BBA 75031

EFFECTS OF DIPHENYLAMINE ON CAROTENOIDS AND MENAQUINONES IN BACTERIAL MEMBRANES

M. R. J. SALTON AND MARGRETH D. SCHMITT

Department of Microbiology, New York University School of Medicine, New York University Medical Center, New York, N.Y. (U.S.A.)

(Received July 28th, 1966)

SUMMARY

When *Micrococcus lysodeikticus* was grown in the presence of 12.5, 25 and 50 μg diphenylamine per ml medium, the formation of the more conjugated carotenoids in the membranes was inhibited to the extent of 66–97%; the corresponding percentage inhibition values for *Sarcina lutea* membranes ranged from 83% to 98%.

Menaquinone-9 was identified in the membranes of *M. lysodeikticus* and *S. lutea* and the contents of this compound in the isolated membranes were 7.8–12.1 μmoles/g and 3.8–4.4 μmoles/g, respectively, thus constituting up to about 4% of the membrane lipid. *Bacillus licheniformis*, *Bacillus megaterium* and *Bacillus subtilis* possessed menaquinone-7 in their membranes and the contents were 3.2, 8.4 and 10.0 μmoles per g membrane, respectively. At diphenylamine concentrations of 25–50 μg per ml medium, which reduced the carotenoid contents by 90% or more, the effects on the menaquinone contents of *M. lysodeikticus* and *S. lutea* membranes were variable, showing a reduction of 3–23% at a diphenylamine concn. of 50 μg per ml while at the 12.5 μg diphenylamine per ml level the contents were 12–34% higher than those of the control preparations. With the non-pigmented *B. megaterium*, diphenylamine concentrations of 12.5 and 25 μg per ml medium reduced the content of menaquinone-7 by about 10%.

Diphenylamine was taken up by both growing cells and washed cell suspensions and was detected in the membrane lipid fraction. Diphenylamine was specifically localized in the membrane fractions isolated from cells grown in its presence and from washed cells incubated in buffer containing diphenylamine. *B. megaterium* grown in the presence of 25 μg diphenylamine per ml contained up to 1 mg diphenylamine per g membrane.

INTRODUCTION

It has been known for some time that many microorganisms can achieve normal growth yields in media containing concentrations of diphenylamine which completely suppress the formation of the more unsaturated carotenoids[1–4]. In

addition to this action, other effects attributed to diphenylamine have included interference with ubiquinone (coenzyme Q) biosynthesis[5], inhibitory effects on electron transport systems in plant mitochondria[6] and modification of the fatty acid composition of bacterial membrane lipids[7]. From all of these earlier studies it is apparent that diphenylamine acts, by mechanisms as yet unknown, upon membrane-localized cellular constituents or systems such as those of the electron transport chain which are part of, or intimately associated with, cell membranes.

In a previous study we showed that several organisms in which the more unsaturated carotenoids were localized in the membrane structures, were able to form normal amounts of membrane in the presence of diphenylamine[8] although the formation of the pigments was completely inhibited. Thus, from our studies[7-9] it would appear that these more conjugated carotenoids are not absolutely essential for the formation or normal functioning of the bacterial membranes. As diphenylamine is obviously influencing the formation of certain membrane constituents, a study was undertaken to compare its relative inhibitory effects upon the membrane carotenoid and another class of compounds, the menaquinones (IUPAC–IUB Commission on Biochemical Nomenclature[10]), which have been shown to be largely confined to the bacterial membrane or envelope structures[11]. In addition, we have investigated the possibility that diphenylamine is taken up selectively into the membrane structures.

MATERIALS AND METHODS

Bacteria and growth conditions

Strains of *M. lysodeikticus* (NCTC 2665), *S. lutea* and *B. licheniformis* were those used in previous studies[7-9]; in addition *B. megaterium* strain KM and *B. subtilis* (Met$^-$, His$^-$, Ileu$^-$) strain kindly provided by Dr. DAVID DUBNAU, were used. The micrococci were grown on a medium consisting of 5% Bacto peptone, 0.1% Difco yeast extract, 0.5% NaCl (adjusted to pH 7.5), and diphenylamine was added as previously described[8]. The Bacillus spp. were grown on a basal salts medium[12] containing 0.5% monosodium glutamate and 0.2% yeast extract in the absence and presence of diphenylamine. The organisms were grown at 30° for 24 h with aeration on a New Brunswick incubator shaker, harvested and washed as described in our recent studies[8,9].

