Oxygen Toxicity in *Astasia*

By NICOLE BÉGIN-HEICK AND J. J. BLUM

Department of Physiology and Pharmacology, Duke University,
Durham, N.C. 27706, U.S.A.

(Received 4 April 1967)

1. Exposure of *Astasia longa* to oxygen + carbon dioxide (95 : 5) at atmospheric pressure leads to an inhibition of growth rate and of respiration. Growth resumes at the normal rate as soon as the oxygenation is discontinued, but respiration recovers more slowly. 2. Mitochondria prepared from cells exposed to oxygen + carbon dioxide (95 : 5) during growth have considerably decreased activities of succinate-cytochrome *c* oxidoreductase, NADH-cytochrome *c* oxidoreductase, succinate dehydrogenase and succinate oxidase activities as compared with mitochondria obtained from cells exposed to air + carbon dioxide (95 : 5). Cytochrome oxidase activity is not appreciably inhibited by exposure of the cells to 95% oxygen. 3. The mitochondrial fraction of *Astasia* contains rhodoquinone. The rhodoquinone concentration increases in cells exposed to 95% oxygen. The content of ergosterol-containing compounds also increases in the mitochondria of cells exposed to 95% oxygen. There is little change in the ubiquinone content of the mitochondrial fraction. The ubiquinone of *Astasia* appears to be ubiquinone-45.

The deleterious effects of oxygen on living organisms, first described by Bert (1878), have been amply confirmed, but Bert's theory that they are due to the formation of a toxic substance has not been accepted generally. Rather, evidence has accumulated suggesting that oxygen has a direct effect on certain enzymes (e.g. Davies & Davies, 1965). Chance, Jamieson & Coles (1965), however, noted that the effects of oxygen on some isolated enzyme systems proceeded too slowly to account for the rapid onset of lesions due to high oxygen pressure in the intact animal. In the same paper these authors made the important observation that in rats exposed to oxygen at high pressures (2.7–10 atm.), most of the NAD in the brain and liver is in the oxidized state. In view of this finding, Chance *et al.* (1965) postulated that the energy-dependent electron flow from succinate to NAD is inhibited by oxygen at high pressure. Further, Jamieson & Chance (1966) demonstrated an effect of high-pressure oxygen *in vitro* on the electron-transport chain of mitochondria, and concluded that the inhibition occurred on the substrate side of cytochrome *c*.

The effects of oxygen have also been investigated on mammalian cells grown in tissue culture, and on bacteria. The growth of HeLa cells is inhibited by oxygen at atmospheric pressure. Cell division is blocked, the synthesis of DNA, RNA and protein is decreased, and glucose metabolism is shifted to an anaerobic pattern (Rueckert & Mueller, 1960).

If, after 48 hr. exposure to 95% oxygen, the cells are exposed to air, these effects are partially reversed. Brosemer & Rutter (1961) obtained similar results with a different cell line. Acetate oxidation in *Azotobacter vinelandii* is inhibited by oxygen at pressures above 0.3 atm., and this may be accounted for by observed inhibitions of acetate kinase, phosphate transacetylase, isocitrate lyase and malate synthetase (Wolin, Evans & Niven, 1955).

Some work has been done also with protozoa. Wittner (1957a) reported that in *Paramoecium* the toxic effect of oxygen varied directly with pressure at temperatures above 5°. Similar results were obtained by Cleveland & Burke (1956) for the protozoa of *Cryptocercus*. Wittner (1957b) also found that the addition of 0.04 M-pyruvate to cultures of *Paramoecium* maintained at high oxygen pressure doubled the survival time, and that the addition of 2,3-dimercaptopropan-1-ol, GSH and cysteine reversed some of the toxic effects of oxygen.

The present work began when, during studies on synchronized cultures of *Astasia longa*, it was observed that cell division was inhibited if oxygen + carbon dioxide (95 : 5) was bubbled through the cultures instead of air + carbon dioxide (95 : 5), and that the effect of high oxygen pressure could be observed in cultures grown at constant temperatures. The sensitivity of *Astasia* to oxygen at atmospheric pressure afforded us a model system to

study the deleterious effects of oxygen on a unicellular organism growing in a chemically defined medium. In this paper, we present the effects of oxygen on the growth and respiration of *Astasia*, and on some properties of mitochondria isolated from these cells.

