stored for over 6 weeks in the freezer compartment. This technique of preservation was later used in nutritional evaluation of leaf protein as a source of β-carotene in rat diets. The use of freshly prepared material, wet or that soon after drying, is the obvious choice because of losses during storage. However, the problems of conserving β-carotene in leaf proteins during ordinary storage still need to be solved.

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* Preliminary communication at 13th Annual General Meeting of the Society of Biological Chemists (India), December 1969, Bombay.


4. Fire, N. W., Personal communication, Rothamsted Experimental Station, Harpenden.


ARREST OF CHLOROPHYLL AND PROTEIN BREAKDOWN IN SENESCING LEAF DISCS OF KALE BY CYCLOHEXIMIDE AND VANILLIN

Some inhibitors of RNA and protein synthesis inhibit yellowing in Brassica and Zea leaves kept in darkness.1-3 The results suggest that yellowing is dependent either on synthesis of some specific proteins or/and on the rate of protein breakdown.

7-mm discs were punched from mature leaves of kale, Brassica oleracea L. var. acephala cv. “niski zelony kedzierzawy”, floated on distilled water or solutions of cycloheximide, vanillin or abscisic acid (ABA), and kept in darkness for 1, 2 or 4 days at 25°. In tests on protein or RNA synthesis the discs pre-treated for 24 hr with the inhibitors were washed and transferred to 1.8 ml of L-leucine-U14C (1μCi/ml; specific activity 90 mCi/mM) or uracil-2,14C (5μCi/ml; specific activity 6 mCi/mM) and fed in light (1400 lx) at 23°. To determine the effects of the compounds in protein breakdown, freshly punched discs were fed with labelled leucine for 3 hours, washed, and transferred to solutions of the compounds to be tested supplemented with L-leucine-12C (10-3M) and kept in darkness for 2 days, the solutions being renewed after 24 hr. All incubation media were supplemented with penicillin G 50 mg/l. Protein and RNA were extracted and estimated according to references 4 and 5, and 6 respectively. Chlorophyll was extracted with 80% acetone and measured spectrophotometrically.7 Radioactivity was counted with approximately 35% counting efficiency.

Table I shows that cycloheximide and vanillin markedly retarded yellowing whereas ABA accelerated it. None of the compounds induced visible symptoms of intoxication. The changes in chlorophyll content were correlated with the changes in protein content as determined after 1 and 2 days, and the rate of protein breakdown as inferred from radioactivity remaining in protein fraction of the discs, preliminary labelled with leucine-14C before treatment with the compounds.

Cycloheximide and vanillin markedly inhibited both protein and RNA synthesis. ABA also reduced incorporation of labelled precursors into protein and RNA, the reduction being correlated with the loss of protein and RNA content. From the data of Table I, it can easily be computed that cycloheximide and vanillin strikingly decreased specific activities of protein and RNA, whereas ABA had little effect on these parameters.

Although cycloheximide and vanillin retarded yellowing in darkness, they accelerated it in light (data not shown).

It has been suggested that cytokinins can retard senescence as a consequence of arrest of protein degradation.8 9 The same seems to be true for cycloheximide and vanillin (table) and for other inhibitors1-10. If yellowing in darkness is being taken as a measure of progress of senescence. On the contrary, compounds which accelerate protein breakdown, like abscisic acid (Table I) or 2-chloroethylphosphonic acid (data not shown) stimulate yellowing.

