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THE PRODUCTION OF FREE SUGARS IN THE MUSCLE TISSUE OF PIGS AND OXEN POST MORTEM

By

J. G. SHARP

Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge and Department of Scientific and Industrial Research.

The early work of Carruthers and Lee in 1935[1] and Mystkowski in 1937[2] showed that extracts of rabbit muscle contained enzymes which hydrolysed glycogen to free sugars apparently quite independently of the reactions of the phosphorolytic cycle. More recently Petrova in 1946-47[3,4], provided further evidence for the presence of α amylase and maltase in extracts of rabbit muscle, and later the presence also of the enzyme amylo-1, 6-glucosidase was proved by both Petrova[5] and Illingworth, Larner and Cori[6].

It would appear that in muscle extracts at any rate, glycogen can be broken down by two quite separate paths. In the Embden-Meyerhof phosphate cycle, the 1-4 glucosidic links along the straight chains in the glycogen molecule are attacked by phosphorylase with the formation of glucose-1-phosphate. This process comes to a stop at the 1-6 glucosidic links which form the branch points in the molecule. At this stage, the amylo-1,6 glucosidase comes into action hydrolysing the 1-6 links with formation of free glucose and freeing further straight chains containing only 1-4 links for the continuation of phosphorylase action.

Assuming that glycogen from pig and ox muscle is similar to horse and rabbit muscle in having an average chain length of 15 glucose units, 14 of which are bound with 1-4 links and 1 with a 1-6 link, on phosphorolysis, one molecule of free glucose should be formed for every 14 molecules of glucose-1-phosphate.

Glycolysis by this path stops, even in presence of residual glycogen, when the muscle reaches its ultimate pH. The main end-products are lactic acid and glucose-6-phosphate, the latter reaching a maximum of 0.5% in glucose equivalents.

From the moment of death, the hydrolysis of glycogen by α amylase and maltase proceeds simultaneously with phosphorolysis and continues after phosphorolylis has ceased at the ultimate pH. The final products of the action of α amylase on glycogen are 19% glucose, 73% maltose and 8% isomaltose[7]. In muscle extracts under suitable conditions, the amylo-1,6 glucosidase will hydrolyse the isomaltose, and maltase will complete the degradation to give 100% glucose.

No information appears to be available on the activity of these hydrolytic enzymes in whole muscle post-mortem. Compared with the enzymes of the phosphate-energy cycle, they are of minor importance in muscle biochemistry but they are of greater importance in meat, since they produce reducing sugars containing carbonyl groups which can react with the amino-nitrogen groups of free amino acids and proteins. This reaction, the well-known browning reaction, may produce desirable results as, for example, in the development of flavour in meat extracts, or undesirable results, as in dehydrated meat causing it to become increasingly unpalatable with time[8].

Dahl has recently published observations on the formation of free sugar from starch filler used in various types of sausage

products[9]. He has shown that the ∝ amylase present in meat does not hydrolyse the starch granules in their native state as in potato flour but is active only after the granules have been altered by cooking.

In the present study, observations were made for the most part on sections of the longissimus dorsi muscle or on isotonic KCl homogenates of the muscle held for periods of one day at room temperature (18-20°C) followed by 5-7 days at 1°C.

Experimental

Changes in the carbohydrates of pig muscle

In Tables 1 and 2 the changes in several carbohydrate fractions in whole muscle and in an isotonic KCl homogenate of a sample of the same muscle are given.* The pH of the muscle in this animal at 2 hours post-mortem was rather low at 5.8, but other experiments on muscles with higher initial pH's gave similar results with regard to amylolysis.

Table 1

Post-mortem glycolysis in pig muscle, (longissimus dorsi), held in nitrogen for 26 hours at 18°-20°C and subsequently at 1°C. Values are mgms glucose equivalents/g. muscle.

Hours post-mortem	pH	Glycogen	(1) T.F.S.	(2) G	(1)-(2) FS not G	YR
2	5.8	7.8	1.40	1.34	.06	.20
7	5.5	3.8	2.51	1.88	.63	.29
12	5.4	1.2	3.98	2.78	1.20	.52
26**	5.4	.37	6.20	5.65	.55	.50
99	5.4	.19	7.20	5.61	1.59	NIL
170	5.4	.14	7.20	5.8	1.4	NIL

** Muscle transferred to 1°C.

* The following contractions will be used throughout to denote the reducing values of free sugar extracts by Somogyi reagent in glucose equivalents:-

TRV - Total reducing value.
TFS - Total sugar fermentable by yeast
YR - Non-fermentable residue.
NR - Residue of TRV left after treatment with notatin.
G - Glucose.
FS not G - Fermentable sugar not glucose (Maltose).

