

Effect of reductant level in skeletal muscle and liver on the rate of loss of thiamin due to γ -radiation

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Abstract. A study was made of thiamin content of the skeletal muscles and livers of pork, chicken and beef after γ -irradiation. γ -Radiation from a ^{137}Cs source was used to irradiate the samples with doses of 0, 1.5, 3, 6 and 10 kGy at 2°C. Samples were also titrated with dichlorophenol-indophenol to determine the reducing capacity of the tissues. The rate of loss of thiamin upon irradiation was found to be about three times as fast in skeletal muscle as it was in liver, and to be a function of the reducing capacity of the tissues, the loss decreasing with increasing reductant titer. For the same amount of thiamin loss, liver could be irradiated to three times the dose as could muscle.

1. Introduction

In a previous publication (Fox *et al.* 1992b) we quantitated the effect of various reductants and free radical scavengers on the loss of thiamin and riboflavin in buffers and pork, and an equation was derived for the effect of the concentration of the scavengers on the reduction of the rates of thiamin and riboflavin loss. Using the equation to calculate the effect of endogenous reductants in meat showed that the concentration of sulphhydryl groups in skeletal muscle was sufficient to account for the protection of the two vitamins in meat against irradiation loss. This does not prove, however, that reducing substances in meat are the protective agents, especially since the addition of further reductants to pork skeletal muscle had no effect. Reductants function by competing with the vitamins for the hydroxyl radicals from the radiolysis of water (Fox *et al.* 1992b) but there may be endogenous compounds which act by inhibiting the oxidation reactions. In skeletal muscle tissue the major radiolytic scavengers are the sulphhydryl groups in the contractile proteins, which occur at a concentration of 15–20 mM (Hamm and Hoffman 1965, 1966). Looking at another tissue with other reductants in other concentrations, for example ascorbate in liver, and different metabolic

environments suggested itself as a way to determine if the effect was peculiar to sulphhydryl groups. We chose liver since it is a rich source of metabolic intermediates. We also performed a reduction/oxidation titration to determine the total reducing capability of the tissues studied.

2. Material and methods

2.1. Sample source and preparation

All meats were irradiated 1 day after the animals were killed. Whereas we used specific muscles from beef (semimembranosus) and pork (longissimus dorsi), was necessary to use all of the leg muscles from chicken to produce sufficient sample for irradiation. Data were obtained from seven chicken, five pork, and seven beef cuts. Inasmuch as metabolites vary in concentration throughout tissues, both muscle and liver were ground through a $\frac{3}{16}$ inch plate and the resulting material mixed until homogeneous. Both operations were carried out in a nitrogen atmosphere to minimize the effect of oxidation of the vitamins. Under these conditions we have seen no differences between ground and whole meat insofar as vitamin loss was concerned. Samples were then packaged in oxygen-permeable (2500 ml/100 inch²/24 h) meat and poultry bags (Mobil Chemical Co., Macedon, NY, USA). Samples were irradiated in a ^{137}Cs source (Lockheed Corp., Marietta, GA, USA; dose rate 0.114 kGy/min) to final doses of 1, 3, 6 and 10 kGy at 0–2°C. Zero dose controls were kept in a refrigerator at 0–2°C during the irradiation process. Dose rate was established using reference dosimeters from the National Physical Laboratory (Middlesex, UK). Dosimetry and dose distribution for this radiation source were described by Shieh *et al.* (1985).

2.2. Thiamin determination

Thiamin was determined by a modification of the AOAC short method used previously (Fox *et al.*

1992a). Portions of each sample (3 g) were weighed into 50-ml polyallomer Oak Ridge-style centrifuge tubes and 27 ml 2% trichloroacetic acid was added. The extracts were homogenized with a Tekmar tissumizer, tubes capped, and placed in a boiling water bath for 15 min. They were then vortex mixed and heated for a further 15 min. The tubes were cooled, then centrifuged at 31 000 g for 15 min. The clear supernatant was filtered through a 0.45-micron filter into tubes in an autosampler and injected into a flowing stream of 93% 0.05 M phosphate buffer, pH 5.5, 7% methanol. The stream was mixed with an equal volume of 0.1% potassium ferricyanide in 2% sodium hydroxide, run through a reaction coil to allow for the oxidation of the thiamin to thiochrome, and the latter determined from its fluorescence, $\lambda_{\text{excitation}} = 365 \text{ nm}$, $\lambda_{\text{emission}} = 460 \text{ nm}$ with a cut-off filter of 440 nm.

