SOME APPLICATIONS OF ACID PERMANGANATE OXIDATION TO THE STUDY OF MOLECULAR WEIGHTS OF CARBOHYDRATES AND TO CARBOHYDRATE ANALYSIS

by

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A THESIS

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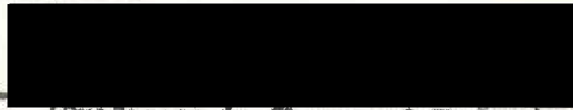

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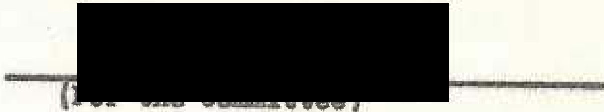

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INTRODUCTION

The purpose of this thesis is to show how potassium permanganate in acid solution may be employed as a tool, firstly for the delineation of the structure of the carbohydrate molecule and its products of oxidation, secondly for the determination of the molecular weight of carbohydrate compounds and thirdly for the quantitative determination of the concentration of carbohydrates in pure solution.

Potassium permanganate has been used for many years in the field of organic chemistry, both in synthetic and analytical capacities. The nature of the reduction of the permanganate ion when treated with an oxidizable organic material has been thoroughly investigated by Schilow (1) and more particularly by Launer (2) and Launer and Yost (3). The latter two workers not only arrived at an understanding of the mechanism of the reaction as regards the permanganate ion, but also, since they studied the oxalate-permanganate reaction of Sorensen (4) with regard to the stepwise products of oxidation, appear to be among the first investigators to account for all the processes and products accompanying the oxidation of an organic compound in acid potassium permanganate solution.

The use of oxidation procedures as an aid in the determination of the structure of carbohydrates is by no means an innovation. The most reliable methods for sugar analysis are applications of oxidation-reduction reactions and various of the methods commonly employed for characterising the chemical groups making up the carbohydrate molecule fall into this class. In general however, these oxidations have been largely confined to reactions in alkaline solution and a search of the literature for reference to the use of acid permanganate solution for the purpose with which this

investigation is concerned was fruitless. The multitudinous references regarding the oxidation of carbohydrate material by acid permanganate solution invariably described qualitative experiments, generally with the object of producing some single compound, an oxidation product of carbohydrate. The value of previous work then lay, not in its guidance for this study, but rather in its suggestion that an unexplored field could be readily approached through the use of acid permanganate.

PART I

RELATION OF PERMANGANATE OXIDATION TO STRUCTURE OF MONOSACCHARIDES AND RELATED COMPOUNDS

The studies of the reactions of potassium permanganate with carbohydrates and related substances were conducted in acid solutions. In such solutions permanganate is exceedingly reactive and reliable, and the monosaccharides and related alcohols and hydroxy acids undergo extensive oxidation with a rapidity closely comparable to that of oxalate, which latter reaction is classically used for the accurate standardization of potassium permanganate solutions. The rapid rate at which oxidation takes place gives to permanganate a unique position among oxidants. At temperatures from 90°-100°C. the reaction is complete in the time necessary for a dropwise titration. When contrasted with the rate of reaction encountered with other oxidants customarily used in carbohydrate study, which generally require several minutes treatment at boiling temperature and subsequent back titration to determine the concentration of unused reagent, the advantage is at once apparent.

It was found that when permanganate is applied to the oxidation of carbohydrate substances, the rate of reaction, within limits, is determined by the acidity of the solution, the temperature and the quantities of carbohydrate and permanganate present. This observation is in accord with the findings of Ridgeway (5) who states that reaction between glucose and permanganate in acid solution is accelerated by an increase in concentration of both glucose and acid.

The first phase of this experiment is concerned with the determination of satisfactory reagent concentrations, conditions for the reaction,

and the development of equipment. The oxidation reactions were studied both from the standpoint of the equivalency of the carbohydrate, expressed in terms of the equivalents of permanganate reduced per mole of sugar or sugar derivative, and the estimation of the probable course of the reaction as shown by analysis of the volatile substances formed.

The possibility of using the permanganate reagent for the quantitative estimation of carbohydrates by direct titration was investigated and will be considered in some detail in a later section.

EXPERIMENTAL

In order to determine satisfactory conditions for the oxidation of simple carbohydrates, experiments were conducted in which the acid and carbohydrate concentrations were varied. Solutions containing glucose in different concentrations were prepared. A volume of permanganate solution, which had been accurately standardized with sodium oxalate according to the method of Fowler and Bright (6) was added to these glucose solutions. Varying amounts of sulfuric acid were added to the mixtures and where possible pH determinations were made with the glass electrode. Experimental data are given in Tables I and II. The mixed test samples in sugar tubes were heated in the water bath for five minutes, rapidly cooled, and potentials determined by the potentiometer using a saturated calomel cell and rotating KCl-agar bridge Fig. I.

Two interesting points were noted as a result of varying the acid concentration. First, a concentration of acid greater than 0.5 N. sulphuric acid was necessary to dissolve the manganous oxide characteristically produced when permanganate ion is reduced, and second, it was noted in the experiment in which 3 N. acid was used that the color of the permanganate was immediately discharged upon placing the tubes into the boiling bath. It was suspected that glucose might react as rapidly with hot acid permanganate solution as does oxalic acid, in which case useful applications of the reaction might be possible.

In order to investigate the direct titration of carbohydrates, solutions of various substances containing 0.4 mg. per ml. were made up. Varying amounts of this stock solution were made up to a volume of 5 ml. and 2 ml. of concentrated sulphuric acid was added. The titrations were

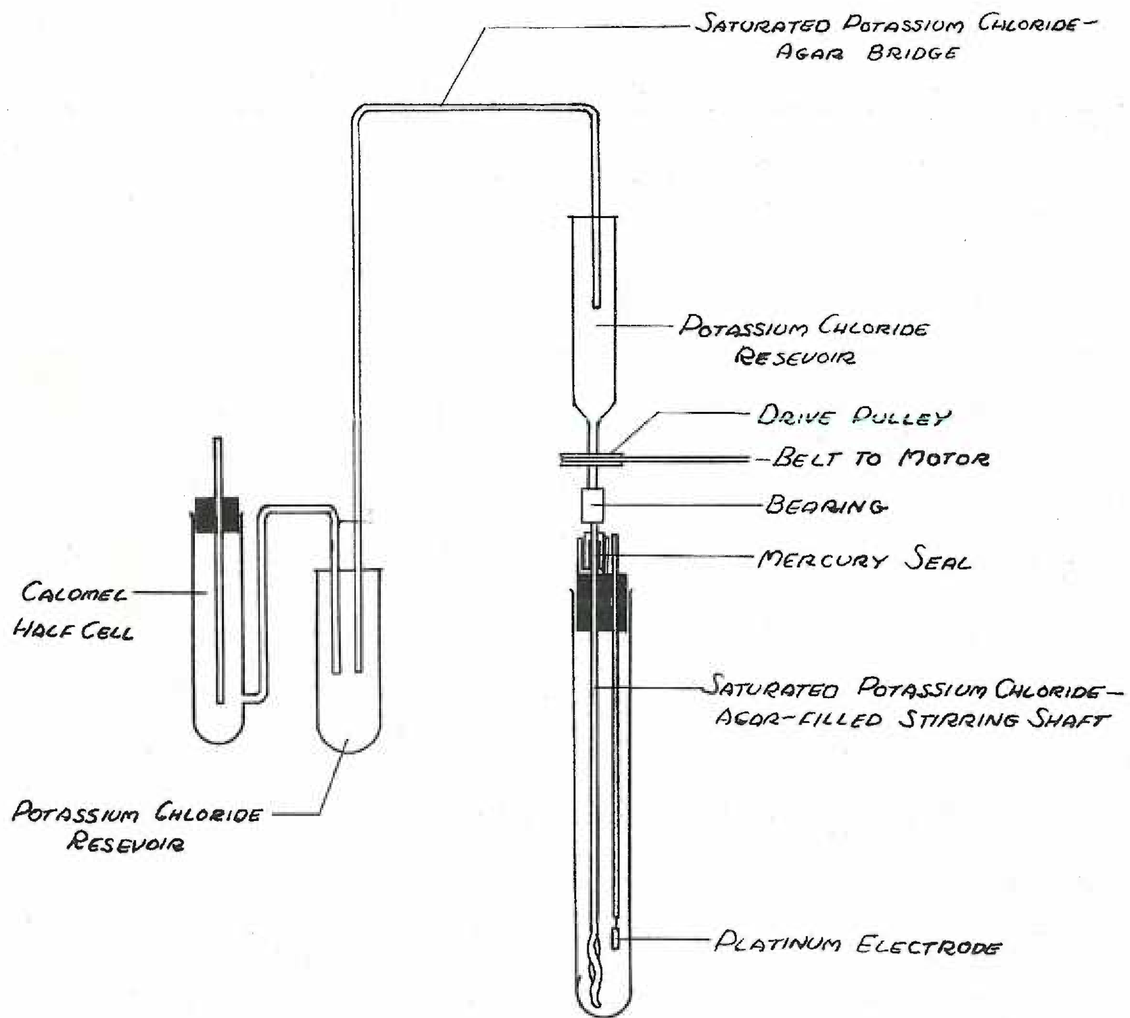


Figure 1

conducted in a 50 ml. Erlenmeyer flask at a temperature between 90°-100° C. and with constant shaking by means of the device illustrated in Fig. 2. Standard permanganate solution was added drop by drop until the first tinge of pink color, stable for thirty seconds, appeared. Tables III-XVI show the results of these titrations and the equivalency of the materials tested. In this case the equivalency represents the number of oxidation equivalent weights of permanganate (one-fifth of the gram molecular weight) used per mole of organic compound. This is true because the reduction of permanganate in acid solution involves the gain of five electrons. It may be calculated in the following equation.

$$E = \frac{Wp\ Mc}{Wc\ Ep} \tag{1}$$

where E is the equivalency as described above, Mc is the molecular weight of the carbohydrate, Wc and Wp are the weights of carbohydrate and permanganate used in the experiment and Ep is the oxidation equivalent of permanganate.

The d-dulcitol, d-sorbitol, d-arabinose and sucrose were obtained from the Pfanstiehl Chemical Company. d-Galactose and pentaerythritol were Eastman Kodak Company preparations. Appreciation is expressed to Dr. C. S. Hudson of the U. S. Bureau of Standards for a sample of d-glucoheptose and to Dr. J. W. E. Glattfeld of the University of Chicago for the samples of dihydroxy butyric acids. The tetramethyl glucose was prepared by the method of West and Holden

All substances were dried at 95°-100° C. in air except the four carbon acids which were assumed to be in a satisfactory condition without treatment.

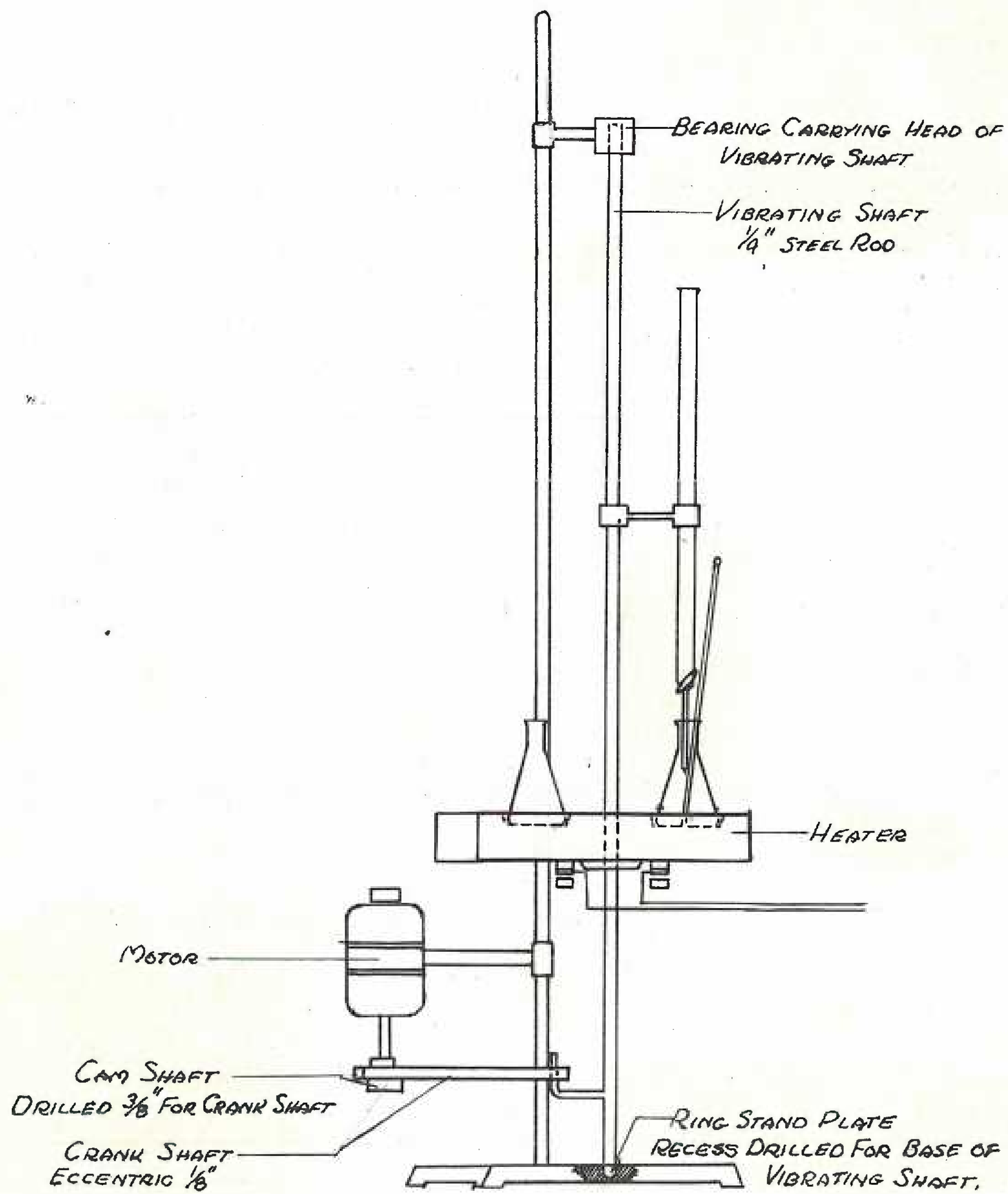


Figure 2

In order to establish some relationship between the degree of oxidation as measured by equivalency and as shown by the products of oxidation the carbon dioxide and volatible acid formed in the reaction were measured. 100 mg. of glucose was oxidised in the same manner as in the previous titrations. The reaction was conducted in a 250 ml. balloon flask connected with a tube containing carbon dioxide-free water to absorb volatile acids, and this tube in turn was connected with an Erlenmeyer flask containing 50 ml. of standard barium hydroxide for the absorption of carbon dioxide. The apparatus is shown in Fig. 3. The glucose solution was titrated to the pink end point with 1.000 normal permanganate. The mixture was boiled for five minutes to sweep carbon dioxide from the reaction flask and acid trap into the absorption flask which was then removed from the apparatus, rapidly covered with a rubber dam to prevent absorption of carbon dioxide from the air, and the excess barium hydroxide titrated using thymol blue as indicator. The contents of the acid trap were combined with the solution from the reaction vessel, which was evacuated to 50 mm. mercury. The mixture was then boiled and distillation continued until about half of the liquid had passed over. The receiving flask was then disconnected and titrated to the phenolphthalein end point with standard base. The flask was again connected, the apparatus evacuated and distillation continued. The receiving flask was again disconnected and titration carried out as above. The process was repeated until no further acid distilled. The quantity of formic acid was determined in the neutralized solution by the method of Fincke (7). The results are shown in Tables XVI-XVIII.

