The Behaviour of *Lactobacillus arabinosus* towards Nicotinic Acid and its Derivatives

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(Received 11 June 1948)

These investigations originated in studying the basis of a method for microbiological assay of nicotinic acid (McIlwain & Stanley, 1948). The method was that in which acid produced from glucose by *Lactobacillus arabinosus* 17-5 is titrated with alkali after a period of incubation of about 3 days. The assay depends on the empirical observation that the quantity of titratable acid produced during incubation is dependent on the quantity of added nicotinic acid. It was found (McIlwain, 1948) that this relationship existed because of changes which took place in nicotinic acid during the assay; the compound was first assimilated to, and later lost from, the cells. Its loss was found to be conditioned by its functioning as a catalyst, and was such that nicotinic acid exhibited a defined catalytic capacity, measured by the ratio: titratable acid formed/nicotinic acid lost (in mol./mol.). Values of the catalytic capacity (about \(5 \times 10^3\)) were relatively independent of the intensity with which nicotinic acid derivatives were functioning as catalysts, which was expressed as a catalytic activity (in mol. titratable acid/hr./mol. nicotinic acid; values ranged from 5 to 15 \(\times 10^2\) hr.

Such observations suggested the need for more detailed investigations of the form in which nicotinic acid derivatives exist in the bacterial cells. We have now examined the nicotinic acid of cells of *Lactobacillus arabinosus*, and found it to be largely in the form of cozymase. *Lactobacillus arabinosus*, in marked distinction from a number of other organisms, reacted only slowly with added cozymase; this, and our interest in the practical problems presented by microbiological assay, directed our attention to metabolic changes occurring in the cozymase of the bacterial cells, and these are described below.

**EXPERIMENTAL**

The assays for nicotinic acid, the preparation of growth media and of materials for assay, and the handling of non-proliferating suspensions of *Lactobacillus arabinosus* were carried out as described by McIlwain (1948). Determination of V-factor and of cozymase, and the extraction of these substances from bacteria were made by the methods used by McIlwain & Hughes (1948).

**RESULTS**

**Assimilation of nicotinic acid by Lactobacillus arabinosus**

1. **Cell content.** Cells of *Lb. arabinosus* from cultures whose growth has not been limited by nicotinic acid may, nevertheless, contain varying quantities of this substance. This is shown in Table 1. Nicotinic acid becomes a limiting factor in growth of the organism at concentrations of about \(2 \times 10^{-7}\) M and the range used in assay is below \(1.5 \times 10^{-7}\) M. The values of Table 1 illustrate also the previous findings (McIlwain, 1948) that the nicotinic acid content of the cells of a given culture falls with time. To approach saturation of the cells with the acid at 26 hr. in the relatively early part of a culture’s growth, a concentration of c. \(5 \times 10^{-7}\) M-nicotinic acid is seen to be required. The cells will thus be unsaturated in this sense during most of their period of incubation in all the concentrations used in assay.

Under the conditions most favourable for assimilation, about 80% of the nicotinic acid added to a culture has been recovered from the cells after 26 hr. growth. As the cells inactivate nicotinic acid during this period, this result gives a minimum estimate only of the completeness with which they can assimilate the acid. The actual concentration of nicotinic acid derivatives in the cells after assimilation can be calculated from the data of Table 1 to be about 2 to \(10^{-4}\) M. In making this calculation the wet weight of the cells was taken as five times their dry weight; this has been found to be a typical ratio. The values recorded in Table 1 for the yield of bacterial cells from unit volume of culture show that the assay medium, though carefully chosen for maximum response to limited nicotinic acid, does not give high yields of bacteria.

2. **Nature of the assimilated nicotinic acid.** Most of the nicotinic acid of the cells existed as derivatives, especially in cells containing limiting quantities of the substance. Table 2 A shows that some 90% of the acid, determined in cells grown from a concentration of the acid within assay range, exists as
relatively inert of its observed nicotinic acid suspensions. 500 binosus did of absence cozymase. May loss wt./hr., < of inorganic salts (organisms from 100 ml. of culture in 50 ml. salt solution) for 72 hr. at 37°, aerobically.

