using a reciprocal grafting experiment. The materials studied were cherry-red and red-free selections from *N. tabacum* cv. 401 and their hybrids with *N. sylvestris*.

Flue-curing decreased the nicotine, total alkaloid content, and the ratio of nicotine to total alkaloid content and increased the nornicotine content in converter entries whereas the alkaloid composition of the nonconverter entry remained essentially unchanged.

Grafting per se did not influence the level of alkaloid production in these materials. The degree of conversion and alkaloid production in a scion or host was not influenced by the other graft component.

The entries serving either as scion or host exhibited increasing conversion capability in the order RF, RFX, CR, and CRX. In their ability to supply scions with alkaloid the entries differed in the increasing order CRX, RFX, CR, and RF.

Nornicotine was not transferred from the scion to host in the case of converter scions developed on a red-free host.

Conversion capability is associated with genotype. Those entries with 2 genes for conversion (CR and CRX) exhibited a greater degree of conversion than the 1 gene type (RFX).

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**Metabolic Changes Associated with the Germination of Corn**

**II. Nucleic Acid Metabolism**

J. Ingle and R. H. Hageman

Department of Agronomy, University of Illinois, Urbana, Illinois

The changes in nucleic acid content which occur during the germination of a variety of plant species have been studied by numerous investigators. The results from such investigations have not, however, led to a clear understanding of the importance of the reserve nucleic acid present in the storage organs of the seed, and the mechanisms whereby this reserve is used by the growing embryo. Oota and Takata (16) suggested that the RNA reserve of bean cotyledons was moved as intact macromolecules into the hypocotyl, whereas Barker and Douglas (1) concluded that the RNA of pea cotyledons was degraded and moved as soluble constituents into the axis during germination. Similar divergent data have been reported for the germination of cereal grain, where the physical boundary between the storage reserves of the endosperm and the embryo presents an additional complication. Matsushita (14) concluded that RNA was degraded in wheat endosperm prior to transport into the embryo, while Ledoux and Huart (12) reported that in germinating barley the RNA moved as macromolecules. An investigation of the changes in soluble nucleotide content of the endosperm during the germination of barley led Ingle (5) to conclude that degradation of the endosperm RNA did occur prior to its utilization by the embryo. This study also showed that the utilization of endosperm reserve could account for only a small percentage of the nucleic acid increase which occurred, indicating the importance of a de novo synthesis of nucleotides and nucleic acids during this early stage of germination.

Degradation of reserve RNA is indicated by the demonstration of an inverse relationship between the

---

**Literature Cited**

level of ribonuclease activity and the RNA content of
the storage organ (11, 14). Furthermore, a large in-
crease in 3' nucleotidase activity, an enzyme intima-
ly associated with ribonuclease in the degradation of
RNA, has been reported during the germination of
wheat (18). Recently, however, it has been shown
that the normal determination of ribonuclease activity
represents the sum of 2 different enzymes (15, 20).
These 2 enzymes have very different properties (21),
and presumably have different metabolic functions
within the cell. These findings, together with the
observations relating ribonuclease activity to growth
(17) and to RNA content (9, 10, 11), suggest the
possibility that ribonuclease may have functions other
than the degradation of RNA.

The distribution and changes in soluble nucleo-
tides, RNA and DNA were determined in the endo-
sperm, scutellum, and axis to ascertain the importance
of the utilization of reserve nucleic acid by the grow-
ing embryo during the germination of corn. The ac-
tivities of ribonuclease and 3' nucleotidase were also
determined in an attempt to elucidate the mechanism
of this utilization, and to consider the significance of
the relationship between ribonuclease activity and
RNA content.

Materials and Methods

Hybrid corn (Zea mays, WF9 M14) was ger-
minated and harvested as previously described (8).
Young, meristematic tissues were obtained from the
root tip (0-2 mm or 0-5 mm) and from the shoot
(5 mm either side of first node, total 10 mm). A tis-
sue containing rapidly elongating cells was obtained
from the root tip (2-4-mm section). Older, mature
tissues were obtained from the root (a 10-mm section
taken 30-40 mm from tip) and from the first internode
of the shoot (a 10-mm section taken 20-30 mm from
the first node).

