A STUDY OF THE RELATIONSHIPS BETWEEN CLIMATE AND STABLE ISOTOPE RATIOS IN TREE RINGS

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ABSTRACT

The main objectives of this work were to find out:

(a) how the stable isotope ratios in wood constituents such as cellulose and lignin vary with changing climate;

(b) whether such variations can be applied to the study of past climate.

Initially rings from a New Zealand grown *Pinus radiata* (Monterey pine) were studied since this is a complacent tree which grows throughout the year and hence over a wide temperature range.

In order to accurately determine the stable isotope ratios in wood components the following techniques were developed:

(a) the preparation of wood constituents on a small scale;

(b) the determination of δD values in waters;

(c) the determination of δD values in plant materials;

(d) the determination of δ^{13}C values in plant materials.

Several possible methods for measuring D/H ratios for cellulose are discussed. It was decided to use a technique which involves heating cellulose with a standard water to fix the D/H ratio of the exchangeable hydrogens. Using this method relative δD values have been determined to a precision of ± 0.9^{o}/oo (1 σ). The δ^{13}C method was developed so that the ^{13}C content of cellulose and lignin samples could be determined to ± 0.04^{o}/oo (1 σ).

A new procedure for determining δ values from raw mass spectrometer results is outlined.

δD determinations on cellulose samples from three growth rings in a specimen of *Pinus radiata* show that the non-exchangeable hydrogens of cellulose are more than 20^{o}/oo depleted in the summer as compared with the winter. These δD values appear to vary with the annual temperature cycle, but in the opposite sense to that expected from thermodynamic considerations. δ^{18}O determinations on the same samples show a summer/winter variation of about 2^{o}/oo and in the opposite direction to the summer/winter variation in the δD determinations.

The δD results from *Pinus radiata* cellulose are compared with δD determinations on cellulose from other trees.

It is concluded that all the measurements to date suggest that an increase in temperature results in a decrease in the D/H ratio of the non-exchangeable hydrogens in cellulose. The results imply a temperature coefficient of about - 3^{o}/oo per °C, which is opposite to that found for precipitation (+ 5.6^{o}/oo per °C). This temperature effect will only be useful for past climate studies if δD variations due to meteoric and/or leaf transpiration changes can be corrected for. This may be possible using δ^{18}O determinations.

A new model to explain the effects of environmental changes on the magnitude of carbon isotopic fractionation in C_3 plants is presented and discussed.

δ^{13}C determinations on both cellulose and lignin from the same *Pinus radiata* that was used for the δD determinations show an enrichment of about 2^{o}/oo in summer wood as compared with winter wood. This summer/winter variation is probably mainly due to changes in air temperature affecting the magnitude of carbon isotope fractionation during photosynthesis. The lignin variations are similar, but significantly displaced in time with respect to the cellulose variations. This is probably the result of secondary lignification.

$\delta^{13}C$ values for cellulose prepared from individual rings in a sample of dendrochronologically dated *Pseudotsuga menziesii* (Douglas fir) are compared with meteorological observations during growth. A correlation between the $\delta^{13}C$ values and mean late spring/early summer temperature is discussed. The temperature coefficient for this correlation is + 0.22°/oo per °C, which is in good agreement with the *Pinus radiata* results.

Similar determinations on individual rings from *Pinus radiata* are difficult to interpret in terms of climatic changes because of a relatively large depletion in ^{13}C towards the centre of the tree studied. A similar depletion in ^{13}C was observed for cellulose prepared from a carbon-14 dated *Agathis australis* (New Zealand kauri) which grew during the last 1000 years. However, no such "age" effect was observed when $\delta^{13}C$ values were determined for cellulose samples prepared from two dendrochronologically dated *Pinus longaeva* (Bristlecone pine) trees which also grew during the last 1000 years.

The short-term fluctuations in the cellulose $\delta^{13}C$ curve for *Agathis australis* are probably temperature related, and therefore represent a record of warm and cold periods in New Zealand's climate. This record is discussed together with information available from other sources. It is concluded that the fluctuations in New Zealand's climate during the last millenium were basically similar to those in the climate of North-western Europe.

The $\delta^{13}C$ variations in *Pinus longaeva* cellulose appear to be climate related, although in the opposite sense to the *Agathis australis* variations. These $\delta^{13}C$ variations in lower forest border *Pinus longaeva* significantly correlate with upper tree line ring width variations for the same species. It is proposed that changing water stress is the underlying cause of the $\delta^{13}C$ variations. If this is the case, the correlation may be the result of a correspondence between periods of water stress at the lower forest border and warm periods at the upper tree line.

It is concluded that $^{13}C/^{12}C$ ratios in both cellulose and lignin vary with changing climate. Results from *Pinus radiata* and *Pseudotsuga menziesii* indicate a temperature dependence of about + 0.2‰ per °C. However, other results from *Pseudotsuga menziesii* and *Pinus longaeva* indicate that, in some situations, this effect is dominated by a larger and opposite effect, attributed in this work to water stress.

Thus there is little doubt that $\delta^{13}C$ variations in the wood components from each tree ring sequence studied reflect climatic changes in that particular area. For some trees the effect of plant physiological changes on such variations is little understood. At present, those trees growing in exposed situations with a plentiful water supply appear to hold most promise for elucidating past climate data from measurements of $^{13}C/^{12}C$ ratios.

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PART I

INTRODUCTION

CHAPTER 1 GENERAL INTRODUCTION

Climatic changes are having an increasing effect on Man's activities. Population growth demands that more marginal agricultural land is brought into production and that water supplies are more fully utilised. Thus the recent drought in the marginal grazing lands south of the Sahara has caused wide-spread famine, while more rain than normal in Australia has resulted in disastrous flooding. The long dry spell in North-western Europe has severely tested resources designed to provide a continuous water supply. Even small changes in long term mean annual temperatures such as the 1 to 2°C cooling between the Medieval Warm Period and the Little Ice Age have left their mark on Man's history (see, for example, Lamb 1965).

Attempts have been made to set up mathematical models of the climate system with the aim of predicting future climate (e.g. Mason 1976, Gordon and Davies 1975). Such models, however, are only as good as the information available to put into them. There is an abundance of data available concerning recent climate, but in most parts of the world few meteorological records longer than 100 years exist. For information concerning climate changes that occurred before records began, proxy methods must be employed.

Palaeoclimatic information has been obtained from, for example, historical records (e.g. Lamb 1965, Ladurie 1972), changes in the abundance or distribution of plants and animals (e.g. LaMarche 1973, Van der Hammen 1974), changes in sea levels (e.g. Mörner 1974), changes in glaciers (e.g. Ladurie 1972), and the composition and stratigraphy of sediments (e.g. Kukla 1975).

Urey (1947, 1948) first suggested that past temperatures might be reconstructed from the isotopic composition of marine carbonates. Since then, Emiliani (e.g. 1955, 1966), Shackleton (e.g. 1975) and other workers have produced palaeotemperature curves from measurements of $^{18}O/^{16}O$ ratios in foraminifera from ocean cores. The oxygen isotope palaeothermometer has recently been applied to speleothems (Hendy 1969, Duplessy *et al.* 1971, Thompson *et al.* 1974, 1976). Variations in the oxygen isotopic composition of ice from the Greenland and Antarctic ice sheets have also been correlated with climatic changes (Dansgaard *et al.* 1969, Epstein *et al.* 1970, Johnsen *et al.* 1972). Such studies have enabled scientists to more accurately assess the effect on climate of, for example, variations in the Earth's orbit (see, e.g., Hays *et al.* 1976).

However, there is a dearth of accurate information concerning the smaller fluctuations in climate that have occurred since the last ice age. Historical records have been used to investigate recent climatic changes in some parts of the Northern Hemisphere (e.g. Lamb 1965), and variations of $^{18}O/^{16}O$ ratios in ice and lake sediments have provided additional information (e.g. Dansgaard *et al.* 1975, Mörner and Walli 1977). However, much more data from all over the world will be required before climatic changes can be unambiguously correlated with such phenomena as the solar cycle or large volcanic eruptions (e.g. Schneider and Mass 1975). Also, the effect on future climate of increased carbon dioxide concentration and aerosols in the atmosphere (e.g. Broecker 1975) can only be properly assessed after the underlying reasons for the world's past climatic changes have been evaluated. The prediction of future climatic extremes is of particular importance. For example, is the north-east of the United States likely to have an even colder winter than the one it has just experienced? If such an event could be forecast, appropriate measures could be taken beforehand.

During the last few years some palaeoclimatologists have been turning to tree rings. The science of dendrochronology has been developed to date the wood making up each tree ring accurately to the nearest year. Chronologies have been built up from several different species of tree in various parts of the world. A chronology based on *Pinus longeava*, which has been developed at the University of Arizona, goes back more than 8000 years (Ferguson 1968, 1972). Moreover, trees that are not sensitive enough for dendrochronological study can be carbon-14 dated to within a few decades. The relationships between ring-width variations and climate have been carefully studied by dendroclimatologists. Correlations are often complex, but some worthwhile palaeoclimate data has been elucidated using multi-variate statistical analysis and computers (e.g. Fritts 1976, LaMarche 1974).

This thesis is concerned with assessing the possibilities of obtaining past climate data from the variation of stable isotope ratios in the constituents of dendrochronologically or carbon-14 dated wood.

CHAPTER 2 TREE PHYSIOLOGY AND BIOCHEMISTRY

Before any relationships between climate and stable isotope variations in wood can be properly assessed, the physiology and biochemistry of wood formation must be considered.

2.1 *WOOD STRUCTURE*

Fig. 1 is a diagrammatic representation of a cross-section through a woody stem showing four seasons' growth. The xylem is the water-conducting tissue which makes up the woody centre of a tree. The phloem is the food-conducting tissue under the bark. Between the xylem and the phloem is a layer of narrow, thin-walled cells called the vascular cambium. These cells are able to divide to make new xylem and phloem cells. All plants with bark also have a cork cambium where the bark is formed.

The annual rings of conifers (gymnosperms) are made up mostly of tracheids, which are long, thin, vertically-orientated cells with relatively thick lignified walls. Earlywood consists of tracheids which are wider and thinner-walled than those in latewood. In consequence earlywood tends to be lighter in colour than latewood so that growth rings are usually readily distinguishable.

The annual rings of angiosperms are made up mostly of vessels, which are thin-walled cells of relatively large diameter. They are normally much shorter than the fibre tracheids which are also usually present. For some trees in this group (e.g. oaks) the demarcation of successive rings can be very distinct due to the formation of a narrow band of exceptionally large earlywood vessels.

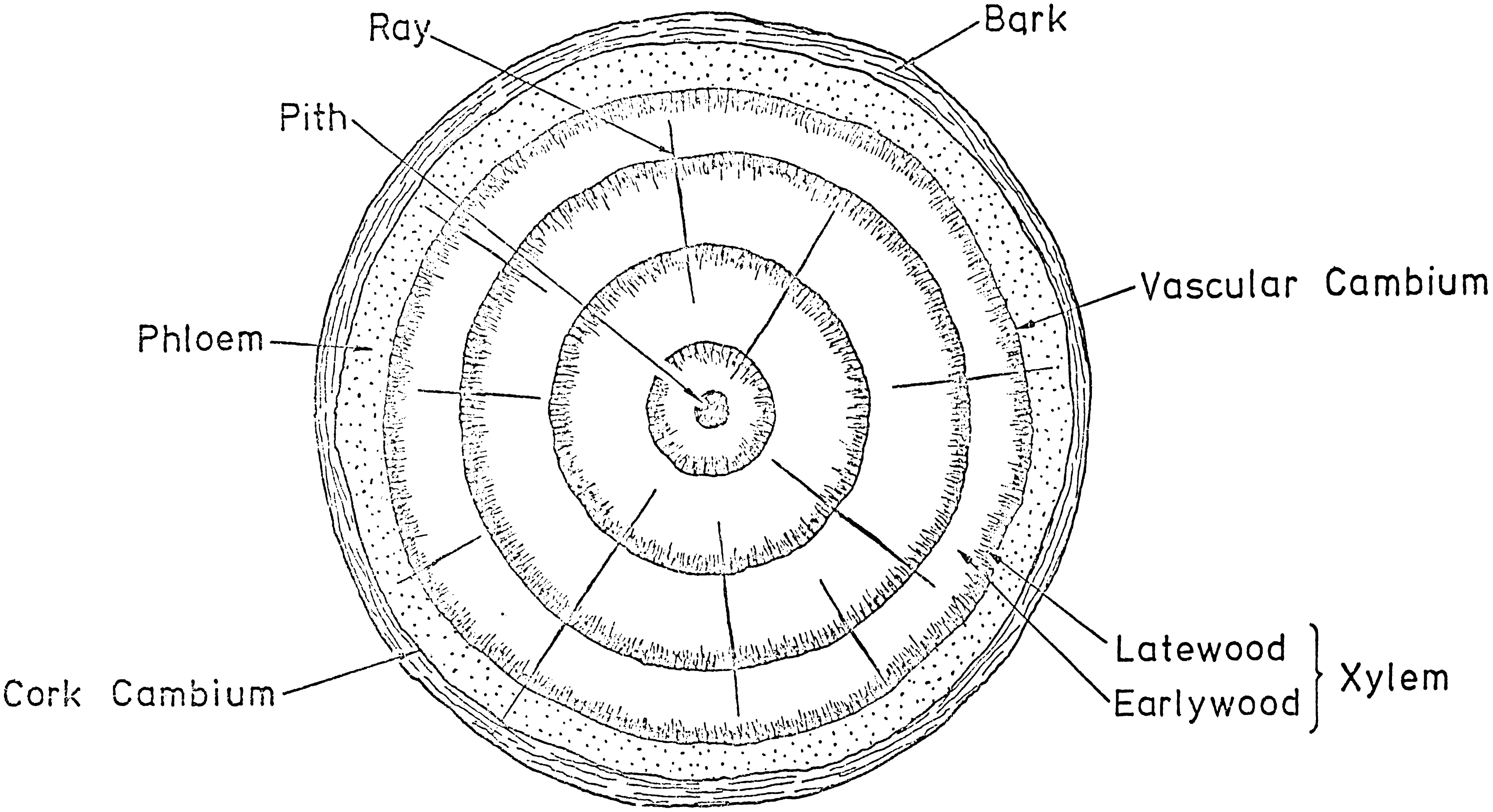


Fig.1. Diagrammatic section through a woody stem showing four season's growth.

Mature trees normally contain a core of dead wood round the pith. This heartwood only provides the tree with mechanical support. The region of xylem between the heartwood and the vascular cambium is often lighter in colour and is known as sapwood. This wood is used for water conduction and the storage of reserve photosynthates. The sapwood region varies from only a few growth rings in some species to nearly 200 in others (Isenberg 1963).

For diagrams and further details concerning wood structure see, for example, Isenberg (1963), Zimmerman and Brown (1971) or Kozlowski (1971).

2.2 *THE FORMATION OF TREE RINGS*

Most mature trees in the temperate zones normally produce only one ring each year. However, multiple rings can be formed during the same season and sometimes certain rings can be completely missing along portions of the tree axis. Multiple rings are especially common in sub-tropical and tropical trees possessing intermittent shoot growth. In such situations a new growth ring often forms corresponding to each new flush of growth (Zimmerman and Brown 1971).

The actual period of wood deposition is under hormonal control. Thus for northern temperate species cambial activity is initiated each spring by auxin which is produced in the developing buds and translocated down the stem (Leyton 1972). In many seedlings and young trees any condition that enhances bud break, rapid shoot growth or continued leaf development results in high levels of auxin production and large diameter cells of the earlywood type. Conversely, low temperatures, drought, or short photoperiods which adversely affect shoot extension and leaf development lower the levels of diffusible auxin and bring about smaller or radially flattened tracheids of the latewood type. This mechanism is, however, probably too simplified to account for the complicated patterns of cellular differentiation in older trees. Seasonal levels of other types of growth promoters and inhibitors must also be important in controlling growth ring formation (Zimmerman and Brown 1971).

Because of the above considerations the period of cambial activity can vary from year to year as a function of differences in the yearly climates, differences in environment from one site to the next and differences in species. For most temperate grown conifers such variations for a particular tree are small and growth occurs over a similar period each year. For example, ring growth in high-elevation, drought-subjected *Pinus longaeva* occurs for approximately 45 days. Cambial activity ceases even though environmental factors appear favourable (Fritts 1976). On the other hand, the cambium of *Pinus radiata* grown in New Zealand does not experience full dormancy although for a short period during the winter no new xylem cells are produced (Barnett 1971). The growing season for most temperate species is somewhere between these two extremes.

In most trees growth, which is most active during the early part of the season, slows down as the season progresses until it ceases totally. The tree's cambium remains dormant until the next spring. Usually growth is most vigorous and the active season longest for young trees, while it is less vigorous and the season shorter for old trees. Thus in *Pinus longaeva* the period of cambial activity is 47-56 days for young trees and 35-43 days for old trees (Fritts 1976).

2.3 PHOTOSYNTHESIS AND RESPIRATION

Photosynthesis is the process by which green plants use energy from sunlight to manufacture carbohydrates from carbon dioxide and water. Oxygen is given off as a by-product. In trees the water is translocated from the roots (see section 2.4) while the carbon dioxide is absorbed from the air via the leaf stomata.

There are two different pathways for photosynthetic carbon dioxide fixation. C_3 plants (e.g. most trees) utilize the Calvin cycle in which ribulose-1 ,5-diphosphate (RuDP) carboxylase is the carboxylating enzyme (fig. 2). On the other hand, the initial carboxylating enzyme in C_4 plants is phosphoenolpyruvate (PEP) carboxylase (see fig. 3). A third type, the CAM plants, utilize both RuDP carboxylase and PEP carboxylase. C_3, C_4 and CAM plants can be distinguished partly by measuring their carbon isotope compositions (e.g. Lerman 1974a). Carbon dioxide fixation has been discussed in detail by many authors including Zelitch (1971), Black (1973) and Kelly *et al.* (1976).

Respiration occurs continuously in all living cells during both the day and the night. In photosynthetic material like leaves respiration in the light is often 3 to 5 times that in the dark. This is due to photorespiration which is specifically associated with substrates produced during photosynthesis. Normal "dark" respiration also occurs in the light, but is a very different biochemical process. Dark respiration involves the breakdown of starch or glucose whereas glycolate is the primary source for photorespiration. Carbon dioxide is a product of both types of respiration (Zelitch 1971).

In trees the rate of gross photosynthesis increases rapidly with temperature to a maximum after which further moderate increases in temperature have little effect. Total respiration increases steadily with temperature even after photosynthesis has reached a maximum. This means that net photosynthesis (gross photosynthesis - total respiration) increases with increasing temperature up to a maximum after which it decreases with any further increase in temperature (see fig. 4). The optimum temperature for net photosynthesis is normally in the range 15 to 30°C (15 to 20°C for temperate plants) (Fritts 1976).

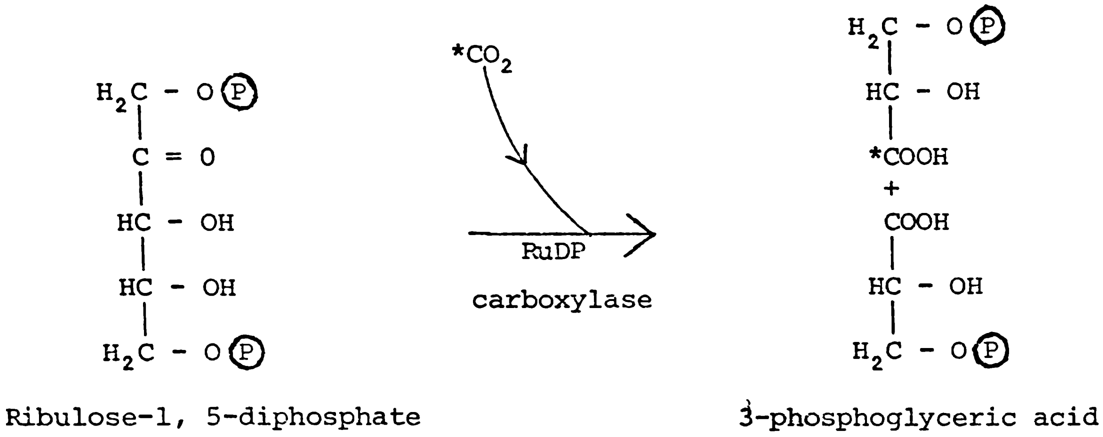


Fig. 2. Carbon dioxide fixation in C_3 plants.

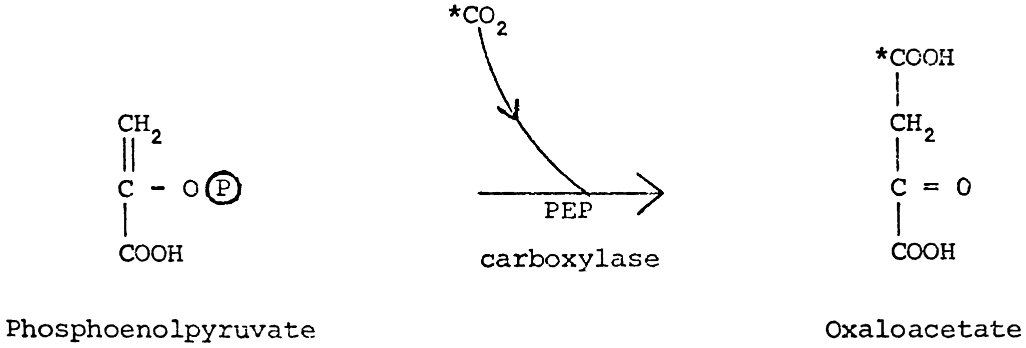


Fig. 3. Carbon dioxide fixation in C_4 plants.

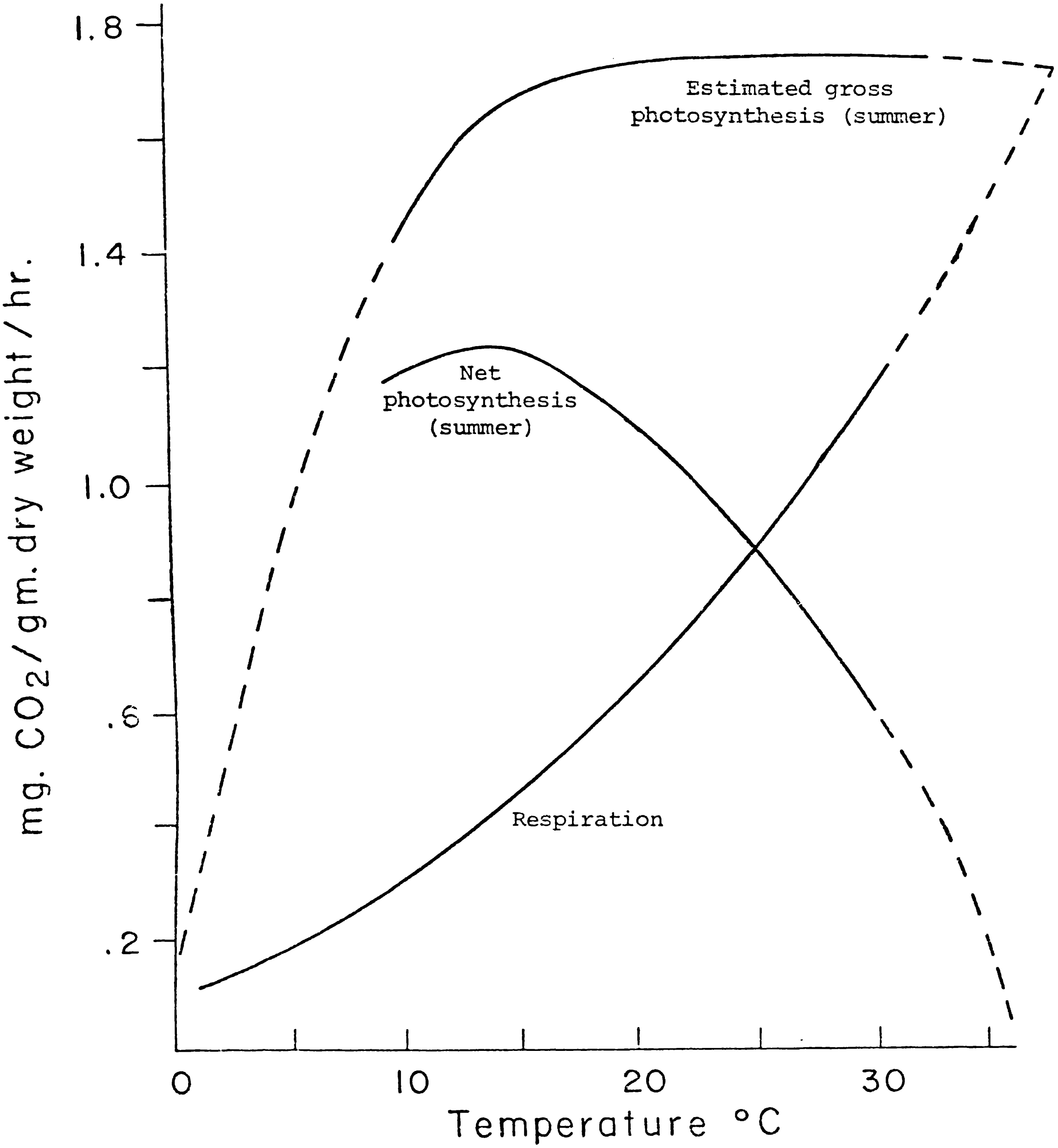


Fig. 4. The relationships between temperature and estimated gross photosynthesis, net photosynthesis and respiration for *Pinus longaeva* during summer (after Fritts 1976).

Apart from temperature the rate of net photosynthesis is controlled by a number of other factors. External factors include light (intensity and quality), carbon dioxide concentration , wind velocity, water supply and nutrient supply. Internal factors include the age of the leaf, chlorophyll content, enzyme factors, leaf water content, leaf structure and stomatal aperture. Many of the internal factors will change in response to external factors (Heath 1969).

For further information one of the many books and reviews on photosynthesis and respiration should be consulted (e.g. Zelitch 1971). Fritts (1976) includes a more detailed discussion of the various factors affecting photosynthesis and respiration in trees.

2.4 *TRANSLOCATION*

Water, taken into a tree through the roots, ascends via the xylem to the leaves. This process is driven by evaporation from the leaves and is regulated by the opening and closing of leaf stomata, although some water is also lost from the epidermal cells. Transpiration during the day (mainly through open stomata) usually removes moisture faster than it is absorbed by the roots and so the tree's trunk shrinks. During the night the leaf stomata are closed and transpiration is normally reduced such that water replenishment exceeds loss and the trunk expands. Temperature changes, humidity, solar radiation levels and wind speed all markedly affect transpiration rates. (Fritts 1976).

Photosynthates (mainly sucrose) from the leaves are transported in the phloem. Unlike transport in the xylem the translocation that occurs in the phloem is bidirectional. Thus mature leaves can translocate to developing leaves above or to the stem and roots below (Larson 1972).

Since photosynthesis often produces more carbohydrates than the phloem can export, the excess is stored by the leaves in the form of starch, ready for translocation during the night after photosynthetic activity has stopped. Photosynthates are also stored for short or extended periods as starch or lipids in the stem and roots. Although wood formation generally occurs during periods of maximum photosynthetic activity, in some trees it can also occur during periods when net photosynthesis is negative, presumably utilizing stored photosynthates (Fritts 1976). Thus it is conceivable that, if a conifer carries out much net photosynthesis during the winter months when there is no cambial activity, some of the wood it lays down in the spring may have originated from winter photosynthates. This could explain why ring widths in some low-altitude, arid-site conifers can be correlated with climatic conditions during the 9 to 10 month period prior to growth (Fritts 1976).

In straight grained wood photosynthates tend to be translocated vertically so that they are used by cambial tissues and roots directly below their branches of origin. In trees exhibiting spiral grain translocation tends to occur in a spiral direction. Thus those portions of an annual ring formed by cambial tissues in the path of translocation streams from heavily foliated branches are often wider than other portions of the same ring (Fritts 1976).

2.5 *WOOD COMPOSITION*

All woods contain the same major components as shown in fig. 5.

About 50% of the wood is normally cellulose. The structure and properties of this substance are discussed in section 6.2. A variable proportion (~15%) of other polysaccharides known as hemicelluloses, are also always present. For detailed discussions on these wood components reviews by Schuerch (1963) and Timell (1964, 1965) should be consulted.

A further ~30% of wood is lignin, which is a complex three

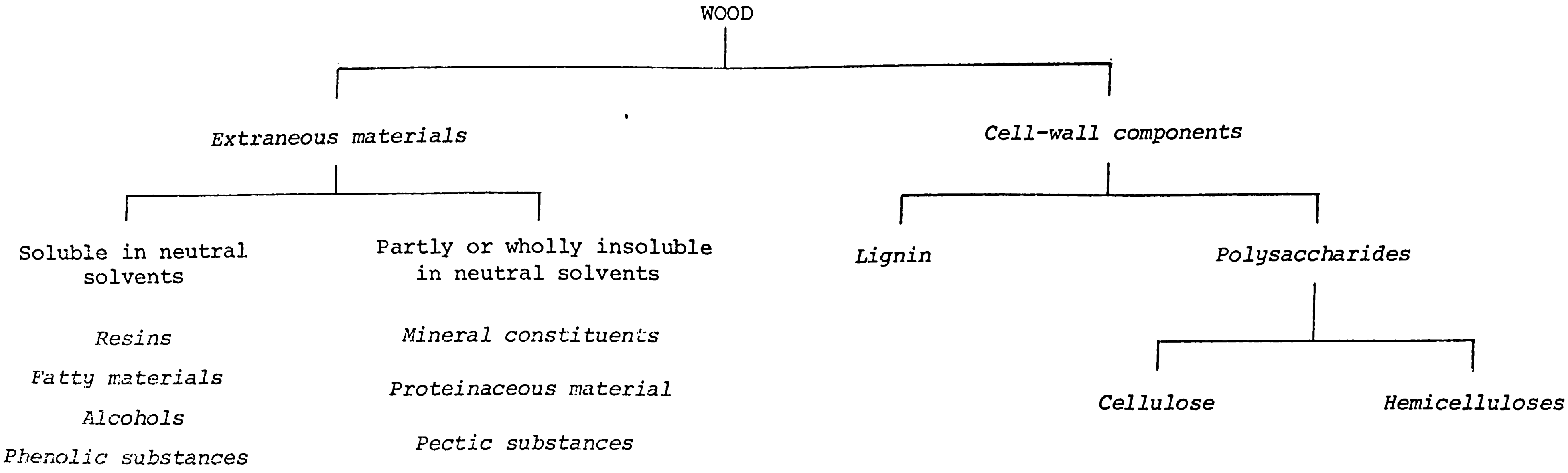
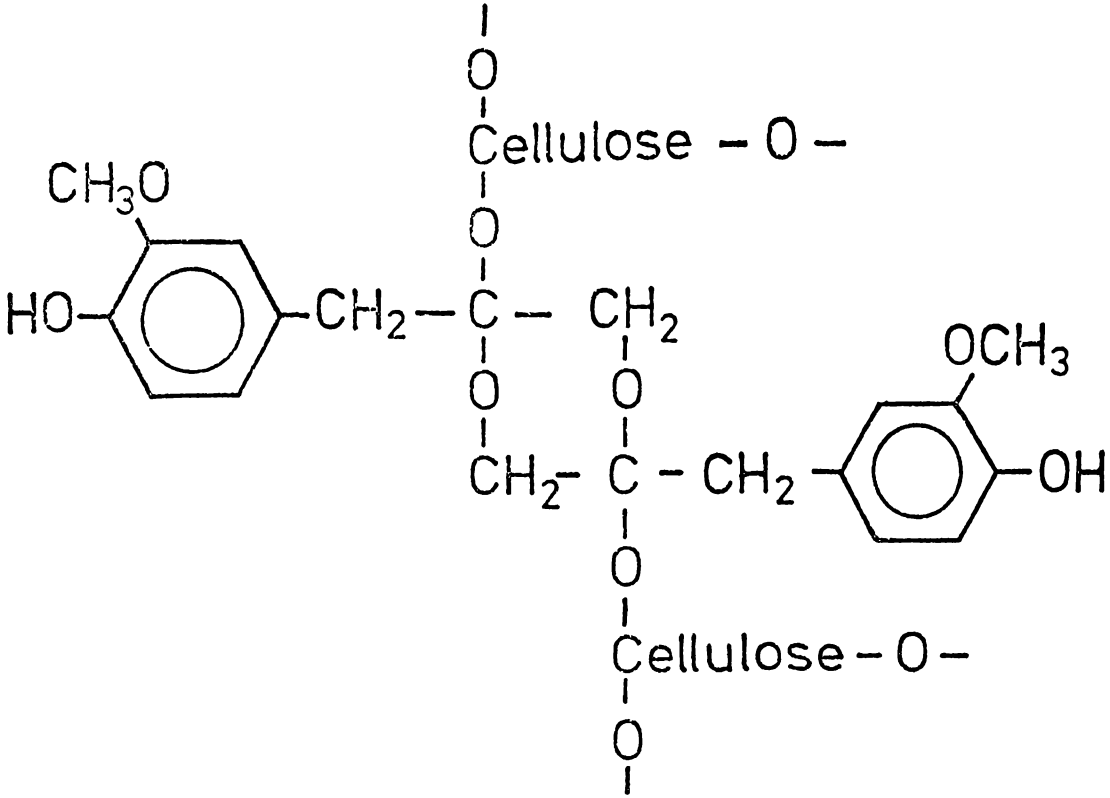


Fig. 5. The composition of wood (after Browning 1967).

dimensional polymer consisting of substituted phenyl propane residues attached to cellulose molecules. A proposed structure is shown in fig. 6 (Brauns and Brauns 1960). Lignin preparations contain mixtures of aromatic compounds formed from the phenyl propane residues. Coniferyl alcohol and syringaldehyde (see fig. 7) are examples of such compounds (Yudkin and Offord 1973). Sarkanen (1963) and Brauns and Brauns (1960) discuss lignin in detail.

Finally, all woods contain extraneous components which are not considered essential parts of the cell wall (Browning 1967). For a detailed discussion concerning extraneous components see Buchanan (1963).

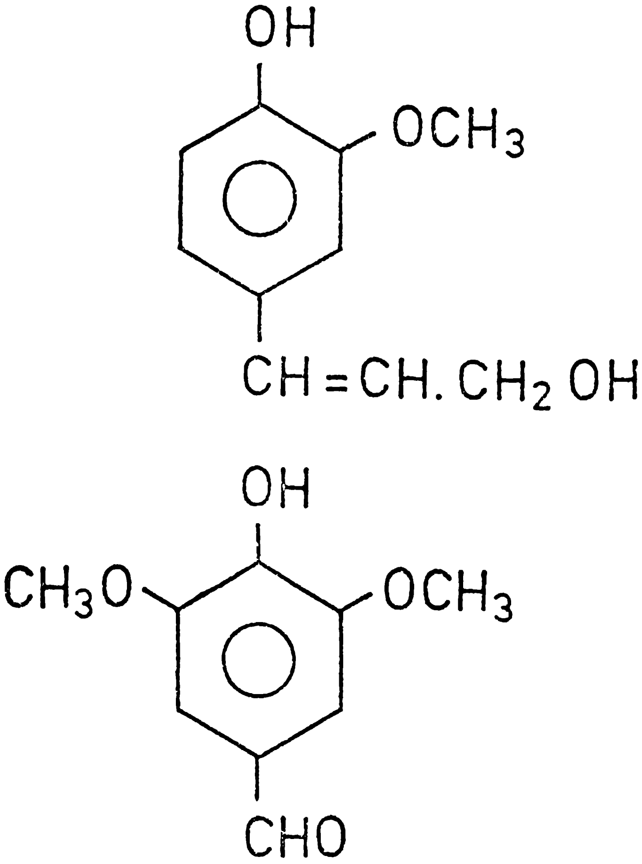
The relative proportions of these wood components depend on the species and also vary within a particular tree (Browning 1967). Such variations in composition occur not only across individual rings (i.e. earlywood compared with latewood), but also up and down and radially across the tree (Narayanamurti and Das 1955). The chemical composition of wood can also vary with age (e.g. Hans 1971, Harwood 1971).



Structure of Protolignin in wood

Brauns and Brauns (1960)

Fig. 6.



Coniferyl alcohol

Syringaldehyde

Fig.7.

PART II

GENERAL EXPERIMENTAL PROCEDURES

CHAPTER 3 MASS SPECTROMETRIC MEASUREMENTS

3.1 THE MASS SPECTROMETER

Unless otherwise indicated the isotope ratios reported in this thesis were determined using a Micromass 602C. This is a precision double collector mass spectrometer designed for the measurement of isotopic enrichment, or depletion, of a sample gas relative to a reference gas. The instrument in our laboratory is a Siamese 602C fitted with two analysers: one for masses 2 to 80, but designed specifically for D/H analysis on hydrogen gas; the other for isotopic analyses of gases with masses 24 to 80, for example, carbon dioxide, sulphur dioxide and nitrogen. In both analysers the ion beams are collected in two Faraday bucket collectors. Full specifications for this mass spectrometer can be obtained from VG Isotopes (see, e.g., 1974).

A considerable length of time was spent setting up and tracking down all the faults in our instrument partly because of the damage it received in transit. This meant that some of the earlier D/H measurements had to be carried out using the Micromass standard 602C at the Institute of Nuclear Sciences, Lower Hutt. The use of their Nuclide Associates (model 6-60-RMS) mass spectrometer for carbon dioxide isotopic analyses was also important during the initial stages of this work although all these earlier measurements were later repeated using our more precise Micromass 602C.

All mass spectrometric results are expressed as the per mil difference between the isotopic ratio of the sample (B) and the standard (A):

$$\delta = \delta_A(B) = \left[\frac{Y - Z}{Z}\right] \times 1000 \ (^{o}/oo) \qquad \text{(i)}$$

where Y and Z are the true isotopic ratios in the sample and standard respectively.

3.2 *CALCULATION OF δ VALUES*

When using the Micromass 602C* a sample gas and a reference gas are connected to each side of the inlet system. The amounts of each gas flowing into the analyser are adjusted to be the same. With the mass spectrometer set on reference, the ratio control box is adjusted until a reading is obtained on the chart recorder. Then the mass spectrometer is switched over to the sample side and, if necessary, the ratio offset control is adjusted to bring the recorder pen back on scale. The Micromass 602C incorporates a digital integrator to obviate the need for measurement from the chart recorder and to improve the accuracy of such measurements. Thus, after setting the integrator delay control to a suitable value, the mass spectrometer is switched to automatic and the digital integrator readings for the reference (R) and the sample (S) are noted for several consecutive change-overs, the number depending on the accuracy of measurement required. Fig. 8 shows a typical chart recorder trace for δ^{45} determination.

The enrichment (δ) of the sample with respect to the reference can then be calculated. A widely accepted method (A.T. Wilson, personal communication; J.R. Hulston, personal communication) for carrying out such calculations is outlined in the Micromass Instruction

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[*] Full details concerning the operation of the Micromass 602C are given in the Micromass Instruction Manual.

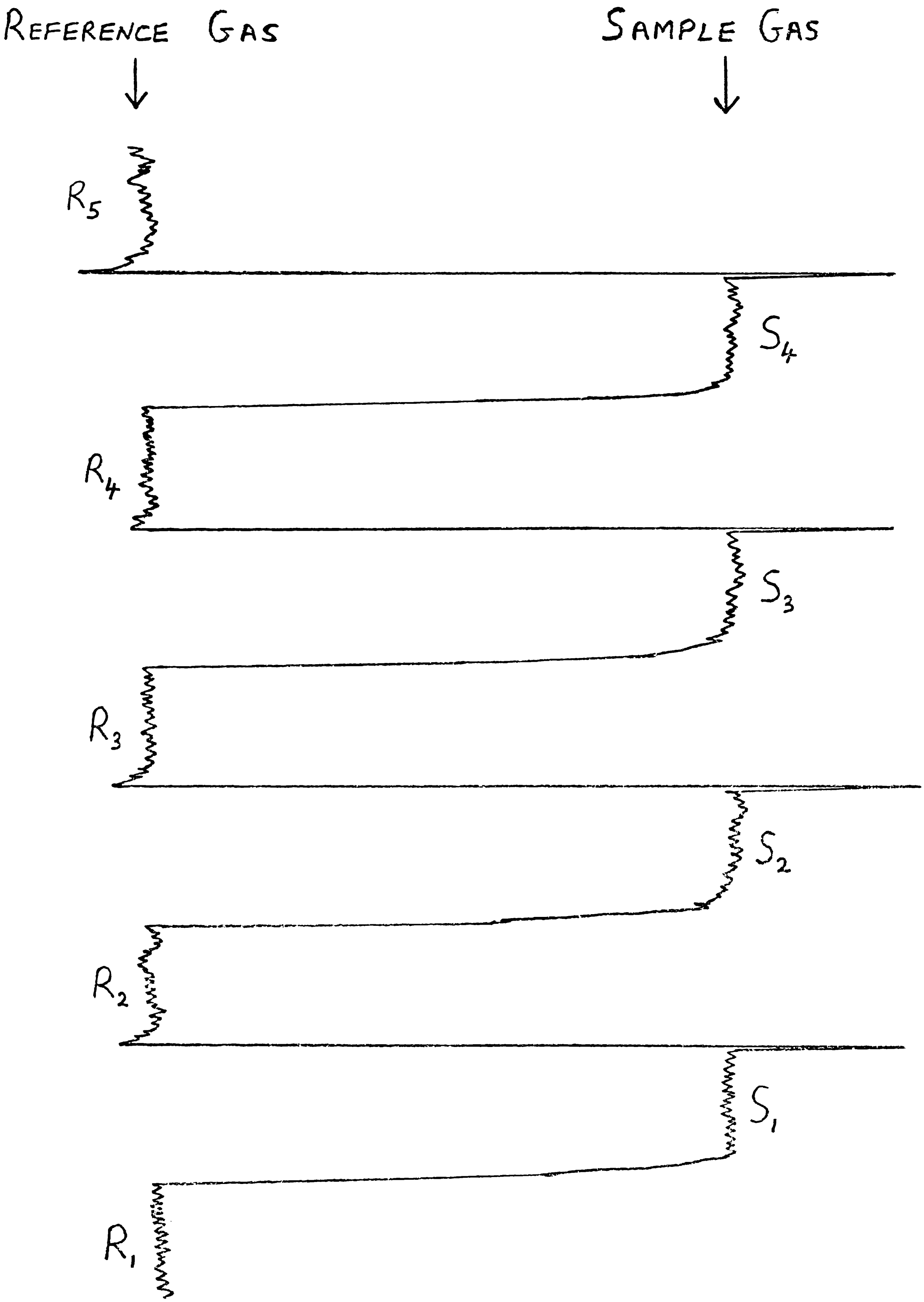

Fig. 8. Chart recorder output from Micromass 602C (mass 45).

Manual. However, a close examination of this method showed it to be statistically incorrect (C. Dyson, personal communication), so a new method was worked out as outlined below.

For each sample measured in the mass spectrometer the following quantities are noted:

R_c = 10^4 x ratio control box setting (the coarse isotopic ratio);

R_o = 10^4 x ratio offset control setting;

$R_1, R_2, \ldots R_{n-1}, R_n$ = digital integrator readings for reference gas (the fine isotopic ratio);

$S_1, S_2, \ldots S_{n-1}, S_n$ = digital integrator readings for sample gas.

For a series of readings starting and ending with a reference, i.e. R_1, S_1, R_2, $S_2, \ldots R_{n-1}$, S_{n-1}, R_n (see fig. 8);

$$\text{take } x_1 = S_1 - \left[\frac{R_1 + R_2}{2}\right]$$

$$x_2 = S_2 - \left[\frac{R_2 + R_3}{2}\right]$$

$$\vdots \qquad \qquad \vdots$$

$$x_{n-1} = S_{n-1} - \left[\frac{R_{n-1} + R_n}{2}\right] \qquad \text{(ii)}$$

This gives n - 1 values of x which are independent estimations of the difference between sample and reference ("S - R").

$$\text{Thus, the mean value of x, } \bar{x} = \frac{\sum_1^{n-1} x}{n - 1} \qquad \text{(iii)}$$

$$\text{Also, the mean value of R, } \bar{R} = \frac{\tfrac{1}{2}R_1 + R_2 + \ldots + R_{n-1} + \tfrac{1}{2}R_n}{n - 1} \qquad \text{(iv)}$$

$$\therefore \quad \delta \text{ (in } {}^{\circ}\!/\text{oo)} = \left[\frac{R_o + \bar{x}}{R_c + \bar{R}}\right] \times 1000 \qquad \text{(v)}$$

$$\text{The variance, } \sigma_a^2 = \frac{\sum_{1}^{n-1} (x - \bar{x})^2}{n - 2} \qquad \text{(vi)}$$

However this value is artificially low because each value of x is smoothed. Thus a statistical correction factor must be applied and the true variance, $\sigma_o^2 = \frac{4}{3}\sigma_a^2$ (vii)

$$\text{Then, the standard error, } \sigma_m = \frac{\sqrt{\frac{\sigma_o^2}{n - 1}}}{(R_c + \bar{R})} \times 1000 \qquad \text{(viii)}$$

The above method should be changed slightly if it is preferred to start the mass spectrometer on the sample instead of the reference gas. Thus, for a series of readings starting and finishing with a sample, the general term in equation (ii) should be changed to

$$x_{n-1} = \left[\frac{S_{n-1} + S_n}{2}\right] - R_{n-1} \qquad \text{(ix)}$$

which gives the maximum number of independent values for x.

Alternatively for a series of readings starting with a reference and finishing with a sample the general term in (ii) should be changed to

$$x_{n-1} = \left[\frac{3}{4} S_{n-1} + \frac{1}{4} S_n\right] - \left[\frac{1}{4} R_{n-1} + \frac{3}{4} R_n\right] \qquad \text{(x)}$$

which gives the maximum number of independent values for x in this case.

Similarly for a series of readings starting with a sample and finishing with a reference the general term in (ii) should be changed to

$$x_{n-1} = \left[\frac{1}{4} S_{n-1} + \frac{3}{4} S_n\right] - \left[\frac{3}{4} R_{n-1} + \frac{1}{4} R_n\right] \qquad \text{(xi)}$$

Apart from its statistical correctness the above method has a further advantage over the old method in that it corrects for any linear drift of S and R. Also the method gives some correction for variable drift since three consecutive readings are taken to compute each x value.

δ values calculated using the method outlined above are essentially the same as those calculated using the old method (as described, for example, in the Micromass Instruction Manual). However, σ_m values are normally slightly larger if the former, more statistically correct method is used.

In this work 5R values and 4S values were generally used to compute δD and δ^{45} (for δ^{13} determinations). Initially the calculations were carried out using an IBM 11-30 computer (see program and example of printout in appendix B). Later a programmable calculator (Texas Instruments SR 52) was used.

3.3 *ISOTOPE RATIO MEASUREMENTS ON CARBON DIOXIDE*

3.3.1 *Notation and Symbols*

All carbon dioxide isotopic ratio determinations are expressed in terms of δ as defined in section 3.1.

In this section the following notations are used for the sample:

Y_{13} = isotopic abundance ratio $^{13}C/^{12}C$;

Y_{17} = " " " $^{17}O/^{16}O$;

Y_{18} = " " " $^{18}O/^{16}O$;

$$Y_{45} = \frac{\text{molecules of mass 45}}{\text{molecules of mass 44}} ;$$

$$Y_{46} = \frac{\text{molecules of mass 46}}{\text{molecules of mass 44}} .$$

The corresponding values for the standard are denoted by Z, using the same subscripts.

Thus from equation (i) we get

$$\delta^{45} = \left[\frac{Y_{45} - Z_{45}}{Z_{45}}\right] \times 1000 \qquad \text{(xii)}$$

and similar relationships for δ^{46}, δ^{13}, δ^{17}, δ^{18}.

Y', Z' and δ' generally refer to measured, uncorrected values.

3.3.2 *Corrections*

Since the Micromass 602C can measure values of δ^{45} and δ^{46} to ± 0.02 (1 σ_m) it was necessary to consider possible sources of error.

To convert δ' (the measured uncorrected value) to δ, the following equation was used:

$$\delta = \delta' (C_T \times C_M \times C_V) + C_E \qquad \text{(xiii)}$$

where C_T = tail contribution correction factor;

C_M = memory effect " " ;

C_V = valve-mixing " " ;

C_E = zero enrichment " " .

(a) Tail contribution, C_T. This arises because the "tail" of the large mass 44 ion peak tends to overlap the relatively small mass 45 and 46 ion peaks, causing the measured δ values to be too small. An analysis of the carbon dioxide spectrum of our instrument was complicated by the existence of a negative electron peak between the mass 44 and mass 45 peaks. However, the tail contribution to mass 45 was clearly less than $1^{\circ}/oo$ and most probably less than $0.5^{\circ}/oo$.* The tail contribution of mass 44 to mass 46 was undetectable. Thus C_T for $\delta^{45'}$ was ~ 1.0005 and C_T for $\delta^{46'}$ was < 1.0002.

