Phospholipids of Bacteria with Extensive Intracytoplasmic Membranes

Abstract. *Examination of the lipids of three species of nonphotosynthetic bacteria with extensive internal membranes revealed phosphatidyl choline (lecithin) in two species. In one of these there was an unusual accumulation of phosphatidyl* N*-dimethylethanolamine. (The relation between lecithin and membrane elaboration in microorganisms is discussed.)*

Recent work indicates that most species of bacteria do not contain phosphatidyl choline (lecithin) (*1*). These studies have been almost entirely confined to bacteria of the order Eubacteriales (*2*). The fine structure of a number of these organisms has been examined and, with the exception of the organelles called mesosomes, intracytoplasmic membranes have generally not been seen (*3*). Recently, extensive internal membranes have been demonstrated in *Hyphomicrobium* (*4*), *Nitrosocystis oceanus*, and *Nitrosomonas europaea* (*5*). In seeking some understanding of these structural differences, we have examined the phosphatide composition of these organisms and have found substantial amounts of phosphatidyl choline and its presumed precursor phosphatidyl *N*-dimethylethanolamine in several strains of *Hyphomicrobium*, smaller amounts of phosphatidyl choline in *Nitrosocystis oceanus*, but none in *Nitrosomonas europaea*.

Four strains of *Hyphomicrobium* were grown on a medium (No. 337) containing methylamine (*6*) in 2-liter indented flasks (500 ml per flask) on a rotary shaker, at 30°C in the dark. *Nitrosocystis oceanus* and *Nitrosomonas europaea* were grown in 14-liter vessels, without organic carbon sources, on ammonium sulfate and other salts (Watson, 6*a*) dissolved in filtered seawater (*Nitrosocystis oceanus*) or distilled water (*Nitrosomonas europaea*).

Lipids were extracted from the wet cells with a mixture of chloroform and methanol (2:1 by volume), washed, and separated by column chromatography on silicic acid. Phospholipids were then chromatographed on thin layers of silica gel G, and lipid phosphorus was estimated after digestion of samples of the separated lipids (*7*).

After comparison of the migration of the phospholipids on thin-layer plates with authentic standards, the phospholipids were identified by paper chromatography of the deacylation products and of the free bases obtained after hydrolysis of the lipids in 1*N* HCl (*7*, *8*) (Table 1).

In order to demonstrate the capacity of these organisms to synthesize phosphatidyl choline, L-methionine-^{14}C-methyl (275×10^3 count/min, 13.2 μc/μmole) was added to 500-ml cultures of each of the four strains of *Hyphomicrobium*. Approximately 10 percent of the methyl carbon from L-methionine (average, 31,000 count/min) was incorporated into the lipids. Essentially all of the incorporated ^{14}C was found in the water-soluble products of hydrolysis in 1*N* HCl. When these products were examined by paper chromatography in solvent 1 (*8*), two radioactive spots were seen on the autoradiograms; these spots corresponded with standards of dimethylethanolamine and choline. On paper chromatography in solvent 3 (*8*) there was one radioactive spot, and this corresponded with both of these bases ($R_F = 0.58$).

Nitrosocystis oceanus was grown in 14 liters of medium containing L-methionine-^{14}C-methyl (7.0×10^7 count/min). Approximately 6×10^5 count/min were found in the phospholipids after column chromatography. When separated by thin-layer chromatography, 92 percent of the ^{14}C was found in the lecithin band and 6 percent in the band containing both the phosphatidyl ethanolamine and the phosphatidyl *N*-methylethanolamine (*7*). After acid hydrolysis and paper chromatography of the water-soluble products, all of the radioactivity was found in choline and *N*-methylethanolamine.

The results of the experiments with L-methionine-^{14}C-methyl indicate that these organisms, like the agrobacteria (*1*, *9*), synthesize lecithin by the stepwise methylation of phosphatidyl ethanolamine. However, it is not possible with the information available to rule out the existence in these organisms of the pathway which utilizes cytidine diphosphate choline (*10*).

A number of differences in the lipids of prokaryotic organisms (bacteria and blue-green algae) and those of eukaryotic cells (fungi, protozoa, algae, plants, and animals) have been observed. Bacteria do not have sterols, polyunsaturated fatty acids, and sphingolipids. Although phosphatidyl ethanolamine and polyglycerol phosphatides are usually found in bacteria, phosphatidyl choline is rarely present (*11*).

