III. EXPERIMENTAL RESULTS

A. Respiratory Metabolism

1. Rate of respiration: Measurement of oxygen uptake by fruits was carried out from the sixth day after flowering (fourth day after anthesis) up to senescence at daily intervals. The fluctuations observed at different stages of development were represented in Figure 2. There was a gradual rise in the rate of oxygen uptake starting from anthesis to twelfth day after flowering. This period of fruit development represents the post-fertilization stage and part of the first active growth period described earlier. Then there was a diminishing rate of oxygen uptake up to 28th day after flowering. This period includes the stages II, III and IV. The decline in the rate of oxygen uptake was very marked in stage IV, i.e., between 20th and 28th day (Fig. 2). After such a fall more or less a sharp rise in the rate of oxygen uptake was observed up to 34th day and this stage corresponds to the maturation or climacteric of the fruit. From the climacteric maximum the rate once again decreased to its minimum during senescent stage of the fruit. Thus the pattern of respiratory drift of pepper fruit showed a typical climacteric type of fruit respiration.

2. Effect of 2,4-Dinitrophenol on the rate of respiration: Fruit tissues at different developmental stages were incubated separately with distilled water or $10^{-4}$M 2,4-dinitrophenol (DNP) for a period of two hours. Measurement of oxygen uptake was done in both the samples. The results of this experiment were represented in Figure 3. The respiratory pattern of DNP
Legends for Figures 2 and 3

Fig. 2. Oxygen consumption by pepper fruit from the onset of anthesis to the attainment of senescence.

Fig. 3. Effect of 2,4-dinitrophenol on rate of oxygen uptake at different developmental stages of fruit.
treated fruit tissue was similar to the control tissue, but with a consistent stimulation of oxygen uptake in the first four developmental stages under DNP treatment. The DNP induced stimulation of oxygen uptake was reduced to its minimum during climacteric stage and no stimulation was observed in senescent stage (Fig. 3).

3. **Esterification of inorganic phosphorus:** Two series of experiments were conducted. In the first series the entire fruit tissue (seed and rind) was incubated for one hour with phosphorus labelled sodium dihydrogen phosphate (approximate activity $8.0 \times 10^5$ cpm). In the second series only the rind portion of the fruit was incubated for one hour with labelled phosphate as above. The two series of experiments were conducted at all the stages of development.

The amount of inorganic phosphorus esterified into organic phosphorus was calculated at each developmental stage in terms of radioactivity incorporated into organic fraction. The results showing the activity incorporated into organic phosphorus in the two series of experiments were given in Table I (page 38).

In the first series of experiments which were conducted with the entire fruit tissue, the capacity of the fruit to esterify inorganic phosphorus into organic phosphorus during stage II was greater than in stage I of development. The ability of the fruit tissue decreased till prematuration stage (stage IV). There was then a substantial rise in the rate of
TABLE I. Rate of esterification of inorganic phosphorus ($P_{32}$) by fruit tissue at different developmental stages (The weight of tissue was 2.0 g in the case of whole fruit and 1 g in the case of rind).

<table>
<thead>
<tr>
<th>Developmental Stage</th>
<th>Total activity supplied $10^5$ CPM</th>
<th>Total activity incorporated into alcohol soluble fraction $10^5$ CPM</th>
<th>Amount esterified into organic $10^5$ CPM</th>
<th>Activity in the organic phosphorous as percent of total supplied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole fruit</td>
<td>Rind</td>
<td>Whole fruit</td>
<td>Rind</td>
</tr>
<tr>
<td>I</td>
<td>8.0</td>
<td>8.0</td>
<td>1.590</td>
<td>0.820</td>
</tr>
<tr>
<td>II</td>
<td>8.0</td>
<td>8.0</td>
<td>1.278</td>
<td>0.224</td>
</tr>
<tr>
<td>III</td>
<td>8.0</td>
<td>8.0</td>
<td>0.926</td>
<td>0.215</td>
</tr>
<tr>
<td>IV</td>
<td>8.0</td>
<td>8.0</td>
<td>0.871</td>
<td>0.230</td>
</tr>
<tr>
<td>V</td>
<td>8.0</td>
<td>8.0</td>
<td>2.403</td>
<td>1.331</td>
</tr>
<tr>
<td>VI</td>
<td>8.0</td>
<td>8.0</td>
<td>3.634</td>
<td>2.933</td>
</tr>
</tbody>
</table>
esterification of inorganic phosphorus during climacteric stage and it was followed by a further increase in the rate during senescent stage.

