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Carbon-14 in Patagonian Tree Rings

Abstract. *The radiocarbon activity found in tree rings from southern Argentina shows secular fluctuations which are synchronous with and of the same amplitude as those known for the Northern Hemisphere. Comparable measurements indicate that the activity in Patagonian trees is about five per mil lower than in European trees.*

Natural radiocarbon allows dating of carbonaceous materials up to about 50,000 years old (1). One of the assumptions on which the method is based is the constancy of the specific activity of the isotope present in atmospheric carbon dioxide, independent of time and locality. A permanent record of the annual activity of the atmosphere has been kept by the carbon assimilated in the growth rings of trees. Analyses of Northern Hemisphere trees showed that the initial C^{14} content varies slightly with time (2-5), causing errors in radiocarbon dating of as much as 150 years

for the last millennium. It was expected that secular variations would show synchronism over the globe, but their existence still had to be demonstrated for the Southern Hemisphere. Furthermore, de Vries (2) reported a "local effect"—the C^{14} activity in a tree from Colorado apparently being about 1 percent higher than that in contemporary samples from central Europe. It was thus deemed desirable to undertake a worldwide survey of samples from different localities and dates. We now summarize our results from some Argentine trees and compare them with

those of other authors (see 2, 4, 6-8).

A survey in the rain forests of the north Patagonian Andes in 1961 was guided by the report of Schulman (9). Only unexplored regions offered possibilities of finding millenary trees (10). The main difficulty was that either the old trees have decayed wood in the center of the trunk or that they are completely hollow. Several specimens were obtained, however (11). They belong to indigenous species, *Fitzroya cupressoides* (lahuán, alerce), *Araucaria araucana* (pehuén, araucaria, pino de los Andes), and *Nothofagus dombeyi* (coigüe or coihue). The samples provided tree rings apparently spanning more than 1700 years (ascertained by ring counting alone). The real ages after A.D. 1400 should, however, be accurate to at most a few tens of years because of the agreement of the different C^{14} analyses at the three selected dates mentioned below. The specimens discussed here are named after the National Parks in which they grew. Los Alerces F 63: *Fitzroya* from the left bank of Arroyo Le Goufre (42°36'S, 72°3'W), west of Lake Cisne, at 550 m altitude. It had a diameter of 2 m and a rotten or missing pith of 40 cm. Its span of preserved year-rings (more than 1700) is the longest known from a Southern Hemisphere tree. It was felled in 1962 (12). Los Alerces F II: *Fitzroya* with a diameter of 65 cm and 300 rings, from the shore at the end of the southwest

