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Myoglobin, Hemoglobin and Iron in Bovine and Porcine Muscle

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Introduction

Myoglobin is regarded as the main muscle pigment which imparts to meat its typical colors. However, in the literature data have been reported again and again indicating that in the skeletal muscle of well exsanguinated slaughter animals 30 to 50 % of the total pigment is present as hemoglobin (e.g. 1,2,3). Therefore, a thorough analysis of both pigments, myoglobin and hemoglobin, in the skeletal muscle of pigs and cattle seemed to be necessary. Model experiments for studying the discoloration of meat are often carried out with a solution of pure myoglobin. If the meat really contains remarkable amounts of hemoglobin, this should be considered in such model experiments because the chemical and physical properties of hemoglobin are somewhat different from those of myoglobin.

Another problem which interested us in connection with these studies on muscle pigments was the distribution of iron within the muscle tissue. Many years ago we observed that apparently a considerable proportion of the muscle iron cannot be bound to the heme pigments (4). If this is true, then the question arises of whether it is justified to determine the iron content instead of the myoglobin content as is sometimes the practice in the field of animal science.

In order to answer these questions, the following experiments were carried out with the same muscle tissue:

1) Determination of the total heme content which includes myoglobin, hemoglobin and minor amounts of other heme compounds such as cytochromes.
2) Quantitative separation and determination of myoglobin and hemoglobin by sephadex chromatography.

3) Isolation of pure myoglobin from the tissue and pure hemoglobin from the slaughter blood of the same animal.

4) Determination of the iron content of the tissue, the blood and the isolated pigments (myoglobin and hemoglobin).

Methods

For the determination of the total heme content of the tissue the method of HORNSEY as previously slightly modified (5) was used.

The separation and quantitative determination of myoglobin and hemoglobin were carried out by chromatography of the watery muscle extract on a column with Sephadex G 75-superfine, recording of the absorption of the eluant at 410 nm, and evaluation according to the method of BUENNIG (6,7). Fig. 1 shows the good separation of myoglobin and hemoglobin obtained by this procedure. The chromatographic isolation of myoglobin preparations from the tissues and of hemoglobin from the blood samples was carried out using the procedure of BUENNIG and HAMM (7).

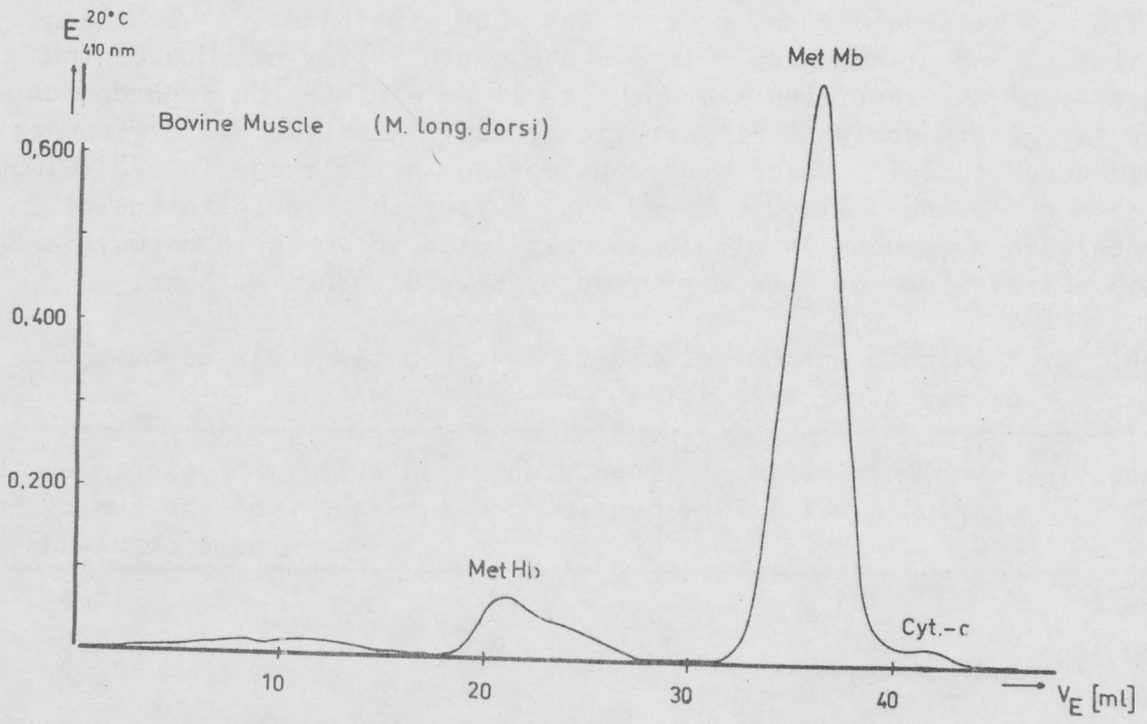

Fig. 1: Separation of Metmyoglobin (MetMb) and Methemoglobin (MetHb) by sephadex chromatography. V_E = volume of the eluant. Cyt.c = cytochrome c.

