Studies on wheat plants using ^{14}C compounds
XIV. Conversion of [1,7-$^{14}C_2$]-α,α'-diaminopimelic acid to [1-^{14}C] lysine*

Two pathways have been suggested for the biosynthesis of lysine, one involving α-aminoadipic acid[1], and the other involving α,α'-diaminopimelic acid[2,3,4]. Since experiments in this laboratory indicate that [6-^{14}C]-α-aminoadipic acid does not lead to the formation of radioactive lysine in wheat plants[5], it was decided to investigate the possibility that α,α'-diaminopimelic acid[2] is converted to lysine in wheat plants.

Wheat plants 84 days old were injected with a solution of [1,7-$^{14}C_2$]α,α'-diaminopimelic acid**[6] (0.1 ml, 0.5 mg, 0.2 μC) and the plants were harvested mature 25 days later. The kernel contents were fractionated to give arbitrary fractions designated as starch, gluten, salt-soluble protein, ether-soluble material, and bran[7].

Lysine, proline, glutamic acid, and aspartic acid were isolated from the acid hydrolyzates of the chaff, gluten, and salt-soluble protein by column chromatography using the technique of HIRS, MOORE AND STEIN[8]. The carboxyl carbon of lysine was obtained as CO_2 from the acid hydrolyzates of the chaff and salt-soluble protein by incubation with lysine decarboxylase[9].

Lysine samples recovered from the plants were found to have a much higher specific activity than other amino acids isolated; and indeed its activity was considerably greater than the tissues from which it was obtained (Table I). These data indicate a fairly direct conversion of diaminopimelic acid to lysine. This conclusion is supported by results which show that the ^{14}C is preferentially incorporated as the carboxyl group of lysine. The high specificity of this incorporation is shown further by the calculation that, although lysine is a minor constituent of wheat protein, 42 % of the radioactive carbon in the salt-soluble protein appeared in the carboxyl

TABLE I

^{14}C CONTENT OF AMINO ACIDS ISOLATED

Substance assayed	*Specific activity (μC/mole CO_2)*		
	Gluten	*Salt-soluble protein*	*Chaff*
Original material	0.66	1.38	5.60
Proline	0.40	—	1.20
Glutamic acid	0.40	1.10	1.40
Aspartic acid	0.24	0.30	1.0
Lysine	12.40	17.50	83.50
C-1 of lysine by ninhydrin decarboxylation	59.0		
C-1 of lysine by lysine decarboxylase*	—	81.0	455.0
% of [^{14}C]lysine in carbon-1	79	77	91

* *Bacterium cadaveris* NCTC 6578 decarboxylase kindly supplied by Dr. F. J. SIMPSON; CO_2 released was collected by the Conway microdiffusion method[10].

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** [1,7-$^{14}C_2$]-α,α'-Diaminopimelic acid was synthesized in this laboratory for these experiments and no attempt was made to separate its isomers before its use. Methods for its preparation will be reported elsewhere.

group of lysine. Similarly, lysine carboxyl groups accounted for 10 % of the radioactivity in the chaff.

Since conversion of [1,7-$^{14}C_2$]-α,α'-diaminopimelic acid to lysine by decarboxylation would be expected to give [1-^{14}C]lysine the above results provide convincing evidence that wheat plants are capable of carrying out this decarboxylation.

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Fatty acid synthesis in heart sarcosomes

Incubation of rat- or rabbit-heart sarcosomes with sodium [2-^{14}C]acetate, ATP, CoA, TPNH and an oxidizable substrate of the tricarboxylic acid cycle leads to the incorporation of the acetate into the long-chain fatty acids of the mitochondria (Table I). Practically no ^{14}C was found in the non-saponifiable fraction. The incorporation in the fatty acids is nearly completely dependent upon oxidizable substrate, and is appreciably less in the absence of added TPNH and CoA. Succinate is much more effective than glutamate, although the two substrates consume oxygen at about the same rate. This, together with the fact that Amytal inhibited the incorporation with succinate by 66 % and that malate gave a greater rate of incorporation than succinate suggests that oxaloacetate formed by the oxidation of succinate or malate is involved in the incorporation reaction. Oxaloacetate is also formed during the oxidation of glutamate[1,2] but is rapidly removed by transamination with the glutamate. The highest rate of incorporation was found with citrate (and isocitrate) as substrate. It is possible that oxalosuccinate has a role similar to that of oxaloacetate. Both compounds fulfil the structural requirements, suggested by SWICK AND WOOD[3], for transfer of CO_2

Abbreviations: ATP, adenosine triphosphate; CoA, coenzyme A; TPN^+, TPNH, oxidized and reduced triphosphopyridine nucleotide.