A study of the effect of fertilizers upon the sugar content of apples, with special reference to a new method of sugar analysis
by Cyril D Evans

A THESIS SuLmitted to the Graduate Committee in partial fulfillment of the requirements for the Degree of Master of Science in Chemistry at Montana State College
Montana State University

Abstract:
1. The percent of sugars in apples increased rapidly during the period of growth.

2. In young apples practically all the sugars are present as reducing sugars.

3. The percent of total sugars increased consistantly over that of the reducing sugars during the period of growth.

4. A close correlation was found to exist between reducing and total sugars during the period of growth.

5. No consistant effect due to any fertiliser treatment was found during the growing period.

6. No relationship was found to exist between the fertilizer treatment and the breakdown of sugars during storage.

7. Individual apples from the same plots were found to have a difference as high as 52.22 percent for reducing sugars and 30.81 percent for total sugars. It should be remembered that the variation of individual apples was not investigated until the apples had been in storage for three months and this might be responsible for the wide variation.

8. The method developed for the determination of sugars was found satisfactory and is believed to be an improvement over other methods in use. It is rapid, simple, convenient, and gave good results.

A STUDY OF THE EFFECT OF FERTILIZERS UPON THE SUGAR CONTENT OF APPLES, WITH SPECIAL REFERENCE TO A NEW METHOD OF SUGAR ANALYSIS

14

by

CYRIL D. EVANS

A THESIS

Submitted to the Graduate Committee in
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In Charge of Major Work

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Chairman Examining Committee

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Chairman Graduate Committee

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A STUDY OF THE EFFECT OF FERTILIZERS UPON THE SUGAR CONTENT OF APPLES, WITH SPECIAL REFERENCE TO A NEW METHOD OF SUGAR ANALYSIS

INTRODUCTION

For a number of years work has been carried on at Montana Experiment Station on the study, "Influence of Plant Foods upon Chemical Composition, Growth, Fruiting, and General Physiological Condition of the Apple Tree", and the work presented in this paper is the result of part of the analytical work done by the author upon the above name project.

The amount of literature is voluminous on the study of carbohydrates in plant materials. However, most of the work is still experimental and the methods are quite largely tentative. Much of the data is directly conflicting and still more can not be duplicated, which it is believed, is largely due to different conditions, methods, and the non-agreement of some fundamental values. This is quite striking of the values one can obtain for the specific rotation of the different sugars. When these values, of such a variance, are used in formulas of optical rotation the results when worked into percentages are discouraging. A good example of this may be had in the following table (see Table I). Sucrose is the only sugar in which consistant values were found and for that reason it has been omitted from the table. It is seen from looking at column 5 a person could have a solution of pure glucose and yet get readings which would give values varying almost twenty per cent. The same applies for the rest of the sugars and undoubtedly there is as much error in the optical rotation of other substances as is here shown for the four sugars.

Table I. Optical Rotation of Different Sugars Found in Standard Reference Works

		1	2	3	4	5	Difference	Error based on the low
		degrees	degrees	degrees	degrees	degrees	degrees	per cent
Glucose	$(\alpha)_D^{20}$	52.74	53.1	52.8	52.5	62.45*	9.95	18.74
Levulose	"	90.72	93.3	92.0	92.0	90.74	2.56	2.82
Maltose	"	138.29	137.5	138.0	137.0	138.39	1.39	1.02
Invert	"	20.02	20.57	19.6	---	18.86	1.71	9.07

These values are all for $(\alpha)_D^{20}$ in a 10 per cent solution.
Values taken from:

Column 1. Landalt, H. Optical Rotation of Organic Substances. p. 491, 495, 589, 592. 2nd Ed. (1902).

Column 2. Mahin, E. G., and Carr, R. H. Quantitative Agriculture Analysis. p. 123 1st Ed. (1923).

Column 3. Allen, A. H. Commercial Organic Analysis. p. 372, Vol. 2, 5th Ed. (1923).

Column 4. Kostychev, S. Chemical Plant Physiology. p. 238, 241, 1st Ed, (1931).

Column 5. International Critical Tables. p. 346, 347, 349, 350, Vol. 2, 1st Ed. (1928).

*This value of $(\alpha)_D^{20} = 62.45^\circ$ is undoubtedly a typographical error, nevertheless, the value is there and it would be quite easy to use this value in a great deal of work before the error would be noticed.

Another point that is very obvious to any worker in carbohydrates is the lack of some modern standard work in the English language. At present the best work available is the "Official and Tentative Methods of Analysis" of the Association of Official Agricultural Chemists. However, it is not comprehensive and is limited in its scope. There are a few good books in English on the different phases of cane and beet sugar industry, but these are quite limited in their scope and are of little value to any one not working in the cane or beet sugar industry.

In 1923 Hagedorn and Jensen[3] proposed a method for determining reducing sugars in which an alkaline solution of ferricyanide is used. The method was first developed was used for blood sugar, but in recent years has been extended to include larger amounts of sugar.[2,4] Blish[2] modified the method for determining the reducing materials in wheat flour and obtained results which indicated that with certain changes the same procedure could be applied to biological fluids. Accordingly an attempt has been made in the present investigation to modify the method so that it could be applied in measuring the reducing substances in apple extracts.

The analytical work done by the author in previous years along this same line has given results which were somewhat doubtful. The results depended on innumerable factors the control of which were in many cases out of the hands of the analyst. Many methods were tried, some giving results which were in good agreement and others in which it was almost impossible to duplicate results. Practically all of the methods when used with a pure solution of only sucrose and glucose gave results which checked closely. However, when these same methods were tried with an apple extract the results obtained varied widely.