(b) Zero enrichment, C_E. Instrumental factors may lead to a small, but constant, zero enrichment value which should be subtracted from δ' value for a sample/reference analysis. This factor was measured by equilibrating the sample between the two parts of the mass spectrometer inlet system. For our instrument C_E was less than $0.02^{\circ}/oo$ and therefore no correction was necessary.

(c) Memory effect, C_M. This can be produced by carbon dioxide gas adsorption onto the metal surfaces of the inlet system or analyser chamber. Under normal operating conditions with clean dry carbon dioxide C_M was less than 1.001 for our instrument. Samples of carbon dioxide contaminated with water produced a significant lasting memory effect which could only be eliminated by a very long pump-out time or preferably a bake-out of the analyser and inlet systems.

(d) Valve-mixing, C_V. The cross-mixing correction factor for the changeover valve on the Micromass 602C is less than 1.001 (VG Isotopes 1974).

Thus, in equation (xiii),

$C_T^{45} \approx 1.0005,$ $\quad C_T^{46} < 1.0002;$

$C_M < 1.001;$

$C_V < 1.001;$

$C_E < 0.02^{\circ}/oo.$

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*Bridger *et al.* (1973) found a tail contribution of $0.7^{\circ}/oo$ for the Micromass 602C.

This means that for differences between reference and sample of up to ± 10‰, $\delta^{45} = \delta^{45'}$ and $\delta^{46} = \delta^{46'}$ within the precision of the mass spectrometer (± 0.02‰). For differences of up to ± 25‰ correction factors C_M and C_V may become significant and therefore the error of measurement will be slightly greater than ± 0.02‰.

For a fuller discussion of mass spectrometer correction factors see Mook (1968), Deines (1970), and Mook and Grootes (1973). For further details concerning isotope measurement with the Micromass 602 series of mass spectrometers see VG Isotopes (1974).

3.3.3 *Evaluation of δ^{13} and δ^{18}*

(a) Ion collector considerations. When the Micromass 602C is set up on mass 45 the minor collector collects ions of mass 45 and the major ions of mass 44. However, when it is set up on mass 46 the minor collector collects ions of mass 46 and the major ions of both mass 44 and mass 45.

$$\text{Thus, } Y'_{45} = \frac{\text{molecules of mass 45}}{\text{molecules of mass 44}} = Y_{45};$$

$$\text{and} \quad Z'_{45} = Z_{45}$$

$$\text{Therefore, from (xii), } \delta^{45} = \delta^{45'} \qquad \text{(xiv)}$$

$$\text{However, } Y'_{46} = \frac{\text{molecules of mass 46}}{\text{molecules of mass 44 + molecules of mass 45}}$$

$$= \frac{Y_{46}}{1 + Y_{45}}$$

$$\text{i.e. } Y_{46} = Y'_{46}\,(1 + Y_{45}) \qquad \text{(xv)}$$

$$\text{and} \quad Z_{46} = Z'_{46}\,(1 + Z_{45}) \qquad \text{(xvi)}$$

$$\text{From (xii), } \delta^{46} = \left[\frac{Y_{46} - Z_{46}}{Z_{46}}\right] \times 1000$$

Using (xv), (xvi), (xii) and (xiv) this becomes

$$\delta^{46} = \delta^{46'} + \frac{Z_{45}.\ \delta^{45'}}{1 + Z_{45}} (1 + 10^{-3}\delta^{46'})$$

For natural samples with a range of up to 50‰ in ^{13}C and ^{18}O, measured on a mass spectrometer accurate to ± 0.02‰, this relationship simplifies to

$$\delta^{46} = \delta^{46'} + Z_{45}\delta^{45'} \qquad \text{(xvii)}$$

(b) Isotopic ion considerations. The ion beams of masses 44, 45 and 46 consist of several different ions originating from the following molecules:

mass 44 - $^{12}C\ ^{16}O\ ^{16}O$;

mass 45 - $^{13}C\ ^{16}O\ ^{16}O$, $^{12}C\ ^{16}O\ ^{17}O$;

mass 46 - $^{12}C\ ^{16}O\ ^{18}O$, $^{13}C\ ^{16}O\ ^{17}O$, $^{12}C\ ^{17}O\ ^{17}O$.

The absolute abundances of these molecules in natural CO_2 are given by Bowen (1966).

Thus, the relationships between the Y values defined in section 3.3.1 are:

$$Y_{45} = \frac{^{13}C\ ^{16}O\ ^{16}O + {}^{12}C\ ^{16}O\ ^{17}O}{^{12}C\ ^{16}O\ ^{16}O} = Y_{13} + 2Y_{17} \qquad \text{(xviii)}$$

and $$Y_{46} = \frac{^{12}C\ ^{16}O\ ^{18}O + {}^{13}C\ ^{16}O\ ^{17}O + {}^{12}C\ ^{17}O\ ^{17}O}{^{12}C\ ^{16}O\ ^{16}O}$$

$$= 2Y_{18} + 2Y_{13}Y_{17} + Y_{17}^2 \qquad \text{(xix)}$$

From (xii), $Y_{45} = Z_{45}\ (10^{-3}.\delta^{45} + 1)$

(The formulae for Y_{46}, Y_{13}, Y_{17}, Y_{18} are similar.)

Substituting into equation (xviii) and rearranging,

$$\delta^{13} = \frac{Z_{45}}{Z_{13}} \cdot \delta^{45} - \frac{Z_{17}}{Z_{13}} \cdot 2\,\delta^{17}$$

Craig (1954b) and Bigeleisen (1952) have shown that the enrichments for ^{17}O and ^{18}O differ by about a factor of 2. Thus,

$$\delta^{13} = \frac{Z_{45}}{Z_{13}} \cdot \delta^{45} - \frac{Z_{17}}{Z_{13}} \cdot \delta^{18} \qquad \text{(xx)}$$

Substituting in equation (xix), rearranging and approximating,

$$\delta^{18} = \frac{Z_{46}}{Z_{46} - Z_{13}Z_{17}} \cdot \delta^{46} - \frac{2\,Z_{13}Z_{17}}{Z_{46} - Z_{13}Z_{17}} \cdot \delta^{13} \qquad \text{(xxi)}$$

Finally, the general equations for δ^{13} and δ^{18} can be obtained from (xiv), (xvii), (xix), (xx) and (xxi) with approximations:

$$\delta^{13} = \frac{Z_{45}(Z_{46} - Z_{13}Z_{17} - Z_{17}Z_{46})}{Z_{13}(2Z_{18} + Z_{13}Z_{17})} \cdot \delta^{45'} - \frac{Z_{46}Z_{17}}{Z_{13}(2Z_{18} + Z_{13}Z_{17})} \cdot \delta^{46'} \qquad \text{(xxii)}$$

and $$\delta^{18} = \frac{Z_{46}}{(2Z_{18} + Z_{13}Z_{17})} \cdot \delta^{46'} + \frac{Z_{45}(Z_{46} - 2Z_{17})}{(2Z_{18} + Z_{13}Z_{17})} \cdot \delta^{45'} \qquad \text{(xxiii)}$$

These equations are accurate to better than ± 0.01^{o}/oo for δ values up to ± 50^{o}/oo relative to the standard (Mook 1968).

3.3.4 *Calculations of δ^{13} and δ^{18} equations for the internal standard, Waikato Pinus radiata (WPR)*[*]

A large quantity of standard carbon dioxide gas (WPR) was prepared and stored in a 9 litre glass bulb as described in section 12.5. A sample of WPR measured on the Nuclide Associates mass

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[*] All stable isotope standards discussed in this section are defined in appendix A.

spectrometer at the Institute of Nuclear Sciences against their Te Kuiti limestone carbon dioxide gas standard ($TKL-CO_2$)* gave the following results:

$$\delta^{45} = -\ 22.97^{\circ}/oo$$

$$\delta^{46} = -\ 10.22^{\circ}/oo$$

Using the formulae of Craig (1957) which apply to $TKL-CO_2$ as well as $PDB-CO_2$* (Blattner and Hulston 1975),

$$\delta^{13}_{TKL}(WPR) = -\ 24.18^{\circ}/oo \quad \text{and}$$

$$\delta^{18}_{TKL-CO_2}(WPR) = -\ 10.45^{\circ}/oo$$

From equation (xʟv),† knowing that

$$\delta^{13}_{PDB}(TKL) = -\ 1.67^{\circ}/oo \quad \text{and}$$

$$\delta^{18}_{PDB-CO_2}(TKL-CO_2) = -\ 4.17^{\circ}/oo \quad \text{(Craig 1957), we get}$$

$$\delta^{13}_{PDB}(WPR) = -\ 25.81^{\circ}/oo \qquad \text{(xxiv)}$$

and

$$\delta^{18}_{PDB-CO_2}(WPR) = -\ 14.58^{\circ}/oo \qquad \text{(xxv)}$$

Using these values, which are correct to ± $0.2^{\circ}/oo$ (M.K. Stewart, personal communication 1976), an approximate equation for WPR can be calculated.

(a) <u>Calculation of approximate equations.</u> Craig (1957) gives the following absolute isotopic ratios for his $PDB-CO_2$ gas standard:

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[*] All stable isotope standards discussed in this section are defined in appendix A.

[†] See appendix C.

$$Z_{13} = 0.0112372$$

$$Z_{17} = 0.0003800$$

$$Z_{18} = 0.0020790$$

Therefore, $$Z_{13}(\text{WPR}) = \frac{1000 + \delta^{13}_{\text{PDB}}(\text{WPR})}{1000} \cdot Z_{13}(\text{PDB})$$

$$= 0.0109472 \text{ (from equation (xxiv))}$$

Similarly, $Z_{18}(\text{WPR}) = 0.0020487$ (from equation (xxv))

Also, $$Z_{17}(\text{WPR}) = Z_{17}(\text{PDB-CO}_2)\sqrt{\frac{Z_{18}(\text{WPR})}{Z_{18}(\text{PDB-CO}_2)}} \quad \text{(Craig 1957)}$$

$$= 0.0003772$$

From equation (xviii), since the relationship is the same for the Z values,

$$Z_{45}(\text{WPR}) = 0.0117016$$

From equation (xix),

$$Z_{46}(\text{WPR}) = 0.00410580$$

Thus for WPR, substituting in equations (xxii) and (xxiii), we get

$$\delta^{13}_{\text{WPR}}(\text{B}) = 1.06855\ \delta^{45'}_{\text{WPR}}(\text{B}) - 0.0345\ \delta^{46'}_{\text{WPR}}(\text{B}) \qquad \text{(xxvi)}$$

and

$$\delta^{18}_{\text{WPR}}(\text{B}) = 1.00105\ \delta^{46'}_{\text{WPR}}(\text{B}) + 0.00956\ \delta^{45'}_{\text{WPR}}(\text{B}) \qquad \text{(xxvii)}$$

where B is the sample measured against WPR.

(b) <u>Correction of the approximate equation for δ^{13}.</u> In order to accurately relate WPR to the PDB scale several portions of the international standard NBS 21* were analysed and δ^{13} values were

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*See appendix A.

calculated using equation (xxvi).

Table 1. Measurement of NBS 21 against WPR (δ^{13})

Standard	$\delta^{13}_{WPR}(B) \pm \sigma_m^{*}$ ‰
	-2.31 ± 0.01
NBS 21	-2.28 ± 0.01
(Spectroscopic graphite)	-2.31 ± 0.01
	-2.30 ± 0.02
Mean value $\pm \sigma^{\dagger}$	-2.30 ± 0.01

From the measurements shown in table 1,

$$\delta^{13}_{WPR}(\text{NBS 21}) = -2.30‰$$

Since,

$$\delta^{13}_{PDB}(\text{NBS 21}) = -28.19‰ \quad \text{(Mook 1968)}$$

using formulae (xLii) and (xLv),[§]

$$\delta^{13}_{PDB}(\text{WPR}) = -25.95‰ \tag{xxviii}$$

which will be more accurate than equation (xxiv).

When equation (xxviii) is used to recalculate Z_{13}, Z_{45} and Z_{46}, equation (xxvi) becomes

$$\delta^{13}_{WPR}(B) = 1.06857\ \delta^{45'}_{WPR}(B) - 0.0345\ \delta^{46'}_{WPR}(B) \tag{xxix}$$

whilst equation (xxvii) is unchanged.

The difference between equations (xxvi) and (xxix) is too small to change equation (xxviii) when this relation is recalculated using

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[*] σ_m = standard error (see section 3.2).

[†] σ = standard deviation.

[§] See appendix C.

(xxix) instead of (xxvi).

Substituting equations (xxviii) and (xxix) into formula (xLv),[§]

$$\delta^{13}_{PDB}(B) = 1.0408\ \delta^{45'}_{WPR}(B) - 0.0336\ \delta^{46'}_{WPR}(B) - 25.95 \qquad \text{(xxx)}$$

This is the equation used throughout this thesis for determining δ^{13} values on a PDB scale from direct isotopic measurements on carbon dioxide samples using WPR as the reference gas.

As a further check several samples of NBS 20[*] were analysed[†] using the method of McCrea (1950).

Equation (xxx) has been used to calculate the δ^{13} values displayed in table 2.

Table 2. Measurement of NBS 20 against WPR (δ^{13})

Standard	$\delta^{13}_{PDB}(B) \pm \sigma_m$ ‰
	-1.18 ± 0.01
NBS 20	-1.13 ± 0.01
(Solenhofen limestone)	-1.12 ± 0.01
	-1.13 ± 0.01
mean value ± σ	-1.14 ± 0.03

Thus,

$$\delta^{13}_{PDB}(\text{NBS 20}) = -1.14 \pm 0.03‰$$

This compares with the value -1.06 ± 0.04‰ given by Craig (1957). The small discrepancy between these two values may be due to correction

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[§] See appendix C.

[*] See appendix A.

[†] By Mr. D.A. Burns.

factors which become non-negligible when reference and sample δ^{13} values differ by as much as 25‰. (See discussion in section 3.3.2.)

(c) Correction of the approximate equation for δ^{18}. Only two accurate determinations of δ^{46} for NBS 20-CO_2 against WPR were made. These were used in conjunction with equation (xxvii) to obtain the values displayed in table 3.

Table 3. Measurement of NBS 20-CO_2 against WPR (δ^{18})

Standard	δ^{18}_{WPR}(NBS 20-CO_2) $\pm \sigma_m$
NBS 20	+ 10.57 ± 0.02
	+ 10.52 ± 0.02
mean value	+ 10.55

Thus,

$$\delta^{18}_{WPR}(\text{NBS 20-}CO_2) = +10.55‰$$

Since $\delta^{18}_{PDB\text{-}CO_2}$(NBS 20-$CO_2$) = - 4.14 ± 0.09 (Craig 1957), using formulae (xLii) and (xLv),[†]

$$\delta^{18}_{PDB\text{-}CO_2}(\text{WPR}) = -14.54 \qquad \text{(xxxi)}$$

which is only slightly different from equation (xxv).

Using (xxxi) to recalculate Z_{17}, Z_{18}, Z_{45} and Z_{46}, equations (xxvii), (xxix) and (xxx) are unchanged. Substituting equations (xxvii) and (xxxi) in formula (xLv),[†] we get

$$\delta^{18}_{PDB\text{-}CO_2}(B) = 0.9865\,\delta^{46'}_{WPR}(B) + 0.0094\,\delta^{45'}_{WPR}(B) - 14.54 \qquad \text{(xxxii)}$$

[†] See appendix C.

(d) Using SMOW* as the international standard for δ^{18}. Equation (xxxii) can be used for determining δ^{18} values on a PDB-CO_2 scale from direct isotopic measurements on any sample using WPR as the reference. If δ^{18} values on a SMOW scale are required the formulae of Mook (1968) can be used:

$$\delta^{18}_{SMOW}(B) = 1.0305\ \delta^{18}_{PDB}(B) + 30.5, \text{ and}$$

$$\delta^{18}_{PDB}(B) = \delta^{18}_{PDB-CO_2}(B) + 10.1; \text{ i.e.}$$

$$\delta^{18}_{SMOW}(B) = 1.0305\ \delta^{18}_{PDB-CO_2}(B) + 40.9$$

Substituting this equation in (xxxii),

$$\delta^{18}_{SMOW}(B) = 1.0166\ \delta^{46'}_{WPR}(B) + 0.0097\ \delta^{45'}_{WPR}(B) + 25.9 \qquad \text{(xxxiii)}$$

3.4 *ISOTOPE RATIO MEASUREMENTS ON HYDROGEN*

All hydrogen isotopic ratio determinations are expressed in terms of δ as defined in section 3.1.

3.4.1 *Corrections*

The Micromass 602C includes an H_3^+ correction control to electronically compensate for the H_3^+ contribution to the mass 3 peak. Using this control, changes in sample pressure produce an error of less than 0.1°/oo on enrichments up to 100°/oo (VG Isotopes 1974). Since the mass spectrometer can measure δ values to only ± 0.3°/oo (1 σ_m) this error is normally negligible.

The zero enrichment factor (see section 3.3.2.b) for hydrogen in our instrument was normally less than 0.5°/oo. However, larger values were experienced necessitating a correction to the measured δ value.

Corrections for tail contribution, memory effect and valve

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[*] See appendix A.

mixing were negligible for enrichments of up to ~200‰.

3.4.2 *Standards*

W1, W1A, W2 and W3 (see section 5.5) were measured against themselves and the international standards V.SMOW, NBS 1, NBS 1A and SLAP. The results are shown in table 4.

Table 4. Intercalibration of water standards for δD determinations

Standard†	δD_{W1} (‰)	$\delta D_{V.SMOW}$ (‰)	Number of determinations	$\delta D^*_{V.SMOW} \pm \sigma$ (‰)
W1	0	- 40.7	3	-
W1A	- 4.2	- 44.7	3	-
W2	-141.9	-176.9	3	-
W3	-318.0	-345.8	3	-
V.SMOW	+ 42.5	0	3	0
NBS 1	- 7.0	- 47.4	3	- 46.7 ± 1.3
NBS 1A	-150.6	-185.2	3	-182.5 ± 1.6
SLAP	-403.0	-427.3	3	-426.8 ± 3.8

In addition a sample of W1 was measured at the Institute of Nuclear Sciences, Lower Hutt, against V.SMOW and their internal standard INS-6 with the following results (M. Cox, personal communication 1975):

$$\delta D_{V.SMOW(direct\ measurement)}(W1) = -41.0‰$$

$$\delta D_{V.SMOW(via\ INS-6)}(W1) = -40.9‰$$

[†] These water standards are defined in appendix A.

[*] Values given by the International Atomic Energy Authority (personal communication 1973) with standard deviations. Other published values include those of Craig (1961), Hagemann *et al.* (1970) and Coplen and Clayton (1973).

These values are in good agreement with those in table 4. In this thesis all δD measurements expressed with respect to SMOW have been calculated assuming that

$$\delta D_{SMOW}(W1) = -\ 40.7 ^{o}/oo \quad \text{(see table 4).}$$

CHAPTER FOUR THE PREPARATION OF WOOD CONSTITUENTS ON A SMALL SCALE

For this work on dissected individual tree rings it was necessary to develop new techniques for preparing cellulose and lignin from small quantities of wood (sometimes less than 0.05 g). The methods finally decided upon are described in this chapter.

4.1 *PRELIMINARY TREATMENT OF WOOD*

Wood chips (matchstick size) were ground to sawdust in a Wiley mill* and about 0.5 g was placed in a small glass extraction tube plugged by glass wool (see plate 1.2). The sawdust was extracted with 2:1 benzene/ethanol for 24 hr and then with 95% ethanol for a further 24 hr using a Soxhlet apparatus. Up to 8 tubes were supported above the bottom of the extraction chamber using glass wool. This allowed good drainage of solvent from the tubes between siphonings and hence a thorough extraction.

4.2 *PREPARATION OF HOLOCELLULOSE*

Holocellulose was prepared using a considerably scaled down version of the chlorite method of Jayme and Wise as described by Green (1963a).

Air-dried extracted wood (~0.1 g) was suspended in 5 ml of distilled water in a 10 ml Erlenmeyer flask. The flask was heated to ~70°C on a hot-plate in a fume cupboard. Glacial acetic acid (~0.02 ml)

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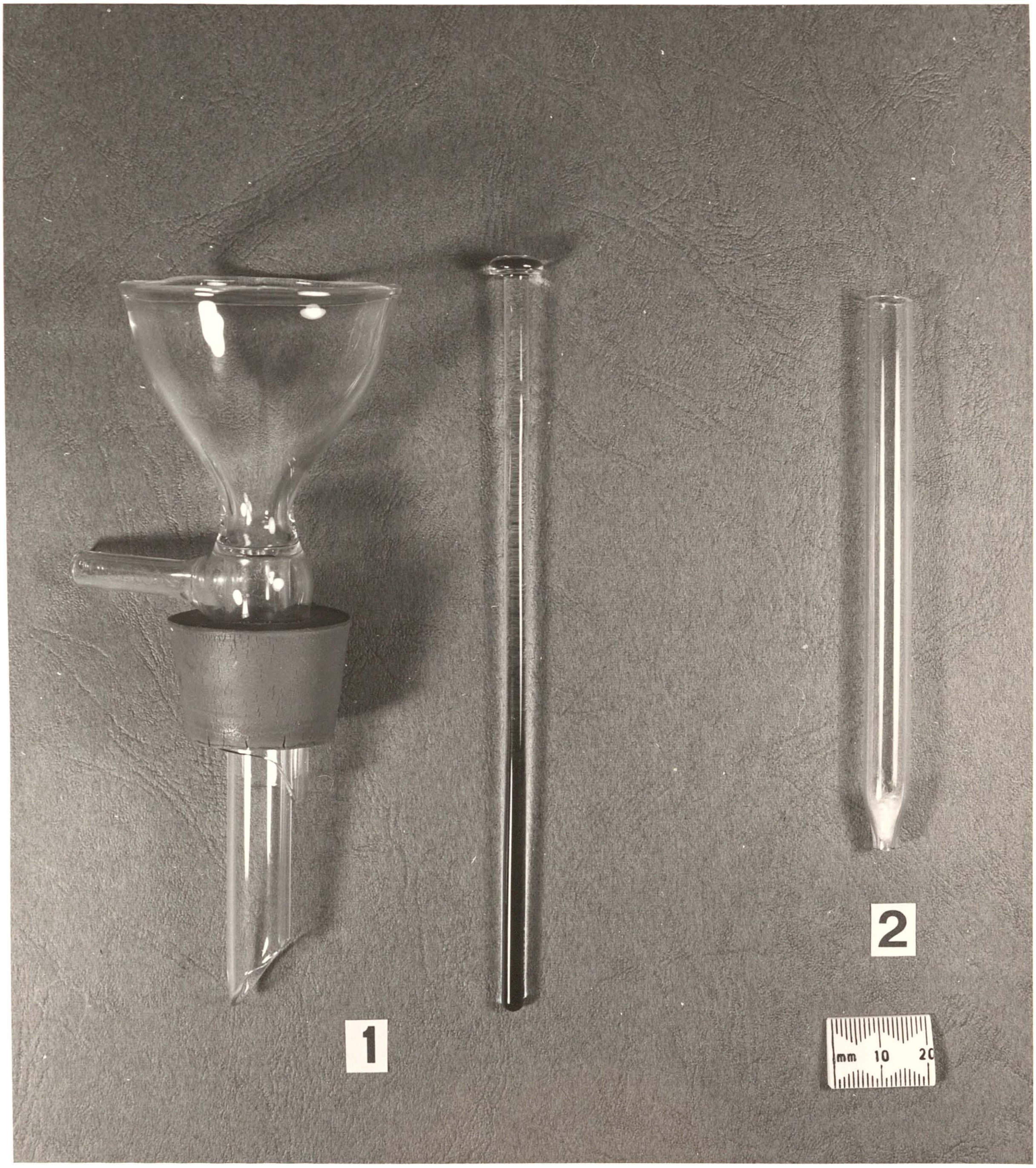
*Intermediate model; A.H. Thomas number 3383-L40; filter number 20 was used.

Plate 1

1.1 Willstätter filter funnel.

1.2 Sawdust extraction tube.


1
2
mm 10 20

was added, followed by technical grade sodium chlorite (~0.05 g), and the flask was covered with a watch-glass. After 40 to 60 min at ~70°C fresh portions of glacial acetic acid (~0.02 ml) and sodium chlorite (~0.05 g) were added, and the heating was continued. A total of 4 or 5 steps were generally required, with a total reaction time of 4 to 5 hr. The residues were either white or slightly yellow.

Up to 15 samples were reacted simultaneously using one hot-plate.

The product mixtures were filtered using a Willstätter filter funnel (see plate 1.1) and the holocellulose was thoroughly washed with cold distilled water. Each sample was air-dried before placing it in a sample bottle.

Holocellulose prepared using this method consists predominately of cellulose, but also contains a small quantity of hemicelluloses. However, it is often referred to as "cellulose" rather than the strictly correct "holocellulose".

4.3 PREPARATION OF LIGNIN

Lignin was prepared using a scaled down version of the method of Adams (1965).

Extracted wood (~0.2 g) was digested for 3 hr in 60 ml of hot distilled water in a boiling water bath to keep it at about 100°C. The suspension was filtered using a Willstätter filter funnel (see plate 1.1), washed and allowed to dry in air. The sample was then transferred to a small sample bottle with a watch-glass cover. 3 ml of cold 73% sulphuric acid was added and stirred for at least 1 min. The sample bottle was clamped in a water bath at 18 - 20°C and left for 2 hr with frequent stirring. The mixture was then washed into a 250 ml Erlenmeyer flask and diluted to 3% acid concentration using 110 ml distilled water in total. The flask was placed on a hot-plate and boiled for 4 hr. Near constant volume conditions were maintained by the frequent addition of hot distilled water. The insoluble lignin was allowed to settle before filtration through a fritted glass crucible. It was thoroughly washed with hot distilled water and air-dried.

4.4 *PREPARATION OF EXTRACTIVES*

The preparation of wood extractives was based on the methods of Selleby (1960) and Kurth (1939).

Sawdust (~10 g) was placed in a cellulose extraction thimble and extracted for 24 hr in a Soxhlet apparatus with diethyl ether (~200 ml), purified using the technique of Vogel (1956). The ether solution was rotary-evaporated to about 10 ml, transferred to a sample bottle and allowed to evaporate to dryness in air (~2 weeks).

PART III

δD MEASUREMENTS

CHAPTER 5

THE DETERMINATION OF δD VALUES FOR WATERS

5.1 *THE REDUCTION SYSTEM*

Water was reduced to hydrogen in a uranium reduction system based on that used by Friedman (personal communication). However, many modifications in apparatus and technique were tried before the system described in this chapter was finalised. The vacuum line is shown in plate 2 and fig. 9.

The uranium pump used in this line has been discussed by Friedman and Hardcastle (1970).

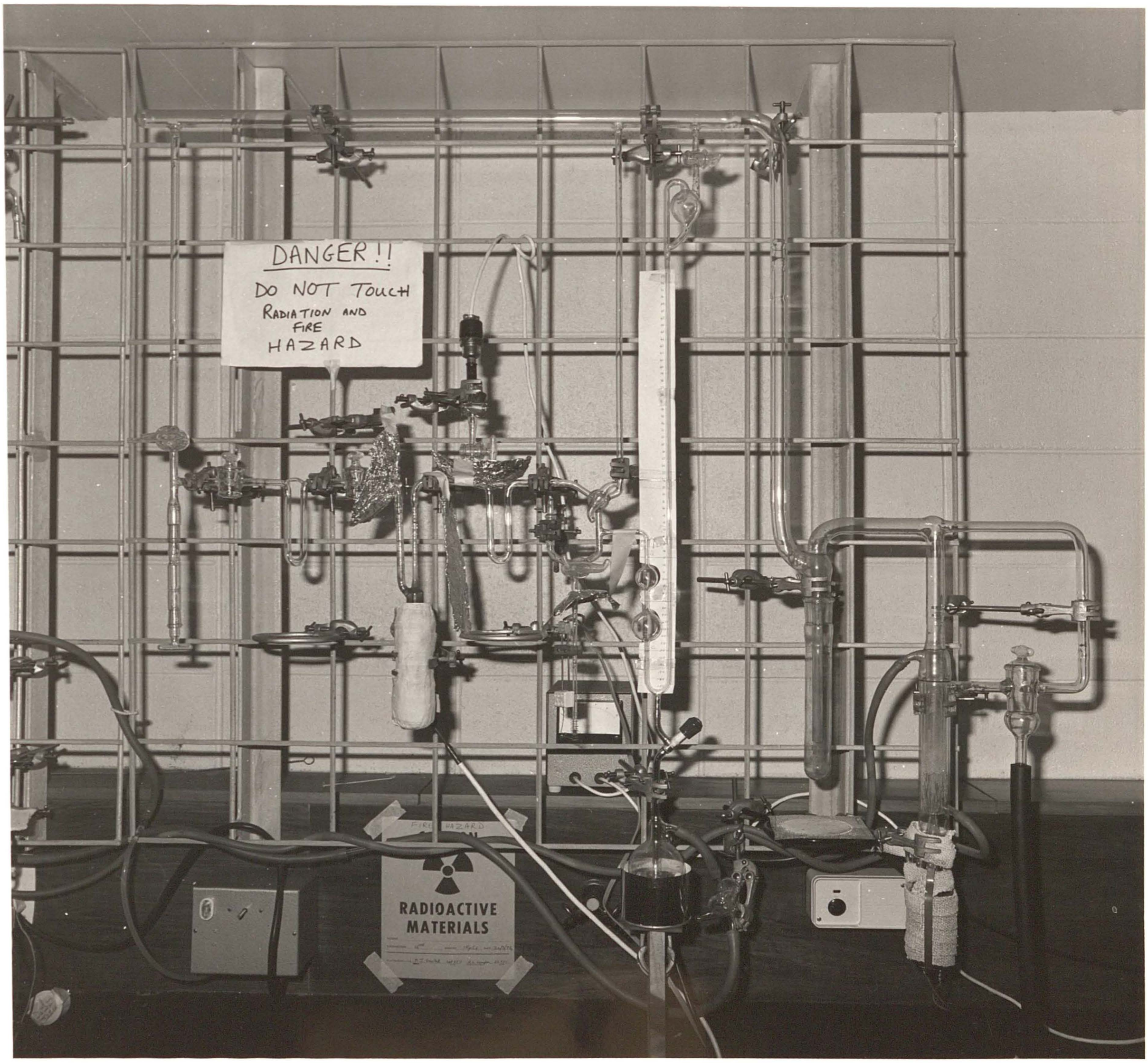
5.1.1 *Working up the Uranium*

Large uranium turnings were broken up under liquid nitrogen (to make them brittle) using a cast-iron mortar and pestle. The smaller pieces were sieved out and stored for use in uranium pump F2 (fig. 9). Concentrated nitric acid was poured onto the larger pieces (~100 g) in a fume cupboard. The reaction was allowed to proceed until the surface of the uranium was clean (~1 min). A large excess of cold water was then added quickly and the turnings were washed several times with distilled water. After rapid drying between filter papers the uranium was introduced into a clean silica furnace (plate 3.3), blown onto the vacuum line (fig. 9) and evacuated as soon as possible since it reacts slowly with air and water at room temperature.

The uranium for pump F2 was worked up in the same way except that the smaller pieces take longer to clean with concentrated nitric acid (3-5 min). The uranium in both F1 and F2 was evacuated for at least

Plate 2

Water reduction vacuum line.


DANGER !!
DO NOT TOUCH
RADIATION AND
FIRE
HAZARD
FIRE HAZARD
RADIOACTIVE
MATERIALS

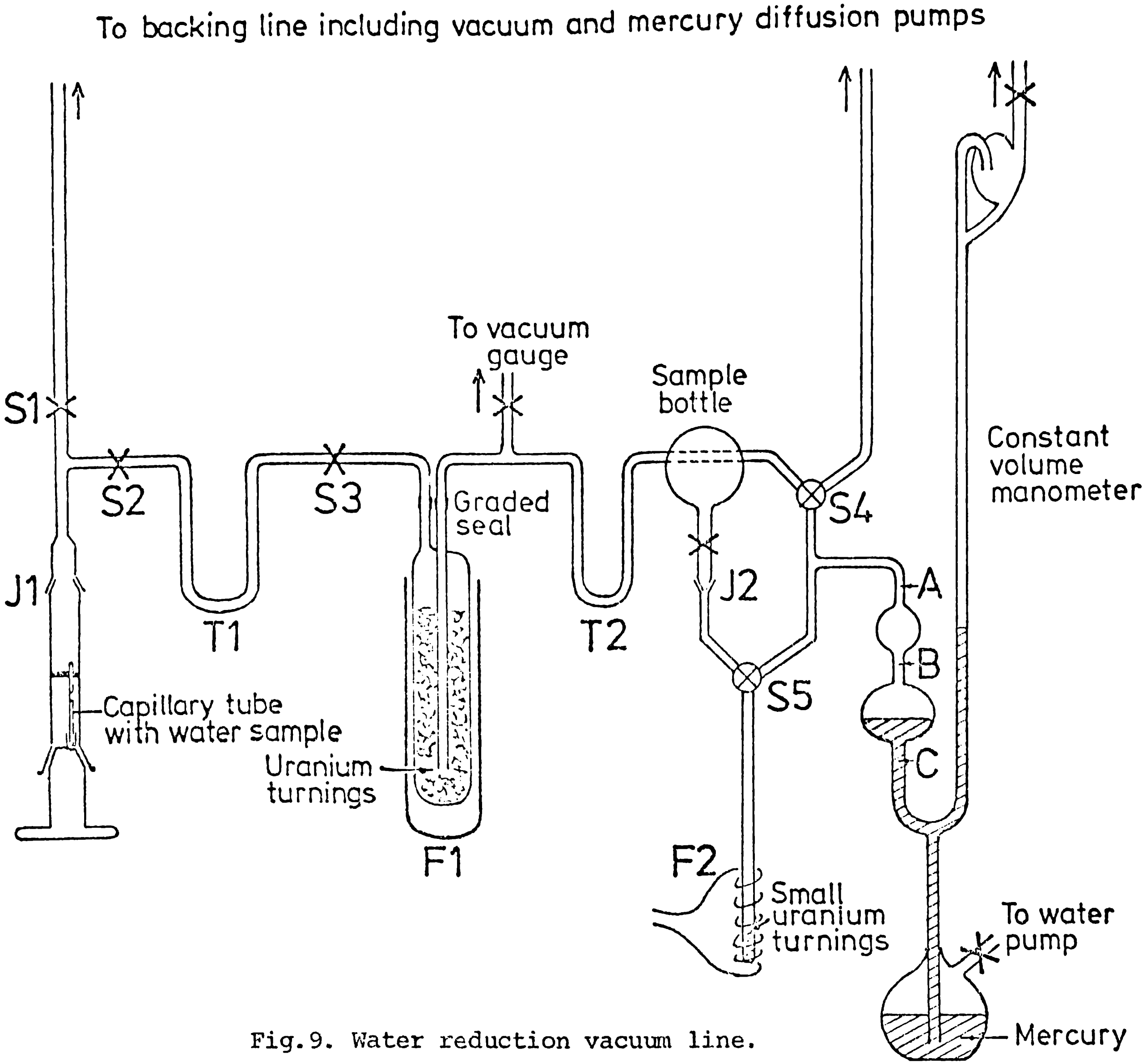


Fig. 9. Water reduction vacuum line.

14 hr before use.

5.1.2 *Conversion of Water into Hydrogen*

The water sample to be analysed was sealed into a fine capillary tube, scratched with a glass knife and placed in a breaker tube, which was then attached to the vacuum line (see fig. 9). After evacuation through S1 the capillary tube was broken and the sample was frozen into trap T1. With 3-way stopcocks S4 and S5 open to the uranium pump F2, S3 was opened (S2 closed) and the water was allowed to distil onto the uranium in F1 (at ~800°C). The hydrogen produced reacted with the uranium in F2 (at ~80°C) to give uranium hydride. Liquid nitrogen around trap T2 held back any unreacted water or other impurities. The mercury level in the constant volume manometer was kept near position A to minimise the "dead" space. The reaction was allowed to proceed until the vacuum gauge needle had almost returned to its fully evacuated position. The latter process took anything from 15 min up to 2 hr depending on the state of the uranium in pump F2.

Normally freshly cleaned uranium turnings had to be activated before use by raising and lowering the temperature of the furnace surrounding them while keeping them in contact with hydrogen gas. Such turnings then reacted very quickly with hydrogen. Gradually, however, they changed to finely divided uranium dust which took longer to react and also tended to blow out of the pump to places where it could have introduced a memory effect. Thus the tube containing the uranium was removed from the vacuum line, cleaned out, and the uranium replaced after every 12 or so samples.

On the completion of the reaction trap T2 was pumped out via S4 while the yield of hydrogen was determined using the constant volume manometer. The hydrogen was driven off the uranium by heating to

~700°C. The maximum possible quantity of hydrogen was removed from the pump tube by lowering the mercury in the manometer to level C. After 5 min S5 was rotated so that the hydrogen in the manometer could pass into a sample bottle (plate 3.1) attached at J2. The mercury level in the manometer was then raised to level A to ensure that the sample bottle was as full as possible.

5.1.3 *Special Precautions Necessary when Working with Uranium*

A problem associated with using uranium in vacuum lines is its pyrophoric nature when clean and dry. On one occasion air was inadvertantly let into furnace F1 while it was hot. This caused a highly exothermic reaction which almost immediately cracked the silica tube containing the uranium, thereby letting in more air and speeding up the reaction still further. The main danger of such an incident, apart from the risk of fire, is the release of finally divided radioactive uranium particles into the atmosphere.

On a smaller scale care must be taken when changing the uranium pump (F2) since finely divided uranium can react exothermically with air even at room temperature.

It should also be noted that the uranium required to fill furnace F1 produces a reasonable amount of radiation (~15μ Ci). In addition the uranium nitrate in solution after reaction with nitric acid is very highly toxic.

Depleted uranium turnings (i.e. with most of the ^{235}U isotopes removed) were used in this study. However, information concerning body burdens, etc., is only readily available for natural uranium (99.28% ^{238}U; 0.72% ^{235}U)* - some of this information is listed below.

(a) $U_{nat.}$ effective energy = 4.43 MeV (α radiation);* other α,

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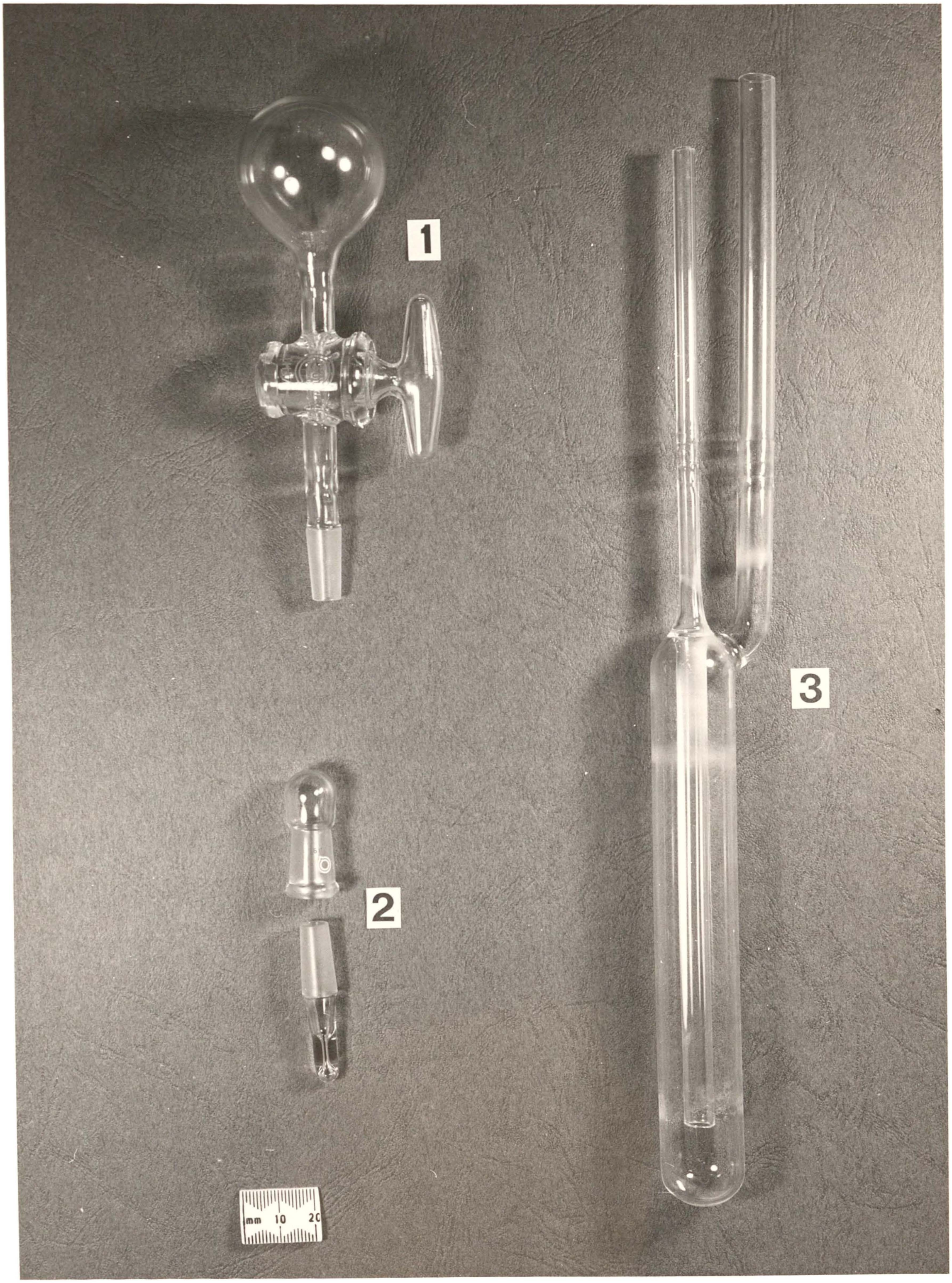
*Information from U.S. Dept. of Commerce (1953).

Plate 3

3.1 Hydrogen gas sample bottle.

3.2 Sample bottle for weighing small quantities of water before reduction to hydrogen.

3.3 Silica uranium furnace tube in water reduction vacuum line.


1
2
3
mm 10 20

β and γ radiations are emitted by daughter products in the uranium decay series.

(b) $U_{nat.}$ maximum permissible body burden = 5×10^{-3} μ C.[†]

(c) $U_{nat.}$ (soluble) maximum permissible concentration in water = 5×10^{-4} μ C per cm^3[†] (critical organ: kidneys[*]).

(d) $U_{nat.}$ (insoluble) maximum permissible concentration in air = 7×10^{-11} μ C per cm^3[†] (critical organ: lungs[*]).

5.1.4 *Water Reduction Line Characteristics*

Two experiments using samples of standard water W1 were carried out before the procedure described in section 5.1.2 was standardised.

First, the length of heating (at ~700°C) of uranium pump (F2) was varied to test for isotopic equilibration of hydrogen in the heat gradient between the pump and the monometer. Fig. 10 shows that equilibration was complete after ~5 min, but fractionation effects occurred if the equilibration time was less than 5 min.

Secondly, the degree of vacuum attained (as indicated by the vacuum gauge needle) before stopping the water to hydrogen reaction was varied, keeping the equilibration time nearly constant. It was found that fractionation effects could occur if the vacuum gauge needle was not allowed to return to close to the backing pressure reading.

5.2 *YIELD DETERMINATIONS*

These were carried out to an estimated accuracy of ± 2% using small aliquots of water weighed in the vessel shown in plate 3.2.

The results in table 5 show that, within the accuracy of

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[*] Information from U.S. Dept. of Commerce (1953).

[†] Information from University of Waikato Radiation leaflet (see bibliography).

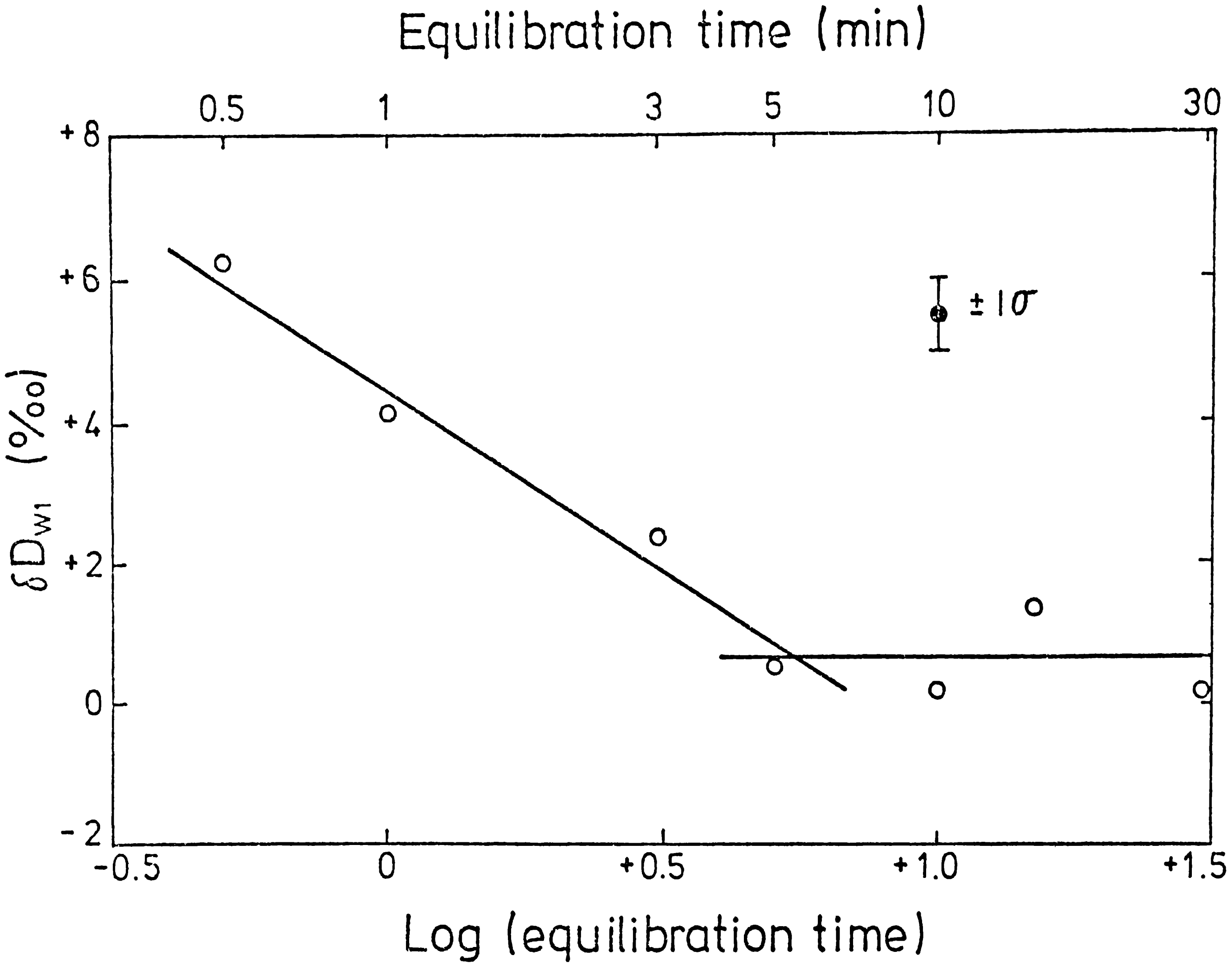

Fig.10. The effect of equilibration time on δD values.

measurement, the reduction line converts water quantitatively to hydrogen.

Table 5. Hydrogen yields from the water reduction line

Weight of water sample W1 (g)	Yield of hydrogen (%)
0.01420	100
0.00654	100
0.00699	100

5.3 *REPRODUCIBILITY*

In order to establish whether the age of the uranium pump (F2) had any effect on the δD values obtained, several samples of standard W1 were run consecutively using a fresh uranium pump. The reproducibility in δD of the first 6 samples was ± 0.3‰ (1 σ), which is the same as that for the mass spectrometer (1 σ_m is generally ± 0.3‰). The reproducibility in δD of the first 12 samples was generally ± 0.5‰ (1 σ), there being a slight tendency to more positive values of δD as the uranium pump was used. If the uranium pump was used more than 12 times on the same sample, the trend to more positive values of δD continued although the reproducibility of a short consecutive series of samples remained about ± 0.5‰ (1 σ). On one occasion the same uranium pump was used more than 60 times. The reproducibility for this set of runs was better than ± 2‰ (1 σ).

Thus for best reproducibility the uranium pump should be changed about every 6 runs. In this study it was normally changed about every 12 runs.

