported by RAMESH et al. (1985). The number of samples analyzed is given by n. The standard errors for m and b are given in parentheses. The linear regressions are all significant at the P=0.005 level, according to the F-test.

Sample	n	m	b	r^2
Linen[1] textiles	29	5.8(0.5)	-274(12)	0.86
Maize[2] stems	15	5.4(1.2)	-178(31)	0.59
Silver fir[3] annual rings				
radius 1 of tree AP2	30	5.8(0.8)	-231(25)	0.63
radius 2 of tree AP2	30	6.0(1.3)	-219(38)	0.44

[1]From *Linum usitatissimum*
[2]*Zea mays*
[3]*Abies pindrow*

meteoric water taken up by the flax plants, since the isotope ratios of the meteoric water reflect mean annual or growing season temperature where the plant grew (EPSTEIN *et al.* 1976; EPSTEIN and YAPP, 1976; YAPP and EPSTEIN, 1982b), and the relationship between the isotopic compositions of meteoric water and of leaf water is influenced by the humidity of the environment (EPSTEIN *et al.*, 1977; ALLISON *et al.*, 1985).

In order to translate cellulose isotope ratios into leaf water isotope ratios, we need to know the isotopic fractionations that occur during the biochemical processes whereby the hydrogen and oxygen of leaf water are incorporated into cellulose. (Although oxygen from CO_2 enters into these reactions, it has no isotopic influence because of exchange occurring with leaf water, as DENIRO and EPSTEIN, 1979, showed.) These fractionation factors can be expressed as α values, where

$$\alpha^{R}_{X-H_2O} = \frac{R_X}{R_{H_2O}}$$

and R is $^{18}O/^{16}O$ and X is cellulose for the fractionation factor for oxygen, and R is D/H and X is cellulose nitrate for that for hydrogen.

The fractionation factor between cellulose and the water used in its synthesis is 1.027 ± 0.003 for $^{18}O/^{16}O$ ratios (DENIRO and EPSTEIN, 1981; STERNBERG and DENIRO, 1983b). Thus, in Fig. 2, we subtracted 27‰ from the linen $\delta^{18}O$ values (by going along the lines E–E_1 and P–P_1) to obtain estimates of the $\delta^{18}O$ values of leaf water.

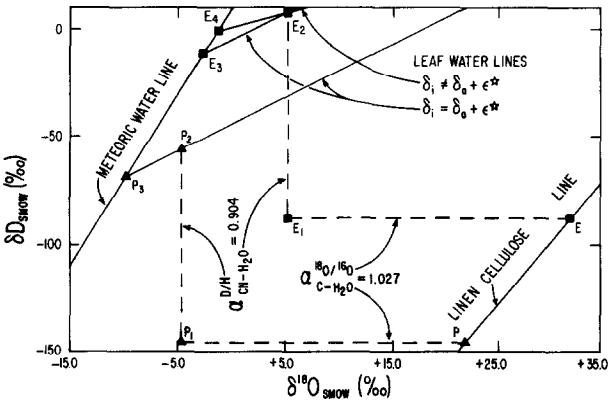

FIG. 2. Example of paleoclimatic reconstruction in which plant cellulose $\delta^{18}O$ values and cellulose nitrate δD values (points E, P) are translated into the corresponding isotope ratios for leaf water (points E_2, P_2) and for meteoric water used by the plants (points E_3, E_4, P_3). All points relating to linen sample JD-10 (Table 1), which is from Egypt, are designated by the letter E with or without subscripts. The letter P is used to identify points relating to linen sample JD-15, which is from Poland. The meteoric water line is from CRAIG (1961). The linen cellulose line is the least squares fit for all linen data presented in this paper (Table 2). The evapotranspiration lines are of two types: those that usually attain in non-arid mid-continental regions in which atmospheric water vapor (δ_a) is in isotopic equilibrium with meteoric (ground) water (δ_i) have slopes (arbitrarily) set at 2.5, while that with a slope (arbitrarily) set at 1.5 represents a situation typical of arid or semi-arid regions in which isotopic equilibrium between the two is not reached (ALLISON *et al.*, 1985). The fractionation factors between cellulose and leaf water for D/H and $^{18}O/^{16}O$ ratios are indicated by dashed lines running parallel to the axes.

