

1930

# Problems related to the commercial production of levulose

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PROBLEMS RELATED TO THE COMMERCIAL  
PRODUCTION OF LEVULOSE

BY

James H. McGlumphy

A Thesis Submitted to the Graduate Faculty  
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subjects  
Food and Sanitary Chemistry  
and  
Plant Chemistry

Approved

Signature was redacted for privacy.

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1930

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PROBLEMS RELATED TO THE COMMERCIAL  
PRODUCTION OF LEVULOSE

INTRODUCTION

Many attempts have been made to place levulose on the market at a price sufficiently reduced to compare favorably with that of sucrose and dextrose. The lack of any considerable demand for levulose, and the many technical difficulties encountered in producing it on a commercial scale, have caused the development to be extremely slow. Since for most purposes, sucrose and dextrose so successfully supply the demand, it is obvious that levulose, to be industrially attractive, must fulfill new requirements which cannot be adequately met by other available sugars.

Levulose possesses distinctive properties which earn for it a place among the commercial sweetening agents. It is the sweetest of all the sugars. Authorities do not agree (1, 2, 3, 4, 5, 6, 7) as to the relative sweetness of the sugars. If the sweetness of sucrose is given an arbitrary value of 100, the sweetness of levulose is reported to be relatively

1. Paul, Chem. Zeit., 45:705(1921)
2. Deerr, Intern. Sugar J., 24:481(1922)
3. Sale and Skinner, J. Ind. Eng. Chem., 14:522(1922)
4. Biester, Wood, Wahlin, Amer. J. Physiol., 73:327(1925)
5. Spengler, and Traegel, Z. ver. Duet. Zucherind., 77:1(1927), *ibid.*, 77:367(1927)
6. Willaman, *ibid.*, 77:365(1927)
7. Richter, Expt. Sta. Record, 58:611(1927)

from 103 to 173, depending upon the method of measurement.

Levulose is one of the most soluble of the sugars, and very (8) difficult to crystallize except from sirups of high purity.

It is somewhat more hygroscopic than sucrose or dextrose. It possesses unique physiological properties, being resorbed directly, assimilated to a larger extent and oxidized more quickly and in larger amounts per time interval than sucrose. (9)

The above properties suggest numerous potential uses for levulose:

1. Uses in the manufacture of food products.

a. The addition of levulose to the list of already available sugars, should give any desired combination of sweetness, texture, and solubility for the production of food products.

1. In jellies, marmalades, and preserved fruits, levulose would prevent the crystallization of sucrose, making impossible dullness, cloudiness, and solidification of the product. (10)

2. Levulose sirups may be had which are quite concentrated and yet possess a relatively low viscosity due to the low molecular weight. This fact should be valuable in improving the texture of ices, ice cream, etc. The presence of levulose might prevent the crystallization

8. Jackson, Silsbee, and Proffitt, Bur. Standards Sci. Papers, 519:614(1926), report 375 g. levulose soluble in 100 g. water at 20°C.

of lactose, which gives considerable trouble in the ice cream industry.

3. A greater degree of delicacy could be obtained in many confectionary products by using levulose alone or in combination with glucose or sucrose. It should be ideal for the manufacture of candies because of its ability to prevent the crystallization of other sugars in its presence.
  4. The use of levulose would give a uniform and permanent flavor to carbonated beverages by preventing the loss of sweetness in the bottled product due to inversion.
  5. Since levulose is somewhat hygroscopic, cakes and other foods might better resist drying out. (11)
  6. Levulose might be used in combination with dextrose for the production of artificial honey.
  7. The use of dextrose could be greatly increased by mixing it with crystalline levulose and thus
- 
9. Daniel, Chem. Zeit., 45:4(1921)
  10. Stein, Chem. Zeit., 32:426(1908): Z. Angew. Chem., 21:1064(1908): Intern. Sugar J. 10:218(1908)
  11. Shoemaker, U. S. Dept. of Agr. Tech. Bull., 33:2(1927)

raising its sweetening power.

8. In the sweetening of cold drinks levulose is desirable because of its high solubility.

b. Many additional advantages may be discovered for levulose when it is produced in sufficient quantities to allow experimentation on its culinary and confectionary applications.

## 2. Medicinal Uses

a. Levulose is an excellent food for the infant. (10)

b. Levulose has found application as a food for the consumptive, where the production of CO<sub>2</sub> in abundance is important. (12)

c. Levulose is effective in the prevention of hyperacidity of the gastric juice. (10)

d. Perhaps the most important use for levulose is in the treatment or prevention of diabetes.

Experimental results have been reported to show levulose assimilation as well as that of its parent carbohydrate, inulin. It seems desirable to present these results in considerable detail (See Historical Part A) due to the possible influence they may have on the ready acceptance of levulose when it may become available. Many diabetic patients tolerate levulose to a remarkable

12. Daniel, loc. cit., (Reference 9)

extent. Even with insulin treatment there is need for carbohydrate foods. Levulose not only supplies, in part, this need, but satisfies the desire for sweets without the use of substitutes which have little or no food value, or which may even be harmful.

Joslin (13) states that one person in seventy-five has diabetes or will develop it. He estimated the total number of cases in the United States to be one million (1915), and suggested that this figure was probably low, because many of those afflicted are unaware of their condition, or the records were incomplete.

There seems to be a direct correlation between the increase in the per capita consumption of sugar (cane and beet), and the increase in the number of deaths per one hundred thousand population, from diabetes. This is shown in Table I and Figure I, the data being taken from the Statistical Abstracts of the United States. (14).

Table I

Death Rate per 100,000 Population from Diabetes Mellitus  
Entire Death Registration Area, Continental United States

Year	Number of Deaths per 100,000 Population	Lbs. Sugar (Cane, Beet) available for Consump- tion per capita
1900	9.7	62.2
1910	14.9	78.9
1911	14.9	78.3

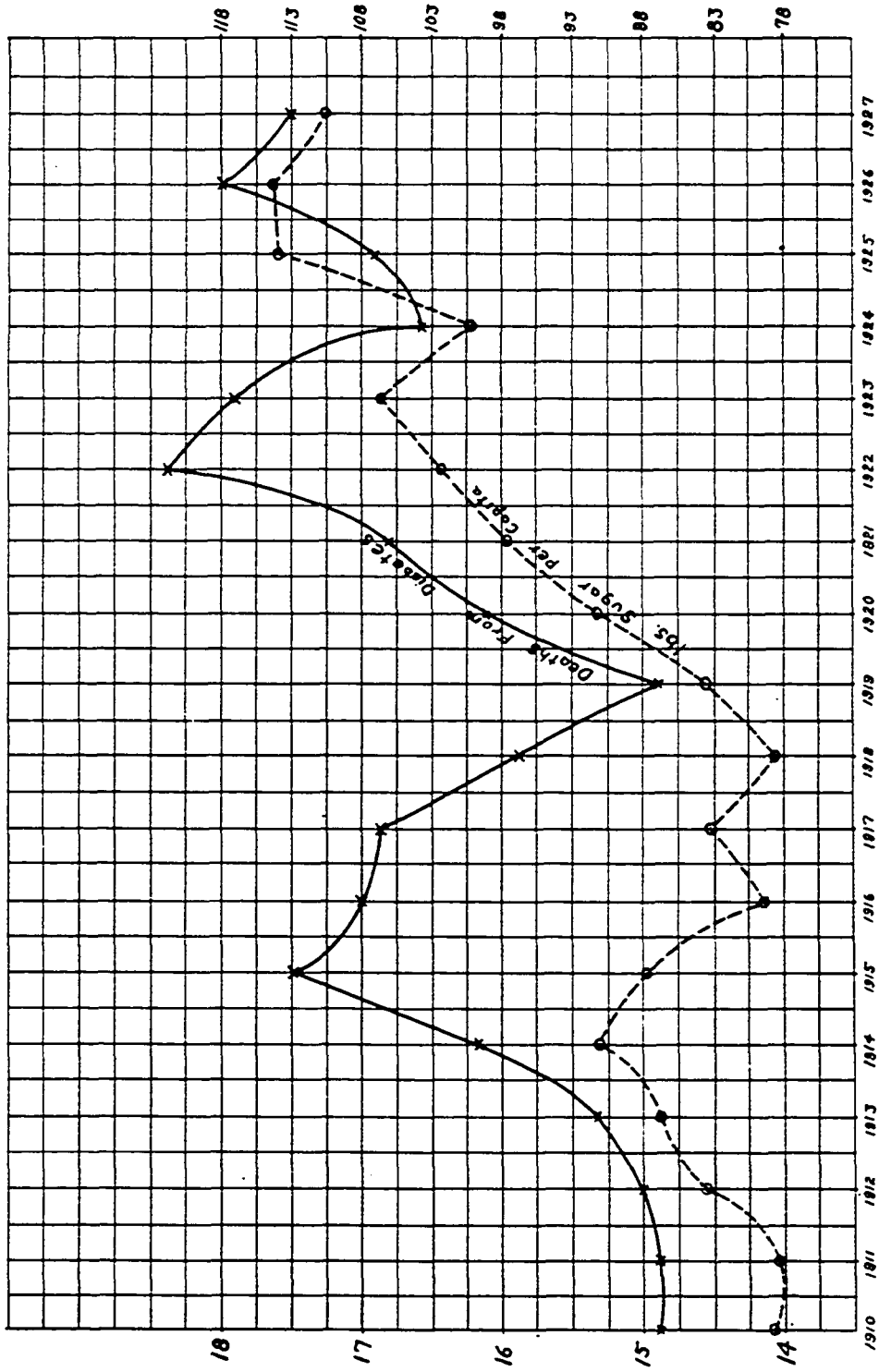
Table I (con't.)

Year	Number of Deaths per 100,000 Population	Lbs. Sugar (Cane, Beet) Available for Consump- tion per capita
1912	15.0	83.9
1913	15.3	86.6
1914	16.2	91.3
1915	17.5	87.9
1916	17.0	79.4
1917	16.9	83.2
1918	15.9	78.5
1919	14.9	83.8
1920	16.1	91.1
1921	16.8	97.6
1922	18.4	102.4
1923	17.9	106.5
1924	16.6	100.2
1925	16.9	114.2
1926	18.0	114.4
1927	17.5	110.6
1928	_____	_____

13. Joslin, "Treatment of Diabetes Mellitus." Lea and Febiger, Phil. and New York, Second Edition, p. 24 (1917)
14. Statistical Abstracts of the United States 42:79 (1919)  
48:75(1925), 49:77(1926), 50:82,666 (1928), 51:82(1929)



Pounds Sugar (Cane and Beet) Available For Consumption Per Capita.



Death Rate From Diabetes and Mellitus Per 100,000 Population.

Fig. 1.

These data may be coincident to the increased accuracy in keeping records and in diagnosis of disease. However, it is of interest to note the decrease in the death rate (Fig. I) in 1924 and the corresponding decrease in sugar consumption, followed in 1925 and 1926 by a correlating increase in each. The methods of recording and diagnosing would not be expected to vary appreciable over this period of time.

Sugar (sucrose) has never been shown to cause diabetes. However, if the condition is present, the fact is well known that it is greatly aggravated by the prolonged use of sucrose. This fact together with Joslin's observation that numerous persons are unknowingly afflicted, make the data of Table I seem reasonable, if not to be expected.

Would not these considerations seem sufficient to merit a more thorough investigation into the effect of replacing sucrose in the diet with levulose, not only for the diabetic but for the normal person as well?

### 3. Fermentation

It is possible that levulose may be used along with glucose or invert sugar in the preparation of alcohol. With the prospect of alcohol relieving the expected fuel shortage, a new source should be welcomed.

There is, then a striking need for levulose, in order that the above suggestions may be subjected to a more thorough

experimental investigation.

The object of this paper is to present experimental data designed to improve upon the previously reported methods for the preparation of levulose and thus make it more quickly commercially available.

HISTORICAL

The history will be treated in chronological order rather than by related subjects. The resulting sequence will stress the fact that most information concerning levulose was obtained at an early date, and, although much repetition will be noted, very few original contributions have been forthcoming in recent years.

The contradictory reports will be discussed in connection with the experimental part of this paper.

Part A

The Therapeutic Use of Levulose, Inulin, and the Jerusalem Artichoke.

A part of the summary following was taken from Joslin's (15) report of 1923. Additions are made to include inulin, and the Jerusalem artichoke, and to list information published since 1923.

Kulz (16) (1874) was apparently the first clinician to advocate seriously the therapeutic use of levulose. He reported that no sugar appeared in the urine of diabetics on an absolute flesh diet after feeding, from 50 to 120 grams of inulin. No inulin was found in the feces. He concluded therefore that inulin was completely assimilated in both mild and severe cases of diabetes.

15. Joslin, "Diabetic Metabolism with High and Low Diets", Carnegie Inst. Pub., 323:211(1923)
16. Kulz, "Diabetes Mellitus", Marburg, 130(1874). Original not seen. Taken from Joslin (Reference 15).

Von Mehring (17) (1876) reported that the use of inulin did not increase the sugar content of the urine.

Minkowski (18) (1890) reported that levulose diminished the protein metabolism in a diabetic dog.

Sandmeyer (19) (1895) fed 80 g. inulin to a depancreatized dog and recovered 46 g. from the feces.

Nakaseko (20) (1900) experimented on the feeding of inulin with reference to glycogen formation.

Bierry and Portier (21) (1900) reported their investigations on the digestion of inulin.

Johansson, Billstrom, and Heijl (22) (1904) gave 100 g. of dextrose, sucrose, and levulose to normal individuals and determined the rise in carbon dioxide expelled as compared with basal values for succeeding hours. They observed that glucose increased the carbon dioxide seven per cent, sucrose fourteen per cent, and levulose (93 g.) fifteen per cent. When the effect of these sugars had passed off, the carbon dioxide values fell below the basal value. The increase in

17. Von Mehring, Jahresber. Tierchem., 6:144(1876)
18. Minkowski, Arch. F. exp. Path. u. Pharm., 26:371(1890)  
ibid., 31:85(1893)
19. Sandmeyer, Z. Biol., 31:32(1895)
20. Nakaseko, Am. J. Physiol., 4:246(1900)
21. Bierry and Portier, Compt. Rend. Soc. Biol., (Paris)  
52:423(1900)
22. Johansson, Billstrom, and Heijl, Skand, Arch. Physiol.,  
16:263(1904)

carbon dioxide, they explain, might be due to increased metabolism or the conversion of carbohydrate into fat, but what percentage of the increase should be assigned to each factor, the experiments did not disclose. The rapidity of storage, they ascribe to the concentration of the given materials in the blood and tissue fluids. With a lower concentration of blood sugar and a low glycogen content in the body, they surmised and proved that oxidation of carbohydrate would go on more slowly and storage more rapidly. They noted that the rapidity of oxidation of levulose was greater and the rapidity of storage as glycogen less than with glucose.

Teyxeira (23) (1905) recommended the addition of inulin to the gluten of wheat as a food.

Persia (24) (1905) stated that inulin was well digested and assimilated by diabetics in large doses and through long periods. He reported that the feces never contained large amounts of inulin.

Miura (25) (1905) found little glycogen formed in rabbits after feeding inulin.

Mendel and Mitchell (26) (1905) found that inulin introduced parenterally into the organism was excreted in the urine, with no evidence of inversion or utilization.

23. Teyxeira, Jahresber. Tierchem., 35:822(1905)
24. Persia, ibid., 25:822(1905)
25. Miura, Z. Biol., 32:263(1905)
26. Mendel and Mitchell, Am. J. Physiol., 14:239(1905)

In a case of levulosuria, Neubaner (27) (1905) found that after feeding 80 g. of inulin the urine showed no increased levulose content. This seemed to indicate that inulin was not converted in the body. No inulin was found in the feces. Strong gas formation in the intestine after the meal indicated bacterial decomposition of the inulin.

Mendel and Nakaseko (28) (1905) found very little glycogen formed after feeding inulin.

Johansson (29) (1908) confirmed the results previously reported. (30) Incidentally he observed that the increase in carbon dioxide varied with the rapidity of absorption of the sugars from the gastro-intestinal tract, and interpreted the results of his experiments as showing that the increase in carbon dioxide varied with the amount of glycogen stored in the body at the beginning of the experiment. The increase of carbon dioxide after the administration of the various sugars began within the first 30 minutes and reached its maximum either in the first or second half hour; the period of increase never exceeded six hours. Levulose increased the excretion of carbon dioxide twice as much as glucose. The reduction of glycogen storage reduced the increase of carbon dioxide excreted after glucose was given, but although the individual similarly tested with levulose had a greater reduction in glycogen storage, the reduction in the excretion of carbon dioxide was no greater. Johansson concluded either that lev-

ulose is less suited for the formation of glycogen or that glycogen formation from levulose does not go on in the same manner or with the same rapidity as that of glucose. He performed several experiments with diabetic patients. In some instances the ingestion of sugar increased the carbon dioxide and in others did not, or increased it to a less extent than with normals. In one diabetic dextrose brought about an increase of 7 per cent in the carbon dioxide elimination, whereas levulose increased the carbon dioxide elimination 13 per cent. In other words, the same relation between glucose and levulose was obtained with this diabetic as with normals.

Pfluger (31) (1908) found that glycogen is formed by the liver from levulose and that only a small amount of the levulose given is eliminated into the urine.

