III. THE HYDROLYSIS OF GLYCOGEN BY DIASTATIC ENZYMES. COMPARISON OF PREPARATIONS OF GLYCOGEN FROM DIFFERENT SOURCES.

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(Received November 23rd 1912.)

For some time considerable uncertainty has prevailed as to whether samples of glycogen prepared from different sources are identical, the evidence on this point being in many respects contradictory. It is agreed that all have the same empirical formula $C_6H_{10}O_5$, the different results obtained by some observers being undoubtedly due to insufficient drying of their glycogen preparations. For Harden and Young [1902] found that the last traces of water can only be removed by heating to 100°C . *in vacuo* over phosphorus pentoxide, a procedure which had not been adopted by previous workers. Very divergent values have however been obtained for the specific rotation of different preparations of glycogen, the results covering a range of about 30° . Thus Cremer [1894] found as the $[\alpha]_D$ of yeast glycogen $+198.9^{\circ}$, while Clautriau's [1895] result was 184.5° . Harden and Young [1902] also obtained for oyster and rabbit glycogens the value 191.2° while with yeast glycogen the mean of several determinations was 198.3° , a result in close agreement with that found by Cremer. It is uncertain however whether these differences are of any real significance, the strong opalescence of glycogen solutions making necessary the use of very dilute solutions and thereby introducing a large experimental error.

Other points of difference have however been noticed by various observers, though again the results are contradictory. Cremer for example states that yeast glycogen gives a darker colouration with iodine than does oyster glycogen, while other workers have obtained exactly opposite results. In a similar way different preparations of glycogen yield solutions with varying degrees of opalescence but these variations are by no means constant either in nature or extent. At present it is impossible to explain these results and

at the same time it is uncertain how far divergences of this nature imply any real differences between the respective glycogens.

A more valuable comparison was made by Harden and Young when they measured the rate of hydrolysis of different glycogen solutions by dilute acids. The course of hydrolysis was followed by estimations of the polarimetric and reducing powers of the solutions. By this method they were unable to detect any differences between rabbit, yeast and oyster glycogens and came to the conclusion that they were in all probability identical.

It seemed possible however that a study of the behaviour of various preparations of glycogen towards diastatic ferments might prove a more delicate means of detecting any difference in their constitution, for it has on many occasions been pointed out what marked influences even small alterations in the molecule may exert on the course of enzyme action. It is the results of such an investigation which are presented in this communication. Four preparations of glycogen have been employed, these being derived from (a) liver of dog, (b) liver of rabbit, (c) oyster and (d) yeast. The enzyme used was an extract of pig's pancreas, this being one of the most convenient sources for obtaining active preparations. Before making any direct comparison, the general conditions of action were studied, for although a great amount of work has been carried out on the behaviour of starch towards diastatic enzymes, the hydrolysis of glycogen has received very much less attention. These experiments will be first described.

PREPARATION OF GLYCOGEN.

A. Dog. A large dog was fed with considerable quantities of carbohydrate for forty-eight hours and then killed. The liver was rapidly removed, minced and heated on the water bath with 60 per cent. caustic potash. The glycogen was precipitated from this solution in the usual way [Pflüger, 1905] and purified by repeated reprecipitation from its aqueous solution, the first two or three precipitations being carried out with solutions rendered faintly acid with acetic acid. Glycogen was in this way obtained in considerable quantity, free from nitrogen and containing only a trace of ash. In the final stages it is frequently difficult to produce a satisfactory precipitation with alcohol alone, the addition of a small quantity of acetone in these cases, however, readily brings down all the glycogen, leaving a liquid which filters well.

B. Rabbit. The method was the same as that described above, a dozen rabbits being used for each preparation. They were previously fed on large quantities of carrot.

