THE METABOLISM OF THE ORGANIC ACIDS OF TOBACCO LEAVES

III. EFFECT OF CULTURE OF EXCISED LEAVES IN SOLUTIONS OF OXALATE

BY HUBERT BRADFORD VICKERY AND MARJORIE D. ABRAHAMS

(From the Biochemical Laboratory of the Connecticut Agricultural Experiment Station, New Haven)

(Received for publication, May 24, 1953)

Oxalic acid has long been regarded as an end-product of oxidative metabolic reactions in leaves. It is widely distributed in plants although the relative quantity present is not commonly great; nevertheless, in certain genera characterized by highly acidic saps, such as Oxalis, Rumex, Begonia, and Rheum, oxalic acid may be the organic acid component present in greatest proportion. In tobacco, a species characterized by a high relative proportion of malic acid in the leaves, oxalic acid usually accounts for about 12 per cent of the total organic acids and for about 2 per cent of the organic solids; in the leaves employed for the present experiments, these figures were, respectively, 9.7 and 1.8 per cent.

When tobacco leaves are excised and subjected to culture in darkness with their bases in water, malic acid diminishes in concentration and citric acid increases. Evidence has been presented in earlier papers (1-3) which suggests that this is an expression of a normal series of metabolic reactions whereby approximately 2 moles of malic acid undergo chemical changes that result in the formation of 1 mole of citric acid. It has also been shown that, if organic acids are taken up by the tissues as potassium salts dissolved in the culture solution, marked effects upon the course and extent of this reaction can be demonstrated. Members of the Krebs tricarboxylic acid cycle are especially effective. Malic acid has been found to stimulate the reaction while citric acid appears to reverse it. It seemed desirable, therefore, to examine the effect of oxalic acid, the third most important organic acid component of tobacco leaves, to see whether any influence is exerted by this substance upon the metabolic reactions of the acids of the leaf cells.

EXPERIMENTAL

Tobacco plants (Nicotiana tabacum, var. Connecticut shade-grown), grown as previously described (3), were sampled by the statistical method (4) 57 days after being transplanted, five samples of twenty leaves each being taken from ten plants. Of these, one was at once dried for analysis.
and the others were subjected to culture in a completely dark room, the temperature of which was controlled at 24° and the relative humidity at 50 per cent. The potassium oxalate solution on which two of the samples were cultured was 0.2 M in concentration and was adjusted to pH 6.05, 1490 ml. being used for each. The other two samples were cultured on water. Culture periods of 24 and 48 hours were arbitrarily adopted. The oxalate solutions remaining were recovered for analysis as nearly quantitatively as possible; they were, respectively, at pH 6.7 after 24 hours and at pH 6.6 after 48 hours. There was no obvious evidence of the growth of microorganisms.

### Table I

**Effect of Culture on 0.2 M Potassium Oxalate upon Composition of Excised Tobacco Leaves**

The figures not otherwise designated represent milliequivalents per kilo of original fresh weight of leaves.

<table>
<thead>
<tr>
<th>Control before culture</th>
<th>Changes during culture in darkness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen, gm...</td>
<td>4.79</td>
</tr>
<tr>
<td>Organic solids, gm......</td>
<td>75.6</td>
</tr>
<tr>
<td>Inorganic solids, gm....</td>
<td>17.6</td>
</tr>
<tr>
<td>Alkalinity of ash......</td>
<td>352</td>
</tr>
<tr>
<td>Oxalic acid equivalent to alkalinity of ash</td>
<td></td>
</tr>
<tr>
<td>Total organic acids......</td>
<td>307</td>
</tr>
<tr>
<td>Oxalic acid.............</td>
<td>29.8</td>
</tr>
<tr>
<td>Citric &quot;..................</td>
<td>41.5</td>
</tr>
<tr>
<td>Malic &quot;...................</td>
<td>144</td>
</tr>
<tr>
<td>Undetermined acid......</td>
<td>90.9</td>
</tr>
<tr>
<td>pH of dry tissue......</td>
<td>5.16</td>
</tr>
</tbody>
</table>

The leaves cultured on water remained fully turgid and increased slightly in fresh weight; the leaves cultured on oxalate, on the contrary, became moderately flaccid within a few hours and had lost 34 per cent of their fresh weight after 24 hours and 59 per cent after 48 hours. However, there was no evidence of mottling until the 2nd day and it was then only slight.

