49. LIVER AMYLASE AND HYPERGLYCAEMIA

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(*Received 26 January 1940*)

The breakdown of liver glycogen which occurs as one of the chief mechanisms controlling the blood sugar level has generally been ascribed to the liver amylase, but it has recently been suggested that the main path of glycogen breakdown may be through a phosphorylating system involving a phosphorylase which forms hexosephosphate and a phosphatase which hydrolyses the hexosephosphate to glucose [Cori & Cori, 1938].

Recent work has added greatly to our knowledge of the phosphorylating mechanism in the liver [Cori *et al.* 1939; Ostern *et al.* 1939], but if the glycogen breakdown *in vivo* is to be ascribed to this system it would leave the significance of the liver amylase in doubt and there is as yet little reason for attributing this function to the one system rather than to the other.

Cori & Cori [1938] have suggested that the amylase found in the liver may be due to the blood contained in the organ and quote in this connexion an older observation of Davenport [1926] that little amylase is found in the liver after thorough perfusion: but other investigators have concluded that perfusion washes the amylase out of the tissues [Lesser, 1920; Willstätter & Rohdewald, 1936]. There is therefore some doubt as to whether the amylase is really present in the liver tissues or not. The question of the exact localization of the amylase is of special interest since Lesser [1921] concluded that the glycogen and amylase are spatially separated in the liver and that adrenaline acts in producing hyperglycaemia by enabling the glycogen and amylase to come together.

The phosphorylating system has been shown to be reversible, catalysing either the breakdown or the synthesis of glycogen. It is unlikely that there would be more than one system that is able to synthesize glycogen and it is therefore probable that this system is concerned in the synthesis of glycogen *in vivo*; but it is not known that the path of glycogen breakdown *in vivo* is the same as that by which it is synthesized. It is also not known that the mechanism concerned in the normal regulation of the blood sugar is the same as that which comes into action in causing the rapid glycogen breakdown which occurs in the various forms of endogenous hyperglycaemia.

In considering the possible significance of the liver amylase and phosphorylase in relation to hyperglycaemia it is clear that much further information is required before it is possible to reach any final conclusions. In the present investigation particular attention was paid to the significance of the liver amylase and an attempt was made to obtain further evidence on the following questions. (1) Is the amylase present in significant amount in the liver tissue? (2) How are the amylase and phosphorylase distributed between the cells, lymph and blood spaces in the liver? (3) How does the activity of the liver amylase compare with the rate of glycogenolysis which occurs during hyperglycaemia *in vivo*?

Methods

The amylase and phosphorylase activities were determined by incubating tissue brei with glycogen and following the rates of glycogenolysis and phosphorylysis by glycogen and phosphate estimations under suitable conditions.

In order to differentiate between the two enzymes use was made of selective inhibitors and a preliminary study of the action of inhibitors on the enzymes was made for this purpose.

Glycogen. In preliminary experiments it was found that commercial samples of glycogen were not hydrolysed as fast as a specimen prepared in the laboratory. It was thought that this might be due to a modification of the glycogen by the strong alkali used in the manufacture and for the present work the glycogen was therefore prepared by a method that avoided the use of potassium hydroxide or other drastic reagents. The livers of rabbits that had recently been fed with sugar were quickly sliced, dropped into boiling water and boiled for 2 min. The liquid was decanted and the boiled liver minced, ground with sand, returned to the liquid and boiled for 10 min. after adjusting to *p*H 5·3 with acetic acid. The mixture was strained through muslin and the glycogen precipitated with 1·5 vol. of alcohol. After 30 min. the clear solution was decanted and the residue centrifuged. The solid was redissolved in a small volume of 1 % acetic acid, the precipitate removed by centrifuging and the glycogen reprecipitated with alcohol, washed well with acetone and dried.

Glycogen estimation. Glycogen was estimated by the micro-method of Heatley [1935] which was modified only in that larger amounts were taken and the sugar estimations were done by the Hagedorn & Jensen [1923] method. For certain purposes it was found sufficiently accurate and much more rapid to estimate the glycogen by the red colour with iodine. The sample of brei (0·5 ml.) containing glycogen was mixed well with 0·5 ml. 20 % trichloroacetic acid in a centrifuge tube. After centrifuging, 0·5 ml. of the solution was added to 1·0 ml. $N/20$ iodine in 2·5 % KI and the colour compared with standards containing 0·25–1·0 mg. glycogen. Samples giving more colour than the standard range could be diluted and estimated after adding more iodine to bring the iodine concentration to the same as before. The standard solutions kept for about a day.

