THE OCCURRENCE IN PEARS OF METABOLIC
GASES OTHER THAN CARBON DIOXIDE

by

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THE OCCURRENCE IN PEARS OF METABOLIC GASES OTHER THAN CARBON DIOXIDE

INTRODUCTION

Investigations on the handling of pears have indicated that factors other than temperature are operative in accelerating the ripening rate. In ripening rooms of canneries, for example, it has been observed that Bartlett pears stored in considerable quantities will soften sooner than when a few boxes of the same material are stored alone. Invariably, also, the fruit in the center of the boxes, or in the center of piles is the first to show indications of attaining an edible condition. Actual cases have been reported where the ripening rate of green pears was markedly accelerated when stored with ripe fruit. Since the presence of the latter apparently has been the only variable in these cases the supposition is made that ripe pears evolve a volatile substance that has a stimulative influence upon the ripening process.

The occurrence of a gas having such marked influence suggests several potentialities. First, there is the possibility that such a substance might be employed to advantage in the ripening of pears for canning purposes or
in the ripening of pears for sale at eastern terminals. Second, there is the possibility that such a substance might be detrimental to the long keeping of pears in storage and that methods for its control or elimination might be necessary.

Scope of investigations. At the inception of this investigation, very little information could be found relative to the evolution or presence in pears of volatile substances other than carbon dioxide and acetaldehyde. The present study, therefore, was undertaken to determine whether there are other volatile products of metabolism in pears that may influence ripening or affect keeping quality. Work was conducted along two major lines as follows:

I. Does there occur in pears metabolic gases other than carbon dioxide and if so, what is the nature of these gases?

II. If such gases do occur what influence do they exert upon the ripening and keeping quality of pears?

HISTORICAL REVIEW

The use of volatile substances artificially applied for the ripening of fruits is apparently not of recent origin. Over three thousand years ago, the ancient
Chinese burned incense in the storage rooms to hasten the ripening of their small, hard, sand pears (15). To remove the astringency from persimmons, the Japanese for many years have followed the practice of placing the fruit in barrels in which rice beer had been fermented (34). For ripening of dates, the Arabs covered the green fruit with sacks, previously soaked in vinegar. Whether or not similar practices have been used in early times for other fruits is not revealed in the literature.

Of more recent origin are the developments leading to the use of ethylene gas for the coloring and ripening of citrus fruits. Previous to the work of Sievers and True (30), the practice in the lemon-curing process for a number of years had been to supply the heat required with oil stoves. Since this practice produced disagreeable smoke and odors, some plants installed steam heating systems, but much to the operators' surprise the lemons failed to color as previously, although the same temperatures and humidities were maintained. When the steam heated plants were abandoned and the old fashioned stoves were again used, the fruit was found to color satisfactorily. A similar difference in the rate of coloring and ripening was found with the innovation of a new type of burner that
produced heat with less smoke and odor.

The chief difference between the two types of stoves was that with the old type, the poor admission of air caused the stoves to burn with incomplete combustion, a condition favorable for the production of unsaturated hydrocarbon gases. As a result of these and similar experiences, the impression soon gained ground that there must be some important relation between the coloring of the fruit and the nature of the atmosphere produced by the stoves. Accordingly, the United States Department of Agriculture detailed Sievers and True to investigate the problem and these investigators found that the pungent, gaseous combustion products given off by the oil stoves brought about the coloring formerly attributed only to the effects of temperature and humidity, (30). Later, Denny, (7) by a process of elimination, found the effective constituents to be unsaturated hydrocarbon gases, chiefly ethylene.

Since the introduction of the ethylene treatment for hastening the coloration and ripening of citrus fruits, numerous investigations have been made regarding the effect of this gas upon the ripening of other fruits, and probably no other subject under investigation in horticulture has
resulted in so many diverse results and contradictory views.

Following Denny's work with lemons in 1924, Rosa (29) found that with tomatoes there was a gain in time of coloring from five to eight days, depending on the variety. Chemical analysis showed that the artificially ripened fruit was poorer in sugar than that ripened on the vine. Harvey (13), however, treated green-mature tomatoes with ethylene and found them sweeter than those untreated. Hibbard (16) and Work (35) also secured positive responses from the use of ethylene on tomatoes.

Similarly, ethylene has been found to stimulate the ripening process in bananas by Harvey (14), Hibbard (16), and others, while Wolfe (34), except in a single case, failed to secure comparable results. Davis and Church (6), working with an astringent and nonastringent variety of Japanese persimmons, found that ethylene stimulated softening, accelerated color development and respiratory activity in both varieties. Chase (4) has reported a loss of astringency in this fruit from treatment but found no change in chemical composition or rate of respiration. Neither could he detect any such change in oranges, lemons, bananas, tomatoes, pomegranates, dates, or avocados; Harvey (15) attributes these contradictory results to the
low concentration of ethylene used.

While it has been demonstrated in many cases that the addition of ethylene in low concentrations to the atmosphere surrounding the fruit will stimulate ripening, the idea that fruit of itself evolves some volatile substance similar to ethylene in effect has until recently never been given serious consideration. As early as 1910, Jamaican investigators (18), from their observations on the ripening of bananas during transit and storage, suggested that some substance was given off by the ripe fruit that hastened ripening in the green fruit. As far as the writer is aware this suggestion was never followed, although Olney (26), 16 years later, states that in the shipment of bananas the observation has been made that some cargoes arrive at the ports too ripe for general distribution, while other shipments apparently handled in the same manner arrived in good condition. From this there has developed a general belief that a few ripe or ripening bananas in a lot of green ones greatly accelerates the ripening of the entire cargo. Kidd and West, (20) in this connection found that bananas placed in a sealed container with a carbon dioxide absorbent, ripened sooner than those held under ventilation where the gases were not allowed to accumulate.
Recently, Elmer (8) has reported that the volatile gases from ripe apples inhibit normal sprout development of germinating potatoes. Peeled and unpeeled Winesap, Jonathan, and Ben Davis apples have caused inhibition, but none resulted from the volatile substances of oranges, bananas, diseased apples, immature fruits, or iso-amyl valerate.