Isolation of membranes

The membranes (plasma–mesosome membrane system) from "normal" and "diphenylamine-grown" cells of *M. lysodeikticus*, *S. lutea*, *B. licheniformis*, *B. megaterium* and *B. subtilis* were isolated by the procedures outlined by SALTON AND FREER[9]. The isolated membranes were dialysed against changes of distilled water at 0–4° and finally freeze-dried and stored in sealed bottles at −10° until ready for extraction of the lipid.

Extraction of membrane lipid

Several extraction procedures were investigated including the chloroform–methanol (2:1, v/v) method of FOLCH, LEES AND SLOAN-STANLEY[13] using lyophilized membranes. With the dry membranes, prolonged extraction or blending with the chloroform–methanol solvent was necessary for complete extraction of the carote-

noids. The more rapid procedure of extracting membranes which had been moistened with distilled water prior to the addition of the 95% methanol or acetone–methanol (7:2, v/v)[14] solvents gave satisfactory results. The rate of extraction of the carotenoids and total lipid was thus much faster for the two solvent systems when moistened membranes were used instead of the dry, lyophilized membranes. With the wet membranes, three extractions with acetone–methanol (7:2, v/v) were adequate for removal of the lipid (determined by weighing), carotenoid and menaquinone (measured spectrophotometrically).

Accordingly, the following procedure was adopted: 50-mg samples of lyophilized membrane were thoroughly rubbed up with 0.5 ml distilled water at 37°. 10 ml of acetone–methanol (7:2, v/v) were added and thoroughly rubbed up with the membrane material and allowed to extract for 30 min at 37° in screw-cap test-tubes. The extracts were decanted off after centrifugation and the membrane residues were extracted twice more with 10-ml portions of acetone–methanol. The extracts were pooled, sparged with nitrogen and held overnight at 0–5° and the small amounts of insoluble residues were centrifuged off and discarded. The lipid extracts were then rapidly taken to dryness in a rotary evaporator, the bath temperature of which was maintained at about 45°. The lipids were dissolved in chloroform–methanol (2:1, v/v) and transferred quantitatively to weighing bottles and the solvent evaporated in a stream of nitrogen. The lipid fractions were stored in the dark in a vacuum desiccator filled with nitrogen and the preparations were examined as soon as possible after isolation from the membranes.

Thin-layer chromatography

Lipid fractions from the membranes isolated from "normal" and "diphenylamine-grown" cells were examined by thin-layer chromatography on 0.25-mm layers of silica gel G using chloroform–methanol–water (65:25:4, v/v)[15] as the solvent for separation of carotenoids and phospholipids. The plates were sprayed with Rhodamine B (0.5% (w/v) in ethanol) and viewed under ultraviolet light.

The menaquinones in the lipid fractions were also identified by thin-layer chromatography as described above but using benzene or isooctane–diethylether (100:30, v/v)[16] as the solvents. Authentic samples of menaquinones-5, -6, -7 and -9, generously given by Dr. O. ISLER, Hoffmann–La Roche and Co. Ltd. (Basle), were used as reference standards. On spraying the thin-layer plates with Rhodamine B, these compounds were readily detectable as dark purple substances and could be distinguished from diphenylamine which gave a purple-brown color. With the isooctane–ether solvent the menaquinones were separable from diphenylamine and from the phospholipids, carotenoids, *etc.* which remained at or near the origin.

