The preparations were then placed in Coplin jars containing paraformaldehyde powder equilibrated with air at 50% relative humidity. The jars were sealed and the preparations exposed to formaldehyde gas at 80 °C for 1 h. Prior to inspections under the fluorescence microscope, the preparations were mounted with non-fluorescent immersion oil.

Results and discussion. Small fluorescent cells were made visible along the whole mounted stretch preparations of the atrioventricular valves of the opossum, prepared according to the Falck-Hillarp method for biogenic amines (figure 1).

These cells were very numerous and exhibit intense yellow-green fluorescence. The nuclei were not visible, and groups of cells were invariably permeated by the dense networks of adrenergic nerves (figure 2). The endings are varicose and similar to those described by others investigators[4,8,11] (figure 3).

Several workers[7–9,12] have used the same technique to visualize monoamine-containing structures in the mammalian heart, and some have reported the presence of fluorescent cells in the atria.

Although it is agreed that the fluorescent reaction product in these cells indicates the presence of a biogenic amine, the precise nature of this substance is controvertial. The study of amine containing cells in several species revealed that they might participate in modulation of ganglionic transmission, since they are in the vicinity of intracardiac parasympathetic ganglia[9]. Ehinger et al.[8] found no evidence of any functional relationship between these cells and the intracardiac ganglia. They are disposed in different topographical localizations within the heart, and it has been demonstrated in different animals that during the phylogeny these cardiac amine-containing cells were more concentrated in the atria. They are thought to function in an adrenergic excitatory control of the heart, at least in lower forms[13]. Using the same histochemical technique, amine-containing cells also have been demonstrated in mammals and in human fetal heart[8,9,13,14]. The literature contains no definitive account of functions of the mammalian amine-containing cells. The cells observed by us showed the characteristics of the small intensely fluorescent (SIF) cells similar to those first described in the sympathetic ganglia[15,16]. These numerous cells suggest humoral rather than neural adrenergic control of the opossum heart.

The well developed neural apparatus and the amine-containing cells found in cusps of atrioventricular valves of the opossum would suggest that, under neural control, both mitral and tricuspid valves are areas representing zones for amine liberation from these cells that might directly affect the receptors localizated in carotic sinus, and a possible mechanism of discharge of afferent stimulations.

11 A. L. Ferreira, J. C. M. Santos and A. M. Rossi, Experientia *31*, 82 (1975).
12 E. M. Krokhina, Acta Anat. *74*, 214 (1969).
13 B. J. Gannon and G. D. Cambell, Z. Zellforsch. *131*, 437 (1972).
14 E. T. Angelakos, Ann. N. Y. Acad. Sci. *156*, 219 (1969).
15 S. Partanen and O. Korkala, Experientia *30*, 708 (1971).
16 O. Eränko, L. Eränko, Prog. Brain Res. *34*, 39 (1971).

Action of alkylating agents on nitrogen fixation by clones of Anabaena doliolum Bharadwaja

V. K. Sharma

Department of Botany, School of Basic Sciences, University of Udaipur, Udaipur-313001 (India),
4 December 1975

Summary. The physiological and mutagenic actions of three alkylating agents have been studied on the process of nitrogen fixation by *Anabaena doliolum* Bharadwaja. The results show that these chemicals presumably switch off the activity of nitrogenase, resulting in the inhibition of fixation.

Few studies[1–6] have been conducted so far on the effects of some alkylating agents on certain blue-green algae. But no reference, except that of Sharma and Kumar[7], is available regarding the physiological and mutagenic actions of these chemicals on the process of nitrogen fixation in a heterocystous species. The present investigation therefore is an attempt in this direction.

