Formation of the β-Carbon of Serine from Formaldehyde.*† (17995)

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Sakami(1) has demonstrated that formate carbon can serve as a source of the β-carbon of serine. Since the conversion of glycine to serine had also been demonstrated(1-3) it was suggested(1) that formate or a formate derivative condensed with glycine to form serine. This is a reversal of the mechanism suggested by Shemin(4) for the conversion of serine to glycine. Kruhoffer(5) further investigated this problem and obtained evidence indicating that formate itself was not a direct precursor of the β-carbon of serine. He found that in a rat liver homogenate carboxyl-labeled glycine was utilized for serine synthesis, whereas the labeled formate was not. This absence of labeling of the β-carbon of serine when C^{14}-formate was used was not due to dilution of the isotopic formate since formate isolated at the end of the experiment had almost as high a specific activity as the added compound. Apparently the enzyme system involved in the conversion of formate to the direct precursor of the serine β-carbon was absent in this preparation, this carbon being supplied by endogenous reactions.

The possibility has been investigated that formaldehyde condenses directly with the α-carbon of glycine to form serine. This has been studied by determining the ability of rat liver homogenate (prepared in the manner of Kruhoffer) to incorporate the C^{14} of labeled formaldehyde into the β-carbon of the serine.

Methods. Labeled formaldehyde was synthesized by reduction of $C^{14}O_2$ with lithium aluminum hydride(6) and oxidation of the resulting methanol to formaldehyde by admixture with dry air and passage over a vanadium molybdenum oxide catalyst(7). The hot vapors were then passed over calcium chloride to remove water formed during the oxidation of the methanol. This prevented moist formaldehyde from polymerizing on the walls of the tubing. The calcium chloride also served to remove unoxidized methanol. The formaldehyde was trapped in water; it contained a trace of methanol (less than 2%) but no formate, and possessed a specific activity of 2.5×10^6 counts per minute per mg carbon. Experiments were conducted in which C^{14}-formate, C^{14}-formaldehyde, and C^{14} carboxyl-glycine were incubated separately with a rat liver homogenate prepared from one part (wet weight) of rat liver and 2.5 parts of modified Krebs-Henseleit medium(8). The homogenate was freed of unbroken cells by low speed centrifugation. Nine ml of homogenate were added to each Warburg vessel together with other substances (Table I) in 0.3 ml volume, making the total volume 9.3 ml. Incubation was carried out at 38°C for 90 minutes in 95% oxygen and 5% carbon dioxide.

After the incubation, 500 mg DL-serine were added as a carrier to each vessel. The homogenates were then deproteinated with trichloracetic acid and the trichloracetic acid

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TABLE I.
Protocol of First Group of Experiments.

Exp. No.		Labeled compound μM	Labeled compound Total activity*	Non-labeled compounds† Glycine, μM	Isolated serine COOH-carbon‡	Isolated serine α-carbon‡	Isolated serine β-carbon‡
1	Na formate	30	1.43×10^6	30	.00	.00	.06
2	Formaldehyde	35	1.07×10^6	30	.02	.01	.62
3	Formaldehyde + heat inactive homogenate	35	1.07×10^6	60	.00	.00	.00
4	COOH-labeled glycine	13	7.7×10^5	—	17.7	.01	.01

* Counts per min. counted as $BaCO_3$.
† 3.0 mg DL-serine were added to each vessel during the incubation.
‡ % of total C^{14} activity added.

TABLE II.
Protocol of Second Group of Experiments.

Exp. No.	Labeled compound used	μM	Total activity*	Non-labeled compounds added† Na formate μM	Formaldehyde μM	Methanol μM	% of C^{14}‡ recovered in serine
1	Na formate	30	1.43×10^6	—	34	34	.07
2	Formaldehyde	35	1.07×10^6	34	—	34	.59
3	Methanol	33	7.2×10^5	34	34	—	.11

* Counts per min. counted as $BaCO_3$.
† 3.0 mg DL-serine and 33 μM glycine added to each vessel during the incubation.
‡ Counted directly on serine (corrected to values for $BaCO_3$).

was removed by ether extraction. The volume of the solution was adjusted to 30 ml and 20 ml of ethanol were added. The samples were heated on a water bath, allowed to cool slowly, and filtered to remove the glycogen. The filtrates were then evaporated to 5 ml and 12.5 ml of ethanol were added to precipitate the serine. This was recrystallized to constant specific activity from ethanol-water solution (6 recrystallizations). The serine was then converted into the p-hydroxyazobenzene sulfonate. The activity of this derivative remained unchanged on recrystallization. In the experiment in which carboxyl-labeled glycine was present, to reduce the possibility of contamination of the isolated serine by labeled glycine, 0.5 mM of non-isotopic glycine was added to the serine after the sixth recrystallization and subsequently removed as the 5-nitronaphthalene-1-sulfonate. Excess 5-nitronaphthalene-1-sulfonic acid was removed from the serine solution by adsorption on the ion exchange resin, Amberlite IR-4B, and the serine was then made into its derivative as described above. The serine isolated and purified in this manner was degraded according to the method of Sakami(1) whereby the carboxyl, alpha, and beta carbons are separately converted to carbon dioxide. The CO_2 samples were counted as barium carbonate and the overall accuracy of the C^{14} determinations was approximately 5%. In the experiment in which labeled formate was used, the C^{14} of the formate remaining at the conclusion of the experiment was determined by the following procedure. Carrier formate was added before the serine isolation. It was then removed by ether extraction and converted to CO_2 by mercuric chloride oxidation. The radioactivity of the carbon dioxide was determined as described above.

