CHAPTER 10

EFFECT OF GLUCOSE INJECTION ON THE ACTIVITIES OF GLYCOGEN SYNTHETASE, GLUCOSE-6-PHOSPHATE DEHYDROGENASE, 'MALIC' ENZYME AND LACTATE DEHYDROGENASE IN THE LIVER OF PIGEON

In birds, carbohydrate metabolism is generally regulated by glucagon while insulin has only a minor role to play (Hazelwood, 1973). Avian pancreas secretes 10 times more glucagon than mammalian pancreas (Vuylsteke and De Duve, 1953). Although avian insulin is capable of lowering the blood sugar level (Hazelwood *et al.*, 1968), the reason for the secondary role of insulin in the carbohydrate metabolism in birds is far from clear. In *in vitro* studies (Chapter 9) it has been reported that acetylcholine rather than insulin has more influence over the glucose uptake by avian hepatic cells. When subjected to a glucose load, the pigeon liver showed more acetylcholinesterase activity indicating an increased

acetylcholine (ACh) secretion at the sinusoidal linings (Chapter 8a). The facts that AChE activity in the pigeon liver was almost double than that was measured in rat liver (Chapters 8a and 8b), histochemical reactivity of AChE at the sinusoidal linings was more in the graminivorous birds (Parakeet, Dove and Pigeon) than in insectivorous, ¢omnivorous or carnivorous birds (Shah et al., 1972b) and that AChE activity was elevated along with an increased glycogen and fat deposition in the pigeon liver during post-hatching development led to a logical conclusion that the pigeon liver employs ACh mediated flow coupled transport for the uptake of glucose more than insulin facilitated chemiosmotic transport (phosphorylation). In Chapter 9, it has also been suggested that a good deal of glucose that enters the hepatic cells might be converted into fat. The avian liver has a tremendous capacity to convert glucose to lipid (see Langslow and Hales, 1971). The lipogenic enzyme such as 'malic' enzyme is activated in the liver when a heavy carbohydrate diet was provided (Goodridge, 1975) and a carbohydrate rich diet leads to a heavy fat deposition in avian hepatic cells (Pilo, 1967; Patel, 1976; Chapter 4). However, neither exogenous insulin nor guinea pig anti-insulin serum affected the induction of 'malic' enzyme or

the increase in the synthesis of fatty acids in the liver of chicks (Goodridge et al., 1974). It is now well documented that avian liver has a very low sensitivity to insulin for glucose uptake and lipogenesis. Both these metabolic responses of the liver could be under the control of other endocrine secretions adjusted by insulin (Hazelwood, 1973) or under the influence of autonomic nervous system (Chapter 8a). The administration of glucose resulted in a peaked response of AChE activity and glycogen deposition in the pigeon liver after 30 minutes (Chapter 8a). In the present experiment, activities of $NADPH_2$ generating enzymes such as G-6-PDH and 'malic' enzyme, as well as glycogen synthetase and lactate dehydrogenase were measured in the pigeon liver following glucose load, to establish a correlation of activities of these enzyme with AChE activity and glycogen deposition.

MATERIALS AND METHODS

Adult domestic pigeons (Columba livia), starved for an overnight period, were injected with glucose (70 mg glucose/100 gm body wt.) as 30% solution in saline intravenously. The control pigeons received the calculated amount of normal saline. Five pigeons were sacrificed at different intervals, viz. 30, 60, 90 and 120 minutes after glucose administration and two pieces of liver were

immediately excised and processed for enzyme assays and protein estimation.

ENZYME ASSAYS

A weighed piece of liver was homogenized in glass distilled water for glycogen synthetase and lactate dehydrogenase, while another piece was homogenized in 0.25 M sucrose solution and the homogenate was centrifuged at 20,000 g for 15 minutes at 4°C in a high speed centrifuge and the supernatant was used for estimation of glucose-6-phosphate dehydrogenase and ~~NADP~~-malic enzyme.

UDPG-α-glucan glucosyl transferase or glycogen synthetase (E.C. 2.4.1.11) activity was estimated according to the method of Leloir and Goldemberg (1962) and protein was estimated using same homogenate according to the method of Lowry et al. (1951). Enzyme activity is expressed as μ mole UDP formed/mg protein/10 minutes.

Activity of glucose-6-phosphate dehydrogenase (E.C.1.1.1.49) was measured by employing the method of Kornberg and Horecker (1965) with modifications suggested by Marks (1966). NADP-malate dehydrogenase (E.C.1.1.1.40) was assayed as descfibed by Hsu and Lardy(1969). The

protein content was estimated by the method of Layne (1957). Activities of both the enzymes are expressed as mμ mole $NADPH_2$ formed/mg protein/minute at 30°C.

The activity of lactate dehydrogenase (E.C.1.1.1.27) was estimated employing the colorimetric method of King as described by Varley (1975). The protein estimation was carried out by the method of Layne (1957). The activity of enzyme is expressed as μ mole lactate oxidised/mg protein/ 15 minutes.