In the second series of experiments which were conducted with the rind tissue, a gradual fall in the rate of esterification of inorganic phosphorus was observed from stage I to stage III. The decrease in rate of esterification was followed by an increase in the rate at stage IV. During stage V as in the entire fruit tissue an abrupt rise in the rate of esterification was observed and the rate approximately doubled itself during the next stage (Table I).

A comparison of the results of two series of experiments shows similar pattern in the rate of esterification in both, except that in whole fruit the rate of esterification was higher in stage II than in stage I while reverse situation appears in the rind. Again in whole fruit, the rate of esterification during stage III was followed by a fall in rate during stage IV, while in rind the rate of esterification in stage III was followed by an increase during stage IV.

B. Pigments

Changes in the amounts of total chlorophyll and carotenoid pigments during development of fruits were estimated. The effect of supply of gibberellic acid to the fruits at different developmental stages on the content of pigments one week after
treatment was also studied.

The fruits at various stages of development while attached to the plants were immersed in a solution of gibberellic acid (500 PPM) for 30 seconds and were allowed to grow on the plants for one week after treatment. Then the fruits were detached and the pigments were estimated. The variations observed in chlorophyll and carotenoid pigments in the control and gibberellic acid treated fruits were shown in Figure 4 and Figure 5 respectively.

The chlorophyll content in the control fruits showed a gradual decline as the fruits reached maturity on unit fresh weight basis and the chlorophyll had completely disappeared in senescent stage. In climacteric stage the fruit contained only traces of chlorophyll pigments. The application of gibberellic acid reduced the chlorophyll content when applied in the initial stages of development, but when gibberellic acid was supplied in later stages the chlorophyll content was more in the treated fruits. The chlorophyll content in gibberellic acid treated fruits also disappeared during senescent stage and practically no change was observed during climacteric between control and gibberellic acid treated fruits (Fig. 4).

Carotenoid content was very low both in the control and in gibberellic acid treated fruits during post-fertilization stage (stage I). Very little change in the carotenoid content was observed in the initial stages of development of the fruit.
Legends for Figures 4 and 5

Fig. 4. Chlorophyll content in control and GA-sprayed fruits at different developmental stages.

Control
GA-sprayed

Fig. 5. Carotenoid content of control and GA-sprayed fruits at different developmental stages.

------ Control
----- GA-sprayed
There was a pronounced rise in the carotenoid pigments during climacteric stage and this level was maintained during senescent stage also with a slight increase. The application of gibberellic acid resulted in a slightly increased carotenoid content in the initial stages but if supplied at later stages a marked inhibition in the synthesis of carotenoid pigments was observed (Fig. 5) when compared with the control fruits.

C. Photosynthesis

Experiments were conducted on \( ^{14} \text{O}_2 \) incorporation by pepper fruits at the six developmental stages. The results showing the variation of photosynthetic capacities of fruits and the amount of photosynthate contributed by fruit tissue to seed (in terms of radioactivity) at different developmental stages were shown in Table II. (Page 44).

The photosynthetic capacity of the fruit as measured by the rate of incorporation of \( ^{14} \text{O}_2 \) as also the amount of photosynthate translocated to the seed decreased with the advancement of age. Maximum carbon dioxide fixation was noticed during the first active growth period. During this period translocation to the seed also was greater than in other stages. No carbon dioxide fixation occurred during senescent stage.

