Effect of vitamin K depletion and restoration on sphingolipid metabolism in *Bacteroides melaninogenicus*

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Abstract *Bacteroides melaninogenicus* requires vitamin K for normal growth. Cells incubated in a vitamin K-free medium form defective cell envelopes. Studies with vitamin K-grown "K(+)" and vitamin K-depleted "K(−)" cells showed that [^{14}C]choline and [^{14}C]glycerol were not taken up, but several amino acids and acetate were incorporated to the same degree by both types of cultures. However, K(−) cells incorporated succinate to a greater degree than did K(+) cultures. The relative incorporation of succinate into ceramide phosphorylethanolamine and ceramide phosphorylglycerol was depressed compared with incorporation into phosphatidylethanolamine in K(−) cultures. *B. melaninogenicus* can be grown in serial subculture in the absence of vitamin K in the presence of 2.5 mg/ml of succinate. Under these conditions the relative incorporation of [2,3-^{14}C]succinate and ^{32}P into ceramide phosphorylethanolamine and ceramide phosphorylglycerol is markedly depressed.

Stimulation of phosphosphingolipid synthesis by vitamin K was shown by comparing the uptake of ^{32}P and lipid phosphorus levels of a succinate-grown, vitamin K-depleted culture supplemented with ^{32}P plus 0.1 μg/ml vitamin K_1 with a similar culture supplemented with ^{32}P only. The phosphosphingolipids from the vitamin K-supplemented cells incorporated greater amounts of ^{32}P and had higher levels of phosphorus than did the ceramide phosphorylethanolamine and ceramide phosphorylglycerol of the culture without added vitamin K. It was further shown that vitamin K added to a vitamin K-depleted culture stimulated synthesis of ceramide phosphorylethanolamine and ceramide phosphorylglycerol 38 min and 60 min, respectively, following the addition of the vitamin; incorporation of ^{32}P into other phospholipids was unaffected.

Supplementary key words cell envelopes · ceramides · succinate

CERTAIN STRAINS OF *Bacteroides melaninogenicus* (*Fusiformis nigrescens*) have an obligate requirement for vitamin K (1, 2). Previous work from this laboratory (3) has shown that in vitamin K-depleted bacteria the cells elongate and form impaired envelopes.

We have also shown (4) that succinate can partially replace this requirement for vitamin K, allowing limited growth of the microorganism in serial subculture. In the work reported here, incorporation of succinate into the phospholipids of the microorganism was altered by growth in the absence of vitamin K. Cells which were serially grown in the absence of vitamin K and in the presence of succinate had depressed levels of phosphosphingolipids. The influence of vitamin K on the synthesis of sphingolipids in vitamin K-deficient *B. melaninogenicus* is the subject of this report. A preliminary report on the effect of vitamin K on the stimulation of sphingolipid synthesis has been published (5).

MATERIALS AND METHODS

The bacterium was a vitamin K-requiring rumen strain of *B. melaninogenicus* used in previous studies of vitamin K metabolism (1–3). The basal medium consisted of trypticase (BioQuest, Cockeysville, Md.) 3%, yeast extract (Difco) 0.3%, and NaCl 0.5%, and was adjusted to pH 7.4. Tubed basal medium (10 ml) was freshly autoclaved before use and was supplemented with 0.05 ml of a 1/20 dilution of horse red cells, laked in distilled water, and 0.1 ml of an aqueous emulsion of vitamin K_1 to give a final concentration of 0.1 μg/ml. Little or no growth occurred in the medium supple-

mented with blood but without vitamin K. Cultures that had been incubated in an anaerobic jar 2 days at 37°C in an atmosphere of 95% H_2 and 5% CO_2 were used as inocula in some of the following experiments.