C. Oyster. A gross of oysters (1500 grms.) were worked up by Pfüger's method and yielded 120 grms. purified glycogen.

D. Yeast. The glycogen was separated and purified from yeast gum by the method recently described by Harden and Young [1912] with the exception that yeast juice was employed as the starting material. Large quantities of yeast juice were boiled, filtered and the filtrate precipitated with alcohol. The precipitate was then heated on the boiling water bath with 60 per cent. caustic potash to remove protein, the solution diluted and again precipitated with alcohol. After thorough washing with 50 per cent. alcohol and reprecipitation, the glycogen was purified from yeast gum by saturation of the solution with ammonium sulphate, which precipitates the glycogen but not the gum. This process was repeated three times and the glycogen then dialysed to remove the ammonium sulphate. The solution was then again precipitated with alcohol and the further purification carried out as in the final stages described above. The preparation is extremely tedious and the yield very variable according to the history of the yeast employed.

The ash content of the four specimens was as follows. Dog glycogen, 0.38 per cent. Oyster, 0.2 per cent. Rabbit, 0.51 per cent. Yeast, 0.87 per cent. The reaction of the two latter was faintly alkaline, while the dog and oyster preparations both yielded slightly acid solutions.

PREPARATION OF ENZYME.

100 grms. of pig's pancreas were minced and well ground up with half the weight of sand. Sufficient kieselguhr was added to form a fairly stiff mass which was then pressed out in a hydraulic press. The extract obtained in this way after filtration yielded a clear yellow-brown liquid which was extremely active. It was usually diluted to five or ten times its volume before use.

EXPERIMENTAL METHODS.

The course of the hydrolysis can be followed in several ways: (i) estimations of the residual glycogen, (ii) determinations of the optical activity, and (iii) estimations of the reducing power of the solution after known intervals of time. The first of these methods however is unsatisfactory owing to the fact that some of the higher dextrans are also precipitated by alcohol as well as the glycogen, for a precipitate is still obtained in this way some time after the disappearance of the iodine reaction. The optical activity of the solution also is due to a complex mixture of substances, the nature of which has not been thoroughly worked out and hence in the majority of the following experiments, the hydrolysis has been

followed by estimations of the reducing power alone, though this method is not altogether free from the same objection. The latter were carried out by Bertrand's method and the reducing power calculated as maltose, preliminary experiments having shewn that under the conditions employed very little if any hydrolysis of the maltose into glucose took place. The reducing power of a maltose solution of known strength was first determined and from the results obtained, a curve constructed from which the maltose corresponding to any found weight of copper could be read off.

The experiments were all carried out in flasks of Jena glass which were used for no other purposes. In most cases toluene was added to the solutions to prevent bacterial contamination.

HYDROLYSIS OF GLYCOGEN BY AN EXTRACT OF PIG'S PANCREAS.

To study the general course of the hydrolysis, 500 c.c. of a 1 per cent. solution of oyster glycogen were incubated with 2.5 c.c. of a pancreas extract with the addition of 5 c.c. toluene as antiseptic. The glycogen and enzyme solutions were each brought to the temperature of the bath (37°) before mixing and then after known intervals of time, samples of the liquid were removed and the following estimations made.

A. Reducing power.

B. Matter precipitable by alcohol (glycogen and higher dextrins). The precipitation was carried out by adding two volumes of alcohol. At the same time the reaction of the solution towards iodine was observed.

The results are collected in the following table.

TABLE I.

Hydrolysis of Glycogen by Pancreatic Diastase.

Time	Duration of action	Wt. of ppt. thrown down by 2 vols. alcohol	Maltose	Iodine reaction	Percentage hydrolysis
12.30	0	4.702 grms.	—	Dark red brown	0
12.35	5 mins.	—	0.223 grm.	Red brown	4.45
12.45	15 "	3.49 "	0.664 "	"	13.32
1.00	30 "	2.74 "	1.156 grms.	"	23.2
1.15	45 "	2.30 "	1.471 "	Pale red brown	29.5
1.30	60 "	1.83 "	1.714 "	Very faint	34.4
2.0	90 "	1.48 "	2.003 "	None	40.2
2.30	2 hrs.	1.20 "	2.145 "	—	43.0
3.30	3 "	—	2.292 "	—	46.0
4.30	4 "	0.65 grm.	2.362 "	—	47.4
5.30	5 "	—	2.434 "	—	48.8
9.30	9 "	Estimation spoiled	2.550 "	—	51.2