Protein, soluble nucleotide, RNA, and DNA were
extracted from the axis (root plus shoot), scutellum,
and endosperm and estimated as previously described
(8).

For the determination of enzyme activities, freshly
dissected tissue was homogenized in 0.05 M Tris,
0.5 M KCl (pH 7.5) at 0°C in an Omni-Mixer (2 min-
utes, full speed). Aliquots of homogenates from axis
and scutellum tissue were used directly for the enzyme
assay; homogenates of endosperm tissue were first
cleared by centrifugation (500 x g, 15 minutes).
Ribonuclease activity was assayed at pH 5.8 by a
modification of the method of Tuve and Anfinsen
described by Wilson (20). The activity at pH 5.8
will subsequently be referred to as total ribonuclease.
The activity thus measured represented the sum of
activities of 2 enzymes, ribonuclease-A and ribo-
nuclease-B, having pH optima at 5.0 and 6.2, respec-
tively (20). The relative activities of ribonuclease-A
and ribonuclease-B were determined by assaying at
pH 5.2 and 6.0, respectively. The ratio of these ac-
tivities gave a qualitative and semi-quantitative esti-
mation of the amounts of the 2 enzymes, based on the
ratio values of 0.60 and 1.7 obtained with pure ribo-
nuclease-A and B, respectively. The ratio values for
pure ribonuclease-A and B show considerable varia-
tion, depending on source of RNA and salt concen-
tration in the assay. The method of Shuster and Gifford
(18) was used to assay 3' nucleotidase. The results
were expressed as u moles of Pi released under the
conditions of the assay. Nonspecific phosphatase ac-
tivity, measured by using adenosine-2-monophosphate
or adenosine-5-monophosphate as the substrate, was
very low with the tissues used in the present inves-
tigation.

Results

Distribution and Changes of Nucleotides and
Nucleic Acids during Germination. Daily changes
in soluble nucleotide, RNA, and DNA content of the
axis, scutellum, and endosperm are shown in table 1.
With growth of the axis the changes of these 3
components followed a similar pattern, with a lag
during the first 24 hours and then a steady increase
over the next 4 days. The relatively small changes
which occurred during the first day were those which
preceded cell division (8), as indicated by the con-
stancy of the DNA content. The large increase in
DNA between the first and second days indicated the
start of cell division, and thereafter the DNA,
RNA, and soluble nucleotide contents increased at
the growth rate (8).

The soluble nucleotide content of the scutellum
nearly trebled during the first 3 days, and then re-
mained constant. The RNA content, after a small
drop during the first 24 hours, increased to a maxi-
mum on the fourth day, and then decreased. The
DNA content of the scutellum increased during the
first 2 days, and then remained constant.

The nucleotide and RNA content of the endosperm
remained practically constant over the germination
period. The variation shown in table I was due to the
experimental error of estimation of these con-
tituents from the endosperm material. Although the
nucleotide and nucleic acid content of the endosperm
appeared large in absolute terms relative to the axis
or scutellum, it represented only 0.03 % of the dry
weight of endosperm in comparison with 2.0 % found
for the embryo.

The changes in the whole seedling showed that a
large de novo synthesis of nucleotides, RNA, and
DNA occurred during this early stage of germination,
accounting for the total increase of these components.

Distribution and Changes in Ribonuclease and 3'-
Nucleotidase during Germination. The total ribo-
nuclease activity of the axis increased 340-fold over the
germination period (table II). The activity in-
creased at a rate much faster than the total protein
for the first 3 days, but then slowed down approxi-
ately to the rate of total protein synthesis. The
changing ratio of ribonuclease activity at pH 6.0 to
pH 5.2 indicated an increase in the ribonuclease-B
component relative to ribonuclease-A. The activity

49
Table I. The Soluble Nucleotide, RNA, and DNA Content of the Axis, Scutellum, Endosperm, and Whole Corn Seedling during a 5-Day Period of Germination