Phosphate estimation. The presence of glycogen reduces the accuracy of phosphate estimation by the usual molybdate method owing to the turbidity of the final solution used for colorimetric comparison. This was overcome by mixing the specimen (0·5 ml.) in a centrifuge tube with 1·5 ml. alcohol, adding 1 ml. 20 % trichloroacetic acid and keeping overnight before centrifuging and estimating in the usual way as described by Denigès [1921] and Atkins & Wilson [1926].

Determination of amylase and phosphorylase activities. Amylase activities were generally measured by incubating the tissue brei with an excess of glycogen in the presence of a phosphorylase inhibitor and following the rate of glycogen disappearance by glycogen estimations at suitable intervals. In most experiments a total volume of 5 ml. contained 2 ml. liver brei, 0·5 ml. $M/9$ Sørensen phosphate buffer *p*H 7 and sufficient glycogen solution to bring the final concentration with the glycogen already present in the tissue to 1–2 %. As phosphorylase inhibitors 6 % glucose, 0·1 mg./ml. copper added as copper sulphate, or 1 % phloridzin were found to be the most effective. In preparing the liver brei the liver was cooled on ice immediately after perfusion, ground thoroughly with sand in a cooled mortar and mixed with an equal volume of water at 0°. The liver brei was kept on ice and used within 3 hr. of preparation.

In determining phosphorylase activities adenylic acid, hydrogen cyanide [cf. Gill & Lehmann, 1939] and magnesium chloride were added to ensure the activity of the phosphorylating system and sodium fluoride was added to inhibit the decomposition of phosphate esters so that the disappearance of inorganic

phosphate could be taken as a measure of the phosphorylase activity. In most experiments a total volume of 5 ml. contained 2 ml. liver brei, 0·1 ml. adenylic acid (0·1 %), 0·1 ml. $MgCl_2$ solution (1 mg. Mg/ml.), 0·5 ml. $M/2$ HCN prepared by neutralizing KCN with HCl, 0·5 ml. $M/9$ phosphate buffer pH 7, 0·5 ml. $M/2$ NaF and sufficient glycogen solution to bring the concentration to 1–2 %. Tubes containing the mixture were incubated in a water bath at 37°. The rate was not increased by shaking. Samples of 0·5 ml. were taken at the beginning and at various time intervals for phosphate estimation. The initial inorganic phosphate in the tubes, with that coming from the liver brei, was usually equivalent to about 1·5 mg. P. The phosphate uptake due to phosphorylation was generally equivalent to 0·3–0·8 mg. P. The phosphate concentration used in these experiments was chosen as being not too far removed from the physiological concentration. This method of estimating the rate of phosphorylysis of glycogen was subject to error in that even in the presence of $M/20$ NaF the liver breis often showed a slow spontaneous liberation of inorganic phosphate coming possibly from the phospholipins or due to the fact that the phosphatase was not completely inhibited, but the errors incurred in this way were generally small.

The sodium fluoride and other additions used did not appreciably affect the amylase activity; the glycogen breakdown due to the amylase and that due to the phosphorylase were frequently measured simultaneously. Glycogen estimations gave the total rate of glycogenolysis due to both enzymes, while phosphate estimations gave the rate of phosphorylysis. The difference between the total glycogen breakdown and the phosphorylysis then gave the rate of "amylolysis", or hydrolysis by amylase. In calculating the glycogen broken down by phosphorylysis it was assumed that each molecule of phosphate led to the phosphorylysis of one $C_6H_{10}O_5$ unit of glycogen and the formation of one molecule of hexosemonophosphate: the phosphorus uptake figures were therefore multiplied by the factor 5·2 to obtain the phosphorylysis figures.

Preliminary experiments with rabbit liver brei in which 2 % glucose was substituted for glycogen under the conditions described showed no measurable uptake of inorganic phosphate: it was concluded that, in agreement with Ostern *et al.* [1939], no phosphorylation of glucose occurs under these conditions and that the phosphate uptake was entirely due to the phosphorylation of glycogen.