To reduce the error of measurement that arises from the above considerations, when unknown samples were measured against hydrogen from standard W1 at least 3 aliquots of the latter were run through the reduction line with each batch of samples. The δD values of the unknown samples were then expressed against the mean δD value of the aliquots of W1.

An overall long-term reproducibility for δD measurements on waters was not accurately computed using a secondary standard. However, most water samples were run two or more times on different occasions. From these results an overall reproducibility of ± 0.7‰ (1 σ) was estimated.

The reproducibility was less than this if samples of widely differing δD were run through the reduction line consecutively. This is because of the existence of a memory effect of ~1‰ per 200‰ difference between consecutive samples. Most samples measured had δD values within 50‰ of each other, so this effect could usually be neglected.

5.4 *STORAGE OF SAMPLES*

In general hydrogen gas was stored in sample bottles with greased stopcocks (see plate 3.1) at room temperature for up to 3 days before measurement. Storage of hydrogen from standard water W1 for various lengths of time up to 4 days at room temperature had no detectable effect on the δD value obtained. Longer storage times (more than 7 days) gave unreliable results. However, storage of hydrogen for as short a time as 3 days often proved impractical because of mass spectrometer breakdowns. Thus a break-seal bottle (fig. 11) was designed for storage of hydrogen samples for long periods of time. Storage times of up to 8 months in these bottles had no detectable effect on the δD values.

5.5 *PREPARATION AND STORAGE OF WATER STANDARDS W1, W1A, W2, W3*

The primary internal standard W1 was made up from local distilled water. This was sealed into a large number of glass McCartney bottles. For added security against exchange each bottle top was sealed with a

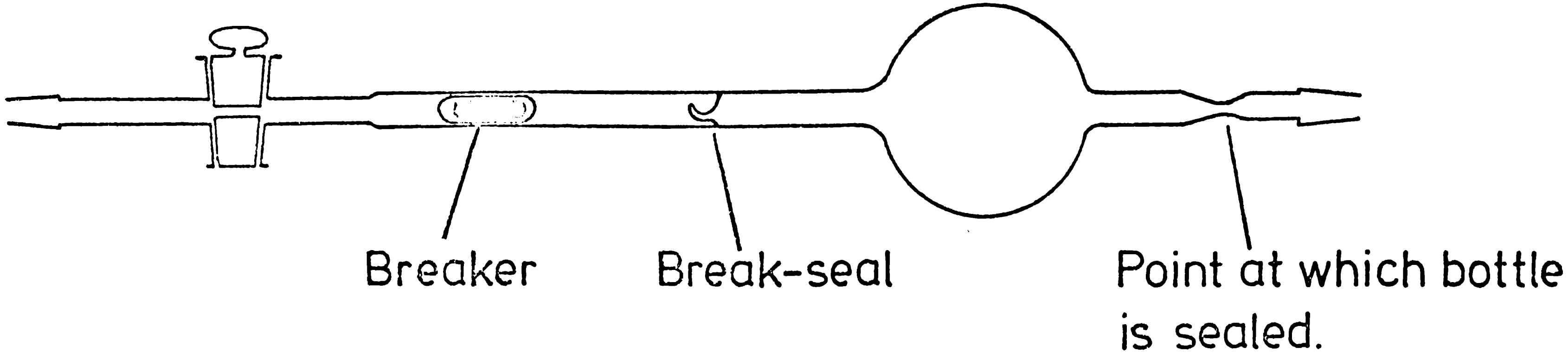

Fig.11. Break-seal bottle for storage of hydrogen samples.

coating of wax[*] before storage in a cupboard. Standard W1A was made up some time later from local distilled water to supplement primary standard W1. Standard W3 was made up in a similar fashion from distilled Antarctic glacier water. Standard W2 was made up by mixing W1 and W3.

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[*] Paraffin wax and bees' wax mixed in a 2:1 ratio.

CHAPTER 6 THE PROBLEM OF DETERMINING δD VALUES FOR PLANT MATERIAL

6.1 *INTRODUCTION*

Despite the large number of δD determinations that have been made since the first precise measurements by Friedman (1953) more than 20 years ago, very few research workers have attempted to measure D/H ratios in plant material.

The first extensive study was carried out by Schiegl and Vogel (1970). They dried their samples of whole plant material at 105°C (1 hr) before combustion in oxygen and subsequent reduction to hydrogen. They found that the hydrogen of both marine and land plants contained several percent less deuterium than the waters in which they grew. The deuterium contents of coal and oil (Schiegl and Vogel 1970), peat (Schiegl 1972), and growth rings in trees (Schiegl 1974) have been measured using the same techniques.

Libby and Pandolfi (1974a, 1974b, 1974c) used a different method for their δD determinations. They liberated hydrogen by heating the raw sawdust with powdered uranium. However, their more recent δD determinations (Libby *et al.* 1976, Libby and Pandolfi 1976) were carried out by combusting the samples and then reducing the water to hydrogen.

Smith and Epstein (1970) used the latter technique. They found a depletion in deuterium of 55‰ for whole plants with respect to environmental water.

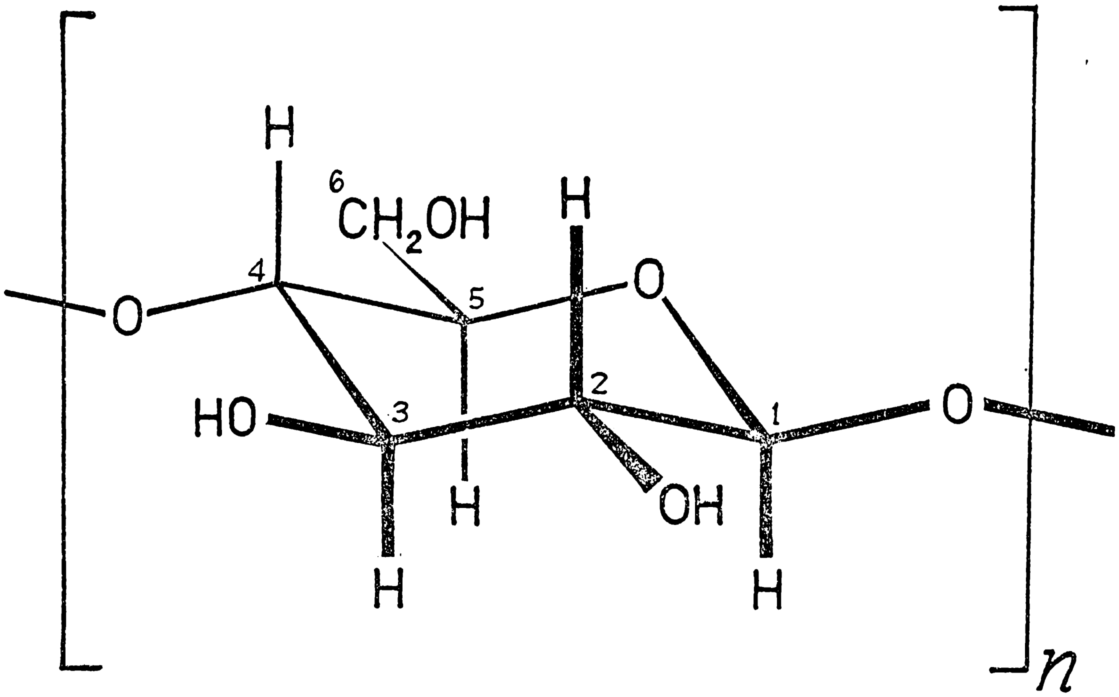
All the above techniques involve the preparation of hydrogen from whole plant material. This limits the usefulness of the results obtained since variations between samples may be due to variations in the proportions of the different chemical constituents in the plant (see section 2.5). Recent studies (e.g. Smith and Epstein 1970, Epstein *et al.* 1976) have shown that individual fractions of the same plant material have widely differing deuterium contents. For example, *Pinus radiata* ether extractives are more than 100‰ depleted in deuterium with respect to cellulose (see section 9.5.2).

An additional limitation of the above work is the probability of isotopic exchange. Exchangeable hydrogen atoms are not only present in cellulose (see section 6.2), but also in whole plant material (e.g. Sumi *et al.* 1964). Thus the measured D/H ratio of a piece of plant material will be partly dependent on the last water it came into contact with. This could well be the water vapour in a laboratory atmosphere.

Because of the above considerations, it was decided to carry out δD determinations only on the individual chemical constituents of plant material, with due regard to isotopic exchange problems. Most work was done on cellulose since it is common to all photosynthetic plants. However, the problem of obtaining meaningful δD values for other plant constituents, e.g. lignin, was also considered (see section 10.2).

6.2 THE PROPERTIES OF CELLULOSE

Cellulose is a polysaccharide containing long un-branched chains made up of glucose units, each one joined by a β-glycoside linkage to C-4 of the next (see fig. 12). Physical methods give molecular weights for cellulose ranging from 250,000 to greater than 1,000,000 (Morrison and Boyd 1966). Wood celluloses have molecular weights as high as 2,700,000 (Goring and Timell 1960). Full discussions concerning the structure of cellulose can be found in the works of



Structure of Cellulose
Fig.12.

Ott *et al.* (1954) and Bikales and Segal (1971).

Native cellulose basically consists of molecules, hydrogen-bonded together in both crystalline and amorphous regions. For example, two types of cellulose O-H groups can be distinguished from vapour-phase deuteration studies monitored by infrared spectroscopy. First, there are those O-H groups that are hydrogen-bonded in an irregular, disordered, amorphous manner, and, secondly, there are those O-H groups that are hydrogen-bonded in a regular, ordered, crystalline manner. Each type gives characteristic absorption bands in the infrared (Mann 1971).

6.2.1 Exchange Properties

The exchange reaction between the O-H hydrogens of cellulose and either deuterium oxide or tritium oxide has been widely studied (e.g. Frilette *et al.* 1948, Mann and Morrison 1956, Lang and Mason 1960). The general character of this exchange reaction (see fig. 13) is essentially similar for all celluloses and is little affected by moderate changes in pH and temperature. The initial fast exchange of the amorphous O-H hydrogens is virtually complete in the first 30 min. Thereafter the amount of exchange increases much more slowly as the crystalline O-H hydrogens react. The intercept value (see fig. 13) for accessibility is generally in the range 71 to 76.5% for a regenerated cellulose and 39 to 44% for a native cellulose (Jeffries 1964). Mann (1971) summarizes the results of other workers in a table showing the percentage of disordered O-H groups in various types of cellulose.

Several workers have tried unsuccessfully to completely deuterate all the O-H groups in the crystalline regions of cellulose (Frilette *et al.* 1948, Mann and Marrinan 1956, Jeffries 1963). Sepall and Mason (1961b) report that they managed to exchange all the O-H groups in

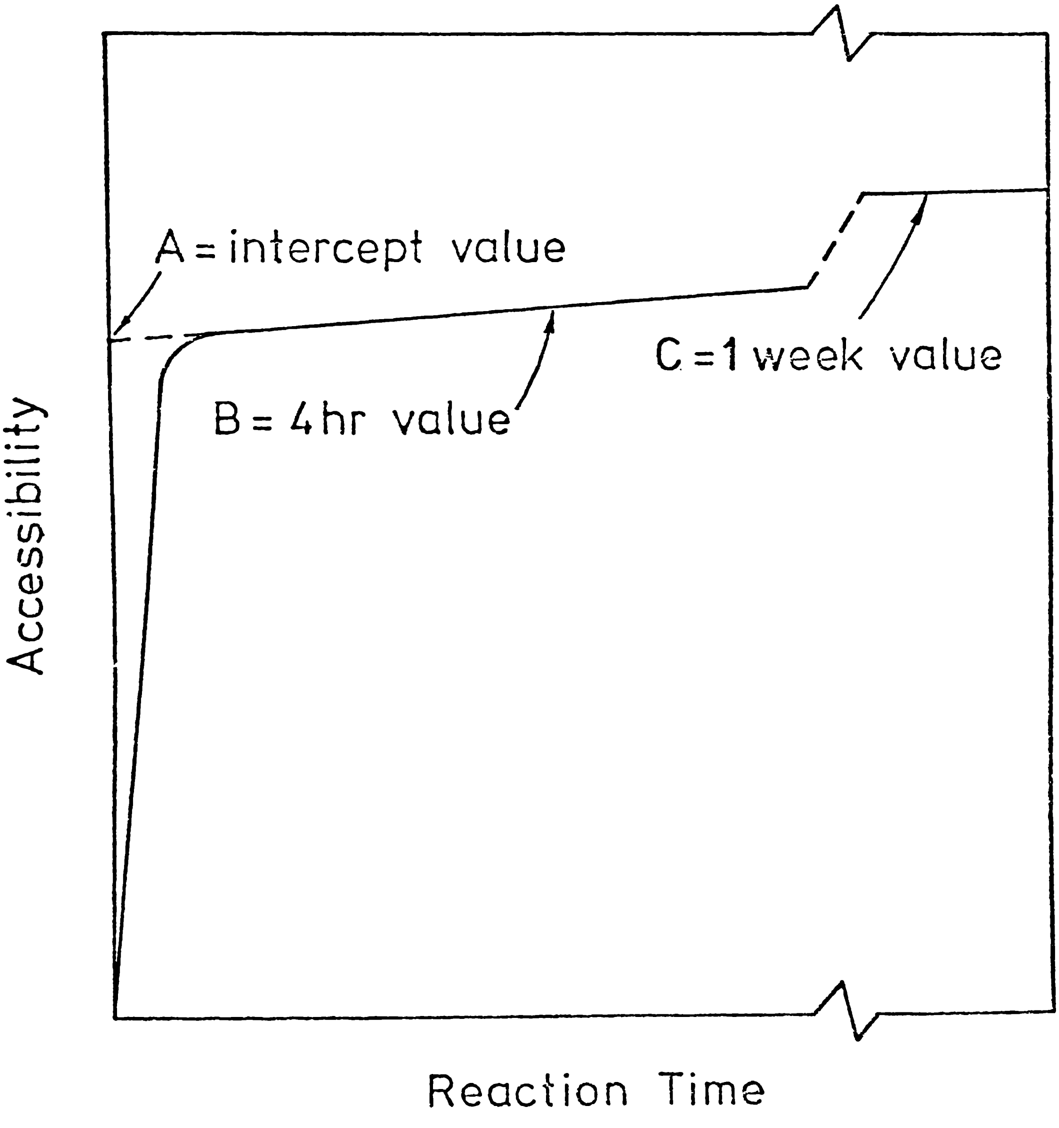


Fig.13. Generalized curve for the exchange reaction between D_2O and cellulose (after Frilette *et al.* 1948).

cellulose prepared from acetate grade soft wood pulp after 100 wetting and drying cycles using tritiated water at 100°C. Prolonged soaking (64 days) of wood cellulose in tritiated water at 100°C exchanged more than 80% of the O-H groups (Sepall and Mason 1961b). Wadhera and Manley (1965) have carried out similar experiments using cellulose from acetate grade soft wood pulp. They agreed with Sepall and Mason (1961b) that after 100 cycles at 100°C there was no further exchange. However, they report that not all the O-H hydrogens were exchanged at this temperature. 100% reaction was not achieved even after 100 wetting and drying cycles at 120°C. Extrapolation of accessibility measurements, based on one 4 hr immersion in tritiated water at various temperatures ranging from 100 to 175°C, suggested that 100% reaction of the O-H hydrogens of wood cellulose may be achievable in one wetting and drying cycle at 190°C (Wadhera and Manley 1965).

6.2.2 *The Hydrogen Atoms in Cellulose*

Studies such as those described above have shown that there are effectively three types of hydrogen in cellulose: first, the C-H hydrogens (70%) which are non-exchangeable with any surrounding water; secondly, the readily exchangeable O-H hydrogens (~15%) in the amorphous regions of the cellulose; and, thirdly, the O-H hydrogens (~15%) which are bound up in the crystalline regions and consequently only exchange very slowly with water. Therefore the cellulose in a sample of plant material will contain C-H hydrogens whose D/H ratio should have remained unaltered since it was synthesised. The D/H ratio of the readily exchangeable O-H hydrogens will be dependent on the deuterium content of the last water that they came into contact with. The D/H ratio of the remaining O-H hydrogens may reflect the deuterium content acquired during cellulose synthesis in the plant.

It was decided to concentrate on developing a method for determining the D/H ratio of the C-H hydrogens in cellulose. This meant that, first of all, the O-H hydrogens had to be either removed by a substitution reaction or exchanged to a known δD value. Because of such problems as those discussed in the previous paragraphs, the production of a tri-substituted derivative of cellulose, using a substituent containing no hydrogen atoms, was first attempted.

CHAPTER 7 THE ATTEMPTED PREPARATION OF TRI-SUBSTITUTED CELLULOSE DERIVATIVES

Cyrot (1959) gives a comprehensive list of cellulose derivatives. The few that may possibly be of use for replacing the O-H hydrogens in cellulose are considered in this chapter.

7.1 *NITRATION*

Cellulose can be nitrated such that less than 4% of the O-H hydrogens remain (Green 1963c). It was decided not to consider this method further for two reasons: first, because of the dangers involved in combusting highly explosive cellulose trinitrate in oxygen (Green 1963c); and, secondly, the possibility that the very strong acid mixture required for the reaction would exchange some of the C-H hydrogens. Recently, however, Epstein and co-workers (1976) have overcome the problems involved in using the nitration technique. They claim to be able to determine the deuterium content of the C-H hydrogens in cellulose to a precision of ± 2‰.

7.2 *TRIFLUOROACETYLATION*

A literature search revealed no claims to have synthesised a fully trifluoroacetylated cellulose. However, Segal *et al.* (1961) claim to have produced a fully esterified n-heptafluorobutyl cellulose ester. They also claim to have used trifluoroacetic anhydride in pyridine to produce a trifluoroacetylated cellulose having a degree of substitution of from 1 to 2 trifluoroacetyl groups per glucose unit.

Geddes (1956) has made a detailed study of the action of pure trifluoroacetic acid on cellulose and cellobise. Dissolution of the cellulose occurred after a period of swelling and micellar dispersion. He suggests that some esterification takes place possibly by a mechanism involving the formation of an intermediate addition compound. He further concludes that the rate of esterification of primary O-H groups is more rapid than that of the secondary O-H groups. Valdsaar and Dunlap (1952) have also studied the dissolution of cellulose in trifluoroacetic acid, but they suggest that the reaction goes no further than the formation of an addition compound.

The trifluoroacetylation of small carbohydrate molecules is much better documented (Bourne *et al.* 1950, Bonner 1961, Anderle and Ková̌c 1970). Esterification is affected by warming the appropriate hydroxy-compound with trifluoroacetic anhydride in the presence of dry sodium trifluoroacetate (Bourne *et al.* 1950). The ester must be isolated under anhydrous conditions. Bourne *et al.* (1950) distilled the reaction mixture several times with dry carbon tetrachloride to remove excess trifluoroacetic anhydride and trifluoroacetic acid. They then extracted the ester from the residual sodium trifluoroacetate using the same solvent or dry petroleum. By this means several fully trifluoroacetylated sugar derivatives including D-mannitol hexakistrifluoroacetate and dulcitol hexakistrifluoroacetate were obtained crystalline. This method, after modification, was later applied to other hydroxy-compounds (Bourne *et al.* 1958). The trifluoroacetates were reasonably stable when pure and dry, but hydrolysed very rapidly in the presence of water (Bonner 1961).

Other workers have prepared trifluoroacetyl polyol derivatives in solution for use in gas-liquid chromatography. Shapira (1969) and Anderle and Ková̌c (1970) left the sugars overnight in contact with trifluoroacetic anhydride in pyridine. Tamura and Imanari (1967) used trifluoroacetic anhydride in methyl cyanide or tetrahydrofuran.

7.2.1 Experimental

Trifluoroacetylation of cellulose was first attempted using trifluoroacetic anhydride in pyridine (after Segal *et al.* 1961, Shapira 1969, and Anderle and Kováč 1970). The pyridine to be used (80 ml) was first dried by refluxing it for 1 hr with barium oxide (15 g). 1 ml of this dry pyridine was added to holocellulose (0.05 g - prepared from *Pinus radiata* as described in section 4.2) in a vial. Trifluoroacetic anhydride (1 ml) was added and the firmly capped vial was left overnight. The cellulose,which had swelled up and turned light brown, but had not dissolved, was filtered, washed with pyridine and then with n-hexane, and left to dry overnight in an oven (75 to 80°C) before weighing. The resulting increase in weight of the cellulose to 0.09 g suggested that it had been partly trifluoroacetylated. However, the product was contaminated with a brown substance. This is probably the compound referred to by Bourne *et al.* (1950) who state that a mixture of pyridine and trifluoroacetic anhydride rapidly darkened and left a brown solid when distilled. However, in view of the number of workers who have used such mixtures experiments were continued.

The above technique was repeated using mercerised cotton* and cupra-ammonium rayon in the hope that these less crystalline celluloses would be more susceptible to trifluoroacetylation. In one experiment a reaction mixture of trifluoroacetic anhydride (2 ml) and a trace of pyridine (0.1 ml) was used. In another, the reaction was carried out as before except that the vial was kept at 0°C. All reaction mixtures were left overnight and then treated as described above except that the products were dried at 105°C for 3 days. Very little increase in

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*Treated with 1 M sodium hydroxide at room temperature for 15 min.

weight was observed.

The method described by Segal *et al.* (1961) was then reproduced more closely. Dry pyridine (8 ml) and trifluoroacetic anhydride (1 ml) were reacted with mercerised cotton (1.2 g) for 2 hr at 50°C. The dark brown mixture produced was filtered and washed with pyridine and then n-hexane before drying at 60°C for 2 days and weighing. A weight increase was observed (as found by Segal *et al.* 1961), but this was, most likely, mainly the result of contamination by the brown solid produced when a mixture of pyridine and trifluoroacetic anhydride is heated (see above discussion and Bourne *et al.* 1950).

The method of Bourne *et al.* (1950) was next attempted. Holocellulose (0.05 g) was mixed with sodium trifluoroacetate (0.15 g) and trifluoroacetic anhydride (3.5 ml), and refluxed for 24 hr. No change was observed except a slight yellowing. The problem here may have been the presence of small quantities of water associated with the cellulose since the latter was only oven-dried at 60°C. The work of Lang and Mason (1959) shows that cellulose can only be properly dried if it is evacuated for a long period at an elevated temperature (e.g. ~60 hr at 70°C). Thus it would be interesting to repeat this experiment using a completely dry sample of cellulose.

Finally, a method based on the observations of Geddes (1956) was investigated. Holocellulose (0.018 g) was mixed with trifluoroacetic acid (1.5 ml) and trifluoroacetic anhydride (1 ml), and gently refluxed under a calcium chloride drying tube for 3 days. The cellulose swelled up and turned brown, but did not dissolve. This method was then repeated but this time a stoppered vial at room temperature was used. The cellulose again swelled up, but did not dissolve even after 9 days. When a sample of cellulose (0.01 g) was treated with trifluoroacetic acid (2.5 ml) at room temperature the swelling was more pronounced than in the previous experiment and after

9 days there were signs that a little of the cellulose had dissolved. Moreover, a sample of Whatman's filter paper cellulose (0.01 g), placed in trifluoroacetic acid (2.5 ml) at room temperature, disintegrated after 24 hours and completely dissolved after 5 days at room temperature. Geddes (1956) suggested that such dissolving is due to partial trifluoroacetylation of the cellulose. (See also the discussion at the start of this section.)

Steelink and Jensen have also recently attempted the synthesis of fully trifluoroacetylated cellulose. They used a mixture of trifluoroacetic acid and trifluoroacetic anhydride (Steelink and Jensen, unpublished, 1976). However, rigorous analysis of the reaction product showed it to be polymerised cellulose. All attempts to reduce this effect proved fruitless (C. Steelink, personal communication 1976).

7.2.2 Conclusion

Thus it seems that so far all attempts to fully trifluoroacetylate cellulose have been unsuccessful, and so this method does not promise to be a viable technique for replacing the O-H hydrogens in cellulose. This conclusion was arrived at after many months of work (see above summary) which included an exhaustive review of the literature.

The claim of Segal *et al.* (1961) to have synthesised a fully esterified n-heptafluorobutyl cellulose ester seems questionable since it is based solely on a weight increase which may be partly due to impurities produced by the interaction of the reactants. In any case the combustion of the product, in the manner normally employed for isotope determinations, would probably be difficult since when held in a flame it melted, but did not burn (Segal *et al.* 1961).

7.3 TRICHLOROACETYLATION

Full trichloroacetylation of cellulose seemed less likely than full trifluoroacetylation because of the size of the substituent groups. However, it was attempted.

Dry pyridine (8 ml) and trichloroacetyl chloride (1 ml) were added to mercerised cotton (0.11 g after drying at 105°C for 1 hr) and left in a stoppered vial at room temperature for 30 min. The reaction mixture was then filtered, washed with pyridine and n-hexane, left to dry in an oven at 60°C (2 days), and weighed. No increase in weight was observed.

7.4 METHODS INVOLVING THE DEGRADATION OF CELLULOSE

Methods by which cellulose can be degraded have been reviewed. These include microbiological degradation (Gasgoine and Gasgoine 1960, Reese 1963) and the effect of strong acid or strong alkali (Bikales and Segal 1971). Using such methods glucose or a mixture of oligosaccharides could be produced. The latter could then be fully trifluoroacetylated using the method of Bourne *et al.* (1950).

A more promising, but longer, method might be to first prepare α-cellobiose octaacetate by the reaction between cellulose, acetic anhydride and concentrated sulphuric acid (Braun 1944). This has the advantage that the crystals of octaacetate (formed in 35 to 37% yield) produced are reasonably pure. These crystals could be reacted with sodium in methanol to give crystals of cellobiose (91% yield) (Braun 1944). The method of Bourne *et al.* (1950) could then be used to synthesise fully trifluorocetylated cellobiose.

CHAPTER 8 EXCHANGE METHODS FOR CELLULOSE δD DETERMINATIONS

An alternative approach to the problem of determining the D/H ratio of the C-H hydrogens in cellulose is to exchange the O-H hydrogens to a known δD value. Unfortunately this has not yet proved possible using water alone (see chapter 6). The various solvents for cellulose were therefore considered before further investigating the exchange reaction with water.

8.1 EXPERIMENTS WITH CELLULOSE SOLUTIONS

The objective of the work described in this section was to find out if it was possible to exchange all the O-H hydrogens by dissolving cellulose in a solvent and later reprecipitating it.

The various cellulose solvents are considered in detail by Ott *et al.* (1954) and Bikales and Segal (1971).

8.1.1 "Cuen" Complexing

The copper complex of 1,2-diaminoethane ("Cuen") was synthesised using standard water W1A and the method of Jayme and Lang (1963). Holocellulose standard C1/10 (0.05 g) was dissolved in 0.5 M Cuen solution (5 ml) by stirring for 30 min at room temperature. 1 M hydrochloric acid (17 ml - prepared by diluting concentrated hydrochloric acid with standard water W1A) was then added slowly with stirring (5 min) to reprecipitate the cellulose. This cellulose was such that it clogged up the sinters used for filtration. Therefore the mixture was centrifuged and the supernatant decanted off. The cellulose residue was washed 3 times with standard water W1A and left overnight at room temperature in contact with W1A (~30 ml) in a stoppered tube. The wet sample was then introduced into a combustion line, dried, combusted and the deuterium content of the water measured as described in chapter 5. Two further samples of the same cellulose were treated according to the procedure described above except that standard waters W2 and W3 were used instead of W1A.

The δD values obtained from the above experiment were compared with those obtained by exchanging cellulose standard C1/10 with the same three water standards (~5 ml) at 92 ± 2°C. After 3 days the water was decanted off and the wet samples were inserted into the combustion line as quickly as possible. δD values were determined as described in chapter 5.

Table 6. A comparison of the Cuen and exchange water methods for exchanging the O-H hydrogens in cellulose

Standard water	δD_{W1} (‰)	δD_{W1} (‰) of standard C1/10 after Cuen treatment	δD_{W1} (‰) of standard C1/10 after exchange at 92 ± 2°C
W1A	- 4.2	- 9*	+ 3*
W2	- 176.9	- 40*	- 40*
W3	- 345.8	- 77*	- 93*

The results displayed in table 6 show that the Cuen treatment had exchanged a smaller proportion of the O-H hydrogens in cellulose standard C1/10 than heating at 92 ± 2°C in the presence of water for 3 days. Therefore it is most likely that the crystalline regions in cellulose are less affected by the Cuen solution process than by the

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*These measurements were made before the problems involved in the determination of δD values using a combustion line had been completely resolved (see section 8.3). Thus they are only correct to ± 3‰ (1 σ).

prolonged heat treatment. Such solutions in Cuen are considered to contain cellulose micelles (Jolley 1939). Probably only the glucose units in the micellar surface (presumably from the amorphous regions of the cellulose) are involved in complex formation with Cuen (Jolley 1939).

8.1.2 Other Solvents

In view of the results discussed above and since the solution processes are similar, it is unlikely that dissolving cellulose in any of the many other possible transition metal complexes (Ott *et al.* 1954, Bikales and Segal 1971, Jayme and Lang 1963) will exchange a significant proportion of the O-H hydrogens in the crystalline regions.

The use of strong acids to dissolve cellulose may be more hopeful. Some degradation generally occurs, and with sulphuric and phosphoric acids esterification takes place to some extent (Jayme and Lang 1963). This may, however, help to open up the crystalline regions and so facilitate the exchange of more C-H hydrogens. Cellulose can be regenerated from such solutions simply by diluting them with water.

8.2 OTHER METHODS

One promising method for fixing the D/H ratio of all the O-H hydrogens involves preparing a derivative and then regenerating the cellulose. Thus cellulose triacetate could first be prepared using, for example, the method of Tanghe *et al.* (1963). Treatment of the triacetate with sodium hydroxide using the conditions described by Green (1963b) regenerates the cellulose.

Alternatively cellulose trinitrate, prepared, for example, as described by Green (1963c) might prove a more suitable intermediate. Denitration can be accomplished using ammonium hydrosulphide. However, some degradation occurs and the final cellulose contains about 1%

nitrogen.

At this stage, however, it was decided to develop the method by which most of the O-H hydrogens in cellulose are exchanged with a standard water at elevated temperatures. This method is discussed in detail in the next section. Friedman and co-workers (personal communication) have been developing a similar technique for exchanging samples of extracted wood.

8.3 THE EXCHANGE WATER METHOD

8.3.1 Description

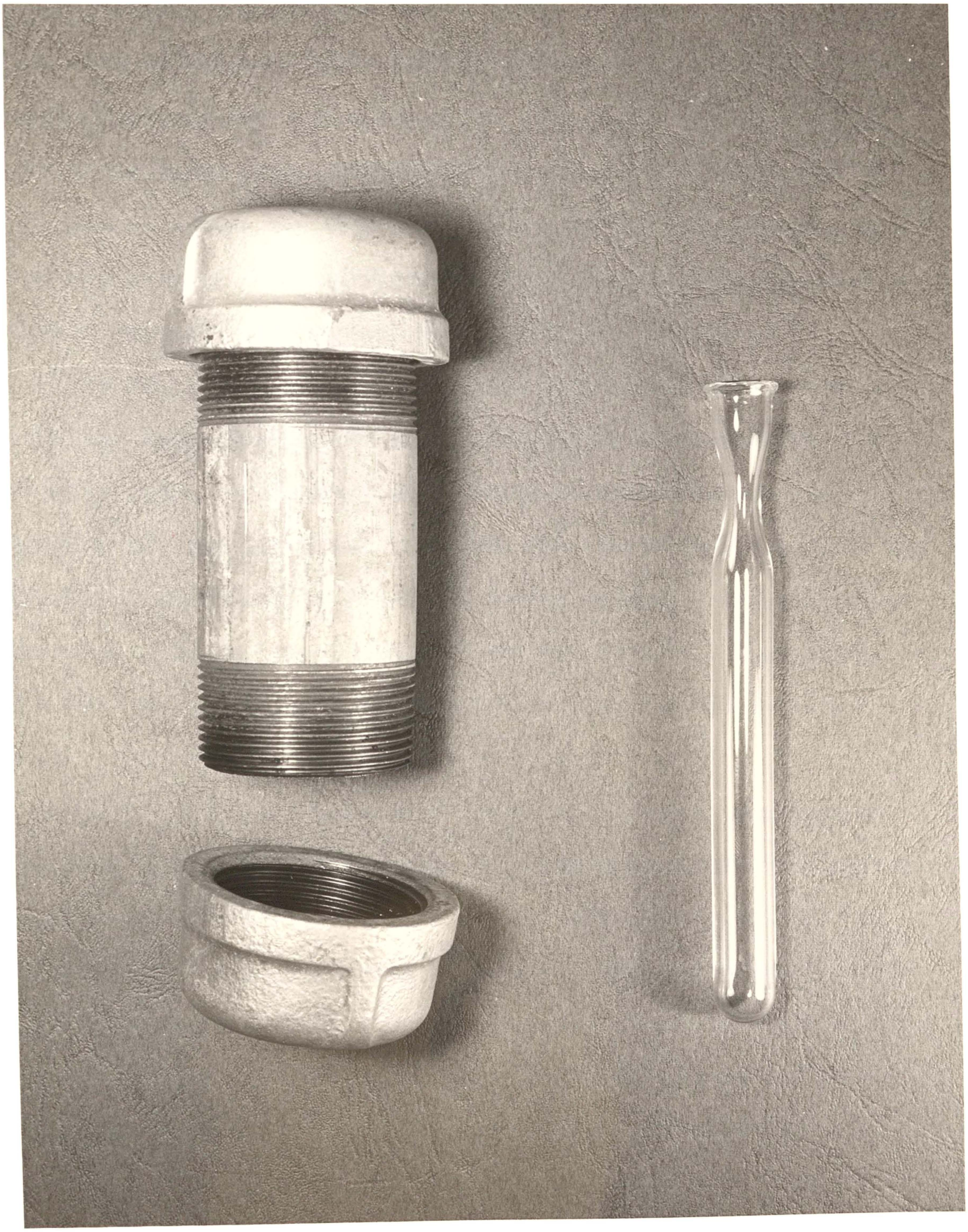
Each sample of holocellulose (0.01 to 0.02 g) was sealed up with standard water (~5 ml) in a glass vial (plate 4). This vial was placed in a metal tube (plate 4) which was then left in an oven at 92 ± 2°C. After 3 days the vial was removed and placed in a second oven at 25°C. After 24 hr the vial was cracked open, the water decanted off, and the wet cellulose rapidly transferred to a combustion line (fig. 24). It was freeze-dried (21 ± 3 hr) with the aid of a liquid nitrogen trap around T8 (fig. 24) for the first 2 to 3 hours to ensure that, while there was still water associated with the cellulose, it remained frozen. The sample was then combusted as described in section 12.1.

After the removal of carbon dioxide the water left in trap T5 (fig. 24) was frozen into a small sample bottle, connected at J2, using liquid nitrogen (stopcocks S10, S7 and S11 closed; S8, S9 and S12 open). This sample bottle was connected to the water reduction line at J1 (fig. 9) and the water was converted to hydrogen as described in section 5.1.

The triplication of combustion furnaces enabled three samples to be dried simultaneously in one combustion line.

Plate 4

Metal tube and glass vial used in exchange water method.



8.3.2 Discussion

Before the above technique was finalised each stage was considered in detail. Thus the sample was equilibrated at 92 ± 2°C because at higher temperatures the cellulose became discoloured, but the exchange reaction did not significantly speed up nor did significantly more O-H hydrogens react. Also there was the risk that any defective glass vials could explode if heated much above 100°C.

A time of 3 days was used because most of the O-H hydrogens that will exchange using this technique had done so after such a period of heating at 92 ± 2°C (see section 8.3.5).

If the vial was cracked open as soon as possible after removal from the hot oven, the reproducibility of the δD determinations was found to be poor. As long a time as 24 hr in the 25°C oven was convenient, but not necessary. However, the sample should be left for at least 1 hr since the fractionation factor between water and O-H hydrogens at 25°C will be different from that at 92°C. The readily accessible O-H hydrogens in the amorphous regions of the cellulose will take a few minutes to re-equilibrate (see fig. 13).

As water evaporates isotopic fractionation occurs (Merlivat *et al.* 1963, Craig *et al.* 1963, Majoube 1971). Therefore, to prevent further exchange, it is important to keep the water associated with the wet cellulose frozen while its vapour is pumped away. The evacuation of wet cellulose for 21 ± 3 hr reduces its water content to about 2% by weight (Lang and Mason 1959). For absolute dryness, heat and longer evacuation times are required. If the cellulose is not dried completely (as in the above method), it is essential that each sample is evacuated for about the same time.

During the development of the exchange water method some deuterated cellulose samples were combusted (see section 8.3.6). After this experiment great difficulty was experienced in removing all traces of heavy water from the combustion vacuum line. The reason for this is probably the fact that water molecules, adsorbed by silica glass at high temperatures, react to produce hydroxyl groups in the atomic network of the glass (Moulson and Roberts 1960). Such hydroxyl groups become buried in the glass surface and so exchange with more water only very slowly. This produces a small, but persistant, memory effect between individual sample combustions. In addition the vitreous silica used has an O-H content as high as 0.04% by weight when it is produced (Hetherington and Jack 1962). The influence of this O-H content on the properties of vitreous silica is discussed by Hetherington and Jack (1962) and Bell *et al.* (1962).

Only the replacement of the original silica combustion tubes eliminated the heavy water contamination of the vacuum line. The sample-to-sample memory effect was later shown to be less than 1°/oo for samples differing by not more than 100°/oo. Every stage of each combustion was carried out reproducibly so that any exchange effects between the water produced and the silica glass were kept as constant as possible.

The Hastings-Raydist compensated thermocouple vacuum gauge* (Hulston 1961) used in the combustion line was also found to adsorb water that came into contact with it. Therefore it was found essential to keep stopcock S10 closed (fig. 24) when freezing the combustion water into a sample bottle.

8.3.3 Yield Determinations

Yields of hydrogen from sucrose samples were calculated directly, to an estimated accuracy of ± 2%, by weighing the samples before combustion. Yields of hydrogen from cellulose samples were calculated,

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*Type DV-5M.

to an estimated accuracy of ± 3%, using the measured carbon dioxide yields from the combustion line (assumed to be 100% - see section 12.2) to calculate theoretical yields of hydrogen. The cellulose was assumed to have the formula $(C_6H_{10}O_5)_n$.

Such yields were determined for every sample analysed. Any samples which gave a lower than normal yield of hydrogen were rejected.

The results obtained for the sucrose samples and for selected cellulose samples are shown in table 7. Clearly nearly all the hydrogen in the original samples is recovered for mass spectrometric analysis. Since the water reduction line gives yields of 100% (section 5.2), a small amount of hydrogen must have been lost as water in the combustion system possibly by sticking to the silica glass (see section 8.3.2) or by reaction with small particles of cold copper oxide outside furnace F3 (fig. 24). In any case each combustion tube was evacuated overnight between samples to remove such traces of moisture.

8.3.4 Reproducibility

To estimate overall reproducibility one sample of cellulose (standard C1/4 prepared from *Pinus radiata*) was analysed several times using the techniques described above.

The results displayed in table 8 show an overall standard deviation for δD determinations on cellulose of ± 0.9‰.

8.3.5 Long-Term Cellulose/Water Exchange Experiment

Several samples of cellulose standard C1/4 were sealed up in glass vials (plate 4) with standard water W1, placed in metal tubes (plate 4), and left in an oven which, over the period of experiment, maintained its temperature in the range 92 ± 3°C. Samples were removed at various times and placed in a second oven at 25°C ready for D/H analysis using the technique described in section 8.3.1.

Table 7

i) Yields of hydrogen from sucrose samples:

Weight of sample combusted (g)	Yield of hydrogen (%)
0.0171	97
0.0200	97
0.0168	94
0.0134	95
mean yield	96

ii) Yields of hydrogen from cellulose samples:

Date	Yield of carbon dioxide (g)	Yield of hydrogen (%)
21/2/76	0.0246	96
8/3/76	0.0145	97
20/5/76	0.0132	95
2/6/76	0.0161	97
5/7/76	0.0096	98
28/9/76	0.0153	96
23/11/76	0.0119	98
	mean yield	97

Table 8. δD measurements on cellulose standard C1/4

Date	δD_{SMOW} (‰)
23/5/76	- 31.7
	- 34.0
28/5/76	- 33.5
	- 34.9
	- 34.1
5/7/76	- 34.3
6/7/76	- 33.1
24/11/76	- 32.9
	- 33.6
	- 34.1
Mean ± σ	- 33.6 ± 0.9

Table 9. Long-term cellulose/water exchange experiment

Time of equilibration at 92 ± 3°C (days)	Number of δD determinations	$\delta D_{SMOW} \pm \sigma_m^*$ (‰)
0	4	- 67.9 ± 0.5
1	1	- 40.8 ± 0.9
2	1	- 34.5 ± 0.9
3	10	- 33.6 ± 0.3
5	1	- 31.5 ± 0.9
10	2	- 30.5 ± 0.6
30	2	- 29.1 ± 0.6
~160	2	- 29.4 ± 0.6
~230	3	- 30.0 ± 0.5

The results are displayed in table 9 and fig. 14. It can be seen that, within the precision of sample analysis, cellulose standard C1/4 had fully equilibrated with water after about 30 days at 92 ± 3°C. This leaves about one-fifth of the O-H hydrogens unexchanged (6% of the total hydrogen atoms in cellulose). Thus a possible simplification of the exchange water method as described in section 8.3.1 would be to leave all cellulose samples at 92°C for 30 or more days. Such samples could then be analysed when convenient without fear of further changes in their D/H ratios.

8.3.6 Calibration of the Exchange Water Method

In order to calculate the D/H ratio of the C-H hydrogens in a

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[*] σ_m, the standard error $= \frac{\sigma_1}{\sqrt{n}}$ where σ_1 = standard deviation for sample analysis = ± 0.9‰ and n = number of determinations.

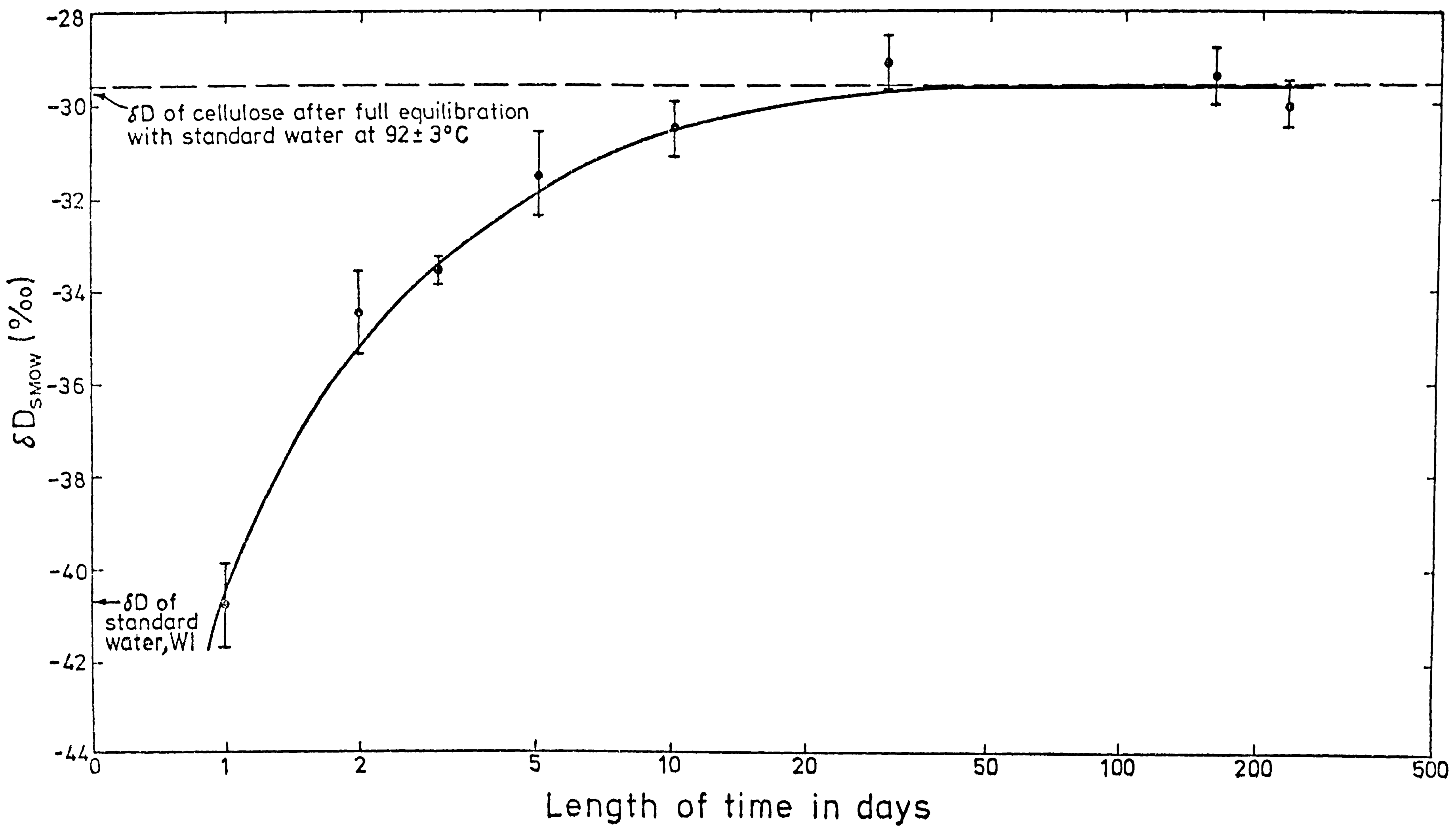

Fig.14. The effect of equilibration time on hydrogen exchange between cellulose and standard water Wl.

sample of cellulose from the D/H ratios obtained using the method described in section 8.3.1, a correction for the O-H hydrogens must be applied. To simplify this calculation the small proportion of O-H hydrogens that do not exchange after 3 days at 92 ± 2°C (about 8% of the total hydrogens in cellulose - see below) have been included with the C-H hydrogens.

Thus,

$$N = xfP + (1 - x)\,Q \qquad \text{(xxxiv)}$$

where N = measured cellulose D/H ratio,

P = exchange water D/H ratio,

Q = non-exchangeable hydrogen D/H ratio,

x = fraction of exchangeable hydrogens in cellulose,

f = exchange water/cellulose O-H hydrogen fractionation factor.

From (xxxiv), if the same cellulose is exchanged, under the same conditions, with two different exchange waters with D/H ratios P_1 and P_2, the measured cellulose D/H ratios N_1 and N_2 are related by the equation:

$$xf = \frac{N_1 - N_2}{P_1 - P_2} = \frac{n_1 - n_2}{p_1 - p_2} \qquad \text{(xxxv)}$$

where n_1, n_2, p_1, p_2 are δD values with respect to a standard.

In order to calculate Q from N and P in equation (xxxiv) we must first determine the values of x and f under the conditions used in the exchange method.

(a) Calibration using results from the nitration method of Epstein *et al.* (1976). Epstein and Yapp (1976) have determined the deuterium content of the C-H hydrogens in cellulose nitrate prepared from a series of *Pinus longaeva* (Bristlecone pine) samples supplied by C.W. Ferguson (personal communication). Wood samples from the same trees* were supplied to this laboratory for $\delta^{18}O$ and $\delta^{13}C$ determinations (see section 13.4). A few samples were also analysed for deuterium using the exchange water method. The results of these analyses are compared with those of Epstein and Yapp (1976) in table 10.

Table 10. δD determinations using both the nitration method and the exchange water method

Pinus longaeva samples - dendrochronological years of growth (A.D.)	δD_{SMOW}(°/oo) of C-H hydrogens computed from Epstein and Yapp (1976) †	δD_{SMOW}(°/oo) of cellulose after 3 days exchange with W1	δD_{SMOW}(°/oo) of cellulose after 3 days exchange with W3
1180 - 1210	- 104	- 43.3 (2)§	- 132.0 (1)§
1210 - 1240	- 101	- 39.6 (2)	-
1420 - 1450	- 96	-	- 117.7 (1)
1540 - 1570	- 107	- 38.8 (2)	- 119.6 (1)

Neglecting the small proportion of non-exchangeable O-H hydrogens in cellulose, and knowing the δD values of W1 and W3 (table 4), mean values for x and f can be calculated using equations (xxxiv) and (xxxv). This gives:

$$x = 0.224 \tag{xxxvi}$$

and

$$f = 1.23 \tag{xxxvii}$$

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[*] University of Arizona reference numbers TRL67-3 and TRL74-101 (C.W. Ferguson, personal communication 1976).

[†] These values represent the means of 3 determinations since Epstein and Yapp analysed 10 year samples.

[§] The numbers in brackets indicate how many times each 30 year sample was analysed.

These are, of course, only estimates of the true values of x and f because in the calculation approximately 8% of the hydrogens in cellulose have been neglected. Also holocellulose prepared using the chlorite technique (see section 4.2) may differ significantly from the cellulose analysed in the nitration technique, although this is unlikely.