In Fig. 1 the results are shown graphically, curve A representing the degree of hydrolysis and curve B the residual glycogen and dextrins precipitable by two volumes of alcohol. It will be seen that the action commences at a high initial velocity and proceeds for a very brief period in a linear direction. By the time the iodine reaction has disappeared, the rate has slowed down considerably and finally a long period of extremely slow hydrolysis follows. It would appear that the rapid initial stage is chiefly concerned with the conversion of the glycogen into dextrins with simultaneous

Fig. 1.

production of sugar. These dextrins, which are very resistant to further hydrolysis, are then slowly broken down to sugar. It is obvious however that in the above experiment this second stage would never have reached completion and this seems to be the general rule unless very high enzyme concentrations are employed. The iodine reaction disappeared when about 40 per cent. of the glycogen had been completely hydrolysed to maltose. The action in short closely resembles the hydrolysis of starch but is slower and less complete.

INFLUENCE OF TEMPERATURE.

A series of flasks each containing 50 c.c. of 1 per cent. glycogen were incubated with 0.5 c.c. enzyme solution, at temperatures varying from 25° to 50°. Thirty minutes after the addition of the enzyme, the action was stopped by adding 5 c.c. of N. KOH, the liquid was at once cooled and the volume made up to 100 c.c. The reducing power of 20 c.c. of this dilution was then estimated. The results are shewn below in Table II and graphically in Fig. 2. For the sake of comparison the results of a starch hydrolysis with the same enzyme preparation are given also,

TABLE II.

Influence of Temperature.

Temperature of incubation	A. Glycogen		B. Starch	
	Total maltose	Percentage hydrolysis	Total maltose	Percentage hydrolysis
25.5°	0.1070	20.2	—	—
27.5°	—	—	0.1310	24.7
34°	0.1212	22.9	—	—
37°	0.1375	25.9	0.1545	29.2
39.5°	0.1330	25.1	—	—
42°	0.1310	24.7	0.1700	32.1
46°	0.1260	23.7	0.1895	35.7
50.5°	0.1190	22.5	0.1760	33.2

Fig. 2.

Hence with the enzyme employed in these experiments, the optimum temperature for glycogen hydrolysis was about 37°C. and this value was unchanged by substituting different preparations of glycogen. For starch hydrolysis on the other hand the optimum temperature found with the same enzyme solution was 46°C. This result therefore lends some support to the hypothesis that the two actions are brought about by two distinct enzymes.

INFLUENCE OF ENZYME CONCENTRATION.

Three dilutions of the enzyme solution were prepared and 0.5 c.c. of each added to a series of flasks each containing 50 c.c. of a 1 per cent. glycogen solution (oyster)+1 c.c. toluene and previously brought up to 37°. Altogether three flasks were used with each dilution.

After 5, 15, and 25 minutes' incubation one flask of each series was removed from the bath and the action stopped by the addition of 5 c.c. N. alkali. The reducing power of each solution was then determined and the results are shewn in Table III below.

TABLE III.

Influence of Enzyme Concentration.

Each flask contained 50 c.c. 1 per cent. glycogen+1 c.c. toluene+0.5 c.c. enzyme.

Exp.	Dilution of enzyme solution	Reduction after time given, mgrms. Cu from 20 c.c. liquid		
		5 mins.	15 mins.	25 mins.
A	1/10	7	21.5	32.0
B	1/20	3.35	10.0	—
C	1/40	1.7	5.2	9.0

These results shew very clearly two things. (i) Under the above conditions the rate of hydrolysis is directly proportional to the concentration of enzyme, and (ii) the action is a linear one, the reduction after 15 minutes' being almost exactly three times that after five minutes' incubation and so on. These results only obtain however when very dilute enzyme preparations are used and during the initial part of the experiment—in other words while there is a considerable excess of unchanged glycogen. Similar results have been obtained by Evans [1912] in the hydrolysis of starch by saliva.

