MATERIAL AND METHODS

*Impatiens balsamina* L. var. Rose belonging to the family Balsaminaceae and *Phaseolus mungo* belonging to the family Leguminaceae were used as experimental materials in this investigation.

**Preparation of hypocotyl cuttings**

Uniform seeds of *Impatiens balsamina* were germinated on thin cotton pads in Petri-dishes in the dark in experiments 1-6, 8, 9, 13 and 14 but in diffused light in experiments 1, 7-12 and 14. Cuttings were made when the seedlings were 6-8 cm long in experiments 1-6, 8, 9, 13 and 14 and 3.5-4.0 cm in experiments 1, 7-12 and 14 by excising the roots and hypocotylar parts 3 cm below the cotyledonary node. The cotyledons and apex were also excised in experiments 1-6, 8, 9, 13 and 14 but were left intact in experiments 1, 7-12 and 14. The hypocotylar cuttings were planted vertically in test solutions in holes on polythene sheets stretched over Petri-dishes (10 cm dia.) and were held in position by rubber bands.

Seeds of *Phaseolus mungo* were sown in Petri-dishes (15 cm dia.) at 28°C and seedlings were raised either in...
the dark (experiments 15, 18 and 19) or in the light (experiments 15–19). When the seedlings had elongated about 8 cm above the cotyledons, these were made into 9 cm long cuttings by excising the cotyledons and roots leaving behind 6 cm long epicotyl together with 3 cm of hypocotyl. The primary leaves and the apical bud were also excised in experiments 15, 18 and 19 but were left intact in experiments 15–19. The cuttings were planted in holes on tin foil covering the grade tubes (7.5 x 3.0 cm) containing 20 ml of test solutions and only the hypocotylar portion was dipped in the test solution. The details of the nature and concentration of test solutions and light conditions to which the cuttings were exposed subsequent to treatment, are described in each experiment.

Temperature and light conditions

All the experiments were carried out in growth chambers with controlled light and temperature conditions. For continuous light (CL), the cultures were exposed to artificial light (3200 lux) obtained from fluorescent tubes with incandescent bulbs in between, fitted on light banks, and for dark (CD) these were kept in dark cabinets in the same air-conditioned room. The temperature was maintained at 28±2°C.

Preparation of stock solutions

The stock solutions of indole-3-acetic acid (IAA), ribonucleic acid (RNA) and guanine were prepared by dissolving the weighed quantity of the chemical in 1.0 ml of alcohol or dil HCl and of deoxyribonucleic acid (DNA) in 1.0 ml of dil alkal
An equivalent quantity of alcohol, dil HCl or alkali was added to water to serve as control. The nucleic acids used were calf thymus DNA and yeast RNA and these were obtained from Sigma Chemical Co., USA. The solutions of glucose (Glu), sucrose (Suc), ribose, 2-deoxyribose, 5-fluorodeoxyuridine (Fudr), actinomycin-D (act-D), cycloheximide (cyc), 5-fluorouracil (FU), 8-azaguanine (Aza-gun), 8-azacytidine (Aza-cyt), 6-azadenine (Aza-ad), adenine, cytosine, thymine and uracil were prepared directly in distilled water. 10 mg of each was dissolved and the volume was then made to 100 ml with distilled water. The stock solutions were suitably diluted to prepare solutions of requisite concentrations.

Observations

Observations recorded in different experiments were as follows:

(A) Morphological

Observations on the number of rooted cuttings, the number and length of roots and length of hypocotyls were recorded at periodic intervals upto 14 days in experiments 1-14 and 7 days in experiments 15-19. The time taken for the emergence of roots was also recorded in all cases. Visual observations on callus formation was also recorded.

(B) Anatomical

Anatomical studies were made of cuttings of some treatments in experiment 4 according to Johansen (1940) with minor adjustments.

Fixing: The basal 1 cm parts of cuttings were fixed in
FAA (45 ml rectified alcohol + 45 ml water + 5 ml formaldehyde + 5 ml glacial acetic acid for one day and were then transferred to 70% alcohol till the sections were cut.

