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RESPIRATION OF FRUITS AND GROWING PLANT
TISSUES IN CERTAIN GASES, WITH REFERENCE
TO VENTILATION AND FRUIT STORAGE

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GEORGE RICHARD HILL, JR.

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RESPIRATION OF FRUITS AND GROWING PLANT TISSUES IN CERTAIN GASES, WITH REFERENCE TO VENTILATION AND FRUIT STORAGE*

GEORGE R. HILL, JR.

Two or three days before ripening, peaches are very hard. At this time, if handled quickly and carefully they may be shipped long distances without great injury. They owe their hardness to a cellulose-like carbohydrate known as pectose. The cells of the flesh of the peach are relatively very large and the cell walls are thin, but the large quantity of pectose in the cells gives them considerable rigidity. As the peach ripens the pectose is hydrolyzed to pectin, which is a jelly-like gum soluble in the cell sap. This hydrolysis is rapid, and in hot weather a hard peach may become in two or three days so soft that the thin, unsupported cell walls are unable to sustain even the weight of the peach and a flat indentation or bruise is formed on the underside of the fruit.

Mature hard peaches are designated as "market-ripe." When such peaches are placed in cold-storage the rate of softening is greatly decreased; peaches are often kept by this means for two or three weeks, and in some cases longer. At ordinary temperature, about 80° F., a day is often sufficient for them to become as soft as they would in a week in cold-storage at 35° F.

Even though the rate of hydrolysis of pectose can be much decreased by refrigeration, peaches of the best quality cannot be put on the market in distant localities for the following reasons: first, because the temperature in an iced refrigerator car is usually above 10° C. (50° F.), at which point the softening of the peaches is still somewhat rapid; second, because of so-called "ice-sca'd"; and third, because, in order to be placed on the market in hard condition, the peaches must be picked so green that they have not acquired that richness of flavor found only in peaches ripened on the tree.

If some means of treatment could be found which would arrest this hydrolysis of pectose without otherwise affecting the peach, it would be possible to let the fruit remain on the tree until ripe enough to be really desirable, and still get it to market without serious damage. The demand for peaches would then be greatly increased and the season for them could be extended over a period long enough to avoid glutting the market.

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In September, 1909, the writer was present at an auction sale of a carload of Elberta peaches in Chicago. The fruit had left Palisade, Colorado, in excellent condition. Each peach had been wrapped separately in paper and carefully packed so as to avoid any bruising. The car had been loaded in the customary manner, with an air space around each box in order to insure ventilation. The peaches had come direct to Chicago except for a short stop at Omaha for inspection. When they were taken out of the car at Chicago they were apparently in good condition, although they had softened considerably. They were yellower than when shipped and they brought a good price. In the afternoon of the same day the writer saw some of the peaches on a fruit stand on South Water Street. The fruit appeared slightly darker than in the morning. Some of the peaches were bought, and it was found that the flesh just under the skin was brown in spots. There was no sign of any bruising. The original paper wrappers were on most of the peaches in the box and these peaches showed the browning as much as did those that had been unwrapped. It was plainly not a matter of bruising. The flavor of the peaches was not bad; they were merely insipid and inclined to be mushy, and had the appearance of having been half-cooked. The writer was informed by the dealer that this was "ice-scald" and that it was not uncommon, the brown spots appearing a few hours after the peaches had become warmed to the temperature of the street.

Since the peaches had probably never reached a temperature below 7° C. (45° F.) injury from cold was inconceivable, and the question as to what was the cause of this "ice-scald" was a rather inviting one. Peaches that had been kept in cold-storage at a temperature 5° C. lower than that in the car for a longer period of time were found not to have been so affected.

Injury to peaches shipped in refrigerator cars is common. Not infrequently entire carloads are ruined. The injury is most frequent in the central part of the top tiers of boxes in the car, and it decreases toward the bottom of the car. Since cold air from the ice bunkers is led along the bottom of the car and cools the car by diffusion and circulation upward, the better condition of the peaches in the bottom of the car has been attributed to the lower temperature there. That small differences in temperature between the top and the bottom of the car are sufficient to cause marked differences in the keeping quality of peaches is to be expected, since it is well known that evolution of carbon dioxid and other metabolic phenomena are usually doubled, and not infrequently trebled, in rate by a rise in temperature of 10° C. Oxygen absorption is intensified by a rise in temperature, to about the same degree as is evolution of carbon dioxid. The refrigerator cars are usually kept closed tight and

there is little circulation of air in them. Under such a condition, with a rapid use of oxygen and evolution of carbon dioxid, would not a dearth of the former and an accumulation of the latter probably result? What would be the effect on the peaches of a dearth of oxygen and an accumulation of carbon dioxid? Might the injury to the peaches be due in part to these factors, as well as to the intensified rate of metabolism at the higher temperatures?

From these observations the writer was led to a study of the behavior of fruits and seeds under aerobic and anaerobic conditions. Is the hydrolysis of pectose associated in any way with the intake of oxygen or the evolution of carbon dioxid? Might it be inhibited or retarded by surrounding the fruit by an inert gas such as hydrogen or nitrogen? Relatively, how strong is the anaerobic respiration of fruit as compared with the aerobic? Since carbon dioxid is one of the products of respiration, what would be the effect of its accumulation on the absorption of oxygen and on the metabolic changes mentioned above? What is "ice-scald," and is it connected in any way with aerobic or anaerobic respiration? Is the relation of oxygen to the respiration and metabolism of ripe fruits the same as to that of growing plant tissues? How important is ventilation in the storage and handling of fruit? An attempt is herein made to answer some of the above questions.

SURVEY OF LITERATURE

The following is a brief review of some of the literature of the important phases of anaerobic respiration, together with some current theories offered in explanation of it and of its relation to aerobic respiration.

The continued evolution of carbon dioxid in the absence of oxygen was first observed by Rollo (1798), who at that time was studying the formation of sugar from "mucilages végétaux" in barley grains. In attempting to determine the relation to oxygen of this formation of sugar, he accidentally discovered that the barley grains gave off a considerable quantity of carbon dioxid for several days in the absence of oxygen. De Saussure (1804) noted that *Lythrum* and other green plants gave off carbon dioxid in an atmosphere of nitrogen. Since that time the relation of oxygen to the living organism has been the subject of considerable study, for the reason that this element seems to be the one most indispensably connected with life. Fruits have been used extensively in this study. Several investigators early called attention to the formation of alcohol in fruits that were kept in a chamber containing no oxygen, but these fruits were not kept free from contamination by yeasts; hence, the production of alcohol by the tissues of the fruit was not established.

(1798) Rollo, —. Expériences et observations sur le sucre. Ann. d. chim. 25: 37-50.

(1804) De Saussure, Th. Des plantes qui peuvent végéter dans le gaz azote. Recherches chimiques sur la végétation, 197-208.

Bérard (1821) noted that green fruits ripened when kept in air. When oxygen was withheld, the fruits were unable to ripen; the ripening process was resumed, however, when the fruits were again placed in the air, if they had not been kept too long in the oxygen-free atmosphere.

Cahours (1864) studied oranges kept in air and in nitrogen and noted that carbon dioxid was evolved in both atmospheres.

Lechartier and Bellamy (1869) found that apples kept in an oxygen-free chamber evolved carbon dioxid for about eight months and that alcohol also was formed almost equaling the carbon dioxid in amount. Pasteur (1872) called attention to the fact that the ratio of this production of alcohol and of carbon dioxid was the same as that in alcoholic fermentation. Lechartier and Bellamy (1872) immediately repeated their work and succeeded in obtaining alcohol from the center of some apples, to which place it was impossible for yeast to have gone. This is probably the first undisputed proof of the formation of alcohol by higher plant tissues. It is the first indication in literature of the close relationship between anaerobic respiration and alcoholic fermentation.

Pfeffer (1878) proposed the name "intramolecular respiration" for that respiration occurring in the absence of oxygen, because the energy and the carbon dioxid come from the destruction of the molecule from which the carbon dioxid arises. He held that aerobic and intramolecular respiration were genetically connected, and that the existence of the intramolecular respiration was the reason for the aerobic. This view was strengthened when Pasteur found alcohol in the inner cells of an apple that had been kept in air. It has been shown later, however, that this connection is not so close as Pfeffer believed.

Buchner (1897) gave great impetus to the study of the nature of anaerobic respiration when he showed that the yeast organism contains an enzyme, zymase, which is capable of transforming glucose into alcohol and carbon dioxid.

Godlewski and Polzeniusz (1901) attempted to determine whether various seeds contained zymase. They found that in the absence of oxygen, carbon dioxid and alcohol are formed in rather large quantities and in nearly the proportion in which they are formed in alcoholic fermentation. This proportion is expressed in the following equation:

(1821) Bérard, —. Du mémoire sur la maturation des fruits. *Ann. chim. phys.* 16: 225-251.

(1864) Cahours, A. Sur la respiration des fruits. *Compt. rend.* 58: 653-656.

(1869) Lechartier, G., et Bellamy, F. De la fermentation des fruits. *Compt. rend.* 69: 466-469.

(1872) Pasteur, L. Note sur la production de l'alcool par les fruits. *Compt. rend.* 75: 1054-1056.

(1872) Lechartier, G., et Bellamy, F. De la fermentation des fruits. *Compt. rend.* 75: 1203-1206.

(1878) Pfeffer, W. Das Wesen und die Bedeutung der Athmung in der Pflanze. *Landw. Jahrb.* 7: 805-834.

