METABOLIC ROLE OF THE BR FACTOR IN BUTYRIBACTERIUM RETTGERI

LEO KLINE,¹ L. PINE,² AND H. A. BARKER

Department of Biochemistry, University of California, Berkeley, California

Received for publication 30 November 1962

ABSTRACT

KLINE, Leo (University of California, Berkeley), L. PINE, and H. A. BARKER. Metabolic role of the BR factor in Butyribacterium rettgeri. J. Bacteriol. 85:967–975. 1963.—The BR factor, which is replaceable by lipoic acid, was shown to be required for the decomposition of lactate by Butyribacterium rettgeri. Since the factor was not required for the fermentation of pyruvate or glucose by this organism, the results indicate that BR factor is essential only for some reaction involved in the oxidation of lactate to pyruvate, possibly the transport of electrons from lactate to the ultimate anaerobic electron-acceptor systems. The same products, namely, acetate, butyrate, and carbon dioxide, are formed from glucose or pyruvate in the presence or absence of BR factor and from lactate in the presence of the factor. Thus the factor is not required for the conversion of glucose or pyruvate to acetate, butyrate, or carbon dioxide. Although B. rettgeri does not require the BR factor for growth on glucose, growth of lactate-adapted cells on glucose is slow and is markedly stimulated by addition of the factor. Such cells growing on glucose do not show any ability to decompose lactate; consequently, an additional function of the BR factor, probably associated with the conversion of glucose to pyruvate, is indicated. Cells fully adapted to glucose grow rapidly on this substrate and do not respond to the factor. Lactate is formed during growth of glucose-adapted cells on glucose, although such cells cannot decompose lactate.

Butyribacterium rettgeri has been shown to require a growth factor, called the BR factor,

¹ National Institutes of Health predoctoral fellow, 1947–48. Present address: Western Regional Research Laboratory, Albany, Calif.
² Present address: Department of Microbiology, Duke University School of Medicine, Durham N.C.

for growth on a partially synthetic medium containing lactate as the main energy source (Kline and Barker, 1960). The BR factor is replaceable by lipoic acid in the nutrition of B. rettgeri (Kline et al., 1952), and the known chemical properties of the two materials are very similar. Therefore, it is probable that the BR factor is identical with lipoic acid and some of its derivatives.

Since lipoic acid has been shown to be a cofactor in the oxidation of pyruvate by some bacteria (Gunsalus, 1953), it appeared worthwhile to report some experiments on the role of the BR factor in the metabolism of B. rettgeri. These experiments indicate that in this organism the BR factor, and presumably lipoic acid, are not required for the decomposition of pyruvate or glucose but are essential for the decomposition of lactate.

MATERIALS AND METHODS

The media and cultural methods used in this investigation have been described by Kline and Barker (1950). The chemical methods have been described by Pine and Barker (1954). Lactate-1-C¹ was prepared biologically by the use of B. rettgeri; 89.5% of the C¹ was in the 1 position, and the remainder was almost equally divided between the 2 and 3 positions.

In growth studies, and in the preparation of cell suspensions, B. rettgeri was grown anaerobically in the semisynthetic medium of Kline and Barker (1950), with lactate, glucose, or pyruvate as the energy source. Unless otherwise indicated, the carbohydrate was used at a level of 0.5%. The BR factor, where used, was supplied either as a standard yeast extract (Difco) preparation containing 1 unit of activity in 1.2 mg (dry weight), or as a purified concentrate (no. 7) containing 1 unit in 2 μg. For cell preparations, the cultures were harvested after 18 to 48 hr, depending upon cultural conditions, washed twice with 0.05 m potassium phosphate buffer (pH 7) containing 0.03% Na₂S·9H₂O, and resus-
suspended in a volume of buffer equal to 2% of the original culture volume.

Manometric studies were carried out in Warburg vessels containing 2.2 ml of final reaction mixture buffered with phosphate at pH 7.0 to 7.2. Cell nitrogen per vessel varied from 1.6 mg to 3.4 mg according to the study.

Both growth and manometric studies were at 37 C, and unless otherwise indicated were carried out anaerobically under N₂.