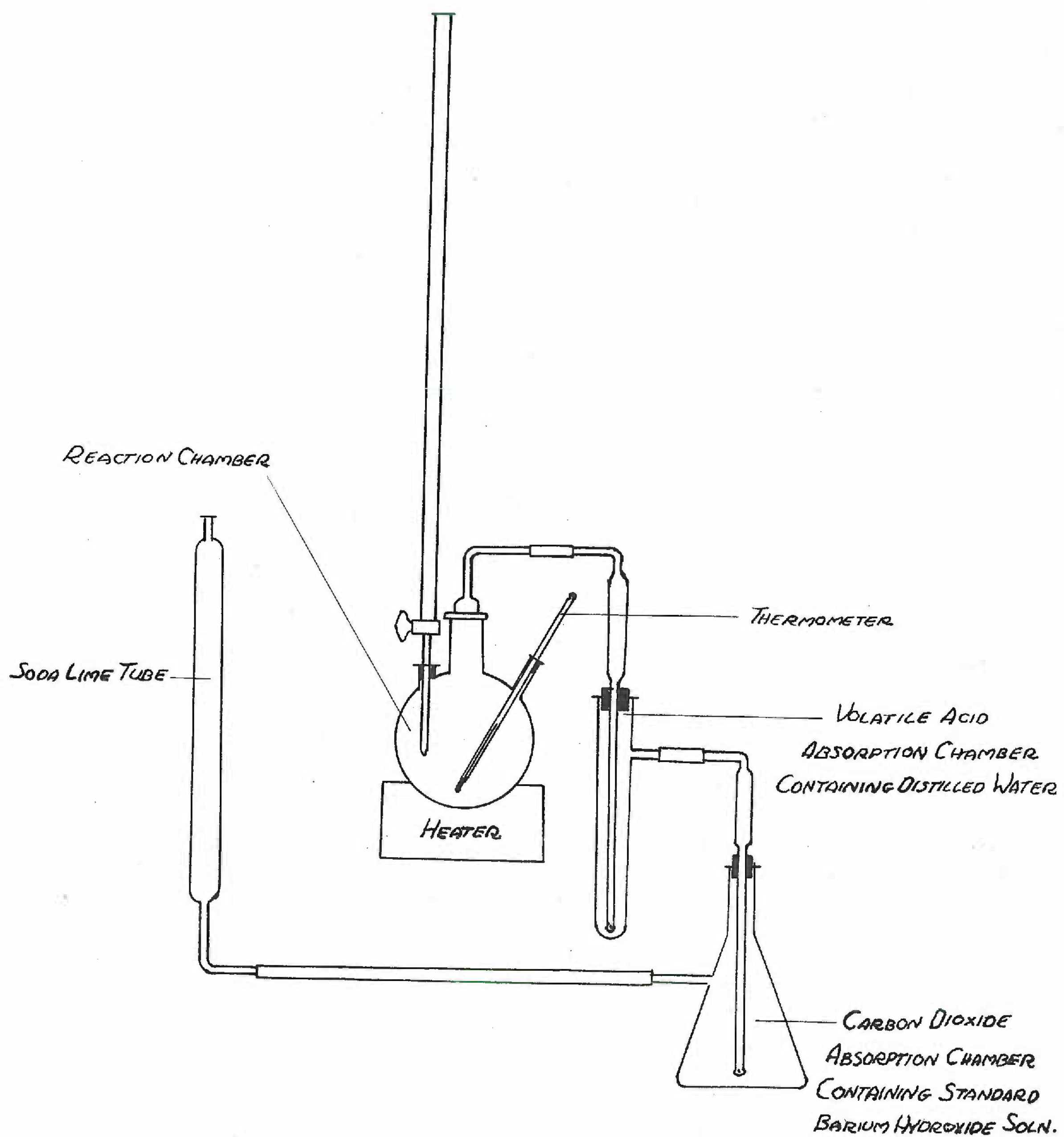


Figure 3

TABLE I

THE EFFECT OF ACID CONCENTRATION ON THE OXIDATION OF 2 mg. OF GLUCOSE BY 2 mg. OF PERMANGANATE*

TEMPERATURE 90°-100° C.

Acid Normality	Potential Volts	Appearance after Heating
0.1	unsteady	cloudy
0.1	"	"
0.5	"	"
0.5	"	"
1.0	0.3392	clear
1.0	0.3360	"
2.0	0.3412	"
2.0	0.2959	"
3.0	0.2700	"
3.0	0.2845	"

*Initial volume of solution 10 ml.

TABLE II

THE EFFECT OF GLUCOSE CONCENTRATION ON THE OXIDATION

OF GLUCOSE BY PERMANGANATE*

TEMPERATURE 90°-100°C.

Glucose mg.	Permanganate mg.	pH of Solution	Potential Volts
0.4	2.0	1.00	1.0986
0.4	2.0	1.00	1.0910
0.8	2.0	1.00	0.9000
0.8	2.0	1.00	0.8922
1.2	2.0	0.96	0.6613
1.2	2.0	0.96	0.6623
1.6	2.0	0.99	0.4173
1.6	2.0	0.99	0.4184
2.0	2.0	0.96	0.3872
2.0	2.0	0.96	0.3728

*Initial volume of solution 10 ml.

TABLE III

MACROTITRATION OF d-GLUCOSE WITH ACID PERMANGANATE

TEMPERATURE 90°-100° C.

Glucose in 5 ml. of Solution mg.	0.0126 Normal Permanganate ml.	Permanganate mg.	Equivalents of Permanganate per Mole of Glucose
0.4	1.92	0.764	
0.4	1.90	0.757	10.88
0.4	1.92	0.764	
0.8	3.81	1.519	
0.8	3.80	1.514	10.84
0.8	3.80	1.514	
1.2	5.70	2.272	
1.2	5.80	2.312	10.97
1.2	5.84	2.321	
1.6	7.68	3.060	
1.6	7.70	3.067	10.94
1.6	7.68	3.060	
2.0	9.61	3.824	
2.0	9.61	3.824	10.90
2.0	9.60	3.821	
		Average	10.91

TABLE IV

MACROTITRATION OF d-GALACTOSE WITH ACID PERMANGANATE

TEMPERATURE 90°-100°C.

Galactose in 5 ml. of Solution mg.	0.0126 Normal Permanganate ml.	Permanganate mg.	Equivalents of Permanganate per Mole of Galactose
0.4	1.92	0.764	
0.4	1.97	0.784	11.02
0.4	1.94	0.772	
0.8	3.85	1.532	
0.8	3.85	1.532	10.72
0.8	3.85	1.532	
1.2	5.82	2.320	
1.2	5.82	2.320	11.02
1.2	5.84	2.323	
1.6	7.88	3.140	
1.6	7.76	3.095	10.80
1.6	7.90	3.144	
		Average	10.89

TABLE V

MACROTITRATION OF d-ARABINOSE WITH ACID PERMANGANATE

TEMPERATURE 90°-100° C.

Arabinose in 5 ml. of Solution mg.	0.0126 Normal Permanganate ml.	Permanganate mg.	Equivalents of Permanganate per Mole of Arabinose
0.4	1.96	0.780	
0.4	1.95	0.776	9.23
0.4	1.96	0.776	
0.8	3.68	1.467	
0.8	3.69	1.470	8.73
0.8	3.69	1.470	
1.2	5.34	2.126	
1.2	5.34	2.126	8.57
1.2	5.34	2.126	
1.6	6.92	2.758	
1.6	7.01	2.798	8.18
1.6	6.97	2.780	
		Average	8.68

TABLE VI

MACROTITRATION OF d-GLUCOHEPTOSE WITH ACID PERMANGANATE

TEMPERATURE 90°-100°C.

Glucoheptose in 5 ml. of Solution mg.	0.0096 Normal Permanganate ml.	Permanganate mg.	Equivalents of Permanganate per Mole of Glucoheptose
0.4	2.62	0.794	
0.4	2.63	0.794	13.19
0.4	2.60	0.787	
0.8	5.12	1.549	
0.8	5.14	1.556	12.89
0.8	5.10	1.544	
1.2	7.66	2.315	
1.2	7.72	2.331	13.13
1.2	7.68	2.355	
		Average	13.04

TABLE VII

MACROTITRATION OF d-SORBITOL WITH ACID PERMANGANATE

TEMPERATURE 90°-100°C.

Sorbitol in 5 ml. of Solution mg.	0.0131 Normal Permanganate ml.	Permanganate mg.	Equivalents of Permanganate per Mole of Sorbitol
0.4	2.34	0.963	
0.4	2.34	0.963	13.36
0.4	2.32	0.956	
0.8	4.65	1.917	
0.8	4.69	1.933	13.88
0.8	4.68	1.930	
1.2	6.92	2.850	
1.2	6.95	2.868	13.78
1.2	7.00	2.884	
1.6	9.31	3.840	
1.6	9.30	3.835	13.78
1.6	9.27	3.821	
2.0	11.74	4.835	
2.0	11.76	4.840	14.01
2.0	11.84	4.878	
		Average	13.86

TABLE VIII

MACROTITRATION OF d-DULCITOL WITH ACID PERMANGANATE

TEMPERATURE 90°-100° C.

Dulcitol in 5 ml. of Solution mg.	0.0096 Normal Permanganate ml.	Permanganate mg.	Equivalents of Permanganate per Mole of Dulcitol
0.4	3.24	0.983	
0.4	3.24	0.983	14.18
0.4	3.23	0.980	
0.8	6.38	1.935	
0.8	6.38	1.935	14.02
0.8	6.38	1.974	
1.2	9.51	2.888	
1.2	9.46	2.872	13.83
1.2	9.48	2.879	
1.6	12.48	3.789	
1.6	12.56	3.815	13.72
1.6	12.56	3.815	
		Average	13.95

TABLE IX

MACROTITRATION OF d-GLUCONIC ACID WITH ACID PERMANGANATE

TEMPERATURE 90°-100° C.

Gluconic Acid in 5 ml. of Solution mg.	0.0143 Normal Permanganate ml.	Permanganate mg.	Equivalents of Permanganate per Mole of Gluconic Acid
0.4	1.69	0.764	
0.4	1.69	0.764	11.83
0.4	1.69	0.762	
0.8	3.52	1.594	
0.8	3.35	1.516	12.02
0.8	3.40	1.538	
1.2	4.98	2.255	
1.2	4.94	2.255	11.70
1.2	5.02	2.266	
1.6	6.68	3.025	
1.6	6.54	2.959	11.68
1.6	6.63	3.004	
		Average	11.81

TABLE X

MACROTITRATION OF PENTAERYTHRITOL WITH ACID PERMANGANATE

TEMPERATURE 90°-100°C.

Pentaerythritol in 5 ml. of Solution mg.	0.0131 Normal Permanganate ml.	Permanganate mg.	Equivalents of Permanganate Per Mole of Pentaerythritol
0.4	3.03	1.244	
0.4	3.08	1.264	13.57
0.4	3.05	1.252	
0.8	6.07	2.494	
0.8	6.12	2.508	13.47
0.8	6.15	2.514	
1.2	9.31	3.815	
1.2	9,32	3.819	13.78
1.2	9.37	3.851	
1.6	12.16	4.990	
1.6	12.10	4.968	13.43
1.6	12.06	4.959	
		Average	13.56

TABLE XI

MACROTITRATION OF SUCROSE WITH ACID PERMANGANATE

TEMPERATURE 90°-100° C.

Sucrose in 5 ml. of Solution mg.	0.0131 Normal Permanganate ml.	Permanganate mg.	Equivalents of Permanganate per Mole of Sucrose
0.4	2.06	0.846	
0.4	2.06	0.846	22.99
0.8	4.06	1.670	
0.8	4.11	1.690	22.90
0.8	4.16	1.711	
1.2	6.16	2.534	
1.2	6.17	2.537	22.93
1.6	8.42	3.460	
1.6	8.40	3.455	23.48
1.6	8.40	3.455	
2.0	10.30	4.219	
2.0	10.41	4.273	23.39
		Average	23.14

TABLE XII

MACROTITRATION OF TETRAMETHYL d-GLUCOSE WITH ACID PERMANGANATE

TEMPERATURE 90°-100°C.

Tetramethyl Glucose in 5 ml. Solution mg.	0.0130 Normal Permanganate ml.	Permanganate mg.	Equivalents of Permanganate per mole of Tetramethyl Glucose
0.4	1.54	0.632	
0.4	1.58	0.649	11.98
0.8	3.24	1.333	
0.8	3.19	1.312	12.42
0.8	3.21	1.321	
1.2	4.62	1.899	
1.2	4.57	1.880	11.82
1.6	6.35	2.602	
1.6	6.29	2.587	12.13
		Average	12.09

TABLE XIII

MACROTITRATION OF d,l-THREO 1, 2, DIHYDROXY BUTYRIC

ACID WITH ACID PERMANGANATE

TEMPERATURE 90°-100°C.