Metabolic studies were made with a specially adapted anaerobic jar (6) consisting of a 250-ml Erlenmeyer flask sealed to the side of the jar, with a short tube connecting the flask to the exterior of the jar. This tube was sealed with a vial cap. 200 ml of basal medium was placed in the Erlenmeyer flask, which was then sterilized by autoclaving and cooled. The jar was then assembled, evacuated, and filled with 95% H_2 and 5% CO_2. Cultures were prepared with different supplements and inocula as follows: (*a*) for cells grown in the presence of vitamin K, K(+) cells, 0.3 ml of a 2-day culture plus 0.5 ml of diluted laked blood and 0.2 ml of a 1/100 emulsion of a 10 mg/ml ethanolic solution of vitamin K_1; (*b*) for vitamin K-depleted cells, K(−) cells, 2 ml of culture and 0.5 ml of diluted blood; (*c*) for vitamin K-depleted cells grown with succinate, K(−) + succinate cells, 1.0 ml of culture, 0.5 ml of diluted blood, and 2.0 ml of succinate solution (0.25 g of the sodium salt/ml, sterilized by autoclaving); (*d*) for cells grown with vitamin K plus succinate, K(+) + succinate cells, 0.6 ml of culture, 0.2 ml of vitamin K emulsion, and 2.0 ml of succinate solution. In certain experiments, 40 μCi of [2,3-^{14}C]-succinate (5.84 mCi/mmole, New England Nuclear, Boston, Mass.) was added to 200 ml of basal medium. In others, 300 μCi of $H_3{}^{32}PO_4$ (New England Nuclear), 40 μCi of [^{14}C]acetate (57 mCi/mmole), 12 μCi of [^{14}C]-valine (30 mCi/mmole), 20 μCi of [^{14}C]choline (20 mCi/mmole), or 20 μCi of [^{14}C]glycerol (10 mCi/mmole) was added (without carrier) to the medium. The labeled acetate, valine, choline, and glycerol were obtained from Schwarz BioResearch, Inc., Orangeburg, N.Y. All additives were inoculated into the flask through the vial cap with a syringe. After cultures had been incubated for 16–24 hr, samples were withdrawn from the flasks with a syringe, the turbidity was measured in Klett units (660 nm), and a 0.5-ml aliquot was added to an equal volume of cold 10% trichloroacetic acid. This mixture was filtered through a Millipore membrane, and the retained cells were washed three times with cold 5% trichloroacetic acid. The membranes were placed in 7 ml of scintillation fluid (200 g of naphthalene and 10 g of 2,5-diphenyloxazole diluted to 2 l with 1,4-dioxane) and counted. Bacterial cells were also collected by centrifugation and, after washing once in 0.1 M phosphate buffer, pH 7.0, lipids were extracted from 0.3-g samples (wet wt) by a modification of the technique of Bligh and Dyer (7, 8). The organic phase was reduced to 3 ml under nitrogen, and 50-μl aliquots were spotted on silica gel thin-layer plates, which were developed with chloroform–methanol–water 70:25:4 in the first direction, and with chloroform–methanol–7 M ammonia 60:35:4 in the second (9). After drying, the plates were sprayed with a molybdate–mercury–acid mixture to detect phospholipids (10) or with 0.005% rhodamine G to detect total lipids (11). Phosphate was determined by the method of Norton and Autilio (12) on spots scraped from the thin-layer plates after detection of lipids with iodine vapor.

In other experiments the two-dimensional thin-layer chromatography system of Rizza, Tucker, and White was used (8). Spots were detected by radioautography using Kodak Noscreen X-ray film. They were scraped off the plates and radioactivity was measured in the dioxane-based scintillation fluid. Ceramide phosphorylethanolamine and ceramide phosphorylglycerol were resistant to mild alkaline methanolysis and were identified according to their chromatographic mobilities (8). After mild alkaline methanolysis of the chloroform extract, glycerol phosphate esters were separated on thin-layer plates coated with MN cellulose and they were identified by their mobilities (8).

When standards were available, radioactive spots were further characterized by cochromatography with authentic standards of phosphatidylethanolamine and phosphatidylserine (Applied Science Laboratories Inc., State College, Pa.).

RESULTS

Enhanced uptake of succinate by vitamin K-deficient cells

Previous experiments in our laboratory (3) have indicated that the cell envelope of vitamin K-depleted *B. melaninogenicus* is impaired. Therefore, the uptake of several compounds by cells grown in the presence or absence of vitamin K was compared. Whereas the vitamin K-supplemented cultures in exponential phase grew a maximum of 40 Klett units/hr, cultures of vitamin K-deficient bacteria prepared as described above grew slowly (6 Klett units/hr) and linearly for a period of approximately 16–30 hr. During this period, growth occurred to some degree as a result of cell elongation (3). These vitamin K-depleted cells are viable in that they are capable of normal growth when small inocula are added to a vitamin K-supplemented medium.