When stronger preparations of enzyme are employed the above relations can only be observed for a very brief period of the hydrolysis, but another point now becomes more noticeable. With dilute enzyme preparations, the action comes to an end long before all the glycogen has been completely

converted into maltose. This is apparent in the results quoted in Table I where it will be noticed that after nine hours there was only a 51 per cent. hydrolysis and the action had practically come to an end. When however higher concentrations of enzyme are employed, the end point of the reaction is moved very much nearer to complete hydrolysis as will be seen from the experiments described below.

A series of flasks was incubated at 37°C. containing the following solutions :

A. 100 c.c. 1 per cent. glycogen solution + 4.9 c.c. H_2O + 0.1 c.c. enzyme + 1 c.c. toluene.

B. 100 c.c. 1 per cent. glycogen solution + 4.5 c.c. H_2O + 0.5 c.c. enzyme + 1 c.c. toluene.

C. 100 c.c. 1 per cent. glycogen solution + 4.0 c.c. H_2O + 1.0 c.c. enzyme + 1 c.c. toluene.

D. 100 c.c. 1 per cent. glycogen solution + 0 c.c. H_2O + 5.0 c.c. enzyme + 1 c.c. toluene.

Fig. 3.

The total volume in each case was the same, the only difference being the concentration of enzyme. After $8\frac{1}{2}$ hours the action was almost over in all four solutions and the amount of hydrolysis which had taken place was as follows :

A, 45.6 per cent. B, 55.1 per cent. C, 60.7 per cent. D, 91.3 per cent. The complete course of the hydrolysis in each case is shewn in Fig. 3.

Very similar results were obtained by Philoche [1908] with taka diastase and it is not quite clear what interpretation is to be placed upon them, for, as

will be seen, there appears to be no direct relationship between the concentration of enzyme and the final degree of hydrolysis. The influence of the products formed during the hydrolysis may be partially responsible but judging from experiments described later, it is unlikely that this is the sole cause. The incompleteness of the hydrolysis cannot be ascribed to the destruction of the enzyme, for the latter after 20 hours' incubation rapidly attacked a second quantity of glycogen.

INFLUENCE OF GLYCOGEN CONCENTRATION.

Unless very low concentrations are used—less than 0.5 per cent. with the strength of enzyme I have generally employed—the *initial* rate of hydrolysis is practically unchanged by increasing the glycogen concentration. In the weaker solution, however, the rate naturally begins to fall at an earlier period owing to the glycogen being more rapidly exhausted. These points are illustrated by the results shewn in Table IV.

TABLE IV.

Influence of Glycogen concentration.

Time after addition of enzyme	Reduction, mgrms. Cu from 20 c.c. liquid		
	A. 1% glycogen	B. 2% glycogen	
5 mins.	9.0	9.0	Rates equal.
15 "	26.8	27.0	
30 "	45	52	Rate in A falling, B practically unchanged.
1 hr.	66	89	
4 hrs.	92	147	Both rates falling, but more rapidly in A.
20 "	108	188	
% hydrolysis	49.6	43.5	Action at an end in both.

INFLUENCE OF PRODUCTS.

As previously stated, unless high concentrations of enzyme are employed the hydrolysis is not complete. In order to see whether this was due to the influence of the products the following experiments were carried out.

Influence of Maltose. In a preliminary experiment the action had stopped when about 50 per cent. of the glycogen had been hydrolysed to maltose, i.e. when the solution contained rather over 0.5 per cent. of maltose. This

amount of maltose was therefore added to one flask at the beginning of the experiment and the rate of hydrolysis compared with one containing no sugar at the beginning. The composition of the two solutions was as follows:

Flask A. 100 c.c. 1 per cent. glycogen + 1 c.c. toluene + 0.5 c.c. enzyme.