The analytical results are shown in Table I, the methods mentioned in previous papers (3) being used. The coefficient of variation of the nitrogen content was 2.6 per cent; the samples were therefore satisfactorily

---

1 For the method to determine alkalinity of ash, see Vickery et al. (5).
constant in initial composition. The leaves were somewhat smaller and less well developed than those used for previous experiments (3) and the rate of respiration, as shown by the loss of organic solids from the samples cultured on water, was only about half as great.

Culture on Water—The alkalinity of the ash decreased by about 1 per cent, a negligible quantity, and the total organic acids by 9 per cent in 24 hours and 10 per cent in 48 hours. Although this determination is not highly accurate, the analytical error being of the order of 5 per cent of the quantity measured, the evidence suggests that there was a small loss of acids, possibly by respiration. Oxalic acid increased barely significantly. Citric acid increased and malic acid decreased in the normal manner. These particular leaves were therefore reliable experimental material to be used as controls for the experiment with oxalate, although they were appreciably lower in malic acid than usual. The undetermined acid diminished by about 10 per cent of the total acidity, and in an amount similar to the apparent loss of total organic acids. There was no significant change in the pH of the leaf extract.

Culture on Oxalate—The effect of the influx of potassium oxalate is evident in the smaller decrease of organic solids as compared with the leaves cultured on water and in the marked increase in inorganic solids. Furthermore, analysis of the residual culture solutions showed losses from them, respectively, of 10 and 13 gm. of oxalic acid per kilo of fresh leaves. That substantial quantities of the salt entered the leaves, in spite of their flaccid condition, is most clearly demonstrated by the increase in the alkalinity of the ash. The increase of 148 m.eq. in 24 hours is equivalent to 150 m.eq. of oxalic acid at pH 6.0, the reaction of the culture solution, and that of 249 m.eq. in 48 hours is equivalent to 253 m.eq. The increases of oxalic acid found were, respectively, 164 and 270 m.eq.

The increases in total organic acids were somewhat smaller than those of oxalic acid, and, accordingly, the undetermined acid appears to have diminished. This change was probably significant, for the quantity of undetermined acid in the samples cultured on oxalate was only a little more than one-half that in the control sample analyzed at the start.

As in the samples cultured on water, citric acid increased and malic acid diminished, although the change does not seem to have been as extensive in the first 24 hours as it was in the control samples cultured on water. In 48 hours, however, the changes were quantitatively almost the same as in the controls. The pH of the tissues increased significantly as it has regularly been observed to do when salts of organic acids are absorbed by tobacco leaves from culture solutions.

* These figures are computed from the dissociation curves of oxalic acid.
The sample cultured for 48 hours on oxalate became greatly enriched in organic acids. The oxalic acid increased by a factor of 10 over the control and the total acids from 27.1 per cent of the organic solids to 37.8 per cent, if the undetermined acid is arbitrarily calculated as citric acid.

**DISCUSSION**

Oxalate absorbed from a culture solution by tobacco leaves in darkness does not appear to enter into the metabolic reactions to any significant extent. Within the errors of the methods, the analytical evidence indicates that the salt merely accumulates, for the increases in both cation and anion, expressed in equivalents, were essentially identical. Furthermore, the newly acquired oxalic acid exerted no detectable influence upon the behavior of the malic and citric acids. In the control leaves cultured for 48 hours on water, the gain in citric acid was 23.8 m.eq. (7.93 mM) and the loss of malic acid was 32.1 m.eq. (16.1 mM). The molar ratio of malic acid loss to citric acid gain was therefore 2.0 in agreement with previous results (3). In the leaves cultured for 48 hours on oxalate, the gain in citric acid was 7.63 mM, and the loss of malic acid 16.1 mM, the ratio being 2.1. Because of the small quantities of malic and citric acids which had undergone change at the end of 24 hours, no significance can be attached to the computed ratios of 0.9 and 2.9 for these two experiments, the errors in the analytical determination of malic acid being too great.

