ABSTRACT

M.Sc. Willard W. Chan Horticulture

Occurrence of Sorbitol (D-glucitol) and Related Carbohydrates in *Malus* Fruits

The seasonal variation of sorbitol and its related carbohydrates in *Malus* fruits were studied both in field and in storage during two years. Data obtained by chromatographic analyses revealed that the initially high sorbitol concentration, which was followed by a gradual decline during the active growing period, was coupled with a subsequent rise at the ripening stage. Its progressive accumulation in stored fruits, compared to the fluctuating content of reducing sugars, suggests both an intermediate and a storage role for sorbitol. Its day and night variations were closely related to the season.

Fructose was the most prominent carbohydrate in fruits, while glucose was generally in excess of sorbitol. Starch fluctuated in a bell-shaped curve in growing season, and disappeared during early storage, whereas sucrose and raffinose occurred in limited quantities throughout.

Similar carbohydrate patterns were found in skin tissues, suggesting that the same carbohydrate metabolic reactions occurred both in pulp and skin, while the latter was also preliminarily observed capable of supplying a limited quantity of photosynthate for fruit growth.

OCCURRENCE OF SORBITOL (D-GLUCITOL) AND RELATED CARBOHYDRATES IN *MALUS* FRUITS

by

Willard Wing Lap Chan

A thesis submitted to the Faculty of Graduate Studies
and Research in partial fulfillment of the
requirements for the degree of
Master of Science

Department of Horticulture,
McGill University,
Montreal, Canada

April 1970

ACKNOWLEDGEMENTS

The author wishes to express his deep gratitude and most sincere appreciation to Dr. C.D. Taper, director of this research, professor and Chairman of the Horticulture Option, for his continued guidance, patience, constant encouragement, and unflinching interest throughout the course of this study.

Thanks are also extended to Professor H.R. Murray and Dr. J. David, professors in the Department of Horticulture, for their constant help and advice.

Acknowledgement is due to Dr. P.A. Anastassiadis, Department of Agricultural Chemistry, for his help on analytical methods; to Dr. B. Bible, Department of Horticulture, for his valuable advice on statistical problems; to Mr. C. Chong, fellow postgraduate student, for his help during the routine analytical work and in correcting the computer programme for the statistical analysis of the data.

The author is indebted to Mr. J.D. Laplaine, the former orchard foreman, for his assistance in the collection of fruits; to Mrs. B. Burdett, Secretary, and Mr. E. Gyapay, Senior Technician in the department for their constant and prompt cooperation whenever assistance of any kind was requested. The excellent work of Mrs. P. Nichols in typing this thesis and the help of Mr. M. Levesque with the French abstract are appreciated.

Finally, the assistance of the National Research Council of Canada, Ottawa, in the form of a generous grant to Dr. Taper and a research assistantship to the author, is gratefully acknowledged.

TABLE OF CONTENTS

Table of Contents (continued)

Table of Contents (continued)

GENERAL INTRODUCTION

Sorbitol (D-glucitol), the water-soluble alcohol of D-glucose, is the most wide-spread of all naturally occurring polyols. It is found naturally in plants, ranging from algae to the higher orders, and is prevalent in fruits. This sugar alcohol was first discovered in the fresh juice of the berries of the mountain ash, Sorbus aucuparia L. by Boussingault in 1872 (Hudson, 1945; Barker, 1955; Steuart, 1955; Lohmar, 1957), but its significance in plants was only extensively studied 40 years after the pioneer work begun by Vincent and Delachanal in 1889, who obtained needles of indefinite composition of sorbitol from many fruits of the family Rosaceae. Sorbitol was also found by other researchers to be present in almost all the members of this family (Reif, 1934; Strain, 1937; Donen, 1939; Ash and Reynolds, 1955a; and Sakai, 1961, 1966). Its distribution, presence or absence in various genera of this family is in complete accordance with its taxonomy (Plouvier, 1955, 1963).

The concentration of sorbitol in plants varies with different species. Donen (1939) found 2.8% of sorbitol in the total sugar present in Kelsey plums, while Nitsch (1953) found 1% fresh weight of sorbitol in young jacquin fruits, and 4.5%

in mature fruits. Eaton (1949) found a sorbitol concentration of 0.5% fresh weight in one-year-old apple shoots in the spring, while in the red seaweed Bostrychia acorpoides, Haas and Hill (1932) found its content to be 13.1%. Rees and Reynolds (1958), in a study of Victoria plums, found sorbitol content up to 5% of its dry weight, whereas **Stoll** (1968) found it to be as high as 25% of the dry matter in pears compared to only 4% in apple.

The metabolic role of sorbitol has long been established in mammalian systems, while only a few studies of this kind have been done in plants. Nevertheless, these few investigations have also cast some light and given us an insight into the physiological role of this sugar alcohol in plant tissues. Thus, Martin (1937) and Donen (1939) demonstrated that sorbitol served as a storage form for the hexose sugars during fruit growth when the latter had reached a maximum, while in storage it was firstly utilized as a carbon source for fruit respiration, and consequently disappeared to a minimum (Nitsch, 1953). In the study of the occurrence of sorbitol in Malus, Whetter and Taper (1963, 1966) suggested that sorbitol is a reserve carbohydrate in leaf buds, leaves, seeds and germinating seedlings. This role was ascertained by the subsequent study of Taper and Liu (1969) in apple leaves during growth and in fruits in storage.

The successive isotopic studies performed separately on leaves, fruits and phloem of apple and plum by Hutchinson (1958), Hutchinson et al. (1959), Anderson et al. (1959, 1961, 1962), Webb and Burley (1962), Williams et al. (1967) and Bieleski

(1969) show that sorbitol plays a central role in the metabolism of Rosaceae family. The facts that sorbitol is the most abundant photosynthate in leaves, that it serves as an index for the reducing sugars and their interconversion with each other, and that it is probably the major carbohydrate translocate have been recognized by the preceding researchers.

In the course of studies on the metabolism of sorbitol in plants, certain sugars especially fructose, glucose, sucrose, raffinose and a number of oligosaccharides, have always been found linked with this sugar alcohol (Nuccorini, 1932; Martin, 1937; Whetter and Taper, 1963, 1966; Williams, 1966; Stoll, 1968 and Bieleski, 1969). This is not only because of their close similarity in structure and properties, but also their ready interconvertibility with each other. The interconvertibility of sorbitol and fructose has been well demonstrated by Kidd et al. (1940) and Touster and Shaw (1962), and that of sorbitol and glucose by Hutchinson (1958), Anderson et al. (1962) and Whetter and Taper (1963). Therefore, the study of sorbitol is found inseparable from that of its related sugars.

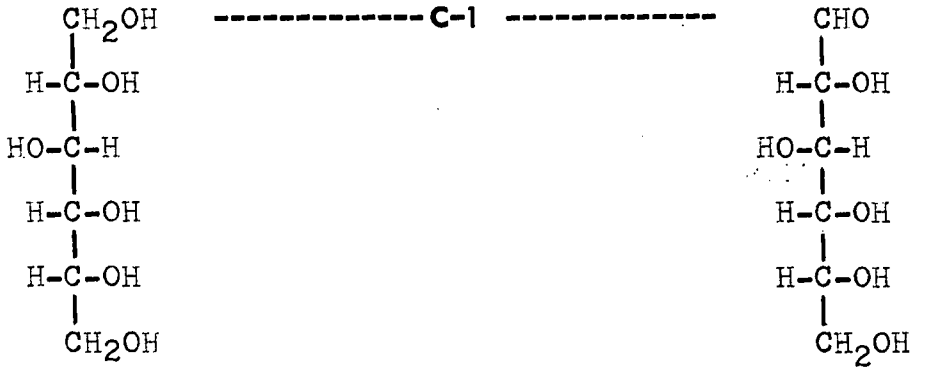
The present study was designed to investigate the seasonal occurrence and variation in content of sorbitol in Malus fruits and its relation to sugars and starch. Samples were taken both day and night, and from the field and storage. The results of experiments conducted over a period of two years, during growth and maturation, were examined and compared. Different groups of trees and a large number of samples taken at close intervals

were used to demonstrate the seasonal variations. Attempts were also made to determine the carbohydrate pattern in the skin of the fruit, for samples both on trees and in storage. A preliminary study on the photosynthetic capacity of the skin in relation to the fruit growth was attempted without much elaboration. The analytical methods used were chosen with the main objective of achieving maximum specificity, accuracy, precision and sensitivity (Anastassiadis and Common, 1968), while at the same time rapidity, economy and convenience were not sacrificed. Paper chromatography was employed to separate sorbitol and related sugars. Periodate oxidation with chromotropic acid was used for quantitative determination of sorbitol, while the phenol-sulfuric acid method was used to determine the contents of other sugars, and the perchloric acid-iodine method for starch estimation.

REVIEW OF LITERATURE

Structure of Sorbitol

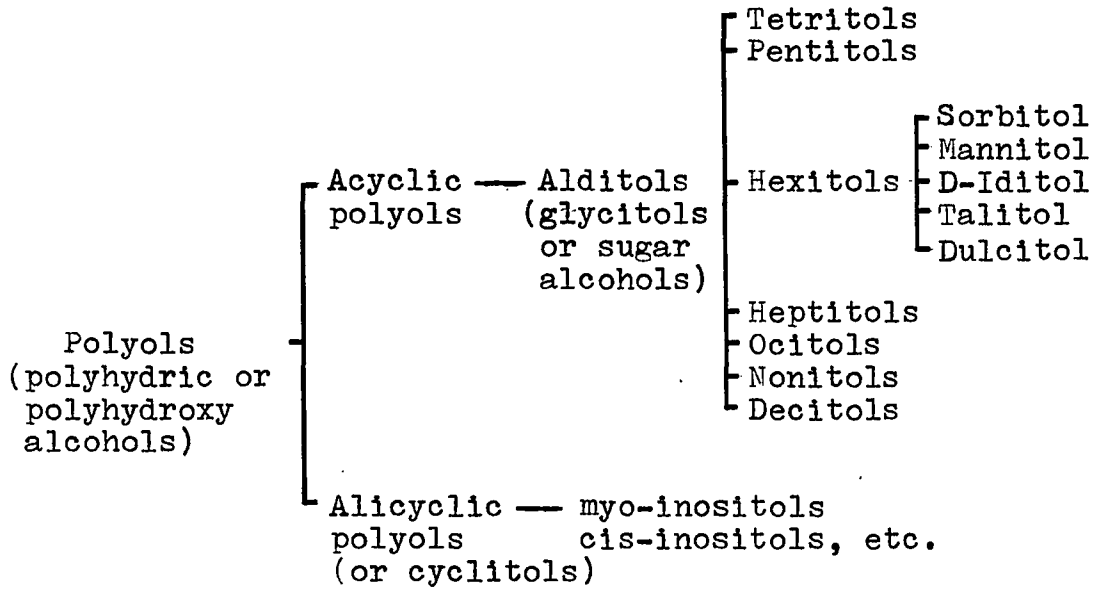
Sorbitol, sometimes referred to as D-glucitol, D-sorbitol, or sorbite, has a chemical structure of a long chain of six carbon atoms. It resembles glucose except for an alcohol group that replaces the aldehyde group of glucose on Carbon 1, thus sorbitol is also called the alcohol of glucose.



Sorbitol (D-glucitol) D-glucose

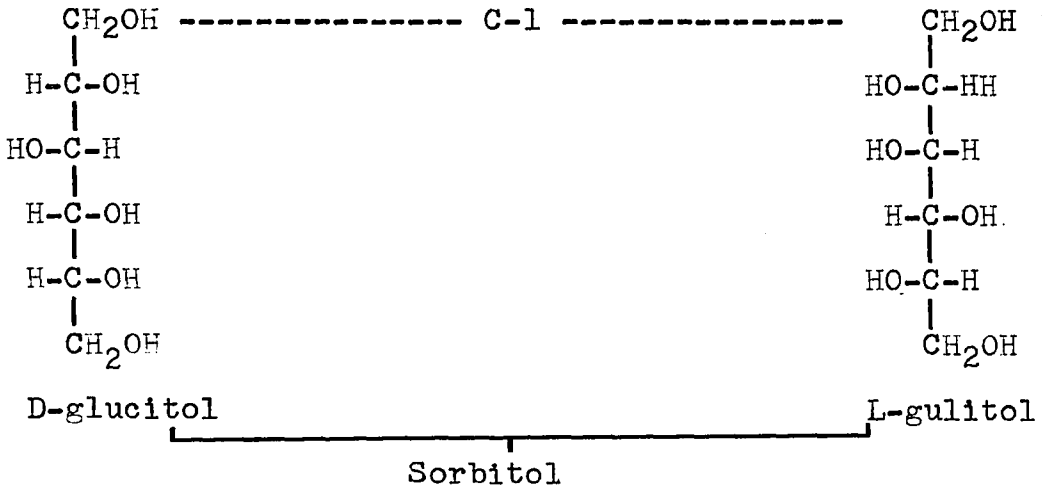
Like other stereoisomeric hexitols, D-glucitol is named by adding the suffix '-itol' to the root of the name of the parent aldose, glucose in this case, and sorbitol is usually

used as its common name. Sorbitol is a member of the family of polyols. This family of polyols, whose name is synonymous with the customary term polyhydric or polyhydroxy alcohols, is divided into two classes, the acyclic and alicyclic polyols, and sorbitol is in the first group, belonging to the division of alditols (or glycitols or sugar alcohols). The family tree of polyols can be shown clearly in the following (Pigman, 1957; Lohmar, 1957):



Since sorbitol is a compound of six carbons and its optically asymmetric end carbons have the same configuration, its allocation to the D- or L-series is faced with ambiguity in Fischer's classification, a classification which is followed essentially today in naming the enantiomorphous compounds. Fischer's method to allocate the D- or L-series of these compounds is made on the basis of the configuration of the bottom-

most asymmetric carbon atom, usually the penultimate carbon. Substances belong to the D-series have the hydroxyl group OH at the right, whereas it is on the left in the case of L-series compounds. The reference group like aldehyde CHO, Carboxyl COOH or Ketone CO is referred to the top. Optically like-end compounds of six or more carbon atoms wherein the end asymmetric carbons have the same configuration usually face this problem, since either of the penultimate (as in the case of sorbitol) carbon atoms can be used for the D, L-nomenclature. Among such compounds with six carbon atoms, only the configuration of sorbitol encounters this problem. Therefore, sorbitol can be called D-glucitol or L-gulitol because of their closely related structures except the different allocation of the hydroxyl groups at carbons 2 and 5. The structures of these two compounds are shown as in the following:



These like-end compounds of the type of sorbitol are called amphi by Rosanoff (1906), since they are related to two optically similar compounds. In naming these compounds, the most important of the two sugars, usually also the parent sugar, from which this compound is first derived, is chosen for the name and the D, L-assignment. Since sorbitol was first derived from D-glucose by reduction in 1890 (Hudson, 1945) and D-glucose is its parent aldose in the cyanohydrin synthesis (Pigman, 1957; Lohmar, 1957), therefore, in this case of sorbitol, the choice is D-glucitol rather than L-gulitol.

This choice is also supported by historical facts as seen from an excerpt from the article by Hudson (1945):

> Sorbitol is one of the few polyhydroxy alcohols which, like perseitol, can be designated either D- or L-, depending upon the arbitrary selection of one of the two aldoses from which it can be derived by reduction. Historically, the reduction of D-glucose to yield natural sorbitol is older than its synthesis by the reduction of L-gulose, and it seems advisable to the writer to use the historical order as the criterion in such cases, thus naming the natural hexitol D-sorbitol, which is in fact the generally accepted designation, first used by Fischer.

The doubts raised by Touster and Shaw (1962) and Liu (1967), who both quoted Lohmar (1957) as saying that if sorbitol was represented by the form D-sorbitol, as did by Hudson (1945) and others, was incorrect. Although they did not elaborate further nor provide evidence to support their arguments, the present thesis writer finds it necessary to elucidate this point more completely. As described above, the term sorbitol is only a trivial name, like levulose for D-fructose and dextrose for

D-glucose, which is based on a major natural source from which it is formed (Hudson, 1945), and is given to a compound long before its configuration is known and chosen. For the sake of simplicity, usage has established the name sorbitol for D-glucitol, like dulcitol for D-galactitol. Yet, this kind of common name could be used more properly without a D- or L-prefix as the name of the naturally occurring isomer (Wolfrom, Lew, Hales, and Goepp, 1945 and Pigman, 1957) despite the fact that many researchers have for long accustomed to using D-sorbitol as a convention name for D-glucitol (Bertrand, 1905; Tutin, 1925; Carr and Krantz, 1945; Hudson, 1945; Gordon et al., 1956; Adcock and Gray, 1957; Jones and Wall, 1960; Plouvier, 1963 and Grasshof, 1964).

Since the establishment of common names such as sorbitol is based on a major natural source from which this compound is derived, the similarity of these two names sorbitol and sorbose, which have both been named from the natural plant species Sorbus aucuparia L., has also given rise to the argument that natural sorbitol should be in the L-series because this L-glucitol is known to be one of the reduction products from L-sorbose (Lohmar, 1957) and the latter is in the L-series. However, the historical facts do not support this view. The first reduction of a sugar to natural sorbitol was from D-glucose by Meunier in 1890 (Hudson, 1945). Sorbose was discovered in the fermented and bacterially oxidized juice of the berries of the mountain ash, Sorbus aucuparia L. in 1852 by Pelouze (Lohmar, 1957),

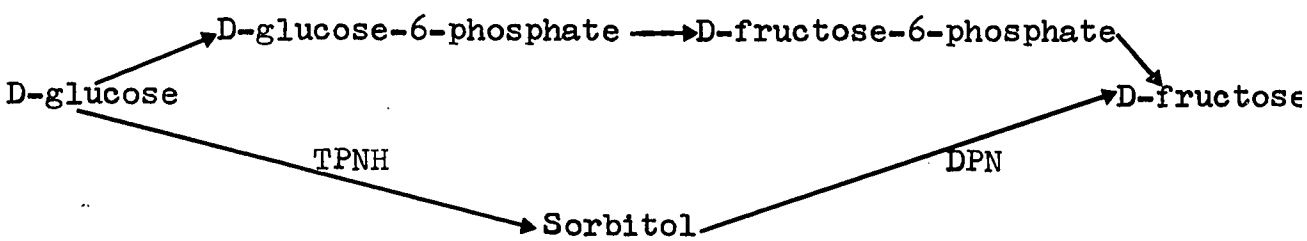
whereas sorbitol was discovered in the fresh juice of the same species by Boussingault in 1872 (Barker, 1955; Steuart, 1955). Although both substances were named from the same species, no chemical relationship between them is involved. Sorbitol was not named from sorbose, nor sorbose from sorbitol. If sorbitol should be in the L-form, i.e., L-glucitol, it would not have been from L-sorbose as suggested but from D-sorbose (Lobry de Bruyn and Alberda van Ekenstein, 1900, cited by Lohmar, 1957). Wolfrom et al. (1946) had also confirmed that L-glucitol was formed only by reduction of L-glucose or D-gulose but not from D-glucose or L-gulose. On the contrary, D-glucitol (sorbitol) is derived from the latter two compounds. Moreover, reporting collectively the properties and syntheses of sorbitol and L-glucitol, Lohmar (1957) pointed out that L-glucitol was not found in nature but it was only a synthetic substance from the reduction of D-gulose by sodium amalgam and by catalytic high pressure hydrogenation (Wolfrom et al., 1946), or it could also be synthesized by means of sodium amalgam (Lobry de Bruyn and Alberde van Ekenstein, 1900, cited by Lohmar, 1957).

Metabolism of Sorbitol and Related Sugars in Animals

Of all the polyhydric alcohols, sorbitol is the only one which has been extensively studied in mammalian systems. The investigation of this substance in animal tissues was initiated and stimulated by the work of Embden and Griesbach (1914) in their study of the metabolism of sorbitol in the liver of fasted or phloridzinized dogs. They found that a perfusion of this polyol in the liver tissues resulted in the formation of a mixture of fructose and glucose, and they suggested that glucose was not the primary product of this perfusion but it was subsequently transformed from fructose. This presumption was supported by the works of Anschel (1930) and Seeberg, McQuarrie and Secor (1955). By administering sorbitol to an individual with fructosuria, Anschel observed that there was a large increase in the excretion of fructose in the blood. Similarly, Seeberg and his co-workers found a prompt rise in blood reducing sugar when sorbitol was injected into dogs and rabbits. This reducing sugar was found to be D-fructose. When the amount of sorbitol in the blood was observed to be only moderate, it was presumed that this ketose was formed directly from this sugar alcohol. These findings, together with the close structural relationship between sorbitol and fructose, may well have explained the ready interconvertibility of them and that sorbitol is a normal metabolic intermediate reading utilized in animal tissues.

Williams-Ashman and Banks (1954), in their study of the relationship of sorbitol and fructose, found that in the various components of the reproductive organs of rats an enzyme was extracted and its function was found to be similar to "sorbitol dehydroglucose", an enzyme responsible for the interconversion of fructose and sorbitol. They suggested that this enzyme might be involved in the formation of D-fructose, the characteristic sugar of seminal plasma, and the mechanism of this sorbitol-fructose conversion was probably operated in these reproductive tissues. Earlier, Mann (1946, 1949) had proposed that, in sheep, this seminal fructose was formed through the successive conversion of glucose, glucose-6-phosphate and fructose-6-phosphate. With the last phosphate finally hydrolyzed by a phosphatase to fructose. This relates glucose to these complicated transformations and also places this hexose in its relationship to sorbitol. This relationship was later elaborated by Hers (1956 and 1960) in his alternate mechanism for the formation of fructose from glucose that glucose was first reduced to sorbitol in the presence of TPNH, followed by dehydrogenation of this polyol to form fructose. These two pathways as suggested by Mann and Hers are compared and summarized in the following:

Mann (1946 and 1949)



Hers (1956, 1960)

The pathway suggested by Mann (1946 and 1949) from D-glucose to D-fructose is indeed the reversion of the same pathway found by Blakley (1951) in liver slices of fasted rats, at the time when he studied the metabolism and antiketogenic effect of sorbitol in these tissues. He found that the D-glucose largely transformed from sorbitol was through the intermediates of fructose. This work further links these three carbohydrates together and demonstrates their close relationship with each other in the metabolic tissues:

sorbitol $\rightleftharpoons$ D-fructose $\longrightarrow$ D-fructose-6-p $\rightleftharpoons$ D-glucose-6-p $\longrightarrow$ D-glucose

Further, Blakley demonstrated that the above transformation of sorbitol to glucose was blocked if sorbitol dehydrogenose and coenzyme 1 were involved. Sorbitol was then directly oxidized to L-sorbose and D-fructose in this DPN-linked reaction without the deposition of D-glucose:

$$\text{sorbitol} \xrightarrow[\text{Co I}]{\text{DPN}\ \text{sorbitol dehydrogenase}} \text{D-fructose and L-sorbose}$$

This finding is essentially a reflection of part of Hers' alternate pathway for D-glucose and the initiation of the study of Seeberg et al. (1955) described above.

In his continued search for a better solution for the glucose-sorbitol-fructose pathway and relationship, Hers (1956 and 1960) stated that he found a large quantity of fructose in

the foetal blood of sheep and a glucose-reducing enzyme, aldose reductose was found present in the placenta of the same animal. This enzyme was not detected in other tissues except those involving in the production of seminal fructose. Sorbitol, at the same time, was also identified in both the foetal blood and seminal vesicles. These observations prompted Hers (1960) to suggest a following mechanism of fructose formation: the first step of this whole pathway being a reaction involving the reduction of glucose to sorbitol by aldose reductase and subsequent oxidation of this product to fructose by ketose reductase. Triphosphopyridine nucleotide (TPN) was involved in the first reaction while diphosphopyridine nucleotide (DPN) was used in the second aerobic glycolytic-breakdown oxidation. The first reaction occurred in the placenta while the second one took place in the foetal liver. The sequence of reactions was concluded by Hers as in the following:

(Reduction in placenta)	$\text{Glucose} + \text{TPNH} + \text{H}^+ \xrightleftharpoons{1} \text{Sorbitol} + \text{TPN}^+$	1-aldose reductase
(Oxidation in foetal liver)	$\text{Sorbitol} + \text{DPN}^+ \xrightleftharpoons{2} \text{Fructose} + \text{DPNH} + \text{H}^+$	2-ketose reductase
Sum:	$\text{Glucose} + \text{TPNH} + \text{DPN}^+ \rightleftharpoons \text{Fructose} + \text{TPN}^+ + \text{DPNH}$	

The same pathway was explained by Sherman and Stewart (1966) and Gabbay *et al.* (1966) in mammalian nerve.

A similar fate of these three carbohydrates including their utilization was explained by Adcock and Gray (1957). In their investigation of the metabolism of (U-^{14}C) sorbitol and related sugars in human subjects, they found that more than 70%

of orally administered labelled sorbitol were metabolized to carbon dioxide by normal and diabetic human subjects and that part of this sorbitol was converted into glucose. The fructose content in the blood was found not exceeding 2 mg./100 ml. and a conclusion was thus arrived at that the radioactivity of the blood sugar was mostly, if not entirely, due to glucose. Oral sorbitol therefore was probably converted in the liver into fructose, a proportion of which is converted into glucose, which might partly be stored temporarily as glycogen and finally released into the blood stream as glucose. That part of fructose not converted into glucose may be directly metabolized by the glycolytic pathway via fructose-6-phosphate. The mechanism for this pathway is similar to that of Blakley (1951) described above.

Initiated by the work of Cori and Shine (1936) who studied the formation of glucose from hexoses and trioses in the liver of the rat, Kreb and Lund (1966) worked further to compare the rates of this glucose formation from various precursors in kidney cortex of the same animal, and found that the rates of formation from hexoses, pentoses, polyols and related compounds were of the same order of magnitude as those in liver, an organ that converted various carbohydrates into glucose (Cori and Shine, 1935, 1936). Fructose was found to be the most active compound in forming glucose among these compounds tested. Next in effectiveness were D-glyceraldehyde and dihydroxyacetone. Sorbitol, along with D-galactose, D-sorbose and others gave low rate of glucose formation. They noted that the substrate concentration had an important effect on this formation wherein the

rate of formation of glucose from sorbitol and other polyols increased markedly when their concentration rose above 10 mM. The rates of various precursors for glucose transformation in sheep kidney were essentially the same as in rat kidney except fructose, dihydroxyacetone and glyceraldehyde which reacted rapidly in rat kidney to form glucose.

Metabolism of Sorbitol and Related Sugars in Plants

Occurrence and distribution

Since its first discovery from natural source by Boussingault in 1872 and the later confirmation by the works of Vincent and Delachanal (1889) and Bertrand (1905), sorbitol has been successively identified and isolated out in apple, members of the Rosaceae family and other plants or micro-organisms for the past 65 years. In the family Rosaceae, sorbitol was found in the genera Pyrus, Sorbus, Photinia, Crataegus, Pyracanta and Cotoneaster and the richest sources are the Sorbus and Crataegus (Lohmar and Goepp, 1949). In apple, Malus pumila, sorbitol has been identified in various parts, ranging from vegetative parts to reproductive organs: in leaves (Strain, 1937; Bradfield and Flood, 1949; Hutchinson et al., 1959; Sakai, 1961; Webb and Burley, 1962; Whetter and Taper, 1963; Lee, 1965; Williams, 1966; Williams et al., 1967; Bieleski, 1969; Taper and Liu,

1969), in buds (Whetter and Taper, 1963), in shoots (Eaton, 1949), in dormant shoots (Bradfield and Flood, 1950; Sakai, 1966), in phloem, twig bark (Webb and Burley, 1962; Sakai, 1966; Bieleski, 1969), in germinating seeds and seedlings (Whetter and Taper, 1966), in sieve-tube exudate (Zimmermann, 1961; Webb and Burley, 1962), in fruits (Tutin, 1925; Nuccorini, 1932; Reif, 1934; Strain, 1937; Simada, 1939; Ash and Reynolds, 1955a; Hutchinson, 1958; Hulme, 1958; Williams, 1966; Williams et al., 1967; Stoll, 1967a, 1967b, 1968; Buchloh and Neubeller, 1969; Taper and Liu, 1969), in fruit cortex (Gorrod, 1961), in fruit spur (Williams et al., 1967), in flowers (Strain, 1937).

In commercial apple products, sorbitol was detected in cider (Tutin, 1925; Strain, 1937; Steuart, 1955; Minsker, 1962); juice (Steuart, 1955; Tanner and Duperrex, 1968), apple wine (Hennig and Flintje, 1955), and candies, jellies and jams (Genest and Chapman, 1962).

The characteristics and contents of sorbitol have also been extensively studied in other members of Rosaceae family: in peach bark (Sakai, 1961, 1966; Rohrbach and Leupschen, 1968a; Buchloh and Neubeller, 1969), pear fruit (Muttelet, 1930; Reif, 1934; Martin, 1937; Strain, 1937; Kidd et al., 1940; Ash and Reynolds, 1955a and 1955b; Stoll, 1967a, 1967b, 1968; Buchloh and Neubeller, 1969), plum fruit (Donen, 1939; Donen and Roux, 1939; Rees and Reynolds, 1958; Buchloh and Neubeller, 1969), peach fruit (Buchloh and Neubeller, 1969), cherry fruit (Haeseler

and Misselhorn (1966), sloe (Lewis, 1963), quince, greengage, apricot (Vincent and Delachanal, 1889; Muttelet, 1930; Reif, 1934; Donen, 1939; Ash and Reynolds, 1955a, 1955b), fruits and leaves of medlar (Stanimirovic et al., 1963a, 1963b, 1964), twig bark of 19 species of fruit trees (Sakai, 1961, 1966), mountain ash (Boussingault, 1872; Vincent and Delachanal, 1889; Bertrand, 1905; Strain, 1937), sorbus commixta, Hudlund, (Asahina and Shimoda, 1930).

In other plants, ranging from lower plants to higher orders, sorbitol has been found in red yeast (Deinema and Landheer, 1960), young embryo of fern (DeMaggio and Wetmore, 1961), osmophilic yeast (Spencer and Sallans, 1956; Spencer and Shu, 1957), fungi (Vining and Taber, 1964; Wright and Le Tourneau, 1964), red algae (Haas and Hill, 1932, 1933), coconut meat (Hause et al., 1962), coconut milk (Pollard et al., 1961), raisin, currant and sultana (Reif, 1934), wheat stalk (Reisener et al., 1962; Bradfield and Flood, 1950; Reif, 1934), oak (Lippmann, 1927), leaves of copper beech (Olsen, 1948), squash fruit (Adcock, 1957), petioles of common plantain (Galkowski et al., 1966).

The role of sorbitol in plants in relation to its fluctuation, utilization and interconversion with its related sugars

When the role and metabolism of sorbitol in mammalian tissues have been extensively studied since the start of this century, its metabolism in plants has received little attention.

Most of the studies on sorbitol are restricted to its occurrence, isolation, and distribution (Barker, 1955; Bourne, 1958). However, for the past fifteen years, books on the physiology and biochemistry of sorbitol in plants had gradually acquired importance, and many valuable researches and investigations have been undertaken and reported. Among the people who have reviewed sorbitol in various aspects, Barker (1955) reported elaborately the general distribution of this sugar alcohol in various genera and species of plants. Lohmar (1957), while examining the origin of this polyol, related sufficiently to its structure, properties, uses and synthesis. Hulme (1958) reviewed extensively the role of sorbitol on fruits of various species with reference to its variations under different conditions. Lewis and Smith (1967a), in their study of sugar alcohols in fungi and green plants, related the natural occurrence of sorbitol and other polyols to their metabolism in lower and higher plants. The methods and techniques for identification and quantitative determinations were also described in detail.

In relating to the role and function of sorbitol in plant tissues, the work of Treboux (1909) is probably the earliest reference available to show the metabolism of this sugar alcohol in plants. After keeping some leaves from plants of the family Rosaceae in the dark to deprive them of starch, Treboux floated these destarched leaves in a solution of sorbitol and starch was again detected. Steele (1934) reported that this ability of sorbitol to form starch was specific for the family

Rosaceae, not mannitol nor dulcitol could be utilized to perform this function. She also stated that the Oleaceae could utilize mannitol and that Adonis vernalis could utilize ribitol but not any other. Experiments done by Trip, Nelson and Krotkov, (1965) further proved that this specificity in that when sorbitol was introduced into the leaves of white ash, only 1% of the total radioactivity moved out of the leaves and only 7% was detected in other compounds. This experiment therefore showed that sorbitol, unlike mannitol, which is specific for these species of white ash and lilac, was hardly translocated at all. These two findings indicated clearly the specificity of the metabolic systems of various families and their high selectivity for metabolites and conducting elements. This indeed is the original suggestion of Treboux and would be very useful for plant taxonomists in their assessing of plant classification, because the appearance of these polyols are in complete accordance with the taxonomy of the plants (Plouvier, 1955, 1963).

The close structural relationship of sorbitol, fructose and glucose has prompted various investigations done on them in animal tissues. However, in plants, this relationship has also been closely studied and extended with their respective linkages to oligosaccharides and polysaccharides. In their reviewing of polyols, Touster and Shaw (1962) stated that plant polyols originated from sugars by reduction and the reoxidation to sugars was the first step in their metabolism. Therefore, Nuccorini in 1932 found that sorbitol was transformed to glucose

and fructose in the detached fruits of sorbus domestica which were subsequently used for respiration. A similar transformation was found in Bosc pear of Martin (1937) who suggested that this conversion accompanied by a preferential oxidation of glucose in respiration, would lead to an excess of fructose over glucose in the fruit. In his study of the after-storage ripening of the same variety of pear, Martin (1937) reported that sorbitol decreased during the ripening process. Of all three pickings, the decreases in the non-sugar soluble solids were accompanied by corresponding decreases in sorbitol content. The most rapid decreases were found to take place in the first six to eight days when both sucrose and fructose were observed to rise with glucose remaining constant. During the next four days, sorbitol again declined together with sucrose, while fructose and glucose were found increased in content. Martin suggested that this inverse relationship of sucrose and these two reducing sugars might probably be due to the hydrolysis of the former to the latter.

In Donen's study (1939) of the changes in the contents of sorbitol and related sugars during the growth of the Kelsey plums, the concentration of sorbitol was found to increase constantly during the period of cell enlargement. Most of the sorbitol was observed to accumulate during the latter part of its growth cycle after the stone growth was completed and when the concentration of reducing sugars had reached a high amount. The ripe fruit contained 2.8 gm. of sorbitol per 100 gm. fresh

weight, or in some instances, as high as 4.5%. These observations led Donen (1939) to suggest that sorbitol was used as a form of storage for these two hexoses, i.e., fructose and glucose when they had reached a maximum concentration. In his continued work with Roux (Donen and Roux, 1939), they stated that the loss of respirable materials from stored plums could be related to the depletion of sorbitol, sugar and acid. Sorbitol and sugars were both lost inside storage but the loss of the latter were dependent upon the initial concentration of sorbitol. Plums of originally low sorbitol content showed marked sugar loss only when most of the sorbitol had disappeared. On the other hand, when plums were moved from 1°C. to 7.5°C. or 20°C., a 10-15% increase was observed in the total sugars but this increase again was dependent upon the sorbitol content and its rate of exhaustion from storage. These findings all seem to come to an agreement that sorbitol is an important metabolite in the fruits and its metabolism with that of other sugars was intimately interrelated.

This intimate relation of sorbitol to other sugars as shown by Martin (1937, Donen (1939) and Donen and Roux (1939) was further elaborated by the work of Kidd, West, Griffiths and Potter (1940), who found a considerable amount of sorbitol in the Conference pear fruit. During storage at 10°C., it was found that much of the sorbitol present was converted to fructose; this was the original idea of Martin (1937), but this sorbitol-fructose conversion was not a direct process as suggested by him

but an indirect one, as was modified by Donen (1939). Similar steps had been previously demonstrated in animal systems as follows:

Sorbitol ⟶ Intermediates ⟶ reducing sugars

This was also the mechanism of conversion of sorbitol to reducing sugars found by Strain (1937) in his study with developing pears.