CHEMICAL METHODS

As has been previously stated the alkaline ferricyanide solution was tried out as a suitable reagent for the determination of sugar in apple extract. In a review of the literature Eiddowson[8] has applied the method of Hagedorn and Jensen for the determination of small quantities of mixed reducing sugars and its application to the products of hydrolysis of starch. Hanes[4] extended the original method of Hagedorn and Jensen to about ten times the amount of sugar, namely, 3.8 mgs. in 5 cc. of solution. Both of the above mentiond workers used quite dilute solution (0.01N ferricyanide and .01N sodium thiosulphate) and obtained results which were in close agreement. Hanes[4] investigated the method for maltose and glucose and gives tables and graphs of his work. He also gives data as to the effect of the time of reduction and effect of dissolved oxygen. Widdowson[8] has applied the method to mixtures of maltose, glucose, fructose and to the products of hydrolysis of starch in view of determining these sugars in young apples. The effect of using basic lead acetate and sodium phosphate as clarifying agents is given, and also the effect of boiling a solution of the different sugars with charcoal. Nothing, however, is given as to the effect of basic lead acetate upon fructose and nothing is said of using any other clarifying agent and apparently this point has been disregarded,

Sobotka and Reiner[7] have used the alkaline ferricyanide method for determining the relation of reducing power to configuration. They found that the titration curves for fructose, glucose and glactose, are

practically all a straight line, which is not in accord with the findings of Hanes.[4]

Blish[2] has extended the method to include 17 mgs. of maltose in 10 cc. of solution, and he summarizes the method as a superior one from the standpoint of accuracy, reliability, simplicity, and convenience to the technician. This statement shows the results and satisfaction obtained at the Nebraska Agricultural Experiment Station.

Preliminary attempts to apply the alkaline ferricyanide method of Hagedorn and Jensen to sugars in apples gave indications that the method possessed many features not embodied in other methods used in common practice.

Solutions of the strength recommended by Blish[2] were decided upon and the method was standardized against the United States Bureau of Standards Dextrose sample. The purity of the sample was tested by means of the polariscope and found to be 99.467%. For the study of invert sugars a commercial sample of sucrose was used and its purity was found by the same method to be 99.952%.

SPECIFICATION OF ADOPTED PROCEDURE

Alkaline Ferricyanide Solution. 16.5 grs. of pure $K_3Fe(CN)_6$ and 22 grs. of C.P. anhydrous Na_2CO_3 were dissolved in 500 cc. of distilled water and let stand for a few days. It was then filtered and made up to a 1000 cc. The solution maintains its strength quite well when stored in dark bottles. The above solution gives a strength of approximately N/20.

N/20 Sodium Thiosulfate Solution. 12.41 grs. of C.P. $Na_2S_2O_3 \cdot 5H_2O$ were dissolved per liter of redistilled water. If special precautions are used in the preparation of this solution it will retain its normality for a long time. However, it should be checked occasionally as quite commonly it slowly deteriorates upon standing.

Acetic Acid Reagent. A solution was made up to contain 200 cc. of C.P. glacial acetic acid, 70 grs. of C.P. KCl, and 20 grs. of $ZnSO_4 \cdot 7H_2O$ per liter.

Potassium Iodide. A solution was made of 50 grs. C.P. Kl per 100 cc. of distilled water and 1 drop of concentrated NaOH to prevent the decomposition of the KI and the liberation of Iodine. If the solution becomes colored a few drops of concentrated NaOH may be added and the solution shaken occasionally and it will again become colorless. The solution must be colorless or it is unfit for use.

Starch Solution. A 1% solution of starch was made up using Litner's Soluble Starch. It was found better to make up a small amount of starch solution and do it quite frequently than to add $ZnSO_4$, NaCl, and numerous other salts that are recommended in the literature. A weak solution of starch, approximately 1%, was used, otherwise a more concentrated solution will give a curdled effect and it is impossible to reach an end point. The starch apparently curdles around some of the iodine and the solution has a speckled appearance.

Neutral Lead Acetate Solution. A solution was made up to contain 450 grs. of C.P. $Pb(C_2H_3O_2)2$ per liter.

Potassium Oxalate. A solution was made up to contain 240 grs. of C.P. $K_2C_2O_4$ per liter.

REDUCING SUGARS

The procedure was carried out in test tubes of 22 mm. x 175 mm. These tubes were thoroughly cleaned and free from grease so that none of the solution would collect in drops along the sides. Into these test tubes was introduced 10 cc. of alkaline ferricyanide solution and an amount of sugar solution whose strength is to be determined. If this solution was not 10 cc., water was added to make up that volume. Hanes[4] stresses this point, but the writer has found no difference with N/20 solution. Far more error is due to dirty glassware and to not mixing the two solutions. It is always a safe procedure to incline the test tube and rotate it slowly thus accomplishing a removal of any drops clinging to the sides of the tube.

After thoroughly mixing the solutions in the test tubes they were placed in a boiling water bath for exactly 30 minutes. Hanes[4] has investigated the rate of reduction and from his work it is shown that for solutions of that concentration the reduction is practically complete in 10 minutes. Blish[2] used 20 minutes and since the boiling point is somewhat lower at the altitude at which this work was carried on the solutions were heated for 30 minutes. No investigation was made as to any other time for the period of reduction since the method was to be standardized and tables made for this particular temperature. Blish[2] emphasizes the time-temperature effect and specifies the time exactly.

After removal from the boiling water bath the tubes were cooled in a stream of running water. The tubes should still have a yellow tint and if they are white all of the ferricyanide has been reduced, in which case the reducing power of the sugar has exceeded the oxidizing capacity of the ferricyanide. The determination must be repeated using a smaller amount of the sample. After cooling, the contents of the test tubes were poured into 250 cc. erlenmyer flasks. The tubes were then rinsed out with portions of 25cc. of the acetic acid reagent until a total of 25 cc. had been added. A slight tendency will be noted here towards the formation of Prussian blue, however, it was never present in sufficient quantities to cause trouble with the end point of the starch iodine color. After all of the tubes had been washed out, (the determination was usually run in sets of six), the solutions were ready for the iodine hypo titration.