**Table 1. Nicotinic acid content of cells of Lactobacillus arabinosus after growth of varying types**

<table>
<thead>
<tr>
<th>Nicotinic acid of growth medium (m x 10⁻⁷)</th>
<th>Period of incubation (hr.)</th>
<th>Yield of cells (mg. dry wt./100 ml. culture)</th>
<th>Nicotinic acid content of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(mumol./cells of 100 ml. medium)</td>
<td>(mumol./mg. dry wt.)</td>
</tr>
<tr>
<td>1.2</td>
<td>30</td>
<td>7.0</td>
<td>6.2</td>
</tr>
<tr>
<td>2.4</td>
<td>26</td>
<td>10.4</td>
<td>13.9</td>
</tr>
<tr>
<td>4.9</td>
<td>26</td>
<td>9.5</td>
<td>37</td>
</tr>
<tr>
<td>4.9</td>
<td>42</td>
<td>10.7</td>
<td>33</td>
</tr>
<tr>
<td>4.9</td>
<td>48</td>
<td>12.0</td>
<td>28</td>
</tr>
<tr>
<td>12.2</td>
<td>26</td>
<td>9.8</td>
<td>53</td>
</tr>
</tbody>
</table>

* Calculated, assuming a water content of 80%.

Organisms were grown in quantities of 200–500 ml. of the assay medium (cf. McIlwain & Stanley, 1948) with nicotinic acid added in the quantities indicated.

**Table 2. Nicotinic acid derivatives in cells of Lactobacillus arabinosus, and in material liberated from cells to solution**

<table>
<thead>
<tr>
<th>Nicotinic acid in growth medium (mumol./100 ml.)</th>
<th>Period of incubation in growth (hr.)</th>
<th>Yield of cells (mg. dry wt./100 ml. culture)</th>
<th>Later treatment</th>
<th>Content (mumol./cells of 100 ml. culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nicotinic acid</td>
</tr>
<tr>
<td>A. Material in cells</td>
<td></td>
<td></td>
<td></td>
<td>6.9</td>
</tr>
<tr>
<td>12.2</td>
<td>30</td>
<td>13.0</td>
<td>Washed</td>
<td>2.7</td>
</tr>
<tr>
<td>12.2</td>
<td>30</td>
<td>13.0</td>
<td>Washed, and in saline mixture† at 37°, 6 days</td>
<td>19</td>
</tr>
<tr>
<td>45</td>
<td>28</td>
<td>10.9</td>
<td>Washed</td>
<td>43</td>
</tr>
<tr>
<td>122</td>
<td>48</td>
<td>12.8</td>
<td>Washed</td>
<td>43</td>
</tr>
<tr>
<td>B. Material liberated on exposure of cells in salt mixture†</td>
<td></td>
<td></td>
<td></td>
<td>6.1</td>
</tr>
<tr>
<td>45</td>
<td>28</td>
<td>10.9</td>
<td>Cells washed and</td>
<td>16-4</td>
</tr>
<tr>
<td>112</td>
<td>48</td>
<td>12.8</td>
<td>exposed†</td>
<td></td>
</tr>
</tbody>
</table>

* The inorganic salts of the medium of Barton-Wright (1946) at pH 6.8 and in the concentrations of the final assay medium.
† In the inorganic salts (organisms from 100 ml. of culture in 50 ml. salt solution) for 72 hr. at 37°, aerobically.

cozymase. The amount determinable as V-factor (probably equivalent to nicotinamide ribosides; for discussion see McIlwain, 1947) was also high.