Table 2

Post mortem glycolysis in homogenate of pig muscle, (longissimus dorsi), held for 25 hours at 18°-20°C and subsequently at 1°C. Values are mgms. glucose equivalents/g. muscle. Time taken from addition of glycogen to homogenate.

Sample No.	Time Hours	pH	Glycogen	(1) TFS	(2) Glucose	(1)-(2) FS not Glucose	Non-fermentable residue (YR)
1	0	5.8	16.5	5.34	3.6	1.7	1.8
2	6	5.8	NIL	10.0	7.8	2.2	3.5
3	20*	5.8	-	15.3	11.1	4.2	1.7
4	44	5.8	-	19.2	13.4	5.8	NIL
5	93	5.8	-	18.7	14.6	4.1	NIL
Total	Δ		-16.5	+13.4	+11.0	+2.4	-1.8

* Homogenate transferred to 1°C.

It is clear from the values in Table 1 that after phosphorolytic glycolysis has ceased at pH 5.4, there is a slow continuous breakdown of glycogen with formation of glucose, fermentable sugar not glucose, and non-fermentable reducing residue. These changes are shown more clearly by the homogenate values, (Table 2). In this case, the homogenate was held for 3½ hours at 18°-20°C to allow the changes associated with rigor mortis to be completed. Only a trace (.005%) of glycogen remained. 16.5mg. of glycogen per gm of muscle were added and the changes in the several carbohydrate fractions followed. The 16.5 mg. of added glycogen which were lost in less than 6 hours were ultimately accounted for within the accuracy of the methods of analysis by the production of 11.0mg of glucose and 2.4mg of FS not G, (maltose), equivalent to 4.8mg of glucose, giving a total recovery of 15.8mg of glucose. The initial 1.8mg of non-fermantable residue were not recovered as glucose. The results shown in Fig. 1 demonstrate clearly the course of the enzymic hydrolysis of the glycogen.

The extracts containing the free sugars were analysed by paper chromatography, and gave three distinct fractions two of which were identified as glucose and maltose whilst the third proved to be composed of dextrins. There was no evidence of the presence of any other sugar such as ribose, for example, which accumulates in the muscle of certain fish to the extent of 0.55% by the action of a nucleotidase[10]. It would appear from preliminary observations on muscle from rabbits and pigs that although the production of IMP from ATP takes place rapidly, the further breakdown of IMP is relatively slow producing first of all inosine and inorganic phosphate and ultimately hypoxanthine and ribose. The longissimus dorsi of a rabbit showed a rise of only 0.3mgm. free ribose per g. after being held for 36 hours at 18°-20°C and 7 days at 1°C.

Further analyses of the sugar extracts taken after 6 and 20 hours' storage and analysed after hydrolysis in N HCl are given in Table 3. The reducing value was shown to be due entirely to glucose and by deducting the original glucose value plus twice the original

Table 3

Analysis of free sugar extracts Nos. 2 and 3 in Table 2 before and after hydrolysis in HCl. Values are mgms glucose equivalents/g muscle.

Sample No.	TRP	YR	NR	TFS	G	FS not G (Maltose)
2.(Before hydrolysis)	13.6	3.6	5.8	10.0	7.8	2.2
(After hydrolysis in N HCl)	28.5	Nil	Nil	28.5	28.5	Nil
△ on hydrolysis	+14.9	-3.6	-5.8	+18.5	+20.7	-2.2
3.(Before hydrolysis)	17.0	1.7	5.9	15.3	11.1	4.2
(After hydrolysis in N HCl)	29.2	Nil	Nil	29.2	29.2	Nil
△ on hydrolysis	+12.2	-1.7	-5.9	+13.9	+18.1	-4.2

Sample 2. Allowing that the original FS not G is maltose then the original dextrins of the YR of 3.6 produce on hydrolysis 20.7 - (2 x 2.2) = 16.3 mg G.
i.e. the dextrins contain on the average not less than 5 glucose units.

Sample 3. Similarly the original YR of 1.7 produces on hydrolysis 18.1 - (2 x 4.2) = 9.7 mg G.
i.e. the dextrins contain on the average not less than 6 glucose units.

maltose value from the total reducing value, an estimate of the hydrolysed dextrins is obtained. It would appear that the dextrins contain on the average at least 5 to 6 glucose units.