In agreement with the findings of Martin (1937) and Donen (1939), Nitsch (1953), in his collective review, stated that sorbitol was probably the substrate used in respiration during storage as well as a storage form for the reducing sugars in fruits, like plums and pears. Yet in contrast to the suggestion of Martin (1937), Nitsch stressed that there was no obvious relation between the concentration of sorbitol as that of sucrose in the plum and that the changes in concentration of sucrose paralleled the rate of respiration of the fruits during their development more closely than those of glucose and fructose. His suggestion that sucrose and sorbitol were not related might contrast with the work of Gorrod (1961). This worker was able to show that when an extract of an acetone powder preparation from the fruit cortex of Miller's Seedling apples was treated with sorbitol in the presence of boiled yeast extract, an acid labile non-reducing sugar was formed and it was probably sucrose. The difference between the findings of these two researchers may be due to the difference in the purpose of their individual studies, one being concerned with the metabolism

in the living tissues (plum), while the other investigated the synthesis of sucrose in the presence of apple extract and yeast. The use of yeast may contribute largely to this sucrose synthesis. McCready Hassid (1941) reported that while many free sugars like glucose, fructose and mannose, etc. were capable of being converted to sucrose to some extent in barley leaves, neither mannitol nor sorbitol were converted to this disaccharide in the same way. These workers, therefore, suggested that in barley there were enzymes which could convert monosaccharides to disaccharides but no particular enzymes which could oxidize these sugar alcohols to their corresponding aldohexoses.

In contrast to the finding of Donen (1939) that sorbitol maintained a constant increase during plum growth, Rees and Reynolds (1958) reported that in his developing Victoria plum that the concentration of sorbitol appeared in a seemingly constant level of 5 gm. per 100 gm. dry weight all through the whole growing season. The first of the two growing periods was characterized by the absence of sucrose and higher glucose concentration than fructose. However, sucrose appeared in the second period when fruits were ripe, while glucose and fructose, at this time, were found to slightly decline. Glucose was the major sugar during the whole growing period, whereas it was replaced by sucrose during the over-ripe stage. While the general pattern of sugar changes in this finding is essentially that of Donen (1939), the accumulation of sucrose during the over-ripe stage is in contradiction with that of Martin (1937)

and the constant level of sorbitol during growth is also diversed from that of Donen and Roux (1939). If sorbitol was not accumulated when the other hexoses had reached a maximum level, as in the case of Rees and Reynolds (1958), it could probably not be regarded as a storage form for these sugars. However, when the technique of Rees and Reynolds for the quantitative determination of sorbitol was examined, his measurement done on a spot area basis for the quantity of sorbitol was not as accurate as that used by Donen (1939) or the periodate oxidation method recommended by Lewis and Smith (1967b) for paper chromatography. The present thesis writer also noticed in his experimental work that the quantity of sorbitol or other sugars did not necessarily correspond to its spot area revealed on the paper chromatogram. Therefore, the accuracy of the quantitative changes of sorbitol and related sugars as reported by Rees and Reynolds was probably not clearly shown and should not be necessarily used as a standard for comparison.

In the study primarily concerned with phloridzin, Hutchinson, Taper and Towers (1959) found D-glucitol C^{14} to be the largest single ethanol-soluble carbohydrate in metabolizing apple leaf disks following the incorporation of D-glucose-C^{14} or $C^{14}O_2$. When D-glucose-1-C^{14} was incorporated, the distribution of radioactivity for D-glucitol-C^{14}, sucrose and glucose were found to be in the proportion of 7.0:2.2:0.2 in the 80% ethanol-soluble fraction of these leaf disks. These results

were able to furnish important evidence for their suggestion that sorbitol played a central role in the carbohydrate metabolism in Malus leaves. Subsequent work on apple was done by Whetter and Taper (1963) who reported that sorbitol content exceeded that of glucose and sucrose in leaf buds and leaves of Malus, by Whetter and Taper (1966) who observed the appearance of sorbitol in the apple seeds before germination, during germination and in all organs of the developing seedlings and by Taper and Liu (1969) who found sorbitol the most abundant ethanol-soluble carbohydrate in Malus leaves. The findings of these researchers all confirmed that sorbitol was the most important metabolite in Malus and that it plays a central role in the metabolism of this species.

In their study of sorbitol occurrence in apple leaf buds and leaves, Whetter and Taper (1963) found that the concentration of sorbitol gradually increased in mature leaves of Malus as cessation of growth approached. This observation was confirmed by a similar datum of Taper and Liu (1969) who found also a progressive accumulation of this sugar alcohol in the leaves from July to September. It was also observed that there was a constant level of sorbitol in the leaf buds prior to leaf expansion and photosynthesis (Whetter and Taper, 1963). Accidentally, this presence of sorbitol in non-developing tissues resembled the work of Bradfield and Flood (1950), who detected sorbito in dormant apple shoots. By summarizing the above results, and recalling the finding that a large amount of D-glucitol-C^{14} was

found in the ethanol-soluble fraction of metabolizing leaf disks (Hutchinson, et al., 1959), these workers (Whetter and Taper, 1963; Taper and Liu, 1969) strongly suggested that sorbitol was used in Malus as a reserve form of carbohydrate.

In view of the close structural similarity of sorbitol and glucose, Whetter and Taper (1963) demonstrated in another experiment using C^{14} labelled compounds incorporating into apple leaf disks that sorbitol was found in disks fed D-glucose-C^{14} and glucose in those fed D-glucitol-C^{14}. In a subsequent experiment with Malus seeds and seedlings (Whetter and Taper, 1966), these workers found that sorbitol was accumulated in light but not in the dark. This suggests that this large amount of sorbitol formed after germination may depend on the production of excess sugars during photosynthesis and that, because of the interconvertibility of glucose and sorbitol, a rapid conversion of this hexose formed during the photosynthetic process to sorbitol is highly probable. Apart from the demonstration of the interconvertibility of these two carbohydrates, the role of sorbitol as a reserve carbohydrate for other sugars when in excess was again shown.

A similar demonstration of sorbitol-glucose relationship has been made by Anderson, Andrews and Hough (1962), who found a rapid synthesis of D-glucitol from D-glucose when D(1-C^{14})-and D-(6-C^{14})-glucose was fed into plum leaves. About 40% of the C^{14} was found in D-glucitol after five hours, and the labelling patterns obtained showed that this conversion occurred probably

without the rupture of the carbon chain. This again stressed the intimate relationship of sorbitol and glucose and this was in accordance with the statement that this polyol was the only product from glucose by direct reduction (Touster and Shaw, 1962).

In studies involving the photosynthesis assimilation of $C^{14}O_2$ by plum leaves, Anderson, Andrew and Hough (1959, 1961) showed a rapid fixation of $C^{14}O_2$ into sorbitol than starch during photosynthesis. Fifteen per cent was newly synthesized from labelled precursors in three hours after photosynthesis in a high $C^{14}O_2$ concentration. Despite the large amount of sorbitol accumulated, it was observed that there had been a rapid turnover of this polyol so that there was no net change in level during a 20-hour period. Sorbitol was presumably at this time equilibrated with the other primary products of photosynthesis and the utilization of this sugar alcohol in plum leaves was also slow. In their investigation of the fate of C^{14} labelled D-glucose, D-glucusonic acid and D-glucitol in metabolism (Anderson et al., 1962), these workers further demonstrated this slow rate of uptake of D-glucitol by incorporating D(1-C^{14})-glucitol into plum leaves. After five hours, 82% of the labelled sorbitol fed was still present in the same form and only 18% of the activity was detected in the leaves in other compounds. This slow utilization of sorbitol by plum leaves to form other compounds, when compared with D-glucose and D-glucuronic acid, was termed by these researchers an insufficient metabolic process

in plum leaves. This finding was similar to that of Trip et al. (1965) who introduced sorbitol into the leaves of white ash and lilac and observed that only 7% were found in other compounds; and among these compounds only three corresponded to known substances. This slow utilization and translocation of sorbitol in these plants was probably due to the high selectivity of a particular species for metabolite (Plouvier, 1963), in which mannitol was selected rather than sorbitol. However, the exact reason for this slow metabolic activity of this sugar alcohol in plum leaves, a species in which it usually plays a large part (Andrews and Hough, 1958; Anderson et al., 1961, 1962), remains obscure. There is a possibility that sorbitol here may act as a form of reserve carbohydrate in the leaves for other sugars as suggested by Whetter and Taper (1963, 1966).

When studying the seasonal variations of polyhydric alcohols and sugars in fruit trees, Sakai (1961) found a considerable amount of sorbitol and mannitol in the twig bark of many species. Sorbitol was found to about 1% wet weight in apple tree, gardenia, pomegranate and mountain ash. The concentration of these two polyhydric alcohols increased by two to three fold in winter than that in summer and their total amount ranged from 40% to 55% of the total sugar content. From these results, Sakai concluded that these sugar alcohols might in some way relate to the frost-hardiness in woody trees. In his subsequent work (Sakai, 1966), he observed again a similar pattern of sorbitol and mannitol in the same fruit trees from

summer to winter. Although Sakai finally did not conclude definitely their role on frost hardiness because of the lack of accurate quantitative methods, he commented that the presence of these polyols required close attention for their roles on the various physiological aspects of fruit trees.

Agreeing with the findings of Whetter and Taper (1963) that a gradual accumulation of sorbitol was found in mature leaves, Williams (1966) reported in his study of sorbitol content in relation to watercore formation in apple fruits that in periods of leaf senescence, the gradual accumulation of this sugar alcohol from summer was replaced by a decline. This decline in turn was coincided with an increase in sorbitol content and watercore in the fruits. This was particularly obvious in the variety Red Delicious which contained over 8% of sorbitol and was badly watercored. The vascular tissue samples collected showed an increase in the level of sorbitol and total sugars with advancing maturity. While the sugar content in the exudate of watercored apples was only one-fourth that of non-watercored fruits, watercored fruits had four times as much sorbitol as did the normal fruits. Williams concluded that this watercore development in apple fruit could be attributed to the accumulation of sorbitol in the intercellular spaces of the fruit tissues. This accumulation was intimately connected with the transfer of this polyol from the leaves where it was found as the major photosynthate, particularly at the maturing stage. The observation from Harley (1938) that the amount of watercore in fruits was directly proportional to the leaves per apple further

provided evidence to support this leaf-to-fruit transfer of sorbitol as a cause of this adverse development.

However, the finding of Williams (1966) that apple leaves contained as much as 10% sorbitol, in which it was the most abundant photosyhthate. This was supported by the recent data of Taper and Liu (1969). They reported that sorbitol was the most prominent and fructose the least abundant constituent in apple leaves whereas the opposite was true in the fruits. The concentration of sorbitol in leaves at the time of harvest was reported by them at a level of 21.76 mg. per gram of fresh fruit tissue.

Echoing the investigations of sorbitol in fruit trees by Webb and Burley (1962), Whetter and Taper (1963) and Williams (1966), Stoll (1967a, 1967b, 1968) in Germany have successively investigated the changes of this sugar alcohol and sugar content in many varieties of apple and pear fruits during growth and storage. Sorbitol content in pear varieties was usually many times as that in apple fruits and its concentration in pear fruits might be as high as constituting 25% of its dry matter compared with only 4% in apples (Stoll, 1968). During the period of growth, the increase of sorbitol in most of these two species almost paralleled their fruit enlargement. This increase of sorbitol during growth was coincided with the picture observed by Donen (1939). In storage, the sorbitol content in pear rose at the expense of the other carbohydrates which, as explained by Stoll (1968), was caused by a considerable

proportion of the assimilates accumulating in the forming of sorbitol. This conclusion supports the suggestion by previous workers that sorbitol and its related sugars are interconvertible, and the gradual accumulation of sorbitol in storage further confirms the proposal of sorbitol being a reserve carbohydrate.

While Williams (1966) found a large quantity of sorbitol in the vascular tissues and cores of watercored apples, Stoll (1967b) found this distribution in an opposite direction in normal pear and apple fruits. He reported that the distribution of this sugar alcohol in these fruits was not homogenous, the highest concentration was found near the epidermis and the least near the core. This was consistent in the three varieties each of apple and pear fruits. Partly because of this observation, together with the similar findings by Martin (1936) and Siegelman (1954), the present thesis writer was inspired to investigate the pattern of sorbitol and related sugars in the skin of the apple fruits and the results obtained are presented subsequently under the appropriate headings.

Stoll (1967b) was probably the first researcher to observe that there was a relationship between C-A storage conditions and the content of sorbitol. He reported that there was a marked decrease in sorbitol content in apple and pear during ordinary cold storage, but when the atmosphere of the stored room was modified with carbon dioxide, sorbitol content was shown to have decreased less, and a slight increase was observed at the

end of the storage period. It is understood that the main objective of the C-A storage is to slow down the rate of respiration of the stored materials by applying a high carbon dioxide to the atmosphere. Sorbitol, the probable respirable substrate for fruits in storage as suggested by Martin (1937), Donen and Roux (1939) and Nitsch (1953), was accordingly reduced in consumption in Stoll's fruits. Moreover, the slow consumption of sorbitol and also the subsequent interconversion between it and its related sugars may probably account for its slight increase at the end of the storage.

In an isotopic study of the fate of sorbitol and related sugars in apple trees, Williams, Martin and Stahly (1967), showed that, apart from the fact that the rate of sorbitol translocated in apple tree was more rapid than that of sucrose, the resulted autoradiogram of the treated lyophlized tissues indicated also that the apple fruits received more of the sorbitol-C^{14} than the new leaves on the same spur, and that this movement into the fruits was very rapid in all three sorbitol-C^{14} applications. After watercore had developed, sorbitol continued to move into the fruit tissues and subsequently the watercored area contained the most radioactivity. These results also appeared to support the suggestion raised by Williams (1966) that sorbitol accumulation in the vascular tissues contributed to the development of watercore. However, the present thesis writer doubts if the sorbitol accumulation inside the fruits is the major cause for this disorder, or the inflow of this sugar alcohol into the fruit

is a result of the watercore development. Moreover, it was noticed that the concentration of sucrose in the vascular tissues, juice and exudate of watercored fruits as shown in Williams' data (1966) exceeded that of all other carbohydrates, including sorbitol itself. Will it finally be shown that the metabolism of sucrose inside the fruit, or the fact as reported by Williams et al. (1967) that high susceptibility for watercore development was observed in places where the growing season was short, contributes more to this development? Or will it be shown that the high metabolic sink formed by the accumulation of sucrose, other sugars and solutes inside these aged tissues causes the influx of this sugar alcohol, and in turn accounts for its watercore development? Since sorbitol is a non-polar polyol (Lewis and Smith, 1967a) and it is metabolically more inert than sucrose and glucose once it is accumulated, such as in the phloem tissues (Bieleski, 1969), its presence in the fruit tissues does not seem a highly probable cause for this watercore development rather than a result of it.

In comparing the rate of accumulation of carbohydrates in fresh or aged phloem tissues, Bieleski (1969) demonstrated that sorbitol, sucrose and glucose were actively accumulated by both tissues; but the rate was much higher in aged tissues -- about 13 times of that of fresh tissues, and this accumulation in aged tissues was more notable for sorbitol than for the other two. Further, the carbohydrates were most rapidly accumulated from the higher external concentration of sugar and this, too,

was most notable for sorbitol. The rate of sorbitol accumulation from 10^{-2} M sugar solution was approximately 29 times that from 10^{-2} M solution for both aged and fresh tissues.

Sorbitol in fresh phloem tissues was observed remaining in its original form without changing into other sugars, amino acid, lipids or polysaccharides (Bieleski, 1969). Yet, in the aged tissues, sorbitol was found to convert into sucrose, probably via glucose, thus the concentration of sorbitol in these tissues decreased markedly with a rise of sucrose. This high amount of sucrose, if the metabolic pathway in the phloem tissues is the same as that in the fruit tissues, may probably account for the high level of sucrose in the watercored (physiologically aged) fruits of Williams (1966). Moreover, the fact reported by Bieleski that sorbitol was accumulated more rapidly in aged tissues than in fresh ones seems to provide another evidence for the suggestion raised by the present thesis writer that the high level of sorbitol accumulated in the watercored apples of Williams (1966) is not necessarily the cause of the disorder, but a result of it. These physiologically aged tissues in watercored fruits, as described by Williams (1966), tend to accumulate large quantities of sorbitol from other organs of the trees, notably from its leaves. Once this sugar alcohol is accumulated, it is metabolically more inert than sucrose or glucose. Only when there develops a system which can convert sorbitol to sucrose does sorbitol enter the metabolic pathways (Bieleski, 1969). Therefore this pool of sorbitol appears to

behave more like a storage form of sugar which is drawn in by the aged tissues. It does not appear to contribute to the aging process and to watercore development.

The role of sorbitol in plant translocation

For the past twenty years, in the studies of carbohydrate translocation in plants, sucrose was generally regarded as the most important carbohydrate translocate. Using an isotope technique to investigate this translocation in sugar beet, Turkina (1954) found that when $C^{14}O_2$ was fed to the leaves, sucrose was the first radioactive substance entering the phloem and that it was quickly translocated to the roots. Similarly, Pavlinova (1954) demonstrated that sucrose was rapidly synthesized in the leaves and conductive tissues of this sugar beet plant from fed C^{14}-labelled hexoses and it was also the principal substance transported from the leaves down to the roots. Ziegler (1956) reported that sucrose was the only sugar present in the sieve-tube exudates of 12 species of European trees under investigation. The same was found true by Zimmermann(1957). Swanson and El-Shishiny (1958), Swanson (1959) and Zimmermann (1960) observed that the C^{14}-hexose/C^{14}-sucrose ratio was decreased downward from the supply leaf of grape, and suggested that sucrose was the only sugar translocated in the grape and hexoses were only secondary hydrolytic products. In the study of sorbitol and related sugars in germinating seeds and developing seedlings of Malus, Whetter and Taper (1966) noticed that

the changes in sucrose concentration followed closely the growth of the seedlings, and significant amounts were found in roots in both the daylight and dark experiments. Therefore, they suggested, also, that sucrose was an important translocate in apple seedlings.

However, although evidences for sucrose-translocating were overwhelming in most of the literatures, a suspicion that hexoses or other forms of carbohydrates were the translocates in the plants persisted for many years. In 1952, Dana reported that when $C^{14}O_2$ was supplied to leaves of dwarf Yellow Delicious apple trees, most of the radioactivity recovered from bark sample was present in glucose, while sucrose was only detected in the secondary amount and fructose the least. This work prompted the subsequent deatiled study of Webb and Burley (1962) in their assessment of sorbitol as one of the important translocates in apple phloem. Eaton (1949) found a sorbitol content of 0.5% in one-year-old apple shoots in the spring, while Bradfield and Flood (1950) reported that sorbitol and other related sugars were present in the dormant shoots of apple, pear, and plum. Later, Zimmermann(1957, 1958) detected a large amount of sugar alcohols in the sieve-tube exudates, notably mannitol in the Oleaceae and sorbitol in the Rosaceae (Zimmermann, 1961) and this finding led him to suggest that these sugar alcohols might be important translocates in the plants. The above findings all seemed to indicate that sorbitol served in one way or another the role of carbohydrate translocate in plants, especially in species like

apple and members of Rosaceae family. Nevertheless, this role was subsequently confirmed and demonstrated by the works of Hutchinson (1958) and Kursanov (1961) who showed respectively that in the apple and sugar beet, sorbitol was the translocatory form for the hexose sugars.

Inspired by the work of Dana (1952) and Zimmermann(1958 and 1961), Webb and Burley (1962) further searched for a better experimental method for identifying and demonstrating that sorbitol was the major translocatant in apple phloem. By rigorous chromatographic procedures, they found that sorbitol was the compound containing the major portion of radio-activity in the stem of apple after the leaves were exposed to $C^{14}O_2$. They reported that most of the activity originally thought to be in glucose as reported by Dana (1952), was probably present in sorbitol. Therefore, they concluded that sorbitol, along with sucrose, was probably the principal carbohydrate transport material in the phloem of apple and in other species of the Rosaceae.

Sakai (1961, 1966) reported that in the twig bark of 12 species of fruit trees, especially pomegranate, gardinia, apple and mountain ash, mannitol and sorbitol were found in considerable amounts, which increased by two to three times in winter over its amount in August. Fructose, glucose, sucrose, raffinose and stachyose were also detected, along with these sugar alcohols. Although further work was not carried out because of the lack of accurate experimental methods, Sakai, however, pointed out

that more consideration should be given to the role of these polyhydric alcohols in the transport of soluble organic substances in the plants and in other physiological problems.

In support of the suggestion put forward by Webb and Burley, Williams et al. (1967) elaborated in their investigation on the fate of sorbitol and related sugars in the apple tree and fruits that sorbitol was absorbed and translocated much faster than sucrose. More than twice as much radioactivity was extracted from the sorbitol-treated plant as from the sucrose-treated one. Movement of both sorbitol and sucrose tended to go upward to the active growing points. Although some downward movement was observed below the point of C^{14} application, no radioactivity was detected in the roots in either chemical-treated plants. This finding may deny in some way the questions raised by Liu (1967) who asked what happened to sorbitol and sucrose after they had reached the roots. Since no labelled sorbitol,nor labelled sucrose,nor compounds in other forms were found in the roots in this study, it would seem probable that sorbitol was readily translocated to and metabolized, not in places of mature tissues, but in the actively growing regions, and the direction of this translocation was more favoured, towards the top than downward to the roots (Bieleski, 1969).

In an extensive study of the accumulation and translocation of sorbitol in apple phloem, Bieleski (1969) summarized all the previous investigations done on this subject and

demonstrated his suggestions for the fate of this sugar alcohol and its related sugars in apple with regard to its utilization and accumulation. He agreed with Webb and Burley (1962) that sorbitol was translocated in the phloem of the plant, not in the cortical tissues, and it was a primary translocate. He demonstrated that phloem, when separated from all other tissues, contained over 70% of the total soluble radioactivity in the form of sorbitol. Sorbitol was always found as the major translocatory product in the phloem samples harvested within a time of ten minutes after the start of photosynthesis. This again confirmed that sorbitol was the major translocate in the apple.

In an attempt to elucidate whether the role of sorbitol in phloem transport is an active or passive process, Bieleski (1969) first demonstrated that the distribution of sorbitol in cortex, wood, leaf and phloem was predominantly over other carbohydrates. If sorbitol was passively drawn into the phloem, Bieleski explained, that serine and phosphate esters, the major early photosynthates after short periods of photosynthesis in the leaf, would also be present in these tissues. But these two compounds were not detected, indicating that sorbitol was translocated into the phloem by itself, or in other words, through an active process. The same view of active sorbitol transport was shared by Williams (1965) in his personal communication with Liu (1967) that when C^{14} was fed into leaves in the period just after fruit set, sorbitol was observed to transport rapidly to the developing fruits. Since the fruit became labelled much faster and to a larger extent than the leaves on the same spur,

Williams concluded that this movement was highly probably not a passive transport but rather an active one. However, Bieleski admitted that in phloem this sorbitol transport was taking place mostly after the process of accumulation had begun. At high concentration, sorbitol was accumulated as readily as sucrose or glucose in the phloem, and the ratio of this entry of carbohydrates into the phloem resembled their ratio in the leaf. Once these carbohydrates were accumulated, there was very little tendency for sorbitol to be converted to sucrose or fice versa. Therefore, the composition of the translocation stream, with sorbitol predominant, assured the active movement of these carbohydrates in the phloem. Seeing this restricted utilization and sparing metabolism of sorbitol in the phloem tissues, Bieleski suggested that this sugar alcohol was the logical and important translocatory substance. Further, this worker concluded that the active growing regions consumed sorbitol readily and low concentration was therefore found at those places. This finding echoed a similar picture shown previously in the developing stem and cotyledons of Whetter and Taper (1966), and in the active growing fruits of Taper and Liu (1969). Bieleski continued that maturing tissues tended to lose this ability of mass utilization and sorbitol was therefore observed accumulating in these tissues at high levels. This accumulation of sorbitol probably coincided with the same picture in the maturing pear and apple fruits of Stoll (1967b, 1968), in the maturing leaves of Whetter and Taper (1963) and Taper and Liu (1969) and in the watercored apple tissues of Williams (1966).

In conclusion, the functions and role of sorbitol in apple, as described and interpreted above and in previous chapters, could be well summarized by an excerpt from Bieleski (1969), who contentedly concluded:

> It is therefore felt that there is no conflict between the results of Webb and Burley (1962) and Whetter and Taper (1966), but rather that each represents a different view of a single situation. Sorbitol is a major translocation product because it is a major photosynthesis product. It can be accumulated as such by the phloem cells, and its conversion to or formation from other compounds can depend on the state of the tissue. Its function is that of a somewhat unusual storage compound: there appear to be interesting parallels between the behaviour of sorbitol in the apple, and that of mannitol, ribitol, and other polyols in lichens (Lewis and Smith, 1967a; Richardson and Smith, 1968).

Uses of Sorbitol

Sorbitol is perhaps the most abundantly used of all the hexitols. Forty years ago, the cost of sorbitol obtained from natural sources limited considerably its general application in various fields. However, since the development of a process for laboratory syntheses of sorbitol from D-glucose by reduction with sodium and amalgam or by pressure hydrogenation using platinum or nickel, sorbitol is no longer a chemical curiosity, but an established cheap chemical compound of commercial importance. Its usage is observed in various fields such as pharmaceutical, food and commercial applications.

As early as 1929, sorbitol was introduced into therapeutics in Europe under the name of "Sionin" by Thannhauser and Meyer. Since then, various workers had investigated the

possibility of sorbitol as a substitute carbohydrate in diabetes. Kaufmann (1929) reported that sorbitol exhibited a protein-sparing action and was a useful adjunct in the diabetic diet. Gottschalk strongly recommended sorbitol's use in diabetes because it had an "insulin-enticing" action (cited by Carr and Krantz, 1945). Sorbitol owes its value to diabetes because it is capable of being stored as glycogen in the liver and its subsequent depolymerization and utilization fail to supply the blood a plethora of glucose which would produce hyperglycemia (Carr and Krantz, 1945). Nowadays, sorbitol enjoys a wide use in the clinical field as an acute diuretic. If it is intravenously injected in high dosage, it will have a laxative effect, and this property led to its use as an adjunct to therapy with ion-exchange resins (Touster and Shaw, 1962). Sorbitol may also be used to promote dehydration in overhydrated patients, and to lower or eliminate the requirements for B vitamins in human and animal diets. It is also able to increase the absorption of Vitamin B_{12} in rats and human subjects, and in pregnant rats it promotes the transport of this Vitamin B_{12} from maternal tissues to the foetus (Touster and Shaw, 1962).

With the production of the cheap crystalline glucose, from which sorbitol is derived and that sorbitol is moderately sweet and hygroscopic in nature with a caloric value of four calories per gram (Olmsted, 1953), this sugar alcohol is widely used in the food industry as the low-calorie component in manufacturing confectionery, candies, jellies and jams, etc.

(Genest and Chapman, 1962), and an addition of 1% of sorbitol is said to increase the sweetening power of saccharin three fold in canned fruits (Walker, cited by Steuart, 1955). In the detection of the degree of adulteration of grape wine and vinegar, fruit wine is added and the measurement of the amount of sorbitol in it gives the extent of this adulteration (Steuart, 1955; Minsker, 1962). Since the oxidation of sorbitol by microorganism is usually the best method for its conversion to ketose and the yield is high, this biochemical reaction is therefore often used as the preferred commercial process for converting sorbitol to L-sorbose, which in turn is the intermediate in the synthesis of ascorbic acid, Vitamin C. By means of hydrogenolysis, sorbitol in a mixture of other polyhydric alcohols, was used in Germany during World War II to make "glycerogen" which is a humectant. The monoesters of sorbitol have surface active properties and contain sorbitan, the dehydrated form of sorbitol, which make them useful to be emulsifiers and stabilizers under the commercial name "Span" and "Tween" (Lohmar, 1957; Graham, 1963). Moreover, nitrate esters of sorbitol are important explosives and pharmaceuticals.

In plants, sorbitol plays many other roles beyond those described in previous sections. When it is infiltrated into the leaves of oats, it will serve as one of the most efficient carbohydrate precursors of ascorbic acid (Devyatnin, 1950). Tandon and Grewal (1954) found that sorbitol was the best carbon source for the growth in culture of Alternaria tenuis isolated

from apple. Sorbitol can also be utilized for polysaccharide synthesis: it is converted to starch in the detached leaves of Rosaceae (Steele, 1934; Barker, 1955), and utilized in the biosynthesis of cell wall carbohydrates (cellulose as xylan) in the wheat plants (Brown and Neish, 1954; Altermatt and Neish, 1956).

Sorbitol has been reported as a major constituent of coconut milk and may be present in other liquid endosperms (Pollard, Shatz and Steward, 1961). Since polyols have rarely been proved very effective in embryo culture (Lewis and Smith, 1967a), its presence in coconut milk may not be a sole nutrient for the development of embryo. It is interesting to observe that young embryo of the fern, Todea barbara, which might not rely on endospermous nutrition, could complete their development when excised in a mixture of sorbitol and inositol, instead of coconut milk (DeMaggio and Wetmore, 1961).

The presence of sorbitol in plant tissues can, however, give rise to some adverse effects; Silverman (1960) demonstrated in his study in stem rust on wheat leaves that sorbitol, and to a lesser extent mannitol, induced blackening and abnormal uredospores in Puccinia graminis when infected wheat leaves were placed in solution of these polyols in the dark. Williams (1966), as described above, reported that the accumulation of sorbitol in fruit tissues might contribute to the development of water-core in apple fruits. Although Rohrbach and Luepschen (1968a) could not find out any clear evidence to show that the occurrence

of sorbitol, together with mannitol, had a positive relation to the pycnidiospore germination and to the susceptibility of cytospora canker infection in peach bark tissues, sorbitol was found to be about three to four times that of mannitol throughout the sampling season and significant amounts of these two polyols were also found in the early season of the three late maturing varieties under investigation. Since a definite enhancement of pycnidiospore germination was observed with mannitol *in vitro*, and to a lesser degree with sorbitol (Rohrbach and Luepschen, 1968b), their presence in peach bark indicates that they may be related to this susceptibility.

EXPERIMENT 1

Variability in the Concentrations of Sorbitol, Related Sugars and Starch in *Malus* Fruits during Periods of Growth and in Storage

A. Materials and Methods

Introduction

The materials and experimental procedures for the three sections under Experiment 1 were identical. These procedures included sampling of fruits, extraction, clarification, chromatography, elution and quantitative determination. The fruit samples were collected according to a statistical design for two sample-groups and at ten-day intervals from both field and cold storage. A sample at a fixed fresh weight was extracted with 70% ethyl alcohol for a continuous 8 hours. The preliminary extract, after successive filterings and washings, was evaporated down to a specific level to evaporate off the alcohol, and then made up to a final volume as required. This known volume of extract was subsequently clarified, using lead acetate and sodium oxalate, and the final clear extract obtained was spotted on the paper chromatogram. After developing and spraying the

chromatogram, each of the separated carbohydrates on the paper was cut out and eluted with distilled water. Color reaction tests were performed withthe eluates, using appropriate methods, and the resultant transmission readings of the various colored solutions were taken in the spectrophotometer at different and appropriate wavelengths. The quantitative result for each of the carbohydrates at a corresponding transmittance was calculated from the individually pre-constructed standard graph.

Sampling of fruits

In sampling apple leaves for the determination of sorbitol and related sugars, Whetter (1962) carried out an elaborate statistical experiment on the population variability of these carbohydrates in the leaves, and showed that their contents could be accurately determined statistically using seven samples in a group with each sample taken from an individual tree. In sampling fruits in the present experiment, it was decided to follow her method as described in her thesis. However, since the size of the fruits varied with the season, being very small at the beginning and later becoming much larger, it was not possible to fix the number of fruits per sample without considering their weights. It was decided to take samples from 14 trees of Malus pumila, cultivar McIntosh in two rows, each containing a group of seven trees. An equal number of fruits was picked from each tree on each sampling date, both day and night. The number of fruits taken from each tree each time decreased

with the increasing size of the apples, but each sample provided a minimum of 50 gm. analytical material. The samples from the two groups of trees were analyzed separately under identical experimental conditions, and the results obtained were fed into a computer at the end of the experiment. They were compared by the 'paired T-test', a statistical test of significance that is used to reveal differences between two paired sets of values (Goulden, 1952; Steel and Torie, 1960).

The two groups of trees chosen were in Row 11 (sample-group I) and Row 6 (sample-group II) in Block 9 of the Macdonald College Orchard. These trees were all nineteen years old, and uniform with respect to size and shape. They were planted in the orchard in the same soil type and cultivated under the same sod-mulch-system. In order to minimize experimental errors, all fruit samples were taken from the south side of each tree. Uniform fruits of approximately the same size, free from disease and injury were selected and labelled the night before sampling. All fruits were from two-year-old shoots 3 to 6 feet above the ground.

Since the number of fruits taken for analysis decreased with their increase in size, more fruits were taken during the first few sampling dates than later. For example, on the first sampling date, 35 fruits, five from each of the 7 trees in the same sample-group, were taken, making 70 fruits in total for the day samples and 70 for the night. As the season advanced, the number was reduced progressively. From July 16, 1968 onward,

each sample-group consisted of seven fruits, one from each of the 7 trees in this group.

In 1968, sampling was first started on June 6, about a week after full bloom, when little fruits were about the size of peas. Fruits were picked at ten-day intervals, both day and night at 10:00 a.m. and 10:00 p.m., respectively. Samples were placed in plastic bags and brought to the laboratory as soon as possible. They were then weighed, and immediately frozen at -12°C. for later analysis. Since fruits in early season were relatively small, they were frozen in toto after the petioles were removed and the fruits cleaned. As fruits became bigger in later days, it was deemed inappropriate to use all the fruit tissues for analysis. Therefore, after July 16, only pulp was used. The fruits were cleaned, peeled, quartered and cored, with seed removed. Samples were packed in plastic bags with labels and stored at -12°C. to inactivate enzymes.

After harvest on September 22, selected fruits from all 14 trees were placed in numbered boxes in refrigerated storage at 32°F. Sampling was from groups, as in the field; and also at ten-day intervals until the beginning of May, 1969.

During the summer of 1969, samples were taken according to the procedure used in 1968. However, the first sample was taken on June 16, 1969, about two and a half weeks from full bloom because fruit set was delayed by cold weather. Sampling time was at the same ten-day intervals and fruits were selected in the same manner as in 1968. Nevertheless, no night samples were collected in 1969. Because of the reduction of the night

sample load, fruits collected from the field could immediately be processed for extraction without going through the frozen stage. The first sample consisted of 35 fruits while seven fruits were collected on September 4 and thereafter. No fruit samples were examined from storage during 1969.

Extraction

Fruit samples were individually blended in a Waring Blendor and the blended materials were thoroughly mixed. From each sample, 50 gm. of the blended fruit materials were taken for extraction.

After weighing, each 50 gm. fresh blended sample was washed by a stream of 70% ethyl alcohol through a funnel into a 500 ml. round-bottomed flask with joint. The latter was then connected to a Graham condenser, with inner and outer T-joints. The sample was just covered with 70% ethyl alcohol. In this dilution of ethanol, Whetter (1962) found sorbitol more soluble than it was in 80%, and the present author also found it satisfactory (see Techniques, An Investigation and Appraisal). The sample was then refluxed for a continuous eight hours. Ten to twelve samples were refluxed at the same time. The extract was then cooled, filtered through Whatman No. 1 or 41 filter paper, using a Buchner filter under vacuum. The residues on the filter were first washed well with 70% ethanol and afterwards with distilled water. After complete rinsing of the residues, the filtrates and washings were combined in a 500 ml. beaker and

evaporated on a hot plate to a volume a little below 100 ml. (for 1968 field samples), or 50 ml. (for 1968 storage samples) or 25 ml. (for 1969 samples). All alcohol was driven off during this operation. The washed alcohol-insoluble residues in the filter were dried and bottled as described in the section, Starch Determination, for starch analyses.

