THE PREPARATION OF GLYCOGEN BY WATER EXTRACTION

by

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INTRODUCTION

The problem of preparing glycogen in the pure form has held the attention of biochemists for many years. It has been prepared from several sources and purified by a variety of methods. Complex polysaccharides are found everywhere associated with living tissue. Glycogen is usually an animal carbohydrate. It is present in the mammalian body chiefly in the muscles and liver. It is present in quantity in some invertebrates, especially in sea life such as oysters and abalone. Yeast contains appreciable quantities of glycogen.

Under these circumstances of occurrence, glycogen is mixed physically with the other constituents of protoplasm. There is evidence also, in some cases at least, that the glycogen is present as a complex in combination with proteins. The problem of preparation and purification is concerned largely with freeing the glycogen from these other tissue contaminants.

Evidence that glycogen is pure is based largely on its specific rotation in water solution, its reduction value after hydrolysis by acids, and its ash, nitrogen and phosphorus contents.

HISTORY

History of Preparation. As early as 1892 Frankel(1) conducted a series of experiments to determine which method of precipitating proteins from solution was best. His first attempt was to extract the glycogen from ground liver with cold 1% hydrochloric acid and to precipitate the proteins from solution with a $KI-HgI_2$ reagent. The glycogen so obtained was precipitated from solution with alcohol. The next method which he tried was that of extracting the liver in the cold with 2% metaphosphoric acid. The extract was then neutralized with barium carbonate, filtered, and the glycogen precipitated with alcohol. The method which he recommended for large quantities, both because of its low cost and its applicability, was to disintegrate the liver rapidly in 2-4% trichloracetic acid. The digest was then added to distilled water to make a volume of 250 ml for each 100 grams of liver. This was filtered and precipitated with two volumes of alcohol. After twelve hours the glycogen was removed by filtering and washed with 60% alcohol until the washings gave no test for acid. The glycogen was then washed with 95% alcohol, absolute alcohol and finally ether.

In 1902 Harden and Young (2) published a method for preparing glycogen from yeast. They ground yeast with sand for two hours, cooling the mixture during this time with liquid carbon dioxide. The ground mixture was then poured

into two or three times its volume of boiling water and boiled for two hours. The insoluble material was removed by centrifugation and reextracted with boiling water. The two extracts were united and 1% sodium phosphate and 1% calcium chloride added. The solution was neutralized with ammonium hydroxide and heated on a water bath. The calcium phosphate was removed by filtering and the solution concentrated by evaporation. The glycogen was precipitated from the concentrate by adding an equal volume of alcohol. This addition of salts with subsequent precipitation was continued until the glycogen which separated was free from the gummy material present in yeast and was no longer sticky. The glycogen was then dissolved in water, and the solution saturated first with sodium chloride and then with ammonium sulfate. This was allowed to stand in the cold for three days and was then dialyzed. Three grams of potassium hydroxide and ten grams of potassium iodide per 100 ml. were added to the dialysate and the glycogen precipitated by the addition of one-half volume of alcohol. The glycogen was washed with a mixture of 500 ml of alcohol and 400 ml of an aqueous 3% potassium hydroxide solution containing 40 grams of potassium iodide. It was then washed with 50% alcohol. The glycogen was precipitated from a solution made acid with acetic acid by adding one volume of alcohol. This procedure was repeated until the product was ash free.

Later, in 1912, these workers (3) simplified their method of preparation. In this method the yeast was ground with sand, extracted with boiling water, and the glycogen precipitated with an equal volume of alcohol. The precipitate was treated with 6% potassium hydroxide on a boiling water bath, cooled, poured into water, filtered, and the glycogen precipitated with two volumes of alcohol. The precipitate was washed several times with a mixture of alcohol and 60% aqueous potassium hydroxide, and finally with alcohol alone. The glycogen was then redissolved in water and the solution neutralized with acetic acid. The glycogen was precipitated by adding an equal volume of alcohol. It was purified by several precipitations from water solution with alcohol. The "purified" product was separated from contaminating yeast gum by precipitating the glycogen from solution by saturating with ammonium sulfate.