**Dehydration:** The samples were dehydrated by passing through tertiary butyl alcohol grades (TBA). The concentration of TBA along with other components used in each grade and the time for which cuttings were kept in each, were as follows:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Water Spirit</th>
<th>Rectified Spirit</th>
<th>TBA</th>
<th>Absolute Alcohol</th>
<th>Time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>40</td>
<td>10</td>
<td>0</td>
<td>1/2</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>50</td>
<td>20</td>
<td>0</td>
<td>1/2</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>50</td>
<td>35</td>
<td>0</td>
<td>1/2</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>45</td>
<td>55</td>
<td>0</td>
<td>1/2</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>75</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>

**Penetration:** A few drops of molten paraffin wax (m.p. 60-62°C) were added to the tube containing the sample in 100% TBA. After 15 min, half the mixture of the tube was poured off and the molten wax was added. This procedure was repeated 6-7 times, each at an interval of 30 min, till the smell of TBA disappeared. Blocks were made in overnight ripe wax. The sections were cut at 10 μ. The ribbons were mounted on slides with uniformly spread adhesive and stretched using 3% formalin water.

**Dewaxing and staining:** The slides were kept in xylol for 4 hr till the wax was completely removed. These were then passed through xylol and alcohol grades in the order: Pure...
xylol, 75% xylol (in absolute alcohol), 50% xylol, absolute alcohol, 90% alcohol, 75% alcohol, 50% alcohol, safranin in 50% alcohol (16 hr), water, 25% alcohol, 50% alcohol, 75% alcohol, 95% alcohol, 100% alcohol, 50% clove oil (in absolute alcohol), pure clove oil, fast green, clove oil, xylol, and finally mounted in Canada balsam. Photomicrographs of the important stages were taken.

(C) Biochemical

A time course study of the changes in the activity of some enzymes were made. The drifts in the DNA-, RNA-, protein-, free amino acid-, carbohydrate-, water- and dry matter contents were also studied. Polyacrylamide gel electrophoresis was performed to study the transients of RNA and protein species using following methods:

Determinations

I. Chemical methods

Dry weight

Dry wt was determined by drying a known amount of fresh material at 105°C to a constant weight.

Calculations: The percentage dry wt was calculated by dividing the dry wt by fresh wt and multiplying by 100 in each case.

Carbohydrates

Carbohydrates were estimated by the method of Morris (1948), Viles and Silverman (1949) and Loewus (1952).

Extraction: The requisite amount of fresh plant material was suspended in 80% alcohol, incubated at 70°C in a water bath
for 10 min, cooled, filtered through glass wool and the volume made to 5 ml by adding distilled water.

**Estimation:** 2 ml of anthrone reagent (0.2% anthrone in ethyl acetate) and 5 ml of conc H$_2$SO$_4$ were added to 1 ml of the extract in a test tube and its optical density measured with the help of a Bausch and Lomb Spectronic-20 photometer at 610 nm after 10 min.

**Calculations:** The carbohydrate content was expressed in mg/mg dry wt in terms of glucose equivalent values, using glucose as the standard.

**RNA**

The RNA content was estimated by the method described by Mesbeum (1939).

**Extraction:** RNA and DNA were extracted according to the method of Bonner and Zeevaart (1962). The ground sample was suspended in acetone till free from pigments. The residue was dehydrated by 1:1 mixture of acetone ether and was air dried at room temperature for 24 hr. 2 ml of 3N perchloric acid (PCA) previously cooled at 4°C, was added to the acetone powder in test tubes, which were then kept at 4°C for 30 min. The material was centrifuged at 2000 rpm for 10 min and the supernatant collected. The sediment was extracted once more with PCA in the same way. The supernatant was pooled and used for the estimation of RNA and the residue was used for the extraction of DNA.

**Estimation:** 2 ml of orcinol (1.0% orcinol and 0.5% FeCl$_3$ in conc HCl) was added to 1 ml of the supernatant and
the tubes were boiled for 10 min on a water bath. The optical density of the green solution was noted at 610 nm.