Clarification

A combination of the methods described in the official analytical method of the A.O.A.C. (10th Edition, 6.075 and 29.021 (c), 1965) was used for clarifying the colored extracts with fine suspended materials obtained from the preceding extraction. Dry solid basic lead acetate, specifically for sugar analysis, was employed as the major clarifying agent. Before the clarification was made, extracts obtained in the previous section were made up to volume with distilled water, namely, 25 ml., 50 ml. or 100 ml. This was important because a certain volume of the extract tended to be lost during the later lengthy series of precipitations and filtrations.

A small quantity of dry solid basic lead acetate was added to the extract and the mixture was shaken thoroughly. More salt was added and the solution shaken again until a flucculent precipitate was produced and the precipitation was complete. The solution was then allowed to settle for 15 minutes and the supernatant was tested with little solid lead acetate.

The solution was shaken and let stand again if more precipitate formed; if no further precipitate appeared, the solution was filtered through Whatman No. 50 filter paper. Enough solid sodium oxalate was added to the filtrate to precipitate the excess lead. The whole solution was permitted to settle for 10 minutes and filtered. The filtrate was further tested for lead with sodium oxalate until a negative result was shown. The mixture was again allowed to stand for 15 minutes and the supernatant was poured into a centrifuge tube and centrifuged for five minutes in an International Clinical Centrifuge at full speed, 8800r.p.m. The fine suspended particles that had escaped the above filtering process were separated at the bottom of the tube, and the clear supernatant was decanted into a specimen vial used for spotting, or kept frozen until required for chromatography.

Chromatographic separation of sorbitol and related sugars

One-dimensional descending paper chromatography was employed to separate sorbitol and its related sugars on the same chromatogram.

(1) Paper preparation

Chromatographic grade Whatman No. 1 filter paper (thickness 0.16 mm., medium flow rate), size 56 1/2 x 23 cm. was cut into two equal strips of 23 x 56 1/2 cm. and each was used for chromatography purposes. A long, thin pencil line was drawn

along the middle of the strip separating it into two halves from one end to the other. Another line was again drawn perpendicular to the previous one, 10 cm. from the end of the paper that was dipped into the solvent trough during the later developing process. Five dots were marked along this line for future spot-application and they were at a reasonably equal distance with three dots on one half of the strip and two on the other. Therefore, when extracts were applied later, these pencil dots were filled up with the following five spots of extracts:

----------11 1/2 cm.------ ----------11 1/2 cm.------

Dot:	1	2	3	4	5
Extract:	sample A	Mixture of standard compounds under investigation	sample B	sample B	sample A

(2) Spotting

Spots were carefully applied to the pre-marked positions, one drop at a time, with a 5 or 10 µl. Drummond disposable 'microcaps' micropipette distributed by Kensington Scientific Corporation, Oakland, California. The quantity of extract applied varied from 10 to 30 µl., depending on the concentration of the extract, but sometimes as high as 70 µl. were used as required by an individual sample. Each spot was dried immediately with an Oster airjet hair dryer before next application was made. The spots when applied on the paper were kept as small and compact as possible and not exceeding 0.5 cm. in diameter. A marker

spot consisted of a mixture of standard compounds under investigation was applied alongside with the sample spots. Its position on the chromatogram was shown in Section (1) above.

(3) Development of chromatogram

A mixture of methyl ethyl ketone (butanone)-glacial acetic acid-water saturated with boric acid (9:1:1/v:v:v) was used as the solvent system for separating sorbitol and related sugars. This solvent system was proposed by Rees and Reynolds (1958) and first used by Britton (1961) to separate sorbitol from other compounds in animal tissues; and later by Webb and Burley (1962) to separate sorbitol and sugars in the stem bark of apple. It was modified by Genest and Chapman (1962) to detect sorbitol in diabetic foods, and was again used by Whetter and Taper (1966) for sorbitol-sugar separation in apple seeds and seedlings, and by Taper and Liu (1969) in apple leaves and fruits. It was recommended by Lewis and Smith (1967b) as the best solvent for polyol separation on paper chromatograms.

The papers, after spotting, were kept in a Pyrex chromatography jar 12 x 12 x 24 inches, covered with a seamed glass plate, with edges greased with vacuum vaseline and weights on top, and a dish of the solvent mixture was placed at the bottom to saturate the jar overnight. The solvent from a separatory funnel was directed through the four holes drilled on the cover plate to the solvent troughs inside the jar. The jar was placed in the chromatography room where the temperature was always kept constant at 22°C. (room temperature) and free from

outside disturbance. The time for development was 38-40 hours. Overlapping of spots was observed on the chromatogram if the running time was less than 35 hours while sorbitol, the compound with the highest Rg value in this solvent system, ran off the paper when developing time was over 45 hours. The solvent was allowed to drip off the lower edge of the paper and fresh solvent was added through the holes whenever necessary, but usually once every 12 hours. A maximum of four papers were run at the same time. After the development was complete, the chromatograms were carefully taken out of the jar without dripping on the others or contaminating them with dirt. All the chromatograms were then dried in the air until all the pungent odor was gone and the whole paper was dry.

(4) Spot detection

Before any detection procedure was undertaken, the paper strip was first cut into two halves according to the line previously drawn for this purpose. Only that half with the marker spot of standard mixtures was used for spot detection. The other half was kept for later eluting and quantitative determination purposes.

The chromatogram was sprayed with a solution of 1% aqueous solution of potassium permanganate and 2% sodium carbonate (1:1/v:v). The oxidation of sorbitol and sugars with permanganate gave yellow spots on a purple background on standing for 7-10 minutes. The color gradually changed to dark grey or deep brown in a light brown background in about 25 minutes.

The spots were marked with pencil to locate the area of the carbohydrates separated on the chromatogram.

The two components of this spray reagent were prepared separately and kept in brown bottles. They were mixed according to proportion immediately before use. The stock solutions could be used even after storage for several months. Besides being easy to prepare, this reagent detected all the required carbohydrates under investigation on the same chromatogram.

Eluting the carbohydrates from the chromatogram

(1) Elution

The zones in which sugars were contained were traced out on the unsprayed portion of the chromatogram with reference to the detected carbohydrate spots on the sprayed one. Ample space was given to each zone so that no part of the materials contained in the paper was missed. The paper was then cut into different sections with respect to the different carbohydrates, and each was placed in a polystyrene disposable petri dish of size 60 x 20 mm. or 100 x 15 mm., depending on the size of paper sections cut. Five ml. of distilled water were syringed in to completely cover the whole piece or pieces of paper. The dish was then shaken gently by hand or by a mechanical shaker for a continuous five minutes, and then it was shaken from time to time for five hours to have the carbohydrates eluted out (Dubois et al., 1956; Spiro, 1960). All samples were accompanied by blanks of the same sizes cut from plain paper and eluted in the same way. This was used

to eliminate the color that might have developed in the course of the reaction between the dissolved cellulose lint from the paper and the reagents employed in the later quantitative determination of sugars. Nevertheless, the reagents in the method for sorbitol determination was shown not to give rise to any interfering color with cellulose lint.

(2) Centrifugation

Since the eluate obtained after the above elution was contaminated with a fine suspension of cellulose lint from the paper, it was found necessary to clear it by centrifugation. The eluate in the petri dish was decanted into a 5 ml. centrifuge tube and spun for three minutes at full speed in the centrifuge. The clear supernatant was then poured into a specimen vial for immediate quantitative determination or kept in the refrigerator for a brief period until time was allowed for determination.

Quantitative determination

(1) Determination of sorbitol

The method for the determination of sorbitol, initially proposed by Lambert and Neish (1950), is based on the oxidation with periodic acid, in which the excess periodate formed is removed by arsenite in an acid medium and the formaldehyde formed is determined colorimetrically with chromotropic acid. This method has been widely employed for analytical purposes by workers such as Adcock (1957), Sakai (1961, 1966), Anderson

et al. (1961), Whetter and Taper (1963, 1966) and Taper and Liu (1969). This method was highly recommended by Lewis and Smith (1967b).

a. Reagents

Chromotropic acid reagent: One gm. of chromotropic acid (1, 8-dihydroxynaphthalene-3, 6-disulphonic acid) was weighed out accurately and dissolved in 100 ml. distilled water and the solution was filtered. To 150 ml. distilled water was added 300 ml. of concentrated sulfuric acid. This was cooled, and added to the above yellow brown chromotropic acid solution (sulphonic acid mixture). The volume was made up to 500 ml. and kept refrigerated in a brown glass-stoppered bottle. This reagent was prepared fresh every two to three weeks.

Sulfuric acid (10 N): 280 ml. of concentrated sulfuric acid was added cautiously to 600 ml. of water and the volume was adjusted to 1 liter when the solution had been cooled.

Periodic acid (0.1 M): 4.56 gm. of periodic acid was dissolved in water and the volume made up to 200 ml.. The solution was stored in a brown bottle in the refrigerator.

Sodium arsenite (1.0 M): 22.5 gm. of sodium hydroxide pellets and 50 gm. of arsenic trioxide (arsenous acid, As_2O_3) was dissolved in 500 ml. of distilled water and stored in a brown bottle.

b. Procedures

In a 10 ml. volumetric flask, 2 ml. of sample eluate (or 2 ml. solution made up with 1 ml. each of sample eluate and distilled water, depending on the concentration of the eluate) were acidified with 0.1 ml. of 10 N sulfuric acid; 0.5 ml. of 0.1 molar periodic acid was then pipetted into the flask. With mixing, 0.5 ml. of molar sodium arsenite was added in the same way five minutes after the previous addition. Iodine appeared in the solution 10 or 20 seconds later and then faded, which was due to the reversibility of the reaction between arsenite and iodine.

After five to ten minutes, the contents of the flask were made up to mark with distilled water and mixed thoroughly. One ml. of this solution was pipetted into a test tube and 10 ml. of the chromotropic acid reagent was syringed in, by means of a 10 ml. hypodermic syringe without a needle, to get a complete mixing and the tubes were heated for 30 minutes in a boiling water bath to have the color developed. Care was teken to avoid direct light from falling in the tubes during the process of heating. The tubes were then cooled and the percentage transmission was determined at 570 mμ. in the spectrophotometer using standard cuvettes.

A standard curve was prepared from pure standard solutions containing 20 to 200 μg. sorbitol in 2 ml. of solution and sample values were read off from this curve. It was prepared for each analysis to compensate for differences in

the chromotropic acid reagent prepared at different times. Blanks were run with all determinations to obtain the correct 100% transmission setting and samples were done in triplicate.

(2) Determination of sugars (fructose, glucose, sucrose and raffinose)

Since samples in this thesis project were numbered to hundreds, it was found too slow to use different analytical methods for different sugar determinations as was previously done by the researchers in this department. Therefore, before this sugar determination was started, efforts were made to search for a precise, accurate but simple method which was also satisfactory for all the sugars under examination. The phenol-sulfuric acid method (Dubois et al., 1956) employed here was found to give reliable results for all these four sugars and the color produced in the reaction between sugars and the reagents was unusually stable (see Techniques, An Investigation and Appraisal) and possessed a definite absorption peak. The intensity of color formed at a constant phenol concentration was proportional to the amount of sugar present. Moreover, the manipulation was simple and rapid, and the reagents were inexpensive and readily available. The standard curves obtained by plotting the sugar concentration versus the absorbance could be readily reproduced (Dubois et al., 1956). This method was reported by Whetter (1962) to require less stringent conditions than the anthrone method which most researchers used for sugars.

a. Reagents

Sulfuric acid: Reagent grade 95.5-98.0%, conforming to ACS specifications, sp. gr. 1.84.

Phenol: 80% by weight, prepared by adding 20 gm. of distilled water to 80 gm. of redistilled reagent grade phenol. This mixture forms a water-white liquid that is readily pipetted and can be kept for several months.

b. Procedures

Two ml. of sugar eluate (or 1 ml. each of eluate and distilled water), were pipetted into a cuvette and 0.05 ml. of 80% phenol were added. Then 5 ml. of concentrated sulfuric acid was added rapidly by means of a syringe without needle, the stream of acid being directed against the liquid surface rather than against the sides of the tube in order to obtain a good mixing. The cuvette was allowed to stand 10 minutes, and then it was shaken and placed for 10-20 minutes in a water bath at 25-30°C. before readings were taken. The color was stable for several hours.

The absorbance of the characteristic yellow-orange color was measured in a spectrophotometer at 490 mμ. for all the four sugars. Blanks were prepared by substituting distilled water for the sugar solution. The quantity of sugar in the eluate was calculated with reference to a standard curve previously constructed for the particular sugar under examination. All solutions were prepared in triplicate to minimize experimental errors.

(3) Starch determination

The micro-quantitative method for starch determination of Carter and Neubert (1954) was followed. Slight modifications were made wherever they were found most suitable for the present determination.

a. Reagents

7.8 N perchloric acid prepared from 72% perchloric acid, 'Baker Analyzed' Reagent, F.W. 100.46.

5% potassium iodide.

0.01 N potassium iodate.

0.16 N sodium thiosulfate.

b. Procedures

The damp alcohol-insoluble residue in the filter after the sugar extraction was placed in the porcelain evaporating dish and dried in the oven at 100°C. overnight until the weight was constant. Then, this dry residue was weighed and ground in a Swiss DFH 48 Culatti grinder with fine meshes to a very fine powder. This powdered residue was bottled and labelled for analysis.

About 0.5-1.0 gm. of the dry ground powder was weighed into a 100 ml. beaker containing 4 ml. of distilled water, and 20 ml. of 7.8 N perchloric acid ($HClO_4$) (1 and 7)* were added by a syringe with rapid initial stirring to avoid high

---

*1 to 7: Notes regarding techniques can be found in the chapter, Techniques, An Investigation and Appraisal.

acid concentrations. Digestion was allowed to proceed for five to ten minutes at room temperature (2), in which the starch in all samples was completely dispersed with maximum development of color. The mixture was then diluted with distilled water so as to retard hydrolysis and eliminate the neutralization step (Nielsen et al., 1945), and filtered through Whatman No. 1 filter paper (3). Thorough washings of the residue with distilled water were made to get all starch particles down to the filtrate. Thereafter, the filtrate was transferred to a 250 ml. volumetric flask and made up to the mark (4).

In order to obtain fractional transmittance values between 20% and 80%, aliquots of different volumes, 5, 10 and 20 ml. etc. (5) from the 250 volumetric flask were transferred to several 100 volumetric flasks. To each flask, 1 ml. each of 5% potassium iodide and 0.01 N potassium iodate was added and five minutes was allowed to permit the iodine and starch to react. The solution was mixed by shaking vigorously and made up to 100 ml. with distilled water. The fractional transmittance was read at 620 mμ of the red filter in a Coleman Junior Spectrophotometer. With standard cuvettes, the instrument was set at 100% transmittance with a blank prepared from the same aliquot of starch and reagents as the sample and 2 drops of 0.16 N sodium thiosulfate (6) were added to remove the starch-iodine color, which ranged from light yellow to dark green, depending on the

concentration. Of the different mixtures in different 100 ml. volumetric flask, only the one with a transmittance between 20% and 80% was chosen for the results. Each sample was made in triplicate. The percentage of starch in fresh apples was calculated from a standard curve prepared by using pure potato starch powder which was shown to have approximately the same amylose-amyropectin ratio as in McIntosh apple starch (Poapst et al., 1959).

To make the standard curve, the fractional transmittance values were determined from a digest of 0.25 gm. of pure potato starch powder with 4 ml. of distilled water and 20 ml. of 7.8 N perchloric acid for five to ten minutes. The digest was diluted and transferred to a 250 ml. volumetric flask. Different aliquots (0.5, 1.0, 1.5, 5.0, 10.0 ml., etc.) of starch content were transferred to 100 ml. volumetric flasks, 1 ml. each of potassium iodide and potassium iodate reagents, was added as in the above procedure and transmittance readings taken. This method was proved to be rapid, efficient, economical and accurate at micro-levels. Large numbers of samples could be processed within a short period without tedious operations.

B. Results and Discussion

The pattern of variation in periods from early fruit growth to maturation during years 1968 and 1969

(1) Results

By using the solvent system, butanone-glacial acetic acid-water saturated with boric acid (9:1:1/v:v:v) and the detecting reagents as described in Materials and Methods, sorbitol, fructose, glucose, sucrose and raffinose were revealed on the same chromatogram in an order of decreasing R_g* values of 4.08, 1.75, 1.00, 0.50 and 0.10, respectively. Paired T-test for paired variates were performed for comparing the results obtained in sample-groups I and II for the year 1968 and the same was undertaken for the two groups in 1969. Another two sets of paired T-tests were used to compare the results in sample-group I of 1968 and 1969, and in sample-group II of these two years. All these paired T-tests were computed by the computer programmed in the formula specifically for two sets of paired values, as the samples from these two sample-groups were originally set up for paired comparison. The observed T values (see Appendix for computing samples) for all these compounds, namely, sorbitol, fructose, glucose, sucrose, raffinose and starch in these two sample-groups of each year were shown to have no significant difference, indicating a good correlation among these two groups and the validity

---

*R_g=glucose (migration relative to glucose).

of the results of the statistical design for sample collection (see Sampling under Materials and Methods, and Whetter, 1962), and also the reliability of the experimental techniques used for analyzing the various samples at different dates in the same season. A mean datum was then computed from results of these two sample-groups in each year and is presented in Table 1 and shown graphically in Figures 1 and 2 respectively.

a. Variation in 1968

At the beginning of the season when fruits were about the size of peas and leaves had not become fully developed, sorbitol content in the fruits was at highest concentration compared to the rest of the samples taken in the field. For the first two weeks after full bloom and when fruits were just setting, sorbitol increased slightly and had its highest peak in mid-June at 5.38 mg. per gm. fresh weight. Thereafter, when fruits grew rapidly and leaves became fully expanded, sorbitol decreased continuously. This trend was continued until mid-August when sorbitol concentration reached the lowest point in the season at 1.23 mg. per gm. fresh weight, which was several times lower than its initial concentration. However, when growth cessation had started and fruits were ripening, sorbitol content in the fruits rose slightly and remained at a rather constant level until the end of the season. Its concentration in the fruits at the time of harvest was lowered by two to three fold its initial amount when fruits were just formed.

Table 1. Sorbitol, related sugars and starch in *Malus* fruits from early fruit growth to maturation for years 1968 and 1969 (mg./gm. fresh weight, mean data)

Sample no.	Date	Sorbitol	Fructose	Glucose	Sucrose
	1968				
1	6/6	4.36	7.24	1.88	0.72
2	16/6	5.38	6.71	4.02	1.20
3	26/6	2.75	18.47	1.31	0.54
4	6/7	1.85	18.34	1.12	0.91
5	16/7	2.85	24.97	1.77	1.86
6	26/7	2.06	47.96	3.17	1.87
7	5/8	1.50	35.11	3.74	1.49
8	15/8	1.23	38.87	3.49	1.20
9	25/8	2.09	40.95	3.22	0.88
10	4/9	1.50	40.21	3.97	0.71
11	14/9	1.83	49.60	0.72	0.27
	1969				
1	16/6	9.23	12.55	12.69	1.02
2	26/6	5.34	16.84	13.00	1.09
3	6/7	6.42	18.42	18.17	1.30
4	16/7	6.50	32.13	33.25	1.60
5	26/7	6.44	26.63	24.63	1.60
6	5/8	4.66	42.63	23.63	2.38
7	15/8	6.32	29.38	29.00	2.78
8	25/8	4.22	28.75	35.00	2.13
9	4/9	5.60	44.25	42.63	3.19
10	14/9	5.35	48.00	40.00	4.82
11	24/9	5.44	43.75	37.75	3.16

*Reducing sugars: Fructose and Glucose.
**Total sugars: Fructose, Glucose, Sucrose and Raffinose.
***Total carbohydrates, starch excluded: Sorbitol, Fructose, Glucose, Sucrose and Raffinose.

Table 1 (continued).

Raffinose	Starch	Reducing sugars*	Total sugars**	Total carbohydrates***
0.77	0.20	9.12	10.61	14.96
0.21	0.26	10.72	12.13	17.51
0.25	0.23	19.78	20.56	23.30
0.07	2.35	19.45	20.42	22.27
0.00	8.01	26.73	28.59	31.38
0.08	5.86	51.13	53.07	55.12
0.41	5.83	38.85	40.74	42.24
0.11	8.80	42.36	43.66	44.89
0.19	7.25	44.16	45.23	47.32
0.25	4.50	44.18	45.14	46.64
0.34	3.69	50.32	50.93	52.76
0.92	0.12	25.24	27.17	36.40
1.21	0.01	29.84	32.13	37.47
1.25	0.92	36.59	39.13	45.55
2.75	3.31	65.38	69.72	76.22
1.51	9.36	51.25	54.35	60.79
1.37	9.72	66.25	70.00	74.66
1.66	10.20	58.38	62.81	69.13
1.03	8.39	63.75	66.91	71.13
2.35	7.31	86.88	92.41	98.01
0.94	4.06	88.00	91.76	97.10
0.69	0.55	81.50	85.35	90.79

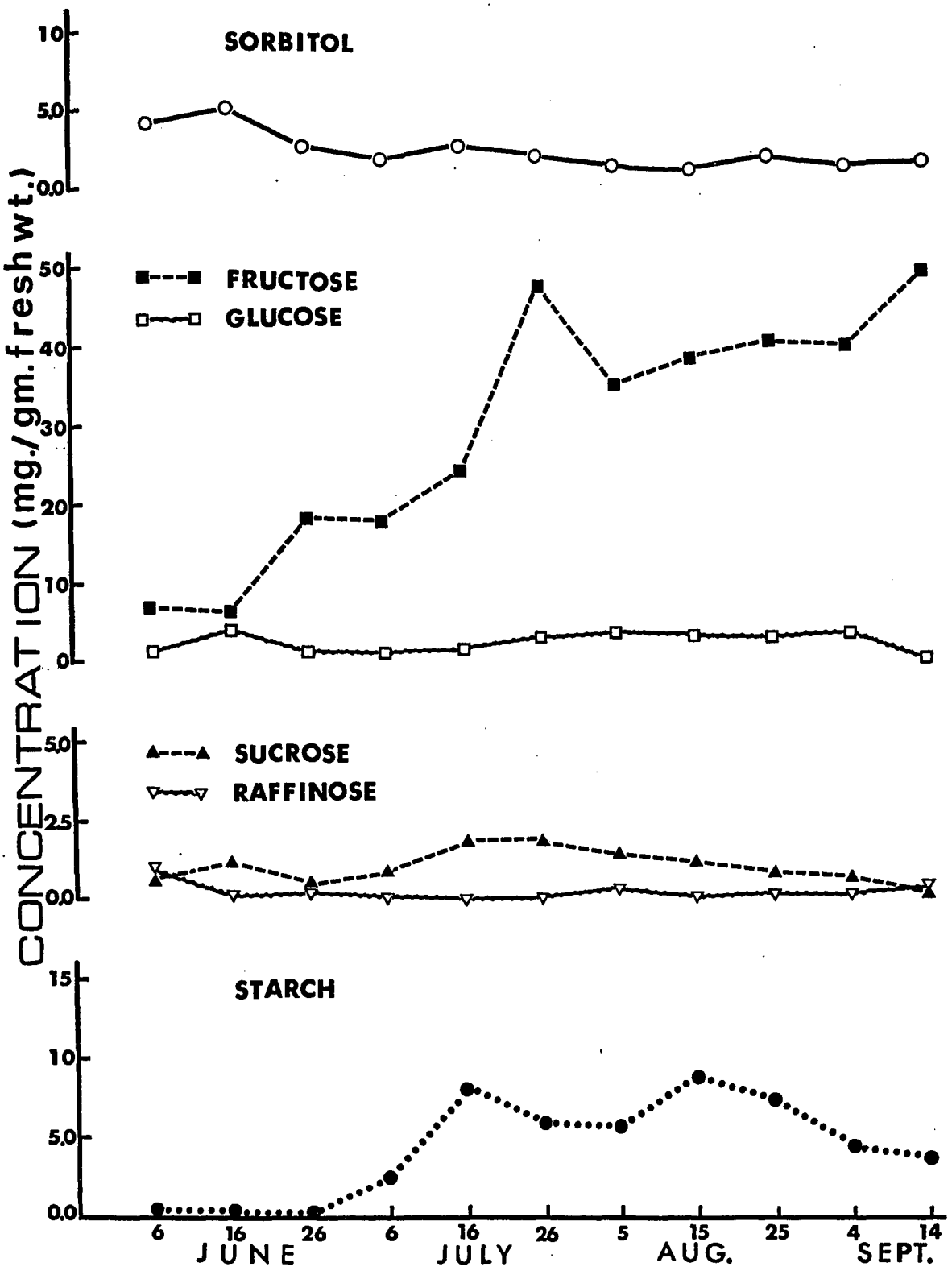


Figure 1. Variations of sorbitol and related carbohydrate contents in Malus fruits from early fruit growth to maturation in 1968.

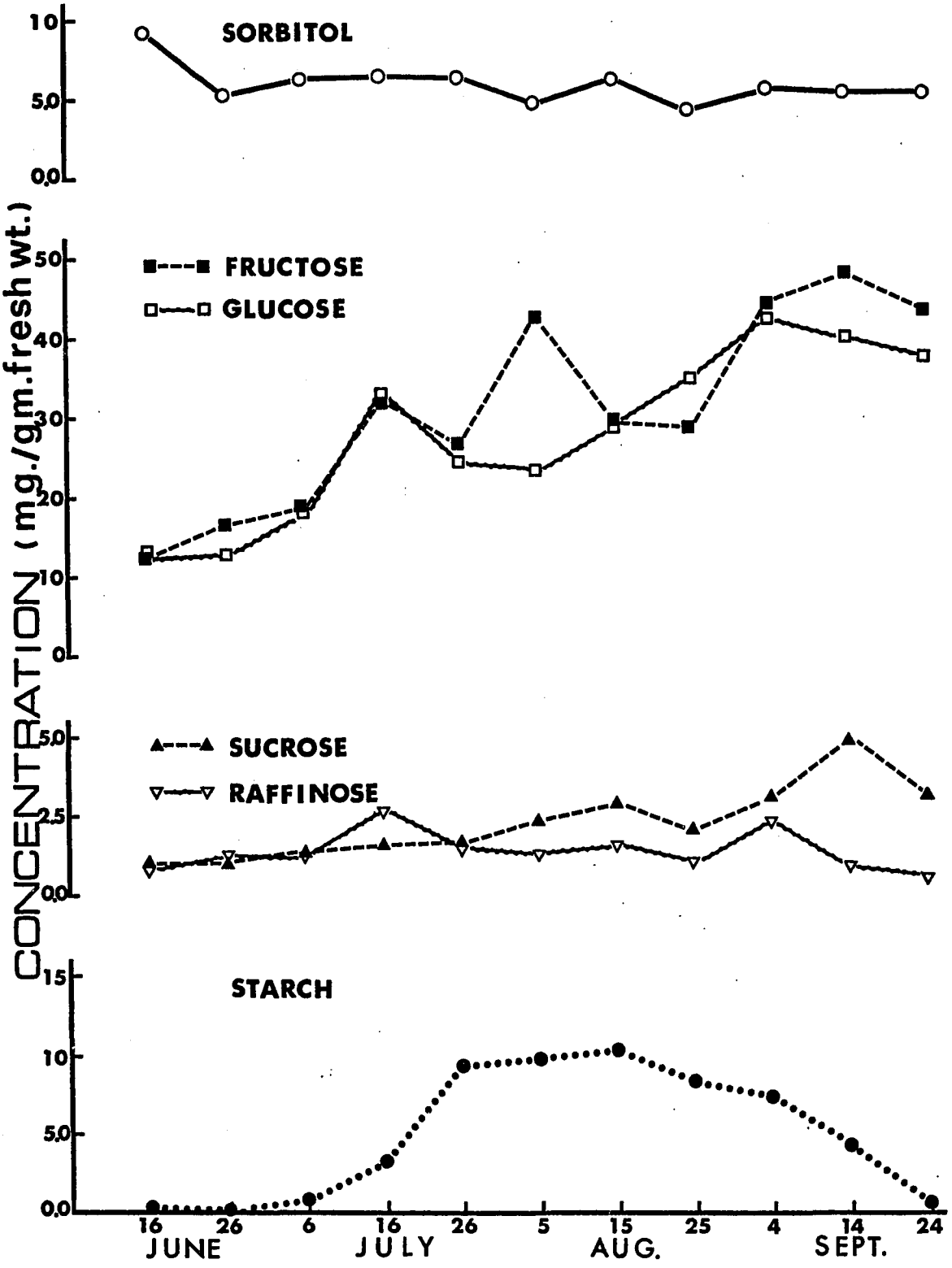


Figure 2. Variations of sorbitol and related carbohydrate contents in Malus fruits from early fruit growth to maturation in 1969.

The appearance of fructose in this year followed an opposite direction of that of sorbitol. The overall picture of its occurrence in the whole season showed a continuously prominent accumulation as the fruits grew in size. The increase of its content almost paralleled the increase of fruit size from fruit set to harvest. Opposite to the higher concentration sorbitol at the beginning of the season, fructose was present in a comparatively low content and the rate of accumulation also appeared to be slow. However, about a month after full bloom and when fruits were larger in size, fructose rose at a very rapid rate and the concentration at the end of June was many times that of the beginning. A prominent peak was reached by the end of July where its concentration was the second highest in the whole season, while at this time sorbitol content was observed to be very low. After this high peak, fructose declined slightly at the beginning of August, but its tendency to increase persisted. At the end of the season when fruits were ripened, its concentration again went up to a peak at 49.60 mg.

In contrast to the picture of glucose accumulation in 1969, the appearance of glucose in summer of 1968 showed a relatively low concentration throughout the growing period. Its behaviour at the beginning of the season resembled that of sorbitol in the initial small rise and then quickly dropped in content. Its concentration present in the fruits in the whole season was approximately equivalent to that of sorbitol, but unlike sorbitol, glucose tended to increase after the first month of

fruit growth. This increase is similar to that of fructose but at a very slow speed. However, this tendency persisted to almost the end of the season when fruits were ripened. Interestingly, the very last decrease in glucose content was observed to match closely with the last rise of sorbitol and fructose.

The content of sucrose fluctuated throughout the season. Its concentration was low by the end of the season and also at the beginning, and the high accumulation was observed during the active fruit growing period when sorbitol content was very low. Raffinose, the oligosaccharide present in the fruits, was in a very limited amount. Its highest concentration was found at the very beginning of the growing period when leaves had not fully developed and fruits were just set. After the initial peak, it disappeared in the middle of the season and reappeared again in a relatively lower concentration than before.

Starch appeared in fruits from the very time of their setting. The amount present initially was smaller than any of the aforesaid carbohydrate at that time. However, its content tended to increase rapidly as fruits grew larger. From June 26 and on, starch accumulated in concentration at a very fast rate and a peak was arrived in mid-July. After this peak, its content subsided slightly and was present almost at a constant level for half a month before it rose again. A second peak which had the highest starch accumulation in the season was reached by August 15 when fruits were very big. As growth cessation had started and fruits ripened, starch concentration declined gradually until

fruits were ready for harvest. However, its content at the last period of the season was still observed to be many times of its initial concentration when fruits had just formed.

b. Variation in 1969

The results of the year 1969 are presented in Table 1 and Figure 2. Each datum represents an average of two sample-groups.

By examining the results of these two years 1968 and 1969 it was found that the picture of the variations of the carbohydrate constituents essentially resembled each other. However, the concentrations of all the carbohydrates in 1969 generally exceeded those in the previous year with the exception of fructose. T-test results showed that significant differences existed between the same sample-group at different years for most of all the compounds under investigation.

Until June, the cold weather in this spring continued and the time for fruit setting was delayed accordingly. Although the date for full bloom was almost the same as that in 1968 at the end of May, sampling was not started until the middle of June. Fruits were collected starting on June 16, 1969, ten days later than the previous year; however, the size of the fruits at the first sampling date was the same like peas as it was in 1968.

At the beginning of the season, unlike the picture of small initial rise in 1968, the concentration of sorbitol in fruits tended to decrease sharply after the first sample in mid-June. After this decline, its content rose slightly and in the

month of July when fruit growth was at its peak, sorbitol stayed at an almost constant level and the concentration at this time almost doubled its content in 1968 at the same period. After the fall in early August, sorbitol rose to a level equivalent to that in July and then decreased again. After reaching the lowest value at the end of August, it rose and stayed once more at a constant level until fruits were harvested. This slight increase at the end resembled the same trend in 1968. When comparing the results of these two years, it was found that fruits in both years had relatively low sorbitol content in August and the lowest sorbitol content in these two years was consistently found in periods when fruits were growing in size and weight; highest concentration was observed at the beginning of the season when fruits were small and leaves had not fully developed. However, sorbitol in 1969 was generally in excess of that in 1968 by twice the amount.

When fruits were small, the content of fructose in them exceeded that in fruits of 1968 (see Table 1). While the overall picture of fructose in 1968 was a gradual rise, the occurrence of this reducing sugar in 1969 fluctuated throughout the season. There were three peaks of fructose accumulation this year and the first peak was observed in mid-July when fruits were rapidly gaining in size. Following a decline at the end of July, the second peak was reached in early August. Thereafter, when growth became slow, fructose content dropped in the month of August. The third peak was observed in mid-September when fruits were

ripened, and this accumulation was the highest during the whole season. This late accumulation at the end of season, like that in fruits of 1968, exceeded by four to five times its initial concentration when fruits were small.

It is interesting to examine the glucose concentrations (Table 1) in these two years. A large difference was obtained in concentration when these two years were compared. In 1969, the concentration of glucose followed closely to that of fructose and was the second largest carbohydrates in the fruits, while the glucose content in 1968 was found only comparable to that of sorbitol. However, it was noticed that the trend of glucose accumulation in these two years after the first month was essentially the same, though a much slower rate was observed in 1968.

In contrast to the behaviour of sucrose in fruits of 1968, the occurrence of this disaccharide in this year tended to increase in concentration with the increase of fruit size. Similar to that of fructose and glucose, its accumulation at the end of the season was higher than the initial concentration. Similarly, raffinose content in fruits of this year was also found higher than that in 1968. While it was found absent or at a very low concentration during the active growing period of 1968, raffinose was present in considerable amount in this period of this year, with peaks both on July 16 and September 4.

The pattern of starch variations was found similar to that of 1968. The initial concentration was very low as before and it gained in bulk rapidly as fruit grew in size. High starch concentration was found all through the active growing period to

that of growth cessation, starting by the end of July to early September. After the highest accumulation in mid-August, starch decreased gradually up to the end of the season. A bell-shaped curve was obtained for its variation in this year.

The appearance of total carbohydrate content in 1969 was generally higher than that in 1968. However, this total carbohydrate concentration in these two years was controlled largely by the two reducing sugars, namely fructose and glucose. Fructose was found to be the largest single carbohydrate present in the fruits throughout the whole season in both years, and raffinose was the least.