[^{14}C]Choline and [^{14}C]glycerol were not incorporated by cells grown in the presence or absence of vitamin K. The uptake of [^{14}C]valine by K(+) cells and K(−) cells is illustrated in Fig. 1. The deficient cells incorporated the same proportion of valine as cells grown with vitamin K, per unit increase in growth as measured by turbidity. Similar degrees of incorporation were obtained with [^{14}C]proline, [^{14}C]leucine, and [^{14}C]acetate.

In contrast to these results, vitamin K-deficient cells were found to incorporate six times more [^{14}C]succinate

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TABLE 1. Incorporation of $H_3{}^{32}PO_4$ and [^{14}C]succinate into phospholipids[a]

Type of Cell Phospholipid	$H_3{}^{32}PO_4$ K(+)	K(−)	K(+) + Succinate	K(−) + Succinate	[^{14}C]Succinate K(+)	K(−)	K(+) + Succinate	K(−) + Succinate
PS[b]	9.1 (7.8)[c]	5.0 (5.6)	12.7 (8.8)	9.4 (5.8)	2.4	3.0	1.6	8.2
CPE	17.7 (10.0)	17.8 (10.7)	20.4 (10.8)	10.6 (5.6)	29.3	8.6	21.1	5.8
CPG	16.8 (9.8)	20.7 (11.7)	17.0 (8.5)	9.6 (4.2)	30.8	27.7	21.1	5.6
PE	56.4 (31.2)	57.8 (31.1)	50.0 (23.0)	71.0 (35.3)	37.5	60.7	56.2	80.4
Total radioactivity in phospholipids (cpm/g cells dry wt)	21,000	20,600	17,200	16,100	680	3130	913	2430

K(+) cultures were grown in 200 ml of medium supplemented with blood and vitamin K_1 (0.1 μg/ml). K(−) cultures were depleted of vitamin K by growth of a large inoculum in medium supplemented only with blood. K(+) + succinate and K(−) + succinate cultures contained vitamin K plus 0.5 g of sodium succinate, and blood plus 0.5 g of sodium succinate, respectively, per 200 ml medium. 500 μCi of $H_3{}^{32}PO_4$ or 40 μCi of [2,3-^{14}C]succinate was added to these cultures as indicated.

[a] Values are percentages of $H_3{}^{32}PO_4$ or [^{14}C]succinate incorporation into phospholipids per g dry wt of cells.

[b] PS, phosphatidylserine; CPE, ceramide phosphatidylethanolamine; CPG, ceramide phosphatidylglycerol; PE, phosphatidylethanolamine.

[c] Numbers in parentheses are lipid phosphorus levels (μmoles/g dry wt of cells).

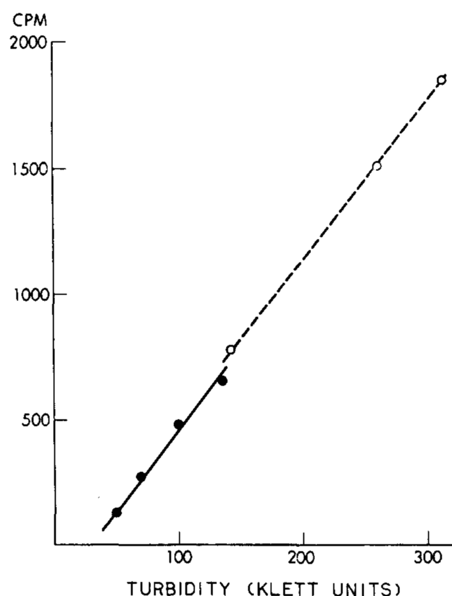

FIG. 1. Incorporation of [^{14}C]valine by cells grown with (○) and without (●) vitamin K.