The organism chosen was *Anabaena doliolum* Bharadwaja, belonging to the family Nostocaceae of Cyanophyta. Many samples of this species were collected from various rice fields and the clones were raised from spores. Only 2 clones, No. 1 and 4, were selected on the basis of their maximum and minimum nitrogen-fixing rates under identical conditions. 3 well-known and potent mutagenic agents, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), diethyl sulphate (DES) and ethyl methane sulphonate (EMS), were chosen. These have been shown to induce gene mutations, deletions and several types of chromosomal aberrations in various plants[8].

A fresh stock solution of MNNG was prepared in dark by dissolving it in phosphate-citrate buffer of pH 5.4, as the mutagenic activity of this chemical is lost upon exposure to light[9]. The concentration used was 500 µg MNNG/ml. The treatment was given for 1 h at an interval of 20 min each. The treated cells were washed thoroughly with sterilized glass-distilled water. 1 ml suspension of each treated clone was then inoculated into culture tubes, containing 20 ml nitrate-free medium of Allen and Arnon[10]. The tubes were incubated under flourescent light intensity of 1.1×10^4 ergs/cm^2/sec and at a temperature of 28 ± 2 °C. Nitrogen contents of the replicate samples were determined after a growth period of 30 days by the method mentioned earlier[11].

1 H. D. Kumar, Can. J. Bot. *43*, 1523 (1965).
2 H. D. Kumar, Beitr. Biol. Pflanz. *45*, 161 (1968).
3 H. D. Kumar, Arch. Mikrobiol. *63*, 95 (1968).
4 R. S. Gupta and H. D. Kumar, Arch. Mikrobiol. *70*, 313 (1970).
5 R. S. Gupta and H. D. Kumar, Beitr. Biol. Pflanz. *46*, 389 (1970).
6 B. D. Sinha and H. D. Kumar, Ann. Bot. *37*, 673 (1973).
7 V. K. Sharma and H. D. Kumar, Z. allg. Mikrobiol. *13*, 517 (1973).
8 C. Auerbach, Science *158*, 1141 (1967).
9 D. R. McCalla, Ann. Protozool. *14*, 480 (1967).
10 M. B. Allen and D. I. Arnon, Pl. Physiol. *30*, 366 (1955).
11 V. K. Sharma and H. D. Kumar, Experientia *31*, 182 (1975).

For DES and EMS treatments, the cells were suspended in *tris*-maleic buffer of pH 7.0 and treated up to a duration of 1 and 4 h respectively; 0.01 ml DES or 0.1 ml EMS was added per 5 ml cell suspension. Other procedural details and the culture conditions remained the same as mentioned above. Nitrogen contents were estimated after 20 and 30 days following treatment with DES and EMS respectively.

These alkylating agents were found to inhibit the process of fixation in both the clones, presumably by switching off of the activity of enzyme nitrogenase. The effect of EMS on fixation was more lethal in comparison to other agents as it inhibited the process altogether. These chemicals altered the permeability of cell membranes, making them almost impermeable to the excretion of nitrogenous substances into the culture filtrates. The percent inhibition by the 3 chemicals was found to be dependent on duration of the treatment and is shown in the Table. However, their exact mode of action on nitrogen fixation is not clearly known.

MNNG is capable of inducing base-pair transitions[12] and frame-shift mutations, besides inducing gross chromosomal aberrations in higher organisms[13]. However, its mutagenic damage shows a high rate of repair. MNNG has been found to inhibit both DNA and ribosomal RNA synthesis in microbial cells at pH 5.5[14]. It is stable at this pH and reacts solely as a mutagenic agent.

Percent inhibition of nitrogen fixation in clones of *A. doliolum* treated with MNNG, DES and EMS

Clone	Treatment time (min)	MNNG	DES	Treatment time (h)	EMS
No. 1				1	78.71
	20	23.21	19.61	2	81.49
	40	39.81	24.08	3	99.19
	60	65.66	42.86	4	100.00
No. 4				1	75.54
	20	25.03	24.16	2	100.00
	40	32.53	26.79	3	100.00
	60	62.50	27.46	4	100.00

SHARMA and KUMAR[7] observed the inhibition of growth and nitrogen fixation in several clones of *Cylindrospermum majus* after treatment with 50 µg MNNG per ml. No mutagenic activity of MNNG was observed even at relatively a high concentration, though it induced certain reversible morphological variations including slight loss of photosynthetic pigments and excessive enlargement of cells into balloon-like structures. These changes were prominent in the beginning up to a period of 10–15 days following MNNG treatment, but disappeared gradually even without sub-culturing the clonal population.