**Calculations:** RNA content (µg/mg dry wt) was calculated from the standard curve made by using ribose.

**DNA**

DNA content was estimated by the method described by Burton (1956).

**Extraction:** To the residue of RNA, was added 2 ml of 3N PCA. It was kept in a water bath at 60°C for 15 min and extracted twice. The material was centrifuged at 2000 rpm for 10 min and the supernatant collected and cooled.

**Estimation:** 2 ml of DNA reagent (1 gm diphenylamine + 1.5 ml conc. H₂SO₄ (AR grade) + 0.5 ml of acetaldehyde diluted 50 times and the total volume made to 100 ml with glacial acetic acid) was added to 2 ml of the supernatant and the tubes were boiled for 30 min in a water bath. The optical density of the bluish green solution was noted at 610 nm.

**Calculations:** DNA content (µg/mg dry wt) was calculated from the standard curve made by using 2-deoxyribose.

**Protein**

Protein content was estimated by the method of Lowry *et al.* (1951).

**Reagents:**

(A) 2% sodium carbonate in 0.1N sodium hydroxide

(B) 0.5% copper sulphate in 1% sodium citrate

(C) 1.0 ml of reagent B is mixed with 50 ml of reagent A

(D) Folin-Ciocalteu reagent
Estimation: The requisite amount of the fresh plant material was suspended in distilled water, 20% trichloroacetic acid (TCA) at 5°C for 24 hr to remove sugars, non-proteins, phenols and soluble nitrogen fractions, and centrifuged. To the residue, 1 ml of the reagent C was added and after 10 min, 0.1 ml of the reagent D was added rapidly and mixed. The optical density of the blue solution was read with the help of a photocolorimeter at 710 nm after an interval of 30 min.

Calculations: The protein content (μg/mg dry wt) was calculated from the standard curve made by using bovine albumin.

Amino acids

Free amino acids were estimated by ninhydrin reaction according to the method of Moore and Stein (1948).

Extraction: The requisite amount of fresh plant material was boiled in 70% alcohol for 10 min in a test tube (10 x 1 cm) on a water bath using air-condensors. The material was centrifuged at 2000 rpm for 10 min and the supernatant collected and evaporated to dryness.

Estimation: While the dried residue was still hot, 2 ml of ninhydrin reagent (0.1 % in 70% ethyl alcohol) was added and the solution was again boiled for 10 min with constant shaking. After cooling, the solution was diluted to 6 ml by iso-propyl alcohol and the optical density was noted with the help of a photocolorimeter at 520 nm.

Calculations: Free amino acid content (μg/mg dry wt) was calculated from the standard curve prepared by using glutamic acid.
II. Polyacrylamide gel electrophoresis

Preparation of gels

The gels were prepared according to the method of Ornstein (1964) and Davis (1964). The following stock solutions were prepared in distilled water and stored in a refrigerator at 15°C till used.

**Solution A**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN HCl</td>
<td>48 ml</td>
</tr>
<tr>
<td>Tris</td>
<td>36.6 gm</td>
</tr>
<tr>
<td>beta-dimethylaminopropionitrile</td>
<td>0.23 ml</td>
</tr>
<tr>
<td>Water to make up</td>
<td>100 ml</td>
</tr>
<tr>
<td>pH</td>
<td>8.3</td>
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**Solution B**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylamide</td>
<td>28.0 gm</td>
</tr>
<tr>
<td>Bis-acrylamide</td>
<td>0.733 gm</td>
</tr>
<tr>
<td>Water to make up</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

**Solution C**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riboflavin</td>
<td>4.0 mg</td>
</tr>
<tr>
<td>Water to make up</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

**Solution D**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium persulphate</td>
<td>0.14 gm</td>
</tr>
<tr>
<td>Water to make up</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

