

Frontier Analysis, Ltd.

TECHNICAL SERVICE RESPONSE NO.: UT012

Subject: Identification of a Red-Brown Residue from an Excised Cow
Discovered 2-21-2001 (Alabama)

Date: June 21, 2001

Requested By: W. C. Levengood
Pinelandia Biophysical Lab.
Grass Lake, MI

Reported By: P. A. Budinger
Analytical Scientist

Objective/Background:

Analyze red-brown particles retrieved from the hide near the anal excision of a mutilated cow. It was purported that the event happened “overnight”, i.e. the cow was found in a grove of trees within 24 hours of the event¹. Other information indicated there was some rain before the animal was found. The carcass apparently fell through trees because limbs were down and a 6” tree broken. The body contained no blood, an udder was removed, and there was a “hole” in the belly. Scuffed up areas on the hide indicated that the cow was possibly strapped.

Conclusions:

The residue found on the excised cow is bovine hemoglobin, a component of blood. This is the second event where hemoglobin has been found². (The first concerned an excised bull found in Red Bluff, California on 1-17-97.) The presence of hemoglobin would indicate a processing of the whole blood has occurred.

As stated in the report of the first event: “The usual procedure for isolating hemoglobin from whole blood is rather complex. It involves separating red blood corpuscles from the lighter plasma components by centrifugation. The plasma is siphoned off and ether is added to the corpuscle paste, causing the cells to burst. Another centrifugation removes the ruptured cell envelopes, and leaves a clear

¹ Personal communication from W. C. Levengood.

² See Frontier Analysis T. S. R. No.: 005.

red solution of hemoglobin.³ It is unlikely that a procedure such as this would be done on site. It is unknown how or why this occurred.”

Procedure:

Sample: The sample was submitted with the following identification.

- KS-05-66 Red-brown particles from a mutilated cow on 2/21/01 in Alabama. They were found on the hide near an anal excision. The sample was received in a small plastic vial.

References:

- Hemoglobin (Hb) Bovine Lyophilized Powder Sigma Cat, No. H-2500
- Whole Bovine Blood, exposed to weathering for nine days on chamois. (June 12 through June 23, 2001)
- Whole Bovine Blood, exposed to weathering for nine days in a small aluminum pan. (June 12 through June 23, 2001)

Infrared spectra were from the residue as well as references of hemoglobin and whole bloods using the Harrick SplitPea™ cell attached to a Nicolet Avatar 360 spectrometer. The ATR crystal used was silicon. Microscope photographs were obtained from the Leica GZ6 stereomicroscope interfaced to a Kodak Digital Science MDS 120 camera.

Results:

Microscope photos of the residue from the Alabama cow and that from an excised bull in California (included for comparison) are shown below. These were taken at approximately 60X magnification.

Residue from Alabama Cow



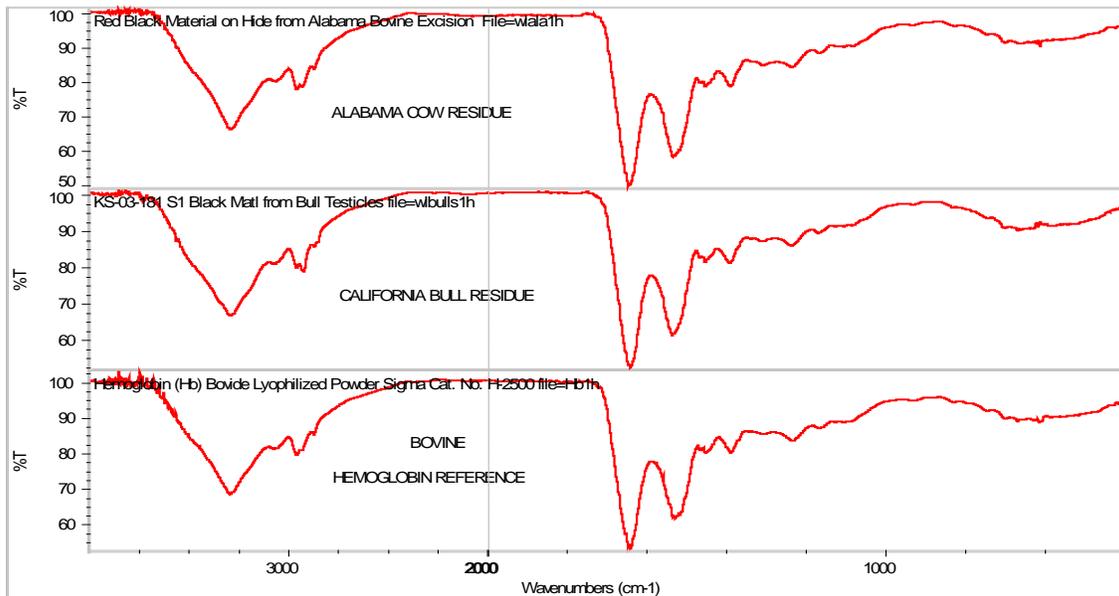
³ Merck Index, 12th Ed., S. Budabari, Ed., p. 794, # 4682 (1996).

