growth of rhizobia, the recovery of viable rhizobia is very low (less than 1%)[5]. The results of this study also show low recovery of viable rhizobia from standard YEM broth on centrifugation (table 1). Improvement was observed when mannitol was used at suboptimal level (1 g/l) in the growth medium, but the results are much better with malt extract, which gave 71–72.53% recovery of viable rhizobia in comparison to 15.18–15.25% with mannitol. Malt extract, when used as sole source of carbon, nitrogen and growth factors in the medium, was found to be better than yeast extract which has been reported to be very good for growth yield and yield recovery of *R. leguminosarum*[5]. It was, however, true only for lower concentration (2 g/l) of malt extract in liquid medium (table 2). At higher concentrations (6–10 g/l) the recovery of viable rhizobia declined, though growth yield improved. With yeast extract, however, recovery yields were good even at higher concentration.

It may be inferred from this study that a large population of viable rhizobia may be recovered from the growth medium for developing cell concentrates if malt extract is used in lower concentration as sole source of carbon, nitrogen and growth factors in the medium. This will not only reduce the cost of production (as malt extract is cheaper than yeast extract) but also improve the efficiency of production of cell concentrates because of faster recovery of cells from growth medium.

28 November 1988; Revised 18 May 1989

1. Thomson, J. A., In: *Methods for Evaluating Biological Nitrogen Fixation*, (ed.) F. J. Bergerson, John Wiley and Sons, New York, 1980, p. 489.
2. Portor, F. E., In: *Developments in Industrial Microbiology*, American Institute of Biological Sciences, Washington, 1969, vol. 10, p. 88.
3. Meade, J., Higgins, P. and O'Gara, F., *J. Appl. Bacteriol.*, 1985, 58, 517.
4. Vincent, J. M., *A Manual for the Practical Study of Root-Nodule Bacteria*, Blackwell Scientific Publications, Oxford, 1970.
5. Ghai, S. K., Hisamatsu, M., Amenura, A. and Harada, T., *J. Gen. Microbiol.*, 1981, **122**, 33.
6. Elsworth, R., *Phase Separation of Suspensions by Centrifuge*, New Brunswick Sci. Co., New Jersey, 1962.

REVERSAL OF CHLORAMPHENICOL-INHIBITED PROTEIN SYNTHESIS BY EDTA AND SULPHHYDRYL COMPOUNDS IN BHENDI SEEDLINGS (*ABELMOSCHUS ESCULENTUS* (L.) MOENCH.)

B. ADINARAYANA and P. GOPALA RAO
Department of Botany, Sri Venkateswara University, Tirupati 517 502, India

It is well established that chloramphenicol inhibits protein synthesis on 70 S ribosomes[1]. Attempts have been made to use this inhibitor to clarify characteristics of protein synthesis in higher plants[2–4]. While chloroplast and mitochondrial ribosomes bind chloramphenicol, cycloheximide blocks protein synthesis on cytoplasmic 80 S ribosomes[5,6]. This communication presents results of experiments to test the ability of EDTA and three sulphhydryl compounds to reverse the effect of chloramphenicol in bhendi seedlings. EDTA is a metal-chelating agent and can conjugate protein[7]. Chemicals like glutathione, cysteine, ascorbic acid and thiourea have been shown to markedly reduce chromosomal aberrations caused by gamma radiation in *Tradescantia* and onion root tips[8,9]. The stability of the disulphide bond makes it an important factor in the tertiary structure of proteins[10]. Sulphhydryl groups and sulphydryl-binding reagents can participate in redox reactions by reversible formation of disulphide bonds.

Seeds of bhendi var. Pusa Savani were surface-sterilized with 5% mercuric chloride for 15 min and washed thoroughly with distilled water. They were then soaked before sowing for 24 h in 200 ppm solutions of EDTA, methionine, cysteine, glutathione or chloramphenicol or combinations of these compounds (see figure 1). Lower concentrations (50 and 100 ppm) were ineffective. Seedlings were allowed to grow in distilled water for 10 days under a light intensity of 2000 $\mu E\, m^{-2}\, sec^{-1}$. Seedling protein was extracted[11] and estimated[12] as described earlier. Increase or decrease in protein was calculated as per cent difference between control and treated seeds.