(2) Discussion

The metabolism of sorbitol in fruits during their growing period has been studied by Donen (1939), who observed that during the growth of the Kelsey plums, sorbitol maintained a constant increase, that a peak of accumulation of this sugar alcohol was found at the time when stone growth had terminated and when the maximal reducing sugars began to level off. This worker thus suggested that, during fruit growth, sorbitol served as a reserve pool for reducing sugars when they had reached a maximum. Rees and Reynolds (1958) found that, during the growth of Victoria plums, sorbitol was present at a seemingly constant level throughout the growth period. Stoll (1968), using a sensitive thin layer chromatography technique in his study of sorbitol in pear and apple fruits, found that the increase in

sorbitol content of Cox's Orange Pippin apples, and Nordhauser Winterforelle pears on the tree, nearly paralleled their increase in size. With Rising Summer pears, the accumulation of this sugar alcohol, with progressive fruit development on the tree, was much less than that of sugars, so that the sorbitol content decreased up to harvest. Taper and Liu (1969) reported that, in their McIntosh apples, sorbitol was the least abundant ethanol-soluble constituent, whereas fructose was the most abundant carbohydrate present. However, on the other hand, sorbitol was found as the major carbohydrate in the leaves. Contrary to its progressive increase in the leaf tissues, there was a progressive decline in sorbitol concentration in the growing fruits.

By comparing the present results during two years with those obtained by the aforesaid researchers, it appears that the pattern shown by the data (Table 1) can hardly be in full agreement with that of Donen,, Rees and Reynolds, nor part of Stoll. However, the present data support those of Taper and Liu (1969) in that a progressive decline of sorbitol was observed in the growing fruits, starting from the beginning of the season, and up to the period of growth cessation in late August. This gradual decline was more marked during 1968 than that in 1969. Incidentally, this decline of sorbitol was also observed in the Rising Summer pears by Stoll (1968). The present data show that the occurrence of sorbitol in fruits at the beginning of the season, when leaves have not become fully expanded, was marked by a higher concentration than in subsequent samples in the

season; and this was consistent for the years 1968 and 1969. When one refers to previous reports, one notices that it has been usually reported that leaves of apple, not the fruits, contain the largest amount of sorbitol (Williams, 1966; Taper and Liu, 1969). Hutchinson et al. (1959) noticed that when D-glucose-C^{14} or $C^{14}O_2$ was incorporated into the metabolizing apple leaf disks, large amount of D-glucitol-C^{14} was formed. When D-glucitol-1-C^{14} was supplied, the highest proportion of radioactivity was again detected in the compound D-glucitol-C^{14}, and, therefore, they stated that D-glucitol (sorbitol) was the largest single ethanol-soluble carbohydrate fraction in the metabolizing leaf disks. The study of Whetter and Taper (1963) also confirmed that in apple leaves sorbitol content was invariably in excess of glucose and sucrose. Therefore, in summing up these studies, one concludes that sorbitol is formed rapidly in the metabolizing leaves, and it is supplied continuously to the fruits for their development. But this production of sorbitol in the leaves at the beginning of the season, as viewed from the data of Taper and Liu (1969), is relatively low, and its supply to the fruits is presumably at a small rate. Thus, when the initial concentration of sorbitol in fruits in the present results is observed at a high level (Table 1), it appears that this sorbitol may not have come solely from the leaves, but also from the buds as well as the phloem. Whetter and Taper (1963) found sorbitol in dormant winter buds where it remained at a relatively constant level prior to the occurrence of leaf expansion and photosynthesis.

Eaton (1949) found a sorbitol concentration of 0.5% fresh weight in one-year-old apple shoots in the spring, while Bradfield and Flood (1950) found sorbitol present in dormant apple shoots. Sakai (1961, 1966) reported that, in winter, the dormant twig bark of apple and other fruit trees contained four times as much sorbitol and mannitol as it had in August, and thus he related this occurrence to a high polysaccharide content in the phloem, and, accordingly, the degree of frost-hardiness shown by the tree. Bieleski (1969) found that sorbitol was quickly translocated from the phloem to the active growing points in the plant for utilization. It appears, therefore, that the high level of sorbitol in these small green fruits accumulated from all these sources before most of the leaves had fully expanded, as shown by the present results, is a normal physiological phenomenon. As soon as the leaves become fully developed, and the temperature becomes warmer, leaves are producing photosynthate in large amount to supply food materials for the various physiological demands in the tree for energy, especially for the growth of the fruits. Since fruits are growing at a tremendously fast rate at this time, and there is high energy demand by the respiratory and other metabolic activities, the demand for sorbitol as a source of energy increases, and the concentration of this sugar alcohol is accordingly reduced in the fruits. This decline persists throughout the whole active fruit growing period, from mid-June to mid-August, until cessation of growth starts in leaves. After the lowest

concentration is attained on August 15 and 25 in 1968 and 1969 respectively, sorbitol begins to rise. As leaves become mature and fruit growth is slowed down at this time, the requirements for carbohydrates from these tissues are gradually reduced. Together with the decreasing utilization of sugar in other organs in respiration and other metabolic activities, sorbitol, reducing sugars, and hence total carbohydrate contents tend to increase. However, this increase, in the case of sorbitol, is only very slight, because partly it is being continuously converted to other sugars, and partly consumed in the metabolic activities of the fruits, which continue to grow up to October, although at a very slow rate. Nevertheless, the slowing down of fruit growth in September gives the leaves, which have stopped growth, a chance to accumulate the products of their own photosynthesis, and hence a high sorbitol content is usually observed in mature Malus leaves (Williams, 1966; Taper and Liu, 1969).

In comparing the decrease of sorbitol in the fruits shown by the present data with that of Taper and Liu (1969), it is noticed that the present decline is not as consistent and continuous as reported by these researchers, who found that sorbitol decreased up to harvest from 7.28 to 6.02 mg./gm. After its low level in the active growing period, sorbitol in the present data is observed to rise, and this slight increase appears to remain at a fairly constant level at the time of harvest. The difference in these two studies may stem largely from the intervals between sampling data. While the present study provides short intervals (10 days) for each sampling date all through the

season, Taper and Liu sampled only at monthly intervals. Since the changes of these compounds, namely, sorbitol and other carbohydrates, in the plant occur usually in days or even hours, long intervals for sampling often result in distorting the true picture of these fluctuations as they occur between the consecutive sampling dates, particularly in the months of July and August when fruits are gaining in size at a rapid rate. Being at a closer interval of sampling,, the present data appear to supplement the missing picture of sorbitol occurrence in that of the aforesaid workers, and more fluctuation of this sugar alcohol is observed.

At the beginning of the season when leaves have not fully developed and photosynthesis in the leaves has not reached a maximum, the appearance of other sugars in the fruits is low. However, after about a month's growth, maximum photosynthesis results in a rapid accumulation of photosynthate, especially in the form of fructose, which is found as the first product of photosynthesis (Calvin, 1962), and the most abundant carbohydrate in the fruits (Taper and Liu, 1969). Hulme (1958), in his collective review of fruit research, summarized that during fruit growth, fructose is the most prominent sugar except during the first few weeks of the development, and glucose is in excess of sucrose. This finding coincides very well with the picture of fructose occurrence in the present data and also that of Taper and Liu (1969). Hulme continued that after the fruits were removed from the trees and placed in storage at various temperatures

fructose remained the most abundant sugar and the total sugar content increased steadily up to, and indeed rather beyond, the time at which the fruit was harvested commercially. This is again in agreement with the present results in which the high accumulation of fructose from the period of growth and maturation continues, together with glucose, when fruits are stored inside refrigerated storage (see variation of carbohydrates in refrigerated storage).

Sorbitol has been shown to be used as a source of energy by higher animals, and when it was administered to dogs and rabbits, a large amount of fructose is seen to appear and increase in the blood stream (Seeberg et al., 1955). An interconversion between these two carbohydrates has been demonstrated by Blakley (1951) and Touster and Shaw (1962); and their relationship has also been elaborated in fruits (Martin, 1937; Donen, 1939; Kidd et al., 1940; Rees and Reynolds; Taper and Liu, 1969). Since fructose has been reported as the least abundant carbohydrate in apple leaves (Taper and Liu, 1969) whereas sorbitol is the most abundant ethanol-soluble constituent present (Williams, 1966; Taper and Liu, 1969), it appears that the occurrence of these two compounds in these leaves does not echo the findings of Calvin (1962) who found that fructose was the first product of photosynthesis. A conversion, therefore, must have taken place in the leaves, in which fructose is converted to sorbitol, leaving its amount at a minimum. Using labelled carbons, Anderson et al. (1962) demonstrated that during

photosynthesis in plum leaves, no net synthesis of sorbitol occurred at first, yet this sugar alcohol was rapidly equilibrated with the primary products of photosynthesis, 15% was newly synthesized from C^{14}-labelled precursors in three hours. Thus, apparently, fructose is converted to sorbitol at a similarly rapid rate in apple leaves, and sorbitol is found as the largest carbohydrate constituent in these tissues. However, this sorbitol is also continuously transported out into the fruits (Williams, 1965), by way of an active process (Bieleski, 1969). After this compound has reached the fruits, this fructose-to-sorbitol pathway quickly operates in an opposite direction where sorbitol is rapidly reconverted to fructose, as well as glucose. These reducing sugars are gradually built up in the fruit tissues, which is reflected in their continual rise in concentrations throughout the whole growing period.

The glucose content in fruits of both years 1968 and 1969 tends to increase as fruits grow in size, though this tendency is shown more obviously in year 1969 (see Table 1 and Figures 1 and 2). Archbold (1932) and Krotkov and Helson (1946), in their study of the changes of sugars during growth of apples, showed that fructose and glucose contents increased in the growing period. This increase was at first rapid, and then slowed down. Following a rapid increase in the first few weeks, glucose content became practically constant for a prolonged period while fructose kept on increasing. The results shown in the present data for the year 1968 agree with their findings

while in the year 1969 the increase of glucose is matched closely with that of fructose; and incidentally, this is similar to the pattern reported by Taper and Liu (1969).

It has been shown in animal tissues that fructose formed glucose more rapidly than did sorbitol (Kreb and Lund, 1966). When sorbitol is oxidized to fructose in rat liver tissues, this reducing sugar was subsequently transformed into glucose; however, this transformation proceeded so rapidly that it appeared that sorbitol was directly converted into glucose without the intermediate of fructose, because only very small amount of fructose was formed at the same time, and not much change was observed in its concentration (Blakley, 1951). Altermatt and Neish (1956) had suggested that in wheat plants, oxidation of sorbitol to fructose was the first step in its conversion to glucose, and this oxidation proceeded only in the presence of a polyol dehydrogenase, probably sorbitol dehydrogenase. Since this enzyme has been found widely in Rosaceae plants (Touster and Shaw, 1962), this sorbitol-fructose-glucose conversion presumably also takes place in a similar way in Malus. The general accumulation of this reducing sugar, glucose, in apple fruits is probably resulted from this sorbitol transformation, by way of fructose, and this reaction proceeds so rapidly that the concentration of the ketose is not affected.

However, direct sorbitol-glucose conversion is probably also taking place at the same time. In the study to investigate the possibility of the ready interconversion of D-glucose and D-glucitol in plum leaves, Anderson et al. (1962) demonstrated

a rapid synthesis of D-glucitol from D-glucose, and the labelling patterns obtained showed that this conversion probably occurred without rupture of the carbon chain. Keeping in mind this evidence and noting the results of Hutchinson et al. (1959), who showed that largest proportion of radioactivity was detected in D-glucitol-C^{14} when D-glucose-1-C^{14} was incorporated into the metabolizing apple leaf disks, Whetter and Taper (1963) were motivated to demonstrate in their experiment that sorbitol was found in disks fed glucose-C^{14} and glucose in those fed sorbitol-C^{14}. These researchers, therefore, proposed that these two compounds, glucose and sorbitol, are readily interconvertible. Although this interconversion is presumably proceeding in all stages of fruit growth, it is not usually susceptible to detection directly because of the complicated interrelating carbohydrate metabolism; however, a slight opposite variation of these two compounds is shown in the fruits at the beginning and at the end of the growing season, notably in 1969.

Rees and Reynolds (1958) showed in their study of Victoria plums that sucrose was absent up to the ripe stage, and glucose was more abundant than fructose. After that, sucrose appeared and increased rapidly while glucose and fructose contents tended to slightly level off. Although the concentration of sucrose found in the fruits in the present results is at a relatively low level compared with that of the reducing sugars, it does not disappear from the fruits during the growing period; but instead, slight increase in the active fruit growing period

is observed, although a drop is subsequently found at time of harvest. Martin (1937) related the increase of fructose and glucose and the decrease of sucrose in pears as the hydrolysis of the latter to the former. While this may explain partly the general low sucrose content shown by the present data, some other unknown reasons must be involved, and the conversion of this sucrose to other low oligosaccharides may be one of them (Lopatecki, 1962). Hulme (1950, 1954) suggested that in apple, changes in starch and in sucrose were regarded as being linked processes. This suggestion is in agreement with the study of Kursanov and Pavlinova (1950), who demonstrated by vacuum infiltration experiments that starch was one of the nearest precursors of sucrose in plants. However, this starch-sucrose linkage is not obviously shown by the present data except to a slight extent at the time of fruit ripening in year 1969, when the drop of starch was coupled with the rise of sucrose; this may probably be attributable to the hydrolysis of starch to the latter. Nevertheless, as viewed from the present data, the occurrence of sucrose in the fruits is probably more related to the reducing sugars than to other carbohydrates, because fructose and glucose have also been demonstrated as precursors of sucrose (Kursanov and Pavlinova, 1950; Axelrod and Seegmiller, 1954; Alexander, 1964), and also, their relationship has been elaborated by many researchers as being important in the metabolism of fruits (Martin, 1937; Rees and Reynolds, 1958).

Although the gradual drop of sucrose in the year 1968, during fruit ripening, and its sudden drop in year 1969 at harvest, may have resulted from its conversion to other carbohydrates; yet, the translocation of this disaccharide, together with leaf sucrose, to the phloem is also possible and probable. In the phloem, sucrose has often been regarded as one of the main translocates (Kursanov et al., 1958; Swanson, 1959; Webb and Burley, 1962; Hartt et al., 1963), Mason and Maskell (cited by Kursanov, 1963) found that in cotton, up to 90% of the assimilates in the phloem flow was sucrose and this sucrose was translocated in the plant from the leaves. Sakai (1966) found that sucrose in the twig barks of fruit trees, along with glucose, was more effective in protecting fruit trees from frost injury than was raffinose, and this sucrose must have come from the metabolic sinks in the plant. These findings seem to support the above suggestion to a certain extent.

The presence of raffinose in the fruits in detectable amounts is found surprising, since it normally is present only in trace quantities. This oligosaccharide has been found in appreciable quantities in dormant shoots of apple (Bradfield and Flood, 1950), in leaves and dormant buds of Malus (Whetter and Taper, 1963), and fruit twig bark in winter (Sakai, 1966). Whetter and Taper (1963) found that raffinose was only present in dormant and developing buds, and again in the leaves from August to October, when it was probably produced for translocation to the stems. The highest quantities of raffinose were

found in the dormant leaf buds, and in the leaves immediately prior to leaf fall. The findings of the aforesaid researchers seem to agree to a point that this oligosaccharide is mainly present in dormant or mature tissues, and its function appears to be a winter storage sugar in the plant more than anything else. Lopatecki (1962) showed that the lower oligosaccharides could be experimentally formed by the action of wheat invertase on an excess of sucrose. Since only raffinose was detected in the present study, but not other lower oligosaccharides; and the amount of sucrose present in the fruits appears not too much different from that of raffinose; moreover, the variations of raffinose and sucrose in the fruit growing season are found following each other, this conversion of sucrose to other oligosaccharides appears not to be possible. However, the generally low content or occasional absence of raffinose during the active growing period agrees in part with the findings of Whetter and Taper (1963) and Sakai (1966) that it is not usually present in active tissues.

In the study of the carbohydrate metabolism of McIntosh apples, Krotkov and Helson (1946) found that starch appeared in apple fruits from the very time of their setting. The amounts present initially were small, and they were declining during the first few weeks, but, starting from mid-June, starch in the fruits began to accumulate, reaching a peak by August. Having reached its peak, it began to subside, and the last traces of it were gone in stored fruits by the end of October or mid-October

in the respective two years of study. Poapst et al. (1959), in their study of the relation of maturation of McIntosh apples to starch loss and abscission, found that starch content and abscission were associated closely, and this association varied with the seasonal temperatures. Starch was found declining progressively in a linear manner during the latter stages of growth and it was observed disappearing from fruits in storage before November. The occurrence of starch in the present data coincides very well with the finding of Krotkov and Helson in that starch content increases after a few weeks of fruit growth and decreases at the end of the growing period. The initial rapid increase of starch is observed following the same increase of reducing sugars and the decline of sorbitol, indicating some conversions may have taken place. The decline at the end of the growing period before harvest is also similar to that of the results of Poapst and his co-workers, and that of Liu (1967) who demonstrated in Malus fruits a same decline of starch in September. However, this decline of starch before harvest is found to be coincidental to a rise of sucrose content, which has been discussed previously as being attributable to the hydrolysis of the former to the latter.

Sorbitol has been shown to be utilized in polysaccharide synthesis, and in converting to starch in detached leaves of Rosaceae (Treboux, 1909; Steele, 1934). It is found to be utilized in the biosynthesis of cell wall carbohydrates, namely, cellulose and xylan, in wheat plants after splitting of the

carbon chain (Brown and Neish, 1954; Altermatt and Neish, 1956). Further, Anderson et al. (1962) explained that, higher proportion of C^{14} was incorporated in the light than in the dark from D-glucitol (sorbitol) into alcohol-insoluble materials found in their experiment, was probably due to the equilibration of D-glucitol with D-glucose or other possible intermediates in polysaccharide synthesis, especially starch, the synthesis of which was favoured in the light. The opposite variation of sorbitol and starch during the active fruit growing period in the present results appears to support the feasibility of this conversion.

The pattern of variation in refrigerated storage immediately after harvest to late spring

(1) Results

Selected fruits used for analysis were moved into refrigerated storage at 32°F. together with other fruits on September 22, 1969. Sampling was undertaken in the same way as the field samples, as described in Materials and Methods. The same carbohydrate pattern detected before was obtained on the chromatogram. Paired T-test was also performed for results from the two sample-groups and the observed values for these two groups were found to be not significantly different. Thereby, a mean datum from these two groups was computed and it is presented in Table 2 and also in Figure 3.

The variations of these carbohydrates, namely, sorbitol, fructose, glucose, sucrose, raffinose, and starch in fruits in storage at 32°F. could be divided into five stages according to

Table 2. Sorbitol and related compounds in *Malus* fruits from harvest to late spring in refrigerated storage (mg./gm. fresh weight, mean data)

Date	Sorbitol	Fructose	Glucose	Sucrose	Raffinose
1968-1969					
25/9	1.63	43.35	17.54	0.67	0.42
5/10	2.24	42.60	23.15	0.54	0.70
15/10	3.78	19.59	56.78	1.96	1.16
25/10	2.11	54.08	29.95	2.16	0.67
4/11	2.63	49.85	24.95	1.53	0.55
14/11	0.67	60.90	9.70	0.90	0.28
24/11	2.22	72.64	6.36	0.55	0.44
4/12	2.31	39.82	47.69	2.23	0.39
14/12	2.87	34.75	49.91	2.32	0.26
24/12	4.56	29.84	72.38	5.73	4.04
3/1	4.26	70.00	31.63	3.69	2.03
13/1	4.26	78.75	26.00	7.13	1.78
23/1	4.13	58.13	34.38	4.82	0.72
2/2	4.01	65.63	27.38	5.13	0.41
12/2	4.00	62.50	30.57	2.19	0.44
22/2	3.82	80.00	20.88	3.10	0.43
4/3	5.25	79.38	38.75	8.35	0.50
14/3	4.15	68.75	37.00	3.69	0.35
24/3	4.94	82.50	29.63	5.38	0.47
3/4	5.82	65.00	31.50	6.10	0.41
13/4	4.44	68.13	36.50	7.22	0.75
23/4	5.05	24.50	54.84	7.25	2.09
3/5	5.71	39.17	42.34	7.54	1.79

*See footnotes under Table 1.

Table 2 (continued).

Starch	Reducing sugars*	Total sugars*	Total carbohydrates*
1.86	60.89	61.97	63.60
0.31	65.75	66.99	69.22
0.12	76.37	79.49	83.27
0.09	84.02	86.85	88.95
0.39	74.80	76.87	79.50
0.06	70.60	71.78	72.45
0.03	79.00	79.99	82.21
0.00	87.51	90.12	92.43
0.00	84.66	87.20	90.10
0.00	102.21	111.98	116.54
0.00	101.63	107.35	111.60
--	104.75	113.66	117.91
--	92.50	98.88	103.01
--	93.00	98.53	102.54
--	93.07	95.69	99.69
--	100.88	104.40	108.21
--	118.13	126.97	131.91
--	105.75	109.79	113.98
--	112.13	117.97	122.91
--	96.50	103.01	108.82
--	104.63	112.60	117.04
--	79.34	88.67	93.72
--	81.50	90.83	96.54

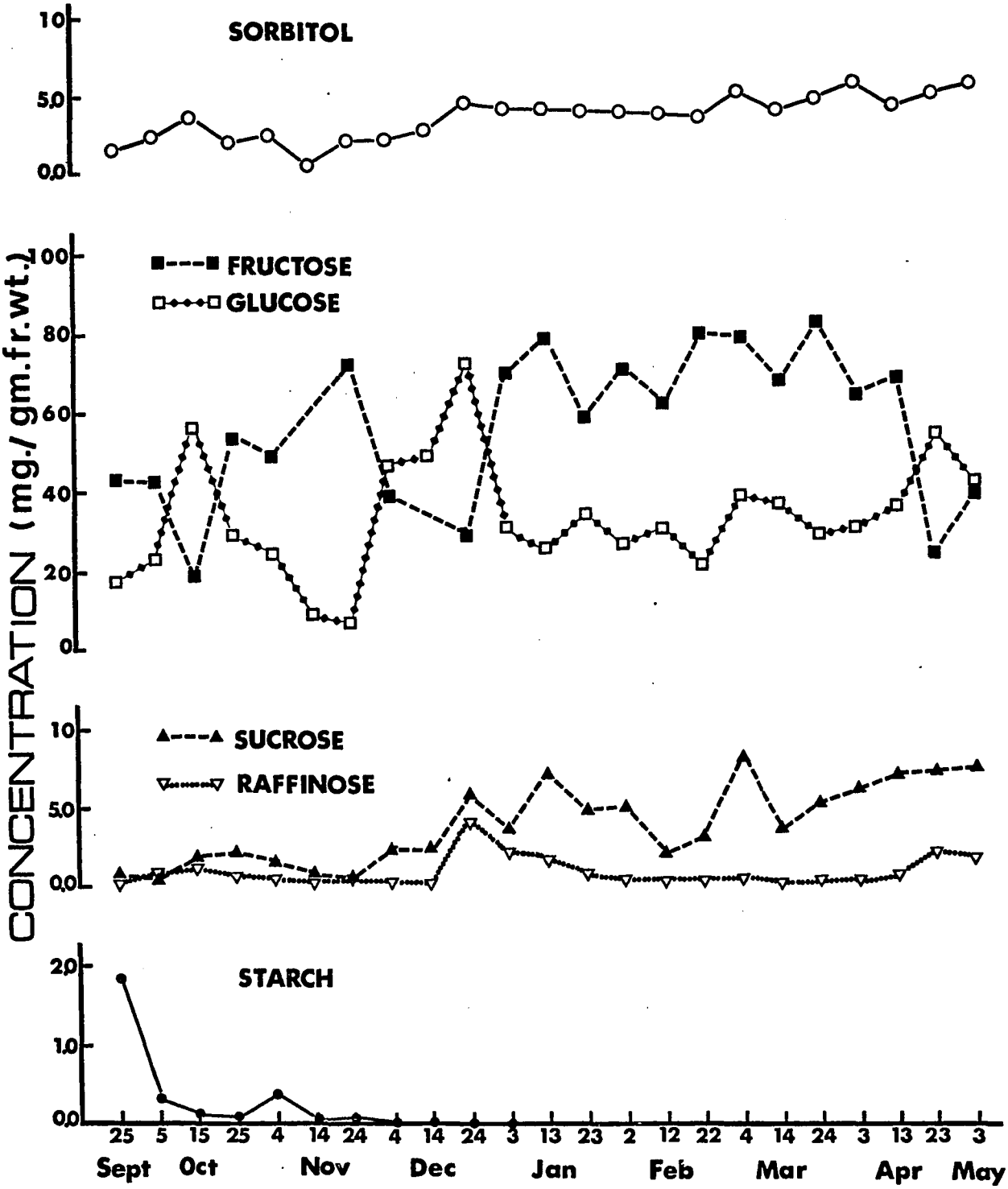


Figure 3. Variations of sorbitol and related carbohydrate contents in Malus fruits in refrigerated storage (32°F.) immediately after harvest to late spring (1968-1969).

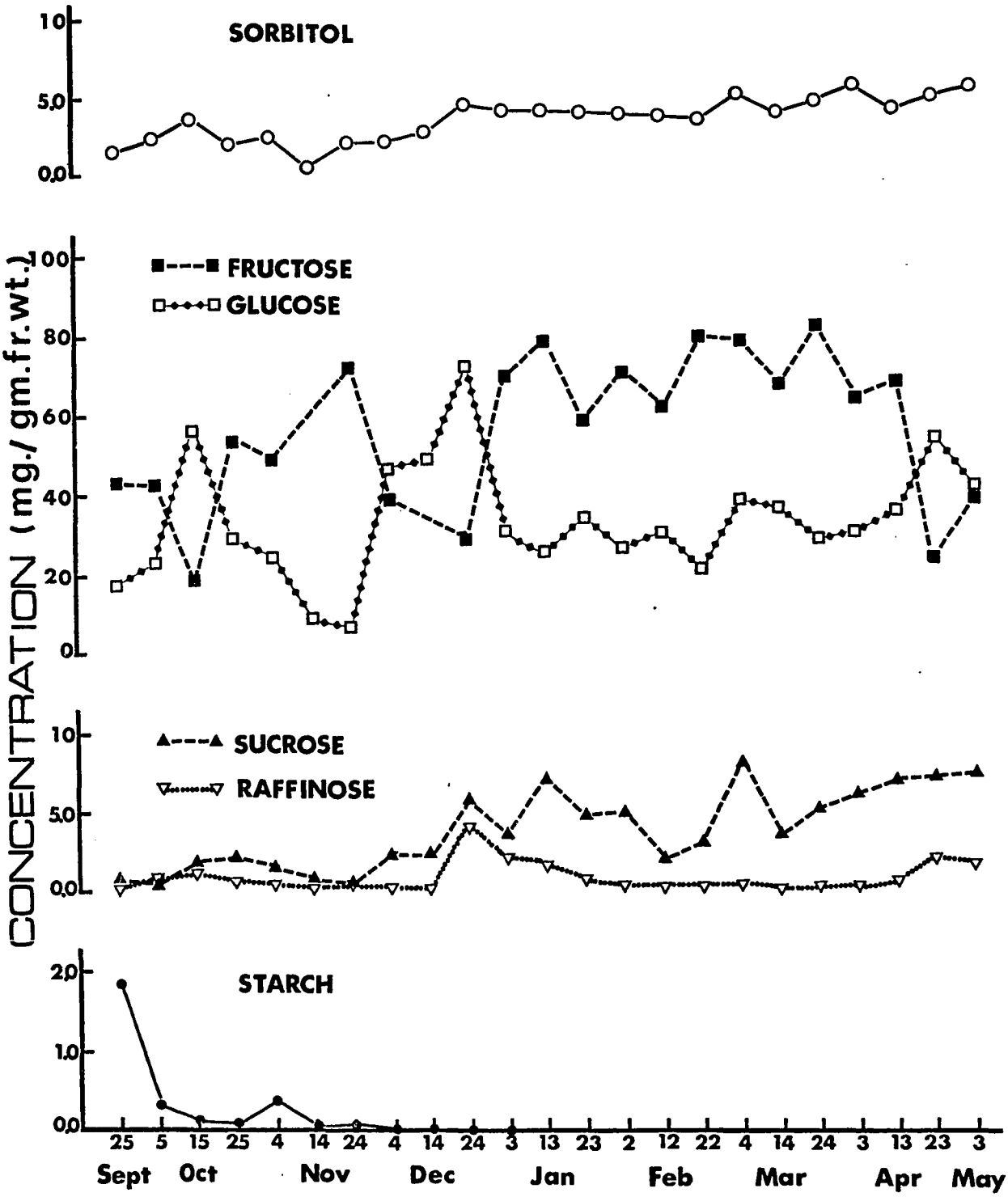


Figure 3. Variations of sorbitol and related carbohydrate contents in Malus fruits in refrigerated storage (32°F.) immediately after harvest to late spring (1968-1969).

their various fluctuations. The first three stages were largely the respective initial three months of storage, namely, October, November and December. The fourth stage started from the end of December to the middle of April in the spring. The last stage included the rest of the storage period from mid-April to early May.

For the first stage of fruit storage (Stage 1) which began by the end of September and ended in mid-October, the behaviour of sorbitol kept close pace with the fluctuations of the reducing sugars in the same way as it was in the field-sampled fruits. Its concentration rose gradually when fruits were just moved into storage until a small peak was observed in the middle of October. During this period, glucose, which had a comparatively low concentration in the growing period, rapidly increased in the first few weeks of fruit storage. Similar to sorbitol, a peak was reached by October 15. At the same time, in contrast to its prominent concentration in fruits during periods of fruit growing and ripening, fructose fell significantly when fruits were first stored in the cold. A lowest concentration was observed in mid-October when sorbitol and glucose at this time were present in high amounts. Notwithstanding the changes of the above carbohydrates, sucrose and raffinose remained at a very low level at this stage, but starch was observed to follow the fall of fructose closely and decreased sharply to almost a minimum.

The second stage (Stage 2) ranged from October 15 to November 24 when fruits were in the storage for about two months. After the initial rise, sorbitol content in the fruits decreased gradually and continuously, together with glucose. The decrease of glucose was very abrupt, and persisted until the end of November when a lowest value in the whole storage period was observed, at 6.36 mg. per gm. of fresh weight, which was about one-ninth of its concentration at the first peak. Sorbitol also reached its lowest point of 0.67 mg. per gm. fresh weight on November 14. However, the decline of these two carbohydrates was reflected in the continuous increase of fructose content. Regaining its previous strength at harvest time, fructose increased at a very rapid rate after its initial fall in October, and quickly reached its high peak of 72.64 mg. per gm. on November 24. Sucrose in this second stage increased slightly but subsided again, while raffinose was still present in very low amounts. Starch continued its decrease and the last traces were gone at the beginning of December.

The third stage (Stage 3) included the period from November 24 to the end of December when fruits were matured sufficiently after three months of storage. Sorbitol was observed at this time accumulating in concentration when fructose fell again. Glucose rose after the initial lowest point and rapidly attained its highest concentration at the end of this stage with a value of 72.38 mg. per gm. The fall in fructose content coincided with the increase of both sucrose and raffinose.

Both of these carbohydrates reached their peaks the first time since fruits had been moved into storage. Starch disappeared in the fruit tissues on December 4, and no trace was detected after this date.

The fourth storage stage (Stage 4) of fruits was from the end of December to the middle of April, in which many of the fruits became soft and some of them began to go rotten. During this period, all the carbohydrates appeared to stay at a fairly constant level with fructose leading in concentration and glucose the second. Sorbitol was observed to stay at a seemingly constant level; this was more notable at the first half of this stage. However, in the second half, sorbitol fluctuated slightly but its concentration was found to reach a relatively high level compared to that of the previous three stages. Fructose, after the second fall in the third stage, began its rapid rise in January, together with a sharp decline in glucose concentration. After a peak in mid-January, fructose did not fall in the same way as before but stayed at a higher level than any other sugars for a period of more than three months, despite occasional slight ups and downs. On the other hand, glucose was present at a constant but relatively low concentration compared with fructose for the same duration of three months. The occasional fluctuation of this reducing sugar was found matching with fructose in an opposite direction. As for sucrose in this stage, it gradually increased in concentration and small peaks were found at the beginning of this stage and also on March 4. Its amount exceeded

its initial concentration in previous periods by many times and this tendency continued up to the end. Raffinose was observed to increase slightly at the start of this period but small amount was still found thereafter.

The fifth stage of carbohydrate variation (Stage 5) included the last three weeks of fruit storage in which most of the fruits were found rotten and soft. Sorbitol was observed slightly rising, and its concentration was found again higher than that in the previous stage. Together with the increase of sorbitol, fructose dropped sharply after more than three months of prominently high accumulation. Glucose was observed at this time to rise again and its concentration at the end of this storage period exceeded that of fructose after a long period of staying behind. Sucrose kept on rising during the last few weeks in storage and raffinose also had a last upheaval after presence in limited concentration for a long time.

Similar to that in the fruits in the field but more prominent, the content of reducing sugars formed the main trunk of the carbohydrate in stored fruits, and the variations of all the carbohydrates appeared to follow closely to their changes. Fructose was found still as the most prominent carbohydrate in storage and glucose the second, while starch was observed to be the least. Sorbitol content in storage was approximately one-twentieth of the average total sugar concentration.

(2) Discussion

It has been shown by many researchers that during fruit growth, fructose is the most abundant carbohydrate in the fruit

and glucose is in excess of sucrose (Krotkov and Helson, 1946; Hulme, 1958; Taper and Liu, 1966). This pattern has also been demonstrated in the results obtained from fruits taken in the field as described in the previous section. Further, it is generally recognized that when fruits are harvested and stored at various temperatures, the concentration of reducing sugars remains high, chiefly in the proportion of fructose. This pattern again coincides with the present data obtained from fruits stored in refrigerated storage at 32°F. (see Table 2 and Figure 3). Since sorbitol is the main carbohydrate studied in this project, efforts are made to relate the present results to other similar studies for a better elucidation of the importance of this sugar alcohol in the carbohydrate metabolism in stored fruits.

The relationship of sorbitol and its related carbohydrates in stored fruits has been studied by many workers. Nuccorini (1932) found a decrease in sorbitol content when Sorb apples were detached from the tree; he then suggested that this decrease was due to the conversion of sorbitol to the reducing sugars, namely, fructose and glucose. Martin (1937) likewise showed a decline of sorbitol in Bosc pears during their after-storage ripening. This decrease was accompanied by an increase of fructose and sucrose concentrations. From these results, this worker suggested that the decline of sorbitol and the rise of sucrose was not a direct process, instead, sorbitol was first converted into glucose and fructose, and then sucrose was formed

by their subsequent transformation. Kidd et al. (1940) found in Conference pears stored at 10°C. a marked decrease in a fraction, 50% of which consisted of sorbitol, this was accompanied by an equivalent rise of fructose, and they therefore suggested that sorbitol was converted into this ketose in these fruits. Donen (1939) reported that though the sorbitol content in Kelsey plums was in some instances, as much as 4.5%, it declined rapidly in storage at 13°C. and 25°C. prior to the other sugars, and thus he referred to this loss as sorbitol was used as a source of energy utilized by the fruits for respiration. This suggestion was supported by the work of Nitsch (1953) who stressed the role of sorbitol as a respiratory substrate for fruits in storage. Stoll (1967b) found that the content of sorbitol in pears and apples decreased markedly during the first three months of ordinary cold storage at 4°C., followed by a slow increase at the end. However, these changes were less marked when the storage atmosphere was modified by carbon dioxide. Again, Stoll (1968), in his third finding, reported that during storage, sorbitol in apples declined at the beginning and during the second month, however, its content rose afterwards at the expense of the carbohydrates.

