of C3H mice injected with the same number of spleen cells from Hcy-treated C57Bl mice was considerably delayed, and most of the group given $20 \times 10^6$ cells survived indefinitely. Two of the mice injected with $50 \times 10^6$ cells survived to the 34th day; one of them died from anesthesia on that day during the graft procedure, and the other died on the 60th day still bearing a healthy skin graft (26 days old). The six survivors of the group given $20 \times 10^6$ treated spleen cells and the four survivors of the control group given $10 \times 10^6$ cells were grafted with C57B1 skin. The control mice rejected the graft with a vigorous reaction of the second set type in 4 to 5 days. The six mice of the group of treated mice also rejected the graft, but this followed a delayed pattern (in 10, 14, 15, 16, 19, and 20 days). Thus, although no tolerance to the donor tissues has been induced in the recipients in this strong histocompatibility combination, the severity of the graft-host reaction has been considerably reduced, and some prolongation of the survival time of the skin grafts has been observed.

In Table 2 are shown the results of the third experiment, that on the combination of Fisher rats and Balb/c mice. The control mice injected with either spleen or bone marrow cells were with one exception dead between the 5th and the 41st day. The rat skin grafted on the survivor was sloughed and rejected in 20 days. Most of the mice injected with cells from Hcy-treated rats survived indefinitely. In the four survivors of the mice given $50 \times 10^6$ spleen cells the rat-skin grafts were sloughed and rejected between the 17th and the 26th day. Of the three grafted mice of the group given bone marrow cells, one died 30 days later with its graft still in a healthy state, while the two others rejected the rat-skin grafts on the 40th and 52nd day after some regrowth of hair on the graft. Thus, 93 percent of the control mice died after the transfer of rat cells, and the one survivor did not become tolerant. Among the mice transfused with cells from Hcy-treated rats only 60 percent died. Most of the survivors rejected their grafts, but three of them showed a significant prolongation of the period of graft survival.

In irradiated mice some survival (3 to 10 percent) has been observed from graft-host reaction up to the 100th day, if the mice had been treated with rat marrow or spleen cells (*9*). On the average, one half of these survivors did tolerate rat tissue grafts for a prolonged period of time (*10*). These results of prior work are rather similar to our findings for the group of control animals. The observation that 40 percent of the mice of the treated group survived the graft-host reaction indefinitely shows that the spleen and marrow cells of the Hcy-treated rats are significantly less reactive than the cells of normal animals.

The aforementioned results further support the idea that a permanent tolerance to foreign tissues could be induced in irradiated adult animals by transfusion of a rather limited number of lymphoid cells. The main obstacle to such a procedure is the activity of these cells against the host. Consequently, if by any means the lymphoid cells of the donor could be rendered less active against the recipient, a sufficiently large number of such cells could be safely injected in order to induce in adult irradiated animals a specific tolerance to the donor tissues. Treating the donors with an unrelated antigen may prove to be one means of attaining the aforementioned goal. Our results show that even in the stronger histocompatibility combinations, an important reduction of the severity of the graft-host reaction can be produced. In these experiments only two commonly available antigens (RGG and Hcy) were used. Other antigenic materials, of bacterial or viral origin for example, might be more effective in this regard. Another possibility is that when the graft-host reaction lasts for a long period, as in our experiments, other forms of medication may facilitate the recovery of recipient animals from the graft-host disease without being prejudicial to the establishment of the specific immune tolerance to the donor's tissues.

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## Vitamin K Compounds in Bacteria That Are Obligate Anaerobes

Abstract. *A naphthoquinone-dependent strain of* Bacteroides melaninogenicus *has been used in a microbiological assay to survey bacteria for compounds of the vitamin K group. Organisms known to contain vitamin K, as well as several bacteria that are obligate anaerobes, produced substances which satisfied the naphthoquinone requirement of the assay organism. Vitamin K was chemically isolated from strains of* Bacteroides melaninogenicus, Bacteroides fragilis, *and* Veillonella alcalescens.