In the early 1900's Pfluger (4) did much work on glycogen and evolved a method which has become standard and which bears his name. In this method liver was heated on a boiling water bath for several hours with 60% potassium hydroxide (one ml of potassium hydroxide per gram of liver). 200 ml of water were added, followed by 400 ml of 96% alcohol. The solution was poured through the filter twice. The glycogen was washed by pouring 96% alcohol through the filter. Water was then poured on the filter until the filtrate no longer showed the presence of glycogen as determined by the lack of turbidity when the filtrate was poured into alcohol.

After concentration of the filtrate on a water bath to a volume of 50 ml, it was neutralized with acetic acid. The solution was then filtered and the glycogen recovered by alcohol precipitation.

Gatin-Gruzewska, a student working in Pfluger's laboratory, published a paper in 1904 entitled "Das Reine Glycogen". (5) In this work she gave a long and complicated procedure for the preparation and purification of glycogen in which she combined several methods. The tissue, liver or muscle, was extracted for five hours with water (200 ml of water per 100 grams of tissue) on a boiling water bath. The extract was filtered and treated twice with a mixture of 800 ml of 10% potassium iodide, 40 ml of 60% potassium hydroxide, and 400 ml of 96% alcohol. It was washed each time with a mixture of 500 ml of 10% potassium iodide, 25 ml of 60% potassium hydroxide, and 250 ml of 96% alcohol. In additio n it was washed each time twice with 66% alcohol and twice 96% alcohol. After these treatments and washings, the glycogen was dissolved on the filter with water and the glycogen precipitated from the filtrate with alcohol. The precipitate was removed by filtration and 30% potassium hydroxide (hot) poured over the filter until the glycogen had dissolved. This solution was heated on a water bath for an hour; then the glycogen was precipitated with two volumes of 96% alcohol, filtered, and washed twice with a mixture of one volume of 15% potassium hydroxide and one volume of 96% alcohol. It was precipitated from water solution with

alcohol five or six times to remove the alkali. The solution was then acidified with acetic acid and the glycogen precipitated with one volume of 96% alcohol. This was repeated three times and was followed by three or four precipitations from neutral solution to remove the acid. It was dried by allowing it to stand for three days with absolute alcohol and three days with anhydrous ether. The dehydration was completed in a vacuum desiccator.

Petree and Alsberg (6), working on the phosphorus content of abalone glycogen, published another method for its preparation in 1929. The abalone was boiled for a few minutes with water. The solution was neutralized to litmus with sodium bicarbonate, as abalone contains a free acid which would hydrolyze the glycogen if not neutralized. The solid was then ground and extracted with water on a boiling water bath for fifteen minutes. The extraction was repeated a third time. The combined extracts were made slightly acid with acetic acid and a saturated, aqueous solution of picric acid added in slight excess of the amount necessary to precipitate the proteins present. The solution was then filtered and the glycogen precipitated by adding two volumes of alcohol. Purification was affected by precipitation from water solution four or five times. The glycogen was dried by washing with 95% alcohol and anhydrous ether. Desiccation was completed in a vacuum over a dehydrating agent.

In the next year Sahyun and Alsberg (7), working also on the phosphorus content of glycogen, prepared the glycogen

from rabbit liver by an adaptation of the old Frankel method. The livers were ground in a mortar with three parts of 3% trichloracetic acid; the liquid was strained off and the solid again extracted with trichloracetic acid. The combined extracts were centrifuged to remove contaminants and the glycogen precipitated with two volumes of alcohol. After dissolving the glycogen in trichloracetic acid, the solution was centrifuged. The solution was then shaken with ether to remove fatty acids present and the glycogen precipitated several times from water solution. Dehydration was carried out in 95% alcohol, anhydrous alcohol and completed in a vacuum desiccator. The authors avoided the use of ether, believing that its use led to too rapid dehydration and made the product hard to dissolve.

