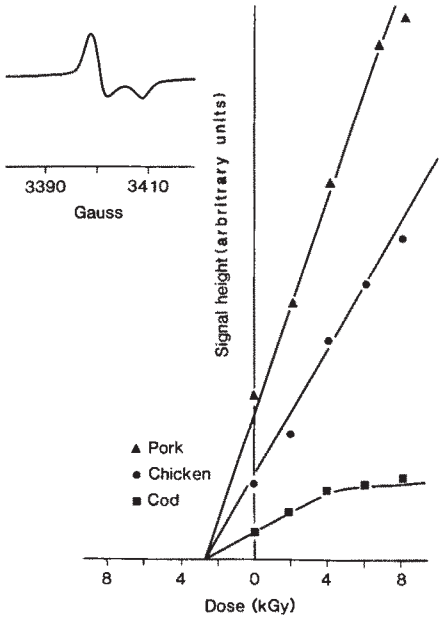
ESR detection of irradiated food

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.

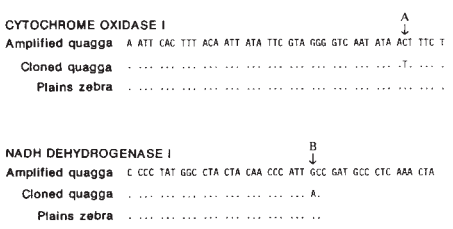
Because *Taq* polymerase may have an overall error frequency of 0.25% in typical amplification reactions[2], there is concern that sequences determined from amplified products may contain artefacts. This is a particular worry in the case of ancient DNA preparations, which contain a wide variety of chemical 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,

will completely block the enzyme. Thus, damaged DNA molecules in the amplification reaction can be expected either not to be replicated at all or to be at a replicative disadvantage, so that intact molecules will amplify preferentially during PCR. This is in sharp contrast to molecular cloning, where bacterial repair and recombination systems will 'salvage' damaged and altered molecules, and thus will introduce a high number of cloning artefacts. In line with this expectation, we find that only short pieces of DNA can be amplified from old or ancient DNA, as all longer templates presumably have been altered so as to be refractory to amplification by PCR.

It should be noted that the high error rate of *Taq* polymerase will be detected if the amplification products are cloned and individual clones are sequenced, whereas all random misincorporations by the enzyme are averaged out when the amplification products are sequenced directly[2]. Only if the amplification is started from an extremely low number (<10) of template



Mitochondrial DNA sequences from a 140-year-old quagga skin determined by direct sequencing of PCR products and from cloned DNA. The corresponding sequences from the plains zebra are shown below. A and B denote the positions of the two cloning artifacts.

molecules, can a particular damaged site be expected to show up in the direct sequencing. To our knowledge, no errors introduced by the *Taq* polymerase have been detected in the now numerous DNA sequences determined by direct sequencing of PCR products in this and other laboratories (H.A. Erlich, U. Gyllensten and T.J. White, personal communication). Thus, the polymerase chain reaction appears not to be a major source of errors but rather offers the possibility of avoiding cloning artefacts.

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Transforming and membrane proteins

SIR—Two recent reports[1,2] reveal a striking sequence homology between phospholipase C and the non-catalytic region of the non-receptor class (*src* class) of tyrosine kinases. Particularly intriguing is the finding of Mayers[2] that a novel oncogene *crk*, which shows common domains with PLC, lacks the tyrosine kinase domain possessed by other members of the *src* group, and yet shows transforming capacity. A hypothesis accruing from these observations is that PLC and the noncatalytic regions of the non-receptor tyrosine kinases share: (1) a similar activity (lipase?) which in oncogene transformed cells might become overexpressed; or (2) interaction with the same or similar cellular components (inhibitor of tyrosine kinase activity) which could be competed out by *crk*.

In recent work (V.-M.W. *et al.*, submitted) we found that α-spectrin has a significant sequence similarity with the non-catalytic domain of *src* and *yes* oncogenes. The homologous domain is in the middle of the α-spectrin molecule and forms a deviant stretch (DI) in a sequence which mostly encodes 106-amino-acid motifs. The homologous domain in c-*src* exactly coincides with the region A (nomenclature from ref. 1) which shows the sequence similarity with PLC. We have not identified any B or C regions in α-spectrin, nor have we observed any sequence homology between α-spectrin and PLC other than that corresponding to A like region in these two molecules. The homology in region A in α-spectrin, PLC, v-*src* and v-*crk* is depicted in the figure.

The function of the DI region in spectrin, in the middle of which the homology occurs, is unknown. We do not think it has any major enzyme function, such as lipase, as such an activity would probably have been observed previously. Rather, we think that DI and its homologous regions in, for example, *src* proteins and PLC, represent interaction sites with a common cellular component. One candidate for such a component is calpactin I (also termed lipocortin II or p36), the major substrate of *src* proteins which is also known to bind to spectrin. Whether p36 also interacts with PLC and whether it serves only as an anchorage point or has more subtle or varying effects on the interacting proteins remains to be elucidated. Of course we cannot exclude that G-proteins or even a new class of regulatory proteins would regulate the function of tyrosine kinases and PLC as suggested by Katan and Parker[4].

Cytoskeletal changes have been recognized as one of the earliest changes seen in *src*-induced transformation[3]. Recently, Warren *et al.*[5] have demonstrated that elevated expression of *src* protein induces a dramatic change in cell shape and especially in membrane plasticity of an epithelial cell line (MDCK-cells) without having a mitogenic effect. These findings, together with the observation on the sequence similarity between *src*, PLC and spectrin, will undoubtedly bring plasma membrane and its underlying spectrin-based membrane skeleton more into focus in studies of oncogene-induced transformation.

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spectrin
D1      (940)  TGKELVLALYDYQEKSPREVIMKKGDILTLLNSTNKDWWKVE-VNDRQ-GFVPAAYVKKLDPAQSASR
                *    +*****   +  ++  ***+ * ++* *  ***    +   * *++* **   *  *
v-src    (81)  GGVTIFVALYDYESWIETDLSFKKGERLQIVNNTEGDWWLAHSLTTGQIGYIPSNYVAPSDSIQAEEW
                *    **+*+    + ** ****+ * *    *  ** *    *  * ** **
v-crk   (368)  EEVEYVRALFDFKGNDDGDLPFKKGDILKIRDKPEEQWWNAE-DMDGKRGMIPVPYVEKCRPSSASVS
                     *+*****+*   + +* * *  *+   +* *  **  +    *    *  ***      *
PLC     (791)  TFKCAVKALFDYKAQREDELTFTKSAIIQNVEKQEGGWWRGD-YGGKKQLWFPSNYVEEMVSPAALEP
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Alignment of similar domains in the chicken α-spectrin, v-*src*, v-*crk* and phospholipase C. Identical residues are marked with stars and conserved substitutions with crosses. Numbers, the first amino-acid residues included in the alignment.

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