Biosynthesis of carnosine and anserine *in vitro*

Several investigators have recently employed ^{14}C-labelled amino acids to study the metabolism of carnosine and anserine in animals[1,2,3]. These dipeptides are abundant only in skeletal muscle. Attempts to detect them in liver by chemical analysis or by radioactive tracer techniques have not been successful[4]. The observation that partial hepatectomy in rats does not decrease the rate of incorporation of labelled β-alanine into muscle dipeptides suggests that the liver is not the site of carnosine and anserine synthesis[1]. However, WILLIAMS AND KREHL[5] reported that carnosine was readily formed in liver slices in the presence of β-alanine and histidine, as judged by a microbiological assay method.

The present note compares liver and muscle preparations *in vitro* with respect to their ability to incorporate β-alanine-1-^{14}C into carnosine-anserine. The ease of complete separation of excess radioactive amino acid from the dipeptide fraction on paper chromatograms formed the basis of a simple and reliable assay procedure.

Weanling rats and 3- to 4-day-old chicks were used. Four to six liver slices, or eight to ten muscle strips (leg and breast for rat and chick, respectively), representing 80 to 100 mg of fresh tissue, were used per flask. The tissue was weighed accurately into flasks containing 1 ml of Krebs-Hensleit bicarbonate buffer (equilibrated with 95% O_2 – 5% CO_2), pH 7.4, and 0.1 ml of amino acid solutions. After flushing with the gas mixture, each flask was shaken at 37° for a specified time. Then the tissue was removed and homogenized in 2 ml of 5% trichloroacetic acid. After centrifugation, the supernatant was extracted three times with ether and then concentrated to less than 0.1 ml. About 20 μg of histidine were added, to improve subsequent definition of the basic zone, and the liquid was quantitatively transferred to Whatman No. 1 paper. The chromatogram was developed for 20 to 24 hours with 75% *n*-butanol–15% formic acid–10% H_2O. After drying, the paper was dipped into 0.1% ninhydrin in acetone, dried again, and heated briefly at 100°. The dipeptide region (1 to 3 cm from the origin) was cut out, and the ^{14}C content of the paper was measured in the flow-gas Geiger counter*. The counts per minute were converted into μg of β-alanine by reference to suitable standards.

Fig. 1 shows that with both rat and chick, several times more β-alanine-^{14}C was incorporated into muscle than into liver tissue. As with *in vivo* experiments, the chick was the more active species. The maximum values represent the formation in 100 mg of tissue of about 0.7 μg of dipeptides in 2 hours, or 1.4 μg in 4 hours (the rate was found to be linear for this time interval).

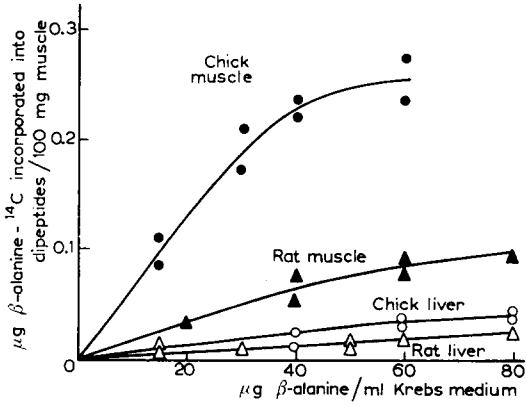

Fig. 1. The effect of varying β-alanine-^{14}C concentration on the degree of labelling of carnosine-anserine. Each flask contained 0.001*M* L-histidine. The incubation time was 2 hours in all cases. The labelled β-alanine (0.05 mc/mmole) was synthesized as previously described[4].

In contrast, WILLIAMS AND KREHL observed the formation of up to 180 μg of carnosine per 100 mg of liver slices in 4 hours under comparable conditions (though with somewhat higher β-alanine and histidine concentrations). Their values are thus about a thousand times larger than our rat liver activities. We are unable to explain this divergence. Higher concentrations of unlabelled histidine or 1-methylhistidine, or of β-alanine-^{14}C caused only slightly greater labelled peptide formation. Tissues of older rats had even lower synthetic ability than those of weanlings.

