The Occurrence in Lichens of the Folic Acid-, Folinic Acid-, and Vitamin B\textsubscript{12}-Group of Factors

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In connection with investigations on the folic acid-, folinic acid-, and vitamin B\textsubscript{12}-group of factors in algae it was considered of interest to study the occurrence of these factors in some lichens, a group of plants known to represent a symbiosis between fungi and algae. The following lichens have been studied using microbiological and bioautographic methods: Cladonia silvatica, Umbilicaria pustulata, Parmelia physodes, Parmelia furfuracea, Cladonia islandica, Evernia prunastri, Alectoria pubata, and Usnea comosa. The lichens were collected at Grönvik, Närmo.

Escherichia coli 113-3\textsuperscript{1} served as a test organism for the vitamin B\textsubscript{12}-factors, Leuconostoc citrovorum ATCC 8081\textsuperscript{2} for the folic acid and Streptococcus faecalis ATCC 8043 for the folic acid tests. The organisms were utilized in the agar cup plate method. For Streptococcus faecalis the Difco folic acid assay medium with 1.6 % Bacto agar was used\textsuperscript{3}. The solvent for the chromatographic separation was sec. butanol saturated with water, and containing 3 % acetic acid and 25 mg KCN/l.

The lichens were carefully cleaned and dried at room temperature. Two grammes of finely ground material were suspended in 25 ml of water or buffer solution. Three different methods of freeing the active substances were tried, namely, extraction with water at 37\textdegree C for 24 hrs, autoclaving for 20 min. at 120\textdegree C in water containing small amounts of KCN, and enzymatic treatment with a chicken pancreas homogenate at 37\textdegree C for 24 hrs at pH 7.5. Autoclaving gave the highest

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Release of Hydantoins from Proteins

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In a note by the author\textsuperscript{1}, the procedure of Fraenkel-Conrat and Fraenkel-Conrat\textsuperscript{2} for the release of hydantoins from proteins was erroneously quoted, the temperature for the reaction being given at 75\textdegree C instead of 36\textdegree C (cf. Fraenkel-Conrat and Fraenkel-Conrat\textsuperscript{3}).


* Correction to Attempted Successive Applications of the Edman Degradation to Insulin\textsuperscript{1}

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**SHORT COMMUNICATIONS**

Table 1. Total activity expressed in μg standard per g dry weight of lichens.

<table>
<thead>
<tr>
<th>Lichenes *</th>
<th>E. coli Vit. B₁₂ std.</th>
<th>L. citrovorum Leucovorin std</th>
<th>S. faecalis Folvite std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladonia silvatica</td>
<td>0.035</td>
<td>0.68</td>
<td>0.26</td>
</tr>
<tr>
<td>Umbilicaria puellula</td>
<td>0.02</td>
<td>0.19</td>
<td>0.24</td>
</tr>
<tr>
<td>Parmelia furfuracea</td>
<td>&lt; 0.005</td>
<td>0.09</td>
<td>0.43</td>
</tr>
<tr>
<td>Parmelia physodes</td>
<td>0.02</td>
<td>2.35</td>
<td>1.16</td>
</tr>
<tr>
<td>Cetraria islandica</td>
<td>&lt; 0.005</td>
<td>1.80</td>
<td>1.16</td>
</tr>
<tr>
<td>Evernia prunastri</td>
<td>0.008</td>
<td>0.06</td>
<td>0.26</td>
</tr>
<tr>
<td>Alectoria pubata</td>
<td>&lt; 0.005</td>
<td>0.01</td>
<td>0.41</td>
</tr>
<tr>
<td>Usnea conoae</td>
<td>0.015</td>
<td>0.28</td>
<td>0.41</td>
</tr>
</tbody>
</table>

* Symbiosis of fungi belonging to Discomycetes, order Lecanorales and algae belonging to Chlorophyceae, order Chlorococcales.

Values for *E. coli*, enzymatic treatment the highest values for *L. citrovorum* and *S. faecalis*.

The results are given in Table 1.

Table 2 shows the various types of growth factors for *S. faecalis* and *L. citrovorum* as revealed after chromatographic separation of the lichen extracts.

The experiments with *E. coli* showed the presence in the lichens of substances having vitamin B₁₂-like activity in a concentration of from less than 0.005 to 0.035 μg B₁₂/g dry weight. This comparatively low activity was found to be due to three different vitamin B₁₂-factors having the same *R*ₖ-values as vitamin B₁₂.

Table 2. *L. Citrovorum* and *S. faecalis* factors in lichens.

<table>
<thead>
<tr>
<th>Factors</th>
<th><em>R</em>ₖ-values</th>
<th><em>L. citrovorum</em> activity in samples</th>
<th><em>S. faecalis</em> activity in samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>ET</td>
</tr>
<tr>
<td>Unidentified</td>
<td>0.07</td>
<td></td>
<td></td>
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<tr>
<td>Pteroic acid</td>
<td>0.10</td>
<td></td>
<td></td>
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<tr>
<td>Unidentified</td>
<td>0.18</td>
<td></td>
<td></td>
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<tr>
<td>Pteroyletriglutamic acid</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unidentified</td>
<td>0.24</td>
<td></td>
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<tr>
<td>Folic acid</td>
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<td></td>
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<tr>
<td>Unidentified</td>
<td>0.37</td>
<td></td>
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<tr>
<td>Formylpteroylglutamic acid</td>
<td>0.45</td>
<td></td>
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<tr>
<td>Folinic acid</td>
<td>0.55</td>
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<tr>
<td>Formylterpico acid</td>
<td>0.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymidine</td>
<td>0.70</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The number of plus signs indicates the intensity of the growth caused by the different factors as observed after chromatographic separation. A = autoclaved, ET = enzyme treated.

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vitamin B12 and factor C, respectively. No evidence was found for the presence of factors having the same RF-values as pseudovitamin B12, vitamin B12, factor A, or factor B. The presence of vitamin B12 is of interest because to date its only other source has been marine algae.

For L. citrovorum four growth factors were found. Two of these have been identified as the natural citrovorum factor (folinic acid) (RF 0.55) and thymidine (RF 0.70). The other two factors had RF-values of 0.18 and 0.24. It seems likely that these factors are identical with two of the citrovorum factors other than folinic acid found in a red marine algae Porcellaria fastigiata.

Altogether nine substances stimulating the growth of S. faecalis were observed. Folinic acid and thymidine, which stimulate the growth of both S. faecalis and L. citrovorum, and factors with the same RF-values as pteroylglutamic acid (Teropterin, Lederle), N-formylfolic acid and N-formylpyrrole acid (Rhizopectin) were found. Only traces of folic acid (pteroylglutamic acid) could be detected. The other factors observed had approximately the RF-values 0.07, 0.18, and 0.37 respectively in this solvent system.

Enzymatic treatment of the extracts did not lead to release of folic acid (pteroylglutamic acid) or to folic acid (N-formyl-tetrahydrofolic acid), but substances with lower RF-values. It was also observed that the enzyme complex of the chicken pancreas homogenate did not seem to release folic acid from a solution of crystalline pteroylglutamic acid (Teropterin, Lederle). This is in agreement with previous findings. Instead it converted the pteroylglutamic acid to a growth factor for L. citrovorum which is not folinic acid. The value of the total activity for

*S. faecalis and L. citrovorum in Table 1 do not therefore represent the true folic acid or folinic acid content of the lichens.

Most lichens investigated contained substances having an antibiotic effect on E. coli, S. faecalis and L. citrovorum, possibly due to usnic acid.

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