γ-Amino Butyrate Pyruvate Transaminase in Plants

While studying the glutamic acid metabolism in certain Indian plants, it was observed that the green leaves of *Trigonella foenum graecum*, fresh seeds of *Momordica charantia* and germinated *Phaseolus mungo* contain an enzyme which is able to catalyse the transfer of the amino group of γ-amino butyric acid to pyruvate, resulting in the formation of alanine. It was observed that the cold water extracts of the sources mentioned above contained the new transaminase along with the glutamate pyruvate transaminase.

The reactions were carried out in the presence of M/15 phosphate buffer pH 7.5, pyridoxal phosphate ($1 \cdot 10^{-3} M$), sodium pyruvate ($5 \cdot 10^{-3} M$), and γ-amino butyric acid ($1\ \mu M$) in final concentrations. The reaction was followed by the circular paper chromatographic method of GIRI and RAO[1].

The enzyme was inactivated on dialysis against ice-cold water. However, the presence of γ-amino butyric acid (at $1 \cdot 10^{-2} M$ concentration) helped to stabilize the enzyme. It was possible to precipitate the enzyme with ice-cold acetone and saturated ammonium sulphate solution.

γ-amino butyric acid is known to undergo transaminase reaction through α-ketoglutarate in beef brain[2]. WILSON et al.[3] injected ^{14}C γ-amino butyric acid into male rats and isolated ^{14}C glutamate, aspartate, alanine and glycogen. According to the latter authors, the metabolism of γ-amino butyric acid proceeds through succinic semialdehyde, resulting in the above-mentioned substances.

The conversion of oxalacetic acid through transamination with γ-amino butyric acid has been reported in plants by CRETOVITCH and GALYAS[4].

In the present investigation, direct transamination between γ-amino butyric acid and pyruvate takes place. It is very interesting to note that both the amino acid and the keto acid are monocarboxylic.

The purification and study of the properties of the new enzyme are in progress and will be described in detail elsewhere[5].

Résumé. Les auteurs ont décelé la présence de la transaminase γ-butarate dans les feuilles de quelques plantes de l'Inde.

M. J. AIRAN, ARUNA P. DHARKAR, and R. W. P. MASTER

Department of Biochemistry, Wilson College, Bombay 7 (India), June 13, 1966.

1 K. V. GIRI and N. A. N. RAO, Nature, Lond. *169*, 923 (1952).
2 C. F. BAYTER and E. ROBERTS, J. biol. Chem. *233*, 1135 (1958).
3 W. E. WILSON, R. S. HILL, and R. E. KOEPPE, J. biol. Chem. *234*, 347 (1959).
4 V. L. CRETOVITCH and E. GALYAS, Dokl. Akad. Nauk USSR, Biochem. Section *124*, 217 (1959).
5 The authors are grateful to Dr. J. W. AIRAN, Principal of Wilson College, Bombay, for his encouragement and his kind interest in this work.

Influence of *Botryodiplodia* Infection on the Ascorbic Acid Content of Two Varieties of Guava[1]

Botryodiplodia theobromae Pat. (previously referred to as *Diplodia natalensis* Pole-Evans[2]) is an important pathogen of guava and other tropical fruits. This fungus causes serious loss of guava fruit during the post-harvest phase. So far no one has studied the influence of *Botryodiplodia* infection on the ascorbic acid content of guava fruit; hence the present investigation is an attempt in that direction.

'Safeda' and 'Apple coloured' varieties of guava fruit of the same age (which were about to ripen) were inoculated with the monoconidial culture of *B. theobromae* and were incubated at 25 ± 1 °C. The ascorbic acid content of the inoculated and non-inoculated fruit was determined at an interval of 48 ± 2 h. For this purpose 2 g of the pulp from the inoculated and non-inoculated fruit was separately crushed with 25 ml of 5% metaphosphoric acid in a ground-glass homogenizer and filtered. The residue was washed twice with 10 ml of metaphosphoric acid and the volume of the total filtrate was finally raised to 50 ml. The filtrate was titrated against previously standardized 2,6-dichlorophenolindophenol reagent and the quantity of free ascorbic acid in different samples was calculated. The data are presented in the Table.

The results indicate that, with an increase in incubation period, there was a decline in the ascorbic acid content of both the healthy and infected fruit, but the rate of decline in the healthy fruit was comparatively less. Such a decline in the ascorbic acid content of guava fruit in storage has also been reported by GHOSH et al.[3]. The rate of decline in the ascorbic acid content of infected fruit was comparatively faster in 'Safeda' (where it could not be traced on days 10 and 12 of incubation) than in 'Apple coloured' variety.

The details of the physiological functions of ascorbic acid are not well known but it is believed to function as one of the biological oxidation-reduction substances. It is known that L-ascorbic acid is easily oxidized to dehydro-L-ascorbic acid by the enzyme ascorbic acid oxidase or by certain other oxidative enzymes like polyphenol oxidase, cytochrome oxidase, peroxidase etc. according to the following reaction:

$$\text{L-ascorbic acid} \xrightarrow[-2H]{\text{oxidase}} \text{dehydro-L-ascorbic acid} \xrightarrow[+H_2O]{} \text{2,3-diketogulonic acid}$$

An oxidative enzyme, specific for L-ascorbic acid, has also been demonstrated by MANDELS[4] in the spores of

1 This research has been financed in part by grant No. P.L. 480, Fg In-133 made by the United States Department of Agriculture, Agricultural Research Service. The authors are thankful to Drs. S. N. BHARGAVA and A. K. GHOSH for their help.
2 M. P. SRIVASTAVA, R. N. TANDON, K. S. BILGRAMI, and A. K. GHOSH, Phytopathol. Z. *50*, 250 (1964).
3 A. K. GHOSH, R. N. TANDON, S. N. BHARGAVA, and M. P. SRIVASTAVA, Naturwissenschaften *52*, 478 (1965).
4 G. R. MANDELS, Archs Biochem. Biophys. *42*, 164 (1953).