(1897) Buchner, E. Alkoholische Gährung, ohne Hefezellen. *Ber. d. deut. chem. Gesell.* 30¹: 117-124.

(1901) Godlewski, E., und Polzeniusz, F. Über die intramoleculare Athmung von in Wasser gebrachten Samen und über die dabei stattfindende Alkoholbildung. *Bul. Acad. Sci. Cracovie* (1901): 227-276.

Since two molecules of each substance are formed, the theoretical ratio in which they may be formed is the ratio of the molecular weight of the carbon dioxid to the molecular weight of the alcohol. This is:

$$\frac{\text{C}_2\text{H}_5\text{OH}}{\text{CO}_2} = \frac{46}{44} = \frac{104.5}{100}$$

This ratio means that for each 100 parts by weight of carbon dioxid produced, theoretically 104.5 parts by weight of alcohol will be produced. These authors found that when soaked peas are placed in glucose or cane sugar solutions, some of the sugar disappears and the increase in the amount of carbon dioxid and alcohol produced corresponds very closely to the amount of sugar lost. This indicates clearly that pea seeds are capable of producing alcoholic fermentation. The authors were unable to isolate zymase from the seeds, but their work gave great impetus to other investigators.

Stoklasa and Czerny (1903), by pulping sugar beets, potatoes, and the like, and by subjecting the pulped tissue to a pressure of 300 atmospheres, succeeded in getting an enzyme possessing the properties of Bucher's zymase. These authors reported also that they had isolated zymase from peas and from the muscle, heart, lungs, liver, and kidney of beef, and since that time Stoklasa and his students have isolated zymase from a wide range of plant and animal tissues.

Nabokich (1903) held that there are two kinds of intramolecular respiration; that one is a true alcoholic fermentation of glucose; and that the other is a fermentation of glucose with the additional use of organic acids, such as lactic acid, and various other organic compounds, resulting in a large excess of carbon dioxid over alcohol. This points to the opinion that alcoholic fermentation is only a part of anaerobic respiration.

Stoklasa, Ernest, and Chocenský (1906) found that the $\frac{\text{C}_2\text{H}_5\text{OH}}{\text{CO}_2}$ ratio for sugar beets, potatoes, apples, and bean and vetch seeds under anaerobic conditions varies not further than 10 to 20 from the theoretical 104.5. From sugar beets these investigators isolated noticeable quantities of lactic acid. They concluded that in most cases anaerobic respiration is an enzymatic process identical with alcoholic fermentation. They found also that in the case of sugar beets the intensity of anaerobic respiration for the temperatures 1° to 3° C., 18° to 20° C., and 30° to 32° C. varies between .358 and .6 of the intensity of normal respiration.

(1903) Stoklasa, J., und Czerny, F. Isolierung des die anaërobe Athmung der Zelle der höher organisierten Pflanzen, und Tiere bewirkende Enzyms. Ber. d. deut. chem. Gesell. 36: 622-634.

(1903) Nabokich, A. J. Über die intramolekulare Atmung der höheren Pflanzen. Ber. d. deut. bot. Gesell. 21: 467-476.

(1906) Stoklasa, J., Ernest, A., und Chocenský, K. Über die glykolytischen Enzym im Pflanzenenergieismus. Zeitsch. physiol. Chem. 50: 303-360.

Several other investigators found in anaerobic respiration a similar production of alcohol and carbon dioxid, which led to the contention that anaerobic respiration is identical with alcoholic fermentation. It has been shown, however, in the papers subsequently cited, that, while alcoholic fermentation does explain a large part of the production of carbon dioxid in anaerobic respiration, it by no means explains all.

Palladin (1905) contends that carbon dioxid arises from plants under anaerobic conditions in three different ways:

1. "Nukleokohlensäure," so called because production of carbon dioxid follows closely the curve of the total nucleo-proteids. This is caused by enzymes, some of which are soluble in the juice and some of which are insoluble. The latter are possibly combined with the protoplasm. Zymase is placed in this group.

2. "Reizekohlensäure," that which is formed directly by the protoplasm itself, due to various stimuli such as quinine hydrochlorid and ether. These two stimuli were applied, one to etiolated vetch shoots in 10-per-cent cane sugar solution and the other to gladiolus bulbs. The series with the stimulus gave about twice as much carbon dioxid in each case as did the check. Both series were then frozen in order to kill the protoplasm, and the stimulants were again applied. They had no effect after the freezing.

3. "Oxydasekohlensäure," that carbon dioxid which is formed by the action of various oxidases. When hydrogen peroxid (H_2O_2) was added to the extracted juice of gladiolus bulbs, a marked increase occurred in evolution of carbon dioxid. When pyrogallic acid was added to this, the evolution of carbon dioxid became very strong.

Palladin and Kostytschew (1906) found that frozen tops of etiolated *Vicia faba* plants in a stream of hydrogen gave the very low $\frac{\text{C}_2\text{H}_5\text{OH}}{\text{CO}_2}$ ratio of 17.1, 18.5, and 8.4, and of only 33 when placed in sugar solution. Other examples are given showing that, while alcohol production is dependent on the amount of zymase present, the production of carbon dioxid is not so dependent.

Kostytschew (1908) found that *Agaricus campestris* gives an abundant evolution of carbon dioxid under anaerobic conditions, but no alcohol. Neither is alcohol produced when a glucose solution is added. This shows that zymase is not present in *Agaricus*; but, since anaerobic respiration continues, it suggests also that alcoholic fermentation is only one phase of the phenomenon.

(1905) Palladin, W. Über den verschiedenen Ursprung der während der Atmung der Pflanzen ausgeschiedenen Kohlensäure. Ber. d. deut. bot. Gesell. 23: 240-247.

(1906) Palladin, W., und Kostytschew, S. Anaerobe Atmung, Alkoholgärung, und Acetonbildung bei den Samenpflanzen. Zeitsch. physiol. Chem. 48: 214-239.

(1908) Kostytschew, S. Zweite Mitteilung über anaerobe Atmung ohne Alkoholbildung. Ber. d. deut. bot. Gesell. 26a: 167-177.

It has also been shown by a number of investigators that a wide range of carbon-containing compounds, not fermentable by zymase, can be used by plant tissue in the anaerobic production of carbon dioxide.

Since zymase seems to be so widely distributed in the tissues of plants, the idea of its having some particular function in aerobic forms cannot but suggest itself. Kostytschew (1908 and 1910) has given considerable experimental data and has proposed some hypotheses with reference to the subject. He showed that alcohol is produced under aerobic conditions only in the parts that are most poorly aerated. If alcoholic fermentation is a step in normal respiration, alcohol should be used by the organism at least as readily as glucose. In Kostytschew's experiments alcohol was found to be used with great difficulty or not at all, and neither could lactic acid be used. Both had a retarding effect on the production of carbon dioxide. Kostytschew concluded that alcoholic fermentation is the first step in normal respiration, but that under aerobic conditions it goes only as far as the formation of an easily oxidizable substance between the glucose and the lactic acid and alcohol. In the absence of oxygen this fermentation continues to the production of lactic acid and alcohol, but not normally under aerobic conditions. Kostytschew called alcohol and lactic acid "Nebenproducte," not "Zwischenproducte," of respiration. He succeeded in showing that a glucose solution, completely fermented by zymase* and freed from any proteins and peptones, contains an easily oxidizable substance that will reduce Fehling's solution. The nature of this substance is unknown. Kostytschew showed also that the production of carbon dioxide is markedly increased by surrounding seeds or other plant tissues with a zymase-fermented solution. Considerable attention is also given by this author to the relation of oxidases to the process of respiration.

Palladin (1909) has attempted to explain the various phenomena of respiration. He divides all such phenomena into two classes — primary and secondary. In the primary class, the materials of respiration are broken down into simple products without the use of oxygen, by means of enzymes. The chemical reactions consist of phenomena of reduction and oxidation similar to those of dry distillation, and occur at the expense of the combined oxygen within the cells. Glucose and other stable substances are used and various more easily oxidizable products arise. Alcohol is produced only in absence of oxygen, but with good aeration the labile intermediate products are oxidized before the alcohol stage is reached. In the secondary class of respiration phenomena Palladin places the

* Zymase is a commercial preparation made by drying yeast cells with acetone and ether. The protoplasm is killed in the process, but the zymase is presumably little affected.

(1908) Kostytschew, S. Über die Anteilnahme der Zymase am Atmungsprozesse der Samenpflanzen. *Biochem. Zeitsch.* **15**: 164-195.

(1909) Palladin, W. Über das Wesen der Pflanzenatmung. *Biochem. Zeitsch.* **18**: 151-206.

(1910) Kostytschew, S. Über den Vorgang der Zuckeroxydation bei der Pflanzenatmung. *Zeitsch. physiol. Chem.* **67**: 116-137.

various oxidation processes. He holds that these are maintained largely by oxidases and similar enzymes.

Since it had been shown by a number of investigators that a wide range of organic compounds were used in anaerobic respiration, and since Palladin had called attention to a production of carbon dioxid in certain cases which varied directly with the nucleo-proteids, and since others had indicated that proteins might be used in anaerobic respiration the same as glucose, Godlewski (1911) undertook to determine to what extent production of carbon dioxid could be correlated with protein decomposition. He concluded that anaerobic decomposition of protein proceeds independently of intramolecular respiration; that it is an enzymatic process which continues long after the evolution of carbon dioxid has ceased, and after the death of the protoplasm. He found also that sugar increases the rate of evolution of carbon dioxid but hinders the rate of protein decomposition. Citric acid markedly decreases the output of carbon dioxid.