Residue from California Bull⁴



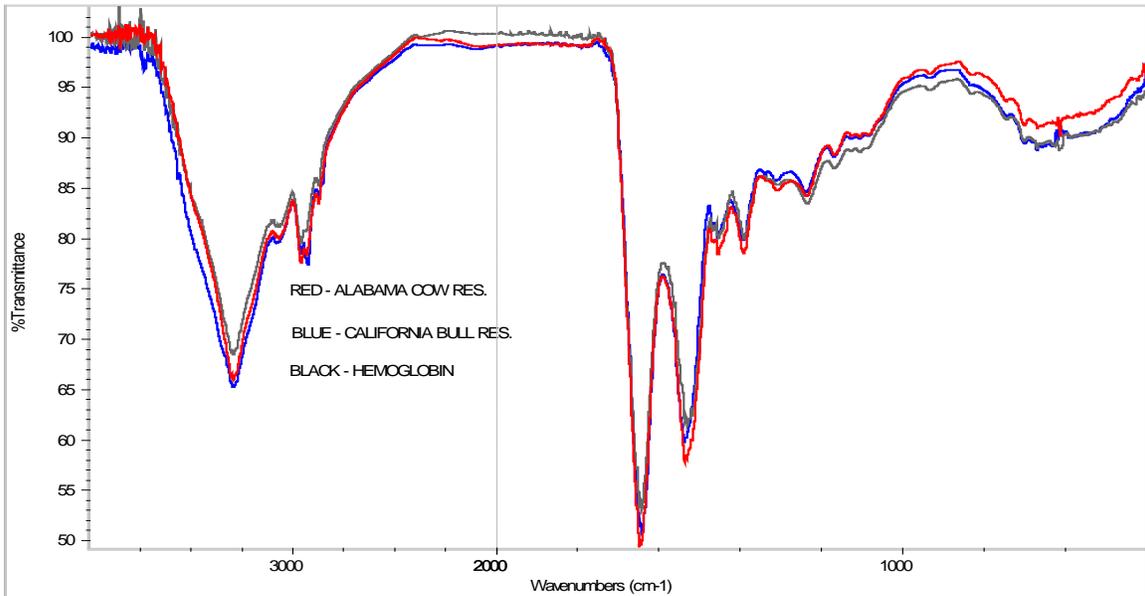
The above photos show the particles visually are similar suggesting they are of the same substance. This was confirmed by the following infrared analysis.

Infrared spectroscopy shows the cow residue is bovine hemoglobin, a component of blood. The infrared spectra match those in a reference of pure bovine hemoglobin purchased from Sigma Aldrich. The spectrum also expectedly matches that of the residue found in the California bull which was also identified as hemoglobin. Following are the spectra of residues from the Alabama cow, the California bull, and a reference of hemoglobin for comparison.

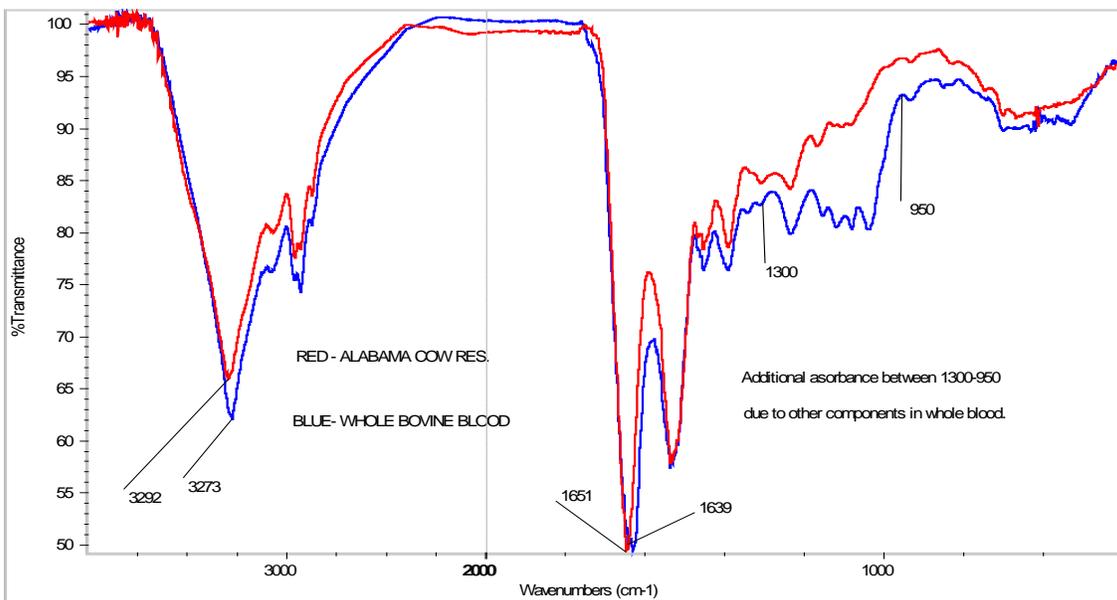


⁴ Ref. 2

The above spectra are superimposed below to more clearly observe the similarities.