Somogyi (8) employed a variation of the Pfluger technic to prepare a product which contained no nitrogen or phosphorus. Liver was digested for three hours on a boiling water bath with 50-60% potassium hydroxide (two ml. per gram of liver). The digest was cooled; the soap which collected on top was extracted with water, and the extraneous material salted out by saturating the solution with sodium chloride. This extract was filtered and the two extracts combined. The important modification which Somogyi made was the concentration of alcohol used in the precipitation. The glycogen was precipitated by adding only 50 ml. of 95% alcohol per 100 ml of solution. The usual amount of alcohol is one to two and a half volumes

for each volume of glycogen solution. By this method he believed that he could prevent the fraction which had the high phosphorus content from precipitating. After the initial precipitation with alcohol, the glycogen was washed with a solution of one volume of 95% alcohol and two volumes of 20% sodium hydroxide until the washings were colorless. The glycogen was then dissolved in water and the solution filtered. 2N. hydrochloric acid was then added until the solution was just acid to congo red paper. The glycogen was then precipitated by adding first 50 ml of alcohol per 100 ml of solution, running this through the filter and then adding more alcohol to a final concentration of 42%. The glycogen was precipitated twice from neutral solution by adding alcohol to a final concentration of 42%. The glycogen was washed twice with 45% alcohol, twice with 95% alcohol, and finally with ether.

Bell and Young (9) in 1934 used a modification of the Frankel method to prepare a glycogen low in ash content. They extracted ground liver with twice its volume of water three times for thirty minutes each on a boiling water bath. The cooled, combined extracts were treated with 40% trichloracetic acid to a final concentration of 4%. The precipitated material was removed by centrifugation and the glycogen precipitated by adding two and one half volumes of alcohol. The glycogen was then freed from ash by four precipitations from 80% acetic acid.

History of Analysis. The amounts of contaminants which various workers have reported in samples of glycogen are very variable. This is to be expected because of the variety in methods used for preparation, the source of the glycogen and the methods used for analysis. Frankel has listed values for the specific rotations of glycogen determined before 1892 which vary from 127 to 238 degrees.

Samec and Sajevic, (10) McDowell, (11) Petree and Alsberg, (6) and Sayhun and Alsberg (7) believed that glycogen contains an irremovable phosphorus content. Somogyi has recently reported the preparation of a sample which is free from this element.

The values reported for ash content have varied from 0.0 to 0.87% Likewise the values for nitrogen content are variable, depending to a great extent upon the degree of purification of the samples.

The values obtained for the reducing power of hydrolyzed glycogen have received considerable discussion. When glycogen is hydrolyzed, water is added on to the glucose residues as they are split off. For this reason the glucose obtained is heavier than the glycogen which was hydrolyzed. Just what factor the glucose should be multiplied by to give the correct value for glycogen originally present has been the subject of considerable discussion. This factor will depend on the number of glucose residues present in a molecule of glycogen, and the completeness of the hydrolysis before destructive action on the generated glucose takes place. Nerking (12) in 1901 published a long list of

analyses to show that the factor 0.9 which had been used for conversion before that time was too low and that the factor 0.927 was more accurate. Since that time workers have been unable to repeat Nerking's work and no one has obtained glycogen values of 100% using this factor. Kerly(13) suggested that the glucose found to be present multiplied by the factor 0.957 gave glycogen values of 100%.

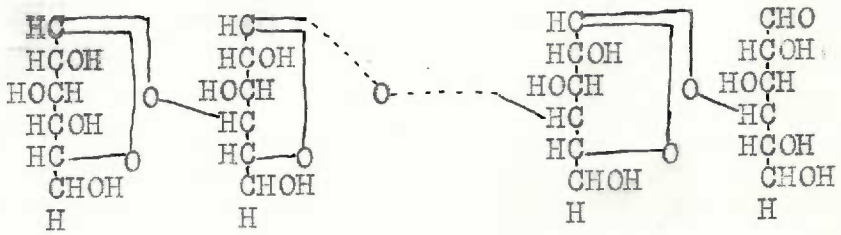
The values which workers have obtained for impurities present in glycogen prepared by various methods and from various sources have been recorded in tabular form (table 1).

