mid R124-J1. Since the mutation(s) conferring increased antibiotic resistance is plasmid determined and affects both the resistances carried by R124, we examined the possibility that R124-J1 might be a copy mutant. To examine copy number, we measured the amount of plasmid DNA and its approximate molecular size in strains M827 pLT2$^-$ R124-J1 and 11G pLT2$^-$ R124-J1. Table 1 shows that strains carrying the mutant plasmid had about twice as much DNA in the ccc plasmid form as strains harbouring the parental R124. Measurements of the sedimentation of the mutant plasmid on alkaline sucrose gradients showed a mol. wt for R124-J1 of ca. 65 md with the mutant plasmid running very similarly to the parental R124 (figure). Such gradients showed no indication of the presence of a larger plasmid in strains carrying R124-J1. The copy number of R124-J1 is therefore greater than that of R124 in strains 11G pLT2$^-$ and M827 pLT2$^-$ (table 1).

R124 behaves like R1[4] in that the number of copies per chromosome equivalent increased with decreasing growth rate. The same is true for the copy mutant R124-J1 and in strain 11G *dnaC* pLT2$^-$ over a range of growth rates the ratio of R124-J1 copy number/R124 copy number remained at about 2. The copy number of R124 in this strain at 30 °C increased from 0.45 in nutrient broth to 0.70 in glucose minimal medium while that of R124-J1 increased from 0.93 in nutrient broth to 1.45 in glucose minimal medium. Strains harbouring copy mutants of R1*drd*19 have a decreased growth rate compared to those harbouring R1*drd*19 itself or free of plasmid[11]. The same proved to be the case for strains harbouring R124-J1. They grew at a slightly reduced rate in all the media tested (table 2).

We have tested the effect of R124-J1 on the maintenance of F*lac* in the *dnaC* mutant. R124-J1 was more effective than R124 in causing F*lac* instability. With strain 11G *dnaC* pLT2$^-$ R124 F*lac*, overnight growth in broth at 30 °C gave rise to 22.4% of Lac$^-$ organisms; under the same conditions 81.3% of organisms from strain 11G *dnaC* pLT2$^-$ R124-J1 F*lac* lost the F*lac* plasmid. Interestingly a copy mutant of R1*drd*19 (mutant R1*drd*19-B42) also destabilizes F*lac* more than R1*drd*19 does in strain 11G *dnaC* whereas another copy mutant of R1*drd*19 (mutant R1*drd*19-B2) allows relatively stable maintenance of F*lac*[12]. R1*drd*19-B42 apparently has an altered replication repressor[4] and therefore the increased copy number of R124-J1 may be consequent upon an abnormality of this kind.

The replication behaviour, incompatibility properties and other characteristics of R124-J1 will be further compared to those of R124. The mol. wt studies suggest that substantial amounts of DNA have not been added to or deleted from R124 in the formation of R124-J1. These 2 plasmids are, however, being further tested for structural differences using restriction endonucleases.

11 B. Engberg, K. Hjalmarsson and K. Nordstrom, J. Bact. *124*, 633 (1975).
12 V. Rodriguez Lemoine and R. J. Rowbury, unpublished observations.

Regulation of ammonia uptake in *Aspergillus nidulans*[1]

J. D. Desai* and V. V. Modi

Department of Microbiology, Faculty of Science, M. S. University of Baroda, Baroda 390 002 (India), 9 May 1977

Summary. The ammonia uptake in *A. nidulans* was found to be linear for about 20 min, and was proportional up to 1.5 mg/ml dry cell density. The transport of ammonia does not involve energy. Normal and biotin deficient *A. nidulans* showed an identical K_m-values of 10.26×10^{-5} M ammonia for uptake. The uptake of ammonium ion has been shown to be regulated by the intracellular concentration of ammonia.

