Enzymatic Synthesis of Anserine in Skeletal Muscle by N-Methylation of Carnosine*

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Anserine biosynthesis may occur in skeletal muscle by two and possibly three pathways: (a) condensation of l-methylhistidine with β-alanine (1, 2), (b) direct N-methylation of carnosine (3, 4), and, possibly, (c) β-alanyl transfer from carnosine to 1-methylhistidine (5). The relative importance of these various routes is unevaluated, although some studies in vivo suggest that species differences are significant, e.g. methionine but not 1-methylhistidine is utilized for the synthesis in vivo of anserine in the rat, in contrast to the chick which can utilize either compound (7, 8).

Winnick and Winnick (3) have shown that S-adenosyl-l-methionine may function as a methyl donor for the synthesis in vitro of anserine and to a lesser degree for synthesis of 1-methylhistidine. The present paper describes a further investigation of the properties and specificity of the enzyme obtained from chick muscle, carnosine-N-methyl transferase, which catalyzes the synthesis of anserine by transfer of the methyl group of S-adenosylmethionine to carnosine. A preliminary report of this work has appeared (4).

EXPERIMENTAL PROCEDURE

Materials

S-Adenosyl-L-methionine-methyl-C14 was isolated as the bromide salt from yeast after incubation with L-methionine-methyl-C14 according to the procedure of Stokol, Anderson, and Weiss (9). Radioactive purity of the bromide salt of S-adenosylmethionine was verified by chromatographing an aliquot on Whatman No. 1 paper in ethanol-acetic acid-water (65:1:34, volume per volume) (10) followed by examination for ultraviolet-absorbing, ninhydrin-reactive, and C14-containing components. A single radioactive spot having an Rf identical with that reported for S-adenosylmethionine by Schlenk and DePalma (10) was obtained. L-Anserine was isolated from fresh cod muscle by chromatographic procedures previously described (11). L-Carnosine, L-1-methylhistidine, and L-homocarnosine sulfate were purchased from the California Corporation for Biochemical Research. The purity of these compounds was verified by chromatographing samples on Whatman No. 1 paper with butanol-acetic acid-water (4:1:1 volume per volume) as the developing agent, and spraying the papers with ninhydrin and with diazonsulfanilic acid reagent (12). L-Methionine-methyl-C14 was obtained from Isotope Specialties Company, Inc. Methylhistamine was prepared by the method of Rothschild and Schayer (13). The dipicrate derivative of L-1-methylhistidine was prepared according to Fargher and Pyman (14).

Methods

Assay of Carnosine-N-methyl Transferase—The assay of carnosine-N-methyl transferase is based upon measurement of the incorporation of C14 from S-adenosylmethionine-methyl-C14 into the 1-methylhistidine moiety of anserine. The enzyme preparation was incubated for 90 to 60 minutes in 12-ml centrifuge tubes at 37° with 40 to 60 μmoles of L-carnosine, 50 μmoles of Tris buffer (pH 8.0), 1.0 μmole of S-adenosyl-L-methionine-methyl-C14 (10,011 c.p.m. per μmole), and other additions (as indicated in individual experiments) in a final volume of 2.3 ml. After incubation, the mixture was deproteinized by the addition of an equal volume of absolute ethanol and heated in a boiling water bath for 1 minute. In some of the earlier experiments, 1.5 ml of 1.5 N perchloric acid were added for deproteinization. As carrier, 10 μmoles of anserine or 1-methylhistidine were added and the mixture was centrifuged to remove protein. The protein precipitate was washed with 1 ml of water and the wash was added to the deproteinized supernatant solution. The sample was then applied to a Dowex 50 X4 (H+) column (0.9 X 15 cm), and the sample was rinsed in with a few milliliters of water. The column was developed with water, 0.2 M pyridine, and 0.2 M 2,6-lutidine according to previously described procedures (6). The peptides displaced by lutidine were either further freed from contaminants by chromatography on a Dowex 50 column saturated with 2,6-lutidine (0.9 X 9 cm), or the peptide fraction was hydrolyzed in 6 N HCl in a sealed tube for 5 to 16 hours and, after repeated concentration of the resulting hydrolysate to remove HCl, the hydrolysate was chromatographed on a Dowex 50 column saturated with α-picoline (11). The peptide fraction or 1-methylhistidine obtained by acid hydrolysis of the peptide mixture was made to a suitable volume with 30% ethanol, and an aliquot was plated and dried on an aluminum planchet and counted as an infinitely thin sample to a ±2% accuracy in a Geiger gas flow counter equipped with a Micromil end window. Results are expressed as millimicro moles of anserine synthesized per mg protein per hour.

