CARNOSINE AND ANSERINE IN MAMMALIAN SKELETAL MUSCLE*

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Two imidazole derivatives, carnosine and anserine, have been isolated from vertebrate skeletal muscle. Carnosine may be readily obtained from the ox (1), horse (2), pig (3), etc. Anserine, a methyl carnosine, has been isolated from birds, a reptile (4), fish (5), and certain mammals (6). The presence of the two compounds in muscles from the same animal has been adequately demonstrated only once: Hoppe-Seyler, Linnneweh, and Linnneweh (4) obtained anserine and a small amount of carnosine from the crocodile. We report below information which, together with the data in a previous paper (6), demonstrates that both carnosine and anserine are present in the muscles of several mammals.

Isolation of Anserine—The procedure of Ackermann, Timpe, and Poller (7) as modified by us (6) was employed for anserine isolations. Further modifications in the original procedure were made by precipitating copper anserine from aqueous solution with acetone and recrystallizing from ammonia, steps used by von Fürth and Hryntschak (8) and Kuen (9) in the estimation of carnosine. In outline, the present isolations were made as follows. A concentrated protein-free aqueous extract of muscle was precipitated with mercuric sulfate and alcohol. The mercury precipitate was decomposed with hydrogen sulfide and the solution was

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then fractionated with barium hydroxide and silver nitrate. The lysine fraction was freed from silver and barium, concentrated, and precipitated with mercuric sulfate and alcohol. The mercury precipitate was decomposed, and the resulting solution, freed from sulfate, was concentrated and treated with copper carbonate. The copper anserine was precipitated by adding 5 volumes of acetone and allowing the mixture to stand 24 to 72 hours at 0°. The copper salt was dissolved in a minimum quantity of ammonium hydroxide (sp. gr. 0.90) and centrifuged. The ammoniacal solution was decanted from the insoluble residue, and diluted with 4 or 5 volumes of water. The ammonia was allowed to evaporate spontaneously, the last traces being removed in vacuo over sulfuric acid. Any decrease in volume of liquid was made up by adding distilled water. When crystallization appeared complete, usually in 24 to 36 hours, the crystals were collected in a centrifuge tube and washed three times with small quantities of distilled water. The copper salt was recrystallized two or three times from ammonium hydroxide and finally dried in vacuo over sulfuric acid. In one preparation (opossum) leaching out the copper anserine with a little dilute ammonium hydroxide reduced the color value from 2.74 to 1.29 per cent.

Isolation of Carnosine—Several difficulties arose in the isolation of relatively pure carnosine from muscles low in the compound. In dog muscle the carnosine determined colorimetrically accounted for only 2 or 3 per cent of the total extractive nitrogen. In the first carnosine fraction obtained with silver nitrate and barium hydroxide, the carnosine (diazo, colorimetric) nitrogen amounted to 20 per cent of the nitrogen present. Two more fractionations with silver and baryta raised the carnosine nitrogen to 60 per cent of that present in the silver precipitate. Owing to losses of carnosine, only two or three treatments with silver and baryta were practicable.

The precipitability of carnosine changed considerably as the isolation proceeded. From a relatively crude extract most of the carnosine precipitated with silver and baryta in the pH range 8 to 11. In the second and third fractionations it precipitated almost completely between pH 6 and 7.5. In the later stages of the preparation it was precipitated by mercuric sulfate much more
readily than in the early stages. In the final precipitation with mercuric sulfate most of the carnosine came down from aqueous solution if the pH were adjusted to 4.0 to 4.5 with barium hydroxide.

The isolation of carnosine from dog and cat muscle was further complicated by the fact that the fractions had been stored for months in the ice box before they were studied. Under such conditions it is our experience that the difficulties of isolation are greatly increased and the yields are low. With these two preparations, attempts to prepare the copper salt resulted in a green amorphous wax. In these instances the material was dissolved in approximately 2 M sulfuric acid and the copper removed with hydrogen sulfide. The resulting solution, freed from sulfide, was treated with about 200 mg. of mercuric sulfate per gm. of carnosine present in 500 cc. volume. After standing overnight, the brownish precipitate was removed by centrifuging. The supernatant fluid, which contained 80 or 90 per cent of the carnosine, was treated with mercuric sulfate in excess and enough barium hydroxide to keep the pH at 4 or 5. This precipitate, containing most of the carnosine, was decomposed, the sulfate removed quantitatively with barium hydroxide, and the carnosine converted into the copper salt. A crop of clean blue hexagons resulted in both preparations.