Earlier work from this laboratory indicated that biotin deficiency in *Aspergillus nidulans* causes significant increase in the cellular synthesis when ammonium nitrate was used as a sole nitrogen source[2,3]. Further, it was demonstrated that the increase in cellular synthesis is associated with the marked increase in the protein content with the concomitant decrease in the lipid content of the mold[2]. Biotin deficiency in this culture showed significant change in the permeability properties of the cell[4]. The glucose[5–7] and phosphorus[8] uptake systems have already been studied in *A. nidulans* and now we report the characteristics and the regulatory aspect of its ammonia uptake system.

Materials and methods. The strain, composition of the basal medium and cultural conditions are the same as described earlier[3,5]. Cultures grown in the presence of 5 units of avidin (General Biochemicals, Ohio, USA) were 65% lower in their fatty acid content and were referred as biotin deficient [3–7]. The method of Brown and Romano[9] previously modified[4,5] was followed for the uptake studies. Ammonia was determined by the method of Fawcett and Scott[10]; 24-h-old cells grown on basal medium were collected, washed and about 100 mg wet cells were added to the fresh medium containing the indicated amount of ammonia. Cells were further incubated on a rotary shaker (200 rpm) at 30 °C for about 24 h and collected. The intracellular concentration of ammonium ion was calculated on the basis of 4 µl water/mg dry cell weight[5,11].

Results and discussion. It has been found that when cells were grown on KNO_3 as a sole nitrogen source, the uptake

* Present address: Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388 120, India.
1 Acknowledgment. The award of research fellowship by the Govt of India, Department of Atomic Energy, Bombay, to JDD is acknowledged.
2 K. K. Rao and V. V. Modi, Can. J. Microbiol. *14*, 813 (1968).
3 J. D. Desai and V. V. Modi, Can. J. Microbiol. *21*, 807 (1975).
4 J. D. Desai and V. V. Modi, Curr. Sci. *44*, 236 (1975).
5 J. D. Desai and V. V. Modi, Ind. J. exp. Biol. *12*, 438 (1974).
6 J. D. Desai and V. V. Modi, Experientia *31*, 160 (1975).
7 J. D. Desai and V. V. Modi, Experientia *33*, 726 (1977).
8 J. D. Desai, Jyotsna Misra and V. V. Modi, Ind. J. exp. Biol. *14*, 198 (1976).
9 C. E. Brown and A. H. Romano, J. Bact. *100*, 1198 (1969).
10 J. F. Fawcett and J. E. Scott, J. clin. Path. *13*, 156 (1960).
11 C. G. Mark and A. H. Romano, Biochim. biophys. Acta *249*, 216 (1971).

Intracellular and extracellular concentrations of ammonia in *A. nidulans*

Initial concentration (mM)		Final concentration (mM) Extracellular	Intracellular
Normal	10	7.9	8.0
	20	16.0	15.2
	30	20.1	19.6
Deficient	10	5.0	5.0
	20	9.8	10.0
	30	15.1	15.0

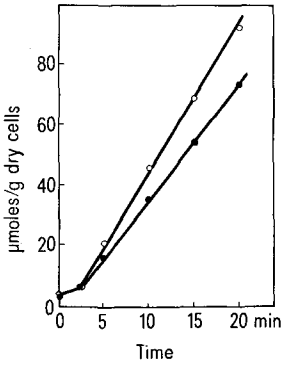

Fig. 1. Ammonium ion uptake by normal (●) and biotin-deficient (○) *A. nidulans*.

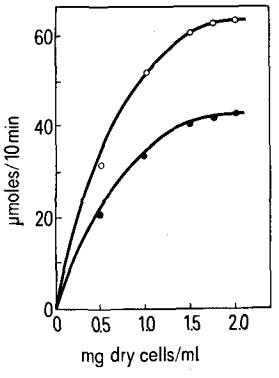

Fig. 2. Effect of cell density on the uptake of ammonium ion by normal (●) and biotin-deficient (○) *A. nidulans*. Experimental conditions are the same as described for uptake studies, except that the cell density was adjusted as indicated in figure and after 10 min of incubation ammonia uptake was measured.