An increase of temperature was found by von Chudiakow (1894) to increase the rate of anaerobic production of carbon dioxid according to the Van't Hoff-Arrhenius law, just as in aerobic respiration. Von Chudiakow came to the conclusion that in anaerobic respiration the total amount of carbon dioxid produced is the same whether at high or low temperatures, the evolution at low temperatures continuing for a sufficiently longer time to make up for the difference in rate. In order to determine the effect of temperature, von Chudiakow heated the object for a given length of time to a given temperature, then raised the temperature to that next desired, and so on. Palladin (1899) pointed out that when a plant is heated to any temperature and then cooled to the original temperature, the rate of respiration will not be the same as that before the heating. The work of von Chudiakow has been objected to on this ground by some investigators, but Kuyper (1909-1910) found that temperature does not become injurious until it goes above 25° C. or thereabout, varying with the kind of tissue used. Below 25° C. Kuyper found the ratios to vary directly with the temperature. This gives weight to the work of von Chudiakow, although there is still the objection that the time interval which he used was rather short.

Pourievitch (1905) has shown that the age of the particular plant and the amount of nutritive material that it contains, together with other

- (1894) von Chudiakow, N. Beiträge zur Kenntniss der intramolekularen Athmung. Landw. Jahrb. 23: 333-389.
 (1899) Palladin, W. Influence des changements de température sur la respiration des plantes. Rev. gén. bot. 11: 241-257.
 (1905) Pourievitch, K. Influence de la température sur la respiration des plantes. Ann. sci. nat. (IX) 1: 1-32.
 (1909-1910) Kuyper, J. The influence of temperature upon the respiration of higher plants. Proc. Sec. Sci. Roy. Acad., Sci. Amsterdam 12: 219-227.
 (1911) Godlewski, E. Über anaerobe Eiweisszersetzung und intramolekulare Atmung in den Pflanzen. Bul. Acad. Sci. Cracovie (1911): 623-717.

considerations, modify rather markedly the sensitiveness of the plant to temperature changes. This would affect von Chudiakow's results, since he used one set of material for several changes of temperature.

The absence of oxygen is very quickly manifested on growth, which ceases almost abruptly. Takahashi (1905), Crocker (1907), Nabokich (1909), Lehmann (1911), Shull (1911), and others have studied growth in the complete absence of oxygen and in small amounts of it. Growth in the absence of oxygen is reported in the cases of a fairly large number of the higher plants, but not in all under the conditions tried. This growth, however, is so slight as to be insignificant, and it seems to be influenced by many factors besides oxygen, such as nutrition, temperature, age, carbon dioxid, and the like.

The reversibility of chemical reactions holds for enzymatic transformations as well as for inorganic chemical ones. The rate of chemical reaction decreases with an accumulation of the products of the reaction until the reaction finally ceases. This is true in respiration. Kostytschew (1910) showed that alcohol materially decreased the rate of anaerobic respiration. The effect of an accumulation of carbon dioxid is manifested in a variety of ways. De Saussure (1804) found that bean plants withered directly in an atmosphere of two thirds or more carbon dioxid. In 50 per cent carbon dioxid they were dead in seven days; in 25 per cent they lived ten days with no growth; and they grew much better in $8\frac{1}{3}$ per cent carbon dioxid than in $12\frac{1}{2}$ per cent, in sunlight. Loproire (1895) studied the effect of carbon dioxid on the growth of molds, yeasts, pollen tubes, and the like, and found considerable variation. When 25 per cent oxygen was present in the gas, carbon dioxid inhibited the growth of pollen tubes only when present in large amounts. Different kinds of pollen showed wide variation. Mangin (1896) placed starchy and oily seeds in various stages of germination in atmospheres of 1 to 3 per cent and 4 to 5 per cent carbon dioxid. Both evolution of carbon dioxid and absorption of oxygen were decreased in the latter case; likewise the $\frac{\text{CO}_2}{\text{O}_2}$ ratio was raised, indicating that the carbon dioxid had decreased absorption of oxygen more than evolution of carbon dioxid.

(1804) De Saussure, Th. Influence du gaz carbonique sur la végétation. *Recherches chimiques sur la végétation*, ..25-34.

(1895) Loproire, G. Über die Einwirkung der Kohlensäure auf das Protoplasma der lebenden Pflanzenzelle. *Jahrb. wiss. Bot.* 28: 531-626.

(1896) Mangin, L. Sur la végétation dans une atmosphère viciée par la respiration. *Compt. rend.* 122¹: 747-749.

(1905) Takahashi, T. Is germination possible in absence of air? *Bul. Col. Agr. Tokyo* 6: 439-442.

(1907) Crocker, William. Germination of seeds of water plants. *Bot. Gaz.* 44: 375-380.

(1909) Nabokich, A. J. Temporäre Anaërobie höherer Pflanzen. *Landw. Jahrb.* 38: 51-194.

(1910) Kostytschew, S. Über den Vorgang der Zuckeroxydation bei der Pflanzenatmung. *Zeitsch. physiol. Chem.* 67: 116-137.

(1911) Lehmann, E. Zur Kenntnis des anaeroben Wachstum höherer Pflanzen. *Jahrb. wiss. Bot.* 49: 61-90.

(1911) Shull, C. A. The oxygen minimum and the germination of Xanthium seeds. *Bot. Gaz.* 52: 453-477.

Gore (1911 and 1912) and Lloyd (1911) have shown that carbon dioxide applied to persimmons has the effect of destroying the astringency, due presumably to a transformation of the tannin into an insoluble compound. The length of time necessary for this transformation varied widely with different sorts. Softening was usually retarded during the process but increased noticeably when the process was finished. Some varieties colored more quickly after the treatment, while others were not thus affected. The flesh of the fruit darkened somewhat after the treatment, particularly if it was carried a little too far. The flavor in a number of cases was slightly inferior to that of the unprocessed fruits. At ordinary temperatures the processed fruit perished in most cases quicker than the unprocessed. These experiments show that carbon dioxide materially affects several of the metabolic functions.

Gerber (1903) noted that an increased percentage of oxygen increased the respiratory quotient and hastened the maturation of unripe bananas, but decreased the respiratory quotient in ripe bananas. Whenever this fell below unity in the case of ripe fruits, the aroma was decreased. This, Gerber thinks, is because the alcohols, which otherwise produce volatile oils, are oxidized.

Powell and Fulton (1905) made a study of apple scald. This, they think, is a physiological breakdown of the tissues of the fruit, probably due to oxidizing enzymes. It is described as a browning of the flesh just under the skin, giving a semi-baked appearance to the fruit. It is very common on some varieties of apples kept in cold-storage, more than fifty per cent of the fruit being affected in some cases. These authors found that immature fruit is much more subject to scald than is mature, well-colored fruit. Fruit that is not stored for several days after having been picked in hot weather scalds badly in storage. In the study here referred to, paper wrappers did not reduce the scald but paraffined wrappers are reported to have done so. Air containing formaldehyde, sulfur dioxide, chlorine, alcohol, ether, chloroform, or turpentine had no effect on the scalding of Ben Davis apples but the apples were injured in several cases. The scald was increased in an atmosphere of moist oxygen, but was entirely prevented in an atmosphere of nitrogen for nine days. It was retarded

- (1903) Gerber, M. C. Influence d'une augmentation momentanée de la tension de l'oxygène sur la respiration des fruits à éthers volatils, pendant la période où, murs, ils dégagent un parfum. *Compt. rend. soc. biol.* 55: 267-269.
- (1903) Gerber, M. C. Respiration des fruits parfumés lors de leur maturation complète, quand on les place à l'état vert et non parfumés dans de l'air enrichi en oxygène. *Compt. rend. soc. biol.* 55: 269-271.
- (1905) Powell, G. H., and Fulton, S. H. The apple in cold storage. *U. S. Dept. Agr., Bur. Plant Indus. Bul.* 48: 1-64.
- (1911) Gore, H. C. Experiments on the processing of persimmons to render them nonastringent. *U. S. Dept. Agr., Bur. Chem. Bul.* 141: 1-31.
- (1911) Lloyd, F. E. Carbon dioxide at high pressure and the artificial ripening of persimmons. *Science n. s.* 34: 924-928.
- (1912) Gore, H. C. Large scale experiments on the processing of Japanese persimmons. *U. S. Dept. Agr., Bur. Chem. Bul.* 155: 1-20.

when the fruits were placed in water or covered with vaseline, paraffin, or olive oil. The apples had been kept in cold-storage for several months prior to the experiments. Neither the duration of these experiments nor the temperature employed is given.

It is not the purpose of the writer to consider the literature critically or to criticise the many interpretations that may be placed on the publications mentioned above. All that is desired is to suggest a few of the concepts which seem to be prominent in the literature.

From this brief review it is apparent that many widely different katabolic processes occur in plant tissues in the absence of oxygen; that these katabolic processes are maintained largely by enzymes; that there is a profuse and frequently long-continued evolution of carbon dioxid as a result of these processes; that the carbon dioxid is produced by a variety of enzymes and probably in some cases partly by the living protoplasm itself; that the carbon dioxid arises principally from the decomposition of sugar by zymase; that a considerable quantity of carbon dioxid arises from the decomposition of fats, of certain cyclic compounds, and of many other organic substances by means of the various enzymes; that, besides the processes producing carbon dioxid, there are others, such as protein decomposition, which proceed independently and in some cases last longer; that alcohol, lactic acid, glycerin, and other materials are produced, depending on the presence of zymase and of suitable carbohydrates; that production of carbon dioxid and other processes are materially affected by various substances, as salts, acids, alkalies, alcohol, carbon dioxid, and various stimulants, and by changes in temperature.

