Table III. Foods Without Detectable (1 γ) Amounts of DDT

Applesauce	Corn[a]	Potatoes, mashed[a]
Beans, string[a]	Corn bread	Rolls[a]
Bean soup	Dry cereal[a]	Root beer
Cake[a]	Grapefruit juice	Steak, pan broiled[b]
Catsup	Gravy[a]	Sirup[a]
Clam chowder	Jello salad	Tea[a]
Coffee, black	Oleomargarine	Toast[a]
Cola beverage	Peas[a]	Tuna fish salad
Combination salad	Potatoes, escalloped[a]	Vegetables, mixed
		Vinegar

[a] Other portions contained a measurable amount of DDT (5 γ or more).
[b] Calculation based on aliquot chromatographed.

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BIOCHEMISTRY OF MYOGLOBIN

Quantitative Determination in Beef and Pork Muscle

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Chemical Studies with Purified Metmyoglobin

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Studies have been initiated to determine the concentration and chemical reactivity of myoglobin, the major heme pigment present in lean meat. A procedure has been developed for the quantitative determination of myoglobin in beef and pork muscle and reactions of purified metmyoglobin (Fe^{+++}) prepared from beef muscle with ascorbate to form myoglobin (Fe^{++}). Reactions of the latter compound with nitrite to form nitric oxide myoglobin were also studied. This work on quantitating the concentration and reactivity of myoglobin is of importance in evaluating the uniformity and stability of myoglobin derivatives in fresh and cured meats during storage or various processing procedures. Chemical changes in the myoglobin of meat attributable to irradiation with gamma rays from a cobalt-60 source are now being studied in this laboratory.

THE HEME PIGMENTS constitute a class of very important biochemical compounds. Much work has been done with hemoglobin and heme catalysts in oxidation (catalase, peroxidase, cytochromes, etc.), but relatively little has been done to elucidate the functions and reactions of myoglobin, the oxygen-carrying pigment in muscle.

The importance of understanding the chemical changes associated with the color of lean meat during irradiation by gamma rays (cobalt-60) or other treatments prompted studies on myoglobin in muscle.

While visual observation of beef and pork muscle indicates that considerable variation exists in the heme pigment content, few quantitative data are available on the myoglobin content of beef and pork muscle. Shenk, Hall, and King (*10*) described a method for the determination of "muscle hemoglobin" (myoglobin) and presented data for beef muscle. Crandall and Drabkin (*1*) and Drabkin (*2*) reported data on the myoglobin content of rat, human, horse, dog, and beef muscle. Husaini (*6*), Hershberger (*5*), and Weiser (*12*), and their associates, have reported twofold variation in amount of total pigment (hemoglobin plus myoglobin) in beef muscle.

As the authors were interested in measuring the changes occurring during irradiation, a method was adopted for the determination of myoglobin in beef and pork muscle.

Work was also undertaken to gain information about the in vitro chemical

reactions of metmyoglobin and myoglobin with ascorbic acid and sodium nitrite. The information obtained from these experiments may be used in the interpretation of changes occurring when myoglobin solutions or muscle tissue are irradiated with gamma rays from a cobalt-60 source. These studies will also be of importance in relation to chemical changes that occur in the meat pigments during curing and other processing or storage experiments.

Quantitative Determination in Beef and Pork Muscle

Experimental The method adopted for myoglobin determinations was based on Theorell's procedure for isolating myoglobin (*11*), Morgan's modification of this procedure (*9*), and Drabkin and Austin's method for the conversion of methemoglobin to cyanmethemoglobin (*3*).