Bierry ( 32) (1910) studied the digestion of inulin. He found that the higher animals do not secrete an enzyme capable of hydrolyzing inulin. The hydrolysis is accomplished by the HCl of the gastric juice. Attempts to hydrolyze inulin in neutral, slightly acid, or slightly alkaline media by means of

27. Neubaner, Munch. Med. Wochschr., 1525(1905)
28. Mendel and Makaseko, Am. J. Physiol., 14:245(1905)
29. Johansson, Skand. Arch. Physiol., 21:1(1908)
30. Johansson, Billstrom and Heijl, loc. cit., Ref. 22
31. Pfluger, Arch. Ges. Physiol., (Pfluger's) 121:559 (1908)



secretions and extracts of the digestive glands of the dog and rabbit were all unsuccessful. The digestive juices of the *Helix pomatia* contain an enzyme which hydrolyzes inulin. It is prepared by diluting the juice with 10 volumes of water and removing the albuminoids by adding  $\text{HgNO}_3$ , neutralizing with  $\text{NaOH}$ , then precipitating the colloids and mercury together with  $\text{H}_2\text{S}$ . The excess  $\text{H}_2\text{S}$  is removed without heating by means of  $\text{CuSO}_4$ . The molluscs secrete an enzyme which splits inulin into levulose.

Swartz (33) (1911) reported that no enzymes found in the higher animals attack inulin.

Straus (34) (1911) experimented with two cases of diabetes, administering from 40 g. to 100 g. pure inulin daily and reported the urine sugar free, and the patients greatly benefited. He recommended the use of vegetables rich in inulin such as artichokes, dandelions, etc., suggesting however, that absorption of inulin would naturally proceed more slowly than from the pure product itself.

Lewis (35) (1912) wrote concerning the value of inulin as a foodstuff. In his conclusion he makes the following statements:

1. Inulin fed to a Healthy man was not eliminated in the feces.

32. Bierry, Compt. rend., 150:116(1910): Biochem. Z., 44:402(1913)

33. Swartz, Tr. Connecticut Acad. Arts and Sci., 16:298(1911)

34. Strauss, Therapic der Gegenwart, 52:337(1911)

35. Lewis, J. Am. Med. Assoc., 58:1176(1912)

2. Marked intestinal fermentation was observed to follow the feeding of inulin.

3. The acidity of the gastric contents of a dog to which inulin was given by a stomach sound was sufficient to hydrolyze inulin partially to levulose in from one to two hours.

"These facts would seem to indicate that any utilization of inulin can occur only after hydrolysis by the gastric juice. The extent of this hydrolysis must vary with conditions in the stomach. If the diet is of such a character that it leaves the stomach soon, the action of the acid gastric juice is checked by the intestinal reaction before the inversion of inulin can proceed far. The acidity of the gastric contents also must influence the rate of inversion. The character of the diet and individual peculiarities both play a role here. Hence the percentage utilization of inulin for any individual must vary and cannot be determined except by experiment. Any inulin which leaves the stomach unchanged is liable to escape utilization and undergo bacterial decomposition in the intestine, a decomposition which results in no formation of carbohydrates. Any inulin which escapes this bacterial action is probably eliminated unchanged in the feces.

In view of these facts, as well as the inability to administer more than comparatively small quantities, the value of inulin as a significant source of energy in human dietaries must be questioned."

Lewis and Frankel (36) (1913) found that inulin administered to phlorhizinized dogs does not give rise to glucose, and that the feeding of levulose to the same animals results in the elimination of a large amount of glucose. They concluded that inulin is not, to any appreciable extent, converted into levulose or any other substance capable of forming glucose in the diabetic organism.

Togel, Brezina, and Durig (37) (1913) observed that levulose increased the metabolism to a greater degree than glucose, that the increase began earlier and in their opinion led more than glucose to the formation of fat. With a patient to whom they gave 30 g. of levulose in hourly doses the respiratory quotient was kept at 1.00 for a long time. They also observed that the respiratory quotient fell for the first few moments after the carbohydrate was given.

Verzar (38) (1914) demonstrated that levulose when given to a depancreatized dog, raised the respiratory quotient for a considerable period after glucose failed to do so, though eventually it too lost the power.

Isaac (39) (1914) reported that fructose is rapidly transformed into glucose in the perfused extirpated liver, contra-

36. Lewis and Frankel, J. Biol. Chem., 17:365(1913)
37. Togel, Brezina, Durig, Biochem. Z., 50:296(1913)
38. Verzar, *ibid.*, 66:75(1914)
39. Isaac, Z. Physiol. Chem., 89:78(1914)

dicting the opinion of Voit that ingested fructose is first converted into glycogen, then into glucose. He stated that glycogen produced from fructose may arise (indirectly) from glucose derived from fructose. No difference between dextrogenetic glycogens and fructogenetic glycogens has ever been noticed.

Goudberg (40) (1914) stated that feeding inulin raises the respiratory quotient for a long time, indicating a slow utilization of this carbohydrate. He concluded that it does not lead to anhepatic utilization with glycogen formation, but is brought about in the following steps: partial hydrolysis in the acid of the gastric juice, absorption of another fraction without hydrolysis, and a slow utilization of this inulin in the tissue, bacterial decomposition of the remainder with the formation of organic acids which do not induce glycogen building, but which are themselves burned. Inulin therefore appears to have just those characteristics valuable in a carbohydrate for diabetics.

Lusk (41) (1915) used the oxygen as well as the carbon dioxide in the study of the effect of 50 g. of various sugars (glucose, sucrose, and levulose) which he gave to a dog. He noted any increases in metabolism in the order named of 36%, 34%, and 37%, but only with levulose did any considerable increase persist throughout the fifth and subsequent hours. The

40. Goudberg, Z. Exp. Path. Therap., 13:310(1914)

41. Lusk, J. Biol. Chem., 20:555(1915)

respiratory quotients rose to 1.00, 1.02, and 1.02, respectively. There was no increase in the metabolism and respiratory quotient with lactose and but little with galactose. Levulose (2.8 g.) appeared in the urine as such in the levulose experiment, and 0.25 g. appeared as sucrose after the sucrose experiment, but the urine was sugar free following glucose. (Joslin suggests that perhaps the particular dog used in Lusk's investigation may have had a low levulose threshold.)

Bernstein and Falta (42) (1916) gave 100 grams of levulose on three successive days to a diabetic patient. No rise in respiratory quotient is recorded, but from the test it would appear that the metabolism tests made were basal tests and upon the mornings after the levulose was given.

Sansum, Wilder, and Woodyatt (43) (1916) reported the intravenous tolerance limit for glucose at close to 0.85 g. per kilogram of body weight hourly and for levulose close to .15 g.

Mandel and Lusk (44) (1917) were unable to confirm the tests of Minkowski (45) when experimenting on a diabetic man instead of a dog.

Benedict and Carpenter (46) (1918) pointed out that in experiments with feeding levulose to normals the peak of oxida-

42. Bernstein and Falta, Duet. Arch. klin. Med., 121:95(1916)
43. Sansum, Wilder, Woodyatt, Proc. Soc. Biol. Chem., J. Biol. Chem., 24:XIX-XX(1916)
44. Lusk, "Science of Nutrition", W. B. Saunders Co., Philadelphia and London, p. 485 (1917)
45. Minkowski, loc. cit., (Reference 18)
46. Benedict and Carpenter, Carnegie Inst. Pub., 261:246(1918)

occurred within 40 to 60 minutes after the levulose was given.

Loeffler (47) (1919) gave 100 g. of levulose to a diabetic patient and at an interval of seven hours repeated the dose. He observed an increase in the metabolism, with an increase in the respiratory quotient, both of which were greater after the second feeding. The increases were not more marked than with glucose, but less of the levulose was excreted in the urine.

Okey (48) (1919) reported that concentrations of HCl such as might be found in the gastric juice, produced very slight hydrolysis of inulin. Experiments in which inulin was fed with water or with egg-white indicated that it left the stomach so rapidly that no considerable hydrolysis was likely to occur, though the small amount remaining was almost entirely hydrolyzed. Sterile extracts of three samples of human feces from three types of diets converted inulin into a reducing sugar, but negative results were obtained with feces from a guinea pig and from a dog.

Daniel (49) (1921) made the observations previously mentioned and stated that fructose effects a saving in fats and proteins.

Schimizu (50) (1921) found no enzyme in macerated intestine and pancreas preparation capable of hydrolyzing inulin. He reported that the addition of inulin to a meat diet resulted in a diminution of the nitrogen output, concluding that inulin is a

47. Loeffler, Z. Klin. Med., 87:309(1919)

48. Okey, J. Biol. Chem., 39:139(1919)

49. Daniel, loc. cit., (Reference 9)

50. Schimizu, Biochem. Z., 117:227(1921): *ibid.*, 245(1921)

digestible and available foodstuff in the digestive canal of a dog and is a protein sparer. The exact portion of the tract or the mechanism whereby they are split was not known.

Spence and Brett (51) (1921) reported results on the use of levulose as a test for hepatic inefficiency. They found that in a healthy adult with a normal liver function, 50 g. of levulose will produce no rise in blood sugar. With diminished liver function a definite rise in blood sugar will result from the ingestion of levulose. They stated that the kidney threshold is lower than that for glucose and varies in different individuals. The inconsistency of the threshold for levulose renders the older method of testing liver efficiency by urinary examination inaccurate.

Folin and Berglund (52) (1922) found that levulose produced no hyperglycemia in the normal individual to whom they gave 200 grams. From the results of this experiment they conclude:

1. That levulose is not directly converted into glucose by the liver, or else the blood sugar would have risen.
2. That levulose did not appear in the urine because, like glucose, it also has a renal threshold which the rare cases of levulosuria illustrate.
3. That the rapidity of its disappearance from the blood is "beyond possibility of plausible explanation in terms of metabolism processes."

51. Spence and Brett, Lancet II, 1562(1921)

52. Folin and Berglund, J. Biol. Chem., 51:213(1922)

4. That the interpretation of the fate of absorbed levulose is as follows: -

"The liver retains fructose as well as every other usable sugar to a greater extent in proportion to its weight than do the general tissues such as muscles. But such retentions by the liver are never even approximately quantitative, and a large fraction of absorbed sugars, possibly the greater part, gets by this organ. Other tissues, such as the muscles, take up sugars from the plasma of arterial blood, and it is this general absorption which prevents excessive accumulations of sugar in the blood. But tissue sugar like blood sugar is normally and predominantly glucose, partly because the major part of our carbohydrate food is made up of glucose, partly because all other usable sugars are gradually converted into this essential sugar. The tissues being relatively well stored with glucose and empty of other sugars, such as fructose, may well be able to absorb these other sugars from the blood so nearly completely that the venous blood used for our analyses shows only traces. The glycogen formation may or may not begin immediately, and at all events need not be the immediate cause for the rapid disappearance of levulose from the blood. Levulose happens to be an excellent glycogen former, but that this is not the immediate cause of its disappearance from the blood is strongly indicated by the results which we have obtained with a much poorer glycogen former--galactose."

5. That the above facts and interpretations suggest the



possibility of making more extensive use of levulose in diabetes, since past failures may have been due to impurities in the levulose which the early experiments avoided because based on the purest obtainable brands.

6. That the "tissues of the diabetic person should contain much higher concentrations of glucose than tissues of normal persons, and this is the immediate reason why the blood sugar rises so high after the intake of glucose in any form. The high concentration of glucose in the tissues would probably have little or no effect on their absorption of fructose. From the giving of fructose we should, therefore, get a much higher concentration of total sugar (glucose plus fructose) in the tissues without any material increase in the sugar of the blood. Because of this higher concentration an increased utilization might well take place. Continuous or excessive use of fructose would, of course, defeat the purpose of the treatment, because gradually this would become equivalent to the loading of all the tissues, with glucose, and, because of the large bulk of the tissues, an excessive amount of glucose would pour into the blood and be eliminated with the urine. Such a series of results has, however, no bearing on what would happen from small doses of pure fructose given at carefully regulated intervals. For under such conditions there is not only a large concentration of sugar in the tissues in relation to the level of the blood sugar, but there is also the possibility that nascent glucose is more easily

utilized than ordinary, preformed glucose. At least, it seems safe to say that until the effects of pure fructose on diabetics have been carefully investigated from this standpoint the subject has not been exhausted."

Carpenter (53) (1922) noted no definite change in the respiratory quotient in four experiments when 25 grams of levulose were given by rectum, but after 50 grams of levulose, there was an increase of 0.03 to 0.05 in two experiments after 1 to 2 hours, and in a third experiment an increase of 0.15 within 3 hours of the injection.

Desgrez, Bierry, and Rathery (54) (1922) stated that the investigation of levulose helps to correct some of the accidents of metabolism. Its use in the diabetic in general furnishes a means of forestalling and combating the elimination of B-hydroxybutric acid. The carbohydrate tolerance of the diabetic being known, it suffices to add to the ration of phosphate and Vitamin B, levulose equivalent to the maximum amount of carbohydrates which the patient is able to assimilate.

Bornstein and Holm (55) (1922) reported that 100 g. of levulose ingested by mouth begins to oxidize in 5 to 8 minutes, while the blood sugar rises but little, if any.

Joslin (56) (1923) made extensive investigations with levulose. He summarized his results as follows: "Fifty-one observations upon the effect of levulose were made. As a rule the quantities ingested were well utilized, (the levulose

utilized, as computed from the carbohydrate balance was: mild cases 100%. moderate cases 99%, severe cases 88%) though of 41 experiments with 19 severe cases, in only 9 did the urine remain sugar free.

Levulose increased the metabolism of the diabetic patients from 5 to 32 per cent, and on the average 17 per cent. This was slightly greater than that found in this Laboratory with normals under similar conditions, and persisted at a higher level for a greater period of time. In part, the wide variation in response of the diabetics to levulose is explained by the quantity of levulose given, since this varied between 28 and 100 grams or 0.90 and 2.5 grams per kilogram of body weight. While variations in the quantity of levulose administered per kilogram of body weight affected the metabolism, the quotient was affected to a much less degree.

The increase in metabolism following the ingestion of levulose with severe cases was greater than with moderate or mild cases or with normal individuals.

The post-absorption quotients obtained in the experiments which served as basal for the levulose experiments varied between

53. Carpenter, Am. J. Physiol., 59:440(1922)
54. Desgrez, Bierry, and Rathery, Compt. rend., 175:536(1922)
55. Bornstein, and Holm, Biochem. Z., 130:209(1922)
56. Joslin, loc. cit., (Reference 15)

0.70 and 0.91, but levulose produced the same effect upon the respiratory quotient and metabolism when the basal quotients of the patients were low as when they were high.

The average quotients of the diabetics for the second half-hour after levulose was 0.84 and thereafter it fell steadily and for the most part uniformly to the basal quotient of 0.79 in the fifth half-hour. Subsequently it was below the standard, in contrast to normals in which the increase in the quotient continues into the ninth half-hour.

The respiratory quotient rose considerably above 1.00 in 4 experiments with 2 cases after the ingestion of 1.55 to 1.90 grams of levulose per kilogram of body weight. The two patients with the highest respiratory quotients were also high.

It appears impracticable to explain the quotients above unity other than as due to a conversion of carbohydrate to fat, though such a conversion may take place at lower levels.

One patient receiving similar quantities of levulose upon three successive days showed average increases in quotient above basal of 0.08, 0.09, and 0.17, respectively.

The average percentage of blood sugar before taking levulose was the same as that 24 hours later or 0.19 per cent. In 8 experiments the blood sugar averaged 0.23 per cent before levulose was given. About three or four hours after the levulose was taken, it was 0.30 per cent, increasing values being found in all but two experiments.

In eight experiments levulose and fat were taken by four severe cases free from acidosis and the metabolism increased 16 percent. The respiratory quotient rose from 0.83 to 0.88 with an average maximum quoteint of 0.92."

Desgrez, Bierry, and Rathery (57) (1923) reported that the transitory influence of insulin was favored and prolonged by a balanced diet. The addition to such a diet either of Vitamin B or levulose or a mixture of these two substances allowed a greater interval of time between the injections of insulin, and increased the efficiency of a single dose. These conclusions were based on the 24 hour output of ketonic substances and of B-hydroxybutyric acid.

Holm, (58) (1923) states that levulose is immediately utilized by man, normal dogs, and by dogs with an Eck fistula (with hardly any rise in the blood sugar).

Schatti (59) (1923) took blood samples from a normal individual after ingesting 20 g. carbohydrate 13 to 15 hours after a standard meal. He also examined the urine in each case, and from the results obtained he arranged the following sugars in order of decreasing alimentary hyperglucemia: glucose, levulose, sucrose, lactose, and galactose. He stated levulose was excreted more quickly and in larger amounts than glucose.

57. Desgrez, Bierry, and Rathery, Compt. rend., 177:795(1923)
58. Holm, Z. Ges. Exptl. Med., 37:43(1923)
59. Schatti, Biochem. Z., 143:201(1923)

Meyer-Bisch (60) (1924) reported that the ingestion of 100 g. of levulose by normal individuals and by hepatopathics leads to an increase in the concentration of serum protein (1.2%) and to a simultaneous increase in the hemoglobin concentration usually within 15 minutes.

In diabetics, concentration of the blood after levulose occurred in only 50% of the cases. Diuresis often occurs and in such cases there is also a loss in body weight.

Nagasuye (61) (1925) reported that the rise in blood sugar following the ingestion of levulose is generally much less than that after glucose. In the fasting organism the ingestion of levulose may cause the blood-sugar level to rise somewhat higher than in one on a normal diet, while on a protein-fat diet, levulose exerts no influence on the blood sugar level. The ingestion of either glucose or galactose in the fasting condition causes but a slight increase in the liver glycogen, while levulose contributes considerably to raising the glycogen content of the liver both in fasting and on a protein-fat diet.

Abelin and Goldener (62) (1925) stated that the ingestion of levulose does not lead to a significant hyperglucemia in normal individuals. A hyperglucemia is produced in diabetics, but not when insulin is given simultaneously. Levulose does not reduce the hyperglucemia that results from insulin poisoning.