EXPERIMENTAL WORK

RESPIRATION OF FRUITS AND GERMINATING SEEDS IN HYDROGEN, NITROGEN, AND AIR

Studies were made of the production of carbon dioxid in hydrogen, in nitrogen, and in air by ripe cherries, blackberries, green peaches, ripe grapes, and germinating wheat. The hydrogen was obtained from a cylinder of the compressed gas furnished by the Department of Physics at Cornell University. The nitrogen, or oxygen-free air, was obtained by passing air over red-hot copper in a combustion furnace. The air was obtained from an automatic electric pump attached to a compression tank. The hydrogen and the nitrogen were each passed through three wash-bottles containing potassium pyrogallol renewed often enough to insure freshness. This removed traces of oxygen and of carbon dioxid. The nitrogen was so free from oxygen that the alkaline pyrogallol was not darkened noticeably by it in a half-hour. The hydrogen was made elec-

trolytically and before passing through the alkaline pyrogallol it contained only a very slight amount of oxygen. The air was passed through a wash-bottle containing a solution of potassium hydroxid (KOH). All three gases were passed through wash-bottles containing water before they entered the respiration chambers. This prevented a backward absorption of the carbon dioxid.

The respiration chambers consisted of glass bottles each having a capacity of 250 cubic centimeters. Each bottle was fitted with a two-holed rubber stopper. A glass tube extending just through the stopper led the gas into the chamber, and another glass tube reaching to the bottom of the chamber drew the gas away. The gas was led from the respiration chamber through a jar of calcium chlorid and then through a Mohr potash bulb, in which the carbon dioxid was collected and weighed. In order to prevent absorption of moisture from the air by the potash bulb, the gas, on leaving the bulb, was led through another jar of calcium chlorid.

The temperature was controlled to within $\frac{1}{2}^{\circ}$ C. by placing the respiration bottles in water in a basin constructed for the purpose. The water was heated by an electric heater of nichrome ribbon; and the temperature was controlled by connecting a mercury thermostat with a gravity cell and a telegraphic relay in such a manner that the current through the heater would be automatically turned off or on at any desired temperature. An electric motor was used for running a stirrer by means of which the temperature of the water was kept uniform throughout.

The respective gases were conducted in glass tubes with very close connections of rubber tubing. The experiments were always run in triplicate. The stream of gas through each chamber was regulated to about 500 cubic centimeters per hour. The potash bulbs were weighed usually twice daily and the hourly rate computed from the weights thus found.

The fruits in each respiration chamber were carefully weighed, and were sterilized when it was possible to do this without injury to the fruit. An equal number of fruits were used in each case, and the amount used generally weighed about fifty grams. The data were then all computed on the one-hundred-gram basis for comparison.

The Roman numerals I, II, and III were used for designating the series in air, in nitrogen, and in hydrogen, respectively, and the letters a, b, and c were used for the individual members in each series: thus, Ia, Ib, and Ic indicate the series in air; IIa, IIb, and IIc, the series in nitrogen; and IIIa, IIIb, and IIIc, the series in hydrogen. The denotation of any series in addition to these will be found with that series.

Respiration of ripe cherries

Well-ripened Duke cherries were used. These were sound cherries, carefully chosen. The flesh was firm and the cherries were sour. Fifteen cherries were chosen for each test, their aggregate weight being about 50 grams. Each stem was cut about one centimeter from the fruit. The fifteen cherries were placed in cheesecloth, dipped in 95-per-cent alcohol then twice in sterile water, and placed in the sterile respiration chambers. The gases were passed through the chambers for one hour before the collection of carbon dioxid began.

There were two series of these cherries. The first was kept in a continuous current of the respective gases. The second was surrounded by the respective gases and a current passed through for one half-hour twice daily, in order to remove the carbon dioxid. The first series (Table 2A) is designated by the Roman numerals and letters Ia, Ib, and Ic for air, IIa, IIb, and IIc for nitrogen, and IIIa, IIIb, and IIIc for hydrogen, as already described; the second series (Table 2B) is designated by IVa, IVb, and IVc for air, Va, Vb, and Vc for nitrogen, and VIa, VIb, and VIc for hydrogen. The temperature was 30° C. throughout.

The series was started on July 13 and continued for two and one half days. Five determinations of carbon dioxid from each were taken. In Table 1 are given the weights of each group of fifteen cherries. In tables 2A and 2B are given the number of hours of each run, the amount of carbon dioxid in milligrams per hour for each 100 grams of cherries, for each period and for the entire period, and the ratios of the weights of carbon dioxid produced in nitrogen and hydrogen to that produced in air.

TABLE 1. WEIGHT OF THE FIFTEEN CHERRIES IN EACH RESPIRATION CHAMBER

Groups	Grams	Groups	Grams
Ia.....	49.7	IVa.....	44.6
Ib.....	51.0	IVb.....	51.2
Ic.....	48.0	IVc.....	46.6
IIa.....	48.5	Va.....	45.9
IIb.....	48.0	Vb.....	46.6
IIc.....	47.7	Vc.....	46.2
IIIa.....	46.8	VIa.....	44.9
IIIb.....	50.0	VIb.....	48.3
IIIc.....	48.2	VIc.....	46.0

TABLE 2A. AVERAGE HOURLY PRODUCTION OF CARBON DIOXID IN MILLIGRAMS PER 100 GRAMS OF RIPE CHERRIES IN AIR, NITROGEN, AND HYDROGEN AT 30° C. IN CONTINUOUS CURRENT OF RESPECTIVE GASES

Period	1	2	3	4	5	Average
	Hours	Hours	Hours	Hours	Hours	Hours
Duration of period....	8.5	10.5	15.0	9.5	14.0	*57.5
Groups in air	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour
Ia	8.8	12.6	12.2	13.9	13.9	12.5
Ib	Lost	8.6	16.8	16.9	18.1	15.6
Ic	12.9	15.4	12.7	13.7	16.6	14.4
Average.....	10.8	12.2	13.9	14.8	16.2	14.2
	Hours	Hours	Hours	Hours	Hours	Hours
Duration of period....	10.0	10.0	15.5	9.5	14.0	*59.0
Groups in nitrogen	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour
IIa	11.2	11.0	10.1	10.4	8.1	10.0
IIb	13.7	13.9	12.8	14.1	11.7	13.1
IIc	17.7	15.7	11.2	14.0	9.0	13.0
Average.....	14.2	13.5	11.4	12.8	9.6	12.0
	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio
An† N	1.31	1.11	.81	.86	.59	.85
	Hours	Hours	Hours	Hours	Hours	Hours
Duration of period....	11.0	10.0	15.5	9.5	14.0	*60.0
Groups in hydrogen	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour
IIIa	11.6	11.5	13.7	12.7	11.8	12.4
IIIb	10.1	12.3	13.4	9.3	10.2	11.3
IIIc	9.9	13.4	9.7	9.7	8.9	10.2
Average.....	10.5	12.4	12.3	10.6	10.3	11.3
	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio
An N	.97	1.02	.88	.72	.64	.80

* Total hours, not average.

† Ratio of anaerobic respiration to normal respiration.

TABLE 2B. AVERAGE HOURLY PRODUCTION OF CARBON DIOXID IN MILLIGRAMS PER 100 GRAMS OF RIPE CHERRIES IN AIR, NITROGEN, AND HYDROGEN AT 30° C. IN CURRENT OF RESPECTIVE GASES FOR ONE HALF-HOUR TWICE DAILY

Period	1	2	3	4	5	Average
	Hours	Hours	Hours	Hours	Hours	Hours
Duration of period....	12.0	9.5	16.5	8.5	14.0	*60.5
Groups in air	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour
IVa.....	10.8	12.8	12.9	14.2	14.2	13.0
IVb.....	Lost	Lost	Lost	9.8	11.5	
IVc.....	11.6	12.0	11.9	11.4	10.4	11.5
Average.....	11.2	12.4	12.4	11.8	12.0	12.2
	Hours	Hours	Hours	Hours	Hours	Hours
Duration of period....	13.5	9.5	16.5	8.5	14.0	*62.0
Groups in nitrogen	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour
Va.....	13.2	13.2	10.6	9.2	8.0	10.8
Vb.....	11.1	10.2	10.0	8.3	6.8	9.3
Vc.....	10.6	11.2	10.3	8.4	7.8	9.7
Average.....	11.6	11.5	10.3	8.6	7.5	9.9
	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio
An N.....	1.04	.93	.83	.73	.63	.81
	Hours	Hours	Hours	Hours	Hours	Hours
Duration of period....	13.5	10.0	16.0	8.5	14.0	*62.0
Groups in hydrogen	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour
VIa.....	13.6	12.9	11.5	10.5	8.3	11.3
VIb.....	10.0	11.8	10.8	10.1	8.7	10.2
VIc.....	12.2	11.6	11.2	11.5	10.1	11.2
Average.....	11.9	12.1	11.2	10.7	9.0	10.9
	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio
An N.....	1.06	.98	.90	.92	.75	.89

* Total hours, not average.