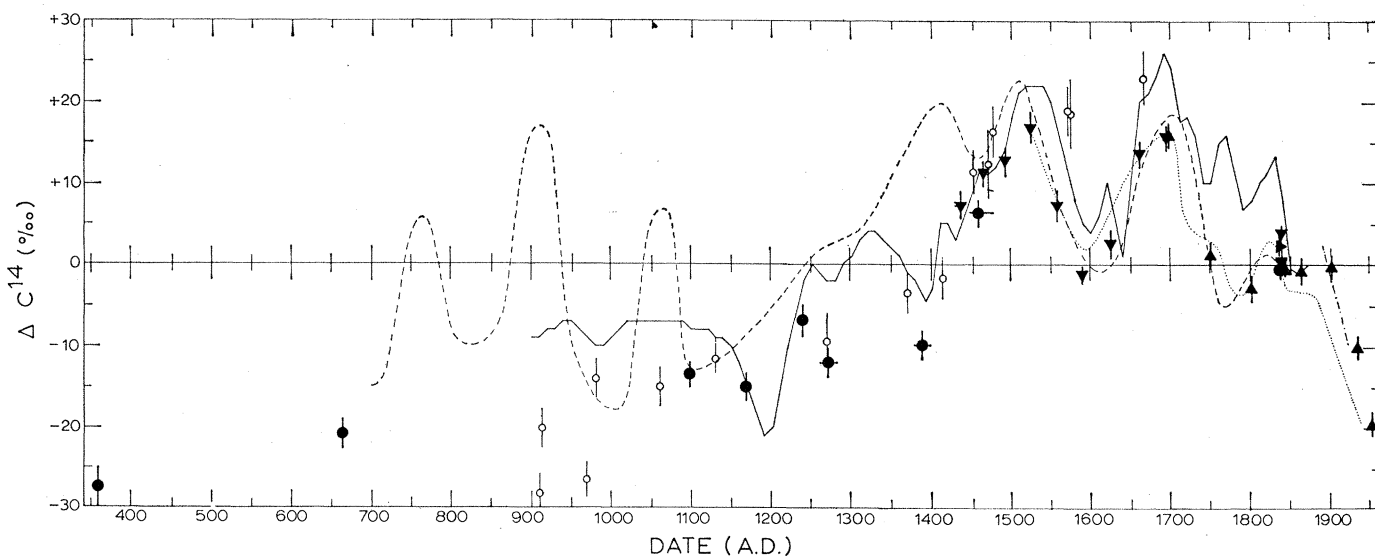


Fig. 1. Secular fluctuations of carbon-14 in tree rings from Patagonia compared with measurements of others. The absolute value of the reference axis for each series of data is explained in the text. The vertical error bars indicate the standard deviations (σ) calculated as the internal error of the mean value. The horizontal bars indicate, when the scale allows for it, the number of rings in the samples. Data from previous publications of Northern Hemisphere (Europe and North America) samples are represented by: dotted line, de Vries (2) corrected; dashed line, Willis *et al.* (4); dash and dot line, Lerman *et al.* (17). The Southern Hemisphere is represented by measurements of a New Zealand tree \circ by Jansen (7, 8), and of the Patagonian trees mentioned in the text: \blacktriangle , Los Alerces F II; \bullet , Los Alerces F 63; \blacktriangledown , Nahuel Huapi B; \blacktriangleright , Nahuel Huapi J; \blacksquare , Lanín.

branch of Lake Menéndez (42°45'S, 72°5'W), at 525 m altitude. It was felled in 1959 for forestry studies. Nahuel Huapi B: *Nothofagus* from near the road between Puerto Blest and Puerto Alegre (41°2'S, 71°49'W), west side, at 780 m altitude. The tree, known as the "Bisabuelo" (great-grandfather), fell during a storm in spring 1954. Its diameter was 2.5 m and the sampled section (12 m above root level) contains more than 530 rings. Nahuel Huapi J: *Nothofagus* with 185 rings, from the same site described for the previous sample. It was felled in 1966 (12). Lanín: *Araucaria* with a diameter of 90 cm and about 1000 rings, from the slopes near Lake Quillén (39°30'S, 71°20'W). It was felled in 1962 for the lumber mill at Estancia La Ofelia.

Each sample consisted of 30 g of wood with a number of rings that depends on their width around the desired date. Most of these analyses correspond to samples containing about ten adjacent rings. Only in a few cases was it necessary to use more rings (Fig. 1).

Each sample was cut into thin pieces, treated with dilute acid and alkali, and burned (13). Radiocarbon activities were determined in a quartz proportional counter with an active volume of about 8 liters (14). The accuracy of each analysis, defined as 1σ (standard deviation), varied from ± 1.5 to ± 2 per mil.

The initial (age-corrected) radiocarbon activity is deduced from the measured activity with the presently accepted half-life of radiocarbon, 5730 years (15). The relative deviation (designated as δC^{14}) of the age-corrected activity of the sample from the standard reference activity (95 percent of the oxalic acid standard sample of the National Bureau of Standards) is then derived. For the counter used, this standard activity is known within an uncertainty of ± 2.2 per mil. The ratio of C^{13} to C^{12} of the analyzed gas is used to correct δC^{14} for isotopic fractionation (16). The corrected deviation (designated as ΔC^{14}) is obtained with the usual formula (17).

Our results have been compared with those of others (Fig. 1). For an absolute comparison a better standardization between laboratories is needed than has been realized thus far.

In Fig. 1 the curve of de Vries (2) has been modified as follows: (i) it is now referred to the oxalic acid radiocarbon standard, and (ii) the correction

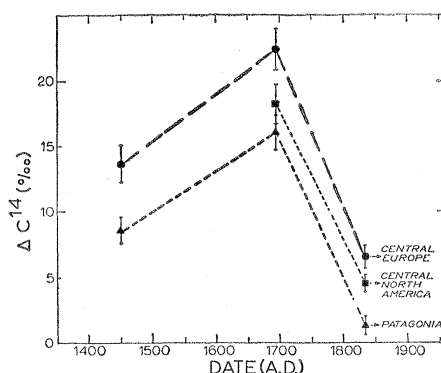


Fig. 2. Continental or latitudinal effect in the C^{14} concentration in the atmosphere as measured in Patagonian and Northern Hemisphere trees. The values represented have been corrected for secular variations. The dashed lines are only intended as a visual aid.

