

### 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,  
August 17, 1936.

<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

maintain their digestibilities when they were redispersed in an artificial environment (phosphate buffer). In the course of this experiment, many interesting points of difference between the two caseins, revealed themselves. As compared with cow's casein it was difficult to redisperse the casein from ass's milk in the M/15 phosphate buffer (pH 7.7); and it is therefore clear that although the same buffer and the same concentration of the two caseins were used for the digestion experiments, the substrates were, therefore, not dispersed to the same extent. Ass's casein is much harder and denser than cow's casein prepared and purified under the same conditions. The casein particle in ass's milk exhibits a powerful tendency to aggregate and the exceptionally high ratio of albumin to casein (1 : 2) is Nature's provision to protect the highly dispersed casein particles and keep them from coalescing.

It has been found that the rate of digestion of albumin from ass's milk is only a fifth of the rate at which ass's casein is digested. This circumstance is of great importance from the point of view of digestion of the casein particle since the albumin continues to offer colloidal protection until the casein attains a reasonably advanced stage of degradation.

K. BHAGVAT.

M. SREENIVASAYA.

Department of Biochemistry,  
Indian Institute of Science,  
Bangalore,  
September 14, 1936.

### An Ageotropic Mutation in X-Rayed Rice.

IN the course of studies on the mutations obtained in X-rayed rice, a plant with a prostrate habit was isolated in the second generation of plants raised from one of the pure lines, Co. 4, the dry seeds of which had been exposed to X-rays for one hour under a Coolidge tube with copper anti-cathode operated at 53 kv. and a tube current of 10-11 m.a. at a target distance of 17 cm. without filter. Only a few seeds were obtained from this plant as by the habit of the plant, most of the seeds had fallen into the field. The seedlings raised from the seeds in the usual swampy seed-bed were of two kinds, the natural erect ones and the prostrate ones like the parent. In the prostrate type the inclination of the shoots did not present any regularity as it was in all directions. The seedlings were later transplanted in

regular plots. Generally rice seedlings grow erect after they strike root, which will be in about a week after transplantation. In this particular progeny it was found that while many of the seedlings were growing in an inclined direction, some were like the normal seedlings growing erect. The segregation was a mono-hybrid one, indicating the dominance of the prostrate habit.

	Observed	Expected
		(3 : 1)
Prostrate ..	28	30
Erect ..	12	10
		$\frac{\text{Dev.}}{\text{S.E.}} = 0.72.$

These prostrate plants are unique and are different from the type described by Ramiah (1930) where the prostrate habit of the plant was due to the spreading nature of the tillers. During the heading time, the tillers bend at the nodes and become erect and later on again assume a spreading habit at maturity. The type described here, on the other hand, presents an appearance of a plant completely or partially lodged without any bending or curving in any of the nodes against gravity. It resembles the 'lazy' plant described by Jenkins and Gerhardt (1931) in maize where the prostrate nature of the plant was suggested by Eyster (1934) and later on by Overbeek (1936) as due to the stem being 'ageotropic' or gravitationally indifferent.

Seeds of one of this prostrate plant and of the normal Co. 4 strain from which this mutation arose, were sown in a line centrally, each in a separate pot. As soon as the tip of the seedlings were visible above the surface of the soil, both the pots were tilted and kept in a horizontal position with the soil surface vertical. While the seedlings from the prostrate plant grew horizontally, the normal seedlings curved up and grew vertically (Fig. 1). In another set both the pots were hung inverted, top downwards soon after germination. The prostrate plant progeny grew downwards while the normal seedlings bent flat in their attempt to grow vertically upwards against gravity.

Fig. 2 shows the same control and prostrate progenies sown in a pot and allowed to grow without any change in the position of the pot. While the seedlings of the control are erect, those of the prostrate show inclination to the vertical. This inclination is more enhanced if these are raised in wet seed-bed.

The progeny of all the plants both erect