tion similar to that obtained with intercellular fluid. Tests with 15 additional isolates of six tabtoxin-producing pathovars of *P. syringae* gave the same result.

The effect of Zn is rapidly evident. If it is added to a 3-day-old culture of pv. *tabaci,* all the tabtoxin present is converted to tabtoxinine-β-lactam within 1 h. If however the culture is maintained at 4 °C for 2 h after the Zn addition, there is only a 66% conversion. These effects strongly suggest that the Zn effect is associated with enzymatic activity in the bacterium.

This supposition was confirmed when a hydrolase for tabtoxin, located in the periplasmic space, was shown to require Zn (personal communication, C. Levi). Woolley's medium contains Zn only as a contaminant, and during bacterial growth the pH rises, commonly to eight or more, under which conditions Zn rapidly becomes unavailable. Thus, there normally is not sufficient Zn in the culture to satisfy the hydrolase's requirement, however the addition of either intercellular fluid or Zn will satisfy it. There is though sufficient Zn in the plant, because the extracted intercellular fluid is more dilute than that found *in planta,* and it has an acidic reaction (pH 6.1) which favors Zn solubility. In support of this, the bacterial isolates responding to Zn were found to produce only tabtoxinine-β-lactam in infected plants[5].

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Growth of *Nostoc muscorum* mutants in the presence of diuron (DCMU) and L-methionine-DL-sulfoximine[1]

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Summary. Diuron (DCMU) is inhibitory to the photoautotrophic and photoheterotrophic growth of the N_2-fixing blue-green alga *Nostoc muscorum* at concentrations of 1.0×10^{-5} M and 2.0×10^{-5} M, respectively. A mutant of this organism resistant to 5.0×10^{-5} M DCMU under its photoheterotrophic growth conditions, with the ability to utilize DCMU as a carbon and nitrogen source for growth, and complete inability to grow photoautotrophically has been isolated. With the apparent defect in its photosynthetic ability, it is suggested that the *DCMU*^{r} mutant lacks the step inhibited by 1.0×10^{-5} M DCMU, and metabolizes DCMU by an existing enzyme system in the absence of such inhibition. That this enzyme may be glutamine synthetase (GS) is explained with the help of a L-methionine-DL-sulfoximine (MSO)-resistant mutant of *N. muscorum* which is able to grow faster with 2.0×10^{-5} M DCMU and is known to contain an altered GS.

Key words. Blue-green algae; *Nostoc muscorum;* diuron; L-methionine-DL-sulfoximine; photoautotrophic growth; photoheterotrophic growth.

Diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), is known to cause the abolition of photochemically generated reducing power ($NADPH_2$), thus blocking CO_2 assimilation without causing any adverse effect on the generation of ATP through cyclic photophosphorylation in oxygenic photosynthetic organisms[2]. Accordingly, while obligate photoautotrophs fail to recover for DCMU inhibition of growth in the presence of an organic carbon supplement, heterotrophs, including the blue-green algae *Anacystis nidulans*[3] and *Nostoc muscorum*[4], show rapid recovery from DCMU inhibition of growth under similar conditions. DCMU contains methylurea groups, and recent genetic studies have shown that organic sources like methylamine (an inhibitor of photophosphorylation in *Nostoc muscorum*)[5] is utilized by the methylamine-resistant mutant of *N. muscorum* like a metabolizable source of carbon[6] and nitrogen[7]. Therefore, in the present investigation an attempt was made to isolate a diuron-resistant (*DCMU*^{r}) mutant of this heterotroph and to study the metabolic fate of DCMU in such a mutant. Studies on the apparent metabolic utilization of DCMU by the *DCMU*^{r} strain of *N. muscorum* have been helped by the use of a previously-isolated *MSO*^{r} mutant of this organism[8].

Methods. Organisms. The axenic clonal cultures of the parent *Nostoc muscorum* and its *MSO*^{r} mutant strain, obtained from Professor H.N. Singh's personal culture collections, Central University of Hyderabad, India, were grown routinely in modified Chu 10 medium[9] under the culture conditions described previously[8]. While N_2 (nitrogen-free, i.e., at the expense of elemental nitrogen)-growing cultures of these two strains form 5–6 and 12–13% heterocysts, respectively, NH_4^+-growing similar cultures remain non-herocystous (heterocysts are specialized cells which are non-photosynthetic, but are sites of elemental nitrogen fixation)[4].