Since GUPTA and KUMAR[4–5] were able to secure mutant strains of *Anacystis nidulans* after treatment with DES both in acidic and alkaline range, it was considered desirable to treat the selected clones of *A. doliolum* at pH 7.0. KUMAR[1] studied the effects of DES on *A. nidulans* and reported this mutagen to be strongly inhibitory to growth. The data presented here show its inhibitory action on nitrogen fixation too.

EMS has been shown to be a highly active mutagen in maize[15], *Drosophila*[16], *Neurospora*[17], bacteria[18], and phage[19]. In addition to causing chromosome breaks, it causes base substitutions in DNA leading to point mutations. KUMAR[2] studied the effects of EMS on *A. nidulans* and observed that it was more toxic in alkaline buffers than in acidic ones. The results reported here indicate that EMS treatment at pH 7.0 inhibited nitrogen fixation but did not induce mutation of any kind in clonal cells.

[12] H. V. MALLING and F. J. DE SERRES, Genetics *60*, 201 (1968).
[13] T. GICHNER, A. MICHAELIS and R. RIEGER, Biochem. biophys. Res. Commun. *11*, 120 (1963).
[14] E. CERDÁ-OLMEDO and P. C. HANAWALT, Biochim. biophys. Acta *142*, 450 (1967).
[15] E. AMANO and H. H. SMITH, Mutation Res. *2*, 344 (1965).
[16] O. G. FAHMY and M. J. FAHMY, Nature, Lond. *184*, 1927 (1959).
[17] M. WESTERGAARD, Experientia *13*, 224 (1957).
[18] A. LOVELESS and S. HOWARTH, Nature, Lond. *184*, 1780 (1959).
[19] A. LOVELESS, Nature, Lond. *181*, 1212 (1958).

Peroxidase activity in mitochondria of Prototheca moriformis

Luisa De Vecchi and Stefania Pellegrini

Istituto di Scienze Botaniche, Università degli Studi, via G. Colombo 60, I-20133 Milano (Italy), 27 July 1976

Summary. Peroxidase activity was investigated by the use of diaminobenzidine method in fixed cells of Prototheca moriformis. A strong peroxidase activity was observed in the mitochondria. DAB staining was unaffected by KCN, aminotriazole and antimycin A, but it was completely inhibited by methanol-nitroferricyanide.

During our cytochemical studies[1], aiming to detect cytochrome oxidase by means of the cytochemical reaction with 3–3′ diaminobenzidine (DAB), we found preliminary evidence that the mitochondria showed also a peroxidase activity in Prototheca, Chlorella and in its yellow mutant CM 20.

Here we present the results of a study in which the characterization of this peroxidase activity – as shown by DAB reaction in the presence of different inhibitors – was undertaken in Prototheca.

Materials and methods. Prototheca moriformis was supplied by the Culture Collection of Algae and Protozoa of Cambridge University and grown on a glucose containing medium[2] for 5 days at 25 °C. The fixation and embedding methods were the same as described in a previous paper[1]. DAB reaction was performed as described for cytochrome c peroxidase staining in baker's yeast[3]. The incubation

1 S. Pellegrini and L. De Vecchi, J. submicrosc. Cytol. *8*, 353 (1976).
2 S. A. Waksman, in: The actinomycetes, p. 193. Ed. Waltham. The Chronica Botanica Co., Mass. 1950.
3 M. M. Todd and E. L. Vigil, J. Histochem. Cytochem. *20*, 244 (1974).