Distribution of amylase in the liver

Perfusion. The possibility of deciding whether the liver amylase is present in the tissues or is mainly confined to the blood spaces in the liver depends on the success with which the blood can be removed by perfusion without causing any loss of amylase from the tissues; considerable attention was therefore paid to the perfusion technique.

Perfusion with distilled water, which was used by Davenport [1926], is liable to cause considerable damage to the tissues owing to the oedema which rapidly occurs and this must hasten the loss of amylase from the tissues. Another source of error is the clotting of the blood in the capillaries through slight damage or chilling of the surface while the liver is being excised. After several different methods had been tried the following was adopted as being the most successful in removing a maximum amount of blood in a minimum of time and with a minimum of damage to the tissues.

Rabbits were anaesthetized with nembutal (about 25 mg./kg. intravenously). The abdominal cavity was opened and a loose ligature rapidly put in position round the portal vein. A hypodermic needle delivering normal saline at 37°

and from a height of 40 cm. was then inserted into the portal vein, the ligature tied round the needle and the vena cava and aorta cut to allow the blood to escape freely. The removal of blood from the liver was hastened by gentle massage. After about 7 min. of rapid perfusion, when the perfusate appeared free from blood, the liver was taken out and immediately cooled on ice.

The efficiency of the perfusion was frequently checked by centrifuging a suspension made by shaking the minced liver in saline: any red blood corpuscles which might be present then formed a fine layer on the surface which enabled the presence of a very small amount of blood to be detected. It was estimated that in none of the experiments was the residual blood as much as 1 % of that originally present or 0·3 % of the weight of the liver; it could therefore be taken that at least 99 % of the blood amylase was removed in this way.

Glycogenolytic activity of perfused liver

The activity of the brei prepared from the perfused rabbit livers in breaking down glycogen was tested under the conditions described. The activity varied greatly from one animal to another: frequently the activity was very high and corresponded to the glycogenolysis of 20–50 mg. glycogen per g. fresh liver per hr., but in many experiments the glycogenolysis did not exceed 1 mg. glycogen per g. liver per hr. The rate of glycogenolysis under these conditions remained constant for several hours or until nearly all the glycogen had disappeared.

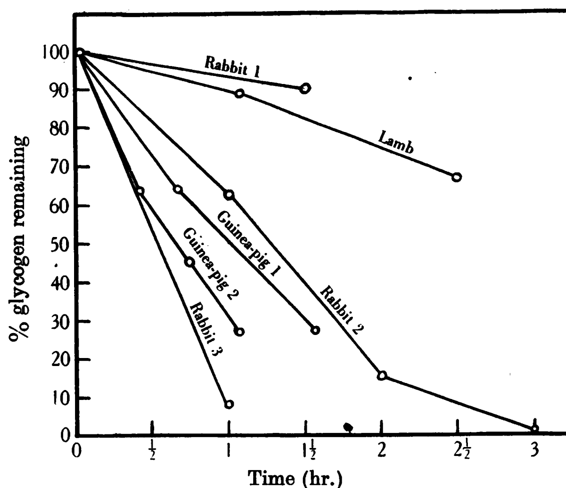

Fig. 1. Glycogen breakdown by liver brei. Guinea-pig 1 and rabbit 2 livers perfused; guinea-pig 2, rabbit 1 and 3 and lamb livers not perfused. Initial glycogen concentration 1 %.

At this stage it was not known whether the glycogenolysis in the liver brei under these conditions was due mainly to an amylase or to the phosphorylase. Experiments were therefore carried out to test this by means of specific amylase and phosphorylase inhibitors.

Amylases are known to be strongly inhibited by maltose, which is a product of their action on glycogen, and phosphorylases are inhibited by phloridzin. Gill & Lehmann [1939] have recently made an extensive study of the action of inhibitors on the muscle phosphorylase. Their observations on the inhibition of muscle phosphorylase by glucose, maltose and copper were confirmed. The

actions of 6 % glucose, 12 % maltose, 1 % phloridzin and 0·1 mg./ml. copper on (*a*) pancreatic amylase, (*b*) blood amylase and (*c*) muscle phosphorylase were then compared with their effects on (*d*) the total glycogenolysis and (*e*) the phosphorylysis measured simultaneously under the same conditions in freshly perfused rabbit liver brei (Table 1).