Compounds of the vitamin K group are widely distributed in plants, animals, and microorganisms. It has been suggested that these compounds function in phosphorylation accompanying electron transport during oxidative metabolism (*1*). This hypothesis is supported in part by what is known of the distribution of vitamin K compounds in various bacteria; in particular, the naphthoquinones are absent from cytochrome-free organisms and chemoorganotrophic anaerobes (*2*, *3*, *4*). The anaerobe, *Chromatium*, has been found to contain naphthoquinones of the vitamin K group, but this organism represents a special case, being a photosynthetic, cytochrome-containing sulfur bacterium (*5*). However, a naphthoquinone was recently isolated from the lactic acid bacterium, *Streptococcus faecalis* (*6*). In addition, certain strains of *Bacteroides melaninogenicus*, a strict anaerobe, require naphthoquinones for growth, which suggests that these compounds may be important in anaerobic chemoorganotrophic metabolism (*7*, *8*). We now report data indicating that compounds of the vitamin K group are present in a variety of obligately anaerobic chemoorganotrophic bacteria.

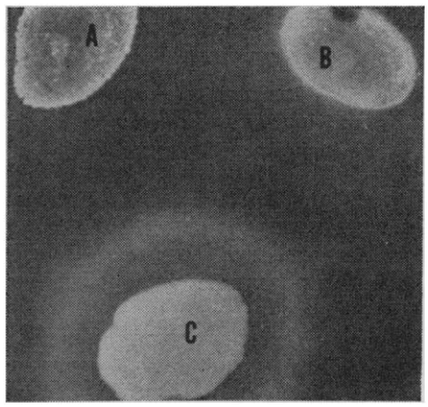


Fig. 1. Agar plate seeded with naphthoquinone-dependent strain of *B. melaninogenicus* (K110). *A*, No growth response to *Clostridium histolyticum; B*, weak or questionable growth response to *B. melaninogenicus* strain CR2A; *C*, marked growth response to *B. fragilis*. Vitamin K was chemically isolated from *B. melaninogenicus* and *B. fragilis*.

A naphthoquinone-dependent strain of *B. melaninogenicus* was used in a microbiological assay to detect vitamin K compounds in bacteria. The reliability of the assay was determined by testing microorganisms, whose naphthoquinone content was known, for their ability to support growth of a naphthoquinone-dependent strain of *B. melaninogenicus* (strain K110). This organism and the conditions for its cultivation have been described (*8*, *9*). The basal medium used contained per liter: trypticase (BBL), 27 g; yeast extract (Difco), 3 g; NaCl, 2 g; $KH_2PO_4$, 2.5 g; $K_2CO_3$, 2 g; and hemin, 5 mg. For routine cultivation, 0.2 $\mu$g of menadione per milliliter was added. This was autoclaved separately under pressure, 10 lb (4.6 kg) for 10 minutes. Menadione-deficient cells were obtained from stock cultures by inoculating the organism in broth free of menadione, where limited growth occurred. Cells so grown were used to seed agar plates of the basal medium, and the plates were used for assays. Cultures to be examined for naphthoquinones were placed in spots on the seeded plates and incubated anaerobically. Zones of growth of *B. melaninogenicus* ranging from 1 to 15 mm were observed adjacent to colonies of organisms which were known to contain compounds of the vitamin K group (Fig. 1 and Table 1).

Of five organisms studied by Bishop *et al.* (*3*), in which vitamin K could not be detected by chemical means, three failed to support growth of the assay organism. However, the remaining two (*Serratia marcescens* and *Aerobacter aerogenes*) produced substances which satisfied the naphthoquinone requirement of *B. melaninogenicus*. This discrepancy may be due to greater sensitivity of the microbiological assay, to differences in bacterial strains utilized, or to a nonspecific response of the assay organism.