(b) Calibration using the exchange water method. Cellulose prepared from *Pinus radiata* (see chapter 4) was exchanged with 99.7% deuterium oxide at 92 ± 2°C. The cellulose was analysed as described in section 8.3.1 except that, to ensure the removal of almost all the deuterium oxide, evacuation was continued for 3 days before combustion. The hydrogen produced was measured on the mass spectrometer against a standard containing 26.26 mole % deuterium.*

For cellulose exchanged for 3 days the δD with respect to this standard was - 180 ± 20‰. Therefore the amount of deuterium present in one mole of exchanged cellulose

$$= \frac{26.26}{100} \times \frac{(820 \pm 20)}{1000}$$

$$= 0.215 \pm 0.005 \text{ mole.}$$

Thus the fraction of exchangeable hydrogens in cellulose,

$$x = 0.215 \pm 0.005 \qquad \text{(xxxviii)}$$

which is in good agreement with (xxxvi).

Several samples of cellulose standard C1/4 were also exchanged with standard waters W1, W2 and W3 using the method described in section 8.3.1.

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*This standard was made up from a mixture of 99.7% D_2O and standard W1.

Table 11. δD of cellulose standard C1/4 after exchange with standard waters

Standard water	δD_{SMOW} (°/oo) of standard water	δD_{SMOW} (°/oo) of exchanged cellulose	Number of determinations
W1	- 40.7	- 33.6	10
W2	- 176.9	- 71.1	2
W3	- 345.8	- 114.8	5

When the results of table 11 are inserted in equation (xxxv) it is found that

$$xf = 0.266 \qquad \text{(mean value)}$$

$$[\text{estimated error} = \pm 0.002 \ (1\ \sigma)]$$

Therefore using (xxxviii),

$$f = 1.24 \pm 0.04 \qquad (\text{ixL})$$

which is effectively the same as (xxxvii).

(c) Calculation of the water/O-H hydrogen fractionation factor for amylose. As a further check some samples of amylose were exchanged with standard waters W1 and W2 using the method described in section 8.3.1. The results are shown in table 12.

Table 12. δD of amylose after exchange with standard waters

Standard water	δD_{SMOW} (°/oo) of exchanged amylose	Number of determinations
W1	- 21.1	2
W3	- 140.2	2

Amylose, like cellulose, consists of long unbranched chains of glucose units, each joined by a glycoside linkage to C-4 of the next one (see cellulose structure in fig. 12). Unlike cellulose, however, the glycoside linkages in amylose are alpha instead of beta (see, for example, Morrison and Boyd 1966). Moreover, all the O-H hydrogens in amylose are fully exchangeable with water (Sepall and Mason 1961a). Therefore for amylose, $x = 0.3$.

Substituting the results shown in table 12 in equation (xxxv), we get

$$f = 1.30 \tag{xL}$$

This value is similar to those obtained for cellulose (xxxvii) and (ixL).

(d) Conclusions. Thus under the conditions used in the exchange method, $x \approx 0.22$ and $f \approx 1.23$. These values can be used in conjunction with equation (xxxiv) to calculate Q, the non-exchangeable D/H ratio of cellulose from measurements of N and P.

Reported values for the isotope distribution factor k for the following equilibrium with alcohols;

$$\mathrm{ROH} + \mathrm{HOD} = \mathrm{ROD} + \mathrm{H_2O}$$

$$\text{where } k = \frac{[D]/[H]\ \text{(alcohol)}}{[D]/[H]\ \text{(water)}}$$

range from 0.96 to 1.25 (Mann 1971). The above value for f cannot, however, be taken as an accurate estimate of the k factor for cellulose for two reasons. First, not all the exchange water is removed before the cellulose is combusted (see section 8.3.2). Secondly, although most of the exchange water is freeze-dried away, a small quantity may become liquid during the latter stages of drying. If this were to happen the evaporation of the remaining water would tend to increase the deuterium content of the exchangeable O-H hydrogens in the cellulose.

8.3.7 A Comparison Between the Exchange Water and Nitration Methods.

Both the nitration method of Epstein *et al.* (1976) and the exchange water method described in this chapter can be used for determining the D/H ratio of the non-exchangeable hydrogens in cellulose. If it is desired to determine absolute values for the D/H ratio of the C-H hydrogens, the nitration technique is probably the more accurate since it is unnecessary to correct for O-H hydrogens. If, however, it is desired to measure the difference between the D/H ratio of 2 samples of cellulose (e.g. for palaeoclimate studies), the exchange water method is more precise since δD values can be determined to ± 0.9‰ (1 σ) compared with ± 2‰ (1 σ) (Epstein and Yapp 1976) for the nitration technique. The exchange water method has an additional advantage in that $\delta^{13}C$ values can be determined at the same time. This would be difficult using the nitration technique because of the problem of removing nitrogen oxides from the combustion products. The exchange method seems to be simpler, and it involves no unstable intermediates, unlike the nitration method. The exchange method is also generally applicable to the determination of D/H ratios in other organic compounds containing exchangeable O-H hydrogens (e.g. lignin).

CHAPTER 9 δD VARIATIONS IN TREES

9.1 INTRODUCTION

9.1.1 Previous Work

Very few workers have investigated the possible correlations between climate and the deuterium content of plant material. The most common approach so far has depended on a direct correlation between the D/H ratio of the hydrogen in plants and the D/H ratio of their source water (normally local precipitation). It was hoped that the variations in the deuterium content of plants would faithfully record the variations in the deuterium content of the precipitation. Dansgaard (1964) has shown that the latter changes by + 5.6‰ per °C rise in temperature for maritime localities (see also Friedman *et al.* 1964).

Schiegl (1972) measured the deuterium content of peat samples and found a correlation with temperatures of + 3.2‰ per °C. He later measured the deuterium content of a series of whole wood samples from a *Picea* from southern Germany and found a similar temperature coefficient of + 3‰ per °C (Schiegl 1974). It is interesting to note that both these temperature coefficients are less than that measured for meteoric waters. This may be due to the existence of a negative coefficient of - 2 to - 3‰ per °C for the fractionation that occurs when environmental water is taken up by the plant and used in photosynthesis. However, it has since been shown that whole wood data may not show meaningful δD trends (Epstein and Yapp 1976). This casts some doubt on the value of Schiegl's data.

Libby and co-workers have investigated the variation in the deuterium content of whole wood samples from several trees. They report a δD temperature coefficient of + 76 ± 10‰ per °C for *Quercus petraea* (German oak) (Libby and Pandolfi 1974a, 1974b) although their later work on *Crytomeria japonica* (Japanese cedar) seems to indicate a δD variation of about + 10‰ per °C (Libby *et al.* 1976). Libby and Pandolfi (1974c) also report a δD temperature coefficient of + 3.3 ± 0.7‰ per °C for the blue-green algae from hot springs. Moreover, Libby and Pandolfi (1974a, 1974b) found a δD range of more than 150‰ in *Quercus patraea* for the last 150 years. This compares with a range of 14‰ as found by Schiegl (1974) for the same time interval in a *Picea*, also from Germany. Such discrepancies are difficult to rationalise unless the techniques used for extracting the hydrogen from plant material are responsible, as has been suggested by Epstein and Yapp (1976, 1977).

Epstein and Yapp (1976) have measured the variation in deuterium content in cellulose nitrate prepared from tree rings in *Pinus sylvestris* from Scotland and *Pinus longaeva* (Bristlecone pine) from California. They report some correlations between the δD values and climate particularly over the period of meteorological observations. Their results indicate a positive temperature coefficient which is probably mainly due to meteoric water variations.

An alternative approach to the problem of relating D/H ratios in plant material to climate is to first determine whether the isotopic fractionation factor between plant hydrogen and source water is constant or changes with changing climate. This approach was suggested by Libby (1972). She calculated a thermodynamic temperature coefficient of + 2‰ per °C for the C-H hydrogens in cellulose.

The work described in this chapter shows that the deuterium isotopic fractionation factor between the C-H hydrogens in cellulose from *Pinus radiata* and their source water does indeed vary with climate. A temperature dependence of - 3‰ per °C is consistent with the results obtained (see section 9.5.1). This compares favourably with the - 2 to - 3‰ per °C computed from Scheigl's measurements using whole plant material (see above discussion).

More recently Epstein *et al.* (1976) compared the δD of cellulose from different species, from a variety of locations and growing in water at different temperatures. They report an increase in deuterium content of 25‰ for a temperature difference of approximately 11°C. However, this result can be regarded as extremely tentative considering the wide variation (about 0 to - 50‰) in the measured fractionation between the C-H hydrogens of cellulose and the environmental water (Epstein *et al.* 1976, this thesis - section 9.5.2).

9.1.2 Other Preliminary Considerations

All the δD determinations discussed so far have shown the importance of the isotopic composition of local precipitation as the major factor determining the deuterium content of plant material. The mean annual D/H ratios of precipitation at a particular site change very little (Dansgaard 1964). However, in the short term the isotopic composition can vary widely, depending on many factors including the temperature history of the air masses which bring the precipitation to an area (Friedman *et al.* 1964).

The effect of the plant's physiology on its deuterium content must also be carefully considered. Tree physiology and biochemistry are discussed in chapter 2. For all studies involving δD determinations on plant material it is particularly important to consider the transpiration processes that take place in the leaves. Such processes can cause very large fractionations particularly in arid environments (Gonfiantini *et al.* 1965, Wershaw *et al.* 1966, Lasaint *et al.* 1974). Also, for isotopic measurements on trees, it is important to consider at what times of the year the material that ultimately becomes wood was fixed by photosynthesis.

9.1.3 *The Choice of Pinus radiata*

The main objective of the study described in this chapter was to investigate whether the isotopic fractionation factor between the C-H hydrogens of tree cellulose and the hydrogens of the water absorbed by the roots is related to changing climate. For this investigation it was decided to study wood from rings of a *Pinus radiata* (Monterey pine) tree which grew in Hamilton, New Zealand, for the following reasons:

(a) In Hamilton *Pinus radiata* grows throughout the year (Barnett 1971, 1973), and thus over a wide temperature range. During the year both the monthly mean maximum daily temperature and the monthly mean daily temperature vary through a cycle with an amplitude of 10°C (see appendix E and N.Z. Met. Service).

(b) Wide rings (2 cm) are laid down (see plate 5) which enable different parts of the ring, and therefore wood laid down at different temperatures, to be sampled.

(c) The isotopic composition of the local precipitation varies only slightly throughout the year (see appendix D).

(d) Hamilton has a relatively humid climate all year round (N.Z. Met. Service) so that leaf transpiration should not be excessive and should not vary too much throughout the year.

(e) Because the tree sampled was complacent and grew all year one would expect the wood to be laid down from current photosynthesis.

9.2 *δD VARIATIONS IN PINUS RADIATA SAP WATER*

The isotopic composition of *Pinus radiata* sap water was measured in order to ascertain whether or not it varied as much as the isotopic

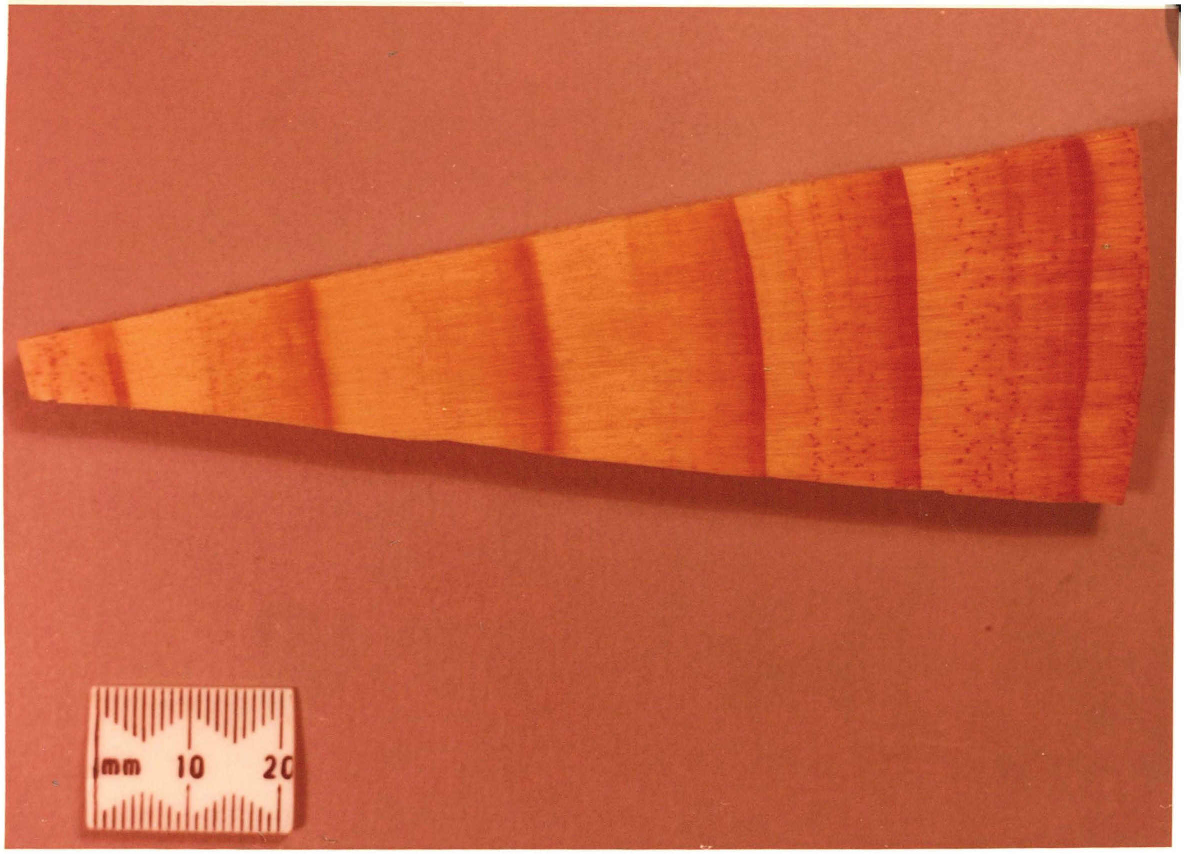

Plate 5

New Zealand grown *Pinus radiata*.

composition of the local precipitation (see appendix D).

A *Pinus radiata* tree growing at Waikato University, Hamilton, was selected for study because it is the same species and grows in a similar situation to the tree used for isotopic determinations on wood components.

Initially the tree was sampled every 2 months in the morning. Later sampling was carried out between 09.15 and 10.20 at monthly invervals. On one day 4 samples were taken between 09.30 and 15.10. At each sampling a small branch (~1 cm in diameter) was cut off the tree, the bark was removed, and the sap water was squeezed† into a small bottle as soon as possible. Each bottle was sealed and stored in the dark.

δD determinations were carried out using the method described in chapter 5.

Table 13. Variation in the isotopic ratio of hydrogen in *Pinus radiata* sap water during one day (1/3/76)

Time of sample collection	δD_{SMOW} (‰)*
09.30	- 39.1
10.50	- 37.7
12.40	- 38.3
15.10	- 38.3
mean of 4 ± σ	- 38.4 ± 0.6

The results in table 13 show that the deuterium content did not change significantly during a 6 hr period in one day. Since this day

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† A number DX-02 die (supplied by the Research and Industrial Instrument Company) and a hydraulic press were used. This type of die is normally used for producing large KBr discs for infrared measurements or sample discs for X-ray fluorescence analysis.

* One determination was carried out for each sample.

was hot and sunny after a cold night, transpiration rates must have increased dramatically over the 6 hr sampling period. However, the isotopic composition of the sap water did not significantly change. This result is in agreement with that of Dongmann *et al.* (1972) who found no significant change over a 48 hr sampling period in the oxygen 18 content of sap water taken from beech and oak trees. Wershaw *et al.* (1966) and Lasaint *et al.* (1974) found that sap water was generally slightly enriched in deuterium compared with the water supplied to the plant. The magnitude of this enrichment varied only slightly (barely outside experimental error) despite the fact that samples were taken from several plant species, at different times, on various days and during widely differing weather conditions. Thus any changes in the isotopic composition of sap water in trees are most likely to reflect changes in the ground water supplying the trees' roots.

In contrast the isotopic composition of leaf water has been shown to change dramatically throughout the day due to changes in transpiration rates (Gonfiantini *et al.* 1965, Wershaw *et al.* 1966, Dongmann *et al.* 1972).

Table 14 and fig. 15 show the variation in the deuterium content of *Pinus radiata* sap water over a 2 year period. No clear patterns emerge although all of the determinations except one lie within a range of less than 6^{o}/oo. This compares with a probable mean summer/winter variation of about 20^{o}/oo (see appendix D) in the deuterium content of precipitation at Hamilton.

Table 14 Variation in the isotopic ratio of hydrogen in *Pinus radiata* sap water over a two year period.

Date of sample collection	δD_{SMOW} (‰)	Number of determinations
9/9/74	-40.5	2
26/11/74	-39.3	2
7/2/75	-40.9	2
25/4/75	-41.1	3
11/8/75	-42.4	2
30/10/75	-41.6	2
1/12/75	-38.6	1
28/12/75	-39.9	2
3/2/76	-37.8	1
1/3/76	-38.4	4
1/4/76	-41.8	1
1/5/76	-34.9	2
1/6/76	-37.3	1
1/7/76	-38.8	1
2/8/76	-38.3	2
6/9/76	-41.3	1
5/10/76	-43.1	2

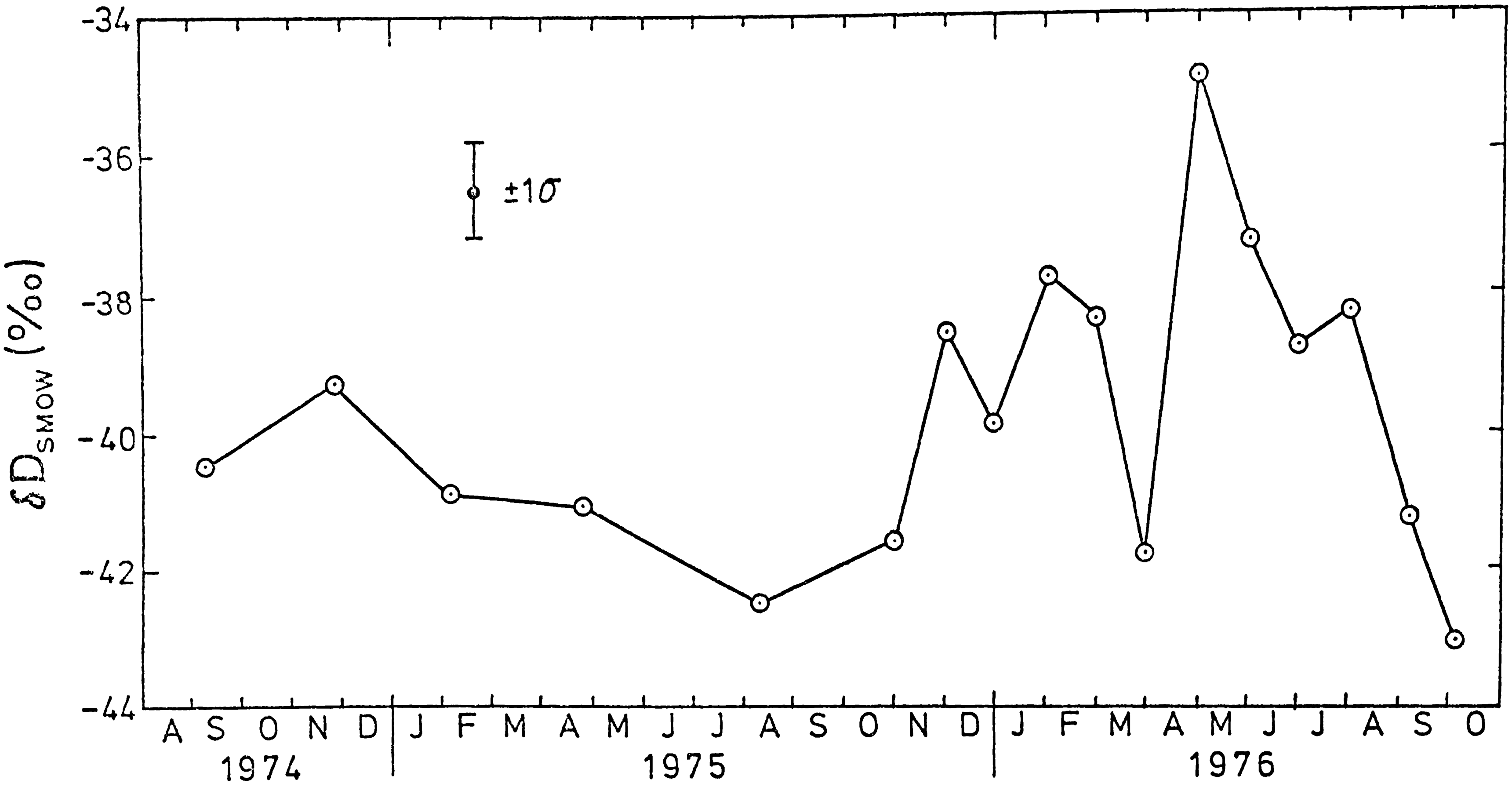

Fig.15. Variation in the isotopic ratio of hydrogen in *Pinus radiata* sap water over a two year period.

9.3 *δD AND $\delta^{18}O$ VARIATIONS ACROSS THREE GROWTH RINGS OF PINUS RADIATA*

9.3.1 *Cellulose δD*

Three rings of *Pinus radiata* heartwood* were divided up into 15 samples. For each sample the isotopic ratio of the hydrogen atoms in cellulose was determined using the exchange method described in section 8.3.

The δD values shown in table 15 and displayed in fig. 16 are those measured for the cellulose samples after exchange with standard water W1. Using equation (xxxiv) with $x = 0.22$ and $f = 1.23$, the mean δD value for the non-exchangeable hydrogens for the 15 samples of *Pinus radiata* cellulose,

$$\delta D_{SMOW}(P.radiata \text{ cellulose}) = -88 ^{o}/oo$$

The δD values of the non-exchangeable hydrogens in each individual sample of cellulose were computed in the same way to give the results displayed in the upper half of fig. 17.† It can be seen that the non-exchangeable hydrogens are more than 20°/oo depleted in the summer as compared with the winter.

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*Ring-counting implied that these rings were laid down from 1918 to 1920 inclusive, and that the first ring of the tree was laid down in 1913. The tree was felled in the summer of 1972-1973.

†These results differ slightly from those originally published (Wilson and Grinsted 1975) for two reasons. First, the δD measurements in the original work were carried out before all the problems of using the exchange water method had been completely resolved. The original measurements are thus only accurate to ± 3°/oo (1 σ). Secondly, the results were expressed on the assumption that the fractionation factor between the hydrogens of H_2O and C-OH was close to 1.0. It was later found that this fractionation factor is about 1.23 (see section 8.3.6).

Table 15 δD determinations for cellulose from three rings of *Pinus radiata*.

Sample Number	δD_{SMOW} (‰)*	Number of determinations
1	-29.6	1
2	-24.5	1
3	-32.6	1
4	-38.6	1
5	-30.4	1
6	-29.4	1
7	-26.1	1
8	-21.7	1
9	-35.6	2
10	-38.4	1
11	-33.5	1
12	-22.3	1
13	-21.3	1
14	-23.3	2
15	-32.7	2
Mean of 15 samples	-29.3	

*As determined for the cellulose samples after exchange with standard water W1.

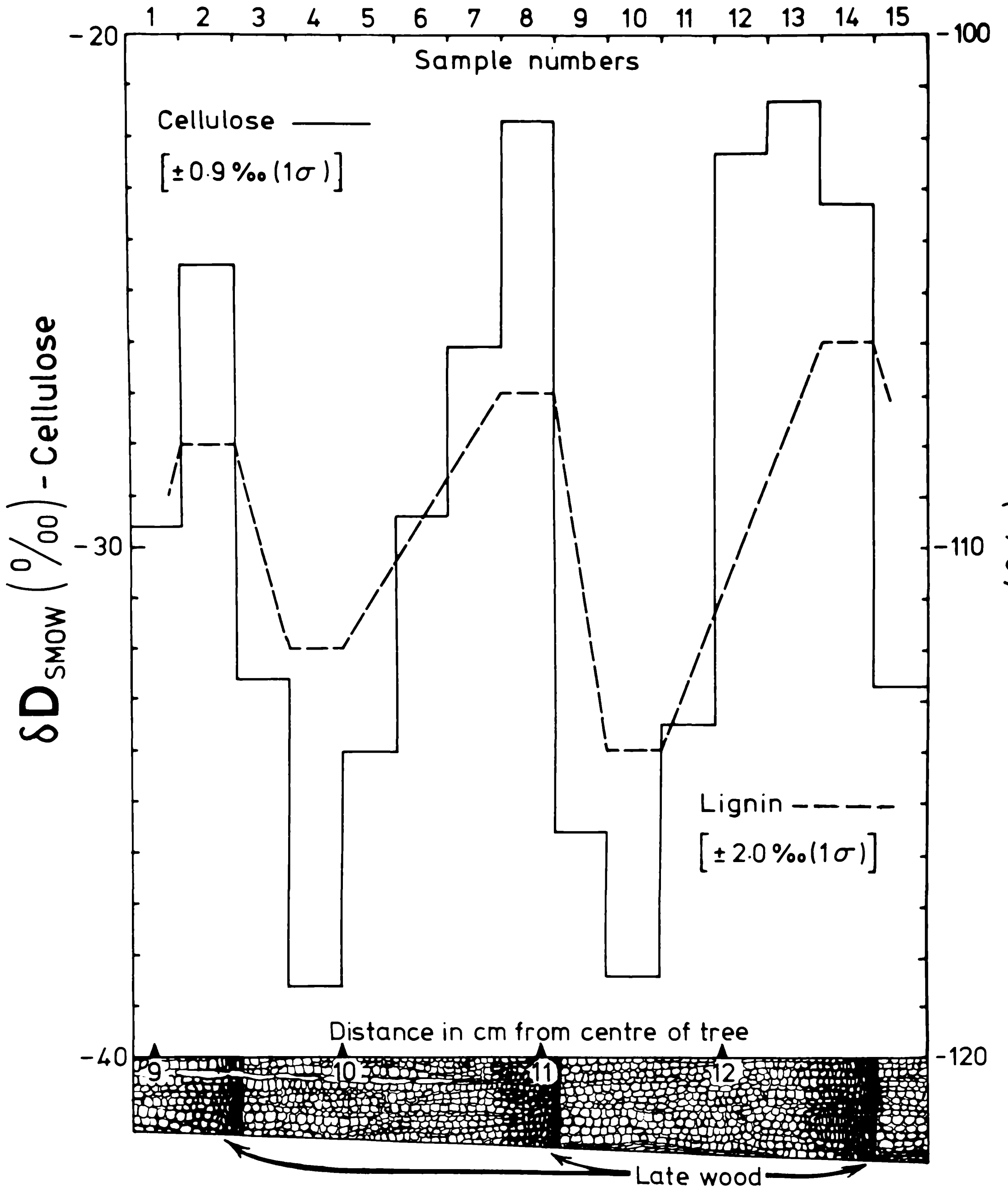

Fig.16. Variation in the isotopic ratio of the hydrogens in cellulose and lignin across tree rings from *Pinus radiata* - raw values after exchange with WI.

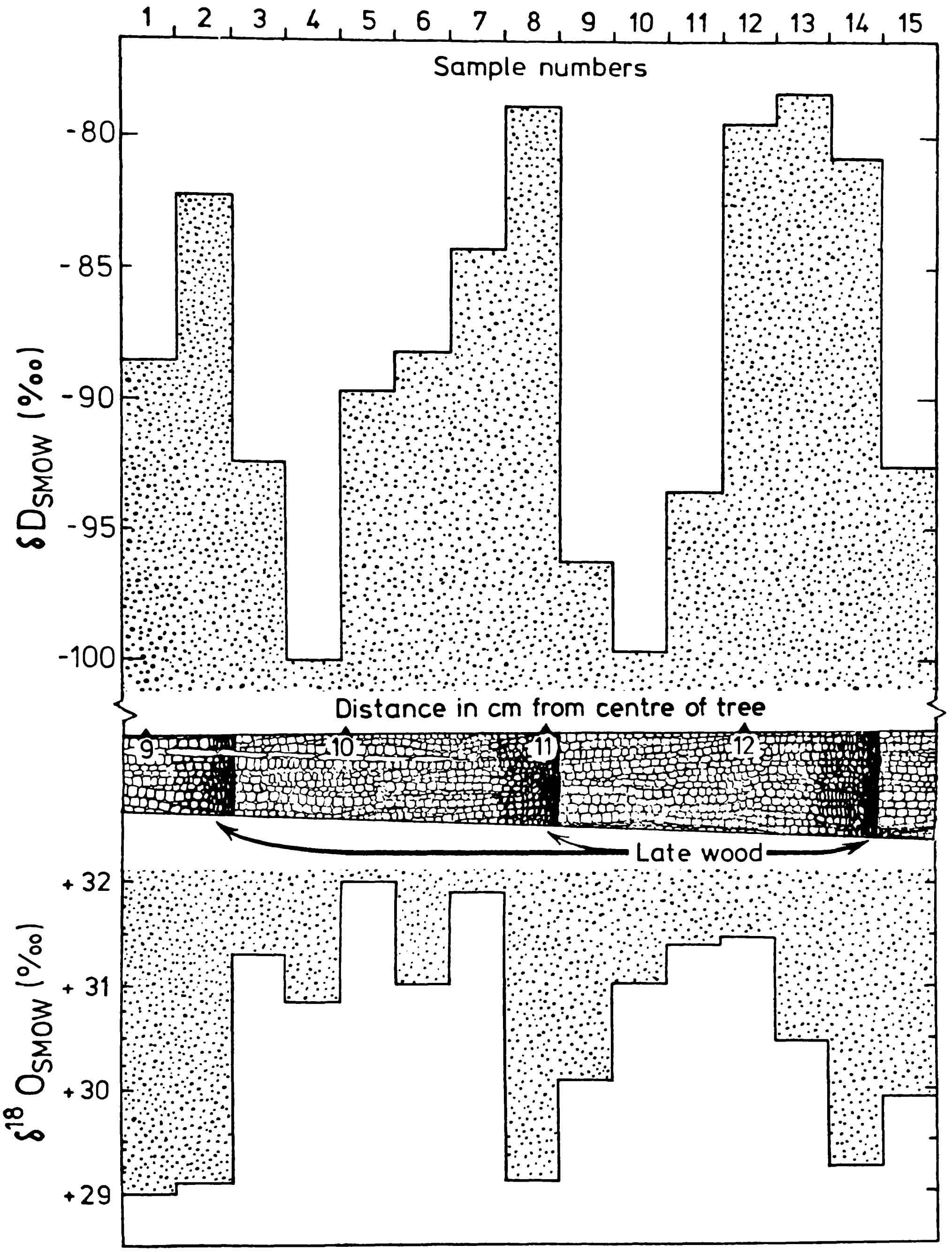

Fig.17. Variation in the isotopic composition of hydrogen and oxygen in cellulose across tree rings from *Pinus radiata*.

9.3.2 Cellulose $\delta^{18}O$

Oxygen isotopic ratios for the same 15 cellulose samples used for δD determinations were measured by Professor A.T. Wilson using a technique developed from the method of Rittenberg and Ponticorvo (1956).

The results in table 17 and the lower half of fig. 17 show that $\delta^{18}O$ varies in the opposite direction to δD. Cellulose oxygen is more than 2‰ enriched in the summer as compared with the winter.

9.3.3 Lignin δD

δD values were determined for lignin prepared from 5 of the samples used for cellulose δD and $\delta^{18}O$ determinations. Because of problems associated with the method used (see discussion in section 10.2), the results shown in table 16 and displayed in fig. 16 can be regarded as rather tentative. However, one conclusion is evident. The non-exchangeable hydrogens in lignin are clearly depleted in the summer as compared with the winter.

Table 16. δD determinations for *Pinus radiata* lignin

Sample number	δD_{SMOW} (‰)* after exchange with W1	δD_{SMOW} (‰)* after exchange with W3
2	- 108	-
4	- 112	-
8	- 107	-
10	- 114	-
14	- 106	- 164

It is possible to apply a correction for the lignin hydrogens that

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*One determination was carried out for each sample. Since the measurements were done using small samples, the figures quoted are only accurate to ± 2‰ (1 σ).

Table 17 $\delta^{18}O$ determinations for cellulose from three rings of *Pinus radiata*.

Sample Number	$\delta^{18}O_{SMOW}$ ($^o/oo$)*
1	+28.99
2	+29.09
3	+31.30
4	+30.84
5	+32.01
6	+31.02
7	+31.90
8	+29.11
9	+30.09
10	+31.02
11	+31.40
12	+31.47
13	+30.46
14	+29.24
15	+29.92
mean value	+30.52

*One determination was carried out for each sample except number 7 which was measured 3 times. The reproducibility of the technique is ± $0.2^o/oo$ (1σ). (A.T. Wilson, personal communication 1976.)

exchange with the standard water. If the additional hydrogens that exchange with the concentrated sulphuric acid during preparation (see section 10.2) are neglected, a very approximate mean δD value for *Pinus radiata* lignin can be calculated using equations (xxxiv) and (xxxv), and the results in table 16.

δD_{SMOW}(*P.radiata* lignin) ≈ $- 160^{o}/oo$

9.3.4 *Lignin $\delta^{18}O$*

$\delta^{18}O$ values for lignin prepared from samples 8 and 11 were determined by Professor A.T. Wilson. The results are shown in table 18.

Table 18. $\delta^{18}O$ determinations for *Pinus radiata* lignin

Sample number	δD_{SMOW} ($^{o}/oo$)
8	+ 11.8
11	+ 7.9
mean value	+ 9.8

9.4 *δD DETERMINATIONS FOR CELLULOSE FROM OTHER TREES*

9.4.1 *The Work of Epstein and Yapp*

Since the completion of the study described in section 9.3, Epstein and Yapp (1976) have published their work on the D/H ratios of the C-H hydrogens in earlywood and latewood cellulose. They studied *Pseudotsuga* (Douglas fir) and *Pinus ponderosa* from Radio Ridge, Arizona, and *Pinus radiata* from the Monterey Peninsula, California. Their results are reproduced in figs. 18 and 19. They are discussed in section 9.5.

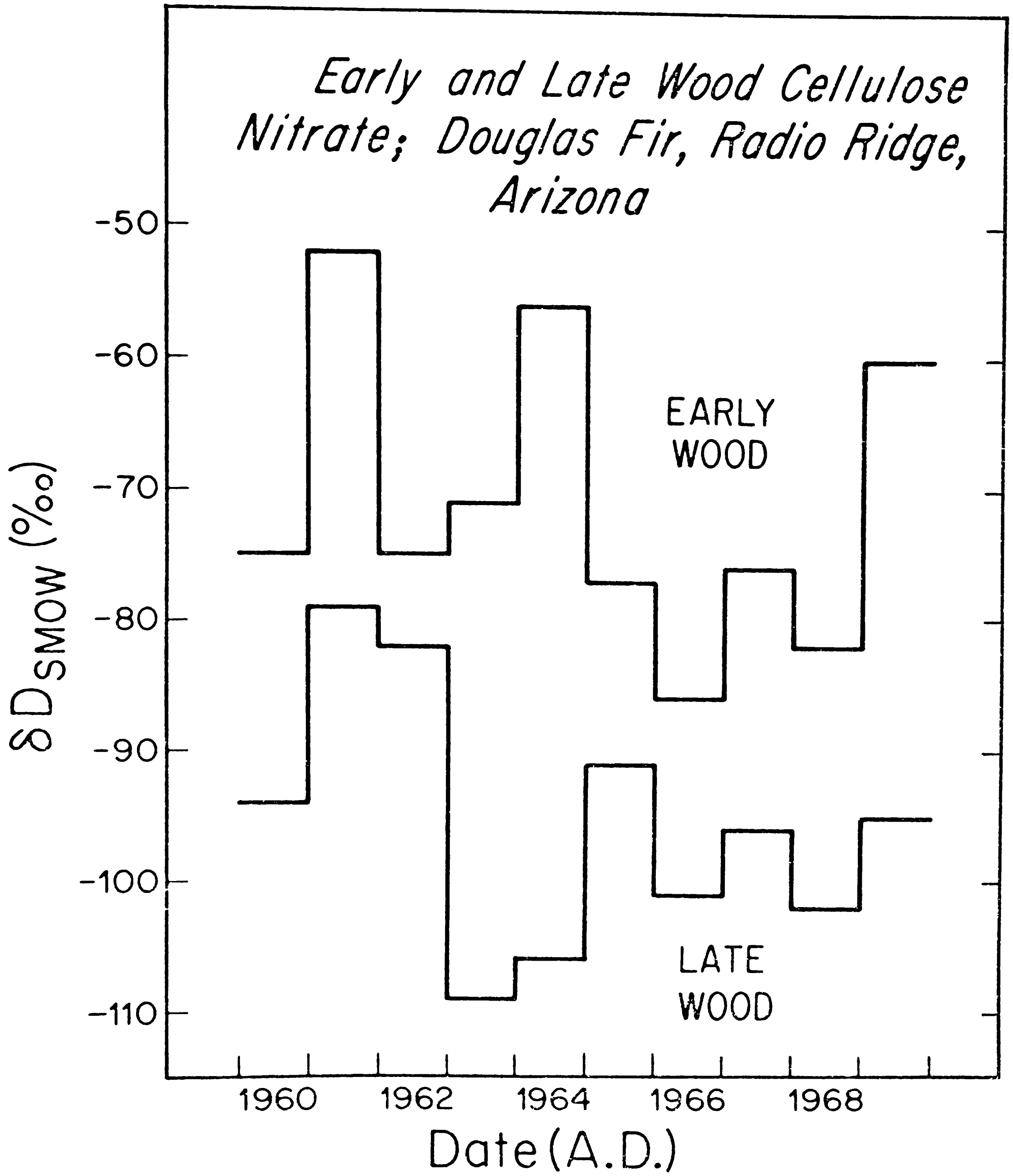

Fig.18. The δD values of the isotopically non-exchangeable hydrogen of cellulose (cellulose nitrate) in early and late wood from single tree rings from a Douglas fir located in Radio Ridge, Arizona, (after Epstein and Yapp 1976).

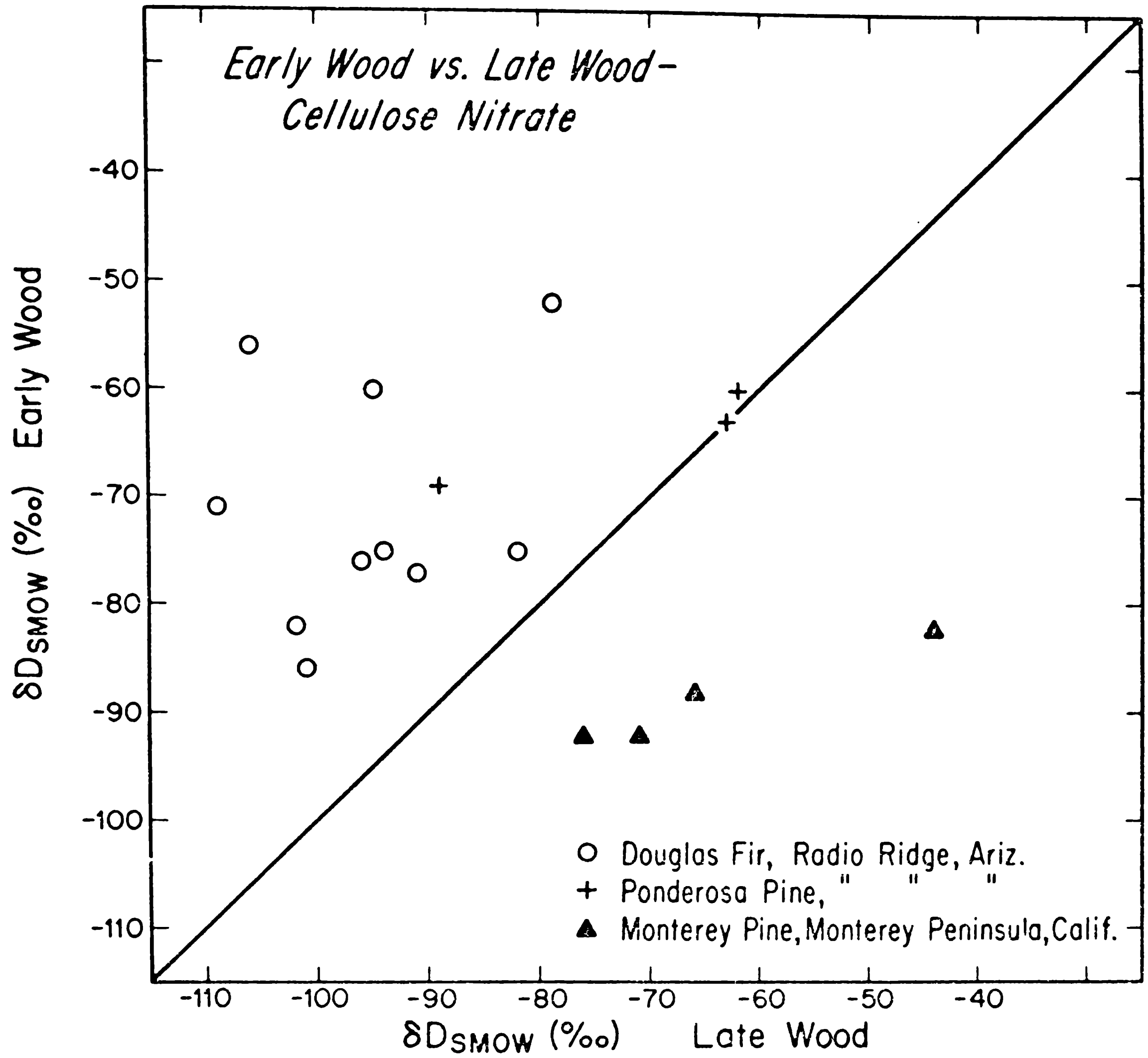

Fig.19. The relationship between the δD of the isotopically non-exchangeable hydrogen of cellulose (cellulose nitrate) in early and late wood. Each point represents the early and late wood formed in a single growing season, i.e., a single tree ring (after Epstein and Yapp 1976).

9.4.2 *Altitude Variations in Pseudotsuga*

According to Ambach *et al.* (1968) the evaporation of falling drops causes the isotopic composition of rain to change by - 0.2°/oo in $\delta^{18}O$ and - 1.6°/oo in δD for every 100 m increase in altitude. This effect may be detectable in the isotopic composition of the wood components from trees growing in the same region, but at different altitudes. However, the results will be complicated if there are any temperature effects on the isotopic fractionation factors between cellulose and the environmental water. Assuming an adiabatic lapse rate of - 0.5°C per 100 m rise in altitude (Lamb 1972), two *Pseudotsuga* trees growing at 1828 m and 2747 m should experience about a 4.5°C difference in mean temperature.

Thus δD and $\delta^{18}O$ values were determined for the cellulose prepared from two samples of wood which grew during the same season, but at two different altitudes. The *Pseudotsuga* trees sampled grew on Mt. Lemmon, Arizona.

Table 19. δD and $\delta^{18}O$ determinations of *Pseudotsuga* cellulose from two different altitudes

Altitude (m)	δD_{SMOW} (°/oo)*	$\delta^{18}O_{SMOW}$ (°/oo)†
1828	- 64.6 (2)§	+ 30.9 (1)§
2747	- 65.9 (2)	+ 28.9 (1)

The $\delta^{18}O$ results (see table 19) differ by 2.0°/oo. This is similar to the theoretical difference in the oxygen 18 content of precipitation,

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[*] The δD values are those of the non-exchangeable hydrogens in the cellulose.

[†] The $\delta^{18}O$ determinations were carried out by Professor A.T. Wilson.

[§] The number of determinations is shown in the brackets.

which should change by about - 1.8°/oo for a 919 m increase in altitude (see above).

The δD results (see table 19) differ by 1.3°/oo. However, the deuterium content of the precipitation should change by - 15°/oo for a 919 m increase in altitude (see above). A possible explanation for this discrepancy is the existence of a temperature effect on the cellulose/environmental fractionation factor of about - 3°/oo per °C rise in temperature.

However, many more samples will have to be analysed before any firm conclusions can be arrived at. Factors such as the source of the trees' water supply (whether it comes from local precipitation or groundwater originating from another area), different earlywood/latewood ratios, different growing periods, and variable transpiration may well complicate matters.

9.5 *DISCUSSION AND CONCLUSIONS*

9.5.1 *δD Variations Between Earlywood and Latewood*

The results in section 9.3.1 show that the non-exchangeable hydrogens in cellulose from three growth rings of a *Pinus radiata* tree from Hamilton, New Zealand, are more than 20°/oo depleted in deuterium in the summer as compared with the winter. This summer/winter variation seems to reflect the annual temperature cycle (see appendix E).

The isotopic composition of cellulose varies in the opposite direction to the mean isotopic composition of local atmospheric precipitation, which is about 20% enriched in deuterium in the summer as compared with the winter (see appendix D). The deuterium content of *Pinus radiata* sap water does not change as much as that of the precipitation since it depends on the deuterium content of the ground water taken in by the tree's roots (see section 9.2). The observed cellulose δD variations are therefore unlikely to be the result of the changing isotopic composition of precipitation or groundwater. Also they do not reflect the expected changes in the isotopic composition of leaf water which would tend to become enriched in the summer due to greater transpiration associated with higher temperatures and lower humidity (N.Z. Met. Service). Indeed it is likely that transpiration fluctuations have reduced the magnitude of the observed summer/winter variation in δD values.

Thus the deuterium content of cellulose in the wood of *Pinus radiata* probably varies with temperature. This may be due to a temperature effect on one or more of the biochemical reactions leading to cellulose. The variation most likely reflects leaf temperature since the C-H bonds are formed during photosynthesis of sucrose in the leaves. The sucrose is translocated to the trunk where it is converted to cellulose (see chapter 2). If the observed variation does indeed depend on temperature, a rise in temperature of one $^{\circ}$C decreases the deuterium content by more than 2°/oo.

The results in section 9.3.3 show that the non-exchangeable hydrogens in lignin are also depleted in the summer as compared with the winter. Since lignin is, like cellulose, synthesised from translocated sucrose, a similarity is to be expected if the above discussion is valid. Thus the lignin results provide support for the conclusions arrived at in the preceding paragraph.

Epstein and Yapp's study of earlywood and latewood (see section 9.4.1) was carried out on trees which grew in regions where the variation in the isotopic composition of the water used by the trees was unknown, and in arid regions where there would be considerable fluctuations in transpiration rates. Their results are therefore more difficult to interpret. However, in the light of the *Pinus radiata* results discussed above an alternative interpretation to that given by Epstein and Yapp (1976) should be considered.

At Radio Ridge, Arizona, earlywood is laid down in the spring and latewood in the summer (Fritts 1976); that is latewood is laid down at higher temperatures than earlywood. Figs. 18 and 19 show that for *Pseudotsuga* (Douglas fir) the earlywood growing under colder conditions is invariably heavier than latewood. Thus as the temperature increases the deuterium content of the non-exchangeable hydrogens of cellulose decreases just as in New Zealand grown *Pinus radiata*. The fact that there is a large variation in the deuterium content between latewood and earlywood in individual years from *Pseudotsuga* is probably related to fluctuations in transpiration rates and the D/H ratio of the water supply. Epstein and Yapp's data on *Pinus ponderosa* (see fig. 19) show either no difference between earlywood and latewood or the same effect as that found for *Pseudotsuga*.

In the well-watered sites on the Monterey Peninsula, California, *Pinus radiata* lays down latewood at lower temperatures than earlywood (MacDougal 1938; J.R. Barnett, personal communication 1976), just as it does in New Zealand. Thus, as expected, Epstein and Yapp's results for *Pinus radiata* from the Monterey Peninsula (see fig. 19) are similar to those found for New Zealand grown *Pinus radiata*.

Therefore, all the data so far compiled concerning D/H ratios in cellulose from earlywood and latewood suggest that as the temperature increases the deuterium content of the non-exchangeable hydrogens decreases. A temperature coefficient in the region of - 3‰ per °C would be consistent with the results obtained.

9.5.2 *The Magnitude of the Fractionation Between Plant Hydrogens and Environmental Water*

The δD determinations in this work support the conclusions of Epstein *et al.* (1976) that plants preferentially concentrate the lighter isotope into their cellulose C-H hydrogens. Thus the mean of the *Pinus* *radiata* cellulose δD determinations was estimated as - 88‰ with respect to SMOW (see section 9.3.1). This compares with a mean of about - 40‰ with respect to SMOW for local *Pinus radiata* sap water (see section 9.2).