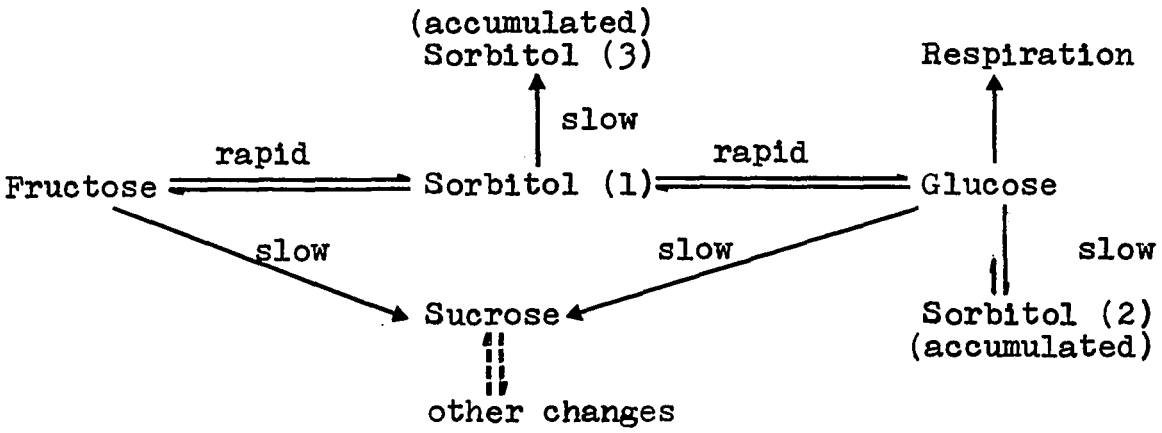
All these findings appear to come to a general conclusion that sorbitol decreases in content when fruits are stored, and this decrease is closely related with the increase of other sugars, mainly fructose and glucose. It is shown in the present data (Table 2) that although sorbitol content does not decrease

significantly throughout the whole storage period, it does decline gradually in Stage 2 (see Results) in November, reaching its lowest point of 0.67 mg./gm. on November 14. This decrease is coupled with a rapid rise of fructose content. The occurrence of sorbitol at this stage agrees with most of the findings of the above researchers. However, apart from its subsequent decline as a respirable sugar, as was suggested by Martin (1937) and Nitsch (1953), this sugar alcohol rises slightly but continuously until the end of the storage period, from an initial concentration of 1.63 mg. to 5.71 mg./gm. at the end (see Table 2). This gradual accumulation of sorbitol has come into general agreement with the data of Taper and Liu (1969) who observed an increase of this sugar alcohol, ranging from 8.39 to 9.66 mg./gm. fresh weight in mature fruits in storage from October to February. While the net increase shown by the present data is several times greater than theirs, the general sorbitol concentration is low when compared to their levels in the fruits. The present results also agree with those of Stoll (1967b) in having a rise of sorbitol in apple fruits toward the end of the storage period. However, the small variation in sorbitol content during the whole storage period, and its eventual accumulation in storage support the suggestion of Taper and Liu (1969) that it is a reserve carbohydrate in Malus fruits.

In agreement with the findings of Krotkov and Helson (1946) and Taper and Liu (1969), fructose, as shown by the present results, forms the single largest carbohydrate in the stored fruits as it does in the growing fruits (see Table 1).

It accumulates in the fruit tissues as fruits become more and more mature in the storage (Table 2). Glucose, similar to its content in fruits on the tree, is the second sugar with respect to content in the fruits during storage; and its general level of concentrations is close to that of fructose. These two reducing sugars are found fluctuating very much in the stored fruits, but it is interesting to note that their fluctuations are in an exactly opposite direction all through the whole storage period. Although sorbitol has been shown to be interconvertible with these two reducing sugars, its consistently low concentration does not seem to be associated in a particular interconversion with either of them; but in the course of the opposite variations of fructose and glucose, sorbitol appears to be in between their fluctuations. Since the conversion of these two reducing sugars to each other is not a direct process (Blakley, 1951; Hers, 1956, 1960), some intermediates must be involved, and sorbitol is the most suitable and the highly possible intermediate for this rapid transformation. Its role as being an intermediate for these reducing sugars can be seen from its general low concentration and almost constant level after the first three months of storage, particularly in Stage 4 (see Results). During this stage, while fructose and glucose stay at opposite high and low levels for a long period without crossing each other as they do in the first three stages, sorbitol content also keeps at a simultaneously constant level for almost three months.

Although sorbitol keeps a low concentration in the fruits and its content appears at a slightly constant increase toward the end, it does vary slightly in Stages 1 and 2 (see Figure 3, and Table 2), but this slight fluctuation is in exact direction with that of glucose and opposite to that of fructose; therefore, some side conversionsfrom either or both of these two reducing sugars must also be taking place at the same time, which is also responsible for the subsequent accumulation of sorbitol at the end. Sorbitol has been demonstrated to interconvert with glucose (Anderson et al., 1962; Whetter and Taper, 1963), and with fructose (Blakley, 1951; Seeberg et al., 1955; Touster and Shaw, 1962), moreover, fructose is found by Kreb and Lund (1966) to form glucose more rapidly than any other substrates tested, including sorbitol. Hers (1960) showed that in animal tissues, the conversion of glucose to fructose required the intermediate of sorbitol and the presence of various enzymes. This same mechanism probably also occurs in Malus tissues, because the enzymes responsible for this conversion have been found widely in the Rosaceae family (Touster and Shaw, 1962). Sorbitol, therefore, found here as a storage compound at the end as shown by its eventual accumulation in concentration, appears also as an intermediate during the rapid interconversion of fructose and glucose. A scheme of these interconversions is proposed as in the following steps:



At the time when fructose and glucose are interconverted with each other, the speed of these transformations is so rapid that glucose appears to convert directly to fructose or vice versa without the presence of sorbitol in between, this is seen in the slight change of the sorbitol content and its generally low concentration. However, during the course of these interconversions, as mentioned previously, some side conversions at a very slow speed, from either of these reducing sugars to sorbitol, probably chiefly from glucose, may take place, which is the frequent occurrence in a complicated metabolism, and sorbitol is therefore slightly accumulated (sorbitol 2, see scheme above). This slight accumulation of sorbitol may also have resulted from the possibility that the amount of sorbitol transformed from fructose is not completely reconverted to glucose, leaving a certain quantity of this sugar alcohol to accumulate in the fruits (sorbitol 3, see scheme above). The reason for this possibility can be attributed to the fact that fructose content is often at an excess of that of glucose, and the amount decrease is not entirely coincidental with the same net increase of glucose content. Although part of the glucose

content is probably consumed in respiration, some other transformations or conversions are also possibly involved, and the accumulation of sorbitol without further converting into glucose may be one of them.

Glucose, being interconverted to fructose through sorbitol, is generally regarded as an energy substrate for fruit respiration. Kidd et al. (1940) suggested that a respiratory drain on the glucose units throughout, and a supplementary drain on fructose units during the post-climateric phase were mainly responsible for the carbohydrate change during storage of Conference pears. Taper and Liu (1969) have shown that glucose decreases in stored fruits at 34°F., which is presumably used in fruit respiration, and is also possibly the cause of the rise of sucrose. The decrease of glucose content in the present data (Table 2) from its maximum on December 24 to the end of Stage 4 (see Figure 3) on April 13, ranges from 72.38 mg. to 36.50 mg./gm., suggesting at least a large part of this decrease is used up in respiration although a small quantity may be used for conversions with other sugars. Its last increase from 36.50 gm. to 54.84 mg./gm. (difference: 18.34 mg) may probably result solely by conversion from fructose which falls from 68.13 mg. to 24.50 mg. (difference: 43.63 mg.).

The contents of sucrose and raffinose are very low at Stages 1 and 2 (see Results) at the beginning of storage life. At this time starch decreases sharply to a minimum, while the two reducing sugars interconvert at a rapid rate with sorbitol

in between. However, sucrose in the last three stages (see Figure 3) increases in concentration, and its variation follows glucose more than fructose. The increase of sucrose when starch quickly disappears may be attributed to the hydrolysis of the latter to the former, as it has been shown that changes of these two carbohydrates are a linked process (Hulme, 1950, 1954). However, the gradual accumulation of sucrose in the last two stages may probably be attributed to its synthesis from fructose and glucose, which have been shown to be precursors of sucrose (Kursanov and Pavlinova, 1950; Axelrod and Seegmiller, 1954; Alexander, 1964). During the continuous interconversion of these two reducing sugars, as shown previously, apart from the accumulation of sorbitol, sucrose is also formed gradually, particularly from glucose, which is observed to decrease markedly after December as sucrose rises (see Figure 3). This increase of sucrose in fruits, coupled with the decrease of reducing sugars, is in agreement with Martin (1937) and Rees and Reynolds (1958). These results also agree with those of Taper and Liu (1969), who found that sucrose tended to increase in stored fruits together with a decline of glucose content. However, the accumulation of sucrose toward the end of the storage as shown in the present results renders an impression that it is a surplus sugar in late stored fruits without further changing into other sugars once it is accumulated.

Raffinose has its small peak on December 24 together with sucrose and glucose (see Figure 3), but its concentration falls

to a very low level during the rest of the storage life. At time of harvest, raffinose, like sucrose, falls markedly, and this decline is probably attributed to its translocation to the phloem. Sakai (1966) found that raffinose appeared first in September and October in twig barks of many fruit trees, including apple, and its concentration increases as winter approaches. From this observation, he suggested that its presence in the phloem might be related to frost-hardiness of the plant. The small amount of raffinose found in the fruits as shown by the present results can probably account for its translocation to the phloem. However, the slight increase of raffinose in late December and early January can be attributed to its synthesis from sucrose, since Lopatecki (1962) had shown that oligosaccharides could be formed from excess sucrose.

The rapid disappearance of starch in storage does not show any positive relation to the content of reducing sugars, but it seems to aid partly in the increase of sucrose in mid-December because starch is one of the nearest precursors of sucrose in plants (Kursanov and Pavlinova, 1950). This disappearance of starch in the storage in December agrees with the findings of Poapst et al. (1959) and Krotkov and Helson (1946) who found starch disappeared in McIntosh apple fruits by the end of October and early November, respectively.

The variation in data from day and night samples from fruit set to maturation (1968)

(1) Results

The average results for each of the day and night variations of sorbitol and its related carbohydrates in the two sample-groups are summarized in Table 3, and are also shown graphically in Figure 4. The data of the day samples are the same as those reported in Table 1, which are shown here specifically for the purpose of comparing with the night variations. Paired T-tests were also performed for results from the two sample-groups I and II at night, and no significant difference was found to exist between them.

The variation of sorbitol concentration in fruit samples taken at night was found similar to the pattern in day samples, but different in concentrations. At the beginning of the season when fruits were small and leaves had not fully become developed, sorbitol was present at high levels in the night fruits as it was in the day fruits, but higher sorbitol content was observed at night than in the day fruits at this time, and the same was true in the period when growth cessation had started and fruits ripened. During the period of active fruit growth, starting from the end of June to early August, sorbitol in night samples was consistently lower than that in the day. However, in summary, both day and night samples were found to possess the same tendency of having relatively low sorbitol content in the rapid fruit growth period but a higher content at the beginning

Table 3. Variations of sorbitol, related sugars and starch in day and night samples from fruit-set to maturation (mg./gm. fresh weight, mean data)

Date (1968)	Sorbitol		Fructose		Glucose		Sucrose	
	day	night	day	night	day	night	day	night
6/6	4.36	8.92	7.24	11.06	1.88	3.36	0.72	1.42
16/6	5.38	5.40	6.71	5.85	4.02	4.89	1.20	1.27
26/6	2.75	2.19	18.47	17.45	1.31	1.72	0.54	0.79
6/7	1.85	1.09	18.34	21.59	1.12	2.11	0.91	1.22
16/7	2.85	0.98	24.97	31.35	1.77	2.22	1.86	1.48
26/7	2.06	1.63	47.96	46.40	3.17	3.03	1.87	1.38
5/8	1.50	0.73	35.11	32.70	3.74	4.26	1.49	0.75
15/8	1.23	2.70	38.87	61.75	3.49	6.40	1.20	1.32
25/8	2.09	3.80	40.95	46.04	3.22	5.86	0.88	1.02
4/9	1.50	1.86	40.21	34.63	3.97	5.07	0.71	0.28
14/9	1.83	1.36	49.60	46.05	0.72	1.24	0.27	0.65

*See footnotes under Table 1.

Table 3 (continued).

Raffinose		Starch		Reducing sugars*		Total sugars*		Total carbohydrates*	
day	night	day	night	day	night	day	night	day	night
0.77	0.00	0.20	0.16	9.12	14.42	10.61	15.83	14.96	24.75
0.21	0.48	0.26	0.38	10.72	10.74	12.13	12.48	17.51	17.88
0.25	0.15	0.23	0.35	19.78	19.16	20.56	20.10	23.30	22.29
0.07	0.22	2.35	2.41	19.45	23.70	20.42	25.13	22.27	26.22
0.00	0.18	8.01	7.43	26.73	33.56	28.59	35.22	31.38	36.19
0.08	0.71	5.86	6.37	51.13	49.43	53.07	51.67	55.12	53.14
0.41	0.20	5.83	10.42	38.85	36.96	40.74	37.90	42.24	38.63
0.11	0.61	8.80	11.23	42.36	68.15	43.66	70.08	44.89	72.78
0.19	0.46	7.25	9.23	44.16	51.90	45.23	53.37	47.32	57.17
0.25	0.46	4.50	4.56	44.18	39.70	45.14	40.43	46.64	42.29
0.34	0.20	3.69	3.41	50.32	47.30	50.93	48.14	52.76	49.49

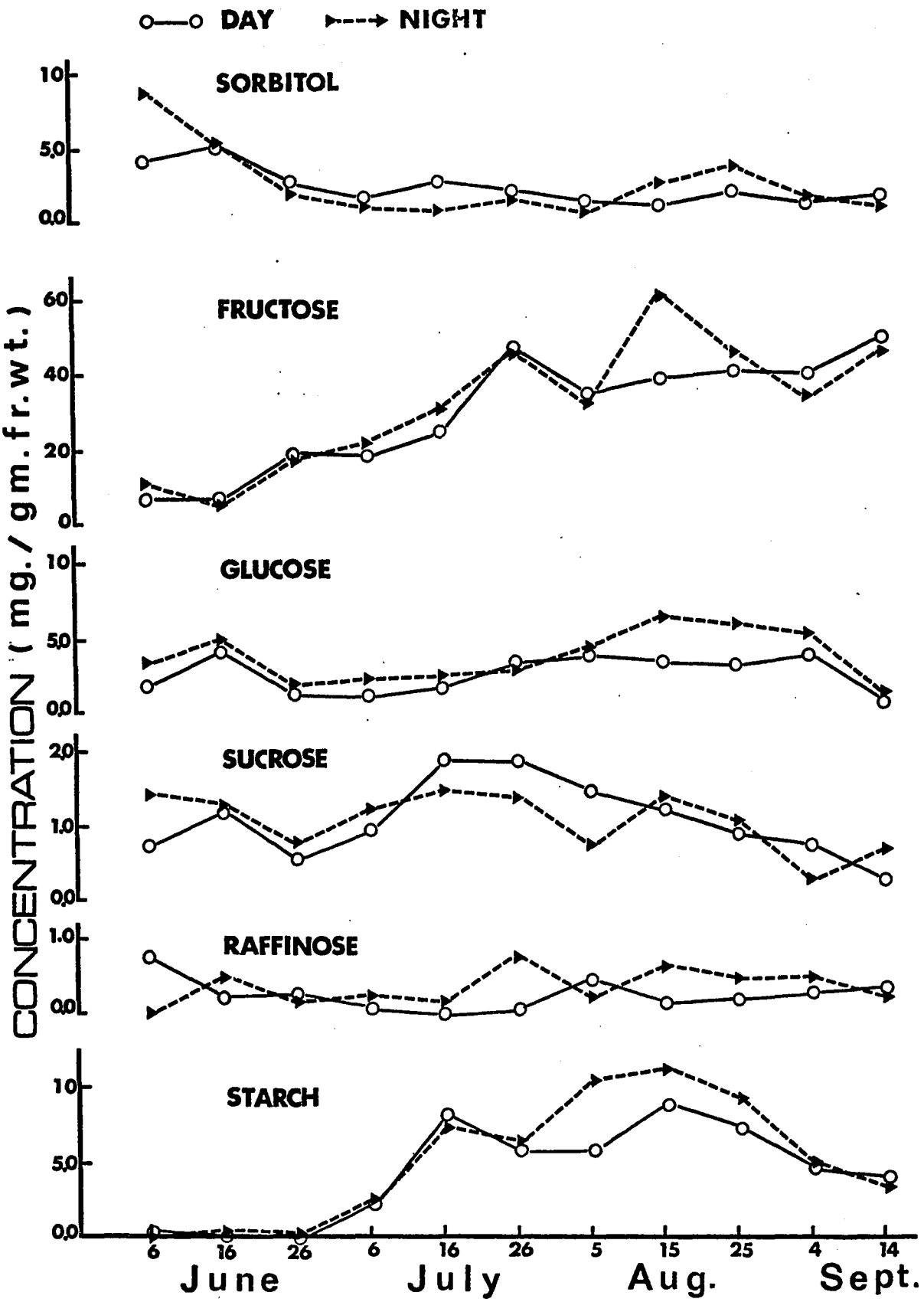


Figure 4. Day and night variations of sorbitol and related carbohydrate contents in Malus fruits from fruit set to maturation (1968).

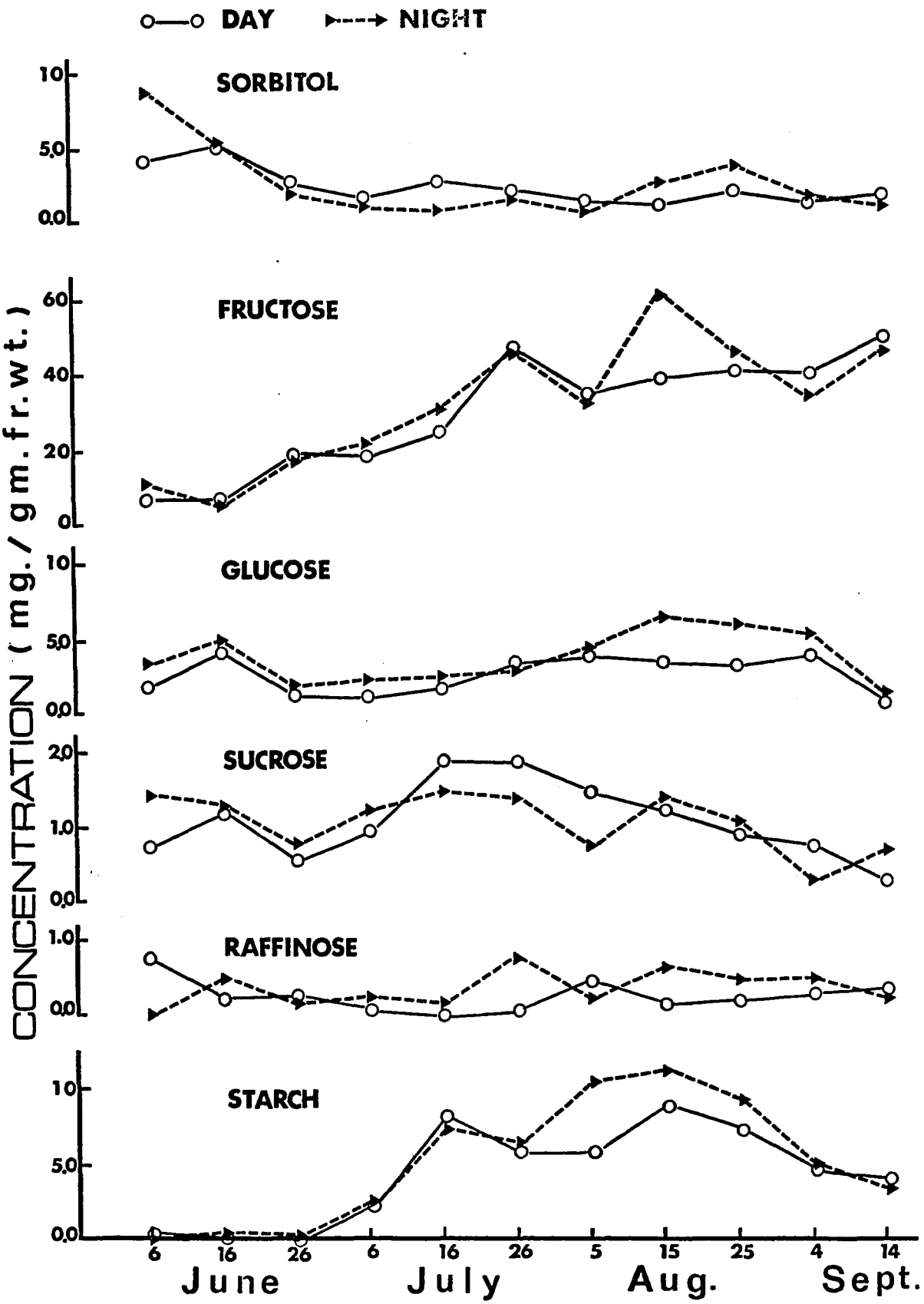


Figure 4. Day and night variations of sorbitol and related carbohydrate contents in Malus fruits from fruit set to maturation (1968).

and at the end of the season, with the night samples showing a better picture of the same variation.

As for the night pattern of fructose, its concentration was observed at an averagely higher level than that in the day. However, the occurrence of this reducing sugar at night in the first two months followed closely the day pattern in a rapid rise of concentration as fruits were gaining in size and weight. The same high peak of fructose accumulation at night was found on the same day as in the day samples, and these two peaks did not differ very much in concentration. After the fall in both night and day samples in early August, the night fructose content rose at a much faster rate than that in the day. A peak of maximum concentration was reached in night samples by mid-August where fructose was found at 61.75 mg. per gm. of fresh weight (see Table 3). During the period of growth cessation and fruit ripening, the day fructose rise was very gradual compared to the sharp decline in night concentration. A considerable increase of fructose content was observed at the end of the season for both day and night fruit samples.

For the first two months of fruit growth, the appearance of glucose at night fruit samples almost paralleled the pattern followed by the day, but at a slightly higher level. After growth had slowed down and fruits began to ripen, glucose rose to a level almost doubling its day concentration, and this level persisted for the following three weeks before it fell again at the end. However, both day and night samples maintained the

tendency to rise at the end of the season when fruits were ripened, and this rise was coincided with the slight increase in sorbitol and the sharp rise of fructose in both day and night samples.

Different from its day variations, sucrose in night fruits slightly fluctuated throughout the whole season. Its amount was higher than that in the day fruit samples for the first one and a half months when fruits were rapidly increasing in size. Then, in the active growing season, it maintained at about the same levels as before while the day samples had generally higher sucrose content. When fruits ripened, sucrose subsided and the same decrease was observed in the day samples. Raffinose was found to be present in a slightly higher amount at night than it was in the day samples. Although it did not disappear during the active growing period, its night concentration at that time was only present in a limited amount.

In the first two months of the growing season, the occurrence of starch in night fruit samples almost coincided with its variation in day. A peak was reached on July 16, the same day as the peak was observed in day samples. However, its concentration in the second half of the season rose at a faster rate than the day, and stayed at a considerably high level over that in the day samples for almost the rest of the season. A second peak was observed on August 15 at 11.23 mg. compared to the peak in day samples of 8.80 mg. After fruits had ripened, starch in both day and night samples fell gradually until fruits were ready for harvest.

In general, the total sugar and total carbohydrate contents in night samples were higher than those in the day fruits in the first month, but during the period of rapid growth, day samples exceeded the night in these contents. As fruit ripened and growth cessation had started, the night total sugar and carbohydrate contents increased and were in excess of those in the day. However, when fruits were ready for harvest, both total carbohydrate and sugar contents in day samples were again present in slightly higher amounts than the night.

(2) Discussion

Since most of the diurnal or day and night studies of carbohydrates changes are centered on the leaves (Davis et al., 1916; Waite and Boyd, 1953; Eagles, 1967), or sometimes on seeds or seedlings (Whetter and Taper, 1966), there is no detailed literature available on day and night studies of sorbitol and other carbohydrates in fruits. However, efforts have been made in this discussion to relate the present results to those obtained in other seemingly similar studies in the hope of furnishing some evidence in support of the present data.

In a diurnal study of carbohydrates in the agronomic grass cocksfoot grown in a controlled environment of 16-hour light and an 8-hour dark period, Eagles (1967) found that the content of the alcohol-soluble sugars in the leaf blades increased three to four-fold during the whole light period. In the dark, these concentrations subsided rapidly from 9.5% to 3.8% of dry weight from the 18th hour to the 24th hour (period

of artificial night). The same pattern was observed in the sheath. Eagles suggested that this decrease in the dark was probably a result of translocation and conversion of simple sugars to fructosan, which is a carbohydrate reserve in grasses when other sugars are in excess. This finding was in contrast with that of Waite and Boyd (1953) who showed in their investigation of water-soluble carbohydrate in rye grass that, when samples were taken during fairly sunny weather in June, sucrose was found rising to a maximum in the late afternoon (3:00 p.m.), while hexoses fell to their minimum at the same time; however, they rose to a comparatively higher level at night and to a very high concentration in the morning. Therefore, these researchers suggested that all the grass should be cut between 9:00 a.m. and 10:00 a.m., at which time hexoses were at maximal concentrations. The total carbohydrate content is shown by the present data (Table 3) to differ from that of Eagles in that, all through the whole season, its night concentration is almost parallel to its day concentration, and usually at an excess. However, the present results agree partly with the finding of Waite and Boyd in that during active fruit growth, day sucrose exceeds its night content and so does sorbitol; but the picture of hexose sugars (glucose and fructose) does not follow their finding of having maximum concentration in the morning. This is particularly untrue in the fruit-ripe period when night hexose content greatly exceeds its day content taken at 10:00 a.m. (see Table 3). This difference is

probably due to the different species of plants and organs used for comparison.

Miller (1924) found that the content of hexoses in the leaves of sorghum and maize remained fairly constant throughout the course of the day, whereas sucrose fluctuated markedly, increasing by day and diminishing by night. From these results, they concluded that sucrose was probably the primary sugar of photosynthesis. However, this theory has since been rejected and fructose is now considered to be the first product of this process (Calvin, 1962). As mentioned previously, sucrose is shown by the present data (Table 3) to have high day and low night concentrations in the period when leaves and fruits are growing rapidly. This view of sucrose coincides with that of the above researchers; and so does the pattern of the two reducing sugars, which remain at a fairly constant level both day and night from the beginning of the season. However, the night concentrations of these hexoses in the fruit-ripe period generally exceeds that in the day which may be due to the constant accumulation and less utilization of these sugars when fruit tissues are mature (see Table 3).

In the investigation of the carbohydrate pattern in the seeds and seedlings in apple, Whetter and Taper (1966) observed that fructose was the largest single soluble carbohydrate present in apple seeds in both light and dark during the first three days of germination. The seeds in the dark contained only a trace of sucrose, but relatively greater amounts of reducing

sugars were found at this time than in seeds in the light. These researchers suggested that the hydrolysis of sucrose had proceeded farther in seeds in dark because of their longer period in storage. The subsequent considerable amount of sucrose in the cotyledons in dark may be attributed to sorbitol reconversion (Whetter and Taper, 1963), possibly augmented by the hydrolysis of seed starch. In the present experiment, this conversion probably took place during the active fruit growing period from July 16 to August 15 when part of the sorbitol was converted to sucrose at a time when the two reducing sugars kept on increasing in concentration. Whetter and Taper further demonstrated that sorbitol was found to accumulate in photosynthesizing cotyledons in light sooner and in larger amounts than sucrose, but that sorbitol was not detectable in the dark. This suggested that the synthesis of sorbitol in higher amounts after germination was dependent on the production of excess sugars during photosynthesis, and then by rapid conversion of these sugars to sorbitol. The same mechanism probably operated during the active fruit growing period and at the fruit-ripening stage in the present experiment, but it was particularly notable at the end of the season when increase of sorbitol was coupled with a decrease of glucose. This is indeed the original suggestion of Donen (1939) who suggested that sorbitol was accumulated at the end of the plum growth period when reducing sugars began to level off. Whetter and Taper (1966) again suggested that photosynthesizing cotyledons were a site of synthesis of sorbitol.

While this finding is in accordance with those of Williams (1966) and Taper and Liu (1969) in their assessment of sorbitol as the most abundant photosynthate in the leaves, Williams (1966) stressed that this pool of sorbitol produced in the leaves was translocated out into the fruits at the time when leaves had become mature or senescent. An influx of sorbitol, though small in quantity, in the fruit-ripening stage in the present data, echoes their suggestion and this is more marked in the night.

Sideris et al. (1948) studied the diurnal changes and growth as associated with ascorbic acid, titratable acidity, carbohydrate and introgenous fraction in the leaves of pineapple Ananas comosus L., and they found that total sugar content, which showed maximal values at 6:00 p.m. and minimal at 6:00 a.m., was in invert order with titratable acidity (malic-citric acid). The results suggested that, during respiration, sugars in the tissues were oxidized, in the absence of light, to malic, citric and possibly other organic acids; the combined values of these acids increased as sugars decreased. The titratable acidity was reduced more in the leaves and fruits of the plants in dark than those in light. This reduction of titratable acidity is shown in the following scheme according to the principle of Le Chatelier (Bennet-Clark, 1933).

$$\text{Carbohydrates} \underset{\text{light}}{\overset{\text{dark}}{\rightleftharpoons}} \text{malic acid} + CO_2 + \text{energy}$$

Although increasing temperature accelerates the rate of organic acid disappearance from tissues and displaces this equilibrium in the direction in which energy is absorbed, light is considerably more effective in this shift (Bennet-Clark, 1933). Since titratable acids have not been analyzed in fruits in the present study, the possibility of this mechanism operating in the fruit tissues is not known. However, the accumulation of total carbohydrates shown by the present data (Table 3) does not agree with this shift, but a higher concentration is often found at night. Since the experimental samples are different, and fruits are not the main site of photosynthesis, this difference is conceivable.

Using a single sample, Liu (1967), in her study of sorbitol in Malus leaves, found a much higher concentration of sorbitol at night on August 27, 1966 in the leaves taken at 10:00 p.m. than on a day sample at 9:00 a.m. She explained this difference by using the results of the experiment conducted by Anderson et al. (1962), that the higher sorbitol content at night was probably attributable to the equilibration of this sugar alcohol with glucose or other possible intermediates in polysaccharide synthesis, especially starch, and the synthesis was favoured in the light. Since Anderson et al. (1959) had also demonstrated in their short term radioactive experiment with plum leaves a rapid synthesis of sorbitol following photosynthesis, and also a conversion of labelled carbohydrates into sorbitol in the dark, Liu, therefore, explained that the

subsequent drop in concentration of glucose and total sugars at night, shown in her data following the rise of sorbitol, was due to their conversion to sorbitol. Although she did not present more data to support her suggestions, the present results found in the fruits in late August agrees with her findings that high night sorbitol concentration is observed with the decline of other carbohydrates; but this is not consistent, and opposite variation is often observed; this may be partly due to the different experimental samples compared, in which the occurrence of these carbohydrates in fruits varies in opposite direction to those in the leaves.

Chong (1970), in his diurnal study of sorbitol in the apple leaves, found that sorbitol and carbohydrate contents decreased during the night, followed by a subsequent rise in the day, and that this was related to the diurnal change in air temperature, relative humidity, and to a lesser extent, solar radiation. This probably explains, for the present results, that the high level of total carbohydrates in the fruits at night may have in part come from the leaves through translocation for a temporary storage, and are again used for utilization in metabolism during the following day.

In summarizing the present results, it is noted that, at the beginning of the season when small fruits are just formed and most of the leaves have not come out or become fully developed, and high production and supply of food materials from

the leaves have not yet attained, sorbitol is found at high concentration in the green fruits. At this time, the plant carries on its normal metabolic activities as usual both day and night. These activities are made possible by utilizing sorbitol and other sugars as a source of energy and the rate of metabolism during the day is comparatively higher than that at night, hence, the rate of energy utilization is also higher. With a small supply of food materials from the leaves, sorbitol and the total carbohydrate contents are therefore observed at a lower level in day than at night. As rapid fruit growth begins after leaves have become fully expanded, large amounts of photosynthates are produced and supplied to the fruits in the day. Although the rate of energy utilization and metabolism is still proceeding at a higher speed in day than at night, the supply of carbohydrates to the fruits in the day exceeds their utilization, forming a higher carbohydrate pool in the day fruits than in those at the night, notably in the form of sorbitol. When growth ceases and fruits ripen and leaves become mature or senescent, the metabolic rate in these tissues is gradually slowed down and reduced, together with a reduced production of photosynthates. As the plant keeps on its physiological activities as usual and the rate in day is higher than at night, this shortage of photosynthate supply to compensate for the high day usage again results in a pattern similar to that at the beginning of the season: being in higher concentration at night and lower in the day, particularly in the form of sorbitol.

EXPERIMENT 2

An Investigation Concerning the Contents of Sorbitol, Related Sugars and Starch in the Skin of *Malus* Fruits during Periods of Fruit Growth and in Storage

A. Materials and Methods

Introduction

In the course of their chemical studies of fruits, some researchers (Martin, 1936; Siegelman, 1954) found that the skin of pear and apple fruits exhibited a carbohydrate pattern, though sorbitol was not included, which was essentially similar to that in the pulp tissues. Stoll (1967b) recently observed that the fruit tissues near the epidermis of pear and apple contained more sorbitol than the rest of the pulp tissues. Motivated by these findings, the present thesis writer felt that these compounds in the skin might in some way affect the life of fruits during their growth, or be related to some physiological disorders that originate from these skin tissues when fruits are stored. Therefore, an examination of these apple skin tissues in order to have a true picture of the carbohydrate pattern in them seemed necessary.

It was also noticed that none of the preceding workers nor other fruit researchers had done experiments concerning the contents of sorbitol and its related carbohydrate in the skin alone, and therefore, there were no established methods which could be followed. However, since skin tissues are an integral part of the fruit, the experimental procedure employed in the investigation of the carbohydrate contents in the fruits can theoretically be used for this carbohydrate determination in the skin. Therefore, most of the experimental procedures described in Experiment 1 are followed in this experiment. Samples were taken both from the fruits in the field and inside refrigerated storage to observe the relative contents of these carbohydrates under the two different environmental conditions.

Sampling of fruits

Skin samples were taken from fruits collected essentially in the same way as in Experiment 1. Five samplings at different dates were made from fruits in late storage period early in 1969 and six samplings were undertaken from fruits on tree during periods of late fruit growth and fruit ripening in summer of 1969. Although this was not a study of seasonal variation of carbohydrate contents in the skin tissues, sampling was nevertheless taken at a ten-day interval as was done for the fruits in Experiment 1.

Seven sample trees, cultivar McIntosh, were selected for sampling. These seven trees were located in Row 1, Block 9 of the Macdonald College Orchard. Sampling from the field commenced on July 26, 1969, when fruits were growing actively, and it was done in the mornings at 11:00 a.m. until September 14, ten days before all the fruits in the block were harvested and moved into cold storage. Fruits were not taken at the beginning of the season because the skin at that time was found hardly differentiated from the pulp. Since the objective of this experiment was mainly to determine the relative content of each carbohydrate constituent in the skin of apple fruit, the fruits were chosen randomly around each tree and the numbers were not strictly counted but usually varied according to their size on any particular date. However, the number of fruits in each sample was normally twice that for Experiment 1, because the quantity of skin tissue per fruit was small relative to the amount of pulp.

Sampling from refrigerated storage at 32°F. was done only for fruits in the late storage, period commencing in March 14 until April 23. These apples were originally from Row 11 of Block 9 harvested in the season of 1968, and skin determinations were made concurrently with the pulp estimation shown under Experiment 1. The number taken for each skin sample was either 14 or 21.

Skin sampling and processing

The apples collected were taken into the laboratory and the skin was peeled with a hand-peeler so as to leave as little adhering flesh as possible. The peeled skin was placed in a Pyrex glass dish and heated for three minutes in a steam bath according to the procedure of Siegelman (1954). All the adherent flesh tissue after the steaming process was carefully removed by scraping with a spatula and the water on the surface was blotted by tissue paper. The fresh skin was weighed and each sample, 3-20 gm. was blended in a small Waring blendor; each sample was then directed by a stream of 70% ethanol into a 500 ml. round-bottomed flask and extracted continuously for eight hours. Thereafter, the extract was filtered, washed, concentrated and clarified as described for the fruit samples used in Experiment 1. General details of chromatography and determination of sorbitol, sugars and starch were the same as in the previous experiment excepting that a large dosage of extract was spotted each time on the paper chromatogram.

