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$^{13}C/^{12}C$ and $^{15}N/^{14}N$ variations among size-fractionated marine particles: implications for their origin and trophic relationships

G. H. Rau[1,*], J.-L. Teyssie[1], F. Rassoulzadegan[2], S. W. Fowler[1]

[1] International Laboratory for Marine Radioactivity, International Atomic Energy Agency, MC-98000 Monaco
[2] Station Zoologique, BP 28, F-06230 Villefranche-Sur-Mer, France

ABSTRACT: Differences of up to 5‰ in $\delta^{13}C$ and $\delta^{15}N$ were consistently observed across a size spectrum (< 3 to > 150 µm) of suspended particulate organic material (SPOM) sampled from a coastal Mediterranian site over a 10 mo period. On each of 4 sampling dates, lowest values of $\delta^{13}C$ and $\delta^{15}N$ (to −25.3 and −0.5‰, respectively) were always found in size fractions < 8 µm, with higher values in larger particles. Relationships among SPOM $\delta^{13}C$, $\delta^{15}N$, and C/N suggest that isotope fractionation associated with plankton C and N cycling is responsible for the isotopic variations observed. The relative ^{13}C and ^{15}N depletions present in the smallest but most abundant SPOM size fractions are consistent with the view that nano- and picoplankton constitute a sizable, low-trophic-level component of marine SPOM.

INTRODUCTION

The combined measurement of ^{13}C and ^{15}N natural abundances has proven useful in identifying sources of marine organic matter (e.g. Peters et al. 1978, Macko 1983, Wada et al. 1987a), sources of nutrition for marine food webs (e.g. Rau et al. 1981, Rau 1985), and trophic relationships among marine organisms (e.g. Rau 1982, Wada et al. 1987b, Fry 1988). Most of these studies, however, focused either on bulk organic material (net plankton, bulk filtrations, total surface sediments, etc.), or on invertebrate or vertebrate consumers. There has been relatively little characterization of either ^{13}C or ^{15}N natural abundances among discrete size classes of suspended particulate organic matter (SPOM), some fraction of which is composed of nano-, pico-, and microplankton.

Given the real or imagined trophic and feeding differences occurring across these plankton size classes (e.g. Sieburth et al. 1978, Azam et al. 1983, Williams 1984, Rassoulzadegan & Sheldon 1986, Sherr et al. 1986, Hagström et al. 1988), there appears to be considerable potential for isotopic heterogeneity to exist within bulk SPOM size classes. This assumes that isotope fractionation effects evident higher in the marine food-web can be extrapolated to smaller organisms, and that these organisms or their remains are quantitatively important constituents of SPOM. Systematic increases in animal $\delta^{13}C$ and especially $\delta^{15}N$ as a function of trophic level have been repeatedly found in both terrestrial and marine food-webs (e.g. McConnaughy & McRoy 1979, Rau et al. 1983, Fry et al. 1984, Gearing et al. 1984, Minagawa & Wada 1984, Schoeninger & DeNiro 1984, Fry 1988). There is some uncertainty as to the cause of these trophic level isotope enrichments, but it seems likely that isotopically selective excretion and respiration of the lighter isotopes of C or N (i.e. ^{12}C or ^{14}N) are largely responsible (e.g. Minagawa & Wada 1984, Checkley & Entzeroth 1985). In any case, these or related isotopically fractionating processes (e.g. Macko & Estep 1984, Macko et al. 1987) associated with heterotrophy within the so-called marine 'microbial' food-web could alter isotope abundances across these trophic levels and thus across plankton and particle size classes.

A second factor relevant to SPOM isotope abundances is that the initial formation of this material (primary production) can occur within several different plankton size classes, most notably those containing cyanobacteria and those containing the larger eucaryotic algae (e.g. Beers 1986). Because isotope effects associated with carbon fixation and inorganic nitrogen assimilation are often large and taxon-specific (e.g. Wong &

* Present address: Institute of Marine Sciences, University of California, Santa Cruz, California 95064, USA

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Sackett 1978, Wada 1980), the size distribution and activity of different phytoplankton species could also cause isotopic differences among plankton and therefore SPOM size classes.