The results (figure 1) indicate that in EDTA-treated seedlings there was about 19.7% increase in protein content on day 10. Chloramphenicol treatment caused a 29.3% reduction in protein content. However, when seeds were treated with EDTA and chloramphenicol, the decrease in protein content was only 4.3% on day 10 of seedling growth. Thus

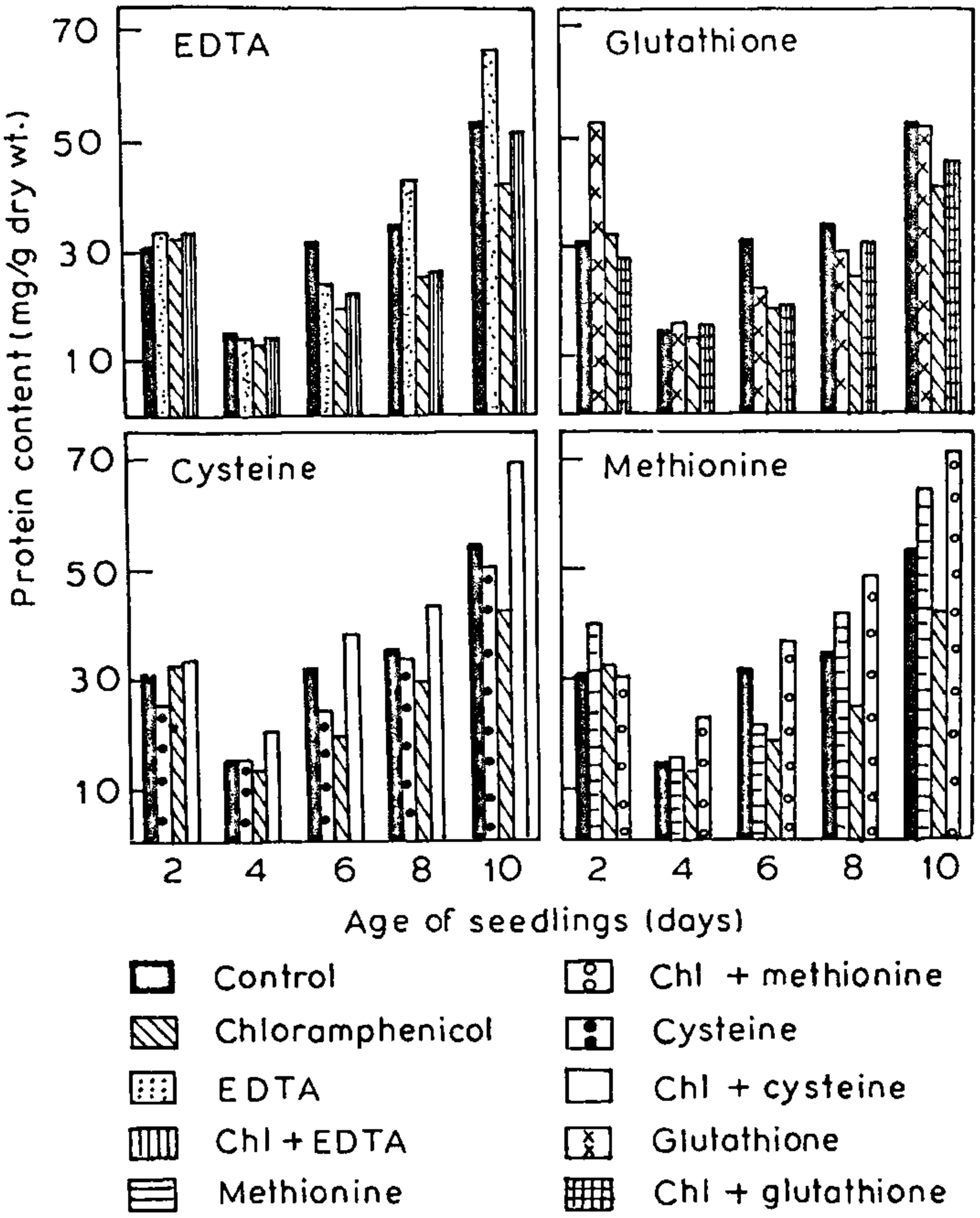

Figure 1. Protein content of bhendi seedlings from seeds treated with EDTA, glutathione, cysteine or methionine, or combinations of these reagents.

EDTA could reverse the inhibition of protein synthesis caused by chloramphenicol.

While methionine caused an increase in protein content on day 10 by about 17.1%, treatment with chloramphenicol and methionine increased protein content by 25.7%. Thus it appears that methionine is highly potent in reversing the inhibition of protein synthesis and also activating the synthesis. Earlier work[13] indicates that methionine altered the composition of the pool of free amino acids. It was also observed that methionine altered the composition of protein during sulphur deficiency. Gatehouse *et al.*[14] stated that genetic potential for increasing the ratio of the sulphur-rich protein legumin to the sulphur-poor protein vicilin, and thus improving storage protein quality by increasing sulphur-containing amino acid content, is limited. The present study might offer a strategy for the improvement of seed protein quality and quantity through a physiological approach.