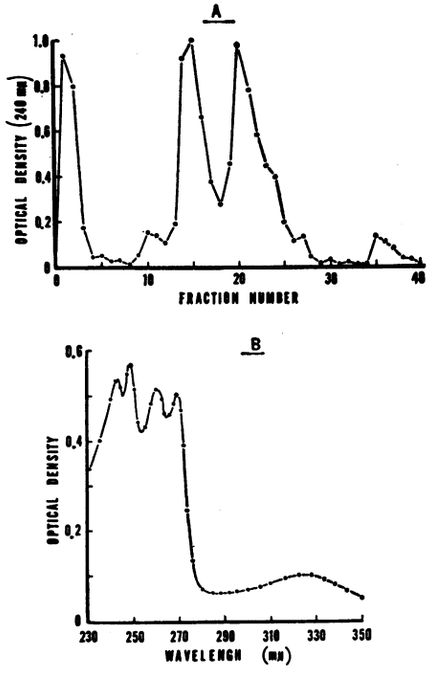


Fig. 2. *A*, Column elution pattern of lipid from *B. melaninogenicus* strain BE1, which was typical of the anaerobes examined. *B*, Ultraviolet absorption spectrum of isooctane solutions of vitamin K present in fractions 13 to 17, after purification by thin-layer chromatography. The absorption spectrum of vitamin K from other anaerobes examined was qualitatively similar.

Table 1. Microbiologic assay of vitamin K in bacteria.

| Organisms tested | Strains tested (No.) | Growth response of naphthoquinone-dependent *B. melaninogenicus* | Reported vitamin K content |
|---|---|---|---|
| | Facultative anaerobes | | |
| *Bacillus megaterium* | 1 | + | + (*3*) |
| *Bacillus subtilis* | 1 | + | + (*3, 4*) |
| *Corynebacterium diphtheriae* | 2 | + | + (*3*) |
| Facultative diptheroids | 2 | + | |
| *Lactobacillus casei* | 4 | − | − (*3*) |
| *Sarcina lutea* | 1 | + | + (*3*) |
| *Staphylococcus aureus* | 2 | + | + (*3, 4*) |
| *Streptococcus mitis* | 2 | − | |
| *Aerobacter aerogenes* | 1 | + | − (*3*) |
| *Alcaligenes faecalis* | 1 | − | |
| *Escherichia coli* | 2 | + | + (*2, 3*) |
| *Proteus vulgaris* | 1 | + | + (*3, 4*) |
| *Pseudomonas aeruginosa* | 1 | − | − (*3*) |
| *Serratia marcescens* | 2 | + | − (*3*) |
| | Obligate anaerobes | | |
| *Clostridium histolyticum* | 1 | − | − (*3*) |
| *Corynebacterium acnes* | 1 | + | |
| Anaerobic diphtheroids | 2 | + | |
| *Peptostreptococcus magnus* | 2 | + | |
| *Peptostreptococcus* species | 2 | + | |
| *Peptostreptococcus* species | 3 | − | |
| *Bacteroides fragilis* | 2 | + | |
| *Bacteroides oralis* | 1 | + | |
| *Bacteroides melaninogenicus* | 1 (BE1) | + | |
| *Bacteroides melaninogenicus* | 1 (CR2A) | ± | |
| *Fusobacterium fusiforme* | 2 | − | |
| *Veillonella alcalescens* | 1 | + | |

Various anaerobic bacteria supported growth of *B. melaninogenicus* strain K110 (Table 1). These were strains of *Bacteroides fragilis, Bacteroides oralis*, naphthoquinone-independent strains of *B. melaninogenicus*, strains of *Veillonella alcalescens*, and certain strains of anaerobic streptococci and diphtheroids.

The presence of vitamin K compounds in anaerobic bacteria, as suggested from the foregoing data, was confirmed in two strains of *B. melaninogenicus* (BE1 and CR2A), and one strain each of *B. fragilis* and *V. alcalescens* by chemical isolation. Each organism was grown in batch culture in large Florence flasks filled to the neck with medium to obtain anaerobic conditions. For *B. fragilis*, the basal medium was supplemented with 0.5 percent glucose and for *V. alcalescens*, with 0.5 percent sodium lactate. Cells were harvested by centrifugation and lyophilized; the dried cells (5 to 10 g dry weight) were extracted three times with a mixture of isooctane and iso-