Identity and purity of samples were verified by ascending paper chromatography in ethanol-water-acetic acid, 65:34:1, (10) and in other systems described previously (8, 11). Protein was assayed by the Lowry-Folin method (15).

Partial Purification of Carnosine-N-methyl Transferase—Pec-
toral muscle from young White Rock chicks, weighing 80 to 90 g, after cutting into small pieces, the muscle was minced in a Latapie mincer and then homogenized for 2 minutes in a large Potter-Elvehjem homogenizer in 5 volumes 0.3 M sucrose. The homogenate was centrifuged in the cold for 6 minutes at 1000 × g to remove nuclei and cell debris. In experiments in which mitochondrial and microsomal fractions were isolated, the Hogeboom-Schneider fractionation procedure (16) was used. Usually, the supernatant solution was obtained by centrifugation of the fluid obtained from low speed centrifugation or of the homogenate itself at 10,000 × g for 10 minutes. This supernatant, which contains about 8 mg of protein per ml, was usually dialyzed for 16 hours against cold distilled water to precipitate some inert protein, and to remove endogenous carnosine and other trace metabolites. With this preparation, there was insignificant incorporation of C14 into anserine in the absence of added substrate. The preparation was divided into portions of 5 ml and stored frozen. Under these conditions, it retained essentially full activity for at least 3 months.

Solid ammonium sulfate was added to 100 ml of ice-cold dialyzed extract adjusted to pH 7 until a 40% saturated solution was obtained. After being mixed in the cold for 30 minutes, the suspension was centrifuged and the 0 to 40% precipitate was discarded. Ammonium sulfate was added to the above supernatant to 75% saturation, mixed, and the resulting 40 to 75% precipitate was taken up in 1 ml of 0.1 M tris buffer (pH 8.0) and stored in the frozen state. For use, aliquots were diluted 10-fold with 0.1 M tris buffer (pH 8.0) and 10 mg of packed calcium phosphate gel were added per mg of protein. After standing in ice for 10 minutes, the suspension was centrifuged for 15 minutes at 1,500 × g and the supernatant was discarded. A volume of 0.1 M phosphate buffer (pH 7.4) equal to the volume of the original solution was added to the gel, the mixture was centrifuged after standing for 10 minutes, and the gel was discarded. The supernatant solution which contained active enzyme was stored at −4°C.

A unit of enzyme activity is defined as the amount of enzyme needed to synthesize 1 μm mole of anserine per hour.

RESULTS

Localization of C14 in 1-Methylhistidine Moiety of Anserine

Three criteria were used to establish purity and to localize C14 in 1-methylhistidine obtained by acid hydrolysis of anserine after incubation of a complete enzymatic reaction mixture. These are (a) ascending paper chromatography of the basic fraction obtained from the Dowex-50lutidine column in ethanol-water-acetic acid (65:34:1), before and after acid hydrolysis, showed that this gave radioactive only in the areas corresponding to standard anserine and to 1-methylhistidine, RF values, 0.43 and 0.39, respectively. (b) A preparation of the dipicrate derivative of 1-methylhistidine obtained after acid hydrolysis of the peptide fraction retained constant specific activity after three recrystallizations from hot water (14). (c) C14 was measured in aliquots from eluant fractions obtained from chromatography on Dowex 50-α-picoline columns of 1-methylhistidine derived from a peptide hydrolysate isolated from a reaction mixture containing 60 μmoles of L-carnosine, 2 μmoles of S-adenosylmethionine-methyl-C14 (10,300 c.p.m. per μmole), 0.8 ml of crude chick extract, and 50 μmoles of tris buffer (pH 7.5) in a final volume of 2.3 ml. The mixture was incubated for 2 hours and 20 μmoles of carrier anserine were added at the end of the incubation. The activity of L-methionine and S-adenosyl-L-methionine to serve as methyl donors in the formation of anserine as catalyzed by undialyzed chick muscle preparations. Results are expressed in terms of C14 incorporation into anserine since these preparations contain significant levels of endogenous carnosine and anserine which were not determined. With a low speed centrifugate prepared from a 1:1 sucrose homogenate, the utilization of L-methionine-C4H9 is largely dependent upon added L-carnosine, and is stimulated by the addition of 20 μmoles of ATP, although considerable activity is obtained without added ATP. This activity may be attributed to the presence of endogenous ATP-generating constituents. The reaction is linear with time for 2 hours and remains constant for at least 3 hours. The ability of L-methionine-C4H9 to function as a methyl donor is undoubtedly due to the presence of methionine transadenosylase (17) since these same preparations are fully active when S-adenosyl-L-methionine-C4H9 is substituted for L-methionine-C4H9 and ATP.