Kidd and West (21), following Elmer’s work, subjected sprouting seeds of pea and mustard to the vapors of a ripening apple and found a growth inhibition very similar to that obtained by the use of ethylene. These gaseous products were also found to increase the respiration rate and decrease the time of ripening of apples. Similar results were obtained from the volatile gases of bananas and tomatoes. Smith and Kane (31), working with the above investigators found that the effective substance was not removed by 20 per cent sodium hydroxide; saturated alkaline or acid potassium permanganate; 3 per cent iodine in potassium iodide; saturated sodium meta bisulfite; saturated acid cuprous chloride; shredded paraffin; 10 per cent warm olive oil; 10 per cent ammoniacal silver oxide; or 1 per cent palladium chloride. Complete combustion over copper oxide removed the active constituent and the yield of carbon dioxide was equal to a concentration of one volume in 28,000 volumes of air. Bromine, ozone, and fuming nitric
and sulfuric acids also removed the active substance. Subsequent work by Kidd and West (21) has substantiated these findings, and recently Gane (9), by passing the gases from Worcester Pearmain apples through pure bromine, has succeeded in obtaining a derivative having a boiling point very close to that of ethylene bromide apparently proving that ethylene gas is actually evolved by apples.

GENERAL METHODS AND MATERIALS

Samples. The pears used in this investigation were Anjou and Winter Melis varieties obtained from the Southern Oregon Experiment Station. The former were picked August 27, 1934 and the latter September 27, the dates corresponding with the commercial harvest period, of these varieties, in the Rogue River Valley. Immediately after picking, the fruit was washed, packed in oil wraps, and stored at 31 degrees Fahrenheit. At intervals throughout the season, lots were removed from storage and treated as indicated in the following experiments.

Ripening containers. The containers, used in the biological tests and as ripening chambers, were 5-gallon glass pickle jars fitted with air-tight lids containing inlet and outlet tubes. A false bottom of one-quarter inch galvanized mesh wire to support the fruit was made to fit inside each jar about six inches from the bottom. This arrangement permitted the use of a potash solution underneath the fruit in case it was desired to absorb the carbon
dioxide evolved.

**Use of Biological Indicators.** The seeds used as biological indicators were the garden pea, mustard, and radish. In making a test the general procedure followed was to soak the seeds in cold water overnight, then place a few of each kind in a petri dish over moistened filter paper. The petri dish with the seeds was then placed in the containers as indicated later. The jars were tightly sealed and left in this condition until germination had proceeded far enough for results to be evident.

The tomato plants used were grown in the greenhouse and transferred to 3-inch pots several days prior to use. Young, vigorous plants about eight to ten inches in height were used. As with the seeds, a plant was simply enclosed in a jar with the fruit or ethylene as designated in individual experiments.

In making these tests in a closed container, every precaution was taken to prevent contamination by foreign gases. The jars, prior to use, were filled with water to displace any gases present. After being emptied they were placed near an open window. As a further precaution, after the fruit and seeds or tomato plant had been enclosed and the jar sealed, air in the jars was exhausted and replaced by fresh air drawn from outdoors.
Ripening tests. The general procedure followed in making the ripening tests was to first thoroughly air the jars to displace any gases present. Five hundred milliliters of 90 per cent KOH were then placed in the bottom of the jar and the false bottom installed. Ten or twelve specimens of green fruit, together with the same number of ripe fruit, were placed in the jars. The check lots were identical in treatment, except that no ripe fruit was included. The lids were screwed tight and tested for leaks against a 5-inch vacuum. The jars were then placed in the ripening room where a constant temperature of 66 degrees Fahrenheit was maintained. Thermograph records show that this temperature seldom varied over one to two degrees. Each morning and evening the pressure within the jars was adjusted to atmospheric conditions by momentarily opening the pinch-cock on the inlet tube. Invariably there would be an intake of air, indicating that no leaks were present, that the CO₂ was being absorbed and that oxygen was being used up. In the case of the check lots, the air was drawn in from outside the ripening room to prevent entry of any gases from ripe fruit that might have been present in the room. At the end of a five-day period, the jars were opened and one-half of the ripening fruit tested with a U. S. pressure tester. The remainder was removed from the jars and placed in the ripening room.
Measuring degree of ripening. Differences in degree of ripeness between treated and untreated lots were measured by the U. S. type pressure tester on pared specimens unless otherwise stated. Since the variations in pressure tests of individual fruits within a lot averaged 0.5 pound, any difference between two lots less than 0.5 pounds was not considered a significant difference.

EXPERIMENTAL

I. Does there occur in pears metabolic gases other than carbon dioxide and acetaldehyde, and if so, what is the nature of these gases?

At the inception of this investigation, the writer was aware of no specific qualitative chemical method for the detection of minute quantities of ethylene, propylene, or similar gases that may occur in pears. Fortunately, however, these gases, especially ethylene, have peculiar physiological effects upon the growth of certain plants that are manifest even in very low concentrations. Crocker (5) and others have noted that mere traces of ethylene in the atmosphere cause epinastic curvature in tomato leaves and retard normal germination development in certain kinds of seeds, particularly pea and mustard. So far as known, ethylene, acetylene, propylene, butylene, and carbon monoxide are the only gases that produce these
effects, (36). Accordingly, biological indicators were used in the preliminary experiments.