Table I

Investigator	Method of Preperation	Source	$(a)_D$	%N	$\%P_2O_5$	% Ash	Reduction
Frankel (1)	Trichloracetic acid in the cold	liver	197.9	none		0.03% 0.07%	
Harden and Young (2)	Water extraction and KI-KOH protein precipitation	yeast	198.3	none		none	97.7%
	Pfluger's	liver	191.1				96.3%
	Pfluger's	oysters	191.2				98.3%
Gatin-Gruzewska (4)	KI-KOH treatment, alcohol precipitation from acetic acid; 16-18 total precip.	muscle	196.6	none		none	96.8%
		liver	196.6	none		0.023%	95.4%
		liver	196.6	none		none	97.7%
Nerking (12)	Water extraction and KI-KOH treatment	muscle		0.046%		0.06%	
	Precipitation of above sample from acetic acid			0.026%		none	100%
Grebe (14)	Pfluger's	muscle					97.2%
Norris (15)	Pfluger's	liver				0.38%	
		oyster				0.20%	
		rabbit				0.51%	
		yeast				0.87%	
Sameo and Sajevic (10)	No Reference	liver			0.721%		
McDowell (11)	Pfluger's and electrodialysis	mussel			0.0343% 0.0368% 0.0361%	0.0827% 0.0467%	
Petree and Alsberg (6)	Picric acid and electrodialysis	abalone			0.039% 0.037% 0.047%	0.069% 0.065%	
Haworth, Hirst, and Webb (16)	commercial (Kahlbaum) electrodialysed		191.4	0.35%	0.14%	0.37%	
Sahyun and Alsberg (7)	Trichloracetic acid	liver			0.032%	0.20% 0.18%	96.4% 99.6%
Sahyun and Alsberg (17)	Trichloracetic acid						96.4% 97.3% 98.7%
Kerly (13)	Trichloracetic acid	liver mussel muscle		0.70%	0.17%		96.9%
	Treatment of above sample with KOH			0.30%			
Bell and Young (9)	Trichloracetic acid and precipitation from 80% acetic acid	liver	196 196 196 196		0.107%	none 0.13% 0.067% 0.028%	
Somogyi (8)	Modified Pfluger's	liver		none	none	none	

DISCUSSION

Haworth and Percival (18) reported that when methylated glycogen was hydrolyzed, trimethyl glucose and tetramethyl glucose were obtained. The tetramethyl glucose constituted about 9% of the yield. They assigned to glycogen a structure made up of glucose residues and upon the basis of the yield of tetramethyl glucose, contended that glycogen contains twelve such units. The linkage was believed to be in this fashion:



As is evident the tetramethyl glucose comes from the residue on one end of the chain. This necessitates, in order for their structure to be valid, the presence of a free sugar group on the other end of the molecule. This being the case, glycogen should react with sugar reagents such as phenylhydrazine, hydroxylamine and alkaline copper.

So far as is known no report is available upon the behavior of glycogen toward any of these reagents except alkaline copper. The reduction values are not of the same order as Haworth's formula would indicate. Bell and Young(9) found reduction values ranging from 0.06 to 1.90 (glucose

equals 100). This means that each glycogen molecule would contain between 50 and 600 glucose residues. Haworth's structure would require a reduction value of approximately 9. It seems likely that if glycogen does contain a free sugar group, the available methods of preparation may be harsh enough to alter its properties during purification. The strong alkali in Pfluger's method would cause enolization and disintegration of the residue containing the free sugar group. The other methods of preparation which make use of acid precipitation might conceivably alter the constitution of the glycogen molecule by hydrolysis.

If glycogen does have the structure which Haworth proposes, it would not only give the reactions with phenylhydrazine and hydroxylamine but would also be subject to oxidation by proper means to the corresponding acids. Various salts could be made from such acids. The extent of reaction of glycogen with phenylhydrazine and hydroxylamine could be determined by the nitrogen content after the reaction had taken place. Likewise, the salts of acids formed by mild oxidation of glycogen could be determined by the ash content. In view of these prospective methods of analysis it would be necessary that the glycogen preparation used have a low content of nitrogen and ash.

In the preparation of glycogen accompanying protein contaminants must be removed. This can be done by hydrolyzing the protein with alkali and removing it in this way. It can be done by precipitating the protein from solution

with such reagents as trichloraectic acid or picric acid. Glycogen with low ash and phosphorus contents has been prepared by both methods. However the possible disadvantages inherent in these methods have been pointed out.

It seemed desirable, therefore, to attempt the preparation of glycogen of low ash, nitrogen, and phosphorus content by means which would not subject the glycogen to harsh treatment. A third class of protein precipitants is the heavy metal ions. Yamakawa (19) has made use of this property of heavy metals in precipitating protein contaminants from solution. His method for quantitative estimation of glycogen in various organs consists in digesting with strong potassium hydroxide followed by acid hydrolysis to form glucose. Ferric hydroxide is then used to free the hydrolysate from contaminants.