The only apparent influence upon the organic acids of culture on oxalate was that upon the undetermined acid. This quantity is computed by difference, and the analytical error is correspondingly large. However, there was a loss of nearly one-half of the undetermined acid during culture on oxalate for 48 hours, the analogous loss in the control experiment being only about one-quarter. No attempt to interpret this observation can be made until more information on the composition and behavior of the undetermined fraction of the organic acids has been obtained. It may, however, be connected with the stimulated respiration of these samples of leaves referred to below and thus may have no bearing upon the metabolism of oxalic acid.

Determinations of isocitric acid were made on all samples. Small negative quantities (of the order of $-3$ m.eq. per kilo) were found and presumably represent the error of the analytical method. This result confirms previous observations that tobacco leaves normally contain no readily demonstrable quantity of isocitric acid.

The present results do not conflict with the view that oxalic acid represents an end-product of oxidative metabolism in leaves. The reactions by which it is formed are clearly not of kinds that are reversed to any significant extent by the mere introduction of a large excess of oxalic acid for, if so, the increase in oxalic acid found in the sample cultured on
oxalate would not have been so closely equivalent to the increase in the alkalinity of the ash. Although no light has been shed upon the nature of the reactions whereby oxalic acid is synthesized in tobacco leaves, it seems clear that these reactions are not reversibly connected with those whereby malic acid is converted to citric acid. This is a further instance of the complexity of the metabolic systems in which the organic acids of leaves are involved.

The observed behavior of oxalic acid bears a close analogy to that of tartaric acid (2). This substance also appears to be freely taken up by tobacco leaves from a culture solution but, so far as present information goes, remains unchanged and exerts no detectable effect upon the metabolism of malic and citric acids.

A word should be added concerning the obvious increase in the respiration of tobacco leaves subjected to culture on potassium oxalate. The leaves lost 3.1 gm. of organic solids during culture on water for 48 hours in darkness. During culture on oxalate for a similar period, they lost 0.8 gm. but, at the same time, acquired 12.2 gm. of oxalic acid which was found as such in the tissues. Accordingly, the respiration must have been stimulated, for approximately 13 gm. of organic substance disappeared from the system. Analogous observations have been made with leaves cultured on malate, citrate, isocitrate, and acetate (3) and similar effects can be seen in the data of Pucher and Vickery (2) for several other organic acids. Turner and Hanly (6) have reported a marked increase in respiration when carrot root tissue slices were suspended in potassium chloride and especially in potassium succinate. Machlis (7) noted increases in the respiration of barley roots treated with potassium salts of malic, succinic, fumaric, and citric acids and also in the capacity of such roots to accumulate bromide ion, a function closely associated with the rate of respiration. However, similar results could be obtained with potassium sulfate, and he pointed out that the effects were probably due to the influx of potassium ions. The present observations may have a similar explanation.

SUMMARY

Leaves of tobacco (Nicotiana tabacum, var. Connecticut shade-grown) cultured for 48 hours in darkness on 0.2 M solutions of potassium oxalate accumulate this salt in considerable quantities. There was no effect, however, upon the conversion of malic to citric acid which normally takes place in such leaves in darkness, and no evidence was found to suggest that oxalic acid is connected by reversible equilibrium reactions with either of these metabolites. The results conform to the view that oxalic acid represents an end-product of oxidation reactions in this species.

Confirmation was obtained of previous observations that approximately
2 moles of malic acid are converted into 1 mole of citric acid during culture in darkness.

The respiration of the leaves, as estimated from the loss of organic solids during the culture period, was stimulated during culture on oxalate, possibly as a result of the presence of abnormal quantities of potassium ions.

BIBLIOGRAPHY

The metabolism of the organic acids of tobacco leaves: III. Effect of culture of excised leaves in solutions of oxalate

Hubert Bradford Vickery and Marjorie D. Abrahams


Access the most updated version of this article at http://www.jbc.org/content/186/1/411.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/186/1/411.citation.full.html#ref-list-1