Potassium iodide was then added to each sample directly before titration, thus avoiding any loss of iodine by volatilization, as was noted by Widdowson[8]. The iodine freed was titrated by .05N sodium thiosulphate, using 1 cc. of starch solution as an indicator. The amount of sodium thiosulphate used in titrating the iodine represents the excess ferricyanide, or the amount not reduced by the sugar. This method is an indirect one and depends upon the titration of the excess ferricyanide, and by subtraction we obtain the amount of ferricyanide reduced by the sugar. Thus by running a blank determination of 10 cc. of ferricyanide solution and 10 cc. of water in the same manner as given previously, a value is obtained for the blank. Hence the value of the sugar determination is subtracted from the value of the blank, and thus is obtained the amount of

ferricyanide actually reduced by the sugar.

Since the concentrations of the solutions used in this investigation were not exactly .05N they were all calculated to this basis for the purpose of comparison. The values obtained (Table II) were plotted in a curve from which can be read the milligrams of glucose per .1 cc. of .05N ferricyanide solution (see figure 1). The values interpolated from this curve were not corrected for the effect of a clarifying agent and are given in Table III.

From previous work with lead acetate as a clarifying agent it was found quite satisfactory for giving a very clear solution and precipitating the proteins. The excess lead is removed by sodium oxalate and no difficulty was experienced in filtering the precipitated lead oxalate. The effects of lead acetate and potassium oxalate upon sugar solutions were investigated for different concentrations of these salts (see Tables IV and V). The results in Table IV are from glucose solutions of two different concentrations with varied amounts of potassium oxalate and potassium acetate. The figures show that very little effect is due to potassium oxalate and it is practically all due to the potassium acetate.[1] The effect of these reagents is seen to increase with their concentration, so a very definite amount to be used as a clarifying agent must be agreed upon. The amount, however, must also be sufficient to precipitate the proteins and give a clear solution. From a number of trials on samples of apple extract solution it was found that 1-2 cc. of the lead acetate solution of 450 grs. per liter was sufficient to give a clear solution. In no case was more found to be necessary. Hence it was decided to use 2 cc. of this

solution for all future clarifications and the correction of .07 cc. of .05N ferricyanide would be applied to all determinations. The correction of .07 cc. was arrived at from a study of Tables IV and V. It will be seen in Table III that .07 cc. of ferricyanide solution equals 1 mg. of glucose. hence this amount must be subtracted from each determination. In a number of determinations it will be easier to construct a new table having the correction applied and then results may be read directly. Table III is given without any corrections being applied because if it is found necessary to use different amounts of the clarifying agent or an entirely new clarifying agent the corrections for these can then be applied.

INVERT SUGAR

Invert sugars were to be determined and after inversion the same method was used as that for establishing the glucose conversion table. Samples of sucrose from 1 mg. to 12 mgs. were used and their weight calculated to the equivalent weight of invert sugar which was in turn used as the basis to plot the curve obtained in figure 2. The results were calculated to the basis of .05N ferricyanide solution.

The rapid method of inversion[6] was used which is as follows: A 50 cc. portion of the lead free filtrate was pipetted into a 100 cc. flask and then 25 cc. of water added. 10 cc. of HCl were then added a little by little, while rotating the flask. (HCl sp. gr. 1.1029 at $20^{\circ}/4^{\circ}$). A water bath was heated to 70° C and the burner regulated so that the temperature of the bath remained approximately at that point. The flask was placed in the water bath, and a thermometer inserted, and it was then

Table II. The Oxidation of Glucose by Alkaline Ferricyanide Solution

Sample number	Weight of Glucose	Hypo titres	Subtraction from blank	Converted to 0.05N
	mg.	cc.	cc.	cc.
1	13.925	9.7 0.7	9.59	9.26
2	11.936	2.06 2.10	8.21	7.93
3	9.946	3.40 3.40	6.89	6.65
4	7.957	4.89 4.80	5.44	5.25
5	5.968	6.18 6.18	4.11	3.96
6	3.978	7.58 7.58	2.71	2.62
7	1.989	8.95 8.94	1.34	1.30
8 (blank)		10.29 10.29		

Normality of hypo = 0.048288

Method of calculation:

$10.29 - 0.70 = 9.59$ cc. $\quad 9.59 \times \frac{0.048288}{0.0500} = 9.26$ cc.