3. **Relative stability of cozymase added to Lb. arabinosus.** McIlwain & Hughes (1948) found that many bacterial species rapidly inactivate added cozymase; even at low concentrations of cozymase (c. 10⁻⁵ M) loss may occur at the rate of 10 mumol./mg. dry wt./hr., and with higher concentrations may rise to over 500 mumol./mg./hr. Such a coefficient means that the organism is inactivating its own weight of cozymase in about 3 hr. *Lb. arabinosus* was, however, relatively inert towards cozymase. Under the conditions of its use in assay, i.e. with cells of between 1 and 3 days growth, and at pH values between 5 and 7, any reaction with 10⁻⁴ or 10⁻⁴ M-cozymase was of < 1 mumol./mg. dry wt. of cells/hr. In attempts to observe a more rapid reaction, experiments were also carried out at pH 4.5 and 7.6, in the presence and absence of phosphates and of glucose, and with some suspensions initially rich and others initially poor in nicotinic acid derivatives. Again, any reaction was of < 1 mumol./mg./hr. It was evident that *Lb. arabinosus* did not inactivate cozymase at the rapid rate observed in several other bacteria. Nevertheless, slow inactivation has been observed (Table 1), and found to be related to the assay (McIlwain, 1948); this process has, therefore, been studied further.

**Change in nicotinic acid in suspensions of Lactobacillus arabinosus**

An attempt was made to reproduce the changes of Table 1 under conditions better defined than those of a growing culture. Table 3 shows that the cellular nicotinic acid of *Lb. arabinosus* can also be very stable. Cells were examined which had been grown in media relatively rich and relatively poor in nicotinic acid; the latter conditions (12 mumol. nicotinic acid/100 ml.) are within the range used in assay. In all cases the cells could be incubated at 37° in salt mixtures at pH 6.5–7 for 6 days without any loss in the total nicotinic acid of the suspension. A small increase, of some 10% of the initial value, was frequently found.

These experiments showed that the loss in nicotinic acid observed during the assay was not brought about by the bacteria when they were incubated in the presence of the majority of the constituents of the assay medium. The components omitted from the suspending fluids during the
experiments quoted were glucose, the casein hydrolysate providing amino-acids to the medium, and in one case sodium acetate. Other results in Table 3 showed sodium acetate to have only a small effect in the change in nicotinic acid; but addition of glucose caused a major change. In each case examined, addition of glucose led to a decrease in nicotinic acid content.

Table 3. Changes in the nicotinic acid derivatives of suspensions of cells of Lactobacillus arabinosus

<table>
<thead>
<tr>
<th>Batch of organisms; conditions of growth</th>
<th>Initial content of nicotinic acid (mμmol./mg. dry wt.)</th>
<th>Conditions of reaction</th>
<th>Change in nicotinic acid during 6 days* (mμmol./mg. dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 26 hr., 122 mμmol. nicotinic acid/100 ml.</td>
<td>5-4</td>
<td>Salts of medium,† pH 6-8</td>
<td>+0-70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>As above, with glucose (0-1 M)</td>
<td>-1-80</td>
</tr>
<tr>
<td>2. 30 hr., 12 mμmol. nicotinic acid/100 ml.</td>
<td>0-88</td>
<td>Salts of medium, pH 6-5</td>
<td>+0-10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>As above, with glucose (0-1 M)</td>
<td>-0-25</td>
</tr>
<tr>
<td>3. 26 hr., 24 mμmol. nicotinic acid/100 ml.</td>
<td>0-72</td>
<td>Inorganic salts of medium,‡ pH 7</td>
<td>+0-08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>As above, with glucose (0-1 M)</td>
<td>-0-14</td>
</tr>
</tbody>
</table>

* The change quoted is in the whole reaction mixture; the kinetics of the change and the partition of nicotinic acid between cells and solution are discussed later.
† NaCl, Na acetate, and inorganic salts A and B of Barton-Wright (1946), at the concentrations used in assay.
‡ As in footnote †, but lacking Na acetate.

The course of changes in nicotinic acid derivatives in suspensions of Lactobacillus arabinosus

1. In absence of glucose. Although the total nicotinic acid of cell suspensions changed little under these conditions, marked changes occurred in its distribution. Fig. 1 shows this in the case of a suspension of cells which were initially rich in the acid. The quantity in the cells fell throughout the experiment, but at a decreasing rate; and the acid which was lost from the cells appeared in the solution. Results similar to those of Fig. 1 have been obtained also with cells which were initially poorer in nicotinic acid (containing 30 or 15 mμmol./100 ml., the latter value being the upper limit of the assay range); but in such cases the proportion of nicotinic acid leaving the cells was smaller than with those initially rich in the acid.

