BBA 45773

QUINONES OF THE CHLOROBACTERIACEAE
PROPERTIES AND POSSIBLE FUNCTION

R. POWLS* AND E. R. REDFEARN**
Department of Biochemistry, University of Leicester, Leicester (Great Britain)
(Received October 28th, 1968)

SUMMARY

1. Quinone concentrations in *Chlorobium thiosuphatophilum* and *Chloropseudomonas ethylicum* have been determined under various growth conditions as a guide to their functions.

2. Three quinones were isolated from both organisms; menaquinone-7, chlorobiumquinone and a polar menaquinone, probably 1'-hydroxymenaquinone-7.

3. The concentration of chlorobiumquinone and the polar menaquinone varied in the two organisms; 87 and 2 mmoles per mole of chlorophyll respectively in *C. thiosulphatophilum*, and 6 and 0.6 mmoles per mole of chlorophyll in ethanol-grown *Cps. ethylicum*. Whereas the menaquinone-7 concentration was of the same order in the two organisms, 70 and 75 mmoles per mole of chlorophyll.

4. Resuspension of *Cps. ethylicum* in autotrophic medium caused a fall in the menaquinone-7 concentration to 46 mmoles per mole of chlorophyll, accompanied by increases of chlorobiumquinone and the polar menaquinone concentrations to 52 and 8 mmoles per mole of chlorophyll respectively.

5. Since chlorobiumquinone is virtually absent in ethanol-grown cells, but appears on adaptation to the sulphide medium, it is likely to be involved in the photooxidation of sulphide. Menaquinone-7 could well be involved in a cyclic phosphorylation pathway of both organisms.

6. The polar menaquinone probably is a precursor of chlorobiumquinone as its concentration is high only when the rate of chlorobiumquinone biosynthesis is high. Moreover the polar menaquinone breaks down into chlorobiumquinone *in vitro*.

INTRODUCTION

The green photosynthetic bacteria, the Chlorobacteriaceae, differ from all other photosynthetic organisms in the chemical nature of their photosynthetic pigments. Their characteristic chlorophylls, the chlorobium chlorophylls, are particularly unusual in having farnesol rather than the usual phytol as the esterified alcohol, as well as a considerably modified porphyrin system[1]. The carotenoids also differ from

Abbreviation: NMR, nuclear magnetic resonance.

* Present address: University of London King's College, Botany Department, 68 Half Moon Lane, London, S.E. 24.
** Deceased March, 1968.

those of other photosynthetic organisms[2]. This originality has also been found to extend to their quinones. FULLER *et al.*[3] found that in contrast to other photosynthetic bacteria, *Chlorobium thiosulphatophilum* lacked ubiquinone. A reducible quinone was present showing maximal absorption at 254 nm. FRYDMAN AND RAPOPORT[4] identified this compound as a derivative of menaquinone-7 in which the first methylene of the normal polyisoprenoid side-chain was omitted in order to explain the unusual features in the ultraviolet and NMR spectra of the quinone. This compound was given the trivial name chlorobiumquinone.

Various experimental approaches have led to the suggestion that quinones are involved in photosynthetic electron transfer. In higher plants and algae, the localization of plastoquinone-9 in the chloroplasts, the extraction and reactivation experiments of BISHOP[5], as well as the demonstration of the photoreduction of endogenous quinone[6] are strong evidence for the implication of plastoquinone as a functional member of the photosynthetic electron-transfer system. It has even been suggested that quinones may be directly involved in the phosphorylation reactions. LEDERER AND VILKAS[7] have suggested a phosphorylation scheme involving the intermediate formation of chromanol 6-phosphate. The only evidence for such an involvement in photosynthetic phosphorylation is the isolation of plastochromanol from many plants[8].

An insight into quinone function can be obtained when they show variations in concentration under different growth conditions. Concentrations of quinones involved in photosynthesis fall when organisms are grown heterotrophically. In *Euglena gracilis* grown in the dark, ubiquinone but not plastoquinone is present; in contrast to the light grown organism which has both quinones[3].