Preparation of gels

<table>
<thead>
<tr>
<th>Solution</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3 parts</td>
</tr>
<tr>
<td>B</td>
<td>9 parts</td>
</tr>
<tr>
<td>C</td>
<td>6 parts</td>
</tr>
<tr>
<td>D</td>
<td>6 parts</td>
</tr>
<tr>
<td>Water</td>
<td>3 parts</td>
</tr>
</tbody>
</table>
The above solutions were warmed up to the room temperature before use, mixed and poured into glass tubes (5 mm x 90 mm) fitted in rubber caps in a perspex stand. The top portions of the tubes (20 mm) were layered gently with distilled water to eliminate a meniscus and to maintain a flat smooth gel surface. The tubes were placed before a fluorescent lamp for 10 min to allow photopolymerization. When the gels were properly set they were stored in distilled water in the refrigerator at 15°C till needed.

**Electrophoretic apparatus**

It consists of two plastic chambers placed in such a way that the base of the upper chamber fitted nicely in the rim of the lower chamber. Eight gel tubes were fitted at the base of the upper chamber, with the help of rubber stoppers in such a way that they dipped in the buffer solution of the lower chamber. Two platinum electrodes were provided, cathode in the upper and anode in the lower chamber. The electrodes were connected to an electrophoretic power supply unit (Model Toshniwal, CMO 1/02 Sr. No. 126) which supplied the required DC current. A constant current of 40 mA (5 mA/tube) was passed through the electrode chambers during electrophoresis.

**Electrophoretic run**

At the time of electrophoretic run, the lower anodic chamber was filled two thirds with the buffer (Lithium hydroxide, 0.025M and boric acid, 0.20M mixed in the ratio 1:1, pH 9.0). The gel tubes were fitted at the base of the cathodic chamber which was then placed on the top of the
anodic chamber. Each gel tube was fed with the extract by equalizing the activity. The material was gently layered with 1-2 drops of 60% urea. The upper chamber was then, filled with tank buffer slowly in order to avoid any disturbance to the material in the gel tubes. A few drops of marker dye (2% bromophenol blue) were added to mark the solvent front. The electrodes were then connected to the terminals of power supply unit and the current passed till the marker dye reached the lower limit marked at 8 cm below the top.

**Removal of gels**

The power supply was disconnected and the gels were then removed from the tubes by running them with the help of a clean sharp needle slowly in between the gel and the tube. During the process, the tubes were kept in a trough filled with ice-cold water to avoid entry of air-bubbles, which hinder the process.

**Calculation of Rf values**

The Rf values were calculated by individually putting the gels in a 10 ml measuring cylinder and noting the position of the solvent front as well as that of the bands, against a fluorescent tube. The position of the solvent front was noted before putting it in the stain because it became indistinguishable later.

Thus, $Rf = \frac{\text{distance travelled by the band}}{\text{distance travelled by the solvent front}}$
(a) **Extraction of RNA for gel electrophoresis**

RNA was extracted by the method of Brawerman and Eisenstadt (1964), Kirby (1967) as follows:

The lipid-free plant material was suspended in ice-cold 0.1 M tris-HCl buffer, pH 9.0, containing 0.5% sodium dodecyl sulphate. The suspension was mixed with an equal volume of ice-cold aqueous phenol (80%), and shaken gently at room temperature for 60 min and was centrifuged for 30 min. The upper aqueous layer was removed by a pipette. To this solution was added (1:0.1, v/v) 10% sodium chloride and 2.5 volumes of absolute alcohol, mixed and kept at 4°C for 48 hr. The tubes were centrifuged and the residue was used for further work.

**Gel electrophoresis of RNA**

RNA solution was prepared by dissolving the above residue in 0.025 M lithium hydroxide-boric acid buffer, pH 8.6. The same buffer was also used in the electrode vessels.

The current was supplied by a DC stabilizer at 5 mA per tube at 250 volts. After completing the run, the tubes were taken out of the glass tube with the help of a needle. They were stained in 0.2% toluidine blue for 18 hr. The destaining of the gels was done using 10% acetic acid till the bands were clearly visible.