Since the results in Experiment 1 showed that the last traces of starch inside the apple fruit tissues had disappeared by early December, no attempt was made to determine the starch content in the skin of apple fruits collected from refrigerated storage in this experiment. However, starch determination for skin samples from the field was undertaken.

B. Results

Using the experimental procedures described in Materials and Methods, the same carbohydrate pattern which had been found in fruit pulp tissues was detected in fruit skin for samples taken both from late storage and late growing season. These carbohydrates were separated on the chromatogram as in Experiment 1 in an order of decreasing R_g values, namely, sorbitol, fructose, glucose, sucrose and raffinose. The spots on the chromatogram were observed darker than those spotted with extracts from pulp tissues. This might be due to the high dosage used in each spot application as it had been so reported under Materials and Methods. Starch was detected from alcohol-insoluble skin residue. The results of these carbohydrate contents on different sampling dates are summarized in Table 4.

Carbohydrate pattern in skin of fruits in late storage

In examining the pattern of carbohydrates in the skin of fruits in storage (see Table 4), it was noticed that sorbitol, sucrose and raffinose were present in a considerably higher amount than their respective concentrations in stored fruit pulp tissues (see Table 2), while fructose and glucose were observed at a relatively low concentration in the skin compared to their prominent amount in pulp tissues as shown

Table 4. Sorbitol, sugars and starch contents in the skin of *Malus* fruit during periods of fruit growth and in storage (mg./gm. fresh weight)

Date	Sorbitol	Fructose	Glucose	Sucrose
		In Late Storage		
14/3/69	4.58	3.75	10.00	7.71
24/3/69	16.46	18.33	24.58	0.00
3/4/69	18.33	1.67	6.67	7.50
13/4/69	8.33	2.22	2.22	5.83
23/4/69	6.11	3.33	1.11	6.94
		Late Growing Season		
26/7/69	16.13	17.50	12.92	3.33
5/8/69	9.15	20.73	10.06	0.00
15/8/69	8.07	37.10	21.77	2.42
25/8/69	5.51	34.33	23.96	6.48
4/9/69	8.72	20.19	16.98	5.05
14/9/69	4.65	19.74	13.08	5.57

*See footnotes under Table 1.

Table 4 (continued).

Raffinose	Starch	Reducing sugars*	Total sugars*	Total carbohydrates*
2.71	--	13.75	24.17	28.75
13.33	--	42.91	56.24	72.70
0.42	--	8.34	16.26	34.59
3.06	--	4.44	13.33	21.66
2.78	--	4.44	14.16	20.27
1.67	1.18	30.42	35.42	51.55
2.74	3.07	30.79	33.53	42.68
6.65	7.54	58.87	67.94	76.01
0.32	5.71	58.29	65.09	70.60
0.00	4.30	37.17	42.22	50.94
0.11	5.68	32.82	38.50	43.15

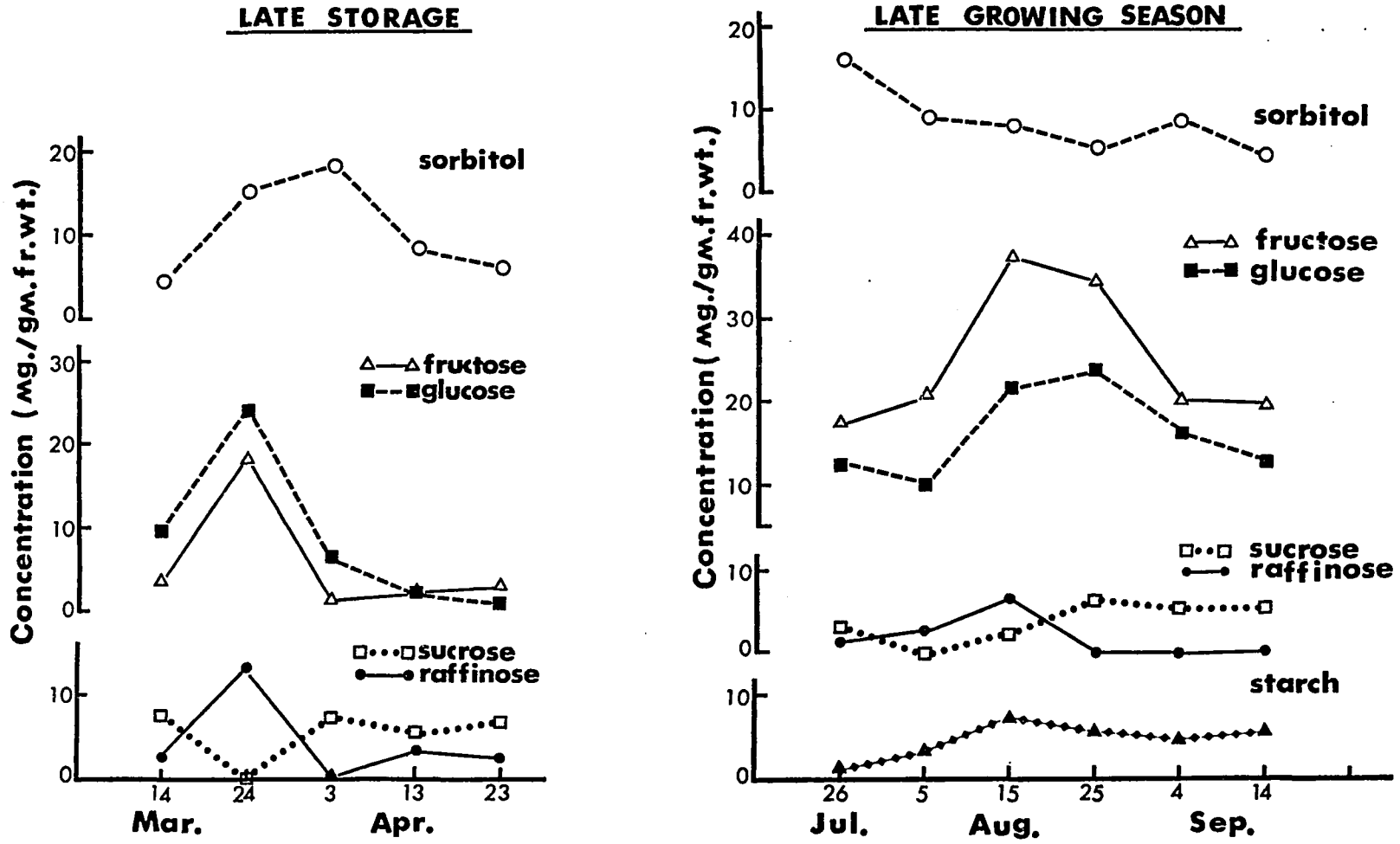


Figure 5. Concentrations of sorbitol and related carbohydrates in the skin of Malus fruit during late fruit growth and in late storage (1969).

before. Of these two reducing sugars, glucose was found in a higher level than that of fructose. However, sorbitol content appeared to be the leading carbohydrate in the skin in the whole late storage period with the exception on March 24 where glucose was the largest.

During this late storage period when most of the fruits had become soft, the occurrence of sorbitol was observed to follow very closely its variation in the pulp tissues at the same time (see Table 2). In the first sample taken on March 14, 1969, when sorbitol content in the pulp tissues at this time was at a very low level, its content in fruit skin was also low compared to other samples in this storage period. As its content in pulp tissues increased, sorbitol in fruit skin rose accordingly, but at a much faster rate, and quickly reached a high concentration which was almost four times as much as its initial content. A peak was reached on April 3 at a level of 18.33 mg., while at this time sorbitol content in pulp tissues was also at its maximum of 5.82 mg. per gm. fresh pulp tissue (Table 2 and Figure 3). After this peak, similar to the two reducing sugars and raffinose, sorbitol fell sharply, and this fall was again coincided with a gradual decline in pulp sorbitol content. However, excepting the first sample taken on March 14, the concentration of sorbitol in all the skin samples exceeded its content in pulp tissues taken at the same time, and this excess was particularly notable on March 24 and April 3.

In contrast to the continuously opposite variations in the pulp tissue as shown in Table 2 and Figure 3, the occurrence of fructose and glucose followed each other closely in the skin tissues. Glucose was generally in excess of fructose in the skin. On March 24, when fructose in pulp tissues rose to its maximum, skin fructose increased in a similar way to a peak which was about six times of its initial concentration ten days before. This increase coincided with the same sharp rise of sorbitol, glucose and raffinose, while sucrose was absent. After this peak, fructose fell abruptly to almost a minimum when its concentration in the pulp at this time still maintained at a considerably high level and was in excess of glucose (see Table 2). Glucose, like fructose, had its peak on March 24, but it fell thereafter to a concentration slightly below the level of fructose.

Among all the carbohydrates, sucrose was the only one that did not rise at the end of March, but instead, it disappeared completely from the skin tissues while at this time its concentration in pulp tissue kept on rising. However, sucrose reappeared again in early April when all the other carbohydrate but sorbitol dropped tremendously. Thereafter, it tended to remain at a constant level until the last sample. As for raffinose, it was interesting to find that its concentration in fruit skin was far in excess of that in the fruit pulp tissues analyzed in the same late storage period. It had a maximal uprise at the end of March when its concentration was

found at 13.33 mg. per gm. fresh weight. Its content in the last three samples was low.

Carbohydrate pattern in skin of fruits during late fruit growing period

In reporting the contents of various carbohydrate constituents in the skin tissues, it was felt necessary to compare them with the same carbohydrates in the fruit pulp tissues taken at the same time in order to have a comprehensive picture of their overall variations in the fruit as a whole. Therefore, as it was done in the previous section, results in this section were often referred to the data obtained in the pulp tissues for fruits taken in the summer of 1969, which were previously presented in Table 1 and Figure 2.

In examining the sorbitol content in skin tissues, it was found that sorbitol was in excess of its concentration in the pulp tissues taken at the same time, notably in the first sample taken on July 26, 1969 when fruits were large and still gaining in weight. The accumulation of this sugar alcohol in the fruit skin was the third largest in concentration. However, both skin and pulp tissues had the tendency to decrease in concentration during this period, and the rate in skin tissues was at first very rapid but gradually slowed down. Similar to its occurrence in the pulp, sorbitol in the skin stayed at an almost constant level thereafter when fruits had ripened.

Fructose and glucose in the fruit skin accumulated in concentration in a similar way as it did in the pulp tissues when fruits gradually gained in size (Table 1). Fructose was the largest carbohydrate in the skin tissues as it was in the pulps, and glucose the second. A peak was reached by August 15 for fructose content and the maximum amount of glucose was also attained at the end of the aforesaid month. Contrary to their ever-increasing concentration in the pulp tissues, these two reducing sugars in the skin declined when fruit ripened which was coincided with a small rise of sucrose content. Raffinose variation in the skin was in an opposite direction to that of sucrose. It was present in a slightly higher amount in the first few samples when fruit growth had slowed down, while sucrose was found at this period to disappear from the skin and to reappear again in a small amount. Then, as raffinose subsided to a minimum, sucrose rose in the fruit ripening period. Generally, these two carbohydrates were all in excess of their concentrations in the pulp tissues. As for starch in the skin, its concentration did not differ very much with that in the pulp tissue and it had its maximum accumulation on the same day as it did in the pulp tissues. Similarly, when fruits ripened, their contents decreased in the same manner as in the fruit pulps.

C. Discussion

When reviewing literature on skin tissues of apple fruits, it was noticed, to the disappointment of the thesis writer, that almost all these researches either concerned the wax content in the skin (Chibnall *et al.*, 1931; Markley *et al.*, 1932; Hall, 1966), its color pigments with relation to fruit maturity and storage (Sims, 1963; Lott, 1965), or skin structure as related to transpiration or polishing (Pieniazek, 1944; Skene, 1963). Apart from the previously reported three papers (Martin, 1936; Siegelman, 1954; Stoll 1967b; see Introduction, Materials and Methods, Experiment 2), no literature was found on carbohydrate distribution or metabolism in the skin tissues. The following discussion, therefore, relates the present results mostly to the findings of those three researchers.

In his study of the distribution of sugars in Bosc pear fruit, Martin (1936) noticed that among the four regions he divided inside a pear, namely, skin, cortical, stone cell and core regions, the concentration of glucose was found present in a larger amount in the skin region than the other three, while fructose and sucrose were the least. Although this same researcher (Martin, 1937) a year later detected sorbitol in the pear and suggested its relation to fructose, glucose and sucrose during the after-storage ripening, he did not report the presence of sorbitol in the fruit nor in the skin in this study. Siegelman (1954) revealed a pattern of carbohydrates, namely,

in an order of increasing R_x* values, sucrose, glucose, fructose, and xylose on his paper chromatogram when spotted with an extract from Grimes Golden apple and Bartlett pear skin. The same pattern of these sugars was detected in various solvent systems. Furthermore, the flavonoid compounds of Grimes Golden apple skin were again shown to consist of quercetin 3-glycosides of the following sugars: glucose, galactose, xylose, arabinose, rhamnose, and rutinose (Siegelman, 1954). Only two of these sugars, glucose and xylose, were also found as free sugars in the skin. This researcher strongly stressed the presence of xylose in these tissues and suggested that its role in the metabolism of apple and pear skin needed further examination, because this sugar had been reported from only a few plants (Bidwell, et al., 1952; Hay and Pridham, 1953). In comparing the R_x values of his carbohydrates with the present ones found in McIntosh apple skin tissues, it was thought that the xylose which had been stressed by Siegelman might probably be sorbitol found on the chromatogram in the present experiment, the reason being that the carbohydrates found in our study are arranged in the same order as that of Siegelman, namely, raffinose, sucrose, glucose, fructose and sorbitol, with increasing R_g values; and sorbitol here appeared to be in the same position as occupied by xylose in Siegelman's chromatogram. Test

---

*R_x = R xylose (migration relative to xylose).

chromatograms using standard compounds revealed that the spot of xylose in the present solvent system was in a position between fructose and sorbitol. However, the amount of xylose in McIntosh apple skin on the chromatogram tested was found to be almost negligible. Since Siegelman did not report its concentration in the skin tissues, no comparison was able to be made.

Stoll (1967b), in his experiment to investigate the distribution of sorbitol in various regions inside pear and apple fruit, reported that the highest sorbitol concentration resided in the area near the epidermis, and the least was found near the core. This was consistent for the three varieties each of apple and pear. When sorbitol was so found in skin region as never done before, the present thesis writer recalled the skin region of Bosc pear where Martin (1936) found glucose in the highest amount. There was some thought that this high glucose accumulation in the skin region of Martin's pear might possibly be sorbitol, as it was found by Stoll (1967b) to be in the same region at high concentration. The sensitive method of Stoll's thin layer chromatography may prove to be more efficient than that used by Martin, because the latter stated that his quantitative results so obtained in the skin were subject to errors arising from interfering substances in the skin and no effort was made to correct it. Since these two compounds, sorbitol and glucose, are structurally alike, and have been proved to be readily interconvertible with each other (Anderson

et al., 1962; Whetter and Taper, 1963), it is conceivable that they are sometimes found together, in a mixed manner, even in their reaction to radioactivity (Dana, 1952; Webb and Burley, 1962). However, if the glucose in the skin region of Bosc pear were actually sorbitol, the findings of Stoll and the present data would be more justified.

In examining the present results of these carbohydrate constituents in McIntosh apple skin (Table 4), it is observed that the presence of sorbitol as the leading carbohydrate in it during storage agrees with the finding of Stoll (1967b), and that glucose as the second largest carbohydrate accords with the findings of Martin (1936). During late storage, all the carbohydrates in the skin in March rise in concentration excepting sucrose, this occurrence coincides with their respective fluctuations in the pulp tissues analyzed at the same time (see Table 2). The subsequent decline of reducing sugars coupled with the increase of sorbitol reflects a same mechanism of the interconversion between these carbohydrates operating in the skin. At the end of the storage period, sucrose is present in excess of the reducing sugars, which also renders the belief that this invert sugar may have formed through conversion from the latter, in a similar pathway that has been established in the pulp tissue. As for the skin samples taken from fruits during period of growth, the appearance of all the carbohydrates in it is shown also to parallel their individual variations in the fruit pulps. Sorbitol decreases in concentration as fruits are

rapidly gaining in size, and it rises in content when fruits are ripened. Similarly, fructose appears as the leading carbohydrate in the skin and its occurrence is matched closely with glucose. Apart from their translocating from the pulp tissues, the general accumulation of these two reducing sugars in the skin may have come directly from the metabolic tissues in the skin where it is suggested that photosynthesis takes place (see Experiment 3), and fructose is the first product of this process (Calvin, 1962). During fruit ripening, all the carbohydrates tend to lose in content, notably in fructose, glucose and sorbitol, while at the same time, these carbohydrates in the pulp tissues increase (see Figure 2). This opposite variation presumably may be attributed to that when fruits are ripened, the red pigments formed on the skin prevent any further photosynthesis to produce photosynthate for accumulation, and/or the presence of the high carbohydrate pools in the pulp tissues at this ripe stage draws most of these carbohydrates inside.

Nevertheless, since the variation of sorbitol and other carbohydrates is seen to follow closely their individual variations in the pulp tissues, it appears that the metabolism of these carbohydrates in the skin, as in the pulp itself, is an integral part of the total metabolism in the fruit. Hence, one has confidence in the previous assumption and may state that skin is an inseparable part of the fruit, but its carbohydrate

pattern may well serve as an index for the changes of the various carbohydrate pools inside.

EXPERIMENT 3

A Preliminary Investigation of the Photosynthetic Capacity of Apple Fruit Skin Compared to that of the Adjacent Leaves during Active Fruit Growth and at Maturation

A. Materials and Methods

Introduction

This experiment was undertaken primarily to determine whether or not the skin of apple fruit is able to photosynthesize, but with a further aim, should such function be established, to conduct studies to determine the skin's capacity to supply photosynthates to the fruit which it encloses and to compare such capacity with that of the adjacent leaves.

If the fruit is isolated by ringing, or other means, from its adjacent leaves and the rest of the tree, it is thus blocked from its usual supply of food materials for its growth. An experiment to determine whether an isolated fruit is still able to continue its enlargement and growth, by manufacturing

its own food through its green or the whole skin surface, should be capable of furnishing evidence concerning the ability of the skin to photosynthesize. This type of experiment may be done by taking readings at fixed intervals on the fruit for its increase of fresh weight, dry weight, diameter, volume and carbohydrate content. This is the original concept of Curtis (1920) and Ryugo and Davis (1959), though they did not include skin investigation per se in their experiment. When data from a number of sampling dates are collected, a general picture of the ability of the skin to supply food for fruit growth may be obtained. Furthermore, if another set of fruit units is similarly isolated, but with the adjacent leaves left within the isolated unit, the ability of these leaves to supply photosynthates for fruit growth can be compared with that of fruit skin when their individual 'net assimilation rates' are computed. This term 'net assimilation rate' is the original concept of Gregory (1917), which is expressed in terms of the amount of dry matter per unit of leaf area (in this case, leaf or skin area) per unit of time. In their experiment to evaluate the photosynthetic ability of peach leaves to supply dry matter requirement of the crop, Ryugo and Davis (1959) compared net assimilation data with the rate of accumulation by the crop and they found that, in regard to the assimilation of dry matter by the fruits, the double-girdled method they used did not show all the photosynthates produced by the leaves, the reason being that the vegetative parts of the tree and the

maturing fruits used a certain amount of photosynthetic product as a source of energy for respiration. This loss was not measured in their experiment. Similarly, in the experiment conducted by the present author, this consumption of part of the photosynthates was not included, and the term 'net' was used throughout to make explicit that only the residual dry matter, or the products of 'apparent photosynthesis', were determined (Ryugo and Davis, 1959).

Ringing and sampling

The double-girdled method of Ryugo and Davis (1959), used to compare the net assimilation rate of peach leaves and the rate of accumulation of dry weight by the fruit, was essentially followed in conducting the present experiment.

On July 4, 1968 when fruits were growing rapidly, five uniform trees of cultivar McIntosh in Row 1, Block 9 of the Macdonald College Orchard were chosen for sampling. From these trees, 150 short fruiting branches of approximately the same size, mostly on the south side, were selected, tagged and numbered, making 30 branches per tree. The branches chosen were not thickly crowded with leaves or small side branches. An experimental unit was isolated by double-girdling on each branch, with each unit containing a single fruit, and all the fruits used were of uniform size. Leaves were removed from one-half (15) of the units on each tree. For the other 15 fruiting units, about 4-6 leaves, either small or large and

free from blemish or darkened spots, were kept within the rings. Data from leafless and leafed units were compared.

The procedure of ringing was as follows: a ring of bark, 3/8 to one inch wide, was removed three inches below and above the fruit in each unit. Only the outer bark and the phloem were removed and no injury was done to the xylem. The rings were covered with black rubber grafting tape to reduce evaporation, and they were checked every ten days to see if the cut was healed; further re-ringing was done whenever necessary.

Sampling was done at 13-day intervals. On each sampling date, five sample fruits, one from each tree, were removed at random from leafless units and another similar five from leafed units, making 10 units at each sampling date for these two kinds of branches. Care was taken to include in the samples all the leaves and fruits in the units and no disturbance was made to the other branches still on the tree. Each sample branch was put in a plastic bag and brought back to the laboratory immediately. Outlines of the leaves removed from the various units were traced on paper and the areas were later measured by means of a planimeter, and expressed in square decimeters.

After the sampling, all these ten samples were combined into two groups, namely, leafed group and leafless group. The leafed group (Group 1) consisted of five fruits and a number of leaves collected from the five leafed units, while the leafless group (Group 2) consisted of five fruits alone, which were taken from the five leafless units where no leaf was left within their girdles.

Fruits from each of these two groups, leafed or leafless, were weighed and their diameters measured. They were then quartered and placed on dishes in an oven at 100°C. and dried overnight; and were re-weighed when the dry weight was constant. The carbohydrate contents in the fruits of either group were determined from the dry materials by the same methods as described in the section, Materials and Methods of Experiment 1, and only total carbohydrate content was reported.

On each sampling date, the median diameter of each fruit still on the tree in each ringed unit was measured to the nearest tenth millimeter by means of a Vernier caliper. Increments in diameter and volume of these fruits were calculated from these measurements for each sampling date.

Sampling was continued until September 20 for leafed fruit units while fruits in the units without leaves were only collected up to the 7th of the aforesaid month because many fruits had dropped off from these units in the latter part of the season.

B. Results and Discussion

Results of this preliminary investigation of the photosynthetic capacity of the fruit skin and that of its adjacent leaves are summarized in Tables 5 and 6. The photosynthetic area in the leafed group is the sum of the areas of leaves and skin of the fruits within the girdles, while that in the leafless

Table 5. Results to show the increment to fresh weight, dry weight, diameter and volume of the two sets of 'ringed' groups during active fruit growth and at maturation

Set	Date 1968	Photosynthetic area (sq. dm.) leaf	skin	total	Total fr.wt. (gm.)	Total dr.wt. (gm.)	Av. diam. of each (cm.)	Av.vol. of each (cu.cm.)
Group 1 (leafed units)	4/7	--	2.35	--	--	--	3.87	30.25
	17/7	7.48	2.97	10.45	149.59	14.92	4.35	43.16
	30/7	8.23	3.73	11.96	214.11	22.87	4.87	60.41
	12/8	8.10	4.60	12.70	327.75	36.26	5.41	82.82
	25/8	7.61	5.39	13.00	411.26	54.45	5.86	105.16
	7/9	7.61	5.95	13.56	505.38	66.16	6.15	121.55
	20/9	7.19	6.47	13.66	549.35	78.47	6.42	138.47
Group 2 (leafless units)	4/7	--	2.23	2.23	--	--	3.77	28.04
	17/7	--	2.37	2.37	143.34	12.17	3.88	30.59
	30/7	--	2.48	2.48	149.59	12.63	3.97	32.72
	12/8	--	2.67	2.67	189.47	13.39	4.12	36.63
	25/8	--	2.89	2.89	206.74	15.05	4.29	41.35
	7/9	--	2.54	2.54	199.89	15.65	4.02	34.02
	20/9	--	--	--	--	--	--	--

Table 5 (continued).

Total carbohydrates (mg./gm.) dr.wt.	Av. increase of fr.wt. (gm.)	dr.wt. (gm.)	diam. (cm.)	vol. (cu.cm.)
--	--	--	--	--
3.04	--	--	0.49 (12.7%)	12.91(42.7%)
28.09	64.52 (43.1%)	7.95 (47.0%)	0.52 (12.0%)	17.25 (40.0%)
17.75	113.64 (53.1%)	13.39 (58.6%)	0.54 (11.1%)	22.41 (37.1%)
31.95	83.51 (25.5%)	18.19 (50.2%)	0.45 (8.3%)	22.34 (27.0%)
42.76	94.12 (22.9%)	11.71 (21.5%)	0.29 (4.9%)	16.39 (15.6%)
41.53	43.97 (87.0%)	12.31 (18.6%)	0.27 (4.4%)	16.92 (13.9%)
--	--	--	--	--
2.32	--	--	0.11 (2.9%)	2.55 (9.1%)
20.56	6.25 (4.4%)	0.46 (3.8%)	0.09 (2.3%)	2.13 (7.0%)
9.54	39.88 (26.7%)	0.76 (6.0%)	0.15 (3.8%)	3.91 (11.9%)
18.98	17.27 (9.1%)	1.66 (12.4%)	0.17 (4.1%)	4.72 (12.9%)
27.09	-6.85 (-3.3%)	0.60 (4.0%)	-0.27 (-6.3%)	-7.33 (-17.7%)
--	--	--	--	--

Table 6. The 'net assimilation rates' (E_a) of the leafed and leafless unit-groups at different dates. Values are expressed as milligrams of dry weight per square decimeter of photosynthetic area per day.

Date	Leafed group		Leafless group		
	E_{a_1} (leaf and skin)	% Leaf area	E_{a_2} (leaf only)	E_{a_3} (skin only)	$\frac{E_{a_2}\text{ (skin)}}{E_{a_2}\text{ (leaf)}} \times 100\%$
30/7	0.0237	68.8%	0.0163	0.0063	38.7%
12/8	0.03619	63.8%	0.0231	0.0198	85.7%
25/8	0.0471	58.5%	0.0276	0.0199	72.1%
7/9	0.0294	56.1%	0.0165	negative	--
20/9	0.0303	52.6%	0.0159	--	--

group includes the skin areas of the fruits only. Since the whole surface of the fruit was assumed to photosynthesize, the surface area from each fruit was computed for the surface of a sphere by using the formula $4\pi r^2$, in which r is the median diameter of the fruit and the resultant area is expressed in square decimeters. The volume of the fruit was calculated by the formula $4/3\pi r^3$, which again is originally designed for the volume of a sphere. Average increase of fresh weight, dry weight, diameter and volume was both reported in actual measurements and percentages. Total carbohydrate content was expressed as milligrams per gram of dry weight, which is a sum of the concentrations of sorbitol, fructose, glucose, sucrose, raffinose and starch.

Appearance of fruit growth in leafless group (Group 1)

By examining the results presented in Tables 5 and 6, this attempt to study the photosynthetic ability of the skin is observed to have been rewarded with some encouraging data. It is shown in the results (Table 5) that fruits keep on growing in the leafless units which are isolated from the normal supply of photosynthates from the adjacent leaves or other sources. The increase of fruit size shown by the increase in volume, and the accumulation of photosynthates expressed in the increase of dry matter are indices of this fruit growth. However, this ability of the skin tissues to supply photosynthates

is shown more elaborately by the net assimilation rate (E_a), as presented in Table 6, which will be discussed later. From Table 5, a continuous increase of dry weight and volume was observed in the period from July 17 to August 25, in which ordinary fruit growth was at the most rapid rate. The largest fruit size obtained was on August 25, at an average volume of 41.35 cu. cm. each, which was about one and one-half that of the fruits when ringing was just started in early July. Also, on the same day, the fruits in the units had the largest fresh weight in the whole season, but the largest dry weight was not attained until 13 days later on September 7. The highest net increase in dry weight occurred in the period from August 12 to August 25, when a 12.4% increase over the immediate last sample was found. However, at the end of August, while the other fruits on the tree were approaching their ripening stage, the fruits in the leafless units became gradually wrinkled on the surface and an unhealthy appearance was subsequently observed. Moreover, many fruits were found dropping from their units, which may partly have been caused by strong wind or heavy rains during that period, but a more important reason was probably attributable to the formation of the abscission layer in the fruit spurs when fruits reached their physiologically aged period earlier, due to inadequate supply of food (Jacobs, 1962). Since these fruits had shrunk in the late sampling dates, the last measurements of their increment are shown in negative results (see Table 5).

Appearance of fruit growth in leafed group (Group 2)

As shown in Table 5, fruits in the leafed units grew in an almost normal manner. Their rate of growth, size and accumulated dry matter exceeded all those of fruits in the leafless units by a considerable margin. The highest accumulation of dry matter was on August 25 when an increase of 18.19 gm. was recorded; the largest volume measurement was also found on the same day. Fruits were found to have a continuous growth inside the girdles from the very beginning of the experimental period up to its end. Unlike those fruits in the leafless units, no fruit in this group was observed to have fallen off at the end of the experimental period. However, at the ripe stage, the fruits in this group were only two-thirds the size of the normally ripe fruits, and they were found not to ripen as other fruits on the tree did. Green or light green color was still found prevailing on the surface and less red pigments than was the case with normal fruits.

A comparison of the net assimilation rates (E_a) in the leafed and leafless groups

The data for the production of dry matter by the leaves and the skin obtained in the double-girdled method expressed in 'net assimilation rate' (E_a) are shown in Table 6. These net assimilation rates are expressed as milligrams of dry weight per square decimeter of photosynthetic area (leaf or skin) per day (Gregory, 1917; Ryugo and Davis, 1959). This rate was computed from the equation:

$$E_a = \frac{W_2 - W_1}{L_2 - L_1} \cdot \frac{\ln L_2 - \ln L_1}{t_2 - t_1}$$, in which E_a is the net assimilation rate, W is the dry weight of the fruits, t is the time and L is either the sum of the area of the leaves between the girdles plus that of the skin of the fruits in the leafed group, or the skin area of the fruits in the leafless group. The symbol E_{a_1} in Table 6 represents the net assimilation rate of leaves plus skin of the fruits in the leafed group: E_{a_2} is that of leaves only in the same group. E_{a_3} represents the net assimilation rate of fruit skin in the leafless group. The last column in Table 6 is a ratio of the net assimilation rate of the leafless group to that of the leafed group expressed in percentage.

In examining the net assimilation rate of the skin tissues in the leafless group in Table 6, it was found that these skin tissues supplied dry matter to the fruits at a high speed, ranging from 38.7% to 85.7% of that of the adjacent leaves. The closest rate of these two groups, leafed and leafless, was observed on August 12 when rapid fruit growth had gradually slowed down, and fruits in both groups were accumulating dry matter. The lowest rate of the leafless group, as compared to that of the leafed group, was observed on the date July 30, when their ratio was approximately 1:3, indicating leaves were still the main site of photosynthesis, in which further photosynthates were being produced before their tissues became mature.

The total carbohydrate content in the fruits, as shown in Table 5, does not have a continuous increase in the period of fruit growth, whereas fluctuation is observed throughout the whole experimental season. It therefore appears impossible to relate this carbohydrate content to one of the indices for fruit growth, or for the ability of these leaf or skin tissues to supply dry matter to the fruits. However, the ups and downs of this carbohydrate content in both groups are observed to follow each other closely, indicating that the supply of carbohydrates to the fruits from either the leaf or the skin is metabolically the same, which relates to the season but differs only in concentration.

When the net assimilation rates of these two groups are found at a generally close range, it appears that it is not logical for skin tissues to supply dry matter to fruits at such a high rate as shown in the data. Leaves are generally regarded as the main photosynthetic site of the plant, in which the photosynthates produced are continuously supplied to various organs for growth or other metabolic activities. When skin tissues are viewed anatomically, it is obvious that there is less chlorophyll content in these tissues than that in the leaves. It was, therefore, anticipated that if skin were able to supply dry matter to the fruit, it should be at a slow rate, much slower than it had been found in this experiment. When this doubt arose, a detailed inspection of the methods and experimental procedures performed in this experiment was

undertaken, and a major discrepancy observed at the ringing step. When the twig bark was ringed at the beginning of the experiment, the cut on the bark was covered with grafting tape, and it was inspected at an interval of ten days to check if the cut was healed; a re-cut was made when healing did happen, and these re-cuts were performed many times. It was observed that these cuts made after the first ringing, were healed a few days before they were inspected at the routine interval of 10 days, and this was found most frequently in the leafless units. Since the cut was healed, the supply of photosynthates from non-adjacent leaves or other sources might have come into these girdled fruits, and thus a large portion of the increase of dry matter in the fruits may be accountable to an influx of photosynthates from sources other than the adjacent leaves or fruit skin. Further, at the time of sampling, all the leaves in the leafed units were collected and their areas were used subsequently as the basis for photosynthetic area in Group 1. However, when these leaves were examined carefully, it was noticed that some of them were yellow or brown in color. While these yellow or brown leaves may not have contributed to the supply of dry matter to the fruits, the addition of their areas into the photosynthetic area of this leafed group for calculation may simply have resulted in an apparent smaller assimilation rate than anticipated, and the E_a calculated in this group is accordingly closer to that of the leafless group. Moreover, the measurement of the fruit skin area may also contribute to this apparently high net

assimilation rate in the leafless group. The fruits used in this experiment were not perfect spheres, but were slightly oval in shape; the use of the formula originally for sphere surface area resulted in a slightly smaller area than the fruit actually had, hence a higher E_a was calculated. Furthermore, the oven used in the experiment for drying apples was not well under control, there was a possibility that the sliced apple fruits were not completely dried before their weight was taken, which may also lead to a higher assimilation in the calculation. Finally, the small number of experimental samples used in this experiment appears not to meet the requirement of a highly-valid statistical representation. While the present experiment was carried out concurrently with the elaborate experiments previously reported, and it was only on a trial basis, the author was limited to the number of samples which a single operator was capable of processing. It is hoped that more elaborate methods will be designed to further this experiment.

TECHNIQUES, AN INVESTIGATION AND APPRAISAL

A. Introduction

This chapter examines and elucidates some of the methods used in the present experiments, and in previous studies conducted in this department in order to attain a better understanding and to improve the effectiveness of these techniques. Some sections deal with a number of precautionary measures which the present writer has found necessary during the course of his own experiments throughout this thesis project, while some paragraphs report the failure of certain techniques because of certain laboratory conditions prevailing at the time, and the nature of the samples used. The writer believes that these observations will help to serve, in one way or another, as an aid or reference for people conducting similar investigations in the future. The suggestions contained herein do not necessarily reject nor substitute for procedures described or carried out by other workers, but rather, within the limits of the writer's capacity, are supplementary to them. It is noted that Mr. C. Chong, a fellow postgraduate student in the Department of Horticulture, collaborated in part of the investigation of the techniques on thin layer chromatography.

B. An Experiment to Observe the Solubility of Sorbitol and Related Sugars in Various Concentrations of Ethyl Alcohol

Whetter (1962), in her experiment dealing with the extraction of leaf samples by ethyl alcohol, found that the solubility of sorbitol in 70% alcohol was greater than in a dilution of 80%, a dilution generally used by many resesearchers for plant extracts (Ash and Reynolds, 1955a, 1955b; Hutchinson et al., 1959; Trip et al., 1965; Williams, 1966). She found up to 30 gm. sorbitol dissolved in 100 ml. of 70% ethanol. This dilution was later used by Liu (1967) in her extraction of apple leaves and fruits. During the routine work of this study, it came to the mind of the present writer that if this dilution of 70% ethyl alcohol could be further increased with more water content, more alcohol would have been saved and expenses reduced, because the amount of alcohol consumed each day for extractions was very large. Moreover, the interest aroused by Whetter concerning the relation of sorbitol solubility to alcohol dilutions prompted the writer to perform a careful investigation of this problem.