Physiological assessments. The NH_4^+-growing non-heterocystous parent organism was harvested, washed and pre-suspended in N_2 medium to an OD of 2.5. 2 ml aliquots were then inoculated into fresh sterile N_2 or 1 mM NH_4^+ medium containing or lacking 3 mM glucose, at different concentrations (0 or 1.0 to 2.0×10^{-5} M) of DCMU. All samples were incubated in the growth chamber and their growth (by optical density determinations) on the 10th day, and heterocyst frequency (by microscopic examination) on the 3rd day were assessed. Phycocyanin content of the samples was estimated on the 3rd day of inoculation by the method used ealier[10].

Mutant isolation and characterization. A mutant of the parent *Nostoc muscorum* resistant to 5.0×10^{-5} M DCMU was sought by plating a heavy suspension of the parental culture on solid (1.2% difco bacto agar) N_2 medium containing 5.0×10^{-5} M DCMU, and incubating the set for two weeks in the growth chamber, according to the general method of mutagenesis studies used in *N. muscorum*[11]. A dozen putative *DCMU*^{r} colonies, appearing after a fortnight's incubation, were raised separately into clonal cultures and examined for their growth in N_2 or 1 mM NH_4^+ medium containing or lacking 3 mM glucose, 5.5×10^{-5} M $NADPH_2$ or 5.0×10^{-5} M DCMU. The newly-isolated *DCMU*^{r} and the previously-isolated *MSO*^{r}

strains of *Nostoc muscorum* were tested for their cross-resistance to MSO and DCMU, respectively, in N_2 or 1 mM NH_4^+ medium. Effects of graded concentrations of DCMU were also examined on the heterocyst-forming capacity and phycocyanin content of the *DCMU*^{r} and *MSO*^{r} strains.

Chemicals. Glucose and $NADPH_2$ (obtained from Sigma Chemical Co., USA), and DCMU (gift of Lakshami Chemical Co., India) were used after filter-sterilization. All other chemicals were obtained from the British Drug House, Poole, Dorset, England.

Results. 1.0×10^{-5} M DCMU completely inhibited heterocyst formation in parent *Nostoc muscorum* in N_2 medium (data not shown), along with the inhibition of its photoautotrophic growth in both N_2 and NH_4^+ media (table 1). These effects were reversed on the addition of 3 mM glucose (table 1). Concurrent with the growth, the phycocyanin content of the parent alga also went on decreasing with the increasing concentrations of DCMU in both N_2 and NH_4^+ media (data not shown). At a concentration of 2.0×10^{-5} M and above, DCMU caused a lysis of the algal filaments growing photoautotrophically or photoheterotrophically in both N_2 and NH_4^+ media (table 1). Unlike parent *N. muscorum*, the *DCMU*^{r} mutant failed to grow photoautotrophically but like the parent it showed a significant photoheterotrophic growth when supplied with 3 mM glucose, 5.5×10^{-5} M $NADPH_2$ or 5.0×10^{-5} M DCMU in both N_2 and NH_4^+ media. DCMU supported the best growth, followed by glucose and $NADPH_2$ in that order (data not shown). Table 2 shows that there was a significant growth of the *DCMU*^{r} mutant with $1.5–5.0 \times 10^{-5}$M DCMU, concomitantly increasing with the increasing concentrations of DCMU above 1.0×10^{-5} M. Also, unlike in the parent *N. muscorum* (table 1), there was a significantly better growth of the *MSO*^{r} mutant with $1.5–2.0 \times 10^{-5}$ M DCMU (table 2). At these concentrations DCMU suppressed the formation of heterocysts and supported an increase in the phycocyanin-yield in both *DCMU*^{r} and *MSO*^{r} mutants (data not shown). In contrast to the parent alga, which is sensitive to concentrations of MSO above 3.70×10^{-5} M, the *DCMU*^{r} mutant had a cross-resistance up to 7.50×10^{-5} M MSO (table 2).

Discussion. Nostoc muscorum is a photoheterotroph[4] and is known to photoassimilate glucose and amino acids[12]. In view of the present results, it can be emphasized that the glucose-reversible DCMU inhibitory action in *N. muscorum* is due primarily to a poor supply of the photochemically generated reducing power during PS II, necessary for CO_2 photoassimilation in blue-green algae[2]. However, the lytic effects of DCMU on the parent organism, at a concentration of 2.0×10^{-5} M and above, may not be conclusively explained at the moment.