Results

Growth on glucose. In preliminary experiments, the ability of B. rettgeri to grow in the absence of added BR factor in a semisynthetic medium (Kline and Barker, 1950) with glucose as an energy source was tested by transferring from a lactate-BR factor medium into a glucose medium devoid of the factor. The growth was relatively slow during the first 24 hr but was heavy after 48 hr. Repeated transfers every 2 or 3 days were made on this medium to minimize any effect caused by carry-over of BR factor in the initial transfer, and 15 subcultures developed with undiminished vigor. Thus, it is clear that the factor is not required for growth on glucose.

During the subculturing on a glucose medium, a striking change in growth rate occurred between the fifth and eighth transfers. The organism suddenly was found to grow much more rapidly, reaching maximal growth in less than 18 hr instead of more than 40 hr, and the growth was also appreciably heavier. This rapid and heavy growth was maintained with each transfer up to the 15th subculture, at which point the transfers were discontinued. This phenomenon, probably resulting from the development of a mutant, was repeated in a second and third set of subcultures, starting again with a transfer from a lactate culture.

The above results indicate that it is desirable to recognize at least three strains of B. rettgeri. Strain L is adapted to grow with lactate as an energy source in the presence of BR factor. Strain G1 is similar to strain L, except that it has been transferred several times in a glucose medium; its growth on glucose is markedly accelerated by the BR factor. Strain G2 is adapted to glucose by many transfers on a glucose medium; it grows rapidly on glucose and does not respond to addition of the BR factor. Strain L was grown in the semisynthetic lactate medium supplemented with 0.05% yeast extract (1.2 mg = 1 unit of BR factor); strain G1 was grown with or without yeast extract in the same medium or with 0.5% glucose and 1.5% potassium phosphate in place of 0.5% lactate and 0.2% phosphate; and strain G2 was grown in the glucose, high phosphate medium without yeast extract.

Influence of inoculum and BR factor on growth on glucose and lactate. The object of this experiment was to see how the growth rates of differently adapted strains of B. rettgeri are affected by addition of BR factor concentrates when glucose or lactate is provided as a substrate. Strain G2 used in this experiment had been subcultured ten times on a semisynthetic glucose medium without yeast extract or other source of BR factor.

Neither strain L nor strain G2 grew on lactate in the absence of BR factor (Fig. 1, curves 1, 1A). Cells adapted to glucose (strain G2) have the ability to develop a lactate-decomposing system when provided with BR factor but they showed a 20-hr lag before growth became apparent (curve 2), in contrast to lactate-adapted cells (curve 2A) which began to grow without delay. The final yield of cells was roughly the same for both strains. The results indicate that glucose-adapted cells are relatively deficient in ability to grow on lactate but they can regain this ability when placed in a medium containing lactate and BR factor.

![Graph](http://jb.asm.org/images/fig1.jpg)

**Fig. 1. Influence of inoculum and BR factor on growth on lactate and glucose. Experimental details are given in Table 1.**
Growth occurred in a glucose medium devoid of BR factor when the inoculum was from a lactate culture (curve 3), but there was a pronounced stimulatory effect of the factor (curve 3A). Addition of the factor also resulted in somewhat heavier growth. Glucose-adapted cells (strain G2), when inoculated into a glucose medium, grew very rapidly in the absence of BR factor (curve 4A) and were not stimulated by its addition (curve 4).

Comparison of curves 2 and 4 and of curves 2A and 3A (Fig. 1) shows that much heavier growth occurs with glucose than with an equal weight of lactate. This is not surprising since the conversion of glucose to lactate is an exergonic process.

Figure 2 shows that the growth response to BR factor in a lactate medium is dependent upon the type of inoculum used. Glucose-adapted cells (curves 1 and 2) required considerably more BR factor for a maximal growth response in the lactate medium than did lactate-adapted cells (curve 3). Thus, with the latter type of inoculum, maximal growth was reached with about 4 μg of concentrate no. 7 or 2 mg of the standard yeast extract, whereas the response of glucose-adapted cells was still increasing with 22 μg of concentrate or 10 mg of yeast extract. The response to equal amounts of BR factor was much less with the glucose-adapted cells.

