whether this change be mediated through enzymatic action or through direct chemical processes. Some evidence has been obtained which suggests that the formation of this bleached form of chlorophyll in these experiments may be an enzymatic process (Table 1). Chloroplast fragment preparations placed in a boiling water bath for 5–7 min do not show this loss in chlorophyll absorbancy or the formation of 740 mμ absorbing material. The initial chlorophyll absorbancy peak nevertheless shows a shift from 678 mμ to 670 mμ in response to the addition of 65 per cent methanol, just as it does in non-boiled samples. Cupric chloride, at a concentration of 5×10^{-3} M or higher, also inhibits the loss of chlorophyll absorbancy and prevents the appearance of material absorbing at 740 mμ. A similar concentration of this salt has been found necessary to inhibit the 'Hill reaction'[4]. No absorbancy changes occur when chloroplast–methanol mixtures are placed at − 26° C. Although the high concentration of alcohol necessary to obtain these changes appears to be very unphysiological, it should be recalled that at least two other enzymes attributed to the chloroplast require similar conditions for activity. They are phosphatidase c[5,6] and chlorophyllase[7].

These results provide conclusive evidence that the solvent-induced changes described in a previous paper are due to chlorophyll and fragments of the plastid structure. The decrease in size of these chloroplast fragments which accompanies the formation of the 740 mμ absorbancy maximum is consistent with the assumption that this change involves a crystallization of the chlorophyll molecules. A reversible bleaching of chlorophyll as a result of these treatments is clearly demonstrated in these investigations. The relation of this bleached form of chlorophyll to the formation of a 740 mμ absorbing band is such that the bleached form may be an intermediate. The effects of boiling, cupric chloride and low temperature suggests that an enzymatic reaction is responsible for the production of this bleaching.

This investigation was aided in part by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

Jacques Aghion
Evelyne Porcile

Laboratoire du Phytotron,
Centre National de la Recherche Scientifique,
Gif-sur-Yvette (Seine et Oise), France.

James A. Lippincott*

Department of Biological Sciences,
Northwestern University,
Evanston, Illinois.

* Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

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Argininosuccinic Acid in Germinating Seeds of *Vicia faba* L.

The ornithine cycle amino-acids have been determined in ungerminated seeds and 6- and 14-day-old seedlings of *Vicia faba* L. grown without an external source of nitrogen.

The amino-acids were identified by: (1) their elution characteristics from 'Zeo-Karb 225' (200–300 mesh, × 9 per cent); (2) their R_F values on two-dimensional paper chromatograms using 80 per cent (w/v) aqueous phenol as the first solvent and n-butanol/acetic acid/water (58 : 180 : 20 v/v/v) as the second; (3) colour tests for arginine[3]; argininosuccinic acid[4]; citrulline[5].

Argininosuccinic acid was separated from the free amino-acids of extracts of 500 g ungerminated beans using a 50 × 2·8 cm column of 'Zeo-Karb 225' (200–300 mesh, × 9 per cent), desalted by the method of Mueller[6] and identified using the following criteria.

(1) It was eluted from cation exchange resins as the anhydride forms and emerged as a discrete peak between leucine and tyrosine[7].

(2) Chromatography with 80 per cent (w/v) aqueous phenol gave two spots with ninhydrin, the R_F values of which, 0·27 and 0·47, corresponded to those of 'active' argininosuccinic acid and its anhydride respectively[4].

(3) The anhydride gave a colour in the Jaffe reaction[4].

(4) It behaved as a dicarboxylic amino-acid on paper electrophoretograms using pH 6·5 pyridine, acetic acid, water, buffer (10 : 0·4 : 90 v/v/v) and a potential of 12 V/cm (20 m.amp).

(5) It was acid stable, but on refluxing with strong barium hydroxide gave equal amounts of aspartic acid and ornithine[4].

Table 1. Changes in mg Nitrogen in the Ornithine Cycle Amino-acids of 100 g Freeze-dried Ungerminated Beans, 6- and 14-day-old Seedlings of *Vicia faba*

The amino-acids were determined colorimetrically (ref. 1) after separation on cation exchange resins under conditions similar to those used by Moore and Stein (ref. 2)

Amino-acid	0 days	6 days	14 days
Arginine	1,123	652·32	197·83
Argininosuccinic acid	2·46	6·53	78·84
Citrulline	0	0	62·71
Ornithine	0	0	31·69

In view of these findings, it is of interest that the enzyme argininosuccinase, which reversibly catalyses the formation of argininosuccinic acid from arginine and fumaric acid, has been shown to be present in peas[8], jack bean meal[9], and green pea, ryegrass and wheat[10]. The occurrence of argininosuccinic acid together with the other acids of the ornithine cycle strongly supports the previous suggestion that part, at least, of the ornithine cycle is involved in the metabolism of plants as well as animals.

When arginine labelled with carbon-14 was vacuum-infiltrated into disks of 10-day-old cotyledon, subsequent extraction and chromatography of the free amino acids showed that argininosuccinic acid and proline were the first amino acids to become labelled. With increasing length of time of incubation of the tissue with ^{14}C-arginine, ornithine, citrulline, glutamic acid, aspartic acid and finally alanine and γ-aminobutyric acid, became radioactive. The importance of these amino acids in the metabolism of germinating seeds of *Vicia faba* L. will be discussed fully in a later publication.

J. T. Barber
D. Boulter

Hartley Botanical Laboratories,
University of Liverpool.

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Alteration of Steroid Dehydrogenase Synthesis in a Mutant of *Pseudomonas testosteroni*

Marcus and Talalay have described two nicotinamide-adenine dinucleotide (NAD) dependent steroid dehydrogenases obtained from induced culture of *Pseudomonas testosteroni* (American Type Culture Collection 11996)[1]. The α-enzyme catalyses the oxidation of 3α-hydroxy