Dihydroxy Butyric Acid in 5 ml. of Solution mg.	0.0097 Normal Permanganate ml.	Permanganate mg.	Equivalents of Permanganate per Mole of Dihydroxy Butyric Acid
0.4	1.84	0.564	
0.4	1.86	0.572	6.06
0.4	1.80	0.552	
0.8	3.59	1.101	
0.8	3.58	1.099	5.94
0.8	3.62	1.111	
1.2	5.28	1.621	
1.2	5.28	1.621	5.84
1.2	5.28	1.621	
1.6	7.26	2.227	
1.6	7.35	2.255	6.04
1.6	7.26	2.227	
		Average	5.97

TABLE XIV

MACROTITRATION OF d.l-ERYTHRONIC ACID LACTONE WITH ACID PERMANGANATE TEMPERATURE 90°-100°C.

Erythronic Acid Lactone in 5 ml. of Solution mg.	0.0097 Normal Permanganate ml.	Permanganate mg.	Equivalents of Permanganate per Mole of Erythronic Acid Lactone
0.8	3.68	1.128	
0.8	3.70	1.135	5.18
0.8	3.70	1.135	

MACROTITRATION OF d.l-ARYTHRO 1. 2. DIHYDROXY BUTYRIC ACID WITH ACID PERMANGANATE TEMPERATURE 90°-100°C.

Butyric Acid in 5 ml. of Solution mg.	0.0097 Normal Permanganate ml.	Permanganate mg.	Equivalents of Permanganate per Mole of Butyric Acid
0.8	3.66	1.123	
0.8	3.67	1.126	6.07
0.8	3.64	1.117	

TABLE XV

SUMMARY OF EQUIVALENTS FROM TABLES III - XIV

Substances	Carbon Atoms in Molecule	Equivalents of Permanganate per Mole of Substance
Monosaccharides		
d-Glucose	6	10.81
d-Galactose	6	10.89
d-Arabinose	5	8.68
d-Glucoheptose	7	13.04
Sugar Alcohols		
d-Sorbitol	6	13.86
d-Dulcitol	6	13.95
Pentaerythritol	5	10.00
Acids		
d-Gluconic Acid	6	11.81
d,l-Threo 1,2 Dihydroxy Butyric Acid	4	5.97
d,l-Erythro 1,2 Dihydroxy Butyric Acid	4	6.07
d,l-Erythronic Acid Lactone	4	5.18
Substituted Monosaccharide		
d-Tetramethyl d-Glucose	10	12.09
Disaccharide		
Sucrose	12	23.14

TABLE XVI

PRODUCTS OF OXIDATION OF d-GLUCOSE WITH PERMANGANATE

TEMPERATURE 90°-100°C.

Glucose mg.	Volatile Acid ml. of 0.1 N	Formic Acid mg.	Carbon Dioxide mg.
100	5.2		23.39
100	4.6	22.12	22.94
100	4.4	22.91	23.37
100	4.4	22.85	22.72
100	4.0	22.28	23.16
100	5.3		23.80
100	5.1		23.55

TABLE XVII

PRODUCTS OF OXIDATION OF GLYCOGEN WITH PERMANGANATE

TEMPERATURE 90°-100° C.

Glycogen mg.	Volatile Acid ml. of 0.1 N.	Formic Acid mg.	Carbon Dioxide mg.
100	5.6	21.65	19.93
100	6.2	21.59	19.84
100	6.5	21.93	19.98
100	6.9	22.10	20.12
100	5.9		19.87
100	6.3		20.05

TABLE XVIII

PRODUCTS OF OXIDATION OF d-SORBITOL WITH PERMANGANATE

TEMPERATURE 90°-100°C.

Sorbitol mg.	Volatile Acid ml. of 0.1 N.	Formic Acid mg.	Carbon Dioxide mg.
100	6.8	12.33	20.00
100	7.2	13.62	20.36
100	7.0	12.48	22.19
100	6.5	11.72	20.46
100	6.2		22.15
100	7.1		23.55

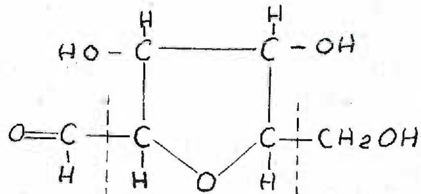
DISCUSSION

The factors of concentration, both of the carbohydrate substance and the sulphuric acid, and their effects upon the oxidation of glucose by permanganate were investigated. The most satisfactory conditions obtain when the proportions of glucose and acid are in the neighborhood of 2 mg. of glucose to 10 ml. of 3 N. sulphuric acid. At this concentration and at a temperature above 90°C. the oxidation takes place rapidly, and reproducible potential readings are obtainable with the modification of the potentiometric apparatus shown in Fig. 1.

A further study of the oxidation reaction with regard to the equivalency of various carbohydrates and related substances resulted in the data as summed up in Table XII.A relationship between structure of the molecule and its equivalency is apparent. In terms of equivalent weights of permanganate per mole of substance oxidized it appears that the pentoses have a value in the region of nine equivalents, hexoses eleven equivalents and heptoses thirteen equivalents. Contrasted with these values are the values of the six carbon sugar alcohols which have a value of about fourteen equivalents. Gluconic acid, the six carbon oxidation product of glucose has an equivalency value between those of glucose and sorbitol which appears to be about twelve equivalents. The titration of four carbon acids resulted in similar results for d-l-threo 1, 2 dihydroxy butyric acid and d-l erythro 1, 2 dihydroxy butyric acid, two isomeric compounds, but gave a difference of about one equivalent in the titration of d-l erythronic acid lactone and the above acids.

Tables XVI-XVIII present the data obtained for the carbon dioxide, total acid, and formic acid produced by the oxidation of glucose, sorbitol

and glycogen. It will be noted that the quantity of these substances recovered in the case of glucose and glycogen closely approach the values which might be expected if glucose in acid solution be assigned the methyl fufural formula.



If oxidation of the above substances took place by first removing the substituents from the furane ring and subsequently converting the hydroxy methyl portion to formic acid and the aldehyde residue to carbon dioxide and water a theoretical yield of 25.6 mg. of formic acid and 24.5 mg. of carbon dioxide would be expected from 100 mg. of glucose. The acidity represented by this quantity of formic acid would be equivalent to 5.5 ml. of 0.1 N. base. By comparison of these values with those obtained experimentally it would appear that glucose is oxidized under the experimental conditions as suggested. Glycogen which evidence shows to hydrolyze to glucose in strong acid solution gave similar results. It should be mentioned at this point that about fifty percent more time is required for the complete titration of glycogen than for glucose. Sorbitol, which does not form a furane derivative in acid solution, appears to be oxidized by a different and perhaps more complex method.

PART II

THE DETERMINATION OF THE MOLECULAR WEIGHT OF POLYSACCHARIDES BY THE USE OF ACID POTASSIUM PERMANGANATE SOLUTION

Numerous attempts have been made to determine the molecular mass of higher polysaccharide molecules. As a result of the varied nature of these studies considerable confusion has arisen regarding the common term "Molecular Weight" as it has been applied to carbohydrates and other high molecular weight compounds.

Early cryoscopic studies of Pringsheim (8) gave to glycogen acetate a molecular magnitude of 486.29. Great care was taken to point out that the material analyzed dissolved in water without opalescence, but showed the unaltered iodine coloration of glycogen and might, therefore, be assumed to be composed of the same number of monosaccharide units as glycogen.

Herzog (9) using resorcinol as solvent for glycogen found a molecular weight of 648.28 by cryoscopic measurement.

Studies by Haworth (10), Bell (11) and Bell and Young (12) which depended upon complete methylation of glycogen followed by hydrolysis of the product and separation of tetramethyl glucose from trimethyl glucose have given values between 1963 and 2935.

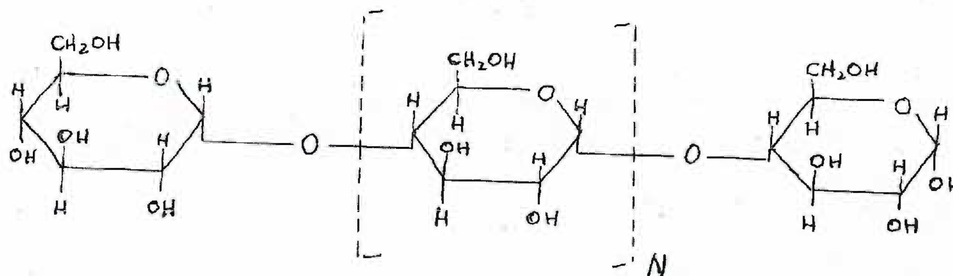
The cryoscopic methods as applied to higher carbohydrate molecules either do not give true molecular weights of the dissolved molecules in the ordinary chemical sense, or if they do, the process of solution in the solvent leads to profound disaggregation if values obtained by other methods are at all valid. The methylation end-group assay method of Haworth depends upon the presence of an extra hydroxyl group in one of

the two monosaccharides constituting the ends of a glucosidic chain, and presupposes a knowledge of the exact chemical constitution of the carbohydrate molecule. Limitations of the end-group assay method have been pointed out by Hess and Lung (13). Molecular weights by this method are considered to represent the masses of the smallest particles held together by primary valence forces, or so-called true molecular weights.

In contrast with the above methods several attempts have been made to determine the micellar size of glycogen aggregates in colloidal solution. These purely physical methods of testing have lead to widely divergent results, varying from Samec's value of 114,000 obtained by osmotic pressure measurements to the recent value of 1,296,560 as determined by Von Ardenne (14) employing the electron microscope and of Hausemann and Ruska (15) who obtained values between 65,000 and 1,500,000 by osmotic measurements. Unfortunately these values have been loosely termed "Molecular Weights". They are preferably considered as "Micellar Weights" which undoubtedly in these cases represent aggregations of fundamental molecules through secondary forces.

It should be pointed out that the values set forth in this thesis were obtained by a chemical method and might therefore be expected to conform with values obtained by previous chemical procedures.

The determination of the molecular weight of glycogen by potassium permanganation oxidation depends upon the well substantiated fact that glycogen is made up of a number of glucopyranoside molecules joined together in a chain-like aggregate by means of a-glucosidic linkages. This type of linkage is accomplished by splitting out one molecule of water in part from each of the glucose units to be joined.



The use of permanganate oxidation for determination of the molecular weight of glycogen and other polysaccharides is based upon a definite oxidation equivalent of permanganate for each monosaccharide unit (such as glucose) of the carbohydrate molecule liberated by hydrolysis in the hot acid solution. Since in this process of hydrolysis water is added at each glucosidic linkage, and the weight of monosaccharide exceeds the weight of polysaccharide by an increment determined by the number of glucosidic linkages in the molecule, the weight of permanganate per unit weight of polysaccharide increases with increasing molecular size.

It will be noted that whereas the molecular weights of hexoses are 180.096 such disaccharides as sucrose or trehalose have molecular weights 2x180.096 - 18.016 in which 18.016 is the value for the molecular weight of the water that is lost in the combination. Since water is a product of the combination of monosaccharides to form polysaccharides its loss in effect leaves the disaccharide molecule more concentrated as regards oxidizable material. The reduction of permanganate by a disaccharide, will then be, not idêntical with that of two molecules of monosaccharide but somewhat greater as shown by the following statements:

$$\frac{\text{2 m.w. monosaccharide}}{\text{2 m.w. monosaccharide - m.w. water}} \qquad (2)$$

or: in case the monosaccharide is a hexose

$$(3) \quad \frac{2 \times 180.096}{(180.096 \times 2) - 18.016} = 1.0527 \text{ times greater}$$

than the value for the hexose in monosaccharide.

A general formula for the calculation of the reducing capacity constant, R, for a given polysaccharide composed of hexose units would take the form:

$$(4) \quad R = \frac{180.096\ Y}{(180.096\ Y) - 18.016(Y-1)}$$

where Y is the number of hexose units in the carbohydrate molecule the factor, R, so obtained when multiplied by the permanganate required for the oxidation of a weight of hexose equal to that of the polysaccharide, represents the theoretical permanganate reduction of the polysaccharide. This equation can be simplified as follows:

$$(5) \quad R = \frac{180.096\ Y}{18.016(9.9964\ Y - Y + 1)}$$

or:

$$(6) \quad R = \frac{9.9964\ Y}{8.9964\ Y + 1}$$

solving for Y

$$(7) \quad 9.9964\ Y = 8.9964\ RY + R$$

$$(8) \quad R = Y(9.9964 - 8.9964\ R)$$

$$(9) \quad Y = \frac{R}{9.9964 - 8.9964\ R}$$

From formula 6 a series of values for the relative reducing power of polysaccharides as compared with their constituent monosaccharides may be calculated. Table XIX shows such theoretical values. It is apparent that factor R, increases progressively with increase in the molecular weight of the carbohydrate, but that the increment of progression grows

TABLE XIX

THE MOLECULAR WEIGHTS AND THEORETICAL REDUCTION CONSTANTS OF POLYSACCHARIDES

Hexose Units Y	Uncombined Molecular Weight (Mm Y)	Combined Molecular Weight of Polysaccharides (Mm Y)-19.016(Y-1)	Reduction Constant R	Weight of Permanganate Reduced by 10 mg.of Carbohydrate
1	180.096	180.096	1.0000	27.332
2	360.192	342.176	1.0527	28.772
3	540.288	504.256	1.0714	29.284
4	720.384	666.336	1.0811	29.549
5	900.480	828.416	1.0869	29.707
6	1080.576	990.446	1.0909	29.816
7	1260.672	1152.576	1.0939	29.898
8	1440.768	1314.656	1.0959	29.953
9	1620.864	1476.736	1.0975	29.997
10	1800.960	1638.816	1.0989	30.035
11	1981.056	1800.896	1.1000	30.065
12	2161.152	1962.976	1.1009	30.090
13	2341.248	2125.056	1.1017	30.112
14	2521.344	2287.136	1.1023	30.128
15	2701.440	2449.216	1.1029	30.142
16	2881.536	2611.296	1.1034	30.158
17	3061.632	2773.376	1.1038	30.169
18	3241.728	2935.456	1.1042	30.180
19	3421.824	3097.536	1.1046	30.191
20	3601.920	3259.616	1.1049	30.199
21	3782.016	3421.696	1.1053	30.210
22	3962.112	3583.776	1.1055	30.216
23	4142.208	3745.856	1.1057	30.221
24	4322.304	3907.936	1.1060	30.229
25	4502.400	4070.016	1.1062	30.235
26	4682.496	4232.096	1.1064	30.240
27	4862.592	4394.176	1.1066	30.246
28	5042.688	4556.256	1.1068	30.251
29	5222.784	4718.336	1.1069	30.254
30	5402.880	4880.416	1.1070	30.257
31	5582.976	5042.496	1.1072	30.262
32	5763.072	5204.576	1.1073	30.265
33	5943.168	5366.656	1.1074	30.267
34	6123.264	5528.736	1.1075	30.270
35	6303.360	5690.816	1.1076	30.273
36	6483.456	5852.896	1.1077	30.276

smaller with each monosaccharide unit added to the molecule. Figure 4 shows the calculated values of R, plotted on the ordinate against the number of hexose units, Y, in the molecule on the abscissa.

