Effect of γ-Irradiation on Beef Myoglobin

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Absorption spectra of γ-irradiated (1.0 Mrad) samples of metmyoglobin, myoglobin, or a mixture of oxy- and metmyoglobin in an oxygen or nitrogen atmosphere suggested that the iron was in the Fe^{3+} form but the normal metmyoglobin structure was destroyed. The Soret absorbance of hemin and, to a lesser extent, myoglobin underwent a progressive decrease as irradiation dose increased from 0 to 6 Mrad. Treatment of myoglobin with H_2S and γ-radiation singly and in combination provided spectral evidence that sulfmyoglobin *per se* cannot account for the absorption spectrum of irradiated myoglobin. The fluorescent spectra of irradiated hemin and irradiated myoglobin which were not similar to that of protoporphyrin were suggested to be due to the presence of a chole-heme protein formed by rupture of the hemin nucleus.

Irradiation using γ rays (γ-irradiation) has been investigated widely as a means of sterilizing or pasteurizing red meat products (Urbain, 1966). With such products, the stability of pigments responsible for the color are as important as freedom from microbiological activity, at least for aesthetic reasons. Therefore, it becomes important to investigate the effect of γ-radiation on the myoglobins, the heme-containing pigments responsible for the color in red meats.

Early studies on the effect of irradiation on myoglobins indicated that the effect was principally on the porphyrin ring, resulting in the formation of a green pigment, with an absorption band between 610 and 620 nm (Ginger *et al.*, 1955; Ginger and Schweigert, 1956). Fox *et al.* (1958) suggested that a new porphyrin-type compound resulted from irradiation. Barron and Johnson (1956) claimed that Soret band absorbance and fluorescence decreased as a result of rupture of the porphyrin nucleus. Schweigert *et al.* (1956) suggested that metmyoglobin is the reaction product. Tappel (1956) reported that irradiation of meats containing metmyoglobin caused regeneration of oxymyoglobin possibly by reaction of metmyoglobin with free radicals.

Brown *et al.* (1964) suggested the possibility of oxidation of oxymyoglobin and the conversion of metmyoglobin to a substance with spectral characteristics similar to those of oxymyoglobin. This suggestion agrees in some way with the work of Ginger, concerning a change in the porphyrin moiety.

Brown *et al.* (1964) demonstrated that two peptide fragments were split from myoglobin irradiated at 500 krads, and Ho (1967) showed that a peptide containing 12 residues was split from myoglobin after irradiation at 500 krad. The peak at 612 to 620 nm was not observed.

The available information suggests that the heme, as well as the protein moiety, is affected by irradiation. However, the evidence for some of these changes is nebulous, and some of the results conflict. Thus, it was decided that further study of the effect of irradiation on myoglobins was warranted.

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MATERIALS AND METHODS

Juice was expressed from cubes of bovine muscle by subjecting them to a pressure of 2000 lb/in.^{2} in a Carver Press. Beef myoglobin was prepared from the expressed juice, according to the method of Kendrew and Parrish (1956).

Hemin was prepared from sheep's blood, according to the method of Labbe *et al.* (1957), and protoporphyrin was made from hemin, according to the method of Ramsey (1953).

Oxidation of myoglobin to metmyoglobin was effected by potassium ferricyanide and reduction of metmyoglobin to myoglobin by using sodium dithionite.

Myoglobin samples were treated with H_2S by passing the gas generated from FeS and HCl through the myoglobin solutions.

Spectrophotometric measurements were made using a Unicam S.P. 800 recording spectrophotometer.

Fluorescence measurements were made using an Amino-Bowman spectrofluorometer.

Irradiation was initially conducted with samples under either an oxygen or nitrogen atmosphere to determine if atmospheric oxidative conditions affected the response of the samples to irradiation. Subsequent samples were irradiated in an air atmosphere.

Irradiation was carried out in a Gammacell 220 (Atomic Energy of Canada Ltd.). Irradiation dose rate was approximately 1.0 Mrad per hr. Samples were maintained in an ice bath during irradiation. Unless otherwise stated, the total dose of irradiation was 1 Mrad.

