67. ADRENALINE HYPERGLYCAEMIA

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ADRENALINE hyperglycaemia is mainly due to an increased breakdown of glycogen in the liver, but how adrenaline produces this effect is not known. Until recently it was believed that adrenaline activates the liver amylase or brings the amylase and glycogen together so that they can interact [Willstätter & Rohdewald, 1936; Lesser, 1921], but it is now known that the liver contains a phosphorylating system which is also able to break down glycogen to glucose, and it has been suggested that adrenaline may act by accelerating the phosphorylysis of glycogen [Cori & Cori, 1938; Cori *et al.* 1939; Ostern *et al.* 1939].

In a previous investigation evidence was obtained which made it appear unlikely that the glycogenolysis in adrenaline hyperglycaemia is due to the liver amylase [Lee & Richter, 1940]. In the present study an attempt was made to obtain further information as to (a) whether the activity of the liver phosphorylase is sufficient to account for the rate of glycogenolysis in adrenaline hyperglycaemia and (b) how the phosphorylysis of glycogen is influenced by factors which might be expected to play a part in regulating the rate of glycogen breakdown in the liver under physiological conditions.

Methods

The activity of the liver phosphorylase was determined by incubating liver brei with an excess of glycogen in the presence of NaF and measuring the uptake of inorganic phosphate due to the formation of hexosemonophosphate. The glycogen was prepared and the glycogen and phosphate were estimated by the methods described by Lee & Richter [1940]. In order to ensure the activity of the phosphorylating system adenylic acid, $MgCl_2$ and, in some experiments, HCN were added. In preparing the liver brei, the liver, which was excised under nembutal anaesthesia except where otherwise stated, was cooled on ice, ground thoroughly with sand in a cooled mortar and mixed with an equal volume of water at 0°. Rabbit livers were generally used. In experiments carried out under conditions "A" a total volume of 5 ml. contained 2 ml. liver brei, 0.1 ml. 0.1% adenylic acid, 0.5 ml. $M/9$ Sørensen phosphate buffer pH 7.0, 0.1 ml. $MgCl_2$ solution (1.0 mg. $Mg/ml.$), 0.5 ml. $M/2$ NaF and sufficient glycogen solution to bring the glycogen concentration, with that already present in the liver, to 1–2%. Tubes containing the mixture were incubated in a bath at 37° and samples of 0.5 ml. were taken at the beginning and at various time intervals for phosphate and glycogen estimations. Experiments carried out under conditions "B" contained 0.5 ml. $M/2$ HCN, prepared by neutralizing KCN with HCl, in addition to the substances present under conditions "A".

The figures giving the phosphorylysis and amylolysis (glycogen breakdown by amylase) are calculated in terms of mg. glycogen broken down per g. fresh liver per hr. The phosphorylysis figures were calculated directly from the uptake of inorganic phosphate due to hexosemonophosphate formation. The amylolysis

figures represent the total glycogen breakdown as given by the glycogen estimations *minus* the glycogen breakdown due to phosphorylysis.

Blood sugar estimations were done by the Hagedorn & Jensen [1923] method. The additions were neutralized when necessary with NaOH or HCl; the figures giving the amounts added are per g. fresh tissue. Phosphate uptakes are given as mg. P per g. fresh tissue.

Rate of glycogenolysis in vivo

Assuming that the rise in blood sugar in adrenaline hyperglycaemia is mainly due to the breakdown of liver glycogen, it is possible to estimate the rate of glycogenolysis in the liver that would be necessary to account for an observed blood sugar rise.

Rabbits that had been well fed with carbohydrate were deprived of food for 18 hr. and blood sugar estimations were done before and after subcutaneous injection of adrenaline (0.04–0.45 mg./kg.). The blood samples were taken from the marginal ear vein and the glucose estimations done in duplicate.

Fig. 1. Adrenaline hyperglycaemia in the rabbit. (Adrenaline doses are shown on curves.)