The nicotinic acid-containing material liberated from the cells consisted at least in part of derivatives of the acid. The solutions yielded were too dilute to give significant values for cozymase in the apozy-
mase system, but their V-factor activity could be
determined. The values obtained (Table 2B) showed
two thirds or one half of the liberated material
to possess such activity and presumably to be
at least of the complexity of a riboside of nicotin-
amide.
2. In presence of glucose. Glucose led not only to
the decrease in total nicotinic acid noted in the
previous section, but in many cases it also caused
(1.22 mµmol./10 ml.) within the assay range and
were reaped at 30 hr. when their rate of acid for-
mation was high. Their initial rate in the salts of the
assay medium, with glucose, was > 10 µmol./10 ml./
hr., and this fell to about 1 µmol./10 ml./hr. on the
sixth day. The rate fell rapidly during the first 2 or
3 days and then more slowly. Changes in the rate
have, therefore, been examined in relation to changes
in cellular nicotinic acid.

Table 4. Effect of glucose on distribution of nicotinic acid in suspensions of Lactobacillus arabinosus

<table>
<thead>
<tr>
<th>Conditions of growth and reaction</th>
<th>Nicotinic acid (mµmol./10 ml. of suspension)</th>
<th>Percentage of nicotinic acid present in cells after 6 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initially</td>
<td>Cells</td>
</tr>
<tr>
<td>As 1, Table 3; no glucose</td>
<td>4.9</td>
<td>0.81</td>
</tr>
<tr>
<td>As 1, Table 3; with glucose</td>
<td>4.9</td>
<td>2.44</td>
</tr>
<tr>
<td>As 2, Table 3; no glucose</td>
<td>3.05</td>
<td>1.06</td>
</tr>
<tr>
<td>As 2, Table 3; with glucose</td>
<td>3.05</td>
<td>1.38</td>
</tr>
<tr>
<td>As 3, Table 3; no glucose</td>
<td>0.88</td>
<td>0.49</td>
</tr>
<tr>
<td>As 3, Table 3; with glucose</td>
<td>0.88</td>
<td>0.29</td>
</tr>
</tbody>
</table>

a marked change in the distribution of nicotinic acid
derivatives between cell and solution. Fig. 2 shows
that, with organisms rich in nicotinic acid, glucose
caused nicotinic acid which otherwise would have
passed into solution to be retained in the cells. The
quantity in solution increased only transitorily, and
then fell in a fashion roughly parallel to that of the
cells. This behaviour was less marked in organisms
which initially contained less nicotinic acid, and
with cells grown from 12 mµmol. of the acid/100 ml.
no considerable change in distribution of the acid was
brought about by glucose (Table 4), but the fall in
total quantity remained marked.

Acid formation from glucose by suspensions
of Lactobacillus arabinosus

1. Fall with time. The changes in cellular nicotinic
acid are relevant to the assay because this depends
on acid formation by the cells, and this again depends
on the presence of nicotinic acid derivatives. It has
already been shown (McIlwain, 1948) that freshly
harvested cells of Lb. arabinosus are capable of fer-
menting glucose at rates almost to the most
rapid reached during growth in nutritionally rich
media. Organisms from nicotinic acid-deficient
cultures were found to produce acid at a lower rate
than those with excess of the acid, and the deficiency
could be made good by addition of nicotinic acid or
cozymase. In these experiments acid formation was
followed for only an hour or two after harvesting.

The course of acid formation from suspensions of
Lb. arabinosus was now followed over periods com-
parable to those of the assay. Fig. 3 shows that
observed in an experiment lasting 6 days. Organisms
had been grown from a quantity of nicotinic acid

Fig. 3. Course of formation of titratable acid in suspensions of Lb. arabinosus, grown in the same way as the organisms of Figs. 1 and 2 but for 30 hr., and suspended in the medium described for Fig. 2.