Table 1. *Percentage inhibitions by maltose, glucose, phloridzin and copper*

Inhibitor	Pancreatic amylase	Blood amylase	Muscle phosphorylase	Liver brei: Total glycogenolysis	Liver brei: Phosphorylysis
6 % glucose	0	2	100; 73; 100	5; 0; 11	82; 100; 41
12 % maltose	85	33	50; 100; 67	66; 45	60
1 % phloridzin	2	8	50	13; 0; 8	56; 47; 24
0·1 mg./ml. copper	8	3	100; 100; 78	0; 0; 0	29; 19; 26

The liver brei was prepared from rabbit liver which gave a breakdown of 15–25 mg. glycogen per g. liver per hr. The copper was added as $CuSO_4$.

The pancreatic amylase was prepared from sheep pancreas and purified by the method of Hanes & Cattle [1938]. Ox blood was used as a source of blood amylase. Muscle phosphorylase was used in the form of an extract made from rabbit muscle as described by Meyerhof [1926]. The enzyme solutions were diluted so as to give a glycogen breakdown of about 50 % in 2 hr. with an initial concentration of 1–2 % glycogen. The amylase experiments were done in the presence of 10 mg./ml. NaCl and $M/30$ phosphate buffer *p*H 7. The conditions of the experiments with muscle extract and liver brei were as described above.

Maltose inhibited the phosphorylase as well as the amylase and so failed to differentiate between them but the inhibitions observed with glucose, copper and phloridzin gave clear evidence that an amylase was present in the liver brei and was mainly responsible for the glycogenolysis observed under these conditions. This was confirmed by the figures giving the phosphorylysis calculated from measurements of the phosphate uptake (Table 2).

Table 2. *Glycogenolysis and phosphorylysis in perfused rabbit liver brei*

Exp. no.	Incubation hr.	Total glycogenolysis mg./g./hr.	Phosphorylysis mg./g./hr.
1	2¼	10·7	1·07
2	2	15·0	0·98
3	1¼	25·6	3·94
4	1¾	3·4	0·86
5	½	24·0	4·23
6	2¼	26·0	1·06

Conditions as described for measuring total glycogenolysis and phosphorylysis simultaneously.

The figures for total glycogenolysis and phosphorylysis represent mg. glycogen broken down per g. fresh liver per hr. The phosphorylysis was always much less than the total glycogenolysis, indicating again that most of the glycogenolysis under these conditions was due to the amylase.

The rates of total glycogenolysis and phosphorylysis were also measured in the same way in the brei prepared from unperfused rabbit liver. The blood was roughly removed by pressing out on filter paper.

The results with unperfused liver (Table 3) were very similar to those obtained with perfused liver and showed that the presence or absence of blood did not produce any very striking difference such as might have been expected if the

Table 3. *Glycogenolysis and phosphorylysis in unperfused rabbit livers*

Exp. no.	Incubation hr.	Total glycogenolysis mg./g./hr.	Phosphorylysis mg./g./hr.
1	1¾	21·6	2·01
2	2	4·8	1·04
3	1	55·0	2·50
4	1½	5·3	2·24
5	1	24·0	2·94
6	1¾	21·6	2·01
7	1	14·4	1·00

Conditions as described for measuring total glycogenolysis and phosphorylysis simultaneously.

blood amylase were an important factor. There was also no evidence of any loss of amylase or phosphorylase activity during the perfusion.

In other experiments with fresh unperfused livers from other species the absence of any considerable inhibition of glycogenolysis by glucose, phloridzin or copper again showed that under these conditions most of the glycogen breakdown in the liver brei was due to "amylolysis" rather than to phosphorylysis.

Animal	Incubation hr.	Total glycogenolysis mg./g./hr.	Percentage inhibition		
			Glucose	Copper	Phloridzin
Guinea-pig	2	12·8	16	0	0
Sheep	2	3·2	0	0	—
Lamb	2	3·2	0	—	0

The sheep and lamb livers were used within 45 min. of slaughtering. The inhibitor concentrations and other conditions were the same as described for rabbit liver.