While these isotope effects associated with marine primary and secondary production could impart differences in C and N isotope abundances across SPOM size classes, other processes may act to ameliorate such differences. As noted in the model of Azam et al. (1983), organic matter can be transferred from larger to smaller organisms via dissolved organic matter (DOM) excretion and subsequent uptake by smaller consumers such as bacteria. Physically or biologically mediated fragmentation of larger particles/organisms could form smaller particles that would presumably retain the isotopic signature of the larger forms. Conversely, smaller particles/biota can aggregate to form larger entities. Such recycling and mixing of organic C and N across particle/consumer size classes would serve to 'homogenize' isotope abundances within the suspended fraction. In the extreme case, such mixing would result in no isotopic trends with plankton or particle size.

There are relatively few data with which to investigate such isotope/particle-size scenarios. Bishop et al. (1977) reported some significant differences in $\delta^{13}C$ in a single upper-ocean profile of < 1, 1–53, and > 53 µm SPOM from the equatorial Atlantic. The lowest values (to −26.4 ‰) were attributed to the presence of bacteria. In a study in Narragansett Bay (Rhode Island, USA), Gearing et al. (1984) observed that the $\delta^{13}C$ of the zooplankton-dominated samples (> 150 µm) averaged 0.6 ‰ higher than phytoplankton-dominated samples (< 64 µm) over a 1 yr period. Unfortunately, no $\delta^{15}N$ measurements were made in either of these studies. Wada et al. (1987a) reported relatively uniform $\delta^{13}C$ values across size fractions ranging from < 63 to > 2000 µm sampled from terrestrially impacted Japanese coastal surface sediments. Considerably greater variability was seen in the associated $\delta^{15}N$ values, with the lowest value found in the < 63 µm fraction. It is not clear whether these trends in sediment isotope abundances are also characteristic of suspended particles. Altabet (1988) found $\delta^{15}N$ differences of up to 2.5 ‰ among upper-ocean SPOM size classes ranging from < 1 to > 75 µm collected from a site in the Sargasso Sea. At any given depth, lowest $\delta^{15}N$ values were usually found in the 1–75 µm size fraction with higher values present in the smaller and larger particles. No $\delta^{13}C$ measurements were conducted.

Given (1) the lack of knowledge about ^{13}C and ^{15}N abundances among SPOM size classes, and (2) the possible information such measurements might offer regarding the origins of marine SPOM and the trophic interactions leading to its formation, we sought to develop and employ a method of size-fractionating suspended marine particles (ranging from < 3 to > 150 µm) in amounts sufficient for stable isotopic analyses.

METHODS

At a site ca 5 km ESE of the port of Monaco (43° 42'N, 07° 29'E), 140 l of surface seawater were obtained by filling 4 copiously rinsed 35 l Nalgene carboys. Full carboys were then returned to the laboratory and their contents filtered through a series of 2 Nytex screens (150 and 20 µm respectively), two 142 mm diameter Nuclepore filters (8 and 3 µm), and finally a 130 mm diameter glass fiber filter (Sartorius). The screens and filtering apparatus (without filters) had been previously flushed with ca 5 l of distilled water. Filters were then introduced and the filtering proceeded by siphoning the contents of each carboy through the 150 and 20 µm screens into a 5 l glass reservoir from which water was simultaneously removed by vacuum and passed successively through the 8 µm, 3 µm, and glass fiber filters. The filtrate was collected in a downstream 10 l glass reservoir kept under vacuum (down to 630 mm of Hg below atmospheric pressure) by mechanical vacuum pump. When this reservoir became full the filtering process was stopped by first isolating the filter manifolds using 2 stopcocks and then stopping the siphoning from the carboy with a clamp. The vacuum pump was then stopped and vented and the filtrate in the reservoir discarded. The filtering process was restarted by reversing the stop-filtering procedure. After ca 70 l had been filtered as above, the Nuclepore filters were carefully removed from each manifold and replaced with new filters. The filters containing particulates were each placed in separate glass flasks containing 1 to 2 l of filtered seawater. This allowed resuspension of the particulates initially retained by the Nuclepore filters. Based on our own observations, this process liberated 90 to 100 % of the particles originally retained on the Nuclepore filters. This resuspension process was repeated with the second batch of filters after filtration of the remaining 70 l of seawater.