The values of the factor, R, as calculated above may be applied to the actual determination of the factor, Y, or to the determination of the molecular weight of a polysaccharide by first determining the quantity of permanganate reduced by a definite weight of the pure hexose which composes the polysaccharide and by a similar weight of the polysaccharide in question. If the weight of permanganate reduced by the monosaccharide is multiplied successively by each of the values of R, a column may be added to the above table which will state directly the weight of permanganate which must be used for the oxidation of the series of polysaccharides made up of increasing numbers of units of the monosaccharide whose equivalency was originally determined. From such a table the number of monosaccharide units may be read from the weight of permanganate reduced, and the molecular weight may be determined by simple multiplication.

An equation for the direct calculation of the molecular weight, Mp, of a polysaccharide made up of hexose sugars can be derived from equation 5 and from the fact that the number of units Y, in the carbohydrate times the molecular weight of the hexose, Mm, less the weight of water lost in the combination is equal to the molecular weight, or:

$$(10) \quad Mp = Mm\ Y - 18.016(Y - 1)$$

combining equations 5 and 10

$$(11) \quad Mp = Mm \frac{R}{(9.9964 - 8.9964\ R)} - 18.016 \frac{R}{(9.9964 - 8.9964\ R)} - 1$$

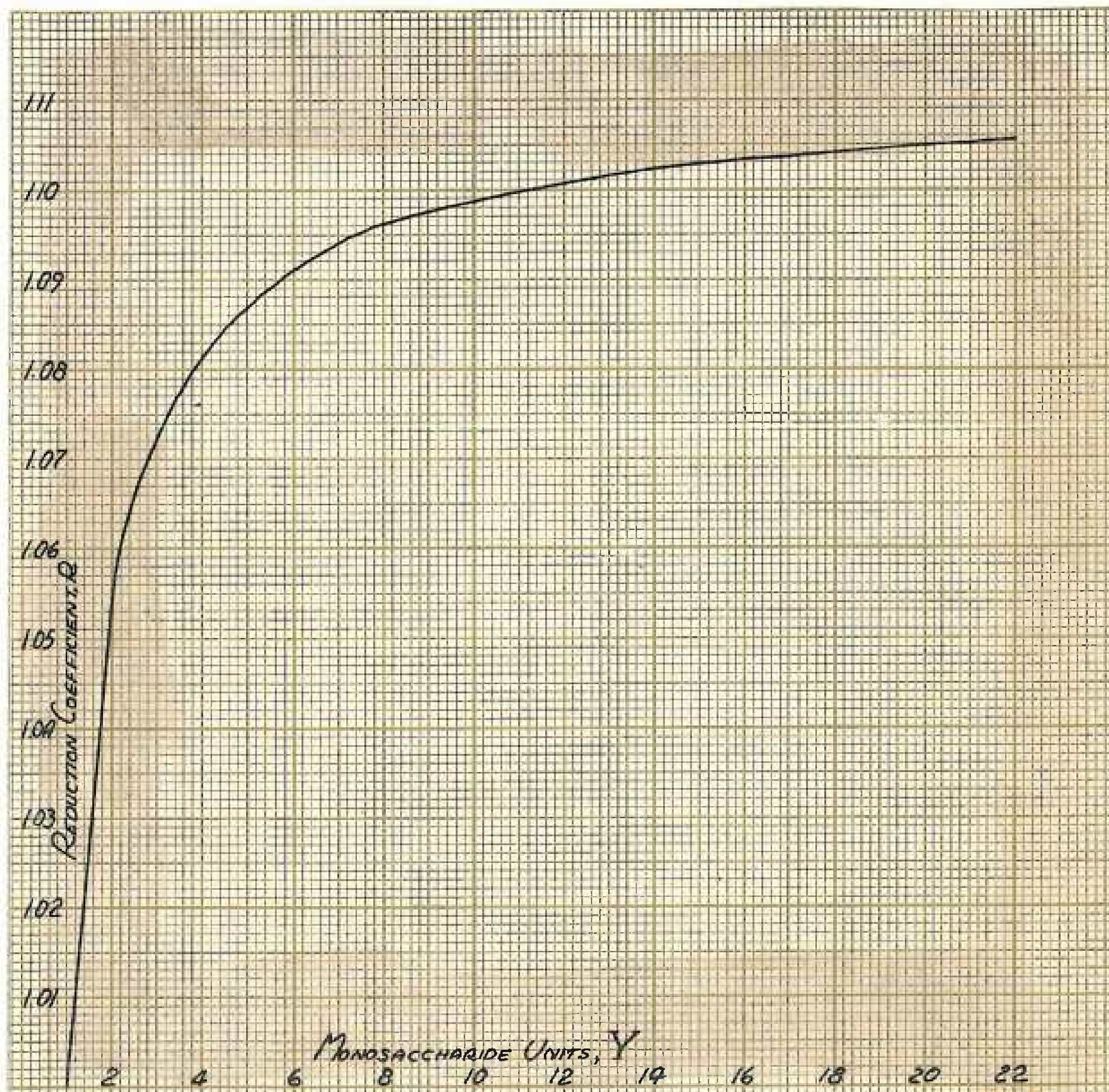


Figure 4

The last column of Table XIX gives calculated weights of permanganate for polysaccharides of various molecular size. These weights were calculated from the values of R, and the experimentally determined weight of permanganate required for the oxidation of 10.000 mg. of glucose.

The following sections present work on the molecular weights of sucrose and trehalose, two well characterized disaccharides and demonstrate that the experimental method employed is capable of yielding accurate values as calculated by the method described. Data regarding a number of carefully purified glycogens obtained from oysters and from the livers of rats and rabbits fed on glucose and galactose are also presented as well as a determination of the molecular weight of inulin.

MOLECULAR WEIGHT OF SUCROSE AND TREHALOSE

It was pointed out above that the difference in reducing capacity between compounds composed of more than six units of monosaccharide is relatively small. This difference decreases as the carbohydrate molecule becomes more complex. The actual difference in the weights of permanganate reduced by 10 mg. of a 17 and an 18 hexose unit compound would be about 0.010 mg. In terms of 0.003 N. permanganate solution this weight would represent very nearly 0.1 ml. of volume since a 0.003 N. permanganate solution contains 94.818 mg. per 1000 ml. of solution. The quantity 0.010 mg. is well within the range of the Kuhlmann micro-balance and it is not impossible to obtain significant results in comparing differences in volumes of 0.1 ml. if a satisfactory micro-burette is used. In order to insure a maximum of accuracy, however, it was believed that a statistical method in which the final value is taken as the average of numerous titrations of separate samples would give the best results. This method was used throughout the molecular weight studies of higher carbohydrates.

Table XIX indicates that a given weight of a disaccharide should have a reducing power 1.0527 times as great as that of a similar weight of the monosaccharide of which it is composed. In order to determine whether or not theoretical values could be obtained experimentally, trehalose and sucrose, which are composed of glucose and glucose, and of glucose and fructose respectively, were selected. Monosaccharides used were glucose obtained from the U. S. Bureau of Standards which had been dried three days at 76°C. in vacuo over phosphorous pentoxide. This material contained 0.03% ash. Eastman Kodak Company fructose was recrystalized several

times with dry alcohol and contained after purification 0.017% ash. The trehalose used was obtained from the Pfanstiehl Chemical Company and was found to contain 0.081% of ash. The sucrose was from the Eastman Company. It was purified by means of the method of fractional electrical transport (16) and contained 0.023% of ash. Before titration the carbohydrates were dried at 76°C. over phosphorous pentoxide until free of moisture.

EXPERIMENTAL

The method used depended upon the titration of 10,000 mg. of the carbohydrate by its equivalent of acid permanganate. To overcome the use of excessive volumes of solution, 10,000 mg. of carbohydrate was weighed out on the micro-balance and a weight of dry permanganate, sufficient to bring the oxidation of the carbohydrate almost to completion, was added. Oxidation was completed by titration with standard permanganate solution. 5 ml. of redistilled water was added gradually in 45 seconds, followed by 2 ml. of Kahlbaum sulfuric acid, which was added dropwise over a period of one minute. The mixture was then placed on the heater and maintained at 95°C ±5°C. Exactly fifteen minutes elapsed from beginning of the addition of water until the first of the permanganate solution was run in. This solution was made up to about 0.003 normal, the accurate value is given with the experimental data, and was added in quantities of 0.50 ml. every minute. To accomplish this the shaker was stopped, the solution run in, and the shaker started, a process which invariably took very close to four seconds. The potential of the cell formed between the burette-tip electrode and a platinum electrode immersed in the solution titrated was read off by means of a potentiometer 30 seconds after the addition of the permanganate solution. Thirty seconds elasped between observation of potential and addition of permanganate. Thus a complete cycle, of adding solution and noting the potential, took place every 60 seconds.

The end point of the titration was indicated by a sharp drop in potential reading, providing the carbohydrate and permanganate, added dry to the flask at the beginning, were so balanced that from 7 ml. to

18 ml. of the standard permanganate was adequate to complete the oxidation. When the two substances were not present in this ratio it was impossible to reach a sharp end point, and numerous preliminary experiments were necessary in order to determine the ratio.

The burette-tip cell as illustrated in Figure 5 gave excellent potential readings when immersed in a solution which was being constantly stirred. It will be seen from the figure that the cell is made up of a platinum wire which is so placed in the tip of a micro-burette that it makes contact with the solution being titrated through a column of standard permanganate. The reproducibility of this electrode was demonstrated by employing it in the determination of the concentration of permanganate solutions by oxidation of sodium oxalate. In these standardizations the same quantities of water and sulphuric acid were added to the oxalate as in the method used for the carbohydrate determination. This not only served to indicate the strength of the permanganate solution, but also to obviate the necessity of titrating blanks made up of water and acid in the quantities used throughout the molecular weight experiments. The potentiometric standardization of the permanganate checked with the ordinary visual oxalate titration.

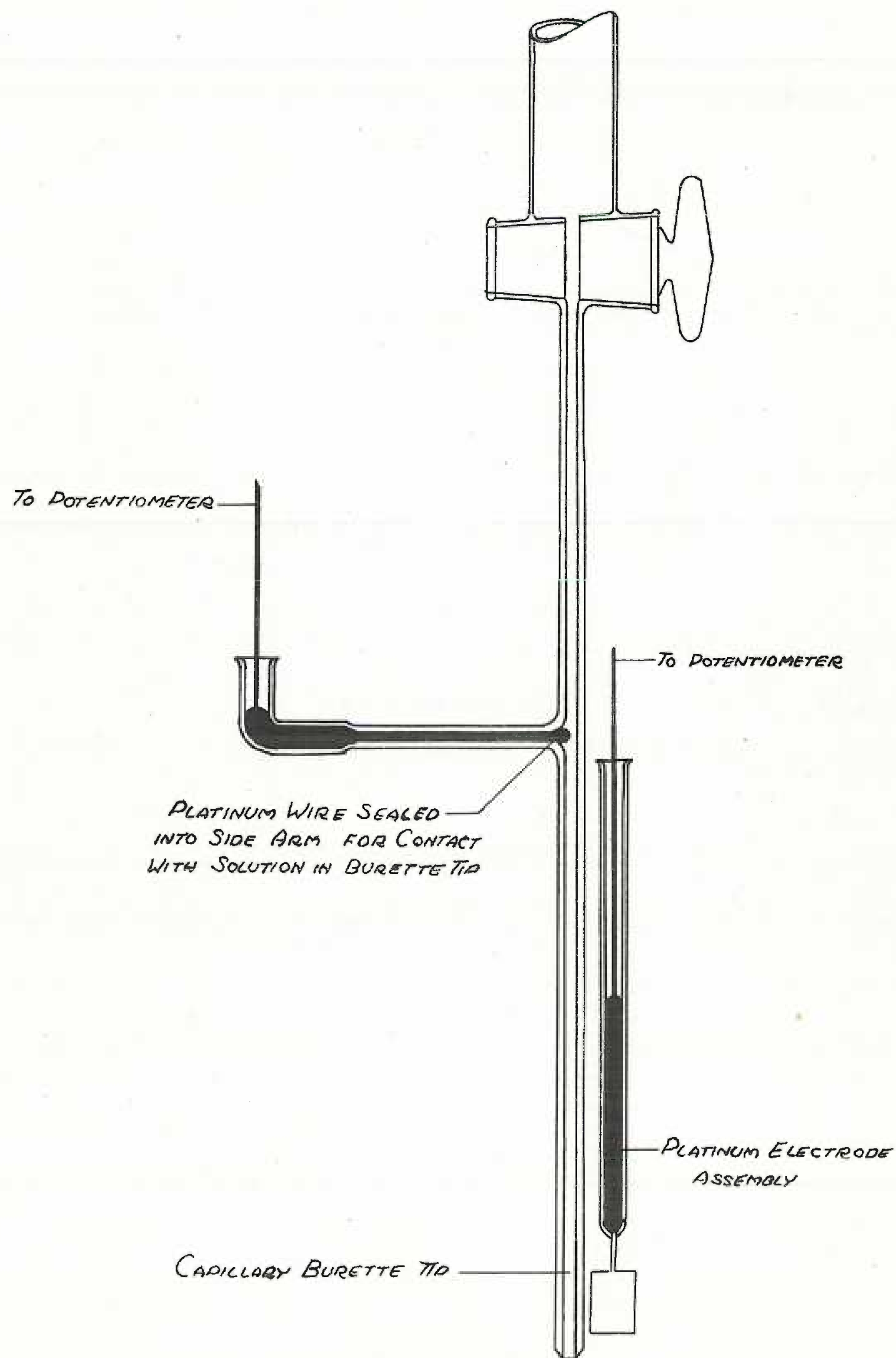


Figure 5

DATA

The results of the experiments are presented in the form of composite curves. In preparing this type of curve the data from each titration in the experiment was plotted on the same sheet of paper. The result was a number of lines grouped sufficiently close together that some difficulty arose in interpreting the figure. It was believed that an average curve would present the data in a clearer fashion. Accordingly, the entire area enclosed between the curves giving the highest and lowest values has been blacked in while the average of all the titrations has been left as a white line through the composite curve. The total width of the heavy black line shows the maximum variations that were obtained in the experiment. The end point selected is the midpoint in the break of the average curve and this point has been marked out by a horizontal line which is labelled to show the weight of permanganate reduced by 10.000 mg. of the carbohydrate in question. The number of hexose units making up the polysaccharide molecule is obtained from this value and the data of column five in Table XIX.

