Direct repeats

SIR—The identification of a retinoic-acid response element by de Thé *et al.*[1] led to the interesting observation that this element contains a perfect direct repeat of the sequence motif GTTCAC. We wish to point out that the thyroid hormone response element (T3RE) from the rat growth-hormone promoter[2–4] contains two copies of an imperfect direct repeat of the motif AGGTAA. Mutation of this sequence to a perfect direct repeat leads to a substantial increase in T3 responsiveness[2–4]. Furthermore, we have shown[3] that this perfect direct repeat by itself is a functional T3RE. The rat growth hormone T3RE also contains a third imperfect copy of the hexamer inverted relative to the other two[4,5]. Making this sequence a better match to the T3RE consensus sequence AGGT(C/A)A also increases T3 response[4]; it has also been shown that a perfect inverted repeat of this consensus sequence by itself functions as a strong T3RE (ref. 5). Remarkably, a functional element conferring response to T3 (or retinoic acid) can be constructed from two copies of a hexameric recognition site arranged as either direct or inverted repeats.

Although the retinoic-acid receptor can induce transcription of rat growth hormone, it is clear the proposed consensus hexameric T3 response motif is only distantly related to the proposed retinoic-acid receptor motif GTTCAC. Thus, it would be interesting to know if the T3 receptor can bind to and induce expression via this latter sequence. Further, how can a single protein bind to sequence elements arranged as either direct or inverted repeats? How is the function of hormone response elements affected by the number and orientation of such repeats? Do response elements that contain multiple copies of such repeats, such as those found in rat growth hormone, osteocalcin and laminin, bind mixed oligomers of different receptor monomers?

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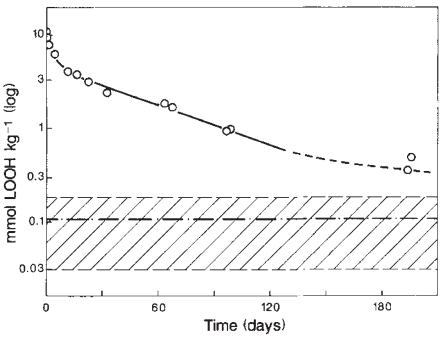
Lipid test

ALTHOUGH the irradiation of foodstuffs has been approved in many countries, as an important method for preventing food spoilage and foodborne diseases, worldwide acceptance and trade in irradiated foods have been hindered by the lack of an appropriate means of control[1]. Although several methods for the identification of irradiated foods have been proposed, radiation-induced changes are generally very small. The most easily measured changes are those produced by chain reactions, whereby the initial chemical change is multiplied many times. We have accordingly explored the potential of the radiation-induced oxidation of lipids with the formation of hydroperoxides as a suitable chain reaction for identification.

We propose the use of lipid hydroperoxides (LOOH) as indicators of the irradiation of egg powder, which is a potential vector of salmonellosis, whose low bulk and high monetary value provide the necessary economic and technical incentives for radiation processing, and which contains high and approximately equal proportions of proteins and lipids (see figure).

Several batches of commercially available spray-dried eggs obtained from two manufacturers were irradiated by gamma-rays at a dose rate of about 14 kGy h^{-1} under three distinct sets of conditions: in open, sealed and evacuated polyethylene pouches, respectively. After irradiation, lipid was extracted by a chloroform: methanol mixture (2:1 v/v) and the amount of hydroperoxides determined directly by a modified ferrous thiocyanate method[2].

The formation of hydroperoxides appears to depend only on the radiation dose. There is at first an exponential increase of hydroperoxide with dose, followed, beyond about 4 kGy, by a linear increase at least with samples to which the atmosphere has access. The linear regions of these curves, when extrapolated backwards, intercept the dose axis at about 2 kGy, which is interpreted as the dose



Time-dependence of radiation-induced lipid hydroperoxides LOOH in whole egg powder irradiated with 4 kGy in equilibrium with air. Shaded area, background level of LOOH.

required to overcome natural radioprotective capacity. With samples sealed from the atmosphere, on the other hand, hydroperoxide formation is found to saturate at about 3 kGy, but the hydroperoxide concentration (about 1.5 mmol LOOH per kilogram of lipid) is more than ten times higher than in unirradiated samples.

We have also investigated the stability of radiation-induced hydroperoxides over a period of more than 6 months. After an initially rapid decay for about 10 days, the first-order decay of LOOH proceeded for about three half-life periods (see figure). The rate of the first-order decay was approximately proportional to the dose received by the sample. Thereafter, decay continued more slowly; the level of LOOH remaining 6 months after irradiation was still higher than the background value of unirradiated samples (0.11 +0.07 mmol kg^{-1}).

We have shown that reduction factors for *Salmonella* of between 10^4 and 10^5 can be achieved by irradiating dehydrated eggs up to 3 kGy in the presence of air, and up to 5 kGy in the absence of air, without adversely affecting the organoleptic properites[3,4]. At these dose levels, the irradiated samples can be unambigously identified by the elevated hydroperoxide levels at least 6 months after the treatment. Hot storage, the only alternative method for elimination of *Salmonella* from egg powder (1 to 2 weeks at 49–55 °C)[5] does not generate hydroperoxides significantly above the background level. On the other hand, boiling irradiated samples in water for several minutes does not destroy radiation-induced hydroperoxides.

The advantage of the proposed method is that the measured parameter is inherent to the food itself. Other dehydrated foods rich in lipids and with an extensive inner surface, such as dehydrated milk and soya flour, exhibit similar behaviour. But before a method can be developed to measure the irradiation absorbed by a material, further work is necessary to characterize the effects of environment on the response and its time-dependence.

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