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.

Acknowledgment. This research was supported by the Belgian Ministry for Science Policy Programming.

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.

0014-4754/85/010099-03$1.50 + 0.20/0

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

K. Tanabe

Department of Medical Zoology, Osaka City, University Medical School, Asahi-machi, Abeno-ku, Osaka 545 (Japan),
6 February 1984

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.

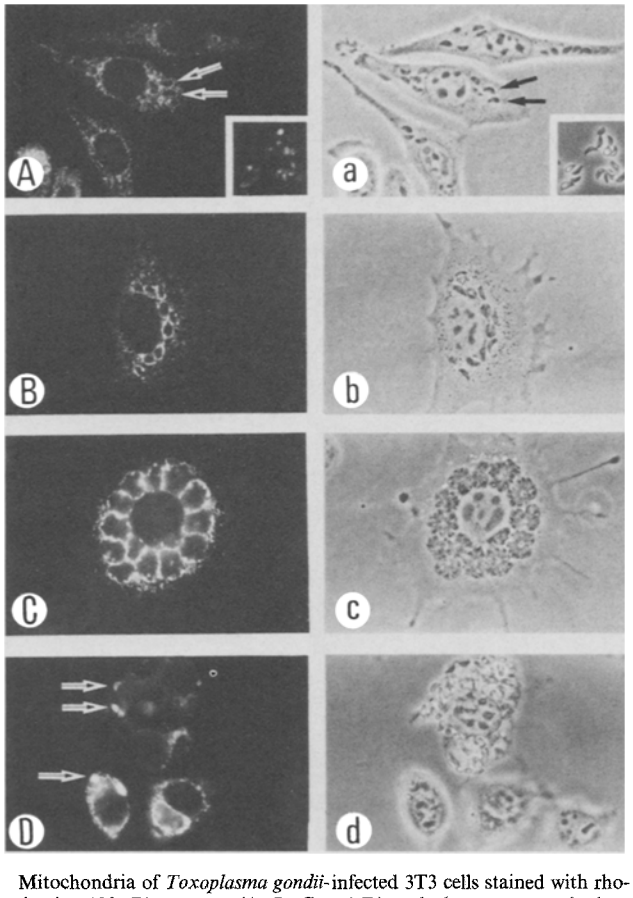
Materials and methods. The RH strain of *T. gondii* was used. The infection was maintained as described earlier[5]. Parasites harvested from infected peritoneal exudate were centrifuged at 1600 × g for 5 min and washed twice with phosphate buffered saline (PBS: 135 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 5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

cells were grown in RPMI 1640 medium (GIBCO) supplemented with 10% foetal calf serum (FCS: GIBCO) at 37 °C in 5% CO_2 in air.

Cell monolayers were dissociated by Ca^{2+} and Mg^{2+} free PBS containing 0.02% trypsin (Miles Lab.) plus 0.02% EDTA (Sigma Chem.). Dissociated cells (4×10^4) were plated onto 35-mm plastic dishes (Falcon) and incubated in 2 ml of RPMI medium with 10% FCS. One day after culture, the cells were washed twice with PBS and inoculated with 2×10^7 parasites in 1 ml of PBS. The parasites were allowed to settle onto the host cells at room temperature for 10 min. The culture dishes were then shifted to 37 °C and incubated for another 10 min to initiate parasite invasion. The free parasites were then removed by pipetting. The infected cells were washed with RPMI medium and cultured in 2 ml of RPMI medium with 10% FCS at 37 °C for various periods of time in 5% CO_2 in air.

A stock solution of R123 (1 mg/ml) was freshly prepared with distilled water before the experiments. R123 (20 µl) was added to the cell culture, which was incubated at 37 °C for 30 min. The cells were then washed with PBS several times and covered with a 22-mm round cover slip. Cells stained with R123 were examined with a fluorescence microscope as described earlier[6]. Photographs were taken with Kodak Tri-X film at an exposure time of 45 sec.