In contrast, L-methionine-C4H9 is a poor methyl donor when a 10,000 × g centrifugate prepared from a 1:5 sucrose homogenate is used as shown in Table I. However, this preparation is active when S-adenosyl-L-methionine-C4H9 is used as the methyl donor and it is dependent upon the presence of carnosine for activity.

Distribution of Carnosine-N-methyl Transferase Activity—A number of tissues from several species were examined for carnosine-N-methyl transferase activity, including skeletal muscle, liver, kidney, brain, heart, spleen, and lung from young chicks, rats, and rabbits, and from mature guinea pigs and cat. The results are shown in Table II. Results are expressed as per-
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Incorporation of C<sup>14</sup> from L-methionine-C<sup>14</sup>H<sub>3</sub> and S-adenosyl-L-methionine-C<sup>14</sup>H<sub>3</sub> into anserine catalyzed by chick muscle extracts

Incubation mixtures: 20 μmoles of L-carnosine, 100 μmoles of Tris buffer (pH 7.5), 20 μmoles of phosphate buffer (pH 7.5), 10 μmoles of MgCl<sub>2</sub>, 10 μmoles of L-methionine-methyl C<sup>14</sup> (100,000 c.p.m.) or 10 μmoles of S-adenosyl-L-methionine-C<sup>14</sup> (103,000 c.p.m.), 1 ml of chick muscle extract. Final volume, 3.8 ml. Incubated 2 hours at 37°.

<table>
<thead>
<tr>
<th>Type of preparation</th>
<th>Additions</th>
<th>Methyl donor</th>
<th>Incorporation of C&lt;sup&gt;14&lt;/sup&gt; per 10 μmoles of anserine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant from 1:1 homogenate centrifuged at 900 × g</td>
<td>Carnosine + ATP</td>
<td>L-Methionine-methyl-C&lt;sup&gt;14&lt;/sup&gt;</td>
<td>254 c.p.m.</td>
</tr>
<tr>
<td></td>
<td>No carnosine, + ATP</td>
<td>L-Methionine-methyl-C&lt;sup&gt;14&lt;/sup&gt;</td>
<td>31 c.p.m.</td>
</tr>
<tr>
<td></td>
<td>Carnosine, no ATP</td>
<td>L-Methionine-methyl-C&lt;sup&gt;14&lt;/sup&gt;</td>
<td>146 c.p.m.</td>
</tr>
<tr>
<td></td>
<td>Carnosine, no ATP</td>
<td>S-Adenosyl-L-methionine-C&lt;sup&gt;14&lt;/sup&gt;H&lt;sub&gt;3&lt;/sub&gt;</td>
<td>319 c.p.m.</td>
</tr>
<tr>
<td>Undialyzed supernatant from 1:5 homogenate centrifuged at 10,000 × g</td>
<td>Carnosine + ATP</td>
<td>L-Methionine-methyl-C&lt;sup&gt;14&lt;/sup&gt;</td>
<td>26 c.p.m.</td>
</tr>
<tr>
<td></td>
<td>Carnosine, no ATP</td>
<td>S-Adenosyl-L-methionine-C&lt;sup&gt;14&lt;/sup&gt;H&lt;sub&gt;3&lt;/sub&gt;</td>
<td>523 c.p.m.</td>
</tr>
<tr>
<td></td>
<td>No carnosine, no ATP</td>
<td>S-Adenosyl-L-methionine-C&lt;sup&gt;14&lt;/sup&gt;H&lt;sub&gt;3&lt;/sub&gt;</td>
<td>12 c.p.m.</td>
</tr>
</tbody>
</table>

Results are expressed as percentage of activity relative to chick pectoral muscle.

