possibility that this difference can be ascribed to the presence in the peritoneal cells of a much higher proportion of phagocytic cells than are found in suspension of cells from the thymus and lymph nodes, was tested by removing glass adherent cells by culturing these cells in plastic petri dishes or in siliconized glass tubes. Similar results were obtained in both types of vessel, and the data shown in Table II are for cells maintained in plastic vessels. The difference in RNAse content is very great, the non-adherent cells from the peritoneum contain some twenty times as much RNAse than do those from the thymus.

The non-adherent cultured cells from both peritoneum and thymus contain more RNAse (see Table II) than the average of the cells before separation, the values for which are shown in Table I. The non-adherent cells recovered after culturing are, by morphological criteria, mostly composed of lymphocytes, yet their RNAse content varies widely and suggests that different populations of lymphocytes may be distinguished on the bases of the amount of alkaline RNAse they contain.

We have shown previously* that after high doses of cortisone (2.1 mg per mouse), which leads to a marked loss of lymphoid cells from the thymus and lymph nodes, the RNAse content expressed per mg of tissue rises sharply. The data shown in Table I confirm these results but show in addition that the already high RNAse content of the peritoneal lymphocyte is raised still further by treatment of the mice with cortisone. The results in Table II show that cortisone does not significantly alter the RNAse content of the adherent cells. There is a 160 fold difference between the RNAse content of non-adherent cells from the peritoneum of cortisone-treated mice as compared with that of the non-adherent cells from the thymus of normal mice. In terms of 6 marker, the proportion of T-cells will be greatest for the thymus and less in the lymph nodes and peritoneal cells.\(^\text{13}\)

The RNAse content of the adherent cells from the peritoneum is considerably greater than that of the adherent cells from thymus and lymph nodes, but this data is difficult to interpret. While the adherent peritoneal cells are made up of at least 95% of macrophages (the peritoneal cells when taken from a non-stimulated cavity contain approximately equal numbers of lymphocytes and mononuclear phagocytes\(^\text{14}\)), the nature of the relatively few adherent cells separated from the thymus and lymph nodes is uncertain. It is noteworthy that cortisone pre-treatment does not affect the RNAse content of any of the adherent cell populations studied.


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Evidence for the Allosteric Nature of IAA Oxidase System in Phaseolus mungo Hypocotyls

The synergistic effect of sodium metabisulfite with IAA in the production of adventitious roots in hypocotyl cuttings of Phaseolus mungo reported earlier from this laboratory1 lends support to the view that IAA effects are caused through IAA oxidation products\(^\text{12,14}\). This is contrary to the view of other workers\(^\text{12,14}\), who consider that IAA oxidase causes detoxification of IAA in the plant system.

In in-vitro-experiments that are carried out to determine the activity of IAA oxidase in tissue homogenates, the concentrations of IAA that are used as substrate are fairly high (10⁻⁴ M). If the destruction of IAA in vivo also occurs at this rate, the plant tissue will be depleted of its endogenous IAA within 1–10 min, as its biosynthesis is considered to proceed at a very slow rate\(^\text{12}\). As the maintenance of a proper balance between the synthesis and

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Table II. Differences in the ribonucl ease activity of adherent and non-adherent cells cultured from the peritoneum and the thymus of normal and cortisone-treated mice

<table>
<thead>
<tr>
<th>Cells obtained from</th>
<th>RNAse content of cells after 2 h in culture(^ab)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adherent cells</td>
</tr>
<tr>
<td>Peritoneum from normal mice</td>
<td>12.0 ± 5.0</td>
</tr>
<tr>
<td>From mice 1 day after cortisone</td>
<td>13.2 ± 4.4</td>
</tr>
<tr>
<td>From mice 2 days after cortisone</td>
<td>10.8 ± 2.7</td>
</tr>
<tr>
<td>From mice 3 days after cortisone</td>
<td>14.4 ± 4.6</td>
</tr>
<tr>
<td>Thymus from normal mice</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>From mice 1 day after cortisone</td>
<td>0.34 ± 0.3</td>
</tr>
<tr>
<td>From mice 2 days after cortisone</td>
<td>0.60 ± 0.2</td>
</tr>
<tr>
<td>From mice 3 days after cortisone</td>
<td>0.63 ± 0.1</td>
</tr>
<tr>
<td>Lymph node from normal mice</td>
<td>1.5 ± 0.5</td>
</tr>
</tbody>
</table>

