ences between the megakaryopoietic pattern of MC-treated femurs and that of the controls; some megakaryocytes were detectable, but they were not in any particular arrangement; they were not grouped, but distributed singly throughout the marrow.

The histological examination of the liver showed some FC aggregates, but in our model we could not demonstrate hepatic hemopoiesis in any group.

We measured the cellular and nuclear area of megakaryocytes from both experimental groups by an electronic planimetric system (Videoplan, Kontron. At least 50 cells evaluated for each day and each group). We could not demonstrate any difference at various times between the MC-treated and control groups. Moreover there was no difference in nuclear lobulation number. Taken together, all these data do not allowed us to postulate any difference in megakaryocyte ploidy, at the different times, between the experimental groups.

Discussion. Our study, performed using the ESC technique, demonstrated on day 10 a statistically significant increase in ESC number in the MC-treated group, which is in line with observations in a preceding study[7], carried out by the exogenous CFU-S technique.

As a matter of fact, data are available in the literature suggesting that functional conditions of RES may affect the hemopoietic process. Blockade of RES by administration of carbon particles[11] or polystyrene latex particles[12] before sublethal irradiation in mice, as well as RES stimulation by bacterial endotoxins[13], have been shown to increase the number of ESC. In carbon-treated mice this effect seems to depend on some humoral activity present in the serum[14].

The large increase in splenic megakaryocyte production in MC-treated mice should be accounted for a splenic and reticulo-endothelial trapping of platelets, but this hypothesis is in contrast to the observation in bone marrow, where we could not demonstrate an increase in megakaryocytes in the MC-treated group. Ebbe reported furthermore that chronic thrombocytopenia at the time of irradiation appears to increase the radiosensitivity of thrombopoietin-responsive cells[15]. On the other hand, Crandall and Boggs observed in MC-treated irradiated mice that megakaryopoietic foci are more resistant than the erythroid ones[16].

We want to emphasize that in the femural bone marrow of MC-treated mice we observed a small number of FC in comparison to the large number detectable in the spleen. The constant topographical relationship between FC groups and megakaryocytic clones observed in the spleen suggests the possible role of macrophages, activated by phagocytosis, in the control of megakaryopoiesis at a microenvironmental level. Recently it was observed that monocyte-macrophage lineage cells elaborate an activity enhancing murine megakaryopoiesis in vitro[17], and that could support our hypothesis.

Table 2. Number of megakaryocytic clones per section and megakaryocytes per clone evaluated on day 10. Two midline sections for every spleen were completely scored. C = Control group. MC = Methylcellulose treated group. *$p < 0.01$. Original data were transformed: $X_t = \sqrt{X + 0.5}$ for the analysis of variance.

Group (n of mice)	C (9)	MC (9)
N of meg. clones mean ± SE	6.5 ± 2.03	31 ± 6.7*
N of meg. per clone mean ± SE	4.6 ± 0.9	5.31 ± 0.57

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2 Divisione Ematologia.
3 Istituto Anatomia Patologica.
4 Cattedra Ematologia.
5 Istituto Radiologia.
6 Palmer, J.G., Eichwald, E.J., Cartwright, G.E., and Wintrobe, M.M., Blood *8* (1953) 72.
7 Stang, H.D., and Boggs, D.R., Am. J. Physiol. *233* (1977) H234.
8 Teom, T.B., J. Path. Bact. *81* (1961) 33.
9 Pfirmer, W., Joice, R.A., Turner, R., and Boggs, D.R., Blood *52* (1978) 610.
10 Karnovky, M.J., and Roots, L., J. Histochem. Cytochem. *12* (1964) 219.
11 Mori, K.J., Radiat. Res. *56* (1973) 494.
12 Mori, K.J., Seto, A., and Ito, Y., Experientia *31* (1975) 112.
13 Smith, W.W., Brecher, G., Budd, R.A., and Fred, S., Radiat. Res. *27* (1966) 610.
14 Mori, K.J., Nakamura, S., Seto, A., and Ito, Y., Radiat. Res. *18* (1977) 225.
15 Ebbe, S., and Stohlman, F., Jr, Blood *35* (1970) 783.
16 Crandall, T.L., and Boggs, D.R., Exp. Hemat. *8* (1980) 25.
17 Williams,. N., Jackson, H., Ralph, P., and Nakoins, I., Blood *57* (1981) 157.