The gel electrophoresis of RNA was also tried using different buffers at various pH values. A number of stains were also used for staining the RNA bands.
(b) **Extraction of proteins for gel electrophoresis**

The plant material was suspended in ice-cold 0.067 M phosphate buffer, pH 7.0. Supernatant was collected after centrifuging 2-3 times at about 3000 rpm to remove the sand and debris. The extract obtained was either used immediately or stored at 0°C till further analysis.

**Disc electrophoresis of proteins**

Electrophoresis was carried out on 10% polyacrylamide gels at 4°C. Lithium hydroxide-boric acid buffer, pH 9.0 was used in the electrode compartments. Crude extract containing a known amount of protein was placed on the gel at the top and was overlayered by 60% urea solution. The buffer was then carefully placed in the chamber so as not to disturb the extract. Lower chamber was connected to +ve terminal and the upper chamber to -ve terminal of constant DC supply power unit.

Gels were incubated in 10% TCA for 1 hr, washed thoroughly with distilled water and then stained for 18 hr in the following stains:

1. 1.0% amido black in 7% acetic acid (Davis, 1964).
2. 0.2% coomassie blue in 7% acetic acid (Chrambach, 1967).
3. 1:1 1.0% amido black and 0.2% coomassie blue.

After staining the gels were destained using 7% acetic acid till the bands were clearly visible.

**Assay of enzymes**

**Extraction of the crude enzymes**

The cuttings were washed 5-6 times with distilled water
and were then ground in pre-chilled glass pestle and mortar at 4°C in a ice-bath with acid-washed sand. Extraction of the crude enzymes was done either with 0.067 M phosphate buffer, pH 7.0 or citrate buffer, 0.1 M, pH 5.5. Supernatant was collected after centrifuging 2-3 times at about 3000 rpm, to remove the sand and the unbroken cells. The cell-free extract was stored in a deep freezer till used.

The buffers used for the extraction of different enzymes were as follows:

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Buffer/Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA-oxidase, peroxidase, amylase,</td>
<td>Phosphate buffer 0.067 M, pH 7.0.</td>
</tr>
<tr>
<td>invertase, polyphenol-oxidase</td>
<td>Citrate buffer 0.1 M, pH 5.5</td>
</tr>
<tr>
<td>ATPase, alkaline and acid</td>
<td></td>
</tr>
<tr>
<td>phosphatase, ribonuclease,</td>
<td></td>
</tr>
<tr>
<td>deoxyribonuclease</td>
<td></td>
</tr>
</tbody>
</table>

**ACTIVITY**

**Peroxidase**

The activity was assayed by the method of Mitra et al. (1970).

An aliquot of the extract of known protein value was incubated with 1 ml of 0.025% benzidine in 25% acetic acid mixed with an equal volume of 0.02% hydrogen peroxide for 5 min at 30°C in the dark. The activity is expressed as units per mg of protein as determined by using Folin reagent (Lowry et al. 1951).

**IAA-oxidase**

The activity was determined by the modified method of Gordon and Weber (1951).
The amount of the extract equivalent to the amount of protein was incubated with 0.5 ml of the 1 mM IAA in 5 mM MnCl₂ and 1.0 ml of 5 mM 2,6-dichlorophenol (DCP) and 0.06 M phosphate buffer (pH 6.0) for 1 hr at 30°C in the dark. 4.0 ml of Salkowski’s reagent was added and the mixture incubated for 20 min more. The optical density of the pink solution was measured at 530 nm and the activity is expressed as percentage of IAA oxidised per mg of protein.

Amylase

The activity was determined by the method described by Bernfeld (1951) and modified by Dure (1960).

The reaction mixture consisting of 0.2 ml of the extract, 0.067 M phosphate buffer (pH 7.0) together with 1.0 ml of 0.2% starch, was incubated at 30°C. The reaction was stopped after 30 min. The tubes were boiled in water-bath for 5 min, cooled and the intensity of the colour determined at 540 nm. A control was maintained each time using 0.2 ml of the extract after boiling it and the amount of maltose that was released was obtained by subtracting the colorimeter value. The activity of the enzyme is expressed as units of maltose released per mg of protein.