RESULTS AND DISCUSSION

The visible spectra of metmyoglobin, myoglobin, or a mixture of oxy- and metmyoglobin, γ-irradiated in either an oxygen or nitrogen atmosphere were similar with absorption maxima at 411, 542, 582, and 620 nm (Figure 1).

The peaks at 542 and 582 nm can be attributed to oxymyoglobin, but the peak at 620 nm was not present in any of the unirradiated samples, and is therefore a result of the irradia-

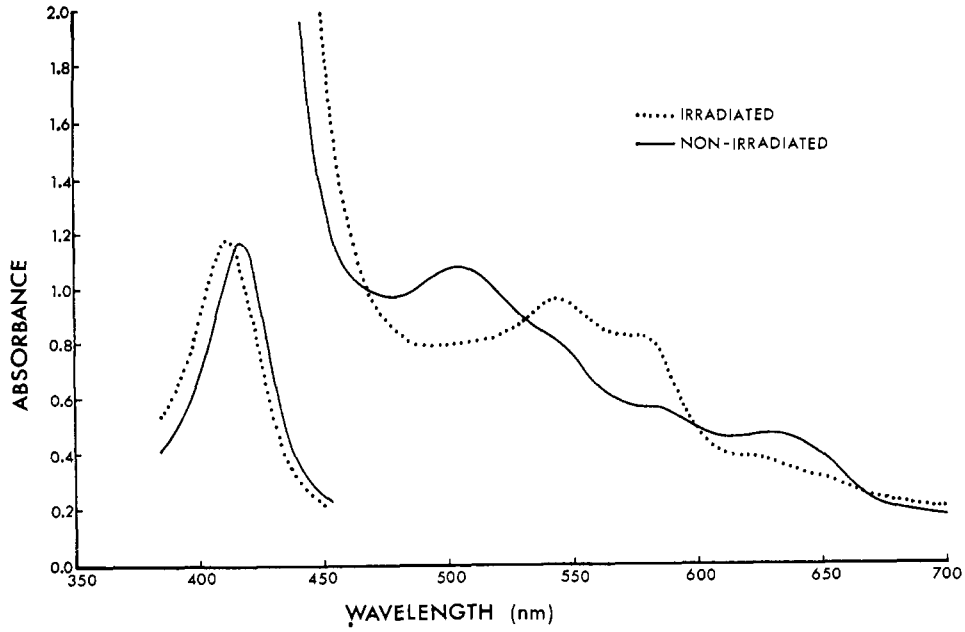

Figure 1. Absorption curves for irradiated (1 Mrad) and nonirradiated samples of a mixture of myoglobins prepared from expressed meat juice

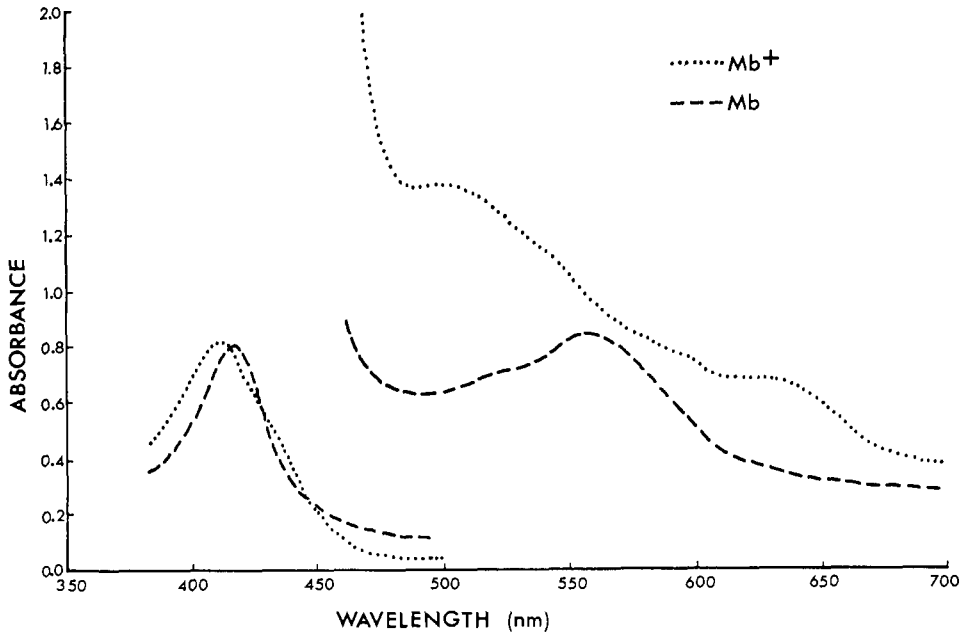

Figure 2. Absorption curves showing characteristic Soret peaks and absorption maxima for nonirradiated myoglobin (Mb) and metmyoglobin (Mb^+) reference samples