The different conditions under which the Chlorobacteriaceae can be cultured offer scope for looking at any differences in their quinone concentrations. *C. thiosulphatophilum* is an obligate sulphur bacterium which grows autotrophically although it will utilise acetate as a carbon source, provided sulphide or thiosulphate is used as a source of reducing equivalents and CO_2 is also available[9]. The recently discovered *Chloropseudomonas ethylicum*[10] can grow in strictly autotrophic media, however, it also shows photoheterotrophic tendencies being able to utilise ethanol and other carbon compounds even in the absence of sulphide. This approach has been used to suggest roles for the quinones in these organisms. A preliminary report of some of this work has already been published[11].

METHODS

Organisms and culture conditions

Two organisms were used, *C. thiosulphatophilum* NCIB 8346 and *Cps. ethylicum* 2K a gift from Dr. HOOGENHOUT, The State University, Leiden. The culture and maintenance of *C. thiosulphatophilum* was as described by LARSEN[12], *Cps. ethylicum* as described by BOSE[13]. The suphide suspending medium for *Cps. ethylicum* was the same as the ethanol medium apart for the absence of ethanol and an increase in the sulphide concentration to 0.5 g/l.

After inoculation with about 250 ml of an actively growing culture of either organism, extra medium was added to completely fill 5-l glass bottles, the necks of which were sealed with rubber bungs. Four such bottles were placed 4 feet from two

60-W incandescent bulbs and incubated at 30°. Growth was usually complete in 5–6 days, when the cells were harvested in a Sharples supercentrifuge. The bacterial cell paste was either extracted immediately or stored at —20° prior to extraction.

Lipid extraction

The bacterial lipid was extracted by homogenization of the cell paste with ice-cold methanol in a Potter–Elvehjem homogenizer. The cell debris was removed by centrifugation and the extracts further clarified by passage through a sintered-glass funnel. The debris was re-extracted with methanol until no further chlorophyll could be extracted. Four extractions with methanol were sufficient. The dark grey cell mass was extracted with diethyl ether. To the combined extracts, in a separating funnel an equal volume of diethyl ether was added followed by the careful addition of excess 2% aqueous $(NH_4)_2SO_4$ solution. By gently swirling the lipid was transferred into the ethereal phase, the methanol being washed out into the aqueous phase. The ethereal extract was washed twice with $(NH_4)_2SO_4$ solution, followed finally with water, before being dried over anhydrous Na_2SO_4. After 30 min the Na_2SO_4 was filtered off and the extract taken to dryness *in vacuo* at room temperature using a rotary evaporator. The final small volume of ether was removed under a stream of N_2.

Chromatography

Chromatography on a column of silicic acid–Celite (2:1) was employed for a preliminary fractionation of the lipid. The lipid was dissolved in the minimum volume of diethyl ether, light petroleum (b.p. 40–60°) was added until the resulting solution had a composition of 25% diethyl ether and 75% light petroleum. This solution was applied to the column, chlorophyll and other polar lipids were adsorbed whereas the non-polar lipid passed through. The column was washed with diethyl ether–light petroleum (3:7, by vol.) until all the lipids less polar than chlorophyll had been eluted. The eluted lipid was taken to dryness as described previously.

In the case of *C. thiosulphatophilum* where the electron donor had been sulphide, the lipid contained considerable sulphur. This could be removed by further chromatography. The lipid was applied to the column in light petroleum and the column washed with diethyl ether–light petroleum (1:99, by vol.), when the chlorobactene moved slowly down the column as a deep orange band. All material eluted prior to the carotenoid was discarded and the column then eluted completely with diethyl ether. When this fraction was taken to dryness it was found to be completely free of sulphur. This step was not necessary with ethanol-grown *Cps. ethylicum*.

The sulphur-free lipid was separated into its constituents by thin-layer chromatography with diisopropyl ether–light petroleum (15:85, by vol.) on 400-μ layers of silica gel. Ultraviolet absorbing compounds were located by their quenching of fluorescence in ultraviolet light after spraying the whole of the plate with 0.01% sodium fluorescein in ethanol. At the same time reduced naphthaquinone compounds could be visualized by their blue fluorescence. Compounds of interest were removed from the adsorbent by three extractions with diethyl ether. The ether was then removed under a stream of N_2.

Quinone isoprenologues were identified by thin-layer chromatography on silica gel impregnated with liquid paraffin or on thin layers of polyamide.

