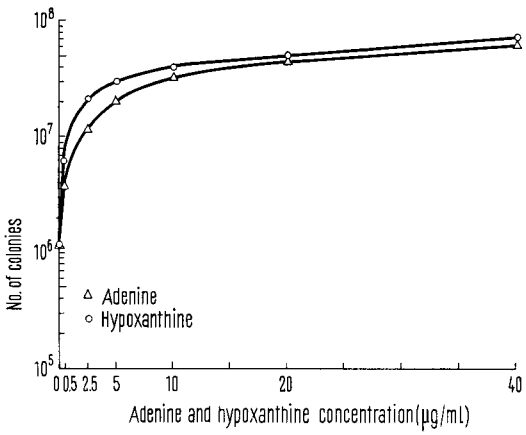
An Adenine-Requiring Mutant of *Azotobacter vinelandii* Blocked in Inosinic Acid Synthesis

Biochemical mutations in *Azotobacter* are of rare occurrence, even with the use of chemical and physical mutagens, and where such mutations have been reported they have been found to be very unstable[1]. The cells of strain *A. vinelandii* were subjected to treatment with NTG (N-methyl-N'-nitro-N-nitrosoguanidine, Aldrich Chemical Co.), 10 µg/ml for $^1/_2$ h at 37 °C, followed by removing the NTG by centrifugation, washing and resuspending the cells in Burk's nitrogen-free medium[2] enriched with purines, pyrimidines, amino acids and B-vitamins, and heating the cells at 45 °C for 20 min (to inhibit any enzymatic recovery that might occur; heating also killed the cells by a factor of 2). The suspension was diluted to 10 times its volume with enriched medium and incubated for 4 h, after which dilution platings were done on enriched medium. The colonies were tested for auxotrophs using velvet replication[3]. 3 mutant strains were isolated in this manner, of which 2 strains had lost the ability to fix nitrogen, these were unstable and reverted back to normal after a number of successive transfers. The third mutant strain designated (Ad-116), requiring adenine, proved to be stable. The growth of this mutant could also be satisfied with hypoxanthine (Figure). Other purines or their intermediate metabolites were unable to support the growth of this mutant. Attempts to transform this culture to parent type (Ad^+) by means of DNA-induced transformation were not successful. The adenine-requiring strain under suboptimal conditions of growth accumulated a metabolite which gave an orange-colored reaction product having a peak absorption at 500 nm with the BRATTON and MARSHALL test[4] for diazotizable amines. The accumulated product also had a non-specific end absorption in the UV-region. The parent culture (wild type) under similar conditions did not accumulate any product identical to the one accumulated by Ad-116. The color reaction and pattern of UV-absorption was identical to that of 5-aminoimidazole ribotide[5]. It seems from the data that this mutant had a genetic block in the conversion of 5-aminoimidazole ribotide to 5-amino-4-imidazolecarboxylic acid ribotide, leading thereby to the accumulation of the former[6].



Growth of adenineless mutant (Ad-116) in the presence of different concentrations of adenine and hypoxanthine after 24 h of incubation at 33 °C on a reciprocating shaker.

Zusammenfassung. Mangelhaft ernährte Mutationen in Azotobakterien sind schwierig zu erlangen; die meisten sind zudem unbeständig. Eine Adenin erheischende Mutation wurde mittels Nitrosoguanidin und Hitzebehandlung gewonnen. Dieser Mutant erwies sich als unbrauchbar für die Snythese der Inosiniksäure.

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Mutagenic Action of Ethylene Halogenhydrins

It has been found that fumigation of spices and other materials with ethylene oxide may lead to the formation of ethylene chlorohydrin[1]. Since the latter substance might possess mutagenic properties, and therefore constitute a public health hazard, we investigated ethylene chlorohydrin (BDH) and such related substances as ethylene bromohydrin (Koch-Light), ethylene iodohydrin (Koch-Light) and ethylene cyanohydrin (Koch-Light).

The fluctuation test[2] was used to find out whether any of the substances being examined are mutagenic. As a test organism we used a mutant of *Klebsiella pneumoniae* requiring for growth uracil and proline. Nutrient broth containing the substance under test was seeded with 100 bacteria/ml, and divided in 105 portions of 3 ml each. After overnight incubation at 37 °C, the total number of streptomycin-resistant and streptomycin-dependent bacteria was determined in 100 portions by a pour-plate technique using nutrient agar supplemented with 100 µg/ml of dihydrostreptomycin. After 3 days incubation at 37 °C, the colonies in the dihydrostreptomycin containing agar were counted. The number of bacteria present in the 5 remaining portions was determined using

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