Quantitative estimation of the menaquinones and diphenylamine

Menaquinones were determined after separation by thin-layer chromatography. Known amounts of lipid fractions dissolved in chloroform–methanol (2:1, v/v) were placed in the form of a band on the thin-layer chromatography plates which were then developed in jars containing isooctane–diethylether (100:30, v/v) for the separation of menaquinones[16]. The solvent front was allowed to migrate a distance of about 10–14 cm and markers of authentic compounds were employed. The menaquinones were visible as pale yellow bands as the solvent dried off and they were

accordingly marked immediately after taking the plates out of the chromatography jars. The silica gel was carefully removed and transferred to a screw-cap tube and extracted twice with 10 ml anhydrous diethylether. The extracts were centrifuged, supernatant solutions decanted into clean beakers, and the ether was evaporated under a stream of nitrogen. The menaquinone was then dissolved in isooctane and the spectrum was determined in a Cary recording spectrophotometer, Model 15. A molar absorbance coefficient of $19 \cdot 10^3$ (ISLER *et al.*[17]) was used for computing the contents in the bacterial membranes after determining the absorbance at the 248-mμ peak from the ultraviolet-absorption spectra.

The separation of known amounts of menaquinone-7 and -9 by thin-layer chromatography followed by extraction from the gel as described above, gave recoveries of 95%.

Diphenylamine was separated from the menaquinones using the thin-layer chromatography system as described above. The average distance of migration of diphenylamine in the isooctane–diethylether (100:30, v/v) solvent was 0.7 that of menaquinone-7. For quantitative determinations of the diphenylamine contents of the lipid fractions, the gel areas corresponding to side markers of diphenylamine revealed with Rhodamine B, were removed and extracted with 95% ethanol. The diphenylamine was estimated spectrophotometrically in 95% ethanol ($E^{1\%}_{1\,cm}$ 1200 in ethanol at 284 mμ after centrifuging off the gel. Known amounts of diphenylamine subjected to thin-layer chromatography were recovered to the extent of 90–95% by this method .

RESULTS

Influence of diphenylamine on carotenoids in membranes

Membranes from *M. lysodeikticus* and *S. lutea* were isolated from the organisms harvested after growth in the presence of diphenylamine concentrations ranging from 12.5 to 50 μg per ml medium. Extraction of the membranes with the acetone–methanol solvent removed the more conjugated carotenoids leaving a buff-colored residue devoid of the yellow pigments. The spectra of the total lipid extracted from the membranes of normal and diphenylamine-grown cells of *M. lysodeikticus* showed that growth in the presence of diphenylamine resulted in a reduction of the contents of the more unsaturated carotenoids in the membranes. Similar results were obtained with the *S. lutea* membranes.

The carotenoids of *M. lysodeikticus* have been re-investigated in more detail recently by ROTHBLAT, ELLIS AND KRITCHEVSKY[18] and following saponification at least seven pigments were separable by column chromatography. Our results are in general agreement in that both *M. lysodeikticus* and *S. lutea* membranes contain a mixture of carotenoids. In the present studies, thin-layer chromatography of the total (unsaponified) lipid extracts revealed the presence of four principal yellow pigments with traces of an additional yellow substance in membranes from normal cells of both organisms. Spectra of the four compounds eluted from the gel indicated that all were of carotenoid nature. In the chloroform–methanol–water solvent system, the menaquinones present in the lipid extracts separated from the carotenoids and migrated close to the solvent front, thus forming a sixth yellow compound detectable by thin-layer chromatography. On spraying the thin-layer plates with

Rhodamine B there were no obvious qualitative differences in the variety of lipids present in the membranes of normal and diphenylamine-grown cells of either *M. lysodeikticus* or *S. lutea*.

The effects of growing the yellow-pigmented micrococci in the presence of diphenylamine on the contents of the carotenoids in the membranes has been determined by extraction of the isolated membranes with acetone–methanol and by measuring the absorbance at 468 mμ. The results for *M. lysodeikticus* and *S. lutea* are presented in Table I and they clearly show that the formation of the more con-

TABLE I

EFFECTS OF GROWTH IN THE PRESENCE OF DIPHENYLAMINE ON CAROTENOID CONTENTS OF MEMBRANES ISOLATED FROM *Micrococcus lysodeikticus* AND *Sarcina lutea*

Relative carotenoid content expressed as absorbance at 468 mμ per 10 ml acetone–methanol (7:2, v/v) extract from 50 mg membrane. Diphenylamine added expressed as final concentration of diphenylamine per ml medium in which organisms were grown.