In order to obtain δD values of leaf water, it is necessary to know the fractionation factor between cellulose nitrate and leaf water for D/H ratios. This α value is not as well constrained for hydrogen as it is for oxygen. Values ranging from 0.85 to 1.07 have been observed in various aquatic plants (DENIRO and EPSTEIN, 1981; YAPP and EPSTEIN, 1982a). In order to estimate the α value for linen plants, which have not been studied, we took the following approach. The isotopic composition of the meteoric water used by flax plants growing in Poland (Point P_3 in Fig. 2) was estimated from the δD value of water in the River Weichsel (FRIEDMAN *et al.*, 1964) and the equation for the meteoric water line (CRAIG, 1961). After meteoric water is taken up by roots, it is transported to leaves, where it undergoes evaporation. During this evapotranspiration process, leaf water becomes enriched in deuterium and ^{18}O relative to meteoric water (ALLISON *et al.*, 1985). For plants growing in midcontinental non-arid environments in which atmospheric water vapor is in isotopic equilibrium with meteoric water, leaf waters have δD and $\delta^{18}O$ values lying along lines with slopes of ~2.5–4 that pass through the source (meteoric) water point (ALLISON *et al.*, 1985). Thus, we assumed a slope of 2.5 to construct the line that passes through points P_3 and P_2 in Fig. 2. This line is the locus of the isotope ratios for leaf waters in flax plants growing at different humidities in Poland using the meteoric water P_3. We then projected vertically upward from point P_1 until we crossed line P_3–P_2 at point P_2, which identifies the isotopic composition of leaf water in the flax plants that gave rise to the linen with δD and $\delta^{18}O$ values indicated by point P. The difference in δD values between points P_1 and P_2 can be used to estimate the fractionation factor between cellulose nitrate and leaf water in flax plants for D/H ratios, which is 0.904 in this case. We used this α value to determine the δD value of leaf water for the flax plants growing in Egypt that gave rise to linen with isotopic composition indicated by point E by moving 97‰ in the vertical direction up from point E_1. In this case, the leaf water isotopic composition is given by point E_2. The line E_2–E_3 is drawn to have a slope of 2.5, which might have been the case if the flax grew in an environment in which atmospheric water vapor and meteoric water were in isotopic equilibrium, and intersects the meteoric water line at a value indicated by E_3, which is within the range of $\delta^{18}O$ values reported for meteoric waters in Alexandria, Egypt (DANSGAARD, 1964; INTERNATIONAL ATOMIC ENERGY AGENCY, 1981). However, the line E_2–E_3 probably does not accurately reflect the effect of evapotranspiration on leaf water isotopic composition for plants that grew in Egypt. Accordingly, we drew the line E_2–E_4, which is the locus of the isotope ratios of leaf waters that might be expected for flax plants growing at different humidities in an environment in which isotopic equilibrium between meteoric water and atmospheric water vapor was not attained. In this case, the slope is less than that for the situation of plants growing in the midcontinental non-arid climates discussed above (we arbitrarily set it at 1.5) and the intersection of the leaf water line and the meteoric water line, point E_4, cannot be used to predict the composition of the meteoric water where the plants grew because it does not coincide with values of the source water the plants used (ALLISON *et al.*, 1985). The fact that the distance E_3–E_2 and the distance E_4–E_2 are both greater

than the distance P_3–P_2 is consistent with the presumably greater aridity of the climate in which the flax plants grew in Egypt compared with those that grew in Poland, regardless of the model chosen to represent the evapotranspiration effect for the Egyptian plants (ALLISON *et al.*, 1985).

By following the steps discussed above, we were able to arrive at reasonable estimates for the humidity of the environments in which flax plants grew in Egypt and in Poland, at least on a qualitative basis. We also were able to produce reasonable estimates for the isotopic composition of the meteoric (source) waters the plants used, at least if they grew in environments in which atmospheric water vapor and meteoric water were in isotopic equilibrium. (Ignore for the moment the fact that we used the isotopic composition of meteoric water in Poland to constrain the value for the hydrogen isotope fractionation factor between cellulose nitrate and water. As we discuss below, it should be possible to determine this value directly in the laboratory.) From these meteoric water values it would be possible to estimate the mean annual or growing season temperatures at which the plants grew. A rigorous paleoclimatic reconstruction based on isotopic analysis of fossil plant material must involve similar backtracking from the δD and $\delta^{18}O$ values of cellulose nitrate and cellulose. Yet, the information needed to perform the backtracking operations is not available at present, as summarized briefly below.