BIOLOGICAL EVIDENCE

Effect on germination of seeds. To determine the effect of the presence of pears on the germination of garden pea, mustard, and radish seeds, and to compare this effect with that obtained from treatment with apple vapors and ethylene, an experiment was set up as follows:

Jar A — seeds alone
Jar B — seeds with 10 ripe Anjou pears
Jar C — seeds with ethylene gas 1:1000
Jar D — seeds with ten ripe Delicious apples

Germination was allowed to proceed for a period of six days before examination was made. From this experiment it is evident that the presence of pears produced effects on germinating seeds similar to those produced by the presence of apples and of ethylene gas.

In jar A, normal germination had taken place in all three kinds of seeds. The peas had formed hypocotyls averaging about an inch in length on which secondary roots were forming. The epicotyls were elongating and the primary leaves unfolding. The mustard and radish seeds had developed long fibrous roots, and the epicotyls averaged about three-fourth inch in length. In jars B, C, and D,
the seeds all showed abnormal development. The growth of the epicotyls of the peas was markedly suppressed. The most striking feature was the short, swollen, and stumpy appearance of the epicotyl. The radish and mustard seeds were mostly germinated, but root growth was not as great as that of the check plants, and the epicotyls were strikingly curled in a complete spiral. The seeds germinated over apples and pears were similar in every respect to those germinated in the presence of ethylene. Figure 1 shows a photograph of the pea seeds after six days treatment.

**Effect on tomato plants.** To compare the effect of the presence of pears with that of ethylene gas on the growth of tomato plants, an experiment was set up as follows:

- **Jar 1** — tomato plant alone
- **Jar 2** — tomato plant with ethylene gas 1:1000
- **Jar 3** — tomato plant with 10 ripe Anjou pears.

From the results of this experiment it was again evident that the presence of pears produced effects similar to those produced by ethylene gas. The plant enclosed with peas, however, developed decided symptoms of injury that were identical with those shown by the plant treated with ethylene.

During a period of 24 hours a complete spinastic
effect had occurred and the petioles had grown completely
downward from a horizontal position (figure 2). Micro-
scopic sections made through the area showing the curva-
ture at the base of the petioles, showed that cell growth
and elongation on the dorsal side had been stimulated, so
that the greater growth in this area had forced an epine-
astic curvature. This phenomenon has been reported by
other investigators (5, 36) for tomato plants treated with
ethylene.

Detection of gases in pears held in storage. To det-

To determine if the gas given off by pears ripened at high temp-
eratures was produced also by fruit held in cold storage,

To determine if the gas given off by pears ripened at high temp-
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thirty Anjou pears with wraps removed were placed in a jar,
which was sealed and left in the cold room. A tomato
plant was placed in a second jar and observed for 24 hours
to make sure that no contaminating gases were present.
The air was partially exhausted from this jar, and after-
wards connected by a glass tube to the jar containing the
pears. Thus, on opening the pinch-cock the gases from
the pears were drawn into the atmosphere surrounding the
tomato plant. After closing the inlet tube, the jar was
removed from the cold room.

As in the case of the fruit ripened at 65° F., the
pears in the cold room caused an effect similar to that
produced by ethylene gas. After six hours the leaves
began to grow downward, and in 24 hours marked curvature
of the petioles was apparent, although not as pronounced as with exposure to fruit held at high temperatures. This experiment was repeated with Anjou pears at intervals throughout the storage period, and once with wrapped Winter Nelis pears. A positive test was obtained in all cases.

The occurrence of cases in pears that are past the stage when they will ripen. When pears are held in cold storage beyond their normal storage life, they will not ripen upon removal to ripening conditions. To determine whether pears in this state affect tomato plants in a manner similar to that of normal pears, a lot of 10 Bose pears which, by early January, had reached the condition described, were enclosed with a tomato plant.

Unlike the previous tests the fruit used in this experiment failed to produce deleterious effects upon the tomato plant. Similar experiments carried on with Bose as late as April 15 gave like results. Apparently, the gas responsible for the effects on tomato plants already described is evolved only so long as the fruit remains "alive" and continues to carry on normal respiration activities. When, however, respiration practically ceases as was the case with the pears used in this experiment, evolution of the gas also declines or ceases.

Effect on tomato plants of volatile substances that are known to occur in pears. In order to be sure that
no volatile substances which are known to occur in pears
were producing the above effect, the responses of tomato
plants to carbon dioxide and acetaldehyde were determined.
The effect of iso-amyl acetate was ascertained also, since
this ester is thought to occur in pears.

To determine the effect of these substances on tomato
plants, an experiment was set up as follows:

Jar A --- CO₂ (50 per cent)
Jar B --- acetaldehyde (1 ml. per liter of space)
Jar C --- iso-amyl acetate (1 ml. per liter of space)

The results demonstrate that these substances have
no perceptible effect upon tomato plants. After thirty-
six hours exposure, the tomato plants were apparently
normal in every respect, showing no indications of epi-
nasty as was evident with pears and ethylene gas.

Chemical Evidence

The results obtained by biological tests indicate
that metabolic gases other than carbon dioxide and acetaldehyde occur in pears. As far as known, ethylene, acet-
ylene, propylene, carbon monoxide and butylene are the
only gases that produce the peculiar injury observed in
germinating seeds and tomato plants. Zimmerman, Hitchcock,
and Crocker (36) tested thirty-nine gases on tomato
plants, including many constituents of illuminating gas,
alcohols, aldehydes, anaesthetics, etc., and found that
only the five enumerated above induce leaf epinasty. Since
these are the only gases known to produce this effect, and
since the presence of pears caused an effect identical to
that of these gases, the indications are that one or more
of these effective gases occur in pears.