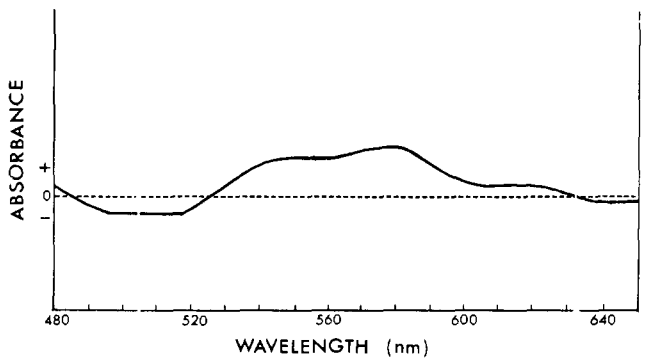

Figure 3. Differential absorption spectrum for an irradiated (1 Mrad) sample of a mixture of myoglobins with the nonirradiated sample as the reference. The horizontal portion of the differential spectrum between approximately 495–515 nm indicates the lower limit of the instrument

tion. The Soret band at 411 nm precludes the possibility of the compound being oxymyoglobin, as this would give a Soret band at 418 nm (Tappel, 1956).

The Soret band at 411 nm is quite similar to that given by metmyoglobin, at 410 nm (Figure 2) but the other metmyoglobin maxima at 500 and 635 nm were not present (Figure 1).

When the respective nonirradiated sample was used as a spectrophotometric reference, there were negative regions corresponding to the 500 and 635 nm bands, and positive bands at 542, 582, and 620 nm (Figure 3). This is indicative of destruction of the metmyoglobin, an enhancement of oxymyoglobin-type absorption, and the presence of a new compound absorbing at 620 nm.

These results suggest that the changes taking place due to 1 Mrad of γ-radiation are quite complex, with the iron of the hemin in the Fe^{3+} form, but the normal metmyoglobin structure destroyed. It was, therefore, thought that changes

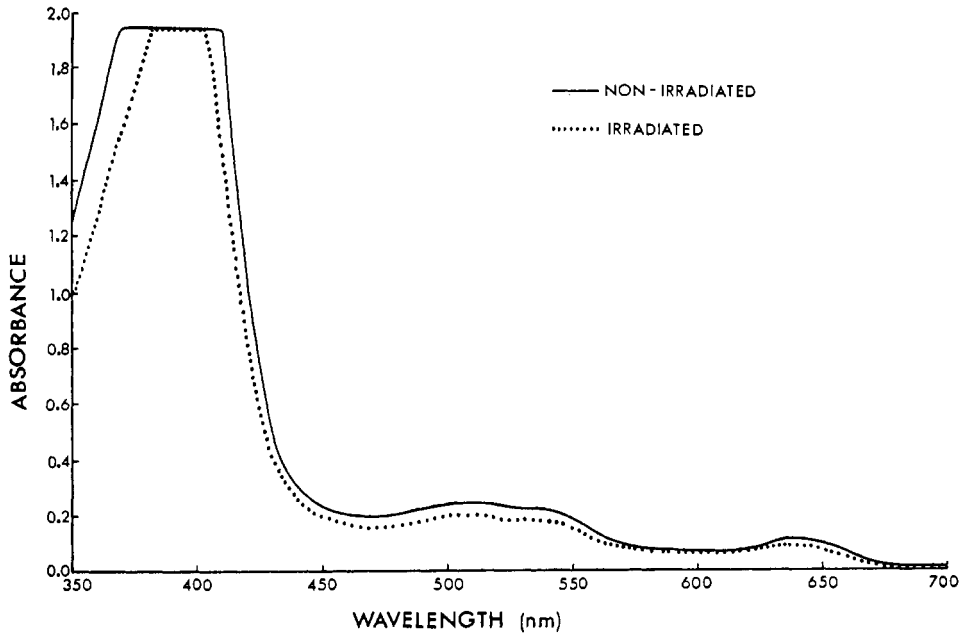

Figure 4. Absorption curves for hemin cleaved from irradiated (1 Mrad) and nonirradiated myoglobin samples. The horizontal portion of the spectrum around 400 nm indicates the upper limit of the instrument