In table 20 mean δD values for cellulose, lignin and diethyl ether extractives* in *Pinus radiata* are compared with those for cellulose and benzene/methanol extractives in *Pinus longaeva* as found by Epstein *et al.* (1976).

Table 20. δD measurements on *Pinus radiata* and *Pinus longaeva* wood constituents

	Cellulose δD_{SMOW}(‰)	Lignin δD_{SMOW}(‰)	Extractives δD_{SMOW}(‰)
Pinus radiata (this work)	- 88	- 160†	- 233
Pinus longaeva (Epstein *et al.* 1976)	- 106	-	- 255

9.5.3 $\delta^{18}O$ *Variations in Pinus Radiata*

The results in section 9.3.2 show the oxygen atoms in cellulose from *Pinus radiata* to be more than 2‰ enriched in ^{18}O in the summer as compared with the winter. The variations seem to reflect the changing isotopic composition of the local precipitation (see appendix D), although the sap water is likely to have varied by no more that 1‰ in $\delta^{18}O$.§ The result may reflect changes in leaf transpiration rates which would be greater in the summer due to higher temperature and lower humidity (N.Z. Met. Service).

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*For the method of preparation see section 4.4.

†Very approximate - see section 9.3.3.

§This has been deduced from the observed variation of about 6‰ in the δD content of *Pinus radiata* sap water (see section 9.2).

It should be noted that the cellulose oxygen is greatly enriched in ^{18}O. It is about + 35 °/oo heavier than the local ground water (H. Baber, personal communication 1976) and local precipitation (I.A.E.A. 1969 to 1975). Most of this fractionation may be a result of carbon dioxide/water equilibration in the leaf before the carbon dioxide is incorporated into the photosynthetic cycle. At equilibrium carbon dioxide oxygen is about 40 °/oo heavier than the water oxygen (Bottinga and Craig 1969). The fractionation factor for the carbon dioxide/water equilibrium has a temperature coefficient of 0.2 °/oo per °C (Bottinga and Craig 1969). Thus the annual variation in $\delta^{18}O$ shown in fig. 17 may be partly a result of this temperature coefficient.

Gray and Thompson (1976) found a similarly large enrichment for the cellulose oxygen in *Picea glauca* (white spruce) from Edmonton, Canada. They also found a correlation with temperature for $\delta^{18}O$ in cellulose of + 1.3 ± 1 °/oo per °C. Such a large temperature dependence seems to be incompatible with the 2 °/oo change found in *Pinus radiata* for a 10 °C temperature cycle. A possible explanation is that Gray and Thompson's tree was subjected to much larger variations in transpiration rates and in the isotopic composition of the source water.

9.5.4 The Use of a Function $\delta D - x\delta^{18}O$

If there is no ground water evaporation or leaf transpiration, the function $\delta D - 8\,\delta^{18}O$ should remain constant (Craig 1961)* even though the isotopic composition of precipitation and hence leaf water changes. Thus any variations in this function must be due to another factor. At the other extreme, if all the variation in leaf water isotopic composition is due to transpiration effects and none is meteoric, the function $\delta D - 3\,\delta^{18}O$ should remain constant (Lasaint *et al.* 1974).*

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*See also section 10.4.

Thus any variations in this function must also be due to another factor. In practice there will be both meteoric and transpiration variations. Thus the true value of x in the function $\delta D - x\delta^{18}O$ will lie somewhere between 3 and 8. If we could determine the true value of x for a particular system at a particular time we should be able to correct the δD values for both meteoric and transpiration variations using the $\delta^{18}O$ values.

In practice it would be very difficult to determine accurate values of x. However, if the function $\delta D - x\delta^{18}O$ is plotted against sample number for *Pinus radiata* cellulose (see fig. 20), a highly significant annual variation is evident for any value of x between 3 and 8. This variation is probably the result of the annual temperature cycle.

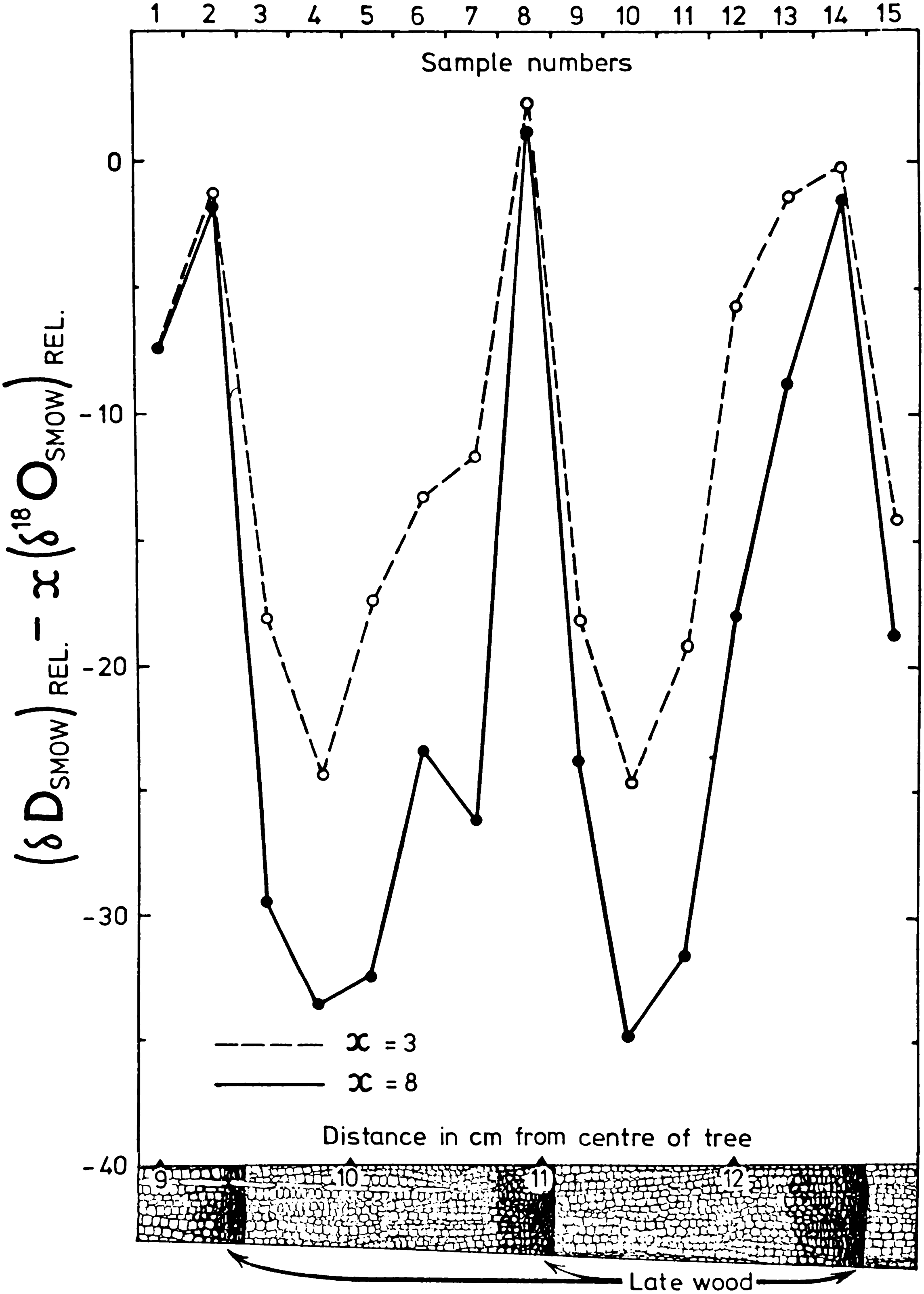

Fig.20. The functions $[(\delta D_{SMOW})_{REL}-3(\delta^{18}O_{SMOW})_{REL}]$ and $[(\delta D_{SMOW})_{REL.}-8(\delta^{18}O_{SMOW})_{REL.}]$ plotted against sample number for cellulose in *Pinus radiata*.

CHAPTER 10 SUGGESTIONS FOR FURTHER WORK

10.1 INTRODUCTION

In chapters 7 and 8 several possible alternatives to the exchange water method are considered, but not all have been closely studied. Using one of these methods it may be possible to determine the D/H ratio of the C-H hydrogens in a sample of cellulose to a greater precision than is possible using the technique described in section 8.3. However, there are many other potentially more rewarding possibilities for further work. Some of these are considered in this chapter.

10.2 THE DETERMINATION OF δD VALUES FOR LIGNIN[*]

So far very little work has been done on the problem of obtaining meaningful D/H ratios for lignin. A good method would have many applications since lignin is present in most plants.

It is possible to determine the difference in the δD values of two or more samples of lignin by using a method similar to the exchange technique for cellulose (see section 8.3). However, if the lignin is prepared using 72% sulphuric acid (see section 4.3), hydrogen atoms other than the O-H hydrogens will exchange (with $(H^+)_{aq.}$ from the sulphuric acid). Such hydrogen atoms do not re-exchange when heated with a standard water.[†]

The few lignin δD values reported in this thesis (section 9.3.3)

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[*] The structure of lignin is discussed in section 2.5.

[†] This was shown by labelling a sample of lignin with tritium using tritiated 72% sulphuric acid. The label could not be removed by water exchange.

were determined using an isotope dilution technique. Each wood sample was treated similarly using the same reagents so that any hydrogen atom exchange should be common to all samples of lignin prepared. Each sample was exchanged with standard water and analysed as described for cellulose in section 8.3. However, although it was shown that treatment with 72% sulphuric acid exchanges some of the C-H hydrogens in lignin, the proportion of such hydrogen atoms was not accurately determined. Thus, although the differences between the measured δD values will be proportional to actual differences between the D/H ratios of unreacted wood lignin, absolute δD values on a SMOW scale can only be estimated. The estimation of such values would require the determination of not only the proportion of hydrogen atoms that exchange, but also the fractionation factor between the hydrogen atoms of sulphuric acid and lignin.

The potential of this method for making accurate determinations of the D/H ratio of *in situ* wood lignin is not great. More precise measurements may be obtainable by first preparing lignin from the wood using a technique which will not exchange the C-H hydrogens. For example, "native lignin" could be isolated by extracting it from wood using solvents such as 96% ethanol (Brauns 1939, 1954). Alternatively "milled wood" lignin could be prepared by first grinding the wood and then extracting the lignin using acetone (Browning 1967). A third possible method involves breaking down the cellulose in the wood using enzymic digestion (Browning 1967).

If lignin is prepared using the above techniques, only the O-H hydrogens are likely to exchange. These could be re-exchanged with a standard water as in the method described for cellulose (see section 8.3). Then, just as for cellulose, the fractionation factor between the O-H hydrogens of lignin and water and hence the D/H ratio of the

C-H hydrogens could be determined by exchanging the same lignin sample with various standard waters (see section 8.3.6).

10.3 THE DETERMINATION OF δD VALUES FOR METHOXYL GROUPS

The methoxyl ($-OCH_3$) groups in wood represent a promising system which has not yet been investigated by isotope dendroclimatologists. Such groups are present in both the lignin and polysaccharide portions of the wood.

Methoxyl groups can be converted to methyl iodide by reaction with concentrated hydriodic acid (Browning 1967):

$$ROCH_3 + HI \rightarrow ROH + CH_3I$$

The carbon and hydrogen atoms in the methyl group remain unexchanged in this reaction. The volatile methyl iodide could be collected and converted to carbon dioxide and hydrogen for mass spectrometric measurement.

10.4 THE DETERMINATION OF δD VALUES FOR PAST PRECIPITATION

10.4.1 The Use of Cellulose C-H Hydrogen δD Determinations

Approximate δD values for past precipitation can be derived from fossil plant material by measuring the D/H ratios of the C-H hydrogens of cellulose preparations. However, several factors must be taken into account. First, the fractionation factor between environmental water and the C-H hydrogens of cellulose is not constant. The work of Epstein *et al.* (1976) and that described in this thesis (section 9.5.2) show that this fractionation can be anything from 0 to about - 50‰. Secondly, the magnitude of this fractionation for a particular plant varies with climate (see section 9.5). Thirdly, transpiration effects must be considered particularly if the plants grew in arid environments (see also sections 2.4 and 9.1.2). The importance of such effects is well illustrated by Epstein and Yapp's work (1976) on *Pinus longaeva*. They found δD values ranging from − 90‰ to − 120‰ SMOW for wood growing during the last 1000 years. However, precipitation in this area has a δD of − 160 ± 20‰ (I. Friedman, personal communication 1976). The large enrichment in deuterium for *Pinus longaeva* cellulose, which compares with the depletion found for most plants (see above), is probably the result of rapid transpiration in an arid environment.

It should be possible to determine the magnitude of the cellulose/water fractionation factor and its variation with climate for a particular species. Then the measured δD values for cellulose from fossil plants of the same species could be corrected for this fractionation.* This should give an estimate of the deuterium content of the leaf water at the time that the carbohydrate was fixed. The ^{18}O content of the leaf water could be estimated by correcting the measured $\delta^{18}O$ value of the cellulose for biochemical fractionation in a similar way. The estimated δD and $\delta^{18}O$ values for leaf water could then be plotted on a δD vs. $\delta^{18}O$ diagram (see fig. 21). If the resultant plot was on or near the meteoric line,† the plant probably grew in a non-arid environment and so the δD and $\delta^{18}O$ values should reflect those of the precipitation at that time. If the plot was below the meteoric line,an extrapolation could be made (see fig. 21) onto the meteoric line along a line of slope 3 (transpiration line of Lasaint *et al.* (1974)) in order to obtain the isotopic composition of the precipitation. The length of this extrapolation should give a

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*Information concerning climate may be obtained from the ^{13}C content of the fossil plant's cellulose - see chapter 13.

†The meteoric line (see fig. 21) represents the relationship between the δD and $\delta^{18}O$ values of natural meteoric waters from all over the world as long as such waters have not undergone excessive evaporation (Friedman 1953, Craig 1961, Dansgaard 1964).

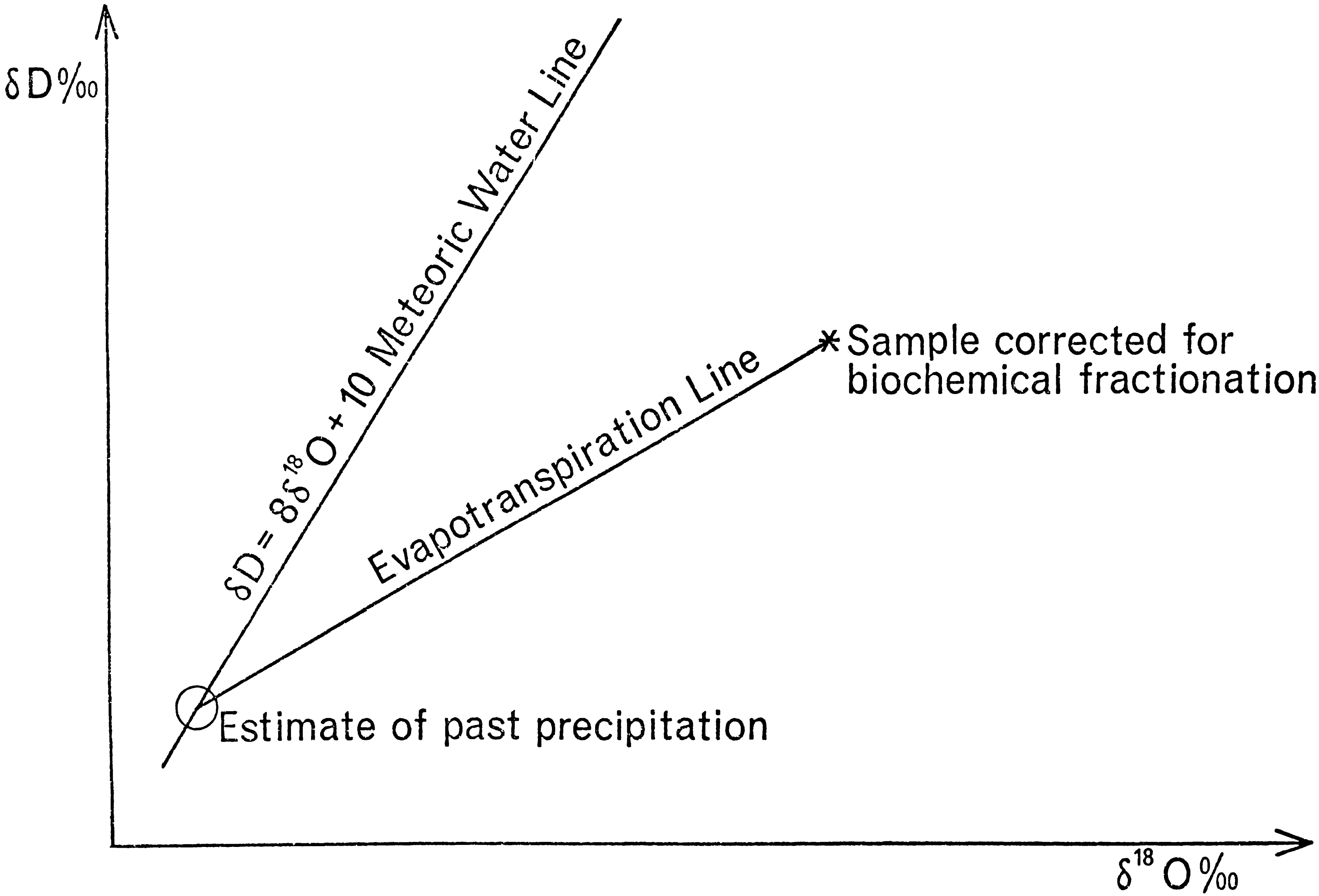

Fig. 21. δD vs $\delta^{18}O$ Diagram

measure of the aridity of the climate at the time the photosynthates were fixed from the atmosphere.

10.4.2 Other Methods

An alternative approach to the problem of obtaining δD values from fossil plant material relies on the non-exchangeable O-H hydrogens in cellulose. From the discussion in section 6.2 it can be seen that only about 40% of the O-H hydrogens are readily exchangeable with water at room temperature. Even prolonged soaking at 92°C only exchanges about 80% of this hydrogen (see section 8.3.5). The D/H ratio of the remaining non-exchangeable O-H hydrogens probably depends on the D/H ratio of the cellular sap water in which the cellulose was biosynthesised from sucrose. The studies of Wershaw *et al.* (1966) and Lasaint *et al.* (1974) show that the D/H ratio of sap water is generally almost the same as that of the local ground water.

It should be possible to determine the D/H ratio of the non-exchangeable O-H hydrogens using the nitration technique of Epstein *et al.* (1976) in conjunction with the exchange technique (see section 8.3). The latter technique would have to be modified so that a smaller proportion of the O-H hydrogens is exchanged. However, the non-exchangeable O-H hydrogens will still amount to less than 20% of the total hydrogen in cellulose. Thus the precision of any δD values obtained in this way will be relatively low. A δD value for the local ground water could be estimated by correcting for the fractionation factor between the sap water and the O-H hydrogens.

A third approach to the problem arises from the fact that wood contains water molecules bound up in its structure. Long evacuation times at elevated temperatures are required to remove such water (e.g. Browning 1967). Thus it is probable that the most tightly bound water has not exchanged with the sap water since the wood was laid down. If this water could be isolated, its D/H ratio should reflect the D/H ratio of the sap water and hence the local ground water at the time the wood was laid down.

10.5 EXPERIMENTS UNDER CONTROLLED CONDITIONS

Plants should be grown under controlled conditions, for example in growth chambers, so that the fractionation factors between leaf water and cellulose can be more accurately estimated. The effect of environmental conditions such as temperature, humidity and light level should also be carefully studied. To these ends an attempt was made to grow *Pinus radiata* seedlings under controlled conditions, but unfortunately this experiment had to be abandoned (see appendix F).

PART IV

$\delta^{13}C$ MEASUREMENTS

CHAPTER 11 PRELIMINARY CONSIDERATIONS[†]

11.1 $\delta^{13}C$ VARIATIONS IN PLANTS

11.1.1 Introduction

The existence of systematic differences in the $^{13}C/^{12}C$ ratios of various carbonaceous materials was first demonstrated by Nier and his co-workers (Nier and Gulbransen 1939, Murphey and Nier 1941). However, accurate measurement of small differences in isotope abundance ratios was not possible until Nier (1947) designed a new mass spectrometer which was later improved by McKinney *et al.* (1950). Since then innumerable $^{13}C/^{12}C$ measurements have been made. The stable isotope biogeochemistry of carbon has been reviewed by many workers including Degens (1969), Schwartz (1969) and Hoefs (1973).

Early measurements of $^{13}C/^{12}C$ ratios in plants include those of Wickman (1952), Baertschi (1953) and Craig (1953, 1954). These and later studies showed that plants can have a wide range of $\delta^{13}C$ values. The reason for this only became clear after plant physiologists discovered a photosynthetic carbon fixation cycle different from the traditional pathway of Calvin (see Hatch and Slack 1966). Soon after this, Bender (1968) studied some grasses and suggested that the highest depletion in ^{13}C ($\delta^{13}C \simeq -27‰$[†*]) corresponds to the Calvin cycle (C_3 plants) and the lowest depletion ($\delta^{13}C \simeq -13‰$[†*]) to the Hatch-Slack cycle (C_4 plants). $^{13}C/^{12}C$ ratios in most plants have since

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[†] All $\delta^{13}C$ values reported in this chapter are with respect to PDB - see definition in appendix A.

[*] Atmospheric carbon dioxide has a $\delta^{13}C$ value of about $-7‰$ (see section 11.2).

been related to such differences in photosynthetic pathways (e.g. Bender 1971, Smith and Epstein 1971, Olsson and Osadebe 1974). Many plants have been found to have intermediate $\delta^{13}C$ values due to the presence of both Calvin cycle and Hatch-Slack cycle enzymes. These have been termed CAM (Crassulacean Acid Metabolism) plants. The ranges of $\delta^{13}C$ values that have been found for these three types of plant have been reviewed by Lerman (1972a, 1974b) and Troughton (1972). Their photosynthetic pathways are considered in more detail in section 2.3.

Most of the plant $^{13}C/^{12}C$ ratio measurements reported in the literature have been carried out using whole plant material. This is unfortunate since many studies have shown that individual plant constituents can have widely differing carbon isotope ratios (e.g. Abelson and Hoering 1961, Degens *et al.* 1968b, Whelan *et al.* 1970, Smith and Epstein 1970, Smith 1972, Olsson *et al.* 1972). For example, the lipid fraction of plants can be depleted in ^{13}C by as much as 8‰ compared with whole plant material (Park and Epstein 1961). Even the cellulose and lignin fractions can differ by as much as 3.5‰ (this thesis - section 13.2.1).

Thus $\delta^{13}C$ determinations that have been carried out on whole plant material are only of limited value. The observed variations between such determinations may well be due to variations in the proportions of the various chemical constituents, rather than such factors as the changing isotopic composition of atmospheric carbon dioxide or the effect of environmental conditions (e.g. temperature) on the magnitude of the enzymic fractionation. Despite this fact most of the workers in this field are still measuring whole plant $\delta^{13}C$ values. Thus the environmental effects discussed in the next section may be partly or wholly due to changing chemical compositions. More meaningful results can be obtained by measuring direct environmental effects for individual plant constituents such as cellulose.

11.1.2 The Effect of Environmental Conditions on Plant $\delta^{13}C$ *Values*

Since Urey (1947, 1948) first suggested that the abundance of ^{13}C in organic matter might vary with temperature, many workers have looked for such a relationship. Craig (1953) suggested that $^{13}C/^{12}C$ ratios in plants should be affected by carbon dioxide exchange with the sea which is temperature dependent. Libby (1972) calculated a thermodynamic temperature coefficient of + 0.36^{o}/oo per °C for photosynthetic carbon isotope fractionation between carbon dioxide and plant cellulose.

Sackett *et al* (1965) determined the $^{13}C/^{12}C$ ratios of several specimens of marine plankton growing at temperatures between 0 and 25°C. They found a temperature dependence of + 0.23^{o}/oo per °C. A similar temperature dependence (+ 0.35^{o}/oo per °C) was later reported for plankton grown in a laboratory (Degens *et al.* 1968b). Eadie (1972) reported a temperature coefficient of + 0.5^{o}/oo per °C for phytoplankton from the Antarctic marine ecosystem. However, Deuser *et al.* (1968) found that if large quantities of carbon dioxide were administered to growing plankton no temperature effects were noticeable. Measured $^{13}C/^{12}C$ ratios at all temperatures corresponded to those found in plankton from cold water environments. They concluded that the effect of water temperature on the magnitude of carbon isotopic fractionation may be due to carbon dioxide availability (solubility in seawater, etc.).

The results of Park and Epstein (1960) support this contention. They observed that tomato plants grown on 1.5% carbon dioxide exhibited about 3^{o}/oo greater fractionation than plants grown on 0.015% carbon dioxide. Abelson and Hoering (1961) found that *Chlorella* grown on 5% carbon dioxide exhibited a carbon isotopic fractionation of 25.8^{o}/oo when vigorously aerated, but only 11.3^{o}/oo when poorly aerated. Degens *et al.* (1968b) observed a similar effect for *Skeletonema*.

The relationship between carbon dioxide concentration and isotopic fractionation was more clearly demonstrated by Calder and Parker (1973; see also Pardue *et al.* 1976). They showed conclusively that photosynthetic fractionation of carbon isotopes by three species of blue-green algae in laboratory culture is dependent on the carbon dioxide concentration in the feed gas. They found that the fractionation was maximum at high carbon dioxide concentration and minimum at a relatively low concentration (about 0.2% carbon dioxide in the feed gas mixture). They observed no temperature effect.

A negative temperature coefficient (- 0.6°/oo per °C) found for hot spring algae has been attributed to the presence of bacterial methane (Libby and Pandolfi 1974c).

The use of $^{13}C/^{12}C$ ratio analyses to estimate the proportions of C_3 to C_4 plants in organic remains has been suggested by Hendy *et al.* (1972) and Lerman (1974a). The relative biomass of these two types of plants seems to vary with environmental conditions (Hendy *et al.* 1972). Preliminary studies on the organic material in peat support this contention (J.C. Lerman, personal communication 1976).

The ^{13}C content of some CAM plants may also vary with environmental conditions (Lerman 1974a, Troughton *et al.* 1974c). However, Sutton *et al.* (1976) found little change in $^{13}C/^{12}C$ ratios between winter and summer for three CAM plants from a desert environment.

Recent studies have shown that the ^{13}C content of organic matter in lake and soil sediments may vary with fluctuations in climate (Nakai 1972, Stuiver 1975, A. Long personal communication 1976).

Many workers have grown plants in constant temperature growth chambers. However, the results of such experiments are difficult to interpret unambiguously, for a number of reasons. First, it is hard to keep the $^{13}C/^{12}C$ ratio of the growth chamber atmosphere constant.

Unless it is controlled, the ^{13}C content of the source carbon dioxide will vary due to fluctuations in the local atmosphere, whether the laboratory is situated in an urban or a rural area (Keeling 1958, 1961a). Also, greenhouse and growth chamber plants often contain less ^{13}C than field grown plants of the same species due to recycling of respired carbon dioxide (Smith *et al.* 1975). Secondly, leaf temperatures often differ from the surrounding air temperature. Downes (1969) found that this difference ranged from about - 1°C to + 7°C depending on environmental conditions and plant species. Thirdly, carbon isotope fractionation seems to depend on the age of the plant (Smith *et al.* 1973, Lerman *et al.* 1974). Fourthly, most workers analyse whole plant material despite the fact that the proportion of each plant constituent will vary if the environmental conditions are varied (see section 11.1.1).

Points such as those outlined in the preceding paragraph must be considered before any firm conclusions can be drawn from the results of growth chamber experiments. This means that many of the studies described in the literature are of limited value.

Several workers (e.g. Troughton *et al.* 1974a, Smith *et al.* 1973) have used growth chambers to study the effect of temperature on the carbon isotope fractionation of plants. Their results show a wide variation. However, in general the ^{13}C content increases with increasing temperature up to about 20 to 30°C. When the temperature is further increased the ^{13}C content decreases. Thus, the magnitude of carbon isotopic fractionation between growth chamber carbon dioxide and the plant is generally least at optimal growth conditions. As the extent of the deviation from optimal conditions increases plant $\delta^{13}C$ values decrease by up to about 4‰.

Recently, the effect of temperature on ^{13}C discrimination by cell-free extracts has been studied. Whelan *et al.* (1973) reported a large temperature effect (+ 1.2‰ per °C) for a ribulose-1, 5-diphosphate carboxylase preparation from sorghum. However, Christeller *et al.* (1976) found no significant temperature effects for a cell-free preparation of the same enzyme extracted from soybean.

Studies concerned with the effect of environmental conditions on $^{13}C/^{12}C$ ratios in trees are discussed in section 13.1.

11.2 $\delta^{13}C$ VARIATIONS IN ATMOSPHERIC CARBON DIOXIDE

The first accurate determinations of the ^{13}C content of atmospheric carbon dioxide were made by Craig (1953). His suggestion of - 7‰ as a reasonable $\delta^{13}C$ value for carbon dioxide in air over the oceans was later confirmed by Keeling (1958, 1961a). Considerations of the effect of atmospheric N_2O on the measured isotopic composition of atmospheric carbon dioxide led Craig and Keeling (1963) to revise this figure to about - 6.4‰.

Keeling (1958, 1960, 1961a) has studied the variations in concentration and isotopic abundances of carbon dioxide from both rural and marine air. He found that, at locations far removed from terrestrial plants and the influence of cities, the concentration and ^{13}C content of carbon dioxide in the air was nearly constant. He showed the annual fluctuation in ^{13}C content for various locations near the Pacific coast of the United States to be about 0.4‰. However, large diurnal fluctuations in $\delta^{13}C$ of up to 4‰ were found for forest air. These fluctuations have been attributed to the activity of land plants (e.g. Keeling 1960). A correlation between the ^{13}C content and the concentration of atmospheric carbon dioxide was also observed.

The increase in carbon dioxide concentration arising from Man's combustion of fossil fuels since the Industrial Revolution has been detected in many parts of the world (e.g. Bolin and Bischof 1970, Machta 1972a, Ekdahl and Keeling 1973). Many authors have discussed the possible atmospheric warming effect of such an increase (e.g. Machta 1972b, Broecker 1975, Wang *et al.* 1976).

A corresponding change in the ^{13}C content of atmospheric carbon dioxide is probable since fossil fuels are depleted in ^{13}C by about 18‰ relative to the atmosphere. No long term direct measurements of $^{13}C/^{12}C$ ratios in atmospheric carbon dioxide have so far been reported in the literature. However, Freyer and Wiesberg (1975) and Farmer and Baxter (1974) have attempted to measure the magnitude of this anthropogenic effect indirectly by determining $^{13}C/^{12}C$ ratios in wood. Their results suggest that the ^{13}C content of the atmosphere has decreased since 1900, but the magnitude of this decrease is difficult to assess since other factors can influence $^{13}C/^{12}C$ ratios in wood.*

11.2.1 The Role of the Oceans

The oceans probably play a major part in regulating not only the concentration but also the isotopic composition of atmospheric carbon dioxide. Estimates of the total quantity of carbon dioxide released to the atmosphere by the burning of fossil fuels indicate that at least half has been removed mainly to the sea and partly to the terrestrial biosphere (e.g. Bolin and Bischof 1970, Broecker *et al.* 1971, Machta 1972a). The mean atmospheric residence time for carbon dioxide exchange with the oceans has been variously estimated as between 5 and 10 years (e.g. Craig 1957b, Broecker 1963, Rafter and O'Brien 1969, Baxter and Walton 1970, Gulliksen and Nydal 1972). This exchange presumably regulates the average isotopic composition of atmospheric carbon dioxide, but its quantitative effect is not yet certain. In this context it is interesting to note the temperature effect for equilibrium ^{13}C exchange in the system $CO_{2\,(gas)}/CO_{2\,(aq.)}/HCO^{-}_{3\,(aq.)}$.

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*For a fuller discussion see chapter 13.

This is known to be about + 0.08‰ per °C (Deuser and Degens 1967, Mook *et al.* 1974). However, carbon dioxide exchange with the ocean has been shown to be too slow to establish local equilibrium (Bottinga and Craig 1969, Kroopnick 1974, Kroopnick *et al.* 1976).

Kroopnick *et al.* (1976) have measured the ^{13}C content of atmospheric carbon dioxide over the Pacific Ocean along longitude 150°W, from latitude 63°S to 57°N, during April and May, 1970. They found the $\delta^{13}C$ values of the Southern Hemisphere samples to be very similar to each other (about - 7.1‰*) whilst the $\delta^{13}C$ values of the Northern Hemisphere samples decreased from about - 7.1‰* at the equator to - 8.0‰* at latitude 57°N. This could be the result of the combustion of fossil fuels which occurs mostly in the Northern Hemisphere, or it may be related to the activity of land plants. Further measurements of the ^{13}C content of marine atmospheric carbon dioxide at various seasons are required before any firm conclusions can be arrived at. Kroopnick (1974) and Kroopnick *et al.* (1976) also found correlations between ^{13}C content and carbon dioxide concentration in oceanic surface waters and the atmosphere.

Possible changes in the ^{13}C content of oceanic and atmospheric carbon dioxide over geologic time have been discussed by several authors (e.g. Weber 1967, Veizer and Hoefs 1976). However, carbonate rocks of similar ages often have widely differing $\delta^{13}C$ values. Therefore such long term variations in the ^{13}C content of oceanic and atmospheric carbon dioxide remain speculative.

11.2.2 Localised Effects of Respiratory Carbon Dioxide

The $^{13}C/^{12}C$ ratio of respired carbon dioxide has been shown to be similar to that of the plant's tissue, i.e. about - 28‰ for C_3 plants

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*These $\delta^{13}C$ values have not been corrected for the N_2O contribution - see Craig and Keeling (1963).

(Baertschi 1953, Park and Epstein 1960, 1961, Jacobsen *et al.* 1970, Smith 1971). The effect of such respiration on the ^{13}C content of the local atmosphere is shown by Keeling's measurements (1958, 1961a). Diurnal and annual variations in the gross respiration of a forest and of agricultural crops have been investigated by Woodwell and Dykeman (1966) and Verma and Rosenberg (1976).

The results of Harris and van Bavel (1957) suggest that the principal contributor to soil atmospheric carbon dioxide is root and mycorrhizal respiration, although the decay of soil organic matter probably makes a small contribution. More recent work (e.g. Wiant 1967a, 1967b; Reiners 1968) supports this inference. Galimov (1966) determined $\delta^{13}C$ values for carbon dioxide in several different temperate soil atmospheres. He found a mean ^{13}C content of about $-25^{o}/oo$ which is comparable with the ^{13}C content of the local vegetation. Lerman (1972b) determined $\delta^{13}C$ values for soil atmospheres under CAM vegetation. He found that the isotopic composition of the soil carbon dioxide matched the composition of the corresponding vegetation cover ($\delta^{13}C \approx -16^{o}/oo$). His results indicated a much higher contribution of carbon dioxide from root respiration than from organic matter decay. Other workers have found similar correspondences in $\delta^{13}C$ values between vegetation cover and soil atmospheres (e.g. Hendy 1971).

Such root and mycorrhizal respiration will tend to deplete the atmosphere near the ground, particularly in a forest. Thus small plants growing in the lower stratum of forests tend to be depleted in ^{13}C (Munnich and Vogel unpublished, Lerman 1974a). This effect may also explain the depletion in ^{13}C found for the inner rings of large trees (see sections 13.1, 13.2.2, 13.5).

11.3 MODELS TO EXPLAIN CARBON ISOTOPIC FRACTIONATION IN C_3 PLANTS

11.3.1 *Introduction*

Although the depletion in ^{13}C that occurs during photosynthesis has been known for over 25 years, some details concerning the origin of this fractionation are still not certain.

Wickman (1952) suggested that the wide range of measured plant $^{13}C/^{12}C$ ratios is mainly the result of variations in the $^{13}C/^{12}C$ ratio of the carbon dioxide in the atmosphere surrounding each plant. However, Keeling's measurements (1958, 1961a) show that, although variations in the ^{13}C content of atmospheric carbon dioxide do occur, they are not large enough to fully account for the measured range of plant $\delta^{13}C$ values. Craig (1954) suggested that most of the fractionation of the carbon isotopes takes place inside the plant. This hypothesis has since been supported by other workers (e.g. Park and Epstein 1960, Troughton 1972) and is now generally accepted. The largest differences in ^{13}C content between individual plants are explained by the existence of two different pathways of photosynthetic carbon fixation (in C_3, C_4 and CAM plants - see sections 2.3 and 11.1.1). However, some of the measured variations in the $^{13}C/^{12}C$ ratios of, for example, the C_3 plants are clearly the result of variations in the $^{13}C/^{12}C$ ratio of the local atmosphere, as advanced by Wickman (1952). Such variations have been discussed by Keeling (1961b) - they are considered in more detail in section 11.2. Other variations in the ^{13}C content of C_3 plants are related to variations in chemical composition (see section 11.1) and probably variations in climate (see chapter 13).

11.3.2 *The Model of Park and Epstein*

Park and Epstein (1960) suggested two main steps to explain the fractionation between atmospheric carbon dioxide and plants (see fig. 22). They also suggested that the magnitude of the fractionation at each of

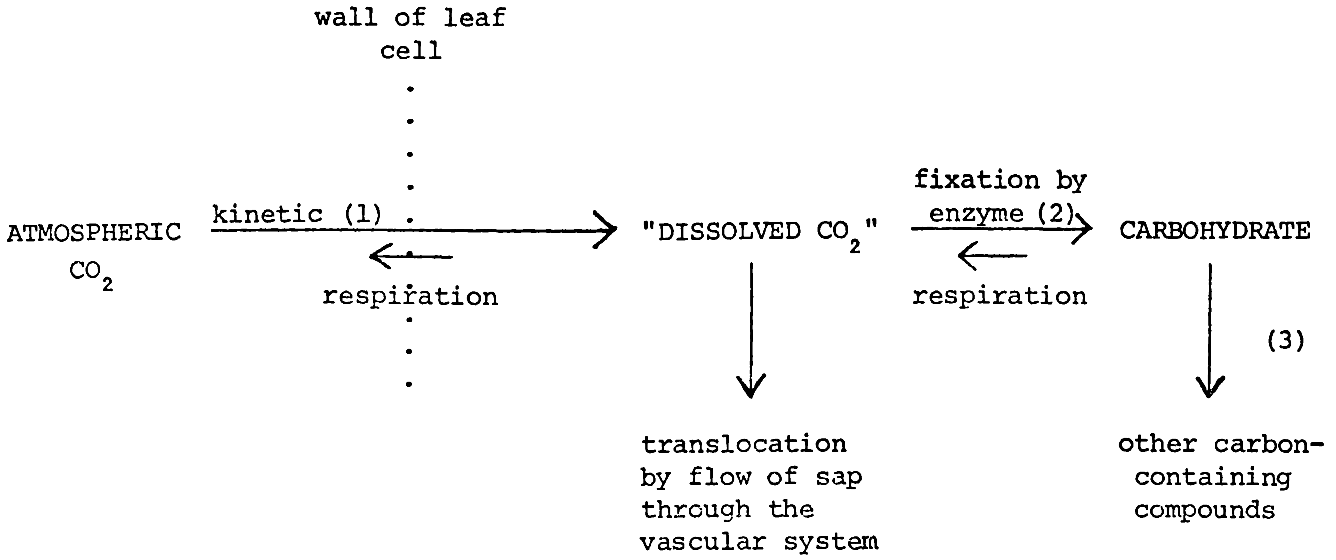


Fig. 22. Park and Epstein's model (1960) to explain carbon isotopic fractionation in plants.

these steps would vary and so affect the overall fractionation.

In the light of more recent experiments, Park and Epstein's model requires some modifications. The fractionation at their step (2) is now known to depend primarily on the type of enzyme system in the plant. In addition, their mechanism requires that carbon dioxide enriched in ^{13}C should be translocated to the roots to be expelled by respiration. However, the work discussed in section 11.2.2 shows that carbon dioxide from root respiration probably has the same $\delta^{13}C$ value as the photosynthates. Also, Riech (1970 - reported in Zelawski *et al.* 1970) found that basipetal translocation of photoassimilated atmospheric $^{14}CO_2$ in pine seedlings was very slow.

11.3.3 The Role of Respiratory Carbon Dioxide

This is one aspect of photosynthetic carbon dioxide fixation which has so far been little considered by stable isotope biochemists. The work of Zelawski *et al.* (1970) with $^{14}CO_2$ and *Pinus elliotti* seedlings shows that respiratory carbon dioxide can be re-used in photosynthesis. This is supported by Andre and Richaud's work (1971) with $^{13}CO_2$ and leaves from spinach and sugar cane. Their results suggest that carbon dioxide traffic due to photorespiration can in some cases be largely internal. In fact, plant physiologists have for some time accepted that part of the respiratory carbon dioxide is always refixed under photosynthetic conditions (e.g. Heath 1969, Zelitch 1971). This is not surprising since in some situations carbon dioxide production by photorespiration and "dark" respiration can exceed carbon dioxide assimilation by photosynthesis (e.g. Fritts 1976).

Troughton (1972) has suggested that photorespiration may explain up to 5‰ of the difference in ^{13}C content between C_3 and C_4 plants. Such a large effect seems unlikely and in any case cell-free preparations of phosphoenolpyruvate carboxylase and ribulose-1,5-diphosphate carboxylase (Whelan *et al.* 1973, Deleens *et al.* 1974, Christeller *et al.* 1976) suggest that the difference can be explained almost entirely by the fractionation factors of different enzymes.

11.3.4 *An Alternative Model*

The model shown in fig. 23 is thus proposed for carbon isotopic fractionation in C_3 plants.

On the basis of this model, the overall fractionation between atmospheric carbon dioxide and carbohydrate will depend mainly on three different factors:

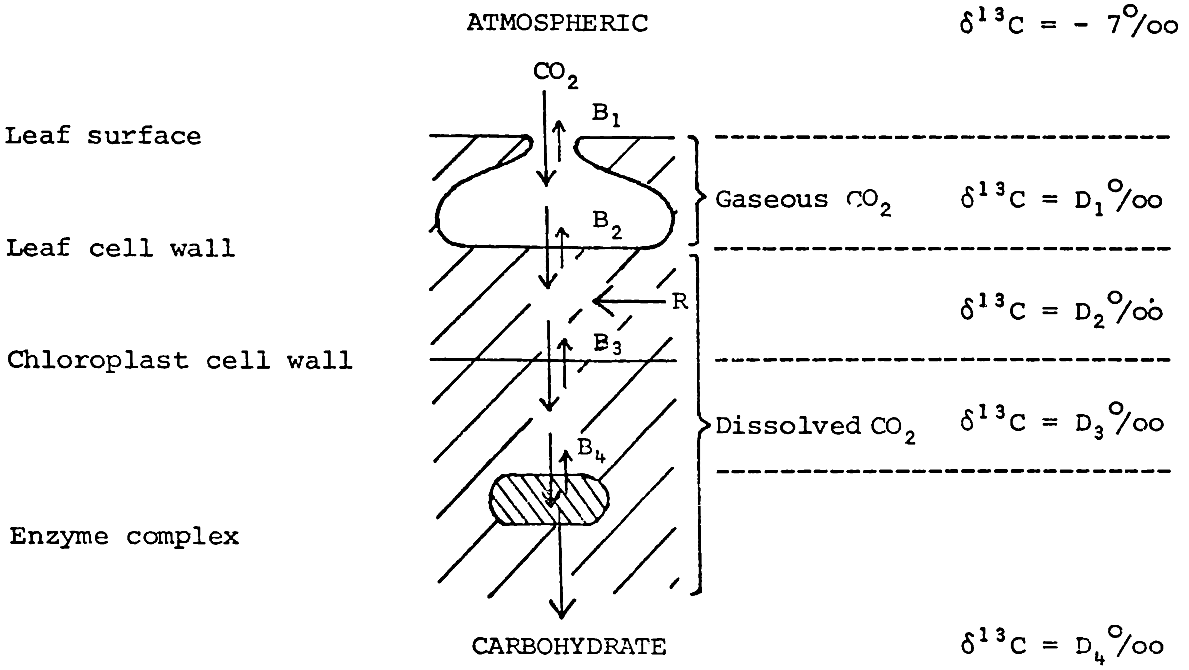
(a) the magnitude of the discrimination effect against ^{13}C by the enzyme complex;

(b) the extent to which movement of carbon dioxide across B_1, B_2, B_3 and B_4 departs from thermodynamic equilibrium;

(c) the contribution of respiratory carbon dioxide, R.

To assess the effect of each of these factors it is useful to consider some hypothetical situations.

(a) Thermodynamic equilibria across B_1, B_2 and B_3. If the movement of carbon dioxide across boundaries B_1, B_2 and B_3 is in thermodynamic equilibrium, the $\delta^{13}C$ value of the carbon dioxide between the leaf surface and the leaf's cell wall should be the same as the atmosphere. The equilibrium $CO_{2\,(gas)} \rightleftharpoons CO_{2\,(aq.)}$ across B_2 will lead to a fractionation of about - 1°/oo (e.g. Mook *et al.* 1974). In order not to displace the thermodynamic equilibria across B_1, B_2 and B_3, the carboxylation reaction at B_4 is assumed to take place only very slowly. The reaction will then be equivalent to that for cell-free extracts of ribulose-1, 5-diphosphate carboxylase supplied with an "infinite" quantity of $CO_{2\,(aq.)}$. Several workers have attempted to measure the carbon isotope discrimination of this enzyme. Unfortunately their



At B_1 - Movement of $CO_{2\,(gas)}$ across the leaf surface via stomata.

At B_2 - Movement of $CO_{2\,(gas \rightleftharpoons aqueous)}$ across the leaf cell boundary.

At B_3 - Movement of $CO_{2\,(aqueous)}$ across the chloroplast cell wall.

At B_4 - Conversion of $CO_{2\,(aqueous)}$ to carbohydrate by the enzyme complex.

R - Respiratory CO_2 (light + dark respiration).

Notes: (1) "Enzyme complex" includes all enzymes and co-factors involved in carboxylation.

(2) "Dissolved CO_2" is described as "$CO_{2\,(aqueous)}$" since the latter is probably the active species for the enzyme complex in C_3 plants (Cooper *et al.* 1969).

Fig. 23. A model to explain carbon isotopic fractionation in C_3 plants.

measurements of the magnitude of this discrimination range from ~17‰[*] (Park and Epstein 1960) through ~28‰[†] (Christeller *et al.* 1976, Whelan *et al.* 1973) to ~70‰[*] (Deleens *et al.* 1974). Clearly more work needs to be done.

Assuming ^{13}C discrimination by the enzyme complex to be - 28‰ for an "infinite" supply of $CO_{2\,(aq.)}$, the $\delta^{13}C$ value in fig. 23 will be as follows:-

D_1 = - 7‰[§]
D_2 = - 8‰
D_3 = - 8‰
D_4 = -36‰

(b) <u>Thermodynamic equilibrium across B_1, but not across B_2, B_3; no respiration effect.</u> In this case the concentration of $CO_{2\,(aq.)}$ inside the leaf cell during photosynthesis would be less than that for thermodynamic equilibria across B_2 and B_3.[¶] Thus kinetic fractionations will be introduced. However, such effects are likely to be dominated by the carboxylation reaction. The enzyme complex will discriminate against ^{13}C and so tend to enrich the carbon dioxide in the surrounding solution. This effect and the lack of thermodynamic equilibria across B_2 and B_3 will result in $\delta^{13}C$ values such as the following:-

D_1 = - 7‰
D_2 = - 3‰

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[*] For $HCO_{3\,(aq.)}^{-}$ → carbohydrate.

[†] For $CO_{2\,(aq.)}$ → carbohydrate.

[§] With respect to PDB (see appendix A).

[¶] The existence of such a concentration gradient in photosynthesising leaves is discussed by Zelitch (1971).

D_3 = + 5‰

D_4 = -23‰

(c) Thermodynamic equilibrium across B_1, but not across B_2, B_3; respiration effect. In addition there will clearly be a respiration effect as discussed in the preceding section (11.3.3). This will introduce carbon dioxide of about - 25‰ at R in fig. 23. The result will be $\delta^{13}C$ values such as the following:-

D_1 = - 7‰

D_2 = - 5‰

D_3 = + 3‰

D_4 = -25‰

The proposed model would thus predict that a leaf photosynthesising in optimal growth conditions will have internal carbon dioxide $\delta^{13}C$ values of the order of those given in situation (c). Park and Epstein (1960) have attempted to measure the carbon isotopic composition of dissolved carbon dioxide in photosynthesising tomato plants. However, no firm conclusions can be drawn from their results because of possible contamination by respiratory carbon dioxide. This effect makes unambiguous $\delta^{13}C$ values for dissolved carbon dioxide at a particular instant in an actively photosynthesising leaf, very difficult to determine.

Many of the observed environmental effects on carbon isotopic ratios can be explained using the proposed model.