MATERIALS AND METHODS

Cells. The cells used were *Astasia longa* (Jahn strain). They were grown axenically at constant temperature (25–27°) in a modified Cramer–Myers medium (Blum & Padilla, 1962) with acetate as the sole source of carbon and energy. Cells were counted with a Coulter counter (Coulter Co., Hialeah, Fla., U.S.A.). The cultures were gassed either with air + CO₂ (95 : 5) or with O₂ + CO₂ (95 : 5). In a control experiment, the O₂ + CO₂ mixture was bubbled through the medium for 24 hr. before inoculation. In this case, the cells grew normally. This rules out the possibility that oxygen damages the medium.

Measurement of Q_{O_2} . The cells were collected by centrifugation for 2 min. at 800g, and resuspended in fresh medium at a concentration of about 3×10^6 cells/ml. Measurements were carried out at 27° in a standard Warburg respirometer with a total incubation volume of 2 ml. When the incubations were performed under oxygen the manometers were gassed for 10 min. and then equilibrated for 7 min. before the first reading was taken.

Chemical determinations. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). RNA and DNA were determined by a modification of the Schmidt and Thannhauser procedure (Blum & Padilla, 1962). Ultraviolet and visible spectra were measured in a Beckman model DBG recording spectrophotometer.

Chromatography. Glass plates (20 cm. $\times$ 20 cm.) were coated with 250 μ - or 500 μ -thick layers of silica gel G (Brinkman Instruments Inc., Westbury, N.Y., U.S.A.). The plates were dried in air and activated at 110°. The samples to be analysed were applied to the gel as bands and the plates were developed in the appropriate solvents. For reversed-phase chromatography, the activated plates were dipped in a solution of liquid paraffin (5%, v/v) in ether, dried in air, and, after application of the samples, developed in acetone–water (9 : 1, v/v) and sprayed with 10% (w/v) phosphotungstic acid in 90% ethanol. Ergosterol (Mann Research Laboratories, New York, N.Y., U.S.A.) was recrystallized from hot ethanol. Ubiquinones-30 and -50 (Sigma Chemical Co., St Louis, Mo., U.S.A.) and ubiquinone-45 isolated from rat liver mitochondria were used as standards. (The numbers of the ubiquinones indicate the number of carbon atoms they contain.)

Preparation of mitochondria. Mitochondria were prepared by the methods of Buetow & Buchanan (1964) as modified by Kahn & Blum (1967). Briefly, the cells were collected in a continuous-flow system, suspended in ice-cold buffer (0.25 M-sucrose–5 mM-EDTA–24 mM-tris–HCl, pH 7.4) and centrifuged at 1000g for 5 min., and the cell pellets were ground in a chilled mortar with glass beads (Superbrite, type 100–500S; Minnesota Mining and Manufacturing Co., St Paul, Minn., U.S.A.) for 1 min. The cell extract was then eluted from the glass beads with buffer, and centrifuged at 1000g for 5 min. The supernatant fraction was centrifuged for 10 min. at 3000g and again at 27000g for 15 min. This

supernatant was discarded and the sediment was resuspended in buffer and again centrifuged at 27000g for 15 min. The mitochondrial pellet was finally suspended in buffer at a protein concentration of about 3 mg./ml.

Enzyme assays. The enzyme assays were done on at least two different concentrations of mitochondrial protein, each in duplicate. The results quoted are averages of these determinations. The protein concentrations chosen were in the region where activity was proportional to protein concentration.

Succinate–cytochrome c oxidoreductase. This activity was assayed by adding 15 μ moles of sodium succinate to a cuvette containing mitochondria (10–50 μ g. of protein), 1 μ mole of KCN, 60 μ moles of cytochrome c (type III or VI from horse heart; Sigma Chemical Co.) and 100 μ moles of sodium phosphate adjusted to pH 7.4 with 0.2 N-NaOH, in a total volume of 3 ml. The increase in E_{550} was recorded on a Gilford absorbance recorder equipped with a Beckman DU optical system (Gilford Instrument Co., Oberlin, Ohio, U.S.A.). The results are expressed as $m\mu$ moles of cytochrome c reduced/min./mg. of protein, 18.5×10^3 l./mole/cm. being taken as the molar extinction coefficient of reduced cytochrome c in the presence of oxidized cytochrome c (Margoliash, 1954).