It will be seen from Table 1 that there was considerable diversity in the weights of the several groups of 15 cherries, notwithstanding the fact that the cherries had been chosen with a view to uniformity. The cherry stones were more nearly uniform in weight than were the whole cherries, consequently the proportion of flesh in Ib, weighing 51 grams, was greater than in IIIa, weighing 46.8 grams. The cherries were not all from the same tree. Some were from shaded parts of a tree and some from well-lighted parts. Some came from heavily loaded limbs and others from limbs bearing only a few cherries. All such factors cause the water content, the acid content, the sugar content, and presumably the enzymatic content of the fruit to vary; and since all such factors influence the production of carbon dioxide a wide variation is to be expected, no matter how uniformly the fruits may be grouped together after having been picked. The same applies to all the other fruits used, and is probably sufficient to explain a large part of the individual variations in the rates of evolution of carbon dioxide.

Another cause of individual variation was as follows: The flow of gas through each chamber was regulated by a pinchcock so as to give about two to three bubbles per second through the potash bulbs. Sometimes, in the absence of the operator, a jar of calcium chloride or a potash bulb would become sufficiently clogged to decrease this rate by perhaps half and increase the others correspondingly. This would slightly increase the amount of carbon dioxide collected in the one case and slightly decrease it in the others. In the following run the carbon dioxide that had accumulated in the chambers through which the gas flow was slowest would be caught, and this would give those chambers a correspondingly higher rate for that period. At times the temperature of the room was considerably colder than at other times. While this would not affect the respiration chambers, it would affect the temperature of the gases in the tanks and thus cause a variation in the rate of flow.

The method of calculating and expressing the data in tables 2A and 2B and the other similar tables is as follows: The different periods are expressed by the numerals 1, 2, 3, 4, and 5 in a horizontal row, and just under the number of each period is given the number of hours duration of that period. The total number of hours of the entire period is given at the right of this row. The members of each series, Ia, Ib, Ic, or IIa, IIb, IIc, and so on, are arranged in a column, and the number of milligrams of carbon dioxide produced by each member in each period is placed under the corresponding period number. In the last column the average of each member for the entire period is given. Below the members of the series is given the average of the triplicates for each period. In the nitrogen and hydrogen series, the ratio of anaerobic production of carbon dioxide to normal production, expressed as $\frac{A_n}{N}$, is given for each period.

This was obtained by dividing the average rate for each period in those gases by the corresponding rate in air.

In Table 2A a number of things are shown very markedly, despite the individual variations:

1. About the same amount of carbon dioxide was produced in nitrogen as in hydrogen. Hence neither of these gases hinders anaerobic respiration of ripe cherries more than the other.

2. Approximately as much carbon dioxide was produced during the first thirty-six hours in both nitrogen and hydrogen as in air. The production of carbon dioxide in ripe cherries is apparently caused by processes quite independent of the absorption and use of oxygen, since the rate is maintained so well in hydrogen and nitrogen and is as great as that in air to begin with.

3. The hourly rate of evolution of carbon dioxide during the sixty hours decreased only slightly in nitrogen and scarcely any in hydrogen, while it increased rather markedly in air. The increase in air was probably not due to a yeast or other infection, since such an infection at once gives a very rapid increase in evolution of carbon dioxide, such as was not found in this case. It is possible that at 30° C. the ripening of fruit is attended by a production of various carbon-dioxide-producing enzymes which is favored by oxygen, and that an increased production of these enzymes might account for the increased rate of evolution of carbon dioxide in air.

In order to obtain the ratio of anaerobic respiration to normal respiration, the average hourly production for each period in nitrogen and in hydrogen was divided by the corresponding average in air. The ratio is written $\frac{An}{N}$. It declines markedly in both nitrogen and hydrogen after the second period, although it was slightly greater than unity during that period. The apparent decline is due not so much to a reduction in the hourly rate in nitrogen and hydrogen as to an increase in the rate in air.

The vertical column of averages at the right was obtained by dividing the total amount of carbon dioxide produced in each case by the total number of hours. The average rate in both nitrogen and hydrogen is seen to be about the same as the individual rates in those series.

In Table 2B are given the results with a series identical with those used for Table 2A, except that the gases were drawn through the respiration chambers twice daily for one half-hour only, in order to remove the carbon dioxide for determination.

In all three members of the nitrogen series there is a decline in the hourly rate after the first two periods. There is a slight decline in the hydrogen series. In air the rate is more nearly uniform, with a tendency to rise, and the hourly rates for the first two periods in air are again about the same as in nitrogen and hydrogen. The average rates are slightly lower than in the corresponding series shown in Table 2A. This is probably due to an accumulation of carbon dioxide in the chambers between the runs.

At the end of the experiments the cherries kept in nitrogen and in hydrogen gave a strong iodoform test for alcohol and also had a slightly fermented flavor. They were a trifle bleached in spots; otherwise, no difference could be seen between them and those kept in air.

These experiments seem to show that for a considerable period of time the respiratory processes in ripe cherries are about as active in the absence of oxygen as in the presence of it. This hypothesis seems to suggest that these processes might be maintained for the most part by enzymes and probably not to any great extent by the living protoplasm. In spite of the high probable error due to difference in the hourly rate of evolution of carbon dioxid, some reasons for which have already been pointed out, the triplicate experiments herein described seem to point consistently to the above conclusions.

Respiration of blackberries

A series of experiments similar to those with cherries was run with ripe blackberries. The surfaces of these fruits were so uneven that they could not be sterilized. The first period of their respiration, however, gave a yield of carbon dioxid as great in both nitrogen and hydrogen as in air. No tables for the blackberries are given for the reason that because of micro-organisms the evolution of carbon dioxid became too irregular after the first period to be of any value.

Respiration of green peaches

A series with green peaches taken just at the time when the stone was hardening, at which time the fruits were about half grown, was run in order to see the behavior of the green growing tissue. The same respiration chambers were used as with the other fruits.

Three peaches were placed in each respiration chamber. The weight of the peaches in each chamber is given in Table 3:

TABLE 3. WEIGHT OF THE THREE GREEN PEACHES IN EACH RESPIRATION CHAMBER

Groups	Grams
Ia.....	57.4
Ib.....	58.8
Ic.....	55.4
IIa.....	55.6
IIb.....	56.1
IIc.....	55.2
IIIa.....	55.5
IIIb.....	51.8
IIIc.....	56.9

No attempt was made to sterilize the peaches. The series was run at 30° C. and the details of manipulation were the same as in the series

with cherries. The experiments extended from July 20 to July 26. They were run for eight periods (90.5 hours) in the respective gases and then they were all run in air for four periods (49 hours).

A summary of the results is given in Table 4. The general make-up of the table is the same as that of tables 2A and 2B. The results include the hourly rates of evolution of carbon dioxide per 100 grams of peaches and the ratios of anaerobic production to aerobic. The ratios as they appeared when the nitrogen and hydrogen were replaced by air are given also, in order to show the tendency toward a return to the normal.

In Table 4 is shown a different type of production of carbon dioxide from that shown in tables 2A and 2B. Here the effect of the absence of oxygen is manifested from the first in a greatly decreased production of carbon dioxide. The average ratio $\frac{A_n}{N}$ is less than .5. The amount and rate of production of carbon dioxide is approximately the same in both nitrogen and hydrogen. In air there is a steady decline for the first six periods, after which the hourly production remains almost constant. The point of constant production is reached about two or three periods earlier in nitrogen and hydrogen. This constant rate is less than half the rate during the first period.

After being kept for eight periods in nitrogen and hydrogen the peaches on being transferred to normal air did not regain the normal rate of production of carbon dioxide. The ratio $\frac{A_n}{N}$ was increased, however, from approximately .5 to .7.

These experiments show that the respiratory processes are very different in green fruits from those in ripe fruits. The former are dependent on an immediate supply of oxygen to the extent of fifty per cent of their activity. After four days in the absence of oxygen the tissue had been so injured that it was unable to return to its normal rate of respiration. There was a marked tendency to return, however, and this suggests that there is probably a point at which the return would be complete. It suggests further that growing tissues are able to continue their functions in the absence of oxygen for a time without injury.

At the close of the experiment the peaches were perfectly turgid. There were no visible signs of the growth of micro-organisms, and the regular rates of production of carbon dioxide preclude the possibility of their action to an appreciable extent. The flavor of the fruit was not noticeably different. At the time when the peaches were picked, an examination of the seed showed that there was a considerable quantity of endosperm surrounding the growing cotyledons and the testa was perfectly white. At the close of the experiment the testa was browned and the endosperm had disappeared. These effects were as noticeable in the peaches kept in air as in those kept in the other gases.

TABLE 4. AVERAGE HOURLY PRODUCTION OF CARBON DIOXID IN MILLIGRAMS PER 100 GRAMS OF GREEN PEACHES IN A CONTINUOUS CURRENT OF AIR, OF NITROGEN, AND OF HYDROGEN AT 30° C.