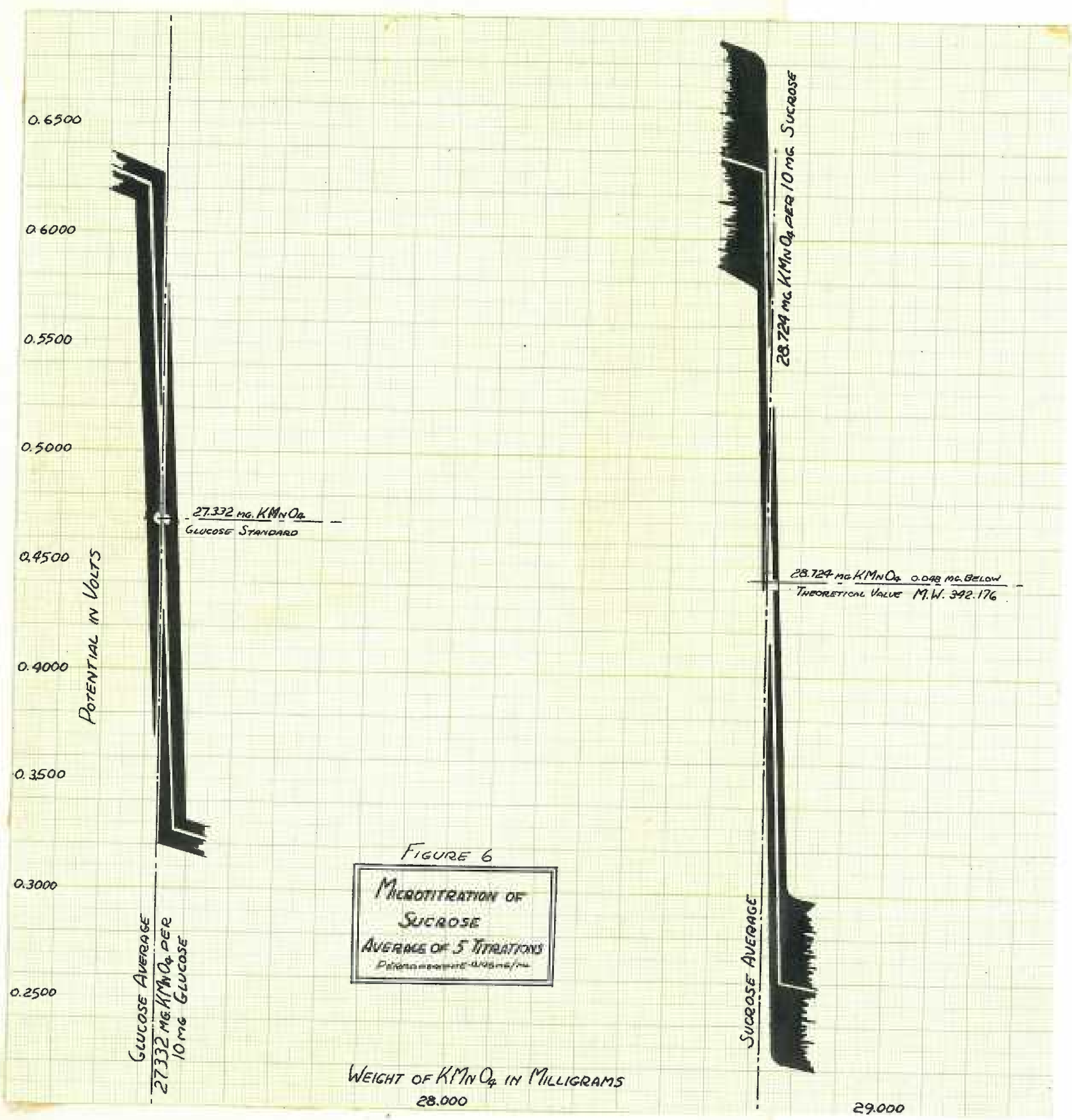
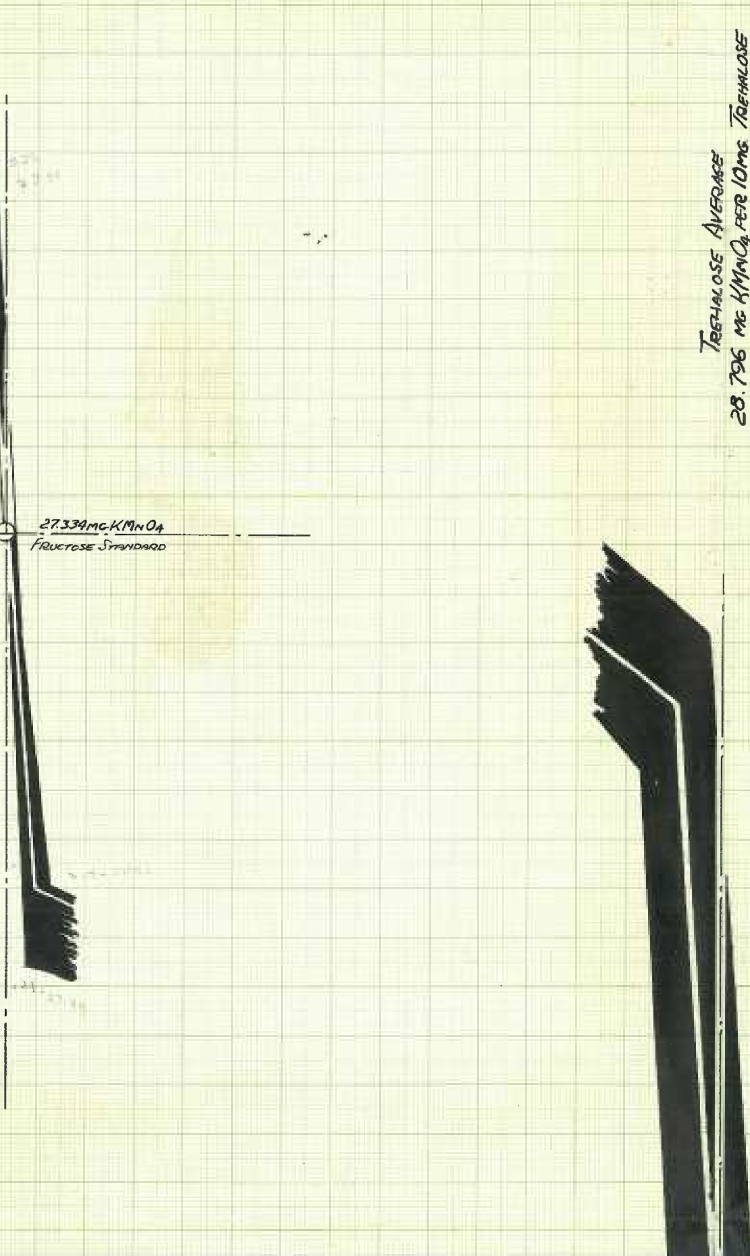
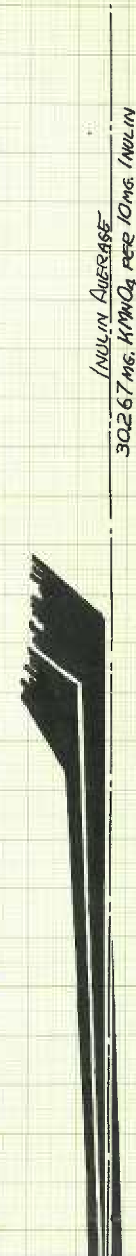
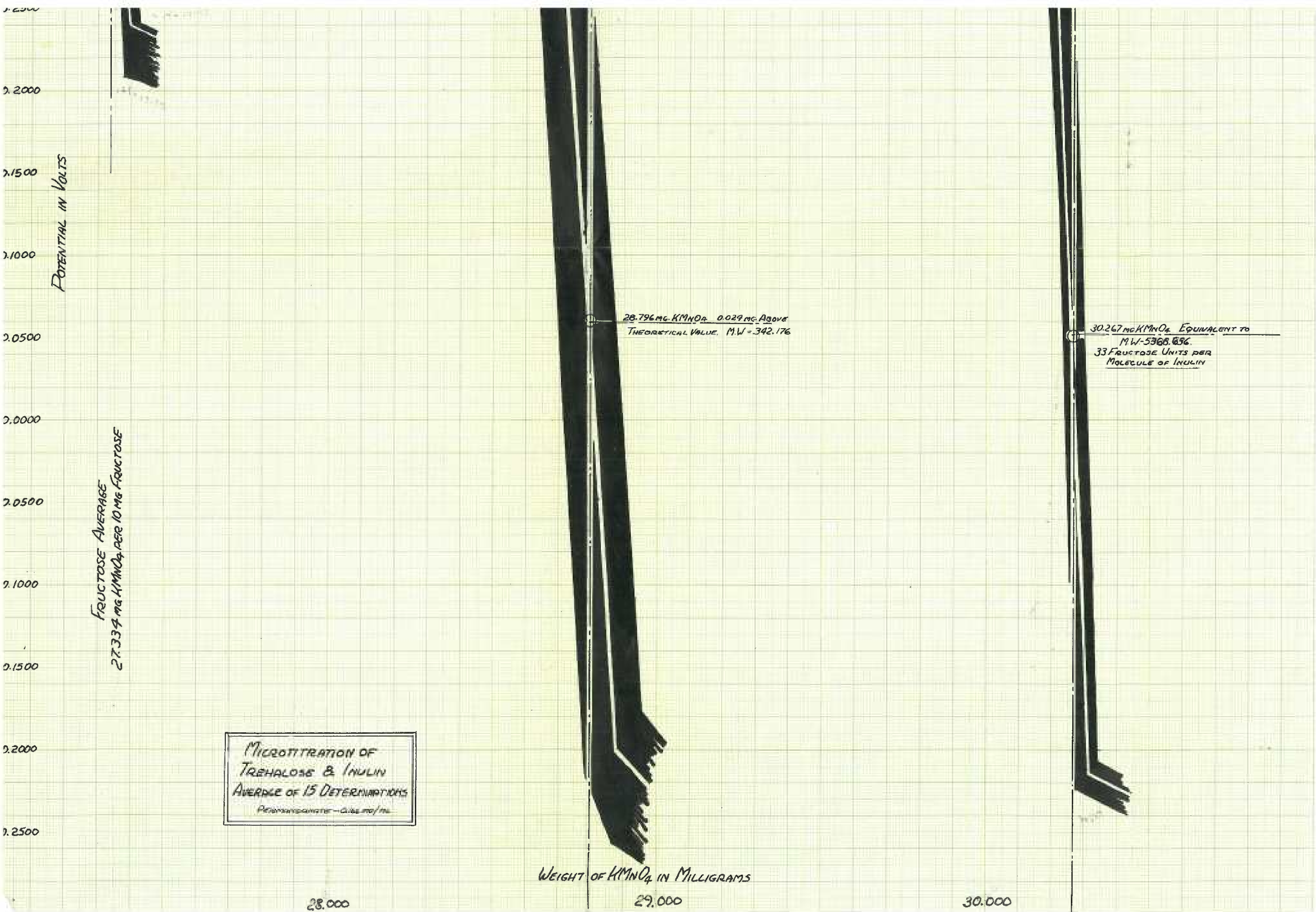


Figure 6
Microtitration of
Sucrose
Average of 5 Titrations
Potential in Volts
0.6500
0.6000
0.5500
0.5000
0.4500
0.4000
0.3500
0.3000
0.2500
27.332 mg. KMnO4
Glucose Standard
28.724 mg. KMnO4 per 10 mg. Sucrose
28.724 mg. KMnO4 0.048 mg. Below
Theoretical Value M.W. 342.176
Glucose Average
27.332 mg. KMnO4 per
10 mg Glucose
Sucrose Average
Weight of KMnO4 in Milligrams
28.000
29.000


0.6000
0.5500
0.5000
0.4500
0.4000
0.3500
0.3000
0.2500
0.2000
+0.1500
0.1000
POTENTIAL IN VOLTS
27.334 MG. KMnO4
FRUCTOSE STANDARD
TREHALOSE AVERAGE
28.796 MG. KMnO4 PER 10 MG. TREHALOSE


INULIN AVERAGE
30.267 MG. KMnO4 PER 10 MG. INULIN



.2000
.1500
.1000
.0500
.0000
.0500
.1000
.1500
.2000
.2500
Potential in Volts
Fructose Average
27.334 mg KMnO4 per 10 mg Fructose
28.796 mg. KMnO4 0.029 mg. Above
Theoretical Value. M.W = 342.176
30.267 mg KMnO4 Equivalent to
M.W-5368.656.
33 Fructose Units per
Molecule of Inulin
Microtitration of
Trehalose & Inulin
Average of 15 Determinations
Weight of KMnO4 in Milligrams
28.000
29.000
30.000

MOLECULAR WEIGHT OF OYSTER GLYCOGEN

Two samples of oyster glycogen, one prepared according to the method of West and Scott (17) and the other by the method of Bell and Young (12) were employed in the experiments. The materials as produced by these methods were further purified by means of the method of fractional electrical tranport of Williams (16). Equipment of the type shown in Figure (8) was found to be satisfactory. This cell is made up of five pyrex Erlemeyer flasks of 50 ml. capacity joined together by four inverted Y tubes. Platinum electrodes were placed in the end flasks. The cell was charged by placing redistilled water in all except the central flask and filling the latter with the glycogen solution to be purified.