The investigators mentioned have determined that the
minimum concentrations of each gas necessary to induce
epinasty in tomato leaves is as listed:

- Ethylene — 1 part in 10,000,000
- Acetylene — 1 part in 20,000
- Propylene — 1 part in 20,000
- Carbon Monoxide — 1 part in 2,900
- Butylene — 1 part in 200

From this data it is apparent that the gas or gases
in pears producing the epinastic effect observed with to-
mato plants, must be present in at least the minimum con-
centrations shown above. In case they are present in
amounts less than these minima, it is reasonable to assume
that they did not produce the effects noted. Thus, for
example, if the amount of carbon monoxide present was
determined and found to be 1 part in 40,000, then this
gas could not have been the cause of the effect observed,
for to be effective, a minimum concentration of 1 part in
2,000 is necessary. The following experiments were con-
ducted, therefore, to determine which of the five gases
occur in pears in amounts sufficient to induce the effects
noted.

Quantitative determination. The writer is aware of
only two methods that might be applicable to the determination of small amounts of these five gases: the *combustion method*, and the *iodine pentoxide method* (23). Since the equipment necessary for combustion analyses was not available, the latter method was used.

The iodine pentoxide method depends upon the reduction of iodine pentoxide with the liberation of iodine, which can be detected by chloroform or determined by absorption in potassium iodide solution and titrated with standard sodium thiosulfate. This method is a standard procedure for the determination of small amounts of carbon monoxide in the atmosphere. Although its reaction with the unsaturated hydrocarbon gases is known (17, 19, 23), this method has not been used for their quantitative determination. According to Kinnicutt and Sanford (23), carbon monoxide can be determined in quantities as low as 1 part in 40,000. Since ethylene or the other unsaturated gases would have a reducing value towards iodine pentoxide greater than carbon monoxide, the method should be adaptable to much smaller amounts of these gases. Preliminary trials showed that ethylene in at least 1 part in 50,000 could be readily detected. Moreover, the gases drawn from 30 pounds of pears gave a color reaction with chloroform, indicating that the gases present could be determined quantitatively. This color test was also given after passing the gases thru saturated sodium bi-sulfite, demonstrating that the test
shown was not due to acetaldehyde present. Accordingly, the experiment was carried out as follows.

Thirty-two kilograms (70.5 pounds) of unripened Anjou pears were removed from cold storage and placed in an air tight container, fitted with inlet and outlet tubes. To prevent any contaminating gases entering the jar, the air was drawn from out-of-doors and was first passed through concentrated sulfuric acid and also through 20 per cent KOH solution to maintain the proper degree of humidity in the chamber.

Before the actual determinations were made, a jar enclosing a tomato plant was connected to the container and the air drawn through at the rate of 9 liters per hour to be certain that the concentration of gases, drawn from the pears at this rate would be sufficiently great to cause epinasty in tomato leaves. After 6 hours, such an effect was apparent. Accordingly, determinations were made to ascertain which gas or gases were present in sufficient amounts to induce this effect.

The procedure followed in making the determinations was the same as that reported by Kinnicutt and Sanford (23). The iodine pentoxide used was previously heated at 150° C, for 5 days to remove any free iodine present and was then tested with chloroform in the absorption bottle of the apparatus to make certain that no free iodine was left in the material. The U-tube containing the iodine
pentoxide was connected through two calcium chloride tubes to the chamber containing the pears. The air was drawn through at the rate of 9 liters per hour as measured by a flow-meter. Each determination was conducted for 2 hour's time, and after each run, the line to the chamber containing the pears was disconnected and a blank determination made for the same length of time.

The liberated iodine absorbed in the potassium iodide solution was titrated with .005 N sodium thio-sulfate, and the titration values determined calculated to equivalent milliliter volumes of carbon monoxide, ethylene, acetylene, propylene and butylene. The carbon monoxide equivalent was calculated from the equation

$$5CO \cdot I_{205} = 5CO_2 \cdot I_2$$

and for ethylene from the equation

$$5C_2H_4 \cdot 6I_{205} = 10CO_2 \cdot 10H_2O \cdot 6I_2$$

The equivalent volume for acetylene was calculated similarly as for ethylene, and for propylene and butylene on the basis of oxidation of only the double bond. The calculated volume of each gas equivalent to one milliliter of .005 N sodium thiosulfate is as follows:

- Carbon monoxide --- .28 ml.
- Ethylene --------- .047 ml.
- Acetylene --------- .047 "
- Propylene --------- .062 "
- Butylene --------- .066 "

The results of six determinations made over a period of 6 days are shown in Table I. The concentrations are
expressed in parts by volume per volume of air.

Table I

Concentration of Gases in Pears Reducing Iodine Pentoxide.

<table>
<thead>
<tr>
<th>Date</th>
<th>Ml. .005 N sod. thio. required</th>
<th>Parts by volume of air as (thousands omitted)</th>
<th>CO</th>
<th>C₂H₄</th>
<th>C₂H₂</th>
<th>C₃H₆</th>
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<tr>
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According to these calculations, ethylene is the only gas that could have been present in concentrations great enough to have caused the epinastic effect observed in the biological tests. The concentrations of the other gases as calculated are shown to be present in amounts far too low to have caused any effect. This would indicate that ethylene gas is produced by pears and is the specific substance responsible for producing the effects observed when tomato plants were enclosed with pears. This does
not preclude, however, the possibility of other gases being present.