Flask B. 100 c.c. 1 per cent. glycogen solution containing 0.5 gm. maltose + 1 c.c. toluene + 0.5 c.c. enzyme.

TABLE V.

Influence of Maltose.

Time after addition of enzyme	A. Glycogen alone	B. Glycogen + maltose		
	Mgrms. Cu from 20 c.c.	Mgrms. Cu from 20 c.c.	Cu due to added maltose	Cu due to maltose formed
15 mins.	27	136	108	28
30 "	46	154	"	46
1 hr.	66	173	"	65
2 hrs.	80	188	"	80
4 "	92	199	"	91
8 "	100	207	"	99
20 "	108	212	"	104
% hydrolysis 49.6		47.6		

It will be seen from these results that the rate of hydrolysis was practically unchanged by the maltose added and the final degree of hydrolysis lowered by only two per cent. The dextrins formed during the course of the reaction, however, seemed to have some retarding influence, this being illustrated by the following experiment.

Influence of Dextrin. 100 c.c. of 2 per cent. glycogen were incubated at 37° with 0.5 c.c. enzyme until the solution no longer gave the iodine reaction. The action was then stopped by heating the flask in a boiling water-bath. The solution thus obtained was free from glycogen and in addition to the dextrins contained about 0.9 per cent. of maltose. It is referred to below as "products of hydrolysis." The following mixtures were then made up and incubated at 37°.

A. 50 c.c. 2 per cent. glycogen + 50 c.c. "products of hydrolysis" + 1 c.c. toluene + 0.5 c.c. enzyme.

B. 50 c.c. 2 per cent. glycogen + 50 c.c. H₂O + 1 c.c. toluene + 0.5 c.c. enzyme.

Incubation was continued for six hours, the reducing power of the two solutions being estimated from time to time. The results are collected in Table VI.

TABLE VI.

Influence of Products of Hydrolysis.

Time after addition of enzyme	B. Glycogen alone	A. Glycogen + products		
	Reduction, mgrms. Cu from 20 c.c. liquid	Reduction, mgrms. Cu from 20 c.c. liquid	Cu due to sugar present at start	Cu due to maltose formed
15 mins.	26	110	84	26
30 "	45	128	"	44
1 hr.	66	149	"	65
2 hrs.	81	159	"	75
4 "	90	166	"	82
6 "	97	170	"	86
% hydrolysis 44.6				39.3

In this case although there was again no difference in the initial rates, after one hour the hydrolysis in A began to lag behind that in B and after six hours only 39.3 per cent. of the glycogen in A had been hydrolysed as against 44.6 per cent. in B. Moreover in A the action had almost stopped while in B it was still proceeding at a fair rate. We have here then a marked hindering action which must apparently be due to the dextrin-like substances formed during the hydrolysis.

INFLUENCE OF ACIDITY OR ALKALINITY.

The ash almost always present in samples of glycogen consists to a large extent of phosphates and according to the nature of these phosphates the solution may have either an acid or alkaline reaction. Before comparing different samples of glycogen it was therefore necessary to determine to what extent this difference in reaction would influence the rate of hydrolysis. It is obvious moreover that the optimum dose of acid will vary according to the source and proportion of the enzyme used, for the latter as well as the solution acted on may contain substances capable of neutralising some of the acid. On the other hand the optimum hydrogen ion concentration is a constant independent of these factors. In the latter case we obtain a measurement of the true optimum reaction of the solution, while in the former we have only an apparent value, the degree of accuracy of which will depend on the amount and nature of the salts present in the solution. Thus Sørensen [1909] working with three different preparations of invertin found the optimal dose of sulphuric acid (apparent acidity) to be very different for the three preparations but the optimum hydrogen ion concentration (true

acidity) was the same in each case. Hence in all the following experiments the reaction of the solution has been determined by measurements of the hydrogen ion concentration.

