BIOGENESIS OF ASCORBIC ACID IN SPROUTING LEGUMES

ON the basis of observations that vitamin C formation is augmented in legume embryos grown in nutrient media containing glucose and mannose,[1] and that germination in the dark also results in stimulation of ascorbic acid formation along with increase in reducing sugars through enhanced amylolysis,[2] sugars have been suggested as active precursors of vitamin C. This has been demonstrated more directly with the rat, especially when chloretone-fed,[3,4,5] adequate thiamine nutrition being a prerequisite in this case.[6] Chloretone, however, is not utilised directly for vitamin C synthesis[7] and presumably exerts its effect upon the enzyme systems which control the oxidation of glucose.[8] Studies on the partial degradation of the biosynthetic ascorbic acid after administration of labelled glucose have provided evidence that the total transfer of C^{14} from glucose to ascorbic acid was approximately equivalent to total conversion of dietary carbohydrate to ascorbic acid by weight.[7] The observations would suggest that the carbon chain of glucose may not be broken before being converted to ascorbic acid. However, the possibility of recombination of the fragments without a major dilution effect could not be ruled out and would seem the probable route from other observations[9] on the stimulatory effects of glyceraldehyde and pyruvate in *in vitro* studies with liver slices of chloretonised rats. This is also inferable from the facts that chloretone administration to rats results in simultaneous increased urinary excretion of ascorbic and glucuronic acids[3,4,5] and that glucuronic acid is formed from C_3 substances.[10] Recent work from this laboratory would lend

support to the view that conversion of glucose to ascorbic acid takes place through mediation of the glycolytic route rather than through a direct oxidative pathway. Among the various observations made, using sprouted *mung* seeds or embryos therefrom, may be mentioned the following:

(1) Vitamins of the B group, particularly thiamine, riboflavin and nicotinic acid, catalyse the oxidative breakdown steps that result in the formation of ascorbic acid from glucose.

(2) The acids involved in the intermediary metabolism of glucose, particularly fumaric and succinic acids, stimulate biogenesis of vitamin C.

(3) There is a close parallelism between the elaboration of ascorbic acid and of nicotinic acid under a variety of experimental conditions. The latter vitamin is partly found as pyridinoprotein enzymes although on account of the high DPNase activity in seedling extracts they are not estimable in this form except when using nicotinamide during extraction.[11]

(4) Use of selective inhibitors of certain enzymes concerned in glucose breakdown, such as azide, iodoacetate and fluoride and in phosphorylation such as 2 : 4 dinitrophenol and atabrine, adversely affect ascorbic acid formation. The inhibitors similarly influence nicotinic acid, phosphatase and particularly pyrophosphatase activities.

(5) Malonate, a competitive inhibitor of succinic dehydrogenase depresses ascorbic acid synthesis.

(6) In proper concentrations, certain mitotoxic agents such as chloretone, urethane and coumarin favour vitamin C synthesis. This results presumably from a metabolic shunting of glucose intermediates away from the normal pathway and towards steps leading to ascorbic acid synthesis.

Details are being published elsewhere. One of us (S. P. B.) is grateful to the Lady Tata Memorial Trust for the award of a Research Fellowship.

Dept. of Chem. Technology, University of Bombay, *March* 29, 1952.

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