Crude preparations of copper anserine and copper carnosine contain too little nitrogen and show low decomposition points, so that correct values for each constitute good evidence of freedom from unknown impurities. Carnosine may be freed from anserine with ease from solutions not too concentrated, because carnosine precipitates with silver and baryta, while anserine does not. It is far more difficult to remove small amounts of carnosine from anserine. The color values (6) of copper anserine indicate the extent of admixture of copper carnosine. That the copper carnosine preparations were relatively free from copper anserine was indicated by the appearance of the crystals and by the color values. The color values for the preparations of copper carnosine mentioned in this paper varied from 100 to 108 per cent of the expected values calculated from our standard carnosine solutions. The reason for the high values is uncertain.
Dog. Copper Carnosine—Typical blue hexagons were obtained which decomposed at 215–216°. The preparation was recrystallized three times from ammonia and dried at 120° for analysis.

\[
\text{C}_2\text{H}_4\text{N}_2\text{O}_2\cdot\text{CuO. Calculated. N 18.36} \\
\text{Found. " 17.97 (Kjeldahl)}
\]

Cat. Copper Carnosine—The typical blue hexagons decomposed at 218–219°.

\[
\text{C}_2\text{H}_4\text{N}_2\text{O}_2\cdot\text{CuO. Calculated. N 18.36} \\
\text{Found. " 18.36 (Kjeldahl)}
\]

Deer (Odocoileus virginianus) —5.06 kilos of clean skeletal muscle were obtained mainly from the chest and forelegs of two animals. The tissue was kept at 5–10° for 48 hours prior to extraction.

Copper Anserine—4.39 gm. of typical lilac-red crystals which decomposed at 220° and gave a color value (6) of 0.6 per cent (carnosine) were isolated.

\[
\text{C}_\text{18}\text{H}_\text{12}\text{N}_\text{3}\text{O}_\text{3}\cdot\text{CuO. Calculated. N 17.53} \\
\text{Found. " 17.32 (Dumas)}
\]

Copper Carnosine—0.92 gm. of typical blue hexagons which decomposed at 219° was isolated.

\[
\text{C}_\text{4}\text{H}_\text{14}\text{N}_\text{2}\text{O}_\text{2}\cdot\text{CuO. Calculated. N 18.36} \\
\text{Found. " 18.58 (Kjeldahl)}
\]

Opossum (Didelphis virginiana)—Seven animals, killed in the laboratory, were dissected and the skeletal musculature was worked up immediately. The weight of muscle was 5.22 kilos.

1 This was obtained from silver precipitates of the extract which yielded the anserine described in a previous paper (6).

2 All decomposition temperatures have been corrected. The decomposition points are of value in showing the absence of certain impurities present in crude preparations. However, the decomposition points are unsatisfactory for demonstrating the presence of copper anserine in copper carnosine preparations and vice versa.

3 The recrystallization of copper carnosine, by dissolving it in strong ammonia and diluting after removal of some insoluble foreign material, is not ideal. The analyses of the preparations improved but the decomposition points became less satisfactory.
Copper Anserine—2.25 gm. of lilac-red crystals which decomposed at 229° and gave a color value of 1.29 per cent were isolated.

\[ C_{13}H_{14}N_3O_4 \cdot CuO \]

Calculated. N 17.53
Found. " 17.52 (Dumas)

Copper Carnosine—0.33 gm. of typical blue hexagons which decomposed at 220–221° was isolated.

\[ C_{13}H_{14}N_4O_3 \cdot CuO \]

Calculated. N 18.36
Found. " 18.0 (Dumas)

Gnu (Connochtes taurinus)—A healthy animal, injured in an accident, was killed by its keeper and the carcass kept in an ice box 16 hours prior to dissection of 8 kilos of muscle from the hind legs. The diazo method applied to this tissue indicated 0.017 per cent carnosine, the lowest value which we have obtained on mixed skeletal muscle from any mammal. It is rather striking that an animal closely related to the ox should show only a trace of carnosine and large amounts of anserine.

Copper Anserine—9.95 gm. of typical lilac-red crystals which decomposed at 225–227° and gave a color value of 0.31 per cent were isolated.

\[ C_{13}H_{14}N_3O_4 \cdot CuO \]

Calculated. N 17.53
Found. " 17.70 (Dumas)

Copper Carnosine—33 mg. of typical blue hexagons which decomposed at 216–220° were isolated.

\[ C_{13}H_{14}N_4O_3 \cdot CuO \]

Calculated. N 18.36
Found. " 18.0 (Dumas)

Llama (Lama glama)—A healthy animal was killed and the muscles dissected from the hind legs immediately. The total weight of muscle used was 6.8 kilos.

Copper Anserine—4.94 gm. of typical lilac-red crystals which decomposed at 225° and gave a color value of 2.7 per cent were isolated.

\[ C_{13}H_{14}N_3O_4 \cdot CuO \]

Calculated. N 17.53
Found. " 17.70 (Dumas)

This isolation was carried out by Mr. John Zapp.
Carnosine and Anserine in Muscle

DISCUSSION

We have found both carnosine and anserine in the skeletal muscles of five mammals including two carnivores, a marsupial, and two ruminant herbivores, whereas carnosine alone has been found in the herbivorous horse and ox. Of these two compounds, anserine was certainly present in excess in the muscles of the dog and gnu. This is shown in Table I by a comparison of the compositions of the muscles, the calculations being based on (1) the recoveries of anserine, which yield data naturally much too low, and (2) the diazo color determinations for carnosine, which are probably a little too high. With figures based on the yields of carnosine obtained by isolation, the comparison is even more striking, is possibly more nearly correct, and suggests that the mixed muscles from the cat, deer, and opossum may also contain as much (or more) anserine as carnosine.

SUMMARY

Data presented in this and a previous paper demonstrate that anserine and carnosine are present together in the skeletal muscles of the dog, cat, deer, gnu, and opossum. Anserine has been isolated from the llama.

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