Distribution of amylase between tissue and blood

The foregoing experiments gave evidence of the existence of an active amylase in the liver tissue. The similar results obtained with perfused and unperfused livers made it improbable that the amylase found in the liver could be attributed to traces of blood remaining in the liver after the perfusion, but it was desired to have more definite evidence on this point and in a number of experiments the amylase contents of the blood and perfused liver from the same animal were tested under the same conditions and at the same time.

Table 4. *Amylase activities of liver and blood from the same animal*

Exp. no.	Animal	Incubation time min.	Additions	Glycogenolysis		Remarks
				Liver mg./g./hr.	Blood mg./g./hr.	
1	Rabbit	65	—	9·2	10·1	—
2	,,	65	—	55·0	50·0	Not perfused
	,,	65	6% glucose	52·0	50·0	,,
3	,,	105	—	3·4	5·7	—
	,,	105	1% phloridzin	3·4	5·7	—
4	,,	150	—	10·7	9·8	—
	,,	150	6% glucose	9·8	9·8	—
5	Guinea-pig	60	—	42	99	—
	,,	60	1% phloridzin	45	99	—
6	,,	65	—	23·4	27·6	Not perfused

The livers were perfused except where stated. Conditions as described.

In five out of six experiments (Table 4) the amylase activities of the blood and liver, expressed as mg. glycogen broken down per g. per hr., were very similar.

In vivo about 30 % of the liver weight is due to blood, but after excision and pressing out this figure may be reduced to 5–10 %. The amount of blood remaining after perfusion was estimated as less than 0·3 %. It was therefore evident that the amylase activity of the blood left in the organ could not possibly account for the activity found in the liver. It was clear that the amylase must be present in the liver tissue and that the concentration in the tissue was similar to that in the blood.

Similar experiments in which the distribution of the phosphorylase was tested showed that it was present exclusively in the tissues: no phosphorylase activity was found in the blood.

Distribution of amylase between cells and intercellular fluid

In stained sections prepared from cat, rabbit and pig livers after fixation in formalin the volume of the intercellular or lymph spaces between the cells and sinusoidal membranes always appeared to be small; but this may be due to shrinkage of the membranes on to the cells in the fixing and staining. There is evidence that the ratio of intercellular space to cell volume may vary considerably *in vivo* under different physiological conditions and the marked change in liver volume produced by adrenaline suggested that such a change might be related to the hyperglycaemic action and made it appear desirable to try to obtain evidence as to whether the amylase is normally present in the cells or is present only in the intercellular fluid.

In ordinary liver brei prepared by grinding in a mortar the texture is generally uneven, and microscopic examination after staining with toluidine blue shows a considerable proportion of damaged cells and cell fragments. After several different methods had been tried it was found that by gently pressing liver slices of about 1–2 mm. thickness first through a coarse net of 25 holes per sq. cm. and then through a fine net of 170 holes per sq. cm. and suspending in normal saline a cell suspension could be obtained consisting mainly of single cells and small clusters of cells in which very few damaged cells or cell fragments could be found. The cells quickly separated out on centrifuging and in this way a rough separation of the cells from the intercellular fluid was obtained. The suspending fluid may be expected to contain most of the enzymes present in the lymph and intercellular fluid as well as any enzymes washed out of the cells.

For testing the amylase activities the liver which was cooled on ice immediately after perfusion was sliced as rapidly as possible, pressed through net into a cooled centrifuge tube, shaken rapidly with an equal volume of normal saline and the suspension immediately centrifuged at high speed. The time taken from starting to cut the slices to centrifuging was about 2 min. The separated cells were ground with sand and made into a brei with an equal volume of water. The amylase activities of the cells and suspending solution were then tested in the usual way. In most experiments 6 % glucose or 1 % phloridzin was added to inhibit the phosphorylase, but this did not appear to be necessary as the phosphorylase was relatively inactive under the conditions used, which were not optimal for showing phosphorylase activity. Two experiments are given in which 6 % gum acacia in normal saline was used as a suspending medium for the cells (Table 5). Control experiments showed that the gum acacia did not inhibit the amylase.