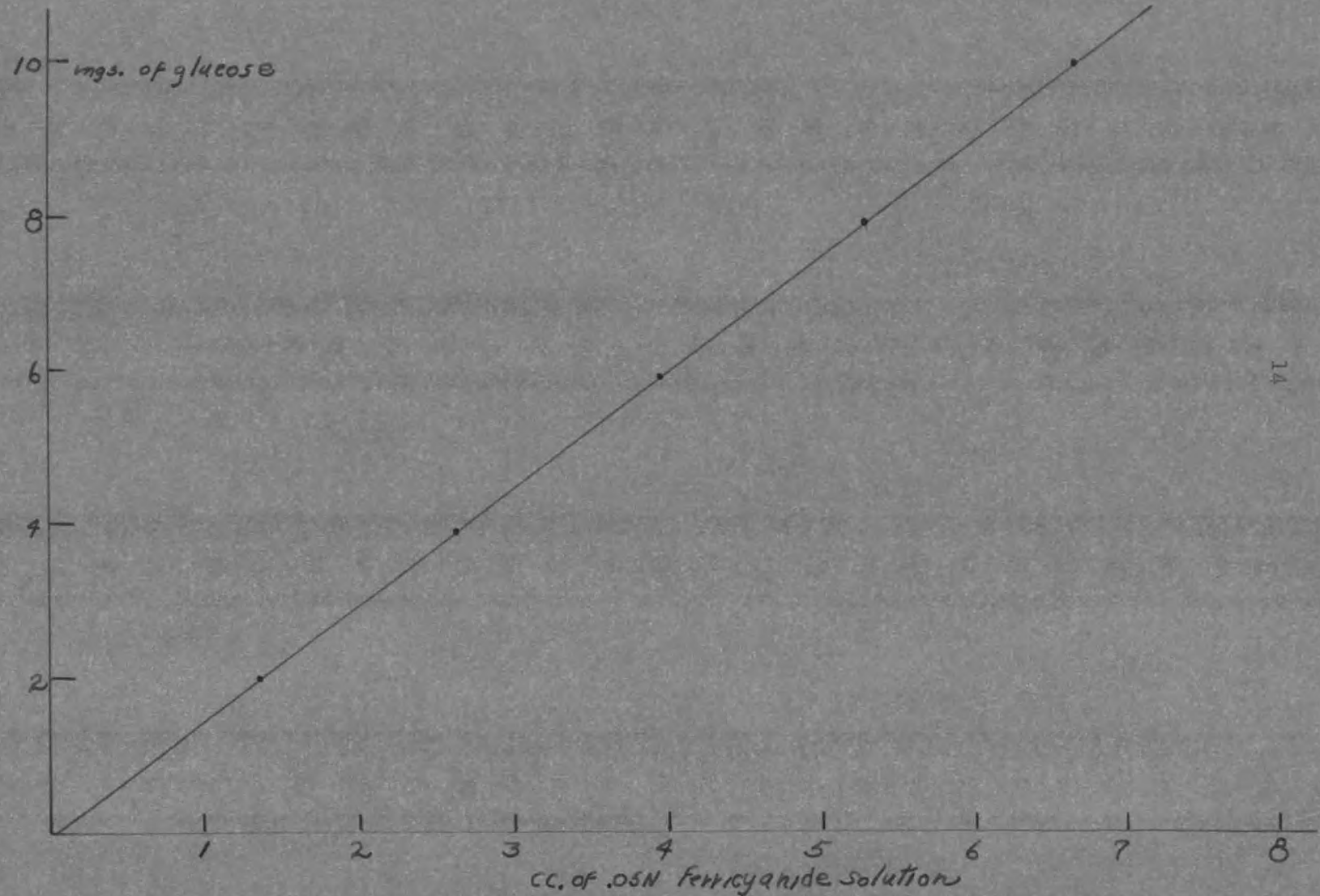

Figure 1 – Curve obtained by plotting milligrams of glucose against cubic centimeters of .05N ferricyanide solution

Table III. Glucose Conversion Table

0.05 N Ferricyanide reduced	Glucose equivalent	0.05 N Ferricyanide reduced	Glucose equivalent	0.05 N Ferricyanide reduced	Glucose equivalent
cc.	mgs.	cc.	mgs.	cc.	mgs.
0.1	0.15	3.4	5.10	6.7	10.05
0.2	0.30	3.5	5.25	6.8	10.20
0.3	0.45	3.6	5.40	6.9	10.35
0.4	0.60	3.7	5.55	7.0	10.50
0.5	0.85	3.8	5.70	7.1	10.65
0.6	0.90	3.9	5.85	7.2	10.80
0.7	1.05	4.0	6.00	7.3	10.95
0.8	1.20	4.1	6.15	7.4	11.10
0.9	1.35	4.2	6.30	7.5	11.25
1.0	1.50	4.3	6.45	7.6	11.40
1.1	1.65	4.4	6.60	7.7	11.55
1.2	1.80	4.5	6.75	7.8	11.70
1.3	1.95	4.6	6.90	7.9	11.85
1.4	2.10	4.7	7.05	8.0	12.00
1.5	2.25	4.8	7.20	8.1	12.15
1.6	2.40	4.9	7.35	8.2	12.30
1.7	2.55	5.0	7.50	8.3	12.45
1.8	2.70	5.1	7.65	8.4	12.60
1.9	2.85	5.2	7.80	8.5	12.75
2.0	3.00	5.3	7.95	8.6	12.90
2.1	3.15	5.4	8.10	8.7	13.05
2.2	3.30	5.5	8.25	8.8	13.20
2.3	3.45	5.6	8.40	8.9	13.35
2.4	3.60	5.7	8.55	9.0	13.50
2.5	3.75	5.8	8.70	9.1	13.65
2.6	3.90	5.9	8.85	9.2	13.80
2.7	4.05	6.0	9.00	9.3	13.95
2.8	4.20	6.1	9.15	9.4	14.10
2.9	4.35	6.2	9.30	9.5	14.25
3.0	4.50	6.3	9.45	9.6	14.40
3.1	4.65	6.4	9.60	9.7	14.55
3.2	4.80	6.5	9.75	9.8	----
3.3	4.95	6.6	9.90	9.9	----

Table IV. The Effect of Neutral Lead Acetate as the Clarifying Agent on the Determination of Glucose

Glucose	Potassium oxalate	Potassium acetate	Hypo titre	Difference in titre
cc.	cc.	cc.	cc.	cc.
8	0.0	0.0	4.84 4.84	---
"	0.0	0.5	4.84 4.85	---
"	0.0	1.0	4.84 4.82	0.02
"	0.0	2.0	4.80 4.82	0.03
"	0.5	0.0	4.84 4.84	---
"	0.5	1.0	4.82 4.82	0.02
"	0.5	2.0	4.78 4.78	0.07
"	1.0	0.0	4.85 4.85	---
4	0.0	0.0	7.35 7.35	---
"	0.0	0.5	7.30 7.30	0.05
"	0.0	1.0	7.30 7.30	0.05
"	0.0	2.0	7.30 7.30	0.05
"	0.0	6.0	7.20 7.22	0.14
"	0.5	0.0	7.35 7.35	---
"	1.0	0.0	7.35 7.35	---
"	2.0	0.0	7.33 7.33	0.02
"	6.0	0.0	7.30 7.30	0.05
"	0.5	1.0	7.30 7.30	0.05
"	0.5	2.0	7.28 7.28	0.07
"	0.5	6.0	7.20 7.20	0.10

Table V. The Effect of Neutral Lead Acetate as the Clarifying Agent on the Determination of Levulose