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Chemical control of eucaryotic and blue-green algae in anaerobic photoreactors culturing Rhodospirillaceae

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Summary. To control the growth of eurocaryotic and blue-green algae in anaerobic reactors of photosynthetically grown Rhodospirillaceae, the effect of algae inhibitors with different modes of action was examined. Tests were performed with mixed populations of green algae and blue-green algae, besides strains of the purple nonsulphur bacteria *Rhodopseudomonas capsulata, Rhodospirillum rubrum* and *Rhodomicrobium vannielii.* Chloroxuron, a urea-derivative, was found to inhibit completely growth of green and blue-green algae at 5 ppm. When it was applied to the Rhodospirillaceae cultures, growth was not reduced and nitrogenase activity was not inhibited.

Key words. Algae, green; algae, blue-green; Rhodospirillaceae; algae inhibitors; algae control.

Since hydrogen production was observed in *Rhodospirillum rubrum* by Gest and Kamer[1,2] the hydrogen evolving photosynthetic bacteria have been studied in detail, as hydrogen is considered a promising fuel for the future. Many strains belonging to the Rhodospirillaceae convert different organic acids into hydrogen with high efficiency using light energy[3–5]. Recently, applied studies aimed at producing hydrogen over longer periods in outdoor conditions were reported[6–9]. The

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maximum life-span of continuous photosynthetic reactors producing hydrogen is about one month. One of the problems is the growth of oxygen-producing algae in the reactor. Despite slow growth rates, they develop in the reactor liquid, and also adhere firmly to the reactor walls. Van Niel[10] suggested the use of short IR illumination (800–1000 nm) as source of radiant energy to eliminate algal growth. As photoreactors are expected to use sunlight, one has to filter the shorter sun radiance. A more elegant method is the use of growth inhibitors. The product must control growth of eucaryotic and blue-green algae, but should not affect growth nor nitrogenase activity in photosynthetic heterotrophic bacteria. For this purpose four growth regulators with different modes of action were examined: copper sulfate, cycloheximide, nystatin and chloroxuron.

Materials and methods. Rhodospirillaceae. *Rhodopseudomonas capsulata* ATCC 23782, *Rhodopseudomonas capsulata* ST 407 (obtained from Judy D. Wall, Univ. of Missouri, Biochem. Department, Columbia, USA), *Rhodospirillum rubrum* ATCC 11170 and *Rhodomicrobium vannielii* ATCC 17100 were grown axenically in 40-ml glass tubes of 1.50 cm internal diameter. The tubes were placed in a glass anaerobic jar providing complete anaerobiosis. The growth media contained (per l of water) 4.0 g malic acid, 1.3 g sodium glutamate, 0.6 g KH_2PO_4, 0.9 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.05 g $CaCl_2 \cdot 2H_2O$, 0.02 g EDTA, 0.02 g $FeSO_4 \cdot 7H_2O$, 0.1 g $MnCl_2 \cdot 4H_2O$, 10 mg yeast extract, 1 ml of the trace element solution and 1 ml of a vitamin solution[5,11]. The latter solution contained (per l of water) 1 g nicotinic acid, 15 mg biotin and 1 g thiamine HCl. The pH of the growth medium was adjusted to 6.9 with NaOH.

Green and blue-green algae. Algae were grown non-axenically in 20-ml glass test tubes containing 10 ml inoculated growth medium. Two distinct media were used. The medium A contained (per l of water) 100 mg KNO_3, 100 mg $Ca(NO_3)_2$, 40 mg K_2HPO_4, 30 mg $MgSO_4 \cdot 7H_2O$, 0.5 mg $FeCl_3 \cdot 6H_2O$, 10 ml soil extract (10 g soil in 100 ml physiological solution, shake 24 h, filter and sterilize at 120 °C, for 20 min). Medium B contained (per l of water) 25 mg $CaCl_2 \cdot 2H_2O$, 75 mg $MgSO_4 \cdot 7H_2O$, 75 mg K_2HPO_4, 18 mg KH_2PO_4, 25 mg NaCl, 5 mg $FeCl_3 \cdot 6H_2O$ and 1 ml of the trace element solution of Segers and coworkers[11]. The inoculum of green algae added to medium A contained a mixed culture with dominance of two types of Chlorophyceae: a flagellated Chlamydomonas and a nonflagellated Chlorella species. The inoculum of blue-green algae added to medium B was also a mixed culture and dominated by heterocyst containing filamentous species.

