with 50\% (v/v) ethanol and radioactivity determined by liquid-scintillation counting. The separation procedure used is capable of separating cyclic AMP from all known naturally occurring adenine nucleotides. Results showed that the 'total' membrane fraction from barley leaves possesses adenylate cyclase activity equivalent to 26 pmol of cyclic AMP formed/min per mg of membrane protein. The 100,000 g supernatant possessed negligible adenylate cyclase activity. Constancy of the $^3$H/$^{14}$C ratio of the isolated sample through several purification steps (Table 1) and during hydrolysis by ox heart phosphodiesterase confirms that the product of the enzymic activity determined is cyclic AMP.


### Aromatic Amino Acid Decarboxylase: pH-Dependence of Substrates and Inhibitors

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The enzyme, aromatic amino acid decarboxylase (EC 4.1.1.28) is a key enzyme in the production of biogenic amines. It is necessary for the production of 5-hydroxytryptamine and dopamine (3,4-dihydroxyphenethylamine) and thence adrenaline and noradrenaline.

Inhibition of the enzyme has been used clinically in the treatment of hypertension and in conjunction with L-dopa (3,4-dihydroxyphenylalanine) in the treatment of Parkinsonism. Despite the fact that the enzyme has been purified from pig kidney apparently as a single entity, (Christenson et al., 1970; Lancaster & Sourkes, 1972), with activity towards both dopa and 5-hydroxytryptophan there are a number of properties which require clarification. For example, it has been stated that it has different pH optima towards different substrates and also shows markedly different changes in activity towards different substrates on treatment with denaturants (Clarke et al., 1954; Bender & Coulson, 1972). Properties such as these led to the suggestion that two different enzymes were responsible for the production of dopamine and 5-hydroxytryptamine, until the purification of the enzyme seemed to have resolved the point.

In the experiments described below, the kinetics of the rat liver enzyme have been studied in relation to both dopa and 5-hydroxytryptophan as substrates and also in relation to several inhibitors. Evidence is presented to show that the binding of the enzyme and substrate or inhibitor is markedly dependent on pH for phenylalanine-related structures but not for the indole-related structures. The significance of these findings is discussed.

The enzyme used was a partially purified preparation from rat liver, by the method of Christenson et al. (1970) up to the dialysis stage after heat treatment in the presence of substrate. Assays of the enzyme activity were carried out by the method of Bender & Coulson (1972). Buffer solutions ranging from pH 6.8 to 7.8 were prepared from sodium and potassium phosphate, being 0.1 M with respect to phosphate and 16 $\mu$M with respect to pyridoxal phosphate. All solutions were made up in freshly boiled water and were gassed thoroughly with O$_2$-free N$_2$ before storage. Incubations were gassed with O$_2$-free
N₂ and covered with Oxoid caps to decrease oxidation. Incubations were for 5 min at 30°C with 250 μg of protein from the enzyme preparation. In most experiments duplicate incubations were carried out and duplicate chromatograms from each incubation were run. The results plotted were then means of these four determinations (s.d. values are shown as bars on the graph). Lineweaver–Burk plots were computer-fitted by least-squares procedure from unweighted data. Each of the plots shown in Fig. 1 was from two separate experiments both of which were repeated on several occasions. The values for Kᵢ shown in Table 1 were determined from plots of 1/v against [I] at several substrate concentrations, the values quoted being the means (± s.d.) of all observed intercepts.

The report that dopa and 5-hydroxytryptophan exhibit different pH optima with this enzyme was investigated by determining the dependence of reaction velocity on substrate concentration for a range of pH from 6.8 to 7.8. The Lineweaver–Burk plots for these data are presented in Fig. 1. The plots for 5-hydroxytryptophan resemble a typical non-competitive-inhibition situation in which the proton corresponds to the inhibitor. This suggests an effect on either the product leaving or the catalytic reaction.

5-Hydroxytryptamine shows no significant change in Kᵢ from pH 6.8 to 7.8 so the change in velocity reported above is unlikely to be due to product leaving and must be attributed to an effect on the catalytic process. On the other hand, decarboxylase activity towards dopa shows a sudden change in kinetic parameters above pH 7.2 (Fig. 1), having a lower Vₘₐₓ and a lower Kₘ above this pH.

Inhibitor studies with the product, dopamine, show that it also shows a very marked change in binding between pH 6.8 and 7.8, but in this case it binds less well at the higher pH. Both dopa and dopamine show a pH-dependent change in absorbance in this pH range, associated with their oxidation, but the fact that their affinities change in opposite ways makes a common oxidation step an unlikely cause of these changes. To further elucidate this situation, a study of the pH-dependence of the dopa decarboxylase inhibitors, α-methyldopa and α-methyl-m-tyrosine was undertaken. The results are summarized in Table 1.

