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Letter to the Editor

Production of tyrosine isomers in mice by therapeutic doses of ^{60}Co irradiation

Sir,

There is no good evidence that *o*- or *m*-tyrosine (Tyr) occur in mammalian systems although a number of related phenols are known which could arise from these amino acids [1]. No important food source for these amino acids is known [1]. Their production via an enzyme-mediated pathway has not been demonstrated in vivo but it has long been known that they may be produced by non-specific hydroxylation of phenylalanine (Phe) [2]. This may be via a free radical oxidation or, more likely in vivo, a Fenton-type reaction. The former is known to occur during irradiation which causes water radiolysis [3].

The production of the *o*- and *m*-Tyr isomers during the irradiation of food has been demonstrated and has been proposed as a convenient marker for monitoring food irradiation [4]. Although food irradiation is carried out with radiation levels a thousand-fold greater than are used for radiotherapy an approximately linear dose–response relationship is expected for the formation of products of any radiochemical reaction. We have examined the plasma of mice irradiated with doses of the same order of magnitude as used in ^{60}Co radiotherapy to see whether at these lower levels of radiation it was possible to detect the unnatural Tyr isomers.

EXPERIMENTAL

Male mice (Parks outbred, weight 45–50 g) were exposed to ^{60}Co γ-irradiation (dose rate 1.8 Gy min^{-1}) in purpose-built perspex chambers. They were anaesthetised and exsanguinated by cardiac puncture 15 min after irradiation. Plasma was extracted and analysed for the Tyr isomers using the high-performance liquid chromatographic (HPLC) method described previously [1]. Standard curves of *o*- and *m*-Tyr were prepared from solutions containing 1

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mg/ml *p*-Tyr, which is approximately the normal plasma concentration and a constant level of α-methyl-*m*-Tyr (internal standard). The area under the *m*-Tyr peak, which is not completely resolved in our system from the *p*-isomer in the mixture, was calculated using tangent skimming. The standard curves were used to estimate approximate yields of the *o*- and *m*-isomers formed by irradiation.

Solutions of saline containing Phe, *p*-Tyr, or Phe plus *p*-Tyr at the levels found in normal plasma (approximately 1 mg/ml) were irradiated: as prepared, after ultrafiltration followed by helium sparging and after vigorous shaking to ensure maximum saturation with air.

RESULTS AND DISCUSSION

Solutions of Phe irradiated with a dose of 5 Gy contained an approximately equal mixture of the three Tyr isomers providing oxygen was present. The relative proportions were $o > p > m$ (Fig. 1A) and not the ratio 2:1:2 expected if the hydroxylation was truly non-specific. No detectable amounts of the tyrosines were formed in the 'oxygen-free' solutions. 3,4-Dihydroxyphenylalanine was produced by similar irradiation of the oxygenated Tyr solutions. Other dihydroxyphenylalanine isomers were either not resolved in the chromatography system used or were masked by the large tyrosine peak. These levels of radiolysis products were not found in vivo. No plasma sample contained more than traces of the isomers and these were discernible only at a dose above 4 Gy of radiation (Fig. 1B and C).

We have demonstrated that small amounts of the unnatural compounds *o*- and *m*-Tyr are formed by therapeutic levels of radiation. The compounds are detectable following irradiation doses of 4–12 Gy. However, the levels are too

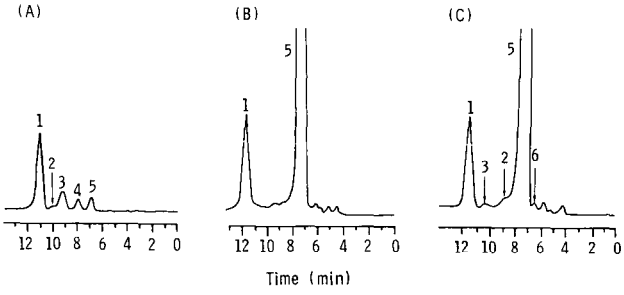

Fig. 1. (A) Irradiation of Phe solution (5 Gy) (1 mg/ml); (B) control mouse plasma; (C) plasma from mouse irradiated with 5 Gy. Peaks: 1 = α-methyl-*m*-Tyr; 2 = Phe; 3 = *o*-Tyr; 4 = *m*-Tyr; 5 = *p*-Tyr; 6 = 3,4-dihydroxyphenylalanine. Fluorescence detection: excitation at 275 nm, emission at 305 nm. (Note: in panel A monochromator settings for maximum phenylalanine sensitivity would be 258 and 288 nm for excitation and emission, respectively.)

low as to be measured quantitatively by a simple chromatographic technique and therefore could not easily be used to monitor any clinical treatment.

MRC Toxicology Unit,
Woodmansterne Road,
Carshalton, Surrey SM5 4EF (U.K.)

R.C. BROWN
C.E. EVANS
J.A. HOSKINS*

Institute of Cancer Research,
Royal Marsden Hospital,
Belmont, SM2 5NG (U.K.)

J. PEACOCK

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