**Influence of BR factor on the products of glucose fermentation.** Glucose-adapted (strain G2) cells gave essentially the same yields of acetic and butyric acids from glucose in the presence or absence of BR factor (Table 1). With lactate-adapted (strain L) cells, on the contrary, omission of the factor caused sharply decreased yields of volatile acids, particularly acetic acid. Several balance experiments have demonstrated two other characteristic features of glucose fermentations by lactate-adapted cells in the absence of the factor, namely, a considerable accumulation of lactic acid of the order of 10 to 15 moles per 50 moles of glucose decomposed and an increased formation of unidentified products other than volatile acids, lactate, and carbon dioxide, resulting in a low carbon recovery, usually 65 to 73% of theoretical. The addition of the BR factor greatly decreased the yield of lactate and increased the carbon recovery to 85 to 95% under otherwise identical conditions. Several attempts to identify the compounds containing the missing carbon in the BR factor-free medium were unsuccessful.

**TABLE 1. Influence of type of inoculum and BR factor supplementation on fermentation products formed from glucose and lactate**

<table>
<thead>
<tr>
<th>Culture</th>
<th>Substrate</th>
<th>BR factor</th>
<th>Inoculum strain</th>
<th>Final pH</th>
<th>Acetate</th>
<th>Butyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lactate</td>
<td>-</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td>Lactate</td>
<td>-</td>
<td>G2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Lactate</td>
<td>+</td>
<td>G2</td>
<td>6.8</td>
<td>26.1</td>
<td>46.7</td>
</tr>
<tr>
<td>2A</td>
<td>Lactate</td>
<td>+</td>
<td>L</td>
<td>6.7</td>
<td>27.3</td>
<td>50.3</td>
</tr>
<tr>
<td>3</td>
<td>Glucose</td>
<td>-</td>
<td>L</td>
<td>6.1</td>
<td>24.4</td>
<td>24.4</td>
</tr>
<tr>
<td>3A</td>
<td>Glucose</td>
<td>+</td>
<td>L</td>
<td>5.8</td>
<td>46.6</td>
<td>28.9</td>
</tr>
<tr>
<td>4</td>
<td>Glucose</td>
<td>-</td>
<td>G2</td>
<td>5.8</td>
<td>33.4</td>
<td>30.2</td>
</tr>
<tr>
<td>4A</td>
<td>Glucose</td>
<td>+</td>
<td>G2</td>
<td>5.8</td>
<td>35.6</td>
<td>29.7</td>
</tr>
</tbody>
</table>

* All cultures were grown anaerobically in test tubes (10 ml) on semisynthetic medium of Kline and Barker (1950) with indicated substrates, inocula, and BR factor additions. The BR factor, where used, was 22 μg of concentrate no. 7 per tube. Analyses made after 50 to 70 hr. Acetate and butyrate yields are expressed as μmoles per 50 μmoles of glucose or 100 μmoles of lactate decomposed.
The growth rate on pyruvate is essentially independent of the presence of BR factor, although the yield of cells is slightly greater in the presence of the factor (Fig. 3). Growth on pyruvate is not the result of the carry-over of traces of the factor from the lactate inoculum, since it has been shown to persist without noticeable change through 15 subcultures on a pyruvate medium devoid of BR factor. Growth also occurs readily when a pyruvate medium is inoculated with glucose-adapted cells which have been grown without BR factor for 12 subcultures.

**Products of pyruvate fermentation.** The influence of BR factor on the products of pyruvate fermentation with different types of inocula was determined by analyzing the media at the termination of the growth experiment described in Fig. 3. The data in Table 2 show that, just as in a glucose fermentation, the BR factor has no conspicuous effect on the products of pyruvate decomposition. However, it may be noted that with a lactate-adapted inoculum (strain L) but not with a glucose-adapted inoculum (strain G2) a little lactate accumulates in the absence of the factor. Also, with both types of inocula, the carbon recovery and the yield of volatile acids are a little higher with than without the factor. A pyruvate-adapted inoculum gave essentially the same results as a lactate-adapted inoculum.