The iron content of the tissue, the blood and the preparation of myoglobin and hemoglobin was determined after digestion of the sample in perchloric acid-H_2O_2 by a slightly modified sulfosalycilic acid procedure (8).

Results and Discussion

The longissimus dorsi muscle of normally exsanguinated cattle contained only small amounts of hemoglobin equal to about 3-6 percent of the total pigment (Table 1) which is contrary to heart muscle in which we found about 50 % of the total pigment as hemoglobin. The hemoglobin content of the longissimus dorsi muscle of pigs ranged between 3 and 12 % of the total pigment (Table 1). A higher hemoglobin content (15.8 %) was found in the psoas muscle of the pig.

We conclude from this result that hemoglobin contents in the skeletal muscle of 30 % or more of the total pigment, which are reported in the literature, are caused by the use of inaccurate procedures.The methods used most for determination of myoglobin and hemoglobin in tissue are based on a photometric determination of all heme pigments in watery or buffered extracts after their transformation to CN-, CO-, O_2- or Met-derivatives, which gives the value of total pigments. After elimination of other accompanying proteins - by precipitation with lead acetate - and of hemoglobin in 3 M phosphate buffer at pH 6.0, the content of the remaining heme protein is determined and regarded as the "myoglobin content". It surprising that this type of "difference procedure" is still being used considering that already in 1954 GINGER, WILSON and SCHWEIGERT (9) showed that during the precipitation of hemoglobin myoglobin is also co-precipitated in variable amounts and this observation has been confirmed by several other authors.

Table 1. Myoglobin and hemoglobin contents in the skeletal muscle of slaughter animals

species	myoglobin content mg/100 g wet tissue	hemoglobin content mg/100 g wet tissue	hemoglobin as the % of the sum of both heme compounds
pig*)	154.0	29.0	15.8
pig	117.9	6.7	3.0
pig	86.6	8.1	8.6
pig	86.9	8.0	8.4
pig	69.8	9.9	12.4
cattle	297.2	11.1	3.6
cattle	284.5	17.4	5.8
cattle	400.5	19.7	4.7

*)Psoas muscle; all the other samples: Longissimus dorsi muscle.

Table 2 shows the distribution of iron in the muscle tissue. The total heme iron was calculated from the total heme content. The iron values for myoglobin and hemoglobin follow from the quantitative determination of both pigments by sephadex chromatography and from the iron contents of the isolated pigments.

The values of total iron corresponded nearly to the sum of myoglobin and hemoglobin iron (72 - 100 %). This good correspondence indicates that the methods used are reliable. In porcine muscle about 26 % and in bovine muscle about 58 % of the total iron was found bound to myoglobin.

Contrary to data reported in the literature only 0.75 % (porcine muscle) and 0.74 % (bovine muscle) of the total iron was found as hemoglobin iron.

Whereas in the blood of the animals investigated 97 to 99 % of the total iron was present as heme iron, in the skeletal muscle this proportion was much lower. The non-heme iron content was as high as 68 % of the total iron in porcine muscles and 29 % in bovine muscles. The type of binding of this non-heme iron and its physiological role are not yet exactly known. It is improbable that this iron is bound to heme compounds which are not determined by the method used. As we found in earlier work (4,10), no free iron ions are present in muscle tissue; and in bovine muscle 28 % of the total iron was not bound to soluble pigments but to myofibrillar proteins. This value is in good agreement with the non-heme iron content of bovine muscle reported here (29 %).