Spectroscopy

Ultraviolet spectra were determined with a Unicam SP. 800 recording spectrophotometer. Spectra involving quantitative determinations were checked with the Unicam SP. 500. Spectroscopic-grade ethanol or cyclohexane was used in all cases.

Menaquinone-7 was estimated from the difference in the absorption of its cyclohexane solution between 270 and 290 nm using $\Delta\varepsilon$ of 15 900 (ref. 14),

Chlorobiumquinone was estimated from its maximal absorption at 251 nm in cyclohexane using ε of 16 300 (ref. 4).

The polar menaquinone was estimated from the absorption difference at 246 nm before and after reduction of a buffered ethanol solution with $NaBH_4$ (ref. 15). The $\Delta\varepsilon$ of 20 700 found for desmethylmenaquinone was used.

The fluorescent chromanol-like compound which had a similar absorption spectrum to that of phyllochromanol was estimated from its maximal absorption at 245 nm in cyclohexane using ε of 26 600 determined for phyllochromanol[16].

Chlorophyll was estimated by taking an aliquot of the diethyl ether extract after drying, prior to chromatography. The chlorophyll of both organisms was chlorobium chlorophyll 660 and was estimated directly from its absorption at 660 nm in diethyl ether. A mean molecular weight of 864 (ref. 1) and a specific absorption coefficient of 112.5 (ref. 18) were used.

Polarography

Oxidation–reduction potentials of quinones were determined as described by MORET, PINAMONTI AND FORNASARI[19]. As the oxidation and reduction was found to be reversible at the electrode the half wave corresponded to the oxidation–reduction potential of the quinone at the pH used (7.0).

RESULTS AND DISCUSSION

Thin-layer chromatography with diisopropyl ether–light petroleum (15:85, by vol.) of the lipid from both organisms enabled four compounds to be isolated. Three of these compounds absorbed ultraviolet light whereas the fourth (R_F 0.52) had a strong blue fluorescence in ultraviolet light. The three compounds were identified as a menaquinone (R_F 0.68), chlorobiumquinone (R_F 0.34) and a polar menaquinone (R_F 0.11) from their ultraviolet spectra.

Menaquinone

After elution of the least polar ultraviolet absorbing compound, crystallization from ethanol yielded pale yellow crystals (m.p. 50–51°). These crystals dissolved in cyclohexane had the typical menaquinone spectrum, λ_{max} 243, 248, 261, 270 and 327 nm. Reversed phase thin-layer chromatography with water–acetone (5:95, by vol.) failed to separate the isolated compounds from authentic menaquinone-7 (R_F 0.50). Positive identification as menaquinone-7 was achieved by NMR. Determination of the oxidation–reduction potential gave a value of —0.081 V.

Chlorobiumquinone

Recent work from this laboratory[20] has shown that the published structure[4] for chlorobiumquinone to be in error and this compound to be 1′-oxomenaquinone-7.

Thin-layer chromatography with chloroform–benzene (1:1, by vol.) separated chlorobiumquinone into two ultraviolet absorbing components (R_F 0.82 and 0.71). Both these had a typical chlorobiumquinone spectrum, λ_{max} 251 nm with pronounced shoulders at 245, 257 and 267 nm in cyclohexane. In ethanol the absorption maximum shifts to 254 nm and is accompanied by a marked shoulder at 265 nm. On reduction with $NaBH_4$ in buffered ethanol, the absorption at 354 nm decreased, being replaced by the typical naphthaquinol absorption at 247 nm. FULLER *et al.*[3] and also FRYDMAN AND RAPOPORT[4] report only an inflexion at 251 nm on reduction, but as no precautions were taken to prevent a rise in pH, probably alkali destruction of the naphthaquinone occurred. On the basis of their absorption at 251 nm, the more polar compound chlorobiumquinone-2, represented 85% of the total quinone. The remaining 15% being accounted for by chlorobiumquinone-1. These two compounds were inseparable by thin-layer chromatography on polyamide, R_F 0.36 with methanol–methyl ethyl ketone–water (20:10:3 by vol.), and also on paraffin-impregnated silica gel, R_F 0.71 with water–acetone (5:95, by vol.). Chlorobiumquinone-2 crystallized readily from ethanol as pale yellow crystals (m.p. 47–48°), whereas crystallization of chlorobiumquinone-1 was more difficult and gave darker crystals (m.p. 42–43°). Since these compounds could not be separated by partition thin-layer chromatography they cannot be isoprenologues. A similar phenomenon has recently been reported[21]. The menaquinone isolated from *Mycobacterium phlei* has been separated into two components by adsorption thin-layer chromatography although it behaved as a single entity on partition thin-layer chromatography. These compounds have been identified as geometric isomers in the ring-terminal isoprene unit. They can be differentiated by their NMR absorption. The *cis*-isomer absorbs at 8.36 τ, the *trans*-isomer at 8.26 τ. Unfortunately these values cannot be used for chlorobiumquinone, as the oxo group in the ring-terminal isoprene unit considerably interferes with this absorption. As a result the absorption of the relevant methyl group in chlorobiumquinone-2 is at 7.78 τ (ref. 20). However in comparing the chromatographic properties and relative abundance of the *M. phlei* menaquinone isomers it seems likely that chlorobiumquinone-1 is the *cis*-isomer and chlorobiumquinone 2 the *trans*-isomer. This identification must remain tentative until enough chlorobiumquinone-1 has been obtained for NMR studies.