2.3. Reduction/oxidation titration

2,6-Dichlorophenol-indophenol (DPIP) is used for the determination of ascorbic acid (AOAC 1990), and has been used for nicotinamide adenine dinucleotide (NAD)-containing-enzyme assays (Mahler 1955). In general, DPIP is readily reduced by mild reductants, including sulphhydryl groups. Titration of standards and samples was carried out at 70–80°C under a flowing stream of nitrogen to prevent re-oxidation of the DPIP. Solutions of DPIP were standardized with ascorbic acid, cysteine, and dithiothreitol. Ascorbic acid titrated to a rosy-pink end-point whereas the sulphhydryl compounds titrated to a greyish-blue end point.

2.4. Calculation of results

The rate constants for the loss of thiamin due to irradiation were subjected to an analysis of variance, grouping the muscle and liver values separately. The parameters for the dependence of the rates of thiamin loss of the DPIP titers were determined for the previously-derived equation for the reduction in the rate of thiamin loss due to competition for the hydroxyl radical by reductants (Fox *et al.* 1992b):

$$k_m = k_o / (1 + k_r[R]), \quad (1)$$

where k_m is the measured rate constant for a given sample, k_o is the theoretical value for meat with no reductant, k_r is a constant related to the effectiveness

Table 1. Effect of γ -radiation on the thiamin content of liver and skeletal muscle

Species	Rate constants (kGy^{-1})		
	Muscle		Liver
	Breast	Leg	
Chicken			
Supermarket			0.0561†
Kosher	0.0300		
	0.0374	0.0627	0.0453
	0.0436		0.0405
	0.0735	0.0744	0.0290
	0.0440	0.0691	0.0386
Pork		L. dorsi	
		0.093	0.0053
			0.0406
		0.119	0.0265
		0.104	0.0304
		0.107	0.0284
Beef		semimembranosus	
			0.0215
			0.0215
			0.0250
			0.0252
		0.167	0.0239
		0.165	0.0467
		0.0911	0.0284
		0.0651	0.0131

† For all of the data c.v._{pooled} = 6.5%.

of the reductant and [R] is the concentration of the reductant, in this case measured by the DPIP titration.

Using the GLM curve fitting program of the SAS (1987). For the regression of Figure 2 the observed k_m values were plotted as a function of the term, $1/(1 + k_r[R])$, to obtain a linear relationship.

3. Results

The results of the determinations are presented in Table 1 and a plot showing the difference in rates of loss in beef semimembranosus and liver is shown in Figure 1. The pooled coefficient of variation for the rate constants was 6.5%. The rates of thiamin loss were smaller for liver than for muscle for all three meats. Data were analysed by a paired *t*-test (Snedecor and Cochran 1980); the Student's *t*-test value was 5.96, corresponding to $p < 0.0001$ for 11 d.f. Liver values showed a smaller variation than did

skeletal muscle values, but it cannot be concluded that leg values were different from breast values. The mean of the muscle values was $0.084 \pm 0.042 \text{ kGy}^{-1}$; the mean of the liver values was $0.031 \pm 0.012 \text{ kGy}^{-1}$; the populations were significantly different ($p < 0.001$).

Table 2 presents the rate constant figures for those runs for which DPIP titrations were performed; the pooled coefficient of variation of the DPIP values was 3.0%. The regression line of Figure 2 is the least-squares plot of the data. The values of the parameters for the regression of equation (1) using the data of Table 2 were $k_0 = 0.557 \text{ kGy}^{-1}$ and $k_r = 0.462 [\text{R}]^{-1}$. The Student's *t*-test values for the intercept and slope of the curve were 0.204 and 8.10, respectively, for 15 d.f. The intercept was not significantly different from zero, but the slope was highly significant, ($p < 0.0001$). Since the factor $1/(1 + k_r[\text{R}])$ is essentially zero at very high concentrations of the scavenger, the plot indicates that there would have been no loss of the vitamin had the concentrations of reductants been high enough.

4. Discussion

One observation was made which bears on the question of the reducing compounds responsible for protecting thiamin against radiation destruction. When titrating the standards it was noted that sulphhydryl groups titrated to a greyish-blue colour and that both muscle and liver titrated to the same colour, an indication that the principal components

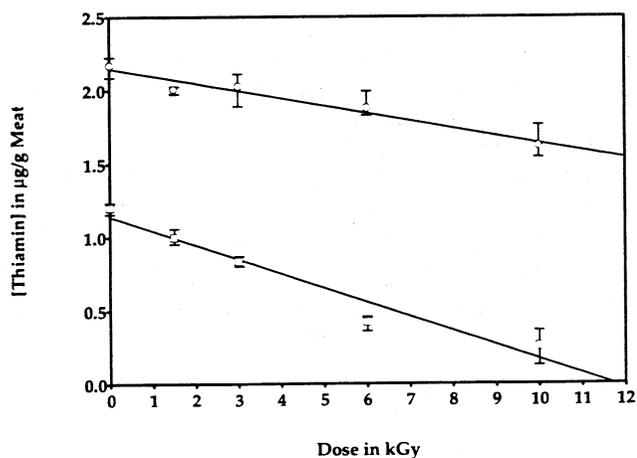


Figure 1. Concentration of thiamin remaining in irradiated beef gluteus maximus (\square) and liver (\circ) as a function of radiation dose. Bars represent S.E. for $n = 4$.