Autoradiograms of chromatograms developed with the alcohol soluble fraction at the first five developmental stages were
TABLE II. $^{14}$C incorporation into alcohol soluble fraction of pepper fruit rind and the translocation into seed at different developmental stages.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Total activity introduced $10^5$ CPM</th>
<th>Amount incorporated into alcohol soluble fraction of rind $10^5$ CPM</th>
<th>Amount of activity translocated into seed $10^5$ CPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6.6</td>
<td>1.213</td>
<td>0.035</td>
</tr>
<tr>
<td>II</td>
<td>6.6</td>
<td>1.398</td>
<td>0.048</td>
</tr>
<tr>
<td>III</td>
<td>6.6</td>
<td>0.929</td>
<td>0.027</td>
</tr>
<tr>
<td>IV</td>
<td>6.6</td>
<td>0.700</td>
<td>0.019</td>
</tr>
<tr>
<td>V</td>
<td>6.6</td>
<td>0.263</td>
<td>--</td>
</tr>
</tbody>
</table>
shown in Figure 6. In order to compare the pattern of photosynthetic carbon fixation of fruit with that of leaf of same plant, leaf was also fed with labelled carbon dioxide and was analysed. The total fixation into alcohol soluble fraction in leaves was much greater than in fruits. Both in leaf and fruit the major activity was found to be incorporated into sucrose and the pattern of the formation of photosynthetic products was same in both the materials. The incorporation of activity into organic acids was low in the leaf when compared with the fruit with a corresponding high rate of incorporation into sugar fraction. Figure 7 shows the products of photosynthesis by leaf of pepper plant. The number of photosynthetic products formed decreased with the advancement of age in fruits.

The individual compounds were eluted from the chromatograms and the activities incorporated into them were measured.

When chromatograms developed from fruit extracts were sprayed with aniline phthalate, among the active compounds two discrete spots in addition to sugar phosphates reacted to the spray. These two aniline phthalate positive spots did correspond with sucrose and glucose based on Rf and color reactions. When the sheets were sprayed with aniline-diphenylamine reagent, the suspected sucrose spot gave brown color and the spot which was suspected as glucose was blue to the reagent. This afforded additional confirmation of the identity of sucrose and glucose. The confirmation of identity of citric acid was made by spraying
Legends for Figures 6 and 7

Fig. 6. Products of $^{14}O_2$ incorporation into pepper fruit after 1 hour photosynthesis at different developmental stages.

a. Stage I  
b. Stage II  
c. Stage III  
d. Stage IV  
e. Stage V

Fig. 7. Products of $^{14}O_2$ incorporation into pepper plant leaf after 1 hour photosynthesis.
the chromatogram with p-dimethylaminobenzaldehyde reagent which gave pink color. All the sugar phosphates gave blue on the chromatograms to ammonium molybdate reagent.

The photosynthetic products in each developmental stage of fruit and the activities incorporated into those are shown in Table III (Page 50).

In the initial stages the major activity was found to be present in sugar fraction (sucrose + glucose). It was greater in sucrose than in glucose. As fruit ages, the fixation into sugar fraction decreased with a corresponding increase into sugar diphosphates and sugar monophosphates particularly during stage IV. In climacteric stage of the fruit the activity in the sugar monophosphates and glucose was highest relative to other constituents.

D. Gibberellin-like substances

Gibberellin-like substances were extracted separately from seed and rind at the six stages of development and their biological activity was measured using two bioassay systems: (1) cucumber hypocotyl extension, (2) rice leaf sheath elongation.