Polarography gave an oxidation–reduction potential of + 0.39 V.

Polar menaquinone

An ultraviolet absorbing compound R_F 0.11 in diisopropyl ether–light petroleum (15:95, by vol.), running with a carotenoid was isolated from the lipid of both bacteria. Further thin-layer chromatography with chloroform–benzene (1:1, by vol.) completely separated this compound (R_F 0.42) from the carotenoid. The purified compound, which was a pale yellow oil, showed λ_{max} 244.5 and 250 nm with small peaks at 261, 270 and 330 nm. In some respects this spectrum resembled that of the menaquinones, but it did show significant differences (Fig. 1). The benzenoid absorption bands at 245 and 250 nm are virtually the same as the typical menaquinone. The quinonoid bands, despite their similar position are extremely weak, a shoulder at 270 nm and an inflexion at 260 nm, in contrast to the sharp bands of the menaquinones[22]. However, there is no doubt that this compound is a quinone as its buffered ethanolic solution shows the spectral changes typical of the reduction of naphthaquinones (Fig. 2). The

infrared spectrum of this compound resembled that of menaquinone-7 apart for the presence of a hydroxyl group which would account for the increased polarity.

Hydroxyphylloquinone isolated from *Anacystis nidulans* had an R_F of 0.30 on thin-layer chromatography with chloroform–benzene (1:1, by vol.) in contrast to that of the polar menaquinone from the bacteria, R_F 0.42. Hydroxyphylloquinone has an identical ultraviolet absorption spectrum to that of phylloquinone. This is only to be expected as the hydroxyl group is considered to be located in one of the isoprene units distal to the nucleus[23]. For this reason it is probable that the hydroxyl group of the polar menaquinone is very close to the nucleus. Especially as its ultraviolet spectrum differs far more from the typical menaquinone spectrum than does 3'-hydroxyphylloquinone[24].

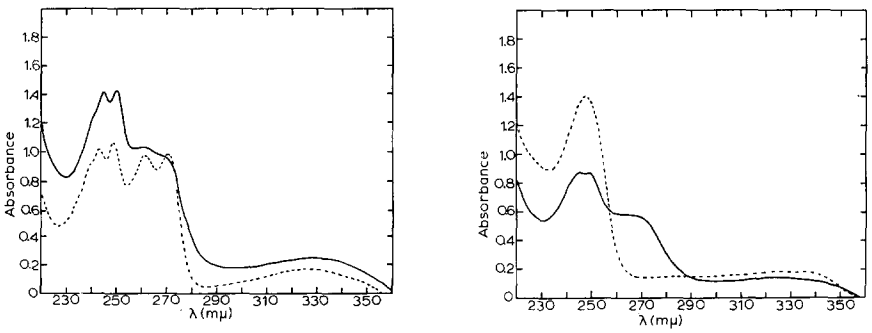

Fig. 1. Ultraviolet absorption spectra of cyclohexane solutions of the menaquinones isolated from the Chlorobacteriaceae. ·····, menaquinone-7; ————, polar menaquinone.