Membrane preparations	*Relative carotenoid contents*			
	Expt. 1		*Expt. 2*	
		Percentage inhibition		*Percentage inhibition*
M. lysodeikticus				
Normal	0.865	0	1.260	0
12.5 μg diphenylamine per ml	0.290	66	—	—
25 μg diphenylamine per ml	0.050	94	0.035	97
50 μg diphenylamine per ml	0.025	97	0.040	97
S. lutea				
Normal	0.905	0	0.730	0
12.5 μg diphenylamine per ml	0.150	83	0.065	91
25 μg diphenylamine per ml	0.060	93	0.050	93
50 μg diphenylamine per ml	0.020	98	0.050	93

jugated carotenoids is inhibited to the extent of 90% or more when the organisms are grown in media containing diphenylamine concentrations above 12.5 μg/ml.

Although the carotenoids are conspicuous membrane constituents they are obviously minor components of the lipid fraction. Assuming an $E_{1\,cm}^{1\,\%}$ of $3 \cdot 10^3$ for the carotenoids[14], an approximation of the amount in normal cells of these organisms would account for about 0.4% (by weight) of the membrane lipid and about 0.1% of the whole membrane structure.

Identification and estimation of menaquinones in membranes and the effects of diphenylamine

Compounds belonging to the menaquinone group were the most conspicuous substances detectable by ultraviolet spectroscopy of the total lipid fractions of the membranes dissolved in isooctane. The lipid extracts from the membranes isolated from both normal and diphenylamine-grown cells contained menaquinones. The spectra of the membrane lipid from the organisms grown in the presence of diphenylamine showed additional ultraviolet-absorbing material between 270 and 310 mμ. The absorption characteristics of this material did not appear to correspond to either

phytoene or phytofluene which accumulate in the presence of diphenylamine in certain other bacteria[14]. The difference between the normal and diphenylamine preparations was most pronounced for *B. megaterium* membranes and the ultraviolet-absorption spectra of the total lipid fractions dissolved in isooctane are shown in Fig. 1. The spectrum of diphenylamine showing a maximum absorption at 284 mμ (in 95% ethanol) was very similar to that of the material detected in the *B. mega-*

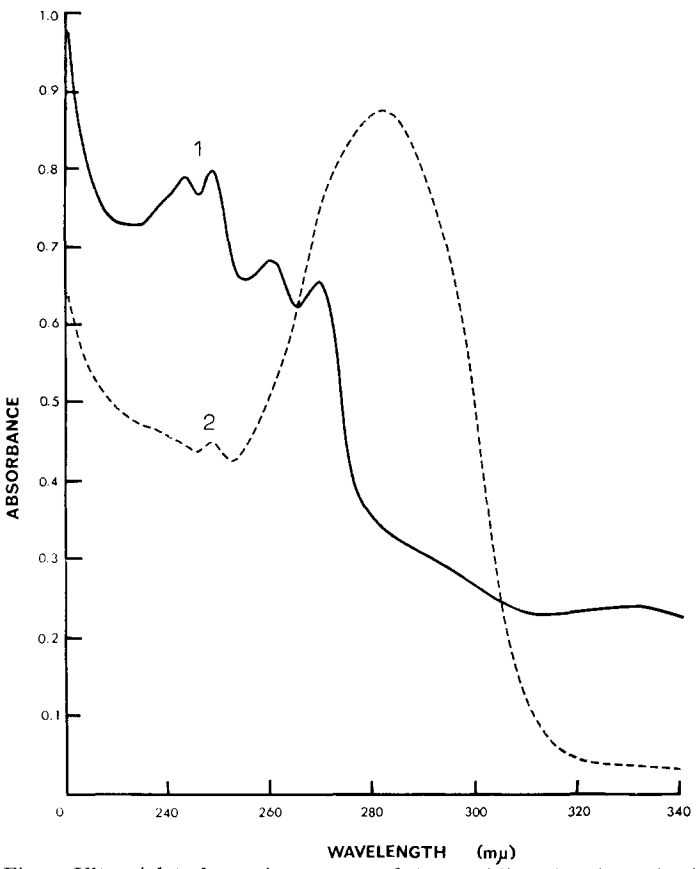

Fig. 1. Ultraviolet-absorption spectra of the total lipid fractions dissolved in isooctane, extracted from isolated membranes from *B. megaterium* normal cells (—) and cells grown in the presence of 25 μg diphenylamine per ml medium (- - - -). Curve 1, normal membrane lipid, 0.53 mg per ml isooctane; Curve 2, membrane lipid from diphenylamine-grown cells, 0.23 mg per ml isooctane.

terium membrane lipid and its presence in the lipid fractions was confirmed (as indicated below) by thin-layer chromatography.

