

Injection of cerebral ganglia extract results in a sharp fall in the total blood sugars. There is a significant rise in the glucose level within 30 min and a decline later (Figure 2). This suggests the presence of a strong 'hypoglycaemic' factor and a 'hyperglycaemic' factor in the cerebral ganglia. The effect of ventral ganglia extract is a rise in the blood sugar level which returns to normal level after 1 h. There is a gradual significant rise in the blood glucose level as well, suggesting the presence of 'hyperglycaemic' factors in the ventral ganglia. When nerve-ring extract is injected, the 'hyperglycaemic' factors of cerebral and ventral ganglia predominate. Evidently there is some factor in the nerve-ring extract suppressing the effect of 'hypoglycaemic' factor of the cerebral ganglia. Both cerebral and ventral ganglia extracts cause a sharp rise in the free amino acid levels suggesting the presence of factors responsible for protein catabolism. Significant lowering of free amino acid levels upon injection of nerve-ring extract shows that these factors are suppressed in the extract (Figure 1).

Discussion. Not much is known about the neuroendocrine regulation of metabolism in molluscs. The presence of a 'hyperglycaemic' factor in the albumin gland of *Helix aspersa* has been suggested²¹. The present work indicates that metabolism of the snail *Ariophanta* Sp. is highly controlled and regulated. The data suggest that there is a complicated interplay of different controlling factors from the central nervous system in the regulation

of metabolism. The presence of two 'hyperglycaemic' factors one for glucose, another for sugars, and a strong 'hypoglycaemic' factor in the central nervous system indicates that the carbohydrate metabolism is highly regulated in this snail. This agrees with the earlier work where it has been shown that the metabolism of the tissues of *Ariophanta* is carbohydrate-oriented²⁴. The presence of principles influencing blood free amino acid levels suggests that protein metabolism is also regulated by the central nervous system²⁵.

Résumé. Contrôle neuroendocrinien du métabolisme d'un *Ariophanta* Sp., mollusque pulmoné. Les résultats indiquent la présence de deux facteurs hyperglycémiques dans le système nerveux central, l'un pour le glucose et l'autre pour les autres sucres. Les ganglions cérébraux contiennent un facteur hypoglycémique très actif et des facteurs qui sont responsables du catabolisme des protéines.

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²⁴ R. RAMAMURTHY and O. V. SUBRAMANYAM, in preparation (1971).

²⁵ The author wishes to thank Dr. R. RAMAMURTHY, S.V. University, Tirupati and Dr. K. V. NAIDU for all the encouragement.

Induced Seed Protein Mutant of Barley

In recent years an intense search for protein-improved barleys has been carried out. A lysine increase is especially searched for because, when used as feed for non-ruminants, lysine is the first limiting essential amino acid of barley. Many variants have been found^{1,2} with only modest protein improvements, followed by yield decreases of approximately 20% when compared with normal trade varieties. One of the most promising high-lysine barleys found is Hiproly^{3,4}. It has 1. a genetically dependent 20 to 30% increase in lysine per 16 g of nitrogen, 2. modest changes in protein composition and in the content of the remaining amino acids⁵ and 3. a yield depression of approximately 60%, based on single plants and compared with a normal lysine, high yielding variety⁴.

Among the protein variants found by us, one seems really extraordinary. The aim of this paper is to report on this variant and make it available to others.

The Risø mutant 1508 was found in 1970 by screening for deviating relative dye-binding capacity (DBC)⁶ in the Danish two-rowed spring barley variety Bomi treated with ethyleneimine. After a preliminary test of the mutants' protein composition in 1971, a line of mutant 1508 was established and compared with Bomi in 1972 under normal field conditions. The mutant was about 10% inferior to Bomi in grain yield. Apart from slightly smaller seeds, mutant 1508 resembles the parent variety very closely in performance.

The lysine content of the protein ($6.25 \times N$) varied from 5.18 to 5.42% in 4 replicates of mutant 1508 as compared with a variation from 3.64 to 3.82% in Bomi. The results given below (Tables I and II) derive from analysis on one replicate of the field grown material in 1972. Investigations carried out on single plants from 1971 gave similar results.