**Further evidence on the occurrence of Ethylene.** To obtain further evidence on the occurrence of ethylene in pears, the solubility of the evolved gases in mercuric nitrate solution was determined. The reaction of the ethylene with mercury salts is well known through the work of Hoffman and Sands (24). According to the investigations, an ethanol mercury compound is formed in which the ethylene group is held in combination in some manner analogous to the way CO is combined with cuprous chloride. The structure of the compound is represented thus: \( \text{C}_2\text{H}_4\text{Hg} \). By addition of HCl the ethylene is liberated in gaseous form according to the following reactions:

\[
\begin{align*}
(1) & \quad \text{C}_2\text{H}_4\text{Hg} \cdot \text{HCl} = \text{C}_2\text{H}_4\text{HgCl}_2 \cdot \text{H}_2\text{O} \\
(2) & \quad \text{C}_2\text{H}_4\text{HgCl}_2 \cdot 2\text{HCl} = \text{HgCl}_2 \cdot 2\text{HCl} \cdot \text{C}_2\text{H}_4
\end{align*}
\]

The reaction of ethylene with certain of the mercury salts is apparently specific. Curme (24) has described a method for separating ethylene in pure condition from gas mixtures by use of mercuric sulfate, and Treadwell and Hall (32) recommend mercuric nitrate solution as a specific absorbent for ethylene. If the gases evolved by pears, then, were passed through a mercuric nitrate solution, and epinasty resulted when a tomato plant was subjected to the gases liberated by addition of HCl to the solution, then, strong evidence would be offered that the gas evolved by
pears is ethylene. To ascertain if such a result could be obtained, the following experiment was undertaken.

A tomato plant was placed in a jar which was sealed tight and observed for 24 hours. Since no curvature of the leaves occurred in this time, it was considered that the jar was free of contaminating gases. The gases from 30 pounds of ripe Anjou pears were drawn through 50 ml. of 20 per cent mercuric nitrate solution prepared according to the directions of Treadwell and Hall, and contained in a Truog absorption tower. The air in the jar containing the tomato plant was then partially exhausted and the jar connected to the bottle to which the mercuric nitrate solution had been transferred. To this solution was added 50 ml. of dilute HCl through a dropping funnel and the pinch-cock to the jar containing the tomato plant was opened. The partial vacuum in this jar would thus draw any gases liberated by the addition of the acid into the atmosphere surrounding the tomato plant. The pinch-cock on the inlet tube was then closed.

After 6 hours exposure to the gas absorbed and liberated in the above manner, the tomato plant developed the full symptoms of epinasty. To obtain a check on these results, the experiment was duplicated, and again similar effects were noted. A blank test was also run to make sure that epinasty was not caused by substances released by the solution. In this case, the tomato plant remained
normal in all respects.

Further tests apparently preclude the possibility of any gas but ethylene causing the effects observed on germinating seeds and tomato plants. A test for acetylene using Illosov's reagent which, according to Pietsch and Kotowski (27), will detect $3.7 \times 10^{-4}$ per cent, failed to reveal any traces of acetylene in gases evolved from as much as 60 pounds of pears. Passing the gases from these same pears through 87 per cent sulfuric acid to remove propylene and butylene and into a jar containing a tomato plant, failed to prevent the development of the epinastic condition.

Discussion

It is fully realized that these experiments do not prove beyond doubt that the substance occurring in pears is ethylene gas. That this is the case, however, is strongly indicated. Biological tests have shown that some gas evolved by pears produced abnormal conditions in germinating seeds and epinasty in tomato leaves. In view of the chemical evidence obtained, it appears that this gas must be ethylene, since the only other gases known to produce these effects are carbon monoxide, acetylene, propylene, and butylene, which on the basis of these experiments cannot be present in pears in sufficiently large amounts to have been the cause. Moreover, the non-
occurrence of acetylene as shown by a specific test, the elimination of propylene and butylene by specific absorption, and the extremely high concentrations of carbon monoxide necessary to produce epinasty, indicate that only ethylene gas can be present to produce the effects observed. Further, very strong evidence that the gas is ethylene is that the substance evolved by pears and absorbed by mercuric nitrate solution, a specific absorbent for ethylene, produced epinasty in tomato leaves after being released with hydrochloric acid. Then, also, the positive evidence for the occurrence of ethylene in apples (9) suggests that this gas might also be present in pears, since the metabolic processes in the two fruits are similar in many respects.
Part II - Does the Gas Produced by Pears Have any Effect Upon Ripening Rate?

Since ethylene gas is known to be a ripening agent for many fruits, it is natural to suppose that if ethylene or a similar gas is given off by pears, the ripening rate of pears in general would be affected. Ripe pears evolving this gas, for example, might be expected to start the ripening of unripe pears if the two were enclosed in the same chamber. Particularly is this true, since in the case of ethylene, the amount that seems to be present in pears is sufficient to affect the ripening of some fruits (7, 23). To determine what effect the gases found to occur in pears have upon ripening, the following experiments were carried out.

Effect of Carbon Dioxide upon Ripening Rate. Since the procedure followed in making ripening tests was to enclose the fruit in air-tight containers, it is evident that the carbon dioxide evolved by the fruit would build up to considerable quantities during the period of treatment unless provisions were made for its absorption. Undoubtedly, this accumulation would have an inhibiting effect upon ripening, which would tend to counteract any accelerating effect of ethylene or a gas similar in effect evolved by pears. That carbon dioxide does have an inhibiting effect upon ripening was indicated in a preliminary
experiment (Table III) where provisions for its removal were apparently not adequate. To determine if such an effect is actually produced by this gas, the following experiment was conducted.

Ten green Anjou pears were placed in each of two jars. In jar A was placed 500 ml. of 20 per cent KOH solution. In jar B no carbon dioxide absorbent was included. To maintain a comparable humidity in jar B, a beaker containing 300 ml. of 15 per cent sulfuric acid was included. The results are shown in Table II.

<table>
<thead>
<tr>
<th>Lot</th>
<th>Pressure Test After 5 days Treatment</th>
<th>Days to Ripen After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.1</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>6.6</td>
<td>5</td>
</tr>
</tbody>
</table>

It is very evident that the natural accumulation of carbon dioxide in the containers has a marked inhibiting effect upon the rate of ripening. At the end of five days the fruit in container A had almost reached an edible condition, while that in jar B was hard-ripe and still had the characteristic astringency of green pears. This marked effect of carbon dioxide in retarding the ripening processes causes one to wonder just how many cases of negative results that have been reported for ethylene treatment can
be explained on the basis of failure to prevent accumulation of this gas in the ripening containers. As one specific example, Wolfe, (34) experimenting with the effects of ethylene treatment upon bananas, allowed the concentration of carbon dioxide evolved during ripening to build up to as high as 10 per cent in some cases, which may partially account for his failure to secure positive results from ethylene treatment.

