

control eyes, a pupil is present and the mesenchyme is organized to form an anterior chamber and a cornea.

Our results confirm the suggestion that the lens plays an important part in corneal morphogenesis<sup>3-5</sup>. At the same time they agree with the views put forward by Giroud<sup>6</sup> and Stroeve<sup>7</sup>, who emphasize a strong organo-genetic connexion between the iris and the lens.

J. M. GENIS-GALVEZ

Departamento de Anatomía y Embriología,  
Facultad de Medicina,  
Universidad de Salamanca,  
Spain.

<sup>1</sup> Hamburger, V., and Hamilton, H. L., *J. Morphol.*, **88**, 49 (1951).

<sup>2</sup> Genis-Galvez, J. M., and Santos Gutierrez, L., *Anal. Desarrollo*, **11**, 481 (1963).

<sup>3</sup> Amprino, B., *Roux' Arch. Entwicklung.*, **144**, 71 (1949).

<sup>4</sup> Neyfakh, A. A., *C.R. Acad. Sci. U.S.S.R.*, **85**, 987 (1952).

<sup>5</sup> Genis-Galvez, J. M., *Anal. Desarrollo*, **10**, 249 (1962).

<sup>6</sup> Giroud, A., *Acta Anat.*, **30**, 297 (1957).

<sup>7</sup> Stroeve, O. G., *J. Embryol. Exp. Morphol.*, **8**, 3 (1960).

## RADIOBIOLOGY

### Manganese Free Radicals in Cobalt-60 Gamma-irradiated Strawberries

THE formation of free radicals in biological systems exposed to ionizing radiation is well established<sup>1-7</sup>. Some of the induced radicals, as well as some of those occurring naturally<sup>8</sup>, have long lives<sup>9-11</sup>. The surprising stability of the induced radicals may have far-reaching biological implications. As an example of stability, cystine, on irradiation *in vacuo* at room temperature, gave electron paramagnetic resonance signals even after 35 days<sup>12</sup>. Numerous investigations in connexion with irradiation damage of proteins and amino-acids confirm the stability of these radicals<sup>13-18</sup>. This stability must be considered carefully in assessing the wholesomeness of foods preserved by ionizing radiation. Such assessment involves the concepts of stored chemical energy and consequent radiomimetic hazard<sup>19-20</sup>. Chromosomal aberrations have been shown to occur in germinating barley and onion seeds following treatment with orange or apple juice, which had been irradiated with 200 Krad of  $\gamma$ -rays<sup>21</sup>. Although the radiomimetic precursors generated by irradiation of fruit juices have not been ascertained, preliminary studies indicate that peroxides may be implicated.

From research in our laboratory, strawberries appear to be one of the most promising fruits for application of irradiation to extend shelf life<sup>22</sup>. This communication reports on an investigation to determine whether long-lived organic free radicals were present in irradiated fruits. Greenhouse-grown 'Shasta' strawberries were irradiated at 200 Krad at 0° C, and maintained at this temperature for 64 h. Samples taken at regular intervals, starting immediately after irradiation, were held in evacuated quartz tubes submerged in liquid nitrogen, until electron paramagnetic resonance measurements. The spectra were determined with a Varian electron paramagnetic resonance spectrometer having a 9-in. magnet system, over the temperature range of -196°-0° C. The scan was varied from 500 to 1,000 G at roughly  $g$  2. The samples were evacuated continuously during the measurements.

We found no organic free radicals and were only able to detect  $Mn^{2+}$  in the samples. Similar results were recorded from commercially available fruit irradiated over a dose range of 200-600 Krad. Atomic absorption spectroscopy showed 19 p.p.m.  $Mn^{2+}$  (dry weight basis) in the greenhouse strawberries.

Preservation of foods by radiation, as a novel concept, led to extensive investigations concerning the wholesomeness of irradiated foods. Among the various general principles used to evaluate the biological safety of such foods, sub-threshold toxicity and delayed response are

particularly significant when considering wholesomeness in relation to long-lived free radicals.

Due to the absence of any long-lived organic free radicals, the foregoing concept is of no significance in irradiated strawberries. Comprehensive feeding trials of the irradiated foods have shown no adverse effects in test animals. Likewise, in trials conducted by the United States Army Quartermaster Corps, troops fed sterilized rations showed no ill effects. Manganese resonance also has been detected in various non-irradiated plant materials, such as fallen oak leaves and pine needles and naturally dried ivy stems.