The steepest part of each curve gave the maximal rate of blood sugar rise. The rate of rise in the blood sugar is known to increase with increasing adrenaline doses until it reaches a maximal rate which is not further increased by giving larger amounts of adrenaline [Griffith *et al.* 1939]; this may be due to the rate of rise being limited by the maximum possible rate of glycogenolysis in the liver. Taking the liver weight as 4.5 % of the body weight and assuming that under these conditions the blood sugar is in equilibrium with 20 % of the body weight [cf. Evans *et al.* 1931], the total amount of sugar (x) passed into the blood in a given time (t) and hence the rate of glycogenolysis (y) in the liver could be estimated ($y = \frac{x}{100} \times \frac{20}{4.5} \times \frac{60}{t}$). The amount of sugar passing into the urine under these conditions was taken as being negligible.

Exp. no.	Adrenaline mg./kg.	Time interval min.	Blood sugar rise mg./100 ml.	Glycogenolysis rate in liver mg./g./hr.
1	0.04	12	124 → 180 = 45	10
2	0.33	10	239 → 311 = 72	19
3	0.45	11	161 → 218 = 57	14
4	0.33	14	249 → 375 = 126	24

In this way it was estimated that, in the intense hyperglycaemia induced by adrenaline, the system responsible for glycogenolysis in the liver must be capable of breaking down 15–25 mg. glycogen per g. liver per hr. These figures can be taken as giving only a very rough estimate of the rate of glycogenolysis that may be expected to occur *in vivo*, but they are of the same order as the figures obtained by Evans *et al.* [1931] who carried out direct glycogen estimations on liver samples removed during adrenaline hyperglycaemia in the decapitated cat.

Activity of liver phosphorylase

Lability of phosphorylase. Cori & Cori [1938] found that the liver phosphorylase was easily inactivated during dialysis; it was frequently confirmed in the present work that the activity diminished in a few hours at 0° and generally disappeared completely if the liver or brei was left overnight in the ice-chest. In this respect the phosphorylase differed markedly from the liver amylase, which remained active on keeping for several days:

Exp. no	Age of brei	Incubation hr.	Amylolysis mg./g./hr.	Phosphorylysis mg./g./hr.
1	4 days at 4°	2	13.0	0
2	1 day at 4°	3	2.14	0
3	1 hr. at 37°	1½	6.7	0

The rate of inactivation of the phosphorylase was much greater at 37° and little or no phosphorylation could be detected in liver breis that had been kept at 37° for 1–2 hr. The observed inactivation was not due to the accumulation of glucose or other reaction products since attempts to reactivate the enzyme by dialysis were unsuccessful.

These experiments were carried out with rabbit liver under conditions "B", and under the same conditions the fresh liver showed an average phosphorylysis rate of 1–3 mg. glycogen per g. liver per hr. during an incubation of 1–3 hr. The times of incubation were similar to those used by other investigators who have studied glycogenolysis in the liver, but it was evident that experiments of such long duration could give little indication of the true activity of a system so labile as the liver phosphorylase.

When phosphate estimations were done after much shorter incubation times it was seen that most of the phosphate uptake occurred in the first few minutes of the incubation (Fig. 2). After about 1 hr. there was even a diminution in the phosphate uptake, which indicated that the decomposition of phosphate esters was not negligible under these conditions in experiments of long duration. In this respect the liver system showed a difference from the muscle system, a difference which may be attributed to the very active phosphatase present in liver.

In a number of experiments in which the activity of the liver phosphorylase was estimated in different animals by incubating the liver brei under similar conditions for periods of 10–20 min., much higher phosphorylysis rates were

obtained (Table 1). Since the present investigation was completed Ostern *et al.* [1939] have also reported experiments showing high phosphorylation rates in liver brei.