The most striking feature of these results is that all the phenylalanine-related substrates and inhibitors (not just the catechols) show changes in binding with pH, whereas the indoles do not. This suggests that the change of binding with pH is not due to oxidation of the catechols. Whether it is due to there being separate sites for the two structures or merely to different groups in the same site being involved in the binding is not clear.
### Table 1. Inhibition constants for various inhibitors of aromatic amino acid decarboxylase

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Substrate</th>
<th>pH 6.8 $K_i$ (μM)</th>
<th>Type of inhibition</th>
<th>Substrate</th>
<th>pH 7.8 $K_i$ (μM)</th>
<th>Type of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>Dopa</td>
<td>0.08 ± 0.02</td>
<td>Competitive</td>
<td>Trp(5OH)*</td>
<td>0.54 ± 0.03</td>
<td>Competitive</td>
</tr>
<tr>
<td>5-Hydroxytryptamine</td>
<td>Dopa</td>
<td>0.22 ± 0.04</td>
<td>Competitive</td>
<td>Trp(5OH)</td>
<td>0.18 ± 0.02</td>
<td>Competitive</td>
</tr>
<tr>
<td>Methyl-m-tyrosine</td>
<td>Dopa</td>
<td>0.32 ± 0.07</td>
<td>Competitive</td>
<td>Dopa</td>
<td>0.06 ± 0.015</td>
<td>Competitive</td>
</tr>
<tr>
<td>Methyl-m-tyrosine</td>
<td>Trp(5OH)</td>
<td>0.23 ± 0.04</td>
<td>Non-competitive</td>
<td>Trp(5OH)</td>
<td>0.124 ± 0.008</td>
<td>Non-competitive</td>
</tr>
<tr>
<td>Methyldopa</td>
<td>Dopa</td>
<td>0.15 ± 0.04</td>
<td>Competitive</td>
<td>Trp(5OH)</td>
<td>0.04 ± 0.007</td>
<td>Competitive</td>
</tr>
</tbody>
</table>

* Abbreviation: Trp(5OH), 5-hydroxytryptophan.
Clarke et al. (1954) did show that when the enzyme had been treated with semicarbazide it had decreased activity towards both substrates, but on addition of pyridoxal phosphate the activity towards dopa was fully restored, whereas activity towards 5-hydroxytryptophan was not.

Lancaster & Sourkes (1972) reported that the activity of their purified pig kidney enzyme towards dopa and 5-hydroxytryptophan was not enhanced by added pyridoxal phosphate, but that the activity towards o- and m-tyrosine was enhanced up to threefold by such addition.

These results are compatible with there being two binding sites and also two molecules of pyridoxal per molecule of enzyme.


Deoxyribonucleic Acid Polymerase and Deoxyribonuclease Activities in Pea Seedlings

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Cells of pea (Pisum sativum) seedlings and of other higher plants contain two major DNA-dependent DNA polymerases (Srivastava & Grace, 1974; Robinson & Bryant, 1975b; Stevens et al., 1975; Stevens & Bryant, 1976; Stevens, 1976). One is tightly bound to the chromatin, and is somewhat similar to the DNA polymerase-β of vertebrates (Dunham & Cherry, 1973; Srivastava & Grace, 1974; Stevens & Bryant, 1976; Stevens, 1976). The other is soluble, and is very similar to the DNA polymerase-α of vertebrates (Srivastava & Grace, 1974; Gardner & Kado, 1976; Stevens & Bryant, 1976; Stevens, 1976). There is no direct evidence for the roles of these enzymes in pea, other higher plants or vertebrates. However, it is widely held that DNA polymerase-α is involved in DNA replication, whereas DNA polymerase-β is involved in repair (e.g. Keir et al., 1977).

Pea seedlings also contain deoxyribonucleases. One of the deoxyribonucleases is a soluble Mg²⁺-dependent enzyme that is apparently confined to senescent cells where it degrades DNA during cell lysis (Bryant et al., 1976). In non-senescent cells we have detected chromatin-bound deoxyribonuclease which is stimulated by Ca²⁺, but not by Mg²⁺. Characterization of the hydrolysis products, released from DNA by crude preparations of chromatin-bound nuclease, suggests that both exo- and endo-nuclease activities are present, since we are able to detect oligodeoxyribonucleotides of varying length, and monodeoxyribonucleotides. Chromatin-bound or nuclear deoxyribonucleases have been detected in other higher plants (Srivastava, 1968; Kligman & Takats, 1975). In Tradescantia, at least part of the activity is known to be endonucleolytic (Kligman & Takats, 1975). As with DNA polymerases, there is no direct evidence for the roles of these nuclease, although endonucleolytic 'nicking' of DNA is regarded as a possible major regulatory step in the initiation of DNA replication (Pederson, 1972; Burzio & Koide, 1973), and further it is clear that DNA repair must involve nucleolytic activity (Kornberg, 1974).

To obtain circumstantial evidence on the roles of DNA polymerase and deoxyribonuclease, we have studied the activities of the enzymes in relation to the cells' ability to replicate DNA. We have used two experimental systems: (i) germinating pea seeds; (ii) roots of established pea seedlings. During germination at 22°C there is no net synthesis of DNA in the embryonic axis for the first 29 or 30 h. From 30 to 80 h, net DNA synthesis occurs at a rate of 0.5 μg/h per embryo axis (Robinson & Bryant, 1975a). Chromatin-bound DNA polymerase activity per embryo axis increases 3-fold between 6 and 22h,