The ultraviolet-absorption spectra of the total lipid fractions from the membranes suggested that the compounds belonged to the menaquinone group. In order to tentatively establish the identity of the membrane menaquinones, the lipid fractions were subjected to thin-layer chromatography in benzene and in isooctane–diethylether and the ultraviolet-absorption spectrum of each separated compound was determined as described in METHODS.

The ultraviolet spectra of the menaquinones isolated by thin-layer chromatography of the membrane lipids from *M. lysodeikticus*, *S. lutea* and *B. megaterium* are compared with that of authentic menaquinone-7 in Fig. 2. As shown in Fig. 2 all of the compounds showed the principal absorption peaks or inflections at 239, 243, 248, 260, 269 and 326 mμ, characteristic of menaquinones[17]. Absorption spectra of the membrane compounds from *B. licheniformis* and *B. subtilis* were indistinguishable

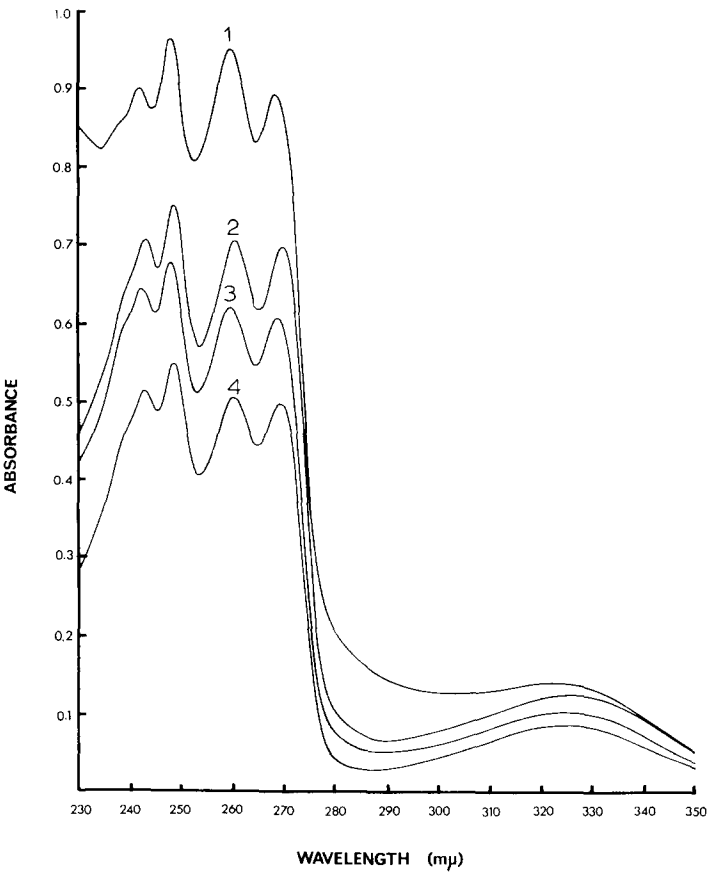

Fig. 2. Ultraviolet-absorption spectra in isooctane of menaquinones separated by thin-layer chromatography of the lipid extracted from isolated membranes compared with that of authentic menaquinone-7. Curve 1, menaquinone-9 from *S. lutea* membranes; Curve 2, menaquinone-9 from *M. lysodeikticus* membranes; Curve 3, menaquinone-7 from *B. megaterium* membranes; Curve 4, authentic menaquinone-7, 20 μg per ml isooctane.

from the reference compounds thus indicating their identity with those of the menaquinone group. The anomalous behavior of the spectrum of the *S. lutea* compound reported by BISHOP, PANDYA AND KING[19] was not encountered under the conditions of extraction and isolation used in this study.