Table 5. *Percentage distribution of amylase between liver cells and suspending fluid*

Exp. no.	Animal	Glyco-genolytic activity mg./g./hr.	Additions	Incubation time min.	Distribution of amylase (%) Cells	Fluid	Remarks
1	Guinea-pig	27·2	—	60	66	34	—
	,,	27·2	1% phloridzin	60	67	33	—
2	,,	24	—	95	53	47	Cells washed
3	,,	10·2	1% phloridzin	60	72	28	Cells washed
4	Rabbit	4·8	—	60	52	48	—
5	,,	24·0	6% glucose	60	54	46	Not perfused
6	,,	25·6	—	75	85	15	Gum saline
7	Lamb	3·2	6% glucose	150	66	34	Gum saline; not perfused

In exps. 3 and 4 the cells were washed by rapidly resuspending in saline and recentrifuging before estimating the amylase activity. Conditions as described.

In every experiment more amylase was found in the cells than in the suspending fluid containing the lymph and intercellular fluid belonging to them. The activity of the suspending fluid was probably due in part to amylase washed out of the cells, as by washing the cells at the ordinary temperature the amylase was soon removed from them; but in two experiments in which the cells were rapidly resuspended in saline at 0° and centrifuged off again the amount of amylase left in the cells was still greater than that in the original suspending fluid.

It was concluded that the amylase occurs to a greater extent inside the liver cells, or closely attached to them, than in the intercellular fluid or lymph.

Distribution of phosphorylase

Similar experiments carried out to test the distribution of the phosphorylase indicated that it is also mainly present in the cells. In these experiments sodium fluoride was added and the phosphorylase activity was measured by the inorganic phosphate uptake under the conditions described.

Exp. no.	Animal	Incubation time hr.	Phosphorylysis mg./g./hr.	Distribution of phosphorylase (%) Cells	Fluid	Remarks
1	Rabbit	1	2·9	62	38	—
2	,,	½	6·3	72	28	—
3	,,	1¼	3·9	63	37	Gum saline

Effect of drying with acetone on glycogenolytic system

Many previous investigators have used "acetone powders" made by grinding the liver with acetone in studying the properties of liver "glycogenase". Hodgson [1936] claimed that the acetone powder prepared from liver is free from blood amylase since the blood amylase is destroyed in this process. It appeared of interest to test whether a real separation of the enzymes could be obtained in this way.

A dry acetone powder was prepared from rabbit liver as described by Hodgson. The powder (1 g. in 5 ml.) was then incubated with glycogen and the total glycogen

breakdown and phosphorylysis measured under the conditions described for the fresh liver brei.

Time of incubation hr.	Total glycogenolysis mg./g./hr.	Phosphorylysis mg./g./hr.
1½	0·65	0·43

The glycogenolysis and phosphorylysis figures, which give the activities per g. of the original fresh liver, indicated that the preparation contained both amylase and phosphorylase with relatively more phosphorylase than is usually present in fresh liver; but the activity of the preparation was low and suggested a considerable loss during the preparation. Contrary to Hodgson, an acetone powder prepared in the same way from blood showed considerable amylase activity.

Amylase in muscle and other organs

Gill & Lehmann [1939] have suggested that the amylase found in muscle may provide an alternative path of glycogen breakdown to the normal phosphorylysis. Parnas [1937] on the other hand was unable to find any amylase in muscle, which suggests that, as in liver, the amount of amylase present may be very variable.

We were able to confirm the presence of amylase in muscle extracts prepared by the method of Meyerhof [1926] from the leg muscles of rabbits which had been perfused *in vivo* with saline through the descending aorta:

Exp. no.	Incubation time hr.	Total glycogenolysis mg./g./hr.	Phosphorylysis mg./g./hr.	Remarks
1	1½	12·0	1·3	Perfused
2	3	6·7	0·53	Not perfused
3	3	5·3	0·74	Perfused

Under the conditions of these experiments, which were the same as those described for measuring glycogenolysis and phosphorylysis simultaneously in liver, the phosphorylysis was much too low to account for the total glycogenolysis. It should perhaps be emphasized that the low phosphorylysis rates shown in these experiments, as in those with liver, do not mean that active phosphorylases were not present in the tissues. The phosphorylysis rates appear low because the conditions were chosen as being optimal for the amylase and were unsuitable for optimal phosphorylase activity. The phosphorylases are very labile and soon destroyed under these conditions, but by using short incubation periods and higher phosphate concentrations high rates of phosphorylysis of over 15 mg. per g. per hr. were easily obtained with both liver and muscle [Lee & Richter, 1940].