The strict photoheterotrophic growth characteristics of the *DCMU*^{r} mutant strongly suggest that this strain has lost the capacity to utilize CO_2 photosynthetically, and that glucose, $NADPH_2$ and DCMU serve as a readily-assimilable source of carbon and/or reductant for its only possible mode of photoheterotrophic growth and differentiation. That DCMU is also serving as a nitrogen source in this mutant is clear in view of the significant increase in the phycocyanin content of the *DCMU*^{r} and *MSO*^{r} mutants resulting from the application of DCMU (at a concentration not less than 1.5×10^{-5} M) under both N_2- and NH_4^+-mediated conditions, in a manner remarkably similar to those of the combined inorganic nitrogen sources[13,14]. This is further strengthened by the fact that at these concentrations DCMU led to a complete suppression of heterocyst formation in the two mutants, like the inorganic nitrogen sources[11]. It is important to mention at this point that although DCMU inhibited heterocyst formation in the parental organism as well, it was essentially not due to the metabolic utilization of DCMU as a nitrogen source; it was apparently fatal as a result of the inhibition of the photosynthetic assimilation of inorganic carbon[4].

Table 1. Data* on photoautotrophic and photoheterotrophic growth** of parent *Nostoc muscorum* in variously diuron (DCMU)-supplemented N_2 or 1 mM NH_4^+ medium ± 3 mM glucose

Nitrogen source	DCMU concentration ($n \times 10^{-5}$ M)	Growth Photoautotrophic (− glucose)	Photoheterotrophic (+ glucose)
N_2	0.0	0.635 ± 0.010	0.413 ± 0.016
N_2	0.5	0.301 ± 0.025	0.408 ± 0.014
N_2	1.0	0.0	0.375 ± 0.012
N_2	1.5	0.0	0.304 ± 0.017
N_2	2.0	0.0	0.0
NH_4^+	0.0	0.750 ± 0.013	0.514 ± 0.014
NH_4^+	0.5	0.330 ± 0.021	0.410 ± 0.033
NH_4^+	1.0	0.0	0.403 ± 0.026
NH_4^+	1.5	0.0	0.304 ± 0.018
NH_4^+	2.0	0.0	0.0

*The values are the means of five independent readings with their respective SE. **Increase in optical density at 663 nm on the 10th day of inoculation; initial OD in all cases was 0.005; decrease in OD is given as 0.0; Similar photoheterotrophic growth-yield was observed in the *DCMU*^{r} mutant growing with 3 mM glucose, 5.5×10^{-5} M $NADPH_2$ or 5.0×10^{-5} M DCMU; *DCMU*^{r} mutant could not grow photoautotrophically.

Table 2. Data* on growth** of the *DCMU*^{r} and *MSO*^{r} mutant strains of *Nostoc muscorum* in N_2 or 1 mM NH_4^+ medium, supplemented with graded concentrations of DCMU and/or MSO

Concentration of (a) DCMU and/or (b) MSO ($n \times 10^{-5}$M)	Growth *DCMU*^{r} N_2	NH_4^+	*MSO*^{r} N_2	NH_4^+
Nil	0.0	0.0	0.475 ± 0.013	0.485 ± 0.026
(a) 1.0	0.0	0.0	0.475 ± 0.024	0.486 ± 0.017
(a) 1.5	0.261 ± 0.017	0.284 ± 0.013	0.572 ± 0.031	0.592 ± 0.011
(a) 2.0	0.286 ± 0.015	0.314 ± 0.017	0.590 ± 0.016	0.598 ± 0.015
(a) 3.0	0.315 ± 0.022	0.341 ± 0.012	0.0	0.0
(a) 4.0	0.326 ± 0.016	0.343 ± 0.015	0.0	0.0
(a) 5.0	0.328 ± 0.014	0.345 ± 0.037	0.0	0.0
(b) 3.70	0.0	0.0	0.476 ± 0.014	0.484 ± 0.016
(b) 5.00	0.0	0.0	0.476 ± 0.022	0.485 ± 0.017
(b) 37.00	0.0	0.0	0.476 ± 0.013	0.484 ± 0.019
(a) 5.0 + (b) 3.70	0.305 ± 0.018	0.337 ± 0.015	0.0	0.0
(a) 5.0 + (b) 5.00	0.265 ± 0.016	0.295 ± 0.013	0.0	0.0
(a) 5.0 + (b) 7.50	0.182 ± 0.017	0.202 ± 0.016	0.0	0.0
(a) 5.0 + (b) 10.0	0.0	0.0	0.0	0.0

*The values are the means of five independent readings with their respective SE. **Increase in optical density at 663 nm on 10th day of inoculation; decrease in OD is given as 0.0; initial OD in all cases was 0.005; parent *Nostoc muscorum* was sensitive to concentrations of MSO above 3.70×10^{-5} M.