Cysteine, another sulphhydryl-containing amino acid caused a decrease in protein content on day 10 by about 8.2%, but when it was used with chloramphenicol there was an increase in protein content by about 22.5%. How cysteine alone caused reduction in protein content is not clear.

According to Macnicol[15] sulphur deficiency severely affects the composition of the free amino acid pool of developing *Pisum sativum* seeds, causing a marked decrease in cysteine but no change in methionine. In the present study the response to exogenous amino acids varied with the amino acid, i.e. decrease in protein content with cysteine and increase with methionine.

Glutathione treatment caused a reduction in protein content on day 10 by about 1.3% while treatment with glutathione and chloramphenicol caused a further reduction (14.5%). However, glutathione could partially reverse the inhibition by chloramphenicol (29.3 to 14.5%). Thus, when compared to methionine and cysteine, glutathione appears to be weak in counteracting the effect of chloramphenicol. Glutathione disulphide was found to be involved in reversing inhibition of glucose-6-phosphate dehydrogenase by NADPH[16].

The authors thank Prof. V. S. Rama Das for guidance and encouragement.

22 April 1988; Revised 1 February 1989

1. Hess, D., *Plant Physiology*, Springer-Verlag, New York, 1975, p. 22.
2. Molotkovskii, Yu, G. and Smirov, A. M., *Fiziol. Rast.*, 1963, **10**, 2325.
3. Parthier, B., *Nature* (*London*), 1965, **206**, 783.
4. Osipova, O. P., Nikolaeva, M. K. and Khein, Kh. Ya., *Fiziol. Rast.*, 1967, **14**, 210.
5. Hoober, J. K. and Blobel, J., *Mol. Biol.*, 1969, **41**, 121.
6. Reger, B. J., Smille, R. M. and Fuller, H. C., *Plant Physiol.*, 1972, **50**, 19.
7. Martell, A. E. and Calvin, M., *Chemistry of the Metal Chelate Compounds*, New York Publications, 1952, p. 385.
8. Mikelson, K., *Proc. Natl. Acad. Sci. Wash.*, 1957, **40**, 171.
9. Riley, H. P., *Am. J. Bot.*, 1955, **42**, 765.
10. Clarkson, D. T. and Hanson, J. B., *Annu. Rev. Plant Physiol.*, 1980, **31**, 239.
11. Key, J. J., *Plant Physiol.*, 1964, **39**, 365.
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **93**, 790.
13. Baratt, D. H. P., *Ann. Bot.*, 1984, **54**, 21.
14. Gatehouse, J. A., Croy, R. R. D. and Boulter, D., *Biochem. J.*, 1980a, **185**, 497.
15. Macnicol, P. K., *FEBS Lett.*, 1983, **156**, 55.
16. Buchanan, B. B., *Annu. Rev. Plant Physiol.*, 1980, **31**, 361.

PYRUVATE OXIDATION IN TOAD GASTROCNEMIUS MUSCLE AFTER ANODIC OR CATHODIC STIMULATION

K. SUBRAHMANYAM*, K. S. SWAMI and C. S. CHETTY

Department of Zoology, Sri Venkateshwara University, Tirupati 517 502, India

**Department of Zoology, Ideal College of Arts and Science, Kakinada 533 004, India*

THE anatomical basis of metabolic compartmentation in the central nervous system[1-6] is attributed to neuronal elements, glial components and astrocytes. Within the neuron, regionalized compartmentation, involving perikaryon with central functions, mitochondrial elements in the axoplasm with supporting functions and terminal units with synaptic functions, has been suggested[7-9].

The metabolic systems involving oxidoreductases possess cathodal characteristics[10], while those involving protein turnover, proteases, transaminases and acetylcholinesterase activities possess anodal characteristics. These systems are demarcated in terms of cathodal or anodal charge-based metabolic compartments[11-13]. If the activity patterns of these metabolic systems are under the control of the respective polarity characteristics, exogenous supplementation of identical characteristics is expected to activate the respective metabolic events. This has been tested in the present study on toad gastrocnemius muscle.

Medium-size (30 ± 5 g) *Bufo melanostictus* were collected in and around Tirupati and were maintained in clean glass troughs containing wet sand and leaves. The bed in the troughs was changed once every day. The toads were fed cockroaches *ad libitum* and acclimatized to laboratory conditions for one week. For electrical stimulation the toads were positioned on a Perspex base with a soft rubber band and made immobile. One platinum electrode (0.2 mm thickness) was placed superficially on the skin in the region of the gastrocnemius muscle of the right leg and the other electrode (0.2 mm) was placed in the midventral region of the abdomen. The skin of the animal between the electrodes was wiped to minimize surface wetness and reduce the possibility of short-circuit effects across the skin. An electric gradient of 0.8 V/cm (DC) was applied for 5 min every day for five days. The electrode contact regions

*For correspondence.