The biological activities of the gibberellin-like substances in the seed and rind in respect of cucumber hypocotyl extension and rice leaf sheath elongation were shown in Figures 8 and 9 respectively. These represent the activities
TABLE III. Radioactivity incorporated into individual compounds by fruits at various developmental stages.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Component</th>
<th>Per cent activity incorporated into individual compounds out of total recovered in CPM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stage I</td>
</tr>
<tr>
<td>1.</td>
<td>Sugar diphosphates</td>
<td>2.73</td>
</tr>
<tr>
<td>2.</td>
<td>Sugar monophosphates</td>
<td>5.54</td>
</tr>
<tr>
<td>3.</td>
<td>PGA</td>
<td>1.59</td>
</tr>
<tr>
<td>4.</td>
<td>Sugars (Sucrose + Glucose)</td>
<td>64.51</td>
</tr>
<tr>
<td>5.</td>
<td>Cystine</td>
<td>1.54</td>
</tr>
<tr>
<td>6.</td>
<td>Aspartic acid</td>
<td>1.67</td>
</tr>
<tr>
<td>7.</td>
<td>Glutamic acid</td>
<td>2.59</td>
</tr>
<tr>
<td>8.</td>
<td>Glycine and serine</td>
<td>3.1</td>
</tr>
<tr>
<td>9.</td>
<td>Histidine</td>
<td>0.4</td>
</tr>
<tr>
<td>10.</td>
<td>Alanine</td>
<td>3.93</td>
</tr>
<tr>
<td>11.</td>
<td>Tyrosine</td>
<td>2.26</td>
</tr>
<tr>
<td>12.</td>
<td>Tryptophan</td>
<td>--</td>
</tr>
<tr>
<td>13.</td>
<td>Valine</td>
<td>--</td>
</tr>
<tr>
<td>14.</td>
<td>Phenylalanine</td>
<td>0.71</td>
</tr>
<tr>
<td>15.</td>
<td>Glutamine</td>
<td>1.78</td>
</tr>
<tr>
<td>16.</td>
<td>Citric acid</td>
<td>1.54</td>
</tr>
<tr>
<td>17.</td>
<td>Malic acid</td>
<td>2.19</td>
</tr>
<tr>
<td>18.</td>
<td>Glycolic acid</td>
<td>0.25</td>
</tr>
<tr>
<td>19.</td>
<td>Succinic acid</td>
<td>0.75</td>
</tr>
</tbody>
</table>
Fig. 8

Fig. 9
Legends for Figures 8 and 9.

Fig. 8. Amounts of gibberellin-like substances in seed and rind portions (per gram fresh weight) at different developmental stages: Cucumber hypocotyl bioassay.

Fig. 9. Amounts of gibberellin-like substances in seed and rind portions (per gram fresh weight) at different developmental stages: Rice leaf sheath bioassay.
of the whole extracts.

(1) Results based on cucumber hypocotyl bioassay

Using cucumber hypocotyl bioassay, a relatively high activity was observed in the seeds of post-fertilized fruits. This was followed by a gradual decline in the activity during the active growth period of fruit and there was a rise in the activity in stages IV and V. During senescent stage the seed gibberellin content was found to be at its lowest (Fig. 8).

As in seeds, a high concentration of gibberellin-like substances was present in the rind of post-fertilized fruits (stage I). In stages II and III, only an inhibition was observed of the extension of cucumber hypocotyls in the rind extracts. Then there was a slight growth promoting activity in the extracts of rind in prematuration and maturation stages. A marked inhibition was observed in the growth of the cucumber hypocotyl by the extract of rind at senescent stage (Fig. 8). Thus gibberellin content of rind was found to be highest in the post fertilized fruits, thereafter the rind had practically no gibberellin. On the other hand, the seed continued to possess gibberellins throughout the stages except in the senescent stage of the fruit.

(2) Results based on rice leaf sheath bioassay

The estimation of gibberellin-like substances using the rice leaf sheath bioassay showed same pattern in the concentrations
of those substances in the seed and rind extracts.

Both in seed and rind, a gradual decrease in activity was observed from post-fertilization stage up to the end of the active growth period of the fruit. There was then a gradual rise during prematuration and maturation periods of fruit followed by a decline in activity during senescent stage. The seed gibberellin content during post-fertilization, prematuration and maturation stages, however, was much greater than in the rind. The amount of seed gibberellins was also greater than the rind gibberellins even during stages II and III but the seed itself has relatively low content when compared to other stages (Fig. 9).

To study the variations in the amounts of individual (or groups of) gibberellin-like substances, chromatograms were developed of the extracts of seed and rind at each developmental stage. The chromatogram sheets were cut into four equal segments from the origin to the solvent front. The growth promoting substances present in individual segments were designated as factors 1, 2, 3 and 4 with Rf values 0.00 to 0.25, 0.25 to 0.50, 0.50 to 0.75 and 0.75 to 1.00 respectively. The segments were separately eluted and the biological activity of the eluates was measured with the two bioassay systems as were used for the whole extracts.
(a) Results based on cucumber hypocotyl bioassay

The relative amounts of factors 1, 2, 3 and 4 in seed and rind as measured with the cucumber hypocotyl bioassay were represented in figures 10a, 10b, 10c and 10d respectively.