Effect of the Presence of Pears at Different Stages of Maturity. An experiment was planned to determine whether or not the presence of ripe pears accelerated the ripening rate, and if so, at what stage of ripeness the fruit has the greatest accelerating influence. Accordingly, 10 Bosc pears were removed from cold storage every four days during a period of 16 days and placed in the ripening room. Thus, when the experiment was set up on the sixteenth day there were 4 lots of fruit; one over-ripe, one prime-ripe, one semi-ripe, and one green. The over-ripe lot, which had been ripening for 16 days, was beginning to show scald and breakdown. The semi-ripe lot eight days out of storage was beginning to soften but had not reached prime eating condition. A check lot was included, containing only green pears to be ripened with no others. Each of the other lots was placed together with 10 green Anjou pears just removed from storage. In this experiment 200 ml. of 40 per cent KOH solution were used in each jar instead of 500 ml. of 20
The data indicate that Bosc pears at various stages of ripeness have no effect upon increasing the ripening rate of green pears. In fact, lot E in which no ripe fruit was included, showed lower tests than B, C, or D where ripe fruit was present.

In all subsequent trials, the presence of ripe fruit failed to influence the ripening of green fruit. A summary of the results obtained are shown in Table IV.
Table IV

Summary of Experiments to Determine the Effect of Ripe Pears on Rate of Ripening

<table>
<thead>
<tr>
<th>Date of experiment</th>
<th>Pressure Test</th>
<th>Days to Ripen</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 5 days</td>
<td>Including Period of Treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>with ripe pears</td>
<td>check</td>
<td>with ripe pears</td>
</tr>
<tr>
<td>Dec. 16</td>
<td>6.1</td>
<td>5.3</td>
<td>9</td>
</tr>
<tr>
<td>Jan. 14</td>
<td>3.2</td>
<td>3.4</td>
<td>8</td>
</tr>
<tr>
<td>Jan. 20</td>
<td>4.0</td>
<td>3.5</td>
<td>7</td>
</tr>
<tr>
<td>Mar. 4</td>
<td>5.0</td>
<td>5.2</td>
<td>7</td>
</tr>
<tr>
<td>Mar. 5#</td>
<td>8.0</td>
<td>8.5</td>
<td>13</td>
</tr>
<tr>
<td>Mar. 20#</td>
<td>6.3</td>
<td>6.4</td>
<td>11</td>
</tr>
</tbody>
</table>

*Winter Nelis*

Effect of gases evolved by green pears upon rate of ripening. Since it has been shown that green pears held in cold storage evolve ethylene or a similar gas, there is the possibility that where such fruit is removed to high temperatures and enclosed in a container, the concentration of gas built up would be great enough to cause an acceleration of the ripening rate without the additional amount that would be produced by the presence of ripe fruit. To determine if the accumulation of gas in the jars containing only green fruit is great enough to accelerate ripening, the following experiment was carried out.
Ten green Anjou pears were placed in each of two jars. Jar A was sealed after the addition of the carbon dioxide absorbent. Jar B was placed in the same room, but instead of sealing the jar, an air stream was drawn through at a rate sufficiently rapid to prevent any accumulation of gases in the container. The results are shown in Table V.

Table V.
Effect of Gases Evolved by Green Pears
Upon Rate of Ripening

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pressure Test at end of 5 days</th>
<th>Days to Ripen including period of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. No ventilation</td>
<td>3.5</td>
<td>7</td>
</tr>
<tr>
<td>B. Rapid ventilation</td>
<td>3.4</td>
<td>7</td>
</tr>
</tbody>
</table>

The results show that the fruit in the tightly closed jar where the gases were allowed to accumulate, did not ripen at a faster rate than did the fruit in the jar wherein the gases were not allowed to accumulate. It seems, then, that the gases evolved by the green fruit had no effect upon the ripening of this fruit.

**Effect of the addition of ethylene to the containers upon the rate of ripening.** Since preliminary experiments have shown that the presence of ripe pears have had no apparent effect in increasing the ripening rate, there is the possibility that pears do not respond to as low concen-
Concentrations of ethylene or similar gases as do some other fruits. To determine if this is the case, an experiment was set up in which varying concentrations of ethylene were used in the ripening jars. The treatment given and the results obtained are contained in Table VI.

Table VI.
Effect of Ethylene on Ripening of Anjou Pears

<table>
<thead>
<tr>
<th>Lot no.</th>
<th>Concentration</th>
<th>Pressure test at end of 5 days treatment</th>
<th>Days to ripen including period of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>1:500</td>
<td>3.6</td>
<td>7</td>
</tr>
<tr>
<td>B.</td>
<td>1:1000</td>
<td>3.7</td>
<td>7</td>
</tr>
<tr>
<td>C.</td>
<td>1:5000</td>
<td>3.8</td>
<td>7</td>
</tr>
<tr>
<td>D.</td>
<td>no ethylene</td>
<td>4.2</td>
<td>7</td>
</tr>
<tr>
<td>E.</td>
<td>10 ripe pears</td>
<td>3.8</td>
<td>7</td>
</tr>
</tbody>
</table>

The results indicate very clearly that the failure to obtain increase in rate of ripening with the presence of ripe pears is not due to an insufficient concentration of the gas in the containers. Even in lot A with a concentration of 1:500 there was no significant increase in rate of ripening over Lots D or E which had not been subjected to ethylene treatment.
DISCUSSION

The results obtained show that during the period from December to April, the presence of ripe fruit has no significant effect upon the ripening rate of Anjou or Winter Nelis pears, even though ethylene or a gas similar in effect has been shown to occur in these varieties during storage and ripening. No data were obtained on fruit ripened immediately following picking or during the early storage period, since the experiment was started after the fruit had been in storage several months. Analysis of the data of other workers (22) indicate that during this comparatively short time, the fruit probably passed through a climacteric period, auto-induced by ethylene naturally occurring, and after which normal ripening proceeds without stimulus.