On the basis of the evidence available, it is logical to assume that wholesomeness of irradiated strawberries is not affected by the presence of  $Mn^{2+}$  free radicals. We have been unable to locate any references in the literature as to the fate of ingested transition metal free radicals. More definitive evidence regarding the biological safety of  $Mn^{2+}$  free radicals will come from such an investigation.

J. SHAH\*

E. C. MAXIE

University of California,  
Davis, California.

Varian Associates,  
Palo Alto, California.

W. C. LANDGRAF

\* Permanent address: Pakistan Atomic Energy Commission, Karachi.

<sup>1</sup> Weiss, J., *Nature*, **157**, 584 (1946).

<sup>2</sup> Gray, L. H., *Prog. Biophys.*, **3**, 240 (1950).

<sup>3</sup> Rabinowitch, E. I., *Photosynthesis and Related Processes* (Interscience Publishers, New York, 1945).

<sup>4</sup> Calvin, M., *Chem. and Eng. News*, **31**, 1735 (1952).

<sup>5</sup> Evenari, M., and Stein, G., *Experientia*, **9**, 94 (1958).

<sup>6</sup> Ingram, D. J. E., *Free Radicals as Studied by Electron Spin Resonance* (Butterworths, London, 1958).

<sup>7</sup> *Free Radicals in Biological Systems* (Academic Press, New York and London, 1961).

<sup>8</sup> Commoner, B., Townsend, J., and Pake, G. E., *Nature*, **174**, 689 (1954).

<sup>9</sup> Truby, F. K., *Detection of Radiation-induced Free Radicals by Paramagnetic Resonance*, Report No. 16, Contract DA 19-129-QM-378 (1957).

<sup>10</sup> Ehrenberg, A., and Ehrenberg, L., *Arkiv. Fysik*, **14**, 133 (1958).

<sup>11</sup> Zimmer, K. G., Ehrenberg, L., and Ehrenberg, A., *Strahlentherapie*, **103**, 3 (1957).

<sup>12</sup> Gordy, W., Ard, W. B., and Shields, H., *Proc. U.S. Nat. Acad. Sci.*, **41**, 983 (1955).

<sup>13</sup> McCormick, G., and Gordy, W., *Bull. Amer. Phys. Soc.*, **1**, 200 (1956).

<sup>14</sup> Gordy, W., and Shields, H., *Bull. Amer. Phys. Soc.*, **1**, 267 (1956).

<sup>15</sup> Rexroad, H. N., and Gordy, W., *Bull. Amer. Phys. Soc.*, **1**, 200 (1956).

<sup>16</sup> Shields, H., and Gordy, W., *Bull. Amer. Phys. Soc.*, **1**, 267 (1956).

<sup>17</sup> Shields, H., Ard, W. B., and Gordy, W., *Nature*, **177**, 984 (1956).

<sup>18</sup> *Free Radicals in Biological Systems* (Academic Press, New York and London, 1961).

<sup>19</sup> Ehrenberg, L., *Proc. FAO/WHO/IAEA, Tech. Meeting on the Wholesomeness of Irradiated Food*, Brussels (1961).

<sup>20</sup> Westermarck, T., *Risø Report No. 16*, 28 (1960).

<sup>21</sup> Chopra, V. L., Natarajan, A. T., and Swaminathan, M. S., *Radiat. Bot.*, **3**, 1 (1963).

<sup>22</sup> "Radiation Technology in Conjunction with Post-harvest Procedures as a Means of Extending the Shelf Life of Fruits and Vegetables". UCD-34P80-2, AEC Res. and Development Report, Contract No. AT(11-1)-34, Project Agreement No. 80.

### Strontium-90 Content of First Bicuspid

THE tooth crown, once formed, is a stable calcified structure removed from direct equilibrium with body fluids and therefore undergoes minimal remodelling, exchange or turnover of mineral elements. During development and calcification, the mineral elements of the crown are in equilibrium with body fluids and, indirectly, with the diet. The concentration of strontium-90 in the tooth crown is, therefore, a measure of the equilibrium between the tooth and dietary strontium-90, and the tooth crown represents a permanent record of the equilibrium existing at the time of tooth formation.

We have recently reported<sup>1,2</sup> that the accumulation of fall-out strontium-90 in deciduous teeth of children is adequately described by a linear equation of the form  $C_T = K C_D$ , where  $C_T$  and  $C_D$  are the strontium-90 concentrations expressed as pc. strontium-90/g calcium in the tooth crown ( $C_T$ ) and the diet ( $C_D$ ). The slope,  $K$ , was