for isotopic fractionation has been recalculated on the basis of new C^{13}/C^{12} analyses (14). The dashed line is the curve of Willis *et al.* for a *Sequoia gigantea*. Although it was originally intended as a visual aid in following the fluctuations (4), it is reproduced here to compare the new results with the general trend. The normalization refers to their sample of A.D. 1859. The solid line drawn in the same figure represents a smoothed version of Suess' results for North American and European trees. This curve has been referred to the average age-corrected activity of a number of tree rings dating between A.D. 1860 and A.D. 1880 (6). The open circles show the C^{14} fluctuations in a Kauri tree (*Agathis australis*) from New Zealand. These values have been recalculated from the original analyses of Jansen (7), on the assumption that the section had lost 150 instead of 30 rings by the action of fire (8). They have been normalized to the oxalic acid standard. The dash and dot line represents the average activity around A.D. 1910 as determined in rings of a Norwegian tree (17).

The new measurements differ from those of Suess (6) and Willis *et al.* (4) for the period before A.D. 1400. In general our points lie lower than theirs, and we found no deviation above the reference axis. This apparent depletion of C^{14} might result from missing rings in the analyzed radius of the *Fitzroya*.

After A.D. 1400 the number of measurements is larger. They show fluctuations similar to those found by de Vries and Suess. Little can be deduced from the fact that they do not exactly coincide, due to difficulties in comparing

results from different laboratories to an accuracy better than a few per mil, and because the statistical uncertainties of the measurements are of the same order of magnitude. Since an accurate comparison has to be undertaken in one and the same counter, the present analysis has been complemented by measuring tree rings of the same age from several other localities. Three dates were selected for this purpose: about A.D. 1835, 1695, and 1450. The latitudinal or continental differences can be only briefly summarized here. In Fig. 2 the radiocarbon deviations of three somewhat restricted regions are shown: central Europe (Germany), central North America (Colorado and Arizona), and north Patagonia (Argentina) (14). The Patagonian samples have a radiocarbon content that is on the average 5.3 ± 1.3 per mil lower than in tree-ring samples from Europe; the North American samples deviate by about half of this value (1 per mil less C^{14} is equivalent to apparently 8 years older) (18).

We conclude that (i) after A.D. 1400 the secular variations in Patagonia are, within the limits of accuracy of our measurements, coincident in phase and amplitude with those of the Northern Hemisphere, and (ii) there is a statistically significant indication that the radiocarbon content in Patagonian trees is lower than in European trees.

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- The tree samples were secured (1961–63) by the Groningen (Netherlands), Uppsala (Sweden), and Bariloche (Argentina) physics laboratories. The Argentine Comisión Nacional de Energía Atómica and Universidad de Cuyo offered the facilities of their Centro

- Atómico and Instituto de Física, located at San Carlos de Bariloche, Argentina, which were used as base for the operation led by Ake Nilsson-Vinterbäck, from Uppsala, then visiting professor at Bariloche. I. U. Olsson, of Uppsala, helped with the selection of the samples. The Argentine Dirección General de Parques Nacionales offered the assistance of the three Intendencias of Parque Nacional Los Alerces, Lanín and Nahuel Huapi. B. Arschanow of the last park joined some expeditions and analyzed the cores, and E. Rodríguez of the Administración General de Bosques, Buenos Aires, did the first ring counting of the sections, later recounted by M. Munaud (Louvain).
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X-ray Resistant Cell Required for the Induction of in vitro Antibody Formation

Abstract. *Mouse spleen cells were separated into two populations on the basis of adherence to plastic. The recombination of these two populations was required for the in vitro antibody response to sheep erythrocytes. By separating the two populations from x-irradiated mice and combining each with the other population prepared from normal mice, it was demonstrated that the immunologic function of the nonadherent population only was highly sensitive to x-ray injury. In contrast, x-irradiation in doses as high as 1000 roentgens had no measurable effect on the immune function of adherent cells, that is, the population which first interacts with antigen and is composed principally of large phagocytic cells.*

The remarkable sensitivity of the immune response to x-ray (1) has been attributed to injury to macrophages, specifically to antigen "processing" by such cells (2). The present experiments, in which the in vitro response of mouse spleen cells to sheep erythrocytes was used (3), fail to support this suggestion.