The menaquinones separated by thin-layer chromatography were revealed as deep-purple substances by spraying with Rhodamine B. A comparison of the R_F

values of the *M. lysodeikticus* and *S. lutea* membrane compounds with those of the reference substances has tentatively identified them as menaquinone-9, while those from *B. licheniformis*, *B. megaterium* and *B. subtilis* are identical to menaquinone-7. These results are in accord with previously published data[11,19].

In addition to detecting menaquinones in the lipid extracts from diphenylamine membranes subjected to thin-layer chromatography on silica gel G in the isooctane–diethylether solvent, a substance identical in behavior to diphenylamine was detected. Both diphenylamine and the membrane-bound diphenylamine gave purple compounds on spraying the thin-layer plates with Rhodamine B, but unlike the menaquinones, on standing they became a brown color. Compounds corresponding to the behavior of phytoene or phytofluene were not detectable by spectrophotometric examination of extracts nor by thin-layer chromatography.

Menaquinone contents of the membranes were accordingly determined for normal and diphenylamine-grown bacteria by separating the compounds on thin-layer plates as described in METHODS. The results are presented in Table II. These quantitative determinations indicate that the menaquinones may account for up to about 4–5% of the weight of the membrane lipid. Extraction of the protoplasmic fractions of *M. lysodeikticus*, *S. lutea* and the Bacillus species revealed that the menaquinone contents were substantially lower than 0.5 μmole/g, thus showing that

TABLE II

MENAQUINONE CONTENTS OF MEMBRANES ISOLATED FROM NORMAL AND DIPHENYLAMINE-GROWN BACTERIA

Diphenylamine added expressed as final concentration of diphenylamine per ml medium in which organisms were grown. Values in parentheses represent the per cent of the "normal" values.

Membrane preparations	*μmoles menaquinone per g membrane*	
	Expt. 1	*Expt. 2*
M. lysodeikticus		
Normal	12.1 (100)	7.8 (100)
12.5 μg diphenylamine per ml	13.5 (112)	—
25 μg diphenylamine per ml	9.4 (78)	5.0 (64)
50 μg diphenylamine per ml	9.3 (77)	4.0 (51)
S. lutea		
Normal	4.4 (100)	3.8 (100)
12.5 μg diphenylamine per ml	5.9 (134)	4.1 (108)
25 μg diphenylamine per ml	5.7 (130)	3.6 (97)
50 μg diphenylamine per ml	3.7 (84)	3.6 (97)
B. megaterium		
Normal	8.4 (100)	
12.5 μg diphenylamine per ml	7.6 (90)	
25 μg diphenylamine per ml	7.6 (90)	
B. licheniformis		
Normal	3.2	
B. subtilis		
Normal	10.0	

more than 95% is in the membrane, an observation in agreement with that of BISHOP AND KING[11].

At the higher diphenylamine concentrations there is a variable reduction in the menaquinone contents. This reduction did not appear to be compensated for by a greater amount of "unattached" menaquinone in the protoplasmic fraction. Indeed, extraction of the lyophilized protoplasmic fractions obtained after separation of the membranes showed that there was no excess menaquinone in this fraction of the diphenylamine-grown cells. As with the normal cells, at least 95% of the menaquinone was found to be localized in the membranes. The failure to detect an accumulation of phytoene and phytofluene in the presence of diphenylamine is puzzling and differs from the effects reported for Gram-negative bacteria and fungi[4,14].

Localization of diphenylamine in membranes

Since diphenylamine obviously affects cellular components present in membrane systems[4-8], and since it was detected in the membrane lipid fractions (Fig. 1), the possibility that it is taken up selectively into the membrane structure was tested. That the compound responsible for the ultraviolet absorption in the total lipid extracts was diphenylamine was confirmed by separation, by thin-layer chromato-

TABLE III

DIPHENYLAMINE CONTENTS OF ISOLATED MEMBRANE AND PROTOPLASMIC FRACTIONS FROM *B. megaterium*, *M. lysodeikticus* AND *S. lutea* GROWN IN THE PRESENCE OF DIPHENYLAMINE

Organism and diphenylamine level in growth medium	*Diphenylamine contents of cell fractions (μg diphenylamine per 100 mg)*		
	Isolated membranes		*Protoplasmic fraction*
B. megaterium			
12.5 μg diphenylamine per ml	23	20.5	0*
25 μg diphenylamine per ml	53	41	0
M. lysodeikticus			
25 μg diphenylamine per ml	49		0
S. lutea			
25 μg diphenylamine per ml	31		0