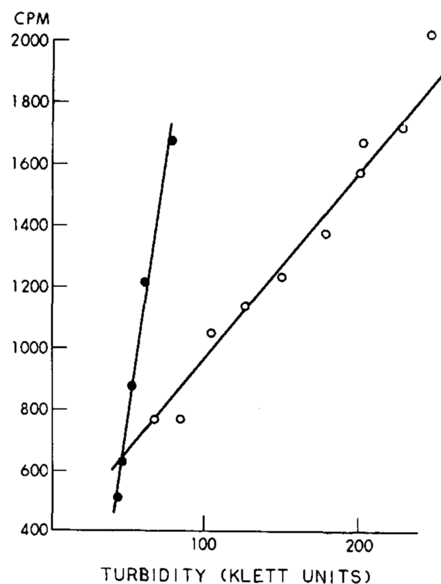

FIG. 2. Uptake of [2,3-^{14}C]succinate by cells grown with (○) and without (●) vitamin K.

per gram of cells than the vitamin K-grown cells (Fig. 2). Moreover, the increased degree of incorporation occurred from the earliest stage in the growth of the vitamin K-deficient culture, i.e., at a very low turbidity.

Incorporation of [2,3-^{14}C]succinate and $H_3{}^{32}PO_4$ into lipids

Vitamin K-deficient bacteria grown in the presence of small amounts of carrier-free [^{14}C]succinic acid incorporated more labeled material into total phospholipids (Table 1) than did corresponding cells grown with vitamin K. When the extracts were analyzed for phospholipids, seven major spots were visible after radioautography (Fig. 3). The largest of these cochromatographed with authentic phosphatidylethanolamine and spot PS migrated as phosphatidylserine. The vitamin K-deficient bacteria incorporated seven times more succinate carbon into phosphatidylethanolamine than did the cells grown with vitamin K when extracts from equal cell mass (dry wt) were analyzed. Incorporation of labeled succinate into other phospholipids from the vitamin K-deficient preparation was greater than that found in corresponding extracts from cells grown in the presence of vitamin K. In addition, one lipid (spot no. 5, Fig. 3) not containing phosphorus had three times more ^{14}C per gram dry weight of cells in the K(−) cells than in the K(+) cells. The percentage incorporation of [^{14}C]-succinate and $H_3{}^{32}PO_4$ into the various phospholipids is shown in Table 1.

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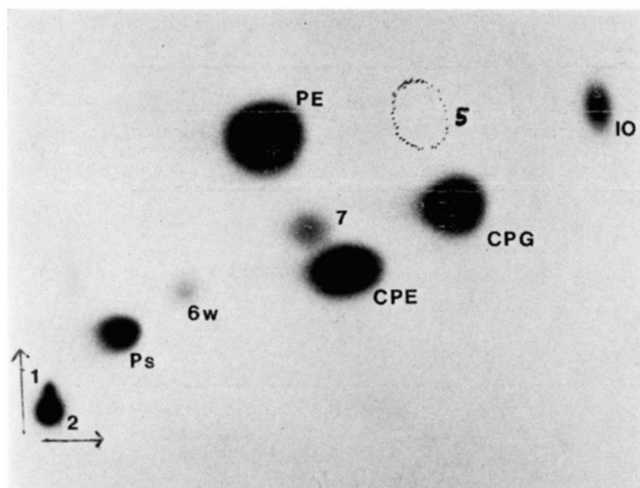

FIG. 3. Major phospholipids of *B. melaninogenicus* shown by radioautography after thin-layer chromatography of lipids from a culture labeled with $H_3{}^{32}PO_4$; spot 5 is a major lipid that does not contain phosphorus.

In contrast to the incorporation of succinate, ^{32}P was incorporated to the same degree into the phospholipids of cells grown in the presence and absence of vitamin K. Phosphatidylethanolamine and other phospholipids were labeled to a similar degree in both preparations (Table 1). The greater degree of succinate incorporation into lipids of deficient cells than into those of the vitamin K-grown cells, with essentially the same level (per gram dry weight of cells) of incorporation of ^{32}P in both preparations, suggests that lipid metabolism is deranged in vitamin K-deficient cells. This conclusion was also suggested by the number of highly labeled lipids found after radioautography of an extract of K(−) cells grown with [^{14}C]succinate; such a pattern of labeling was not found when corresponding extracts of K(+) cells were examined.

In the above experiments, vitamin K depletion was produced by growth of a large inoculum of cells for 16–30 hr in a vitamin K-free medium. Under these conditions vitamin K is progressively depleted as the culture grows.