Table 2. Rates of thiamin destruction and the DPIP titre of liver and skeletal muscle rate constants (kGy^{-1})

Species	Muscle		Liver
	Breast	Leg	
Chicken	0.0374†	0.0627	0.0405
	16.3 ‡	15.8	25.6
	0.0436		0.0290
	16.3		25.6
	0.0735	0.0744	0.0386
	20.7	13.4	42.55
Pork	0.0440	0.0651	0.0287
	24.2	19.9	20.6
		L. dorsi	
		0.107	0.0284
		19.7	31.1
		G. maximus	
Beef		0.0911	0.0284
		16.5	29.8
		0.0651	0.0131
		19.1	35.6

All determinations were run in duplicate and the coefficients of variation of all samples were pooled.

† Values in normal type: first order rate constants (kGy^{-1}), c.v._{pooled} = 6.5%.

‡ Values in bold type: DPIP titre ($\mu\text{mols/g tissue}$), c.v._{pooled} = 3.01%.

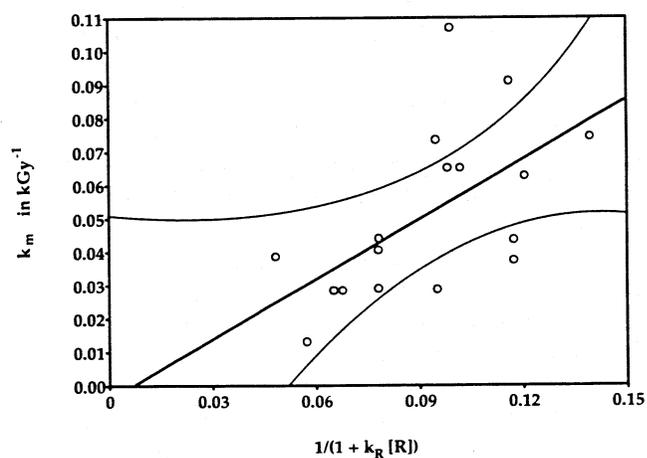


Figure 2. Rate constants for the loss of thiamin in chicken, pork and beef skeletal muscle and liver as functions of the reducing capability of the tissues as determined by dichlorophenolindophenol titration. The expression in the abscissa comes from equation (1) and transforms the concentration dependence into a linear function. The lines above and below are the 95% confidence limits.

Table 3. Cystine, ascorbate and thiamin content ($\mu\text{g/g}$) of chicken, pork and beef skeletal muscle and liver

Meat	Cystine	Ascorbate	Thiamin	
			HNIS†	Ours
Chicken				
Liver	4250	338	1.38	4.70 ± 1.57 ($n=5$)‡
Leg	2580	32	0.79	1.44 ± 0.53 ($n=6$)
Breast	2960	12	0.70	
Pork				
Liver	4040	253	2.86	2.99 ± 0.22 ($n=5$)
Loin	2130	7	7.97	13.84 ± 1.79 ($n=4$)
Beef				
Liver	3070	220	2.60	2.17 ± 0.44 ($n=7$)
Round	2280	0	1.00	1.33 ± 0.26 ($n=3$)

† HNIS (1979, 1982, 1990).

‡ Mean \pm SD of all samples in study.

in the tissues reacting with DPIP were sulphhydryl compounds.

4.1. Source of tissue

While the rates of thiamin destruction were different for liver and muscle when analysed as different meats, when the rates were plotted as functions of the DPIP titres they were essentially the same family of values, that is the liver values as a group were different only in that they had greater reducing capacity. The reducing capacity of these tissues is a function of sulphhydryl groups, ascorbate, sugar, reduced coenzyme content, etc. of the tissues. Table 3 lists values for the cystine, ascorbate and thiamin contents for the various tissues studied (HNIS 1979, 1982, 1990). Values we determined for the thiamin content of beef are essentially the same as those given in the HNIS tables, but the chicken and pork values are higher. In a previous study we found that the method usually reported for the determination of thiamin in chicken yielded low results (Fox *et al.* 1992a) due to loss of the vitamin in one of the steps. Higher and more consistent results were obtained by modifying and shortening the procedure. Using the latter we have consistently obtained higher thiamin concentrations values for pork than are reported in the literature. The ascorbate content of liver listed in Table 3 corresponds to 1–2 mM for the several livers, whereas the skeletal muscle ascorbate content is negligible. However, the decreased rate of thiamin loss in liver cannot be accounted for by its ascorbate content, since 1–2 mM ascorbate would reduce the unprotected (buffered) rate of 12 kGy^{-1} to *c.* 1 kGy^{-1} , but not to the observed rate of 0.031