Table III shows the results of a fractional centrifugation of a sucrose homogenate of chick muscle. Clearly the bulk of the activity is retained in the soluble fraction with little or no activity in the mitochondrial and microsomal fractions. The apparent activity in the microsomal fraction is magnified by using a 20-fold concentrated suspension. Repeated freeze-thawing of a mitochondrial preparation in order to disrupt structural integrity (18) failed to alter activity.

Purification of Chick Pectoral Muscle Carnosine-<i>N</i>-methyl Transferase

The results of a partial purification of the enzyme are shown in Table IV. The starting extract was prepared by homogenizing a pectoral muscle mince from four chicks with 5 volumes of 0.3 M sucrose and centrifuging it for 10 minutes at 10,000 × g. A typical preparation contained 7.9 mg of protein per ml with a specific activity of 4.8 units per mg of protein. After freezing and 24-hour dialysis against cold distilled water, there was loss of some inert protein to give 4.8 mg of protein per ml with some percentage of activity relative to a comparable wet weight of chick pectoral muscle.

The effect of age of the tissue on enzyme activity.

Chick pectoral muscle is the best source of the enzyme, although a significant level of activity was found in chick liver and in leg muscle from all species. It has been observed that chick muscle is much more active than muscle from mature chickens; therefore, the results reported for the cat and guinea pig may require modification after systematic investigation of the effect of age of the tissue on enzyme activity.

Table III shows the results of a fractional centrifugation (16) of a sucrose homogenate of chick muscle. Clearly the bulk of the activity is retained in the soluble fraction with little or no activity in the mitochondrial and microsomal fractions. The apparent activity in the microsomal fraction is magnified by using a 20-fold concentrated suspension. Repeated freeze-thawing of a mitochondrial preparation in order to disrupt structural integrity (18) failed to alter activity.

Table III

**Partial species and tissue distribution of carnosine N-methyl transferase**

Results are expressed as percentage of activity relative to chick pectoral muscle.

Incubation mixture (see "Methods"): 0.8 ml of extract obtained from 1:5 sucrose homogenate centrifuged for 20 minutes at 3,000 r.p.m. (International centrifuge PR-2). Incubated 1 hour at 37°. Protein concentration, 20 to 25 mg per ml of extract.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Chick</th>
<th>Guinea pig</th>
<th>Rat</th>
<th>Cat</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle (pectoral)</td>
<td>100</td>
<td>18.4</td>
<td>16.8</td>
<td>8.0</td>
<td>22.4</td>
</tr>
<tr>
<td>Skeletal muscle (leg)</td>
<td>22.1</td>
<td>0</td>
<td>2.8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>7.2</td>
<td>1.9</td>
<td>14.0</td>
<td>2.7</td>
<td>2.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.7</td>
<td>0</td>
<td>4.7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>4.7</td>
<td>4.0</td>
<td>7.8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0</td>
<td>2.2</td>
<td>4.6</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>0</td>
<td>5.3</td>
<td>10.3</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Table IV

**Partial purification of carnosine N-methyl transferase from chick pectoral muscle**

Incubation mixture: 40 μmoles of L-carnosine, 1 μmole of S-adenosyl-L-methionine-methyl C<sup>14</sup> (16,611 c.p.m. per μmole), 50 μmoles of Tris buffer (pH 8.0), 0.7 ml of cell preparation in 0.3 M sucrose. Final volume, 2.3 ml. Incubated 1 hour at 37°.

<table>
<thead>
<tr>
<th>Cell fraction</th>
<th>Anserine formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole homogenate</td>
<td>33</td>
</tr>
<tr>
<td>Mitochondria (fresh)</td>
<td>0</td>
</tr>
<tr>
<td>Mitochondria (freeze-thaw)</td>
<td>0</td>
</tr>
<tr>
<td>Microsomes*</td>
<td>4</td>
</tr>
<tr>
<td>Soluble fraction</td>
<td>21</td>
</tr>
</tbody>
</table>

* Microsomes suspended in 1/2 of the volume of 0.3 M sucrose used for other fractions.
FIG. 2A. Effect of incubation time on anserine formation. Reaction mixture contained enzyme preparation, 40 µmoles of L-carnosine, 1 µmole of S-adenosyl-L-methionine-methyl-C\textsuperscript{14} (16,611 c.p.m. per µmole), 50 µmoles of Tris buffer (pH 8.0), final volume, 2.3 ml.