Table 1. Lipids of bacteria with internal membranes.

Component	*Hyphomicrobium* NQ-521*	*Nitrosocystis oceanus*	*Nitrosomonas europaea*
	Percentage dry weight		
Lipid content	10	20	18
	Percentage free lipid		
Phospholipid	75	80	75
	Percentage phospholipid phosphorus		
PE†	22	67	78
PDME‡	36	<1	<1
PC§	30	3	<1
Polyglycerol phosphatides	10	28	17

* The lipids of three other strains of *Hyphomicrobium* (H-526, M-552, and ZV-580) were examined in less detail. The phosphatide compositions were qualitatively and quantitatively similar. † Phosphatidyl ethanolamine. ‡ Phosphatidyl *N*-dimethylethanolamine. § Phosphatidyl choline.

With regard to membrane structure, examination of thin sections of heterotrophic bacteria has usually revealed only minor elaborations of the cytoplasmic membrane in comparison with the complex intracellular membranes of eukaryotic cells. The mesosomes found in a number of gram-positive, heterotrophic bacteria provide one exception to this general rule. On the other hand, intracytoplasmic vesicular structures observed in photosynthetic bacteria several years ago are now known to be a general feature of these cells (*3*). Intracellular lamellar and vesicular membranes have recently been found in several autotrophic (*5*) and aerobic organotrophic bacteria (*4*). These features appear to differ in structure from the mesosomes of heterotrophic bacteria.

Although not all the bacteria with extensive intracellular membranes contain phosphatidyl choline, a large proportion of those studied do contain this phospholipid. In addition to our finding of phosphatidyl choline in *Hyphomicrobium* and *Nitrosocystis oceanus*, it has also been found in substantial amounts in *Rhodopseudomonas spheroides* (*12*) and in two other members of this genus, but not in *R. gelatinosa, Rhodospirillum rubrum*

(*13*), or *Azotobacter agilis* (*14*), which also have internal membrane systems. Phosphatidyl choline has also been found in several species of *Agrobacterium* (*1*), but no details of the membrane structure of these organisms are available. Since phosphatidyl choline is the major phospholipid of the endoplasmic reticulum of higher organisms, its presence in a number of bacteria with extensive intracytoplasmic membranes is suggestive of an important role for this phospholipid in the elaboration of cytoplasmic membranes (*15*).

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References and Notes

1. M. Kates, *Advances Lipid Res.* **2**, 17 (1964); H. Goldfine and M. E. Ellis, *J. Bacteriol.* **87**, 8 (1964).
2. R. S. Breed, E. G. D. Murray, N. R. Smith, *Bergey's Manual of Determinative Bacteriology* (Williams and Wilkins, Baltimore, ed. 7, 1957).
3. R. G. E. Murray, in *General Physiology of Cell Specialization*, D. Mazia and A. Tyler, Eds. (McGraw-Hill, New York, 1963), p. 28; W. van Iterson, *Bacteriol. Rev.* **29**, 299 (1965).
4. S. F. Conti and P. Hirsch, *J. Bacteriol.* **89**, 503 (1965).
5. R. G. E. Murray and S. W. Watson, *ibid.*, p. 1594.
6. P. Hirsch and S. F. Conti, *Arch. Mikrobiol.* **48**, 358 (1964).
6a. S. W. Watson, in preparation.
7. N. A. Baumann, P-O. Hagen, H. Goldfine, *J. Biol. Chem.* **240**, 1559 (1965).
8. H. Goldfine, *Biochim. Biophys. Acta* **59**, 504 (1962).
9. T. Kaneshiro and J. H. Law, *J. Biol. Chem.* **239**, 1705 (1964).
10. E. P. Kennedy, *Fed. Proc.* **20**, 934 (1961).
11. K. Bloch, in *Taxonomic Biochemistry and Serology*, C. A. Leone, Ed. (Ronald Press, New York, 1964), p. 377; J. H. Law, in *Specificity of Cell Surfaces*, B. D. Davis and L. Warren, Eds. (Prentice-Hall, Englewood Cliffs, N.J., in press).
12. J. Lascelles and J. F. Szilágyi, *J. Gen. Microbiol.* **38**, 55 (1965).
13. B. J. B. Wood, B. W. Nichols, A. T. James, *Biochim. Biophys. Acta* **106**, 261 (1955).
14. T. Kaneshiro and A. G. Marr, *J. Lipid Res.* **3**, 184 (1962).
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Tetraethylammonium and Tetrodotoxin: Effects on Cochlear Potentials