Factor 1 was in highest concentration in seeds during post-fertilization period (stage I) followed by a gradual decrease more or less till the end of stage III, thereafter rising again. In senescent stage a small decrease in amount of factor 1 over the previous stage was observed.

But factor 1 of rind showed a slightly different pattern. There was a gradual decrease in amounts in first two stages. The eluates of segments in stages III and IV corresponding to factor 1 in Rf however had an inhibitory effect on the growth of the cucumber hypocotyl. During stages V and VI small amounts of factor 1 were found to be present. The eluates of segments corresponding to factor 1 in seed showed no inhibition at any stage, but in rind the eluate in the zone corresponding to factor 1 in stages III and IV showed inhibition. In all the stages the amounts of factor 1 in seed were greater than the same in rind (Fig. 10a).

The amount of factor 2 in cucumber hypocotyl bioassay was greater in the seed during stage II than stage I followed by a decrease during stage III. The amount of factor 2 again increased gradually during stages IV and V in seed. The content
STAGES OF DEVELOPMENT

FIG. 10.a

STAGES OF DEVELOPMENT

FIG. 10.b
STAGES OF DEVELOPMENT

FIG. 10, c

STAGES OF DEVELOPMENT

FIG. 10, d
Legends for Figures 10a, 10b, 10c, 10d.

Figs. 10a - 10d. Amounts of factors 1 (0.0 to 0.25), 2 (0.25 to 0.50) 3 (0.50 to 0.75) and 4 (0.75 to 1.00) in seed and rind portions at different developmental stages per gram fresh weight (cucumber hypocotyl bioassay).

10 a  Factor 1
10 b  Factor 2
10 c  Factor 3
10 d  Factor 4
of factor 2 in seed decreased during senescent stage of the fruit.

But the amount of factor 2 in rind showed a gradual decline from stage I to stage III. There was an increasing concentration of factor 2 during stage IV and stage V. A considerable fall in the concentration of factor 2 in rind during stage VI when compared to stage V was observed.

The amount of factor 2 in seed was very markedly higher than that of rind in stages I, II and V. In all the stages the content of factor 2 was greater in the seed than in the rind. Stage VI was an exception where there was a small increase in the amount of factor 2 in rind than in seed (Fig. 10b).

Factor 3 of seed was found to be in a high concentration in stage I followed by a more or less gradual decrease in amount upto the end of stage III. Then there was an increase in the amount of factor 3 during stages IV and V followed by a decrease in concentration in senescent stage (Stage VI).

Factor 3 of rind showed a slightly different pattern. A small increase in amount of factor 3 was observed in stage II than stage I. Then a fall in the concentration of factor 3 was observed during stage III and the amount in stage IV was almost equal to what was noticed in stage III. A rise in the content of factor 3 during stage V was followed by a decrease in stage VI was noticed.
Except in stages II and VI where greater amounts of factor 3 were observed in the rind than in seed, factor 3 was lower in all other stages in the rind when compared to seed (Fig. 10c).

Factor 4 in seed showed a gradual decline in concentration in first two stages followed by a small rise during stage III. Eluates of the zone corresponding to factor 4 in seed showed only an inhibitory effect in the later stages of development (i.e., in stages IV, V and VI). In the rind, eluates of the zone corresponding to factor 4 showed only an inhibition in all the stages. The maximum inhibition was observed during climacteric stage (Stage V; Fig. 10d).

(b) Results based on rice leaf sheath bioassay

The relative concentrations of factors 1, 2, 3 and 4 in seed and rind at different developmental stages, as measured with rice leaf sheath bioassay were shown in Figures 11a, 11b, 11c and 11d respectively.

As in cucumber hypocotyl bioassay the amount of factor 1 in seed was very high during post-fertilization stage in this bioassay system also. The high concentration of factor 1 in stage I was followed by a decrease during stages II and III and the concentration rose again during stages IV and V. In stage VI, a decrease in the amount of factor 1 was observed (Fig. 11a)

Similarly in the rind there was a decrease in concentration of factor 1 from stage I to stage III. The amount of factor 1
STAGES OF DEVELOPMENT

Fig. 11a

STAGES OF DEVELOPMENT

Fig. 11b
Legends for Figures 11a, 11b, 11c, 11d.