60. Meyer, Bisch, *Klin. Wochschr.*, 3:60(1924)

61. Nagasuye, *J. Biochemistry*(Japan), 5:449(1925)

62. Abelin and Goldner, *Klin. Wochschr.*, 4:1733(1925)

Emerique (63) (1925) reported that pure inulin given to mice, rats, and guinea pigs was assimilated in small or moderate quantities, but was completely assimilated when the whole vegetable (Jerusalem artichokes) was given.

Root and Baker (64) (1925) reported their results from experiments using Jerusalem artichokes in the treatment of diabetes. Diabetic patients who used them for six months were benefited. Glucosurea was neither induced or increased where already present and in some cases urine was rendered sugar free when artichokes were substituted for other carbohydrates. Patients were able to increase other components of diet and gain weight with only slight increase of insulin dosage. The respiratory quotient was increased in all cases, also the blood sugar increased.

Snapper, Grunbaum, and Van Creveld (65) (1926) reported a case of genuine levulosuria in a seventeen year old girl. The blood sugar content was not increased and did not rise even following the administration of fructose, no diabetic troubles were present.

Cori (66) (1926) presented curves showing the rate of glycogen formation in the liver of the rat during the absorption of glucose, fructose, and galactose. The first two were

63. Emerique, *Ann. physiol. phys. chim. biol.*, 1:123(1925)  
*Chem. Abstr.*, 20, 2522(1925)
64. Root and Baker, *Arch. Internal. Med.*, 36:126(1925)
65. Snapper, Grunbaum, and Van Creveld, *Nederl. Tijdschr. Geneeskunde*, 70, 1, 1600(1926), *Chem. Abstr.*, 20:3730(1925)

shown to be on a par as formers of glycogen despite the slower absorption of fructose. The maximum sugar retention by the liver occurred in four hours and amounted to 17% of the total absorbed with glucose and 39% with fructose.

Kronenberger and Radt (67) (1927) reported that experimental evidence points to an early and massive appearance of levulose in the blood on feeding this carbohydrate. This was ascribed to a rapid resorption of the sugar.

Cori and Cori (68) (1927) found that a surplus of insulin leads to an increased oxidation of fructose in rats.

King (69) (1927) reported a study of the levulose tolerance test for hepatic efficiency. A series of 53 cases showed the test to be consistent and reliable.

Dunton (70) (1927) recommended the Jerusalem artichoke and milk as a good possible combination for furnishing bone-building materials. He suggested that inulin is probably not available to the human organism and therefore, the artichoke should be a desirable crop for diabetes and obesity.

Bodey, Lewis, and Huber (71) (1927) reported that the ad-

66. Cori, J. Biol. Chem., 70:577(1926), Proc. Soc. Exptl. Biol. Med., 23:459(1926)
67. Kronenberger and Radt, Biochem. Z., 190:161(1927)
68. Cori and Cori, J. Biol. Chem., 73:555(1927)
69. King, Lancet, I, 385(1927)
70. Dunton, Forecast, 34:295, 296, 332(1927), Expt. Sta. Record 58:290(1927)
71. Bodey, Lewis and Huber, J. Biol. Chem., 75:715(1927)



ministration of fructose or inulin in butter oil to fasted rats led to a deposition of hepatic glycogen.

Ciaccio (72) (1927) tried the effect of levulose on hyperglucemia. Normal and starved dogs were given intraperitoneally small doses of fructose and glucose, 30 minutes afterwards large doses of glucose were given by mouth. Blood sugar determinations made at intervals showed clearly that fructose exerts a definite inhibiting action on glucose hyperglucemia. The inhibiting action is probably connected in some way with the internal secretion of the pancreas.

Steinberg (73) (1927) divided the organs into four groups as regards their power to utilize levulose:

1. Heart, pancreas, and small intestine using it but to slightest extent.
2. Skeletal muscle and kidney using it about as readily as glucose.
3. Salivary glands intermediate to No. 1 and No. 2
4. Liver and lung assimilate levulose more readily than glucose.

Cori and Cori (74) (1928) stated that the configuration of sugar determines the rate of absorption and mode of utilization in the animal (rat) body.

72. Ciaccio, Bull. soc. ital. biol. sper., 2:1029(1927)  
73. Steinberg, Arch. ges. Physiol.(72. Chem. Abstr., 22:  
(Pfluger's) 217:686(1927) 2601(1928) )  
74. Cori and Cori, J. Biol. Chem., 76:755(1928)

	<u>Rate Absorption</u>	<u>Utilization 4 hours</u>		
		<u>Oxidized</u>	<u>Liver</u>	<u>Body</u>
		<u>Glycogen</u>	<u>Glycogen</u>	<u>Glycogen</u>
Glucose	100	44%	18%	25%
Fructose	51	36%	38%	12%
Dihydroxyacetone	65	51%	21%	15%

Turcatti (75) (1928) reported that levulose may be transformed by the blood of diabetics. He experimented on the glucolysis of glucose, and levulose in the blood of normal dogs and those with experimental diabetes. Glucolysis in normal blood after 6 hours ranged from 27% to 59%. On the addition of levulose the glucolysis was greater than on the addition of glucose. In diabetic blood the glucolysis was generally less than that of normal blood. Upon the addition of glucose the glucolysis was decreased and in some cases disappeared. Upon the addition of levulose to diabetic blood the glucolysis was generally diminished, but in some cases it was unchanged or increased.

Solarino (76) (1928) found that inulin produces in dogs a variable but definite hyperglucemia, it has an inhibiting action on glucose hyperglucemia not as marked as starch, but comparable to that of levulose.

Carpenter and Root (77) (1928) kept a diabetic patient for

75. Turcatti, Compt. rend. soc. biol., 98:175(1928)
76. Solarino, Boll. soc. ital. biol. sper., 3:108(1928)  
(Chem. Abstr., 22:2621(1928).)
77. Carpenter, Root, Arch. Internal. Med., 42:64(1928)

six days on a diet of eggs, meat, vegetables, butter, oil, bran, and baked potatoe. On five days Jerusalem artichoke furnished 100 g. of the carbohydrates or 95% of the hydrolyzable sugar, on the sixth day it was replaced by potato. The average daily intake was 76 g. protein, 271 g. dry nitrogen and fat free substance. The average daily absorption (feces) was 50 g. protein, 40 g. fat, 205 g. carbohydrate, or a total of 1500 calories. Glucosuria was absent on the artichoke diet. Replacement of artichoke by potatoe was followed by glucosuria, increased blood sugar, nitrogen excretion, and heat production. The symptoms disappeared when the artichoke diet was resumed.

Corley (78) (1929 reported some experiments on the metabolism of levulose. He found that after internal administration of levulose to rabbits, it appeared in the blood in small amounts and mild poisoning with hepatotoxic agents had little influence on the amount of levulose in the blood after internal ingestion. After intravenous injection of 2 g. levulose per kilogram of body weight levulose practically disappeared from rabbit blood in 90 minutes and liver poisons had little influence on the rate of disposal except in massive doses. If injected simultaneously with insulin, levulose protected the rabbit from the effect of insulin without there being any striking influence on the rate of removal of the circulating levulose. Insulin shock was observed on several occasions and levulose disappeared more rapidly from the blood if insulin was given subcutaneously or

intravenously an hour or more previous to the intravenous injection of levulose.

Solarino (79) (1929) found that inulin fed in 5 gram quantities to starved dogs produced a hyperglucemia. Inulin showed marked inhibiting action on glucose hyperglucemia, being comparable to that of levulose.

Bertram (80) (1929) reported that peroral administration of levulose results in a less marked rise of blood sugar than the same amount of dextrose. Although levulose is absorbed more slowly, it is oxidized more quickly. He stated that insulin is not essential for the utilization of levulose and suggested a number of possible explanations for the differences in the metabolism of levulose and dextrose.

Westcott and Wise (81) (1929) reported that a diabetic patient was unable to utilize dried artichoke powder. They stated that the glucose tolerance was not increased and that artichokes have no advantage as a diabetic food.

Silberstein and Wachstein (82) (1929) stated that feeding levulose to rabbits has in no instance produced a typical insulin effect. Some dogs showed insulin production following oral administration of levulose while others did not. They discussed

79. Solarino, Arch. sci. biol., 13:31(1929), Chem. Abstr. 23:2761(1929)
80. Bertram, Z. ges. exptl. Med., 64:295(1929)
81. Westcott, and Wise, Arch. Internal. Med., 44:362(1929)
82. Silberstein and Wachstein, Biochem. Z., 213:501(1929)

the fact that levulose is not an adequate food for carnivara.

Arujama and Takahasi (83) (1929) listed the assimilation per 100 grams body weight of various carbohydrates and mixtures of carbohydrates, giving levulose the value of 0.5 g., sucrose 0.76 g., glucose 1.04, maltose 1.5 g., and inulin 1.56 g. The urine of rats fed inulin reduced only after hydrolysis. The tolerances for maltose, glucose, sucrose, and levulose were given as about the same. They stated that maltose, fructose, glucose, starch, and dextrin lead in nutritive value followed by galactose, mannose, arabinose, xylose, lactose, saccharose, insulin and glycogen.

Carpenter and Fox (84) (1930) reported that the maximum respiratory quotient was found mostly during the second half hour after levulose was given to human subjects. They presented considerable data on the gaseous exchange of the subject as affected by levulose.

83. Arujama, Takahasi, *Biochem. Z.*, 216:269(1929)
84. Carpenter, Fox, *J. Nutrition*, 2:359(1930), (*Chem. Abstr.*, 24:2784(1930):)

## Part B

The Discovery and Methods of Preparation of Inulin and Levulose.

Since the main portion of this paper is to deal with the Jerusalem artichoke as the source of inulin and levulose, connecting references would be appropriate. The first written notice of the Jerusalem artichoke was by Champlain (85) who reports seeing it in the gardens of the Indians at Mallebarre (now Manset Harbor, Cape Cod, Mass.) on July 21, 1605. A substance was found by Rose (86) in the rhizome of elecampane (*Inula helenium*) in 1804, which was named "Inulin" by Thomson (87) in 1811.

Funcke (88) is sometimes erroneously credited with the discovery of inulin. His work appeared, however, in 1810, in which he found that he could prepare the peculiar "starch" from a water extract of the root of *Inula helenium*.

In 1813 and 1814, John (89) reported 36.7% inulin in inula or elecampane and 40% in *anacyclus officinalis* Hayne.

De Claubry (90) published his study of inulin in 1815. He reviewed the previous work, adopted the name inulin and prepared it from roots by extracting with hot water, filtering, evaporating, and adding cold water. He described its properties, believing it

85. Champlain "The Voyages and Explorations of S. De Champlain", C. Scribner's Sons, New York, p. 53 (1922)
86. Rose, Neuse Allg. J. Chem., 3:217(1804), Nicholson's J. of Nat. Philos. Chem. and the Arts, 11:97(1804), Gehlen's J. III:217(1804). Original not seen. Taken from Harding (Reference 147).

to be similar to starch. It formed an insoluble compound with Barium, was insoluble in cold water and soluble in hot water: was precipitated by ethyl alcohol, and could be oxidized with nitric acid to malic, oxalic, and acetic acids. He found it difficult to separate from filter paper if allowed to dry.

Payen (91) first prepared inulin from the dahlia bulb in 1823. He suggested as a method: to pulp the bulbs, mix with water, add 1/2% CaCO<sub>3</sub>, boil 1/2 hour, filter and press, rewash and repress the residue, evaporate the combined filtrates to 3/4 their original volume, clarify with carbon, filter and evaporate. He called the inulin "dahline".

Inulin was found in the Jerusalem artichoke by Braconnot (92) in 1824. He obtained 8.88 grams of what he thought to be inulin from 500 grams of artichoke tubers. He believed Payen's "dahline" to be inulin.

Liebig (93) (1832) confirmed the above observations of Braconnot.

Marquart (94) (1834) reported the preparation of inulin from dahlias and artichokes but did not detail his method.

87. Thomson, System d. Chemie, 5:744(1811)
88. Funcke, Ann. de Chemie I 76:98(1810)
89. John, Chemische Tabellen des Pflanze analysen, Nurnberg. Schrag p. 17(1814)
90. De Claubry, Ann de Chemie I, 94:200(1815)
91. Payen, J. Pharm. Chim., II 9:383(1823)
92. Braconnot, Ann. Chim. phys., 25:358(1824)
93. Liebig, Ann. 2:235(1832)
94. Marquart, ibid., 3:10(1834), ibid., 2(1834)

Parnell (95) (1841) worked on the constitution of inulin.

Crookewitt (96) (1843) was the first to observe that inulin could be converted to an uncrystallizable sugar by heating for fifteen hours in a water solution.

Soubeiran (97) (1843) discovered that dextrose was not the sole product of the hydrolysis of sucrose. He found that a levo-rotatory sugar which fermented readily was also formed.

Woskressensky (98) (1846) used chicory as the raw material for the preparation of inulin. He used 380 grams of the powdered root and obtained 110 grams of inulin.

Bouchardat (99) (1847) differentiated between inulin and dextrin by their specific rotations and by the difference in the sugars they yield on acid hydrolysis.

Dubrunfaut (100) (1847) observed that one component of hydrolyzed sucrose could be isolated as an insoluble calcium salt. This discovery was to become the basis for the preparation of levulose for many years, and at the present time still holds the most promise of becoming the successful method for purifying levulose sirups.

Dubrunfaut stated that as early as 1830 he had noted the presence of this component sugar in invert sugar, that it rotated

95. Parnell, *ibid.*, 39:213(1841)
96. Crookewitt, *ibid.*, 45:184(1843)
97. Soubeiran, *J. de Pharm.* III 4:347(1843)
98. Woskressensky, *J. prakt. Chem.*, 37:309(1846)
99. Bouchardat, *Compt. rend.*, 25:274(1847)
100. Dubrunfaut, *Ann. Chim. phys.*, 21:169(1847)



negatively three times as strongly as invert sugar and fermented more slowly than glucose, thus betraying its presence.

Dubrunfaut (101) (1847) in a second article confirmed his former observations, stating that the presence of a strongly levo-rotatory sugar could be shown as glucose crystallized or was fermented out of invert sugar.

Dubrunfaut (102) (1856) presented his proof that invert sugar was composed of two sugars, one dextrose, and the other identical with the sugar formerly prepared from inulin.

Dubrunfaut (103) (1869) gave in detail his technique for the separation of levulose from dextrose as the calcium levulosate. He used 100 cc. of sirup containing ten grams of previously inverted sugar, added six grams of powdered calcium hydroxide at a low temperature with stirring. The precipitated calcium levulosate was filtered off and the filtrate as well as the precipitate treated with oxalic, sulfuric or carbonic acid until neutral. The sugar thus obtained in the filtrate had a final rotation of  $+52^{\circ}$  and was dextrose, the sugar from the precipitate rotated at about  $-45^{\circ}$ . The method was described as being very reliable. The levulose was not obtained in crystalline form.

Ferrouillat and Savigny (104) (1869) prepared inulin from the dahlia bulb. Their method was to pulp the bulbs,

101. Dubrunfaut, Compt. rend., 25, 307(1847)
102. Dubrunfaut, *ibid.*, 901(1856)
103. Dubrunfaut, *ibid.*, 69:1366(1869)
104. Ferrouillat & Savigny, *ibid.*, 68:1571(1869)

boil with water for one hour, filter, precipitate with lead acetate, filter, remove the excess lead with hydrogen sulfide, concentrate and allow to stand.

Dragendorff (105) (1870) published a review of the previous work on inulin. This was a very complete study of the subject with many references given. He recommended especially the dahlia or chicory root as a source of inulin, suggesting that one half to one hour extraction with water was sufficient. He also described a method in which he pressed the dahlia bulbs and precipitated the material fractionally with alcohol.

Loscoeur and Morelle (106) (1878) prepared inulin from the dahlia, inula helenium, and chicory, and reported the specific rotations and chemical properties to be identical from the three sources.

Peligot (107) (1880) described a modification of Dubrunfaut's method for the separation of levulose from dextrose. a six to eight per cent solution of invert sugar was agitated with an excess of lime at 0°C., the calcium levulosate thus formed filtered off and decomposed with oxalic acid. A sample of the dried levulosate analyzed to be  $C_{12}H_{20}O_{11}$ ,  $3CaO$ . No crystalline levulose was obtained.

Jungfleisch and Lefranc (108) (1880) reported the crystal-

105. Dragendorff, "Monograph on Inulin", F. Schmitzdorff, St. Petersburg, (1870)
106. Loscoeur, Morelle, Compt. rend., 87:216(1878)
107. Peligot, *ibid.*, 90:153(1880)
108. Jungfleisch, Lefranc, Bull. soc. chim., II, 34:675 (1880), J. pharm. chim., V, 4:437(1880), Compt. rend., 93:547(1880).

lization of levulose for the first time. They gave a review of previous work, crediting Berthelot with giving levulose its name. Their sirup was prepared by Peligot's modification, of the Dubrunfaut method. (107). The concentrated sirup was washed repeatedly with absolute alcohol and was eventually sufficiently water free to crystallize when abandoned. Fine silky needles were observed to form slowly. These writers were the first to observe that the specific rotation of levulose varies materially with differences in temperature.

Kiliani (109) (1880) gave a long historical review of previous work on inulin. He prepared a crystalline sugar identical in properties with Dubrunfaut's levulose by hydrolyzing inulin in water. He gave the final specific rotation as  $-92^{\circ}$  to  $-93^{\circ}$  and claimed a 96.7% conversion of inulin to levulose.