Periods			In the respective gases												All in air						Average for all periods														
			1		2		3		4		5		6		7		8		Average				9		10		11		12		Average				
Hours		Mg. per hour	Hours		Mg. per hour	Hours		Mg. per hour	Hours		Mg. per hour	Hours		Mg. per hour	Hours		Mg. per hour	Hours		Mg. per hour	Hours		Mg. per hour	Hours		Mg. per hour	Hours		Mg. per hour						
Duration of period.....			7.0	10.5	12.5	13.5	10.0	14.5	8.5	14.0	*90.5													12.0	12.0	12.0	11.5	13.5	*49.0						
Groups in air			Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour						
Ia.....			24.7	18.7	13.6	12.3	11.6	9.4	9.9	8.8	12.9	7.8	9.4	8.8	13.0	9.9	8.8	13.0	9.4	8.8	13.0	9.9	8.8	13.0	9.9	8.8	13.0	9.9	8.8						
Ib.....			25.8	18.0	13.9	10.5	9.9	7.3	8.3	7.9	11.8	6.7	8.3	7.9	11.8	6.7	8.3	7.9	8.0	6.8	8.3	7.9	6.8	8.3	7.9	6.8	8.3	7.9	6.8						
Ic.....			27.6	21.7	14.6	14.0	12.7	13.3	11.5	13.0	15.4	10.0	13.3	13.0	15.4	10.0	13.3	13.0	12.6	13.1	13.4	12.3	13.4	12.3	13.4	12.3	13.4	12.3	13.4						
Average.....			26.0	19.5	14.0	12.3	11.4	10.0	9.9	9.9	13.4	8.2	10.0	9.9	13.4	8.2	10.0	9.9	10.0	9.6	11.6	9.9	11.6	9.9	11.6	9.9	11.6	9.9	11.6						
Groups in nitrogen			Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour						
IIa.....			14.0	9.8	6.1	6.1	7.1	4.9	6.0	6.8	7.2	6.2	9.8	7.7	6.8	8.0	7.3	6.2	9.8	7.7	6.8	8.0	7.3	6.2	9.8	7.7	6.8	8.0	7.3						
IIb.....			13.2	8.3	7.5	4.8	4.3	3.6	4.8	4.7	6.0	5.0	6.5	6.7	6.6	6.2	6.2	5.0	6.5	6.7	6.6	6.2	6.2	5.0	6.5	6.7	6.6	6.2	6.4						
IIc.....			14.2	9.6	7.3	6.4	4.1	2.9	3.2	4.7	6.1	4.9	8.1	7.1	7.0	6.8	6.4	4.9	8.1	7.1	7.0	6.8	6.4	4.9	8.1	7.1	7.0	6.8	6.4						
Average.....			13.8	9.2	7.0	5.8	5.2	3.8	4.7	5.4	6.4	5.4	8.1	7.2	6.8	7.0	6.6	5.4	8.1	7.2	6.8	7.0	6.6	5.4	8.1	7.2	6.8	7.0	6.6						
An			Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio						
N			.53	.47	.50	.47	.46	.38	.47	.55	.48	.66	.81	.75	.59	.71	.55	.66	.81	.75	.59	.71	.55	.66	.81	.75	.59	.71	.55						
Groups in hydrogen			Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour						
IIIa.....			13.3	8.2	5.2	5.6	3.4	4.5	7.2	3.6	5.9	5.1	7.0	7.3	6.8	6.5	6.1	5.1	7.0	7.3	6.8	6.5	6.1	5.1	7.0	7.3	6.8	6.5	6.1						
IIIb.....			14.3	8.5	6.3	5.4	4.4	3.9	7.2	4.5	6.2	3.0	8.4	9.2	9.7	7.2	6.7	3.0	8.4	9.2	9.7	7.2	6.7	3.0	8.4	9.2	9.7	7.2	6.7						
IIIc.....			13.8	7.9	6.0	5.3	4.4	4.9	6.4	4.6	6.2	4.7	8.0	7.9	7.8	7.1	6.5	4.7	8.0	7.9	7.8	7.1	6.5	4.7	8.0	7.9	7.8	7.1	6.5						
Average.....			13.8	8.2	5.8	5.4	4.1	4.4	6.9	4.2	6.1	4.3	7.8	8.1	8.1	6.9	6.4	4.3	7.8	8.1	8.1	6.9	6.4	4.3	7.8	8.1	8.1	6.9	6.4						
An			Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio						
N			.53	.42	.41	.44	.36	.44	.70	.42	.46	.52	.78	.84	.70	.70	.53	.52	.78	.84	.70	.70	.53	.52	.78	.84	.70	.70	.53						

* Total hours, not average.

Respiration of ripe grapes

From October 26 to October 30 an experiment was made with ripe Concord grapes. The experiment was the same in arrangement as those already described. The Concord grapes were bought at a grocery store, therefore their history is not known. They were in excellent condition. Each grape was carefully taken from the bunch by cutting its stem about three millimeters from the fruit. Only perfect berries were used and these were carefully chosen for uniformity. Twenty berries were used in each case. They were immersed in 95-per-cent alcohol, then rinsed in sterile water and placed in the sterilized respiration chambers. The grapes were kept at 30° C.

From November 8 to November 10 an experiment was conducted with Catawba grapes. It was identical in method with the experiment with Concord grapes, except that the temperature used was 37° C.

The weights of the several groups of twenty Concord grapes used and the weights of the groups of Catawba grapes are given in Table 5. In Table 6 are given the data obtained from the Concords and from the Catawbas.

TABLE 5. WEIGHT OF THE TWENTY GRAPES IN EACH RESPIRATION CHAMBER

Concord grapes		Catawba grapes	
Groups	Grams	Groups	Grams
Ia.....	52.5	Ia.....	47.1
Ib.....	48.4	Ib.....	48.7
Ic.....	50.4	Ic.....	47.5
IIa.....	48.8	IIa.....	50.5
IIb.....	49.7	IIb.....	45.2
IIc.....	49.3	IIc.....	43.8
IIIa.....	44.8	IIIa.....	47.4
IIIb.....	55.2	IIIb.....	42.8
IIIc.....	51.3	IIIc.....	47.5

It will be seen in Table 5 that the weights of the several groups of twenty grapes varied considerably from the 50-gram average, as did the weights of the groups of cherries.

From Table 6 it is clear that ripe grapes respire as rapidly in both nitrogen and hydrogen as in air. This was true of both Concords and Catawbas, in the experiment here described. It was true of the former at 30° C. and the latter at 37° C., and the higher temperature gave an increase in the rate of evolution of carbon dioxid quite in accord with the

TABLE 6. AVERAGE HOURLY PRODUCTION OF CARBON DIOXIDE IN MILLIGRAMS PER 100 GRAMS OF RIPE GRAPES IN A CONTINUOUS CURRENT OF AIR, OF NITROGEN, AND OF HYDROGEN

Periods	Concord grapes at 30° C.										Catawba grapes at 37° C.			
	1	2	3	4	5	6	7	8	9	Average	1	2	3	Average
	Hours	Hours	Hours	Hours	Hours	Hours	Hours	Hours	Hours	Hours	Hours	Hours	Hours	Hours
Duration of period.....	10.0	21.0	10.5	13.5	10.5	14.5	9.5	13.5	11.5	*114.5	11.5	10.0	12.5	*31.0
Groups in air	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour
Ia.....	8.9	4.1	4.8	4.8	5.5	4.9	4.2	3.9	5.8	5.1	11.0	9.3	8.3	9.5
Ib.....	9.8	4.5	4.7	4.9	4.9	4.9	4.3	3.7	5.6	5.1	11.7	9.5	8.9	10.0
Ic.....	10.8	Lost	3.7	5.9	5.3	3.7	4.9	4.8	6.4	5.5	11.2	9.0	10.5	10.3
Average.....	9.8	4.3	4.4	5.2	5.2	4.5	4.5	4.1	5.9	5.2	11.3	9.3	9.2	9.9
Groups in nitrogen	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour
IIa.....	10.1	5.2	5.5	7.2	4.4	4.3	5.0	4.1	5.4	5.5	9.1	8.8	5.4	7.7
IIb.....	Lost	Lost	5.7	Lost	Lost	3.5	3.2	3.2	3.9		9.3	Lost	Lost	Lost
IIc.....	10.9	5.1	Lost	7.3	6.1	7.3	6.3	5.4	7.3	6.9	12.1	12.3	9.7	11.3
Average.....	10.5	5.1	5.6	7.2	5.2	5.0	4.8	4.2	5.5	6.2	10.2	10.5	7.6	9.5
An.....	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio
N.....	1.07	1.19	1.27	1.38	1.00	1.11	1.07	1.02	.93	1.19	.90	1.14	.83	.96
Groups in hydrogen	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour
IIIa.....	10.8	5.5	7.2	6.8	6.2	8.1	6.9	6.4	9.4	7.3	10.8	10.6	9.2	10.1
IIIb.....	9.8	6.0	8.1	8.7	7.5	7.3	8.4	6.5	6.1	7.4	13.4	Lost	Lost	Lost
IIIc.....	Lost	5.6	6.5	5.9	7.0	6.2	8.0	6.2	5.5	7.1	11.3	10.8	8.4	10.1
Average.....	10.3	5.4	7.3	7.1	6.9	7.2	7.8	6.4	7.0	7.3	11.8	10.7	8.5	10.2
An.....	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio
N.....	1.05	1.26	1.66	1.37	1.33	1.60	1.73	1.56	1.19	1.40	1.04	1.15	.92	1.03

* Total hours, not average.

Van't Hoff-Arrhenius law, if we assume that the two varieties would respire similarly under the same conditions. It will be further noted in Table 6 that in the case of the Concord grapes the respiration in the first period is nearly double that of some of the succeeding periods and after the first period the rate is almost constant throughout the succeeding periods. The increased evolution during the first period is possibly due to the release of carbon dioxide on the transference of the groups from a condition of room temperature to that of the experiment, since carbon dioxide has a lesser solubility in the cell sap at the higher temperature.