Under a potential ranging from 15,000 volts at the start of electrolysis to 5,000 volts toward the close of treatment, and a current of from 300 to 5 milliampers, the ash content decreased to 0.03% and 0.012% respectively. The pH values of the cells at the start and finish of treatment were taken by means of the Beckman glass electrode. The glycogen failed to migrate under the influence of the current and was recovered from the central cell, and the solution evaporated in vacuo over flake sodium hydroxide as the dissicant. The clear glassy glycogen was finely pulverized and dried for three days in vacuo over phosphorous pentoxide at a temperature of 76° C The following table shows typical pH values of the five cells after seventy-two hours treatment. The difference in pH between the cells (Table XX) show that appreciable concentrations of acid and base ions were removed from the glycogen solutions upon application of the electric current.

TABLE XX

EFFECT OF ELECTROLYSIS UPON pH OF SOLUTIONS

IN ELECTROLYTIC CELLS

West and Scott Glycogen

Cell	pH before treatment	pH after treatment	Remarks
1	5.80	6.18	Cathode
2	5.79	5.86	
3	5.68	5.76	Glycogen
4	5.79	5.68	
5	5.79	4.94	Anode

Bell and Young Glycogen

Cell	pH before treatment	pH after treatment	Remarks
1	5.92	7.49	Cathode
2	5.92	6.86	
3	5.65	5.70	Glycogen
4	5.92	5.21	
5	5.92	4.21	Anode

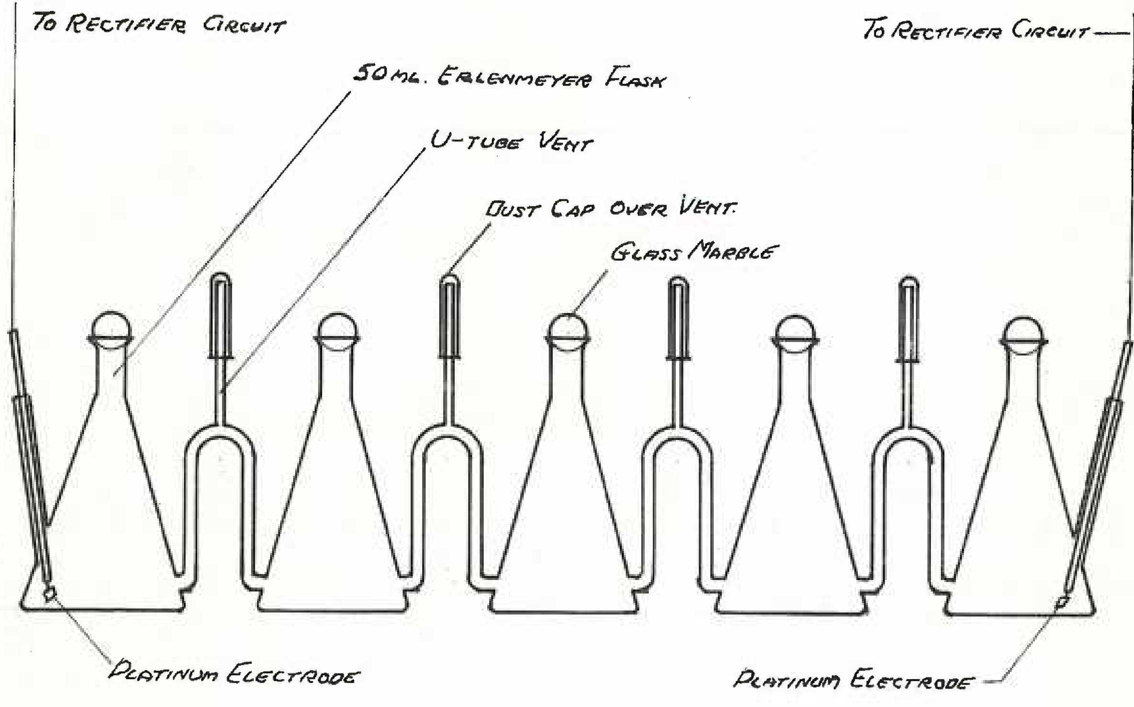


Figure 8

The titration method used was identical with that previously described. Pure dry glucose was taken as the standard for the determination of the monosaccharide reducing power.

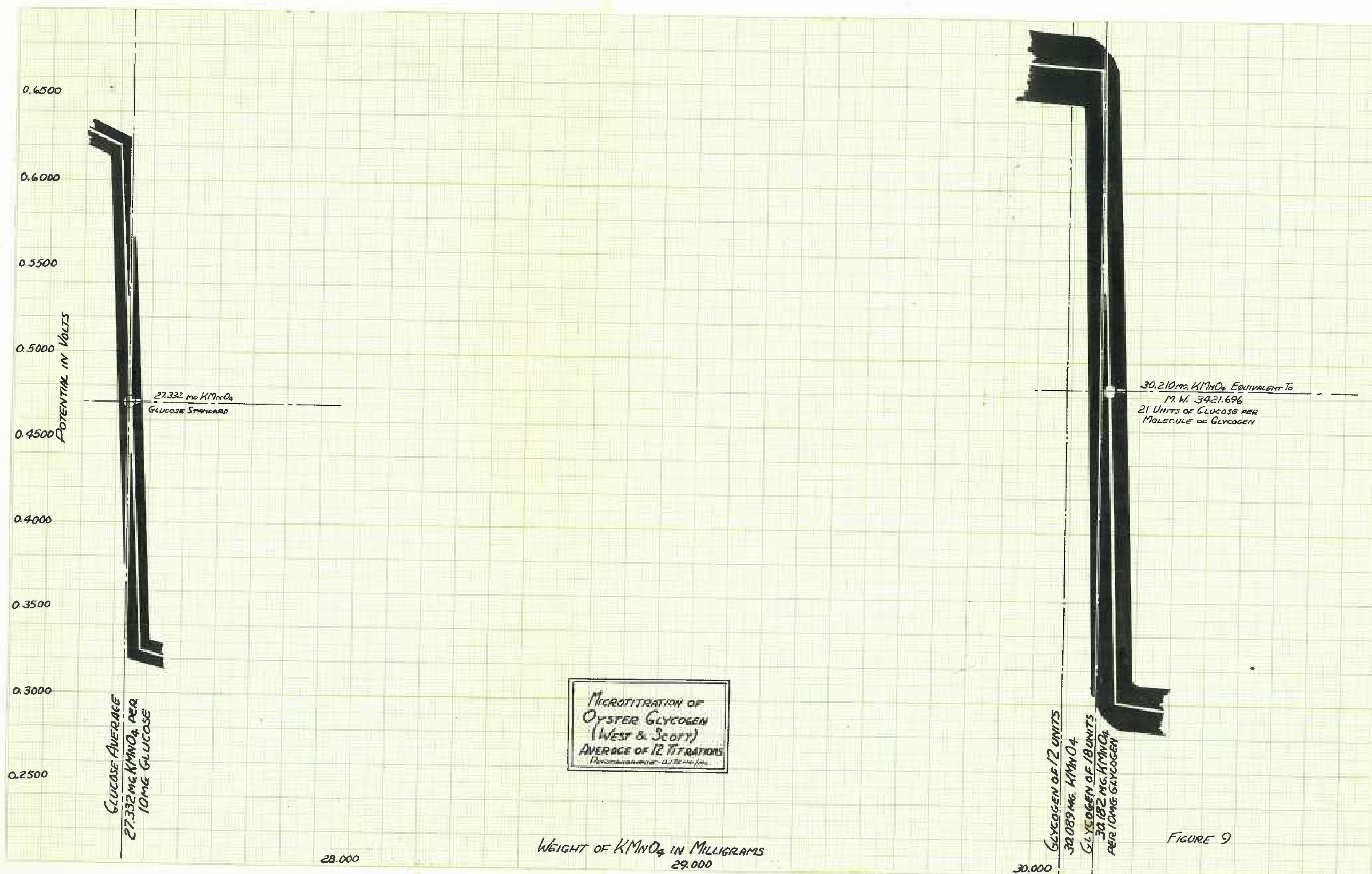


FIGURE 9

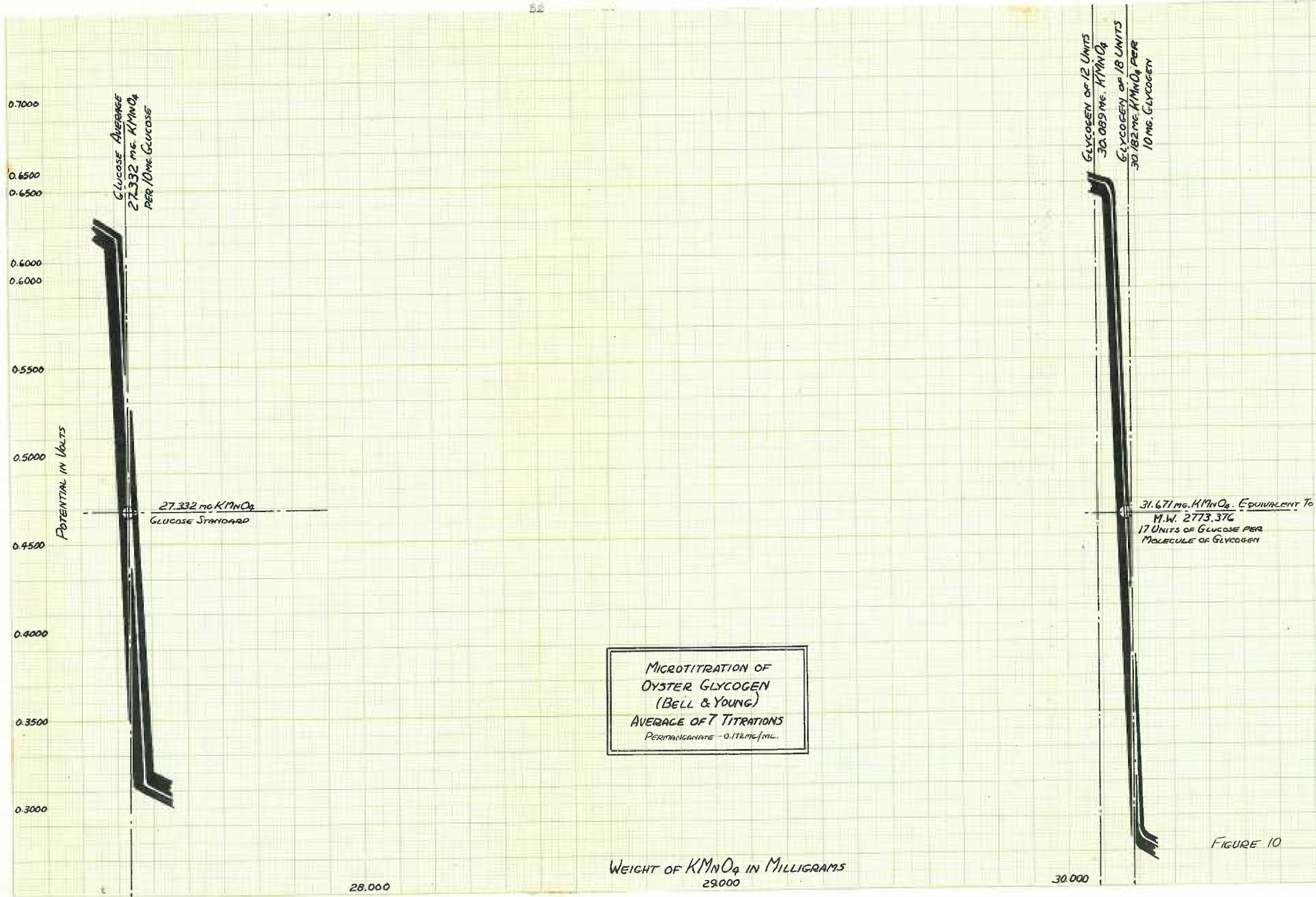


Figure 10

MOLECULAR WEIGHT OF MAMALIAN LIVER GLYCOGENS

Recent work of Bell and Young (12) has indicated that the glycogen obtained from the livers of different animals might vary greatly in molecular weight. According to their report rabbit liver glycogen obtained from animals fasted for périods of 24 or 48 hours, then fed 5 grams of galactose per kilogram of body weight showed 18 units of glucose per molecule of glycogen, whereas rabbits on a normal diet, in which glucose was presumably the monosaccharide from which liver glycogen was derived, showed the presence of 12 glucose units per molecule of glycogen.

The permanganate oxidation method was applied to the two kinds of glycogen in an attempt to secure further evidence regarding this peculiar observation.

EXPERIMENTAL

Both rats and rabbits were employed for the study, and since the suggestion had been made that glucose and galactose produce different glycogens in the case of the rabbit, it was considered best to treat two groups each of rats and rabbits in identical ways with respect to everything except the type of monosaccharide administered. In the experiment with rabbits, animals were fasted for 48 hours after which 5 grams of glucose per kilogram of body weight was given to two of the animals by means of a 50% solution introduced by a stomach tube, while the remaining two animals were given 5 grams of galactose in a similar manner. Two hours were allowed for absorption of the carbohydrate after which the animals were killed and their livers extracted as rapidly as possible and treated according to the method of Bell and Young.

In carrying out the experiment on rat liver glycogen, 10 adult rats were selected for each of the two types of monosaccharides, the times of fasting, absorption and methods of treatment being the same. Following the extraction of liver glycogen the electrical method, previously described, was employed for further purification, and the glycogen was dried at 76° C. over phosphorous pentoxide in vacuo for three days. The samples of glycogen gave the following results when analyzed:

Source of glycogen	Ash	Protein
Rat - glucose fed	0.019%	Below 0.005%
Rat - galactose fed	0.025%	Below 0.005%
Rabbit - glucose fed	0.014%	Below 0.005%
Rabbit - galactose fed	0.011%	Below 0.005%

The protein test used was the Ninhydrin test of Harding and MacLean (18). The glycogen samples were hydrolised with dilute sulfuric acid and in order to overcome any variation that might be caused by the presence of glucose along with protein hydrolysate in the solution, a proportionate quantity of glucose was added to the standard alanine solution before comparison. The concentration of protein is reported as below the value of 0.005%, lower because in the 80 mg. sample of glycogen available for analysis, the protein concentration was below 0.005 mg. per ml. which is the minimum necessary to give a positive test.