The results show that the major biochemical processes involved in pyruvate breakdown,

**TABLE 2. Effect of type of inoculum and BR factor supplement on the products of pyruvate fermentation**

<table>
<thead>
<tr>
<th>Product</th>
<th>Pyruvate decomposed (moles per 100 moles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain L</td>
</tr>
<tr>
<td></td>
<td>No factor</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.4</td>
</tr>
<tr>
<td>Acetate</td>
<td>46.2</td>
</tr>
<tr>
<td>Butyrate</td>
<td>26.7</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>47.6</td>
</tr>
<tr>
<td>Per cent carbon recovery</td>
<td>85</td>
</tr>
<tr>
<td>Per cent of original carbon as volatile acids</td>
<td>66</td>
</tr>
</tbody>
</table>

* Experimental conditions as in the legend to Fig. 3.
namely, conversion of pyruvate to acetate and carbon dioxide, synthesis of butyrate from acetate, and synthesis of acetate from carbon dioxide, are not dependent upon the addition of the BR factor. However, the slightly higher carbon recoveries and fatty acid yields in the presence of the factor suggest that it may have an indirect effect on pyruvate metabolism.

Test of BR factor synthesis during growth on glucose. Since the BR factor is required for growth on lactate but not for growth on glucose or pyruvate, it is of interest to determine whether the factor is synthesized in a glucose medium. For this purpose, 5-ml portions of a 24-hr tenth subculture on a glucose medium without the factor and of a 48-hr lactate broth culture containing 0.05% yeast extract were adjusted to pH 0.5 or pH 6 and autoclaved at 120°C for 1 hr. At pH 0.5, but not at pH 6, complex forms of the BR factor are broken down under these conditions to the form utilized by *B. rettgeri* as a growth factor in a lactate medium. After autoclaving, the suspended material was removed by centrifugation, and 1-ml samples of the supernatant solutions were neutralized to pH 7 and assayed for BR factor activity (Kline and Barker, 1950). Only the lactate-yeast extract culture, autoclaved at pH 0.5, showed BR factor activity. The free factor was not present in the lactate culture, and neither free nor combined factor was detected in the glucose culture. Evidently the BR factor is not synthesized during growth in a glucose medium.

Absence of a lactate-degrading system in glucose-grown cells. Since the BR factor is required for growth on lactate, indirect evidence for the possible presence of the factor in glucose-grown cells in a combined form, not determinable by the usual assay procedures, could be obtained by growing the bacteria in a factor-free medium containing both glucose and lactate. If the factor were formed by growth on glucose, lactate should also be decomposed. Figure 4A shows that lactate-grown cells, inoculated into a glucose medium or a medium containing both glucose and lactate, devoid of BR factor, grew only to the extent permitted by the available glucose. When BR factor was added, both glucose and lactate were rapidly consumed, and the cell yield was approximately doubled, indicating that the lactate-degradation system is readily developed in such cells. With glucose-adapted cells as the inoculum (Fig. 4B), growth was very rapid in all media, but up to 70 hr of incubation time it appears to have been restricted to utilization of glucose even in the presence of added BR factor. This finding is consistent with the previously observed lag exhibited by an inoculum of glucose-adapted cells (strain G2) growing on a lactate medium.

A further check on the utilization of lactate by cells growing on glucose without BR factor seemed desirable, particularly in view of the fact that under these conditions lactate is frequently formed from glucose. This was done by allowing glucose-adapted cells to grow in 0.1% glucose medium without BR factor but in the presence of 1% lactate-1-C¹⁴. The concentration of lactate did not change significantly during 48 hr of incubation. Most of the C¹⁴ (99%) was found in the lactate, the remaining 1% being nearly equally distributed between carbon dioxide and fatty acids. This experiment supports the previous conclusion that lactate is not appreciably decomposed when BR factor is not added to the medium, and indicates that enzymatically active forms of the factor are not synthesized during growth on glucose.