In further experiments, cells were grown continuously in the absence of vitamin K in a medium containing succinate (2.5 mg/ml) and 40 μCi of [2,3-^{14}C]succinate, and the lipids of these K(−) + succinate cells were compared with those in cells grown in the presence of vitamin K plus succinate. In extracts of the K(−) + succinate cells, the incorporation of succinate into phosphatidylethanolamine was increased disproportionately compared with incorporation into other lipids. Incorporation into phosphatidylserine was also increased, but incorporation into ceramide phosphorylethanolamine and ceramide phosphorylglycerol was significantly decreased. In these experiments, more than 80% of the succinate incorporated into the phospholipids of the vitamin K-depleted cells was found in phosphatidylethanolamine (Table 1). When cells were grown in succinate-supplemented medium in the presence of $H_3{}^{32}PO_4$, the incorporation of ^{32}P into phosphatidylethanolamine and the level of phosphatidylethanolamine increased, whereas incorporation of ^{32}P and levels of the ceramides were reduced. This pattern of incorporation and level changes was similar to the incorporation of [^{14}C]succinate in K(−) + succinate cells (Table 1, and Table 2, Expt. 4).

Effect of vitamin K supplementation on distribution of phospholipids

The above experiments indicate that vitamin K can affect the phospholipid content of *B. melaninogenicus*. In order to test further the effect of vitamin K, K(−) + succinate cultures were inoculated with vitamin K and $H_3{}^{32}PO_4$ simultaneously and incubated for 2 hr (i.e., less than one generation time). A control culture was inoculated with $H_3{}^{32}PO_4$ only and incubated. Incorporation into individual phospholipids was then determined.

The results of two such experiments are shown in Table 2 (Expts. 1 and 2). Vitamin K supplementation significantly increased incorporation of ^{32}P into both ceramide phosphorylethanolamine and ceramide phosphorylglycerol. The distribution of phospholipids of a vitamin K plus succinate culture inoculated with $H_3{}^{32}PO_4$ alone (Expt. 3) is shown for comparison; the distribution is almost identical with that of the vitamin K-supplemented culture of Expt. 2.

When $H_3{}^{32}PO_4$ was included in the medium of a K(−) + succinate culture before inoculation, the effect of vitamin K_1 added later on phospholipid distribution was similar to that of Expts. 1 and 2, although these effects were less marked (Expt. 4).

The effect of vitamin K on ceramide phosphorylethanolamine and ceramide phosphorylglycerol was studied in a further experiment. One culture of cells was grown deficient in vitamin K in the presence of succinate; it was incubated with $H_3{}^{32}PO_4$ and vitamin K simultaneously, whereas a control culture was inoculated with $H_3{}^{32}PO_4$ alone. At 45, 90, 135, and 180 min thereafter, 25-ml samples were withdrawn, the cells were washed, and lipids were extracted. Radioactive spots on silica gel thin-layer plates were detected by radioautography, scraped off, and counted.

The results are shown in Fig. 4. After the addition of vitamin K there was a linear increase in incorporation of ^{32}P into ceramides phosphorylethanolamine and phosphorylglycerol. This increase was apparent 45 min after the addition of vitamin K, and when extrapolated to zero it is estimated that the increase in ceramides phosphorylethanolamine and phosphorylglycerol synthesis

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TABLE 2. Effects of vitamin K_1 on the incorporation of ^{32}P into the phospholipids of vitamin K-depleted *B. melaninogenicus*[a]

Phospholipids	Expt. 1		Expt. 2		Expt. 3	Expt. 4	
	K(−) + Succinate[b]	K(−) + Succinate + K[c]	K(−) + Succinate	K(−) + Succinate + K[c]	K(+) + Succinate[c]	K(−) + Succinate	K(−) + Succinate + K[c]
PS[d]	25.6	14.6	21.9	17.6	17.9	4.6	5.4
CPE	9.3	24.5	10.7	26.0	25.6	7.2	13.5
CPG	1.6	7.9	2.2	13.5	12.2	5.0	7.7
PE	62.6	52.2	63.3	42.4	42.3	81.0	71.3
6w[e]	0.9	0.8	1.8	0.7	0.9	0.2	0.2
Total phospholipid counts	2318	2170	2379	4550	3157	22,485	14,134

In Expts. 1 and 2, vitamin K and 300 μCi of $H_3{}^{32}PO_4$ were added simultaneously to 200 ml of culture K(−) + succinate + K. K(−) + succinate received only 300 μCi of $H_3{}^{32}PO_4$. In Expt. 3, $H_3{}^{32}PO_4$ was added to a log-phase succinate culture (25 mg/ml), and cells were extracted after a 2-hr incubation. In Expt. 4, 300 μCi of $H_3{}^{32}PO_4$ was added to two cultures prior to inoculation. K(−) + succinate + K was supplemented with vitamin K for 1.5 hr.