Table 1. *Rate of phosphorylysis in liver brei*

Exp. no.	Animal	Conditions	Incubation time min.	Initial phosphate mg. P/g.	P uptake mg. P/g.	Phosphorylysis mg./g./hr.
1	Rabbit	B	20	1.94	0.88	13.7
2	"	B	15	1.23	0.87	7.5
3	"	A	10	1.79	0.43	13.4
3	"	B	10	1.87	0.53	16.6
4	Guinea-pig	A	5	2.10	0.20	13.2
5	"	A	5	1.06	0.26	16.4
6	"	B	15	1.87	0.81	12.6
7	"	A	5	3.23	0.32	20.0

Fig. 2.

Fig. 2. Phosphate uptake at different times. (Conditions "A" and "B".)

Fig. 3.

Fig. 3. Rate of phosphorylysis observed with different incubation times. (Conditions "A" and "B".)

The falling off in the phosphorylation rate with longer incubation times might be attributed to the drop in phosphate concentration, which would be expected to reduce the rate; that the falling off was in fact mainly due to the inactivation of the enzyme was shown by comparing the activities of samples to which the same amount of phosphate was added after keeping at 37° and at lower temperatures.

Exp. no.	Treatment of brei	Incubation time min.	Phosphorylysis mg./g./hr.
1	15 min. at 10°	15	6.7
	15 min. at 37°	15	2.7
2	15 min. at 0°	15	4.5
	15 min. at 37°	15	1.2
	30 min. at 37°	15	0

Curves obtained by plotting the observed phosphorylysis rate against the incubation time (Fig. 3) indicated that the phosphorylase activities observed with shorter incubation periods were still below the true initial activities in the brei: by extrapolation to zero time higher phosphorylysis rates of the order of 20–30 mg./g./hr. were obtained. It was therefore evident that the activity of the liver phosphorylase was sufficient to account for the rates of glycogenolysis observed in adrenaline hyperglycaemia *in vivo*.

Factors affecting the rate of phosphorylysis

Glucose. In considering the mechanism by which the activity of the liver phosphorylase is controlled *in vivo*, the observation of Gill & Lehmann [1939] that the muscle phosphorylase is inhibited by glucose suggested that a similar mechanism might operate *in vivo* in the liver, and the phosphorylysis of glycogen in the liver might be controlled by the local glucose concentration. The inhibition by glucose of the phosphorylysis in muscle extract was readily confirmed:

Exp. no.	Age of muscle extract days	Incubation time hr.	Initial phosphate mg. P/g.	Phosphate uptake mg. P/g.	Inhibition by 6% glucose %
1	3	3	2.10	0.74	100
2	2	3	1.95	0.44	73
3	6	3	2.06	0.31	100
4	1	3	1.96	0.46	100

The muscle extract was prepared by the method of Meyerhof [1926]. Each tube contained 2.5 ml. muscle extract, 0.3 ml. *M/9* Sørensen phosphate buffer pH 7.0, 1 ml. 5% glycogen, 0.1 ml. 0.1% adenylic acid, 0.1 ml. $MgCl_2$ solution containing 1 mg. Mg/ml., 0.5 ml. *M/2* HCN, 0.1 ml. 5% NaCl and 1 ml. water or glucose solution in a total volume of 5.6 ml. The initial phosphate and phosphate uptake figures are expressed as mg. P in each tube.

The phosphorylysis of glycogen in liver brei was also inhibited by glucose, but the inhibition was generally less than with the muscle extract and showed considerable variation from one animal to another (Table 2).

Table 2. *Inhibition of phosphorylysis in liver by 6% glucose*

Exp. no.	Animal	Conditions	Incubation time hr.	Initial phosphate mg. P/g.	Phosphate uptake mg. P/g.	Inhibition by 6% glucose (%)
1	Rabbit	A	1	0.96	0.39	41
2	"	B	1	2.23	0.28	100
3	"	B	1	1.47	0.56	82
4	"	B	2½	1.32	0.49	43
5	"	A	1½	1.23	0.35	49
6	"	B	1½	1.16	0.44	59
7	Guinea-pig	B	2	1.24	0.63	46

Fig. 4. Inhibition of phosphorylysis by glucose at different concentrations. (Conditions "B".)