**Experiments with cell suspensions.** To obtain further information concerning the role of the BR factor in *B. rettgeri*, the decomposition of glucose, lactate, and pyruvate was studied with washed-cell suspensions under a variety of experimental conditions. Preliminary tests
showed that, whereas glucose and pyruvate are readily decomposed by suitably grown cells, lactate is often not attacked under anaerobic conditions when added to a washed suspension of cells just harvested from a lactate medium. However, lactate is rapidly fermented by the same cells on addition of a small amount of the fermented culture medium. It was found that the fermented medium can be effectively replaced by a mixture of acetate and pyruvate or glucose. For example, the fermentation of 10 μmoles of lactate in 2 ml of phosphate buffer, which is slow and incomplete when either 2 μmoles of sodium pyruvate or 10 μmoles of sodium acetate are added separately, becomes rapid and complete when both compounds are added together. A possible explanation of this “sparking” action is that both an acetyl donor, such as acetyl coenzyme A, and free acetae are required to form the hydrogen acceptors that are essential for the initial oxidation of lactate to pyruvate. The acetyl donor may be provided by the dismutation of pyruvate. Once a suitable hydrogen acceptor is formed and the oxidation of lactate gets started, the fermentation of lactate becomes a self-perpetuating process.

Experiments with cells grown with lactate plus BR factor. Figure 5 shows data on gas production from pyruvate, glucose, and lactate under anaerobic conditions. Gas formation is calculated as carbon dioxide, since no alkali-insoluble gas is formed by lactate-adapted cells under these conditions. The figure illustrates (curves 3 to 8) the influence of acetate and pyruvate or glucose on the decomposition of lactate. The results are typical except with respect to the response to pyruvate (curve 5) which, contrary to the usual experience, was less effective than glucose (curve 3) in sparking lactate decomposition. This may be a concentration effect since the same molar quantity (2 μmol) of glucose and pyruvate was used, and 1 mole of glucose (2 C₂ units) is roughly equivalent to 2 moles of pyruvate.

Pyruvate, glucose, and lactate (under suitable conditions) are decomposed readily by the lactate-grown cells. The rate with pyruvate (curve 1) is about twice that with glucose (curve 2). Thus, approximately 35 min were required for 50% decomposition of 20 μmoles of pyruvate, whereas more than 70 min were required with 10 μmoles of glucose. The initial rate of lactate decomposition, in the presence of acetate and a little glucose, appears to be about the same as when glucose alone is added as a supplement.

The relative rates of aerobic oxidation of lactate, pyruvate, and glucose by lactate-adapted cells were determined in separate experiments that will not be described in detail. The rates of oxygen uptake with pyruvate and glucose were nearly the same. With lactate, the initial rate was about twice as great as with pyruvate, but, after about half the total oxygen was consumed, the rate declined to that observed with pyruvate, suggesting that the latter compound had accumulated.

The above experiments establish that cell suspensions of lactate-adapted cells can decompose glucose, pyruvate, and lactate under both aerobic and anaerobic conditions.

Experiments with cells grown with glucose and without BR factor. The results obtained with the
glucose-adapted cells are quite different from those obtained with lactate-adapted cells. Figure 6 shows that lactate (curves 1 and 2) is not oxidized by glucose-adapted cells at an appreciable rate, and addition of BR factor does not affect lactate oxidation. The aerobic decomposition of pyruvate by these cells is relatively slow and is not accelerated by the addition of BR factor during a period of 80 min (curves 3 and 4). Glucose is oxidized rapidly at first (curve 5) and the rate later declines to that observed with pyruvate, suggesting that pyruvate accumulates. Added BR factor has no effect on the initial rate of glucose oxidation (curve 6), but appears to prolong somewhat the period of rapid oxygen uptake.

In anaerobic experiments, essentially similar results were obtained with cells of the same type. Lactate was not decomposed, glucose was oxidized rapidly, and pyruvate was oxidized somewhat more slowly. Addition of the BR factor did not materially influence the results.

Experiments with cells grown with glucose plus BR factor. Cells grown with glucose in the presence of BR factor were almost identical in their behavior to cells grown on a glucose medium devoid of the factor. Thus, they decomposed glucose and pyruvate rapidly (Fig. 7, curves 1 and 2) but showed no ability to degrade lactate.
even in the presence of pyruvate or glucose plus acetate and BR factor (curves 3, 4, and 5). The obvious conclusion is that growth on a glucose medium containing the factor does not cause formation of the lactate-degrading system. Both lactate and the factor, and possibly the absence of glucose, are necessary for formation of this system.

**Discussion**

The evidence presented in this paper clearly establishes the fact that *B. rettgeri* requires the BR factor for growth on lactate but not for growth on glucose or pyruvate. If no further information were available, this result could be interpreted to indicate that the factor is formed during growth on glucose or pyruvate but not during growth on lactate.