For example, Park and Epstein (1960) showed that the ^{13}C content of tomato plants decreases with increasing carbon dioxide concentration (see also section 11.1.2). A similar effect has apparently been observed by Vogel (see discussion in Freyer and Wiesberg 1975). An increase in external carbon dioxide concentrations would increase the internal concentrations and so reduce the $CO_{2\,(aq.)}$ enrichment effect of the carboxylation reaction. This will lower D_3 (see fig. 23) and hence decrease the ^{13}C content of the carbohydrate.*

The model can be used to explain the observed temperature effects on the ^{13}C content of growth chamber grown plants (see section 11.1.2). At low temperatures total photosynthesis is slow (see, for example, fig. 4) and so the dissolved carbon dioxide will tend towards thermodynamic equilibrium with the atmosphere, and therefore the plant will become depleted in ^{13}C compared with one grown under optimal conditions. Moreover, at very low rates of photosynthesis the respiration effect will be appreciable. This will also tend to deplete the dissolved carbon dioxide used by the enzyme complex. An increase in temperature will increase the ^{13}C content of the fixed carbohydrate as a result of increased photosynthetic rate. Between about 20 and 30°C, however, the rate of gross photosynthesis in C_3 plants usually increases only very slowly (see, for example, fig. 4). In this temperature range the steady increase in the rate of respiration becomes important. This provides an increasing proportion of ^{13}C depleted carbon dioxide at R in fig. 23. Thus a maximum plant $\delta^{13}C$ value is reached near the optimal growth temperature. A further increase in temperature results in lower plant $\delta^{13}C$ values. Any temperature effect on the extent of ^{13}C discrimination by the enzyme complex will of course also effect the resultant plant $\delta^{13}C$ value.

It is well known (e.g. Zelitch 1971) that net photosynthesis can be suppressed in the leaves of C_3 plants by raising the concentration of oxygen in the external atmosphere. This effect has been associated with inhibition of the carboxylating enzyme and with the stimulated production of photorespiratory carbon dioxide (e.g. Osmond and Björkman

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*Carbon dioxide availability has recently been used to explain carbon isotopic fractionation effects observed for plankton and algae (see section 11.1.2).

1972). Berry *et al.* (1972) found that a change in the oxygen concentration also affected the ^{13}C content of *Atriplex patula* (a C_3 plant). They reported that the carbon isotope fractionation at 4% oxygen concentration was - $19^{o}/oo$ whereas that at 21% oxygen concentration was about - $22^{o}/oo$. This result can be readily explained using the proposed model. Thus at the higher oxygen concentration there will be a larger contribution of photorespiratory carbon dioxide at R (see fig. 23) and hence a larger carbon isotope fractionation effect. The model implies that a reduction in the rate of enzymic fixation of $CO_{2\,(aq.)}$ (for example, due to inhibition by higher oxygen concentrations) could also explain the results obtained.

The effect of a small stomatal aperture on $^{13}C/^{12}C$ ratios would be difficult to predict since the reduced internal concentrations of carbon dioxide will tend to increase the ^{13}C content of the chloroplasts. On the other hand, the increased contribution of respiratory carbon dioxide will tend to decrease the ^{13}C content of the leaf cell and hence the chloroplasts.

(d) Conclusions. The proposed model predicts that the ^{13}C content of the primary photosynthates in a C_3 plant is dependent on three factors, in addition to the carbon isotope discrimination effect at the enzyme complex:

(a) the rate of enzyme fixation of $CO_{2\,(aq.)}$ (affected by, for example, temperature, light level, oxygen concentration, $CO_{2\,(aq.)}$ concentration) - an increased rate increases the $\delta^{13}C$ value of the photosynthates;

(b) the rate of total respiration (affected by, for example, temperature, light level, oxygen concentration) - an increased rate decreases the $\delta^{13}C$ value of the photosynthates;

(c) carbon dioxide availability to the leaf cell (affected by, for example, the opening and closing of stomata) - increased availability decreases the $\delta^{13}C$ value of the photosynthates.

CHAPTER 12 THE DETERMINATION OF $\delta^{13}C$ VALUES FOR ORGANIC SAMPLES

12.1 *THE COMBUSTION SYSTEM*

Two similar combustion systems were constructed for preparing carbon dioxide (for $\delta^{13}C$ determinations) and water (for δD determinations) from organic material. Since it was desired to carry out isotopic measurements to a very high degree of precision, considerable time and effort was put into the development of these systems and the techniques involved in using them, before the finalised versions described in this section were decided upon.

Fig. 24 is a diagrammatic representation of one system except that the combustion tube section (in the dotted rectangle) was triplicated. The other system was essentially the same except for the lack of a constant volume manometer (see plate 6). Trap T8 (fig. 24) was replaced by a stopper if only the ^{13}C content of the sample was to be determined.*

A sample (generally in the range 0.002 to 0.02 g) was placed in a quartz boat lined with platinum foil. This was introduced through J1 (fig. 24) into a silica tube fitted with a moveable furnace F2 and pumped out for at least 60 min through stopcock S6. Meanwhile, medical grade cylinder oxygen was slowly introduced via S1, passed through furnace F1 (at ~600°C) and collected in trap T1 using liquid nitrogen. When sufficient liquid oxygen had collected, S1 was closed and the oxygen was distilled into T2 using a slush bath containing

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*See section 8.3 for the determination of δD in organic material using the same vacuum system.

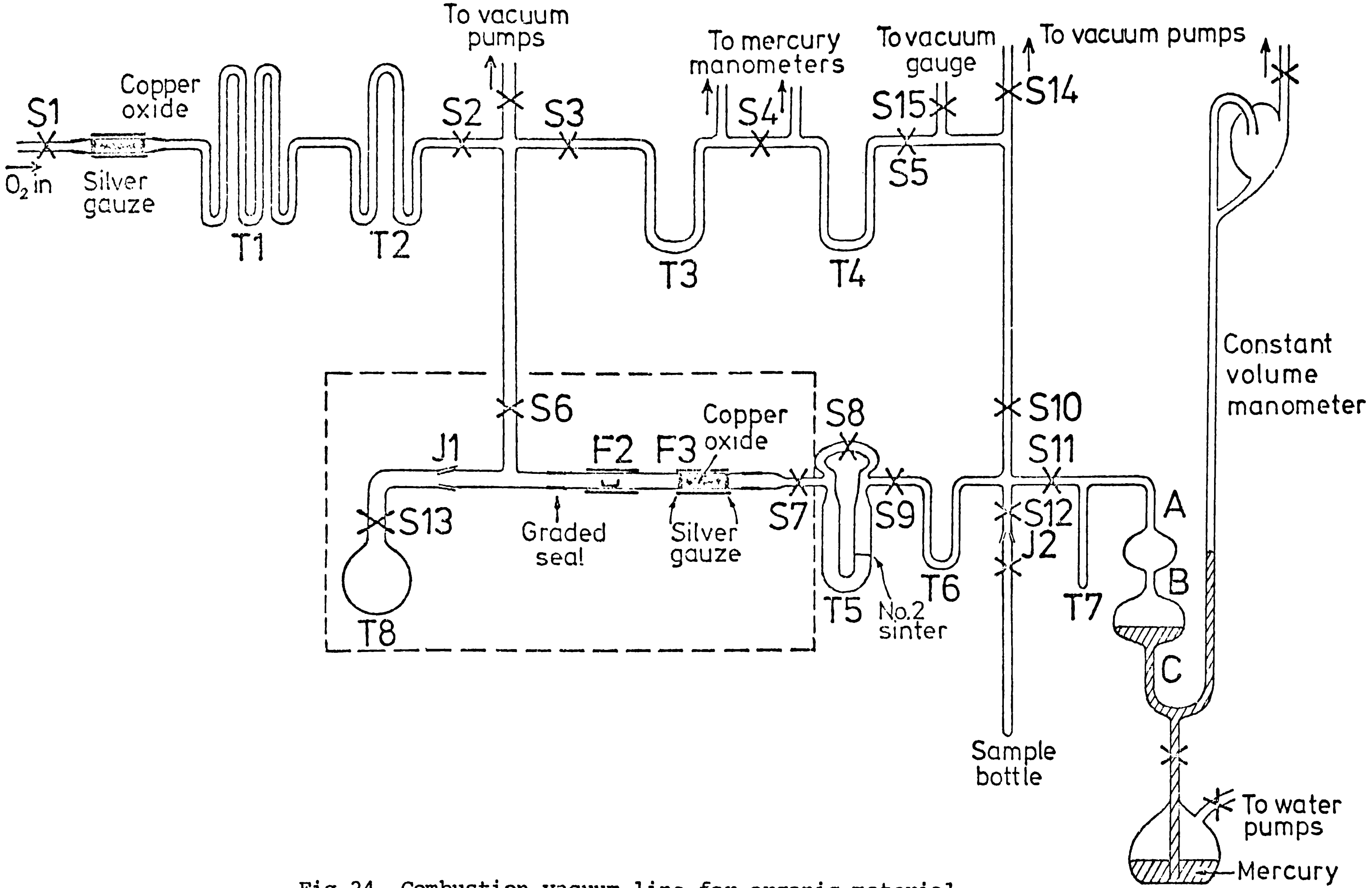
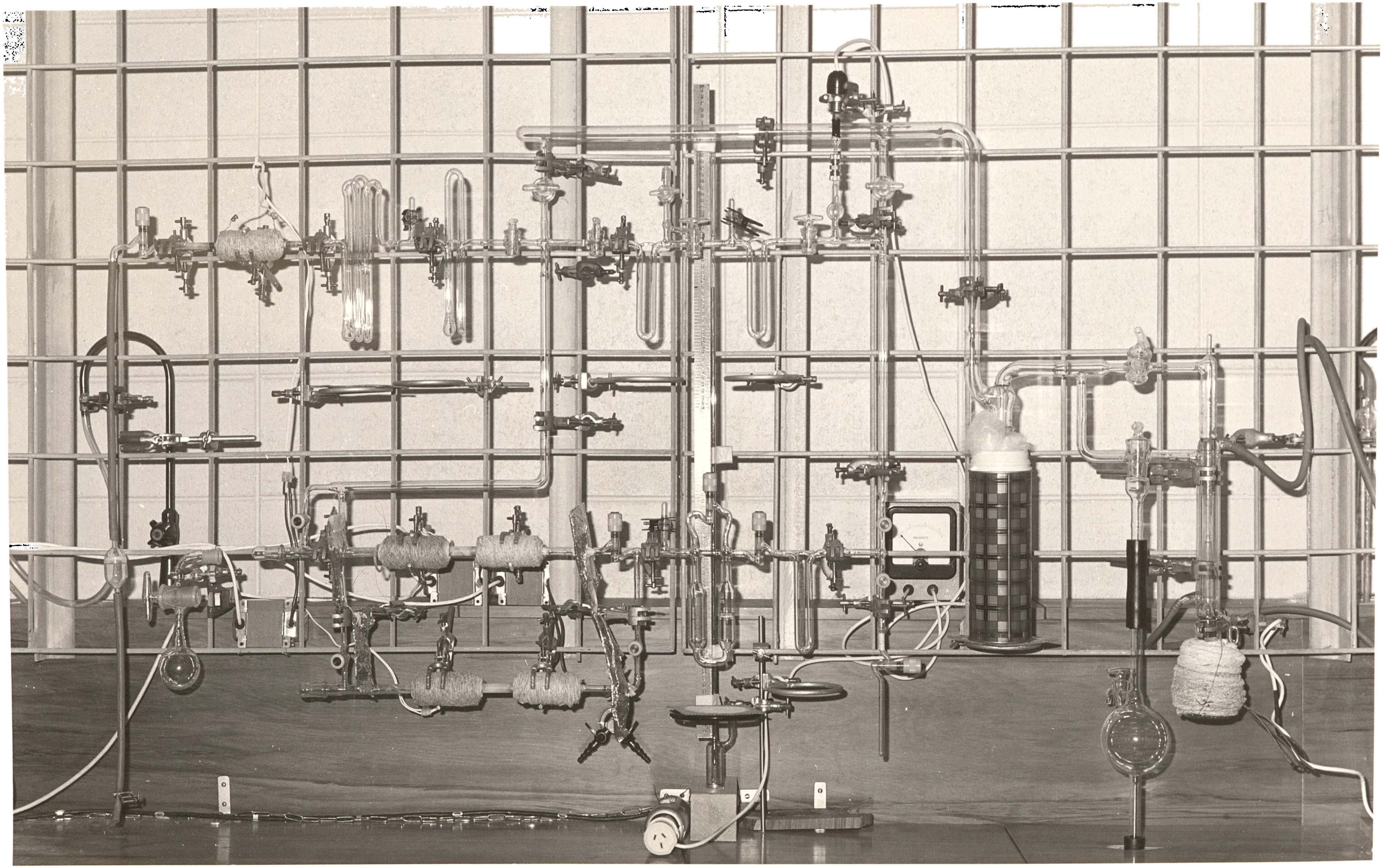


Fig.24. Combustion vacuum line for organic material.

Plate 6

Combustion vacuum line.



50% n-propanol and 50% absolute alcohol by volume (~ - 140°C) to retain the carbon dioxide and water impurities in the gas. After the distillation was complete this bath was frozen with liquid nitrogen.

The oxygen to be used for combustion was then slowly distilled over into T3. The combustion tube was filled with oxygen to a pressure of ~0.5 atm through S6 and the sample was combusted in furnace F2 at 800 - 900°C. The combustion products were slowly passed into T5 (liquid nitrogen), with S8 and S9 closed, through furnace F3 (~600°C), which contained silver to react with any halogens, and through copper oxide to complete the conversion to carbon dioxide and water. More oxygen was then slowly distilled from T3 into T5 to flush through the combustion products. Any tar deposit outside F2 was burnt off at this stage using a blow-torch. After closing S7, the excess oxygen in T5 was distilled into T4 via S9 (S8 closed). Care was taken to ensure that T4 did not heat up much above liquid nitrogen temperature. Any carbon dioxide vapour was thus re-sublimed at the sinter which also prevented blow-through of solid particles.

The oxygen in T4 was slowly distilled round to T5 via S6. Any carbon monoxide that passed through F3 was thus cycled round with the oxygen for a second pass through the furnace. Also, the combustion tube was again flushed out. The oxygen was cycled once more before it was slowly pumped away through S14 with liquid nitrogen around T5 and T6. When most of the oxygen had been removed S8 was opened to allow a faster pump out of the remainder than was possible via the sinter. Any carbon dioxide still mixed with the oxygen was frozen out in T6.

The contents of T6 were transferred to T5 before distilling over the carbon dioxide in T5 to T6 using a slush bath containing 95% ethanol (~ - 120°C) (S7, S8 closed). The sinter held back any water, thus producing a dry sample of carbon dioxide. This carbon dioxide was either transferred into T7 for yield determinations in a constant volume manometer or frozen straight into a sample bottle attached at J2. The manometer had three calibration marks; A, B and C, to enable widely differing quantities of carbon dioxide to be measured (fig. 24).

12.2 *YIELD DETERMINATIONS*

The constant volume manometer enabled yield determinations to be carried out to an estimated accuracy of ± 2%. Cellulose is unsatisfactory for such determinations because its affinity for water (see section 8.3.2) makes determination of sample size difficult. For example, cellulose in an air-dry condition contains about 5% moisture (Green 1963b). Thus 5 samples of sucrose were combusted.

Table 21. Carbon dioxide yields from sucrose

Weight of sample (g)	Yield of carbon dioxide (%)
0.0144	102
0.0171	100
0.0200	100
0.0168	103
0.0134	102
mean % yield ± σ	101 ± 1

The results in table 21 show that, within the accuracy of measurement, the combustion reaction goes to completion as far as carbon dioxide is concerned.

12.3 *REPRODUCIBILITY*

As a periodic check on the combustion systems and to provide an estimate of overall reproducibility one sample of cellulose (standard Cl/4, prepared from *Pinus radiata*) was analysed several times. The results displayed in table 22 show an overall standard deviation (σ) for sample analysis no larger than ± 0.04^{o}/oo and a standard error (σ_m) of ± 0.01^{o}/oo (i.e. less than the mass spectrometer standard error - see chapter 3).

12.4 STORAGE OF SAMPLES

In general the carbon dioxide from the combustion system was stored in sample bottles (see plate 7) for up to 3 days at ~6^{o}C before isotopic measurement on the mass spectrometer.

One sample of carbon dioxide was divided between several of the sample bottles (see plate 7) and stored at 6 ± 1^{o}C. No significant change in δ^{13}C value was observed over a 7 day period.

In another experiment small portions of the internal carbon dioxide standard (WPR - see section 12.5) were sealed into several small break-seal tubes which had been pumped out overnight. Two of the tubes were stored in a cupboard at room temperature for 6½ months, and two more were stored at room temperature for 4 months and then at 95 ± 5^{o}C for 2½ months, before measurement on the mass spectrometer. The δ^{13}C values of these samples were all within ± 0.02^{o}/oo of the bulk WPR standard.

12.5 THE PREPARATION AND STORAGE OF CARBON DIOXIDE STANDARD, WPR

Pinus radiata wood was extracted for 24 hr with diethyl ether and then burnt in a stream of oxygen in a vacuum line designed for preparing large quantities of carbon dioxide for carbon-14 determinations. The combustion products were passed through a 2 M solution of silver nitrate; a silver/copper oxide furnace at ~600^{o}C; an 0.5 M solution of iodine in 10% potassium iodide; a second 2 M solution of silver nitrate; chromic acid; and, finally, silica gel. The carbon dioxide formed (~9 litres at S.T.P.) was transferred to a glass storage bulb (fig. 25) and

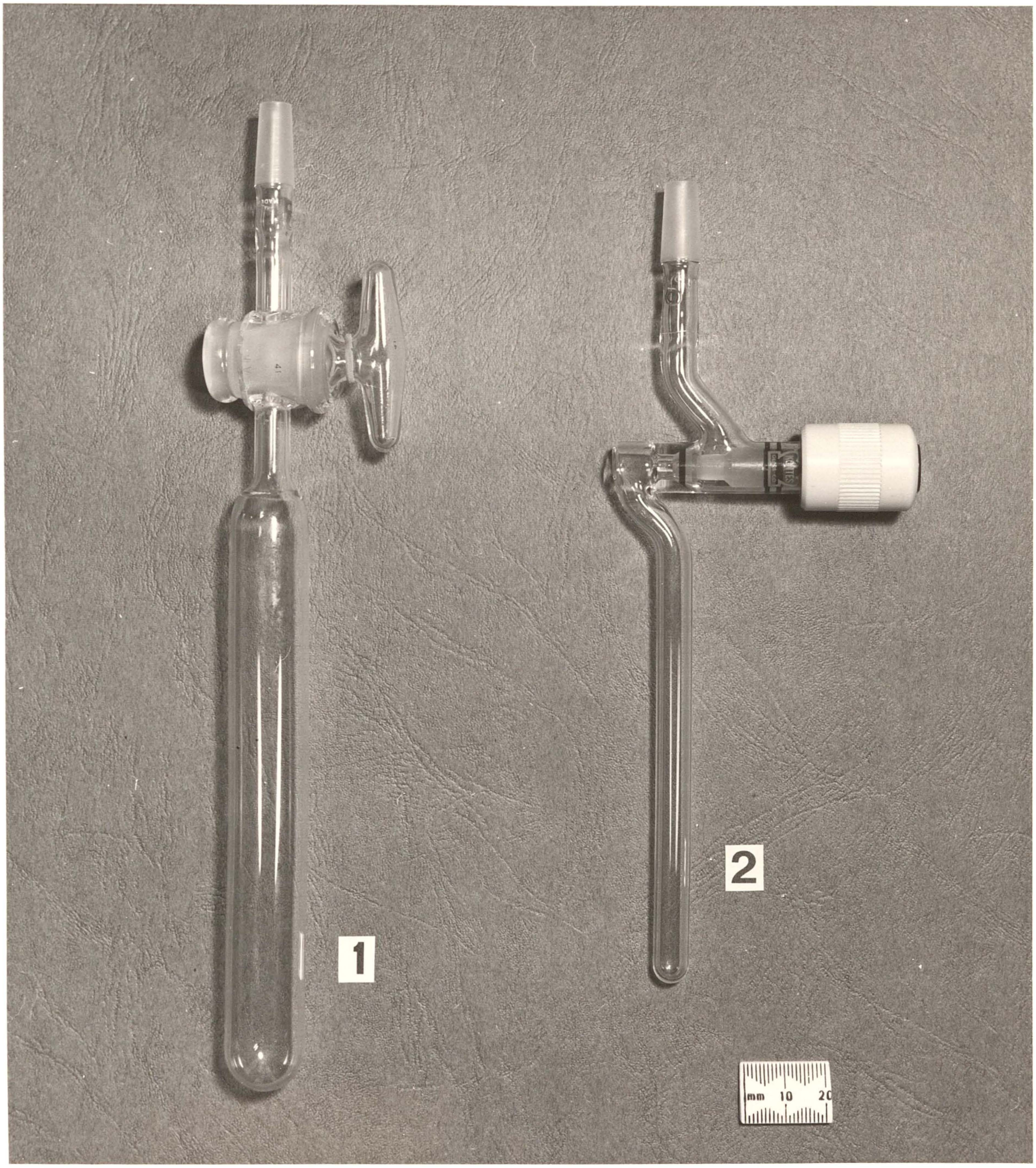
Table 22 $\delta^{13}C$ measurements on cellulose standard C1/4.

Date	$\delta^{13}C_{PDB}$ (‰)	
	Individual measurements	Day means
12/4/76		-25.02*
27/5/76	-25.02	
	-25.01	-25.01
	-25.00	
28/5/76	-25.05	
	-25.03	-25.02
	-24.98	
5/7/76	-25.11	
	-24.95	-25.03
	-25.06	
6/7/76	-25.00	
	-25.04	-25.03
	-25.05	
15/10/76	-25.03	
	-25.05	-25.03
24/11/76	-25.00	
	-25.00	-25.01
	-25.03	
mean values ± σ	-25.02 ± 0.036	-25.02 ± 0.009

[*] This value represents the mean of 5 samples run on the Nuclide Associates mass spectrometer at the Institute of Nuclear Sciences, Lower Hutt.

Plate 7

Carbon dioxide gas sample bottles.


1
2
mm 10 20

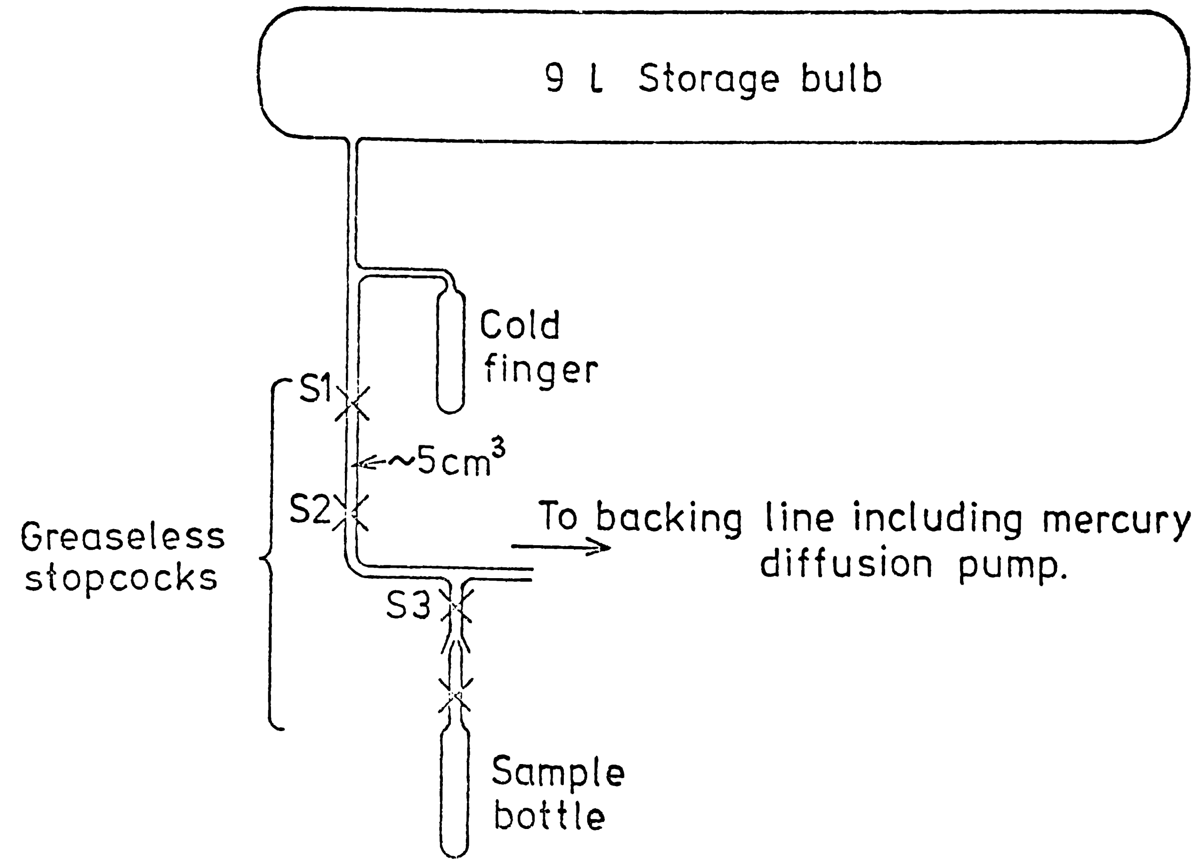


Fig.25. Apparatus for storing carbon dioxide gas standard WPR.

purified by adsorbing it onto calcium oxide (prepared by heating analar calcium carbonate to ~1000°C) at ~700°C, pumping on the calcium carbonate formed (~4 hr at ~450°C), and liberating the carbon dioxide by re-heating at ~1000°C.*

Small aliquots (5 cm^3 at S.T.P.) of the standard were sampled using the volume between the two greaseless stopcocks S1 and S2 (fig. 25). Each sample was frozen into a sample bottle (plate 7) through S3.

This primary laboratory gas standard (WPR) was periodically measured against a secondary cellulose standard (C1/4). There was no significant change in the $\delta^{13}C$ values (see table 22).

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*The method is based on that of Rafter (1955).

CHAPTER 13 δ^{13}C VARIATIONS IN TREES†

13.1 PREVIOUS WORK

Craig (1954a) was the first to study variations in the $^{13}C/^{12}C$ ratios of tree growth rings. He analysed whole wood samples from individual rings of a *Sequoia gigantea* which grew over a 2,500 year period. He found that the wood near the centre was depleted in ^{13}C compared with the remainder of the tree. $\delta^{13}C$ fluctuations in most of the tree were relatively small (less than 2°/oo) and showed no correlations with climate variations. Many, although not all, of the narrow rings were relatively depleted in ^{13}C.

Jansen (1962) found a similar depletion in the inside rings of an *Agathis australis* (kauri) tree (see fig. 34). In addition, Smith *et al.* (1973) found that variations in the carbon isotopic composition of barley, pea and rape correlated with shoot weight. Thus the depletion of ^{13}C in young trees may be partly related to physiological and biochemical differences from older trees of the same species. Also, root and mycorrhizal respiration is known to reduce the ^{13}C content of the local atmosphere near the ground, particularly in a forest (see section 11.2.2).

A seasonal cycle in $\delta^{13}C$, varying from - 22°/oo in early spring to - 28°/oo in late autumn, has been observed in maple leaves growing under natural conditions (Lowden and Dyck 1974). A similar cycle was found for grasses at the same location. Such variations seem too large to be

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†All $\delta^{13}C$ values reported in this chapter are with respect to PDB - see definition in appendix A.

explained by a diurnal $\delta^{13}C$ cycle for atmospheric carbon dioxide (see section 11.2.2). They may be related to changes in chemical composition and the increased photorespiration in leaves as they age.

$^{13}C/^{12}C$ ratios for European wood over the period 1900 to 1970 have been measured by Dequasie and Grey (1971), Farmer and Baxter (1972, 1974a, 1974b), Freyer and Wiesberg (1973, 1974, 1975) and Lerman (1974a).

Freyer and Wiesberg (1974) report 5 year mean $\delta^{13}C$ values derived from the analysis of cellulose samples from 10 free-standing trees. The $\delta^{13}C$ trends found for these trees are basically similar to those found by Farmer and Baxter (1974b) for whole wood samples taken from a free-standing *Larix decidua* (larch) and a forest *Quercus robur* (oak). Dequasie and Grey's analysis of United States poplar wood and Lerman and co-workers' analysis of a poplar from Norway are also comparable. All the trees show a general decrease in ^{13}C content from 1900 to 1970 which is probably related to the anthropogenic effect of Man's combustion of fossil fuels (see section 11.2). More minor variations in the curves may be climate related.

Freyer and Wiesberg (1974, 1975) also report variations in the ^{13}C content within a single tree ring. $\delta^{13}C$ values for cellulose prepared from wood samples taken from the same ring, at the same height, but from a different radius, varied by as much as 1^{o}/oo. The variation of such $\delta^{13}C$ values with height was found to be much smaller. Moreover, different increment-core samples from the same tree were found to show slightly different trends in their $^{13}C/^{12}C$ ratios. A correlation between ^{13}C content and ring width was only evident for one of the trees analysed.

Libby and Pandolfi (1974, 1975) have analysed whole wood samples laid down between 1700 and 1950. Their published results show a total range in $\delta^{13}C$ values of 8^{o}/oo. However, according to recent reports (personal communication to A.T. Wilson, 1974; Freyer and Wiesberg 1975) their range of measured $\delta^{13}C$ values is, in fact, considerably less.

$\delta^{13}C$ variations for whole wood samples from three *Arthrotaxis selaginoides* (King Billy pine) trees which grew from 1850 to 1970 have been studied by Pearman *et al.* (1976; see also Fraser *et al.* 1976). They report a significant correlation between $\delta^{13}C$ values and mean maximum summer temperatures with a coefficient of + 0.4^{o}/oo per ^{o}C. Their growth chamber experiments on *Pinus radiata* and *Pinus pinasta* show an increase in ^{13}C content with increasing temperature to a maximum at $27^{o}C$, and then a sharp decrease when the temperature is further increased to $30^{o}C$. These results are similar to those found for many other C_3 plants (see section 11.1.2).

Thus, $^{13}C/^{12}C$ ratios in tree rings have been correlated with both changes in climate and changes in the carbon isotopic composition of the atmosphere. Clearly much more work needs to be done before information concerning past climate or past atmospheric carbon dioxide changes can be elucidated from $^{13}C/^{12}C$ ratios in wood. The main aims of the work described in this chapter were to assess the relationships between climate and $^{13}C/^{12}C$ ratios in wood cellulose and lignin, and to apply such knowledge to the elucidation of past climate data from $\delta^{13}C$ measurements on sub-fossil wood. $\delta^{13}C$ determinations were carried out on wood constituents rather than on whole wood for the reasons outlined in section 11.1.1.

13.2 δ^{13}C VARIATIONS IN PINUS RADIATA

13.2.1 δ^{13}C Variations Within Individual Growth Rings

In order to assess the effect of large changes in climate on $^{13}C/^{12}C$ ratios in wood, it was decided to study New Zealand grown *Pinus radiata* which grows throughout the year and hence over a wide temperature range. The advantages of studying this species are discussed in detail in section 9.1.3.

Three rings* of a *Pinus radiata* from Hamilton, New Zealand, were divided up into 15 samples. Cellulose and lignin were prepared from each sample as described in chapter 4. The method described in chapter 12 was used to determine δ^{13}C values for each of the samples, which were the same as the samples used for the δD and δ^{18}O study (see section 9.3). The results are shown in tables 23 and 24 and displayed in fig. 26.

It can be seen that there is a depletion in ^{13}C for late (winter) wood as compared with early (summer) wood for both cellulose and lignin. Keeling's measurements (1960, 1961a) of the isotopic ratio of atmospheric carbon dioxide over the Pacific Ocean show an annual fluctuation of about 0.4°/oo, the atmosphere becoming isotopically heavier in the late summer and autumn (see section 11.2). The annual change shown in fig. 26 is of different phase and much greater than this. Moreover, the annual fluctuation in the ^{13}C content of the atmosphere in the Southern Hemisphere is likely to be even less than 0.4°/oo, since the annual fluctuation of carbon dioxide concentration is only ~2 ppm (D.C. Lowe, personal communication 1976; P. Fraser, personal communication 1976) compared with ~6 ppm for Northern Hemisphere Pacific Ocean air (e.g. Machta 1972a). The Southern Hemisphere

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*Ring-counting implied that these rings were laid down from 1918 to 1920 inclusive, and that the first ring was laid down in 1913. The tree was felled in the summer of 1972-1973.

fluctuation has been attributed to the activity of land plants in the Northern Hemisphere (D.C. Lowe, personal communication 1976). Therefore Keeling's relationship (1958) between the ^{13}C content and the concentration of atmospheric carbon dioxide may hold. If so, the annual fluctuation in the ^{13}C content of the Southern Hemisphere's atmosphere would be only about 0.1‰. Local fluctuations in atmospheric $\delta^{13}C$ values due to plant activity near Hamilton, New Zealand, are likely to be small and irregular since it is a windy place.

Thus the variations in $\delta^{13}C$ shown in fig. 26 are probably mainly the result of changes in air temperature affecting the amount of carbon isotopic fractionation during photosynthesis. In Hamilton both the monthly mean maximum daily temperatures and the monthly mean daily temperatures vary through an annual cycle with an amplitude of 10°C (see appendix E and New Zealand Meteorological Service records). Thus the carbon isotopic composition of both cellulose and lignin appears to vary with temperature with a coefficient of at least 0.2‰ per °C.

Fig. 27 presents the cellulose and lignin data superimposed. Several conclusions are evident. First, the amplitude of the $\delta^{13}C$ variations for cellulose is very similar to that for lignin. This probably can be taken as evidence that the temperature dependent step (or steps) leading to both cellulose and lignin is early in the sequence, i.e. in the leaves. Thus any temperature being monitored is that of the leaves rather than the trunk of the tree.

Secondly, although the cellulose and lignin curves are significantly different, these differences are probably too small to be of any use in palaeoclimatology with present mass spectrometric techniques.

The third interesting result is the phase relationship between the cellulose and lignin curves. It is generally believed by plant physiologists that newly-formed xylem cells contain only relatively small amounts of lignin. Most of the lignin is laid down some time later (Fritts 1976). The result of this secondary lignification can be seen in fig. 27 since the lignin curve is significantly displaced in time with respect to the cellulose curve. This supports the general interpretation given above.

Table 23 $\delta^{13}C$ determinations for cellulose from three rings of *Pinus radiata*.

Sample Number	$\delta^{13}C_{PDB}$ (°/oo)	Number of determinations
1	-25.12	3
2	-25.92	3
3	-25.41	3
4	-24.54	1
5	-24.67	2
6	-24.69	3
7	-24.41	1
8	-25.87	1
9	-25.51	2
10	-24.77	1
11	-24.16	3
12	-24.37	2
13	-25.19	2
14	-26.68	3
15	-26.01	6

Table 24 $\delta^{13}C$ determinations for lignin from three rings of *Pinus radiata*.

Sample Number	$\delta^{13}C_{PDB}$ (‰)	Number of determinations
1	-28.64	1
2	-29.65	2
3	-28.65	1
4	-27.98	1
5	-28.20	3
6	-28.23	1
7	-28.20	1
8	-29.58	3
9	-28.68	2
10	-28.00	2
11	-27.73	2
12	-28.11	1
13	-28.95	1
14	-30.29	1
15	-29.25	2

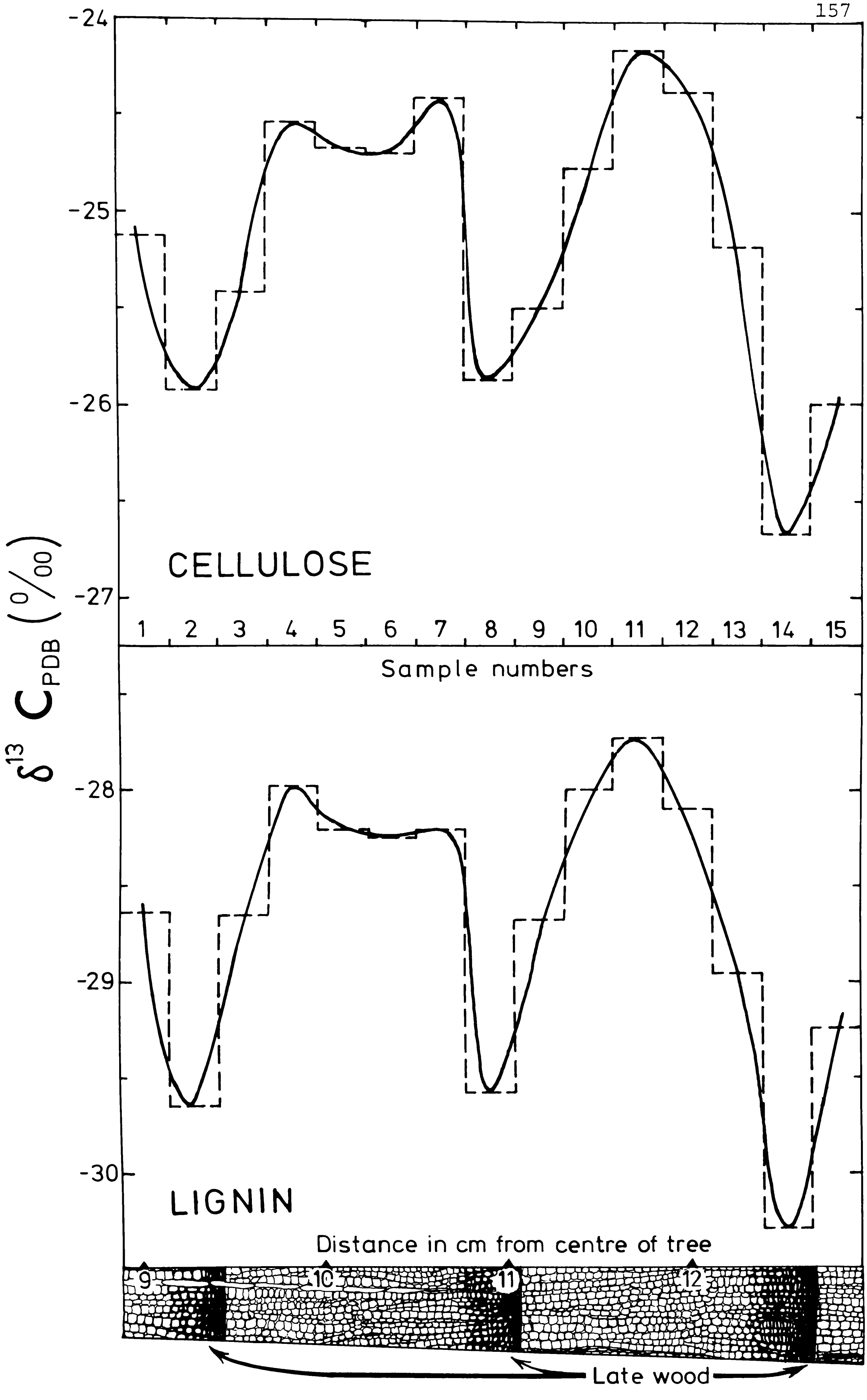

Fig.26.Variation in the isotopic ratio of the carbon in cellulose and lignin across tree rings from *Pinus radiata*.

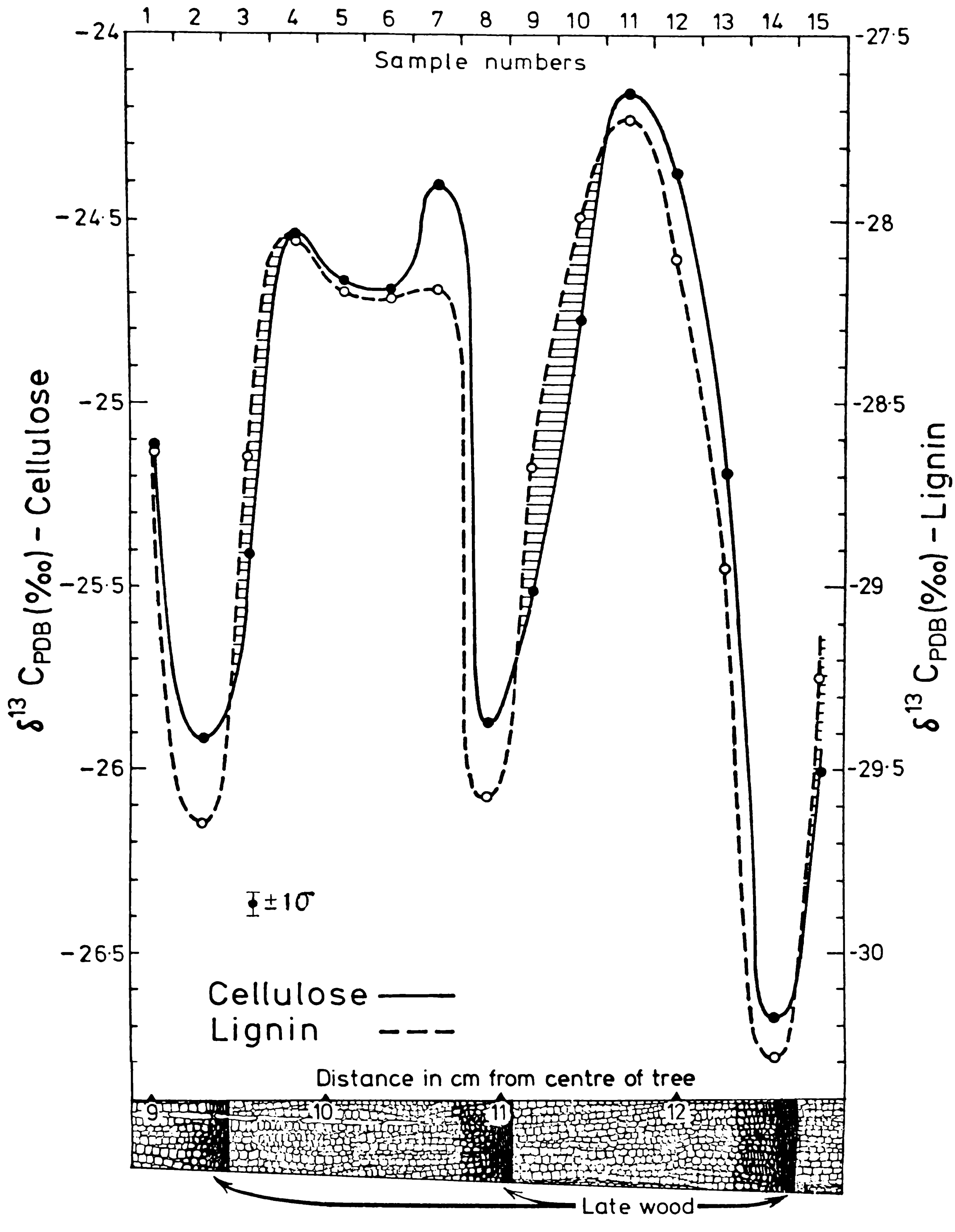

Fig. 27. Variation in the isotopic ratio of carbon across tree rings from *Pinus radiata* – a comparison between cellulose and lignin.

13.2.2 *$\delta^{13}C$ Variations Between Individual Growth Rings*

The objective of this study was to look for possible correlations between the $\delta^{13}C$ values of mid-ring cellulose and spring/summer climate. On the basis of the results described in the preceding section it was decided to analyse wood from the middle 50 to 60% of each ring.* Ten samples of the same specimen of *Pinus radiata* that was used in the previous study were selected. The years in which these samples grew (according to ring-counting) are shown in table 25. Cellulose was prepared from each of these samples (see section 4.2) and $\delta^{13}C$ values were determined (see chapter 12). The results are shown in table 25. These results, together with the mean $\delta^{13}C$ values for the middle of rings 1918-1919 and 1919-1920 (calculated from table 23), are displayed in fig. 28.

Table 25. Cellulose $\delta^{13}C$ determinations for 10 individual ring samples of *Pinus radiata*

Spring/summer of growth (A.D.)†	$\delta^{13}C_{PDB}$ (‰)§
1923/1924	- 24.79
1927/1928	- 23.57
1934/1935	- 22.00
1936/1937	- 22.39
1937/1938	- 22.60
1939/1940	- 22.31
1943/1944	- 22.06
1946/1947	- 21.89
1949/1950	- 21.60
1951/1952	- 22.18

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*For example, that equivalent to samples 4, 5 and 6 in fig. 26.

†According to ring-counting.

§Two determinations were carried out for each sample.

A large depletion in ^{13}C for the inside rings as compared with the outside rings is immediately evident. This effect is similar in magnitude to that found by Craig (1954a), but rather smaller than that found by Jansen (1962) (see fig. 34 and section 13.1). In view of the fact that this *Pinus radiata* grew some distance from any other trees, and in a fairly exposed situation, this effect is unlikely to be the result of the respiration of nearby plants. It is more likely the result of physiological and/or biochemical changes in the tree itself as it ages.

Because of the presence of this "age" effect it is not possible to obtain any meaningful correlations between the $\delta^{13}C$ values and climate. However, one result is perhaps worthy of comment. This is the fact that the spring/summer of 1934-1935 was exceptionally hot (mean maximum temperature about $26^{o}C$) and dry, whereas the spring/summer of 1951-1952 was exceptionally cold (mean maximum temperature about $21^{o}C$) and humid. In fig. 28 it can be seen that the $\delta^{13}C$ value for 1934-1935 cellulose is well above the general trend in $\delta^{13}C$ values, whilst the $\delta^{13}C$ value for 1951-1952 cellulose is well below the general trend. This is to be expected in view of the summer/winter variations in $\delta^{13}C$ values discussed in the preceding section.

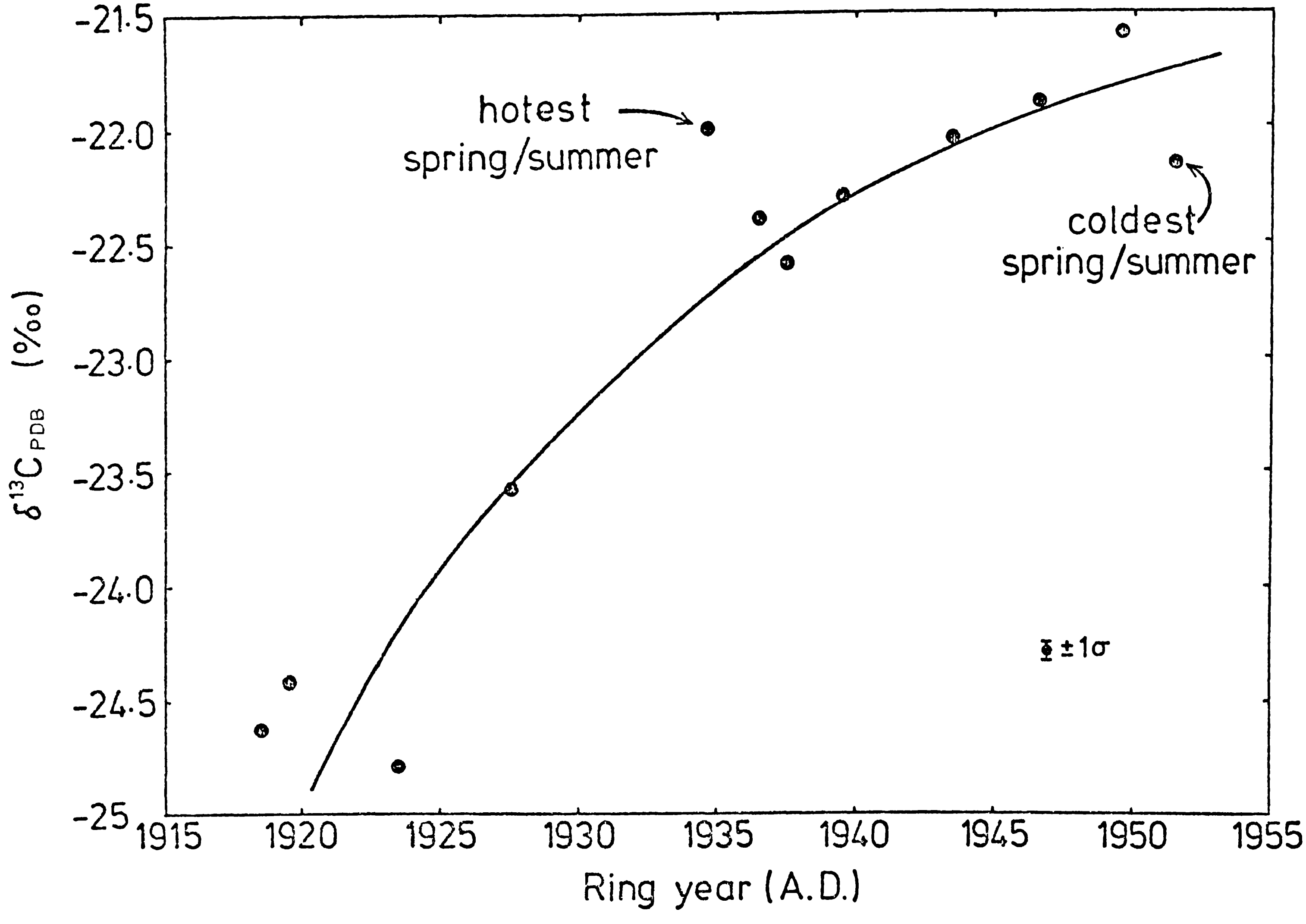

Fig.28. $\delta^{13}C$ values for cellulose samples prepared from selected rings of *Pinus radiata*.

