39 Estimation of heme oxidation in carboxymyoglobin. R. Mancini*[1], S. Surendranath[2], and C. Faustman[1], [1]*University of Connecticut*, *Storrs*, [2]*University of Kentucky*, *Lexington*.

Traditional methodology used to determine the in vitro redox status of myoglobin does not account for carboxymyoglobin (COMb) as these equations are based on wavelength maxima for oxymyoglobin (OMb), deoxymyoglobin (DMb), and metmyoglobin (MMb). The nearly identical spectra of COMb and OMb also make it difficult to determine pigment oxidation in solutions containing COMb. Fundamental research in COMb redox chemistry is needed as modified atmosphere packaging containing carbon monoxide is approved for use in the US. Thus, our objective was to determine a suitable method to estimate heme oxidation in COMb solutions in vitro.

To create 100% COMb, equine DMb was bubbled with a gas mixture of 0.4 % CO, 69.6% CO2 and 30% N2 for 40 min. The conversion of DMb to COMb was monitored spectrophotometrically, with no change in absorbance spectra occurring after 40 min. Residual hydrosulfite was removed using a PD-10 column, and desalted COMb samples were re-bubbled with 0.4% CO for an additional 10 min to minimize column-induced OMb formation. Oxymyoglobin was prepared by sodium hydrosulfite-mediated reduction of MMb. All myoglobin derivatives were prepared at pH 7.4 using 50 mM sodium phosphate buffer.

Variable proportions (0 to 100%, with increments of 10%) of (1) COMb and MMb, (2) COMb and OMb, and (3) OMb and MMb were placed in split-chamber cuvettes and absorbance was recorded. Heme oxidation was characterized by the actual amount of each myoglobin form added to each compartment of the split-chamber cuvettes. Correlation analyses were performed using the Proc Corr procedure of SAS.

In split-chamber cuvettes containing variable proportions of COMb and OMb, the ratio of A543/A581 successfully differentiated between samples containing 100% COMb and 100% OMb. For 100% COMb samples, absorbance at 543 nm was greater than absorbance at 581 nm (A543/A581 was greater than 1), whereas the reverse was true for 100% OMb samples (A543/A581 was less than 1). The absorbance peak at 581 nm was prominent irrespective of different COMb-OMb proportions and was adopted as a reference wavelength maximum for both COMb and OMb.

In split-chamber cuvettes containing either (1) COMb and MMb or (2) OMb and MMb, the magnitude of the peak at 581 nm decreased, whereas the peak at 503 nm (corresponding to the wavelength maxima of MMb) increased with increasing amounts of MMb. The actual proportion of MMb added to each chamber was strongly correlated with A503/A581 ($r = 0.94$).

In split-chamber cuvettes containing COMb and OMb, A503/A581 remained constant (A503/A581 = 0.39) as COMb and OMb content changed. Thus, it is likely that A503/A581 is not influenced by COMb-OMb interconversions, but rather, characterizes changes in redox state that occur as COMb is oxidized to MMb.

Based on the actual amounts of each myoglobin derivative added to compartments within the split-cuvettes, the proportion of red colored non-MMb pigments decreased as A503/A581 increased. Thus, our results suggest that A503/A581 can be used to assess heme oxidation in COMb solutions. In addition, A543/A581 may be useful for differentiating between 100% COMb and 100% OMb. Further research is being undertaken to assess the usefulness of reflectance data as a means of determining COMb oxidation on the surface of meat samples.