Levulose	Potassium oxalate	Potassium acetate	Hypo titre	Difference in titre
cc.	cc.	cc.	cc.	cc.
4	0.0	0.0	7.40 7.40	---
"	0.0	0.5	7.40 7.40	---
"	0.0	1.0	7.37 7.36	0.035
"	0.0	2.0	7.36 7.35	0.05
"	0.0	6.0	7.25 7.25	0.15
"	0.5	0.0	7.40 7.40	---
"	1.0	0.0	7.40 7.40	---
"	2.0	0.0	7.38 7.38	0.02
"	6.0	0.0	7.35 7.35	0.05
"	0.5	1.0	7.38 7.38	0.02
"	0.5	2.0	7.33 7.33	0.07
"	0.5	6.0	7.30 7.25	0.125

Table VI. The Effect of Hydrogen-Ion Concentration upon the Alkaline Ferricyanide Titration Resulting from the Use of Different Indicators in Neutralizing the Acid Required for Inversion

Sample number	Reduction of 0.05 N. Ferricyanide by sugars		
	A	B	C
	cc	cc	cc
1	9.05	8.30	8.05
	9.05	8.40	8.05
2	7.45	6.95	6.85
	7.35	6.85	6.85
3	9.10	8.25	7.95
	9.25	8.25	7.95

A - Methyl orange indicator used in neutralizing the acid.

B - Methyl red indicator used in neutralizing the acid.

C - Phenolphthalein indicator used in neutralizing the acid.

Table VII. The Oxidation of Invert Sugar by Alkaline Ferricyanide Solution

Sample number	Weight of sucrose	Weight of invert sugar	Hypo titre	Subtraction from blank	Corrected to value to pipette and to 0.05 N solution
	mgs.	mgs.	cc.	cc.	cc.
1*	11.994	12.625	1.24 1.24	9.05	8.77
2	9.995	10.521	2.88 2.88	7.41	7.19
3	7.996	8.417	4.28 4.28	6.01	5.82
4	5.997	6.312	5.66 5.68	4.63	4.49
5	3.998	4.208	7.00 7.04	3.27	3.17
6	1.999	2.104	8.56 8.50	1.76	1.706
7	0.999	1.052	9.36 9.36	0.93	0.903
blank			10.29 10.29		

Normality of hypo = 0.048288

*Note error of 0.15 cc. This figure was not used in constructing figure 2 or table VIII.

Method of calculation:

$$10.29 - 1.24 = 9.05 \text{ cc.}$$

$$9.05 \times \frac{0.048288}{0.500} = 8.74 \text{ cc.}$$

$$8.74 \times \frac{10.00}{9.962} = 8.77 \text{ cc.}$$

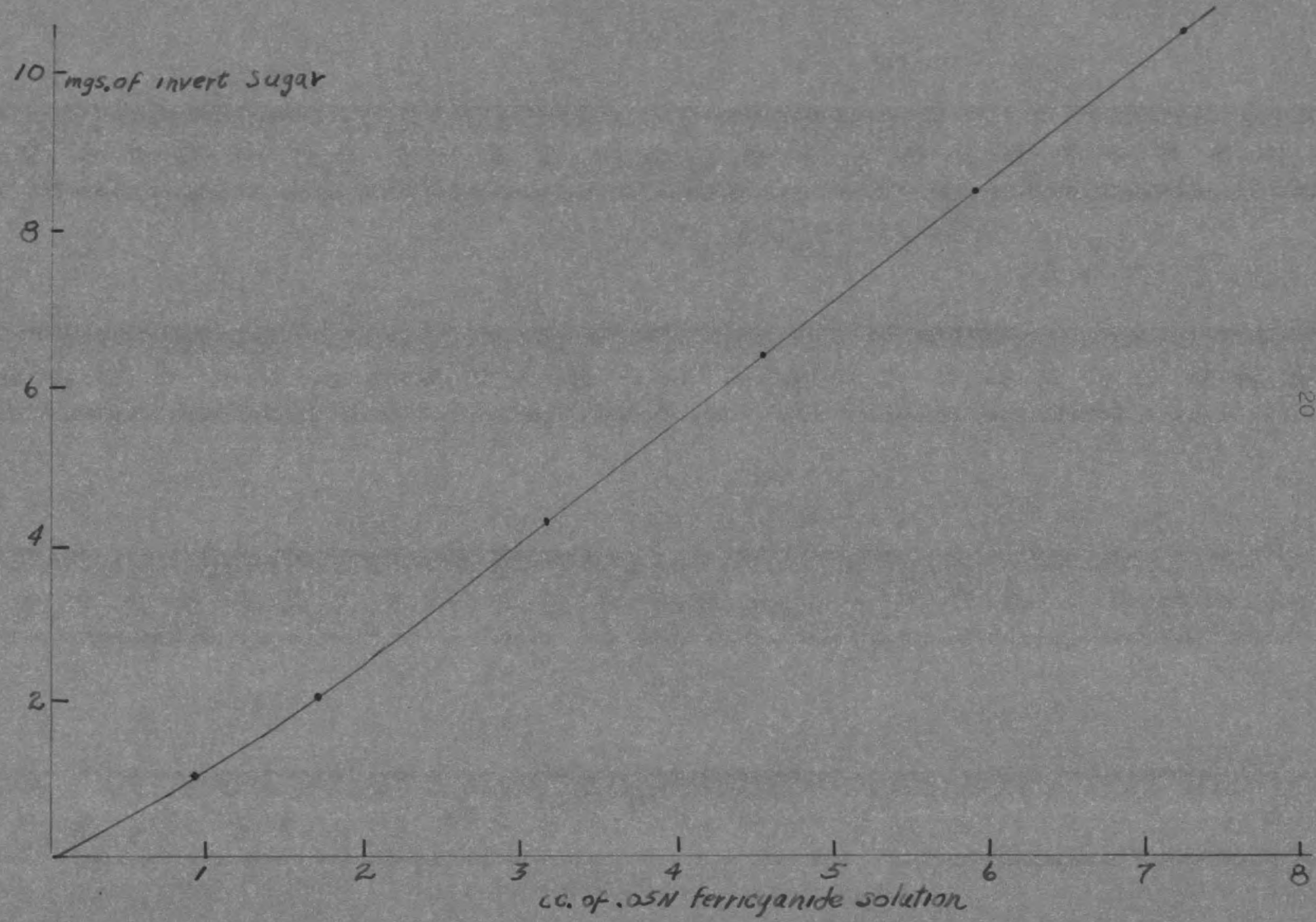

Figure 2 – Curve obtained by plotting milligrams of invert sugar against cubic centimeters of .05N ferricyanide solution