In a number of other experiments the rates of glycogenolysis were measured in brei prepared from other animal organs. The conditions were as described for measuring amylase activity; under these conditions phosphorylysis was always very small and the glycogen breakdown was mainly due to amylolysis:

Organ	Incubation time hr.	Glycogenolysis mg./g./hr.	Organ	Incubation time hr.	Glycogenolysis mg./g./hr.
Ox blood	¾	31·8	Lamb brain	2	0
Rabbit blood	2½	34·5	Lamb pancreas	¼	578,000
Lamb blood	2¼	0	Lamb liver	2¾	4·3

It appeared that evidence of the ability of the amylase to diffuse through membranes might be obtained by testing if the blood amylase is able to pass through the haemotoencephalic barrier into the cerebrospinal fluid. Marchionini [1935] has described the presence of amylase in the C.S.F. and in order to confirm this three specimens of C.S.F. which had been sent to this laboratory for Wassermann tests and found to be normal were incubated (*a*) with starch and (*b*) with glycogen at 37°. Each tube contained 1 ml. *M*/15 phosphate buffer *p*H 7·4, 1 ml. normal saline, 0·2 ml. 1 % starch or glycogen, 1 ml. C.S.F. or water, 1 ml. 50 % glucose or water and 0·02 ml. toluene. When tested with iodine a slow disappearance of starch or glycogen, which was not inhibited by glucose, was observed in the tubes containing C.S.F.

Discussion

Origin of liver amylase. Evidence has been obtained that an amylase occurs not only in the blood contained in the liver but also in the liver tissue. Previous investigators have generally concluded that the amylase activity is much greater in the blood than in the liver [Macleod, 1926; Davenport, 1926] but this may be due to the loss of liver amylase during the perfusion or through incomplete extraction. In five out of six experiments in which the amylase activities in the liver and blood from the same animal were measured at the same time the amylase activities, expressed as mg. glycogen broken down per g. tissue per hr., showed a closed agreement (Table 4).

Macleod & Pearce [1909], who compared the blood and liver amylase activities in a number of animals, found that the blood was generally, but not always, the more active and they failed to find any relation between them except for noting that in three experiments in the pig, rabbit and sheep the blood and liver amylase activities appeared to run parallel. The failure of Macleod & Pearce to observe a more definite relationship between the blood and liver amylase activities may be due to their generally omitting to make the comparison at the same *p*H, for in four experiments carried out for another purpose in which buffer was added [Macleod & Pearce, 1911] their figures, recalculated in terms of mg. glycogen broken down per g. per hr., agree with the present observations in indicating a similarity in the blood and liver activities:

Exp. no.	Glycogenolysis in blood mg./g./hr.	Glycogenolysis in liver mg./g./hr.
1	48·6	41·8
2	45·0	53·0
3	34·7	16·1
4	27·2	27·6

It is generally accepted that the blood amylase is derived mainly from the pancreas, since the blood amylase activity is reduced by pancreatectomy and is considerably affected in disease of the pancreas. If, as has been widely believed, the liver amylase is specially synthesized in the cells and has no connexion with the amylase in the blood, there would be no reason to expect any relation between them and the ratio of their activities might lie almost anywhere within a wide range. The observation that in spite of the great individual variation in their absolute amounts the amylase concentrations in the blood and liver so frequently agree can hardly be due to chance. It is concluded that the two are probably related and that the liver amylase is derived from, and normally in equilibrium with, that in the blood.

If the amylase concentration in the liver is maintained equal to that in the blood simply by diffusion from the blood, the amylase must be able to diffuse into the liver cells which occupy the greater part of the liver volume. Independent evidence that the amylase occurs in the cells was obtained by estimating the distribution of amylase between the cells and intercellular fluid. It may be questioned whether it is possible for an enzyme to pass through the cell membranes into the cells, but the amylase is known to diffuse from the pancreas into the blood and through the membranes of the kidney into the urine: it is also found in the lymph and can pass through the haematoencephalic barrier into the cerebrospinal fluid. This gives evidence that the amylase is particularly easily diffusible. The amylase is known to diffuse rapidly out of the tissue when liver slices are suspended in normal saline [Willstätter & Rohdewald, 1936] and there is evidence that it may diffuse out of the liver on perfusion. It does not therefore appear impossible that the amylase may diffuse at a significant rate in the reverse direction so that in the course of time the concentration in the cells *in vivo* may approximate to that in the blood.