General culture conditions. For all cultures, the light intensity (Sylvania Gro-Lux Fluorescent lamps F40/T12, equally supplemented with incandescent lamps) was about 150 $\mu E \cdot m^{-2} \cdot sec^{-1}$ at the surface of the culture vessels. The temperature was 30 °C.

Assay for selective inhibitors. The following products were examined for growth regulation: the inorganic chemical $CuSO_4 \cdot 5H_2O$, the antibiotics nystatin and cycloheximide (Janssen Chimica, Beerse, Belgium), and the pesticide chloroxuron (IUPAC name 3-[4-(4-chlorophenoxy)phenyl]-1.1-dimethylurea; trade mark Tenoran, Ciba-Geigy AG); the formulation Tenoran 50 WP (Liro Belgium, B–1720 Asse) was used in this study. All concentrations are expressed as active ingredients. Concentrated stock solutions of nystatin and cycloheximide (1000 ppm) were filter-sterilized and stock solutions of $CuSO_4 \cdot 5H_2O$ and chloroxuron (1000 ppm) were sterilized by autoclaving and added to the inoculated media to obtain final concentrations of (in ppm): 0.00 as blank, 0.01, 0.05, 0,10, 0.50, 1.00, 5.00, 10.0 and 50.0. Harvest of the Rhodospirillaceae and determination of growth and gas production took place 5 days after inoculation. As an indicator of growth, suspended solids were determined gravimetrically after centrifugation (15 min at 20,000 × g). Green and blue-green algae were harvested after 10 days of incubation, and the samples were analyzed for growth visually and by using a Polyvar (Reichert-Jung) light microscope with fluorescence equipment to determine the nonfluorescent heterocysts in the filaments of blue-green algae. All experiments were done with four repetitions.

Results and discussion. The principal aim of the work was the selective total inhibition of green and blue-green algae. The concentrations required for total inhibition are shown in table 1 for the different inocula. Obviously, for a number of combinations, no total inhibition was observed.

Copper sulfate. This chemical is commonly employed for the control of algae. Complete inhibition occurred at 10 ppm of $CuSO_4 \cdot 5H_2O$ (about 6 ppm of $CuSO_4$) for both algal inocula tested. This value is higher than the commonly reported tolerance limit of 1 ppm, but in agreement with the review of Kraus[12] who also stated that some algae can tolerate considerably higher levels. However, $CuSO_4$ was found to be very toxic for the Rhodopseudomonas species also.

Cycloheximide. Cycloheximide, an antibiotic belonging to the group of the glutarimides, is selective against eucaryotes, as it inhibits protein synthesis on the 80 S ribosome[13]. As shown in the table, indeed only the green algae could be controlled with cycloheximide applied at 1 ppm. Growth was not reduced in any of the procaryotic cultures.

Nystatin. The polyene antibiotic nystatin has been considered as a pore former in sterol-containing membranes. As sterols appear to be of general occurrence in eucaryotes, it is believed to be active against all eucaryotes[13–15]. With this chemical no complete growth inhibition of the green algae was achieved, although growth rates at 50 ppm were reduced to about 10%. This result ties in with other recent reports that the inhibitory effect of nystatin cannot be generalized[16,17].