Table VIII. Invert Sugar Conversion Table

0.05 N Ferri-cyanide reduced	Invert equiva-lent	0.05 N Ferri-cyanide reduced	Invert equiva-lent	0.05 N Ferri-cyanide reduced	Invert equiva-lent
cc.	mgs.	cc.	mgs.	cc.	mgs.
0.1	0.08	3.4	4.63	6.7	9.74
0.2	0.20	3.5	4.77	6.8	9.89
0.3	0.32	3.6	4.93	6.9	10.05
0.4	0.40	3.7	5.09	7.0	10.20
0.5	0.53	3.8	5.24	7.1	10.35
0.6	0.65	3.9	5.40	7.2	10.51
0.7	0.76	4.0	5.55	7.3	10.66
0.8	0.88	4.1	5.71	7.4	10.81
0.9	1.00	4.2	5.86	7.5	10.97
1.0	1.11	4.3	6.02	7.6	11.12
1.1	1.23	4.4	6.17	7.7	11.27
1.2	1.37	4.5	6.33	7.8	11.42
1.3	1.49	4.6	6.48	7.9	11.58
1.4	1.61	4.7	6.64	8.0	11.73
1.5	1.74	4.8	6.79	8.1	11.88
1.6	1.86	4.9	6.95	8.2	12.04
1.7	1.99	5.0	7.10	8.3	12.19
1.8	2.14	5.1	7.26	8.4	12.35
1.9	2.30	5.2	7.41	8.5	12.50
2.0	2.48	5.3	7.57	8.6	12.65
2.1	2.60	5.4	7.72	8.7	12.71
2.2	2.71	5.5	7.88	8.8	12.96
2.3	2.87	5.6	8.04	8.9	13.12
2.4	3.03	5.7	8.19	9.0	13.27
2.5	3.19	5.8	8.35	9.1	13.42
2.6	3.35	5.9	8.50	9.2	13.58
2.7	3.51	6.0	8.66	9.3	13.73
2.8	3.67	6.1	8.82	9.4	13.89
2.9	3.84	6.2	8.97	9.5	14.04
3.0	4.00	6.3	9.12	9.6	14.19
3.1	4.16	6.4	9.27	9.7	14.35
3.2	4.31	6.5	9.43	9.8	----
3.3	4.47	6.6	9.59	9.9	----

heated with constant agitation until the thermometer in the flask indicated 67°C. This preliminary heating period should require 2½-2 3/4 minutes. From the moment the thermometer in the flask indicated 67°C, the flask was left in the bath for exactly 5 minutes longer, during which time the temperature should rise to about 69.5°C. The flask was at once plunged into cold water, the thermometer removed from the flask and rinsed, and the flask left to cool. For use in the ferricyanide method the acid used for inversion must be neutralized and this was accomplished by neutralizing with C.P. NaOH solution using methyl red as an indicator. After the flask had cooled, two drops of methyl red indicator were added and a rather concentrated solution of NaOH added until most of the acid had been neutralized, but leaving the solution still acid to methyl red. This point will have to be predetermined. The flask was again cooled and when cold the neutralization was completed with a dilute solution of C.P. NaOH. The flask was allowed to stand for some time, or until it had attained room temperature, after which it was filled to the mark and mixed well.

Table VI shows the importance of the indicator used and the difference in the amounts of sugar found by using the various indicators. The method of inversion as previously outlined was used, using methyl red as an indicator. The data obtained is given in Table VII.

The weights of sucrose used were calculated to read in weights of invert sugar which is $\frac{360.196}{342.18}$ times weight of sucrose.

Table VIII was obtained from a curve, similar to figure 6, but larger, by interpolation and reads in milligrams of invert sugar per .1 cc. of .05 N ferricyanide solution. The values interpolated from the curve

were not corrected for a clarifying agent. The effects of lead acetate and potassium oxalate as clarifying agents were investigated for levulose and glucose (see Tables IV and V). The effect is practically the same for both sugars, thus the difference of .07 cc. must be applied as a correction to either the titration, or a new table constructed with the .07 cc. sugar equivalent correction applied.

Another factor of great importance in this method is the effect of impure sodium carbonate upon the alkaline ferricyanide solution. This effect was discovered accidentally upon the making up of two new solutions of ferricyanide. Solutions A and B (see end of Table IX for description and concentration) were made up and the blanks checked exactly. However, when run with 10 cc. of glucose solution 9.36 cc. of ferricyanide was reduced for solution a, and only 8.51 cc. of solution B, a difference of .85 cc. which in terms of milligrams of sugar is equal to 1.28 mgs. A mixture of equal parts of solutions A and B was tried, (solutions A and B, Table IX), but the results were not intermediate as expected.