Results. Staining of 3T3 cells with R123 revealed mitochondria of granular to rod-like shape throughout the cytoplasm. Following invasion, parasites made no direct contact with the host cell cytoplasm, but were surrounded by a parasitophorous vacuole (PV) membrane[7], even though this could not be shown by phase contrast microscopy. Figure A shows that host cell mitochondria moved to some PVs (arrows). After 30 min of incubation, nearly 40% of the PVs were surrounded by mitochonria. More than 80% of the PVs were surrounded at 1 h of incubation. In contrast to host cell mitochondria, those of intracellular parasites did not incorporate R123, whereas those of the extracellular parasites did (figs A and a, inlets). At 4 h of incubation, some of the parasites had already multiplied, and host cell mitochondria surrounded all the PVs and incorporated R123 extensively (figs B and b). However, there was no further dye uptake by those mitochondria not surrounding the PV. At 22 h, the parasites had multiplied several times and showed a rosette-shaped array in the PV (fig. c); mitochondria in the peri-PV continued to take up the dye (fig. C). Mitochondria of cells on the verge of rupture incorporated no R123 (fig. D, upper cell). Liberated parasites regained their ability to accumulate the dye (fig. d, arrows).



Mitochondria of *Toxoplasma gondii*-infected 3T3 cells stained with rhodamine 123. Fluorescent (A, B, C and D) and phase contrast (a, b, c and d) micrographs taken 30 min (A and a), 4 h (B and b) and 22 h (C, c, D and d) after parasite invasion. Inlets of A and a show extra-cellular free parasites. Note the localization of intra-cellular parasites (right) and host cell mitochondria (left). × 260.

Discussion. It was shown previously that *T. gondii* failed to multiply in differentiating erythroid cells[8]. In these cells, host cell mitochondria were lost during the process of cell maturation. *T. gondii* seemed to require ATP synthesized by host cell mitochondria for its growth. Recently, Schwartzman and Pfefferkorn have shown conclusively that *T. gondii* incorporated exogenous ATP[9], but they also noted that ATP was extensively degraded before entering the parasite and that *T. gondii* grew normally in cell mutants defective in aerobic respiration[9]. This indicates that host cell mitochondria in peri-PV apparently play no significant role in parasite growth.

R123 selectively accumulates in mitochondria of living cells[3] and monitors the mitochondrial membrane potential[4]. The present study demonstrated that the mitochondria of *T. gondii*-infected mouse fibroblasts accumulated at the cytoplasmic surface of PV and increased the uptake of the dye in the peri-PV as the parasite multiplied. The latter finding suggests that the mitochondria in the peri-PV consequently take on increased membrane potential. These alterations of mitochondrial function and distribution probably reflect changes in cellular physiological conditions due to *T. gondii* infection. R123 was found to penetrate the PV membrane[10]. The failure of intracellular parasites to take up R123 consequently suggests that their mitochondrial electron transport system scarcely functions. Thus, intracellular parasites probably obtain ATP only from glycolysis. Elucidation of the biochemical processes involved in the metabolic changes of both intracellular *T. gondii* and its host cell will provide further insights into obligatory intracellular parasitism.

1 The author wishes gratefully to acknowledge the work of Miss Mayumi Kato and Mr. Isao Kimata in preparing the photographs. The publication cost was supported by a grant of the Ohyama Health Foundation.

2 Jones, T.C., and Hirsh, J.G., J. exp. Med. *136* (1972) 1173.
3 Johnson, L.V., Walsh, M.L., and Chen, L.B., Proc. natl Acad. Sci. USA *77* (1980) 990.
4 Johnson, L.V., Walsh, M.L., Bockus, B.J., and Chen, L.B., J. Cell Biol. *88* (1981) 526.
5 Tanabe, K., Kimata, I., Tabuse, Y., Furusawa, M., and Takada, S., Exp. Parasit. *46* (1978) 72.
6 Tanabe, K., Protozoology *30* (1983) 707.
7 Sheffield, H.G., and Melton, M.L., J. Parasit. *54* (1968) 209.
8 Tanabe, K., Kimata, I., and Takada, S., J. gen. Microbiol. *118* (1980) 45.
9 Schwartzman, J.D., and Pfefferkorn, E.R., Exp. Parasit. *53* (1982) 77.
10 Tanabe, K., and Murakami, K., J. Cell Sci. (in press).

0014-4754/85/010101-02$1.50 + 0.20/0