<table>
<thead>
<tr>
<th>Age of seedling (hr)</th>
<th>4</th>
<th>23</th>
<th>48</th>
<th>71</th>
<th>95</th>
<th>121</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/part.</td>
<td>µg/part.</td>
<td>µg/part.</td>
<td>µg/part.</td>
<td>µg/part.</td>
<td>µg/part.</td>
</tr>
<tr>
<td><strong>Axis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble nucleotide</td>
<td>18.2</td>
<td>24.3</td>
<td>97.1</td>
<td>206.0</td>
<td>413.0</td>
<td>585.0</td>
</tr>
<tr>
<td>RNA</td>
<td>63.3</td>
<td>66.6</td>
<td>142.5</td>
<td>323.0</td>
<td>485.0</td>
<td>710.0</td>
</tr>
<tr>
<td>DNA</td>
<td>6.3</td>
<td>6.4</td>
<td>17.2</td>
<td>39.6</td>
<td>63.5</td>
<td>106.5</td>
</tr>
<tr>
<td><strong>Scutellum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble nucleotide</td>
<td>53.0</td>
<td>74.0</td>
<td>114.0</td>
<td>138.0</td>
<td>141.0</td>
<td>138.0</td>
</tr>
<tr>
<td>RNA</td>
<td>105.0</td>
<td>95.0</td>
<td>177.5</td>
<td>336.3</td>
<td>146.0</td>
<td>114.0</td>
</tr>
<tr>
<td>DNA</td>
<td>12.7</td>
<td>11.4</td>
<td>15.0</td>
<td>15.3</td>
<td>15.1</td>
<td>15.8</td>
</tr>
<tr>
<td><strong>Endosperm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble nucleotide</td>
<td>92.2</td>
<td>107.0</td>
<td>117.0</td>
<td>116.5</td>
<td>121.5</td>
<td>100.0</td>
</tr>
<tr>
<td>RNA</td>
<td>47.5</td>
<td>25.4</td>
<td>28.5</td>
<td>31.7</td>
<td>25.4</td>
<td>25.4</td>
</tr>
<tr>
<td>DNA</td>
<td>21.4</td>
<td>21.0</td>
<td>14.0</td>
<td>11.9</td>
<td>11.9</td>
<td>9.1</td>
</tr>
<tr>
<td><strong>Whole seedlings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble nucleotide</td>
<td>163.4</td>
<td>205.3</td>
<td>318.1</td>
<td>460.5</td>
<td>675.5</td>
<td>823.0</td>
</tr>
<tr>
<td>RNA</td>
<td>215.8</td>
<td>187.0</td>
<td>288.5</td>
<td>491.0</td>
<td>656.4</td>
<td>849.4</td>
</tr>
<tr>
<td>DNA</td>
<td>40.4</td>
<td>38.8</td>
<td>46.2</td>
<td>66.8</td>
<td>90.5</td>
<td>131.4</td>
</tr>
</tbody>
</table>

* Obtained by summation of the individual parts.

of 3'nucleotidase in the axis showed the same general trend as total ribonuclease.

In the scutellum, both total ribonuclease and 3'nucleotidase activity decreased during the first day, and then increased for the next 4 days, the 3'nucleotidase at a slightly faster rate. The ratio of ribonuclease activity at pH 6.0 and pH 5.2 remained remarkably constant, around 0.80, throughout the period.

The endosperm from the 4-hour sampling contained considerable total ribonuclease activity, which increased to a maximum around the fourth day. However, due to the decrease in total protein of the endosperm during the latter part of the germination period (8), the specific activity of ribonuclease continued to increase over the whole period. The ratio of activity at pH 6.0 to pH 5.2 remained constant throughout, with a mean value of 0.64. The 3'nucleotidase activity of the endosperm was initially very low relative to ribonuclease, but it increased more rapidly from the second to the fifth day.