Three more solutions were then made up (see bottom of Table X). Solutions C and D were made up as identical as possible. Solution E was made up using a different brand of sodium carbonate and the results of these three solutions are shown in Table X. From the results given in Table X the agreement is quite close and the error shown in table IX is due to an impure sample of sodium carbonate. It so happened that all of the sample of Baker's reagent grade of sodium carbonate was used in making up solution B, so that no other check could be made on it. However, two solutions of Mercks Ph-B 1898 grade and Mercks C.P. grade, conforming to

Table IX. The Effect of Impure Sodium Carbonate upon Alkaline Ferricyanide Solution

$K_3Fe(CN)_6$ solution	Glucose	Hypo titre	Subtraction from blank
cc.	cc.	cc.	cc.
10 - A	0	10.2	---
		10.2	
"	10	0.84	9.36
		0.84	
"	6	4.45	5.77
		4.40	
"	2	8.30	1.88
		8.33	
10 - B	0	10.2	---
		10.2	
"	10	1.69	8.51
		1.69	
"	6	5.20	5.00
		5.20	
"	2	8.50	1.69
		8.52	
10 - A+B	0	10.2	----
		10.2	
"	10	1.00	9.22
		0.95	
"	6	4.60	5.60
		4.60	
"	2	8.28	1.92
		8.28	

Quantities for one liter of solution:

Solution A - 16.5 grams $K_3Fe(CN)_6$ Mercks pure crystals, 22 grams Na_2CO_3 anhyd. Bakers reagent grade.

Solution B - 16.5 grams $K_3Fe(CN)_6$ Mercks pure crystals. 22 grams Na_2CO_3 anhyd. A mixture of Bakers reagent grade and Mercks Ph-B 1898.

Solution A+B - 50 parts of A. 50 parts of B.

Table X. The Effect of Impure Sodium Carbonate upon the Alkaline Ferricyanide Solution

$K_3Fe(CN)_6$ solution	Levulose	Hypo titre	Subtraction from blank
cc.	cc.	cc.	cc.
10 - C	0	10.3	---
		10.3	
"	10	3.55	6.75
		3.56	
"	6	6.18	4.10
		6.20	
"	2	8.89	2.40
		8.90	
10 - D	0	10.3	---
		10.3	
"	10	3.55	6.75
		3.55	
"	6	6.20	4.10
		6.20	
"	2	8.89	2.42
		8.88	
10 - E	0	10.2	---
		10.2	
"	10	3.42	6.76
		3.46	
"	6	6.10	4.13
		6.03	
"	2	8.70	2.50
		8.70	

Quantities for one liter of solution:

Solution C and D - (made up of separate weighings)
16.5 grams $K_3Fe(CN)_6$ Mercks pure crystals.
22 grams Na_2CO_3 anhyd. Mercks C.P.

Solution E - 16.5 grams $K_3Fe(CN)_6$ Mercks pure crystals.
22 grams Na_2CO_3 Bakers C.P. analyzed.

Standards of Murray, were made in the same concentrations as in the alkaline ferricyanide solution, or that of 22 grs. per liter. Duplicate samples were run adding these solutions in increasing 0.5 cc. portions to the alkaline ferricyanide solution with the following results:

Table XI. The Effect of Adding Different Sodium Carbonate to .05N Ferricyanide Solution.

Sample	Na_2CO_3 solution of 22 grs/liter added	Difference in titre of .05N ferricyanide solution from standard sugar titration. Mercks Ph-B	Mercks C.P.
1	.5 cc.	.050 cc.	.025 cc.
2	1.0	.130	.005
3	1.5	.145	.005
4	2.0	.220	-.005
5	2.5	.240	.015

It is seen from Table XI that the effect is due to the Ph-B grade of sodium carbonate and the effect is directly proportional to the amount added. The time was quite limited when this effect was first noted and no further attempts were made to find out what impurity was giving the trouble.

APPLE INVESTIGATIONS

Collection and Preservation of Materials.

The apples used in this investigation were collected from the trees growing in the Elgin Heights Orchard near Stevensville in the Bitter Root Valley. Ten plots of two rows of three trees each were laid out with a guard row separating each plot.

The fertilizer treatment that each plot has received is as

follows:

Rows 2 and 3, are checks or untreated rows.

Row 4, is a guard row.

Rows 5 and 6, sodium nitrate 2 lbs. and ammonium sulphate 2 lbs. were added to each tree from 1928 to 1932. In 1933, 3 lbs. of sodium nitrate and 2 lbs. of ammonium sulphate were added.

Row 7, is a guard row.

Rows 8 and 9, six lbs. of whale meat per tree were added in 1928. Six lbs. of whale meat and bone, and 6 lbs. superphosphate per tree were added in 1929. Six lbs. superphosphate per tree were added from 1930 to 1933, inclusive.

Row 10, is a guard row.

Rows 11 and 12, two lbs. of potassium chloride per tree were added from 1928 to 1933, inclusive.

Row 13, is a guard row.

Rows 14 and 15, are checks or untreated rows.

Row 16, is a guard row.

Rows 17 and 18, two lbs. of sodium nitrate, 2 lbs. sodium sulphate, 3 lbs. whale meat and bone, and 3 lbs. superphosphate per tree were added in 1928. From 1929 to 1932, inclusive, each tree received 2 lbs. sodium nitrate, 2 lbs. ammonium sulphate and 3 lbs. of superphosphate. In 1933 the sodium nitrate was increased to 3 lbs. per tree.

Row 19, is a guard row.

Rows 20 and 21, two lbs. sodium nitrate, 2 lbs. ammonium sulphate, 3 lbs. whale meat and bone, 1½ lbs. superphosphate and 1 lb. potassium chloride

were added per tree in 1928. From 1929 to 1932, inclusive each tree received 2 lbs. sodium nitrate, 2 lbs. ammonium sulphate, 3 lbs. superphosphate and 1 lb. of potassium chloride. In 1933 sodium nitrate was increased to 3 lbs. per tree and superphosphate was increased to 5 lbs. per acre.

Row 22 is a guard row.

Rows 23 and 24, in 1928-29-30, 6 lbs of whale meat and bone and 2 lbs potassium chloride were added per tree. In 1931-32, 1½ lbs sodium nitrate, 1½ lbs ammonium sulphate and 6 lbs superphosphate per tree were added. In 1933, 3 lbs of sodium nitrate, 2 lbs of ammonium sulphate and 12 lbs of superphosphate per tree were added.

Row 25, is a guard row.