At lower glucose concentrations, such as might be expected to obtain under physiological conditions, the inhibition was relatively small (Fig. 4). It therefore

appeared unlikely that a direct inhibition of the phosphorylase by glucose could play a significant part in controlling the rate of glycogenolysis in the liver.

Oxidizing and reducing agents. Lipmann [1933] showed that the phosphorylase of glycogen by muscle extract is inhibited reversibly by oxidizing agents and suggested that the inhibition of glycolysis by oxygen (Pasteur effect) might be explained in this way. Gill & Lehmann [1939] have extended Lipmann's observations to other oxidizing and reducing agents and reported a marked increase in the phosphorylase rate in the presence of hydrogen cyanide.

The observations of Gill & Lehmann were readily confirmed with muscle extracts under the conditions of the present experiments:

Initial phosphate mg. P	Phosphate uptake alone mg. P	Addition	Phosphate uptake with addition mg. P	Inhibition %	Activation %
1.64	0.20	Sat. quinhydrone	0.00	100	—
1.77	0.31	<i>M</i> /20 HCN	0.80	—	160
2.07	0.25	<i>M</i> /20 HCN	0.46	—	84
1.67	0.14	20 mg. thiolacetic acid	0.43	—	207
1.67	0.14	5 mg. GSH	0.16	—	14
1.67	0.14	50 mg. ascorbic acid	0.30	—	114

Each tube contained 2.0 ml. muscle extract prepared by the method of Meyerhof [1926], 1.0 ml. 5% glycogen, 0.1 ml. $MgCl_2$ (containing 1 mg. $Mg/ml.$), 0.1 ml. 0.1% adenylic acid, 0.1 ml. 5% NaCl, 0.3 ml. *M*/9 Sørensen phosphate buffer pH 7.0, 0.5 ml. *M*/2 NaF, 0.1 ml. *M*/1.5 $NaHCO_3$ and special addition or water to bring the total volume to 5.0 ml. The phosphate figures give mg. P per tube. The incubation time was 1–2 hr.

Owing to the very active reducing systems present in liver brei it was necessary to add a relatively large amount of oxidizing agent. A strong inhibition was obtained when autoxidizable dyes were used and the tubes were rapidly shaken in the water bath:

Initial phosphate mg. P/g.	Phosphate uptake alone mg. P/g.	Addition	Phosphate uptake with addition mg. P/g.	Inhibition %
1.67	0.17	10 mg. 2:6-dichlorophenolindophenol (not shaken)	0.21	0
1.16	0.25	20 mg. 2:6-dichlorophenolindophenol (shaken)	0.00	100
1.32	0.24	20 mg. 2:6-dichlorophenolindophenol (shaken)	0.04	83
1.79	0.45	2 mg. methylene blue (shaken)	0.22	51
1.79	0.45	2 mg. K-indigodisulphonate (shaken)	0.21	53
1.79	0.45	2 mg. α -naphthol-2-Na-sulphonate indophenol (shaken)	0.45	0

Reducing agents such as glutathione, thiolacetic acid and ascorbic acid had little effect on the rate of phosphorylase in liver brei, as might be expected in view of the large amounts of similar reducing agents already present, but a strong acceleration by HCN was observed:

Initial phosphate mg. P/g.	Phosphate uptake alone mg. P/g.	Addition	Phosphate uptake with addition mg. P/g.	Change %
1.12	0.34	5 mg. GSH	0.39	+ 15
1.12	0.34	20 mg. thiolacetic acid	0.26	— 23
1.12	0.34	50 mg. ascorbic acid	0.32	— 6
1.12	0.34	<i>M</i> /20 HCN	0.70	+ 106
1.67	0.17	<i>M</i> /20 HCN	0.56	+ 229