Figs. 11a - 11d. Amounts of factors 1 (0.0 to 0.25), 2 (0.25 to 0.50), 3 (0.50 to 0.75) and 4 (0.75 to 1.00) in seed and rind portions at different developmental stages per gram fresh weight (rice leaf sheath bioassay).

11 a. Factor 1
11 b. Factor 2
11 c. Factor 3
11 d. Factor 4
in rind during stage III was almost negligible. The eluate in
the zone corresponding to factor 1 during stage IV in the rind
inhibited the elongation of leaf sheath. But in later stages
(stages V and VI) there was increase followed a decrease in the
amount of factor 1.

The amount of factor 1 in seed as measured with rice leaf
sheath bioassay technique showed was greater in all the stages
than the same in rind.

High concentration of factor 2 was observed in seed during
stage I. There was again a decrease in the amount of factor 2
upto stage III. It had risen during stages IV and V followed
by a decrease in senescent (VI) stage as usual.

In the rind concentration of factor 2 showed a decrease
during first two developmental stages. A rise in amount of
factor 2 followed by a decrease was observed during stages III
and IV respectively. The concentration of factor 2 in rind
again increased in stage V with a decrease in the next.

Except in stages III and V, the amount of factor 2 in all
the other stages was higher in the seed than in the rind. In
stage III, factor 2 was in higher concentration in the rind
than in the seed and in stage V the amounts were almost equal
in seed and the rind (Fig. 11b). There was an abrupt rise in
the amount of factor 3 in seeds during post-fertilization
stage. A sudden decrease in concentration of factor 3 was
observed during stages II and III. The amount of factor 3
again increased during stages IV and V followed by a decrease during stage VI.

In the rind a small increase in content of factor 3 was observed in stage II over stage I followed by a marked decrease during next stage. Again a gradual rise in the concentration of factor 3 in rind was observed during stages IV and V followed by a decrease during stage VI.

Except in stage II, where the amount of factor 3 in seed and rind were almost equal, the content of factor 3 in seed was consistently greater than in rind (Fig. 11c).

As usual a decrease in the amount of factor 4 of seed was observed during the first two developmental stages. In the later stages the eluates of zone corresponding to this factor had an inhibitory effect in the growth of the rice leaf sheath, whereas in rind, in all the stages the eluate of zone corresponding to factor 4 showed only inhibition of the elongation of the rice leaf sheath (Fig. 11d).

**Effect of GA on the endogenous gibberellin content**

The fruits while attached to the plants were sprayed with 200 PPM gibberellic acid and one week after treatment the gibberellin-like substances were extracted separately from seed and rind at the various developmental stages and their biological activity was measured with the two bioassay systems already described. Histograms showing the relative amounts of
gibberellin-like substances in seed and rind of fruits sprayed with gibberellic acid were represented in figures 12 and 13 using cucumber hypocotyl and rice sheath bioassay systems respectively.

Using cucumber hypocotyl bioassay, a high gibberellin content was observed in the seeds of post-fertilized fruits; gibberellin content then decreased in the next stage with a subsequent rise in the seeds during stage III. A marked decrease in the concentration of gibberellin content was observed in the seeds during stage IV followed by a three fold increase during stage V. The final senescent stage was marked by a fall in the concentration of gibberellin content in seeds.

Gibberellin content in the rind showed a continuous fall from stage I to stage III. A rise in gibberellin content was observed during stages IV and V followed by a fall during stage VI. In the first two developmental stages of fruits gibberellin-like substances in rind were higher in content than in seed while in the remaining four stages seed gibberellin was higher (Fig. 12).

When the biological activity of the gibberellin-like substances of seed and rind of fruits supplied with exogenous GA was measured with rice leaf-sheath bioassay, two peaks of activity in seeds during stage I and stage III were observed with a low level during stage II. The activity again decreased during stage IV followed by a threefold increase during stage V,
Legends for Figures 12 and 13.

Fig. 12. Amounts of gibberellin-like substances in seed and rind portions of GA-sprayed fruits per gram fresh weight. (cucumber hypocotyl bioassay)

Fig. 13. Amounts of gibberellin-like substances in seed and rind portions of GA-sprayed fruits per gram fresh weight (rice leaf sheath bioassay).
followed by a decrease during stage VI.