**Ribbonuclease and 3'Nucleotidase Activity in Root Sections of Differing Physiological Age.** The specific activity of both enzymes was several-fold higher in the elongating cells of the 2- to 4-mm section than in the

Table II. Ribonuclease and 3'Nucleotidase Activity in the Axis, Scutellum, and Endosperm during the Germination of Corn

<table>
<thead>
<tr>
<th>Age of seedling (hr)</th>
<th>4</th>
<th>23</th>
<th>48</th>
<th>71</th>
<th>95</th>
<th>121</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/part.</td>
<td>µg/part.</td>
<td>µg/part.</td>
<td>µg/part.</td>
<td>µg/part.</td>
<td>µg/part.</td>
</tr>
<tr>
<td><strong>Axis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ribonuclease/axis</td>
<td>0.30</td>
<td>0.50</td>
<td>13.45</td>
<td>44.20</td>
<td>87.30</td>
<td>102.50</td>
</tr>
<tr>
<td>Total ribonuclease/mg protein</td>
<td>0.36</td>
<td>0.82</td>
<td>6.60</td>
<td>9.50</td>
<td>10.20</td>
<td>10.70</td>
</tr>
<tr>
<td>Ribonuclease pH 6.0*</td>
<td>0.27</td>
<td>0.83</td>
<td>0.90</td>
<td>1.04</td>
<td>0.99</td>
<td>1.26</td>
</tr>
<tr>
<td>3'Nucleotidase/axis</td>
<td>0.26</td>
<td>0.28</td>
<td>7.94</td>
<td>27.10</td>
<td>57.50</td>
<td>74.20</td>
</tr>
<tr>
<td>3'Nucleotidase/mg protein</td>
<td>0.31</td>
<td>0.46</td>
<td>3.90</td>
<td>5.85</td>
<td>6.70</td>
<td>7.75</td>
</tr>
<tr>
<td><strong>Scutellum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ribonuclease/scutellum</td>
<td>4.36</td>
<td>4.06</td>
<td>9.25</td>
<td>11.20</td>
<td>20.90</td>
<td>22.90</td>
</tr>
<tr>
<td>Total ribonuclease/mg protein</td>
<td>1.30</td>
<td>1.28</td>
<td>2.40</td>
<td>3.00</td>
<td>6.00</td>
<td>6.83</td>
</tr>
<tr>
<td>Ribonuclease pH 6.0*</td>
<td>0.82</td>
<td>0.84</td>
<td>0.77</td>
<td>0.81</td>
<td>0.79</td>
<td>0.81</td>
</tr>
<tr>
<td>3'Nucleotidase/scutellum</td>
<td>1.60</td>
<td>1.41</td>
<td>3.06</td>
<td>4.70</td>
<td>10.40</td>
<td>12.80</td>
</tr>
<tr>
<td>3'Nucleotidase/mg protein</td>
<td>0.48</td>
<td>0.45</td>
<td>0.80</td>
<td>1.26</td>
<td>2.98</td>
<td>3.84</td>
</tr>
<tr>
<td><strong>Endosperm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ribonuclease/endosperm</td>
<td>41.20</td>
<td>63.80</td>
<td>62.81</td>
<td>65.90</td>
<td>68.90</td>
<td>60.60</td>
</tr>
<tr>
<td>Total ribonuclease/mg protein</td>
<td>1.92</td>
<td>3.06</td>
<td>4.18</td>
<td>6.82</td>
<td>14.30</td>
<td>20.70</td>
</tr>
<tr>
<td>Ribonuclease pH 6.0*</td>
<td>0.60</td>
<td>0.66</td>
<td>0.56</td>
<td>0.62</td>
<td>0.72</td>
<td>0.67</td>
</tr>
<tr>
<td>3'Nucleotidase/endosperm</td>
<td>1.07</td>
<td>0.85</td>
<td>1.62</td>
<td>3.17</td>
<td>5.39</td>
<td>7.04</td>
</tr>
<tr>
<td>3'Nucleotidase/mg protein</td>
<td>0.05</td>
<td>0.04</td>
<td>0.11</td>
<td>0.33</td>
<td>1.13</td>
<td>2.42</td>
</tr>
</tbody>
</table>

* Ratio values have been defined in Methods section.
young meristematic cells (table III). Total ribonuclease activity was still higher in the mature cells, whereas 3’nucleotidase activity was less in the mature cells than in the region of cell elongation. The ratio of ribonuclease activity at pH 6.0 to pH 5.2 was considerably higher in both the elongating and the mature cells than in the meristematic region.