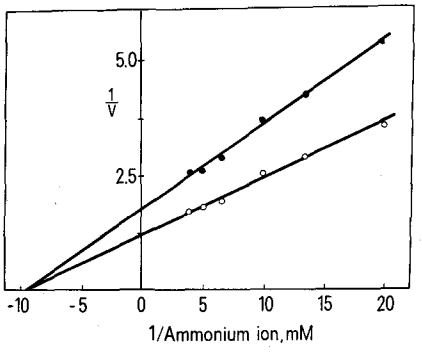

Fig. 3. Lineweaver-Burk plot of uptake of ammonia by normal (●) and biotin-deficient (○) *A. nidulans*.

of ammonia was about 30% and 50% more than that found in the cells grown in presence of NH_4NO_3 and NH_4Cl as a nitrogen source, respectively. This may be due to the repression of ammonium ion uptake system. However, the degree of derepression was very poor as compared to that reported in *P. chrysogenum*[12]. For further studies we have used the cells of *A. nidulans* grown on NH_4NO_3 as a nitrogen source. Figure 1 shows the linearity of ammonium ion uptake as a function of time for about 20 min in both normal and biotin deficient cells. It is interesting to note that biotin deficiency causes about 40% increase in ammonium ion uptake in this culture. The uptake of ammonium ion was found to be proportional to the cell density up to about 1.5 mg/ml dry cells (figure 2).

The intracellular concentration of ammonia was found to be identical to that of extracellular concentration in both normal and biotin deficient *A. nidulans*. Further, the intracellular concentration increased as the extracellular concentration of ammonium ion was increased, and the reverse was also true in the sense that the intracellular concentration of ammonia decreased as the extracellular concentration of ammonia was dereased. It is interesting to note that in all cases the intracellular concentration of ammonia was low in biotin-deficient cells as compared to that in normal cells (table). This observation suggested that the entry of ammonium ion in *A. nidulans* does not occur against the concentration gradient and depends upon the intracellular concentration of ammonium ions. The addition of KCN, 2,4 DNP and NaN_3 to the uptake medium did not cause any significant alteration in the uptake rate of ammonium ion, thus indicating that, for the entry of ammonia in *A. nidulans*, energy is not involved. The above results lead us to suggest that the entry of ammonium ion into the cell is not by the active transport mechanism but by the facilitated diffusion process. The characteristics of the ammonia uptake system are quite different than those of the other transport systems we have studied in *A. nidulans*[5–8], as well as the ammonia uptake system in *P. chrysogenum*[12]. Our results are however in line with those reported by MacMillan[13] and Pateman et al.[14].

The uptake of ammonium ions in relation to the ammonium ion concentration follows Michaelis-Menten kinetics with an identical K_m-value of 10.26×10^{-5} M ammonium ion in both normal and biotin-deficient *A. nidulans* (figure 3). This indicated that the affinity of ammonium ion uptake system for the substrate may not be the cause of higher uptake due to biotin deficiency, as shown in figures 1 and 2. Recently, we have shown that ammonia in *A. nidulans* is assimilated mainly via NADP-glutamate dehydrogenase route, and biotin deficiency in this culture causes significant increase in NADP-glutamate dehydrogenase activity[15]. Thus, from all these results, it can be suggested that the increased uptake of ammonia due to biotin deficiency in *A. nidulans* may be the result of its faster conversion to glutamate thus lowering the intracellular ammonium ion concentration and allowing more uptake of ammonium ions from the medium.

12 S. L. Hackette, G. E. Skys, C. Burton and I. H. Segal, J. biol. Chem. *145*, 4241 (1970).
13 A. MacMillan, J. exp. Bot. *7*, 113 (1956).
14 J. A. Pateman, J. R. Kinghorm, E. Dunn and E. Forbes, J. Bact. *114*, 943 (1973).
15 J. D. Desai and V. V. Modi, Experientia *32*, 423 (1976).