DATA

Here again the results are most conveniently presented in the form of a composite curve, so arranged as to show the relationship between quantities of permanganate reduced by 10.000 mg. of glycogen and by 10.000 mg. of pure glucose. From these values the molecular size of the glycogen may be obtained from the graph in a manner exactly as previously outlined.

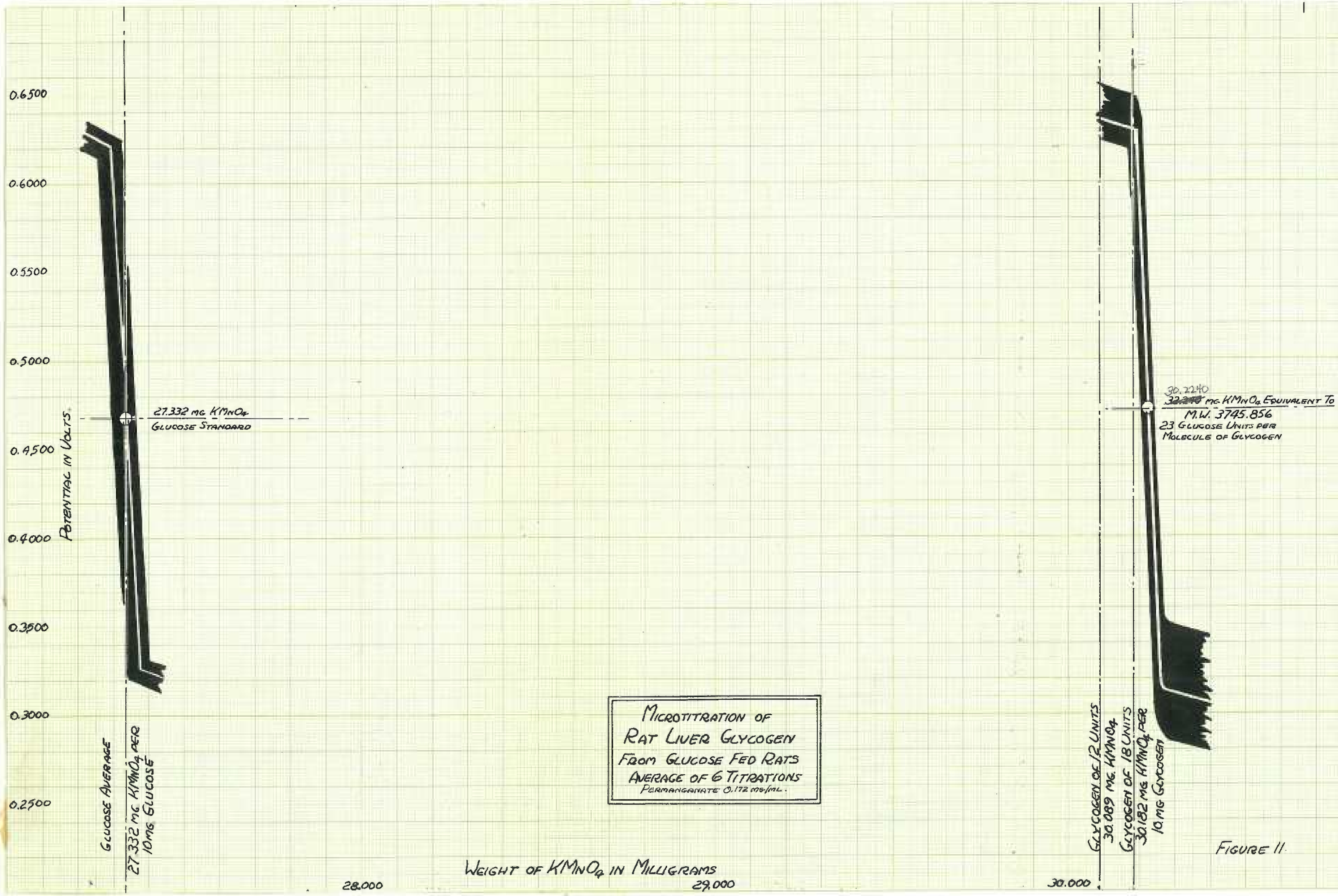
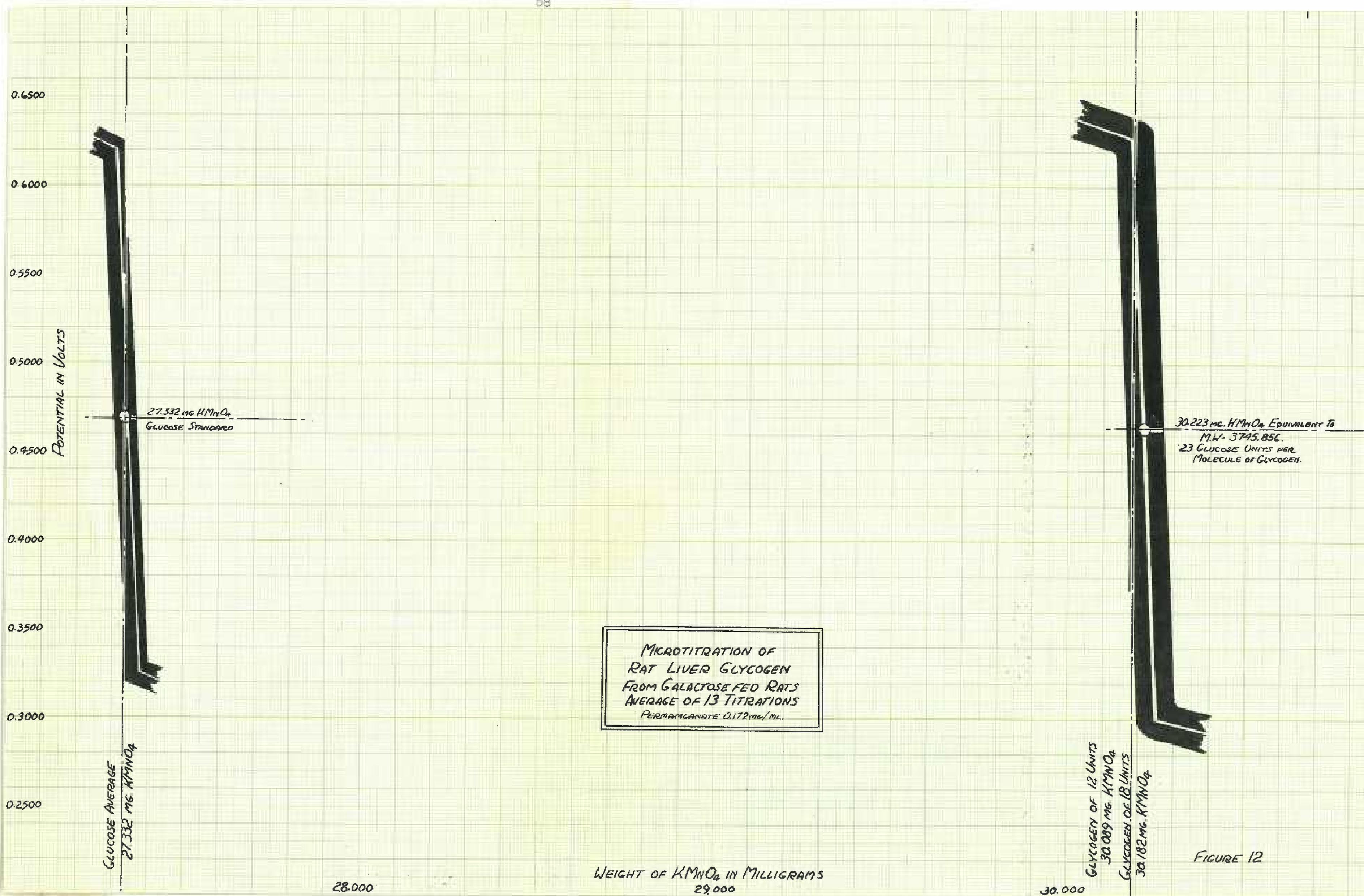
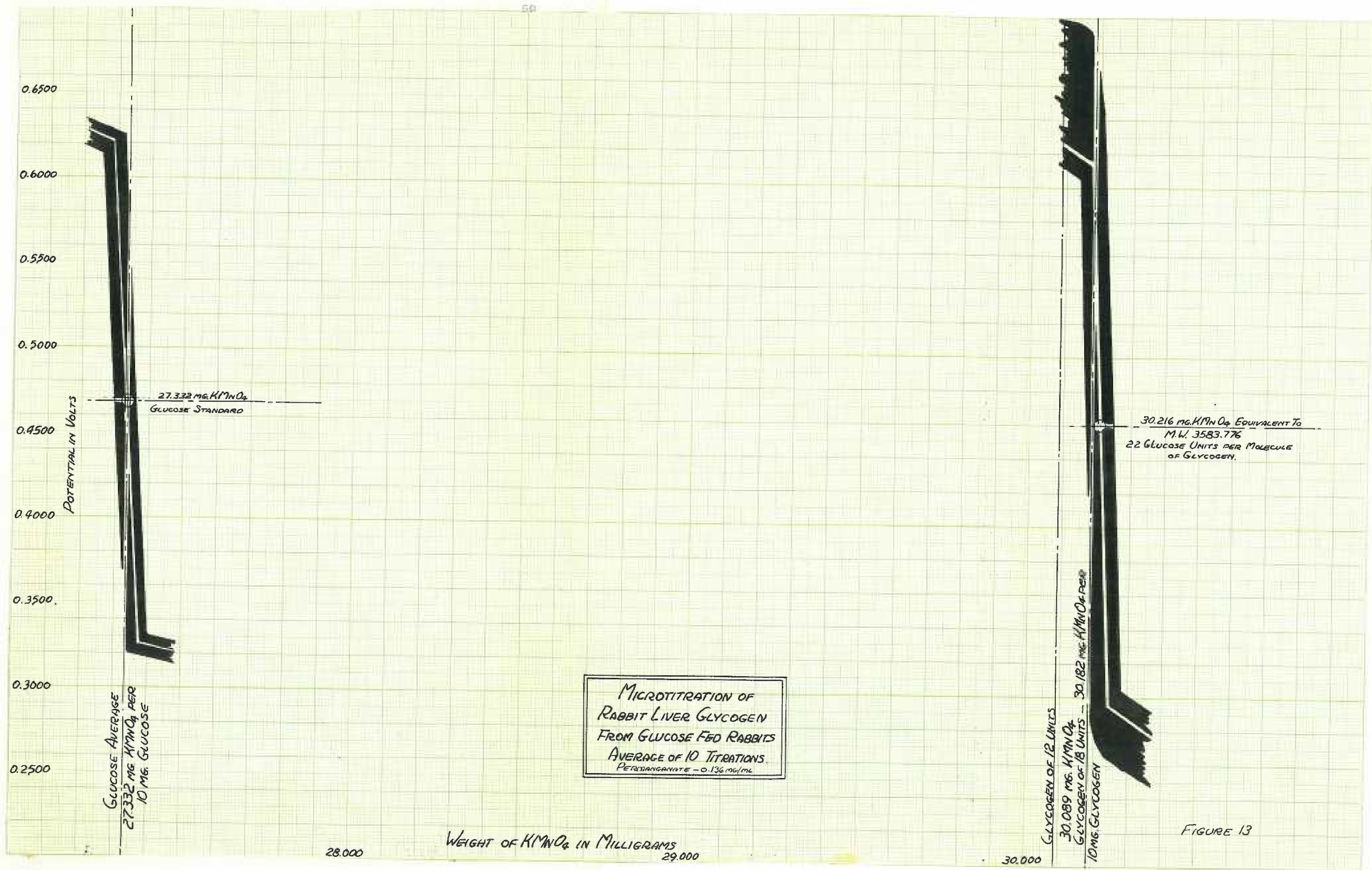


FIGURE 11



0.6500
0.6000
0.5500
0.5000
0.4500
0.4000
0.3500
0.3000
0.2500
Potential in Volts
27.332 mg KMnO4
Glucose Standard
30.223 mg. KMnO4 Equivalent to
M.W.- 3745.856.
23 Glucose Units per
Molecule of Glycogen.
Microtitration of
Rat Liver Glycogen
From Galactose fed Rats
Average of 13 Titrations
Permanganate 0.172 mg/ml.
Glucose Average
27.332 mg. KMnO4
Glycogen of 12 Units
30.089 mg. KMnO4
Glycogen of 18 Units
30.182 mg. KMnO4
Figure 12
Weight of KMnO4 in Milligrams
28.000
29.000
30.000



0.6500
0.6000
0.5500
0.5000
0.4500
0.4000
0.3500
0.3000
0.2500
Potential in Volts
27.332 mg. KMnO4
Glucose Standard
Glucose Average
27.332 mg KMnO4 per
10 mg. Glucose
30.216 mg. KMnO4 Equivalent To
M.W. 3583.776
22 Glucose Units per Molecule
of Glycogen.
Glycogen of 12 Units
30.089 mg. KMnO4
Glycogen of 18 Units
30.182 mg KMnO4 per
10 mg. Glycogen
Microtitration of
Rabbit Liver Glycogen
From Glucose Fed Rabbits
Average of 10 Titrations.
Permanganate - 0.136 mg/ml
Weight of KMnO4 in Milligrams
28.000
29.000
30.000
Figure 13