Release of inhibition by HCN

The effect of HCN in increasing the rate of phosphorylysis was very striking. It indicated that the rate of phosphorylysis was not maximal in fresh liver brei, but that the system was partially inhibited. The effect of HCN in releasing that inhibition was studied further in the hope of obtaining information as to the factors which normally inhibit the phosphorylysis of glycogen under physiological conditions. The HCN effect was confirmed in a great many experiments under different conditions, using perfused and unperfused livers, different incubation times and the livers from different species. The magnitude of the effect differed from one animal to another, but there was nearly always a marked increase in the phosphorylysis rate (Table 3).

Table 3. *Effect of HCN on phosphorylysis in liver brei*

Exp. no.	Animal	Perfusion	Incubation time hr.	Initial phosphate mg. P/g.	Phosphate uptake mg. P/g.		Increase with HCN %
					Alone	With HCN	
1	Rabbit	—	1½	1.03	0.48	0.70	46
2	"	—	1	1.84	0.69	0.85	23
3	"	Perfused	1½	0.97	0.14	0.32	128
4	"	"	2	1.16	0.25	0.41	64
5	"	"	½	1.61	0.26	0.72	177
6	"	"	½	1.12	0.32	0.44	37
7	"	"	½	1.79	0.45	0.70	56
8	Guinea-pig	—	2	1.24	0.25	0.63	152
9	"	—	½	1.53	0.48	0.81	69

Perfusion with saline as described by Lee & Richter [1940].

The rate of glycogenolysis in the tissues is known to be increased by anoxaemia, and it was thought that HCN might be acting by inhibiting the transfer of O_2 by the cytochrome-cytochrome oxidase system. Against this it was found that added cytochrome c produced no effect, and no acceleration was observed with 0.1 % NaN_3 or $M/1000$ HCN, which are sufficient to produce a very considerable inhibition of the cytochrome system. Experiments in which the air was evacuated from the tubes or replaced by coal gas also gave no significant change in the phosphorylysis rate (Table 4).

Table 4. *Relation of the cytochrome system to phosphorylysis*

Exp. no.	Incubation time hr.	Initial phosphate mg. P/g.	Phosphate uptake alone mg. P/g.	Addition	Phosphate uptake with addition mg. P/g.	Change %
1	½	1.79	0.45	1.5 mg. cytochrome c	0.46	+ 2
2	½	1.97	0.15	3 mg. cytochrome c	0.13	- 13
3	½	1.61	0.26	Evacuated	0.28	+ 8
4	½	1.97	0.15	Coal gas	0.14	- 7
5	½	1.79	0.45	1% NaN_3	0.38	- 15
6	½	1.32	0.24	"	0.17	- 29

Conditions "A"; rabbit liver used.

Phosphorylysis is known to be strongly inhibited by traces of Cu and other heavy metals, but it was unlikely that the HCN was acting by removing Cu,

since the addition of sodium diethyldithiocarbamate, which specifically combines with Cu, produced no effect:

Initial phosphate mg. P/g.	Phosphate uptake alone mg. P/g.	Addition	Phosphate uptake with addition mg. P/g.	Change %
1.53	0.48	Na diethyldithio- carbamate (5 mg.)	0.42	-12

Guinea-pig liver used; incubation period $\frac{1}{2}$ hr.

A further possibility to be considered was that the HCN was acting by removing an aldehyde or ketone which was serving as an inhibitor. In support of this view, a number of aldehydes were found to inhibit the phosphorylysis; but semicarbazide or hydroxylamine failed to show any acceleration such as was produced by HCN.