Gibberellin-like substances in rind showed a gradual decline in content up to stage IV followed by a small rise during stage V. Stage VI was marked by a decrease in the amount.

In all the stages except II seed gibberellins were higher in amounts than the rind gibberellins. In the post-fertilization stage (Stage I) the gibberellin content was very high when compared with other stages and the content both in seed and rind were almost equal. The application of gibberellic acid increased the content of gibberellin-like substances in seed during stages III and V and reduced the activity during stage IV (Fig. 13).

The gibberellin-like substances of seed and rind portions of the control fruits and fruits sprayed with GA as measured by cucumber hypocotyl bioassay are shown in figures 14 and 15 respectively.

The application of GA increased the seed gibberellin content in all the stages except IV. The increase was very marked in stages I, III, V and VI. There was a small decrease in activity of gibberellin-like substances in seeds of GA sprayed fruits when compared with the control fruits during ripening (Fig. 14).
Legends for Figures 14 and 15.

Fig. 14. Gibberellins in seed portions of control and GA-sprayed fruits per gram fresh weight. (cucumber hypocotyl bioassay)

Fig. 15. Gibberellins in rind portions of control and GA-sprayed fruits per gram fresh weight. (cucumber hypocotyl bioassay)
The application of GA also resulted in an increase in the rind gibberellin content in all the stages but increase was very high during stages I, II and V. No inhibition of hypocotyl extension was observed in any stage in the rind of GA sprayed fruits unlike in the rind of control fruits (Fig. 15).

The amounts of gibberellin-like substances in seed and rind of control and GA sprayed fruits measured with rice leaf-sheath bioassay are shown in Figures 16 and 17 respectively.

As in cucumber hypocotyl bioassay, in all the stages GA increased the content of endogenous gibberellin-like substances. A marked decrease was observed in the seeds of control fruits when compared with the seeds of fruits that received an exogenous supply of gibberellic acid during III and V stages. Unlike with the cucumber hypocotyl bioassay during stage IV the seeds of GA sprayed fruits showed higher content than the seeds of control fruits when measured with rice leaf-sheath bioassay (Fig. 16).

Gibberellin content of rind also had increased at all the developmental stages when the activity was measured with rice leaf-sheath bioassay. A marked increase was observed in stages I, II and V of rind gibberellin content in GA sprayed fruits over the controls.

**E. Nucleic acids:** Nucleic acids were estimated and expressed as the amount of nucleic acid phosphorus per gram
Legends for Figures 16 and 17.

Fig. 16. Gibberellins in seed portions of control and GA-sprayed fruits per gram fresh weight. (rice leaf sheath bioassay)

Fig. 17. Gibberellins in rind portions of control and GA-sprayed fruits per gram fresh weight. (rice leaf sheath bioassay)
(fresh weight) of material.

A batch of fruits were sprayed with 200 PPM gibberellic acid and after one week, nucleic acid content was estimated separately in the seed and rind portions of fruits thus treated and in the controls which have not received GA.

The variations in the amount of nucleic acid phosphorus of the seed and rind portions of control and GA sprayed fruits are shown in Table IV (Page 76). The nucleic acid content was high in the seeds during post-fertilization stage in the controls. With the advancement of age there was a decline in the nucleic acid content upto stage III. The content increased during the next stage followed by a further marked increase during maturation stage. The seed nucleic acid content had fallen during senescent stage.

As in the seed, in rind portion the nucleic acid content was high in the post-fertilization stage. Unlike the seed, here a gradual decline in the amount of nucleic acids was observed till stage IV followed by a small rise during climacteric and senescent stages.