The pH of optimum activity of ∝ amylase in pig muscle

The activity of the ∝ amylase present in pig muscle (longissimus dorsi) as shown by the breakdown of added glycogen was observed over a range of pH in whole homogenates in 0.16 M KCl and in the fibril free supernatant fractions after dialysis. The rate of glycogen loss at pH 5.4 was arbitrarily taken as 1.0 and the relative rates at other pH's calculated accordingly. The results given in Figure 2 together with unquoted free glucose values showed that there was no significant difference between the two systems, whole homogenate and dialysed supernatant, either in the rate of loss of glycogen or in the rate of accumulation of free glucose.

The optimum pH lies between pH 6.8 and 7.2 which corresponds with the activity of pancreatic amylase[11]. The optimum rate of accumulation of glucose lies within the same range although the optimum pH of maltase itself is stated to be 5.6 - 6.8 in rabbit muscle and 6.6 in blood serum[12].

Activity of amylolysis in pig muscle

The rates of loss of glycogen and accumulation of glucose at pH 6.2 in homogenates of muscle from 4 pigs are given in Table 4. The mean rates in mg./g./hr. are 5.4 and 0.88 for loss of glycogen and accumulation of glucose respectively.

Table 4

Activity of ∝ amylase in the longissimus dorsi of individual pigs. Values are loss of glycogen and gain in free glucose in mgms./g. muscle/hr. in homogenates of muscle in 0.16M KCl at pH 6.2 at 17.°C.

Pig No.	Glycogen loss	Glucose gain	Ratio $\frac{\text{Glucose gain}}{\text{Glycogen loss}}$
2	3.4	0.85	0.25
3	8.5	1.17	.14
4	5.4	0.77	.14
5	4.3	0.71	.17
Average	5.4	0.88	.18

It is difficult to assess the rate of amylolysis in whole muscle since it proceeds steadily under changing conditions from the moment of death through the development of rigor mortis, accompanied by formation of lactic acid, falling pH and accumulation of hexose phosphate esters. Although amylolysis continues after phosphorolytic glycolysis has ceased at the ultimate pH, the glycogen residue remaining at this stage in the muscle of the pigs available for this study was usually too small for measurement of rates of change. The highest values of free glucose attained in whole muscle after the glycogen stores were exhaused were 5.8 and 3.3 mg./g. after 24 hours at 17°C. In the great majority of animals, the glycogen content at death was only sufficient to give an ultimate pH of 5.4 - 5.7 and a glucose content of 1-2 mg./g. after 48 hours at 10°C.

The initial free glucose content at approximately 1 hour post mortem of the longissimus dorsi muscle of well-fed, rested pigs, killed without struggling, was about 0.5 mg./g. but if the animal struggled before death, the initial glucose content was increased to 1 to 1.5 mg./g. and in addition smaller intermediary fractions of maltose and dextrins were present.

From the results of a study of the amylolytic activity in rabbit muscle, in which rabbits killed under myanesin were compared with rabbits allowed to struggle before death, it would appear that ante-mortem activity has no effect on the degree of amylolytic activity in the muscles.

Amylolysis in beef muscle

Although the course of hydrolysis of glycogen in ox muscle was found to be similar to that observed in pig muscle, the activity of the enzymes was very much lower. It was possible in this case to measure the rate of change in whole muscle since ample glycogen was left in the muscle after the ultimate pH had been reached. In whole muscle at 20°C, the rates of loss of glycogen and accumulation of glucose at pH 5.5 were .046 and .034 mg./hr./g. respectively. In isotonic KCl homogenates at 20 C, the corresponding rates at pH 6.2 were 0.13 and .04 mg./hr./g. respectively, as compared with 5.4 and 0.88 mg./hr./g. respectively for homogenates of pig muscle under similar conditions.

Despite the lower rate of amylolysis, however, the actual glucose level reached in ox muscle after 5-7 days at 1°C was as high as in pig muscle generally, 1-2 mg./g. Over longer periods, glucose will continue to accumulate slowly in ox muscle due to the presence of residual stores of glycogen which are not available in pig muscle after rigor mortis.

Summary and conclusions

1. It has been shown that in pig and ox muscle post-mortem, in addition to the loss of glycogen by phosphorolytic glycolysis, there is loss due to hydrolytic breakdown by the three enzymes, α amylase, amylo-1,6 glucosidase and maltase.

2. The pH of optimum activity of the system in pig muscle lies between pH 6.8 and 7.2 corresponding to the activity of pancreatic amylase.

