search Facilities and Resources. This work was carried out during the tenure (F.S.) of an Established Investigatorship of the American Heart Association, Inc.

Department of Muscle Research, Retina Foundation, F. SRETER
Institute of Biological and Medical Sciences; N. IKEMOTO
Department of Neurology, Massachusetts General Hospital; J. GERGELY
and Departments of Neuropathology and Biological Chemistry,
Harvard Medical School, Boston, Mass. (U.S.A.)

1 F. A. SRETER AND J. GERGELY, *Biochem. Biophys. Res. Commun.*, 16 (1964) 438.
2 M. E. CARSTEN, *Circulation Res.*, 20 (1967) 599.
3 F. FUCHS, E. W. GERTZ AND F. N. BRIGGS, *J. Gen. Physiol.*, 52 (1968) 955.
4 B. FANBURG AND J. GERGELY, *J. Biol. Chem.*, 240 (1965) 2721.
5 W. VAN DER KLOOT, *Science*, 164 (1969) 1294.
6 N. IKEMOTO, F. A. SRETER AND J. GERGELY, *J. Ultrastruct. Res.*, 23 (1968) 216.
7 A. MARTONOSI AND R. FERETOS, *J. Biol. Chem.*, 239 (1964) 648.
8 G. D. BAIRD AND S. V. PERRY, *Biochem. J.*, 77 (1960) 262.

Received December 1st, 1969

Biochim. Biophys. Acta, 203 (1970) 354–357

BBA 73107

Carotenoid pigments and the stability of the cell membrane of *Sarcina lutea*

The carotenoid pigments of non-photosynthetic bacteria are located within the cell in association with the cell membrane[1–3], and have been shown to protect the cells against lethal photosensitization[4,5]. It has been suggested[6] that one of the ways that carotenoid pigments might function would be by stabilizing such damaged membranes. Indeed, SALTON AND ETHISHAM-UD-DIN[3] and SALTON AND FREER[7] found that occasional preparations of cells of the carotenoid-containing organisms *Micrococcus lysodeikticus* and *Sarcina lutea* whose membrane-associated carotenoids were completely depleted by growing the cells in the presence of diphenylamine lysed more readily, and membranes prepared from these cells appeared to be less stable. These observations and suggestions have prompted us to investigate whether carotenoid pigments play a role in stabilizing the cell membrane of *S. lutea*. In addition to diphenylamine-treated cells, we used a colorless mutant which accumulates the colorless precursors phytoene and phytofluene, the same compounds which accumulate in DPA-treated cells.

The organisms used in this study were a lysozyme-sensitive strain of *S. lutea* obtained from Dr. R. Y. Stanier, and a colorless mutant of this strain, mutant 2wm induced by exposure of the wild type to ultraviolet light. The cells were grown in nutrient broth (Difco) on a rotary shaker at 30°. For those experiments in which inhibition of production of colored pigment was desired, diphenylamine was added to the nutrient broth at the time of inoculation to give a final concentration of 0.1 mM. Diphenylamine was obtained from Distillation Products Industries, and lysozyme from Nutritional Biochemical Corp.

In order to compare the relative stability of whole cells of wild type, diphenylamine-treated wild type and mutant 2wm to repeated centrifugation and resuspension, the following experiment was performed. Cells of each strain were suspended in 0.1 M phosphate buffer at pH 7.0, and were centrifuged at 5000 × *g* for 15 min, then resuspended in the same supernatant, and centrifuged again. This was done for a total of 10 times. After the last centrifugation, an aliquot of the supernatant was removed for protein determination[8], the cell pellet was then resuspended in the rest of the supernatant, and the viable count was determined. In all three cultures the viable count and protein leakage into the supernatant solution were unaffected by repeated centrifugation and resuspension, indicating that the colorless cells were no more susceptible to lysis under this mechanical stress than were the pigmented wild-type cells.