When the above was completed, each of the solutions containing resuspended particles were separately filtered through a 47 mm diameter, precombusted, Whatman GF/F filter. This filtration was followed by a rinse of ca 10 ml of distilled water through the filter prior to isolation from vacuum and removal of the filter. Similarly, the particles retained on the 150 and 20 µm Nytex screens were rinsed into glass containers and these solutions each filtered through separate GF/F filters as above. Each of the above filters together with the remaining Sartorius glass fiber filter and several unused (blank) filters were then placed in separate

clean filter containers, submerged in 0.5 N HCl to remove inorganic carbon, dried at 50 °C for at least 24 h, and stored in a desiccator prior to isotope analyses. The above sampling and filtration process was conducted on 4 dates; 1 July, 29 September, 16 December 1987, and 6 April 1988.

Because of concerns over possible sample alteration (and subsequent isotopic changes) imparted by the filtration procedure, we tested what effects vacuum intensity and distilled water rinsing may have on the isotopic abundances in suspended particulates. Two types of particle solutions were chosen for this test, one being local surface seawater and the second a suspension of *Dunaliella tertiolecta* cells. In the first case, 10 l of fresh surface seawater was filtered through a precombusted, 47 mm diameter, Whatman GF/C glass fiber filter using maximum vacuum (down to −630 mm Hg). Air was allowed to be drawn through the filter at the end of the filtration, and this filtering was followed by a filter rinse under vacuum with ca 10 ml of distilled water. The filter was then removed from the manifold, a new filter inserted, and the sampling replicated with another 10 l of fresh surface seawater. A second set of replicate filtrations was conducted under reduced vacuum (< 250 mm Hg below atmospheric) without allowing air to be drawn through the filter at the end of the filtration, and without rinsing with distilled water.

In the second test, 250 ml aliquots of seawater containing 5×10^3 *Dunaliella tertiolecta* cells ml^{-1} were filtered under conditions identical to the above. An additional third set of replicate filtrations was conducted using a vacuum that did not go below −25 mm Hg, without air passage, and without distilled water rinsing at the end of the filtration. All of the preceding filters and several unused (blank) filters were then treated and stored as for the filters from the 140 l seawater filtrations.

Subsequently, each sample or blank filter (or subsamples in the case of the 130 mm glass fiber filters) was loosely encased in precombusted silver foil and placed in a precombusted quartz tube (7 mm i.d., 30 cm long, one end sealed). Approximately 1 g each of organic-free CuO and Cu particles (30 mesh) was also placed in each tube and the contents put under vacuum for about 4 h. The tubes were then sealed using a gas-oxygen torch, and heated to 800 °C for 4 h in a muffle furnace. The resultant CO_2 and N_2 gases within each sample tube were then cryogenically purified and separated, and their volume measured manometrically. The stable isotope abundances in these gases were analyzed using a Nuclide 6–60 ratio mass spectrometer whose inlet system had been modified to accomodate small-volume gas samples. By convention, the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of each sample is reported as the relative per mil (‰) difference between the sample ratio and the ratio of a standard. That is:

$$\delta X = [R(\text{sample})/R(\text{standard}) - 1] \times 1000\ (‰)$$

where X = ^{13}C or ^{15}N; R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$; and standard = PDB carbonate or air N_2, respectively. The analytical precision (1 standard deviation) of these measurements was < 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. Raw m/z 45:m/z 44 ratios were corrected for extraneous m/z 45 in the form of $^{12}C^{16}O^{17}O$. Additionally, each N_2 gas sample was 'scanned' for the presence of NO and O_2, and in no case were these molecules found in abundances significantly above background. While this method determines the $\delta^{15}N$ of the total particulate N in each sample, we assume that the nitrogen analyzed is predominantly if not entirely organic N. Blank filters typically yielded 5.0 and 0.3 µmol of CO_2 and N_2, with $\delta^{13}C$ and $\delta^{15}N$ values averaging −27.9 and +0.2‰, respectively. All raw isotope values were subsequently blank-corrected.