When the rates of evolution of carbon dioxide in grapes are compared with those in cherries it will be noticed that the cherries respire markedly faster per unit weight than do the grapes. This suggests that the rate of evolution of carbon dioxide is more or less proportional to the rate of spoiling of ripe fruit, since cherries spoil more quickly at the same temperature than do grapes. And since these processes seem to be quite independent of oxygen, they are probably mostly enzymatic, and hence the rate at which fruit spoils is more or less proportional to its enzyme content. This suggests that if the factors controlling the production of enzymes in fruits were sufficiently understood, these factors might be artificially controlled at a low enough cost to effect a great saving in the handling and storing of fruit. Such control would also materially prolong the fruit season.

Respiration of germinating wheat

From November 27 to December 1 an experiment was conducted with wheat. The wheat was soaked for twenty-four hours. It was then sterilized by immersion in 95-per-cent alcohol, and was rinsed in sterilized distilled water. Fifty grams of wheat were put into each respiration chamber and the methods were as before. A summary of the results is given in Table 7. The temperature during this experiment was 25° C.

From December 2 to December 6 a similar experiment was made with germinating wheat. The methods were the same as in the preceding experiment, except that the wheat was sterilized in a solution of formalin, 1 part to 600 parts of water, for 15 minutes, instead of being immersed in alcohol. The results are given in Table 8 (A). In Table 8 (B) are given the results of a set treated exactly as those in 8 (A) except that the respiration chambers were closed tightly with pinchcocks after being set up and were allowed to stand for seven days, at the end of which time the CO₂ was drawn off and measured.

It will be seen in tables 7 and 8 that respiration is much slower in hydrogen and nitrogen than in air. The rate of respiration of seeds sterilized in formalin was greater than the rate of respiration of seeds sterilized in alcohol. This increased rate obtains only in the continuous

TABLE 7. HOURLY AVERAGE PRODUCTION OF CARBON DIOXID IN MILLIGRAMS PER 100 GRAMS OF WHEAT IN A CONTINUOUS CURRENT OF AIR, OF NITROGEN, AND OF HYDROGEN AT 25° C. SEED STERILIZED IN 95-PER-CENT ALCOHOL

Period	1	2	3	4	5	6	7	Average
	Hours	Hours	Hours	Hours	Hours	Hours	Hours	Hours
Duration of period.	11.5	12.0	10.0	11.0	14.0	9.0	14.0	*81.5
Groups in air	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour
Ia.....	16.2	12.9	17.6	13.3	9.4	4.0	7.1	11.4
Ib.....	17.1	14.1	14.4	13.4	8.9	6.9	6.2	11.4
Ic.....	17.7	18.4	17.9	16.6	12.3	16.2	13.2	15.6
Average.....	17.0	15.1	16.6	14.4	10.2	9.0	8.8	12.8
Groups in nitrogen	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour
IIa.....	8.2	6.7	8.5	6.2	3.9	4.5	3.1	5.7
IIb.....	9.4	7.9	5.8	6.1	5.9	4.4	3.5	6.1
IIc.....	9.8	7.8	6.9	6.6	5.3	3.8	3.4	6.2
Average.....	9.1	7.5	7.1	6.3	5.0	4.2	3.3	6.0
	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio
$\frac{A_7}{N}$	.54	.50	.43	.44	.49	.47	.37	.47
Groups in hydrogen	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour
IIIa.....	11.0	8.8	6.8	5.8	5.0	4.5	4.0	6.5
IIIb.....	10.9	9.4	6.2	6.0	4.2	6.0	4.1	6.6
IIIc.....	8.9	9.5	6.6	6.1	5.2	5.2	3.9	6.4
Average.....	10.3	9.2	6.5	6.0	4.8	5.2	4.0	6.5
	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio
$\frac{A_n}{N}$	.61	.61	.39	.42	.47	.58	.45	.51

* Total hours, not average.

streams of air and of nitrogen. In the hydrogen the rate of respiration of formalin-sterilized seed and of alcohol-sterilized seed was approximately the same. This is suggestive of the work of Nabokich (1903), who found that sterilization of seeds by chemicals such as mercuric chlorid ($HgCl_2$) resulted in an increased rate of production of carbon dioxid which may continue for two or three days. The increased production of carbon dioxid in air and in nitrogen shown in Table 8 over that shown in Table 7

(1903) Nabokich, A. J. Über den Einfluss der Sterilisation der Samen auf die Atmung. Ber. d. deut. bot. Gesell. 21: 279-291.

was not due to contamination, since wheat that had been killed by boiling and then sterilized the same as the others, when kept in a continuous current of air showed no noticeable production of carbon dioxide during the interval of these experiments. Contamination at once manifests itself by a very marked increase in the rate of evolution of carbon dioxide. These experiments indicate, then, either that alcohol reduces the production of carbon dioxide or that formalin stimulates it. If the alcohol is injurious the effect is purely local, for the wheat in air germinated and grew rapidly. Since the seeds were rinsed in three different vessels of sterile water, the alcohol or formalin contained would be very slight, for the interval of sterilization in alcohol was only a second and in formalin only fifteen minutes.

TABLE 8. AVERAGE HOURLY PRODUCTION OF CARBON DIOXIDE IN MILLIGRAMS PER 100 GRAMS OF WHEAT IN AIR, NITROGEN, AND HYDROGEN AT 25° C. SEED STERILIZED IN FORMALIN

A									B Left in respective gases for seven days. No current	
In continuous current of respective gases										
Period	1	2	3	4	5	6	7	Average	Total carbon dioxide produced per 100 grams of wheat (in milli- grams)	Total carbon dioxide produced per 100 grams of wheat (in milli- grams)
Duration of period....	Hours	Hours	Hours	Hours	Hours	Hours	Hours	Hours		
	11.00	10.00	14.00	11.50	14.75	7.50	14.75	*83.50		
Groups in air	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour		
Ia.....	23.0	26.8	33.4	41.9	30.9	28.1	34.5	31.6	2,640.8	449.0
Ib.....	18.8	27.7	43.0	45.6	30.1	29.2	29.0	32.4	2,703.4	378.4
Ic.....	20.4	34.2	45.8	49.5	31.4	31.4	37.1	36.6	3,054.6	360.8
Average.	20.7	29.6	40.7	45.7	30.8	29.6	33.5	33.5		396.1
Groups in nitrogen	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour		
IIa.....	17.1	8.1	9.1	10.6	8.2	6.7	7.2	9.5	796.0	414.6
IIb.....	8.0	7.5	5.6	5.3	7.8	6.1	5.4	6.5	543.8	363.8
IIc.....	7.7	9.1	9.1	9.6	4.8	7.7	5.5	7.4	617.4	348.2
Average.	10.9	8.2	7.9	8.5	6.9	6.8	6.0	7.8		375.5
An	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio		
N.....	.53	.28	.19	.19	.22	.23	.18	.23		
Groups in hydrogen	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour	Mg. per hour		
IIIa.....	3.5	6.0	6.4	8.4	4.5	4.8	3.7	5.3	444.2	425.2
IIIb.....	6.6	7.9	9.9	8.9	7.2	7.2	5.3	7.6	633.4	452.8
IIIc.....	6.8	6.6	7.1	6.5	4.4	6.1	7.0	6.3	525.0	493.6
Average.	5.6	6.8	7.8	7.9	5.4	6.0	5.3	6.4		447.2
An	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio		
N.....	.27	.23	.19	.17	.18	.20	.16	.19		

* Total hours, not average.

The decrease in respiration in a continuous current of hydrogen and of nitrogen below that in a continuous current of air was about 50 per cent in seeds sterilized in alcohol and about 80 per cent in seeds sterilized in formalin. In these tissues containing actively growing protoplasm, the absence of oxygen results in a marked decrease in the respiratory rate. The same was shown also with green peaches (Table 4), in which the decrease was about 50 per cent. On the other hand, the series with cherries, blackberries, and grapes indicate that in tissue which is mature and not in an actively growing condition, such as the pulp of ripe fruits, the oxygen relation is very different from that in actively growing tissues and the respiratory rate is little affected by it. There seems to be in fruits a direct relationship between protoplasmic activity and the effect of oxygen in the production of carbon dioxid.

Although oxygen does not appear to directly affect the production of carbon dioxid in ripe fruits, it does greatly affect other metabolic activities, as is shown later in this bulletin.

The rate of anaerobic respiration of germinating wheat at 25° C. is about the same as that of grapes and green peaches at 30° C., but it is considerably less than that of cherries at 20° C. The rate of normal respiration is slightly slower for wheat seed sterilized in alcohol than the rate for green peaches, but it is considerably faster in the case of wheat seed sterilized in formalin.

In the light of the literature of this subject, these experiments seem to show that the production of carbon dioxid in anaerobic respiration is due to agents, probably enzymes, which work independently of oxygen, and that these are practically the only carbon-dioxid-producing agents in ripe fruits. On the other hand, in tissues containing actively growing protoplasm the production of carbon dioxid seems to be due as much to processes that are dependent on oxygen as to those independent of that gas. The latter processes may be enzymatic, but it is probable that the direct metabolism of the living protoplasm itself plays a considerable part in them.

METABOLISM AND KEEPING QUALITY OF FRUITS IN NITROGEN, HYDROGEN, CARBON DIOXID, AND AIR

Red Astrachan apples and Wiggins, Late Crawford, Crosby, and Elberta peaches were used in these experiments. Sound, carefully chosen fruits were placed in large sterilized glass jars of four liters capacity. Bottles containing sulfuric acid (H_2SO_4) and potassium hydroxid (KOH) were placed in the jars in order to prevent the accumulation of moisture and carbon dioxid. The apples were dipped in 95-per-cent alcohol and then rinsed in sterile water before being placed in the jars, so as to render them as nearly sterile as possible. No attempt was made to sterilize the peaches.