Girard (110) (1880) described a method for the preparation of crystalline levulose which was essentially the Dubrunfaut method. He hydrolyzed a 10% sucrose solution (700 g. sucrose) in seventeen hours at  $60^{\circ}$  C., with 20 cc. HCl per liter of solution. The solution was cooled to  $-5^{\circ}$ C, and six grams of lime added to each ten grams of sugar. The mixture was agitated while the temperature was held below  $+2^{\circ}$  C. The resulting precipitate was pressed out in a hand press, suspended in water and decomposed with oxalic acid, which was found to be preferable to carbonic acid. The final concentration was made by freezing

109. Kiliani, Ann., 205:145(1880)

110. Girard, Bull. soc. chim., I, 33:154(1880)

and very white levulose resulted.

Herzfeld (111) (1884) obtained 75 g. of levulose (rotation  $-94.7$ ) from 2250 g. of inulin by dehydrating his sirup with ether and alcohol.

Lehmann (112) (1884) prepared levulose from inulin by acid hydrolysis and from invert sugar by the lime precipitation method, in order to compare the reducing properties of the sugar from the two sources. He gave many bibliographical citations.

Winter (113) (1887) reported the specific rotation of levulose to be  $-71.4^{\circ}$  in a 20% solution at  $20^{\circ}$  C. and reasoned from this that invert sugar consisted of four parts, levulose and three parts dextrose.

Honig and Schubert (114) (1887) reported the crystallization of levulose from inulin. After hydrolysis with sulphuric acid, barium hydroxide was added, the filtered sirup concentrated and alcohol added. The following year the same authors (115) gave the specific rotation of their levulose from inulin as  $-113.936^{\circ}$  at  $20^{\circ}$  C. They very positively declared invert sugar to be a mixture of equal parts of dextrose and levulose.

Weizsaecker (116) (1890) described in detail the method of

- 111. Herzfeld, Z. Ver. dnet. Zuckerind., 34:430(1884)
- 112. Lehmann, *ibid.*, 34:993(1884)
- 113. Winter, *ibid.*, 37:796(1887)
- 114. Honig and Schubert, Monatsh., 8:529(1887)
- 115. Honig and Schubert, *ibid.*, 9:562(1888)
- 116. Weizsaecker, J. Fabr. Sucre, 34(1890)

Jungfleisch because of difficulties encountered by some in the preparation of crystalline levulose. 100 g. of pure sucrose was boiled for 5 minutes in one liter of distilled water containing one gram of concentrated  $H_2SO_4$ . The solution was cooled to  $32^{\circ}C.$ , 50 g.  $Ca(OH)_2$  added, and the mixture stirred for two minutes before filtering. On cooling the filtrate the calcium levulosate appeared. This was filtered off after 24 hours, decomposed while suspended in water at  $30^{\circ}C.$  by oxalic acid to exact neutrality, filtered and concentrated in vacuo. The resulting very thick sirup was dehydrated with alcohol and the levulose rather readily crystallized.

Wiechmann (117) (1891) reported the preparation of some levulose from inulin after hydrolysis with sulfuric acid.

Tanret (118) (1893) found both inulin and levulose present in the juice extracted from the Jerusalem artichoke. He isolated and estimated the amount of each of the several condensation products of levulose present.

Schering (119) (1893-1894) published his work on levulose. He secured a patent upon what was essentially the old Dubrunfaut method of precipitating the lime levulosate and treating it with carbon dioxide. He used molasses as the raw material.

Young (120) (1898) reported that inulin could be precipitated from water solution by the addition of magnesium or ammonium sulfate.

- 117. Wiechmann, Zeit. Rubenz. Ind., 41(n. f. 28)531(1891)
- 118. Tanret, Bull. soc. chim., (3)9:227, 622(1893), Compt. rend., 117:50(1893), J. Pharm chim., V:28, 57(1893), Compt. rend., 116:514(1893)

Wolff (121) (1899) made inulin from chicory in which he estimated 12 to 13%. He heated 100 g. of the pulped root, with a little  $\text{CaCO}_3$  added, in one liter of water, boiled for ten minutes, pressed out the liquors, evaporated the filtrate to 100 - 200 cc., and precipitated the inulin by the addition of eight volumes of 90% alcohol. Upon reprecipitation 2.3 g. was obtained. Wolff suggested the use of inulin for feeding diabetics. He stated that the dried chocory root contained at least 50% inulin.

Dean (122) (1904) published an extensive work on inulin. He compared Tanret's method of preparation with Dragendorff's and, starting with the same amount of raw material, prepared 14 g. of inulin with a specific rotation of  $-36.8^\circ$  by the former, and 22.5 g. with a specific rotation of  $-34.2^\circ$ , by the latter. He tried most of the methods previously published. His usual method was to boil the pulped roots with water containing  $\text{CaCO}_3$ , express the juice, clarify with lead, and precipitate inulin with alcohol or by freezing.

The Levulose Company of England (123) was granted a patent in 1905. Inulin and levulose were prepared by heating the pulped raw material to 50 - 70°C. to dissolve out the inulin,

119. Schering, Oest. Hung. Z. F. Zucherind., 22:108(1893),  
Zeit. Rubenz. Ind., 33:72(1894)
120. Young, J. Physiol., 22:401(1898)
121. Wolff, Ann. Chim. anal., 4:157(1899), *ibid.*, 187.
122. Dean, Amer. Chem. J., 32:69(1904)
123. Levulose Co. of Eng., British Patent 353,670(1905)  
Chem. Abstr., 1:1075(1905)

taking care to keep the solution neutral, filtering, getting rid of albuminoids by centrifuging, recovering inulin by freezing, and finally transforming it into levulose by acid hydrolysis.

Stein (124) (1908) criticized the old Schering and Dubrunfaut methods as expensive and difficult, and suggested the preparation of levulose from the dahlia containing 10 - 12% inulin, or chicory root containing 6 - 11%, or the artichoke. He suggested steaming the pulp after the addition of some lime, filtering, clarifying the juice, and freezing. The inulin thus crystallized could be centrifuged, hydrolyzed with dilute acid and concentrated to sirup. Various uses of levulose were listed.

Adler (125) (1909) prepared diglucose benzidide, diarabinose benzidide and dimaltose benzidide by boiling the respective sugars with benzidine and alcohol. No crystalline derivative could be obtained for levulose. He suggested that it was possible to separate glucose from its mixtures with levulose by means of benzidine, thus purifying the levulose. Excess of the bases should be employed, and that which remains uncombined could subsequently be precipitated by sulfuric acid.

Bierry, Henri, and Ranc (126) (1910) tried the effects of ultraviolet rays on d fructose and reported it to be completely decomposed into formaldehyde and carbon dioxide.

124. Stein, loc. cit., (Reference 10)

125. Adler, Ber., 42:1742(1909)

126. Bierry, Henri, and Ranc, Compt. rend., 151:316(1910)

Fernbach and Schoer (127) (1912) described a method of production of levulose by biochemical methods. An anerobic bacillus, called "gommobacter" was discovered, which, in nutritive medium, attacks sucrose and gives a gum, which is precipitated by alcohol, acetone or  $Ba(OH)_2$ . This gum, (approximately 50% of the sucrose is used) is hydrolyzed by a trace of acid to the same weight of levulose. The gum is probably a levulan. It is produced only from sucrose. Although the first action is an inversion of the sucrose, no gum is obtained from invert sugar or an equimolecular mixture of dextrose and levulose. The other half of the sucrose is changed into volatile and gaseous products.

Ranc (128) (1914) reported again the action of untraviolet rays on levulose.

Nef (129) (1914) treated d fructose with various alkaline substances and reported the dissociation observed.

Wolff (130) (1916) found a substance in the roots of the dahlia and chicory which rapidly coagulated the inulin in juices from these roots. He named the substance inulo-coagulose.

Daniel (131) (1917) was issued a patent on the prepatation of inulin and levulose.

- 127. Fernbach and Schoer, Compt. rend., 155: 84(1912)
- 128. Ranc, Biochem., Z., 64:257(1914)
- 129. Nef, Ann., 403:204, 383(1914)
- 130. Wolff, Compt. rend., 162:514(1916)
- 131. Daniel, British Patent, 109, 813(1917)  
(Chem. Abstr., 12:156(1917):)



This called for the production of inulin first, from vegetable saps, the saps being purified from albuminous matter etc., by heating with alkaline reagents such as the hydroxides of Na, K, NH<sub>4</sub>, Ba, Sr, Ca, or Mg, or the carbonates of Na, K, and NH<sub>4</sub>, or Pb(Ac)<sub>2</sub>. The purified saps may then be concentrated to obtain the inulin, which may be converted to levulose.

Daniel (132) (1918) obtained another patent on a process of preparing inulin from plants in a form suitable for therapeutic use. He specified keeping the solutions strongly alkaline.

Irvine and Steele (133) (1920) made inulin from dahlias for their investigation of its relation to levulose. They separated it by freezing, washed it by decantation (which is as effective for freeing it from ash as dialysis), and reported the specific rotation as  $-34.21^{\circ}$ .

Herzfeld and Klinger (134) (1920) gave briefly a method for preparing inulin in pure form which did not differ markedly from previous methods.

Wolff and Geslin (135) (1920) discussed some of the properties of inulin and changes in its physical state. They found that inulin prepared from chicory or dahlia was more soluble in water than that obtained from other sources after it had been precipitated from alcohol.

132. Daniel, Swiss patent 77,857(1918): (Chem. Abstr., 13:164(1919):)
133. Irvine and Steele, J. Chem. Soc., 117:1474(1920)
134. Herzfeld and Klinger, Biochem. Z., 107:268(1920)
135. Wolff and Geslin, Bull. soc. chim. biol., 2:19(1920)

Willaman (136) (1920) proposed the production of levulose sirup from the Jerusalem artichoke, suggesting that the yield was large per acre and the inulin content from 12 - 14%. He gave certain figures as to production, etc., to attract commercial and industrial attention.

Daniel (137) (1921) recommended the manufacture of levulose from chicory roots which give good yields per acre. He suggested using the exhausted chips and leaves as stock food, converting the molasses into a coffee substitute and into dye-stuff.

Pringsheim and Aronowsky (138) (1921) made inulin from dahlias and chicory by Dragendorff's method. They reported a specific rotation of  $-36^{\circ}$ . An attempt to purify commercial inulin by Tanret's  $Ba(OH)_2$  method failed.

Bourquelot and Bridel (139) (1921) studied the products of fermentative hydrolysis of inulin and concluded that inulin contains fructose but no glucose molecules.

Harding (140) (1922) published what he classed as "the first real departure from the much imitated technique of Dubrunfaut", for the preparation of levulose. His method was based upon the use of glacial acetic acid as a solvent

136. Willaman, Science, 52:351(1920)
137. Daniel, loc. cit., (Reference 9)
138. Pringsheim and Aronowsky, Ber. 54B:1281(1921)
139. Bourquelot and Bridel, Compt. rend., 172:946(1921)
140. Harding, J. Am. Chem. Soc., 44:1765(1922)

in the preparation of glucose from levulose. The method included the recovery of the glucose. Sucrose was the raw material used and levulose was obtained in yields of 25.5 to 28% of the weight of sucrose taken, and the dextrose in yields of 36 to 37.5%. Harding stressed that the method was successful on a laboratory scale only.

The same year Willaman gave (141) specific directions for the preparation of inulin from the Jerusalem artichoke. He ground the tubers as fine as possible, and put them into boiling water containing calcium carbonate. For each kilo of tubers he used 1300 cc. of water and 30 g. of  $\text{CaCO}_3$ . This was boiled 15 to 20 minutes, the juice extracted with a press, reboiled with 1000 cc.  $\text{H}_2\text{O}$  and 10 g. of  $\text{CaCO}_3$ , extracted and extracts combined. The juices were clarified with lead acetate, avoiding a large excess, and centrifuged or filtered. The lead was removed with ammonium oxalate and centrifuged again. The clear liquor was then treated with decolorizing charcoal if necessary, and evaporated under vacuum to a content of 40 to 60% solids. The sirup was allowed to cool slowly, then kept at 0 to 50C for several hours, thoroughly stirred with an equal volume of ice water and centrifuged. The crystals were then redissolved in about 3 volumes of water, filtered hot, concentrated to about twice the volume of original crystals, and allowed to crystallize in the cold as before. The crystals were again stirred with

ice water, filtered on paper or silk bolting cloth with suction, keeping everything cold. The crystals were washed with cold water, then with 20, 50, 80, and 95% alcohol and ether, and dried in an oven at 100°C. (The specific rotation should be  $-33^{\circ}$  at least. It is useless to try to obtain a higher rotation than  $-38^{\circ}$  or  $-39^{\circ}$ .) Willaman stated that Jerusalem artichoke tubers are not a satisfactory material for the preparation of true inulin, although they are good for the study of the whole group of inulin substances. He supported Dean's (142) hypothesis that inulin is a group of substances with large loosely bound molecules, and not a single substance. He suggested the following as a possible procedure for the manufacture of levulose sirup.

- a. Extraction of juice by diffusion.
- b. Clarification by means of lime, phosphoric acid, and carbon.
- c. Acid hydrolysis of all the inulin bodies.
- d. Precipitation of Ca-fructosate.
- e. Decomposition of Ca-fructosate and evaporation of the fructose solution to sirup.

Pringsheim and Lassmann (143) (1922) worked with inulin, determining the molecular weight of inulin acetate.

Pringsheim and Aronowsky (144) (1922) stated that inulin

142. Dean, loc. cit., (Reference 122)
143. Pringsheim and Lassmann, Ber. 55B:1409(1922)
144. Pringsheim and Aronowsky, ibid., 1414(1922)

in solid form and in its colloidal solution is an association product of a triply polymerized anhydrotri-fructose.

Micksch (145) (1922) discussed the advantages and uses of levulose (fruit sugars) recently prepared from chicory.

Daniel (146) (1922) obtained a patent for the clarification of juices from the dahlia roots. The juices are heated to about 80° for 30 to 90 minutes and treated with  $\text{Na}_2\text{CO}_3$  solution in excess, filtered and evaporated to crystallize the inulin.  $\text{CO}_2$  may be used to precipitate the impurities before filtration.

Harding (147) (1923) presented an historical sketch on the discovery and preparation of levulose, along with an account of his own work on the preparation of dextrose and levulose from sucrose by a combination of the glacial acetic acid method (141) and the old Dubrunfant procedure. Later the same year Harding presented also the history of inulin and described a method which he found reliable for preparing 10 to 15% of good inulin from the dried commercial chicory root. He suggested this as the most easily accessible source for ordinary purposes, and stated that the method could be applied to 100 pounds of the dried root at a time. The method was described as follows:

145. Micksch, Z. ges. Kohlensaure Ind., 28:263(1922)
146. Daniel, U. S. Patent 1,399,544(1922): Chem. Abstr., 16:850(1922)
147. Harding, Sugar, 25:406(1923): ibid., 636(1923). A portion of the present summary was taken from these two historical sketches.

One kilo of finely ground chicory root is mixed with 5 liters of water, and this mixture boiled for one hour and filtered. Repeated trials demonstrated that the hydrolysis during this boiling is not sufficient appreciably to effect the ultimate yield so no carbonate is added. The chicory residue is thoroughly washed with hot water, and the juice finally expressed. The filtrate, after cooling to at least 40° c., is treated with basic lead acetate solution to complete precipitation with the slightest possible excess, and the precipitate is separated by filter pressing. The addition of a little Norite, and sometimes a few cubic centimeters of basic lead acetate solution just before passing hydrogen sulfide, practically always prevents the formation of the troublesome colloidal precipitate. A little CaCO<sub>3</sub> may be added and sufficient Norite to decolorize as much as possible. All the color will not usually disappear. The filtrate is concentrated to a volume of about 500 cc. and 2 volumes of 80% alcohol added. If placed in the cold over night 10 to 15% of inulin settles out before morning. The ash may be lowered either by dialysis or by washing by decantation. Reprecipitation as follows also tends to reduce the ash content.

One hundred and fifty grams of crude inulin are dissolved in one liter of hot 40% alcohol. When solution is complete, Norite is added, and the solution should filter clear and colorless. Two liters of 80% alcohol containing a little HNO<sub>3</sub>, (about 1% by volume) are added, and the solution is set in the ice box over night. The inulin settles out, is filtered off and dried

in vacuo at 95°F., and the yield on reprecipitation is about 70%.

Jackson, Silsbee, and Proffitt (148) (1924) announced that white crystalline fructose had been prepared from the Jerusalem artichoke (and could possibly be made similarly from the dahlia and chicory) by extracting the juice, hydrolyzing with dilute  $H_2SO_4$ , defecating with lime, filtering, precipitating the Ca-fructosate, carbonating, filtering, and evaporating the sirup in vacuo to 91% solids, crystallizing in motion, centrifuging and drying. The first massecuite gave 51% by weight as crystals. A second crop of white fructose was obtained by dropping the purity further to 69. The artichoke juice contained 10.7 - 12.4% fructose mostly in polymerized form and 80 - 85% of this was obtained as crystalline fructose.

In 1925, Bates (149) wrote concerning some research work on dextrose and levulose, giving the progress of the investigations of the Bureau of Standards.

In 1926, Lippmann (150) reported an interesting incident of only theoretical importance. He found after a long autumn hot spell suddenly followed by an unexpectedly sharp frost, that a large number of large, half-ripe tomatoes still hanging on the plants showed peculiar excrescences consisting of a

- 148. Jackson, Silsbee, and Proffitt, J. Ind. Eng. Chem., 16:1250(1924)
- 149. Bates, Facts About Sugar, 21:250(1925)
- 150. Lippmann, Ber. 59B:348(1926)

mucilaginous nucleus permeated with a multitude of pointed needles, which he found to be pure crystalline fructose.

