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Identification of β-Alanyl-Lysine in Chick Muscle[1]

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WHILE studying the metabolism of lysine in growing chicks fed a diet containing 35% casein, an unidentified ninhydrin-positive peak was observed in acid extracts of muscle analyzed for free amino acids. The compound was hydrolyzable and was eventually identified as a peptide, composed of β-alanine and lysine.

There is little information in the literature concerning such a peptide and no details concerning its presence in avian species. Other related peptides, carnosine (β-alanylhistidine) and anserine (β-alanyl-1-methylhistidine) have been recognized as constituents of muscle for many years. Kalyankar and Meister (1959) have described the enzymatic synthesis of carnosine, anserine and several related peptides, including β-alanyl-lysine. They used a cell-free system obtained from chick pectoral muscle. Meister (1965) referred to an unpublished observation that a peptide with the properties of β-alanyl-lysine occurs in the muscle of chicks fed diets containing 2% lysine. Recently α-(β-alanyl)-lysine has been isolated from rabbit muscle (Matsuoka *et al.*, 1969).

EXPERIMENTAL PROCEDURES AND RESULTS

Previous experiments had shown that extracts of muscle from chicks fed casein based diets contained an unidentified compound which eluted at the same position as the internal standard, α-amino-γ-guanidobutyric acid. To isolate the compound, a chick, fed a practical corn-soybean meal diet for 16 days, was fasted for 4 hours and fed a 35% casein diet (O'Dell and Savage, 1966) for 24 hours. Lysine-^{14}C (uniformly labeled) was injected intraperitoneally at a dose of 15 μCi. per 100 grams of body weight (44.6 μCi. total). Immediately after

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TABLE 1.— *Distribution of radioactivity among exhaled carbon dioxide, excreta and the non-protein fraction of tissues*

Component	Distribution
Co_2	7.7% recovery of ^{14}C-dose
Excreta	3.3% recovery of ^{14}C-dose
Plasma	1.31×10^5 c.p.m./ml.
Muscle	4.34×10^5 c.p.m./gm.
Liver	1.59×10^5 c.p.m./gm.
Kidney	4.61×10^5 c.p.m./gm.

44.6 μCi. of L-Lysine-^{14}C(μl.) were given intraperitoneally and samples taken after 2 hours.

TABLE 2.— *Distribution of radioactivity among the amino acid fractions of the sulfosalicylic acid extracts*

Tissue	Acidic and neutral amino acids	Lysine	Unidentified peak
	%	%	%
Plasma	13	87	—
Muscle	10	54	36
Liver	5	95	—
Kidney	19	81	—

See Table 1 for conditions.

administration of the isotope the chick was placed in an all glass metabolism cage (Delmar-Roth) and the CO_2 collected in 2N NaOH during a 2-hour period. The total urinary and fecal excreta were collected in 10 ml. of 10% sulfosalicyclic acid so as to minimize bacterial action. At the end of the period the chick was sacrificed and the radioactivity in the acid-soluble fraction of breast muscle, liver and kidney was determined. Approximately 1 gram of tissue was homogenized with 4 volumes of 10% sulfosalicylic acid, the precipitated protein removed by centrifugation and the clear supernate retained. The plasma proteins were precipitated with an equal volume of 10% sulfosalicylic acid. For liquid scintillation counting one ml. aliquots of the tissue extracts and diluted excreta were placed in 10 ml. of Bray's solution. The $^{14}CO_2$ was prepared for counting by suspension of 1.0 ml. of the sodium hydroxide solution in 10 ml. of Bray's solution containing 4% (W/V) of the thixotropic gel, Cab-O-Sil.[3] The distribution of radioactivity in the exhaled carbon dioxide, excreta and nonprotein fractions of plasma, muscle, liver and kidney is presented in Table 1. Assuming that 40% of the chicken's mass is muscle, it is clear that at this time the majority of the carcass radioactivity was present in the non-protein fraction of muscle.

[3] Godfrey L. Cabot, Inc., Cambridge, Mass.

The sulfosalicylic acid extracts of the tissues were analyzed for both radioactivity and amino acid content by use of a Beckman[4] amino acid analyzer and a scintillation flow cell placed in a Tri-Carb liquid scintillation spectrometer.[5] The shorter column of the 2-column Beckman system separates the basic amino acids but the acidic and neutral amino acids are eluted first, as a composite. The distribution of radioactivity in the amino acid fractions of the tissue extracts separated by this column is shown in Table 2. Only in the case of muscle was an unidentified peak observed, and here it accounted for a significant amount of the total radioactivity (36%). Determination of the acidic and neutral amino acids was made but the radioactivity was too low to give precise identification.

An aliquot of the muscle extract was hydrolyzed with 6N HCl at 110°C. for 20 hours. It was then filtered, evaporated to dryness, made to a 5-ml. volume and an aliquot analyzed for amino acids. The unidentified peak disappeared and the concentration of lysine as well as the radioactive peak for lysine increased. This indicated that the unidentified peak was a peptide containing lysine. In addition the concentrations of 1-methylhistidine, histidine

[4] Beckman Instruments, Inc., Fullerton, Calif.

[5] Packard Instrument Co., Inc., Downers Grove, Ill.

and β-alanine, increased. An increase in these amino acids would result from hydrolysis of the peptides, anserine and carnosine. Because of the high concentration of β-alanine it was not possible to determine the proportion that arose from hydrolysis of the unidentified peptide.

To further identify the unknown peptide an aliquot of the extract was applied to the short column of the amino acid analyzer and the radioactive fraction was collected from the flow cell before it passed to the color developing component of the analyzer. The fraction was rechromatographed in order to exclude small amounts of contaminating amino acids. Following acid hydrolysis of the purified fraction β-alanine and lysine were found in a ratio of 1:1.04. This is in good agreement with the value of 1:1.03 observed for the components of β-alanyl-lysine in rabbit muscle (Matsuoka *et al.*, 1969). Simultaneous recordings of the ninhydrin color and radioactivity of the basic amino acids extracted from muscle are presented in Figure 1. The peptide eluted after carnosine and before arginine.

Although the data presented do not rigorously prove the structure of the peptide, its chromatographic characteristics suggest that it is α-(β-alanyl)-lysine. Nothing is known concerning its physiological function, but it is also true that the function of other muscle peptides, carnosine and anserine, is unknown. Davey (1960) has suggested that they serve as buffers which stabilize the pH during the accumulation of lactic acid. Casein contains a high concentration of lysine and an excess of this amino acid may promote the formation of β-alanyl-lysine. It is possible that peptide formation acts to detoxify lysine or the peptide itself may affect muscle function.

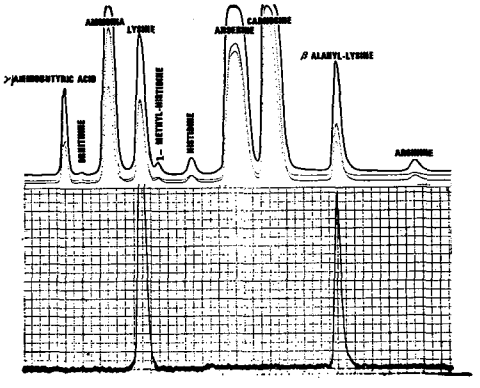

FIG. 1. Simultaneous recordings of the absorbance of the ninhydrin products (top) and radioactivity (bottom) of a sulfosalicylic acid extract of chick muscle showing the elution pattern and relative position of β-alanyl-lysine.

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