**Relationship between Total Ribonuclease Activity and RNA Content.** The ratios of the total ribonuclease activity to the RNA content for the axis, scutellum, and endosperm over the germination period are given in table IV. This ratio increased 40-fold in the axis during the first 4 days of germination. In the scutellum, the ratio showed a 5-fold increase, while in the endosperm it remained practically constant after an increase during the first day.

The distribution and relationship of total ribonuclease activity and RNA content in the axis of a 4-day-old seedling is shown in table V. As already noted in table III, the mature region of the root contained considerably more total ribonuclease activity than the young meristematic region, but less RNA; thus, the ratio of these 2 components was 10 times higher in the mature than in the meristematic region. Similar ratios resulted from a comparison of young and mature regions of the shoot.

**Table III.** *Ribonuclease and 3’Nucleotidase Activity in Root Sections of Differing Physiological Age*

<table>
<thead>
<tr>
<th>Root section</th>
<th>0-2 mm</th>
<th>2-4 mm</th>
<th>30-40 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ribonuclease/mg protein</td>
<td>6.7</td>
<td>14.8</td>
<td>17.8</td>
</tr>
<tr>
<td>Ribonuclease pH 6.0</td>
<td>0.85</td>
<td>1.10</td>
<td>1.08</td>
</tr>
<tr>
<td>Ribonuclease pH 5.2</td>
<td>4.9</td>
<td>18.2</td>
<td>12.3</td>
</tr>
<tr>
<td>3’Nucleotidase/mg protein</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

The data show that little nucleotide or nucleic acid was stored in the corn grain (var. WF9 × M14). The increase of nucleotides, RNA, and DNA, in the growing axis was therefore by de novo synthesis. The scutellum and endosperm did not serve as a reserve source of nucleic acid or nucleotides for the embryo, based on changes in nucleic acid and nucleotides of these organs over the experimental period. There was, in fact, an increase in RNA and soluble nucleotide content of the scutellum, which paralleled the changes in dry weight, protein, soluble protein, and amino acid content previously reported (8), indicating the synthetic activity of the scutellum during germination. The results suggest that the synthesis of nucleotide material occurs entirely in the growing axis, although the possibility of synthesis in the scutellum or endosperm followed by translocation into the axis cannot be ruled out.

Nucleic acid metabolism therefore differs fundamentally from protein metabolism during germination, since the seed normally contains sufficient endogenous protein to support growth of the embryo over this period. These data and the previous work with barley (5) suggest that the nucleic acids stored in the endo-

**Table IV.** *The Relationship between Total Ribonuclease Activity and RNA Content in the Axis, Scutellum, and Endosperm during the Germination of Corn*

<table>
<thead>
<tr>
<th>Age of seedling (hr)</th>
<th>4</th>
<th>23</th>
<th>48</th>
<th>71</th>
<th>95</th>
<th>121</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axis</td>
<td>4.7</td>
<td>7.5</td>
<td>94.5</td>
<td>137.0</td>
<td>180.0</td>
<td>145.0</td>
</tr>
<tr>
<td>Scutellum</td>
<td>41.5</td>
<td>42.8</td>
<td>79.0</td>
<td>82.4</td>
<td>143.0</td>
<td>201.0</td>
</tr>
<tr>
<td>Endosperm</td>
<td>870</td>
<td>2,510</td>
<td>2,210</td>
<td>2,080</td>
<td>2,700</td>
<td>2,380</td>
</tr>
</tbody>
</table>