This property of the ferric ion in slightly alkaline solution has been made use of in an attempt to prepare glycogen in a pure form.

EXPERIMENTAL

Preparation . One pint of oysters was cut up into small pieces with scissors and heated for ten minutes with 600 ml of water on a boiling water bath. This coagulated the protein and made grinding much easier. The solid was then strained off and ground three times in a meat grinder. The ground oysters were then extracted with the original water for one-half hour on a boiling water bath. The solid was removed by straining through muslin and extracted again with 300 ml of water for one-half hour on a boiling water bath. The extracts were united and cooled; 2 ml of 60% ferric sulfate for every 100 ml of extract was them added with stirring. The solution was made just alkaline to litmus with sodium bicarbonate. To facilitate the removal of the ferric hydroxide and precipitated protein, 15 grams of filter aid and 25 grams of norite were added and mixed in thoroughly. These substances were removed by filtration and centrifugation . The solution was made just acid to congo red paper with citric acid and the glycogen precipitated by adding alcohol to a final concentration of 48%. The precipitation was repeated twice more from neutral solution using the same concentration of alcohol.Drying was carried out by washing twice with 95% alcohol and with

ether. Final desiccation was completed in a vacuum desiccator over a dehydrating agent (drierite).

The solutions from which the glycogen was precipitated were kept cold while acid to preclude any chance of hydrolysis. Precaution was also taken to allow no solution of glycogen to stand over night, since bacterial action might conceivably give rise to sugars of smaller molecular size and yield a glycogen of mixed structure.

The yield of dry glycogen by this method was about 20 grams per pint of oysters during the winter months. During the summer months when the oysters are spawning, a sample prepared from a pint gave 4.5 grams.

Discussion of the Method of Preparation. Before completion of the method several modifications were tried. The concentration of the original extracts to a volume of 300-400 ml was tried. It was found that the procedure gave very little if any increase. Since subjection of the glycogen to several hours of heating might alter its constitution, this was eliminated.

The amounts of filter aid and norite were varied. It was found that the amounts given were most satisfactory; the larger amounts aided in the removal of iron and did not adsorb enough glycogen to lower the yield.

The amount of iron added was varied in an attempt to determine its effect on the ash content. Smaller amounts than that listed gave no lowered ash content. Because of this and the fact that the larger amount gave better

removal of the protein, this quantity of ferric sulfate was used.

Originally the acidification of the solution before precipitation of the glycogen with alcohol was done with oxalic acid. This was changed to citric acid. Both citric and oxalic acids form undissociated complexes with metallic ions. This combination tends to lower the ash content of the preparation. Citric acid, however, has a much lower dissociation constant than oxalic acid which allows the use of a greater amount of citric acid without lowering the pH below that of congo red end point.

The effect of the number of alcohol precipitations on the ash content was studied. The lowering of the ash content was not commensurate with the greater labor and loss of yield which more than four precipitations entailed.

An attempt to adapt Somogyi's (8) alcohol concentrations to this method failed. Somogyi recommended the original precipitation from an alcohol solution of approximately 33%. This concentration failed to give flocculation in the iron method, presumably because the salt concentration was not nearly so large as it was in Somogyi's procedure.

Preparation from Liver. Several attempts were made to prepare a glycogen low in nitrogen content from liver. A preparation made from liver using the method described

for oysters had a nitrogen content of about ten times that of the oyster preparation. The use of two iron treatments gave no better results.

It was believed that the use of a negatively charged ion to precipitate the protein might be of value. Toward this end liver was ground and extracted with water. It was then precipitated with alcohol and the precipitate extracted with alcohol and ether. The glycogen was dissolved in water and the solution acidified to congo red paper with 20% sulfosalicylic acid. The glycogen was precipitated three times from neutral solution. Nitrogen determinations showed values of the same order as was found for products prepared by the iron method.

The use of potassium ferro-cyanide as the precipitating agent failed due to the formation of prussian blue which became adsorbed on the glycogen and could not be removed.