Polyphenol oxidase

The activity was determined by the methods of Bastin and Unlüer (1972).

The reaction mixture that consisted of 0.2 ml enzymes extract, 2 ml of 0.06 M phosphate buffer (pH 6.5) and 0.5 ml of 0.01 M DOPA, was incubated at 30°C for one hr. The rise
In optical density was measured at 430 nm at 2-min intervals. The activity was expressed as DOPA µg per mg of protein per min.

**Invertase (Sucrase)**

The method of Hatch and Glassiou (1963) was used for invertase assay.

The amount of the extract equivalent to the amount of protein was added to the reaction mixture containing 1 ml of 0.25% sucrose and 1 ml citrate buffer (pH 5.3) at 30°C for 30 min, the enzyme was inactivated by adding 2 ml of 5% perchloric acid. The solution was made up to a constant volume (10 ml). 1 ml of this solution was used to develop colour by anthrone method (Mcready et al., 1950). The exact amount of glucose produced was calculated from the following regression equation that was obtained by taking the known concentrations of glucose:

\[ X = 0.19 Y - 0.0212 \]

where \( X \) is the amount of glucose (mg) and \( Y \) the O.D.

The enzyme activity was expressed as mg of glucose released per mg of protein per min.

**ATPase**

The activity was determined by the method described by Kasamo and Yamaki (1974).

0.2 ml of the enzyme extract was incubated with 1 ml of 0.2 mg/l of ATP containing 2 ml of 0.1 M acetate buffer (pH 5.6) for one hr. The enzyme was precipitated with 1 ml of ice-cold 10% trichloroacetic acid and centrifuged. The
The volume of the supernatant was made up to 5 ml with acetate buffer. 2 ml of the supernatant was used for the estimation of phosphorus released by the method of Fiske and Subbarao (1925).

**Calculations:** ATPase activity was calculated from a standard curve prepared by taking potassium di-hydrogen orthophosphate as standard. It was expressed as μg phosphate released per min per mg enzyme protein.

**Acid phosphatase**

0.2 ml of enzyme extract was incubated with 2 ml of 1% sodium beta glycerophosphate prepared in acetate buffer (pH 5.0) at 37°C for 30 min. The enzyme was precipitated by the addition of 0.5 ml ice-cold TCA and centrifuged. The inorganic phosphate released was determined by the method of Fiske and Subbarao (1925).

**Calculations:** Same as for ATPase.

**Alkaline phosphatase**

The enzyme extract was incubated with 2.0 ml of 1% sodium B-glycerophosphate in Tris-HCl buffer, pH 9.5 and incubated at 35°C for 30 min. The enzyme was precipitated by adding 1.0 ml of ice-cold 10% trichloroacetic acid and centrifuged. The inorganic phosphate released was determined by the method of Fiske and Subbarao (1925).

**Calculations:** Same as for ATPase.

**Ribonuclease**

The activity was estimated according to the method of Tanaka (1961).
The enzyme extract was incubated with 4 ml of RNA (1 mg of yeast RNA/ml of Tris-HCl buffer) at 37°C for 30 min. Unhydrolysed RNA was precipitated by the addition of 0.25 ml perchloric acid containing 0.75% uranyl acetate. After allowing the reaction to stand for 30 min at 4°C, the samples were centrifuged for 15 min at 5000 g. 1.0 ml of the supernatant was used for the estimation of RNA by the method of Mezbaum (1939). The enzyme activity is expressed as µg of RNA hydrolysed per mg protein per min.

Deoxyribonuclease

The activity was determined by the method of Bernardi (1967).

0.2 ml extract was incubated with 4 ml of DNA (100 µg Calf thymus DNA, Sigma grade) in 2.0 ml acetate buffer, (pH 5.0) and 1 ml of EDTA buffer at 35°C for 20 min. Unhydrolysed DNA was precipitated by the addition of 1 ml of ice-cold 12% perchloric acid, centrifuged, and the supernatant was used for the estimation of DNA content by the modified method of Burton (1956). The enzyme activity is expressed as µg of DNA hydrolysed per mg protein per min.