The method of Daniel (151) was tried on a factory scale in 1926 by Hoche (152). Chicory was the raw material used. The roots were cut in a beet slicer and extracted in a diffusion battery at 75 - 80°C. The raw juice contained 12 - 14% dry substance. Inulin was first crystallized out after liming and treating with sulfur dioxide. Perfectly white inulin in yields of 2% of the original chicory were obtained at one factory while 6 - 8% were obtained at another factory. The difference in yields was credited to the second factory being better equipped. The inulin was mixed to a paste with 50% water, 0.1% HCl, and heated at 90 - 97°C. for 1 1/4 to 1 1/2 hours. Hydrolysis was considered complete when addition of an equal volume of alcohol gave no turbidity. The solution was neutralized by NaOH to only slight acidity, treated with "Eponite" and filtered. A golden yellow sirup at 80° Brix containing 0.26% of ash was obtained. Solutions of 84-88° Brix crystallized on seeding and holding at 40°C.

Jackson, Silsbee, and Proffitt (153) reported also in 1926, their detailed method for the preparation of levulose from the Jerusalem artichoke, and the dahlia. The levulose

151. Daniel, loc. cit., (Reference 9)
152. Hoche, Z. Ver. d. Zuck. Ind., 76:821(1926),  
Sugar, 29:181(1927)
153. Jackson, Silsbee, and Proffitt, loc. cit.,  
(Reference 8).



was separated from the converted artichoke juices by the old Dubrunfaut lime precipitation method, revised to produce larger and more easily filterable crystals. The inulin was obtainable directly from the dahlia juices by freezing, and converted to levulose sirups of 86 - 90% purity. A study of the solubility of levulose in water indicated the feasibility of crystallization from aqueous solutions. They reported that pure white levulose is capable of forming aqueous masseccites which can be purged centrifugally with a facility approaching that of sucrose. Their method followed very closely the procedure suggested by Willaman (154) in 1922.

Schering (155) secured a patent on the preservation of the inulin content of sliced chicory roots or carbohydrates of other plant materials, by treatment with chloroform vapor or other narcotic gases such as ethylene bromide, toluene, acetic ester, CO<sub>2</sub>, CO, water gas, or HCN. Also a patent on the treatment of an aqueous pulp of inulin with a volatile organic acid such as formic, acetic, or CO<sub>2</sub>, decolorizing the sirup with charcoal and concentrating to obtain the fructose direct. When CO<sub>2</sub> is used the process is carried out in autoclaves.

Arsen (156) secured a number of patents in 1927. These covered the purification of inulin by Mg(OH)<sub>2</sub>, the purification

- 154. Willaman, loc. cit., (Reference 141).
- 155. Schering, Brit. Patents 266,695, Feb. 26(1926), 272,876(1926), French patent 634,363(1927).
- 156. Arsen, U. S. Patents 1,616,164: 1,616,167: 1,616,169: 1,616,165: 1,616,172: 1,616,171: 1,616,170:1,616,166 (1927). Chem. Abstr., 21:1026, 1027(1927)

by  $\text{Ca}(\text{OH})_2$ ,  $\text{CaCl}_2$ , boneblack, and finally  $\text{Na}_2\text{CO}_3$ ; the hydrolysis of inulin by tartaric acid or other acids until rotation passes through maximum, decreases, and passes through a second maximum even greater than the first; obtaining fructose from inulin by  $\text{HCl}$  at pH to 0.01N at 100, concentration of fructose sirup from purified inulin by use of less than 70%  $\text{H}_2\text{O}$  and an organic acid such as tartaric at pH of 0.05 or 0.015N; specifies the use of  $\text{HCl}$  at a concentration of 0.04N for 5 minutes at 100° C; clarification by adding a basic substance such as  $\text{NaOH}$ ,  $\text{KOH}$ ,  $\text{NH}_4\text{OH}$  to an acid aqueous extract containing inulin until the acid extract contains 0.00001 g. equivalent of acid per liter, impurities then precipitated when extract is heated and solution can be used to prepare pure fructose.

In 1928, Waterman, Rooseboom, and Oberg, (157) reported that Calcium fructosate, which is easily prepared by adding  $\text{Ca}(\text{OH})_2$  to a 5% solution of fructose and allowing to crystallize, is partially decomposed on drying. They stated that if fructose is to be prepared the fructosate must be worked up while wet, and suggested that fructose could be prepared from invert sugar and beet molasses.

The same year Arsem (158) secured two additional patents, the first covering the clarification of inulin bearing juice

- 157. Waterman, Rooseboom, and Oberg, Chem. Weelsblad, 25:50(1928)
- 158. Arsem, U. S. Patents, 1,663,233: 1,663,234(1928)  
Chem. Abstr., 22:1700(1928)

to form a mixture of inulin and other carbohydrates in solution. These are then hydrolyzed to convert into fructose and an enzyme such as pepsin is added to remove protein impurities. The second patent specifies hydrolyzing the polysaccharides contained in the residue after inulin has been recovered from the juice of plants such as the dahlia or the Jerusalem artichoke and separating the fructose formed.

Haworth and Learner (159) (1928) worked on the structure of inulin.

Vogel and Pictet (160) (1928) recorded some observations on the depolymerization of inulin.

Schluback and Horst (161) (1928) described the synthesis of the "basic substance" of inulin.

Fringsheim, Reilly, and Donovan (162) (1929) reported further concerning the structure of inulin and Reilly and Donovan (163) stated that their experimental results pointed to disfructose anhydride as being the structural unit of inulin.

159. Haworth and Learner, J. Chem. Soc. I, 619(1928)
160. Vogel and Pictet, Helvetica Chim. Acta, 11:215(1928)
161. Schluback and Horst, Ber., 61B:2358(1928)
162. Fringsheim, Reilly, and Donovan, Ber., 62B:2378(1929)
163. Reilly and Donovan, Sci. Proc. Roy. Dublin Soc., 19:409(1930)

EXPERIMENTAL

A: Analysis of Jerusalem Artichoke Tubers. (164)

The sugars were determined by Ost's cupro-carbonate method as modified by Nyns (165) and reviewed by Jackson (166), Oliver (167), and Traub, Thor, Willaman, and Oliver (168).

Due to the unavailability of Nyns' (165) article, his table with additions by Oliver is repeated here. (Table II) (167).

The analytical results are summarized in Table III.

164. The author is grateful to Dr. E. S. Haber of the Horticulture Department, Iowa State College, for furnishing the Jerusalem artichokes and chicory used throughout these experiments.
165. Nyns, Bull. assoc. ecole sup. brasserie Louvain, 25:63(1925): (Chem. Abstr., 19:1236(1925).)
166. Jackson, J. Assoc. official Agr. Chem., 9:178(1926)
167. Oliver, "The Development of Methods for the Analysis of Inulin Bearing Plants". (1927). (Unpublished Master's Thesis. Copy on file Univ. of Minn. Libr.)
168. Traub, Thor, Willaman, and Oliver, Plant Physiol., 4:123(1929).

Table II

Copper Values for Glucose and Fructose in the Cupro-  
Carbonate Method

Column I is for use when reduction is carried out on a sand bath, II in boiling water, III in water bath at 48.5°-49.0°C.

Sugar Dextrose and Levulose	Fructose			Glucose
	I	II	III	II
mg.	mg.	mg.	mg.	mg.
5	14.5	14.5	11.5	12.72
6	17.5	17.5	15.0	16.13
7	20.5	20.5	18.0	19.56
8	23.5	23.5	21.5	22.98
9	26.5	26.5	25.0	26.41
10	29.5	29.5	28.5	29.82
11	33.5	33.5	32.0	33.26
12	37.5	37.0	35.0	36.69
13	41.5	40.5	38.0	40.4
14	45.0	44.5	41.0	43.51
15	48.5	48.0	44.0	46.67
16	52.5	52.0	47.5	49.79
17	56.0	53.5	51.0	52.50
18	59.0	59.0	54.0	55.79
19	62.5	63.0	57.5	59.00
20	66.0	67.0	61.0	62.10
21	69.5	71.0	64.0	65.30
22	73.0	75.0	67.0	68.41
23	76.0	78.5	70.0	71.21
24	79.0	81.5	73.0	74.97
25	82.0	84.5	76.5	78.21
26	85.0	87.5	80.0	81.70
27	88.0	90.5	84.0	85.00
28	91.0	93.0	87.5	88.30
29	94.0	96.0	91.0	91.60
30	97.0	99.0	95.0	94.90
31	100.0	102.0	99.0	97.8
32	103.0	105.0	104.0	100.7
33	105.5	108.0	109.0	103.6
34	108.0	111.0	114.5	106.6
35	111.0	114.0	120.0	109.7
36	114.0	117.0	123.0	113.0
37	116.5	120.0	127.0	116.0
38	119.0	123.0	130.5	119.3

39	122.0	126.5	134.0	122.3
40	125.0	129.5	137.0	125.7
41	128.0	132.0	140.0	128.8
42	131.0	134.5	142.5	131.9
43	134.0	137.0	145.0	135.0
44	136.5	140.0	147.5	137.8
45	139.0	142.5	150.0	140.5
46	141.0	145.0	153.0	143.9
47	143.0	148.0	156.0	147.1
48	145.0	150.5	159.0	150.2
49	147.0	153.0	162.0	153.2
50	149.0	155.5	164.5	156.5
51	152.0	158.0	167.0	159.8
52	155.0	160.5	169.5	163.0
53	158.0	163.0	172.0	165.5
54	161.0	165.5	175.0	167.9
55	164.0	168.0	178.0	170.5
56	166.0	170.5		173.1
57	168.0	173.0		176.1
58	170.0	176.0		179.0
59	172.0	178.5		181.9
60	174.0	181.0		184.9
61	176.5			187.7
62	179.0			190.7
63	181.5			193.7
64	184.0			196.5
65	186.0			199.8
66				202.2
67				204.8
68				207.2
69				210.2
70				213.2
71				215.8
72				218.2
73				221.1
74				223.8
75				226.5
76				229.3
77				232.1
78				234.9
79				237.3
80				239.6

Table III

## Analysis of Artichokes Grown at Iowa State College, 1929

Variety	Date Dug	Date Analyzed	Moisture	Dry Matter	Fructose	(169) Glucose	Total Sugar	Fructose Glucose	Fructose T. Sugar
Mammoth French White (Vaughn's Seedling)	10-15	10-19	77.45	22.55	11.94	2.85	14.79	4.19	0.807
Wild (Native to Iowa)	10-23	10-23	69.00	31.00	15.02	2.02	17.04	7.44	0.881
Purple (Minn. Seedling) Hill 9	10-20	10-24	74.31	25.69	16.64	2.89	19.53	5.75	0.851
Purple (Minn. Seedling) Hill 6	10-20	10-26	75.58	24.42	15.61	1.39	17.00	11.23	0.918
U. S. D. A. (Minn. Seedling) Hill 11	10-20	10-25	78.53	21.47	14.27	4.14	18.41	3.44	0.775
U. S. D. A. (Minn. Seedling) Hill 14	10-20	10-25	76.83	23.17	14.31	4.02	18.33	3.56	0.780
Mammoth French White (170) (Vaughn's Seed.)	10-20	7-31	76.21	23.79	10.14	4.32	14.46	2.35	0.701

169. Glucose refers to all sugar present except levulose.

170. From Vaughn's Seed Store, Chicago. Sample stored in stoppered bottle at 80°C.

B. Analysis of Jerusalem Artichoke Tops

Since the artichoke produces abundant tops (approximately 15 tons (171) per acre, green weight) uses will have to be found for this portion of the plant. With this in view the chemical composition is of interest.

Traub, Thor, Zeleny, and Willaman (172) have reported their analysis of the tops, along with a comparison of other worker's results. They confined their examination to the determination of moisture, ash, sugars, starch, pentosans, protein, and crude lipides (chloroform extract).

The present report will include only moisture, ash, lignin, and cellulose. The samples were prepared by grinding the air dried stocks in a wiley mill through a 200 mesh sieve.

The lignin was determined by the method given by Schorger (173) and as applied to the corn stock by Peterson and Hixon. (174) The cellulose was determined by the chlorination method of Cross and Bevan. (175)

The following average values were found: Moisture - 10.15%  
Ash - 3.61%, lignin - 14.35%. The cellulose was determined on the oven dried sample (100°C), and the value found to be 27.09%.  
The values for ash and lignin expressed as percentage of dry

171. Schoth, Agr. Exp. Sta., Oregon State Agr. College  
Circular No. 89, 9(1929)

172. Traub, Thor, Zeleny, Willaman, Jour. Agr. Research  
39:551(1929)

173. Schorger, "Chemistry of Cellulose and Wood", McGraw-  
Hill Book Co., New York, p.524 (1926)



weight are 4.02% and 15.97% respectively.

C. The Preparation of Inulin

1. From chicory

a. Two hundred grams of dried chicory chips (moisture 5.2%) were treated with one liter of water, 10 g. calcium carbonate added, and the mixture boiled for one hour. The juices were expressed in a hand press and the pulp treated with 700 cc. of water, boiled for 30 minutes, the juices expressed and added to the first extract. The combined extract was boiled with Norite (20 g.) for 15 minutes, 30 g. of filter cell added and the solution filtered on a Buchner funnel. The juices were quite dark in color, having a refractive index of 1.3472 at 28°C. (10.5% solids). This solution was seeded with 0.05 g. inulin and kept in an electric refrigerator at 8°C for five days. No crystallization occurred.

The solution was then treated with 10 g. of sodium carbonate, 20 g. of Norite added and kept at 80° - 90° C. for 30 minutes. Twenty g. filter cell was added and the solution filtered on a Buchner funnel. (The filtration was accomplished very quickly with the filter cell present, but a previous trial without filter cell was unsuccessful.) The filtrate was neutralized to slight alkalinity with HCl and concentrated in vacuo (35 mm.

174. Peterson and Hixon, J. Ind. Eng. Chem., Analytical Edition, 1:65(1929)

175. Cross and Bevan, Cellulose, Longmans, Green and Co., N.Y. London p. 94(1916)

pressure) on a water bath at 60°C. to a volume of 700 cc. This solution was quite dark in color, with a refractive index of 1.3751 at 28°C. (27.3% solids)

A 5 cc. sample of the inulin containing solution was treated with a few drops of a saturated solution of ammonium sulfate. No immediate precipitation of inulin occurred. After standing for 8 hours at 26°C. a slight precipitate was observed.

A second 5 cc. portion of the solution was treated with a few drops of a cold water extract of the peelings from Jerusalem artichoke tubers. No coagulation of inulin was observed even after standing at 26°C. for 8 hours.

The remainder of the solution was placed in an electric refrigerator at 8°C. and allowed to remain for 3 days. Considerable inulin had crystallized and was separated and washed with cold water(10°C) in a centrifuge. The yield was 22.4 g. or 11.2%. The specific rotation of the inulin was found to be -32.5° at 20°C:

## 2. From the Jerusalem Artichoke

a. The sample used was the wild variety, native to Iowa. 600 g. of the washed tubers were ground through a food chopper, treated with one liter of water, 20 g. of calcium carbonate added, and the mixture heated on a water bath (100°C) for one hour. The juices were expressed and the pulp extracted with another one liter portion of water and heated on the water bath for two hours. The combined extracts were treated with ten grams of sodium carbonate, heated to boiling, and filtered

through a small hand filter press. The filtrate was quite dark in color. It was neutralized to slight alkalinity with HCl and concentrated in vacuo on a water bath at 60° C. to a thin sirup. On standing for two days this sirup deposited 8 g. of inulin which was filtered and washed with difficulty on a Buchner funnel; this amounted to a 1.3% yield. The product, however, was dark in color and a small sample yielded considerable ash on ignition. A second crystallization from water gave 4.5 g. (or 0.75%) of white inulin with a relatively low ash content.

b. The juices were expressed from 50 g. of fresh Jerusalem artichoke tubers by means of a hand press, and heated to boiling. The coagulated material was filtered off and the cooled filtrate, which was quite clear, but red in color, was treated with ten drops of the cold water extract from the Jerusalem artichoke peelings. No coagulation of inulin was observed even after allowing the solution to stand for 24 hours.

D. The Preparation of Levulose Sirup from Fresh Jerusalem Artichoke Tubers by direct Methods.

1. One kilo of freshly dug tubers (Vaughn's seedlings Oct. 10, 1929) were chopped in a chopping bowl, treated with 500 cc. of water containing 2.3 cc. of concentrated H<sub>2</sub>SO<sub>4</sub> (to make solution approximately 0.1 N). This mixture was kept at 75° - 80° C. on a water bath for four hours. The juices were expressed in a hand press, the pulp was extracted with a second portion (500 cc.) of boiling water and the extracts combined.

The total volume was then 1500 cc. (pH = 3.5 - 4.0). A sample of this solution was clarified with normal lead acetate, deleaded with disodium phosphate and polarized in the saccharimeter (200 mm. tube). The observed reading was (-14). The solution was treated with 1 cc. of concentrated  $H_2SO_4$ , and allowed to stand until the following day. (pH = 3.2). A clarified sample was placed in the saccharimeter and the observed reading was (-15.5). Another 1 cc. portion of concentrated  $H_2SO_4$  was added and the solution heated to  $80^{\circ}C$  for 1/2 hour. (pH = 2.8, rotation -24). The rotation remained constant at (-24) after the heating had been continued for 1/2 hour longer.