The term "climacteric" has been used to designate critical changes occurring at certain periods in the life of higher plants and animals. Blackman and Parija (2) were apparently among the first to note the occurrence of critical periods in plants. They observed this phenomenon while studying the respiration of cherry-laurel leaves. Gustafson has noted an increase in metabolic rate at senescence in leaves and also in tomatoes (10). Kidd and West (21) have used the term climacteric in referring to a certain stage preceding ripening in apples and bananas. Ranjan & Khan (28) have noted a similar phenomena in
paidium guava.

The term as used here designates a critical period which apparently occurs naturally in pears during late maturation and is a transition stage preceding senescence. After the changes occurring in this period have taken place, actual ripening processes are able to proceed; but the indications are that until these changes have occurred ripening will not occur or at least is markedly delayed.

The evidence suggesting the occurrence of a climactic in pears is based upon the following facts and observations:

1. The marked response of newly-picked fruit to ethylene treatment as contrasted to total lack of response to treatment after storage.

2. The significant difference in time to ripen between newly-picked fruit and fruit ripened after storage.

3. The similarity between the increase in respiration rates resulting from ethylene treatment and that occurring naturally during late maturation.

The marked response of fruit to ethylene treatment immediately after picking as contrasted to total lack of response to similar treatment after storage has been shown by Allen (1). A rearrangement of a portion of his data is contained in Table VII. A study of the table shows that newly picked Anjou pears treated after harvest ripened
in 7 to 16 days sooner than those untreated, but after being held in storage for a short length of time, pears from the same lot showed no increase in the rate of ripening over untreated lots.

Table VII.

<table>
<thead>
<tr>
<th>Date Picked</th>
<th>After Harvest</th>
<th>After 10 days at 50°F</th>
<th>After 12 Wks. at 32°F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated</td>
<td>Check</td>
<td>Treated</td>
</tr>
<tr>
<td>Anjou:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/15</td>
<td>10</td>
<td>26</td>
<td>6</td>
</tr>
<tr>
<td>8/15</td>
<td>10</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>Winter Nelis:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/30</td>
<td>12</td>
<td>*</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12#</td>
</tr>
</tbody>
</table>

*Failed to ripen after 30 days.
# 15 weeks at 32°F.

Allen's results with Winter Nelis are even more striking. Untreated lots of newly picked fruit failed to ripen in 30 days, while treated lots ripened in twelve. But after storage, ethylene treatment had no effect upon ripening rate, untreated lots ripening in the same length of time as the treated.

That ethylene treatment is effective in hastening ripening only during a period shortly following picking is shown also by investigations on other fruits. Work
applied ethylene treatment to tomatoes at various dates from blooming and found that the greatest response was at the age of 30 to 40 days from blossom. Hibbard (16) also obtained similar results with tomatoes. Davis and Church (6) working with Fuyu and Hachiya persimmons found that the magnitude of response to ethylene treatment decreased from early to late picking, until a point was reached where there was no response. Wolfe (35) working with mature bananas failed to note an increase in ripening rate from ethylene treatment, while others using less mature fruit, observed marked increases in the rate of ripening.

The significant difference between time to ripen for newly-picked fruit and fruit ripened after storage is shown very clearly in the data of Hartman, et al. (11). A rearrangement of a portion of their data is compiled in Table VIII.
Table VIII.

Relation of Time of Picking and Storage Upon the Length of Ripening Period

Days to Ripen After Picking

<table>
<thead>
<tr>
<th>Anjou</th>
<th>Winter Nelis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Date</strong></td>
<td><strong>Days</strong></td>
</tr>
<tr>
<td><strong>Picked</strong></td>
<td><strong>To Ripen</strong></td>
</tr>
<tr>
<td>8/11</td>
<td>22</td>
</tr>
<tr>
<td>8/16</td>
<td>19</td>
</tr>
<tr>
<td>8/21</td>
<td>17</td>
</tr>
<tr>
<td>8/26</td>
<td>17</td>
</tr>
<tr>
<td>8/31</td>
<td>18</td>
</tr>
<tr>
<td>9/5</td>
<td>19</td>
</tr>
<tr>
<td>9/10</td>
<td>17</td>
</tr>
<tr>
<td>9/16</td>
<td>18</td>
</tr>
<tr>
<td>9/21</td>
<td>14</td>
</tr>
</tbody>
</table>

Days to Ripen After Storage

<table>
<thead>
<tr>
<th>Anjou</th>
<th>Winter Nelis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Date</strong></td>
<td><strong>Length of Storage Period</strong></td>
</tr>
<tr>
<td><strong>Stored</strong></td>
<td><strong>90</strong></td>
</tr>
<tr>
<td>8/16</td>
<td>8</td>
</tr>
<tr>
<td>8/21</td>
<td>8</td>
</tr>
<tr>
<td>8/26</td>
<td>9</td>
</tr>
</tbody>
</table>
A study of the table clearly indicates that during a comparatively short period of time during late maturation on the tree and in early storage, some change in the fruit has occurred which markedly decreased the time to ripen. Thus, in the case of the Anjou, for example, there was a gradual decrease from 22 days to 14 days in time to ripen in fruit picked at successive intervals throughout the harvest period. This would indicate that during this period some substance necessary for ripening was gradually being formed in the fruit, or possibly that some condition preventing ripening was being gradually overcome. Whatever change took place was evidently completed during the early storage period, because there was no further decrease in time to ripen even after 210 days storage.