Exp. no.	Phosphate uptake alone mg. P/g.	Addition	Phosphate uptake with addition mg. P/g.	Change %
1	0.48	1% acetaldehyde	0.14	-71
2	0.48	1% <i>n</i> -butaldehyde	0.12	-75
3	0.48	1% octaldehyde	0.16	-67
4	0.48	2% Na pyruvate	0.39	-19
5	0.48	<i>M</i> /2.5 semicarbazide	0.46	-4
6	0.48	<i>M</i> /2.5 hydroxylamine	0.41	-15
7	0.48	<i>M</i> /20 HCN	0.81	+69

Conditions "A"; guinea-pig liver. Incubation time 15 min. Initial phosphate 1.53 mg. P/g. liver.

Gill & Lehmann [1939] concluded that the phosphorylase is inactive under oxidizing conditions and that HCN and the other reducing agents activate it by reducing —SS— groups in the enzyme to —SH. It would appear doubtful, however, whether the phosphorylase may be always inactive under oxidizing conditions, since it is believed to be concerned in the resynthesis of glycogen from glucose, which occurs only in the presence of O₂. The evidence suggests rather that HCN may not act directly on the phosphorylase, but indirectly by removing an inhibitory reaction product.

Effect of adrenaline on phosphorylysis

In several experiments in which varying amounts of adrenaline were added to the liver brei it was found, in agreement with Ostern *et al.* [1939], that no effect on the rate of phosphorylysis could be observed.

If adrenaline works *in vivo* by activating the liver phosphorylase it appeared that it might be possible to show an increased phosphorylase activity in samples of liver rapidly taken out during adrenaline hyperglycaemia. In order to test this, two glass pestles and mortars containing sand were set up in a covered water bath at 37°. A guinea-pig or rabbit was anaesthetized with nembutal, the abdomen opened and a lobe of liver tied off with cotton and removed. The lobe was rapidly weighed and ground with sand in a mortar at 37°. The weighing occupied less than 1 min. After grinding for 1 min., a solution containing phosphate, glycogen and NaF kept at 37° was added, and the phosphorylase activity was tested by measuring the rate of phosphate uptake in the usual way.

After the removal of the first lobe of liver the abdomen was closed, the animal was kept warm by surrounding with cotton wool and placing near an electric heater and adrenaline was injected subcutaneously. 5 min. later a second lobe

Table 5. *Phosphorylase activity of liver removed during adrenaline hyperglycaemia*

Guinea-pig 1			Guinea-pig 2			Rabbit 1		
Incuba- tion min.	Lobe 1 (1.9 g.) normal	Lobe 2 (2.1 g.)	Incuba- tion min.	Lobe 1 (3.5 g.) normal	Lobe 2 (2.8 g.) normal	Incuba- tion min.	Lobe 1 (10.0 g.) normal	Lobe 2 (10.2 g.)
		5½ min. after adrenaline						17 min. after adrenaline
5	13.2	11.25	5	16.4	14.0	8	7.3	—
10	13.2	9.35	10	11.0	11.4	16	4.6	4.0
15	10.0	8.3	15	9.6	8.3	24	3.7	3.3

Phosphorylase activities expressed as mg. glycogen broken down per g. fresh liver per hr. Guinea-pig 1 received 0.3 mg. adrenaline per kg. Guinea-pig liver lobes were ground with 2.5 ml. 5% glycogen, 2.0 ml. *M*/2 NaF and 0.25 ml. *M*/1.5 Sørensen phosphate buffer pH 7.0. Rabbit 1 received 0.4 mg. adrenaline per kg. The rabbit liver lobes were ground with double these quantities of glycogen, etc.

of liver was removed and tested in the same way as the first. A slight diminution in phosphorylase activity was observed in the successive lobes and there was the usual decrease in activity during the incubation. The experiments showed that the phosphorylase in the normal liver tested under these conditions was already very active, and no further activation by adrenaline was observed.

Effect of adrenaline on inorganic phosphate and adenosine triphosphate in liver

Apart from a direct activation of the phosphorylase, adrenaline might increase the rate of phosphorylase in the liver (*a*) by increasing the concentration of inorganic phosphate, (*b*) by catalysing the decomposition of adenosine triphosphate and so liberating phosphate and adenylic acid which activates the phosphorylase or (*c*) by hastening the removal of glucose-1-phosphate or other inhibitory reaction products.