NADH–cytochrome c oxidoreductase. This activity was determined as for succinate–cytochrome c oxidoreductase, except that succinate was omitted and the reaction was started by the addition of 1.5 μ moles of NADH (International Chemical Corp., City of Industry, Calif., U.S.A.). The results were calculated as for succinate–cytochrome c oxidoreductase.

Cytochrome c–oxygen oxidoreductase. Cytochrome c was reduced according to the procedure of Smith (1954). The E_{550}/E_{565} ratio was always between 6 and 10. For the spectrophotometric assay, each cuvette contained 100 μ moles of tris adjusted to pH 7.4 with 0.2 N-HCl, 250 $m\mu$ moles of reduced cytochrome c and mitochondria (200–600 μ g. of protein), in a total volume of 3 ml. The decrease in E_{550} was recorded for 10 min., and then 10 μ l. of 0.1 M-KCN was added to stop the reaction. The total amount of reduced cytochrome c present was determined by adding a crystal of K₃Fe(CN)₆ and recording E_{550} . The activity was calculated according to the method described by Smith (1955).

Succinate oxidase activity. The reaction mixture contained 6 μ moles of sodium succinate, 2 μ moles of ADP, 3 μ moles of KF, 2 μ moles of MgCl₂, 5 μ moles of sodium phosphate buffer, pH 7.5, 5 μ moles of tris–HCl buffer, pH 7.4, and mitochondria (200–600 μ g. of protein), in a total volume of 1 ml. The rate of oxygen consumption was measured with a Clark oxygen electrode attached to an oxygraph (Gilson Medical Electronics Co., Madison, Wis., U.S.A.) at 22°. Activity was expressed as $m\mu$ moles of O₂/min./mg. of protein.

Succinate dehydrogenase. This enzyme was assayed essentially according to the method of Arrigoni & Singer (1962) with 150 μ moles of sodium phosphate buffer adjusted to pH 7.6 with 0.2 N-NaOH, 60 μ moles of sodium succinate, 3 μ moles of NaCN, 90 $m\mu$ moles of 2,6-dichlorophenol-indophenol and 1 mg. of phenazine methosulphate, in a total volume of 3 ml. The temperature was 22°. The results were computed from recordings of the decrease in E_{600} , by using 21×10^3 l./mole/cm. as the molar extinction coefficient of 2,6-dichlorophenol-indophenol (Savage, 1957).

Lipid extraction. Mitochondrial lipids were extracted according to the methods of Pumphrey & Redfearn (1960) from samples of mitochondria containing 50–100mg. of protein. The light petroleum extracts were evaporated to dryness and the residues were dissolved in ethanol and applied to a thin-layer plate. The chromatograms were developed in hexane-ethyl acetate (9:1, v/v). After the chromatograms were developed and dried, the various regions desired were scraped off and eluted with ethanol. The silica gel was then removed by centrifugation, and the spectrum of each fraction was recorded on a Beckman DBG spectrophotometer.

RESULTS

Effects of oxygen on the growth rate, respiration, and nucleic acid and protein content of Astasia. Cells in the logarithmic phase of growth were

Fig. 1. Effect of O₂ + CO₂ (95:5) on growth of *Astasia*. Cultures were grown at 25° in defined medium; the doubling times of the cells grown in air + CO₂ (○) and in O₂ + CO₂ (●) were 8.5 hr. and 16.5 hr. respectively.

transferred to Erlenmeyer flasks fitted with sintered-glass bubblers and incubated at 25° with either air + carbon dioxide (95:5) or oxygen + carbon dioxide (95:5) for 45 hr. After 45 hr. the cells were collected and their respiration rate was determined by the standard Warburg technique, with 0.20M-acetate as substrate. Although the growth rate of the oxygen + carbon dioxide-treated cultures was considerably inhibited compared with that of the air + carbon dioxide-treated control (Fig. 1), no significant difference could be detected in the DNA, RNA or protein contents of the cells in the two cultures (Table 1). A considerable decrease was noted in the content of RNA and of protein of these cells during logarithmic growth, as observed for *Euglena* by Buetow & Levedahl (1962). The effect of oxygen treatment on the growth rate is rapid, and occurs with no noticeable delay (Fig. 1).