The jars were filled with the respective gases and all except those containing air were sealed. The jars containing air were plugged with cotton stoppers.

The fruits were left in these jars for several days at laboratory temperature, which ran from 21° to 23° C. Notes were taken from time to time as to the general appearance of the fruit. When the jars were opened, notes were made of the color, texture of skin and flesh, flavor, and other characters.

Behavior of Red Astrachan apples in air, nitrogen, and hydrogen

Seven apples were placed in each jar. The apples in each jar were as nearly like those in the other jars as careful selection could make them. In each jar some apples were fairly ripe and these graded back to some that were rather green. The experiment lasted from August 5 to August 18, 1911.

Jar I was in air; jars IIa and IIb were in nitrogen; jars IIIa and IIIb were in hydrogen.

After four or five days the apples in nitrogen and those in hydrogen began to have a bleached appearance. The red color then gradually disappeared. Finally the apples acquired a brownish tinge. The apples in air remained a beautiful red.

When the jars were opened the apples in air had a fine apple odor and the appearance of very ripe Astrachan apples. They were much less sour to the taste than when placed in the jar. No soft rots had developed, but two apples were nearly destroyed by what appeared to be brown rot. The other apples were in good condition.

The apples in both nitrogen and hydrogen looked as if they had been about half baked in an oven with a slow fire. They had entirely lost their red color. The skin in places was elevated in small blisters. The flesh was light brown at the surface and white inside, but when exposed to the air it browned very rapidly — many times more rapidly than did the flesh of the apples that had been kept in air. The apples had no bad flavor. The taste was that of half-baked apples, according to five persons who tasted them. The "baking" was greatest near the surface and gradually lessened toward the core. The natural flavor was almost entirely gone.

In order to learn whether the browning and the apparent baking were due to some organism, sterile agar tubes were inoculated with pieces of the browned flesh taken from both the nitrogen and the hydrogen series. No growth developed from them at all, showing that these effects were the result of anaerobic metabolism of the fruits themselves. Some agar tubes were inoculated with the rotted tissue from one of the apples in the air series and an abundant growth of a mold resulted.

This series of experiments shows that apples need aeration and that they cannot be satisfactorily held in inert gases such as nitrogen and hydrogen.

Behavior of Wiggins peaches in air, nitrogen, hydrogen, and carbon dioxid

The peaches were large and market-ripe. They had white flesh and some were beginning to show a light red blush. They were all hard. The surface of peaches is such that it was deemed inadvisable to attempt to sterilize them. They were chosen for uniformity. Ten peaches were placed in each sterile jar. The treatment was the same as with the apples. The experiment was begun on August 19, 1911.

Jars Ia and Ib were in air with potassium hydroxid (KOH) and sulfuric acid (H_2SO_4) as absorbents; jars IIa and IIb were in nitrogen and jars IIIa and IIIb in hydrogen with the same absorbents; and jars IVa and IVb were in carbon dioxid with sulfuric acid (H_2SO_4) as the only absorbent.

Seven days after the experiment was begun, jars Ia, Ib, IIa, IIIa, and IVa were opened and examined. There were only three peaches in jars Ia and Ib that had not rotted. The three good peaches were of a beautiful cream color. The flesh was juicy and soft and of excellent flavor. The peaches contained no more red, however, than at the beginning. The rotted peaches were covered with molds, brown rot being the chief one.

No molds could be found in jar IIa, IIIa, or IVa, and the peaches looked about as they did when placed in the jars. They had acquired a bad flavor, however, just strong enough to spoil them for eating purposes. When they came in contact with the air they darkened somewhat rapidly. The epidermis, however, retained its green color for some time.

Jars IIb, IIIb, and IVb were kept in their respective gases for three weeks longer. At the end of that time the peaches in nitrogen and those in hydrogen were mostly brown and soft. The peach aroma and flavor were entirely gone. There were some hard green spots on some of the peaches and these had a decidedly bad flavor. The soft brown parts had a snappy alcohol flavor and persons who were unacquainted with the treatment that they had received pronounced them brandied peaches. This flavor was not particularly unpleasant.

The peaches in carbon dioxid were mostly green and as hard as when placed in the jar four weeks earlier. The flavor was very bad. It was not the snappy alcohol flavor of the peaches kept in nitrogen, but was bitter and nauseating. The hard green spots that were noted on the peaches in nitrogen and in hydrogen also had this flavor, but it was not so strong in them. When the peaches from the jar of carbon dioxid were placed in air they turned brown very rapidly, but they did not soften markedly.

It seems to be evident that carbon dioxid considerably decreases the hydrolysis of pectose, since peaches in that gas did not soften. In this case, as with the apples, the need for good aeration is apparent, since the

flavor was spoiled in the gases containing no oxygen and since in a closed space without ventilation the carbon dioxid resulting from the respiration of the fruit accumulates so rapidly as to displace the air in a short time.

Behavior of green, market-ripe, and ripe peaches in air, nitrogen, hydrogen, and carbon dioxid

In order to determine the effect of the degree of ripeness on the rate of softening of peaches, some very ripe Elbertas, some market-ripe Crosbys and Late Crawfords, and some hard green Crosbys were treated in the same way as were the Wiggins peaches and were allowed to remain in the gases for two weeks. The experiment was begun on September 20. The peaches in air retained their qualities and ripened well, but the ripe Elbertas and some of the others deteriorated from brown rot after a few days.

At the end of the two weeks the ripe Elbertas, which were soft at the beginning, had a very bad flavor in carbon dioxid and almost as bad a flavor in nitrogen and in hydrogen. They had darkened noticeably. As soon as they were taken out into the air they darkened rapidly under the skins and had the appearance of peaches that had been badly injured in shipment in refrigerator cars.

The market-ripe Crosbys and Late Crawfords softened somewhat, but not so much as in air. Their flavor was very bad and they showed browning, which increased very rapidly when they were placed in air. The hard Crosby peaches remained hard in all three of the oxygen-free gases.

DISCUSSION

Since fruits produce carbon dioxid very rapidly, as has been shown, and since they brown and lose their flavor when they are not supplied with oxygen, the need for thorough ventilation becomes apparent. The peaches that were described at the beginning of this bulletin as having been injured by "ice-scald" had each been wrapped separately in paper. With respiration as rapid as it has been shown to be, it is a matter of only a few hours until all the air within the paper wrapper would be displaced by the carbon dioxid given off by the peach. From the experiments described, it is quite probable that "ice-scald" is injury due to poor aeration and to an accumulation of carbon dioxid.

Certain packing companies provided with refrigerator cars have appreciated this need of plant tissues for fresh air and have conducted some experiments in the ventilation of their cars in transit. They have obtained some very satisfactory results with several kinds of fruits and vegetables. The results have been so satisfactory that one large packing company is putting a special ventilator, which can be opened or closed as

the shipper prefers, over the doors of its refrigerator cars. When the car is moving, air will be drawn out through these ventilators and will be replaced by fresh air that has been cooled in its passage through the ice bunkers at each end of the car.

The question of wrappers for fruits is worthy of an extended investigation from the standpoint of ventilation. The good points in favor of a separate wrapper for peaches, apples, and other fruits are numerous. But such wrappers allow only a very small air space around each fruit. Some type of perforated or porous wrappers has been suggested as a possible means of combining the desirable qualities of the wrappers with better ventilation of the fruit.

It seems that to some extent peaches can be prevented from softening by inert gases, and especially by carbon dioxide; this is of no economic value, however, until some method can be found by which the softening will be prevented and in the use of which the flavor will be retained.

Preliminary experiments for an extended study of the relations between carbon dioxide and oxygen in fruits, vegetables, and other plant tissues have been made by the writer, and he is hoping to continue this work, particularly in its relation to various temperatures, during the coming season.

SUMMARY

1. The respiration of ripe fruits, as well as that of green fruits, is rapid.
2. The anaerobic production of carbon dioxide by ripe cherries, blackberries, and grapes is as rapid as the aerobic production for a considerable length of time.
3. Ripe fruits that spoil quickly, such as cherries, have a higher respiratory rate than those that do not spoil so quickly, such as grapes. This is due possibly to a higher enzyme content.
4. Fruit tissues that respire as actively anaerobically as aerobically seem to be those that have finished their growth and are ripe.
5. Growing tissues, such as green peaches and germinating wheat, respire more than twice as rapidly aerobically as anaerobically. The activity of the protoplasm would seem to be connected with this more direct use of oxygen in the production of carbon dioxide.
6. If growing tissues, such as green peaches, are placed in an oxygen-free gas for a few days and then brought back into air, the rate of production of carbon dioxide does not entirely return to the normal. This would indicate a permanent injury to the protoplasm or to some of the enzymes, due to insufficient oxygen.
7. Ripe apples lose their color, texture, and flavor, and take on the qualities of half-baked apples, by being kept for a sufficient length of time in oxygen-free gases. This emphasizes the need of good aeration for apples.

8. The softening of peaches seems to be decreased greatly by carbon dioxid and to a considerable extent by hydrogen and nitrogen.

9. Peaches become brownish and acquire a very bad flavor when oxygen is withheld from them.

10. "Ice-scald" seems to be injury due to insufficient oxygen and to an accumulation of carbon dioxid within the paper wrappers in which peaches are so often shipped. With good ventilation in conjunction with good refrigeration, such injury may be greatly reduced. This applies to fruits in storage as well as to those in transit.

11. Good ventilation in conjunction with refrigeration is of prime importance for the successful storage of fruit.

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