Rows 26 and 27, from 1928 to 1932, inclusive, each tree received 2 lbs sodium nitrate, 2 lbs ammonium sulphate and 1 lb potassium chloride. In 1933 the sodium nitrate was increased to 3 lbs per tree. The amounts of other fertilizers remained the same.

Row 28, is a guard row.

Rows 29 and 30, are checks, or untreated rows.

The superphosphate used was the ordinary 17 to 18 per cent phosphoric acid (P_2O_5). The whale meat and bone referred to contained 2.59 per cent N as NO_3, 7.70 per cent as NH_3 and 2.03 per cent as organic nitrogen. The total P_2O_5 was 8.93 per cent, of which 0.43 per cent was water soluble, and 4.43 per cent reverted. The K_2O content was 9.00 per cent.

These trees were sampled on June 25, 1933, August 18, 1933, September 7, 1933, and September 26, 1933. The apples collected for chemical analysis were sliced as soon as picked and stored in 95 per cent

alcohol for shipment to the laboratory. It was attempted to obtain a sample of apples weighing between 50 and 60 grs, as this weight would given an alcoholic solution of about 80 per cent which is the amount specified for storage of plant samples[5]. At the time of sampling a portion was taken from each apple and placed in two tared jars to serve as a determinator of the average moisture in all samples.

At the date of the last sampling, which was the time of picking of the crop, a sufficient quantity of apples was taken from each plot for studies to be carried out during the winter. These apples were packed and shipped to Bozeman where they were placed in storage at a temperature of 2°-3°C.

For the determination of sugars the sample was extracted four times with 200 cc. portions of 80 per cent alcohol. The extractions were carried out in a hot water bath maintained at a temperature to have a slow boiling of the alcohol. Each extraction was run over night with a new portion of alcohol. No $CaCO_3$ or precipitated chalk was added to these samples either during extraction or storage, as earlier work carried out in the Experiment Station showed that the chalk had a tendency to destroy part of the sugar. A number of second extractions were carried out from time to time and no sugar could be found in these and it was assumed that complete extraction had taken place in every case.

After extraction the samples were made up to 1000 cc. and mixed well and a 50 cc. portion of this was used in analysis. It is well to analyze the same as soon after extraction as possible because of a slow change taking place in the sugars. Samples stored over a period of four

months showed a change of .4 mg in 5.56 mgs of reducing sugar. Duplicate samples stored with precipitated chalk showed practically the same change.

In the analysis of the extract for sugar all samples were run in duplicates. Two 50 cc. portions of the extract were pipetted into a suction flask and 50 cc. of distilled water added to each. The flasks were then placed in a heated water bath and the alcohol driven off at reduced pressure and the volume was lowered to 25 cc. The flask was then removed and the sides washed down and 2 cc. of lead acetate added to precipitate the proteins and clarify the solution. The flask was shaken and the precipitate allowed to settle before filtering. The solution was filtered and washed with hot water several times. The lead was precipitated by the addition of 2 cc. of potassium oxalate solution after which it was well shaken and the lead oxalate allowed to settle. The solution was filtered into a 200 cc. volumetric flask. If care is taken to decant some of the supernatant liquid on to the filter paper first no trouble will be experienced in filtering the lead oxalate. The solution was made up to volume and mixed and a 50 cc. portion taken for inversion and analyzed for total sugars. This method of the inversion used is given on page 12 and it will not be repeated here. A second portion of the solution was taken for analysis of reducing sugars and a volume of either 5 or 10 cc. was used depending on the amount of sugar present. The complete detains of this method are given in the discussion of Reducing Sugars, on page 9.

PRESENTATION OF RESULTS

The chemical data secured from this investigation are presented in tabular and graphic forms as percentage of sugar on wet basis. This seemed desirable, since it would express the results on a truer basis of existing conditions. On this plan moisture was not taken for each set of plots, but a composite from all plots at each time of sampling.

The percentage of reducing sugar in apples during the growing period is given in Table XII, and presented in graphic form in figure 3. The curves show a tendency to follow each other with fair regularity. The large gap between the lower curve and the other two is due to the elapse of fifty five days between the samplings, while the period between the two upper curves is only twenty days. The highest percents of reducing sugars shown at the first date of sampling are both from plots receiving high nitrate fertilizers (plots 2 and 3, and 26 and 27). This same lead is maintained at the second date of sampling with approximately the same increase over the other plots. In looking at figure 4 which gives the total sugars over the same period, the same general relationship occurs there, the nitrate plots showing a higher percentage in the first two dates of sampling.

At the third date of sampling, September 7, 1933, the plots receiving potassium chloride showed the greatest percentage of both total and reducing sugars. This fact was expected as many authors have shown that potassium is necessary for sugar formation and that the use of potassium chloride increased the amount of sugar in sugar beets, tomatoes, potatoes, and grapes.[7] The effect of potassium on the samples collected on

September 7, 1933, was very pronounced as is seen in figures 3 and 4. Such results were looked for in later samplings, but this effect did not show up although an increase in sugar occured in all samples.

The curves for reducing and total sugars in figures 3 and 4 follow each other with close regularity. It is seen that the total sugars at the time of the first sampling are practically the same as the reducing sugar or in other words all the sugars present in the young apples are present as reducing sugars. The amount of total sugars increased regularly over the reducing sugar until the time of maturity (figures 3,4, and 5).

Samples collected at the time of harvest, September 26, 1933, (graphically shown in figure 5), were a complete reversal as to the indications given by the three preceeding samplings. Plot 23 and 24 stood out as having abnormally high sugar content both for reducing and total sugars. This plot had received a varied treatment of fertilizers from 1928 to 1932, but in 1933, 12 pounds of super phosphate, 3 pounds of sodium nitrate and 2 pounds of ammonium sulphate were added which constituted a heavy fertilizer treatment and this may partially account for the high sugar content. Why this abnormal jump in sugars should happen in a course of 19 days with no previous indication is hard to explain. In the three previous samples the sugar content of the apples from this plot was that of an average sample. The rest of the samples showed nothing striking or indicative and were all quite similar.