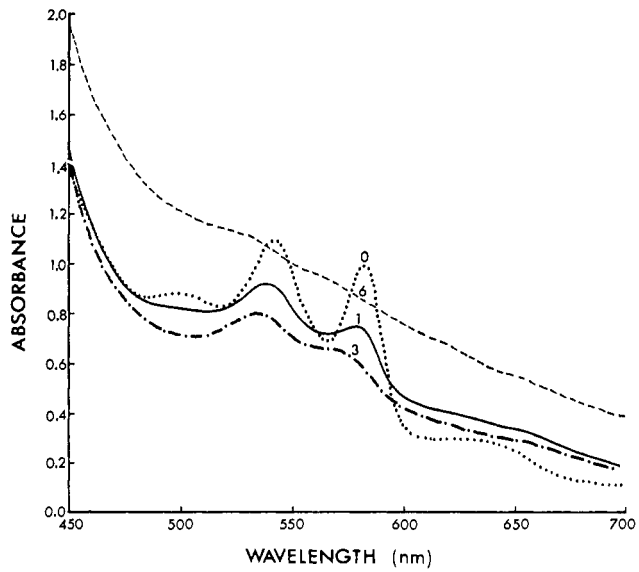

Figure 5. Absorption curves for irradiated (1, 3, and 6 Mrad) and nonirradiated (O) samples of a mixture of myoglobins

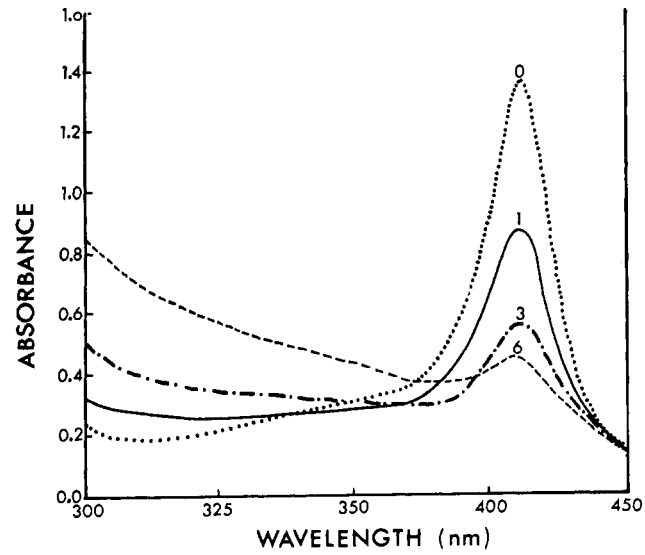

Figure 6. Soret peaks for irradiated (1, 3, and 6 Mrad) and nonirradiated (O) samples of a mixture of myoglobins

taking place in the hemin itself could have resulted in the above spectra changes.

Hemin cleaved from irradiated and nonirradiated myoglobins, according to the method of Labbe *et al.* (1957), showed similar spectra, with maxima at 400, 510, 540, and 638 nm. There was a slightly weaker absorbance due to irradiation (Figure 4).

Barron and Johnson (1956) have reported that X-radiation has a detrimental effect upon myoglobin, with a decrease in the Soret band and a decrease in fluorescence, suggestive of destruction of the porphyrin nucleus. Therefore, to obtain a more comprehensive view of the effect of γ-radiation on myoglobin, samples were irradiated with doses from 1 to 6 Mrad, and both fluorescent and visible spectra measured. There was a pronounced change in the visible spectra (Figures 5 and 6). As previously reported, the bands at 500 and 635 nm disappeared. The minimum which developed at 500 nm, the normal oxymyoglobin minimum at 565 nm, and the peaks at 542 and 582 nm became less evident with increased radiation dose, resulting in a very broad absorption band at the higher dose levels.

