cells, human diploid fibroblasts and others but the principle is the same.

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DR FARRANT *et al.* REPLY—We believe that the protection of living cells against freezing damage given by cooling rate techniques or by a period at a constant subzero temperature are both due to the effects on the cells of the time of exposure to the subzero conditions.

We have evidence that a continuous reduction in temperature (cooling rate) is not essential for protection. The conclusive point is the onset of protection against subsequent rapid cooling damage with time at a subzero holding temperature once that temperature has been reached. This can be seen (1) in our data[1], where the recovery of lymphocytes and tissue culture cells thawed from −196 °C increased with time (between 1 and 10 min) after −26 °C had been reached; (2) in other work on increases in protection with time at a constant subzero temperature in spermatozoa[2,3], red blood cells[4], platelets[5], tissue culture cells[6], nematodes[7], and mulberry twigs[8]; (3) from unpublished work with Chinese hamster cells cooled in capillary tubing to −26 °C within 17 s, in which protection against rapid cooling to −196 °C is absent for the first 1 min and yet increases to a maximum at 2.5 min; and also (4) from the data of Rüdiger *et al.*[9], where there is an improvement in survival of diploid cells with time at −26 °C.

The argument put forward by Rüdiger *et al.*[9], that cooling rate is essential for protection depends solely on their data showing very low survival of the cells cooled to −26 °C within 30 s. It is possible that cooling a 1-ml sample with liquid nitrogen and relying on an 'electric thermometer' of unspecified thermal mass to determine when the sample has reached −26 °C (ref. 9), in fact cools regions of the cell suspension to a much lower temperature, thus producing intracellular ice before the period at the holding temperature. This should be checked in control samples thawed immediately from the holding temperature, which in our experiments gave high survival. Also, with a different cell type, amount of cryoprotective additive (including serum) and the slower thawing rate unavoidable with a larger sample, the optimal conditions of time and holding temperature are different (our unpublished work). The conditions we described originally[1] therefore do not constitute a universal recipe.

In short, cooling rate is important but survival using cooling rate techniques can be explained in terms of the cells being exposed for different times to different subzero temperatures: however, protection acquired with time at a constant subzero temperature cannot be dependent on cooling rate.

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Long-term periodicities in the sunspot cycle

COHEN and Lintz[1] claim that their work shows the presence of a 179-yr periodicity in sunspot cycles, and that this is a beat phenomenon. Another method of analysis can be used to demonstrate more clearly that a period of approximately this length exists in the data, and is indeed the result of beating between two more rapid oscillations. Cohen and Lintz used the term "beat frequency" in the sense of half the difference frequency, but here the beat frequency will be taken to be the difference frequency. Whatever definition is adopted, successive maxima in the beats resulting from frequencies with periods 10.9 and 9.7 yr (ref. 1, Fig. 2) occur at 83-yr intervals, taking the frequency separation to be 0.012 cycle yr^{-1} as they do. The 167-yr interval mentioned by Cohen and Lintz is approximately a waveform repetition period.

To study beat phenomena it is advantageous to consider the transformed sunspot numbers obtained by changing the sign of the numbers for alternate cycles in the way proposed by Bracewell[2] and others. Cole[3] has carried out a spectral analysis on such data by standard methods and the results in his Fig. 1 shows that the resulting power spectrum is much simpler than that obtained by using the ordinary, positive, sunspot numbers. It should therefore be much easier to make deductions about the behaviour of sunspot numbers from this type of spectrum. For instance, as Cole's spectrum (in his Fig. 1*d*) shows three main peaks, the largest with a period of 22.2 yr and two smaller ones with periods of 19.7 and 18.3 yr, one would expect to find beat frequencies with periods of 175 and 104 yr in the data.

If one uses the ordinary sunspot numbers, any period appearing in the envelope of the maxima will also appear as that of a genuine spectral component, because the envelope of the sunspot minima is almost constant (nearly zero). This need not happen if the transformed sunspot numbers are used, and indeed does not happen in Cole's spectrum. Therefore the 175-yr period in the maxima is certainly a beat phenomenon.

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DRS COHEN AND LINTZ REPLY—We had hoped that use of the term "beat" would help the casual reader to better understand the phenomena observed. Given the confusion that has arisen, however, we probably should have discussed the sunspot cycle only in terms of the waveform repetition period (or frequency). In doing so, the data indicate that the waveform should repeat about every 179 yr.

Specifically, through correlation analysis, and guided by the periods of significant signatures in the maximum entropy (MESA) spectrum for the data interval 1750–1963, we find that the spectrum of the 12-month smoothed

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