At first attempts were made to do this by means of the colorimetric method described by Sørensen. The strong opalescence of the glycogen solutions however rendered this method impracticable and hence the electrical method has been used throughout. The apparatus employed was that of Michaelis and Rona [1909] which is especially suitable since only small quantities of solution are required. In this method, the electrodes are kept in a still atmosphere of hydrogen and connection is made between the two cells by means of a tape soaked in a solution of potassium chloride. By making two determinations using different concentrations of KCl, e.g. 1.75 N. and 3.5 N., the contact potential due to the salt can be calculated and corrected for and, as equilibrium is established almost immediately, the method is both rapid and convenient. The determinations were all made at 37°, the temperature at which the hydrolyses were carried out, the two operations being conducted in the same thermostat. In each case the standard solution against which the glycogen solutions were compared was N/10 HCl and from the value of the E.M.F. thus set up and determined, the hydrogen ion concentration was calculated in the usual way¹.

The latter is denoted by the sign p_H , this being the logarithm of the reciprocal value of the factor of normality. p_H being known, the hydroxyl ion concentration can be calculated from the dissociation constant of water. By graphical interpolation from a series of determinations by Lundén [1907], the value at 37° has been taken as $2.45 \times 10^{-14} = 10^{-13.61}$. Hence in a N/100 solution of hydrogen ions where $p_H = 2$ the hydroxyl ions will have a concentration at 37° of $10^{-11.61}$ and so on. In the same way at absolute neutrality the concentration of both ions will be $10^{-6.8}$.

DETERMINATION OF OPTIMUM REACTION.

To a series of flasks each containing 100 c.c. of a 1 per cent. glycogen solution, were added varying amounts of N/100 NaOH or H₂SO₄. The total volume of the solutions, however, was kept the same in every case. Each flask was then in turn placed in the bath at 37° and when the solution had acquired that temperature 0.5 c.c. of a dilute enzyme preparation was added.

¹ A full account will be found in Sørensen's paper [1909] where he also discusses the possible sources of error in the method.

After thorough mixing, a sample was removed and the hydrogen ion concentration at once determined. Thirty minutes after the addition of the enzyme, a second sample was taken, the action stopped by the addition of a small quantity of N. alkali and the reducing power estimated. In this way the true reaction and the degree of hydrolysis was determined in each case. This process was repeated with all four preparations of glycogen. In these experiments no toluene or other antiseptic was used, Sørensen having found that these substances interfere somewhat in the hydrogen ion determinations, equilibrium not being so readily established. As the duration of each experiment, however, was only 30 minutes it is highly improbable that this in any way influenced the results. These are collected in Table VII and also shewn graphically in Fig. 4.

TABLE VII.

Influence of hydrogen ion concentration on the rate of hydrolysis.

Experiment	Source of glycogen	C.c. of N/100 acid or alkali added	E.M.F. volts	pH	Reduction, mgrms. maltose in 20 c.c.	% hydrolysis in 30 mins.
1	Dog liver	3.0 H ₂ SO ₄	0.2055	4.43	7.37	3.7
2	"	1.0 "	0.2306	4.84	33.5	16.8
3	"	0.5 "	0.2754	5.57	52.9	26.6
4	"	0 "	0.2940	5.87	59.0	29.6
5	"	0.25 NaOH	0.3018	6.00	61.4	30.8
6	"	0.5 "	0.3217	6.32	60.3	30.3
7	"	1.0 "	0.3410	6.63	54.0	27.1
8	"	3.0 "	0.3755	7.19	37.1	18.6
9	"	6.0 "	0.4078	7.72	12.1	6.1
14	Oyster	0.5 H ₂ SO ₄	0.2260	4.76	28.0	14.1
15	"	0 "	0.2730	5.53	40.4	20.3
16	"	0.5 NaOH	0.2870	5.76	44.2	22.2
17	"	1.0 "	0.3019	6.00	44.4	22.3
18	"	3.0 "	0.3322	6.49	32.8	16.5
19	"	6.0 "	0.3980	7.56	10.7	5.4
22	Rabbit liver	6.0 H ₂ SO ₄	0.2492	5.14	4.8	2.4
23	"	5.0 "	0.2750	5.56	35.6	17.9
24	"	4.75 "	0.2850	5.72	45.2	22.7
25	"	4.5 "	0.3010	5.97	47.5	23.9
26	"	4.0 "	0.3180	6.26	47.0	23.6
27	"	3.0 "	0.3412	6.64	42.3	21.3
28	"	0 "	0.3818	7.30	24.9	12.5
29	Yeast	6.0 H ₂ SO ₄	0.2748	5.55	25.5	12.8
30	"	5.0 "	0.2940	5.87	39.5	19.8
31	"	3.0 "	0.3120	6.16	40.0	20.1
32	"	1.5 "	0.3245	6.36	39.5	19.8
33	"	0 "	0.3580	6.91	27.1	13.6