Fig. 2. Ultraviolet absorption spectrum of a buffered ethanolic solution of the polar menaquinone. ————, oxidized form; ·····, after reduction with $NaBH_4$.

Previously purified samples of the polar menaquinone were found to consistently undergo a partial breakdown into chlorobiumquinone on storage. This suggests that the polar menaquinone is probably 1'-hydroxymenaquinone-7, oxidation of which would give chlorobiumquinone. The proximity of the hydroxyl group to the nucleus in this compound would be expected to modify considerably the typical menaquinone absorption spectrum.

Fluorescent compound

We had previously identified this compound as menachromanol-6 (ref. 25) however it now appears that this was in error. Although the ultraviolet spectrum resembled that of phyllochromanol it was not identical. On purification the bacterial compound showed λ_{max} 245, 321 and 334 nm; whereas synthetic phyllochromanol had the same spectrum except that it was displaced 4 nm towards higher wavelengths.

Oxidation of phyllochromanol with either $HAuCl_4$ or $FeCl_3$ gave 3'-hydroxyphylloquinone. However in the case of the bacterial compound neither of these oxidizing agents gave the hydroxyquinone. Unexpectedly they both yielded a quinone with R_F 0.62 on thin-layer chromatography with diisopropyl ether–light petroleum (1:10, by vol.). This quinone after crystallization from ethanol as pale yellow crystals was found to show λ_{max} 244, 249, 255, 264 and 327 nm, an identical spectrum to that of synthetic desmethylphylloquinone. The polarity of the oxidation product in

partition chromatography indicated that it was desmethylmenaquinone-7. These oxidation reactions are very puzzling, at present no structure can be put forward for the bacterial compound which would account for these reactions. It is unlikely to have a nuclear methyl group in view of its oxidation to desmethylmenaquinone.

Effect of growth conditions on quinone concentrations

In contrast to SHAPOSHNIKOV, KONDRATIEVA AND FEDOROV[10] who first isolated the organism we could not get any growth of *Cps. ethylicum* in autotrophic medium. It is interesting that CALLELY AND FULLER[27] were also unable to grow this organism autotrophically, finding that a two-carbon unit was required for growth.

However it was found that lipid extracted from this organism after inoculation into the autotrophic medium contained more chlorobiumquinone than did the original ethanol-grown inoculum even though no growth had occurred. Resuspension of the ethanol-grown cells had the same effect. In order to determine the quinone concentrations under the different growth conditions, ethanol-grown *Cps. ethylicum* was harvested and washed with the autotrophic sulphide medium. A portion of these cells were taken for quinone estimation and the remainder resuspended in the sulphide medium using a mechanical Potter–Elvehjem homogenizer. On illumination a precipitation of sulphur occurred as the organism oxidized the sulphide. After 38 h the sulphide-adapted cells were reharvested, extracted and the quinones estimated as previously described.

The concentrations of the quinones in the two bacteria under the different growth conditions are shown in Table I.

In these organisms the concentration of menaquinone-7 was of the same order as that of plastoquinone in higher plants[26]; 70 and 75 mmoles per mole of chlorophyll in *C. thiosulphatophilum* and *Cps. ethylicum* respectively. The cytochrome concentration in *C. thiosulphatophilum* has been shown to be 6.5 mmoles per mole of chlorophyll. Thus the concentration of the quinones is considerably in excess of the cytochromes as in higher plants.

TABLE I

CONCENTRATION OF QUINONES IN THE CHLOROBACTERIACEAE

Mean values ± S.E. with number of experiments in parentheses.

Species and growth conditions	*Quinone concn. (mmoles/mole chlorophyll)*			
	Menaquinone-7	*Chlorobium-quinone*	*Polar menaquinone*	*Fluorescent chromanol-like compound*
C. thiosulphatophilum on sulphide and bicarbonate	70.5 ± 3.4 (3)	86.9 ± 5.8 (3)	2.3 ± 0.4 (3)	23.1 ± 1.1 (3)
Cps. ethylicum on ethanol and bicarbonate	75.5 ± 4.3 (6)	6.0 ± 2.0 (6)	0.6 ± 0.1 (6)	19.6 ± 2.7 (6)
Cps. ethylicum on ethanol and bicarbonate, transferred to sulphide and bicarbonate	46.6 ± 5.3 (6)	51.8 ± 7.1 (6)	7.9 ± 2.2 (6)	21.6 ± 2.0 (6)

