Detection of global carbon dioxide effects

SIR—The quest for clear evidence of effects of global carbon dioxide (CO_2) has been a high priority for a number of years. Climatologists have been diligently searching for signs of the worldwide warming predicted by various numerical models of the CO_2 greenhouse effect, while others have looked for the photosynthetic stimulation of the biosphere of which there have been experimental demonstrations.

Although most responsible climatologists are careful to say that the empirical climatic evidence, while consistent with their predictions, is by no means proof of them, there have been several valid claims of detection of a biological signal, until recently all based on the discrimination of an increasing amplitude in the seasonal CO_2 cycle, usually in the Mauna Loa, Hawaii, record, but also in a few other datasets. Now, however, a published study (*J. geophys. Res.* **92**, 5497; 1987) by I.G. Enting presents an important new piece of evidence for the reality of global photosynthetic stimulation due to the rising atmospheric CO_2 concentration.

Enting has analysed this interannual variation in the seasonal cycle of CO_2 concentration at Mauna Loa, verifying the tendency toward larger amplitudes in recent years and finding a strong correlation between amplitudes of spring peaks and the fall troughs that follow. But there is correlation between amplitudes of fall troughs and the following spring peaks.

After eliminating several other possible mechanisms, including fossil carbon release, ocean–carbon exchange and various atmospheric transport processes, Enting notes that, "of all the processes that significantly influence atmospheric carbon dioxide, only biotic processes seem to be capable of providing the requisite seasonal asymmetry". His interpretation of the spring-peak/fall-trough correlation, together with the fall-trough/spring-peak non-correlation, is that "the interannual variation in the amplitude of the seasonal cycle is associated with variations in individual summer growth seasons and that the carbon that is assimilated into the biota is released to the atmosphere over several years", which we know to be true.

The case for global CO_2 effects on worldwide vegetative productivity would therefore appear to be firm. We know, for instance, that the terrestrial biota is responsible for the seasonal cycle itself and that the amplification of the cycle with time appears to be explicable only in terms of CO_2-induced stimulation of photosynthetic activity. Now, it also appears that a unique asymmetry in the interannual variation in the seasonal cycle is also explicable only in terms of photosynthetic variations. Hence, we appear to have little recourse but to acknowledge the reality of this ubiquitous phenomenon, as many have already done. Indeed, as Morison (*Nature* **327**, 566; 1987) has recently noted with respect to a number of these studies, they emphasize "that the global rise in CO_2 is already having important effects on the biosphere".

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Detection of sickle cell anaemia and thalassaemias

SIR—Prenatal diagnosis for sickle cell anaemia and α-thalassaemia, first introduced[1,2] in 1975, now depends on the analysis of DNA, which is acquired either from fetal cells safely by amniocentesis at week 15 of gestation, or at week 7–10 by chorionic villus biopsy[3,4]. The chief impediment to widespread prenatal diagnosis has been that current techniques, based on Southern blot analysis or dot blot hybridization with radioactive globin-gene or oligonucleotide probes, are complicated and difficult to implement. We hope that the technique we describe here, which is simple, rapid and does not require radioactive isotopes, will be particularly useful in many of the countries where these two disorders are common.

The method is based on the recently described procedure for amplifying DNA *in vitro* by what is known as the polymerase chain reaction (PCR)[5,6]. By using the DNA polymerase from the thermophilic bacterium *Thermus aquaticus*[7], DNA synthesis can be performed at a high temperature, which so increases the specificity of the oligonucleotide priming that the amplified sequence can be directly visualized after staining as a discrete band on gel electrophoresis, circumventing the need for DNA hybridization or radioactive probes.

The usual mutation responsible for the severe form of α-thalassaemia, homozygosity for which results in the lethal condition of hydrops foetalis[9], is a deletion of approximately 23 kilobases of DNA within the α-globin gene cluster[10]. To detect this deletion, we primed DNA synthesis in the PCR with a pair of oligonucleotides that produce amplification of a 136-base-pair (bp) region of the α-globin gene cluster between the $\psi\alpha$ and α2-globin genes. This region was chosen because it lies outside the repeat regions[11] of a α-globin gene cluster and its amplification yields low background. We also added to the reaction mixture two primers that amplify a 110-bp segment of the β-globin gene, which served as a control. In amplified DNA from individuals with the Southeast Asian form of homozygous α- thalassaemia, only the control 110-bp β-globin gene fragment is detected, whereas in normal individuals both this and the 136-bp α-globin gene cluster fragments are visualized (Fig. 1).

To detect the sickle cell mutation, we amplified a 294-bp segment of the β-globin gene which extends from nucleotide −36 through the first exon to nucleotide 117 of the first intervening sequence. (The two primers used to amplify this segment will not amplify the δ-globin gene.) After amplification, the DNA fragments were digested with a restriction endonuclease (*Oxa*NI) that has a recognition site which is abolished by the A→T mutation at codon 6 in the sickle gene[12,13]. Thus, the segment derived from normal DNA is cleaved into two fragments of 191- and 103-bp, while the 294-bp fragment amplified from the DNA of a sickle cell anaemia patient remains uncleaved. In the heterozygote, all three fragments are visualized. These fragments can be detected either by ethidium bromide staining (Fig. 2, left), which requires visualization

Fig. 1 Polyacrylamide gel electrophoresis of amplified DNA from normal control (N) and two fetuses with hydrops fetalis (Hy). The amplified α-globin gene cluster fragment was 136 bp in length and the β-globin gene fragment 110 bp.

Methods Genomic DNA was isolated from white blood cells and amniotic cells [15]. The polymerase chain reaction was performed using a protocol modified from ref. 8 and New England Biolabs. Fifty picomoles of each pair of oligonucleotide primers was mixed with 1 µg of genomic DNA in a 100-µl volume reaction mixture according to the suggestions of the manufacturer (New England Biolabs). The mixture was incubated at 95°C for 5 min to separate the DNA strands, spun in a desktop centrifuge for 10 s and 2–3 units of *Thermus aquaticus* DNA polymerase were added. The mixture was sealed under 35 µl of mineral oil and incubated first at 63°C for 45 s, then at 93°C for 30 s, and then 50°C for 30 s. This heating cycle was repeated 30 times before reducing the temperature to 4°C for storage. Ten-µl aliquots from the amplification mixture were loaded on a 10×10×0.1 cm 12% polyacrylamide gel in 1× Tris-borate, *p*H 8, and run at 45 mA for 45 min. The gel was stained with 1 µg/ml ethidium bromide for 5 min and the DNA bands visualized under ultraviolet light. The synthetic oligonucleotide primers used to amplify a segment of the α-globin gene cluster were 5′-TACTGTAGATACCCGTGTACAA-3′ and 5′-ATCATGGAAACATAGTAAT-3′. The sequences of the primers for amplification of the β-globin gene were 5′-ACACAACTGTGTTCACTAGC-3′ and 5′-CAACTTCATCCACGTTCACC-3′.

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