If the respiration rate of cultures grown in air was measured in an atmosphere of oxygen + carbon dioxide, there was a definite decrease in the respiration rate noticeable within 10 min. Conversely, the respiration rate of oxygen-grown cells was slightly higher after 20 min. of exposure to an air atmosphere (Fig. 2). To examine the recovery rate in air more closely, experiments were performed in which cells were grown at 27° under oxygen + carbon dioxide and, at various intervals, samples were taken from the oxygen-grown culture and transferred into fresh medium with air + carbon dioxide as the gas phase. The cells immediately resumed logarithmic growth with the normal doubling time (9–10 hr. at 27°), independent of the length of time they had been exposed to the oxygen + carbon dioxide gas phase (Fig. 3). Cells kept under oxygen + carbon dioxide for the full duration of these experiments (44–48 hr.) had a doubling time of about 15 hr. Although growth resumed immediately upon transfer to a medium gassed with air + carbon dioxide, the effects of prior exposure to oxygen on the respiration rate were of longer duration. As

Table 1. *Effects of oxygen on the growth rate, nucleic acid content and protein content of Astasia*

Cultures in defined media (see the Materials and Methods section) were started at different cell densities so that they would be at comparable levels throughout most of the experiment. The cultures were gassed with air + CO₂ (95:5) or with O₂ + CO₂ (95:5) at 25°. Cell counts (Coulter counter) and chemical determinations (see the Materials and Methods section) were made on culture samples taken at the given times.

Time (hr.)	10 ⁻³ × Concn. of cells (cells/ml.)		RNA content (μg./10 ⁶ cells)		DNA content (μg./10 ⁶ cells)		Protein content (μg./10 ⁶ cells)	
	Air	O ₂	Air	O ₂	Air	O ₂	Air	O ₂
0	57.5	92.5	48.0	—	4.1	—	362	—
19	272	218	39.0	37.4	4.9	4.1	341	339
25.5	382	257	35.3	36.6	3.8	4.2	324	300
45.5	918	478	30.0	27.4	4.2	3.9	201	223

Fig. 2. Effects of oxygen on respiration of *Astasia*. The broken lines represent cultures exposed to air+CO₂ at 25° for 45 hr. during growth in defined medium and then incubated in the Warburg vessels in either air+CO₂ (●) or O₂+CO₂ (○). The solid lines represent cultures exposed for 45 hr. to O₂+CO₂ during growth and then incubated in the Warburg vessels in either air (▲) or O₂+CO₂ (△). All incubations were done in duplicate; except for the cells grown in O₂+CO₂ and incubated in O₂+CO₂ (△) the duplicate points were essentially identical, and only one point at each time is shown.

the length of exposure to oxygen increased (and, hence, the time in air before the respiration rate was measured decreased), the Q_{O_2} decreased. The Q_{O_2} values were plotted against the time in oxygen for several such experiments; the respiration rate decreased with increasing time of exposure to oxygen, even though the respiration measurements were carried out up to 46 hr. after exposure to oxygen. The line drawn in Fig. 4 was obtained by a least-squares computation. The regression coefficient was -0.78 and the P value for 18 degrees of freedom was <0.001 . It was concluded that the recovery of respiration rate after exposure of the cells to oxygen was a slow process, whereas the rate of cell division recovered very rapidly.

Effect of exposure to oxygen on the mitochondria of Astasia. To see whether short exposures to oxygen would affect isolated mitochondria, the respiration of mitochondria isolated from organisms grown under air+carbon dioxide was studied in the Warburg respirometer with either air or oxygen as the gas phase. The substrate (succinate) was tipped in after equilibration. Succinate-cytochrome *c* oxidoreductase and NADH-cytochrome *c* oxidoreductase activities were also measured on mitochondria isolated from air+carbon dioxide-grown cultures. The measurements were made in two