The concentration of chlorobiumquinone is of the same order as that of the menaquinone in *C. thiosulphatophilum* whereas in ethanol-grown *Cps. ethylicum* there is a fifteen-fold drop in the chlorobiumquinone concentration. The concentration of the polar menaquinone showed a similar difference in the two organisms. When the ethanol-grown *Cps. ethylicum* was resuspended in the sulphide medium considerable changes in the concentrations of the quinones occurred. There was a fall in the concentration of menaquinone-7 whereas the concentration of both chlorobiumquinone and the polar menaquinone increased, the latter surprisingly even to a concentration in excess of that found in *C. thiosulphatophilum.*

Cps. ethylicum is unable to grow in the sulphide medium since it requires a two-carbon unit for growth[27]. In contrast to *C. thiosulphatophilum* it is unable to synthesize a three-carbon unit when CO_2 is the only source of carbon. When ethanol-grown *Cps. ethylicum* is resuspended in the illuminated sulphide medium it photoxidizes the sulphide producing a copius precipitate of sulphur. Under these conditions there is a vast increase in the chlorobiumquinone content which was virtually absent in the ethanol-grown cells. This suggests that chlorobiumquinone is not involved in the photoxidation of ethanol in *Cps. ethylicum* but is probably involved in the transfer of electrons from sulphide to chlorophyll. The presence of chlorobiumquinone in *C. thiosuphatophilum* supports this hypothesis as this organism was using sulphide as the sole electron donor.

As the concentration of menaquinone-7 is of the same order in ethanol-grown *Cps. ethylicum* as in *C. thiosulphatophilum* this compound may be involved in a cyclic photophosphorylation pathway or alternatively a pathway which is common to the photoxidation of sulphide and ethanol. In view of this suggestion it would be interesting to see if a decrease in the concentrations of menaquinone-7 and chlorobiumquinone occurs when *C. thiosulphatophilum* is grown with hydrogen rather than sulphide as the electron donor. In this case reducing power in the form of reduced ferredoxin would be formed from hydrogen, mediated by hydrogenase. Under these conditions light would only be required to produce ATP, probably by a cyclic pathway.

The polar menaquinone partially decomposed to chlorobiumquinone *in vitro*. It probably also acts as a precursor *in vivo*, its concentration being high only when that of chlorobiumquinone is also high. This would explain the very high concentration in *Cps. ethylicum* when resuspended in the sulphide medium, since under these conditions chlorobiumquinone synthesis is higher than at any other time. As the increase in chlorobiumquinone on adaptation to the sulphide medium is accompanied by a decrease in menaquinone-7 concentration, it is possible that this compound is also acting as a precursor of chlorobiumquinone. It is noticeable, however, that the sum of the menaquinone-7 and the chlorobiumquinone concentrations is appreciably higher after adaptation to the sulphide medium than it was in the ethanol-grown cells. This can be explained by conversion into chlorobiumquinone of precursors, the organism had already synthesized whilst growing on ethanol. However, on resuspension in the sulphide medium no further precursors can be synthesized as no assimilatable carbon compounds are then available. Consequently the organism mobilizes its menaquinone-7 and converts this into chlorobiumquinone which the organism requires to photoxidize the sulphide. This interconversion probably also occurs by way of the polar menaquinone.

In contrast to all the quinones the concentration of the fluorescent chromanol-

like compound was the same in the two organisms and no change occurred when *Cps. ethylicum* was resuspended in sulphide medium.

During this work no evidence was obtained for the presence of any of the intermediates or derivatives of them, suggested in the phosphorylation scheme of LEDERER AND VILKAS[7]. Menachromanol was never found, the polar menaquinone isolated was not 3′-hydroxymenaquinone which would be formed by the oxidation of the chromanol. Attempts to verify such a scheme (*e.g.* ref. 28) in various phosphorylating preparations have proved to be unsuccessful. Furthermore all current mechanisms for oxidative and photosynthetic phosphorytlaion (*e.g.* ref. 29) exclude the formation of phosphate derivatives of the electron-transfer components.