[a] Results are expressed as percentage of ^{32}P incorporation into phospholipids per g dry wt of cells.
[b] These cells were grown in the presence of succinate plus blood, and were depleted of vitamin K.
[c] Vitamin K_1 (0.1 μg/ml final total concentration) was added to these cultures.
[d] Abbreviations as in Table 1.
[e] See Fig. 3.

began 38 min and 60 min, respectively, after the addition of vitamin K.

Vitamin K supplementation had no effect on the incorporation of $H_3{}^{32}PO_4$ into phosphatidylethanolamine (Fig. 5). Phosphatidylserine showed an increased incorporation in the vitamin K-supplemented culture after 90 min (Fig. 5), corresponding to the increased rate of growth at this time (Fig. 6). During the first 90 min of the experiment, growth of the vitamin K-supplemented

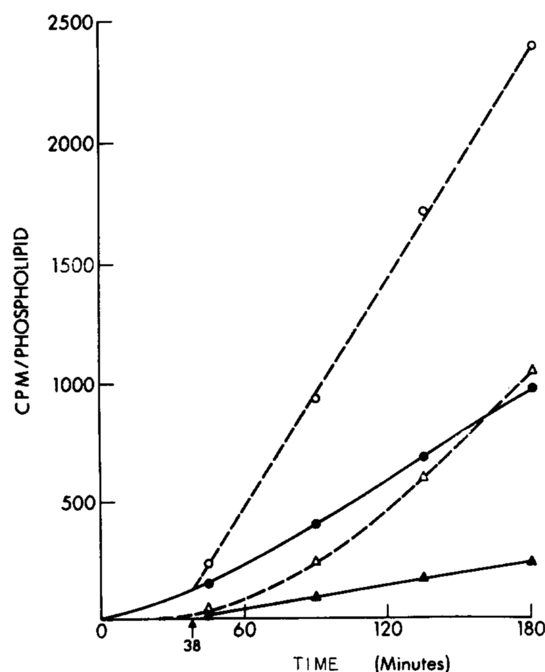

Fig. 4. Stimulation of synthesis of ceramide phosphorylethanolamine and ceramide phosphorylglycerol after addition of vitamin K at time 0 to a K(−) + succinate culture. The values are total cpm for each phospholipid spot from the thin-layer plate. Vitamin K-supplemented culture: ○, ceramide phosphorylethanolamine; △, ceramide phosphorylglycerol. Vitamin K-deficient control culture: ●, ceramide phosphorylethanolamine; ▲, ceramide phosphorylglycerol.

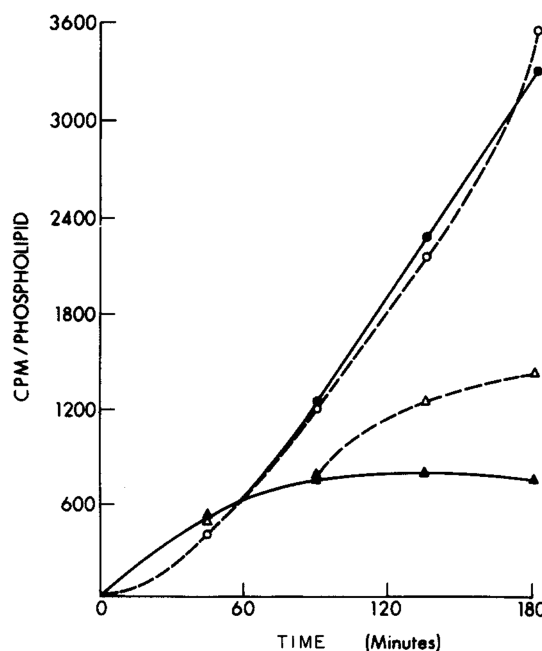

Fig. 5. Effect of added vitamin K (0.1 μg/ml) at time 0 on the incorporation of ^{32}P into phosphatidylethanolamine (○, plus vitamin K; ●, no vitamin K) and phosphatidylserine (△, plus vitamin K; ▲, no vitamin K). The values are total cpm for each phospholipid spot from the thin-layer plate. Increased incorporation of $H_3{}^{32}PO_4$ into phosphatidylserine occurred 90 min after the addition of vitamin K, corresponding to the increased growth of the culture at this point (Fig. 6).