Fig. 3. Effect of exposure to oxygen on the rate of growth on subsequent incubation in air. The parent culture of *Astasia* was grown at 27° in defined medium with exposure to O₂+CO₂ (○), and had a doubling time of 15 hr. After 0 hr. (▲), 3 hr. (□) and 24 hr. (△) of exposure to oxygen, cells were transferred from the parent culture and grown with exposure to air. The three cultures growing in air had a doubling time of about 9.5 hr.

ways: first, by bubbling air+carbon dioxide or oxygen+carbon dioxide directly into the assay mixture in a Thunberg-type cuvette; secondly, by exposure of the mitochondrial fraction to 2 atm. of oxygen+carbon dioxide in a pressure chamber for 1 hr. before assaying in ordinary cuvettes. No appreciable effects on the respiration with succinate as substrate of the isolated mitochondria, or on the succinate-cytochrome *c* oxidoreductase or NADH-cytochrome *c* oxidoreductase activities were observed.

Mitochondria from cells grown under oxygen+carbon dioxide were therefore compared with mitochondria prepared from control air+carbon dioxide-grown cultures (Table 2). Mitochondria prepared from cells exposed to oxygen+carbon dioxide for about 24 hr. had greatly decreased succinate oxidase, succinate-cytochrome *c* oxidoreductase and NADH-cytochrome *c* oxidoreductase activities, as well as succinate dehydrogenase activity. The cytochrome oxidase activity, however, was only slightly inhibited. Several authors have been unable to demonstrate cytochrome oxidase activity in the mitochondria of *Astasia*

with phosphate buffer in the assay mixture (Webster & Hackett, 1965; V. Kahn & J. J. Blum, unpublished work). We also found very little activity in a

phosphate buffer, but in tris-hydrochloric acid buffer of the same pH (7.4) and molarity (33mM) the activity was easily measurable. The optimum conditions for the assay of the cytochrome oxidase activity of *Astasia* mitochondria were not determined.

Effects of oxygen treatment on some of the lipid constituents of Astasia mitochondria. Mitochondria from air- and oxygen-grown *Astasia* were extracted for ubiquinone by the method of Pumphrey & Redfearn (1960). The light petroleum extracts after evaporation and dilution in ethanol gave spectra characteristic of ergosterol. The extracts were therefore chromatographed with hexane-ethyl acetate (9:1, v/v) as the developing solvent. Two fractions were visible in daylight, a violet one with R_F 0.52 and a yellow one with R_F 0.71. The violet compound had a broad absorption band in the visible region, centred at about 500m μ , and a sharp absorption peak at 283m μ in the ultraviolet region, with a shoulder at 312m μ . On addition of borohydride, absorption in the visible region disappeared and the 283m μ peak shifted to 290m μ . Addition of sodium hydroxide or hydrochloric acid did not alter the spectrum of the original compound. The spectral properties are characteristic of rhodoquinone-45, which was isolated from *Euglena gracilis* by Powls & Hemming (1966a). This pigment was not found in rat-liver mitochondria extracted by the same method, but was found in mitochondria prepared from a streptomycin-bleached strain of *E. gracilis* (Blum & Bégin-Heick, 1967).

The yellow compound, R_F 0.71, had a sharp absorption peak at 273m μ that shifted to 290m μ and decreased in extinction on the addition of borohydride. When examined by thin-layer chromatography with several solvents, this compound ran with ubiquinone-30 and ubiquinone-50.

Fig. 4. Effect of growth in oxygen followed by growth in air on the respiration rate of *Astasia*. Cells were exposed to $O_2 + CO_2$ at 27° in defined medium for the time shown on the abscissae, and then exposed to air until 44 or 48 hr. (total in O_2 and air) had elapsed. Then the respiration rate of all the samples were measured in an air atmosphere. The two points shown with open triangles (Δ) are from different experiments in which cells were exposed to $O_2 + CO_2$ for 24 hr. and their respiration rate measured (in air) immediately. Each number represents an experimental point from one of the five experiments done.

Table 2. *Effect of oxygen on the activity of some respiratory-chain enzymes in Astasia mitochondria*

Assays for the enzymes are described in the Materials and Methods section. The units are defined as follows: succinate-cytochrome *c* oxidoreductase and NADH-cytochrome *c* oxidoreductase, m μ moles of cytochrome *c* reduced/min./mg. of protein; succinate oxidase, m μ moles of O_2 taken up/min./mg. of protein; succinate dehydrogenase, m μ moles of 2,6-dichlorophenol-indophenol reduced/min./mg. of protein.