ACKNOWLEDGMENTS

We thank the Science Research Council for financial support and for the award of a Research Fellowship to R.P. We also thank Dr. P. D. J. WEITZMAN for determining quinone redox potentials and Miss J. M. FARR for technical assistance.

REFERENCES

1 A. S. HOLT, in L. P. VERNON AND G. R. SEELY, *The Chlorophylls*, Academic Press, New York, 1966, p. 111.
2 L. JENSEN, E. HEGGE AND L. M. JACKSON, *Acta Chem. Scand.*, 18 (1964) 1703.
3 R. C. FULLER, R. M. SMILLIE, N. RIGOPOULOS AND V. YOUNT, *Arch. Biochem. Biophys.*, 95 (1961) 197.
4 B. FRYDMAN AND H. RAPOPORT, *J. Am. Chem. Soc.*, 85 (1963) 823.
5 N. I. BISHOP, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 1696.
6 F. L. CRANE, B. EHRLICH AND L. P. KEGEL, *Biochem. Biophys. Res. Commun.*, 3 (1960) 37.
7 E. LEDERER AND M. VILKAS, *Vitamins Hormones*, 24 (1966) 409.
8 K. J. WHITTLE, P. J. DUNPHY AND J. F. PENNOCK, *Biochem. J.*, 96 (1965) 17 C.
9 D. S. HOARE AND J. GIBSON, *Biochem. J.*, 91 (1964) 547.
10 V. N. SHAPOSHNIKOV, E. N. KONDRATIEVA AND V. D. FEDOROV, *Nature*, 187 (1960) 167.
11 E. R. REDFEARN AND R. POWLS, *Biochem. J.*, 106 (1968) 50 P.
12 H. LARSEN, *J. Bacteriol.*, 64 (1952) 187.
13 S. K. BOSE, in H. GEST, A. SAN PIETRO AND L. P. VERNON, *Bacterial Photosynthesis*, Antioch Press, Yellow Springs, Ohio, 1963, p. 501.
14 D. H. L. BISHOP, Thesis, University of Liverpool, England, 1962.
15 R. L. LESTER, D. C. WHITE AND S. L. SMITH, *Biochemistry*, 3 (1964) 949.
16 R. H. BAUM AND M. I. DOLIN, *J. Biol. Chem.*, 240 (1965) 3425.
17 L. H. CHEN AND R. D. DALLAM, *Nature*, 198 (1963) 386.
18 R. Y. STANIER AND J. H. C. SMITH, *Biochim. Biophys. Acta*, 41 (1960) 478.
19 V. MORET, S. PINAMONTI AND E. FORNASARI, *Biochim. Biophys. Acta*, 54 (1961) 381.
20 R. POWLS, E. R. REDFEARN AND S. TRIPPETT, *Biochem. Biophys. Res. Commun.*, 33 (1968) 408.
21 P. J. DUNPHY, D. L. GUTNICK, P. G. PHILLIPS AND A. F. BRODIE, *J. Biol. Chem.*, 243 (1968) 398.
22 D. T. EWING, J. M. VANDENBELT AND O. KAMM, *J. Biol. Chem.*, 131 (1939) 345.
23 C. F. ALLEN, H. FRANKE AND O. HIRAYAMA, *Biochem. Biophys. Res. Commun.*, 26 (1967) 562.
24 P. MAMONT, P. COHEN, R. AZERAD AND M. VILKAS, *Bull. Soc. Chim. France*, (1965) 2513.
25 R. POWLS AND E. R. REDFEARN, *Biochem. J.*, 102 (1967) 3 C.
26 E. R. REDFEARN, in R. A. MORTON, *Biochemistry of Quinones*, Academic Press, New York, 1965, p. 149.
27 A. G. CALLELY AND R. C. FULLER, *Biochem. J.*, 103 (1967) 74 P.
28 C. E. HORTH, D. MCHALE, L. R. JEFFRIES, S. A. PRICE, A. T. DIPLOCK AND J. GREEN, *Biochem. J.*, 100 (1966) 424.
29 E. C. SLATER, in M. FLORKIN AND E. H. STOTZ, *Comprehensive Biochemistry*, Vol. 14, Elsevier, Amsterdam, 1966, p. 327.