Chloroxuron. Chloroxuron is a representative of the urea derivatives used as herbicides. All urea derivatives suppress the Hill reaction. In 1939 Hill discovered that isolated chloroplasts of spinach can liberate oxygen on illumination in the presence of a suitable hydrogen acceptor. The Hill reaction has been demonstrated in algae and blue-green algae[18]. This oxygen-liberating reaction is absent in the group of Rhodospirillales, because they lack a 2nd photosynthetic system exciting electrons at the high positive redox potential where the reaction mentioned above takes place. In the presence of chloroxuron growth of green algae and blue-green algae was inhibited at 5 ppm, but growth of the Rhodopseudomonas species was normal. In an additional experiment, four different representa-

Table 1. Concentration ($mg \cdot l^{-1}$) required for total inhibition of growth of different photosynthetic organisms

Growth regulator	Inoculum *Rhodopseudomonas capsulata* ATCC 23782	ST 407	Green algae Medium A	Blue-green algae Medium B
$CuSO_4 \cdot 5H_2O$	5	5	10	10
Cycloheximide	> 50	> 50	1	> 50
Nystatin	> 50	> 50	> 50	> 50
Chloroxuron	> 50	> 50	5	5

Table 2. Effect of chloroxuron on the selective growth of inoculum and photosynthetic bacteria

Days of incubation	Fish pond water without chloroxuron	River water without chloroxuron	Soil without chloroxuron	Manure without chloroxuron	Compost without chloroxuron	Fish pond water with 5 ppm chloroxuron	River water with 5 ppm chloroxuron	Soil with 5 ppm chloroxuron	Manure with 5 ppm chloroxuron	Compost with 5 ppm chloroxuron
2	–	–	–	–	–	–	–	–	–	–
6	–	R	–	–	–	–	R	–	–	–
13	R	R;A	R	–	–	R	R	R	–	–
20	R	R;A	R	–	–	R	R	R	–	–
31	R;A	R,A	R	–	–	R	R	R	–	–

R, Rhodospirillaceae; A, algae; –, no growth.

tives of the Rhodospirillaceae were grown in the absence and presence of 50 ppm chloroxuron. No significant difference in the yield of suspended solids could be observed. After 3 days of incubation, the yield in both cultures was 1.34 $g \cdot l^{-1}$ for *Rhodopseudomonas capsulata* ATCC 23782, 1.50 $g \cdot l^{-1}$ for *Rhodopseudomonas capsulata* ST 407, 1.02 $g \cdot l^{-1}$ for *Rhodospirillum rubrum* ATCC 11170 and 1.22 $g \cdot l^{-1}$ for *Rhodomicrobium vannielii* ATCC 17100. Furthermore, at the end of the logarithmic growth phase all cultures showed intensive hydrogen gas production indicating an active nitrogenase enzyme complex.

To verify the selectivity of chloroxuron, an additional experiment with heterogenous inocula was set up. Samples of water from a fish pond and from a river, as well as samples from garden soil, a manure reservoir and domestic refuse compost were used as inocula for an enrichment test. Of each of the inocula, 3 g were added to 30 ml of growth medium, respectively with and without 5 ppm chloroxuron. Each experiment was done in two repetitions. The tubes were regularly inspected for growth. The results are summarized in table 2. The manure and compost samples contained no photosynthetic propagules or completely inhibited their growth. The three other inocula yielded dense cultures, with complete exclusion of algae in the presence of chloroxuron. It should be noted that the oral and acute dermal LD 50 of chloroxuron for rats is > 3000 $mg \cdot kg^{-1}$ [19]. Feeding rats daily at 10 $mg \cdot kg^{-1}$ for 120 days or dogs 15 $mg \cdot kg^{-1}$ for 90 days produced no ill-effects[19]. Also low toxicity was reported in several fish species and, furthermore, the chemical is known to dissipate quickly to non-phytotoxic concentrations[19]. The experimental findings together with the latter information suggest that chloroxuron could be a useful product to control eucaryotic and blue-green algae in anaerobic reactors of photosynthetic Rhodospirillaceae.

