most soils are fungistatic most of the time. Also, our research has shown that ethylene production in soil is relatively insensitive to heat treatments, which rules out *M. hiemalis* as a major producer because it is killed by even mild heat treatments.

Claims that fungi are insensitive to ethylene must be interpreted carefully because in my original paper it was pointed out that the fungistatic action can be overridden by nutrients. Thus, results obtained with ethylene in pure culture cannot be extrapolated to unsterilised soil.

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tRNA and ageing

HOFFMAN and McCoy[1] have reported on the nucleoside composition of tRNA from mature and old mosquitoes and mature and old mice. They reported that no alterations in the total population of tRNAs occur during ageing. The authors "point out the danger of interpreting chromatographic changes in tRNA or changes in activity of tRNA methylases in terms of the final degree of modification without actually analysing the tRNA". I could not agree with them more but write to point out that they did not analyse the tRNAs whose chromatographic profiles are changed. They analysed the crude mass of tRNAs which in metazoa may number more than 100. The isoaccepting tRNAs which differ in a variety of systems such as tumour tissue and differentiating tissue, are invariably very few in number and often they are minor species. For example, it has been shown in Ames' laboratory that in *Salmonella* $tRNA^{His}$ is the control agent for the histidine pathway operon. In a mutant in which the operon is always open with the consequent constitutive status of the pathway, there is an isoaccepting $tRNA^{His}$ which differs from its counterpart in the wild type by the lack of conversion of two Us to ψs[2]. If we assume only 64 tRNAs in *Salmonella* and an average of four ψs per tRNA no analytical method could have established the absence of two ψs out of 256. Under the circumstances, a mass analysis of tRNA mixtures in which the preponderance of the population has not changed is meaningless.

The senior author has shown earlier in aged mosquitoes a changed elution pattern in but five tRNAs[3]. Only an analysis of any of these from young and aged mosquitoes would have any meaning. This, of course, is technically impossible at present. The authors refer to earlier work by Frazier and Yang who found no difference in elution patterns of tRNAs in young and old mouse liver[4]. An examination of that paper reveals that only nine tRNA profiles were compared and most of these were done with the RPC-2 system which is known to have inadequate resolving power for the separation of isoaccepting tRNAs. May I emphasise again to those who are not conversant with this field, that a very few, indeed sometimes but one regulatory tRNA, can have profound physiological effects. Thus, for example, Jacobson has shown that a single tRNA can control the eye colour of *Drosophila*[5].

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DR HOFFMAN REPLIES: I concur with Dr Borek when he emphasises that a deficiency in one modified nucleoside in one regulatory tRNA can have profound physiological effects. I contend, however, that if that deficiency is due to altered activity of a tRNA-modifying enzyme, then all tRNAs which are substrates for that enzyme will show the same nucleoside deficiency as the regulatory tRNA. It is then quite likely that nucleoside composition analysis of total tRNA would show a significant deficit in one compartment.

The work of Singer *et al.*[1], which Dr Borek quotes, is an excellent example. It is true that lack of conversion of two Us to ψs in the $tRNA^{His}$ of the *his T* mutant of *Salmonella* is directly responsible for constitutive derepression of the histidine operon. It is equally true, however, that many other *his T* tRNAs lack this conversion. Singer *et al.*, showed directly that $tRNA^{Tyr}$ and $tRNA^{Leu}$ I and II from *his T* have altered chromatographic mobility when compared with the wild type. They also pointed out that nearly half (5/11) of the *E. coli* tRNA sequences known at that time contained ψ in the same position as in wild type *Salmonella* $tRNA^{His}$, presumably as products of a *his T*-like enzyme. If one applies that fraction to Dr Borek's assumed 64 *Salmonella* tRNAs, then in *his T* mutants 32 of them would each be missing two ψs for a total of 64 (not two) out of 256, a 25% deficit in ψ which would be readily apparent in a comparison of the nucleoside composition of total tRNA from *his T* and wild type *Salmonella*.

Dr Borek also refers to work on the tRNA involved in suppression of the vermilion mutation in *Drosophila*[2]. In a detailed study of the mechanism of this suppression, Twardzik, Grell and Jacobson[3] suggest that it is due to lack of an enzyme which modifies $tRNA^{Tyr}$. They also state that "other tRNAs may be expected to be altered by the hypothetical modifying enzyme". Again I would predict that nucleoside composition analysis of total tRNA from the suppressor strain would show one component significantly deficient in comparison with wild type *Drosophila* tRNA.

Our work on tRNA and ageing has been strongly influenced by Dr Borek's pioneering research on tRNA methylases, in particular the report by Mays, Finch and Borek[4] on the decline in activity of these enzymes during senescence of rodents. In that report tRNA methylase activity in crude protein fractions, as measured *in vitro* by methyl saturation of total *E. coli* tRNA, declined with age. This decreased activity was attributed to increased glycine N-methyltransferase activity which would produce S-adenosyl-homocysteine, an inhibitor of tRNA methylases. The methods used and the discussion given in this paper were directed toward total tRNA methylase activity and hypomethylation of total tRNA during ageing. Based on this and my above discussion, I still maintain that it was proper for us to search for deficiencies of modified bases in total tRNA as a function of age. Having found no such deficiencies, I must stand on our previous conclusion that tRNA modifying enzymes have no significant role in biological ageing[5].

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