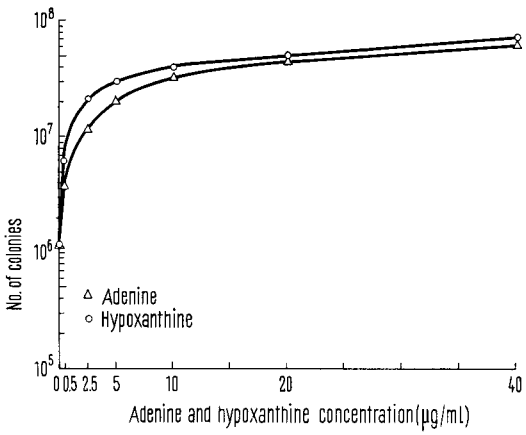
An Adenine-Requiring Mutant of *Azotobacter vinelandii* Blocked in Inosinic Acid Synthesis

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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The mutagenic action of ethylene halogenhydrins to *Klebsiella pneumoniae*

	Compound	Concentration	No. of bacteria/ml after 20 h incubation	Portions without mutants	No. of mutants in median portions	Mutation rate	Increase
I	Ethylene chlorohydrin	0.15 *M*	8.4×10^8	0/100	103	6.23×10^{-9}	× 33
	Ethylene chlorohydrin	0.015 *M*	15.5×10^8	0/100	6	0.401×10^{-9}	× 3.4
	Ethylene chlorohydrin	0.0015 *M*	12.1×10^8	39/100	–	0.179×10^{-9}	–
	Control		15.3×10^8	42/100	–	0.131×10^{-9}	–
II	Ethylene chlorohydrin	0.015 *M*	13.8×10^8	0/100	10	0.648×10^{-9}	× 5.5
	Ethylene bromohydrin	0.015 *M*	8.2×10^8	0/100	272	14.8×10^{-9}	× 125
	Ethylene iodohydrin	0.015 *M*	2.3×10^8	0/100	69	17.5×10^{-9}	× 148
	Ethylene cyanohydrin	0.015 *M*	16.1×10^8	55/100	–	0.086×10^{-9}	–
	Control		18.4×10^8	53/100	–	0.080×10^{-9}	–

nutrient-agar without dihydrostreptomycin. From the number of portions without streptomycin-resistant or dependent mutants the mutation rate was calculated by the Poisson distribution. If in all portions mutants were present, the mutation rate was estimated by the number of mutants in the median portion according to LEA and COULSON[3]. The results of the experiments are shown in the Table.

The increase of the mutation rate was calculated against the average spontaneous mutation rate of the blanks of 14 experiments. It amounts to 0.1183×10^{-9} (95% confidence levels 0.051×10^{-9} to 0.186×10^{-9}).

From the results it appears that ethylene iodohydrin, ethylene bromohydrin and ethylene chlorohydrin are potent mutagenic agents. Probably the carbon halogen bond is involved. If so, the decrease of the bond dissociation energy from the C–I to the C–Cl bond is in accordance with the decrease of mutagenic action from ethylene iodohydrin to ethylene chlorohydrin.

In other experiments we found for the mutation rate induced by coffeine (2 mg/ml): 0.396×10^{-9} (increase × 3.4), and by 5-bromouracil (1 mg/ml): 1.181×10^{-9} (increase × 10).

The results of these experiments demonstrate that the substances investigated have mutagenic properties.

Zusammenfassung. Eine stark mutagene Wirkung wird durch Äthylenchlorhydrin, Äthylenbromhydrin und Äthylenjodhydrin auf *Klebsiella pneumoniae* ausgeübt.

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Cytological Effect of Gonadotropin Releasing Factor Activity from Beef Hypothalamic Extract of the Pituitary Cells in vitro

Accumulated evidence indicates that the hypothalamic-releasing factor stimulates the release of gonadotropin by acting directly at the level of the anterior pituitary gland[1]. The activity of the gonadotropin-releasing factor has been demonstrated by incubating the adenohypophysis in vitro. Extracts of hypothalamus (rat, pig, sheep, beef etc.) added to the medium may act directly on this gland and induce secretion of gonadotropins[2–4].

In the present experiment, fragments of rats anterior pituitary glands were incubated in order to study whether an extract of beef hypothalamus may modify the cytological features of pituitary cells. Preparation of crude (HEc) and semipurified (HEsp) hypothalamic extracts were obtained following the method of SCHALLY et al.[4]. The brain cortical extract (BCE) prepared in the same manner served as control. Incubation of glands was made according to MITTLER's technique[5]. The activity of both extracts in terms of the release of FSH and LH from hypophysis was evaluated in castrated testosterone pretreated male rats[6,7] and in castrated, estrogen and progesterone pretreated female rats[8,9]. For cytological studies the anterior pituitary glands of normal adult male Wistar rats (200–250 g) were used; the incubation procedure is detailed in the Table. Brain cortical extract, saline, medium TC 199 and vasopressin were assayed as controls. The hypophysis fragments were fixed in formol-Cl_2Hg, dehydrated and embedded in paraffin. Sections were stained with PAS-colloidal-iron-Kernechtrot and aldehyde-thionin to differentiate the types of basophilic cells.

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