Orientating experiments failed to show any very striking difference from the normal in the inorganic phosphate or adenosine triphosphate concentration in livers taken out after administering adrenaline. In these experiments the liver samples were removed with the greatest possible speed and ground with trichloroacetic acid. In the guinea-pig experiments, the carotid arteries were cut immediately after giving the animal a blow on the head; the amount of

Table 6. *Phosphate and adenosine triphosphate concentrations during adrenaline hyperglycaemia*

Exp. no.	Animal	Adrenaline dose (mg./kg. subcut.)	Duration of adrenaline action min.	Inorganic phosphate + phosphagen mg. P/g.	A.T.P. mg. P/g.
1	Guinea-pig	—	—	0.262	0.148
2	"	—	—	0.318	0.104
3	"	0.2	20	0.269	0.153
4	"	1.0	30	0.299	0.104
5	Rabbit	—	—	0.293	0.054
5	"	0.4	17	0.289	0.049

Inorganic phosphate was estimated by the method of Denigès [1921] which includes creatine phosphate; but the amount of creatine phosphate in the liver is relatively small [Takahisa, 1936; Wajzer & Lippmann, 1938]. Exp. 5 was done by removing lobes of liver before and after adrenaline injection. A.T.P. was estimated by the method of Lohmann [1928].

adrenaline reaching the liver from the adrenals before the carotids were cut was probably negligible. The amount of inorganic phosphate found in the trichloroacetic acid filtrate should be sufficient to produce a rapid rate of phosphorylysis if present in the free form *in vivo*.

DISCUSSION

Liver amylase and phosphorylase. It has been estimated that the system responsible for the glycogenolysis in the liver during adrenaline hyperglycaemia must be capable of breaking down 15–25 mg. glycogen per g. liver per hr. The activity of the amylase found in the liver is frequently too low to account for so rapid a rate of glycogenolysis, and in a previous paper other reasons have been given for believing that the amylase is not concerned to any significant extent in glycogenolysis in the liver [Lee & Richter, 1940].

The liver phosphorylase is very labile and therefore may appear to be inactive in experiments of long duration, but the activity of the phosphorylase in the fresh liver tissue is very great, being similar to that in muscle and amply sufficient to account for the rate of glycogenolysis observed during adrenaline hyperglycaemia *in vivo*.

Normal blood sugar control. Under normal conditions there must be a balance between glycogen formation from glucose and glycogenolysis in the liver. Assuming that the liver amylase is not involved, the fasting blood sugar level must be maintained by a rate of phosphorylysis which is normally relatively slow: under normal conditions the phosphorylysis of glycogen must be largely inhibited *in vivo*.

It has been shown that the blood sugar level is controlled to a considerable extent by the liver alone, apart from any nervous or hormonal mechanism, since the blood sugar level is maintained approximately constant after denervating the liver, after sympathectomy or after removal of the pituitary gland [Etcheverry, 1937; Soskin *et al.* 1939; Brouha *et al.* 1939]. This means that the rate of phosphorylysis in the liver must depend on the blood sugar level, and phosphorylysis must be increasingly inhibited as the blood glucose rises.

The secondary rise in the blood sugar produced in response to the injection of insulin and many other stimuli is believed to be mediated by adrenaline. Adrenaline, insulin and the pituitary hormones adjust the position of the balance between glycogen synthesis and glycogen breakdown, but they do so by acting on a regulatory mechanism in the liver cells, a mechanism which would appear to depend on the inhibition of phosphorylysis by glucose.