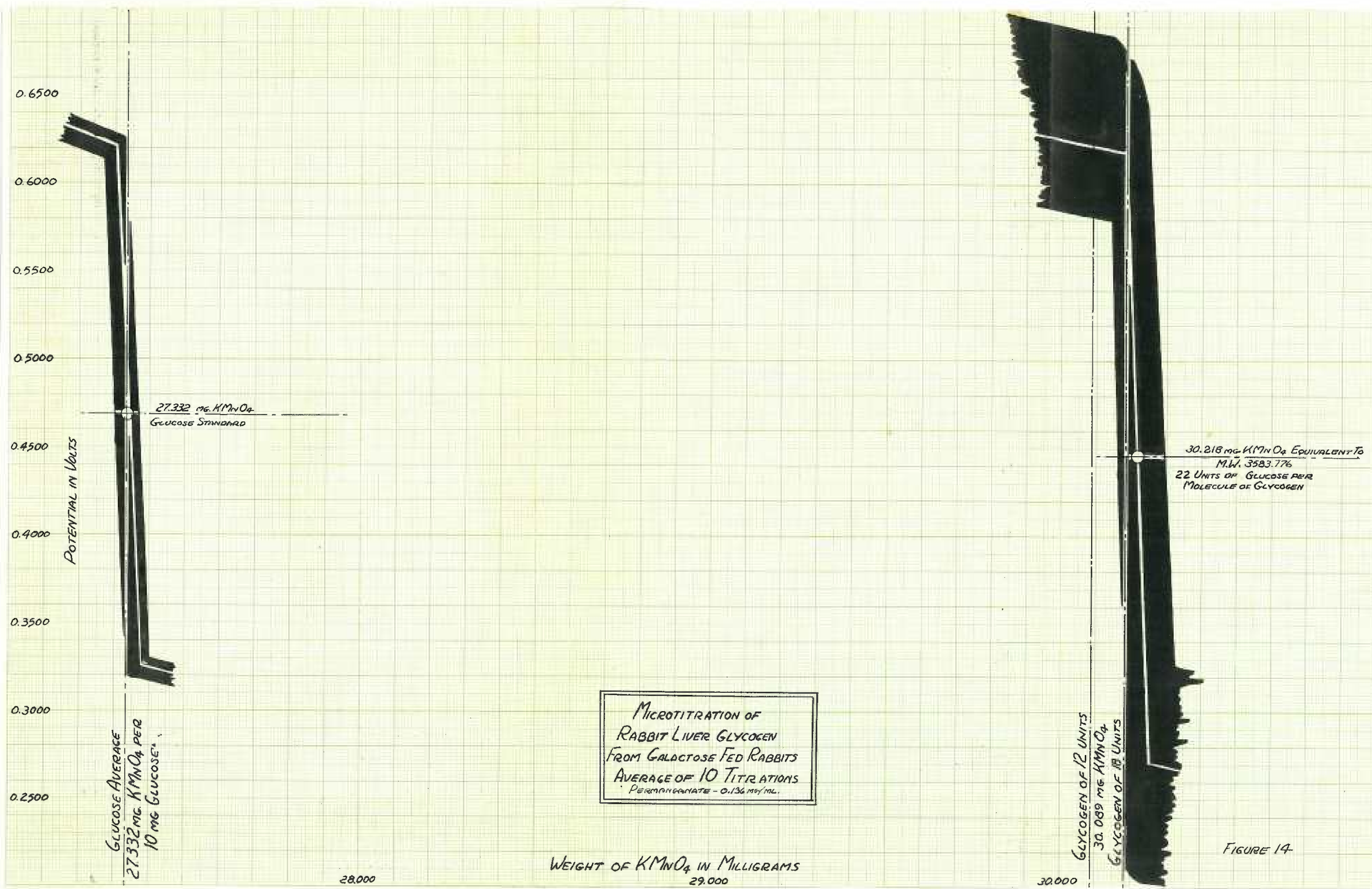


Figure 14

THE MOLECULAR WEIGHT OF INULIN

Previous estimations of the molecular weight of inulin have varied in much the same manner as in the case of glycogen. Results appear to have varied between 162 and 164,000. The most reliable values appear to have been determined by means of the methylation end-group assay procedure of Haworth. Irvine and Montgomery (19) obtained values from 4,700 to 4,900 and Haworth and Coworkers (20) obtained values from 3,300 to 5,100, both by means of the end-group assay method. Westfall and Landis (21) employing the vapor pressure method of Hill (22) and Baldes (23) obtained values from 4,694 to 5,615 with an average value of 5,101.

EXPERIMENTAL

The materials used in this experiment were inulin obtained from the Pfanstiehl Chemical Company which contained 0.016% ash after six washings with dry alcohol. The fructose, used as the monosaccharide standard, was the same as that employed in the section on the molecular weights of sucrose and trehalose.

The experiment was conducted in exactly the same manner as previous experiments relative to the drying, weighing and titration of samples. Figure 7 shows the results obtained for the molecular weight of inulin.

DISCUSSION

From the fact that oxidation by permanganate in acid solution gives values closely approaching theory for the molecular weights of disaccharides, it seems reasonable to expect the procedure to give valid results in case of more complicated carbohydrates which are similarily constituted. If such is the case then, the above obtained values for the molecular weights of the various glycogens may be assumed to approach reasonably close to the true chemical molecular weights of these compounds as they were prepared by the methods selected.

The average molecular weight of glycogen, as determined by the permanganate oxidation procedure, was found to be in the region of 3,200 and to represent 20 ± 3 units of glucose per molecule. This was true regardless of the source of glycogen. It would appear from the variation of values obtained for any one glycogen that the limit of accuracy of the method lies in the region of ± 3 glucose units.

Ordinary liver glycogen is at present considered to be composed of about 12 glucose units, representing a molecular weight of around 2,000, while oyster glycogen and liver glycogen formed from galactose are considered to be made up of about 18 units of glucose, representing a molecular weight of about 3,000. Our results failed to distinquish any marked differences in these glycogens, and do not support the contention of Bell and Young that fasted animals fed glucose and galactose produce glycogens of different molecular size.

Results for inulin indicate a molecule composed of 33 fructose units, corresponding to a molecular weight of 5,300 which is in agreement with the values of the authors previously cited.

PART III

QUANTITATIVE DETERMINATION OF CARBOHYDRATES IN PURE SOLUTION BY MEANS OF POTASSIUM PERMANGANATE

It has been shown in previous sections that permanganate in strongly acid solution readily oxidises complex polysaccharides and that reproducible results in the ratio of polysaccharide oxidised to permanganate reduced may be obtained. To extend the study of the quantitative applications of the reaction the conditions for the direct titration of cellulose and glycogen were determined and a method for the analysis of glycogen in solution was devised. The method was applied to a study of the degree of hydration of air-dry glycogen and to the concentration of glycogen in fresh rat livers.

EXPERIMENTAL

Cellulose Titration

40 mg. of Whatman filter paper was dissolved in 30 ml. of 50% sulfuric acid by gently warming to a temperature not over 30°C. and stirring the mixture. Under these conditions a perfectly clear and colorless solution is obtained. If the heating during the treatment is too intense a golden yellow solution results which is believed by Pringsheim to contain products of oxidation of carbohydrate and to be unsuitable for analysis. The clear solution was made up to 200 ml. and aliquot portions were titrated at 90°-100° C.

The concentration of sulfuric acid in the test solutions was varied from 4.0 M. to 10.0 M. The reaction takes place slowly and an unsatisfactory end point is obtained if the initial acid concentration of the solution is below 10.0 molar. The rate of the reaction increases with increased acid concentration and in the region of 10.0 molar compares with that of the simpler carbohydrates. The end point becomes sharp and reproducible. Results of the experiment are shown in Table XXI.

Glycogen Analysis

40 mg. of air-dried oyster glycogen, prepared by the method of West and Scott, was dissolved in 40 ml. of 30% sulfuric acid by carefully warming to a temperature not over 30°C. and stirring the mixture. This solution was diluted to 200 ml. and varying volumes were taken for analysis. The initial acid concentration was made up to 7.3 M. by adding the proper volume of concentrated sulfuric acid before titration. The solutions were titrated to the visual permanganate end point.

Glycogen from the same source was dried at 76°C. over phosphorous pentoxide in vacuo for three days, at which time it was found that continued drying under these conditions failed to remove further moisture. Test solutions were prepared and titrated as was the air-dried material. The values obtained for the titration of glycogen samples are shown in Tables XXII and XXIII. The percentage of moisture in air-dried glycogen, as determined by the permanganate titration method is obtained from values of the dry and hydrated glycogens and is equal to 10.99% which is in agreement with the values of Meier and Meyerhof (24) who have stated that dry and air-dried glycogens correspond to the formulas $C_6H_{10}O_5$ and $C_6H_{10}O_5H_2O$ respectively. The latter formula implies a concentration of water equal to 9.95%. The formula $C_6H_{10}O_5H_2O$ obviously does not represent any possible molecular or empyrical formula of hydrated glycogen, but only a close approximation. If the glycogen molecule is composed of 20 glucose units linked serially by loss of water between hydroxyl groups, and if the equivalent of one molecule of water of hydration is attached to each of these glucose units in the glycogen molecule, the percent water of hydration would be:

$$(12) \qquad \frac{20 \times 18.012}{(162.096) \times 19 + 180.096 + 360.240} = 9.95\%$$

Obviously the above calculation is valid only if the conditions postulated are met. This method of calculation would give a higher percentage of water of hydration with increased glucose units in the molecule and vice versa. Also more than one molecule of water may be attached to some of the glucose units, and this seems probable in that data cited in Part II of this thesis indicate the presence of about 20 glucose

TABLE XXI

DIRECT TITRATION OF CELLULOSE WITH PERMANGANATE

TEMPERATURE 90°-100° C.

Initial volume of test solution 5 ml.

Weight of Cellulose mg.	Initial Acid Concentration Molarity	Volume of 0.0126 N. Permanganate Solution ml.	Average Weight of Permanganate per mg. of Cellulose mg.
0.4	4.0	0.75	0.77
0.4	4.0	0.78	0.80
0.8	4.0	1.50	0.77
0.8	4.0	1.48	0.76
0.4	8.0	2.24	2.31
0.4	8.0	2.28	2.34
0.8	8.0	4.45	2.29
0.8	8.0	4.56	2.35
0.4	10.0	4.22	4.30
0.4	10.0	4.19	4.33
0.8	10.0	8.37	4.32
0.8	10.0	8.46	4.36

TABLE XXII

DIRECT TITRATION OF AIR-DRIED GLYCOGEN WITH PERMANGANATE

TITRATION AT 90°-100°C.

Initial volume of test solution 10 ml.

Weight of Glycogen	Volume of 0.0126 N. Permanganate Solution ml.	Average Volume Permanganate per mg. of Glycogen ml.
0.2	1.32	
0.2	1.32	6.58
0.2	1.31	
0.4	2.72	
0.4	2.74	6.82
0.4	2.74	
0.6	3.94	
0.6	3.92	6.57
0.6	3.96	
0.8	5.27	
0.8	5.27	6.58
0.8	5.27	

TABLE XXIII

DIRECT PERMANGANATE TITRATION OF GLYCOGEN DRIED OVER PHOSPHOROUS PENTOXIDE IN VACUO

TITRATION AT 90°-100°C.

Initial volume of test solution 10 ml.

Weight of Glycogen mg.	Volume of 0.0126 N. Permanganate Solution ml.	Average Volume Permanganate per mg. of Glycogen ml.
0.2	1.49	
0.2	1.48	7.45
0.2	1.49	
0.4	2.98	
0.4	3.01	7.47
0.4	2.98	
0.6	4.48	
0.6	4.47	7.47
0.6	4.48	
0.8	5.96	
0.8	5.97	7.46
0.8	5.97	

units in the glycogen molecule.

In order to determine the applicability of the titration method to the actual analysis of glycogen, samples of rat liver were rapidly removed from the recently killed animal, ground and heated with 30% potassium hydroxide until the tissue was disintegrated. The glycogen was precipitated from the alkaline solution by adding alcohol to 60% by volume. The mixture was centrifuged and the precipitate purified by dissolving in water and reprecipitating with alcohol made up to 60% by volume. This reprecipitation from solution was carried out four times. A small amount of sodium sulfate was added before the alcohol to facilitate precipitation of the glycogen. The purified material was dissolved in 100 ml. of water and 5 ml. taken for analysis by titration. Samples of glycogen obtained in a similar way from the same rat livers, were also hydrolysed with dilute hydrochloric acid, neutralised and analyzed by means of the conventional Shaffer-Hartmann method. Results of analyses by the two methods are shown in Tables XXIV and XXV.

TABLE XXIV

DIRECT TITRATION OF RAT LIVER GLYCOGEN

Animal Number	Volume of Glycogen Solution ml.	Volume of 0.0126 N. Permang-anate Sol-ution. ml.	Weight of Permang-anate mg.	Weight of Glycogen per 5 ml. mg.	Weight of Liver Analyzed gm.	Glycogen Percentage
4	5.0	2.76	1.131	.362	1.810	.40
	5.0	2.77	1.136			
3	5.0	7.12	2.920	.928	1.999	.89
	5.0	7.15	2.933			
11	5.0	4.79	1.963	.624	3.199	.39
	5.0	4.70	1.927			
6	5.0	4.85	1.988	.646	2.465	.52
	5.0	4.86	1.993			

TABLE XXV

SHAAFFER-HARTMAN TITRATION OF GLYCOGEN HYDROLYSATE

Animal Number	Volume of 0.005 Normal Sodium Thiosulfate ml.	Weight of Glucose in 5 ml. of Hydrolysate	Weight of Glycogen per gram of liver mg.	Percentage of Glycogen
4	16.62 16.60	0.35	4.52*	0.44
3	11.90 11.94	0.89	8.97	0.93
11	13.96 13.96	0.63	4.18	0.42
6	14.26 14.22	0.59	5.63	0.58

* Glucose values must be multiplied by 0.93 to obtain values for glycogen.

DISCUSSION

The similarity of results in the titration of glycogen with permanganate and by the usual method of determining the reducing sugar in solutions of hydrolyzed glycogen demonstrate the possibilities of the acid permanganate reagent in the field of carbohydrate analysis. The direct titration method has the advantage, in the case of glycogen determination, of requiring considerably less time for a complete analysis than the ordinarily used methods involving preliminary acid hydrolysis. Its disadvantage lies in the necessity for painstaking purification of glycogen samples.

The observation that cellulose may be rapidly oxidized in strongly acid solution and reproducible results obtained indicate the possible use of the reaction for the analysis of cellulose and cellulose products.

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