The amino acid composition of the mutant proteins, as determined by ion exchange chromatography (Table I), shows, besides the change in lysine, a 36% increase in

threonine, which is supposed to be the second limiting essential amino acid. Also the contents of His, Arg, Asp, Gly and Ala have increased considerably, whereas especially the contents of Glu and Pro and, to a smaller extent, of Cys and Phe have decreased compared with the parent variety.

Barley proteins are divided into 3 major groups: 1. albumins/globulins, 2. prolamines and 3. glutelins⁷. An important aim in breeding for higher nutritional quality is to minimize the content of the lysine-poor prolamine fraction. Fractionation⁸ of 2-gram samples of ground, defatted seeds (10% water) showed that the contents of albumin/globulin in the mutant increased from 27% to 46% of the total protein ($N \times 6.25$) (Table II), while the prolamine content decreased from 29% in Bomi to 9% in the mutant. The glutelin content was 39% in both. Higher amounts of non-protein nitrogen components in the mutant are indicated by the reduced percentage of nitrogen recovered as amino acids in the mutant (Table II).

The lysine content of the prolamines and of the glutelins are 192% and 36% above the respective values for the

¹ H. DOLL, in *Induced Mutations and Plant Improvement* (IAEA, Vienna 1972), p. 331.

² J. INGVERSEN, A. J. ANDERSEN, H. DOLL and B. KØIE, in *Nuclear Techniques for Seed Protein Improvement* (IAEA, Vienna 1973), p. 193.

³ A. HAGBERG and K. E. KARLSSON, in *New Approaches to Breeding for Improved Plant Protein* (IAEA, Vienna 1969), p. 17.

⁴ L. MUNCK, K. E. KARLSSON, A. HAGBERG and B. O. EGGUM, *Science* 168, 985 (1970).

⁵ L. MUNCK, K. E. KARLSSON and A. HAGBERG, in *Barley Genetics*, II, Proc. Sec. Int. Barley Gen. Symp. (Washington State University Press 1971), p. 544.

⁶ R. MOSSBERG, in *New Approaches to Breeding for Improved Plant Protein* (IAEA, Vienna 1969), p. 151.

⁷ T. B. OSBORNE, *J. Am. chem. Soc.* 17, 587 (1895).

⁸ J. INGVERSEN and B. KØIE, *Phytochemistry* 12, 73 (1973).

Table I. Amino acid composition of whole seeds and protein fractions from the Risø mutant 1508 and the mother variety Bomi

| | Whole seed | | | Albumin/globulin | | Prolamine | | Glutelin | | Insoluble rest | |
|------------------|------------|------|--------|------------------|-----|-----------|-----|----------|-----|----------------|-----|
| | Mutant | Bomi | Mutant | Mutant | | Mutant | | Mutant | | Mutant | |
| | a | a | b | a | b | a | b | a | b | a | b |
| Lys | 7.0 | 4.7 | 151 | 7.5 | 96 | 2.9 | 292 | 5.9 | 136 | 4.9 | 90 |
| His | 3.6 | 2.8 | 127 | 3.5 | 111 | 2.8 | 156 | 3.6 | 118 | 3.6 | 110 |
| Arg | 8.3 | 5.9 | 140 | 10.3 | 107 | 5.0 | 149 | 7.8 | 250 | 5.8 | 86 |
| Asp | 11.4 | 7.5 | 154 | 13.7 | 102 | 4.6 | 244 | 9.9 | 140 | 11.1 | 88 |
| Tre | 5.4 | 4.0 | 136 | 5.0 | 94 | 4.1 | 191 | 5.6 | 120 | 6.5 | 130 |
| Ser | 5.8 | 5.3 | 111 | 5.2 | 99 | 5.3 | 130 | 6.2 | 110 | 6.0 | 92 |
| Glu | 21.3 | 30.5 | 70 | 18.5 | 106 | 33.6 | 75 | 20.3 | 72 | 17.0 | 107 |
| Pro | 7.8 | 13.8 | 56 | 7.2 | 109 | 14.5 | 69 | 7.5 | 64 | 13.7 | 155 |
| Gly | 7.1 | 4.8 | 149 | 7.4 | 98 | 5.2 | 314 | 6.7 | 135 | 8.0 | 103 |
| Ala | 6.9 | 5.1 | 136 | 7.0 | 94 | 5.4 | 254 | 6.5 | 125 | 7.0 | 87 |
| Cys ^a | 1.3 | 1.5 | 90 | 2.4 | 81 | 1.7 | 111 | 0.8 | 95 | 0.0 | — |
| Val | 6.9 | 6.4 | 108 | 7.0 | 98 | 5.6 | 121 | 7.5 | 103 | 7.1 | 92 |
| Met ^a | 2.1 | 1.8 | 113 | 2.1 | 86 | 2.2 | 160 | 2.1 | 107 | 2.0 | 90 |
| Ileu | 4.3 | 4.4 | 97 | 3.9 | 97 | 3.6 | 89 | 4.9 | 100 | 4.3 | 85 |
| Leu | 8.8 | 9.0 | 98 | 7.6 | 92 | 10.0 | 129 | 9.6 | 99 | 9.2 | 86 |
| Tyr | 3.2 | 2.9 | 112 | 3.8 | 95 | 4.4 | 119 | 4.5 | 104 | 2.2 | 99 |
| Phe | 5.3 | 6.4 | 82 | 4.7 | 108 | 5.8 | 72 | 5.7 | 92 | 5.5 | 84 |