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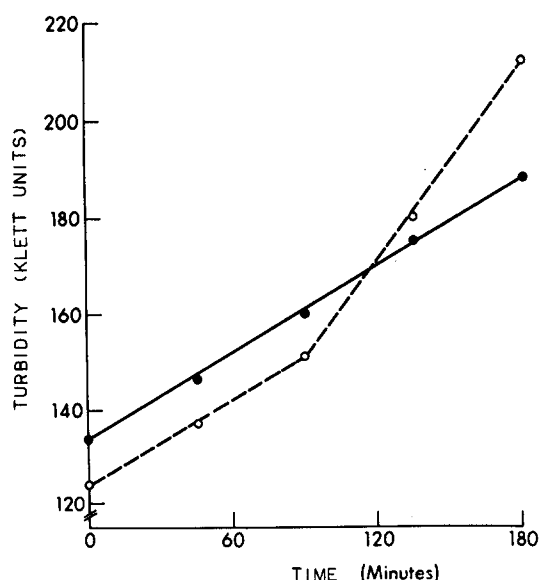

Fig. 6. Growth response of K(−) + succinate culture to which $H_3{}^{32}PO_4$ was added at time 0 (●), and a similar culture to which $H_3{}^{32}PO_4$ and 0.1 μg/ml of vitamin K_1 were added simultaneously (○).

culture paralleled that of the control, demonstrating that the increase in phosphosphingolipid synthesis at this time was not a reflection of an increase in general cell metabolism. The vitamin K-stimulated incorporation of ^{32}P into the ceramide phosphorylethanolamine and ceramide phosphorylglycerol of *B. melaninogenicus* could represent increased synthesis or increased turnover of these compounds. Phosphorus determinations were made on individual lipids extracted from the vitamin K-depleted culture which had been incubated with vitamin K for 180 min (from the experiment quoted above [Figs. 4 and 5]). These determinations were compared with those made on lipids from an unsupplemented, depleted culture. Levels of lipid phosphorus (μmoles/g dry wt of cells) for the vitamin K culture were phosphatidylserine, 8.7; ceramide phosphorylethanolamine, 12.4; ceramide phosphorylglycerol, 7.6; and phosphatidylethanolamine, 24.8. The depleted culture contained phosphatidylserine, 5.1; ceramide phosphorylethanolamine, 6.9; ceramide phosphorylglycerol, 6.2; and phosphatidylethanolamine, 24.0. Thus, the addition of vitamin K stimulates the synthesis of phosphosphingolipids and also the later synthesis of phosphatidylserine but does not affect that of phosphatidylethanolamine. A summary of the lipid changes found under various conditions of growth is shown in Table 3.

DISCUSSION

Previous work on succinate as a growth factor for *B. melaninogenicus* has shown that this compound will replace in part the vitamin K requirement of the microorganism, enabling a limited degree of growth. This growth factor property of succinate was utilized in the study of the mode of action of vitamin K; in some experiments, cells which had been maintained in a vitamin K-deficient state by serial growth in the presence of succinate were compared with cells grown with vitamin K also in the presence of succinate.

The early, greater, and linear uptake of succinate by vitamin K-deficient bacteria indicates that the increase in uptake is closely correlated to the vitamin deficiency. The greater incorporation of succinate into the phospholipids of K(−) as compared with K(+) cultures, and the same degree of incorporation of ^{32}P into the phospholipids of K(+) and K(−) cultures, suggests that lipid metabolism is deranged in vitamin K-deficient cells when a precursor such as succinate is supplied. This derangement is manifested as a greater degree of incorporation into phospholipids and other lipids.