Materials and methods

This experiment was conducted to compare the solubility of sorbitol and related sugars in different dilutions of ethyl alcohol within the range of 55% to 80%.

Ethyl alcohol (95%) was diluted into aliquots of 55%, 60%, 70%, 75% and 80% concentrations which were placed separately in five different glass jars. For each percentage dilution a constant quantity of 1 gm. each of sorbitol, glucose, sucrose and raffinose was placed in respective 10 ml. beakers, and a constant volume of 2.5 ml. of ethyl alcohol of the selected dilution was syringed into each beaker to dissolve the carbohydrate inside. Because of its great solubility, 2 gm. of fructose were used in a similar procedure. Each solution was vigorously but carefully stirred with a fine stirring rod to enable the solute to dissolve as much as possible. The whole solution plus residue was carefully poured onto a pre-weighed Whatman No. 50 filter paper placed inside a Buchner funnel and filtered under vacuum. Each filter paper with residues was taken out cautiously and dried in the air and weighed. By subtracting the weight of residue left on the filter paper from the original weight of carbohydrate used, the quantity of a particular carbohydrate dissolved in each dilution of ethyl alcohol was accurately determined. The results, after converting into percentages, are shown in Table 7.

Results and discussion

In examining the results shown in Table 7, it was observed that the dilutions of ethyl alcohol of 55%, 60% and 70% were ideal for sorbitol extraction from plant tissues, because

Table 7. Percentage of carbohydrates dissolved in various concentrations of ethyl alcohol at room temperature (22°C.)

Ethyl alcohol, %	Carbohydrates dissolved, %				
	Sorbitol	Fructose	Glucose	Sucrose	Raffinose
55%	100%	52.5%	38.8%	100%	12.1%
60%	100%	96.3%	37.3%	94.6%	7.8%
70%	100%	96.0%	20.7%	89.7%	5.1%
75%	93.4%	87.3%	2.1%	36.7%	5.9%
80%	14.5%	77.3%	0.0%	37.5%	3.2%
Amount of carbohydrate (mgm.) in 2.5 ml. of ethyl alcohol	1.0	2.0	1.0	1.0	1.0
Equivalent amount in 100 ml. of alcohol	40.0	80.0	40.0	40.0	40.0

it was readily soluble in each of them. The gross amount of sorbitol dissolved in these dilutions was shown up to 40 gm. per 100 ml. of alcohol, 10 gm. more than the quantity found by Whetter. Fructose and sucrose were also very soluble in each of the above three dilutions. Fructose attained its greatest solubility in ethanol at 60% dilution while sucrose reached its maximum in 55% alcohol. The solubility of glucose was observed fair at these dilutions reported, with the largest amount dissolved in 55% of ethyl alcohol and the lowest at 70%. Raffinose, only sparingly soluble in each of these alcohol dilutions, had a solubility inversely proportional to the quantity of alcohol present in the solution. This inversely proportional relationship was found to be true for all carbohydrates but sucrose in the dilutions of 75% and 80%, in which the reduction of alcohol in the solution was marked by an increase in carbohydrate solubility. Glucose, which had a solubility of only 2.1% at 75%, did not dissolve at all at 80%. Raffinose was the least soluble at 80% alcohol dilution, with sorbitol the second (14.5%); however, fructose was found still relatively soluble in this dilution. The overall picture of these results rendered an impression that the solubility of sorbitol and related sugars increased with the increase of water content; and at 55%, rather than at 70%, the solubility of all carbohydrates with the exception of fructose, attained the highest level in all the dilutions under investigation. The levels at 60% were also relatively satisfactory and were better than that at 70%.

Since water is generally regarded as the best solvent for carbohydrates, and the purpose of selecting a particular solvent here is to obtain maximum efficiency in the extraction of these compounds from the samples, it seems quite unreasonable not to use water as an extracting medium. Nevertheless, the chemical properties of certain compounds present in the present samples, or which ultimately became present due to reaction, did not easily permit the use of water as a solvent. The fruit tissues were found to change into a dark brown color under heating when water alone was employed. This was due to the dark complex called melanoidin formed during the reaction of sugars and amino acids inside the fruit tissues, which is generally known as the Maillard Reaction (Danehy and Pigman, 1951). This darkening of the tissues may eventually interfere with the separation of the individual compounds on the chromatogram.

However, this darkening, in Maillard's own words (cited by Danehy and Pigman, 1951), can be decreased by lowering sufficiently the moisture content of any solvent used for extraction, hence the less water there is present, the lesser the extent of this reaction.

Bearing this in mind, water alone was avoided as an extractive medium, and a water dilution of ethyl alcohol, the usual solvent for this type of sample extraction was retained. Indeed, experiment results showed also that samples extracted in 80% ethyl alcohol, with respect to suppression of Maillard

Reaction, were likely to give the most satisfactory results; and that effectiveness in this regard, decreased with decreasing alcohol content.

Therefore, it can be concluded that, although the dilutions of 55% and 60% alcohol have a high ability to dissolve large amounts of sorbitol and other sugars, they fail to lower the browning of the tissues in the reacting solution, whereas the dilutions of 75% and 80% are sufficient to lower the Maillard Reaction, but not satisfactory in their ability to dissolve a large quantity of the aforesaid carbohydrates. Thus, the dilution of 70% becomes the only satisfactory compromise. Its ability to dissolve sorbitol, the most important carbohydrate in this study, is shown by this experiment to be 100%, and the content of alcohol in it is sufficient to reduce the browning reaction to a considerable extent. In view of these facts, Whetter's recommendation, namely, that 70% ethyl alcohol be used, was retained and used to extract the tissues of apple fruit in the experiments already described in this thesis. The heating step in the course of extraction did help to compensate for the comparatively lower solubility of other sugars in this dilution (70%) as compared to their solubilities at room temperature (22°C.) in the 55% and 60% ethanol solutions.

C. A Test to Examine the Suitability of Roe's Method for Fructose Determination as Compared to that of Phenol-Sulfuric Acid Method

Before the thesis project was started, it was found that a fast and convenient method was necessary to determine the sugars in the apple fruit tissue. Since a large number of experimental samples were collected in this thesis study, it was impossible to use different methods for different sugars as done by Liu (1967). In her determination of sugars in apple leaf and fruit tissue, Liu employed the anthrone method for glucose and sucrose, and Roe's method (1934) for fructose. However, it was found that sugar alcohols reacted with this anthrone reagent (Graham, 1963); further, this reagent required fresh preparation almost every time, and a large quantity of sulfuric acid was consumed. It was also noticed that the method of Roe, which was originally used to determine fructose in blood and urine, required a lengthy manipulation. More importantly, it was observed that the color developed by the reagents in Roe's method was very unstable, even within a short time interval. Roe pointed out, in describing his procedures, that the red color obtained was dependent on the velocity of the reaction at an elevated temperature, and the percentage transmission should therefore be read immediately after the cooling step, because this color began to fade after standing for 30 minutes. This test was intended to study the color

changes in this method at different time intervals, and to compare its suitability for fructose determination with that of phenol-sulfuric acid method. All materials and methods and experimental procedures are identical to that reported by Roe (1934) or by Liu (1967).

Results and discussion

In the course of testing the validity of this method, it was noticed that something more than the color fading was involved. The color developed in the reaction was observed to change 10 minutes after the whole series of experimental procedures was completed. Instead of fading to lighter colors, as described by Roe, the readings obtained at time intervals of 10, 30 and 60 minutes showed a decrease in the percentage of light transmission, which in other words, indicated an increase of color intensity. The transmission readings taken at these time intervals are summarized in Table 8.

It is quite surprising to see the color developed began to change only 10 minutes after the experiment. The difference obtained from transmission readings taken at 30 and 60 minutes was very large, particularly when fructose content was high. At low concentration, however, a decrease in percentage light transmittance was observed to be in accordance with an increase of the concentration of fructose. Yet, when the concentration was up to 140 μg., this decrease in light transmittance stopped and a rise was observed with the increase

Table 8. Transmission readings taken at different time intervals in the quantitative determination of fructose by Roe's method (average of triplicate readings)

(A)	Blank	20	40	60	80	100	120	140	160	180	200
(B)	100	81.0	71.8	65.5	64.0	62.0	58.0	59.0	59.2	57.0	56.0
						10 minutes					
(C)	100	81.0	71.8	65.5	63.0	60.5	56.3	58.3	59.0	57.3	56.8
						30 minutes					
(C)	100	81.3	71.8	65.0	60.0	57.0	54.5	55.8	57.8	56.8	56.3
						60 minutes					
(C)	100	80.8	70.3	61.7	56.0	52.5	48.5	50.3	49.8	48.8	48.8
						24 hours					
(C)	100	83.8	75.3	68.0	61.3	57.8	54.0	52.0	50.0	47.8	46.3

(A) : Concentrations of fructose in μg.
(B) : % transmittance at 520 mμ. taken immediately after the whole series of experimental procedures.
(C) : % transmittance at 520 mμ. taken at various time intervals as indicated after the experiment.

of fructose content. This behaviour was found consistent for the first four time periods as reported in Table 8, but not in the 24-hour period. This abnormal increase in percentage transmittance with increase of fructose concentration did not obey Beer-Lambert Law, in which light transmittance of a certain substance in solution is inversely proportional to its concentration. Since this decrease obtained was not linear, it would obscure the later quantitative calculation if it were used as a standard. Nevertheless, this abnormality shown in the results did not persist long, a linear decrease was observed again at concentrations of 180 µg. or higher.

In view of this unstability of color in the solution, transmission readings for the samples after the experimental procedures have to be taken rapidly. Since a large number of samples are used in this thesis experiment, thirty or forty samples are usually required to analyze at the same time to minimize any experimental error that may arise, it is impossible to handle them within a period of five to ten minutes in order to avoid the color change. Moreover, the abnormality of light transmission occurred at 140 µg., which, as a consequence, does not allow the use of fructose solution beyond this concentration. Since fructose is present as the most abundant sugar in the fruit tissues, and further dilution to obtain a suitable low concentration requires lengthy manipulation, this method does not appear to be a good and convenient one for the determination of this sugar, and therefore it is not recommended.

On the contrary, the use of phenol-sulfuric acid method for the determination of fructose was found to eliminate all the shortcomings so reported for the above method. The results in percentage transmittance of several trials on fructose determination are summarized in Table 9. It is observed that much consistent results were obtained at different time intervals. Although a slight difference was still found at the 60-minute period, this was not shown to be significantly different. The overall curve obtained at either of the time intervals agreed well with Beer-Lambert Law and an abnormal appearance was observed at high fructose concentration. Besides the fact that consistent results can be obtained by this method, it has been proven good also for the determinations of glucose, sucrose and raffinose. Furthermore, the manipulation of this method is very simple and only one reagent is needed to prepare, which can be used for several months.

D. Notes on Starch Determination

During the determination of starch, some special techniques and precautionary measures were observed and found to be worth recording as a guide to a better future handling of this method. The following seven points are directly referred to in the procedure numbered with an asterisk in the section Starch Determination, in the chapter Materials and Methods of Experiment 1.

Table 9. Transmission readings taken at different time intervals in the quantitative determination of fructose by the phenol-sulfuric acid method (average of triplicate readings)

(A)	Blank	10	20	30	40	50	60	70	80	90	100
(B)	100	88.0	77.8	65.0	64.9	54.5	47.0	45.0	40.8	35.5	31.8
					5 minutes						
(C)	100	88.0	77.8	65.0	64.9	54.5	47.0	45.0	40.8	35.5	31.8
					60 minutes						
(C)	100	88.0	77.8	65.0	64.5	54.0	47.0	44.5	40.8	35.5	31.5

(A) : See footnotes (A) under Table 8.

(B) and (C) : See footnotes (B) and (C) under Table 8 excepting that the wavelength used here is 490 mμ.

1. For colorimetric analyses it is essential to disperse the starch in a fine, stable colloidal suspension with minimum hydrolysis to sugar. High perchloric acid concentration, high temperature and increased time of digestion tend to favor this starch hydrolysis. Diluting 72% perchloric acid to about 7.8 N avoids, to a large extent, the hydrolytic effect and the temperature rise due to heat of dilution (Nielsen and Gleason, 1945), and more consistent results are thus obtained.

2. Five to ten minutes' digestion completely disperses the starch in all samples with maximum color development; 3 minutes' digestion is inadequate for dispersing the starch in some samples; while less color, presumably due to hydrolysis, is obtained in samples digested 15-30 minutes (Carter and Neubert, 1954).

3. Whatman No. 2 filter paper filters off starch (Nielsen, 1943).

4. The size of the volumetric flask depends on the approximate amount of starch in the samples. Carter and Neubert (1954) found that in their study, 500 ml. flask was suitable for 1.5-3.0% starch, 250 ml. for 0.7-1.5% starch and 100 ml. for a trace to 0.7% starch. In Table 1 of their paper, the percentage of starch in different varieties of apple was shown at around 1.4%, thus a 250 ml. volumetric flask was chosen for this purpose.

5. The volume of aliquot here is not essential, the important point is to make sure that there is enough

concentration of starch in the aliquot to react with iodine and not over, so as to obtain a reading within the range of 20-80% of transmittance.

6. Sodium thiosulfate solution should not be over-used or applied too early since it clouds the solution instead of clearing it.

7. In handling perchloric acid, extreme care is required, since it is a very dangerous acid, which explodes on contact with oxidizable or combustible, dehydrating or reducing agents. It is sold always in 72% and 60% to avoid this danger. A reading of the article "Note on perchloric acid and its handling in analytical work" prepared by the Analytical Method Committee is recommended (Analyst, 84, 214-216, 1959).

E. A Brief Report on the Application of Thin Layer Chromatography in the Study of Carbohydrates in *Malus* Tissues

Within the past ten years, thin layer chromatography has gradually developed into an important analytical technique of high adaptability which has been successively modified for quantitative analysis in microscales (Stahl, 1965; Kirchner, 1967). Workers in this field have used a variety of names for this technique. It has been referred to as surface chromatography, open-column chromatography, thin layer chromatography, thin film chromatography, coated glass chromatography, laminar chromatography and strip or plate chromatography. However,

thin layer chromatography is the most common term and often it is abbreviated as TLC (Stahl and Kaltenbach, 1961a, 1961b).

The modern basis of TLC was laid by the extensive studies of Stahl and his co-workers in Germany in 1956 (Stahl and Kaltenbach, 1961a; Stahl, 1965). They first developed a practical laboratory technique for producing uniform thin layers of various powdered materials on glass plates. The term 'thin layer' was introduced to indicate that a layer of slurried material had been applied to a sheet of glass and that this layer was about 0.25 mm. thick, thus differentiating this technique from paper chromatography. This development is a major advance in the techniques for separating the constituents of a mixture from each other. Thin layer chromatography was first presented to the European Convention on Chemical Engineering, held at Frankfurt, Germany in 1958. Much interest was attracted from scientists, laboratories and industries. It thus became generally known, and as a result, it soon became widely employed as a research technique.

Thin layer chromatography has found usage in the study of a broad range of compounds, including phenol, lipids, terpene derivatives, vitamins, steroids, amino acids and nucleic acids, etc. (Stahl, 1965). Unfortunately, its application in respect of carbohydrates is limited and often not extensively elaborated in books concerning this technique (Hay et al., 1963). However, some research is also being done in the carbohydrate field, in the separation of simple sugars (Stahl and

Kaltenbach, 1961a), digitalis and podophyllum glycosides (Stahl and Kaltenbach, 1961b), methylated glycosides (Gee, 1963), acetates (Tate and Bishop, 1962), commercial sugars (Bracher and Bauly, 1965), lipids (Morris, 1962, Wren, 1960), polyols and their derivatives (Hay et al., 1963) and so on. Nevertheless, the separation of polyols by thin layer chromatography has been rarely used (Lewis and Smith, 1967b).

Before the thesis project was started, the author had the chance to conduct a series of tests on the suitability of this technique on the fruit and leaf extracts used in this study. The following report concerns a number of trials conducted in an effort to apply thin layer techniques to the chromatography of such extracts.

Materials and methods

(1) Chromatoplates

a. Plain silica gel plates

A slurry of 28 gm. silica gel G (E. Merck, A.G., Darmstadt, Germany) in 60 ml. of distilled water was shaken vigorously for 90 seconds and applied to smooth glass plates (20 x 20 x 0.3 cm.) at a thickness of 0.25 mm. using a standard applicator. The plates were left to dry at room temperature until the gel had set, then heated to 135°C. for one hour. The serrated plates were scraped with a knife to remove adsorbent from the ridge peaks.

b. Bisulfite-impregnated silica gel G plates

A slurry of 40 gm. of silica gel G in 80 ml. of 0.1 M sodium bisulfite solution (Adachi, 1965) was applied to the glass plates. The plates were allowed to stand for 30 minutes at room temperature, then dried in an oven at 120°C. for an hour.

c. Borate-impregnated silica gel G plates

A slurry of 30 gm. of silica gel G was shaken vigorously with 60 ml. of borate buffer (0.02 M, pH 8.0 : 100 ml. 0.02 M boric acid solution, pH 5.9, and 3.0 ml. of 0.02 M sodium tetraborate solution, pH 9.3.) (Jacin and Mishkin, 1965) for 60-90 seconds. Glass plates were coated to the usual 0.25 mm. thickness with the applicator. The plates were kept at room temperature until they were set and then dried for 30 minutes at 100°C. They were stored at room temperature in the dessicator; and like other plates, they were activated for 30 minutes at 100°C. before use.

(2) Spotting and developing

The extracts were spotted by a 5 µl. Drummond disposable pipette and plates were developed in a pre-equilibrated jar, with a dimension specifically for thin layer plates, by one-dimensional ascending chromatography. The solvent front was allowed to migrate up the plate for 15 cm.

(3) Solvent systems

Data concerning the various solvent systems attempted in these trials are tabulated below:

No.	Solvent systems	Proportions (by volume)	Time for migrating to 15 cm. (min.)	References
A	n-butanol-acetic acid-ethyl ether-water	9:6:3:1	105	Hay et al., 1963
B	n-butanol-acetic acid-water	6:3:1	60	Stahl, 1965
C	Ethyl acetate-isopropanol-water	16:6:3	45	Prey et al., 1963
D	Isopropanol-ethyl-acetate-water	54:7:2	55	Wasserman and Hanus, 1963
E	Methyl ethyl ketone-acetic acid water	3:0.5:1.5	90	Adachi, 1965
F	n-butanol-acetic acid-water	5:4:1	120	Jacin and Mishkin, 1965

(4) Spraying reagents

The plates after running in the various solvent systems were heated in the oven at 120-135°C. for approximately 10-15 minutes before they were sprayed with appropriate spraying reagents. These spraying reagents are described as in the following:

a. Mixture of 0.5% potassium permanganate in 0.1 N NaOH (1:1/v:v)

This alkaline permanganate spray readily detects all compounds with free hydroxyl groups or compounds which are derived with alkali-labile groups and hence gives good detection of polyols and sugars (Hay et al., 1963). The carbohydrates detected by this spray on the chromatoplates were observed to appear as bright yellow spots in a light purple background which faded to white spots on a brown background on standing.

b. Phenol-sulfuric acid reagent

This solution contains 3 gm. of phenol and 5 gm. of concentrated sulfuric acid in 95 ml. of ethyl alcohol. The carbohydrates, when sprayed, appeared as red brown spots. The color of these spots could be intensified by further heating. This reagent could still be used when several days old.

Results and discussion

The R_f values of the five carbohydrates, namely, sorbitol, fructose, glucose, sucrose and raffinose recorded in different solvent systems are summarized in Table 10.

In the course of these trials on thin layer chromatography, it was found that the solvent system, n-butanol-acetic acid-ethyl ether-water (9:6:3:1) used by Hay et al. (1963) was the best one for the separation of the aforesaid five carbohydrates, therefore effort in this test was centered on the

Table 10. R_f values of five carbohydrates present in apple fruit and leaf extracts obtained on the chromatoplates developed in different solvent systems, using different plates and spraying reagents

		Solvent A								
	Standard compounds (Hay et al., 1963)	Fruit extract					Leaf extract			
Sample No.	1	1	2	3	4	5	1	2	3	4
Glucose	0.49	0.49	0.44	0.49	0.47	0.46	0.48	0.47	0.33	0.35
Fructose	0.51	0.43	0.37	0.44	0.44	0.37	0.41	0.44	0.31	0.34
Sucrose	0.25	0.35	0.35	0.34	0.35	0.32	0.34	0.35	0.23	0.24
Sorbitol	0.39	0.31	0.26	0.23	0.29	0.23	0.31	0.29	0.22	0.22
Raffinose	0.13	0.15	0.14	0.15	0.13	0.09	0.12	0.13	0.09	0.11
Spray Reagents	a	a	a	a	a	b	a	a	a	a
Plates	w	w	w	w	w	w	w	w	w	w

Solvent systems A B C D E and F: See solvent systems listed previously in this chapter.
Spraying reagents: a -- Mixture of potassium permanganate and sodium hydroxide.
b -- Phenol-sulfuric acid reagent.
Plates: w -- Plain silica gel plate
y -- Bisulfite-impregnated silica gel plate
z -- Borate-impregnated silica gel plate.

Table 10 (continued).

Solvent B	Solvents C	D	Solvent E		Solvent F
Leaf extract	Fruit extract		Fruit extract		Fruit extract
1	1	1	1	2	1
0.40	no		0.43	0.39	0.29
0.38	separation		0.46	0.45	0.31
0.30	was		0.29	0.33	0.28
0.29	observed		not shown	not shown	not shown
0.15			0.22	0.23	0.08
a	a	a	b	b	b
w	w	w	y	y	z

use of this solvent to separate these carbohydrates from fruit and leaf extracts. Unfortunately, it was noticed that while Hay and his co-workers succeeded in separating a mixture of the five standard carbohydrates required, the present attempt failed to separate them from plant extracts with sufficient efficiency to give definite and reproducible R_f values. When examining the chromatoplates, it was found that the spots on the plate overlapping each other. This overlapping was particularly conspicuous for spots of fructose and glucose, because they had adjacent R_f values. Extracts of fruit and leaf were found to show the same overlapping pattern on the chromatoplates. However, the separation of sorbitol and sucrose from fruit extract was observed fairly satisfactory, but their spots overlapped each other in leaf extract. It was also noticed that yellow bands were trailing these carbohydrate spots, and very often they were observed to extend from the base line to the solvent front. These yellow bands were seen sometimes by the naked eye before the spray operation, when they were present in high concentrations. Since these bands possessed an almost similar color to those carbohydrate spots on the chromatoplate, their appearance often obscured spot separation. When examined, they were thought probably to be pigments or tannins that had escaped the previous process of clarification. Since these bands were not shown on the paper chromatogram, their appearance on the thin layer chromatoplates could probably be attributed to the latter's high

sensitivity which detects all impurities even at very low concentrations (Hay et al., 1963; Heftmann, 1967).

The solvent system B was found also to give overlapping spots on the chromatoplates and the R_f values of the individual carbohydrate spots were very close to each other. Solvents C and D were found not to be able to separate the carbohydrates in the present fruit or leaf extract, because spots were seen not to migrate up the thin layer with the solvent. The use of the other two chemical impregnated silica gel G plates, namely, bisulfite- and borate-impregnated plates in solvent systems E and F, respectively, was attempted and the sugar spots were found separated out at a fairly definite pattern without the interfering substances in between, despite these spots being tooth-shaped. But, to the disappointment of the author, sorbitol, the most important carbohydrate in the thesis study, was not detected on the chromatoplates.

The discrepancies observed in these trials, when examined carefully, were found to have resulted from many factors. The fact that no suitable solvent system was discovered to allow a good separation of all the aforesaid carbohydrates in fruit or leaf extract appears to be the first problem. The uneven thickness of the silica gel layer on the plates and the presence of pigments in the extracts are also important reasons. Moreover, the layering apparatus currently used in the department does not allow the production of smooth and uniform thin layers on the plates, which is very critical

with respect to spot separation. As for the pigments, the extracts used have been clarified by lead acetate and sodium oxalate before as described in Materials and Methods, Experiment 1, no attempt is made to clarify them again by other clarifying reagents, because it is feared that the composition of the extract will be affected. Since it is noticed that no interfering substances are detected on paper chromatogram when the same extracts are used, the clarifying step is regarded satisfactory as far as paper chromatography is concerned. Other factors which may be connected with the difficulty in separating these carbohydrates on thin layer plates include the possibility of spotting an excessive volume of extract on the layer, and carelessness in the handling or controlling of the drying and activation procedures.

Since paper chromatography had been proven previously in this department to be a useful tool in the separating of carbohydrates in fruit and leaf extracts, and no pigment or yellow bands were found on the paper chromatogram, and because of the convenience of using paper for the larger number of experimental samples in this project study, decision was finally made to go back to paper chromatography. However, there are also some other reasons why thin layer chromatography was not selected. It is noticed that, with the limited facilities in the department with respect to thin layer chromatography, only a few samples can be processed at a time, when TLC is employed. Also, since the silica gel

layer on the plate is not permanent, it breaks when subjected to slight external disturbance, an inconvenience thus arises that no plate is able to be prepared well before its use; or if this is possible, the only dessicator in the department can only provide storing space for a limited number of plates. Further, time is required to prepare these plates, and all the plates so prepared are not necessarily applicable for spotting, because uneven thickness is very often observed on some plates. When paper chromatography is employed, this procedure of preparing layers is automatically eliminated, and all the paper chromatograms are of a uniform size. Moreover, the application of thin layer chromatography requires a much more expensive maintenance than that of paper chromatography, particularly in this thesis project when large numbers of samples are needed to process. It, therefore, appears that the selection of paper chromatography over TLC for this thesis study is a logical approach.

F. Suggestions for Chromatography

Lewis and Smith (1967b), in their review of analytical methods for sugar alcohols, predicted that gas-liquid chromatography would eventually become a standard practice for quantitative analysis of sorbitol and other polyols from plants. It is considered by the present writer that if this technique were adopted in a project such as the one described

in the preceding pages, a rapid and accurate assay of the experiment samples could be carried out free of the necessity for those stringent steps of separation, identification and quantitative determination used in paper or thin layer chromatography. Since gas chromatography does not require a lengthy operation in which experimental errors are likely to occur, and qualitative and quantitative data can be obtained at the same time, it is suggested that the future analyses of sorbitol and its related carbohydrates may profitably employ this technique to facilitate a rapid, efficient, and accurate determination of the samples and to minimize possible interference of certain components by extraneous materials during the usual experimental processes. It is also recommended that sorbitol be separated in gas chromatography as its tri-methyl-trimethyl-silyl derivative, as was done by Williams (1966) and recommended by Lewis and Smith (1967b).

SUMMARY

The seasonal occurrence of sorbitol and its related carbohydrates in the fruits were studied in a two-year period during fruit growth and in storage. Night variation of these carbohydrates was followed in the first year during the fruit growing period. Sorbitol was observed at highest level when fruits were just set, while the other sugars were comparatively low in content. The gradual increase in reducing sugars and starch during the active fruit growing period was coupled with a decline of sorbitol content. In the ripe stage, a slight rise of sorbitol was echoed by a rapid accumulation of reducing sugars, whereas sucrose, raffinose and starch were observed to decrease. In refrigerated storage, sorbitol decreased initially, levelled off for a time, and subsequently increased slightly. Reducing sugars were found to interconvert rapidly throughout the storage period. Sucrose was accumulated towards the end of storage, while raffinose remained low and starch disappeared completely in December. Night occurrence of sorbitol in the season was reflected in an initial higher level than the day content. This was followed by a low level during active fruit growth. However, all carbohydrates, including

sorbitol, were at higher concentration at night at the time of growth cessation and fruit-ripening.

Fructose is the most prominent carbohydrate in fruits both during the growing period and in storage, while glucose is ordinarily in excess of sorbitol. The rapid decline of sorbitol during period of active fruit growth suggests its utilization as an energy source for plant metabolism, whereas its accumulation in the ripening stage and in late storage indicates its role as a storage form of carbohydrate in fruit tissues. The matching pattern of sorbitol, fructose and glucose in storage suggests that sorbitol was an intermediate in the interconversion of the latter two reducing sugars, and also its ready interconvertability with each of them.

An experiment on the skin tissues from fruits during the growing period and in late storage revealed a carbohydrate pattern similar to the pulp tissues. The resemblance of the variations of carbohydrates in both skin and pulp tissues suggests that the metabolic reactions affecting carbohydrates in these tissues are identical.

Results from the preliminary experiment on skin photosynthetic ability showed that skin was able to supply food materials for continued fruit growth, despite the fact that such supply was at a slower pace compared with that of the adjacent leaves. When discrepancies were found in the methods, more elaborate techniques were expected to be designed in furthering this experiment.

Various techniques were discussed in the last chapter to evaluate the suitability of their applications in the present project. The dilution of ethyl alcohol at 70% and the phenol-sulfuric acid method for sugar analysis were found highly applicable. While paper chromatography was recommended for application over thin layer chromatography, the handling of the techniques in starch determination was given precautionary modification.

SUGGESTIONS FOR FURTHER RESEARCH

1. Since sorbitol has been detected in apple skin tissues in stored fruits in considerable amounts, which agrees with the observations of Stoll (1967b), and its presence has also been related to watercore, a physiological disorder in fruits, some storage disorders of apple that originate from the skin or its surrounding area may probably be related to this sorbital accumulation. Suggestions are made to study the occurrence of sorbitol in the skin as well as in different areas in the apple fruit pulp under different storage temperatures and conditions in order to study the preceding phenomena.
2. Apart from the previous suggestion of using gas chromatography for sample analyses, isotopic techniques are recommended for future studies of the variation of this sugar alcohol and its related carbohydrates in the fruit, stem and leaves. Hourly or bi-hourly samplings for consecutive periods are suggested for diurnal studies, and weekly samplings for seasonal studies. In the course of the diurnal study already described, more attention should have been directed to establishing a time period in which these carbohydrates are in maximal quantities, so that a future time table for harvest could have been determined in accordance with the carbohydrate content of the fruits. To

plan future experiments with this in mind will be helpful.

3. Isotopic techniques are suggested for the study of the photosynthetic capacity of fruit skin. It is recommended that an experiment be conducted in the greenhouse using potted dwarf fruiting apple trees.
4. Apple used for sorbitol studies should be extended to varieties other than McIntosh. Cortland variety is recommended for future study.

BIBLIOGRAPHY

Adachi, S.

1965 Thin-layer chromatography of carbohydrates in the presence of bisulfite. J. Chromatog. 17, 295-299.

Adcock, L.H.

1957 The determination of sorbitol. Analyst, 82, 427-34.

Adcock, L.H. and Gray, C.H.

1957 The metabolism of sorbitol in the human subject. Biochem. J., 65, 554.

Alexander, A.G.

1964 Biosynthesis of sucrose in sugar cane. J. Agr. Univ. Puerto Rico, 48 (4), 265-83. Chem. Abst., 62, 9470c.

Altermatt, H.A. and Neish, A.C.

1956 The biosynthesis of cell wall carbohydrates. III. Further studies on formation of cellulose and xylan from labelled monosaccharides in wheat plants. Can. J. Biochem. Physiol., 34, 405.

Anastassiadis, P.A. and Common, R.H.

1968 Some aspects of the reliability of chemical analyses. Analyt. Biochem. 22, 409-423.

Anderson, J.D., Andrews, P. and Hough, L.

1959 The incorporation of C^{14} into the sorbitol of plum leaves. Biochem. J., 72, 9 p.

Anderson, J.D., Andrews, P. and Hough, L.

1961 The biosynthesis and metabolism of polyols. Sorbitol (D-glucitol) of plum leaves. Biochem. J., 81, 149.

Anderson, J.D., Andrews, P. and Hough, L.

1962 The metabolism of C^{14}-labelled D-glucose, D-glucuronic acid and D-glucitol (sorbitol) by plum leaves. Biochem. J., 84, 140.

Andrews, P. and Hough, L.

1958 The biosynthesis of polysaccharides Part II. Incorporation of $C^{14}O_2$ into plum-leaf polysaccharides during photosynthesis. J. Chem. Soc., 4483-4491.

Anschel, N.

1930 Klin Worchschr. 9, 1400, cited by Touster, O. and Shaw, D.R.D., 1962.

Archbold, H.K.

1932 Chemical studies in the physiology of apples. XII. Ripening processes in the apple and the relation of time of gathering to the chemical changes in cold storage. Ann. Bot., London, 46, 407.

Asahina, Y. and Shimoda, H.

1930 Chemical composition in fruits of Sorbus commixta, Hedlund. J. Pharm. Soc. Japan, 50, 1.

Ash, A.S.F. and Reynolds, T.M.

1955a Water soluble constituents of fruit. III An examination of the sugars and polyols of apricots, peaches, pears and apples by paper chromatography. Aust. J. Chem. 8, 276.

Ash, A.S.F. and Reynolds, T.M.

1955b Water soluble constituents of fruit. V. Sugars and polyols of the apricot fruit. Aust. J. Chem. 8, 444.

Axelrod, B. and Seegmiller, C.G.

1954 Metabolism of apples. Conversion of glucose to sucrose in apple tissue. J. Agr. Food Chem., 2, 1329.

Barker, S.A.

1955 Acyclic sugar alcohols. "Modern methods of plant analysis". (Ed. by K. Paech and M.V. Tracey). Berlin, Springer-verlag, II, 55-63.

Bennet-Clark, T.A.

1933 The role of the organic acids in plant metabolism. Part II. New Phytol. 32, 128-161.

Bertrand, G.

1905 Bull. Soc. Chim., (3) 33, 166, 264. Cited by Barker, S.A. 1955.

Bidwell, R.G.S., Krotkov, G. and Reed, G.B.

1952 Paper chromatography of sugars in plants. Can. J. Bot., 30, 291-305.

Bieleski, R.L.

1969 Accumulation and translocation of sorbitol in apple phloem. Aust. J. biol. Sci., 22, 611-20.

Blakley, R.L.

1951 The metabolism and antiketogenic effects of sorbitol. Sorbitol dehydrogenase. Biochem. J., 49, 257-71.

Bourne, E.J.

1958 The polyhydric alcohols. Acylic polyhydric alcohols; "Encyclopedia of Plant Physiology". (Ed. by W. Ruhland). Berlin, Springer-verlag, VI, 345-362.

Boussingault, J.

1872 Sur la Sorbite, Matiere sucree analogue a la mannite trouvee dans le jus des baies du sorbier des oiseleurs.
Compt. rend. Acad. Sci. (Paris) 74, 939,
Cited by Barker, S.A. 1955.

Bracher, C. and Bauly, L.E.

1965 Thin layer chromatography of the commonly occurring commercial sugars.
Food manufactur. 40, 8, 38-39.
Chem. Abst. 63, 13549e, 1965.

Brafield, A.E. and Flood, A.E.

1949 Ann. Rep. East Malling Res. Sta. Kent, 100-103.

Bradfield, A.E. and Flood, A.E.

1950 Soluble carbohydrates of fruit plants.
Nature, 166, 264-65.

Britton, H.G.

1961 Hexitols in foetal sheep blood.
Biochem. J. 78, 26 p, 1961.
S.A.

Brown and Neish, A.C.

1954 The biosynthesis of cell wall carbohydrates. Glucose-C^{14} as a cellulose precursor in wheat plants.
Can. J. Biochem. Physiol., 32, 170.

Buchloh, G. and Neubeller, J.