FIG. 2B. Effect of protein concentration on anserine formation. Same incubation conditions as described above. Results obtained using supernatant from centrifugation of chick muscle extract at 10,000 \( \times \) g for 10 minutes. Dialyzed against cold distilled water for 24 hours.

FIG. 3. Effect of pH on the enzymatic synthesis of anserine catalyzed by carnosine-\( N \)-methyl transferase. Incubation mixture consisted of 40 µmoles of L-carnosine, 1 µmole of S-adenosyl-L-methionine-methyl-C\textsuperscript{14} (16,611 c.p.m. per µmole), 50 µmoles of Tris or phosphate buffer, enzyme (2.0 mg of protein) in final volume of 2.3 ml. Incubated 1 hour at 37°.

Some Properties of Carnosine-\( N \)-methyl Transferase—Under standard conditions of assay, the formation of anserine is linear with time for at least 4 hours as shown in Fig. 2A. Fig. 2B shows that anserine formation is linear with increasing protein concentration with use of a dialyzed 10,000 \( \times \) g supernatant as the source of enzyme up to at least 2.0 mg of protein under standard assay conditions, so ensuring an adequate procedure for determination of transferase activity.

The enzyme has a high affinity for S-adenosylmethionine, half-maximal synthesis of anserine occurring with a final concentration of 9 \( \times \) 10\textsuperscript{-3} M. Much higher levels of carnosine are required, with 4 \( \times \) 10\textsuperscript{-4} M final concentration giving half-maximal synthesis of anserine. With dialyzed, partially purified preparations, no reaction occurs in the absence of added carnosine, and the formation of anserine is totally dependent on the presence of enzyme.

FIG. 3 shows the effect of pH on enzyme activity. The enzyme shows maximal activity between pH 7.5 and 8.5 with a peak at about pH 8.0. Similar results are observed in phosphate and Tris buffers at a final concentration of 0.022 M. The transferase is stable to freezing and may be stored frozen without loss of activity. It may be heated for 5 minutes at 50° without loss of activity if it is incubated for 10 minutes with carnosine. After heating for 10 minutes, there is a 70% loss of activity. Complete loss of activity occurs after heating at 50° for 3 minutes in the absence of substrate.

No evidence as yet exists for an ion requirement. No loss of activity occurs in the presence of 10\textsuperscript{-3} M EDTA, or after dialysis of the enzyme against 1 \( \times \) 10\textsuperscript{-3} M EDTA for 18 hours, although occasionally some stimulation occurs in the presence of EDTA. Glutathione has no effect on enzyme activity. Preliminary experiments have revealed an extreme sensitivity to several cations, including Zn\textsuperscript{2+}, Co\textsuperscript{2+}, and Mn\textsuperscript{2+}, with 70% inhibition of anserine synthesized at a level of 0.01 µmole under standard assay conditions. This inhibition is regularly obtained and is overcome by added EDTA. This aspect of the problem is under investigation.

Substrate Specificity

Several compounds structurally related to carnosine were examined as possible methyl group acceptors with the standard incubation mixture and 40 µmoles of substrate. Under these conditions, homocarnosine is inactive with chick pectoral muscle extracts, but is about 10% as active as carnosine with weanling rat skeletal muscle extracts. Guinea pig and rat brain extracts show a measurable activity with approximately 7.5 µmole of homocarnosine synthesized per g of wet weight tissue per hour. Similar results were found with brain and muscle tissue obtained from young rats which had been treated by injection (intraperitoneally) with 0.1 mmole of homocarnosine sulfate 44 hours before killing. These findings are of interest in view of the occurrence of homocarnosine as a normal constituent of beef and rat brain (19).

Contrary to the findings of Winnick and Winnick (3), histidine is essentially inactive as a methyl group acceptor with chick pectoral muscle preparations under the experimental conditions used here. However, guinea pig and rabbit skeletal muscle preparations catalyze methylation of histidine in the order of 20% of the levels observed with chick pectoral muscle preparations when carnosine acts as the acceptor.

Histamine, uracil, and imidazole are inactive as methyl group acceptors with chick pectoral muscle extracts.