The tissue to be analyzed was put through a meat grinder several times to assure homogeneity. Ten grams of the finely ground tissue were mixed with 10 ml. of water and allowed to remain overnight in the cold (3° to 5° C.). After centrifugation, the supernatant containing the myoglobin was adjusted to pH 7.0. Saturated basic lead acetate equal to one-fourth the volume of the supernatant was then added and the precipitated foreign proteins were removed by centrifugation. Mono- and dibasic potassium phosphate were added to the filtrate from the lead acetate precipitation to bring the phosphate concentration to 3*M* and the pH to 6.6. This precipitated the hemoglobin (as shown in other experiments with additions of hemoglobin) as well as other proteins. Myoglobin was left in solution (*9*). The precipitate was removed by filtration through a fine filter paper. An aliquot was then taken, and potassium ferricyanide and sodium cyanide were added to provide final concentrations after dilution of 0.6 and 0.8 millimole per liter, respectively. The absorbance of the resultant cyanmetmyoglobin was then read in the Coleman Junior spectrophotometer at 540 mμ. The concentration of myoglobin in millimoles per milliliter was obtained from the equation, $E = \frac{O.D.}{C \times l}$, where the extinction coefficient was assumed to be 11.5, the same as that of cyanmethemoglobin (*3*). In converting to milligrams per gram, a molecular weight of 16,500 was assumed. Equal distribution of myoglobin between the extract and residue was ascertained by repeated extraction and this was taken into account when calculating the dilution factor.

The beef muscle samples were from cuts of rib and round obtained at a local market. The pork muscle samples were from pigs weighing approximately 210 pounds, and both light- and dark-colored muscles were used in separate determinations. The samples for analysis were taken from a 1-inch slice from the center of the hams. The latter samples were of particular interest, in that visual observations of fresh and cured pork cuts indicated considerable variation in pigment concentration of different muscles. The results for beef muscle are presented in Table I along with the average figures for the muscle hemoglobin (myoglobin) content of ribeye muscle of beef animals presented by Shenk, Hall, and King (*10*) and the figure for the myoglobin content of beef muscle reported by Drabkin (*2*).

There is good agreement between the figures presented by Shenk, Hall, and King and those presented in this paper. The figure presented by Drabkin is somewhat lower than the lowest values obtained in either of the other two studies. Shenk, Hall, and King state that the differences they observed in muscle hemoglobin content of the samples cannot be due to nutritional causes but may be explained by the assumption that animals which have more exercise have a higher muscle hemoglobin content (the animals in their experiments which gave high values were pasture-fed, hence getting more exercise than the others which were grain fed).

Results The results of analyses on representative samples of fresh pork muscle are shown in Table II.

These results give a quantitative confirmation of visual observation of differences in color of muscles in a particular cut of ham or loin. A color difference has also been observed within a particular muscle. While the variation in muscle color may be attributed to difference in amount of exercise this does not explain the variations observed within a particular muscle. The ratio of the myoglobin content of beef muscle, as compared to pork muscle, averaged 4.7 to 1 for the light-colored pork muscle samples and 2.6 to 1 for the dark-colored pork muscle samples. Further work on the distribution, purification, and chemical reactivity of myoglobin is being carried out (*8*).

Myoglobin analyses were also made for beef and pork heart to serve as a guide for source material in the isolation and chemical studies with myoglobin. Muscle was as high as heart in myoglobin content (Tables I and II).

Summary When quantitative determinations of myoglobin in beef and pork muscle were carried out, a twofold difference was observed in the content of different pork muscles from the same cut. The ratio of myoglobin content in beef muscle as compared to pork muscle averaged 4.7 to 1 for light-colored pork muscle and 2.6 to 1 for dark-colored pork muscle.

Table I. Myoglobin Content of Beef Muscle and Beef Heart

(All values expressed as mg. of myoglobin per gram of fresh tissue)

Sample	This Paper	Av. Shenk and Associates (10)	Drabkin
Beef muscle	3.70	3.32	1.69
	3.68	3.87	
	3.98	4.54	
	2.79		
	3.69		
	4.22		
	5.41		
	2.26		
Average	3.7		
Beef heart	2.10		

Table II. Myoglobin Content of Pork Muscle and Pork Heart

(All values expressed as mg. of myoglobin per gram of fresh tissue)

	Sample No. 1	2	3	4	5	6	7	8	
Pork muscle									
Light[a]	0.80	0.66	0.98	0.87	0.84	0.61	0.75	0.82	
								Av.	0.79
Dark[a]	1.48	1.20	1.44	1.48	1.77	1.04	1.46	1.68	
								Av.	1.44
Pork heart	0.92								

[a] The light-colored muscle was the *Biceps femoris*, and the darker-colored samples were a composite of the *Rectus femoris*, *Vastus lateralis*, *Vastus intermedius*, and *Vastus medialis* muscles. These studies were conducted in collaboration with W. J. Aunan, Department of Animal Husbandry, University of Minnesota.