1969 Zur qualitativen und quantitative Bestimmung von Zuckern und Zuckeralkoholen in linigen Obstfrüchten mittels Gaschromatographie.
Erwerbsobstbau, 11, 22-7.
Hort. Abst. 4279, 1969.

Calvin, M.

1962 The path of carbon in photosynthesis.
Science, 135, 879-89.

Carr, C.J. and Krantz, J.C.

1945 Metabolism of the sugar alcohols and their derivatives.
Advan. in carbohydrate chem. 1, 175.

Carter, G.H. and Neubert, A.M.

Rapid determination of starch in apples.
J. Agr. and Food Chem., 2, 1070-1072.

Chibnall, A.C., Piper, S.H., Pollard, A., Smith, J.A.B. and Williams, E.F.

1931 The wax constituents of the apple cuticle.
Biochem. J. 25, 2095.

Chong, C.

1970 A quantitative study of sorbitol and related carbohydrates in Malus.
M.Sc. Thesis, Department of Horticulture, McGill University.

Cori, C.F. and Shine, W.M.

1935 The formation of carbohydrate from glycerophosphate in the liver of the rat.
Science, 82, 134.

Cori, C.F. and Shine, W.M.

1936 The conversion of hexoses and trioses to glucose in the liver of the rat..
J. Biol. Chem. 114, xxi.

Curtis, O.F.

1920 The upward translocation of foods in woody plants.
I. Tissues concerned in translocation.
Am. J. Bot. 7, 101-124.

Dana, M.N.

1952 Physiology of dwarfing in apple.
Ph.D. Thesis, Iowa State College, Ames.

Danehy, J.P. and Pigman, W.W.

1951 Reactions between sugars and nitrogenous compounds and their relationship to certain food problems.
Advan. Food Res. 3, 241-90.

Davis, W.A., Daish, A.J. and Sawyer, G.C.

1916 Studies of the formation and translocation of carbohydrates in plants.
I. The carbohydrates of the mangold leaf.
J. Agri. Sci. 7, 255-326.

Deinema, M.H. and Landheer, C.A.

1960 Extracellular lipid production by a strain of Rhodotorula graminis.
Biochim. et. Biophys. Acta. 37, 178.

DeMaggio, A.E. and Wetmore, R.H.

1961 "Morphogenetic studies on the fern, Todea barbara.
III. Experimental embryology."
Am. J. Bot., 48, 551.

Devyatnin, V.A.

1950 Precursors in the synthesis of ascorbic acid in plants.
Biokhimiya, 15, 325.

Donen, I.

1939 The role of sorbitol in the carbon-metabolism of the Kelsey plum.
I. Changes in chemical composition during growth and storage.
Biochem. J., 33, 1611-20.

Donen, I. and Roux, E.R.

1939 The role of sorbitol in the carbon-metabolism of the Kelsey plum.
II. Relation of carbohydrate and acid loss to CO_2 production in stored fruit.
Biochem. J. 33, 1947-56.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, R.A. and Smith, F.

1956 Colorimetric method for determination of sugars and related substances. Analyt. Chem. 28, 350.

Eagles, C.F.

1967 Diurnal variation of carbohydrates in cocksfoot (Dactylis glomerata). J. Sc. Food Agr. 18, 186-187.

Eaton, J.K.

1949 The organic constituents of the apple tree. Preliminary work. Rep. E. Malling Res. Sta. for 1949, pp. 93-98.

Embden, G. and Griesbach, W.

1914 Z. physiol. chem., Hoppe-Seyler's 91, 251. cited by Blakley, R.L., 1951.

Gabbay, K.H., Merola, L.O. and Field, R.A.

1966 Sorbitol Pathway: presence in nerve and cord with substrate accumulation in diabetes. Science, 151, 209.

Galkowski, T.T., Gauvin, P.N., Santurri, P.R.

1966 Sorbitol from common plantain. A sugar alcohol, D-glucitol, has been isolated from the leaf petioles of common plantain. J. Agr. Food Chem., 14, 324.

Gee, M.

1963 Thin layer chromatography of some methylated glycosides. Analyt. Chem. 35, 350.

Genest, C. and Chapman, D.G.

1962 Extraction and identification of sugar alcohols and other carbohydrates in dietectic foods. J. Asso. Off. Agr. Chem., 45, 422.

Gordon, H.T., Thornburg, W., Werum, L.N.

1956 Rapid paper chromatography of carbohydrates and related compounds.
Analyt. Chem., 28, 849.

Gorrod, A.R.N.

1961 Sorbitol metabolism in apple fruits.
Nature, 190,190,1961.

Goulden, C.H.

1952 Methods of statistical analysis. Second edition.
John Wiley and Sons, Inc., New York,
Chapman and Hall, Ltd., London.

Graham, H.D.

1963 Reaction of sugar alcohols with the anthrone reagent.
J. Food Sci., 28, 440.

Grasshof, H.

1964 Dünnschichtchromatographische Bestimmungen von Zuckern und Zuckeralkoholen auf Magnesiumilikat.
J. Chromatog., 14, 513.

Gregory, F.G.

1917 Physiological conditions in cucumber houses.
Exp. and Res. Sta., Cheshunt.
3rd Annual Report.

Haas, P. and Hill, T.G.

1932 The occurrence of sugar alcohols in marine algae.
II, Sorbitol.
Biochem. J., 26, 986.

Haas, P. and Hill, T.G.

1933 Observations on the metabolism of certain seaweeds.
Ann. Bot. London., 47, 55.

Hall, D.M.

1966 A study of the surface wax deposits on apple fruit.
Aust. J. biol. Sci. 19, 1017-25.

Haeseler, G. and Misselhorn, K.

1966 Determination of sorbitol and mannitol in cherry pulps. (Translated name)
Z. Lebensmittelunters. U. Forsch. 129, 71-75, 1966.
Hort. Abst., 442, 1967.
J. Sci. Fd. Agr., 17, ii-139, 1966.

Harley, C.P.

1938 Some associated factors in the development of watercore.
Proc. Amer. Soc. Hort. Sci. 36, 435-439.

Hartt, C.E., Kortschak, H.P., Forbes, A.J. and Burr, G.O.

1963 Translocation of C^{14} in sugar cane.
Plant Physiol., 38, 305-18.

Hause, J.A., Hubicki, J.A. and Hazen, G.G.

1962 Determination of sorbitol as its hexa-acetate by gas-liquid chromatography with an ionization detector.
Analyt. Chem. 34, 1567.

Hay, G.W., Lewis, B.A., and Smith, F.

1963 Thin-film chromatography in the study of carbohydrates.
J. Chromatog., 11, 479-486.

Hay, J.G. and Pridham, J.B.

1953 Free xylose in fruits.
Nature, 172, 207.

Heftmann, E.

1967 "Chromatography".
Second edition.
Reinhold Publishing Corporation, New York.

Hennig, K. and Flintje, S.M.

1955 Chromatography on paper and retention analysis of acids in wine.
Wein-Wiss. Beihefte Deut. Weinbau, 9, 1-16.

Hers, H.G.

1956 Le mécanisme de la transformation de glucose en fructose par les vésicules seminales.
Biochim. et. Biophys. Acta, 22, 202.

Hers, H.G.

1960 Le mécanisme de la formation du fructose seminal et du fructose foetal.
Biochim. et. Biophys. Acta. 37, 127.

Hudson, C.S.

1945 The Fischer cyanohydrin synthesis and the configurations of higher-carbon sugars and alcohols.
Advan. in carbohydrate chem. 1, 1.

Hulme, A.C.

1950 Data for the study of the metabolism of apples during growth and storage.
Sec. II.
J. Hort. Sci., 25, 277.

Hulme, A.C.

1954 Studies in the nitrogen metabolism of apple fruits.
The climacteric rise in respiration in relation to the equilibrium between protein synthesis and breakdown.
J. Exptl. Bot., 5, 159.

Hulme, A.C.

1958 Physiology, biochemistry and technique of apple and pear.
Advan. Food Res., 8, 297-413.

Hutchinson, A.

1958 Biochemical and physiological studies of Malus rootstocks.
Ph.D. Thesis, Department of Horticulture and Botany, McGill University.

Hutchinson, A., Taper, C.D. and Towers, G.H.N.

1959 Studies of phloridzin in Malus.
Can. J. Biochem. Physiol., 37, 901-10.

Jacin, H. and Mishkin, A.R.

1965 Separation of carbohydrates on borate-impregnated silica gel G plates.
J. Chromatog., 18, 170-173.

Jacobs, W.P.

1962 Longevity of plant organs. Internal factors controlling abscission.
Ann. Rev. Plant Physiol., 13, 403.

Jones, J.K.N. and Wall, R.A.

1960 The separation of sugars on ion exchange resins. II. The separation of monosaccharides.
Can. J. Chem., 38, 2290.

Kaufmann, E.

1929 Klin. Wochschr., 8 66.
cited by Carr, C.J. and Krantz, J.C. 1945.

Kidd, F., West, C., Griffiths, D.E. and Potter, N.A.

1940 An investigation of the changes in chemical composition and respiration during the ripening and storage of conference pears.
Ann. Bot., London, N.S., 4, 1.

Kirchner, J.G.

1967 "Thin-layer chromatography".
Interscience Publishers, New York.

Krebs, H.A. and Lund, P.

1966 Formation of glucose from hexoses, pentoses, polyols and related substances in kidney cortex.
Biochem. J., 98 (1), 210-14.

Krotkov, G. and Helson, V.

1946 Carbonhdrate metabolism of McIntosh apples during their development on the tree and in cold storage. Part 1. General trends.
Can. J. Res., C 24, 126-44.

Kursanov, A.L.

1963 Metabolism and the transport of organic substances in the phloem.
Advan. Bot. Res., 1, 209-78.

Kursanov, A.L., Chailakhyan, M.K., Pavlinova, O.A., Turkina, M.V. and Brovchenko, M.I.

1958 Translocation of sugars in grafted plants.
Plant Physiol. (Translation of Fiziologiya Rastenii), 5, 3-15.
Cited by Kursanov, A.L., 1963.

Kursanov, A.L. and Pavlinova, O.A.

1950 The nearest precursors of sucrose in plants.
Biokhimiya, 15, 52-57.
Chem. Abst., 441-5436c.

Lambert, M. and Neish, A.C.

1950 Rapid method for estimation of glycerol in fermentation solutions.
Can. J. Res., B 28, 83-9.

Lee, F.W.L.

1965 Acceleration of juvenility in Malus seedlings.
M.Sc. Thesis, Department of Horticulture, McGill University.

Lewis, D.H.

1963 The uptake and utilization of substances by beech mycorrhiza.
D. Phil. Thesis, University of Oxford.

Lewis, D.H. and Smith, D.C.

1967a Sugar alcohols (polyols) in fungi and green plants.
I. Distribution, physiology, and metabolism.
New Phytol., 66, 143-184.

Lewis, D.H. and Smith, D.C.

1967b Sugar alcohols (polyols) in fungi and green plants.
II. Methods of detection and quantitative estimation in plant extracts.
New Phytol., 66, 185-204.

Lippmann, E.O.

1927 Ber. dtsch. chem. Ges. 60b, 161.
Cited by Barker, S.A. 1955.

Liu, P.S.

1967 Sorbitol in Malus.
M.Sc. Thesis, Department of Horticulture,
McGill University.

Lohmar, R.L.

1957 The polyols.
"The carbohydrates" (Ed. by W. Pigman),
pp. 241-298, New York.
Academic Press, Inc., publishers, New York.

Lohmar, R.L. and Goepp, R.M.

1949 The hexitols and some of their derivatives.
Advan. in Carbohydrate Chem., 4, 211.

Lopatecki, L.E.

1962 Personal communication with Whetter, J.M.
1962, M.Sc. Thesis, Department of Horticulture,
McGill University.

Lott, R.V.

1965 Relation of skin color of Golden Delicious
apples to quality changes during maturation
and ripening.
Proc. Amer. Soc. Hort. Sci., 86, 61-9.

Mann, T.

1946 Studies on the metabolish of semen.
3. Fructose as a normal constituent of seminal
plasma. Site of formation and function of
fructose in semen.
Biochem. J. 40, 481.

Mann, T.

1949 Metabolism of semen.
Advan. in Enzymol. 9, 329.

Markley, K.S., Hendricks, S.B. and Sando, C.E.

1932 Further studies on the wax-like coating of apples.
J. Biol. Chem. 98, 103.

Martin, W.E.

1936 Distribution of certain sugars in Bosc pears.
Plant physiol., 11, 139.

Martin, W.E.

1937 Chemical study of the ripening process of Bosc pears.
Bot. Gaz., 99, 42.

McCready, R.M. and Hassid, W.Z.

1941 Transformation of sugars in excised barley shoots.
Plant physiol., 16, 599-609.

Miller, E.C.

1924 Daily variation of the carbohydrate in leaves of corn and sorghums.
J. Agr. Res. 27, 785-808.

Minsker, F.C.

1962 Determination of sorbitol in vinegar.
J. Asso. Off. Agr. Chem., 45, 562.

Morris, L.J.

1962 Separation of higher fatty acid isomers and vinylogues by thin layer chromatography.
Chem. Ind., London, 1238.

Muttelet, C.F.

1930 Detection of pectic juices in apple jellies.
Ann. Fals. 23, 79-81, 1930.
Chem. Abst., 24, 3289. 1930.

Nielsen, J.P.

1943 Rapid determination of starch.
An index to maturity in starchy vegetables.
Ind. Eng. Chem., Analyt. Ed., 15, 176.

Nielsen, J.P. and Gleason, P.C.

1945 Rapid determination of starch.
Factors for starches and comparison with acid and enzymic hydrolysis methods.
Ind. Eng. Chem., Analyt. Ed., 17, 131.

Nitsch, J.P.

1953 The physiology of fruit growth.
Ann. Rev. Plant Physiol., 4, 207-27.

Nuccorini, R.

1932 Chemical investigations on fruits.
VII. Experiments on the ripening of Sorb apples. Boll. Inst. Sup. Agar. Pisa, 8, 277-290.

Olmsted, W.H.

1953 The metabolism of mannitol and sorbitol: their use as sugar substitute in diabetic therapy.
Diabetes, 2, 132.

Olsen, C.

1948 The mineral, nitrogen and sugar contents of beech leaves and beech-leaf sap at various times.
Compt. rend. trav. lab. Carlsberg,
Sér. chim. 26, 197-230, 1948.
Chem. Abst., 42, 6423, 1948.

Pavlinova, O.A.

1954 Application of the isotopic method of study to the synthesis of sucrose in the sugar beet plant.
Biokhimiya, 19, 364-72.
Chem. Abst., 48, 11560c.

Pieniazek, S.A.

1944 Physical characters of the skin in relation to apple fruit transpiration.
Plant Physiol. 19, 529-36.

Pigman, W.

1957 Introduction: Structure and stereochemistry of the monosaccharides. "The Carbohydrates" (Ed. by W. Pigman), P. 1, Academic Press, Inc., Publishers, New York.

Plouvier, V.

1955 Sur le sorbitol des Rosacées.
Compt. rend. Acad. Sc. (Paris), 241, 1220-1223.

Plouvier, V.

1963 Distribution of oliphatic polyols and cyclitols.
"Chemical Plant Taxonomy" (Ed. by T. Swain),
p. 313. New York. Academic Press.

Poapst, P.A., Ward, G.M., and Phillips, W.R.

1959 Maturation of McIntosh apples in relation to
starch loss and abscission.
Can. J. Plant Sci., 39, 257-263.

Pollard, J.F., Shatz, E.M. and Steward, F.C.

1961 Hexitols in coconut milk: their role in nurture
of dividing cells.
Plant Physiol., 36, 492.

Prey, V., Scherz, H. and Bancher, E.

1963 Beiträge zur Chromatographie organischer
substanzen.
VIII. Die Dünnschicht Chromatographie von
Kohlenhydraten.
Mikrochim. Acta, 567.

Rees, W.R. and Reynolds, T.

1958 A solvent for the paper chromatographic
separation of glucose and sorbitol.
Nature, 181, 767.

Reif, G.

1934 Uber dea Nachweis von Sorbit in Obsterzengnissen
(The determination of sorbitol in fruit pro-
ducts).
Zeitsh, Untersuch. Lebensm., 68, 179-186.

Reisener, H.J., Goldschmid, H.R., Ledingham, G.A. and
Perlin, A.S.

1962 Formation of trehalose and polyols by wheat
stem rust (Puccinia graminis tritici) uredospores.
Can. J. Biochem. Physiol., 40, 1248.

Richardson, D.H.S. and Smith, D.C.

1968 Lichen physiology.
X. The isolated algal and fungal symbionts of Xanthoria aureola.
New Phytol., 67, 69-77.

Roe, J. H.

1934 A colorimetric method for the determination of fructose in blood and urine.
J. Biol. Chem., 107, 15-22.

Rohrbach, K.G. and Luepschen, N.S.

1968a Seasonal changes in sugar alcohols and sugars in peach bark: a possible relationship to cytospora canker susceptibility.
Proc. Amer. Soc. Hort. Sci., 93, 135-140.

Rohrbach, K.G., and Leupschen, N.S.

1968b Environmental and nutritional factors affecting pycnidospore germination of Cytospora leucostoma.
Phytopath., 58, 1134-1138.

Rosanoff, M.A.

1906 J. Am. Chem. Soc., 28, 114.
Cited by Pigman, W., 1955.

Ryugo, K. and Davis, L.D.

1959 The comparison between the net assimilation rate of peach leaves and the rate of accumulation of dry weight by the crop.
Proc. Amer. Soc. Hort. Sci., 74, 134-143.

Sakai, A.

1961 Effect of polyhydric alcohols on frost-hardiness in plants.
Nature, 189, 416-17.

Sakai, A.

1966 Seasonal variations in the amounts of polyhydric alcohol and sugar in fruit trees.
J. Hort. Sci., 41, 207-13.

Seeberg, V.P., McQuarrie, E.B., and Secor, C.C.

1955 Metabolism of intravenously-infused sorbitol. Proc. Soc. Exptl. Biol. Med., 89, 303-305, 1955.

Sherman, W.R. and Stewart, M.A.

1966 Identification of sorbitol in mammalian nerve. Biochem. Biophsi. Res. Commun., 22, 492.

Siegelman, H.W.

1954 The sugars of Grimes Golden apple and Bartlett pear skin.
Proc. Amer. Soc. Hort. Sci., 64, 232-234.

Silverman, W.

1960 The development of stem rust on wheat leaves treated with some sugars and sugar alcohols. Phytomorphology, 50, 114.

Sideris, C.P., Young, H.Y., and Chun, H.H.Q.

1948 Diurnal changes and growth rates as associated with ascorbic acid, titratable acidity, carbohydrate and nitrogenous fractions in the leaves of Ananas comosus L. Merr.
Plant Physiol., 23, 38.

Simada, H.

1939 J. Pharm. Soc. Japan, 59, 621.
Cited by Barker, S.A., 1955.

Sims, E.T.

1963 The influence of diphenylamine, light and warming on color changes in the skin of apple fruit.
Proc. Amer. Soc. Hort. Sci., 82, 64-7.

Skene, D.S.

1963 The fine structure of apple, pear and plum fruit surfaces, their changes during ripening and their response to polishing.
Ann. Bot., London, N.S. 27, 581-7.

Spencer, J.F.T. and Sallans, H.R.

1956 Production of polyhydric alcohols by osmophilic yeasts.
Can. J. Microbiol., 2, 72.

Spencer, J.F.T. and Shu, P.

1957 Polyhydric alcohol production by osmophilic yeasts: effects of oxygen tension and inorganic phosphate concentration.
Can. J. Microbiol., 3, 559.

Spiro, R.G.

1960 Studies on fetuin, a glycoprotein of fetal serum.
I. Isolation, chemical composition, and physico-chemical properties.
J. Biol. Chem., 235, 2860-2869.

Stahl, E.

1965 "Thin layer chromatography", a laboratory handbook.
Springer-Verlag, Berlin,
Academic Press, Inc., New York.

Stahl, E. and Kaltenbach, U.

1961a Dünnschicht-chromatographie.
VI. Mitteilung. Spurenanalyse von Zuckergemischen auf. Kieselgur G. shichten.
J. Chromatog. 5, 351.

Stahl, E. and Kaltenbach, U.

1961b Dünnschicht-chromatographie.
IX. Mitteilung. Schnelltrennung von Digitalis und Podophyllum-Glycosidgemischen.
J. Chromatog. 5, 458.

Stanimirovic, S.G., Stanimirovic, D.L. and Nastic, D.R.

1963a Identification of organic acids and saccharides in fruits and leaves of medlar (Mespilus germanica).
Glasnik Hem. Drustva, Beograd. 28(5-6), 327-35, 1963.
Chem. Abst., 63, 3310b, 1965.

Stanimirovic, S.G., Stanimirovic, D.L., and Nastic, D.R.

1963b Determination of sorbitol.
Glasnik Hem. Drustva, Beograd 28(7), 377-82, 1963.
Chem. Abst. 63, 2854f, 1965.

Stanimirovic, D.L., Nastic, D.R., and Stanimirovic, S.G.

1964 Quantitative variations of glucose, fructose, mevalolactonyl-B-glycoside, sorbitol and organic acids in the leaf of Mespilus germanica during the vegetation period.
Archiv. Farm. (Belgrade) 14(5), 283-7, 1964.
Chem. Abst., 62, 1965h, 1965.

Steel, R.G.D. and Torrie, J.H.

1960 "Principles and Procedures of Statistics".
McGraw-Hill, New York.

Steele, C.C.

1934 "An Introduction to Plant Biochemistry".
London. Bell and Sons Ltd.,

Steuart, D.W.

1955 The sorbitol test.
J. Sci. Food and Agr., 6, 387-90.

Stoll, U.

1967a Nachweis und Bestimmung des Sorbits in Früchten.
Mitt. Geb. Lebensmittelunters. u. Hyg. 58, 56-61.

Stoll, U.

1967b Das Verhalten des Sorbits in Kernobstfruchten.
(Sorbitol in pome fruits.)
Mitt. Geb. Lebensmittelunters. u. Hyg. 58, 145-150.
Hort. Abst., 39, 2230, 1969.
J. Sc. Fd. Agr. 19, ii-242, 1968.

Stoll, U.

1968 Sorbit- und Zuckergehalte in Apfel- und Birnensorten.
(Sorbitol and sugar contents of apple and pear varieties.)
Der Erwerbsobstbau, 10, 27-9.
Hort. Abst., 38, 7164, 1968.

Strain, H.H.

1937 Sources of D-sorbitol.
J. Amer. Chem. Soc., 59, 2264-66.

Swanson, C.A.

1959 Translocation of organic solutes.
"Plant Physiology, a Treatise".
Vol. 2 (Ed. Steward, F.C.), p. 48.
Academic Press, New York.

Swanson, C.A. and El-Shishiny, E.D.H.

1958 Translocation of sugars in the Concord grape.
Plant Physiol., 33, 33-37.

Tandon, R.N. and Grewal, J.S.

1954 Studies on carbon selectivity from some organic compounds, and its effect on growth and sporulation of Altenaria tenuis strain B. causing core rot of apples.
Proc. Natn. Acad. Sci.
India, B. 24.

Tanner, H. and Duperrex, M.

1968 Ueber die Bestimmung von Sorbit in Sortenreinen Birnen-, Apfel und Kirschensäften.
(The determination of sorbitol in the juice of single varieties of pear, apple and cherry.)
Schweiz. Z. Obst.-u. Weinb., 104, 508-12, 1968.
Hort. Abst. 39, 2231, 1969.

Tate, M.E. and Bishop, C.T.

1962 Thin layer chromatography of carbohydrate acetates.
Can. J. Chem. 40, 1043.

Taper, C.D. and Liu, P.

1969 Sorbitol occurrence in Malus leaves and fruits.
Can. J. Plant Sci., 49, 97-98.

Thannhauser, S.J. and Meyer, K.H.

1929 Münch, med. Wochschr., 76, 356.
Cited by Carr, C.J. and Krantz, J.C., 1945.

Touster, O. and Shaw, D.R.D.

1962 Biochemistry of the acyclic polyols. Physiol. Rev., 42, 181.

Treboux, T.

1909 Starkebildung aus Sorbit bei Rosacein. Ber. Deut. Botan. Gesell., 27, 507-11.

Trip, P., Nelson, C.D. and Krotkov, G.

1965 Selective and preferential translocation of C^{14}-labelled sugars in white ash and lilac. Plant Physiol., 40, 470.

Turkina, M.V.

1954 The synthesis and transportation of sucrose in the sugar beet plant. Biokhimiya, 19, 357-63. Chem. Abst., 48, 11560a.

Tutin, F.

1925 Chemical investigations of fruits and their products. I. Apple juice as a source of sorbitol. Biochem. J., 19, 416-417.

Vincent, C. and Delachanal.

1889 Compt. rend. Acad. Sci., 109, 676. Cited by Lohmar, R.L., 1957.

Vining, L.C. and Taber, W.A.

1964 Analysis of endogenous sugars and polyols of Claviceps purpurea by chromatography on ion exchange resins. Can. J. Microbiol., 10, 647.

Waite, R. and Boyd, J.

1953 The water-soluble carbohydrates of grasses. I. Changes occurring during the normal life-cycle. J. Sci. Fd. Agr. 4, 197.

Wassermann, L. and Hanus, H.

1963 Dünnschicht-chromatographie von Zuckern und Zuckeralkoholen. Naturwissenschaften, 50, 351.

Webb, K.L. and Burley, J.W.A.

1962 Sorbitol translocation in apple. Science, 137, 766.

Whetter, J.M.

1962 A study of sorbitol in Malus. M.Sc. Thesis, Department of Horticulture, McGill University.

Whetter, J.M. and Taper, C.D.

1963 Note on seasonal occurrence of sorbitol (D-glucitol) in buds and leaves of Malus. Can. J. Bot., 41, 175-77.

Whetter, J.M. and Taper, C.D.

1966 Occurrence of sorbitol (D-glucitol) and certain related sugars in germinating seeds and developing seedlings of Malus. Can. J. Bot., 44, 51-55.

Williams, M.W.

1965 Personal communication with Liu, P.S., 1967. M.Sc. Thesis, Department of Horticulture, McGill University.

Williams, M.W.

1966 Relationship of sugars and sorbitol to watercore in apples. Proc. Amer. Soc. Hort. Sci., 88, 67-75.

Williams, M.W., Martin, G.C. and Stahly, E.A.

1967 The movement and fate of sorbitol-C^{14} in the apple tree and fruit. Proc. Amer. Soc. Hort. Sci., 90, 20-24.

Williams-Ashman, H.G. and Banks, J.

1954 The ketose reductase of rat liver and accessory sexual organs. Arch. Biochem. Biophys. 50, 513.

Wolfrom, M.L., Lew, B.W., Hales, H.A. and Goepp, R.M.

1946 Sugar interconversion under reducing conditions.
IV. D, L-glucitol.
J. Amer. Chem. Soc., 68, 2342.

Wren, J.J.

1960 Chromatography of lipids on silicic acid.
J. Chromatog. 4, 173.

Wright, J.R. and Le Tourneau, D.

1964 Production of mannitol by the onion pink-root organism.
Phytopathology, 54, 913.

Ziegler, H.

1956 Untersuchungen über die Leitung und Sekretion der Assimilate.
Planta, 47, 447-500.

Zimmermann, M.H.

1957 Translocation of organic substances in trees.
I. The nature of the sugars in the sieve tube exudate of trees.
Plant Physiol., 32, 288-91.

Zimmermann, M.H.

1958 Translocation of organic substances in trees.
III. The removal of sugars from the sieve tubes in white ash (Fraxinus americana L.)
Plant Physiol., 33, 213-17.

Zimmermann, M.H.

1960 Transport in the phloem.
Ann. Rev. Plant Physiol., 11, 167-190.

Zimmermann, M.H.

1961 Movement of organic substances in trees.
Science, 133, 73-79.

APPENDIX

The following pages are a few samples of computer workings for paired T-tests of the data obtained in Experiment 1. The whole series of these workings was filed in the Department of Horticulture, Macdonald College, McGill University.

T-TEST OF PAIRED SAMPLES

OCCURRENCE OF SORBITOL AND RELATED CARBOHYDRATES

IN APPLE FRUITS

SAMPLE(FIELD) -DAY, SETS I AND II,1968

SORBITOL
(MGM/GM OF FRESH WEIGHT)

SET I	SET II	DIFF(I-II)
4.700	4.010	0.690
5.600	5.160	0.440
3.960	1.530	2.430
1.070	2.620	-1.550
3.080	2.520	0.560
1.830	2.280	-0.450
1.420	1.570	-0.150
1.280	1.180	0.100
2.180	1.990	0.190
1.780	1.210	0.570
1.930	1.730	0.200

SUM OF THE DIFFERENCES = 3.0300

MEAN DIFFERENCE = 0.2755

STANDARD DEVIATION OF DIFFERENCES = 0.9535

STANDARD ERROR OF MEAN DIFFERENCES = 0.2875

DEGREES OF FREEDOM = 10

T (OBSERVED) = 0.9581

T (TABLE) = 2.2280 (P=0.05), 3.1690 (P=0.01)

T (TABLE) X STANDARD ERROR = 0.6405 (P=0.05)
0.9111 (P=0.01)

NO SIGNIFICANT DIFFERENCE

T-TEST OF PAIRED SAMPLES

OCCURRENCE OF SORBITOL AND RELATED CARBOHYDRATES

IN APPLE FRUITS

SAMPLE(FIELD) -NIGHT, SETS I AND II,1968

SORBITOL
(MGM/GM OF FRESH WEIGHT)

SET I	SET II	DIFF(I-II)
9.860	7.980	1.880
3.700	7.100	-3.400
1.830	2.550	-0.720
1.120	1.050	0.070
0.760	1.190	-0.430
1.330	1.920	-0.590
1.030	0.430	0.600
3.170	2.230	0.940
3.620	3.980	-0.360
2.250	1.460	0.790
1.660	1.060	0.600

SUM OF THE DIFFERENCES = -0.6200

MEAN DIFFERENCE = -0.0564

STANDARD DEVIATION OF DIFFERENCES = 1.3592

STANDARD ERROR OF MEAN DIFFERENCES = 0.4098

DEGREES OF FREEDOM = 10

T (OBSERVED) = -0.1375

T (TABLE) = 2.2280 (P=0.05), 3.1690 (P=0.01)

T (TABLE) X STANDARD ERROR = 0.9130 (P=0.05)
1.2987 (P=0.01)

NO SIGNIFICANT DIFFERENCE

T-TEST OF PAIRED SAMPLES

OCCURRENCE OF SORBITOL AND RELATED CARBOHYDRATES

IN APPLE FRUITS

SAMPLE(FIELD) -DAY, SETS I AND II,1969

SORBITOL
(MGM/GM OF FRESH WEIGHT)

SET I	SET II	DIFF(I-II)
9.760	8.670	1.090
3.670	7.000	-3.330
6.080	6.750	-0.670
6.250	6.750	-0.500
6.130	6.750	-0.620
4.940	4.380	0.560
5.750	6.880	-1.130
3.690	4.750	-1.060
5.750	5.440	0.310
5.250	5.440	-0.190
5.880	5.000	0.880

SUM OF THE DIFFERENCES = -4.6600

MEAN DIFFERENCE = -0.4236

STANDARD DEVIATION OF DIFFERENCES = 1.2250

STANDARD ERROR OF MEAN DIFFERENCES = 0.3694

DEGREES OF FREEDOM = 10

T (OBSERVED) = -1.1470

T (TABLE) = 2.2280 (P=0.05), 3.1690 (P=0.01)

T (TABLE) X STANDARD ERROR = 0.8229 (P=0.05)
1.1705 (P=0.01)

NO SIGNIFICANT DIFFERENCE

T-TEST OF PAIRED SAMPLES

OCCURRENCE OF SORBITOL AND RELATED CARBOHYDRATES

IN APPLE FRUITS

SAMPLE(FIELD) -DAY, SET I, 1968 VS 1969

SORBITOL
(MGM/GM OF FRESH WEIGHT)

-1968-	-1969-	DIFF(68-69)
4.700	9.790	-5.090
5.600	3.670	1.930
3.960	6.080	-2.120
1.070	6.250	-5.180
3.080	6.130	-3.050
1.830	4.940	-3.110
1.420	5.750	-4.330
1.280	3.690	-2.410
2.180	5.750	-3.570
1.780	5.250	-3.470
1.930	5.880	-3.950

SUM OF THE DIFFERENCES = -34.3499

MEAN DIFFERENCE = -3.1227

STANDARD DEVIATION OF DIFFERENCES = 1.9394

STANDARD ERROR OF MEAN DIFFERENCES = 0.5848

DEGREES OF FREEDOM = 10

T (OBSERVED) = -5.3402

T (TABLE) = 2.2280 (P=0.05), 3.1690 (P=0.01)

T (TABLE) X STANDARD ERROR = 1.3028 (P=0.05)
1.8531 (P=0.01)

SIGNIFICANT DIFFERENCE (P=0.01)

T-TEST OF PAIRED SAMPLES

OCCURRENCE OF SORBITOL AND RELATED CARBOHYDRATES

IN APPLE FRUITS

SAMPLE(FIELD) -DAY, SET II, 1968 VS 1969

SORBITOL
(MGM/GM OF FRESH WEIGHT)

-1968-	-1969-	DIFF(68-69)
4.010	8.670	-4.660
5.160	7.000	-1.840
1.530	6.750	-5.220
2.620	6.750	-4.130
2.520	6.750	-4.230
2.280	4.380	-2.100
1.570	6.880	-5.310
1.180	4.750	-3.570
1.990	5.440	-3.450
1.210	5.440	-4.230
1.730	5.000	-3.270

SUM OF THE DIFFERENCES = -42.0099

MEAN DIFFERENCE = -3.8191

STANDARD DEVIATION OF DIFFERENCES = 1.1269

STANDARD ERROR OF MEAN DIFFERENCES = 0.3398

DEGREES OF FREEDOM = 10

T (OBSERVED) = -11.2403

T (TABLE) = 2.2280 (P=0.05), 3.1690 (P=0.01)

T (TABLE) X STANDARD ERROR = 0.7570 (P=0.05)
1.0767 (P=0.01)

SIGNIFICANT DIFFERENCE (P=0.01)

T-TEST OF PAIRED SAMPLES
OCCURRENCE OF SORBITOL AND RELATED CARBOHYDRATES
IN APPLE FRUITS

SAMPLE(STORE) -SEPT.,1968 - MAY,1969

SORBITOL
(MGM/GM OF FRESH WEIGHT)

SET I	SET II	DIFF(I-II)
1.080	2.170	-1.090
2.700	1.770	0.930
4.470	3.090	1.380
1.960	2.250	-0.290
3.280	1.980	1.300
0.550	0.790	-0.240
2.240	2.200	0.040
1.980	2.640	-0.660
3.050	2.680	0.370
5.440	3.670	1.770
4.130	4.380	-0.250
5.380	3.130	2.250
4.630	3.630	1.000
3.880	4.130	-0.250
4.250	3.750	0.500
3.130	4.500	-1.370
4.500	6.000	-1.500
3.880	4.500	-0.620
5.000	4.880	0.120
6.750	4.880	1.870
4.630	4.250	0.380
3.920	6.170	-2.250
5.580	5.830	-0.250

SUM OF THE DIFFERENCES = 3.1400

MEAN DIFFERENCE = 0.1365

STANDARD DEVIATION OF DIFFERENCES = 1.1441

STANDARD ERROR OF MEAN DIFFERENCES = 0.2386

DEGREES OF FREEDOM = 22

T (OBSERVED) = 0.5723

T (TABLE) = 2.0740 (P=0.05), 2.8190 (P=0.01)

T (TABLE) X STANDARD ERROR = 0.4948 (P=0.05)
0.6725 (P=0.01)

NO SIGNIFICANT DIFFERENCE