The values given for the whole seeds are the mean of 4 analyses, whereas the fractions are the mean of 2 analyses. a) gives gram amino acid per 100 g recovered protein, b) is the relative content of the amino acids in the proteins of the mutant 1508 compared with Bomi. ^a Measured on non-oxidized samples.

Table II. Distribution of the seed nitrogen on, and estimates of the protein contents in the protein fractions of the Risø mutant 1508 and Bomi

| | Whole seed | | Albumin/globulin | | Prolamine | | Glutelin | | Insoluble rest | |
|---|------------|------|------------------|------|-----------|------|----------|------|----------------|------|
| | Mutant | Bomi | Mutant | Bomi | Mutant | Bomi | Mutant | Bomi | Mutant | Bomi |
| Nitrogen (% of seed) | 1.75 | 1.72 | | | | | | | | |
| Total nitrogen in the fractions (%) | 100 | 100 | 46 | 27 | 9 | 29 | 39 | 39 | 6 | 5 |
| Nitrogen recovered as amino acids and ammonia (%) | 85 | 90 | 86 | 79 | 85 | 94 | 87 | 91 | 96 | 94 |

parent variety. While the amino acid composition of the mutant albumin/globulin fraction is almost similar to that of Bomi, the amino acid composition of the mutant glutelin and, especially, of the prolamines indicates a greatly changed protein pattern, characterized mainly by a fall in the contents of Glu and Pro and by an increase of most other amino acids, particularly Lys, His, Arg, Asp, Tre, Gly and Ala. The insoluble rest shows an increase in Tre and Pro and a decrease of most other amino acids.

Summing up, the Risø mutant 1508 has 1. a genetically stable 44% increase in the lysine per 16 g of nitrogen, 2. pronounced changes in protein composition as well as in the contents of other amino acids and 3. a yield depression of approximately 10%, based on a preliminary field trial, and compared with the normal-lysine, high yielding parent variety.

ВЫВОДЫ. Стабильный мутант с хорошей агрономической характеристикой побуждено в ядрах ячменя. Содержание лизина в протеине мутанта выше на 51%. Фракция протеина с убогим содержанием лизина (проламин) понижилась с 29% до 9% с сопутствующим увеличением альбумино-глобулиновой фракции. Аминокислотный состав большей части протеиновых фракций значительно изменяется.

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⁹ Seed samples may be obtained upon application to H. DOLL.

Gene-Enzyme Relationships in the Tryptophan Pathway of *Schizosaccharomyces pombe*.

The reaction sequence of the tryptophan pathway involves the same 5 enzymatic steps in all organisms so far tested (Figure). However, the structure and aggregation of the enzymes and their genetic control differ considerably¹. This diversity found in the biochemical organisation of the tryptophan pathway has been used

as an approach to examine possible relationships among a variety of fungi and other microorganisms².

¹ P. MARGOLIN, in *Metabolic Pathway* (Ed. H. J. VOGEL; Academic Press, New York 1971), vol. 5, p. 389.

² R. HÜTTER and J. A. DE MOSS, *J. Bact.* 94, 1896 (1967).