RESULTS AND DISCUSSION

The $\delta^{13}C$ and $\delta^{15}N$ values of the SPOM size classes isolated ranged from −25.3 to −19.8‰ and from −0.5 to +5.7‰, respectively (Fig. 1A, B). The experiments on isotopic variations in SPOM caused by filtration gave no evidence that this isotopic variability was an artifact of filtering. Isotopic variations of < 1‰ (with most $\delta^{13}C$ and $\delta^{15}N$ values varying by < 0.5‰) were observed for both bulk SPOM and *Dunaliella tertiolecta* cells when subjected to widely differing filtering and rinsing procedures. Furthermore, the lack of significant across-particle-size deviations in relative elemental abundance, C/N (Fig. 2C), supports the view that elemental/biochemical fractionation was not produced by our filtration procedure, and therefore cannot explain the isotopic variations observed. We conclude that the isotope abundances we measured are representative of the SPOM size classes within the water samples collected.

The SPOM $\delta^{13}C$ and $\delta^{15}N$ values are well within the range of values previously reported for marine particulates (e.g. Fry & Sherr 1984, Owens 1987). However, it is striking that such wide ranges of values were consistently found within single 140 l seawater samples from the same location in all 4 seasons. For comparison, a mean $\delta^{13}C$ variation of 4‰ across SPOM size classes (Fig. 1A) approximately equals the range of values previously reported for temperate ocean net plankton (e.g. Rau et al. 1982). A comparable variation in SPOM $\delta^{15}N$ (Fig. 1B) also represents a sizable portion of the range commonly encountered in surface water plankton (Owens 1987).

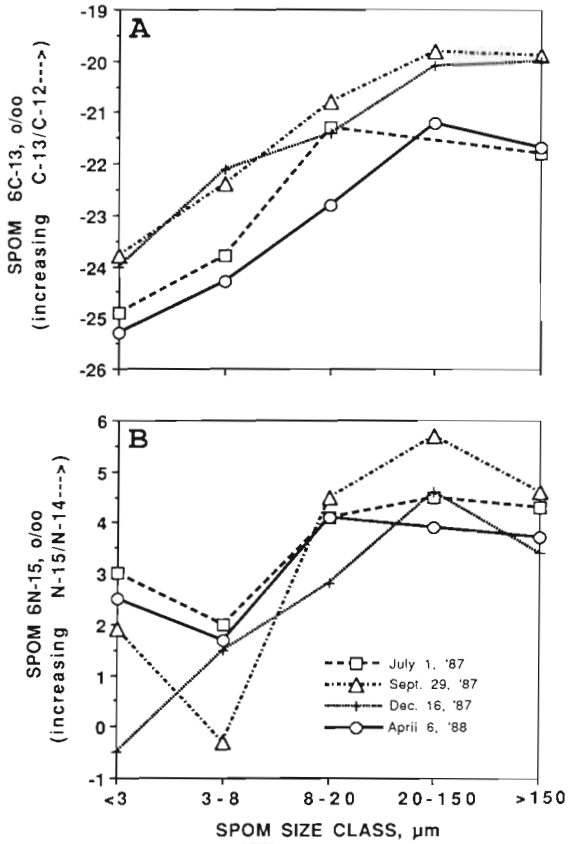

Fig. 1. (A) $\delta^{13}C$ and (B) $\delta^{15}N$ of size-fractionated particles contained in 140 l surface seawater samples collected ca 5 km ESE of Monaco. Isotopic trends across SPOM size classes for each of the 4 sampling dates. Size class ranges in µm denote nominal SPOM size retained or passed by the filters or screens employed

The majority of the carbon and nitrogen analyzed in our samples was contained in the size fractions < 8 µm (Fig. 2A, B), and at each sampling date these fractions were consistently depleted in ^{13}C and ^{15}N relative to the larger particles (Fig. 1). If our data are representative, they suggest that the $\delta^{13}C$ and $\delta^{15}N$ commonly measured for net plankton (e.g. > 150 µm) provide a considerable overestimate of the isotope abundances contained in the total SPOM. We also point out that because of the porosity of the Sartorious glass fiber filters used (similar to Whatman GF/A; nominal particle retention size 1.6 µm), most if not all SPOM < 1 µm in the water samples was not collected and analyzed in this study.