\(^a\) Expressed as 10⁻¹⁰ g of crystalline pancreatic ribonuclease per cell. \(^b\) Cells were pooled from 5 mice for each of the 3 separate experiments (mean of 3 separate experiments ± S.D.). \(^*\) 5 mg cortisone acetate (Frederiksberg Chemical Laboratories Ltd., Copenhagen) given s.c.

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degradation of IAA is necessary for normal physiological function in plants, it seems rather improbable that IAA degradation in vivo can occur at the high rates at which it occurs in vitro experiments with tissue homogenates.

The present paper deals with the results of some experiments that were carried out to shed light on this point, using IAA oxidase system in the tissue homogenates of hypocotyls of Phaseolus mungo.

Seedlings of Phaseolus mungo were raised as described earlier. 3 cm portions below the cotyledonary nodes were used for IAA oxidase activity determined by the method described elsewhere. It was found that the acetone-precipitated proteins that were dissolved in citrate-phosphate buffer at pH 6.0 were able to oxidize only 4.0 µg of IAA in 60 min (Figure 1). The IAA oxidase activity of the acetone-precipitated proteins was then determined at 7 different pH. The activity curve is hyperbolic with a peak at pH 4.0 and decreasing values both with the increasing as well as decreasing pH (Figure 1).

Another experiment was carried out to study the effect of enzyme concentration on its activity in relation to substrate level. 2 concentrations of enzyme proteins, i.e. 437.6 µg and 4,000 µg, were dissolved in citrate-phosphate buffer at pH 4.0 and the concentrations of IAA used as substrate varied from 30 to 150 µg.

The activity curve shows sigmoid tendency with the higher concentration of enzyme proteins (Figure 2). Thus, the oxidation rate increased linearly with the increase in the substrate concentration from 30 to 100 µg IAA but decreased with further increase in substrate level. In marked contrast to this, a considerably higher oxidation rate was recorded with lower (437.6 µg) concentration of the enzyme at lower substrate (30 and 45 µg IAA) levels. However, the activity in this case decreased with the increasing concentration of substrate till 60 µg IAA, beyond which it did not change much.

The results thus show that, at low substrate levels, the rate of IAA oxidation was much higher with lower than with higher enzyme concentrations, although the activity with higher enzyme concentrations exhibited a sigmoid relationship with the change in the concentration of the substrate.

This behaviour of IAA oxidase is characteristic of allosteric enzymes with 2 sites, a primary binding site and a secondary site. This behaviour may be explained on the basis of a model assuming 2 binding sites, or it may be conceived that IAA oxidase system consists of 2 closely associated enzymes, one of which may be an oxidase and the other a peroxidase. These may represent 2 binding sites, as postulated earlier. The primary site has a higher affinity for IAA but low catalytic activity, while as the secondary site has a low affinity for IAA but high catalytic activity. The secondary site (site II) opens as a result of the allosteric transformation, when the primary site (site I) is saturated. Such a model can explain why IAA oxidation is high at low enzyme concentration with low substrate levels but low at high enzyme concentration with the same substrate level.

This model also explains the two views regarding the role of IAA oxidase in plant systems. Most workers have studied the activity of the enzyme in diluted tissue homogenates in vitro experiments, using high concentrations of IAA as substrate. The high activity that is reported in these experiments is due to low enzyme concentration and represents the activity of site II. This may not be the case in intact cells, as the concentration of IAA available endogenously may not be adequate even to saturate the primary site (site I). Due to allosteric transformations, therefore, IAA oxidase system increases or decreases the rate of IAA degradation, depending upon the amount of IAA available in its vicinity.

The presence of such an enzyme system is very significant as site I, the oxidase site, may be considered to be concerned in the production of oxidation products which cause physiological responses, while site II, the peroxidase, may perform a regulatory role and may cause detoxification of the excessive IAA when present in a plant tissue.

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