Naturally, therefore, the metabolic uptake of DCMU is an outcome of a mutational event. The ability of the $DCMU^r$ mutant to metabolize up to 5.0×10^{-5} M DCMU might be responsible for relieving the organism from DCMU-induced cellular lysis, because the mutant obviously appears to have acquired resistance to both forms of DCMU-induced inhibition, i.e., photosynthetic inhibition and cellular lysis. It has probably lost the ability to utilize light energy, leading to the disappearence of the site of inhibition by 1.0×10^{-5} M DCMU. It is, therefore, unlikely that the same mutation can have conferred the ability to synthesize a novel DCMU-metabolizing enzyme system. A possible explanation is that parent *Nostoc muscorum* and the $DCMU^r$ mutant derived from it can both form enzymes capable of metabolizing DCMU, but DCMU can be utilized only when its intracellular level rises above the level which inhibits the photosynthetic assimilation of CO_2. This suggestion is supported by the finding that while DCMU was toxic to the parent organism at a concentration of 1.0×10^{-5} M, it was metabolized by the $DCMU^r$ and MSO^r mutant strains only at concentrations above 1.0×10^{-5} M. Nevertheless, as a readily-metabolizable nitrogen source (as discussed above), DCMU suppressed the formation of heterocysts and supported the increased phycocyanin-yield in both $DCMU^r$ and MSO^r mutants at concentrations strictly above 1.0×10^{-5} M.

MSO is a well known inhibitor of GS activity in blue-green algae[15]. It has been found that a mutation conferring resistance to 3.70×10^{-4} M MSO is associated with an altered GS having higher resistance to MSO inhibition in comparison to the enzyme from the MSO-sensitive parent *N. muscorum*[8]. The present findings that the MSO^r mutant showed a certain degree of cross-resistance for DCMU and vice-versa, suggest that possibly a common site is involved in interactions with DCMU and MSO. The reason why 2.0×10^{-5} M DCMU did not inhibit the growth of the MSO^r strain (as it did in the MSO-sensitive parental strain), may be that a mutation leading to an altered GS (having a higher resistance to MSO) might have been associated with an increased affinity of GS for DCMU. Such an increased affinity leading to the utilization of low concentrations of DCMU might have resulted in the MSO^r mutant having a lower intracellular concentration of DCMU than the parent organism (when the external concentration of DCMU was 1.0×10^{-5} M).

In *Nostoc muscorum* there have been indications of the possible involvement of GS in the metabolism of substrate analogues like methylamine, as a carbon and nitrogen source[10]. It has also been worked out that GS from bacterial[16-19] and eukaryotic[20,21] cells can metabolize methylamine (like ammonia) to some extent. This is further confirmed through radioactive tracer studies[22,23]. It is, therefore, strongly believed that the $DCMU^r$ mutant of *N. muscorum*, as a result of a defect in the photosynthetic mechanism, is capable of utilizing DCMU (containing a methylurea group) as a carbon and nitrogen source (like methylamine) by virtue of an existing enzyme system which may possibly be GS.

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A new simple temperature-controlled membrane oxygenator for the perfusion of isolated rat livers

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Summary. A new temperature-controlled membrane oxygenator for perfusing isolated rat livers was assembled using a combination of heat-exchangeable rubber tubing and silicon rubber tubing. The apparatus supplied enough oxygen to satisfy the requirements of hemoglobin-free perfused livers.
Key words. Membrane oxygenator; perfusion of rat livers.

In an organ-perfusion system, the oxygenator is an essential component. There are two principal types of oxygenators. In one type, the perfusate is oxygenated at large fluid-gas interfaces[2-4], and in the second, oxygen is transferred by diffusion through a membrane and dispersion within the flowing perfusion medium[5-8]. The latter type of oxygenator has some advantages over the former[6,8].

Most perfusion systems employ a thermostatically regulated cabinet with an oxygenator in it. Some research workers[5,9], however, have used a temperature-controlled membrane oxy-