The results shewn above indicate that the value obtained for the optimum hydrogen ion concentration was practically the same with each preparation of glycogen, that from oyster however giving a slightly lower figure than the other three. The optimum reaction therefore for the enzyme I have used was just on the acid side of the neutral point, the hydrogen ion concentration corresponding to an acidity of about $N/10^6$. Alteration of the reaction in either direction produced a considerable reduction in the rate of hydrolysis especially in the case of the dog and oyster glycogens. Hence in comparing the rate of hydrolysis of different glycogen preparations it is essential to see that the reactions of the solutions are as nearly the same as possible, if the results are to be at all reliable.

Fig. 4.

COMPARISON OF GLYCOGENS.

1 per cent. solutions of the four kinds of glycogen were prepared and sufficient $N/100$ acid or alkali added so that each solution after the addition of the enzyme was as nearly as possible at the optimum reaction. The amount to be added was estimated from the results obtained in the experiments last described, the total volume was the same in each case. Immediately after the addition of the enzyme, the hydrogen ion concentration was determined to make sure that the reaction was really correct and then after 30 minutes' hydrolysis, the action was stopped by the addition of alkali and the reducing power estimated. Both the hydrolysis and the hydrogen ion determination were carried out at 37° and no toluene was used for the reason already stated. The results are shewn in Table VIII.

TABLE VIII.

Comparison of Glycogens. I.

Source of glycogen	E. M. F. of solution against N/10 HCl at 37° C.	pH	Mgrms. maltose per 20 c.c. after 30 mins.	Percentage hydrolysis after 30 mins.	Relative rates
Dog	0.2970 volts	5.92	46.10	23.05	100
Rabbit	0.2986 „	5.94	43.25	21.62	93.82
Oyster	0.2956 „	5.90	41.0	20.5	88.9
Yeast	0.3014 „	5.99	39.5	19.7	85.7

COMPOSITION OF SOLUTIONS.

A. Dog glycogen. 100 c.c. 1 per cent. glycogen + 0.25 c.c. N/100 NaOH + 4.5 c.c. H₂O + 0.5 c.c. enzyme.

B. Oyster. 100 c.c. 1 per cent. glycogen + 1.0 c.c. N/100 NaOH + 4.0 c.c. H₂O + 0.5 c.c. enzyme.

C. Rabbit. 100 c.c. 1 per cent. glycogen + 4.5 c.c. N/100 H₂SO₄ + 0.5 c.c. H₂O + 0.5 c.c. enzyme.

D. Yeast. 100 c.c. 1 per cent. glycogen + 4.25 c.c. N/100 H₂SO₄ + 0.75 c.c. H₂O + 0.5 c.c. enzyme.

There is thus quite a marked difference in the rate of hydrolysis of the four preparations of glycogen examined, the least difference being between those from oyster and yeast. A second experiment gave very similar results which are shewn in Table IX.

TABLE IX.

Comparison of Glycogens. II.

