Utilization of the photosensitized oxidation of sulfite for manometric actinometry

A gas-consuming chain reaction, viz. the aerobic oxidation of sulfite[1,-3], which provides a magnified manometric response to the chain-initiating events, was applied to the assay of milk xanthine oxidase[4]. Its utility in this enzymic system suggested its possible use in manometric actinometry. Exploratory experiments indicate that the photosensitized oxidation of sulfite appears to provide the basis for ultrasensitive manometric actinometry.

Conventional Warburg microrespirometers, shaken at 37.5°, with air as the gas phase, were employed throughout. All reactions were performed in a final volume of 2.2 ml buffered by 0.050 *M* potassium phosphate, pH 7.8. Solutions were made with distilled water from which residual ionic impurities were removed by passage through a mixed-bed resin demineralizer. Further control over metallic impurities was achieved by addition of 0.005 % Versene Fe-III and subsequent storage in polyethylene carboys. Illumination was provided by frosted incandescent lamps varying from 7.5 to 100 W which were suspended 4 feet above the bath surface. Illumination intensity at the bath surface was *estimated* with a General Electric photographic exposure meter fitted with a dynacell and used as an incident light meter. Manometric readings, during dark periods, were taken with the aid of a shielded pocket flashlight.

A variety of dyes were tested as photosensitizers of the oxidation of sulfite. Thionine, phenosafranin, toluidine blue, flavin mononucleotide, methylene blue and resazurin tested at a concentration of 0.045 mg/ml were all effective whereas hemoglobin, bromthymol blue, gallocyanin, cresol red and phenol red, tested at the same concentration, were inactive. In Fig. 1 the manometric responses to light of flasks containing 60 μmoles Na_2SO_3 and 0.43 μmole resazurin or 0.02 μmole methylene blue adsorbed onto 50 mg silicic acid are compared to that of the manometric actinometer of WARBURG AND SCHOCKEN[5]. As shown, the resazurin–sulfite system was 10 times more sensitive and the methylene blue–silicic acid–sulfite system 30 times more sensitive than the thiourea–ethyl chlorophyllide system of WARBURG AND SCHOCKEN. The slow, linear oxygen uptake observed with the dye plus sulfite systems in the dark must be subtracted from rates observed in the light to obtain the rate of oxygen consumption caused by illumination. The relationship between intensity of illumination and the manometric responses of the resazurin–sulfite system and the ethyl chlorophyllide–thiourea system are compared in Fig. 2.

Unfortunately, each of the dyes which was an effective photosensitizer of sulfite oxidation was also active as a chain breaker. In consequence, the observed rate of oxygen consumption was decreased if the dye concentrations were greatly raised. The concentrations of dye which gave maximal manometric response to light were too low to assure complete absorption of all light quanta entering the flasks, even at the absorption maxima of the dye used and, thus, represent a compromise between the number of chains initiated by a given quantity of light and the effective length of these reaction chains.

An additional problem was presented by the great reactivity of sulfite. Methylene blue and resazurin slowly react with sulfite in the course of a manometric experiment, which lasts 1 h. The methylene blue-sulfite reaction product shows no absorption in the visible range and, hence, is inactive as a sensitizer in this assay. Consequently,

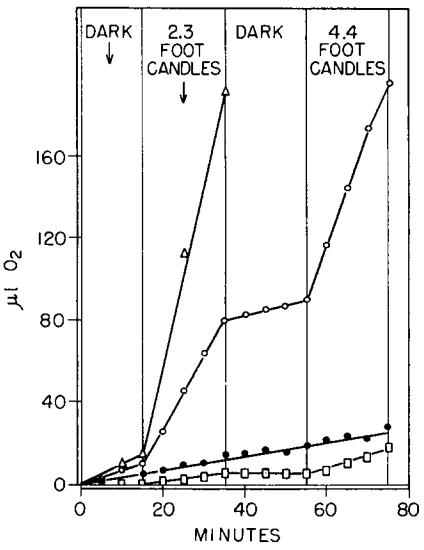

Fig. 1. Manometric response to light by flasks containing: (○) 0.10 mg resazurin and 60 μmoles sulfite in 2.2 ml of phosphate buffer. (△) 0.02 μmole methylene blue, 50 mg silicic acid and 60 μmoles sulfite in 2.2 ml of phosphate buffer. (□) 200 mg thiourea and 1.0 μmole ethyl chlorophyllide in 2.2 ml of pyridine. (●) 60 μmoles sulfite in 2.2 ml of phosphate buffer.

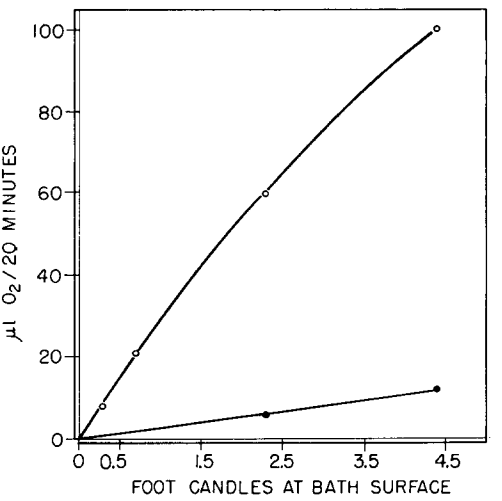

Fig. 2. Relationship of manometric response to light intensity in flasks containing: (○) 0.1 mg resazurin and 60 μmoles Na_2SO_3 in 2.2 ml of phosphate buffer. (●) 1.0 μmole ethyl chlorophyllide a and 200 mg thiourea in 2.2 ml of pyridine.

the rate of oxygen consumption observed in a methylene blue–sulfite system was not linear with time of illumination but decreased steadily. Adsorption of methylene blue onto silicic acid, for which it exhibits a remarkable affinity, greatly retards bleaching by sulfite and thus permits a linear rate of gas uptake by the illuminated system. The resazurin–sulfite reaction product is a red, strongly fluorescent dye whose activity as a sensitizer appears to be the same as that of the unreacted resazurin. Hence the resazurin-sulfite system was observed to give a linear rate of gas uptake under constant illumination. Of course, only those wavelengths absorbed by the sensitizers are effective in causing sulfite oxidation.

This work was supported in part by Contract AT-(40-1)-289 between Duke University and the U. S. Atomic Energy Commission and by Grant RG-91 from the National Institutes of Health.

Department of Biochemistry, Duke University, Durham, N.C. (U.S.A.)

IRWIN FRIDOVICH
PHILIP HANDLER

[1] H. L. J. BÄCKSTRÖM, *J. Am. Chem. Soc.*, 49 (1927) 1460.
[2] H. N. ALYEA AND H. L. J. BÄCKSTRÖM, *J. Am. Chem. Soc.*, 51 (1929) 90.
[3] E. C. FULLER AND R. H. CRIST, *J. Am. Chem. Soc.*, 63 (1941) 1644.
[4] I. FRIDOVICH AND P. HANDLER, *J. Biol. Chem.*, 233 (1958) 1581.
[5] O. WARBURG AND V. SCHOCKEN, *Arch. Biochem.*, 21 (1949) 363.

Received January 16th, 1959