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1 Gest, H., and Kamer, M.D., Science *109* (1949) 558.
2 Gest, H., and Kamer, M.D., Science *109* (1949) 560.
3 Kumazawa, S., and Mitsui, A., in: CRC Handbook of biosolar resources, vol. 1, p. 299. Basic principles, part 1. Eds A. Mitsui and C.C. Black. CRC Press Inc., Boca Raton, Florida 1982.
4 Zürrer, H., Experientia *38* (1982) 64.
5 Segers, L., and Verstraete, W., Biotechnol. Bioengng *25* (1983) 2843.
6 Mitsui, A., in: Proceedings of the subcontractor's review meeting Solar hydrogen production programm, p. 1. Sponsored and organized by Solar Energy Institute Biomass Programm Office for U.S. Department of Energy (1981).
7 Zürrer, H., and Bachofen, R., in: Studies in environmental science. 9. Energetics and technology of biological elimination of wastes, p. 31. Ed. G. Milazzo. Elsevier Scientific Publishing Company, Amsterdam, Oxford, New York 1981.
8 Kim, J.S., Ito, K., and Takahashi, H., Agric. Biol. Chem. *46* (1982) 937.
9 Miyake, J., Tomizuka, N., and Kamibayashi, A., J. Ferment. Technol. *60* (1982) 199.
10 van Niel, C.B, Bact. Rev. *8* (1944) 1.
11 Segers, L., Verstrynge, L., and Verstraete, W., Biotechnol. Lett. *3* (1981) 635.
12 Kraus, R.W., in: Physiology and biochemistry of algae, p. 673. Ed. R.A. Lewen. Academic Press, New York and London 1962.
13 Stanier, R.Y., Adelbergh, E.A., and Ingraham, J.L., General microbiology, 4th ed., p. 871. MacMillan Press, New York, Singapore, Tokyo 1978.
14 Miller, J.D.A., in: Physiology and biochemistry of algae, p. 357. Ed. R.A. Lewin. Academic Press, New York and London 1962.
15 Van Zupthen, H., Demel, R.A., Norman, A.W., and Van Deenen, L.L.M., Biochim. biophys. Acta *241* (1971) 310.
16 Beezer, A.E., and Sharma, P.B., Microbios *30* (1981) 121.
17 Alonso, A., Martinez, E., Struzinsky, R., Michaljanicova, D., and Kotyk, A., Folia Microbiol. *28* (1983) 157.
18 Brody, M., and Brody, S.S., in: Physiology and biochemistry of algae, p. 3. Ed. R.A. Lewin. Academic Press, New York and London 1962.
19 Worthing, C.R., The pesticide manual. Published by the British crop protection council. Printed by the Boots Company Ltd, Nottingham, England 1979.

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Visualization of the mitochondria of *Toxoplasma gondii*-infected mouse fibroblasts by the cationic permeant fluorescent dye rhodamine 123[1]

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Summary. The mitochondria of living mouse fibroblasts infected with *Toxoplasma gondii* were monitored with the cationic permeant fluorescent dye rhodamine 123. Fluorescence microscopy revealed that host cell mitochondria accumulated at the cytoplasmic surface of parasitophorous vacuoles and increased the dye uptake in the periparasitophorous vacuole as *T. gondii* multiplied.
Key words. Rhodamine 123; *Toxoplasma gondii;* membrane potential; mitochondria.

Toxoplasma gondii is an obligatory intracellular protozoan parasite and grows in nearly all avian and mammalian cultured cells. Some workers have found that host cell mitochondria accumulate in the parasitophorous vacuole (PV), in which the parasite grows and multiplies, surrounding its surface[2]. The manner in which the mitochondria accumulate in this vacuole conveys the impression that *T. gondii* incorporates ATP generated by the mitochondria of the peri-PV. It has been recently demonstrated that mitochondria of living cells can be visualized with the cationic permeant fluorescent dye rhodamine 123 (R123)[3]. At physiological pH, R123 selectively accumulates in mitochondria and the specific accumulation appears to result from a high transmembrane potential (inside negative) across the mitochondrial membrane[4]. In the present study, mitochondria of *T. gondii*-infected mouse fibroblasts were monitored with R123.

Materials and methods. The RH strain of *T. gondii* was used. The infection was maintained as described earlier[5]. Parasites harvested from infected peritoneal exudate were centrifuged at 1600 × g for 5 min and washed twice with phosphate buffered saline (PBS: 135 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 5 mM glucose and 10 mM KH_2PO_4-Na_2HPO_4, pH 7.2). Balb 3T3