Experiments were also done to compare formaldehyde and formate uptake into serine with that of C^{14}-methanol. This was done principally to rule out the possibility that the labeling of serine from C^{14}-formaldehyde was due to the small amount of contaminating

C^{14}-methanol. The experiments (Table II) were conducted in a similar manner to the previous ones. The carrier serine was recrystallized to constant activity and counted directly for C^{14} activity to measure the relative uptake of isotope from each of the 3 different labeled one-carbon compounds while in the presence of the unlabeled other 2.

Results. The results of the first group of experiments are shown in Table I. The C^{14} of the formaldehyde labeled the β-carbon of the non-protein serine. In confirmation of Kruhoffer's results there was very slight incorporation of isotope when labeled formate was added although 12% of the added C^{14}-formate was present at the conclusion of the experiment. The C^{14} from the carboxyl-carbon of labeled glycine was more extensively incorporated into the serine than was the C^{14} from the formaldehyde.

In the second group of experiments (Table II) testing C^{14}-labeled formate, formaldehyde, and methanol each individually but in the presence of approximately equal amounts of the other one-carbon compounds which were unlabeled, it was found that C^{14}-formaldehyde labeled serine eight times more than did C^{14}-formate and five times more than did C^{14}-methanol. This would indicate that in the first group of experiments the small amount of contaminating C^{14}-methanol in the C^{14}-formaldehyde solution was not responsible for most of the C^{14} entering into the serine.

Discussion. In our experiments with liver homogenate more C^{14} was incorporated into the non-protein serine β-carbon from formaldehyde than from formate or methanol. Apparently formaldehyde is utilized more readily for the synthesis of serine than is formate or methanol. The isotope from C^{14} carboxyl-glycine, however, was incorporated into serine to a greater extent than that from the formaldehyde; this difference in isotope incorporation between formaldehyde and glycine lends itself to two possible explanations. Formaldehyde might condense directly with glycine in the biosynthesis of serine but there may be a greater dilution of the C^{14}-formaldehyde from unlabeled formaldehyde sources as compared to the glycine dilution. Formaldehyde in mammalian liver has been shown to arise from dimethylglycine(9) and sarcosine(9,10), both of which have been implicated in choline metabolism(11,12), and from the α-carbon of glycine, the methyl carbons of methionine and choline, and the β-carbon of serine(13). These compounds could be the source of dilution of added C^{14}-formaldehyde.

Another possible explanation for the smaller amount of isotope uptake from labeled formaldehyde as compared to that from labeled glycine might be that the C^{14}-formaldehyde, itself, is not diluted to any significant extent but that an intermediate to which formaldehyde must be converted prior to the condensation with glycine is diluted by endogenous sources of this intermediate. Possible sources of this intermediate could be compounds that have been shown to be precursors of the β-carbon of serine. The α-carbon of glycine(14,15), methyl groups of choline(16,13) and methionine(13), and the methyl groups of acetone(17) have been demonstrated in isotope tracer experiments to be sources of the β-carbon of serine. Since these serine β-carbon precursor compounds, with the exception of acetone, are either the same or metabolically related to the same compounds that are known formaldehyde sources it is not unlikely that a common intermediate exists derived from these compounds which is in equilibrium with the β-carbon of serine and with formaldehyde. At the present time there is no data which permits a choice between either of these explanations for the lesser incorporation of formaldehyde isotope into serine as compared to that from the carboxyl-carbon of glycine. Nevertheless, it

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is probable that formaldehyde is closer to the immediate precursor of the β-carbon of serine than either formate or methanol.

Summary. C^{14} from labeled formaldehyde was incorporated into the β-carbon of serine greater than that from labeled formate or methanol. The indications are that formaldehyde is the more direct precursor of the β-carbon of serine.

Addendum. As an extension of the work by Welch and Sakami(18) in which they found that in the intact rat and in liver slices there was incorporation of C^{14} from formate into the methyl groups of methionine and choline we have made some preliminary studies with C^{14}-formaldehyde. Employing the same rat liver homogenate used in these serine studies we have found evidence that formaldehyde is converted into the methyl group of methionine much more extensively than is formate.

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Preparation of Water-Soluble Combination Products of Gossypol and their Toxicity to Aquarium Fish.* (17996)

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The purpose of the investigations here described has been to make combination products of gossypol(1-3) with naturally occurring materials which would have physical properties different from those of the original material and to determine the effect of such changes on some physiological action of gossypol. Gossypol was tested on aquarium goldfish and sharp differences in physiological activity between the original gossypol and the combination products were obtained.

Materials and methods. Pigment glands were prepared from cottonseed by the gland fractionation process(4,5). Gossypol was prepared by acetone extraction of pigment glands and crystallization from a mixture of diethyl ether and light petroleum naphtha (6). Peanut protein was prepared by alkaline extraction of oil-free peanut meal followed by acid precipitation of the protein (7). The casein, amino acids, dextrose, and starch used in the experiments were commercial products.

The general procedure for preparation of the combination products involved two steps, namely: (1) Gossypol and the material to which it was to be combined were solubilized in aqueous solution of pH of approximately 11 in the shortest possible time. (2) The solubilized material was neutralized with hydrochloric acid and dried in the frozen state (lyophilized).

Ten grams of purified peanut protein was added to one liter of distilled water and the

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