Spleen cells were separated into two different populations on the basis of their adherence to plastic (4). The adherent population, which comprises about 10 percent of the total cells, consists primarily of macrophages; the nonadherent population is composed primarily of small lymphoid cells. The recombination of both the adherent and nonadherent populations is required for the in vitro antibody response to sheep erythrocytes. Equally high in vitro responses are obtained whether sheep erythrocytes are added to the recombined populations and remain present during the 4 days of the response or are added only briefly to the adherent population. In this latter procedure, the adherent population is washed free of all detectable extracellular antigen before being combined with the nonadherent population; thus the first cell-antigen interaction is limited to the adherent population.

The following experiment demonstrated that the kinetics of suppression by x-ray was similar for the in vivo and in vitro responses. One group of mice was x-irradiated with doses of from 25 to 250 r and then immunized with sheep erythrocytes; a second group of similarly x-irradiated animals was killed and their spleen cells were immunized in vitro. After 4 days the number of spleen cells releasing hemolytic antibody (5) was measured in the living animals and also in the cultures (Table 1). When the radiosensitivity of the in vitro response was established, the two populations of cells separable in vitro were examined to determine the cell type impaired by x-ray. Two reciprocal experiments were performed: (i) adherent cells from x-irradiated mice were combined with nonadherent cells from normal mice and (ii) adherent cells of normal mice were combined with nonadherent cells from x-irradiated mice. These experiments demonstrated that the immunologic function of the nonadherent population alone was sensitive to x-irradiation.

In one such experiment, spleen cells were pooled from six female DBA/2J mice that had received 500 r total body x-irradiation 1 hour previously (6). The spleens were teased apart in Hanks balanced salt solution. Large cell aggregates, removed by 3-minute gravity sedimentation, were discarded. The suspension of remaining single cells was washed once, resuspended to a concentration of 1×10^7 cells per milliliter in modified Eagle's minimal essential medium with 10 percent fetal calf

Table 1. X-ray suppression of the in vivo and in vitro plaque-forming cell response. Groups of mice were irradiated 1 hour before either being injected intravenously with 2×10^8 sheep erythrocytes or killed and their spleen cell suspensions immunized in vitro with the same antigen. The in vitro and in vivo response were measured 4 days later.

X-ray dose	Plaque-forming cells	
	In vivo*	In vitro†
None	155	145
25	99	58
50	46	42
100	36	12
175	8	3
250	2	0

* The number recorded $\times 400$ is the number of plaque-forming cells per spleen. The number is the average count of six slides; the slides were duplicates from three mice. † The number recorded is plaque-forming cells for 10^8 spleen cells initially cultured per dish. The number is the average count of eight slides; the slides were duplicates from each of quadruplicate cultures.

serum (MEM), and dispersed in 1.0-ml portions in Falcon plastic petri dishes (35 by 10 mm). The dishes were then incubated for 30 minutes (7). Nonadherent cells were removed from these dishes by aspiration, pooled, and reincubated in new petri dishes for 30 minutes to remove any remaining adherent cells. The nonadherent cells were again pooled, sedimented by centrifuga-

Table 2. Plaque-forming cell response of various combinations of cell populations obtained from normal and x-irradiated mice. Adherent and nonadherent cell populations were obtained from mice 1 hour after irradiation with 500 r, or from normal mice. The plaque-forming cell response was measured at 4 days. Each number is the average of counts on six slides; the slides were duplicates from each of the triplicate cultures. In vivo controls included three mice receiving the same dose of x-ray and three normal mice; the six mice were injected intravenously with 2×10^8 sheep erythrocytes. Duplicate slide counts were made on each spleen 4 days after immunization. Spleens of x-irradiated mice contained 60 ± 49 plaque-forming cells; spleens of the control mice contained $56,400 \pm 12,600$ plaque-forming cells.

Combination	Plaque-forming cells
Normal adherent cells + normal nonadherent cells	197
Normal adherent cells + x-irradiated nonadherent cells	18
X-irradiated adherent cells + normal nonadherent cells	156
X-irradiated adherent cells + x-irradiated nonadherent cells	0
Normal adherent cells only	7
Normal nonadherent cells only	7
X-irradiated adherent cells only	0
X-irradiated nonadherent cells only	0
Normal spleen cells unseparated	170
X-irradiated spleen cells unseparated	0
Normal spleen cells unseparated (no antigen)	0
X-irradiated spleen cells unseparated (no antigen)	0