2. Catalytic activity of nicotinic acid in cell sus-
pensions. Relevant results are presented in Table 5.
Here the rate of acid formation in the suspensions is
expressed as a multiple of the quantity of nicotinic
acid in the cells. These values (catalytic activities: in
mol. titratable acid/mol. nicotinic acid/hr.) are seen
to be relatively constant but to fall during the course
of exposure of the cells. The cells are thus changing
during exposure so that nicotinic acid becomes pro-
gressively less effective as a catalyst. It is also
initially much less effective as a catalyst during the
present experiments than in assay; the catalytic
activities of Table 5 are only about one tenth of
those found (McIlwain, 1948) at comparable pH
values during assay.
The higher catalytic activity during assay did not appear to be due to cell proliferation, as the earlier experiments included ones of short duration with harvested and resuspended cells. The difference was found to be due to the organic constituents of the suspending fluid. Reconstituting the assay medium by adding to the suspending fluid of Table 5 casein hydrolysate, ammonium salts, and a mixture of vitamin-like substances restored the catalytic activity to 14,000 mol./mol./hr. at pH 6, or 8000 at pH 5.2. In this effect the vitamin-like substances played little part (Table 5).

Table 5. Catalytic activity and catalytic capacity of nicotinic acid in acid formation by suspensions of Lactobacillus arabinosus

<table>
<thead>
<tr>
<th>Age of suspension (days)</th>
<th>Nicotinic acid content of cells (μmol./10 ml. suspension)</th>
<th>Rate of formation of titratable acid (μmol./10 ml. suspension/hr.)</th>
<th>Catalytic activity (mol. titratable acid formed/mole nicotinic acid/hr.)</th>
<th>Catalytic capacity (mol. titratable acid lost) (×10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5-7</td>
<td>2-2.4</td>
<td>9.1</td>
<td>3700</td>
</tr>
<tr>
<td>2</td>
<td>5-4</td>
<td>2-0.4</td>
<td>4.6</td>
<td>2300</td>
</tr>
<tr>
<td>3</td>
<td>5-3</td>
<td>1-70</td>
<td>2-62</td>
<td>1500</td>
</tr>
<tr>
<td>4</td>
<td>5-25</td>
<td>1-48</td>
<td>2-04</td>
<td>1400</td>
</tr>
<tr>
<td>5</td>
<td>5-2</td>
<td>1-28</td>
<td>1-52</td>
<td>1200</td>
</tr>
<tr>
<td>6</td>
<td>5-2</td>
<td>1-14</td>
<td>1-08</td>
<td>1000</td>
</tr>
</tbody>
</table>

A parallel experiment was carried out with similarly grown organisms in a suspending fluid of the same initial pH but with in addition the aneurin, riboflavin, pyridoxin, pantothenate, p-aminobenzoic acid and biotin of the assay medium. Catalytic activities of 2.5-3.5 × 10⁴ (mean, 2.6 × 10⁴). Metabolism of Nicotinic Acid Derivatives

3. Catalytic capacity of nicotinic acid in cell suspensions. In Table 5 are given also the ratios between the mol. titratable acid formed and the mol. nicotinic acid lost. These values (the catalytic capacities of nicotinic acid) were relatively steady during the experiments and were not far removed from the values observed in assay. Thus, different suspensions gave 2.5-3.5 and 2.0-3.5 × 10⁴, and in assay the capacity was about 5 × 10⁴. It has been observed above that in the present experiments, as in assay, loss of nicotinic acid was associated with the presence of glucose. The values now quoted show that the quantitative relations between the metabolism of glucose and of nicotinic acid are also similar during the assay in non-proliferating suspensions, although the absolute rates of metabolism of each fell in the suspensions to only a fraction of their value in assay.