Similarly, irradiated hemin, under the same conditions showed a decrease in the minimum at 540 nm, leading to a broad band, with decreased absorbance.

Contrary to the results of Barron and Johnson (1956), there was a concomitant decrease in the Soret bands, and an increase in fluorescence of both the myoglobin and the cleaved hemin samples with increasing radiation dose (Table I). The hemin did not suffer the same percentage decrease in Soret absorbance as myoglobin, suggesting that the decrease in absorbance in the myoglobin Soret band was not due solely to hemin rupture, or conversely that the presence of the globin moiety made the hemin more labile.

The fluorescent spectra of irradiated hemin and irradiated

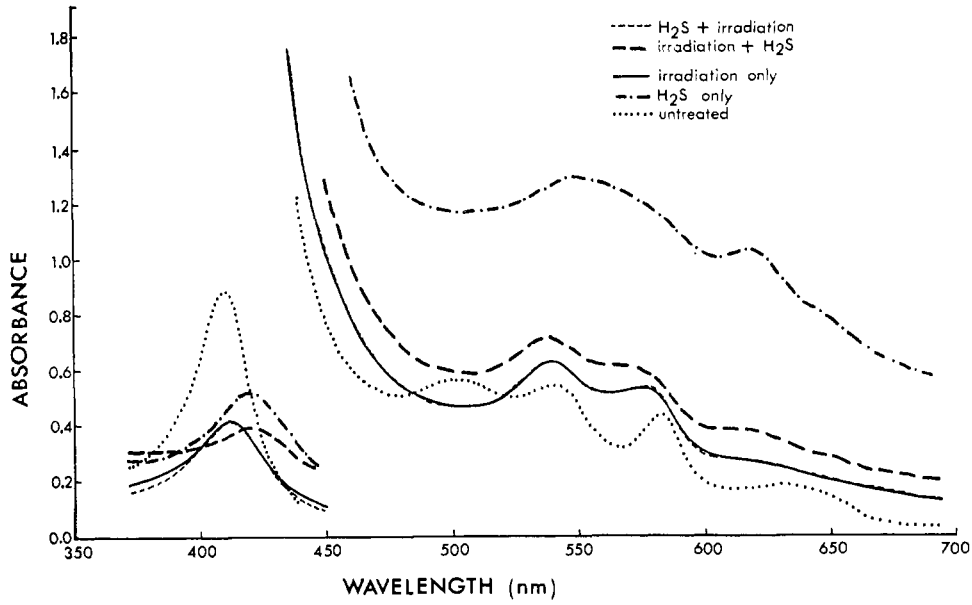

Figure 7. Soret peaks and absorption curves for H_2S treated, irradiated, and control samples of myoglobin

myoglobin were not similar to that of protoporphyrin. At an excitation wavelength of 310 nm, the myoglobin showed a band at 640 nm and the cleaved hemin showed a maximum at 450 nm.

The characteristic absorption of irradiated samples at approximately 615 nm has been attributed by Fox *et al.* (1958) to the formation of sulfmyoglobin, an addition product of hydrogen sulfide and myoglobin. According to Fox, this sulfmyoglobin, under mild oxidative conditions, is converted to metmyoglobin. However, our results indicate that irradiation at low levels does not lead to characteristic metmyoglobin absorption, but to absorption maxima similar to oxymyoglobin.

In addition, the radiative medium is itself oxidative, making it difficult to visualize the formation of sulfmyoglobin, which is very susceptible to mild oxidation.

To check the theory of the formation of sulfmyoglobin as a cause for the 615 nm absorption, a series of myoglobin samples were prepared as follows: (1) Treatment with H_2S then irradiation; (2) Irradiation then treatment with H_2S; (3) Irradiation with no further treatment; (4) Treatment with H_2S but no irradiation; and (5) Untreated myoglobin. Irradiation dose was 1.0 Mrad.

After treatment, the visible and Soret absorption bands were obtained (Figure 7).

The sample which was treated with H_2S and then irradiated had the same spectra as the "irradiated only" sample, with maxima at 411, 540, 580, and 615 nm. The sample which was irradiated and then treated with H_2S showed maxima at 420, 540, 570, and 617 nm. The sample of myoglobin which was treated only with H_2S showed maxima at 420, 550, 620, and a shoulder at 580 nm. Untreated myoglobins showed maxima at 410, 500, 542, 584, and 630 nm.