The pH was adjusted to 5.5 - 6.0 with slaked lime, 20 g. of Norite was added, the solution heated to boiling, and filtered quickly. The filtrate, green in color, was concentrated to 500 cc. in vacuo, on a water bath at  $60^{\circ}C$ ., 10 g. of Norite was added and the solution heated to boiling. This treatment failed to clarify the solution. Slacked lime was added until the pH was 8 and the solution was kept at  $80^{\circ}C$ . for 10 minutes. After filtration the solution was still dark green in color. The pH was adjusted to 5.5 and the concentration in vacuo continued. On being heated the solution suddenly turned a pale yellow color, and became reddish-brown as it became more concentrated. The thick sirup obtained had a bitter saline taste and the odor of strong molasses.

2. One kilo of tubers (same sample as in Sec. 1) were

ground through a food chopper and treated with 500 cc. of water containing 5 cc. of concentrated  $H_2SO_4$ . The solution was kept at  $75^\circ - 80^\circ C.$  for two hours and the juices expressed with a hand press. (pH = 2 - 2.5, rotation -247). Slaked lime was added to adjust the pH to 5.5, 20 g. of Norite added and the solution kept at  $80^\circ - 90^\circ C.$  for two hours. After filtration (in filter press) the solution was light green in color. It was concentrated as in Sec. 1, to a volume of 500 cc., becoming dark green in color. Twenty g. of Norite was added, the solution kept at  $80^\circ - 90^\circ C.$  for 30 minutes and filtered in a filter press. Apparently no color was removed. The concentration in vacuo was continued and a very thick dark green sirup obtained with a taste similar to that from Sec. 1, and an even stronger molasses odor.

3. One kilo of tubers (sample same as in Sec. 1) were ground through a food chopper, heated with one liter of water for one hour (in boiling water bath) and juices expressed. The pulp was extracted with another portion of boiling water (one liter) and the expressed juices combined with the first extract. Five cc. of concentrated  $H_2SO_4$  was added and the solution kept at  $80^\circ C.$  for two hours. Considerable coagulation of material was observed and the solution left light yellow in color. (pH = 2.0 - 2.5, rotation -7 in 10 cm. tube). The heating was continued at  $80^\circ C.$  for four hours and the rotation remained constant. The solution was made alkaline (pH = 8)

with lime and heated to 70°C., 20 g. of Norite was added, the solution filtered in filter press, and pH adjusted to about 6 immediately with H<sub>3</sub>PO<sub>4</sub>. (The total time the solution was alkaline was 30 minutes.) The remaining treatment was identical with that in 2. starting with the concentration. The resulting sirup had a very satisfactory color (light brown) but about the same taste as those of 1. and 2. and not quite so strong an odor of molasses.

E. The Conversion of Inulin with CO<sub>2</sub>.

1. Five grams of pure inulin (Merck) was dissolved in 125 cc. of hot distilled water and the solution divided into two equal parts. The first portion had a rotation (saccharimeter, 200 mm. tube) of -7 at 23°C. One hour later the rotation was found to be unchanged.

The second portion was placed in a pop bottle and treated with CO<sub>2</sub> under a pressure of 100 lbs. with shaking (on a mechanical shaker) for 15 minutes. (Temperature 23°C.) A test showed that the rotation had remained constant at -7. The shaking was continued for another 45 minutes with CO<sub>2</sub> under a pressure of 100 lbs. Again the rotation was found to have remained constant (-7 at 23°C.)

2. Five grams of pure inulin (Merck) was dissolved in 250 cc. of warm distilled water and divided into two equal parts. The first part had a rotation (saccharimeter, 200 mm. tube) of -3 at 35.5°C.; 30 minutes later the rotation was found to be unchanged.

The second part was placed in a pop bottle and heated to a temperature of 85° - 90° C. by means of steam passing through a rubber tubing coiled around the brass shield containing the pop bottle on the mechanical shaker. CO<sub>2</sub> was administered under a pressure of 100 lbs. and the heating and shaking continued for 30 minutes. A test showed the rotation to be the same as the untreated portion (-3 at 33.5° C.)

3. Five grams of pure inulin (Merck) was dissolved in 300 cc. of warm distilled water. The solution had a rotation (saccharimeter, 200 mm. tube) of -2.3 at 25°C. The solution was placed in a pop bottle with CO<sub>2</sub> under a pressure of 150 - 200 lbs. with shaking at 25° C. for 30 minutes. The rotation was found to be -2.4 at 25° C.

4. Five grams of pure inulin (Merck) was dissolved in 125 cc. of warm distilled water and the solution divided into two equal parts. The first part had a rotation (saccharimeter, 200 mm. tube) of -7.2 at 24° C. The solution was kept at a temperature of 85° - 90°C. for 1/2 hour. Distilled water was added from time to time to keep the volume constant. The rotation was found to be -7.4 at 24° C.

The second part was treated as in 2. above except the CO<sub>2</sub> pressure which was 200 lbs. and the time of heating and shaking 1/2 hour. The rotation was found to be -7.39 at 24° C.

F. The Preparation of Levulose Sirup from Fresh Jerusalem Artichoke Tubers by the Lime Precipitation Method.

1. On a small scale

a. Two kilos of tubers (Mammoth French White Variety) were ground through a food chopper and treated with 2 1/2 liters of water and 60 g. of  $\text{CaCO}_3$ . The mixture was heated to boiling and boiled for twenty minutes. The juices were expressed and the pulp treated with 2 liters of water and the mixture kept at boiling temperature for twenty minutes. The expressed juices were added to the first extract, and 46 cc. of concentrated HCl was added, making the solution approximately 0.1 normal. The conversion was accomplished on a water bath at  $75^\circ - 80^\circ\text{C}$ . The temperature was maintained until two successive readings showed the rotation (saccharimeter) to be constant. Considerable material which had coagulated during the conversion was filtered off in a filter press and the filtrate allowed to stand over night. The following day slaked lime was added (pH of 8) and the solution heated at  $80^\circ\text{C}$ . for 15 minutes. Norite was added to decolorize and the solution filtered in a filter press. (Very high pressure was required). The filtrate was a light straw color.

The lime levulate was precipitated in a four l. beaker packed in ice, equipped with a stirrer. The Bureau of Standards (176) procedure was followed for the precipitation. Lime (100g) was slaked, diluted to 1,050 cc. and added in 15 portions of 70 cc. each. (One portion of lime was thus equal to approximately 266 cc. of sugar solution.) Five hundred cc.



of water and one portion of lime were placed in the beaker and cooled to a temperature of 1° to 2° C., 266 cc. of the clarified levulose containing solution was added dropwise, with stirring, another portion of lime added, etc., until the precipitation was complete.

The lime levulate was filtered on two six inch Buchner funnels. Two cakes which were about one inch thick after being pressed down were obtained. The cakes were washed with iced lime water until the washings gave no trace of color. They were pure white after the washing.

The lime levulate was suspended in one liter of iced water and carbonated in an Erlenmeyer flask with slight pressure and shaking. The precipitated  $\text{CaCO}_3$  was filtered on a Buchner funnel, washed, and the filtrate which was colorless was concentrated in vacuo. As the concentration progressed salts precipitated and the solution was found to be alkaline to litmus and yellow in color. The pH was adjusted to 6 with phosphoric acid, the solution filtered and the concentration continued. The resulting thick sirup (100 cc. about 85% solids by refractometer) was light golden brown in color and possessed a very sweet honey-like taste. After seeding and allowing to stand for one week about 3 g. of levulose crystals were obtained. The salts from the water used (and also perhaps from the  $\text{H}_3\text{PO}_4$  acid and  $\text{CaO}$  present) seemed to hinder crystallization markedly.

2. On a large scale

a. Twelve kilos of washed tubers were ground through

a food chopper, placed in a steam jacketed kettle with 16 l. of water and cooked at boiling temperature (100°C.) for 1 1/2 hours. The juices were pressed out in a hand press. (Volume of extract was 18 l.) The pulp was treated with 12 l. of water and the mixture kept at 100°C. for 1/2 hour. The expressed juices were added to the first extract making the total volume 28 liters, 465 cc. of concentrated HCl was added (making the total acidity 0.1923 normal) and the solution allowed to stand for nine hours.

A clarified sample showed a rotation of -4.5 at 24°C. (Saccharimeter, 200 mm. tube) The solution was therefore heated at 80°C. for 3/4 hour. (Rotation -6.4 at 24° C.) After heating again at 80° C. for 1/2 hour the rotation was found to be constant (-6.4) and the solution was filtered through a small hand filter press. The solution was neutralized by lime to a pH of 7 - 8, kept at a temperature of 80° C. for 20 minutes, and again filtered through the filter press. (The press had to be cleaned six times during the filtration of the 28 l. and the time required was 9 hours.) The filtrate was dark in color but clear.

The precipitation of the lime levulate was carried out as on the small scale (Sec. F 1.), using a ten gallon crock packed in ice to receive the juices. The lime (850 g.) was slaked and placed in 6 l. of water. The lime was added in 56 (107cc.)

176. Jackson, Silsbee, and Proffitt, loc. cit., (Reference 8)

portions and the juices in 56 (500 cc.) portions. It was impossible to keep the temperature down to 0°C. The maximum temperature at any time was 15°C. The time consumed by the precipitation was 6 hours.

The lime levulate was filtered on a 15 inch stone suction filter, (requiring 12 hours for the filtration). The cake was washed with iced lime water until the washings were colorless. The cake (pale yellow in color) was suspended in iced water and oxalic acid added until the solution was faintly acid to litmus. The precipitated calcium oxalate was filtered out and the slight excess of oxalic acid precipitated from the filtrate with lime. After filtering again the solution was adjusted to a pH of 6 -6.5 with  $H_3PO_4$  and evaporated in vacuo (on water bath at 60°C.) to a volume of 250 cc. The resulting sirup contained 90% solids (refractometer) but was dark in color and appeared to be caramelized. It failed to crystallize after standing (seeded) for two weeks.

b. Thirty pounds of tubers were treated as in Sec. a. above, cooking for the first time with 18 l. of water for one hour, and the second time with 14.1 l. of water for 1/2 hour. The combined extracts, amounting to 32 l., were treated with 350 cc. of concentrated HCl and kept at 80°C. for one hour. After cooling to room temperature the solution was neutralized to a pH of 8 with lime, 500 g. of filter cell was added and the solution carbonated to neutrality. Filtration was accomplished easily and quickly on the 15 inch stone suction filter.

The filtrate was dark in color but clear.

The lime levulate was precipitated in a 20 gallon galvanized can as in Sec. a. above. The juices were cooled to 5°C. by passing through a glass coil immersed in ice water, before being added to the reaction mixture. The lime (2 lbs. in 6 l. H<sub>2</sub>O) also was cooled to 5°C. before addition. In this way it was found possible to keep the temperature from 4° to 6°C. throughout the precipitation.

It again required about 12 hours to filter out the lime levulate on the stone suction filter. The mixture was added to the filter in small quantities, keeping the excess cooled to 0°C.

The lime cake, which was pale yellow after washing with iced lime water, was suspended in iced water and carbonated (in portions) under slight pressure in a 4 liter Erlenmeyer flask. The CaCO<sub>3</sub> was filtered out and the filtrate acidified to a pH of 6 with phosphoric acid.

The sirup obtained after concentration in vacuo, had a high ash content. It was divided into two portions. The first portion was concentrated to 85% solids, alcohol added to precipitate the salts and the filtrate concentrated to 90 - 92% solids. After five days crystallization took place, yielding 10 grams of white levulose. The sirup after being again concentrated yielded 5 grams of levulose, slightly yellow in color. Four hundred cc. of sirup (82% solids) remained.

The second portion was treated with alcohol before concen-

tration, until no more precipitation occurred. The precipitate was coagulated by stirring and the liquid decanted. The precipitate, tar like in consistency, proved to be an organic salt (water soluble) containing calcium. The liquid after concentration in vacuo yielded 600 cc. of sirup, 93 to 94% solids. This sirup failed to crystallize after standing (seeded) for two months.

G. The Desiccation of Jerusalem Artichoke Tubers.

1. Methods of drying used.

For the drying of small samples a simple dryer was arranged by replacing the door of an ordinary electric drying oven with another door, having an opening at the bottom in which a 6 inch electric fan could be placed, and a slit at the top to allow for circulation. Three electric hot plates were placed in the bottom of the oven to be used when higher temperatures were desired than could be obtained with the heating units already in the oven. Screen wire trays were used.

The tubers were prepared for drying by slicing on a hand vegetable slicer (thickness of slices approximately 1/16 inch). A 500 g. sample (Mammoth French white, Vaughn's seedling) was dried at each of a series of temperatures. The samples are listed here, (Table IV) with temperatures and periods of heating, and will be referred to by number in the analytical work which follows.

Table IV

Sample	Temperature (C <sup>o</sup> )	Time (hrs.)
1	55 - 60 <sup>o</sup>	4.0
2	65 - 68 <sup>o</sup>	3.0
3	70 - 75 <sup>o</sup>	2.5
4	75 - 80 <sup>o</sup>	2.25
5	84 - 85 <sup>o</sup>	1.5
6	90 - 95 <sup>o</sup>	1.25
7	100 <sup>o</sup>	1.0

For the drying of larger quantities of tubers a Proctor and Schwartz dryer was used. The dryer contained twelve trays (3 ft. by 1 1/2 ft.). One tray was suspended from a balance in order that the moisture removed per time interval could be determined. The incoming air was heated by passing over steam coils. The results of a preliminary test to determine the time required for the drying are recorded in Table V. Only the one tray which could be weighed was filled. The tubers were sliced (1/16 inch thick) in a mechanical slicer and spread out quite thinly over the tray.

Table V

Time (Minutes)	Weight Tubers (grams)	Temp. in Dryer (C°)	Temp. Outlet (C°)
0	760	106.5	100
5	710	109	100
10	545	109.5	100
15	505	110	100.5
20	410	111	101
25	350	111.5	102.5
30	290	112	103
35	270	113	103
40	250	113	103.5
45	230	113	104
50	210	113	104
55	210	113.5	104.5
60	210	113.5	104.5

The results of a typical run with a full charge (approximately 1000 g. on each tray) in the dryer are given in Table VI. The temperature was kept below 88°C. since considerable caramelization was observed to occur at the higher temperatures.

Table VI

Time (Minutes)	Weight Tubers (grams)	Temp. in Dryer (C°)	Temp. at Outlet (C°)
0	1000	60	58
10	870	78	72
20	780	84	78
30	650	88	85
40	550	85.5	83
50	470	86.5	83
60	410	86.5	83
70	360	85	80.5
80	320	82.5	78
90	310	83.5	78.5
100	290	84.5	79
110	270	84.5	78.5
120	250	85.5	79.5
130	240	85	79.5
140	230	85	80
150	225	85.5	80
160	220	86	80
170	215	83	77

180	210	81	75
190	210	77.5	73
200	210	76.5	71

A dryer has been designed, (Figure II) and is in the process of construction, for the continuous drying of large amounts of artichokes.

A. is the mechanical slicer, which feeds the sliced tubers directly into the hopper B. J. is a slide to regulate the quantity of chips introduced into the dryer. The framework is made of angle iron, AI, and the trough H is of galvanized iron. D is a six inch metal screw conveyor, 10 ft. in length.

The end view shows the trough curved at the bottom to fit the screw and a screen wire G upon which the chips rest and through which the hot air can be passed.

F is the exit for the dried chips and E a slide to control the amount of chips removed. The screw conveyor D can be turned at a speed of one revolution every three minutes, or faster, by means of the ratchet drive and gear arrangement shown. At this speed the chips will pass through the dryer in one hour; thus approximately two bushels of artichokes may be dried per hour.

For experimental purposes the dryer is divided into three compartments (by slides C and C') in order that three different temperatures may be used. The temperatures employed will have to be determined using this particular type of dryer. However, experiments on a smaller scale seem to indicate that the chips are not harmed if submitted to a temperature as high as 125° C.



Dryer For Artichokes

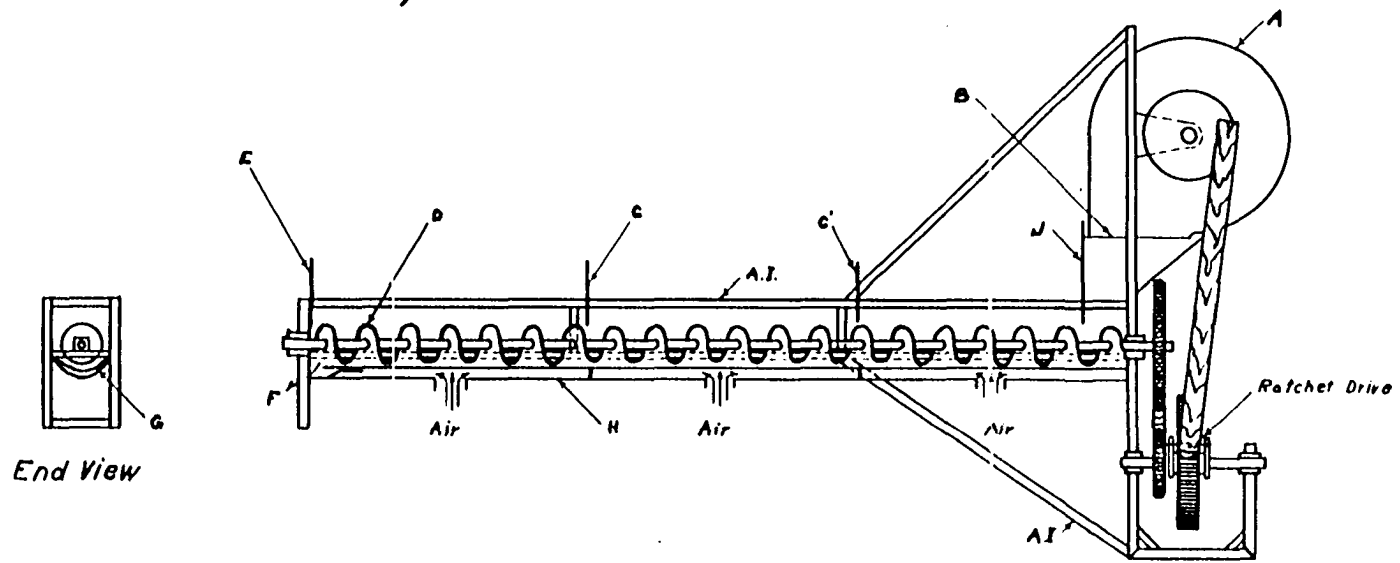


Fig. 2.

during the first stages of the drying, but after most of the moisture has been removed, the temperature must not exceed 80° C. or caramelization will occur.