DISCUSSION

The present studies have given further information on the behaviour of nicotinic acid in Lactobacillus arabinosus, and this behaviour may now be assessed in relation to the use of the organism in assay. The organisms, in growth, assimilated nicotinic acid from very dilute solutions and concentrated it in their cells. The assimilated material has been found to be present largely as cozymase, which is much more stable when added to suspensions of Lb. arabinosus than it is in suspensions of many other organisms. Loss of cozymase from buffered suspensions of Lb. arabinosus was extremely slow, both with respect to cozymase which was added as such, and also to that which was synthesized by the cells themselves from nicotinic acid. Cellular cozymase became markedly less stable in glycolyzing organisms although here also the rate of its loss was by most standards very low, being about 10 μmol./mg. dry wt./hr. It was, therefore, very interesting to observe that this rate bore a relation to the rate of glycolysis similar to that previously observed in more active cells during assay. In both cases the catalytic capacity of nicotinic acid, or the ratio between the rates of change in titratable acid in nicotinic acid, was between 2 and 6 × 10⁴. The possible significance of this has been discussed previously (McIlwain, 1948).

These values give another instance of relative stability in the catalytic capacity of nicotinic acid during marked changes in its catalytic activity (i.e. the rate of reaction which unit quantity of it catalyzes, in this case glycolysis, expressed in μmol./hr./μmol. nicotinic acid). The catalytic activity was markedly affected by the suspending fluid. This is understandable, as glycolysis involves many reactions, and the slowing of any one of these during the 6 days of an experiment could be reflected in the overall rate of reaction. The relative constancy of the catalytic capacity implies that, when glycolysis has fallen in rate, loss of nicotinic acid has also been correspondingly slower.

Data already available from various assay methods for nicotinic acid give further indications of the relative constancy of the catalytic capacity of the acid. As emphasized previously (McIlwain, 1948), the full catalytic capacity of nicotinic acid is
not used during an ordinary assay. In the instance investigated, normal assay practice, which allows reaction to continue until acid formation becomes slow, employs about half the full catalytic capacity of the added nicotinic acid. The standard dosage/response curve of assay, when expressed in mol. titratable acid/mol. nicotinic acid, thus gives a partial catalytic capacity of nicotinic acid. Values of this type have been collected in Table 6. This shows partial catalytic capacities from our own observations with *Lb. arabinosus* to range from 2·6 to 4·8 x 10⁶; those of other observers with this organism, from 1·7 to 2·6, and with *Leuconostoc mesenteroides*, from 4·9 to 6·8 x 10⁶. Fundamental importance is not attributed to the partial catalytic capacities which are expressed immediately by these values, but the latter are considered to indicate that the catalytic capacities themselves are also likely to be of the same order of magnitude under the various conditions chosen by the different observers whose results have been given in Table 6. This increases the significance of the catalytic capacity.

**SUMMARY**

1. *Lactobacillus arabinosus* 17–5, which is used in a microbiological assay of nicotinic acid, assimilated this acid from 10⁻⁸ or 10⁻⁷ M solutions during growth. Its cell content of the substance was then 0·7–5 mμmol./mg. dry wt. of cells, according to conditions of growth.

2. The assimilated substance existed in the cells largely as cozymase. Cozymase in *Lb. arabinosus*, or added to suspensions of this organism, was much more stable than in the presence of many other bacteria.

3. A slow reaction (of 2–20 μmol./mg./hr.), leading to inactivation of cozymase and of its nicotinic acid moiety did, however, occur in *Lb. arabinosus* during glycolysis. Glycolysis altered also the distribution of nicotinic acid between saline solutions and the cells of the organism, leading to greater retention of the acid in the cells.

4. Rates of glycolysis and of loss in nicotinic acid varied in parallel in suspensions of *Lb. arabinosus*, as they had previously been found to do in assay. The ratio between the two rates (the catalytic capacity of nicotinic acid) had approximately the same value, of 3–6 x 10⁶, in the two cases.

5. On the other hand, the activity of nicotinic acid as a catalyst was much lower in non-proliferating suspensions in simple media, than in assay. Catalytic activity and partial catalytic capacity have been used as measures of the behaviour of the organism during assays of the present type.

We are greatly indebted to Miss E. Ellis for assistance during these investigations.

**REFERENCES**