**Table V.** *The Distribution of RNA and Total Ribonuclease Activity in the Axis of a 4-day-old Corn Seedling*

<table>
<thead>
<tr>
<th></th>
<th>Total ribonuclease</th>
<th>μg RNA</th>
<th>Total ribonuclease/mg RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole axis</td>
<td>70.0</td>
<td>377.0</td>
<td>186.0</td>
</tr>
<tr>
<td>Root</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meristematic tissue (0-5 mm)</td>
<td>0.34</td>
<td>12.8</td>
<td>27.0</td>
</tr>
<tr>
<td>Mature tissue (40-50 mm)</td>
<td>0.97</td>
<td>3.0</td>
<td>323.0</td>
</tr>
<tr>
<td>Shoot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meristematic tissue (5 mm either side of first node, total 10 mm)</td>
<td>3.74</td>
<td>86.8</td>
<td>43.0</td>
</tr>
<tr>
<td>Mature tissue (20-30 mm of first internode from first node)</td>
<td>3.54</td>
<td>11.0</td>
<td>322.0</td>
</tr>
</tbody>
</table>

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sperm and scutellum of the grain are of minor importance as a reserve for the developing embryo. The loss of nucleic acid from the endosperm during the germination of barley could account for only 20% of the nucleic acid increase of the embryo, and furthermore, this loss from the endosperm only occurred after a period of intense de novo synthesis of nucleic acid in the embryo (5). The results of Matsushita (13) similarly indicate that the endosperm could supply only a relatively small percentage of the increase in embryo RNA in rice. Studies with germinating barley have indicated that the amino acids of the young embryo could serve as the source of nitrogen for the de novo synthesis of nucleotides and nucleic acids (4).

Certain of the results from this investigation contrast with earlier reports. A rapid degradation of RNA in the scutellum, resulting in a loss of more than half of the RNA (2), and a similar decrease in the RNA content of the endosperm plus scutellum in which 90% of the RNA was lost (19) have been reported. As previously discussed (7), however, it is thought that the method of RNA extraction and estimation used by these workers was unsuitable for the tissues under investigation, and is responsible for the differences in the results.

The large increase in total ribonuclease activity which accompanied germination is in agreement with the work of others (1, 11, 14). At present, there is very little information on the nature of this increase in enzyme activity, whether it is due to a synthesis of the enzyme or to a release or activation of an existing protein. Experiments with corn mesocotyl tissue have, however, shown that the increase of ribonuclease activity is prevented by such inhibitors as actinomycin D, puromycin or 8-aza-quinine (17), suggesting that protein synthesis is required for the increase of activity in embryo tissue. Matsushita (14) concluded that the increase of ribonuclease activity in the wheat endosperm was also due to protein synthesis, since the seeds initially contained a definite activity, which could not be increased by different extraction procedures. The results from the present investigation, however, suggest that the increase of ribonuclease activity in the corn endosperm was due to a release of enzyme, since short incubations with a high salt buffer extracted the maximal ribonuclease activity from the endosperms of ungerminated seeds (6).

The determination of total ribonuclease activity by the degradation of RNA to acid-soluble products represents the sum of at least 2 different enzyme activities. Matsushita (15) found the ribonuclease of the endosperm and of the root cytoplasm to have different properties from the ribonuclease of the microsome fraction of the roots of wheat seedlings. Wilson (20) has recently described the isolation of 2 similar enzymes from corn, and has also studied their specificity of action (21). Ribonuclease-A, the soluble enzyme with pH optimum at 5.0, produced mainly the nucleoside 2', 3'-cyclic phosphates from the digestion of RNA, whereas ribonuclease-B, the particulate enzyme with pH optimum at 6.2, produced largely the nucleoside 5'-phosphates, and furthermore was not specific for RNA, degrading DNA with equal ease. The ratio of ribonuclease activity at pH 6.0 to pH 5.2 enables the relative changes of these 2 enzymes to be followed through the germination period. The constancy of this ratio, at 0.64, in the endosperm, is in agreement with the results of chromatographic analysis of endospermal ribonuclease, which showed the presence of only ribonuclease-A in this organ (6). The ratio of 0.80 observed for the scutellum indicates the presence of some ribonuclease-B, about 10% of the total activity, and the constancy of the ratio during germination shows that both ribonuclease-A and ribonuclease-B increase at approximately equal rates. The change of this ratio during the growth of the axis, from 0.77 to 1.26, indicates the importance of ribonuclease-B during development. This enzyme increases at a much faster rate than the ribonuclease-A, increasing from approximately 10% to 70% of the total ribonuclease activity during the 5 days of growth. The increase of ribonuclease-B activity with development is also indicated from the experiments with root tissues of differing physiological age. The meristematic cells contained largely ribonuclease-A activity, whereas tissues composed of elongating and mature cells contained a much higher proportion of ribonuclease-B activity.