13.3 δ^{13}C VARIATIONS IN PSEUDOTSUGA MENZIESII (DOUGLAS FIR)

In order to obtain further information concerning the relationships between δ^{13}C values and climate, it was decided to study a dendrochronologically dated *Pseudotsuga menziesii.*[*] Individual year samples were supplied by the University of Arizona tree ring laboratory. δ^{13}C values for 21 cellulose samples from the years A.D. 1900 to 1950 were determined[†] using the methods described in section 4.2 and chapter 12. The results are shown in table 26.

The δ^{13}C values were plotted against mean temperatures for the appropriate years including mean annual (August to July) mean March to July, mean March to May, mean May to July, mean previous August to October, and other one-, two- and three-monthly mean combinations of temperature observations.[§] The best correlation was observed between the δ^{13}C values and the mean temperature during the period of wood deposition (approximately May to July) (see fig. 29). The overall correlation for all 21 plots is poor ($r = +\ 0.271$ for a regression line: $\delta^{13}C_{PDB} = 0.091T - 23.53$). However, if the 7 plots enclosed by dashes (see fig. 29) are not considered, the correlation is good ($r = +\ 0.805$ for a regression line: $\delta^{13}C_{PDB} = 0.215T - 26.60$). For such a procedure to be valid a criterion for rejecting the plots enclosed by dashes is required.

13.3.1 Possible Criteria for Rejecting Anomalous δ^{13}C Values

Changing earlywood/latewood ratios is a possibility. However, a close examination of the original wood slice revealed no significant

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[*] See note under table 26.

[†] By Miss L. Moroney.

[§] Computed from the meteorological observations of the University of Arizona weather station, Tucson.

correlations with the $\delta^{13}C$ values (V.C. LaMarche, personal communication 1977).

The $\delta^{13}C$ values show a lack of correlation with the ring width variations[*] in the tree sampled (r = - 0.137), and also with the *Pseudotsuga menziesii* standardised ring indices for the area.[†]

Preliminary $\delta^{18}O$ determinations on some of the cellulose samples show large fluctuations ($\delta^{18}O$ values ranging from + 24‰ to + 32‰ with respect to SMOW), which cannot be simply correlated with the $\delta^{13}C$ determinations. It is perhaps worthy of note that 1947 cellulose has the lowest $\delta^{18}O$ value (2‰ less than any of the other 8 samples so far analysed) and is also the farthest away from the regression line in fig. 29. Further $\delta^{18}O$ determinations should be carried out before this possible method of obtaining a criterion for rejecting the anomalous $\delta^{13}C$ values is abandoned. The large range in $\delta^{18}O$ values may well be the result of large variations in ^{18}O fractionation due to leaf transpiration. Such variations are likely to occur in any trees growing in arid or semi-arid regions (see also sections 9.5.3 and 10.4.1).

It is unlikely that δD determinations alone will throw light on the problem since the predominant reason for any variations will probably be fluctuations in leaf transpiration rates, just as appears to be the case for the $\delta^{18}O$ determinations. However, it is possible that a combination of both δD and $\delta^{18}O$ measurements will supply some useful information (see sections 9.5.4 and 10.4.1).

13.3.2 Possible Explanations for the Anomalous $\delta^{13}C$ Values

During the 6 months (November to April) up to the start of the season of wood deposition the following years had less than 3 inches

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[*] Ring width measurements for this tree were supplied by Professor B. Bannister (personal communication 1976).

[†] Santa Catalina Mountain High chronology listed by Drew (1972).

of precipitation as measured at the University of Arizona weather station: 1900, 1921, 1923, 1934, 1940, 1943, 1947, 1950 (5 out of 8 well away from the regression line in fig. 29). During the season of wood deposition (May to July) the following years had less than 1.4 inches of precipitation as measured at the University of Arizona weather station: 1900, 1905, 1934, 1935, 1939, 1942, 1947 (4 out of 7 well away from the line). In addition, it should be noted that 1932, 1933, 1934, 1935 (3 out of 4 well away from the line) are "dust bowl" era years.

Thus the anomalous years (plots enclosed by dashes in fig. 29) were probably affected by drought. This could cause the tree to use stored photosynthates (fixed during the winter or previous autumn) for wood deposition. However, the tree had its roots in a spring and grew next to a stream. Even when both spring and stream dry up on the surface, there is probably an adequate underground water supply available to the tree except under conditions of extreme drought (A.T. Wilson, personal communication 1977). This supposition is supported by measurements of ring widths (see section 13.3.1) which show the tree to be fairly complacent compared with others in the same region.

Another possible explanation for the anomalous $\delta^{13}C$ values is water stress. A tree growing in still air conditions under a clear sky is likely to have a leaf temperature which is higher than the local air temperature. This overheating could cause rapid transpiration until water can no longer be translocated up the tree's trunk fast enough to cool the leaves. The resulting water stress would cause the leaf stomata to close so as to prevent wilting. Such stomata closure could, on the basis of the model described in section 11.3.4, either raise or lower the $^{13}C/^{12}C$ ratio of the primary photosynthates. However, if increased recycling of respiratory carbon dioxide is the predominant effect an overall depletion in ^{13}C will result. Thus it is proposed that the anomalous $\delta^{13}C$ vs. temperature plots in fig. 29 are the result of long periods of water stress during photosynthesis.

Table 26 $\delta^{13}C$ determinations for 21 cellulose samples from individual years of *Pseudotsuga menziesii*.†

Dendrochronological year of growth (A.D.)	$\delta^{13}C_{PDB}$ (‰)	Number of determinations
1900	-21.36	2
1905	-21.41	2
1908	-21.13	2
1910	-21.02	2
1913	-21.19	2
1917	-21.13	2
1918	-20.91	2
1921	-21.24	2
1923	-21.68	1
1932	-21.47	2
1933	-20.96	2
1934	-21.42	2
1935	-21.70	2
1939	-20.57	2
1940	-21.22	2
1941	-21.05	1
1942	-20.83	3
1943	-20.28	2
1944	-20.59	2
1947	-21.60	3
1950	-20.75	1

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† This Douglas fir is known as the "Hitchcock tree". It grew for 300 years in the Santa Catalina Mountains, Arizona, at 1850 m altitude until 1957.

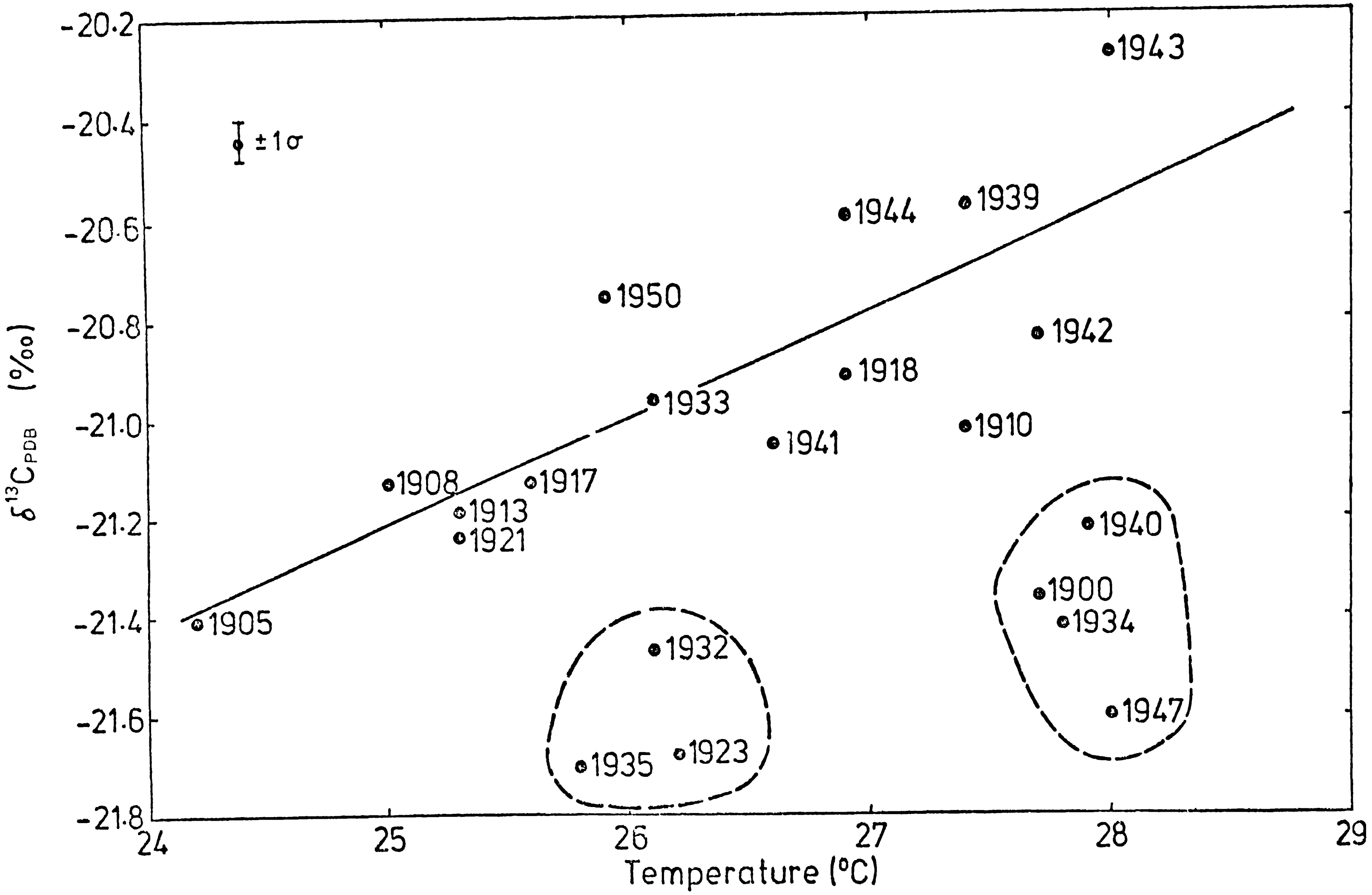

Fig.29. $\delta^{13}C$ values for 21 individual year samples of *Pseudotsuga menziesii* cellulose plotted against mean May-June-July temperature.

13.4 $\delta^{13}C$ VARIATIONS IN *PINUS LONGAEVA* (BRISTLECONE PINE)

Thirty-eight wood samples from two lower forest border *Pinus longaeva* trees which grew during the last 1000 years in the White Mountains of California were supplied by Professor Ferguson of the University of Arizona tree-ring laboratory (see note under table 27). Cellulose was prepared[*] using the method described in section 4.2 and $\delta^{13}C$ values were determined as described in chapter 12. Details concerning the samples analysed and the $\delta^{13}C$ values obtained are given in table 27.

Three time-equivalent samples were analysed. It can be seen from fig. 30 that the $\delta^{13}C$ values for two of these samples are significantly different. However, the differences are small (< 0.6 ‰) compared with the overall variations observed (> 2 ‰). Thus the ^{13}C content of the cellulose in both trees was probably affected by environmental changes in a similar way.

From fig. 31 it can be seen that there is a correlation between 60 year mean $\delta^{13}C$ values[†] in *Pinus longaeva* cellulose and 60 year mean upper tree line ring widths.[§] The correlation is significant at the 5% level.[¶] No significant correlation was found between 60 year mean $\delta^{13}C$ values and 60 year mean lower forest border ring widths.[¶]

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[*] By Professor A.T. Wilson.

[†] The anthropogenic correction that has been applied is, of course, speculative - its magnitude is based on the work discussed in sections 11.2 and 13.1.

[§] The ring width departures plotted in fig. 31 are based on ring indices which have been corrected for the normal overall decrease in ring width as the tree's girth increases (Stokes *et al.* 1973).

[¶] The correlation coefficient for a linear regression analysis of 16 successive comparisons of 60 year mean $\delta^{13}C$ values and 60 year mean upper tree line ring widths is - 0.560. A linear regression analysis of the $\delta^{13}C$ data with 60 year mean lower forest border ring widths gave a correlation coefficient of + 0.023.

Fifty year mean high summer temperatures in central England are also plotted in fig. 31. A comparison between this curve and the $\delta^{13}C$ curve suggests that the $\delta^{13}C$ variations in *Pinus longaeva* cellulose may be climate related. The main peak correspondences in fig. 31 are indicated by the letters A, B and C. It should be noted that the apparent relationship between the $\delta^{13}C$ variations in *Pinus longaeva* cellulose and temperature is much larger and opposite to that observed for the *Pinus radiata* samples (section 13.2.1) and 14 of the 21 *Pseudotsuga menziesii* samples (section 13.3). The effect of climate on the $^{13}C/^{12}C$ ratio in *Pinus longaeva* cellulose may well be similar to that observed for the apparently anomalous samples of *Pseudotsuga menziesii* cellulose.

Thus it is proposed that the $\delta^{13}C$ variations shown in fig. 31 are predominantly the result of long term changes in water stress; where high water stress results in low $\delta^{13}C$ values (see also sections 11.3.4 and 13.3.2). The significant correlation between this work on $\delta^{13}C$ variations in lower forest border *Pinus longaeva* and upper tree line ring width variations for the same species suggests that periods of water stress at the lower forest border may correspond to warm periods at the upper tree line.*

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*Upper tree line ring width variations have been found to be mainly dependent on temperature (LaMarche 1974).

Table 27 Cellulose δ^{13} determinations for 38 samples from two specimens of *Pinus longeava*[†] which grew during the last 1000 years.

Sample Number	Dendrochronological Years (A.D.)	$\delta^{13}C_{PDB}$ (‰)[*]
1	910 - 940	-19.77
2	940 - 968	-19.30
3	968 - 1000	-19.45
4	1000 - 1030	-19.45
5	1030 - 1060	-19.57
6	1060 - 1090	-19.68
7	1090 - 1120	-19.71
8	1120 - 1150	-19.57
9	1150 - 1180	-20.77
10	1180 - 1210	-20.19
11	1210 - 1240	-19.73
12	1240 - 1270	-19.45
13	1270 - 1300	-19.31
14	1300 - 1330	-19.34
15	1330 - 1360	-19.12
16	1360 - 1390	-19.29
17	1390 - 1420	-18.98
18	1420 - 1450	-18.99
19	1480 - 1510	-20.09
20	1510 - 1540	-19.87

Table 27 (continued)

Sample Number	Dendrochronological Years (A.D.)	$\delta^{13}C_{PDB}$ (°/oo)*
21	1540 - 1570	-20.58
22	1570 - 1592	-19.89
20′	1510 - 1540	-20.14
21′	1540 - 1570	-19.99
22′	1570 - 1600	-19.87
23	1600 - 1630	-19.71
24	1630 - 1660	-19.61
25	1660 - 1690	-19.42
26	1690 - 1720	-19.96
27	1720 - 1750	-20.09
28	1750 - 1780	-20.46
29	1780 - 1810	-20.33
30	1810 - 1840	-20.37
31	1840 - 1870	-20.45
32	1870 - 1900	-20.85
33	1900 - 1930	-21.32
34	1930 - 1960	-21.58
35	1960 - 1974	-21.47

[†] The following information concerning these specimens was supplied by C.W. Ferguson (personal communication, 1976):

a) Samples 1 to 22 were taken from University of Arizona tree number TRL67-3. The pith ring for this tree was 905.

b) This specimen was divided at 968 rather than 970 because of a natural crack in mid-968.

c) No sample was supplied for the period 1450-1480 because of a crack in the wood at 1450 - 1460.

d) Samples 20′ to 35 were taken from University of Arizona tree number TRL74-101. The pith ring for this tree was 1485.

e) The samples duplicate the material provided to Professor S. Epstein.

*Two determinations were carried out for each sample except 31 and 33 which were analysed three times.

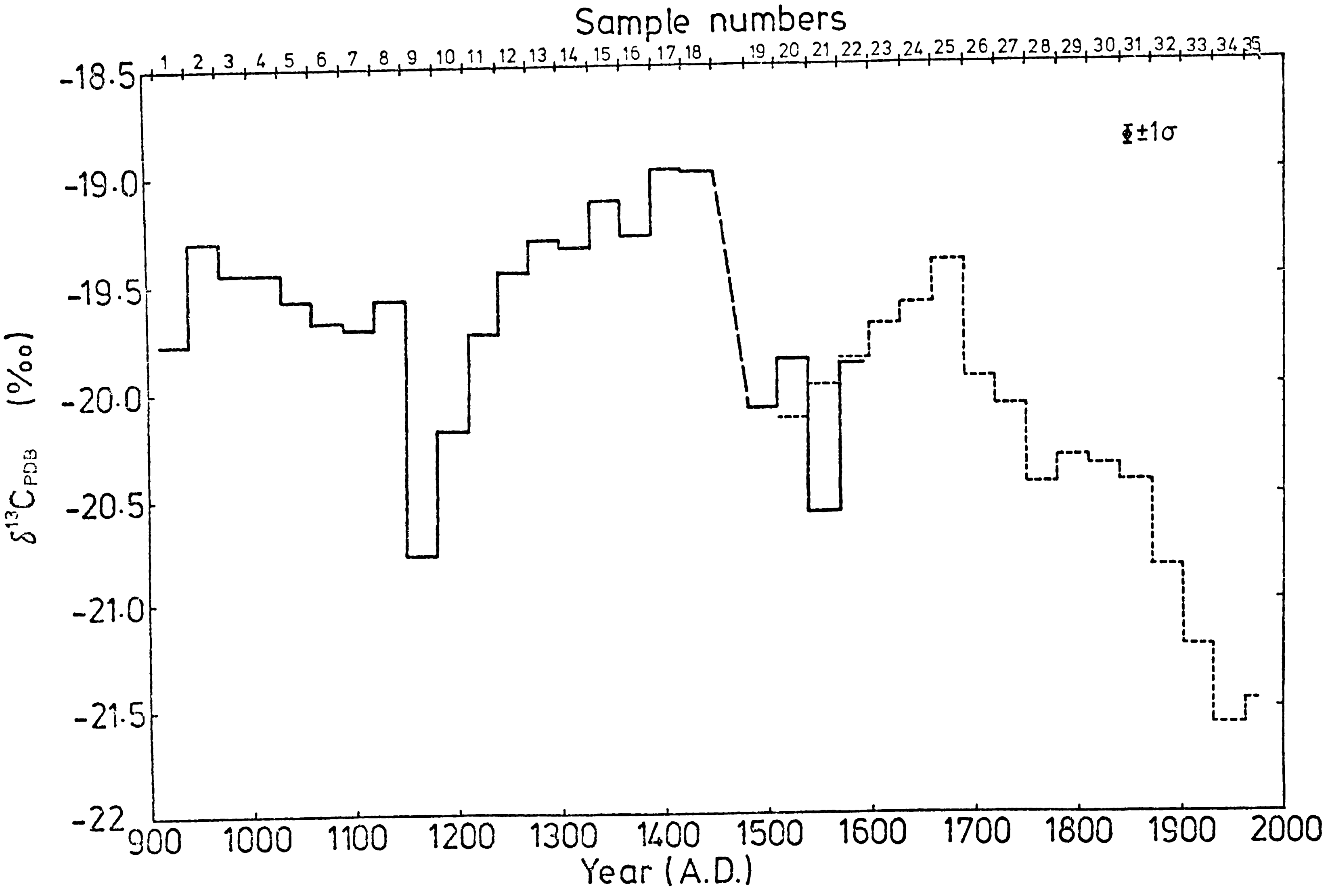

Fig.30. $\delta^{13}C$ variations for the past 1000 years in *Pinus longaeva* cellulose.

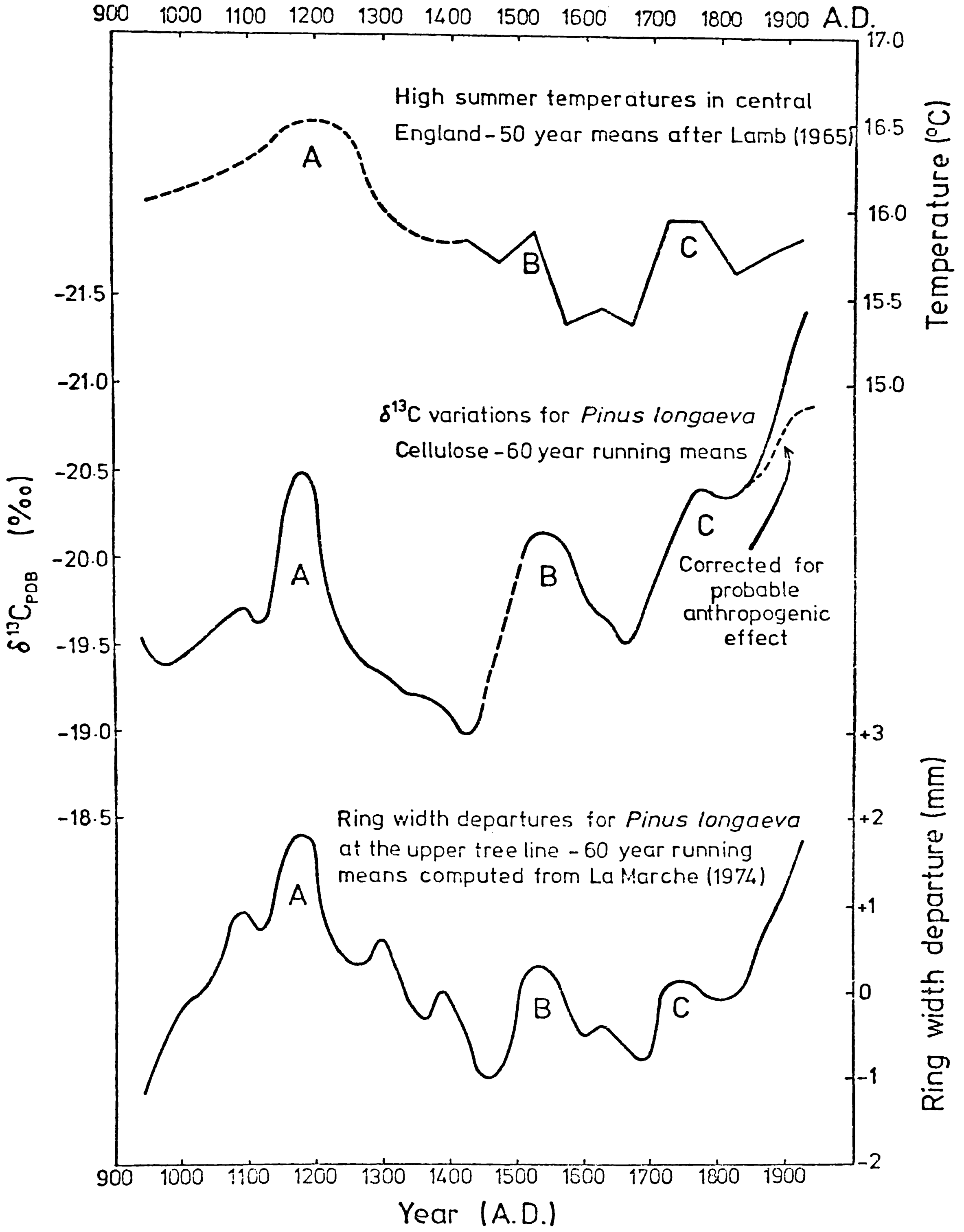

Fig.31. $\delta^{13}C$ variations for *Pinus longaeva* cellulose compared with upper tree line mean ring width departures and high summer temperatures for England.

13.5 $\delta^{13}C$ VARIATIONS IN AGATHIS AUSTRALIS (KAURI)

A wood slice of *Agathis australis** which grew in the Omahutu Forest, North Auckland, New Zealand, was supplied by Mr. H.S. Jansen of the Institute of Nuclear Sciences, D.S.I.R., Lower Hutt. The slice was divided up into 42 samples, each representing approximately 25 years of growth according to carbon-14 dating (see appendix G). Cellulose was prepared and $\delta^{13}C$ values were determined as described in section 4.2 and chapter 12. The width of each sample was also measured and 50 year running mean ring widths calculated. The results are shown in table 28 and figs. 32 and 33.

It should be noted that the general decrease in ring width over the 1000 year period is the result of the increasing girth of the tree. Thus only the peaks and troughs in the two curves should be compared (see fig. 33). Such a comparison reveals a lack of correlation.

The observed general increase in the ^{13}C content of *Agathis australis* cellulose over the last 1000 years may be the result of the "age" effect found by Jansen (1962a, 1962b) for the whole wood analyses of the same tree. Fig. 34 shows Jansen's $\delta^{13}C$ determinations plotted against corrected carbon-14 dates (years A.D.). The $\delta^{13}C$ values and associated error bars are those of Jansen (1962b). Although there is clearly an "age" effect, it is not possible using Jansen's data to estimate the proportion of the tree affected. Two curves that could be fitted to the data are shown in fig. 34. In view of the $\delta^{13}C$ variations found for cellulose (see fig. 32) the dotted curve is perhaps more likely. This "age" effect is much larger than that observed for *Pinus radiata* (see section 13.2.1) or *Sequoia gigantea* (Craig 1954a - see section 13.1). Thus it may well be the result of

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*This slice was taken from the same tree as that carbon-14 dated by Jansen (1962a, 1969) - see appendix G.

both the respiration of nearby plants in the forest and age-related physiological and/or biochemical changes in the tree.

Although the "age" effect probably explains the overall ^{13}C enrichment over the 1000 year period, it is unlikely to be the explanation for the observed peaks and troughs in the $\delta^{13}C$ curve. In fig. 35 it can be seen that these short-term fluctuations correspond with those in Lamb's central England temperature curve. Moreover, they also correspond fairly closely with the peaks and troughs in the $\delta^{18}O$ curve for a New Zealand speleothem particularly if the speleothem time axis is stretched slightly with respect to the *Agathis australis* time axis.* $\delta^{18}O$ variations in speleothem calcite have been correlated with variations in past climate (see e.g. Hendy 1969).

Thus the short-term fluctuations in the $\delta^{13}C$ curve for *Agathis australis* cellulose are probably climate related. If such variations are mainly dependent on temperature, as suggested by the peak correspondences in fig. 35 (see above discussion), the relationship is similar, although greater in magnitude, to that observed for the *Pinus radiata* samples (section 13.2.1) and most of the *Pseudotsuga menziesii* samples (section 13.3). The amplification of the temperature effect may be due to variations in sea surface temperatures affecting ^{13}C exchange between dissolved bicarbonate and atmospheric carbon dioxide. The equilibrium temperature coefficient for such an exchange is about + 0.1‰ per °C (see section 11.2.1), and the mean atmospheric

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*The end of the speleothem curve that corresponds to the present is fixed since calcite was being actively deposited when the speleothem was collected. However, speleothem carbon-14 dating is much less accurate than wood carbon-14 dating because of difficulties in estimating the proportion of calcite carbon originating from limestone rock rather than atmospheric carbon dioxide (see Hendy 1969). Thus the peaks A_1 and A_2 in the speleothem curve (fig. 35) may well correspond in time to peaks A_1 and A_2 in the *Agathis australis* curve. (A.T. Wilson, personal communication 1977.)

residence time for carbon dioxide exchange with the oceans (5 to 10 years - see section 11.2.1) is short enough for changes in the isotopic composition of atmospheric carbon dioxide to be reflected in observed 50 year running mean $\delta^{13}C$ variations in *Agathis australis* cellulose.

If the short-term fluctuations in the cellulose $\delta^{13}C$ curve for *Agathis australis* are temperature related, as seems probable, they represent a record of warm and cold periods in New Zealand's climate during the last millenium (see next section).

Table 28 Cellulose $\delta^{13}C$ determinations and sample widths for 42 samples from a 1000 year specimen of *Agathis australis*.

Sample Number	Ring count "before 1960"§	Years (A.D.)*	$\delta^{13}C_{PDB}$ (‰)†	Width of sample (mm)
1	851 - 833	875 - 900	-21.74	42.0
2	832 - 815	900 - 925	-21.09	31.0
3	814 - 797	925 - 950	-20.43	52.5
4	796 - 778	950 - 975	-20.19	53.5
5	777 - 761	975 - 1000	-20.79	65.0
6	760 - 743	1000 - 1025	-20.03	54.0
7	742 - 725	1025 - 1050	-19.96	52.5
8	724 - 706	1050 - 1075	-20.84	59.0
9	705 - 687	1075 - 1100	-20.56	18.5
10	686 - 668	1100 - 1125	-20.17	26.0
11	667 - 650	1125 - 1150	-20.42	27.0
12	649 - 632	1150 - 1175	-20.08	31.0
13	631 - 613	1175 - 1200	-19.65	26.5
14	612 - 594	1200 - 1225	-19.98	32.0
15	593 - 576	1225 - 1250	-19.56	31.0
16	575 - 558	1250 - 1275	-20.39	19.5
17	557 - 540	1275 - 1300	-20.72	23.0
18	539 - 522	1300 - 1325	-20.42	14.5
19	521 - 503	1325 - 1350	-20.05	22.5
20	502 - 485	1350 - 1375	-20.23	25.5
21	484 - 467	1375 - 1400	-20.09	37.0
22	466 - 449	1400 - 1425	-19.79	34.5
23	448 - 430	1425 - 1450	-19.79	31.5

Table 28 (continued)

Sample Number	Ring count [§] "before 1960"	Years (A.D.)*	$\delta^{13}C_{PDB}$ (‰)[†]	Width of sample (mm)
24	429 - 412	1450 - 1475	-19.36	21.5
25	411 - 394	1475 - 1500	-19.00	14.7
26	393 - 375	1500 - 1525	-19.06	21.5
27	374 - 357	1525 - 1550	-19.36	7.5
28	356 - 339	1550 - 1575	-19.52	5.0
29	338 - 320	1575 - 1600	-19.55	12.3
30	319 - 302	1600 - 1625	-19.60	23.0
31	301 - 284	1625 - 1650	-20.23	21.5
32	283 - 265	1650 - 1675	-19.89	22.5
33	264 - 247	1675 - 1700	-20.06	16.5
34	246 - 229	1700 - 1725	-19.35	18.0
35	228 - 211	1725 - 1750	-19.54	29.5
36	210 - 192	1750 - 1775	-19.55	12.0
37	191 - 174	1775 - 1800	-19.24	9.3
38	173 - 155	1800 - 1825	-18.88	11.0
39	154 - 137	1825 - 1850	-19.63	8.0
40	136 - 119	1850 - 1875	-19.92	19.0
41	118 - 100	1875 - 1900	-19.88	23.0
42	99 - 83	1900 -	-19.90	-

[§] Based on the same ring count that was used by Jansen (1962a).

* These years have been determined using the equation in appendix G. They have been rounded up or down to the nearest 25 year period.

[†] Two determinations were carried out for each sample except 16, 24, 38 and 39 which were analysed three times.

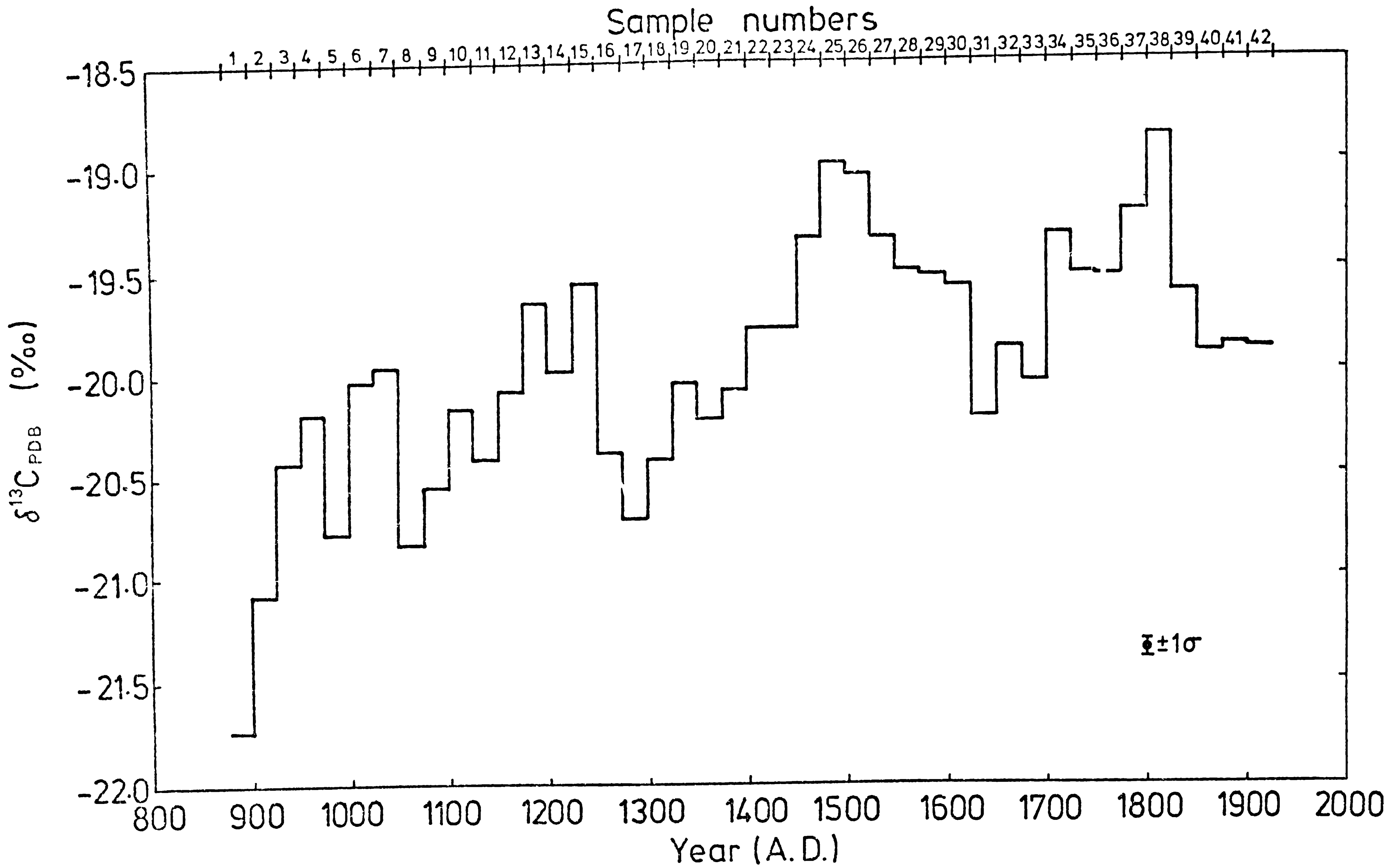

Fig.32. $\delta^{13}C$ variations for the past 1000 years in *Agathis australis* cellulose.

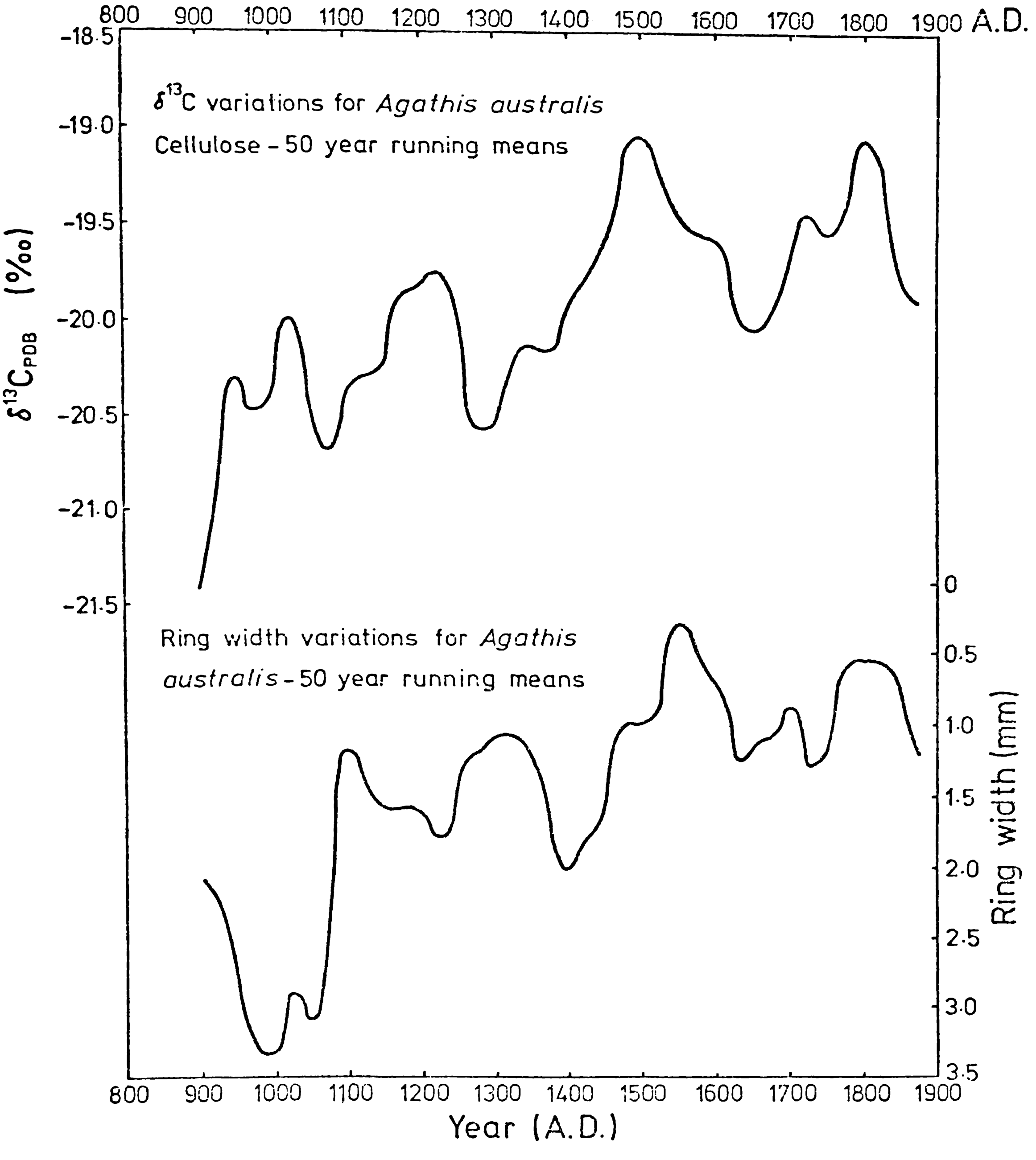

Fig.33. $\delta^{13}C$ variations for *Agathis australis* cellulose compared with mean ring width.

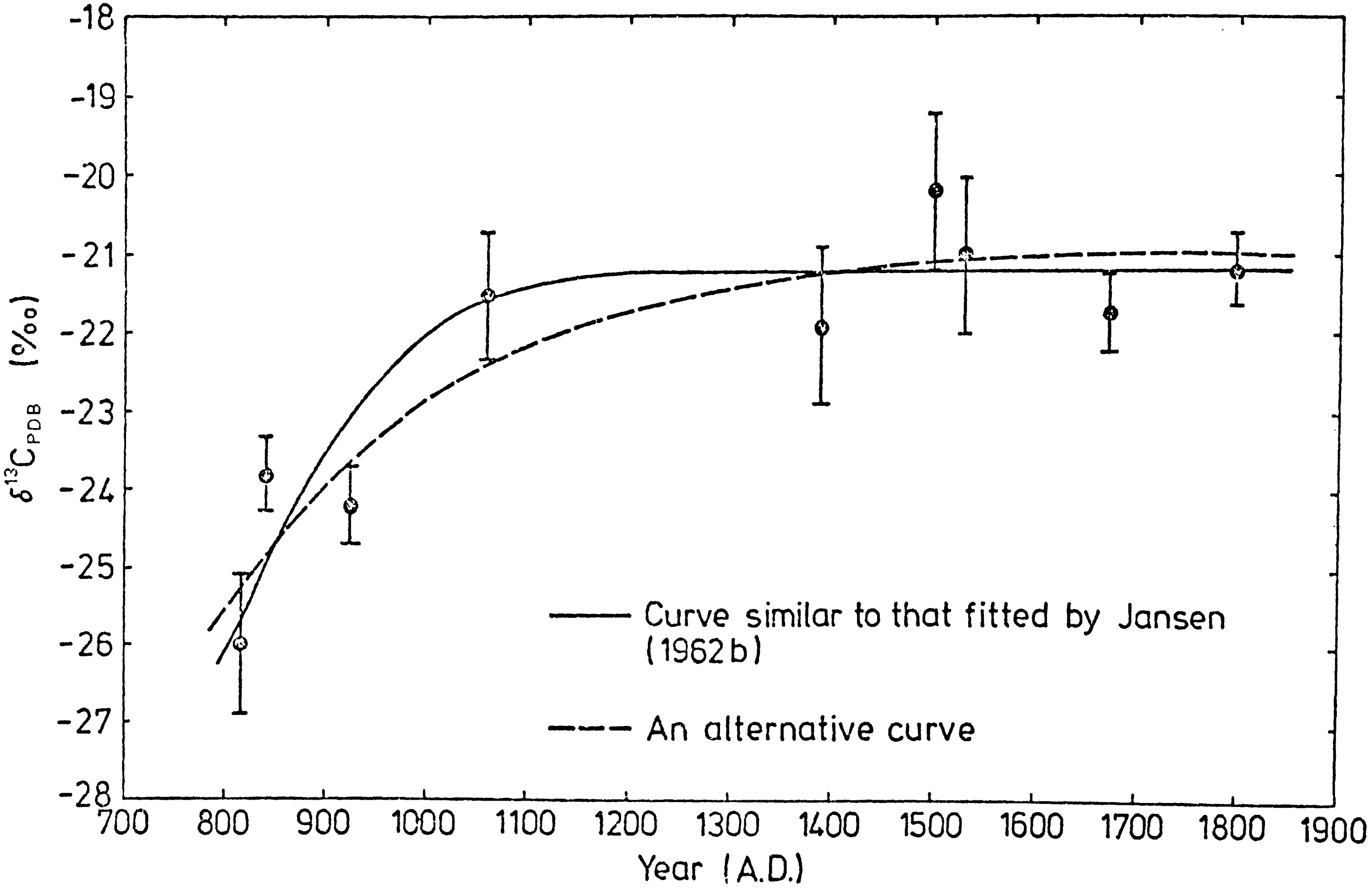

Fig.34. $\delta^{13}C$ variations for *Agathis australis* whole wood after Jansen (1962b) except that his carbon-14 dates (Jansen 1962a) have been corrected using the table of Damon *et al.* (1972).

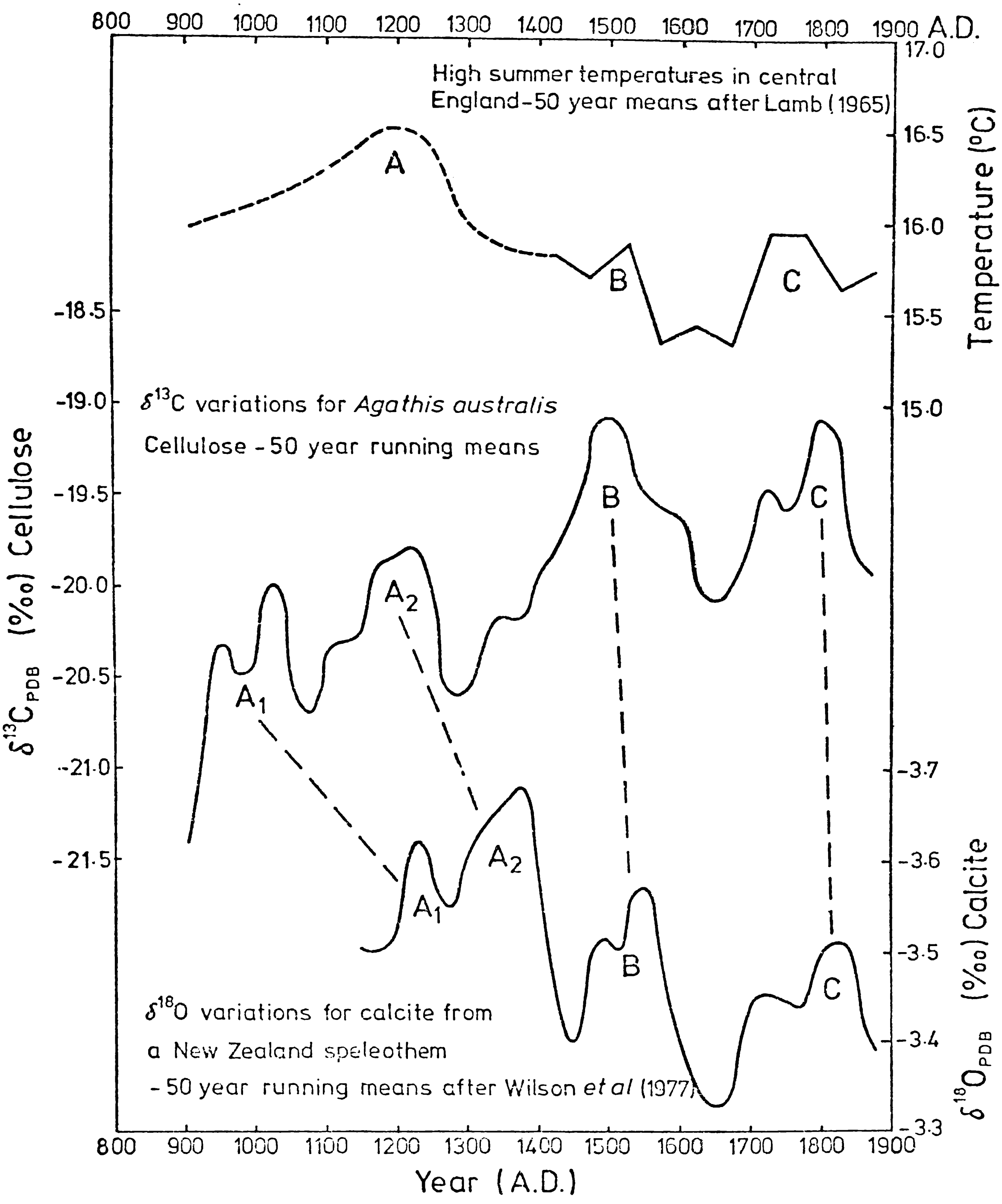

Fig.35. $\delta^{13}C$ variations for *Agathis australis* cellulose compared with high summer temperatures for England and $\delta^{18}O$ variations in a New Zealand speleothem.

13.6 CLIMATIC CHANGE IN NEW ZEALAND DURING THE LAST MILLENIUM

This subject has been exhaustively reviewed by Greenland and Burrows (1977). Other recent reviews include those of Molloy (1969) and Park (1970). Sparrow and Healy (1968) list several hundred references concerned with the meteorology and climatology of New Zealand, but only a few of these deal with climatic change.

Much of the early evidence for palaeoclimatic change in New Zealand has been derived from botanical investigations. For example, Raeside (1948) interpreted changes in vegetation patterns and soil formation in terms of climate periods similar to the Little Ice Age (A.D. 1500-1700) and the Medieval Warm Period (A.D. 1000-1200) which, it is generally accepted, occurred in North-western Europe (see, e.g., Lamb 1965). Holloway (1954) suggested that many of the South Island forests are in an unstable condition as a result of recent climatic changes. Thus in many podocarp forests there is a predominance of mature and senile trees. The existence of a "regeneration gap" has been confirmed by other workers (see review of literature in Greenland and Burrows 1977), but the time of its commencement is in doubt (e.g. Molloy 1969). Wardle (1963) suggested that the least regeneration occurred between A.D. 1600 and 1800 due to drought and cold.

The occurrence of kumara pits in South Canterbury has been put forward as evidence for a relatively warm period during the 13th century (Lockerbie 1950).

Dendroclimatology has been used to obtain information concerning past climate in the Northern Hemisphere (see, e.g. Fritts 1976). However, none of the dendrochronologies worked out to date, using New Zealand trees, extend far enough back in time to be of use in determining past climate. Also, Scott (1972) found that correlations between ring indices and climate were weak for all the species he studied. He concluded that the potential of the method was not great.

