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.

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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

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Ascorbic acid content (in mg/100 g of the fruit pulp) of healthy and infected guava fruit

	Ascorbic acid content							% loss in ascorbic acid after 10 days of incubation
	Days of incubation							
	0	2	4	6	8	10	12	
'Safeda'								
Healthy	305.2	296.0	282.5	260.5	230.0	203.5	190.0	33.3
Infected	–	250.0	177.5	92.0	30.5	–	–	100.0
'Apple coloured'								
Healthy	372.2	349.5	317.5	276.0	238.0	222.0	200.0	40.3
Infected	–	298.0	200.0	195.5	69.5	39.5	–	89.2

Myrothecium verrucaria. Similar oxidation of ascorbic acid during fungal infection is known in the case of rusts [5,6].

It therefore seems probable that the decline in the ascorbic acid content may be due to the production of certain ascorbic acid degenerating enzymes, either by the fungus itself or by the host-parasite interaction as postulated by GHOSH et al. [3]. The comparatively rapid decline in ascorbic acid in the infected tissues may also be attributed to the increased respiration in the infected tissues which may induce rapid oxidation of ascorbic acid. Such an increase in respiration rate under pathogenesis is known to be incited by many fungi – especially powdery mildews and rusts [7–9].

Zusammenfassung. Der Ascorbinsäuregehalt nimmt in reifen Guavafrüchten nach Infektion durch *Botryodiploida theobromae* rasch ab, was wahrscheinlich auf enzymatischer Oxydation der Ascorbinsäure beruht.

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Studies on the Growth of *Prymnesium parvum* Carter (Chrysomonadina) and on the Formation of its Toxin (Prymnesin)

Prymnesium parvum forms a number of toxic principles which are responsible for its ichthyotoxic, hemolytic, and cytotoxic activities [1–3]. Likewise, prymnesin, the purified toxin of the phytoflagellate, inhibits the contraction of the guinea-pig ileum, evoked by a variety of smooth muscle stimulants [4].

For the purification of the toxin, the separation of its components and the study of their chemical properties, large quantities of *P. parvum* are required. ULITZUR and SHILO [5] have grown *P. parvum* on a medium developed by DROOP [6], and have harvested the cells after a period of 30–40 days. In our laboratory, the phytoflagellate has been cultivated routinely at 22 °C in artificial sea-water, enriched with 0.3% (w/v) oxoid liver infusion. Under these conditions, maximum yield of cells and toxin is obtained already within about 20 days (Table, example 1).

Recently a new medium has been developed, containing inorganic salts and 0.5 *M* glycerol [7]. This medium was originally designed to allow the growth of *P. parvum* in the dark, but if the culture was exposed to continuous illumination, the new medium also shortened the growth period considerably [7]. We have found that the results can be further improved by combination of glycerol with amino acids. So far, we have tried glycine, serine and α-alanine (Table). Thus, addition of D,L-serine (example 2) produces an almost threefold increase in cell density and toxin yield as compared with the basal medium (example 1). Examples 2 and 5 also show that, regardless of the conditions of illumination, D,L-serine and D,L-alanine give nearly the same number of cells and comparable yields of crude toxin, while glycine (example 4) is only $^1/_3$ as effective as the other 2 amino acids. Even supplementation of glycine with methionine did not improve growth above the level attained with glycine alone, i.e. the mixture of these 2 amino acids did not simulate the effect of serine. In order to test the stereospecificity of the stimulatory agents, we have examined the L-, the D- and the D,L-forms of α-alanine. All 3 gave approximately the same final yield of cells (Figure). On the other hand, β-alanine did not accelerate growth during the first 7 days,

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