By serial growth of K(−) + succinate cultures, it is possible to obtain cells which are entirely devoid of vitamin K. These cells, however, are still fundamentally vitamin K-requiring and do respond to added vitamin

TABLE 3. Changes in relative incorporation of $H_3{}^{32}PO_4$ or [2,3-^{14}C]succinate into phospholipids under various conditions of growth of *B. melaninogenicus*

Expt.	Medium Supplementation: Vitamin K	Blood	Succinate, 2×10^{-2} M	Label	Relative Incorporation into Lipids[a]: PE	CPE	CPG	PS
1	+		+	[^{14}C]succinate	0	0	0	0
2		+		[^{14}C]succinate	↑	↓	↓	0
3		+	+	[^{14}C]succinate	↑↑	↓↓	↓↓	↑
4		+	+	$H_3{}^{32}PO_4$	↑↑	↓↓	↓↓	↓
5[b]	+ (45–180 min)	+	+	$H_3{}^{32}PO_4$	0	↑	↑	0

↑, increased incorporation; ↓, decreased incorporation; 0, no change.

[a] Incorporation is relative to that of a culture grown in basal medium supplemented with vitamin K and blood. Abbreviations as in Table 1.

[b] Incorporation results are relative to those of Expt. 4.

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K with an enhanced rate of growth. Such cells have an unusual phospholipid complement in that phosphatidylethanolamine is elevated and levels of ceramide phosphorylethanolamine and ceramide phosphorylglycerol are diminished. Since K(+) + succinate cells, on the other hand, have a phospholipid complement similar to that of the K(+) control culture with respect to the major components, vitamin K would appear to affect the proportions of phospholipid synthesized. This involvement of vitamin K was demonstrated more directly by the addition of the vitamin and ^{32}P to deficient bacteria grown with succinate. In these experiments the incorporation of ^{32}P into ceramide phosphorylethanolamine and ceramide phosphorylglycerol was stimulated significantly by the presence of the vitamin. Further experiments showed that ceramide phosphorylethanolamine and ceramide phosphorylglycerol synthesis was stimulated by vitamin K shortly after its addition and that the stimulation of ^{32}P incorporation into these compounds was linear. It was interesting to note that vitamin K had no effect on the incorporation of ^{32}P into phosphatidylethanolamine during the 180-min experimental period; i.e., by its action of ceramide synthesis, vitamin K brings the phospholipid levels to a state approaching those found in fully supplemented cultures.

The mechanism of the relationship of vitamin K to phosphosphingolipid synthesis is not known. Previous work has shown (3) that the cell envelope in vitamin K-deficient cells is impaired, and the relative inability of the vitamin K-deficient bacterium to divide may be a reflection of the depressed levels of ceramide phosphorylethanolamine and ceramide phosphorylglycerol; these compounds may be concerned in the division process of *B. melaninogenicus*. The involvement of phospholipids in bacterial cell division has been discussed by Starka and Moravova (9). Rizza et al. (8) have analyzed the lipids of several other strains of *B. melaninogenicus*. They have shown that phosphatidylethanolamine is the predominant phospholipid and they also have identified phosphatidylserine, phosphatidic acid, and the unusual phosphosphingolipids ceramide phosphorylethanolamine and ceramide phosphorylglycerol or ceramide phosphorylglycerol phosphate, which in their strains accounted for 50–70% of lipid phosphorus. They made the interesting observation that when the growth rate of the bacteria was altered by the levels of added protoheme, the gross lipid composition of the cells was not changed.

B. melaninogenicus, as well as *B. ruminicola* (13), differs from other bacteria in that the membranes contain sphingolipids, substances which are characteristically found in the central nervous system of higher organisms. The depressed synthesis of these compounds in vitamin K-deficient cells and the response of cells to added vitamin K therefore provide a useful biological tool for the study of these sphingolipids in relation to membrane structure and function.

These results on phosphosphingolipid synthesis suggest the possibility that vitamin K could function as a cofactor in the synthesis of these compounds. Another possibility is that vitamin K is involved indirectly; possibly it is the key molecule in the architecture of the cell envelope which could be required to render sites for ceramide synthesis operative. Thus, vitamin K deficiency results in depressed sphingolipid synthesis in *B. melaninogenicus*, and vitamin K has a specific and early effect on the synthesis of these compounds by this microorganism.

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