* None detectable by thin-layer chromatography of total lipid from 100 mg.

graphy, of the menaquinones from the substance suspected of being diphenylamine. The spectrum of the membrane-lipid diphenylamine separated by thin-layer chromatography and extracted into 95% ethanol was identical to that of diphenylamine, both possessing a maximum absorption peak at 284 mμ and its behavior on thin-layer chromatography was identical to diphenylamine. Isolated membranes and the corresponding "protoplasmic" or cell-sap fractions of *M. lysodeikticus*, *S. lutea* and *B. megaterium* grown in the presence of diphenylamine were extracted with the

acetone–methanol solvent and the whole lipid extracts were subjected to thin-layer chromatography as described in METHODS. The diphenylamine contents of the fractions were determined and the results are presented in Table III. Diphenylamine could not be detected in the cell-sap fractions either by elution of the gel areas corresponding to diphenylamine markers or by direct spraying of the thin-layer chromatography plates with Rhodamine B. The cell-sap fraction which includes the lysozyme-digest products of the wall would have included any diphenylamine bound to the cell-wall structure. It is evident that when these organisms are grown in the presence of diphenylamine, it is selectively bound to the membrane structure. The membrane-bound diphenylamine accounted for 0.5% of the total membrane lipid of *B. megaterium* and would correspond to an uptake of about 10^6 molecules/cell.

The specific uptake of diphenylamine into the membrane was further confirmed by shaking washed cell suspensions of *B. megaterium* in 0.067 M phosphate buffer containing 50 μg diphenylamine per ml suspension for 30 min at 30°. The cells were deposited by centrifugation, washed twice in buffer and the diphenylamine was extracted from a lysozyme lysate of whole cells, from the membrane and cell-sap fractions. Virtually all of the diphenylamine taken up by the cell was found in the membrane fraction from the washed cells exposed to diphenylamine. The capacity of washed cells of *B. megaterium* to take up diphenylamine (0.2 μg per mg dry weight) is in agreement with the uptake into the membrane of growing cells (approx. 1 μg per mg membrane) assuming that the membrane accounts for about 20% of the dry weight of the cell[9].

DISCUSSION

Although the more conjugated carotenoids and menaquinones provide very useful "marker molecules" in the bacterial membrane system, it is becoming increasingly apparent that certain constituents normally localized in these structures may not be absolutely essential for the efficient functioning of the cell. The investigations reported here illustrate that diphenylamine has a marked effect on the membrane's carotenoids without any obvious effects on cell growth and membrane formation[8] and that the bacteria used in these studies can also tolerate some variation in the menaquinone contents of their membranes. It is possible that the latter substances may be "essential" membrane constituents, if, as suggested by several authors, these compounds do indeed play a role in electron transport systems[20,21].

These results do reinforce the view that the bacterial mesosome–membrane system is subject to variation in the contents of some of its constituents. Indeed, WHITE[22] has shown that respiratory-chain components can vary widely in the envelope membranes of the Gram-negative organism, *Hemophilus parainfluenzae*, a situation which is in marked contrast to the suggestion that the complement of such constituents is quite constant in mitochondrial membranes[23]. It is hoped that studies such as those of WHITE[22] and the present investigation will lead to a more precise understanding of the minimal requirements with respect to proteins, lipid and electron transport components needed for a functional membrane system in the bacterial cell.

There seems little doubt that the biosynthesis in the bacterial membrane of the

more conjugated carotenoids shows a greater sensitivity to the presence of diphenylamine than does the synthesis of the menaquinones, a finding in accord with earlier reports[24]. However, the exact mechanism of action of diphenylamine on carotenoid biosynthesis still remains obscure. The fact that diphenylamine is selectively taken up into the membrane does make it likely that the enzymes involved in the terminal steps of synthesis of the more unsaturated carotenoids would be localized in the membrane structures, and these observations should now facilitate the study of its mode of action as an inhibitor.