Keeping in mind that vanillin, in comparison to cycloheximide, markedly more effectively inhibited protein synthesis than protein breakdown, it can be concluded that synthesis of some enzymes is also necessary for initiation of senescence processes,
TABLE I

Chlorophyll, protein and RNA in senescing leaf discs of kale treated with cycloheximide
(10⁻¹ M), vanillin (10⁻² M) and abscisic acid (10⁻⁴ M)

<table>
<thead>
<tr>
<th></th>
<th>Values at 0 time</th>
<th>Incubation hours¹</th>
<th>H₂O</th>
<th>Treatment cycleheximide</th>
<th>Vanillin</th>
<th>ABA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophylls, µg²</td>
<td>18.0</td>
<td>96D</td>
<td>5.0</td>
<td>15.0</td>
<td>15.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Protein, µg</td>
<td>87.0</td>
<td>24D + 3L</td>
<td>76.7</td>
<td>85.7</td>
<td>78.3</td>
<td>60.0</td>
</tr>
<tr>
<td>RNA, µg</td>
<td>10.4</td>
<td>24D + 3L</td>
<td>9.6</td>
<td>9.5</td>
<td>7.3</td>
<td>7.6</td>
</tr>
<tr>
<td>Protein synthesis, dpm</td>
<td></td>
<td>(24D) + 3L</td>
<td>13.40</td>
<td>4.416</td>
<td>468</td>
<td>9.183</td>
</tr>
<tr>
<td>RNA synthesis, dpm</td>
<td></td>
<td>(24D) + 3L</td>
<td>426</td>
<td>192</td>
<td>54</td>
<td>324</td>
</tr>
</tbody>
</table>

Tests on protein breakdown

Protein, µg          | 90.0³          | 3L + 48D          | 64.8  | 76.8                     | 70.3     | 41.4|
Protein breakdown, dpm| 12,411³       | 3L + 48D          | 8,430 | 12,033                   | 10,010   | 5,255|

¹ D. darkness; L. Light: + means that discs were transferred from darkness to light or vice versa.
² All data are presented per one disc basis.
³ Measured after feeding for 3 hours with labelled leucine in light. Values differing by more than 14 per cent. are significant at ρ = 0.05.

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ON THE OCCURRENCE OF FREE SULPHURIC ACID IN SPATHOGLOSSUM ASPERUM J. AG.

It is well known that species of Desmarestia are notoriously acid and collectors of marine algae have always avoided placing Desmarestia along with other specimens for fear of injuring them. If injured, the alga turns bluish due to the effect of its acid on the carotenoid pigments. A similar phenomenon has been noticed by the authors in Spathoglossum asperum J. Ag., commonly found at Mandapam and the neighbouring islands. A study was made of the aqueous extract of this alga and it was found that the extract was acid, a 150 ml extract from 220 g of fresh Spathoglossum registering a pH of 1.4 at 25°C. With barium hydroxide, a precipitate of barium sulphate was readily formed, indicating that the acid was sulphuric acid.

To estimate the acid, 220 g of fresh alga was extracted with 150 ml double-distilled water in a blender for one minute, the blend centrifuged at 3,000 r.p.m. for 10 minutes and the clear supernatant was treated with equimolar quantity of barium hydroxide with stirring to precipitate the acid as barium sulphate. The sulphate was filtered, washed and dried at 60°C. From the weight of the dry sulphate, the value of sulphuric acid was calculated. This was 1.6 g or 0.73% of the fresh weight of the alga. The acid could also be titrated with 0.01 N sodium hydroxide and this procedure also gave very similar results.

It was early demonstrated¹ that the acidity of Desmarestia was due to the presence of free sulphuric acid although malic acid² has been credited with causing the acidity. However, Miwa³ has shown that free sulphuric acid is abundant in D. lingulata, D. virides and D. tabacoides. It has been shown⁵ also that in D. manuda, the high acidity is due to the dissociation of 0.025 meq of sulphuric acid and the quantities of organic acids are too low and their pK values too high to account for the observed acidity. The acid has been located⁶ in the vacuole and has been determined to be 0.44 N sulphuric acid.

It is interesting to note the presence of the acid in a member of the Dictyotales. The authors examined also other members of the Dictyotales, viz., Dictyota, Padina and Stoechaspernum but the aqueous extracts of all these alga registered pH between 7.0 and 7.2. Thus, Spathoglossum appears to be the only...