Table 2. Distribution of iron within the muscle tissue

species	total iron mg/100 g wet tissue	iron content as % of the total iron				myoglobin content mg/100 g wet tissue
		total heme iron	non-heme iron(Fe from cytochromes	hemoglobin iron	myoglobin iron	
pig*)	1.74	37.41	62.59	1.50	28.35	154.0
pig	0.86	43.95	65.05	0.62	38.95	117.0
pig	0.92	25.98	74.02	0.74	23.05	86.6
pig	1.01	23.16	79.41	0.69	22.47	86.9
pig	0.86	27.20	72.80	0.94	21.39	69.8
cattle	1.78	67.97	32.03	0.55	51.09	297.2
cattle	1.69	71.01	28.93	0.89	63.96	284.5
cattle	1.92	73.54	26.46	0.87	63.12	400.5

*) Psoas muscle; all the other samples: Longissimus dorsi muscle.

Table 2 also gives some information on the relationship between total iron and myoglobin contents. The psoas muscle of pig with its relatively high myoglobin content also had a high iron content. However, in the longissimus dorsi muscles of pigs the muscle with the highest myoglobin content (117 mg/100 g) showed the lowest iron content (0.86 mg/100 g). For the longissimus dorsi muscle of cattle a closer relationship between iron content and myoglobin content seems to exist. But as these results show it is not justified to accept a priori that with an increased myoglobin content in the muscle tissue the iron content also is increased correspondingly.

APPENDIX

The separation and quantitative determination of myoglobin and hemoglobin by column chromatography on sephadex is much too time consuming for studying the myoglobin/hemoglobin proportion in a great variation of different muscles and species and for investigation of the influence of the exsanguination on the hemoglobin content of meat. Therefore, in our laboratory R.HAMM, B. ROGOWSKI and W. ARNETH developed a fast routine method by transforming the column procedure of BUENNIG and HAMM (7) into a thin-layer chromatographic method on sephadex. This method allows the analysis of about 6 samples on one TC-plate. The proportion of myoglobin and hemoglobin can be determined in situ on the TC-plate without any dying procedures by measuring the natural color of the oxidized pigments using a TC-scanner.

Procedure: The thin-layer is prepared from a sephadex G 75 superfine - tris buffer (0.1 M; pH 7.9) gel and equilibrated with the tris buffer for at least four hours . Meat is extracted by homogenizing with dest. water (1:1) causing homolysis of the erythrocytes; the pigments of the extract are oxydized by $K_3[Fe(CN)_6]$. 10 to 20 µl of the extract are placed on the thin-layer plate. The chromatography is carried out for about 1 hour using the tris buffer as solvent. Immediately after chromatography the remission of the brownish bands of metmyoglobin and methemoglobin at 410 nm are measured and evaluated in situ on the wet plate using a Zeiss-chromatogramm-spectrophotometer and an integrator (CRS-100 A Auswerte-System; Infotronics Corp.). The method is calibrated by measuring solutions of different proportions of pure metmyoglobin and pure methemoglobin in tris buffer.

Fig. 2 shows examples of the separation of myoglobin and hemoglobin in muscle extracts using this method.

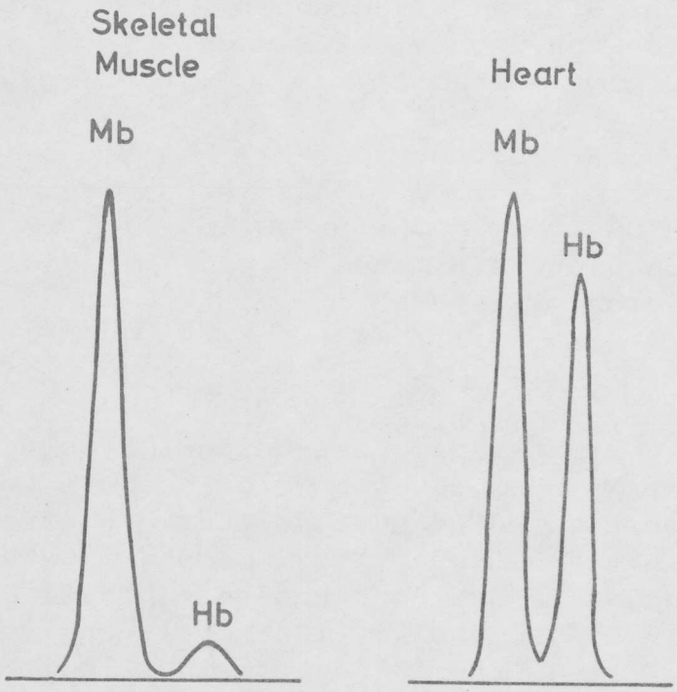

Fig. 2. Sephadex thin-layer chromatograms of watery extracs of bovine heart muscle and bovine skeletal muscle (M. longissimus dorsi)

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