kGy^{-1} . The listed cystine content of muscle tissue corresponds to approx. 20 mM cysteine, most of which is in the contractile proteins and has been shown to be almost all free sulphhydryl (Hamm and Hoffman 1965, 1966). The cystine content of liver is about the same and in pork, and appears to be largely in the free sulphhydryl form; Swatditat and Tsen (1972) reported 18-mM free sulphhydryl in hog liver. This amount of free sulphhydryl in liver is not sufficient either to account for the reduced rate of thiamin loss on irradiation alone, but the combination of increased ascorbate and cysteine concentrations, as well as reducing sugars and coenzymes derived from liver glycogen, can easily account for the reduced rate of thiamin loss and the higher DPIP titer.

4.2. Rate of thiamin loss as a function of DPIP titer

Two observations may be made from the results of this study. The first is that as the concentration of reductant increases, the expression $1/(1+k_1[\text{R}])$ is not significantly different from zero, the conclusion is that if the concentration of reductant had been high enough the loss of thiamin would have been stopped. The second is that when the concentration of reductants was zero, $k_m = k_o = 0.557 \text{ kGy}^{-1}$. The average value for thiamin in buffers with no reductant was $12.1 \pm 0.12 \text{ kGy}^{-1}$, indicating that thiamin in the tissues was being protected from radiation destruction by something other than the titratable reductants. The hydroxyl radical is a much stronger oxidant than DCPPI, however, and many compounds are present in tissues that are oxidized by the former, but not the latter. There is no evidence that any compounds that lower the rate of thiamin loss react in any other way other than reacting with the hydroxyl radical, except for niacin which reacts with the thiamin and riboflavin radicals (Fox *et al.* 1992b). It is also possible that the protection was due to physical factors. The oxidation in buffers took place in a Brownian system, whereas muscle tissue is a highly structured system wherein molecular movement, especially water, is restricted. The restricted movement would result in a lowered rate of oxidation.

4.3. Necessity and sufficiency

In establishing the role of a chemical in a reaction it is necessary that the proposed compound is both necessary and sufficient. The observed rate constant for the loss of thiamin in meat was found to be 0.1 kGy^{-1} . When this value was used in equation (1) to

determine the concentration of sulphhydryl groups that would produce that rate, a value of *c.* 20 mM was obtained (Fox *et al.* 1992b), which is about the concentration of free sulphhydryl groups in muscle tissue. That is, the sulphhydryl groups in muscle tissue are sufficient to account for the observed level of inhibition. Since the oxidation of the sulphhydryl groups eliminated most of the inhibition, it may be concluded that titratable reductants are also necessary to account for the observed level of inhibition in muscle tissue. In liver, the titratable reductants do not fully account for the reduction in the rate of thiamin loss, and therefore while they are necessary to account in part for the total reduction in rate, they are not sufficient.

4.4. Extent of loss

Both pork and chicken have been approved for lower dose irradiation in the USA, pork for up to 1 kGy for elimination of trichinae (FDA 1985, FSIS 1986) and chicken for up to 3.0 kGy for pasturization (FDA 1990). Since liver is more resistant to loss of thiamin than is muscle, it could be irradiated to higher dose levels. In a previous paper (Fox *et al.* 1989) we reported a mean loss of 5.76% of the initial thiamin in pork at a dose of 0.3 kGy. Using the mean values of the rate constants for muscle and liver reported above, a loss of 5.76% would occur in muscle at a dose of 0.71 kGy and in liver at 1.92 kGy. That is, liver could receive about three times the radiation dose given muscle tissues for the same loss of thiamin. Under these circumstances, the pasteurization of livers for storage by irradiation would be a viable procedure.

5. Conclusion

From a study of the rate of thiamin loss due to γ -irradiation in muscle and liver, and the correlation of the rates with the DPIP titer of the tissues, it is concluded that the reductants in the tissues are sufficient to account for the observed reduction in rates and hence are the major, if not only, protective agents in tissue.

Acknowledgements

Mention of brand or firm names does not constitute an endorsement by the US Department of

Agriculture over others of a similar nature not mentioned.

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