Under the influence of anoxaemia the normal inhibition of phosphorylysis is removed: a rapid glycogen breakdown is observed and the blood sugar may rise to a high value without phosphorylysis being inhibited. Phosphorylysis is therefore inhibited by glucose only in the presence of oxygen. A direct inhibition of the phosphorylase by glucose is unlikely since phosphorylysis in the liver brei was inhibited only 50% by 6% glucose, and at glucose concentrations which might be possible under physiological conditions the inhibition was negligible. The inhibitor which operates *in vivo* is apparently a substance which is produced from glucose in the presence of oxygen; this might be glucose-1-phosphate, which is a powerful inhibitor of phosphorylysis even at low concentrations.

Action of adrenaline. Hegnauer & Cori [1934] have shown that adrenaline increases the rate of phosphorylysis in muscle, and it might therefore be expected to act in an analogous manner on the phosphorylating system in the liver. A direct activation of the liver phosphorylase by adrenaline is unlikely since the phosphorylase catalyses the synthesis as well as the breakdown of glycogen, and an activation of the phosphorylase would be as likely to accelerate either process. It is clear that to increase the rate of phosphorylysis adrenaline must alter the concentrations of the reactants by increasing the effective concentrations of phosphate and glycogen or decreasing the concentration of the primary reaction product, which is believed to be glucose-1-phosphate.

The inorganic phosphate concentration found in the liver was generally about 0.3 mg. P per g., which should be sufficient to account for a rapid rate of phosphorylysis, and no rise was observed after adrenaline. A change in the effective glycogen concentration might be produced, as Wajzer [1938] has suggested, by the conversion of desmo- into lyo-glycogen; but most of the glycogen in the liver is already in the lyo-form. It is therefore probable that adrenaline increases the rate of phosphorylysis by accelerating the removal of glucose-1-phosphate or another phosphate ester intermediary between glycogen and glucose-6-phosphate.

The effects of adrenaline on glycogenolysis may be compared with the effects of anoxaemia: in muscle both anoxaemia and adrenaline increase the conversion of glycogen into lactic acid, while in the liver they both cause an increased conversion of glycogen into glucose.

There is a marked reduction in the blood flow due to vasoconstriction when adrenaline is injected into the perfused cat or rabbit liver [Clark, 1928; Neubauer, 1912], and Masing [1912] observed that after adrenaline the blood passing through the perfused liver may be completely reduced. The hyperglycaemic action of adrenaline has been attributed to the anoxaemia produced through vasoconstriction in the liver, but while this effect may be significant in some animals, it cannot be the only way in which adrenaline acts since adrenaline produces hyperglycaemia without a corresponding vasoconstriction in the perfused frog liver [Fröhlich & Pollack, 1914] and it also acts on isolated muscle [Hegnauer & Cori, 1934] and on tissue slices [Cross & Holmes, 1937].

The rate of phosphorylysis is increased by anoxaemia in the intact liver or by reducing agents and HCN in the liver brei. The precise manner in which adrenaline acts is still not clear, but it has been shown that adrenaline is able to act as an oxidation carrier [Green & Richter, 1937] and this suggests that adrenaline might act in producing a similar effect to anoxaemia by coupling the phosphorylating system with the reducing systems of the cell.

SUMMARY

1. During adrenaline hyperglycaemia in the rabbit the system responsible for glycogenolysis in the liver is able to break down 15–25 mg. glycogen per g. liver per hr.

2. The activity of the liver phosphorylase is sufficient to account for the rate of glycogenolysis occurring in adrenaline hyperglycaemia *in vivo*.

3. The phosphorylysis of glycogen is inhibited by glucose, but the inhibition is not sufficient to account for the inhibition which occurs *in vivo*.

4. The phosphorylysis of glycogen in liver brei is inhibited by oxidizing agents and strongly accelerated by $M/20$ HCN.

5. The accelerating effect of HCN is not due to an inhibition of the cytochrome system.

6. No evidence was obtained that adrenaline works by activating the phosphorylase, by increasing the concentration of inorganic phosphate or by liberating adenylic acid from adenosine triphosphate.

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