RESULTS

Table 1 and Fig.1. presents the data on the activities of enzymes studied in the liver of 48-hr starved, normal (overnight starved) and glucose injected pigeons.

GLYCOGEN SYNTHETASE

Activity of glycogen synthetase was 0.2446 unit in the liver of normal pigeon and it was reduced to nearly 10% of the normal or control value in the liver of 48-hr starved pigeon. The glucose administration resulted in a three fold increase at 30 minutes after administration which thereafter declined to nearly normal (0.2839 unit) levels by 120 minutes.

G-6-PDH AND NADP-MALATE DEHYDROGENASE

The activity of glucose-6-phosphate dehydrogenase was found to be 13.83 units in the liver of normal pigeon and it increased slightly (14.57 units) in the liver of 48-hr starved pigeon. Similarly, the liver of glucose injected pigeon too showed an apathetic response to glucose load and reached only ~~at~~ 18.79 units after 120 minutes. The response of 'malic' enzyme to the starvation and glucose load was different from that of the HMP-shunt dehydrogenase. Its activity was found to be very high (83.67 units) in the liver of normal pigeon, which reduced to nearly half the level (45.49 units) in the liver of 48-hr starved pigeon. The liver of glucose injected pigeon registered an increase from 83.67 to 114.75 units within 30 minutes and more or less maintained this high level even at 2-hrs after glucose administration.

LACTATE DEHYDROGENASE

The activity of lactate dehydrogenase was lower (53.35 units) in the liver of normal pigeon than that of either starved (88.88 units) or glucose injected pigeons. After injection, it increased slowly during the first 60 minutes and reached a maximum (141.63 units) at 90 minutes and became slightly lower (106.75 units) at 120 minutes.

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EXPLANATION FOR FIGURE

Fig. 1. Graph showing quantitative analyses of activities of glycogen synthetase, glucose-6-phosphate dehydrogenase, 'malic' enzyme and lactate dehydrogenase in the liver of starved, normal and glucose injected pigeons.

FIG I
ENZYME ACTIVITIES IN LIVER

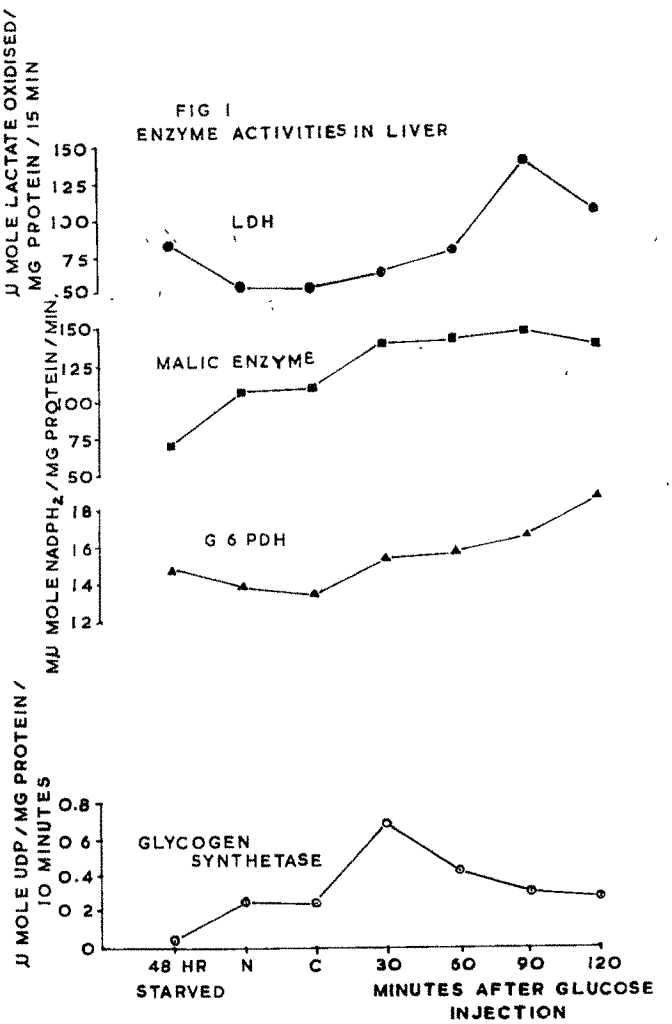

TABLE 1

Activities of Glycogen synthetase, Glucose-6-phosphate dehydrogenase, ~~NADP~~-Malic enzyme and Lactate dehydrogenase in the liver of pigeon after glucose administration. Mean ± S.D.