In order to go from cellulose isotope ratios to leaf water isotopic compositions, the fractionation factors between the two must be known. Most paleoclimatic applications will involve analysis of terrestrial plant matter, yet these fractionation factors have been determined only in aquatic plants (DENIRO and EPSTEIN, 1981; YAPP and EPSTEIN, 1982a; STERNBERG and DENIRO, 1983b). These studies have shown that the fractionation factors differ from species to species, ranging from 1.024 to 1.030 for $^{18}O/^{16}O$ ratios and from 0.85 to 1.07 for D/H ratios, and may themselves be directly responsive to climatic change. Specifically, the fractionation factor between cellulose nitrate and water for D/H ratios in different species display temperature dependences that ranged from −5‰ to +4‰ per °C temperature increase (DENIRO and EPSTEIN, 1981). It is not unreasonable to expect that similar species to species variation and direct responses to climatic variability (*e.g.* temperature change) will characterize the cellulose-leaf water fractionation factors in terrestrial plants. For example, when we performed the graphic exercise shown in Fig. 2 for two maize samples, BM-178 from Massachusetts and BM-1000 from Finland, using isotopic data for meteoric waters from DAANSGAARD (1964) and FRIEDMAN *et al.* (1964) and the equation for the meteoric water line from CRAIG (1961), again assuming a fractionation factor of 1.027 between cellulose and water for oxygen isotopes, we obtained a value of 1.000 for the fractionation factor between cellulose nitrate and water for hydrogen isotopes, a value that differs substantially from the value of 0.904 for flax plants. Therefore, it would seem to be essential that the fractionation factors between cellulose and leaf water be determined directly for the actual species whose fossil representatives will be analyzed in a paleoclimatic reconstruction. Such determinations are best performed in systems in which the effects of evapotranspiration are eliminated, which is the reason they have been studied exclusively in aquatic plants up until now. It should be possible to meet such a restriction for terrestrial plants by growing their cells in aqueous suspension cultures, as we have done with carrots (*Daucus carota*) for other reasons (STERNBERG *et al.*, 1986a), or by growing plants at 100% relative humidity.

If the fractionation factors between cellulose and leaf water for the appropriate terrestrial species were determined as suggested, it would be possible to translate cellulose $\delta^{18}O$ values and cellulose nitrate δD values for fossil plant specimens into the corresponding isotope ratios of leaf waters from which the oxygen and hydrogen in the cellulose were obtained. This, unfortunately, would not produce any paleoclimatic insights, which would be forthcoming only if the isotopic composition of the meteoric water the plants used could also be estimated. To do this, it would be necessary to subtract the effects of evapotranspiration, which cause water in leaves to become enriched in deuterium and ^{18}O relative to the (meteoric) source water a plant takes up from the soil. The problem with subtracting out the effects of evapotranspiration is that in order to do so, one needs to know the climatic regime in which the plant grew. To wit, if the plant grew in an environment in which atmospheric water vapor was in isotopic equilibrium with meteoric (ground) water, the line connecting leaf water δD and $\delta^{18}O$ values and the meteoric water line has a relatively restricted range of slopes (say 2.5–4) and passes through the source water the plant used. However, if the plant grew in an environment in which ground water and atmospheric water vapor were not in isotopic equilibrium, the slope of the evapotranspiration line is not restricted to any narrow range and its intersection with the meteoric water line does not indicate the composition of the source water the plant used. Furthermore, the distance along the meteoric water line between the intersection point and the source water cannot be estimated from first principles (ALLISON *et al.*, 1985). To sum up, in order to go from leaf water values to the meteoric water line (thereby determining the data required to reconstruct paleoclimate), we need to know the paleoclimate. Even if we know the paleoclimate, we do not know what slopes to use in this exercise. Even if we knew the slopes, the exercise is futile in the case of plants that grew in arid and or semi-arid environments, since the point we obtain on the meteoric water line does not tell us anything about the meteoric water the plant used.

Previous workers who have presented paleoclimatic reconstructions based on isotopic analysis of cellulose from fossil or subfossil plant matter have attempted to circumvent the problems discussed above by reducing parts or all of what goes on in plants during the processing of water taken up from the ground into cellulose to constant isotopic fractionations (*e.g.* EPSTEIN *et al.*, 1976; BRENNINKMEIJER *et al.*, 1982; YAPP and EPSTEIN, 1982a; EDWARDS and FRITZ, 1986; RAMESH *et al.*, 1986; DUPONT and MOOK, 1987). We disagree with this approach in which plant biology is reduced to a black box and the abundant data indicating that not all plants are the same in how they fractionate isotopes is ignored. Paleoclimatic reconstruction based on isotopic analysis of fossil plant material is a difficult proposition at best, but ignoring the effects of biology on the entire enterprise does not make it more reliable.