Perhaps the best information concerning climatic changes in New Zealand during the last millenium arises from measurements of $^{18}O/^{16}O$ ratios in a New Zealand speleothem (Wilson *et al.* 1977; see fig. 35) and from studies of glacial moraines (e.g. Burrows 1973, Wardle 1973, Greenland and Burrows 1977). Greenland and Burrows (1977) also found that observations of iceberg irruption in the Southern Ocean since the 1770s generally occurred at the same time as advances of New Zealand glaciers. Glacial events suggest oscillations of about ± 0.5°C in mean temperature during the last 1000 years; with cold peaks in the 12th, 13th,* 15th, 16th, 17th* (probably two), mid 18th,* and mid and late 19th* centuries (C.J. Burrows, personal communication 1977; see also Greenland and Burrows 1977).

This interpretation of glacial events in terms of temperature fluctuations can be compared with the short-term fluctuations in the cellulose $\delta^{13}C$ curve for an *Agathis australis* tree (see fig. 35) and with a stretched $\delta^{18}O$ speleothem curve (see fig. 35 and note in section 13.5). Cold periods in the late 11th (~A.D. 1070), late 13th (~A.D. 1290), 17th (~A.D. 1650) and late 19th (~1870 onwards) centuries are indicated. Somewhat less cold periods are indicated for the mid 18th (~A.D. 1750), and possibly the late 10th (~A.D. 980) and late 14th (~A.D. 1370) centuries. This interpretation agrees well with Burrows' interpretation of the glacial data.

The *Agathis australis* cellulose $\delta^{13}C$ curve (fig. 35) indicates that the warmest periods probably occurred around A.D. 1000 (Peak A_1), A.D. 1200 (Peak A_2), A.D. 1500 (Peak B) and A.D. 1800 (Peak C).

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*These peaks were probably the coldest, but generally all were of similar amplitude (C.J. Burrows, personal communication 1977).

Assuming that the peaks A_1, A_2, B and C in the *Agathis australis* curve do indeed correspond to those in the speleothem curve (see above and section 13.5), the latter curve suggests that the periods around A.D. 1000 and A.D. 1200 were the warmest of the last millenium.

It is concluded that, on the basis of the information available to date, the fluctuations in New Zealand's climate during the last millenium were similar to those in North-western Europe (see, e.g., Lamb 1965 and fig. 35).

13.7 DISCUSSION AND CONCLUSIONS

The results in section 13.2.1 show that the carbon atoms in both cellulose and lignin from three growth rings of a *Pinus radiata* tree from Hamilton, New Zealand, are more than 2‰ enriched in ^{13}C in the summer as compared with the winter. The summer/winter variation is probably mainly due to changes in air temperature affecting the magnitude of carbon isotopic fractionation during photosynthesis. The lignin variations are similar, but significantly displaced in time with respect to the cellulose variations. This is probably the result of secondary lignification.

δ^{13}C values for cellulose prepared from individual rings in a sample of dendrochronologically dated *Pseudotsuga menziesii* (section 13.3) also appear to be related to climate. When these values are plotted against mean late spring/early summer temperatures 14 of the 21 plots are found to lie on or near a line of slope + 0.215. Ring width and earlywood/latewood variations cannot be used as criteria for rejecting the 7 anomalous points. Preliminary δ^{18}O results are not very promising, and it is unlikely that δD determinations alone will help, although a combination of both δD and δ^{18}O measurements may provide the criterion required. It is proposed that the anomalous δ^{13}C vs. temperature plots are the result of water stress during photosynthesis.

The *Pseudotsuga menziesii* tree used in this study contains about 300 rings. Thus, if a good criterion for recognising the anomalous cellulose δ^{13}C values could be found, year-by-year late spring to early summer temperatures could be elucidated back to the middle of the 17th century. Even without such a criterion the data displayed in fig. 29 suggest that cellulose samples with δ^{13}C values greater than, say, - 21.1‰ can probably be directly related to temperature using the regression line. On the other hand, if a cellulose sample is found to have a $\delta^{13}C$ value less than, say, - 21.5°/oo, it is likely that the tree was subjected to an abnormal amount of water stress during that year. However, before such deductions are made the remainder of the rings that grew over the period of meteorological observations should be analysed. Also, the centre rings of the tree should be analysed to find out whether or not there is an "age" effect.

The above results for *Pinus radiata* and *Pseudotsuga menziesii* indicate a temperature dependence for the photosynthetic carbon isotopic fractionation factor between atmospheric carbon dioxide and cellulose of about + 0.2°/oo per °C.

$\delta^{13}C$ determinations on mid-ring cellulose samples from 10 individual rings of *Pinus radiata* (section 13.2.2) are difficult to interpret in terms of climatic changes because of a relatively large depletion in ^{13}C towards the centre of the tree. An even larger such depletion in ^{13}C was observed for cellulose prepared from a carbon-14 dated *Agathis australis* which grew during the last 1000 years (section 13.5). It is suggested that these "age" effects may be the result of age-related physiological and/or biochemical changes in the trees. The *Agathis australis* "age" effect is probably enhanced by the respiration of nearby plants since the tree grew in a forest. No such "age" effect was observed when cellulose $\delta^{13}C$ values were determined for two dendrochronologically dated *Pinus longaeva* trees which grew during the last 1000 years (section 13.4).

The latter $\delta^{13}C$ values from the lower tree line show a significant correlation with *Pinus longaeva* upper tree line ring widths, but not with lower forest border ring widths. The apparent relationship between the $\delta^{13}C$ variations in *Pinus longaeva* cellulose and temperature is opposite and of greater magnitude to that observed for the *Pinus* *radiata* samples and the majority of the *Pseudotsuga menziesii* samples. It is proposed that the $\delta^{13}C$ variations in *Pinus longaeva* cellulose are predominantly the result of long-term changes in water stress. Thus such variations can probably be used to elucidate variations in past climate, where "climate" in this case is a function of both water supply (depends on previous precipitation) and temperature during the photosynthetic fixation of the carbon dioxide that ends up as cellulose.

The "age" effect is not likely to be the explanation for the short-term fluctuations in the $\delta^{13}C$ curve for *Agathis australis* cellulose. These $\delta^{13}C$ variations are probably climate related, being dependent on both a temperature effect on carbon isotopic fractionation during photosynthesis and also changes in the ^{13}C content of atmospheric carbon dioxide which occur due to long-term changes in sea surface temperatures. An increase in temperature appears to correspond with an increase in the ^{13}C content of *Agathis australis* cellulose just as for the *Pinus radiata* cellulose samples and most of the *Pseudotsuga menziesii* cellulose samples. Such a temperature effect reflects the fact that the tree sampled grew in a moist temperate region where it would not have been subjected to the extremes of water stress probably experienced by the *Pinus longaeva* trees, which grew in an arid region.

If the short-term fluctuations in the *Agathis australis* $\delta^{13}C$ curve are indeed temperature related, they represent a record of warm and cold periods in New Zealand's climate. A comparison of this climate record with the information available from other sources leads to the conclusion that the fluctuations in New Zealand's climate during the last millenium were basically similar to those for North-western Europe.

13.8 SUGGESTIONS FOR FURTHER WORK

There are two fundamental problems which require further work. First, there is the postulated effect of water stress on $^{13}C/^{12}C$ ratios in wood components. Secondly, the "age" effect requires more study in order to find out more about its causes and why it is present in some trees, but not in others.

The former problem could be studied by growing trees under controlled conditions, for example in growth chambers, to obtain a greater understanding of the effects of temperature, humidity, light level, water supply, carbon dioxide concentration, etc., on the $\delta^{13}C$ value of, for example, wood cellulose. Due regard must be taken of the points outlined in section 11.1.2. Such experiments could be combined with an investigation of the effects that the same factors have on cellulose δD and $\delta^{18}O$ values (see section 10.5).

The "age" effect in New Zealand *Agathis australis* could be more carefully studied. $\delta^{13}C$ analyses of cellulose samples from free-standing trees should indicate whether the effect is due to physiological and/or biochemical changes in the tree or to the photosynthetic and respiratory activity of nearby plants (see section 13.5). These and similar analyses on forest trees should also establish whether the short-term fluctuations in the $\delta^{13}C$ curve in fig. 32 are common to all specimens of *Agathis australis* which grew during some part of the same time period.

There are, however, several other possibilities for further work which arise directly out of the studies discussed in this chapter. These are considered in the following sections.

13.8.1 Pseudotsuga Menziesii

Some suggestions for further work on individual rings from the "Hitchcock" Douglas fir tree are discussed in section 13.6.

13.8.2 *Pinus Longaeva*

A significant correlation has been found between the cellulose $\delta^{13}C$ variations in lower forest border *Pinus longaeva* over the last 1000 years and upper tree line ring width variations for the same species. The latter variations are presented by LaMarche (1974) as an approximate record of warm-season temperatures in the White Mountains of California. The upper tree line dendrochronology extends back to about 5,400 B.P. (LaMarche 1974). It would be interesting to find out whether cellulose $\delta^{13}C$ variations at the lower forest border vary in a similar way to upper tree line ring widths over the whole of this 5,400 year period. If this were the case, $\delta^{13}C$ measurements of *Pinus longaeva* cellulose could be used to extend the warm-season temperature record back to the start of the lower forest border chronology, which is 8,200 years long (Ferguson 1972).

Another interesting study would be to measure cellulose $\delta^{13}C$ values in *Pinus longaeva* trees from the upper forest. If water stress is a major factor determining the $^{13}C/^{12}C$ ratio of *Pinus longaeva* cellulose, the $\delta^{13}C$ variations are likely to differ from those in lower forest border trees since temperature rather than moisture is limiting to growth in the upper forest (LaMarche 1974).

13.8.3 *Agathis Australis*

If short-term fluctuations in the $\delta^{13}C$ curve presented in fig. 32 are found to be common to all specimens of *Agathis australis* which grew during the same time period, it may be possible to use trees that have been preserved in swamps to extend the record back in time. The specimens that have been carbon-14 dated so far range in age from a few 100 years to 38,000 years B.P. If overlapping specimens are used, only the $^{13}C/^{12}C$ ratios for cellulose from the outermost rings (hopefully not troubled by an "age" effect) need be considered in the construction of a past climate curve. It may be worthwhile also to measure the inner rings of each tree to test for peak correspondences between the $\delta^{13}C$ variations of cellulose in the overlapping sections.

Consider, for example, a tree which has carbon-14 dates that show it to have grown from 400 A.D. to 1400 A.D. A 50 year running mean curve of the $\delta^{13}C$ variations in cellulose prepared from such a specimen should have peaks that correspond with peaks A_1 and A_2 in the $\delta^{13}C$ curve shown in fig. 35. Hopefully, this section of the record will not be troubled by an "age" effect. If this were the case, a palaeoclimate curve for New Zealand over the last 1000 years could be constructed using cellulose $\delta^{13}C$ values for the years 900 to 1400 A.D. from the older tree and 1400 to 1900 A.D. from the tree discussed in section 13.5.

It may be possible to analyse several such overlapping specimens of *Agathis australis* and so obtain a palaeoclimate curve for New Zealand going back several millenia.

13.8.4 Other Work

$\delta^{13}C$ measurements on earlywood and latewood could be extended to other trees with known growth periods in order to obtain more information concerning the effect of seasonal climatic change on cellulose $\delta^{13}C$ values (see section 13.2).

Cellulose $\delta^{13}C$ variations in dendrochronologically and carbon-14 dated wood from elsewhere in the world could also be studied.

$\delta^{13}C$ determinations on wood constituents other than cellulose may provide additional palaeoclimate information. However, the work discussed in section 13.2 showed that, at least for a sample of *Pinus radiata*, $\delta^{13}C$ variations in lignin are similar to those in cellulose.

PART V

CONCLUSIONS

CHAPTER 14 CONCLUSIONS

In this thesis stable isotope ratio variations in wood constituents, and the relationships of such variations to local climate fluctuations, have been studied for several trees.

D/H ratios in the non-exchangeable hydrogen atoms in cellulose samples from three growth rings of a New Zealand *Pinus radiata* tree show a summer/winter variation of more than $20^{\circ}/oo$. The δD values, which are lower in the summer, appear to vary with the annual temperature cycle, but in the opposite sense to that expected from thermodynamic considerations. The observed δD variations are unlikely to be the result of the changing isotopic composition of precipitation or ground water. Also, they do not reflect the expected changes in the isotopic composition of leaf water which would tend to become enriched in the summer due to greater transpiration associated with higher temperature and lower humidity.

Epstein and Yapp's δD determinations (1976) on *Pseudotsuga*, *Pinus ponderosa* and *Pinus radiata* from the Western United States support the interpretation outlined above.

All the data so far compiled concerning D/H ratios in cellulose from earlywood and latewood suggest that as the temperature increases the deuterium content of the non-exchangeable hydrogens decreases. A temperature coefficient in the region of $- 3^{\circ}/oo$ per $^{\circ}C$ would be consistent with the results obtained. This temperature effect will only be useful for past climate studies if δD variations due to meteoric and/or leaf transpiration changes can be corrected for. This may be possible using $\delta^{18}O$ determinations.

The δD determinations in this work support the conclusions of Epstein *et al.* (1976) that plants preferentially concentrate the lighter isotope into their cellulose C-H hydrogens. The fractionation with respect to the environmental water is generally in the range 0 to - 50‰.

The oxygen atoms in the same *Pinus radiata* cellulose samples that were used for δD measurements are more than 2‰ enriched in ^{18}O in the summer as compared with the winter. The cellulose oxygen is about + 35‰ enriched in ^{18}O compared with the local ground water probably as a result of CO_2/ H_2O oxygen isotopic exchange in the leaf. The $\delta^{18}O$ variations are probably due to one or more of the following factors:

(a) the changing isotopic composition of local precipitation;

(b) a small temperature effect on the fractionation factor for CO_2/H_2O oxygen isotopic exchange in the leaf;

(c) changing leaf transpiration rates.

$\delta^{13}C$ values for both cellulose and lignin from the same *Pinus radiata* samples are more than 2‰ enriched in ^{13}C in the summer as compared with the winter. The observed variations are probably mainly the result of changes in air temperature affecting the magnitude of carbon isotopic fractionation during photosynthesis. The amplitude of the $\delta^{13}C$ variations for cellulose is very similar to that for lignin. However, the two curves are significantly displaced in time,probably as a result of secondary lignification which occurs some time later than xylem cell wall formation.

Most of the cellulose $\delta^{13}C$ values for individual rings from a dendrochronologically dated *Pseudotsuga menziesii* from Southern Arizona appear to be related to late spring/early summer temperatures in that region. A good criterion for rejecting certain anomalous $\delta^{13}C$ values has not yet been found. However, it is proposed that these anomalous values are the result of water stress during photosynthesis.

These $\delta^{13}C$ determinations on *Pinus radiata* and *Pseudotsuga menziesii* imply that the photosynthetic carbon isotope fractionation factor between atmospheric carbon dioxide and cellulose is dependent on temperature. Under normal growth conditions for these two trees the temperature coefficient is about + 0.2‰ per °C.

$\delta^{13}C$ determinations on mid-ring cellulose samples from individual rings of the same *Pinus radiata* that was used for the above determinations are difficult to interpret in terms of climatic changes because of a relatively large depletion in ^{13}C towards the centre of the tree. An even larger such depletion in ^{13}C was observed for cellulose prepared from a carbon-14 dated New Zealand *Agathis australis* which grew during the last 1000 years. These "age" effects are probably due to one or both of the following factors:

(a) age-related physiological and/or biochemical changes;

(b) the respiration and photosynthetic activity of nearby plants.

No such "age" effect was observed when cellulose $\delta^{13}C$ values were determined for two dendrochronologically dated Californian *Pinus longaeva* trees which also grew during the last millenium. These $\delta^{13}C$ values are apparently related to climate, but in the opposite sense to $\delta^{13}C$ values for the *Pinus radiata* and most of the *Pseudotsuga menziesii* cellulose samples. It is proposed that the $\delta^{13}C$ variations in these lower forest border *Pinus longaeva* cellulose samples may depend on variations in the degree of water stress to which the tree is subjected. These $\delta^{13}C$ variations show a significant correlation with upper tree line ring width variations for the same species. Such a correlation suggests that periods of water stress at the lower forest border may correspond to warm periods at the upper tree line.

The short-term fluctuations in the $\delta^{13}C$ curve for *Agathis australis* cellulose are probably related to two effects:

(a) the temperature effect on carbon isotope fractionation during photosynthesis;

(b) small changes in the ^{13}C content of atmospheric carbon dioxide resulting from long-term changes in sea surface temperature.

An increase in temperature appears to correspond with an increase in the ^{13}C content of *Agathis australis* cellulose just as is the case for the *Pinus radiata* and most of the *Pseudotsuga menziesii* cellulose samples.

If the short-term fluctuations in the *Agathis australis* $\delta^{13}C$ curve are indeed temperature related, they represent a record of warm and cold periods in New Zealand's climate. A comparison of this climate record with the information available from other sources leads to the conclusion that the fluctuations in New Zealand's climate during the last millenium were basically similar to those of North-western Europe.

Thus there is little doubt that $\delta^{13}C$ variations in the wood components from each tree ring sequence studied reflect climatic changes in that particular area. For some trees the effect of plant physiological changes on such variations is little understood. At present, those trees growing in exposed situations with a plentiful water supply appear to hold most promise for elucidating past climate data from measurements of $^{13}C/^{12}C$ ratios.

Lastly, it should be noted that climate data elucidated by isotope dendroclimatologists is not the same as that measured by meteorologists. Other work has shown that climate deducible from ring width variations in sensitive trees is normally a complex function of several meteorological climate parameters (e.g. Fritts 1976). The results presented in this thesis suggest that there are similar problems when it comes to relating stable isotope ratio variations to climate. Even if, for a particular tree, the temperature during wood deposition is the primary cause of the observed stable isotope ratio variations, past temperatures deduced from such variations are likely to be somewhat different from those measured by meteorologists.

APPENDICES

APPENDIX A GLOSSARY OF STABLE ISOTOPE STANDARDS

A.1 CARBONATE STANDARDS

PDB :	An international calcite standard for $\delta^{13}C$ and $\delta^{18}O$ determinations. It is a Cretaceous belemnite, *Belemnitella americana*, collected from the Peedee formation of South Carolina, U.S.A. (Craig 1957).
PDB - CO_2 :	The CO_2 that is evolved when PDB is treated with 100% H_3PO_4 at 20°C (Craig 1957).
NBS 20 :	An international limestone standard for $\delta^{13}C$ and $\delta^{18}O$ determinations. It is Solenhofen Jurassic limestone, collected in Bavaria by Nier (1950) and distributed by the National Bureau of Standards, U.S.A.
NBS 20 - CO_2 :	The CO_2 that is evolved when NBS 20 is treated with 100% H_3PO_4 at 25°C.
TKL :	A limestone standard for $\delta^{13}C$ and $\delta^{18}O$ determinations It was collected from Te Kuiti, New Zealand, and is distributed by the Institute of Nuclear Sciences, D.S.I.R., Lower Hutt, New Zealand (Rafter 1955).
TKL - CO_2 :	The CO_2 that is evolved when TKL is treated with 100% H_3PO_4 at 25°C.

NOTE - The carbon isotopic composition of PDB is the same as that of PDB - CO_2;

i.e. $$\delta^{13}C_{PDB}(A) = \delta^{13}C_{PDB-CO_2}(A)$$

Also $$\delta^{13}C_{NBS\ 20}(A) = \delta^{13}C_{NBS\ 20-CO_2}(A)$$

and $$\delta^{13}C_{TKL}(A) = \delta^{13}C_{TKL-CO_2}(A)$$

Thus in this thesis only PDB, NBS 20 and TKL are used when discussing carbon isotope analyses, since PDB - CO_2, NBS 20 - CO_2 and TKL - CO_2 are redundant in this case.

However, the oxygen isotopic composition of PDB is <u>not</u> the same as that of PDB - CO_2;

i.e. $\delta^{18}O_{PDB}(A) \neq \delta^{18}O_{PDB-CO_2}(A)$

also $\delta^{18}O_{NBS\ 20}(A) \neq \delta^{18}O_{NBS\ 20-CO_2}(A)$

and $\delta^{18}O_{TKL}(A) \neq \delta^{18}O_{TKL-CO_2}(A)$

Thus it is necessary to distinguish between PDB and PDB-CO_2, NBS 20 and NBS 20-CO_2, and TKL and TKL-CO_2 when discussing oxygen isotope analyses (see chapter 3).

A.2 WATER STANDARDS

SMOW : An international water standard for δD and $\delta^{18}O$ determinations. It is short for Standard Mean Ocean Water and is defined in terms of NBS 1 and NBS 1A - see Craig (1957, 1961), Coplen and Clayton (1973), Hagemann *et al.* (1970) and Baertschi (1976).

V.SMOW : Vienna SMOW. This is the Standard Mean Ocean Water currently distributed by the International Atomic Energy Authority. Hagemann *et al.* (1970) suggest that it has a δD value of - 0.1°/oo relative to SMOW, whilst Coplen and Clayton (1973) suggest - 1.2°/oo relative to SMOW on the basis of their results.

SLAP (or SNOW) : A light international water standard for δD and $\delta^{18}O$ determinations. It is short for Standard Light Antarctic Precipitation and is distributed by the International Atomic Energy Authority.

NBS 1, NBS 1A : Two international water standards for δD and $\delta^{18}O$ determinations. They are distributed by the National Bureau of Standards, U.S.A.

W1,W1A,W2,W3 : Four internal University of Waikato water standards for δD and $\delta^{18}O$ determinations (see section 5.5).

A.3 OTHER STANDARDS

NBS 21 : An international standard for $\delta^{13}C$ determinations. It is spectroscopic graphite and is distributed by the National Bureau of Standards, U.S.A.

WPR : An internal University of Waikato CO_2 gas standard prepared by combusting *Pinus radiata* (see section 12.5).

APPENDIX B

Pages 202 and 203:
FORTRAN program for mass spectrometer calculations.

Page 204:
Example of printout using this program and an IBM 11-30 computer.

```
      INTEGER DELMT,RNO,SNO,END
      DIMENSION RVAL(50), SVAL(50),XVAL(50),IHDR(40),TVAL(50)
      DIMENSION LINE(60)
      DATA LINE/58*'+=','  E','RM'/
      DATA INDEL/'='/
      DATA TVAL/12.706,4.303,3.182,2.776,2.571,2.447,2.365,2.306,2.262,
     12.228,2.201,2.179,2.160,2.145,2.131,2.120,2.110,2.101,2.093,2.086,
     22.080,2.074,2.069,2.064,2.060,2.056,2.052,2.048,2.045,2.042,2.039,
     32.035,2.033,2.031,2.029,2.028,2.026,2.024,2.022,2.021,2.020,2.019,
     42.018,2.017,2.016,2.015,2.014,2.013,2.012,2.011/
      J=3
      K=2
      RND4=0.00005
      RND5=0.000005
      RND6=0.0000005
  110 WRITE(J,101)LINE
  101 FORMAT('1',///,60A2)
      READ(K,2) IHDR
    2 FORMAT(40A2)
      WRITE(J,102)
  102 FORMAT(//' RATIO MASS SPECTROMETER DATA ANALYSIS PROGRAM')
      WRITE(J,58)(IHDR(I),I=1,20)
   58 FORMAT(////' SAMPLE DETAILS     ',5X,20A2)
      WRITE(J,59)(IHDR(I),I=21,40)
   59 FORMAT('0REFERENCE DETAILS ',5X,20A2)
      DO 20 I=1,50
      READ(K,10) R,DELMT
   10 FORMAT(F6.2,3X,A1)
      RVAL(I)=R
      IF(DELMT-INDEL)20,4,20
    4 RNO=I
      GO TO 6
   20 CONTINUE
    6 DO 22 I=1,50
      READ(K,10)S,DELMT
      SVAL(I)=S
      IF(DELMT-INDEL)22,8,22
    8 SNO=I
      GO TO 12
   22 CONTINUE
   12 IF(SNO+1-RNO)14,18,14
   14 WRITE(J,16)SNO,RNO
   16 FORMAT('0BURP - INDIGESTABLE MATERIAL',/,'0NUMBER OF SAMPLE VALUES
     1 IS NOT ONE LESS THAN NUMBER OF REFERENCE VALUES',//,I2,
     2'  SAMPLE VALUES',5X,I2,'  REFERENCE VALUES'//' THIS IS JUST NOT G
     3OOD ENOUGH'//' TUFF - TRY AGAIN')
      GO TO 100
   18 READ(K,24)RC,RD
   24 FORMAT(F6.3)
      XBAR=0.0
      DO 26 I=1,SNO
      I1=I+1
      XVAL(I)=SVAL(I)-((RVAL(I)+RVAL(I+1))/2.0)
   26 XBAR=XBAR+XVAL(I)
      XBAR=XBAR/SNO
      XBARD=XBAR*0.0001
      RBAR=0.5*RVAL(1)+0.5*RVAL(RNO)
      IF(RNO-2)30,30,28
   28 DO 29 I=2,SNO
   29 RBAR=RBAR+RVAL(I)
   30 RBAR=RBAR/SNO
      RBARD=RBAR*0.0001
```

```
      SIG2A=0.0
      DO 32 I=1,SNO
   32 SIG2A=SIG2A+(XVAL(I)-XBAR)**2.0
      SIG2A=SIG2A/(RNO-2)
      SIG2O=(4.0/3.0)*SIG2A
      SIG1=((SIG2O)**0.5)/((RC+RBARD)*10.0)
      DELT=((RO+XBARD)/(RC+RBARD))*10.0**(3)
      ERRDT=SIG1/SQRT(RNO-1.0)
      IF(SNO-1)122,122,120
  120 IF(SNO-50)121,121,122
  121 CONLT=TVAL(SNO-1)*ERRDT
      GO TO 123
  122 CONLT=10000.0
  123 CONTINUE
      WRITE(J,50)
   50 FORMAT('0REFERENCE VALUES'10X' SAMPLE VALUES'19X'X-VALUES')
      DO 34 I=1,SNO
      XVA=XVAL(I)
      XVAL(I)=XVAL(I)+SIGN(RND4,XVA)
   34 WRITE(J,51)RVAL(I),SVAL(I),XVAL(I)
   51 FORMAT(5X,F6.2,19X,F6.2,24X,F9.4)
      WRITE(J,52)RVAL(RNO)
   52 FORMAT(5X,F6.2)
      WRITE(J,53)RC,RO
   53 FORMAT(///' COARSE RATIO SETTING',F6.3,/'0RATIO OFFSET',F6.3)
      XBAR=XBAR+SIGN(RND4,XBAR)
      XBARD=XBARD+SIGN(RND6,XBARD)
      WRITE(J,54)XBAR,XBARD
   54 FORMAT(//' MEAN X = ',F8.4,/'0CORRECTED MEAN = ',F9.6)
      RBAR=RBAR+SIGN(RND4,RBAR)
      RBARD=RBARD+SIGN(RND6,RBARD)
      WRITE(J,55)RBAR,RBARD
   55 FORMAT(//' REFERENCE MEAN = ',F8.4,/'0CORRECTED REFERENCE MEAN = '
     1F9.6)
      SIG2A=SIG2A+SIGN(RND4,SIG2A)
      SIG2O=SIG2O+SIGN(RND4,SIG2O)
      WRITE(J,56)SIG2A,SIG2O
   56 FORMAT(//' VARIANCE A = ' F7.4 /'0VARIANCE O = ' F7.4)
      DELT=DELT+SIGN(RND4,DELT)
      SIG1=SIG1+SIGN(RND5,SIG1)
      ERRDT=ERRDT+SIGN(RND5,ERRDT)
      CONLT=CONLT+SIGN(RND5,CONLT)
      WRITE(J,57)DELT,SIG1,ERRDT,CONLT
   57 FORMAT(//' DELTA = ' F9.4 //' STD DEV OF X-VALUES = 'F9.5//
     1' STD ERROR OF DELTA = 'F9.5,5X,'CONFIDENCE LIMIT (95 PC) = 'F9.5)
  100 WRITE(J,104)LINE
  104 FORMAT(' ',////,60A2)
  105 READ(K,106)DELMT,NEXT,END
  106 FORMAT(9X,3A1)
      IF(DELMT-INDEL)105,107,105
  107 IF(NEXT-INDEL)105,108,105
  108 IF(END-INDEL)110,109,110
  109 CALL EXIT
      END
```

```
=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+= ER

RATIO MASS SPECTROMETER DATA ANALYSIS PROGRAM



SAMPLE DETAILS           C2 BCP1840-1870

REFERENCE DETAILS        WPR (C5)   28/7/76              CO2

REFERENCE VALUES             SAMPLE VALUES                     X-VALUES
     29.57                     62.27                            32.8350
     29.30                     61.94                            32.8600
     28.86                     61.57                            32.7400
     28.80                     61.91                            33.2500
     28.52                     61.46                            33.0750
     28.25


COARSE RATIO SETTING 0.580

RATIO OFFSET 0.000

MEAN X =  32.9520

CORRECTED MEAN =  0.003295

REFERENCE MEAN =  28.8780

CORRECTED REFERENCE MEAN =  0.002888

VARIANCE A =  0.0428

VARIANCE O =  0.0570

DELTA =    5.6532

STD DEV OF X-VALUES =   0.04096

STD ERROR OF DELTA =   0.01832     CONFIDENCE LIMIT (95 PC) =   0.05085


=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+=+= ER
```

APPENDIX C FORMULAE

C.1 FORMULA TO CONVERT $\delta_A(B)$ TO $\delta_B(A)$

From (i), $\delta_A(B) = \left[\frac{Y - Z}{Z}\right] \times 1000$

Therefore, $1000Y = Z\left[\delta_A(B) + 1000\right]$ (xLi)

Similarly, $\delta_B(A) = \left[\frac{Z - Y}{Y}\right] \times 1000$

Substituting (xLi) and rearranging,

$$\delta_B(A) = -\frac{1000\,\delta_A(B)}{\delta_A(B) + 1000} \qquad \text{(xLii)}$$

C.2 FORMULA TO CONVERT $\delta_A(B)$ TO $\delta_C(B)$ KNOWING $\delta_C(A)$

Similarly to (i), $\delta_C(B) = \left[\frac{Y - X}{X}\right] \times 1000$

where X is the true isotopic ratio of standard C.

Therefore, $1000Y = X\left[\delta_C(B) + 1000\right]$ (xLiii)

From (xLi) and (xLiii) $\frac{Z}{X} = \frac{\delta_C(B) + 1000}{\delta_A(B) + 1000}$ (xLiv)

Similarly to (i), $\delta_C(A) = \left[\frac{Z - X}{X}\right] \times 1000$

Substituting (xLiv) and rearranging,

$$\delta_C(B) = \delta_C(A) + \delta_A(B)\left[\frac{\delta_C(A)}{1000} + 1\right] \qquad \text{(xLv)}$$

APPENDIX D ISOTOPIC VARIATIONS IN NEW ZEALAND PRECIPITATION

Since 1961 over one hundred meteorological stations throughout the world have been involved in the International Atomic Energy Authority survey of the isotopic composition of monthly precipitation (I.A.E.A. 1969, 1970, 1971, 1973, 1975). The nearest such station to Hamilton is at Kaitaia, 340 km away in a north-north-west direction. I.A.E.A. publications (1969, 1970, 1971) give monthly data for Kaitaia over the period 1962 to 1966 inclusive. This data was used to calculate the weighted mean δD values for each month's precipitation over the 5 year period as follows:

Weighted mean δD value for a particular month's precipitation

$$= \frac{P_{1962} \times \delta D_{1962} + P_{1963} \times \delta D_{1963} + \ldots\ldots + P_{1966} \times \delta D_{1966}}{P_{1962} + P_{1963} + \ldots\ldots + P_{1966}}$$

where P_{1962}, P_{1963}, ..., P_{1966} are the precipitation totals for the particular month in 1962, 1963, ..., 1966 respectively; and δD_{1962}, δD_{1963}, ..., δD_{1966} are the isotopic compositions of the precipitation for the particular month in 1962, 1963, ..., 1966 respectively.

The results in fig. 36 show that on average precipitation is about 15$^{o}/oo$ enriched in deuterium in the summer as compared with the winter.

If the $\delta^{18}O$ data for Kaitaia are treated in a similar way, it is evident that the precipitation is about 2$^{o}/oo$ enriched in ^{18}O in the summer as compared with the winter.

Isotopic measurements have also been made on precipitation collected at Invercargill. This station is 1100 km south-west of Hamilton. Monthly isotopic data for the period 1962 to 1971 (I.A.E.A. 1969 to 1975) show that on average the precipitation is about 30^{o}/oo enriched in deuterium and about 7^{o}/oo enriched in ^{18}O in the summer as compared with the winter.

The Kaitaia and Invercargill data discussed above indicate that, on average, summer precipitation at Hamilton is likely to be about 20^{o}/oo enriched in deuterium with respect to winter precipitation.

Precipitation data also exists for Kaitoke, Christchurch and Nelson (M.K. Stewart, personal communication 1976). However, this data has been collected for too short a time to show significant summer/winter differences.

It should be noted that the summer/winter variations in isotopic composition of precipitation discussed above have been elucidated from long term averages. Data from individual months show very large fluctuations which are probably related to the direction of origin of storms, although some samples may have evaporated in the rain gauges before isotopic determination.

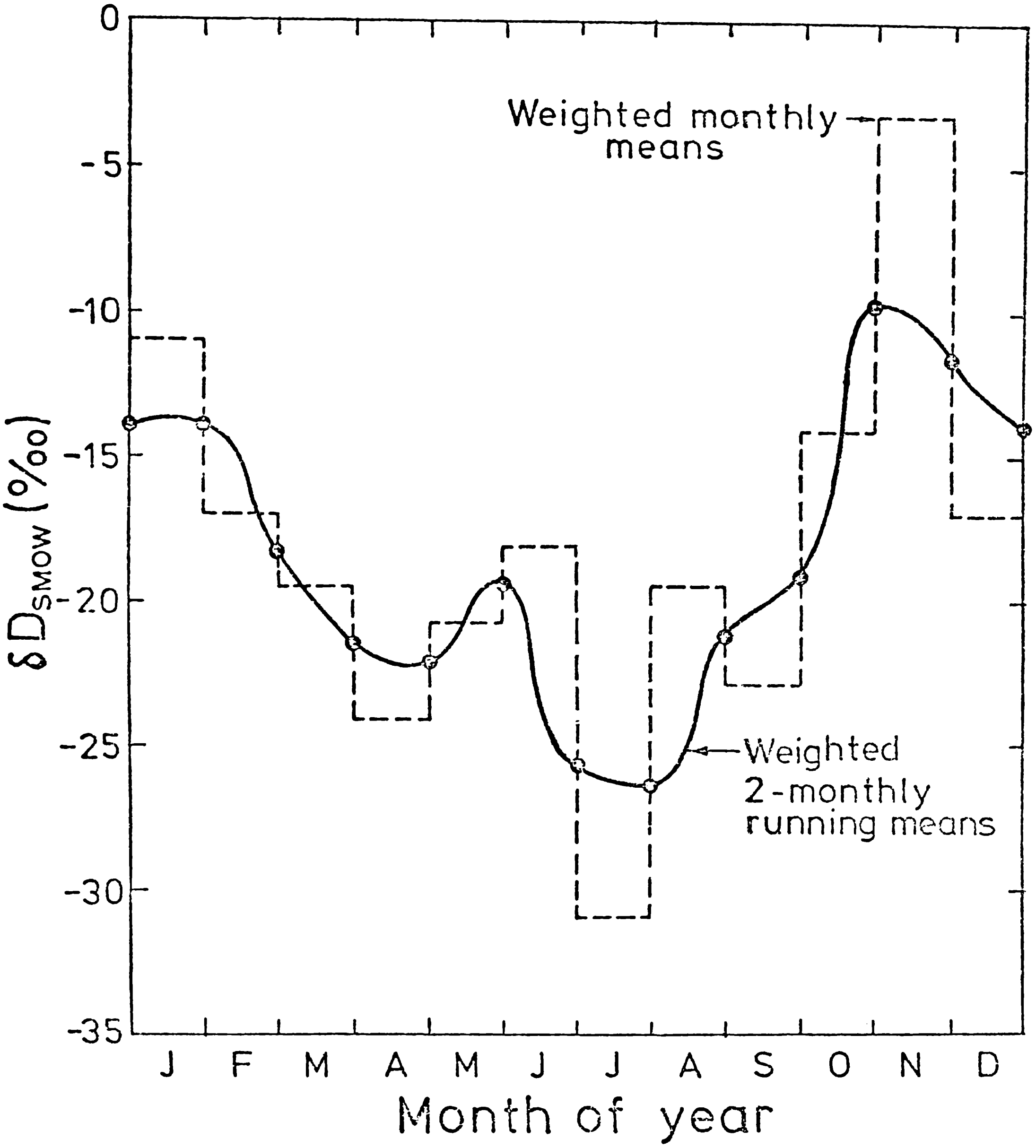

Fig.36. Weighted monthly mean δD values for precipitation at Kaitaia, New Zealand, for 1962 to 1966 inclusive - calculated using the Environmental Isotope Data books 1, 2 and 3 (I.A.E.A. 1969, 1970, 1971).

APPENDIX E TEMPERATURE MEASUREMENTS IN HAMILTON FOR THE PERIOD 1918 TO 1921

The mean temperatures shown in table 29 and fig. 37 were calculated using the observations of Mr. J. Bremner. His maximum and minimum daily temperatures for the centre of Hamilton were published with a few omissions in the *Waikato Times* (1918 to 1921).

Table 29 Temperature measurements in Hamilton, New Zealand, from January 1918 to March 1921.

Month of year	Mean maximum °C	Mean minimum °C	Month of year	Mean maximum °C	Mean minimum °C
1918 J	22.9	18.1	1919 S	16.4	9.3
F	22.9	17.8	O	18.2	11.7
M	23.6	16.2	N	17.6	12.3
A	19.9	14.3	D	19.9	12.9
M	16.1	10.4	1920 J	21.6	15.1
J	13.6	8.5	F	23.0	17.5
J	11.2	5.9	M	23.2	15.1
A	14.1	8.6	A	18.8	12.7
S	16.7	9.3	M	14.8	9.1
O	17.9	11.8	J	13.8	9.3
N	18.2	13.2	J	11.5	6.4
D	20.1	14.1	A	13.4	3.8
1919 J	20.2	14.6	S	16.5	6.0
F	23.5	17.7	O	20.6	6.9
M	23.1	14.1	N	20.9	9.3
A	17.5	10.6	D	25.4	10.1
M	15.1	8.3	1921 J	27.4	11.5
J	12.8	7.9	F	26.9	10.9
J	12.5	7.9	M	25.2	9.5
A	13.3	7.8			

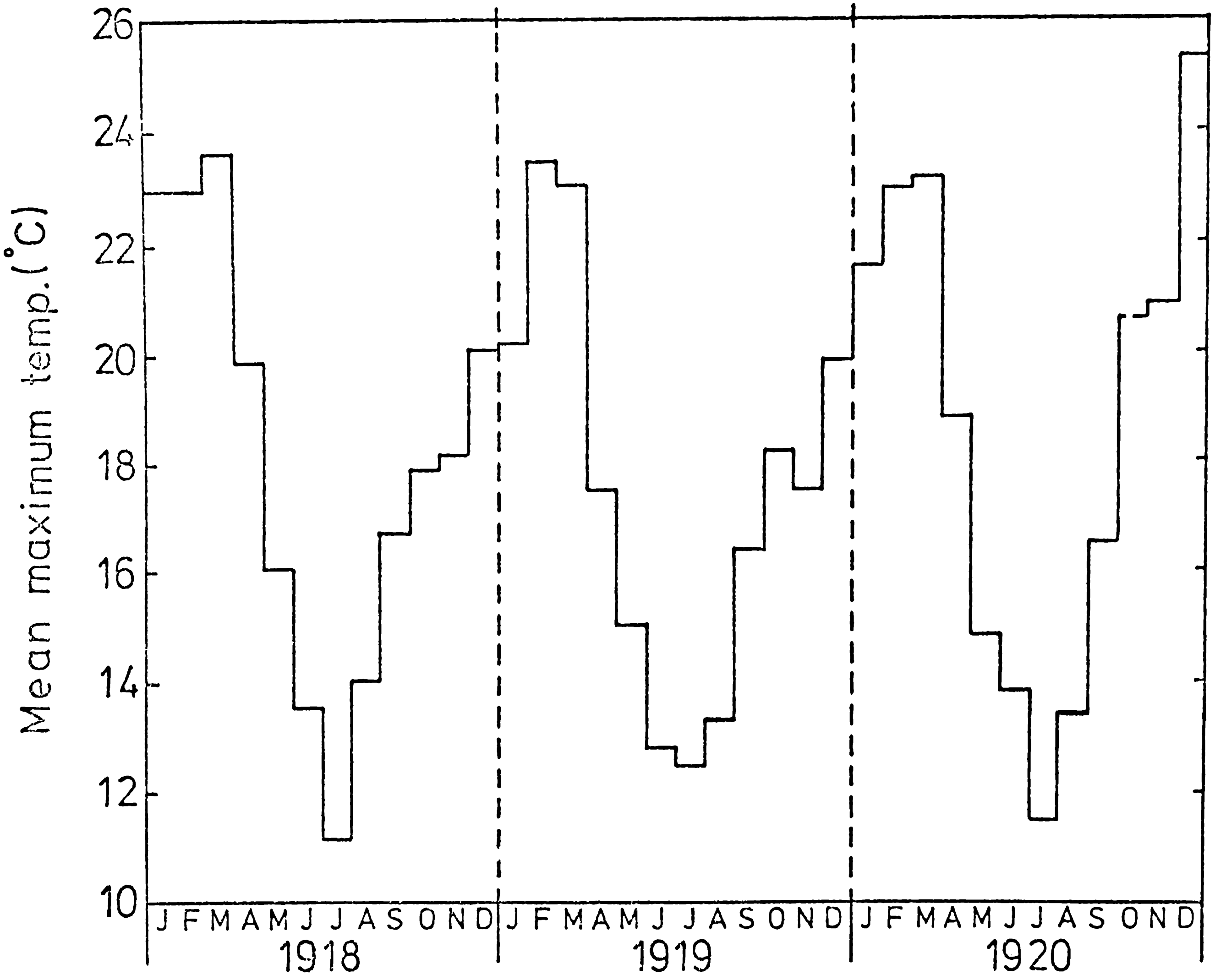

Fig.37. Monthly mean maximum daily temperature in Hamilton, New Zealand, over the period 1918 to 1920.

APPENDIX F GROWTH CHAMBER EXPERIMENT

An attempt was made to grow *Pinus radiata* seedlings under controlled conditions to study more closely the effect of temperature and humidity changes on the isotopic composition of the wood components. The experiments were carried out jointly with Dr. J. Troughton and Mrs. K. Card using their controlled growth chambers at the Physics and Engineering Laboratory, D.S.I.R., Lower Hutt.

The seedlings were grown on Hoagland's solution which was made up using distilled water of known D/H ratio. The humidity and D/H ratio of the growth chamber atmosphere were controlled by bubbling the dry air supply through distilled water with the same D/H ratio as the Hoagland's solution and at the same temperature as the growth chamber. Preliminary experiments showed that a large volume (~10 ℓ) of distilled water was required and that this had to be replaced twice a week in order to keep its D/H ratio virtually constant.

Unfortunately the experiment had to be abandoned because of frequent growth chamber breakdowns.

APPENDIX G CARBON-14 DETERMINATIONS ON *AGATHIS AUSTRALIS*

Jansen (1962a, 1969) has measured the ^{14}C content of the *Agathis australis* wood section that was used for $\delta^{13}C$ determinations (see section 13.5). He found that the shape of his curve relating ^{14}C contents to ring-dates agreed well with that found for many dendrochronologically dated Northern Hemisphere trees. However, the curve was displaced. Jansen (1969) suggested that this effect is either due to major absences of annual rings in *Agathis australis* or to inhomogeneities in the world-wide distribution of atmospheric radiocarbon in the past. Other measurements of the ^{14}C content of atmospheric and oceanic carbon dioxide since the start of nuclear testing show that mixing times for global atmospheric carbon dioxide are of the order of 10 years or less (see also section 11.2.1). Thus the displacement of Jansen's curve is almost certainly due to missing rings. This means that Jansen's carbon-14 dates can be corrected using the Northern Hemisphere measurements, and the relationship between these corrected dates and the ring count can be computed (see table 30 and fig. 38). The linear relationship shown in fig. 38 implies that the proportion of missing rings must be fairly constant throughout the wood section. The equation for the linear regression line is:

$$\text{Ring count "before 1960"} = -0.7325 \times \text{Date A.D.} + 1492$$

(correlation coefficient = 0.994).

This equation has been used to convert ring counts to year (A.D.) in table 30.

Table 30 Carbon-14 determinations on *Agathis australis*.

Ring count[†] "before 1960"		Jansen's (1962a) C-14 date (A.D.)	Corrected date[*] (A.D.)	
784		939	991	
715		1039	1083	
571		1200	1226	
472		1349	1357	
427		1414	1413	
391		1535	1516	
369	368	1583	1557	1572
366		1617	1586	
265	265	1740	1690	1689
265		1738	1688	
175		1865	1795	

[†] Based on the ring count that was used by Jansen (1962a).

[*] Calculated using the table of Damon *et al.* (1972).

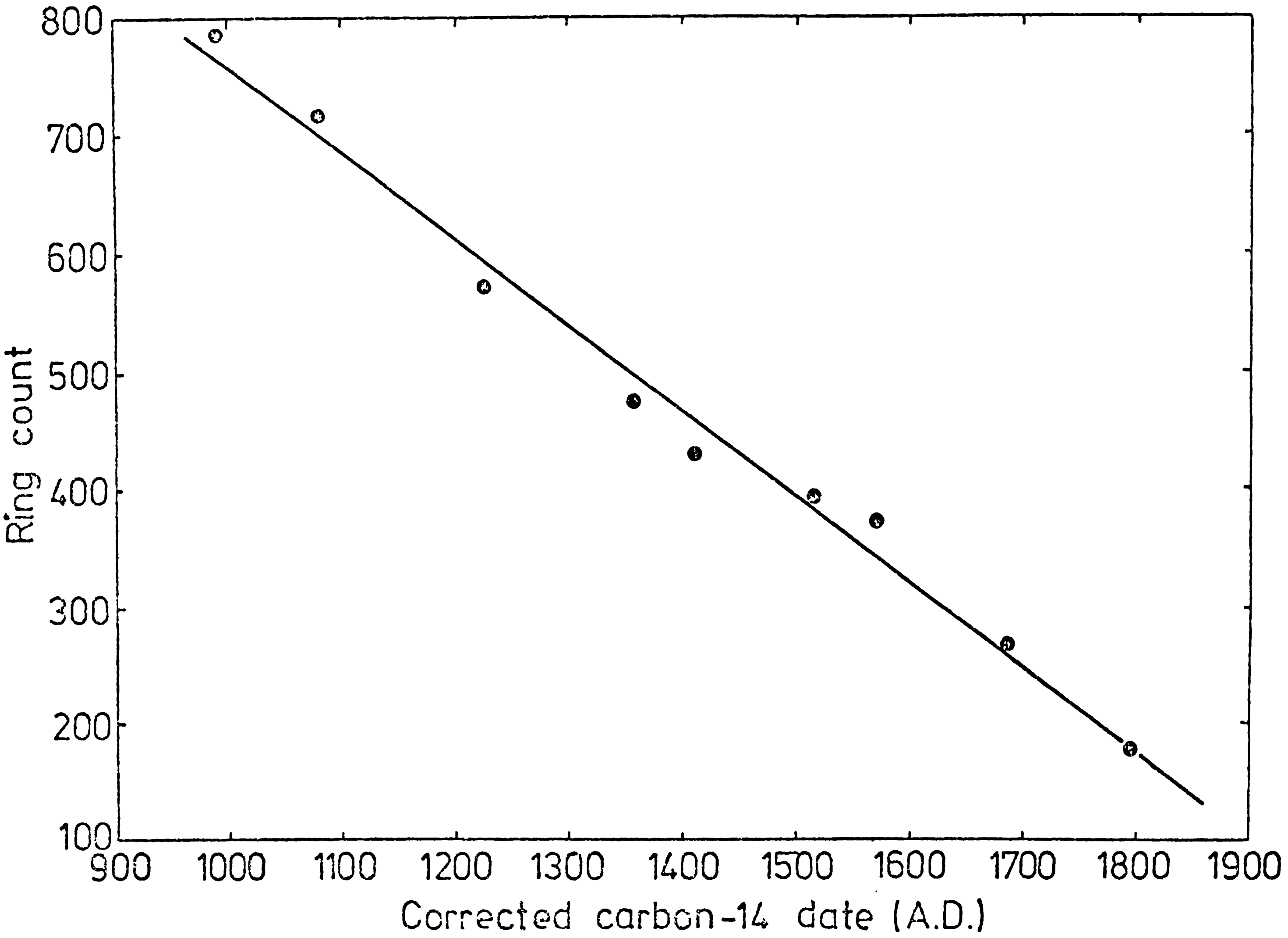

Fig.38. The relationship between corrected carbon-14 date and ring count for an *Agathis australis* tree - the data have been calculated from that of Jansen (1962b).

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