While we reject the hypothesis of isotopic homogeneity across the SPOM size classes we sampled, identifying the cause of the observed isotopic variability is more problematic. Several previous studies using combined $\delta^{13}C$ and $\delta^{15}N$ measurements on marine sedimentary particulates observed a strong positive correlation between these 2 parameters (e.g. Peters et al. 1978, Macko 1983, Wada et al. 1987a). These correlations were interpreted as the result of mixing of terrestrial and marine organic matter, the former having significantly lower $\delta^{13}C$ and $\delta^{15}N$ values than the latter. Such a mixing model may thus also explain the statistically significant positive correlation seen again in our data (Fig. 3), with the slope of the regression line (0.66) similar to that reported by Macko (1983) for coastal and shelf sediments from the NW Atlantic Ocean (slope = 0.58). Certainly, the close proximity of our sampling site to a well-populated shoreline makes it possible that at least some of the suspended particulate matter sampled did not have a marine origin.

However, if terrestrial/marine organic matter mixing were to explain the isotopic variations observed, we would expect concurrent shifts in C/N among particle sizes. Such is not evident in our data; none of the C/N ratios (< 10) are typical of land-derived material (Fig. 2C), and across particle sizes these ratios do not shift in parallel with isotope abundances (compare Fig. 2C with Fig. 1A, B). Significant negative correlations between stable isotope abundances and C/N would be anticipated (e.g. Wada et al. 1987a) when terrestrial/marine mixing is in operation. It therefore seems unlikely that the isotopic variability among our SPOM samples is the consequence of mixing processes involving non-marine SPOM inputs.

As mentioned earlier, correlated $\delta^{13}C$ and $\delta^{15}N$ increases are also typical among invertebrate and ver-

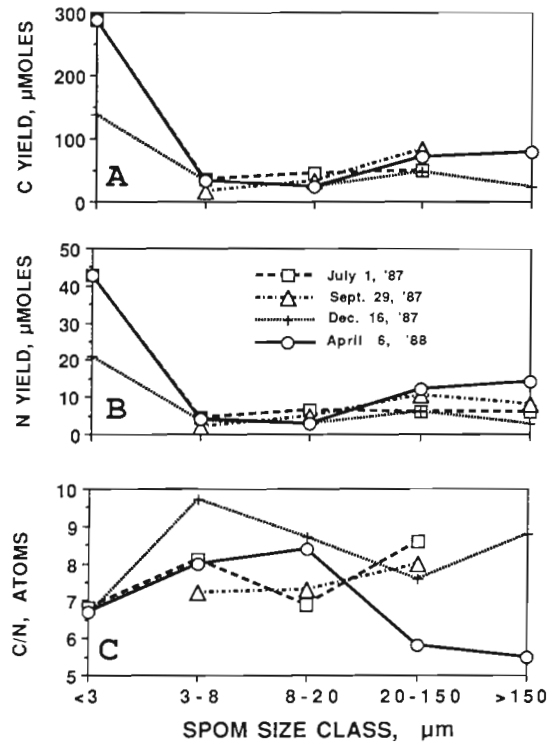

Fig. 2. (A) Carbon and (B) nitrogen yields, and (C) C/N for SPOM size classes indicated. Lines and symbols identify SPOM from one of 4 collection dates as in Fig. 1

tebrate consumers. Perhaps the best example of such a trend is provided by the work of Wada et al. (1987b) where $\delta^{13}C$ and $\delta^{15}N$ analyses were extensively conducted on an Antarctic marine food web that was likely devoid of any non-marine isotopic influences. Here upper-ocean consumer $\delta^{13}C$ and $\delta^{15}N$ values were highly correlated, with a linear regression line slope of 0.6. This relationship is very similar to that seen in our SPOM data (slope = 0.66; Fig. 3).

Previous research on waters near our sampling site (Rassoulzadegan & Sheldon 1986, Sheldon et al. 1986, Rassoulzadegan et al. 1988, Wikner & Hagström 1988) has identified a diverse and active consumer community within the pico- to microplankton size range sampled by this study. Indeed, enumeration of organisms contained in the 3–8 µm and 8–20 µm Nuclepore filter resuspensions from the 16 December sampling provides evidence that the SPOM size fractionations conducted were effective in separating certain biotic groups. In the 8 to 20 µm resuspensions, diatoms, naked oligotrichous ciliates, dinoflagellates, and tintinnids were found in concentrations of, respectively, 12.6, 0.93, 0.66 and 0.50 ($\times 10^3$) ind. l^{-1} resuspension solution (F. Rassoulzadegan unpubl.). In contrast, these organisms were undetectable in the 3–8 µm filter resuspensions.

