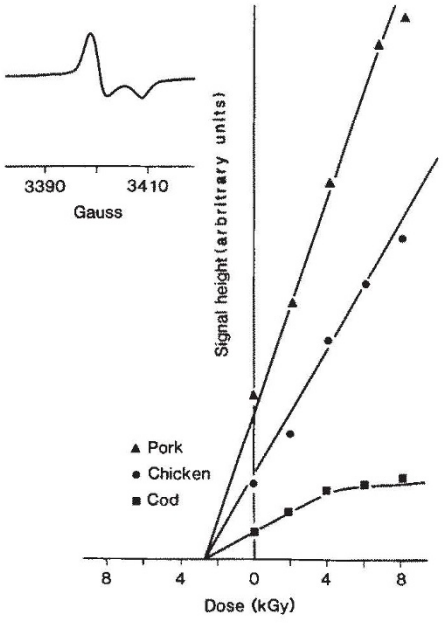
ESR detection of irradiated food

SIR—A wide range of methods for the detection of irradiated food[1] is currently under investigation, as it is unlikely that one method will be applicable to all foodstuffs. At present, only thermoluminescence[2] and electron spin resonance (ESR)[3] are well developed. When applied to irradiated seeds or spices, ESR has no advantage over thermoluminescence, as the signal is generally non-specific and decays rapidly, the rate of decay being dependent on moisture content, thus preventing estimation of dose. On the other hand, as we describe below, samples containing bone or other calcified tissue, such as the shells of molluscs or crustacea,



Dose-response curves for samples of the three species. The initial dose at 2 kGy is shown as the origin with subsequent re-irradiation doses of 2–8 kGy. Extrapolation back to the axis reveals the experimental estimate of the original dose. Inset: typical e.s.r. spectrum of bone.

show an ESR signal that is both stable and characteristic of irradiation.

We irradiated samples of pork, chicken and cod with a dose of 2 kGy, using a 10 MeV electron linear accelerator. The bones were cleaned, powdered and dried and their ESR spectra recorded using a Varian E-9 X-band spectrometer. The spectra vary somewhat between species but all show the characteristic signal of an axially symmetrical radical (see figure), attributed to CO_2^- trapped in a hydroxyapatite matrix[4]. Unirradiated bones showed no detectable signal under the same conditions and thus ESR clearly indicates whether the samples have been irradiated. The height of the signal is greatest in the highly calcified pork, intermediate in the chicken and lowest in the poorly calcified fish bone. Smaller variations in signal height have been observed between samples of the same species.

To estimate the dose received by a sample of bone, it is necessary to re-irradiate to a known dose. The figure shows the signal heights obtained for each sample after re-irradiation with doses of 2, 4, 6 and 8 kGy. Extrapolation to zero signal would indicate that the pork and chicken samples had originally received a dose of 2.6 ± 1 kGy, in agreement with the actual dose. The sample of fish bone shows a non-linear dose-response curve at higher doses, but extrapolation from the lower doses again indicates an original dose of about 2.6 kGy.

For this test to give reliable quantitative results in practice, it is important that there should be no significant decay of the radiation-induced signal under normal conditions of processing and storage. Our work on chicken has shown that there is no significant decay over three weeks at 4°C or several months at −21°C, while the signal from the dried powders is stable indefinitely. Moreover, cooking after irradiation produces no significant loss of signal. The lower limit of detection is 50 Gy. This limit will be higher in fish bone but still below the minimum dose likely to be employed commercially.

These results demonstrate that ESR is a valid method for the detection and quantification of irradiation in a broad class of foodstuffs containing bone or other calcified tissue. It might also be used for other dry foodstuffs such as seeds and spices, its accuracy being comparable to that of thermoluminescence.

Fear has been expressed at the possible harmful effects of radiation-induced free radicals, but radicals *per se* are not harmful and many natural biological processes involve radical formation. Human life itself would be impossible without that ubiquitous radical molecular oxygen. The presence of long-lived radicals in irradiated foodstuffs[5], provides no cause for concern, because they appear either to be indistinguishable from naturally occurring radicals or are trapped in inedible parts of the food.

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Polymerase chain reaction reveals cloning artefacts

SIR—The recently developed polymerase chain reaction (PCR) makes possible the *in vitro* amplification of specific DNA segments bounded by an oligonucleotide primer on each strand[1]. During successive cycles of denaturation and extension of the primers by a heat-resistant DNA polymerase of *Thermus aquaticus* (*Taq* polymerase)[2], large amounts of amplification product can be generated from a few copies of template DNA. This technique holds great promise for forensic science and molecular archaeology as well as population biology.

Because *Taq* polymerase may have an overall error frequency of 0.25% in typical amplification reactions[2], there is concern that sequences determined from amplified products may contain artefacts. This is a particular worry in the case of ancient DNA preparations, which contain a wide variety of chemical modifications[3]. To address this issue directly, we designed primers for the amplification of two regions of the mitochondrial genome from the quagga, an extinct member of the horse family[4]. The DNA sequences are located in the genes for cytochrome oxidase I and NADH dehydrogenase I, respectively, and have been shown by conventional cloning to be identical to those of the plains zebra, the nearest living relative of the quagga, except for two substitutions, one in each of the two clones[4,5]. Because both these substitutions would cause amino-acid replacements at positions which are conserved among all other vertebrates tested, it has been speculated that they represent cloning artefacts caused by post-mortem damage to the old DNA[5].

PCR amplifications from DNA extracts of the same tissue specimen used for earlier cloning yielded products of the expected size. Single-stranded DNA of these amplification products was generated by the unbalanced priming method[6] and sequences were determined by the dideoxynucleotide method. The quagga sequences generated by PCR were identical to the cloned quagga sequences in all positions compared except the two discussed above. At these sites, the PCR products were identical to the orthologous plains zebra sequences (see figure). This confirms the previous assumption that these positions in the cloned sequences represent cloning artefacts.

Although nothing is known about the tolerance of *Taq* polymerase to various types of damage in the template DNA, it can be speculated that the enzyme, like other DNA polymerases, will be slowed down at damage such as baseless sites[7]. Furthermore, many types of damage such as inter- and intramolecular cross-links,