DISCUSSION

Anserine and carnosine are recognized as major nonprotein nitrogenous constituents of skeletal muscle in all vertebrates which have been investigated (20, 21), and Kalyankar and
Meister (1) and Winnick and Winnick (2) have demonstrated their synthesis in chick muscle by condensation of β-alanine with histidine or L-methylhistidine. A second pathway for synthesis of anserine involves a direct methylation of carnosine by S-adenosylmethionine as shown in this paper and in the report by Winnick and Winnick (3). Evidence presented here supports the view that skeletal muscle is the principal site of this transmethylation with S-adenosylmethionine as methyl donor. In addition, skeletal muscle is capable of activation of methionine since crude extracts are able to utilize methionine as a donor. These results indicate that skeletal muscle contains all of the enzymes required to effect synthesis of these muscle peptides. This is in contrast with the synthesis of creatine, which, although abundant and metabolically active in muscle, is synthesized in liver by methylation of guanidinoacetic acid (22) and then is transported to muscle. Little or no creatine synthesis occurs in muscle (3).

The carnosine-methylating enzyme exhibits certain properties which are similar to those of other N-methyl transferases. In common with guanidinoacetate methylase (22) and the imidazole-N-methyl transferase, described by Lindahl (23) and by Brown, Axelrod, and Tomchick (24, 25), which catalyzes methylation of histamine at the nitrogen 1-position, carnosine-N-methyl transferase shows no dependence on metal ions. EDTA fails to inhibit activity of the enzyme even after an 18-hour dialysis against 10^{-4} M EDTA. Numerous attempts to demonstrate a stimulation by cations have been negative, although marked inhibition of activity has been regularly observed on addition of low concentrations of Zn^{2+}, Mn^{2+}, as well as cobalt ions. This behavior as well as a study of the dependence of the enzyme on intact -SH groups awaits further investigation with a more highly purified enzyme preparation. The enzyme shows considerable activity over a pH range of 7 through 8.5, with a maximum at about pH 8 and a greatly decreased activity above pH 9. This approximates the pH range found to obtain for imidazole-N-methyl transferase (23). However, the absence of reaction with histamine and the grossly different tissue distribution appear to differentiate clearly carnosine-N-methyl transferase from the histamine-methylating enzyme.

Whether or not chick pectoral muscle is able to catalyse methylation of L-histidine to form L-1-methylhistidine is not completely clear. Experiments both in vivo (6, 7) and in vitro (1, 2) support the existence of a system capable of synthesizing 1-methylhistidine, and Winnick and Winnick (3) have reported methylation of histidine via methyl transfer from S-adenosylmethionine catalyzed by chick muscle preparations. Our preliminary results (4) supported this finding, but subsequent experiments forced us to conclude that most of the activity which we had observed could be attributed to methylation of endogenous carnosine. Thus, in our preparations, histidine fails to function as a substrate for the chick muscle enzyme. However, rabbit and guinea pig skeletal muscle do exhibit some activity towards histidine and these sources require further investigation.

The recent identification of homocarnosine as a normal constituent of beef and rat brain (19) prompted speculation as to the occurrence and possible synthesis of homoaeserine. The results reported here with homocarnosine as a substrate indicate that some homoaeserine may occur as a trace constituent. This aspect deserves more attention since no attempt has been made to determine optimal conditions for this reaction.

**SUMMARY**

An enzyme, carnosine-N-methyl transferase, has been investigated which catalyzes the transfer of the methyl group of S-adenosylmethionine to the nitrogen in the 1-position of the imidazole ring in carnosine to give anserine. It has been purified 7.5-fold from the soluble fraction of chick pectoral muscle. Activity is confined to the soluble fraction and chick pectoral muscle is the best source of the enzyme. Some activity is observed in skeletal muscle from rat, guinea pig, and rabbit, and in chick liver, but the other tissues examined have insignificant activity.

The chick pectoral muscle enzyme appears to be specific for carnosine as acceptor of the methyl group from S-adenosyl-L-methionine. Histidine, histamine, uracil acid, and imidazole are inactive with this preparation, although histidine is somewhat active when guinea pig and rabbit skeletal muscle extracts are used. The effect of varying the concentration of carnosine and S-adenosylmethionine, the effect of varying time of incubation, and the pH dependence of anserine formation were studied. No ion requirement has been demonstrated for this preparation.

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