Enzyme	Expt. no.	Enzyme activity (units)		Change in activity due to O_2 treatment (%)
		Air	O_2	
Succinate oxidase	1	58.6	15.5	-74
Succinate-cytochrome <i>c</i> oxidoreductase	1	180	34.0	-81
	2	168	45.3	-73
	3	158	35.2	-78
NADH-cytochrome <i>c</i> oxidoreductase	1	405	99	-74
	2	332	139	-58
	3	335	189	-45
Succinate dehydrogenase	3	136	25.5	-81
Cytochrome <i>c</i> - O_2 oxidoreductase	1	0.22	0.20	-9
	2	0.18	0.17	-6

Table 3. *Lipid fractions from mitochondria of Astasia*

Cells were grown in air + CO₂ (95 : 5) or O₂ + CO₂ (95 : 5) and the mitochondria isolated and treated as described in the text. The compounds having the spectra of ergosterol were pooled and extracted from the silica gel with ethanol. Extinction values are given as $E_{1\text{cm}}^{1\text{ml}}$ at $\lambda_{\text{max.}}$ /100 mg. of mitochondrial protein.

Ergosterol		Rhodoquinone	Ubiquinone-45
Compound ...	compounds		
R_F ...	0-0.5	0.52	0.71
Expt.	E_{260}	E_{283}	E_{273}
I Air	3.7	0.56	1.08
O ₂	5.1	1.08	1.44
II Air	3.3	1.31	1.33
O ₂	5.0	3.10	1.42
III Air	—	0.50	1.20
O ₂	—	1.28	1.76

When treated by reverse-phase chromatography, the ubiquinone of *Astasia* ran with ubiquinone-45 in acetone-water (9 : 1, v/v). Ubiquinone-45 has also been found to be the ubiquinone of *E. gracilis* (Threlfall & Goodwin, 1964). (Ubiquinones are numbered by the number of carbon atoms that they contain.)

Several fractions were also seen under ultraviolet light. Those having R_F 0-0.5 have absorption spectra characteristic of ergosterol (peaks at 260, 271, 282 and 293 m μ ; Morton & de Gouveia, 1934). These fractions were not investigated further.

The relative amount of these fractions in mitochondria from air + carbon dioxide- and from oxygen + carbon dioxide-grown cells are reported in Table 3. The ergosterol-like compounds almost doubled in amount during growth in oxygen + carbon dioxide, the ubiquinone increased slightly and the rhodoquinone more than doubled.

DISCUSSION

In many systems, the toxic effects of oxygen are manifested only at high pressures. *Astasia* is sensitive to oxygen at atmospheric pressure and, since it grows on a chemically defined medium, it offers a convenient tool for the study of oxygen toxicity. The effects of oxygen on the growth of *Astasia* are very rapid. Treatment of the cells with 95% oxygen results in a 50% inhibition of growth with no appreciable delay, and similarly, growth resumes at a normal rate immediately after the cells are returned to an air atmosphere. Unlike HeLa cells (Rueckert & Mueller, 1960) *Astasia* undergoes no change in DNA, RNA and protein content when its growth is inhibited by oxygen treatment. The effects of oxygen on respiration, however, are dependent both on the time of exposure to oxygen +

carbon dioxide and on the time allowed for recovery in air + carbon dioxide. Growth under oxygen + carbon dioxide produces a decrease in the rate of respiration of the whole cell that is presumably due to a mitochondrial lesion, as evidenced by the 75% decrease in succinate oxidase activity of the mitochondria. The effect of oxygen on the mitochondria can be further localized at the level of succinate dehydrogenase. There is at least a 75% decrease in the activity of this enzyme, which is sufficient to account entirely for the decrease in activity of the succinate-cytochrome *c* oxidoreductase and succinate oxidase activities. Furthermore, it was found that the activity of cytochrome *c*-oxygen oxidoreductase is very little affected by oxygen treatment. The inhibition of succinate dehydrogenase, however, cannot account for the inhibition of NADH-cytochrome *c* oxidoreductase and the possibility remains that oxygen has more than one site of action on the respiratory chain.