However, the other evidence indicates that the BR factor is not formed during growth on glucose; BR factor could not be detected in significant amounts by bio-assay (Kline and Barker, 1950) in cells grown on a glucose medium without added BR factor. This evidence is not conclusive in view of the fact that control assays of cells grown on lactate plus a low level of BR factor sometimes gave a negative result. However, the bio-assay results are supported by the observation that cells grown for a short time on glucose (G1 cells) are markedly stimulated by small additions of BR factor; therefore, they must be relatively, if not absolutely, deficient in the factor.

If the reasonable assumption is made that lactate is decomposed via pyruvate, then the BR factor, or a metabolically active substance derived from it, presumably must function in the system or in the synthesis of the system transferring electrons from lactate to the final electron acceptors. The fact that the oxidation as well as the fermentation of lactate is dependent upon the availability of BR factor in the growth medium indicates that the factor is involved primarily in lactate oxidation or electron transport from lactate to electron-acceptor systems of the cell.

The slowness with which cells grown in the absence of the factor develop a lactate-degrading system in the presence of the factor, lactate, and an energy source like glucose suggests that utilization of the factor requires one or more synthetic steps. This view is supported by the observation that the factor is present in *B. rettgeri* and other natural sources mainly in a complex form that must be hydrolyzed before it can serve as a growth factor (Kline and Barker, 1950).

When *B. rettgeri* is first transferred from a lactate medium to a glucose medium, growth occurs in the absence of BR factor but is markedly stimulated by addition of the factor. This indicates that glucose decomposition in such cells, in contrast to cells which have been grown for many transfers on glucose, is influenced by the factor. The site of action of the factor is not known. The appearance of measurable amounts of lactate in such cultures, to which no BR factor was added, at first suggested that glucose decomposition proceeds in part through lactate. If the utilization of the lactate then becomes limiting for growth, the function of the factor would be identical to that discussed for growth on lactate. However, there are some important objections to this hypothesis. One objection is that cell suspensions from glucose cultures, either with or without BR factor, do not decompose lactate, even when supplied with glucose or pyruvate supplements, which they readily degrade. Furthermore, cells growing in a medium containing both glucose and lactate, but devoid of the factor, utilize only the glucose, thereby providing additional evidence for the absence of a lactate-degrading system in such cells. It thus appears unlikely that the stimulatory effect of the BR factor on the growth of nonglucose-adapted cells on glucose is the result of the development of a lactate-degrading system in these cells.

A more plausible hypothesis is that the factor, or a derivative of it, may serve as a cofactor for the synthesis or activity of more than one enzyme. Thus, it may be involved both in the conversion of lactate to pyruvate and in a step in the conversion of glucose to pyruvate. The second function must be part of an alternative pathway of glucose breakdown, since the factor is stimulatory but not essential for cells that ferment glucose slowly, and is without appreciable effect on cells that ferment glucose rapidly.

The formation of lactate from glucose by cells growing in the absence of BR factor presents a problem. Since such cells are unable to decompose lactate at an appreciable rate, and since
the action of pyridine nucleotide-linked lactic dehydrogenase is reversible, this enzyme may not be involved in lactate formation. However, it is also possible that lactic dehydrogenase is involved in both lactate formation and decomposition and that the defect which prevents lactate fermentation in the absence of BR factor is associated with the system that transports electrons from lactate to the normal anaerobic electron-acceptor systems.

Since the BR factor is probably identical with lipoic acid (Kline et al., 1952), our experiments throw some light on the role of this compound in the metabolism of B. rettgeri. Although lipoic acid functions as a cofactor in the oxidation of pyruvate by some bacteria (Gunsalus, 1953), it is apparently not required for either glucose or pyruvate decomposition in B. rettgeri. The evidence we have obtained indicates that in this organism lipoic acid is required in the oxidation of lactate to pyruvate and also participates in an optional path of glucose decomposition to pyruvate.

Acknowledgment

This work was supported in part by a research grant from the Division of Research Grants and Fellowships, National Institutes of Health, U.S. Public Health Service.

Literature Cited