For larger installations three units like the one in Figure II should be employed, arranged one above the other so that the exit F of the top unit would feed into the hopper B' of the second unit, and the exit F' of the second unit into the hopper B'' of the third unit. The top unit would then be maintained at the higher temperature. Air could be introduced into the lower unit at the desired temperature (probably 80°C), the exit air from this unit re-heated to a higher temperature (probably 115° C.) and introduced into the middle unit, and the exit air from this unit likewise re-heated to a still higher temperature (probably 125° C.) and introduced into the top unit.

The unit (Figure II) will be inclined at an angle in order that gravity may assist in moving the chips along the trough toward the exit F.

The air will be heated by passing over steam coils and forced through the chips by means of a Buffalo pressure blower.

2. The effect of drying Jerusalem artichokes on the sugar content.

The washed tubers were ground through a food chopper, thoroughly mixed, 30 g. samples taken and either analyzed directly or dried and then analyzed. Table VII gives the data obtained. All analyses were by Ost's cupro-carbonate

method. The percentages given (which are average values) are based on the 30 g. wet sample taken in every case. The percentage moisture in the wet sample was 77.45%.

Table VII

Effect of drying Jerusalem Artichokes on the Sugar Content

No.	Sample	Percent Fructose	Percent Glucose	Percent Total Sugar	Ratio Fructose Tot. Sugar
1	Without drying	11.95	2.85	14.8	0.807
2	Dried at 100° C - 1 hr.	12.04	1.99	14.03	0.858
3	Dried at 90° C - 1 1/4 hr.	12.12	2.43	14.55	0.833
4	Dried at 80° C - 2 1/4 hr.	11.90	2.91	14.81	0.803
5	Dried at 70° C - 2 1/2 hr.	12.21	2.81	15.02	0.812
6	Dried at 125° - 1/4 hr. Then at 75° C.-1 hr.	11.88	2.98	14.86	0.799

3. The effect of drying Jerusalem artichokes on the coagulation of albuminous matter.

The moisture and the total nitrogen were determined in the fresh tubers and in tubers dried at 75° to 80° C. for two hours and also for six hours.

Samples of the fresh tubers and also the dried tubers were extracted with 100 cc. of hot water, using an extractor similar to the one described by Gardner and Kerone (177), and the total nitrogen determined in the extracted pulp. In some cases the coagulable nitrogen (as precipitated by the Stutzer-Barnstein (178) method) was determined in the extract and in other cases the

177. Gardner and Kerone, J. Chem. Education, 7:593(1930)  
 178. Barnstein, Landu. Vers. Stat., 54:327(1900), Chem. Zentr., II:1037(1900)

total nitrogen in the extract was determined.

All nitrogen determinations were made by the Kjeldahl method.

The results are summarized in Table VIII. The numbers in parenthesis are percent albumins (N x 6.25). All percentages are based on the original weight of the sample.

Table VIII

No.	Sample	Percent Moisture	Percent N wet basis	Percent N dry basis	Percent total N in extr. pulp	Percent total N in ex- tract
1	Fresh tubers	80.4	0.403 (2.52)	2.06 (12.88)		
2	Dried 75-80 for two hours	6.69	1.824 (11.4)	1.95 (12.19)		
3	Extracted pulp from fresh tubers		0.12 (0.75)	0.612 (3.83)	29.75	
4	Coagulable al- bumins in ex- tract from fresh tubers		0.138 (0.863)	0.704 (4.4)		34.16
5	Extracted pulp from sample dried at 75-80°C - 2 hrs.		0.639 (3.99)	0.684 (4.28)	35.11	
6	Coagulable al- bumins in extract from sample dried at 75-80°C. - 2 hrs.		0.651 (4.07)	0.697 (4.36)		35.76
7	Dried at 75-80°C for 6 hrs.	4.57	1.90 (11.88)	1.99 (12.44)		
8	Extracted pulp from sample dried at 75-80°C 6 hrs.		0.666 (4.16)	0.697 (4.36)	35.02	

Table VIII (con't.)

9	Coagulable al- bumins in extract from sample dried at 75-80°C. for 6 hours	0.597 (3.75)	0.625 (3.91)	31.40
10	Total extract from a sample dried at 75- 80°C for 6 hrs.	1.184 (7.40)	1.240 (7.75)	62.31
11	Total extract from fresh tubers	0.285 (1.781)	1.443 (9.019)	70.04

4. The effect of drying Jerusalem artichokes on the ease of diffusion.

a. Twenty gram samples of sliced fresh tubers (77% H<sub>2</sub>O) were placed in a series of eight 150 cc. beakers. Fifty cc. of hot (80-85°C.) distilled water was added to the first sample, the beaker placed in a water bath (85-90°C.) and heated for 10 minutes. The extract from this beaker was then poured into the beaker containing the second sample, 50 cc. of fresh water added and both samples were heated for ten minutes, etc., thus obtaining the effect of a diffusion battery on a small scale. Samples were taken from each beaker at the end of every 10 minute period and the refractive index observed as a measure of the total solids extracted. The observations are recorded in Table IX. The readings were all taken at 14°C. at which temperature the distilled water used had a refractive index of 1.3336.

Table IX

The Refractive Indices of the Extracts from the Diffusion of  
Fresh Artichoke Tubers

<u>Sample No.</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>
No. of times extracted								
1	1.3365	1.3380	1.3448	1.3499	1.3512	1.3540	1.3580	1.3588
2	1.3380	1.3410	1.3430	1.3459	1.3491	1.3518	1.3561	1.3570
3	1.3349	1.3389	1.3400	1.3420	1.3460	1.3495	1.3570	1.3575
4	1.3350	1.3366	1.3371	1.3410	1.3429	1.3439	1.3530	
5	1.3350	1.3349	1.3368	1.3374	1.3390	1.3461		
6	1.3341	1.3350	1.3360	1.3369	1.3405			
7	1.3340	1.3341	1.3350	1.3365				
8	1.3335	1.3338	1.3345					
9 (178)	-----	1.3332						
10	1.3331							

b. The same procedure as in Sec. a. above was applied to samples of dried artichoke tubers. The samples used were from No. 4 (see Table IV). The moisture content of these chips was found to be 6.69%. Therefore, 7.1 g. samples were taken (the dry weight thus being the same as in the 20 g. samples of a.). In order that the results might be comparable with those of Table IX, 15 cc. of water was added to each of the eight samples before the diffusion was started. (This was approximately the difference in moisture content of the fresh and dried samples.) Otherwise the procedure was the same except that the refractive index readings were taken at 28°C. ( $H_2O = 1.3320$ ). The results are recorded in Table X.

178. The extract from No. 8 was emptied and the pulp from No. 1. A new sample was added to the series as No. 8 and No. 2 became No. 1, etc.

Table X

The Refractive Indices of the Extracts from the Diffusion of Dried Artichoke Tubers

Sample No.	1	2	3	4	5	6	7	8
No. of times	:	:	:	:	:	:	:	:
Extracted	:	:	:	:	:	:	:	:
1	1.3412	1.3500	1.3580	1.3632	1.3661	1.3700	1.3730	1.3760
2	1.3372	1.3408	1.3470	1.3560	1.3642	1.3700	1.3756	
3	1.3330	1.3365	1.3420	1.3463	1.3540	1.3619		
4	1.3330	1.3341	1.3386	1.3420	1.3471			
5	1.3325	1.3332	1.3361	1.3390				
6	1.3320	1.3329	1.3380					
7	1.3320	1.3328						
8	1.3320							

c. An attempt was made to find the maximum possible density to which the juices could be built up by the diffusion of dried artichoke tubers. Seven gram samples (from No. 4, Table IV) were placed in a series of 150 cc. beakers as in Sec. a., 50 cc. of hot distilled water was added to sample No. 1, and the mixture heated on a water bath (85-90°C.) for 10 minutes. The refractive index of the extract was observed, the extract was poured into beaker containing sample No. 2, 50 cc. of fresh water added to beaker No. 1 and both samples heated for 10 minutes. The refractive index of the extract in beaker No. 2 was then observed. (Reading 2 a. below) The second extract from beaker No. 1 was then poured into beaker No. 2, 50 cc. of fresh water added to beaker No. 1, both beakers were placed in water bath for 10 minutes. The refractive index of the extract in beaker No. 2 was again observed. (Reading 2c. below) Then the extract from beaker No. 2 was poured into beaker No. 3 containing a fresh sample, the extract from No. 1 poured into No. 2,

and fresh water added to No. 1. This process was repeated until 17 samples had been added to the series. The refractive index was determined in each case only on the extract in the last beaker of the series, two readings being made: reading a. after the first portion from the preceding beaker had been added and reading b. after the second portion from the preceding beaker had been added.

The refractive indices (at 28°C.) observed were as follows:

1) - 1.3390: 2) - a. 1.3435, b. 1.3400: 3) - -----: 4) - -----:  
5) - a. 1.3570, b. -----: 6) - a. 1.3610, b. 1.3530:  
7) - a. 1.3760: b. 1.3620: 8) - a. 1.3750, b. 1.3640: 9) - a.  
1.3850, b. 1.3770: 10) - a. -----, b. 1.3970: 11) - a. 1.4020,  
b. 1.3910: 12) - a. 1.4030, b. 1.3930: 13) - a. 1.4070, b. -----:  
14) - a. 1.4130, b. 1.4080: 15) - a. 1.4210, b. -----: 16) - a.  
1.4040, b. -----: 17) - a. 1.4290.

The sample in beaker No. 1 was completely exhausted after the fourth extraction. The refractive index of the last extract (No. 17. a. 1.4290) corresponds to 55% total solids in the solution.

d. By using a series of 12 (150 cc.) beakers as a diffusion battery continuously for a period of time, and observing the refractive index of the effluent the following values were found (7 g. samples ( from No. 4, Table IV ) were used in each beaker): 1) - 1.3550: 2) - 1.3540: 3) - 1.3530: 4) - 1.3540:  
5) - 1.3530: 6) - 1.3490: 7) - 1.3490: 8) - 1.3530: 9) - 1.3490:  
10) - 1.3500.



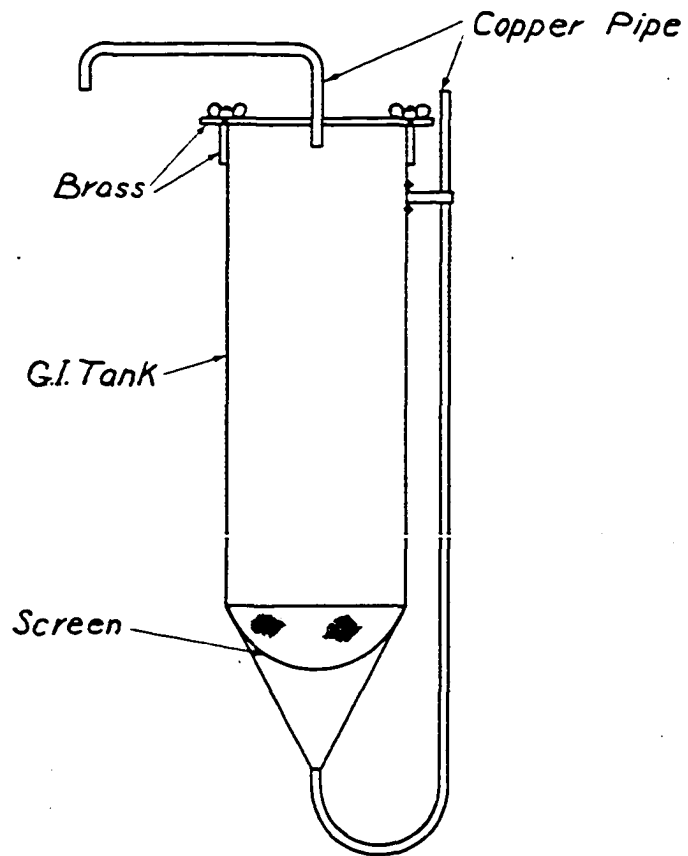
The refractive index of 1.3500 corresponds to 15.25% of total solids.

e. Fifteen Gooch funnels were connected together in series by using drilled rubber stoppers in the mouth of each. Seven gram samples (from No. 4, Talbe IV) were placed in each funnel, hot distilled water (heated by passing through a coil placed in a water bath at 90° C.) was forced through the series and the refractive index of the effluent observed. When a new sample was added as No. 15, No. 1 was emptied, and No. 2 became No. 1, etc., thus operating as a diffusion battery. Enough water was allowed to run until the new sample was covered (No. 15), then turned off for two minutes, and allowed to stand, then again turned on until the extract from No. 15 was replaced. The refractive indices observed ( at 28°C) were as follows: 1) - 1.3490: 2) - 1.3510: 3) - 1.3500: 4) - 1.3495: 5) - 1.3500: 6) - 1.3515: 7) - 1.3500: 8) - 1.3490.

f. An experimental diffusion battery (Fig. III) has been designed and constructed, capable of extracting reasonably large quantities of chips. Eight cells, or more if desired, similar to the one illustrated are connected in series, and in order to extract with a hot solvent all the cells are immersed in a tank of hot water, heated by a steam coil. Each cell is 15 inches high, 5 inches inside diameter, and constructed of galvanized iron. The cells are connected together by means of pressure tubing.

5. The levulose total sugar ratio in dried Jerusalem

*Experimental Diffusion Battery  
Eight Cells Used*



*Fig. 3.*

artichokes after storage.

a. A sample (No. 5, see Table IV) of dried chips was analyzed for levulose and total sugar by Ost's copper carbonate method at the time of drying. The values found were: moisture - 6.1%, levulose - 34.51%, glucose - 7.85%, total sugar - 42.36%, ratio levulose-total sugar 0.814.

The sample was kept in a stoppered bottle for 5 months and the analysis repeated. The values found were: moisture - 6.21%, levulose - 34.35%, glucose - 8.03%, total sugar - 42.38%, ratio levulose-total sugar - 0.810.

6. The effect of drying Jerusalem artichoke tubers on the solubility of undesirable constituents.

a. The total extractable solids and ash (sulfated) were determined on a sample of fresh artichoke tubers. The extraction was made as in G 3. above. The values found were: Total solids 13.85%, Ash 1.13%.

The determination was repeated on the same sample after drying at 75 - 80°C. for 4 hours. Percentages given are based on the original wet sample weight. Total solids - 13.05%, Ash 1.04%.

#### H. The Preparation of Levulose Sirups from Dried Jerusalem Artichoke Chips by Direct Method

200 g. of dried artichoke chips (these chips were dried after having been in storage for 4 months) were treated with one liter of distilled water, boiled for 30 minutes, and juices

expressed in a hand press. The pulp was treated again with 700 cc. of water and heated for thirty minutes. The extracts were combined (2.1), 30 g. of Norite, and 20 cc. of 23.6 N,  $H_2SO_4$  added and the mixture kept at 60 - 80°C. for 1 hour. The solution was filtered through a 6 inch Buchner funnel (filtered in 4 minutes.). The filtrate was almost water clear. Twenty g. of Norite was added and the solution was made faintly alkaline to litmus with slaked lime. This mixture was stirred for 10 minutes, (the temperature never exceeding 45°C.), then filtered through a Buchner funnel (filtered in 10 minutes). The filtrate, which was water clear, was immediately made slightly acid to litmus with  $H_2SO_4$  and allowed to stand over night. The precipitated salts were filtered off and the solution concentrated in vacuo (30 mm. pressure) on a water bath at 60°C. As the concentration progressed salts were found to precipitate and it was necessary to stop the distillation and filter before continuing the concentration. One hundred and ten g. of sirup (92% solids by refractometer) was obtained. The sirup was light brown in color, and although quite sweet, it possessed the characteristic saline taste noticed in the sirups made previously from fresh chips.

The sirup was analyzed for ash and sugars present. The following data were obtained: Ash - 5.14%, levulose - 50.23%, glucose - 29.82%.

I. The Preparation of Levulose Sirup from Dried Jerusalem Artichoke Chips by the Lime Precipitation Method.

Two hundred g. of dried artichoke chips were extracted as in Sec. H. Twenty g. of filter cell was added and the extract limed to a pH of 7.0 - 7.5 at a temperature of 40 - 45°C. After stirring for 5 minutes, the solution was again filtered on the Buchner funnel (time 15 minutes). The clarification was very successful, the filtrate being pale yellow in color. The filtrate was quickly cooled to a temperature of 0°C. by placing it in a beaker packed in an ice-salt mixture.

Sixty g. of lime was slaked, made up to a volume of 200 cc. and cooled to 0°C. The lime paste was poured slowly, with stirring, into the clarified filtrate. The rate of addition of the lime paste was governed by the temperature of the mixture. At no time was the temperature allowed to exceed 5°C. About 10 minutes was required for the precipitation. The stirring was continued for 10 minutes, then the precipitated lime levulate separated by means of a centrifuge, and washed with iced lime water until the washings were free from color. The cake was white.