3. In homogenates of pig muscle at pH 6.2, the rates of breakdown of glycogen by α amylase and accumulation of free glucose are 5.4 and 0.88 mg./hr./g. respectively. In ox muscle, the rates are very much lower, the corresponding values being 0.13 and .04 mg./hr./g. respectively.

4. In the pigs available for this study, the glycogen stores were usually rather low and the final glucose values were 1-2 mg./g. Since the initial glycogen content of ox muscle is higher, similar values are attained provided amylolysis is allowed to proceed over a longer period to compensate for the much lower activity of the enzyme system.

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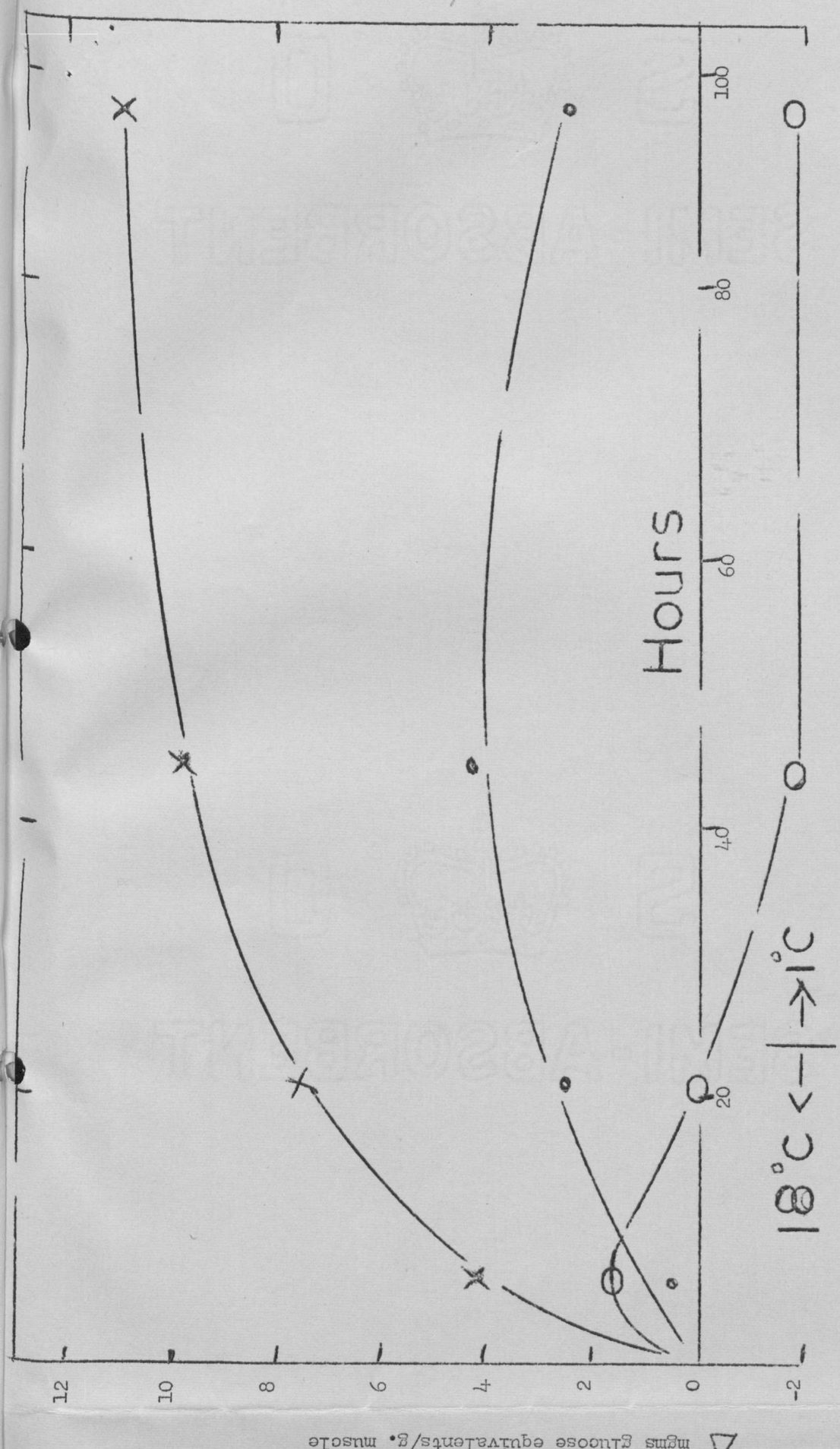

FIGURE 1. Changes in carbohydrate fractions in homogenate of pig muscle held at 18°-20°C for 25 hours and then at 1°C. Values derived from Table 2. Time taken from addition of glycogen to homogenate. Glycogen fell from 16.5 mg./g. to nil in less than 6 hours.