That the reaction product of H_2S and myoglobin gave an absorption maxima at 620 is obvious from Figure 7. However, the remainder of the spectrum was not similar to the spectrum obtained by irradiation of myoglobin.

The easy convertibility of the H_2S addition compound is demonstrated. Samples treated with H_2S and then irradiated gave identical spectra to samples which had only irradiation treatment. If, however, irradiation was followed by treatment with H_2S, the spectrum was markedly different, similar to the H_2S treatment alone.

Table I. Relationship Between Level of Irradiation, Soret Absorbance Band, and the Fluorescence

Irradiation Level Mrad	Soret Band % decrease	Fluorescence % increase
	Myoglobin	
	411 nm	640 nm
1	36	16
2	55	70
3	59	220
4	64	775
5	66	1320
6	67	2000
	Hemin Cleaved from Irradiated Myoglobin	
	388 nm	450 nm
1	19	32
2	22	60
3	23	125
4	24	206
5	27	255
6	30	325

The results indicate that hydrogen sulfide does effectively cause a change in myoglobin which is reflected in spectral differences. However, it is obvious that the irradiation effect is superimposed upon the H_2S effect, and the spectrum after irradiation bears little resemblance to that of H_2S treatment only.

If sulfmyoglobin is formed, it is at best transitory, being very susceptible to oxidative conditions which might also result in oxidative cleavage of the porphyrin nucleus.

In early studies on the peroxide oxidation of heme proteins (Dalziel, 1954a,b) it was suggested that an intermediate product of the addition type is formed between the heme protein and hydrogen peroxide, with an absorption spectrum similar to oxymyoglobin except for a decrease in the Soret band absorbance and wavelength. Opening of the hemin nucleus of this intermediate could then result in the formation of a chole-heme protein.

It has been postulated that the iron atom is necessary for

the rupture of the porphyrin nucleus (With, 1968). Because of this and since the fluorescence obtained was not characteristic of porphyrin, and increased while the Soret band continued to decrease, it therefore seems feasible that this fluorescence was due to a chole-heme compound still principally attached to the denatured globin. The peroxide for the intermediate could be formed easily from the water in the system.

LITERATURE CITED

Barron, E. S., Johnson, P., *Rad. Res.* **5,** 290 (1956).
Brown, W. D., Akoyunoglou, J. A., *Arch. Biochem. Biophys.* **107,** 239 (1964).
Dalziel, K., O'Brien, J. R. P., *Biochem. J.* **56,** 648 (1954a).
Dalziel, K., O'Brien, J. R. P., *Biochem. J.* **56,** 660 (1954b).
Fox, J. B. Jr., Strehler, T., Bernofsky, C., Schweigert, B. S., J. AGR. FOOD CHEM. **6,** 693 (1958).
Ginger, I. D., Lewis, U. J., Schweigert, B.S., J. AGR. FOOD CHEM. **3,** 156 (1955).
Ginger, I. D., Schweigert, B. S., J. AGR. FOOD CHEM. **4,** 885 (1956).
Ho, L., Ph.D. Thesis, University of California, Berkeley, Calif., 1967.
Kendrew, J. C., Parrish, P. C., *Proc. Roy. Soc.* (London) **238,** 305 (1956).
Labbe, R. F., Nishida, G., *Biochim. Biophys. Acta* **26,** 437 (1957).
Ramsey, B., *Biochem. Prep.* **3,** 39 (1953).
Schweigert, B. S., Doty, D. M., Lewis, U. J., Marbach, E., Swilinsky R., Fox, J. B. Jr., Ginger, I. D., Batzer, O., Sribney, M., U.S. Office of Technical Surveys, P.B. 121309 (1956).
Tappel, A. L., *Food Res.* **21,** 650 (1956).
Urbain, W. M., Food Irradiation, Proceeding of Symposium, Karlsruhe, International Atomic Energy Agency, Vienna, pp. 397–410 (1966).
With, T., Bile Pigments, pp. 101–105, Academic Press, New York, 1968.

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