The cake was carbonated and the sirup concentrated as in Sec. F. 1, 52 g. of light colored sirup (refractive index 1.5130 = 90% solids) was obtained. The sirup crystallized in 24 hours without being seeded, yielding 8 g. of white levulose crystals.

J. Soaking Fresh Jerusalem Artichoke Chips in Water Before Use.

1. Effect of soaking chips on the levulose-glucose ratio.

a. The fresh tubers were sliced and 30 g. samples

weighed. The levulose and glucose were determined in the fresh chips. Other samples were allowed to soak in 100 cc. of water for varying lengths of time and at different temperatures, and the sugars determined both in the extract and in the chips. The results are recorded in Table XI.

In another experiment 500 g. of fresh sliced tubers were soaked in 1000 cc. of distilled water at 10 - 15°C. for 1 1/2 hours. Upon analysis 4.4 g. of levulose and 2.79 g. of glucose were found in the extract, while 18.39 g. of levulose and 10.62 g. of glucose remained in the chips.

2. The removal of salts from fresh artichoke chips by soaking in water.

a. 30 g. samples of the fresh sliced tubers (taken from storage in July) were allowed to soak with 100 cc. of distilled water for 24 hours, and for 48 hours at 8°C. The extract was poured off into a 200 cc. volumetric flask, the chips washed with three 20 cc. portions of distilled water, the washings added to the extract and the solution made up to volume. 50 cc. aliquots of this solution were taken for the ash determination. The specific conductance of the solution was also determined.

The total extractable ash (see Sec. G. 6) was also determined, the aliquots taken being the same as for the above determinations. The results are recorded in Table XII. The percentages given are sulphated ash on the basis of the wet sample taken.

Table XI

## Effect of Soaking Jerusalem Artichoke Tubers on the Sugar Content

Treatment	Levulose in extract	Levulose in chips	Total levu- lose	Glucose in extract	Glucose in chips	Total Gluc.	Total Sugars
Fresh tubers (Untreated)			10.14			4.33	14.47
Chips soaked 1 hour at 29°C.	1.27	7.17	8.44	0.984	4.04	5.024	13.46
Chips soaked 2 hours at 29°C.	1.87	5.03	6.90	1.4	4.28	6.68	13.58
Chips soaked 24 hours at 24-28°C. (slightly fermented)	2.9	4.62	7.52	1.73	3.52	5.25	12.77
Chips soaked 48 hours at 8°C.	1.61	8.39	10.00	1.34	3.18	4.52	14.52

Table XII

The Removal of Salts from Tubers by Soaking

	<u>Percentage</u>	<u>Specific Conductance</u>
Total Extractable Ash	1.15 1.11	$2.167 \times 10^{-3}$ $2.125 \times 10^{-3}$
Total Extractable Ash (From dried chips for comparison)	1.05 1.034	$1.940 \times 10^{-3}$ $1.842 \times 10^{-3}$
Ash Extracted in 24 hours at 8° C.	0.212 0.184	$4.682 \times 10^{-4}$ $4.385 \times 10^{-4}$
Ash Extracted in 48 hours at 8° C.	0.168 0.221	$4.139 \times 10^{-4}$ $4.878 \times 10^{-4}$

K. The Preparation of Levulose Sirup from Fresh Jerusalem Artichokes after Soaking the Sliced Tubers.

625 g. of sliced tubers were placed in a 2 l. beaker, covered with 1500 cc. of distilled water and allowed to soak at a temperature of 8°C. for 48 hours. The extract was then poured off, 1500 cc. of fresh water added and the chips allowed to soak again for 72 hours at 8°C. The extract was discarded and sirup made from the chips exactly as in H. (The clarification was quite easily accomplished because much of the color was discarded with the cold extract. The sirup before concentration was water clear.)

60 g. of a golden sirup was obtained (80% solids) having a fairly agreeable taste but still slightly bitter. The sirup was of much better taste and appearance than any previously made without using the lime precipitation method.

Upon analysis the sirup was found to be 45.61% levulose,



22.61% glucose, and 3.2% ash.

L. Analysis of Commercial Levulose

Two brands of levulose were obtained and analyzed by Ost's copper carbonate method.

The first brand, labeled "Levulose for Diabetics", was found to be 83.98% levulose and 18.19% "glucose".

The second brand, labeled "Levulose", was found to be 87.61% levulose and 12.11% glucose.

M. Diabetic Tolerance for Levulose

A medical doctor (179) who was afflicted with a light case of diabetes volunteered to test his tolerance to levulose. The following quotations are extracts from private correspondence with the doctor.

"Being a diabetic myself, (a mild case), I became interested in your work. I found my condition about 18 months ago. I have never used insulin, and can limit the output of sugar pretty well by diet, though any indiscretion will cause it to re-appear, and am having some trouble in keeping my weight and strength up on the limited diet necessary. Can I be of any assistance to you as a trial patient? I have practiced medicine for 48 years, and am aged 76 years. My general health is good, and also my strength."

The doctor was furnished with some levulose (the second

179. The writer wishes to thank Dr. F. S. Smith M. D. of Nevada, Iowa, for making this test.

brand analysis reported in Sec. L). He reported later as follows:

"I had been living on a restricted diet, very little carbohydrate. No potatoes, starch, white bread or sugar, and could keep myself free from sugar in the urine most of the time. I used whole wheat bread, graham crackers, Post's Bran, all the milk products, meat, fats, fish, eggs, and found I could utilize about a teaspoonful of sugar twice a day.

I had found this to be my limit (renal threshold) for pure carbohydrates. Of course, there is some carbohydrate in the diet given above.

With the use of levulose in the same amount or over added I could keep sugar free, and would gain in weight. I felt stronger and more "peppy".

I am convinced that with plenty of levulose I could build up my weight, strength, and endurance.

I could probably do the same with plenty of other carbohydrates and use of insulin. I do not wish ever to be obliged to use the daily injections of insulin.

I believe that if levulose could be produced at a price we could afford, it would be a boon to the adult diabetic."

## DISCUSSION

Many analyses of Jerusalem artichokes have been reported in the literature. The results vary markedly with the variety grown, with the soil and climate, with the length of growing period, and with the period of storage before analysis. The important consideration from the standpoint of the isolation of levulose is the levulose-total sugar ratio. Jackson, Silsbee, and Proffitt (180) found this ratio to be from 0.695 to 0.870 in a large series of analyses. They made no mention of the varieties used, giving only the date of analysis. They apparently found no appreciable decrease in total sugars or in the levulose-total sugar ratio in tubers left in storage over the winter until June 10.

Shoemaker (181) reported levulose-total sugar ratios ranging from 0.746 to 0.926, without giving specifically the varieties analyzed.

Traub, Thor, Willaman, and Oliver (182) made an extensive study of the storage of artichokes and found that from the time of maturity in the fall up to the end of January there is a consistent decrease in the fructose-glucose ratio, and the fructose-total water soluble carbohydrate ratio under all conditions studied. They concluded that from the standpoint of

180. Jackson, Silsbee, Proffitt loc. cit., (Reference 8)
181. Shoemaker, loc. cit., (Reference 11)
182. Traub, Thor, Willaman, Oliver, loc. cit., (Reference 168)

fructose manufacture, harvesting, and use should take place near the time of maturity in November under Minnesota conditions.

Traub, Thor, Zeleny, and Willaman (183) found much lower levulose-total sugar ratios, ranging from 0.165 to 0.69. They reported that the Mammoth French White variety ranks highest, followed by U. S. D. A., Portland, and the purple variety. This is of interest because the data of Table III (Sec. A) were obtained using tubers grown from seedlings obtained from these writers (Minnesota) and it will be noticed that of the varieties above mentioned, the purple variety ranks highest, followed by the Mammoth French White and the U. S. D. A. seedling. Also the ratios and the total sugar content are much higher than found by the above writers.

The present data is in accord with the observations of Traub, Thor, Willaman, and Oliver (182) that the levulose-total sugar ratio decreases when the tubers are allowed to remain in storage.

Chicory seems to be favored by some writers (183) as a source of levulose. Its chief advantage lies in the fact that inulin can be obtained in reasonably pure form and converted directly into levulose, thus avoiding the troublesome lime levulate precipitation. While the single experiment reported

183. Traub, Thor, Zeleny, and Willaman, loc. cit.,  
(Reference 172)

(Sec. C. 1) gave a poor yield of inulin, it is probable that with a more refined technique the yield could be improved. From an agricultural standpoint chicory is less desirable than the Jerusalem artichoke.

The attempt to obtain inulin from the Jerusalem artichoke (Sec. C. 2 a) was made in order to see if this wild variety contained the same ratio of inulin to levulins as had been reported for other varieties. The result substantiates the earlier work of Tanret (184) and Willaman (185), that it is impracticable to obtain inulin from this source.

Since a specific "inulo-coagulase" has been reported (186) to be present in the peelings of the dahlia and chicory tubers, it was interesting to determine if this enzyme is present in the Jerusalem artichoke. (Sec. C 2 b). It is evidently either absent, or there is insufficient inulin present in the artichoke juices to be coagulated.

The absence of this enzyme in the Jerusalem artichoke may account for the low ratio of inulin to the more soluble levulins.

The enzyme may be of importance in the isolation of inulin from the dahlia or chicory juices, since this coagulation would be more simple than the usual concentration or precipitation by alcohol. At the time of this writing dahlia and chicory

- 184. Tanret, loc. cit., (Reference 118)
- 185. Willaman, loc. cit., (Reference 141)
- 186. Wolf, loc. cit., (Reference 130)

tubers were not available for a trial experiment.

The experiments in Sec. D. illustrate the impossibility of obtaining an edible sirup directly by extraction and clarification of the juices from the Jerusalem artichoke.

One of the difficulties observed during the preparation of the sirups was that during the concentration  $\text{CaSO}_4$  continually precipitated and it was necessary to filter intermittently. If the conversion could be accomplished by means of some volatile acid this difficulty would be overcome since the neutralization by lime would be unnecessary, and therefore, no salts formed. With this in view, an attempt was made (Sec. E) to convert inulin with  $\text{CO}_2$ . Schering (187) has obtained a patent for the use of  $\text{CO}_2$  in the conversion of inulin.

Practical temperatures and pressures were used and reasonable time allowed for conversion to at least start, but no conversion whatever occurred. This result was to be expected in view of the fact that Jackson, Silsbee, and Proffitt (180) found inulin to be more than thirteen times as resistant to hydrolytic action as cane sugar.

It seemed unnecessary to try higher pressures than 200 pounds since Moore (188) has shown that  $\text{CO}_2$  solutions reach a minimum and constant pH of 3.2 - 3.3 at 9.5 atmospheres of pressure.

187. Schering, loc. cit., (Reference 155)

188. Moore, "Solution Studies on Carbon Dioxide", (1928)  
(Unpublished thesis. Copy on file Iowa State College Libr.).

The only successful method known at present for obtaining pure levulose from Jerusalem artichokes is by use of the lime precipitation process of Dubrunfaut (190). The Bureau of Standards (181) method of making the precipitation was found to be quite satisfactory on small scale preparations, (Sec. F 1), but very tedious and time consuming if applied to larger quantities of materials (Sec. F. 2 a, and 2 b).

The necessity for the immediate utilization of sugar beets has long been a handicap to the beet sugar industry. Storage of the beets being impractical or entirely unsuccessful, the most natural solution to the problem would be to dry the beets. The tendency, however, has been to build the factories sufficiently large to dispose of the entire crop of beets in season (usually about three months) and close down for the remainder of the year. This naturally caused the investment of large sums of money which would have been unnecessary if the nature of the crop was such that it could be used by a much smaller plant running throughout the year. Only within recent years has the successful and profitable desiccation of sugar beets been accomplished. The problem with its history and solution is discussed by Owen (191).

The advantages claimed for the drying processes are: 1. The juices obtained are purer and much more concentrated than

190. Dubrunfaut, loc. cit., (Reference 100)

191. Owen, "Desiccation of Sugar Beet", His Majesty's Stationery Office, London, (1927).

those obtained from the fresh beet. 2. The process of sugar manufacture is materially shortened. 3. It allows smaller plants to run the entire year. 4. It reduces shipping costs. (Local drying stations remove both dirt and water.)

This same problem is a natural fore-runner of the levulose industry. Only one reference has been found in the literature on the drying of Jerusalem artichokes, and that one appeared since the present work was carried out. Nichols (192) described conditions for the drying of artichokes evidently for the purpose of using them as food.

The drying of sugar beets is complicated by the fact that inversion is liable to occur during the process. This difficulty is not encountered in the drying of artichokes since conversion must take place before levulose can be prepared.

Jerusalem artichokes can be dried without harming the carbohydrates present (Sec. G. 2) provided that the final temperature does not exceed 80°C. The chips should be white when dry. If they are caramelized the total sugar is found by analysis to have decreased, but the levulose to have remained unchanged. Caramelized chips, however, give extracts which are more difficultly clarified. The temperature during the first stages of drying may be as high as 125°C. without injuring the chips.

The drying of Jerusalem artichokes coagulates the albumin-

192. Nichols, Fruit Products, J. and Am. Vinegar Ind., 9:71(1929), Chem. Abstr., 24:440(1930)



ous matter (Sec. G. 3) and causes an increase of about 5% of the total albuminoids to be retained by the pulp upon extraction. The drying also renders the soluble albuminous matter more easily coagulable, and thus simplifies clarification of the extract. Prolonged heating (after drying) at 75-80°C does not increase the amount of coagulation.

The drying of artichokes breaks down the cell structure and makes diffusion more easily accomplished. The maximum obtainable concentration in the extraction of fresh tubers is about 17.5% solids, while with dried tubers an extract of about 55% solids was obtained (Sec. G. 4).

Owen (191) states that "from a superficial examination of the dried product (beet) and the juice extracted therefrom, it seems probable that the heat conditions during drying have an effect in rendering insoluble otherwise soluble pectic matters. This appears to have a direct result in producing a much more easily filterable and purer juice."

A determination of the total extractable solids (Sec. G. 6) from both the fresh and dried artichoke tubers fails to account for any appreciable amounts of pectic matters having been rendered insoluble.

The levulose-total sugar ratio remains unchanged in dried Jerusalem artichokes when stored for 5 months (Sec. G. 5).

While the salt content of the dried chips makes the production of edible sirup by direct extraction impossible (Sec. H.) the clarification for the lime levulate precipitation is

more easily accomplished than from fresh tubers, and the sirup obtained (Sec. I.) is sufficiently pure to crystallize in 24 hours.

The lime levulate is easily precipitated by simply adding the cooled (0°C) slaked lime to the cooled (0°C) juices, with stirring. While the resulting precipitate would undoubtedly be difficult to filter, it is easily separated by means of a centrifuge. Centrifuging has the added advantage that it keeps the lime levulate cold and prevents decomposition of the sugar.

A part of the salts may be removed from fresh Jerusalem artichokes by soaking in water (Sec. J.). This process also removes some levulose and glucose, almost in equal amounts. This would indicate that the glucose is present perhaps, in the form of sucrose. If this is the case it is conceivable that by soaking for the proper time and at the optimum temperature, enough of the glucose might be removed to allow the levulose obtained by direct extraction to crystallize. Of course, some levulose would be lost with the glucose, but it could be recovered by the lime precipitation process, or both the glucose and levulose might be fermented. It was impossible to determine the optimum conditions for the above process because the only available tubers had been in storage for 8 months and the levulose-glucose ratio had decreased to such an extent that the results would be of no value.

The data obtained, however, indicate that the soaking would have to take place at a low temperature since levulose is apparently converted to glucose (Table XI), probably by enzyme action, at room temperatures. It would be of biological interest to know if this is a true conversion of levulose to glucose or simply a conversion of levulose to other reducing substances. The analytical procedure used designated all reducing substances, except levulose, as "glucose".

Table XII gives enough data that curves may be plotted in order that the ash content of juices over limited ranges may be determined by the conductivity method, provided that the juices are prepared in the manner described (Sec. J. 2)

These data will be valuable when the study is continued using freshly dug Jerusalem artichokes. The conductivity method is very convenient and has been applied to beet sugar and beet juices by Lange (193) and Lunden (194), and to cane products by Zerban (195).

Sirup prepared by direct extraction of pre-soaked fresh artichokes (Sec. K.) is of superior quality to any previously prepared by direct methods.

193. Lange, Zeit. Ver. deut. Zuckerind., 60:359(1910)
194. Lunden, *ibid.*, 75:763(1925)
195. Zerban, "Facts about Sugar", 21:278(1926),  
*ibid.*, 21:1158, 1162(1926), *ibid.*, 22:990  
(1927).

Since so many contradictory reports are found in the literature (see Historical Part) relative to the diabetic tolerance for levulose an opportunity to have a medical doctor (a diabetic himself) give it a test was welcomed.) (Sec. K.)

The probable reason for the contradictions in the literature is the fact that many of the tests were made with levulose of unknown quality (at least the report, in many cases, fails to state the quality). The analysis of levulose from two sources indicates that if commercial brands are accepted, without analysis, variations in results are to be expected.

In many of the tests reported excessive amounts of levulose were ingested. The present case is of interest in showing the effect of levulose in amounts that would normally be used.

SUMMARY

Analytical data of value in the commercial utilization of Jerusalem artichokes have been determined.

The optimum conditions for the drying of Jerusalem artichokes have been determined, and many advantages of drying the artichokes before the isolation of levulose have been proven.

The process for the preparation of crystalline levulose from the dried Jerusalem artichoke chips has been presented.

A preliminary investigation of the possibility of removing the undesirable sugars and salts from Jerusalem artichokes by dialysis has been made.