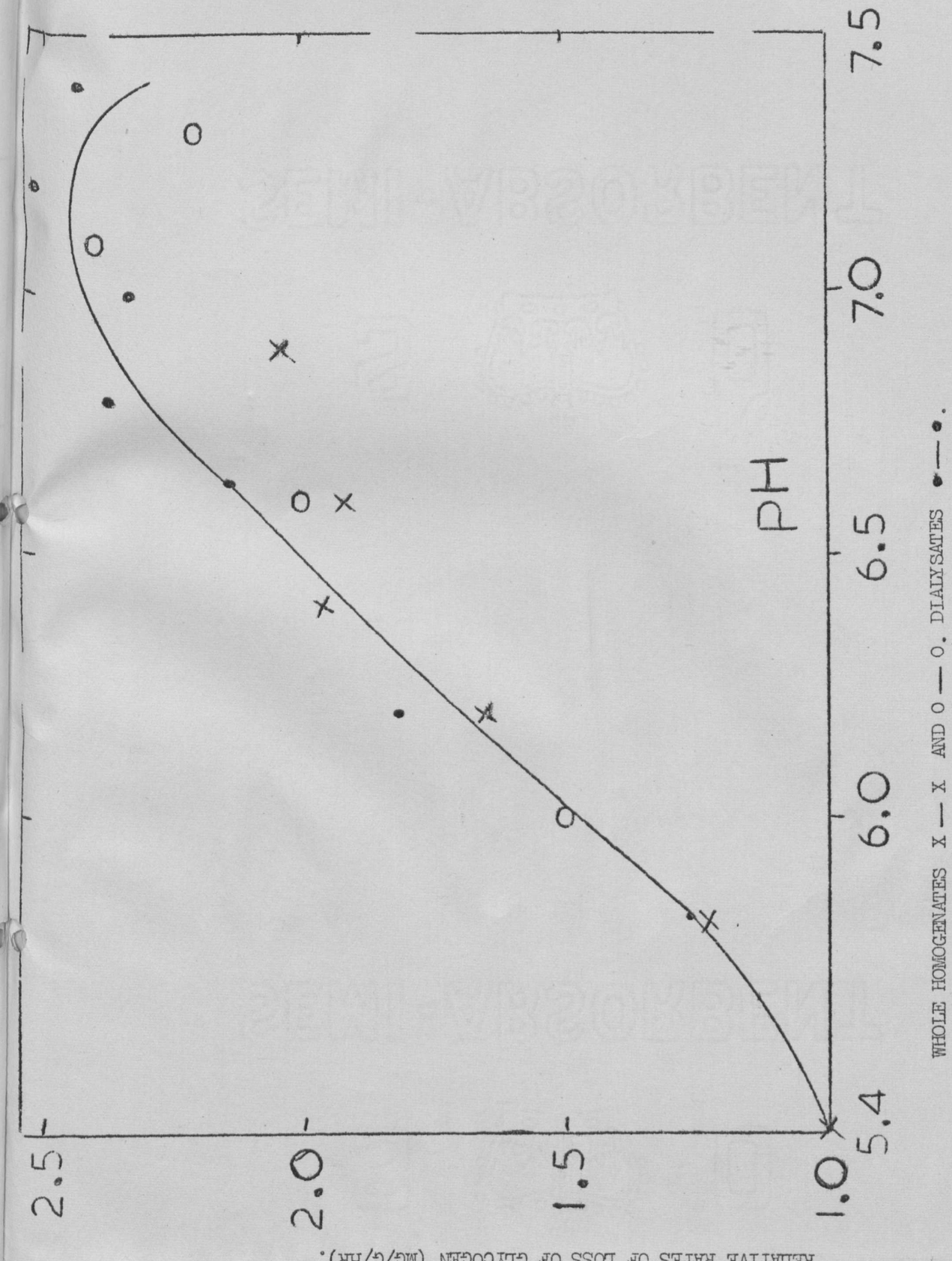

FIGURE 2. RELATIVE RATES OF LOSS OF GLYCOGEN AT DIFFERENT pH's IN HOMOGENATES OF PIG MUSCLE.