The results reported here indicate that while there is considerable separation in the distributions of δD and $\delta^{18}O$ values for cellulose from linen originating in Europe and in the Middle East, there is some overlap (Fig. 1). Two explanations may account for this overlap. First, the provenance of some of the samples may be erroneous. Such errors could arise because it is difficult to determine precisely where many of the linen samples originated (see Appendix) and to eliminate the possibility of trade of linen or of flax by-products (WARDEN, 1968). Second, if the provenances of the samples are correct, the overlap between European and Middle Eastern samples is likely to be caused by growth during periods when climate varied substantially from that which characterizes the present-day climates in the two regions. To give some idea of the magnitude of this type of climatic influence, we note that the δD values for cellulose nitrate from annual rings of a bristlecone pine (*Pinus aristata*) tree determined by EPSTEIN and YAPP (1976) ranged over ~40‰, while the $\delta^{18}O$ values of cellulose in the annual rings of a silver fir (*Abies pindrow*) tree covered ~9‰ (RAMESH *et al.*, 1985). Variations of this magnitude could move the lower lefthand cluster of European samples and the upper righthand cluster of Middle Eastern samples in Fig. 1 toward one another sufficiently to account for the overlap between the two groups that we observed.

In spite of the existence of this overlap, isotopic analysis of cellulose from the Shroud of Turin may still help elucidate its geographic origin. To this end we are continuing discussions with church authorities to obtain a piece of the Shroud. Three possibilities exist if the isotopic analysis described herein is ever performed. First, the point for the Shroud might fall away from any data reported here, suggesting it did not come from either Europe or the Middle East. Second, the D/H and $^{18}O/^{16}O$ ratios might lie clearly in the European cluster or in the Middle Eastern cluster (*i.e.* away from the zone of overlap), permitting reasonable elimination of its geographic origin in one of the two possible regions. Third, the point for the Shroud might lie in the zone of overlap. In this case, it would be necessary to obtain unequivocally provenanced linen samples of the same age as the Shroud (accelerator mass spectrometry radiocarbon dates should be available for the Shroud in the near future) from the two possible geographic regions of origin. Analysis of these properly dated "control" samples would permit the influences of temporal climatic variability to be factored out, so that the isotope ratios of Shroud cellulose could be interpreted strictly in terms of geography. Such an effort would be time consuming, but might permit resolution of the question of geographic origin.

The approach presented here should prove useful in resolving disputed geographic provenance of other cellulose-containing artifacts (*e.g.* baskets, cotton textiles, paper) recovered from historic or prehistoric contexts.

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APPENDIX: PROVENANCE OF LINEN AND MAIZE SAMPLES

JD-1. Linen tapestry lining from Abraham series of tapestries at Hampton Court Palace. The sample was stamped "H:C 1661," together with the series name and number of the tapestry. The tapestries themselves originated in Brussels, but there is no proof that the lining came from there as well. Provided by J. M. Band, Hampton Court Palace, Surrey, England.

JD-2. Linen mummy sheet dated A.D. 100–300, in sarcophagus from Al-Qurna in district of Thebes. Provided by S. Støvring and E. Østergaard, National Museum, Copenhagen, Denmark. See BUHL *et al.* (1982).

JD-3. Linen buffcoat of King D. João I of Portugal, last quarter of 16th century. Provided by J. G. Varna, Instituto de Jose de Figueiredo, Lisbon, Portugal. See DE MENDONCA *et al.* (1973).

JD-4. Linen bandage of a man named Wah, from the 11th Dynasty, Egypt. Provided by N. Kajitani, Metropolitan Museum of Art, New York City.

JD-5. Roman/Coptic medallion-decorated linen garment. *Ca.* 5th century, Egypt. Provided by N. Kajitani, Metropolitan Museum of Art, New York City. MMA 89.18.202.

JD-7. Linen textile from Fatamid Tiraz period. *Ca.* 10th century, Egypt. Provided by N. Kajitani, Metropolitan Museum of Art, New York City.

JD-8. Linen textile from late Fatamid Tiraz period. *Ca.* 12th century, Egypt. Provided by N. Kajitani, Metropolitan Museum of Art, New York City. Kraus #172.

JD-10. Linen rope prepared in 1983 by rope maker who extracted fibers from flax grown in the Nile Delta. Provided by N. Kajitani, Metropolitan Museum of Art, New York City.

JD-11. Linen relining of a Coptic silk from the church of Maaseik. The silk is *ca.* 7th century; the lining was probably added later, possibly in Belgium. Provided by L. Masschelein, Institut Royal du Patrimoine Artistique, Brussels, Belgium.

JD-12. Modern linen. Belgium. Provided by P. Varda, Canvas Specialties, Sacramento, California.

JD-13. Modern linen. Belgium. Provided by P. Varda, Canvas Specialties, Sacramento, California.

JD-14. Modern linen. Poland. Provided by P. Varda, Canvas Specialties, Sacramento, California.

