Indolylacetic acid (IAA) has been postulated as the enzymatically produced endogenous auxin controlling the development of fruit (Muir 1947). Few reports have appeared suggesting that there may, in fact, be other auxins associated with this function particularly during its earliest phase, between pollination and fertilization.

Recently, Lund (1956a, 1956b), in an investigation of the hormonal complement of young Maryland Mammoth tobacco fruit, reported that IAA was the only auxin responsible for the control of processes associated with fruit development, its concentration beginning to rise about 50 hr after pollination, coincident with the beginning of fertilization and the start of ovary enlargement. However, a compound similar to indolylacetonitrile (IAN) was also found in the acid fraction of ether extracts (although its concentration did not vary) while the neutral extracts contained no activity as measured by the *Avena* curvature test. Even though IAN is a neutral compound some of it may enter the acidic fraction (Bennet-Clark and Kefford 1953; Cartwright, Sykes, and Wain 1956). However, if it is present at all, it is unlikely that none of it would be found in the neutral ether extract.

The detection and quantitative estimation of neutral auxins with the curvature test is complicated by the tendency of the neutral compounds towards non-polar or lateral movement (Bentley and Bickle 1952). For this reason the straight growth of *Avena* coleoptiles was adopted in this work as a more suitable means of determining the changes occurring in the acidic and neutral auxin fractions of Little Turkish tobacco ovary tissue during the first 4 days following pollination.

Ovary tissue was lyophilized and ground to pass a 40-mesh screen prior to extraction with dry, peroxide-free ether for 12 hr at \(-20°C\). The ether was partitioned three times with 1 per cent. sodium bicarbonate to remove the acid fraction and the sodium bicarbonate was then acidified and extracted three times with fresh ether. All ether solutions were reduced in volume to 5 ml over a hot water-bath at which time 10 ml distilled water was added. The remaining ether was evaporated, and, following cooling, the water solutions were adjusted to pH 6·0. As a routine procedure, 1 : 10 and frequently 1 : 100 dilutions of the water solutions were assayed with the *Avena* straight-growth test (Muir and Hansch 1953), to be certain that the extracts did not contain supra-optimal concentrations of hormones.

* Manuscript received April 13, 1959.

† Department of Botany, State University of Iowa, U.S.A.; present address: Waite Agricultural Research Institute, University of Adelaide.

‡ Department of Botany, State University of Iowa, U.S.A.
Through analysis of unpollinated ovary tissue at 1, 2, 3, and 4 days after anthesis, it was determined that the free auxin levels (both acidic and neutral) in 200 mg dry weight (about 30 ovaries) were below the limits of sensitivity of the test. However, as shown in Figure 1(a), there is, immediately following pollination (carried out at anthesis), a rapid production of neutral auxin. This exceeds acid auxin production and by the third day following pollination, neutral auxin level is twice that of acid auxin. Four days after pollination, coincident with increasingly rapid changes in dry weight (Fig. 1(b)), a diminution in neutral auxin and a very rapid rise in acid auxin content occur. This is evident on both a per unit tissue and a per ovary basis.

Three interpretations of the results are possible:

1) Neutral auxin functions as a precursor in the formation of acid auxin, conversion being initiated or heightened in conjunction with embryo and endosperm development (beginning in the majority of ovules between the second and third days following pollination).
(2) The biosynthetic pathways lead from a precursor common to both acid and neutral auxin, and the amounts of each present serve as an indication of the success of the competing systems.

(3) The syntheses of acid and neutral auxins are unrelated in so far as different phenomena may control their initiation. The formation of the neutral fraction may be predominantly a pre-fertilization process, while the formation of acid auxin may be largely correlated with post-fertilization processes.

Unfortunately, the data at this time do not allow a distinct choice to be made. There are, however, similarities in all three interpretations; production of neutral auxin in the ovary is initiated by pollen germination and tube growth, and formation of acid auxin in the ovary is stimulated by fertilization and accompanying processes.

**Table 1**

**Tryptophan content of tobacco ovaries following anthesis**

Samples for two successive days are combined

<table>
<thead>
<tr>
<th>Days after Anthesis</th>
<th>Condition</th>
<th>Tryptophan (µg per ovary)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>3 and 4 Unpollinated</td>
<td></td>
<td>5.1</td>
</tr>
<tr>
<td>5 and 6 Unpollinated</td>
<td></td>
<td>13.0</td>
</tr>
<tr>
<td>1 and 2 Pollinated</td>
<td></td>
<td>23.0</td>
</tr>
<tr>
<td>5 and 6 Pollinated</td>
<td></td>
<td>252.0</td>
</tr>
</tbody>
</table>

* Derived by subtracting free from total tryptophan values.

The absence of auxin activity in the neutral fraction, as described by Lund (1956a), may have been the result of a failure of the *Avena* curvature test to assay auxins which are not transported in a strictly polar manner. It may also have been due to a supra-optimal concentration of neutral auxin (Bentley and Bickle (1952) have reported that curvature of *Avena* coleoptiles may disappear completely when IAN is supplied in supra-optimal amounts) since the neutral extracts were neither diluted before assay nor subjected to chromatographic analysis (Lund, personal communication). The slight but constant activity ascribed to IAN in the acid fraction examined by Lund might represent the partition coefficient of IAN between the acid and neutral extracts.

The time at which acidic auxin concentration in the ovary increases agrees well with Lund's results, since he also reports very little change until the pollen tubes enter the ovary and fertilization takes place. The results indicate that the initial phases of ovary enlargement (and possibly the prevention of abscission) may be under the control of neutral auxin, and that the role of acid auxin in the ovary (probably IAA) may not become active until fertilization occurs.