The extraction of liver with alcohol in an attempt to remove the phospholipids before the use of the iron method failed because the presence of the alcohol rendered the iron colloidal and impossible to remove.

The attempts to prepare glycogen low in nitrogen from liver by methods which involved no harsh treatment were all failures. The nitrogen content was never below 0.5%. A probable explanation for this failure lies in the alleged combination between glycogen and proteins. E.M.Mystkawski et al. (20) and St.J.von Przylecki and R.Majmin (21) have

reported complex combinations between glycogen and proteins. The fact that Pfluger's method and adaptations of it give a low nitrogen glycogen from liver is probably due to the fact that in this method the protein which is tied up with the glycogen is hydrolyzed away and the complex thus broken. Glycogen of such high nitrogen content as is obtained from liver would be useless for experiments such as are outlined. For this reason samples were not prepared in quantity for further use.

Analysis. The samples of glycogen were analyzed to determine the ash, nitrogen, and phosphorus contents. Values were also obtained for the specific rotation and reduction values after hydrolysis.

The method used for ash determination was a micro procedure. A small quantity of glycogen (15-25 mg) was weighed in a platinum boat on a micro-balance. The glycogen was burned in an electric furnace at 500-600 degrees in a stream of preheated oxygen. The boat was then weighed again and the weight of the ash determined by difference. For each preparation four or five samples were burned and the ash allowed to accumulate. This gave a check between individual samples and between individual samples and the aggregate.

The phosphorus determination was an adaptation of that given by Peters and Van Slyke (22). The digestion of the sample had to be worked out for application to glycogen.

Glycogen (0.1-0.12 grams) was weighed out, washed into a twelve inch test tube with one ml of fuming sulfuric acid and digested for one-half hour over a micro-burner using a glass bulb over the top to prevent loss of sulfur trioxide. The digestion to a clear solution with sulfuric acid took twenty-four hours or longer. The digestion can be hurried by the frequent addition of small quantities (one-half ml) of concentrated nitric acid. It was found that the irregular acidities which nitric acid gave resulted in erroneous and varying results on known phosphorus samples. Careful neutralization to definite acidity obviated this error but was tedious. It was found that the solution could be cleared rapidly by the following method. To the sulfuric acid digest which had been boiling for one-half hour 2 ml of hydrogen peroxide were added in one ml portions, time being allowed between each addition for the reappearance of sulfur trioxide fumes. This was followed by the addition of 0.5 gram of potassium persulfate. Two more ml of hydrogen peroxide in one ml portions were usually necessary to complete clearance. The remainder of the method was that of Peters and Van Slyke which is colorimetric, using molybdic acid and a reducing agent and comparing the color obtained with that of a known phosphorus sample carried through the same procedure.

The nitrogen determination was carried out with a specially designed micro-kjeldahl apparatus. This method has been published (23).

The specific rotation of the samples was determined in water solution (1%) using the D line of sodium.