Abstract. *Tetraethylammonium chloride, which is believed to decrease potassium conductance, and tetrodotoxin, which apparently decreases sodium conductance in nerve fibers, were introduced iontophoretically into the organ of Corti or the scala media of guinea pig cochlea. The former depressed the direct-current endocochlear potential and also the alternating-current cochlear microphonics (the receptor potential of the ear), but tetrodotoxin was ineffective except on the nerve impulses.*

It is well known that endolymph of the cochlear duct has a high concentration of potassium, nearly 30 times that of the perilymph (*1*). The physiological significance of this pattern of electrolytes is not obvious, yet the richness in potassium seems indispensable for the high sensitivity of the cochlear microphonics (CM) (*2*). Furthermore, some experimental results show that the CM responses may be modified by increasing or decreasing the endocochlear potential by external current or by KCl injected into the scala tympani (*3*).

Katsuki *et al.* (*4*) recently developed the method of iontophoretic introduction of various pharmacological agents in the vicinity of the cochlear hair cells. Using the same technique, we applied tetraethylammonium chloride (TEA) and tetrodotoxin (in the citrate buffer solution, in which tetrodotoxin acts as a cation) to the hair-cell region in order to clarify the ionic mechanism of cochlear responses. We had a frog nerve preparation as a control on the effect of the iontophoretic introduction of tetrodotoxin.

It has been reported that TEA prolongs the spike potential of myelinated nerve fibers of vertebrates (*5*), of muscle fibers of crustaceans and vertebrates (*6*), and of giant axons of squid (*7*); and experiments with *Onchidium* neurons suggest that these effects of TEA probably result from suppression of the increase in K-conductance of the treated cell membrane (*8*).

Tetrodotoxin is known to block impulse activity in nerve and muscle fibers (*9*) and is considered to be a blocking agent for the sodium channel in the membrane (*10*). It has also been shown that in two receptors, the crustacean stretch receptor and the pacinian corpuscle, tetrodotoxin acts selectively on the all-or-none component —that is, it blocks the action potential but leaves the generator potential unaffected. It follows that the spike and the receptor-generator potential in these two receptors are independent events subserved by different mechanisms (*11*).

The CM responses and the neural action potential (N_1) were recorded

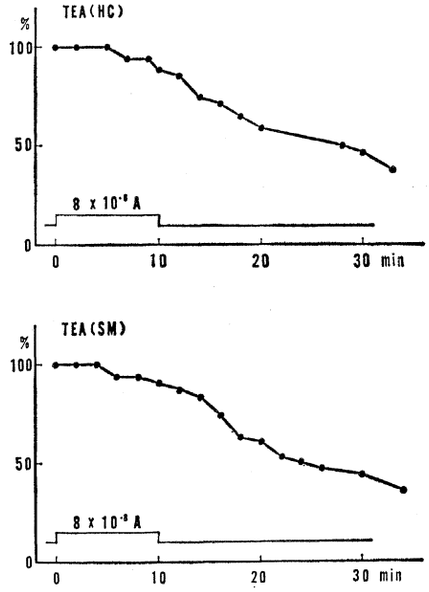

Fig. 1. Effect of TEA on the cochlear microphonics; it was introduced iontophoretically into the hair-cell region (*TEA-HC*) and the scala media (*TEA-SM*) with a current of 8×10^{-6} amp for 10 minutes. Ordinate, amplitude of microphonics as a percentage of that of the control.

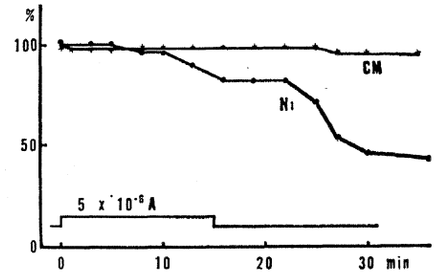

Fig. 2. Effect of tetrodotoxin on the cochlear microphonics (*CM*) and the action potentials (N_1). Tetrodotoxin was introduced iontophoretically into the hair-cell region. *A*, ampere. Ordinate, amplitude as a percentage of that of the control.