propyl alcohol (3 to 1) at room temperature. The extracts were dried at reduced pressure, and the lipid was fractionated on columns containing 10 g alumina (Brockman No. 3). Columns were eluted with portions of hexane (50 ml each) containing 0, 2, 4, 6, and 20 percent of ether. Fractions (5 ml) were collected and scanned for ultraviolet-absorbing material. The elution pattern for *B. melaninogenicus* (strain BE1), which was typical of the anaerobes examined, is shown in Fig. 2*A*. The ultraviolet absorption spectrum of fraction 14 contained peaks at 243, 248, 260, and 269 mμ, characteristic of compounds of the vitamin K group. This material was purified by thin-layer chromatography on activated silica gel HF plates developed with a mixture of isooctane, ether, and acetic acid (94 : 6 : 0.1). Compounds absorbing ultraviolet light were eluted with acetone, and their ultraviolet absorption spectra were determined in isooctane. All four anaerobes examined yielded compounds which had an absorption spectrum typical of 2,3-alkyl substituted naphthoquinones of the vitamin K group (Fig. 2*B*). Reduction of alcoholic solution of each with potassium borohydride ($KBH_4$) resulted in a bleaching of absorption at 260 to 270 mμ, accompanied by an 80 to 100 percent increase in absorption at 245 mμ. The compound from each organism gave a positive reaction in the Irreverre and Sullivan test (*10*) and supported growth of strain K110 of *B. melaninogenicus*. Vitamin K, isolated from *Escherichia coli* in a similar manner, supported growth of *B. melaninogenicus*, wheras ubiquinone failed to do so.

Thus, compounds of the vitamin K group are present in certain strictly anaerobic chemoorganotrophic bacteria and evidently play a vital part in anaerobic metabolism. In the case of the *Bacteroides* species at least, this vitamin must function in processes other than phosphorylation resulting from electron transfer to molecular oxygen, nitrate, or sulfate. The observation that strains of *B. fragilis* synthesize and excrete vitamin K suggests that this organism plays an important role in mammalian nutrition. Synthesis of vitamin K compounds by intestinal bacteria provides the mammalian host with a source of this vitamin (*11*). It has been assumed that the facultative coliform group has been responsible for this synthesis. However, organisms of the *B. fragilis* type are among the most numerous bacteria inhabiting the intestinal tract of man and are present in concentrations 100-fold greater than that of *E. coli* (*11, 12*). Thus, the bulk of vitamin K synthesized by the intestinal microbiota is probably due to these organisms.

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## Allosteric Properties of Glutamate Dehydrogenases from Different Sources

Abstract. *The structure and allosteric properties of glutamate dehydrogenases from different sources have been studied by immunologic means. Despite structural differences detected by electrophoretic mobility and antigenicity, all the vertebrate enzymes responded similarly to allosteric modification. Bacterial glutamate dehydrogenase was immunologically unrelated to the vertebrate enzymes and did not respond to allosteric regulation.*

Certain low molecular weight "allosteric" reagents alter the structure and enzymic activity of glutamate dehydrogenase (GDH) crystallized from bovine liver (see *1*). This enzyme activity is widely distributed among microorganisms and higher forms, and, therefore, it was of interest to find out whether the allosteric regulation observed with the bovine liver enzyme also occurred with glutamate dehydrogenases from other sources. Because immunologic techniques show structural modifications in the enzyme induced by allosteric reagents, even in crude tissue extracts (*2*), we used these methods in the present investigation.

Earlier studies revealed three different antigenic forms of crystalline GDH (*2, 3*). The relative concentrations of these forms were altered by allosteric reagents such as adenosine diphosphate (ADP), guanosine triphosphate (GTP), or diethylstilbestrol. In general, ADP, which favored an antigenic form of the molecule with high GDH activity, also decreased the electrophoretic mobility in agar gel and resulted in heavier enzyme-antibody precipitin lines. Guanosine triphosphate and diethylstilbestrol increased the electrophoretic mobility and had opposite effects from those of ADP on enzyme activity. They favored antigenic forms having little or no GDH activity but having greater alanine dehydrogenase activity. These findings correlated well with the effects of these molecules on the physical and catalytic properties of GDH (*1*).

It was further shown (*2*) that sodium dodecyl sulfate dissociated the enzyme into a single immunologic component that lacked enzyme activity; this component probably represents the fundamental subunit of GDH.

In our present study, crude extracts prepared from commercial "acetone powders" of different beef organs and the livers of other vertebrate species were reacted with rabbit antiserum to GDH in double diffusion (*4*) and immunoelectrophoretic (*5*) experiments. The rabbit antiserum was prepared against crystalline GDH obtained from bovine liver as already described (see *2*).

Figure 1 illustrates two simultaneous immunoelectrophoretic experi-