JD-15. Modern linen. Poland. Provided by P. Varda, Canvas Specialties, Sacramento, California.

JD-21. Linen textile from Italy (?). 19th century. Provided by M. J. Mayorcas, Mayorcas Ltd., London, England.

JD-22. Linen textile from Spain. Late 17th or early 18th century. Provided by M. J. Mayorcas, Mayorcas Ltd., London, England.

JD-23. Linen textile from France. Reign of Louis XIV, about 1700 A.D. Provided by M. J. Mayorcas, Mayorcas Ltd., London, England.

JD-24. Linen textile from Greece or Turkey. 17th or 18th century. Provided by M. J. Mayorcas, Mayorcas Ltd., London, England.

JD-25. Linen paper from France. Modern. Provided by J. Druzik.

JD-31. Linen textile from Austria (possibly Hungary). Modern. Provided by J. Druzik.

JD-41. Linen textile from Uelzen, West Germany. Approximately 50 years old. Provided by A. Schimmelmann, UCLA.

JD-48. Linen map backings, originally on maps of the Württemberg area, near Stuttgart, West Germany, of the last centuries. Provided by R. Grönwoldt, Wurttemberisches Landesmuseum, Stuttgart, West Germany.

JD-50. Linen map backings, originally on maps of the Württemberg area, near Stuttgart, West Germany, of the last centuries. Provided by R. Grönwoldt, Wurttemberisches Landesmuseum. Stuttgart, West Germany.

JD-56. Linen bag from Cave of the Letters, about 135 A.D. Provided by M. Broshi, Shrine of the Book, Israel Museum, Jerusalem. See Plate 90 in YADIN (1963) for a picture of this sample.

JD-57. Linen textile from Nahal Hemar cave. 9,000 years B.P. Provided by T. Shick, Israel Museum, Jerusalem. IM 1203-892 M_1. See BAR-YOSEF (1985).

JD-58. Linen hairnet from Nahal Hemar cave. 9,000 years B.P. or younger. Provided by T. Shick, Israel Museum, Jerusalem. See BAR-YOSEF (1985).

JD-64. Linen textile from burial at Ein Geidi. 1st century B.C., Israel.

JD-65. Linen textile from Nahal Hever. 2nd Temple Period, Israel.

JD-66a. Linen textile from Masada, Israel. 73 B.C.–73 A.D., but probably 50–73 A.D. Provided by A. Schaeffer, Hebrew University, Jerusalem.

JD-66b. Linen textile from Masada, Israel. 73 B.C.–73 A.D., but probably 50–73 A.D. Provided by A. Schaeffer, Hebrew University, Jerusalem.

BM-101. Maize stem from Manilla, Luzon, Phillipines. Collected January 1915. Harvard University Herbarium, 793 (G.H.).

BM-104. Maize stem from Mount Yagan, Mindoro Island, Phillipines. Collected 1953. Harvard University Herbarium, PNH 10733 (A).

BM-106. Maize stem from Hsi-chang, Chijon-shan Kwie-liu District, China. Collected November 1, 1937. Harvard University Herbarium, 28477 (A).

BM-110. Maize stem from San Salvador, El Salvador. Collected August 1922. Harvard University Herbarium, 1157 (G.H.).

BM-111. Maize stem from Rio Grande de Sul, Porto Alegre, Brazil. Collected 1899. Harvard University Herbarium, E. M. Reineek VII 1899 (G.H.).

BM-114. Maize stem from Urubamba Valley, Peru. Collected between 1942 and 1944. Harvard University Herbarium, Cutler #71593-2 ff1254.

BM-116. Maize stem from Urubamba Valley, Peru. Collected between 1942 and 1944. Harvard University Herbarium, Cutler #7165-10 ff1254.

BM-120. Maize stem from Coroico, Bolivia. Collected between 1942 and 1944. Harvard University Herbarium, Cutler #6107-4 823A.

BM-121. Maize stem from Colcaporhua, Bolivia. Collected between 1942 and 1944. Harvard University Herbarium, Cutler #6218B-7 846A.

BM-132. Maize stem from Diomontino, Mato Grosso, Brazil. Collected between 1942 and 1944. Harvard University Herbarium, Cutler #6-748 668.

BM-155. Maize stem from Cambridge, Massachusetts, U.S.A. Collected July 1881. Harvard University Herbarium.

BM-159. Maize stem from Clayton, North Carolina, U.S.A. Collected April 4, 1984.

BM-178. Maize stem from Forest Hills, Massachusetts, U.S.A. Collected 1942.

BM-1000. Maize stem from Helsinki, Finland. Collected 1986.