Wikner & Hagström (1988) found nanoflagellates (< 5 µm) at an ambient seawater concentration of 0.68 cells $ml^{-1} \times 10^3$, with most individuals in the 1–3 µm size range. Similar abundances were found at the mouth of Villefranche Bay (15 km WSE of our sampling site) during our 16 December collection, and based on cell volume, nanoflagellates clearly dominated the resident microbial biomass at that time (F. Rassoulzadegan unpubl.). These organisms are known to be important consumers of bacteria (Rassoulzadegan & Sheldon 1986, Wikner & Hagström 1988), and size-selective predator exclusion experiments by these researchers led to the conclusion that 4 trophic levels (above bacterial) reside in SPOM < 12 µm. Based on measurements of invertebrate and vertebrate food chains (e.g. Fry et al. 1984, Minagawa & Wada 1984), a 4-level system should result in an across-food-chain $\delta^{13}C$ increase of around (4 × 1 ‰ =) 4 ‰, and similarly a $\delta^{15}N$ increase of (4 × 3 ‰ =) 12 ‰. While the observed $\delta^{13}C$ variation across SPOM size classes approximates that predicted (4 ‰; Fig. 1A), the corresponding change in $\delta^{15}N$ (3 to 5 ‰; Fig. 1B) is clearly below that anticipated by this model. A variety of factors may explain this discrepancy. These include: (1) our study sampled and analyzed size-fractionated SPOM (live + detrital organic material) rather than pure live biomass representing discrete trophic levels; (2) few if any organisms < 1 µm, representing important lower trophic levels (Wikner & Hagström 1988), were analyzed by our study; and (3) isotopic selectivity associated with microbial C and especially N uptake and release may be very different from (and more variable than) that observed in higher metazoan systems. The importance of this last point may be evident in the higher seasonal variation of $\delta^{15}N$ (relative to $\delta^{13}C$) in the < 8 µm size fractions analyzed (Figs. 1 and 3). With regard to the presence of live versus detrital organic material, the former component has been observed to comprise 60 to 80 % of the particles > 1 µm in nearby Villefranche Bay (Rassoulzadegan & Sheldon 1986, Sheldon & Rassoulzadegan 1987).

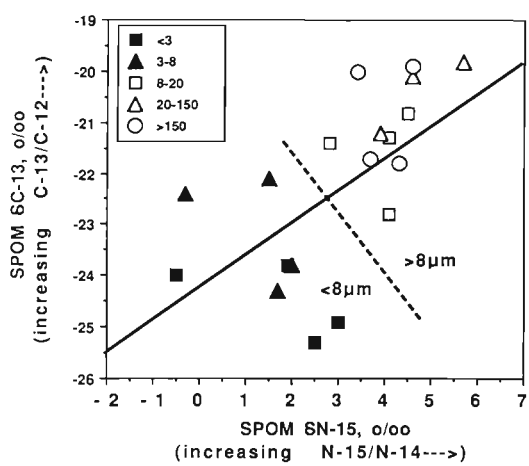

Fig. 3. $\delta^{13}C$ vs $\delta^{15}N$ for the SPOM size classes noted. The correlation coefficient, r, of these data is 0.63 and is significant (Ho: r = 0 is rejected) at the 95 % confidence level. The slope of the least-squares regression line (solid line) is 0.66. The dashed line separates isotopic data for SPOM > 8 µm

In conclusion, significant variations in $\delta^{13}C$ and $\delta^{15}N$ across marine SPOM size classes were found, and it is likely that these variations reflect the nature of C and N cycling and subsequent isotope fractionation within the resident microbial food web. If low $\delta^{13}C$ and $\delta^{15}N$ values are characteristic of low-trophic-level biomass, then it is evident from our data that this biomass is most abundant in the smallest (< 8 µm) size fractions. Further measurements of size-fractionated SPOM and/or associated biota may prove useful in elucidating the sources of and trophic relations among the abundant, small-sized organic particles that are ubiquitous in the marine environment.

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This article was presented by Professor P. J. Wangersky, Halifax, N. S., Canada

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