Preliminary experiments with corn embryos had indicated a very close correlation between 3'nuclotidase and total ribonuclease activity. Furthermore, 3'nuclotidase and ribonuclease-B have certain properties in common: both are very unstable, both are eluted from carboxymethyl-cellulose columns in the same fraction, and both cleave a 3'phosphoester bond. These similarities suggested that the 2 enzyme assays may be different measures of the same enzyme activity. The data presented in table II (endosperm section) suggest, however, that 3'nuclotidase is a separate moiety, since 3'nuclotidase activity per endosperm increased 7-fold during the 5-day period while the ribonuclease pH 6.0:5.2 ratio remained constant. In addition, no ribonuclease-B activity was observed on carboxymethyl-cellulose chromatography of endosperm extracts (6).

Correlation between ribonuclease activity and RNA content has been noted by several workers (1, 10). The results of the present investigation show a similar qualitative correlation, both total ribonuclease activity and RNA content increasing in the corn axis, but the data in table IV show the correlation to be far from quantitative. The total ribonuclease activity initially increases at a rate much faster than RNA content. The results indicate that the ratio of ribonuclease to RNA increases with maturation of the tissue, which agrees with the data from the comparison of young, meristematic tissues with older, mature tissues (table V). Similar changes have been reported by Barker and Douglas (1) for germinating peas, and they suggest that the appearance of ribonuclease activity prior to the increase in RNA indicated a synthetic function for the enzyme. Since the total ribonuclease activity is composed of at least 2 distinct enzymes, it is
necessary to establish whether the synthetic activity is unique to one of them. The distribution of the 2 ribonuclease activities in the seedling, and their relative changes during germination, suggest ribonuclease-B to be the metabolically important enzyme. A similar conclusion was reached by Kessler and Engleberg (9) who observed a positive correlation between RNA content and microsomal ribonuclease activity (ribonuclease-B), but a negative correlation between RNA content and soluble ribonuclease activity (ribonuclease-A) in developing leaves.

The observed correlation between RNA content and ribonuclease activity need not, however, implicate ribonuclease activity in RNA synthesis. The work of Elson (3) has shown ribonuclease activity to be intimately associated with ribonucleoprotein structure, which in itself, could account for such a correlation.

The increases of total ribonuclease activity in the scutellum and endosperm show no correlation with RNA content. The small amount of ribonuclease-B activity present in the scutellum could be related to the metabolic activity of this organ, in terms of the small increase in RNA content during germination. The presence of only ribonuclease-A activity in the endosperm suggests this enzyme to be responsible for the degradation of storage RNA, although the endosperm of corn contains very little such reserve. The high stability of ribonuclease-A activity suggests that it remains in the tissue after its period of metabolic importance, and further investigations are being undertaken on the development and maturation of the corn kernel in order to elucidate the significance of the high enzyme activity.

**Summary**

The soluble nucleotides, RNA, DNA, total ribonuclease, and 3′nucleotidase were determined in the axis, scutellum, and endosperm of corn at daily intervals during a 5-day germination period. The results showed the corn kernel to contain little reserve nucleic acid; the increase of nucleotide and nucleic acid material in the growing axis being due to de novo synthesis.

Large increases in total ribonuclease and 3′nucleotidase activities occurred in the axis, scutellum, and endosperm. A semiquantitative evaluation of ribonuclease-A and ribonuclease-B, showed the latter enzyme to be largely confined to the axis, with small amounts present in the scutellum; whereas the endosperm contained only ribonuclease-A.

Tissues containing young, meristematic cells were found to have low enzyme activities and a low ratio of total ribonuclease to RNA relative to tissues containing older, mature cells.

**Literature Cited**