Source of glycogen	E. M. F. of solution against N/10 HCl at 37° C.	pH	Mgrms. maltose per 20 c.c. after 30 mins.	Percentage hydrolysis	Relative rates
Dog	0.3046 volts	6.0	48.9	24.45	100
Rabbit	0.2950 „	5.9	46.1	23.05	94.3
Oyster	0.2901 „	5.8	42.4	21.2	86.7 *
Yeast	0.3112 „	6.1	40.45	20.2	83 †

* Solution rather too acid.

† Solution rather too alkaline.

OPALESCENCE OF GLYCOGEN SOLUTIONS.

A 0.2 per cent. solution was made up of each of the four preparations of glycogen and the opalescence of these compared. This was carried out in Nessler glasses, the volume of solution being adjusted until the opalescence appeared to be the same in each case. The comparison is not easy and the experimental error is therefore large but the solution of rabbit glycogen was

markedly the most opalescent of the four examined, oyster and dog glycogen being about equal and yeast less opalescent than any. The actual results obtained were as follows:

TABLE X.

Glycogen	Volumes of 0.2 solutions having equal opalescence	Relative opalescence
Rabbit	11 c.c.	4.5
Dog	30 „	1.7
Oyster	33 „	1.5
Yeast	50 „	1

COLOURATION WITH IODINE.

To 10 c.c. of a 0.2 per cent. solution of each of the glycogen preparations was added 1 c.c. of a 1 per cent. solution of iodine. The volume in each case was then made up to 50 c.c. and the colours compared in Nessler glasses with the following results:

TABLE XI.

Glycogen	Volumes of solutions giving equal depth of colour	Relative strength of colouration
Rabbit	10.5 c.c.	4.7
Dog	19 „	2.6
Yeast	32 „	1.5
Oyster	50 „	1

Thus both as regards opalescence and the colouration with iodine there is a distinct difference between the four glycogen solutions.

SUMMARY AND CONCLUSIONS.

The conditions under which glycogen is hydrolysed by a pancreatic extract (pig) have been studied with the following results:

1. The hydrolysis at first proceeds with great rapidity, the glycogen being quickly converted into dextrins with simultaneous production of maltose. The further breaking down of these dextrins takes place with extreme slowness and is as a rule incomplete.

2. The optimum temperature for glycogen hydrolysis of the enzyme employed in these experiments was 37°. Below this there is a rapid fall in the rate, between 37° and 50° this is less marked. With starch the optimum temperature for the same enzyme preparation was 46°.

3. While excess of glycogen is present, the rate of change is directly proportional to the enzyme concentration and the action is a linear one.

4. With low enzyme concentrations, the action is by no means complete; the degree of hydrolysis, however, rises with increasing concentration of enzyme though not in the same ratio.

5. The concentration of the glycogen solution has little influence on the initial rate of hydrolysis unless very low concentrations (less than 0.5 per cent. in my experiments) are employed.

6. The action is hindered to a small extent by the products of hydrolysis, the dextrins probably being the interfering substances.

7. The action is favoured by the presence of small traces of acid, the optimum reaction determined with several preparations of glycogen being a hydrogen ion concentration of 10^{-6} (at neutrality this value is $10^{-6.8}$ at 37°).

8. Samples of glycogen prepared from (i) dog, (ii) rabbit, (iii) oyster, and (iv) yeast are hydrolysed at different rates when compared at the optimum hydrogen ion concentration. Taking dog glycogen as 100, the relative rates of hydrolysis found are, rabbit 94, oyster 88, and yeast 84.

9. The degree of opalescence and the colouration with iodine exhibited by glycogen solutions vary with the source from which the glycogen has been obtained.

10. In view of the above points of difference it is probable that the four preparations of glycogen examined are not identical in constitution, though it is possible these differences might be caused by variations in the colloidal state of the four glycogen solutions. If, however, the glycogens are distinct, the diastatic enzymes obtained from different animals should also be specific and experiments are in progress to test this point.

My thanks are due to Professor Arthur Harden, F.R.S. and to Dr H. MacLean for much useful advice during the course of this research.

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