It is noteworthy that the lipid composition of *Astasia* mitochondria is altered by exposure of the cells to oxygen + carbon dioxide (95 : 5). Although there is little change in ubiquinone content, there are twofold increases in the content of ergosterol compounds and rhodoquinone. Rhodoquinone was found in equal quantities in etiolated and in green *E. gracilis* by Powls & Hemming (1966a), who suggested that it is derived from ubiquinone-45 (Powls & Hemming, 1966b). Powls & Hemming (1966a) could not demonstrate the presence of this pigment in the chloroplasts of *Euglena* and suggested that it might be present in mitochondria and function in electron transport. The present data further support this view, since this pigment has now been found in mitochondrial preparations from streptomycin-bleached *Euglena* and from the naturally achlorotic *Astasia*. The increased content of rhodoquinone in the mitochondria of oxygen-treated *Astasia* is consistent with the suggestion that this pigment functions in electron transport.

We are indebted to Mr Alvernon Hayes and to Mrs Betty Horton for excellent technical assistance. Dr Paul Hochstein provided some of the laboratory facilities and many helpful ideas and suggestions at all stages of this work. This work was supported by Grants GB-2788 and GB-5554 from the National Science Foundation, and by Grant Ro1-HD-01269 from the National Institutes of Health. J.J.B. is the recipient of Research Career Development Award 5-K3-GM-2341 from the National Institutes of Health.

REFERENCES

- Arrigoni, O. & Singer, T. P. (1962). *Nature, Lond.*, **193**, 1256.
- Bert, P. (1878). *Barometric Pressure Researches in Experimental Physiology*, pp. 1021-1024. Translated (1943) by Hitchcock, M. A. & Hitchcock, F. A. Columbus, Ohio: College Book Co.

- Blum, J. J. & Bégin-Heick, N. (1967). *Biochem. J.* **105**, 821.
- Blum, J. J. & Padilla, G. M. (1962). *Exp. Cell. Res.* **28**, 512.
- Brosemer, R. W. & Rutter, W. J. (1961). *Exp. Cell Res.* **25**, 101.
- Buetow, D. & Buchanan, P. J. (1964). *Exp. Cell Res.* **36**, 204.
- Buetow, D. & Levedahl, B. (1962). *J. gen. Microbiol.* **28**, 579.
- Chance, B., Jamieson, D. & Coles, H. (1965). *Nature, Lond.*, **206**, 257.
- Cleveland, L. R. & Burke, A. W. (1956). *J. Protozool.* **3**, 74.
- Davies, H. C. & Davies, R. E. (1965). *Handbook of Physiology*, vol. 3, section 3, p. 1047. Washington, D.C.: American Physiological Society.
- Jamieson, D. & Chance, B. (1966). *Biochem. J.* **100**, 254.
- Kahn, V. & Blum, J. J. (1967). *Biochemistry*, **6**, 817.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Margoliash, E. (1954). *Biochem. J.* **56**, 535.
- Morton, R. A. & de Gouveia, A. J. A. (1934). *J. chem. Soc.* p. 911.
- Powls, R. & Hemming, F. W. (1966a). *Phytochemistry*, **5**, 1235.
- Powls, R. & Hemming, F. W. (1966b). *Phytochemistry*, **5**, 1249.
- Pumphrey, A. M. & Redfearn, E. R. (1960). *Biochem. J.* **76**, 61.
- Rueckert, R. R. & Mueller, G. C. (1960). *Cancer Res.* **20**, 944.
- Savage, N. (1957). *Biochem. J.* **67**, 146.
- Smith, L. (1954). *Arch. Biochem. Biophys.* **50**, 285.
- Smith, L. (1955). In *Methods in Enzymology*, vol. 2, p. 732. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Threlfall, D. R. & Goodwin, T. W. (1964). *Biochem. J.* **96**, 40P.
- Webster, D. A. & Hackett, D. P. (1965). *Plant Physiol.* **40**, 1091.
- Wittner, M. (1957a). *J. Protozool.* **4**, 20.
- Wittner, M. (1957b). *J. Protozool.* **4**, 24.
- Wolin, M. J., Evans, J. B. & Niven, C. F., jun. (1955). *Arch. Biochem. Biophys.* **58**, 356.