

### Amino-Acid Dehydrogenases in Germinating Seedlings.

THE importance of asparagine and glutamine in the nitrogen metabolism of the germinating seedling was established by the classical researches of Schulze and confirmed by the later work of Chibnall and others. It is usually assumed that these amides are formed by the combination of the corresponding acids with ammonia arising from the deamination of other amino-acids, though little is known of the mechanism of such deamination.

Recent work, notably that of Krebs<sup>1</sup> and Bernheim and Bernheim,<sup>2</sup> has demonstrated the part played by dehydrogenases in oxidative deamination in animal tissues; Krebs<sup>3</sup> and Weil-Malherbe<sup>4</sup> have postulated two separate mechanisms involving deaminases which would lead to the formation of glutamine in such tissues, though the presence of the latter in the animal organism has not yet been definitely demonstrated except in certain detoxications. It is obviously of importance to ascertain if there is any evidence of the existence of similar mechanisms in germinating seedlings in which amide synthesis is a well-established fact.

Dehydrogenase activity was tested for by the Thunberg's methylene blue technique. M/10 solutions of the following amino-acids were used as substrates: glycine, *l*(+)-alanine, *l*(-)-leucine, *l*(-)-histidine, *l*(-)-tyrosine, *l*(-)-aspartic acid, and *l*(+)-glutamic acid. The experimental material was prepared by grinding up two days' old seedlings with 0.87% dipotassium phosphate, pressing through muslin and centrifuging the extract.

It was found that the only amino-acids that have a definite accelerating action on the reduction of methylene blue by the extracts are glutamic acid and alanine. The following table shows the results of a typical experiment with the seedlings of *Phaseolus mungo* (green gram). The times given are for the decolourisation of 0.5 ml. of a 1/5000 solution of methylene blue by 0.5 ml. of the plant extract in the presence of 0.2 ml. of M/10 amino-acids.

Substrate added	Period of decolourisation
0.2 ml. Glutamic acid	.. 7 mins.
0.2 ml. Alanine	.. 10 mins.
No substrate	.. 16 mins.

The effect of alanine is not sufficiently marked, the Thunberg factor "I" being less

than one, to infer with certainty the existence of a specific dehydrogenase for this amino-acid. Even the glutamic acid dehydrogenase is limited in its distribution, it being found only in three out of the seedlings of several species examined, *viz.*, in *Phaseolus mungo* (green gram), *Phaseolus radiatus* (black gram), and *Pisum sativum* (pea). From extracts of two day old seedlings of these species, the dehydrogenase can be obtained in cell-free solution by filtration of the centrifuged extract through paper pulp. The optimal reaction for the action of the enzyme is pH 7.8, while below pH 5.8 it has no activity. Manometric experiments show that the enzyme is capable of oxidising glutamic acid in the presence of molecular oxygen also. The products of oxidation as well as the connection of the enzyme, if any, with glutamine formation are being investigated.

M. DAMODARAN,

K. RAMAKRISHNAN NAIR.

University Biochemical Laboratory,  
Chepauk, Madras,  
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<sup>1</sup> Krebs, *Biochem. J.*, 1935, **29**, 1951.

<sup>2</sup> Bernheim and Bernheim, *J. Biol. Chem.*, 1932, **96**, 325; 1934, **106**, 79; 1934, **107**, 275.

<sup>3</sup> Krebs, *Biochem. J.*, 1935, **29**, 1620.

<sup>4</sup> Weil-Malherbe, *Biochem. J.*, 1936, **30**, 665.

### Relative Digestibility of Caseins in their Artificial and Natural Environments.

AN ultra-microscopic study of milks from various species of animals reveals large differences in the dispersion of casein particles. Of the milks examined so far the buffalo's milk has the lowest dispersion of casein while that from the ass has its casein dispersed to the highest extent. The albumin content of a milk generally increases with the extent of dispersion of its casein particles, the higher the dispersion the greater the albumin content. Ass's milk possesses the highest amount of albumin, thereby offering effective protection to the highly dispersed casein particles.

From the point of view of digestion it is reasonable to expect a higher digestibility in the case of milks which exhibit a higher dispersion of their proteins. A comparative study of the *in vitro* digestion of milks from the cow and the ass, has shown that ass's milk is about one and a half times more digestible than that of the cow. It was of interest to determine if the caseins prepared from their respective milks continued to