The above spectra do not compare to that of the dried whole bovine blood. This was previously observed in the analysis done on the California bull residue. Following is a spectrum of the Alabama cow residue and the whole blood.

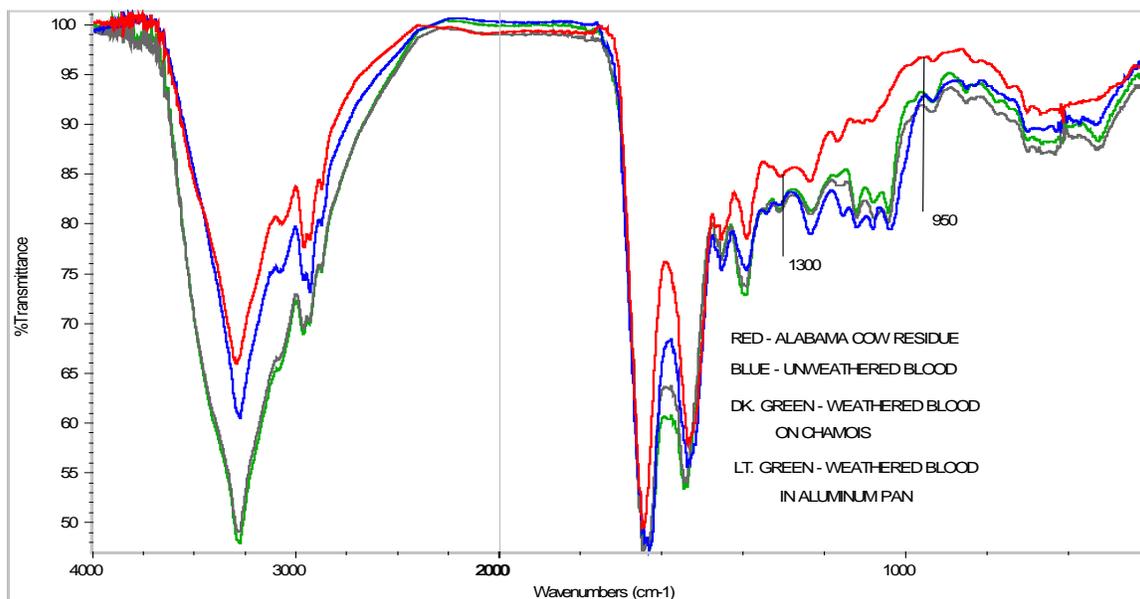


The whole blood spectrum shows additional absorption between 1300-950 cm⁻¹ due to a complex mixture additional components. This additional absorption represents erythrocytes (red cells), leucocytes (white cells), platelets, plasma proteins and serum. Hemoglobin is a protein component found in the

erythrocytes⁵. There are also subtle, but significant, 20 cm^{-1} band shift maxima to the higher frequencies in the strongly absorbing bands ca. 1660-1630 cm^{-1} (II° amide C=O stretch) and 3300-3250 cm^{-1} (amide N-H stretch) in the spectrum of the cow residue spectrum.⁶

At the time of the California analysis, spectra were also obtained of whole blood after exposing it to different environs for comparison to those of the bull residues. It was dried at ambient temperature in the laboratory, exposed to microwaves and dried, and allowed to set for one week before data acquisition. In that analytical report it was noted that none of the spectra compare to those of the residues retrieved from the bull.⁷ See that report for the detailed spectral descriptions. That is obviously true for the residue from the Alabama cow.

Since the “California excised bull” analysis, another experiment on the whole blood was performed in order to determine what (if any) changes occurred after exposure to the “great outdoors”. The blood was weathered under environmental conditions similar to that expected for blood on a carcass, though it is understood that the weather conditions vary widely at the sites where excised animals are found. This experiment was done in order to determine if hemoglobin crystallized/isolated from whole blood for some reason under natural conditions. Following are spectra of whole bovine blood weathered for nine days on chamois leather and in a small aluminum pan. (Note: On weathering the blood “dried” to a solid.) Unweathered dried whole blood and the Alabama cow residue are included for comparison.



⁵ The Condensed Chemical Dictionary, 10th Ed., Gessner G. Hawley, p.140, (1981).

⁶ The spectra were acquired at 4 cm^{-1} resolution.

⁷ Ref. 2.

The spectral data from this experiment reveal that the weathered blood spectra are significantly different from that of the Alabama cow residue by showing additional absorption between $1300\text{-}950\text{ cm}^{-1}$. This absorption is due to the afore-mentioned additional components typical of whole blood. In other words, the spectra of the weathered blood on the chamois and in the aluminum pan show that no isolation of hemoglobin has occurred naturally in this experiment. These spectra, while very similar to that of a spectrum of whole blood, do show very subtle differences due to weathering effects, i.e. decreased absorption at 1153 cm^{-1} and additional absorption in the $1600\text{-}1550\text{ cm}^{-1}$ and $3600\text{-}3000\text{ cm}^{-1}$ regions.

File: UT012.DOC

Phyllis A. Budinger