Function of liver amylase. The activity of the amylase in the rabbit liver was frequently very low and generally too low to account for the rate of glycogenolysis occurring in the more intense forms of hyperglycaemia *in vivo* [Lee & Richter, 1940]; but in many animals the activity of the liver amylase was sufficient to account for a breakdown of 20–50 mg. glycogen per g. per hr.

It is probable however that such high activities do not occur *in vivo* since it has been shown previously that the liver amylase is activated by chloride ions and requires a considerable chloride concentration for optimal activity. The activity rapidly falls off at chloride concentrations below 35 mg. Cl/100 ml. [Eadie, 1927]. Bang [1913] obtained experimental evidence that the chloride concentration in the cells in the frog's liver is well below the optimal for amylase activity since, after perfusing the liver with isotonic sodium sulphate or sugar solution, the amylase was strongly activated by adding sodium chloride. Evidence has recently been accumulating that the concentration of free chloride ions inside the cells in mammalian muscle, liver and other organs is extremely low and it is frequently accepted that practically the whole of the chloride in the tissues is extracellular [Fenn, 1936; Bourdillon, 1937; Amberson *et al.* 1938]. The liver glycogen is known to be confined to the cells, apart from the relatively small amounts present in the blood and in the leucocytes, but there is reason to believe that the amylase present in the cells may be inactive, or much less active than the amylase activities which have been described would indicate, owing to the absence of a sufficient concentration of free chloride ions in the cells.

It has been shown elsewhere that the activity of the liver phosphorylase, when examined under suitable conditions, is very high and sufficient to account for the most intense forms of hyperglycaemia known *in vivo* [Ostern *et al.* 1939; Lee & Richter, 1940]; there is therefore no need to assume that the amylase is concerned in any normal hyperglycaemic mechanism. The blood amylase might possibly have a function in the detoxication of polysaccharides of bacterial origin, but we have no evidence of this and it can hardly be a function that is general to all the mammalia since Macleod & Pearce [1909] found, and we have confirmed, that in the sheep the amylase concentrations in the blood and tissues are always very small and frequently none can be found at all in the blood or liver in the lamb.

If the distribution of amylase is considered in the animal as a whole it is evident that by far the highest amylase concentrations are in the pancreas and salivary glands. In one experiment in the sheep the amylase activity in the

pancreas was found to be 578,000 compared with 3·4 in the liver. It is clear that even the highest activities found in the blood, liver and other organs were only of the order of 1/10,000 of the amylase activity of the pancreas and therefore small in comparison.

The amylases of the pancreas and salivary glands have a clearly defined function in the digestion of starchy foods in the stomach and intestine, but it is difficult to ascribe any important function in carbohydrate metabolism to the relatively small and very variable amounts of amylase that are found in the blood and other organs. There would appear to be no need to regard the small amounts of amylase found in the liver and other organs as consisting of anything more than traces of the very active pancreatic and salivary amylases which have diffused into the blood and throughout the system.

Summary

1. The amylase activity in the liver in the rabbit is very variable. The activity may be high enough to cause a breakdown of 50 mg. glycogen per g. liver per hr.

2. Methods are described for distinguishing between the glycogen breakdown due to the amylase and that due to phosphorylysis.

3. In five out of six experiments the amylase concentration in the liver was similar to that in the blood.

4. The liver amylase is not confined to the blood or lymph spaces, but is present inside or closely bound to the cells.

5. The glycogen phosphorylase also occurs inside or closely bound to the cells.

6. The phosphorylase is present in relatively larger amount than the amylase in the dry powder obtained by treating liver with acetone.

7. It is concluded that the amylase found in the liver is derived from the blood and is not concerned in any normal hyperglycaemic mechanism.

The authors wish to thank Dr H. Lehmann for useful advice and the Rockefeller Foundation for supporting this investigation.

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