The application of gibberellic acid to the fruits resulted in an increase in nucleic acid content in stages I, III and IV of seeds, whereas it has resulted in a loss of nucleic acid content in stages II, V and VI. But contrary to the above, GA application resulted in a decrease in nucleic acid concentration in the first two developmental stages of the fruit in the
TABLE IV. Total nucleic acid phosphorus in seed and rind portions of pepper fruits at different developmental stages and the effect of the application of gibberellic acid on the nucleic acid content in seed and rind.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Seed</th>
<th>Rind</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control ug.P/g fresh weight</td>
<td>GA-sprayed ug.P/g fresh weight</td>
</tr>
<tr>
<td>I</td>
<td>72.00</td>
<td>107.80</td>
</tr>
<tr>
<td>II</td>
<td>40.00</td>
<td>33.00</td>
</tr>
<tr>
<td>III</td>
<td>39.00</td>
<td>47.58</td>
</tr>
<tr>
<td>IV</td>
<td>44.00</td>
<td>68.35</td>
</tr>
<tr>
<td>V</td>
<td>70.00</td>
<td>42.10</td>
</tr>
<tr>
<td>VI</td>
<td>37.00</td>
<td>27.00</td>
</tr>
</tbody>
</table>
rind portion and a marked stimulation during the remaining four stages.

The nucleic acid content of the seed of GA sprayed fruits was higher in all the stages except in stages II and VI than of the rind portion. The reverse was the case in stages II and VI.

F. Pectins

Pectins were extracted from fruit rind at various developmental stages and the amount of pectins present was expressed in terms of the amount of anhydrouronic acids present. The amounts of anhydrouronic acids at the six developmental stages are shown in Figure 18.

An increase in the amount of pectin was observed from post-fertilization period to stage III. A decline in pectin content was noticed during pre-maturation stage of the fruit followed by a four fold increase in concentration during maturation stage. Pectin content decreased to a half during senescent stage (Fig. 18).

G. Cell-wall polysaccharides

Qualitative identification of cell-wall polysaccharides was done in the rind portion and the amount of uronic acid present at each stage was estimated quantitatively.

The compounds were identified by elution from the unsprayed
Legends for Figure 18.

Fig. 18. Total pectins in fruit rind at different developmental stages.
sheets followed by a cochromatography in different solvent systems with the authentic samples.

The presence of uronic acids on the chromatogram was confirmed by the P-anisidine-HCl spray to which the uronic acids gave a cherry red color. In 1 per cent ammonium oxalate fraction, arabinose, galactose and galacturonic acid were identified and they were confirmed by spraying the sheets with aniline diphenylamine reagent which gave greyish brown, blue and green colors respectively after heating.

The monosaccharide composition of individual cell-wall fractions and their identification are shown in Table V (Page 81).

In all the stages same compounds were present in individual fractions: Arabinose, galactose and galacturonic acid were present in pectin fraction. Hemicellulose fraction was composed of arabinose and galactose units and only glucose was present in the cellulose components of walls.

Quantitative determination of uronic acids was done colorimetrically after elution from the developed unsprayed chromatograms. The amount of uronic acids in cell walls of fruits at different developmental stages is shown in Figure 19.

A continuous decline in uronic acid concentration was observed in the cell-walls of fruits from post-fertilization
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fraction</th>
<th>Compound present</th>
<th>Rxylose n-but:acetic acid:water 2:1:1</th>
<th>Color reaction to aniline diphenylamine reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pectin fraction</td>
<td>arabinose</td>
<td>0.976</td>
<td>greyish brown blue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>galactose</td>
<td>0.721</td>
<td>blue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>galacturonic acid</td>
<td>0.283</td>
<td>green</td>
</tr>
<tr>
<td></td>
<td></td>
<td>glucericonic acid</td>
<td>0.537</td>
<td>brown</td>
</tr>
<tr>
<td>2</td>
<td>Hemicellulose fraction</td>
<td>arabinose</td>
<td>0.976</td>
<td>greyish brown blue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>galactose</td>
<td>0.721</td>
<td>blue</td>
</tr>
<tr>
<td>3</td>
<td>Cellulose fraction</td>
<td>glucose</td>
<td>0.754</td>
<td>blue</td>
</tr>
</tbody>
</table>
Optical density per unit dry weight of material.

Stages of development.

Fig. 19
Legend for Figure 19.

Fig. 19. Amount of uronic acid in cell walls of fruit rind at different developmental stages.
stage to the second active growth period (stage III) followed by a gradual rise during pre-maturation and maturation stages. A decrease in the content of uronic acid was observed during senescent stage (Fig. 19).