We have been able to demonstrate that diphenylamine is taken up selectively into the cell membranes of bacteria, although it should be recalled that OLSON AND KNIZLEY[24] were unable to recover it from *Phycomyces blakesleeanus*. It is believed that in moulds such as Phycomyces, carotenoids are found in "lipid globules" rather than in the membranes as in bacteria. However, comparable cell-fractionation studies have not been carried out with these moulds. The uptake of a foreign substance such as diphenylamine into the membrane and its tolerance by the growing cell may also provide us with an additional method of labeling the membrane in studies of membrane development and the distribution of these structures during subsequent cellular generations. Diphenylamine is also growth inhibitory in concentrations higher than those used in the present studies and further investigations of its mode of action may provide useful data on the types of compounds that may have potentialities as selective inhibitors of membrane biosynthesis.

ACKNOWLEDGEMENTS

This work was supported by a Research Grant GB-2877 and continuation Grant GB-4603 from the National Science Foundation. We are grateful to Mrs. MARION SCHOR for technical assistance with some of the experiments.

REFERENCES

1 M. S. KHARASCH, E. A. CONRAY AND W. BLOOM, *J. Bacteriol.*, 32 (1936) 533.
2 G. TURIAN, *Helv. Chim. Acta*, 33 (1950) 1988.
3 T. W. GOODWIN, M. JAMIKORN AND J. S. WILLMER, *Biochem. J.*, 53 (1953) 531.
4 T. W. GOODWIN AND H. G. OSMAN, *Biochem. J.*, 56 (1954) 222.
5 T. SUGIMURA AND H. RUDNEY, *Biochim. Biophys. Acta*, 62 (1962) 167.
6 J. E. BAKER, *Arch. Biochem. Biophys.*, 103 (1963) 148.
7 K. Y. CHO, W. A. CORPE AND M. R. J. SALTON, *Biochem. J.*, 93 (1964) 26C.
8 M. R. J. SALTON AND A. F. M. EHTISHAM-UD-DIN, *Australian J. Exptl. Biol. Med. Sci.*, 43 (1965) 255.
9 M. R. J. SALTON AND J. H. FREER, *Biochim. Biophys. Acta*, 107 (1965) 531.
10 IUPAC-IUB Commission on Biochemical Nomenclature: *Nomenclature of Quinones with Isoprenoid Side-Chains, Biochim. Biophys. Acta*, 107 (1965) 5.
11 D. H. L. BISHOP AND H. K. KING, *Biochem. J.*, 85 (1962) 550.
12 M. R. J. SALTON, *Biochim. Biophys. Acta*, 86 (1964) 421.
13 J. FOLCH, M. LEES AND G. H. SLOAN-STANLEY, *J. Biol. Chem.*, 226 (1957) 497.
14 S. L. JENSEN, G. COHEN-BAZIRE, T. O. M. NAKAYAMA AND R. Y. STANIER, *Biochim. Biophys. Acta*, 29 (1958) 477.
15 H. WAGNER, L. HORHAMMER AND P. WOLFF, *Biochem. Z.*, 334 (1961) 175.
16 M. MCKENNA, M. D. HENNINGER AND F. L. CRANE, *Nature*, 203 (1964) 524.
17 O. ISLER, R. RUEGG, A. LANGMANN, P. SCHUDEL, G. RYSTER AND J. WURSCH, in *Quinones in Electron Transport*, J. and A. Churchill, London, 1961, p. 79.
18 B. H. ROTHBLAT, D. S. ELLIS AND D. KRITCHEVSKY, *Biochim. Biophys. Acta*, 84 (1964) 340.
19 D. H. L. BISHOP, K. P. PANDYA AND H. K. KING, *Biochem. J.*, 83 (1962) 606.

20 A. F. BRODIE, *Federation Proc.*, 20 (1961) 995.
21 D. C. WHITE, *J. Biol. Chem.*, 240 (1965) 1387.
22 D. C. WHITE, *J. Bacteriol.*, 89 (1965) 299.
23 P. V. BLAIR, T. ODA, D. E. GREEN AND H. FERNANDEZ-MORAN, *Biochemistry*, 2 (1963) 756.
24 J. A. OLSON AND H. KNIZLEY, *Arch. Biochem. Biophys.*, 97 (1962) 138.