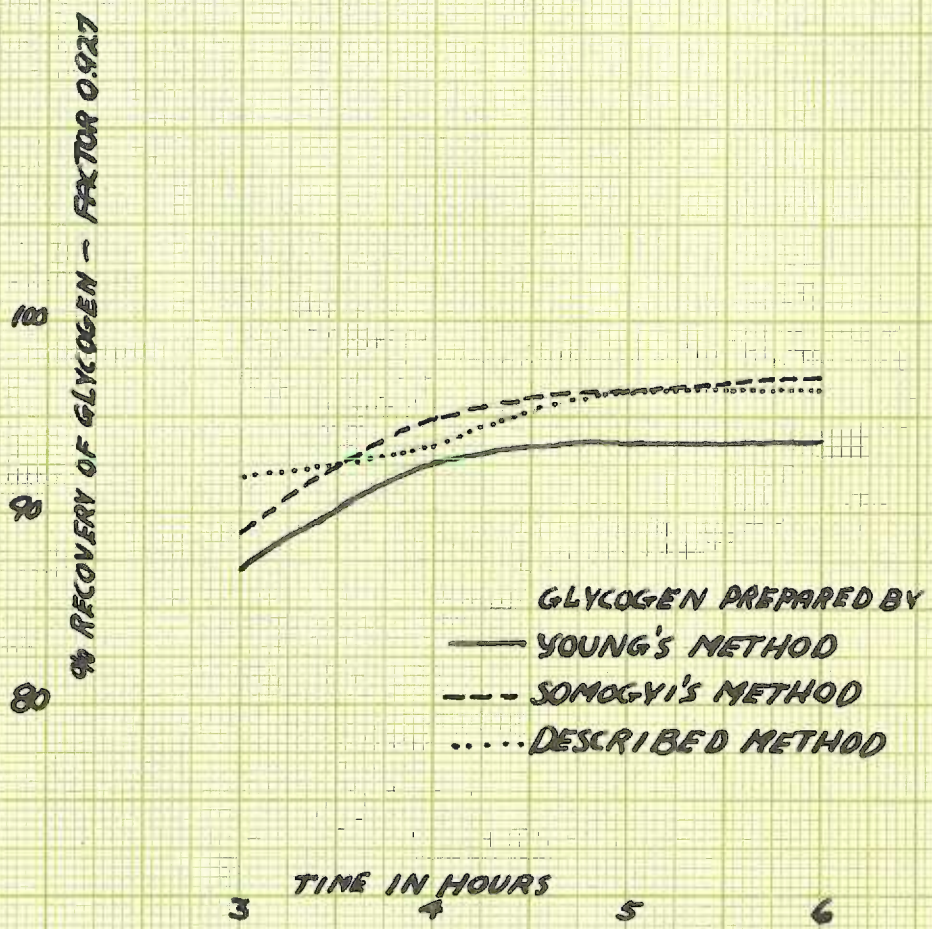
For determination of the reduction values after hydrolysis glycogen (40 mg) was weighed out into a small tube which could be put into an eight inch test tube in its entirety, thus obviating the necessity of transference of the sample after weighing. 40 ml of 0.5N. hydrochloric acid were added and the test tube corked with a rubber stopper into which had been inserted a six inch capillary tube. The capillary tube allowed steam to escape but acted as a condenser and prevented the loss of hydrochloric acid during the hydrolysis. The hydrolysis tube was placed in a boiling water bath and the time necessary for the reduction value to become a constant was determined. The time-reduction curve has been plotted (figure I). After hydrolysis the solution was removed quantitatively to a 200 ml volumetric flask, neutralized with sodium hydroxide and made up to volume. Five ml aliquots of this diluted solution contained approximately 1 mg of glucose. Reduction values were determined by the Schaeffer-Hartman method (24).

It was found that complete hydrolysis was never accomplished, the reduction coming to a constant value at the end of five hours. The figures obtained were in agreement with that given by Kerly (13). The factor which she proposed (0.957) gave 100% recovery (within the limits of experimental error) and led to the conclusion that for glycogen prepared by this method and determined by the Schaeffer-Hartman method, Nerking's

factor (0.927) was too low. Glycogen prepared by Somogyi's modification of Pfluger's method gave reduction values which were in agreement with the results of Kerly. Glycogen prepared by the method of Bell and Young gave reduction values lower than the product prepared by the other methods. This was probably due to the presence of greater amounts of contaminating nitrogen and ash. Such data indicate that reduction values of glycogen prepared by this method also agree with Kerly's factor.

For purposes of comparison glycogen was prepared from liver and oysters by the methods of Bell and Young and Somogyi. Analysis of these samples and those prepared by the method described are shown in tabular form (table II). For all the values given, analyses were carried out in duplicate and the results considered valid only if duplicates checked within 0.01%.

FIGURE I



CONCLUSIONS

From the experimental work done the following conclusions are drawn.

1. Glycogen prepared by the method described has an average analysis of:

rotation	197 degrees
nitrogen	0.048%
ash	0.049%
phosphorus	0.048%
reduction	96.4% (using the factor 0.927)

2. This analysis compares favorably with that of glycogen prepared by the methods of Bell and Young and of Somogyi.
3. The glycogen prepared from liver by the methods described contains a content of nitrogen of 0.5%. This cannot be removed without subjecting the glycogen to harsh treatment such as potassium hydroxide digestion.
4. For glycogen prepared by the methods of Bell and Young, Somogyi, and the method described, Kerly's factor(0.957) gives 100% recovery. The factor of Nerking (0.927) is too low.

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