The results with chicks in Fig. 1 represent a utilization of approximately 0.5% of the total ^{14}C employed, for β-alanine concentrations up to 0.0005*M*. This efficiency is of the same order of magnitude as that observed with intact birds, and seems reasonable in view of the low turnover rates of the dipeptides *in vivo*[1]. With either liver or muscle preparations, only 2 to 4% of the initial ^{14}C was recovered in the β-alanine region of chromatograms, suggesting that extensive decarboxylation resulted, as *in vivo*.

* β-alanine migrated 10 to 12 cm, and hence separated completely from the basic compounds.

The present procedure does not resolve carnosine and anserine, but very probably both became labelled, as occurred with *in vivo* experiments. In any case, there is little doubt that an objective measure of dipeptide synthesis is obtained. With other solvent systems (such as 2:1 pyridine-H_2O), radioactivity invariably appears in the carnosine-anserine region. Also β-alanine-^{14}C can be recovered from acid hydrolysates of chromatographically-isolated peptide fractions.

In conclusion, the data in this note support the view that carnosine and anserine are formed in skeletal muscle, and not in liver. The described muscle strip technique permits better-defined conditions than were hitherto possible, and represents a step towards studies with isolated enzyme preparations.

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Polyene fatty acids in guinea pig tissues

The amounts of dienoic, trienoic, and tetraenoic fatty acids in the tissues of several different animals bear certain similarities. In the rat[1], dog[2], and chick[3,4] the fatty acids in the serum appear to reflect the composition of the liver and also of the heart. Generally, in the normal animal ingesting a diet containing several percent of vegetable oil, the fatty acids of the serum contain about 15–20% of dienoic acid, 0–10% of trienoic acid, and 10–30% of tetraenoic acid. It is of considerable interest that the polyene fatty acid content of guinea pig serum, and also of certain other tissues, is remarkably different from that of other animals.

Guinea pigs 2–4 days old were fed *ad libitum* a purified diet with or without 7.3% corn oil[5]. A few animals were fed 1.3% methyl linoleate, as the urea complex, in place of the corn oil. Symptoms of essential fatty acid deficiency, which appeared in 6–8 weeks in the animals fed the fat-free diet, have been described previously[5]. Guinea pigs fed stock pellets containing 5% total fat were on the diet from weaning. All animals were 24–32 weeks old when killed. The polyunsaturated fatty acids in the tissues were determined by the alkali conjugation method of WIESE AND HANSEN[6]. No corrections for pentaene (347.5 mμ) and hexaene (375 mμ) absorption were made; the absorptions at these wavelengths were usually negligible compared to those for di-, tri-, and tetraene.

The data in Table I show that in animals on a natural stock diet dienoic acid comprised about one-third of the total fatty acids of the serum. On a purified diet containing corn oil, this fraction increased to 48% and was the only polyene fatty acid present to any extent. This high dienoic content was a reflection of the relatively large amounts in the liver, kidney and heart and abdominal muscle. In the rat[1], dog[2], and chick[4], the dienoic acid in these tissues is of the order of 7–15% of the total fatty acids.

The distribution of tetraenoic acid in the guinea pigs fed the stock or purified diet varies considerably. Whereas the contents of the heart and kidney were quite high (12–20%), approaching the percentage of dienoic, the amounts in the liver and abdominal muscle were very low (3–4%). In this respect the serum (0–2%) resembled the liver. In the other species mentioned above the serum fatty acids generally contain 8–30% of tetraenoic acid.

In contrast to the serum, the red blood cell fatty acids contained a high percentage of tetraenoic acid. Recently, EVANS *et al.*[7] reported that human red blood cells contained about twice as much tetraenoic as dienoic acid.

The changes in the fatty acids of the fat-deficient guinea pigs are of interest in several respects. Although the dienoic acid decreased in all tissues, as would be expected, the tetraenoic acid remained the same or decreased only slightly in the heart, liver and kidney. In the rat, dog, and chick, tetraenoic acid in these tissues falls markedly in fat-deficiency. Another contrasting result was the relatively small amount of trienoic acid which appeared in the deficient guinea pigs. The heart muscle showed the greatest increase. The origin of this trienoic acid is of interest since there was no decrease in tetraenoic acid. It has been shown that in rats this acid is probably formed from tetraenoic acid[8].