Apparently, therefore, the "ripening" of pears takes place in two distinct periods:

1. A pre-ripening or climacteric period of short duration during which certain changes take place that enable the fruit to complete the second phase.

2. A post-climacteric or senescent period during which the fruit softens and becomes edible.

Reference to Table VII shows that ethylene treatment was effective only during a short interval following picking. This is a period corresponding to the climacteric. Thus ethylene must be effective only in hasten-
ing the processes occurring during the climacteric and does not have any effect on actual ripening processes, such as were observed in the present investigation.

The marked similarity between the rapid rise in respiration rate resulting from ethylene treatment and the similar rise in respiration rate shown naturally by many fruits during late maturation, suggests strongly that the contributing factors and the fundamental processes taking place are similar. In those cases where ethylene treatment has been effective in increasing the ripening rate, there is always a sharp increase in the respiration rate, which reaches a peak, declines to a certain level and then remains more or less constant. A graph from Hartshorn (12) to illustrate this phenomena in bananas is shown in figure 3. A significant observation of the effects of ethylene treatment is that once this peak in respiratory rate has been reached, ethylene has no further effect either upon ripening or respiration.

A study of the data on the respiration of many kinds of fruits has shown that during the period of late maturation there is invariably a rapid and consistent increase in rate of respiration, differing only from that resulting from ethylene treatment in being more gradual and extending over a longer period. For example, from the data of Burroughs (3) on the respiration rates of apples picked
GRAPH SHOWING CLIMACTERIC RISE IN BANANAS DUE TO ETHYLENE TREATMENT
at various dates during late maturation, it was found that if these initial rates were plotted against successive picking dates, a curve was obtained in every case which corresponded to the respiration curves resulting from ethylene treatment. Two representative curves as plotted are shown in Figure 4. The dotted lines drawn to connect initial rates of respiration during the harvest season show this rise. Bartlett pears (25), persimmons (6), guaras (28), and tomatoes (10), show similar curves. All of these fruits are known to respond to ethylene treatment. Further study of the respiration curves of fruit after picking and in storage show that as with ethylene treatment, once the peak in respiratory rate has been passed, never again in the life of the fruit, even upon removal to high temperature or with ethylene treatments does the respiration rate reach the high level attained at the peak of the climacteric. In other words, the indications are that, whether resulting from ethylene treatment or occurring naturally, the climacteric is the period of greatest metabolic activity in the maturation of the fruit. Thus, it is apparent that the application of stimuli to hasten metabolic processes can be effective only when applied prior to the climacteric, for, obviously, any post-climacteric treatment could not have any effect on the attainment of a condition that had already been reached.
GRAPH SHOWING CLIMACTERIC RISE IN APPLES DURING MATURATION
From the three lines of evidence presented there is strong indication that the failure to obtain positive results either with ethylene or by enclosing ripe pears with green pears, was due to the fact that the fruit used in the experiments had passed the stage when responses can be obtained. Moreover many of the negative results from ethylene treatment reported by other workers can probably be explained on the same basis. In future work, therefore, it would seem highly essential that tests be started immediately after picking and even that fruit be used which has not as yet reached the commercial picking stage.

PRACTICAL SIGNIFICANCE

The results of these investigations indicate that two of the gases produced during the metabolism of pears may be of vital importance: one in hastening certain changes preceding the ripening period, and the other in retarding the ripening. Apparently, therefore, the pear is a dynamic organism producing certain gases that have the effect of accelerating or retarding its own life processes. Potentialities are, therefore, evident in utilization of this phenomenon for practical purposes.

First, the marked inhibiting effect of carbon dioxide on the ripening processes suggests the possibilities of using this gas as a modified pre-cooling method. Further,
it might be employed advantageously in place of cold temperatures for retarding life processes in fruit held for long storage, thus eliminating possible ill effects resulting from small changes in temperatures. In addition carbon dioxide might be used for retarding ripening processes in fruits that are subjected to conditions favorable for ripening during transit, such as delays at market terminals, at ocean docks, and other places where a few hours delay at elevated temperatures may markedly decrease the life of the fruit.

Second, the occurrence of ethylene or a gas similar in effect in pears suggests the utilization of this gas as a ripening agent. Especially might this be of value in commercial canning operations, where rapid and uniform ripening of pears is desirable. The indications are that this end might be accomplished with canning pears to be ripened soon after picking by storing the fruit in considerable quantities in ripening rooms without ventilation and with provisions for removal of carbon dioxide.

Further, a possible means for extending the life of the fruit is suggested. It has been pointed out that possibly with pears, the ripening processes will not take place until certain changes have occurred in the climacteric. If a means for delaying these changes can be found, it is entirely possible that the life of the fruit can be
markedly extended.

Finally, it is suggested that in future investigations full cognizance be taken not only of the probable occurrence of a climacteric, but also of its possible bearing upon the problems connected with harvesting, storage, and ripening of pears.

SUMMARY—

(1) The presence of pears in closed containers has induced epinasty in tomato plants and has retarded the germination of seeds. These conditions occurred at room temperature and in cold storage, but failed to occur in the case of pears that had been held beyond their normal life in storage.

(2) So far as known, the type of injury to tomato plants and germinating seeds observed in these experiments is produced only by ethylene, acetylene, propylene, carbon monoxide and butylene.

(3) Chemical evidence indicates that ethylene is the gas responsible for the effects noted.

(4) Ripening tests failed to show that ethylene or similar gases given off by pears have any influence upon ripening. Failure to obtain positive results, however, may be attributed to the fact that the fruit used in these tests had passed the stage when response could be expected.
LITERATURE CITED