The osmotic fragility of protoplasts of the three types of cells was measured. Protoplasts were prepared by incubating buffered 1.2 M sucrose solutions of cells containing lysozyme (20 μg/ml) for 2–4 h at 30°. After incubation, aliquots were added to tubes containing different molarities of buffered sucrose for 30 min, then, 0.1-ml aliquots were pipetted into tubes containing either 5 ml of buffered 1.2 M NaCl or 5 ml of phosphate buffer alone. The absorbances, at 650 nm, as a function of sucrose molarity are shown in Fig. 1, where it can be seen that there were no significant differences in the osmotic stabilities of the protoplasts produced from the three cultures. In another experiment, protoplasts were produced in sucrose solutions of varying molarities, and similar results were obtained. Thus, in experiments in which the molarities of sucrose solutions were varied either after or during the formation of protoplasts, we could find no significant differences in the stability of protoplasts made from either wild-type, diphenylamine-treated wild-type or mutant 2wm cells.

In the experiments just described, we have studied whole cells and protoplasts of fully pigmented wild-type cells, and two kinds of colorless cells (diphenylamine-treated wild-type cells, and cells of mutant 2wm, both derived from the wild-type cells), which accumulate the same kinds of colorless carotenoid precursors. In neither whole cells nor protoplasts have we been able to find any significant difference in

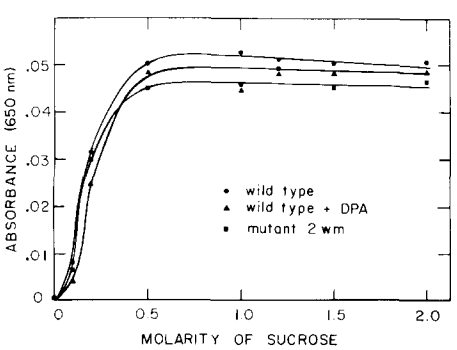

Fig. 1. Stability of protoplasts made from cultures of wild-type *S. lutea*, diphenylamine (DPA)-treated wild-type and mutant 2wm cells. The protoplasts were formed directly in a protoplast-stabilizing concentration of sucrose (1.2 M sucrose in 0.04 M phosphate buffer) and then transferred to varying concentrations of buffered sucrose. Absorbances of appropriately diluted aliquots were measured in 1.2 M NaCl in 0.01 M phosphate buffer, pH 6.8, against a blank of the buffered NaCl solution. Previous experiments with this strain have shown that phosphate buffer caused a minimum leakage of carotenoids from cell fractions[2].

the fragility of these three kinds of cells of *S. lutea*. Therefore, it appears that in *S. lutea*, colored carotenoid pigments play no significant role in stabilizing the cell membrane. The observations of SALTON AND ETHISHAM-UD-DIN[3] and SALTON AND FREER[7] that occasional preparations of diphenylamine-treated bacteria show a decreased membrane stability would therefore appear not to be related to the effect of diphenylamine on carotenoid biosynthesis.

This work was supported in part by grants to M.M.M.-R. from the Medical Foundation, Inc. of Boston, and the National Science Foundation (Grant No. GB-8216), and to N.I.K. from the National Science Foundation (Grant No. GB8093).

Channing Laboratory and Department of Medical Microbiology, Boston City Hospital, Department of Bacteriology and Immunology, Harvard Medical School and Department of Biochemistry, Tufts University School of Medicine, Boston, Mass. (U.S.A.)

MICHELINE M. MATHEWS-ROTH
NORMAN I. KRINSKY

1 A. R. GILBEY, A. V. FEW AND K. MCQUILLEN, *Biochim. Biophys. Acta*, 29 (1958) 21.
2 M. M. MATHEWS AND W. R. SISTROM, *J. Bacteriol.*, 78 (1959) 778.
3 M. R. J. SALTON AND A. F. M. ETHISHAM-UD-DIN, *Australian J. Exptl. Biol. Med. Sci.*, 43 (1965) 255.
4 R. KUNIZAWA AND R. Y. STANIER, *Arch. Mikrobiol.*, 31 (1958) 146.
5 M. M. MATHEWS AND W. R. SISTROM, *Arch. Mikrobiol.*, 35 (1960) 139.
6 N. I. KRINSKY, in A. C. GIESE, *Photophysiology*, Vol. 3, Academic Press, New York, 1968, p. 123.
7 M. R. J. SALTON AND J. H. FREER, *Biochim. Biophys. Acta*, 107 (1965) 531.
8 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 263.

Received December 8th, 1969