(23 U) significantly different, when compared at the 5% confidence level ($t < 2.06$, when $n = 26$).

Discussion. We chose red cells from monkeys as a source of 'standard' transferase for Beutler and Mitchell's[1] assay of human red cell transferase. The blood samples were readily available to us; the enzyme was approximately as active as human red cell transferase and appeared stable when cells were frozen.

The diversity of electrophoretic patterns indicates that the molecular structure of the various species' transferase protein is heterogeneous. We inferred from the different numbers of isozymes displayed that polymer structures were dimeric in some species and either trimeric or indeterminate in others[16].

Similarity of total enzyme activity among species, despite apparent structural heterogeneity, is characteristic of isozyme systems[16]. Since total activity was the parameter we sought, this feature of enzymes further validates our choice of nonhuman cells as a source of 'standard' transferase in the quantitative assay.

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Ammonia assimilatory enzymes in a nif^{-III} mutant of *Azotobacter chroococcum*

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Summary. The levels of ammonia assimilatory enzymes have been studied in a nif^{-III}mutant and a wild strain of *Azotobacter chroococcum* grown in media containing different nitrogen sources. It is suggested that the nif^{-III}mutant may be defective in transport or incorporation of molybdenum.

Studies on nif$^-$ mutants in nitrogen fixing organisms have thrown light on the mechanism and control of biological nitrogen fixation[1,2]. We report here a hitherto nondocumented nif^{-III}mutant of *Azotobacter chroococcum*, possibly defective in the transport or incorporation of molybdenum.

Materials and methods. Azotobacter chroococcum isolated from the root zone of *Cyprus rotandus*, CYP3, obtained from the Advanced Centre for Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, was used as the wild type. This strain was grown in Burk medium[3], and the cells were collected at the exponential phase (56 h), and resuspended in 0.2 M phosphate buffer (pH 7.0) to a final concentration of 10^7 cells/ml. The cell suspension (5 ml) was treated with 300 µg/ml of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), and mutagenesis was carried out according to Adelberg et al.[4]. 4 mutants (nif^{-I}-nif^{-IV}) were identified by scoring out the colonies grown on agar plates with ammonium acetate. *Azotobacter* strains were maintained in Burk agar medium plus 1.1 mg/ml ammonium acetate for the nif$^-$strains or without ammonium for the wild strain[5].

The wild and the nif^{-III} strains were grown in 100 ml of Burk liquid medium in 250 ml Erlenmeyer flasks and also in media supplied with either ammonium acetate, gluta-

Table 1. Nitrogenase and ammonia assimilatory enzymes in wild and nif^{-III} mutant of *A. chroococcum* grown on different nitrogen sources

Nitrogen	No nitrogen		Ammonium acetate		Glutamate		Glutamine		Aspartate	
Enzymes	wild	nif^{-III}	wild	nif^{-III}	wild	nif^{-III}	wild	nif^{-III}	wild	nif^{-III}
Nitrogenase[a]	70.40	No growth	8.20	Not detected	4.24	Not detected	Traces	Not detected	1.50	Not detected
GS[b]	1000	No growth	360	100	300	60	200	90	300	50
GOGAT[c]	300	No growth	110	130	130	120	70	40	250	10
GDH[c]	Not detected	No growth	180	120	70	20	20	20	20	20
GOT[d]	80	No growth	90	160	130	80	150	150	140	100

[a] nmoles of C_2H_4 produced/cell/h; [b] nmoles of γ-glutamyl hydroxamate formed/min/mg protein; [c] nmoles of NADPH oxidized/min/mg protein; [d] nmoles of pyruvate formed/min/mg protein.

mine, glutamate or aspartate. Ammonium acetate was supplied to a final concentration of 1.1 mg/ml and the amino acids to a concentration of 100 µg/ml. All enzyme assays were carried out using cells from the exponential growth phase. Nitrogenase activity in whole cells was determined using the acetylene reduction test according to Hardy et al.[6].

Cell-free extracts in 0.25 M sucrose solution were prepared following the rupture of cells by sonication. The extracts were clarified by centrifuging at 10,000 × g for 10 min in a refrigerated centrifuge. Glutamine synthetase (GS) was assayed by measuring the amount of γ-glutamyl hydroxamate formed in the presence of Mn^{2+} as described by Shapiro and Stadtman[7]. Glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) activities were determined by following the rate of NADPH oxidation at room temperature[8,9]. Glutamate-oxaloacetate transaminase (GOT) activity was determined colorimetrically according to Mohun and Cook[10]. Nitrate reductase (NR) was assayed according to Hageman et al.[11]. The total protein was estimated following the procedure of Lowry et al.[12].

Results and discussion. As shown in table 1, the nitrogenase activity of the wild strain was inhibited to varying degrees in the presence of different nitrogenous compounds. The GS and GOGAT activities were high while GDH activity was not detectable in the wild strain grown in nitrogen-free medium. In the presence of ammonia, although GS and GOGAT activities could still be detected, the GDH activity was found to be high in comparison with nitrogen - free medium and media with other nitrogen sources. The GDH pathway operates at high concentrations of ammonia, while the GS-GOGAT pathway operates at low concentrations[13,14]. Where GS activity was low in nitrogen-supplied cultures, the nitrogenase activity was also low because its synthesis requires active GS[15].

Table 2. Nitrate reductase activity (nmoles of nitrite formed/min/mg protein) of wild strain and the mutant

Nitrogen source	wild	nif^{-III} mutant
No nitrogen	3400	No growth
Ammonium acetate	7800	Not detected
NO_3^-	10,000	No growth

The nif^{-III} mutant could use ammonia as well as glutamate, glutamine and aspartate, but did not show nitrogenase activity in any of the media. The GS, GOGAT and GDH activities showed the same trend as in the wild strain. The GOT activity did not exhibit any significant difference in comparison with the wild strain.

The wild strain showed NR activity on media without nitrogen or with ammonia or with nitrate. The activity was increased in the presence of nitrate. The mutant had no NR activity either in the presence or absence of nitrate (table 2).

The absence of molybdenum-containing nitrate reductase and nitrogenase activites suggests that the mutation may affect the transport of molybdenum or its incorporation into enzyme protein[16]. Hence this mutant may be regarded as phenotypically I^-II^+.

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Glucose and 3-hydroxybutyrate utilization by chick telencephalon during postnatal development[1]

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Summary. The cerebral arteriovenous difference in glucose content remains constant during the whole postnatal development of the chick, whereas that of 3-hydroxybutyrate is 6-9 times as high in the 1-day-old chick as in the 2-30-day-old chick.

In consequence of the high lipid content of maternal milk[2], the infant rat develops a marked ketosis as early as birth[3] and ketone bodies - acetoacetate and 3-hydroxybutyrate - are largely utilized by the brain[4]; indeed, 3-hydroxybutyrate appears to be a very active precursor of amino acids in the brain of the 15-day-old rat[5,6]. The newly-hatched chick is in a state of pronounced nutritional starvation with high fatty acid oxidation in the liver inducing an excessive release of ketone bodies into the blood plasma. This neonatal lipid metabolism is progressively replaced by a predominantly carbohydrate metabolism during the first 5 days after hatching[7]. The 3-hydroxybutyrate is present at very high concentrations in the blood of 1-day-old chicks; it is also a very active precursor of amino acids in the newly-hatched chick telencephalon[8], while glucose is actively incorporated only towards the 4th day after hatching[9]. In order to determine the relative importance of glucose and 3-hydroxybutyrate in the metabolism of chick brain during