Time	Glycogen synthetase[1]	G-6-PDH[2]	'Malic'[3] enzyme	Lactate[4] dehydrogenase
48-hr starved	0.0214 ±0.0021	14.57 ± 2.99	45.49 ± 1.96	88.88 ±14.90
Normal	0.2446 ±0.0629	13.83 ± 2.19	83.67 ± 5.41	53.35 ±11.93
Control	0.2462 ±0.0421	13.26 ± 2.34	86.35 ± 5.03	56.98 ±11.95
Glucose injected 30 minutes	0.7227 ±0.0183	15.32 ± 3.13	114.75 ± 8.23	63.26 ± 7.85
60 minutes	0.4483 ±0.2531	15.79 ± 3.45	115.73 ± 4.89	77.11 ±14.80
90 minutes	0.3058 ±0.0309	16.64 ± 2.20	123.45 ±11.49	141.63 ±13.69
120 minutes	0.2839 ±0.0530	18.79 ± 1.78	113.90 ± 9.80	106.75 ±14.93

1 - μ mole UDP formed/mg protein/10 minutes.

2 & 3 - mu mole $NADPH_2$ formed/mg protein/minute.

4 - μ mole lactate oxidized/mg protein/15 minutes.

DISCUSSION

From the results, it could be seen that increase in the activity of glycogen synthetase in the liver of glucose injected pigeon is sensitive to the concentration of glucose-6-phosphate present in the liver cells. Temporary increase in the activity of glycogen synthetase 30 minutes after glucose administration could be due to the increased conversion of D-form of glycogen synthetase to I-form of active enzyme. In Chapter 8a it has been reported that ACh mediated flow coupled transport of sugar was maximally active at this time, which would naturally increase the concentration of glucose-6-phosphate in the liver cells. So it could be suggested that it is this high concentration of glucose-6-phosphate which induces both new synthesis as well as conversion of D-form into I-form of glycogen synthetase. Increased intake of carbohydrate rich diet by migratory starling (Sturnus roseus) during premigratory period (Pilo, 1967), as well as maximum consumption of grains on the 20th day of post-hatching development in pigeon (Chapter 1) resulted in a deposition of considerable amount of glycogen in the liver of both the birds. Apart from the diet, neuroendocrine factors are also found to influence the activity of glycogen synthetase in the liver. It has also been reported that

stimulation of parasympathetic nerve causes an increase in the total activity of glycogen synthetase, while stimulation of splanchnic (sympathetic) nerve completely counteracted the effect of vagus nerve stimulation and also increased the activity of glucose-6-phosphatase and glycogen phosphorylase in the liver of normal as well as pancreatomized rabbits (Shimazu, 1967; Shimazu and Amakawa, 1968a and b). In the same reports it has also been observed that insulin injection caused an increase in the activity of the total as well as I-form of glycogen synthetase but the response to vagal stimulation was much faster than that of insulin. On the contrary the effects of adrenaline and glucagon are completely opposite to that of acetylcholine and insulin because both adrenaline and glucagon increases intracellular concentration of c-AMP and thus activate glycogen phosphorylase as well as glucose-6-phosphatase. From these facts it could be suggested that 90% decrease in glycogen synthetase activity in the liver of 48-hr starved pigeon is due to severe inactivation of I-form of glycogen synthetase in the presence of very low concentration of glucose-6-phosphate which may be a result of the high concentration of glucagon and epinephrine. When the pigeon was subjected to a glucose load, there was a tremendous increase in the activity of AChE indicative

of cholinergic activation (Chapter 8a). The observed increase in the glycogen synthetase activity in the liver of glucose injected pigeon could be due to the influence of activated cholinergic fibres, the secretion (ACh) of which is shown to stimulate both glucose uptake as well as glycogen synthetase enzyme activity in the pigeon liver.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND 'MALIC' ENZYME

The liver of normal pigeon registered only 13.83 units of G-6-PDH as compared to 83.76 units of 'malic' enzyme. And both of these enzymes showed different responses to the starvation; G-6-PDH surprisingly increased slightly i.e., 14.57 units, while 'malic' enzyme reduced to nearly 50% of normal activity (45.49 units). In the liver of glucose injected pigeon there was no significant increase in the activity of glucose-6-phosphate dehydrogenase. It could only reach a level of 18.79 units at 120 minutes after glucose injection. However, HMP-shunt pathway is always more active in birds that consume more of carbohydrate rich diet. In a study of ~~the~~ two migratory birds, Patel (1976) has reported a comparatively high G-6-PDH activity in the liver of Rosy pastor than that of Wagtail, where the former one consumes carbohydrate rich fruits and grains while the latter was found to feed on protein and lipid rich diet (insects). Since the response of G-6-PDH

is not as prominent as that of 'malic' enzyme following administration of glucose, it is possible that HMP-shunt is not very important in the lipogenesis and this pathway at the best may serve two purposes, (1) to provide small amount of $NADPH_2$ and (2) to provide pentose sugar necessary for synthesis of nucleic acids. The results of the present study then agree with the early report of O'Hea and Leveille (1969) that HMP-shunt dehydrogenase plays only a minor role in lipogenesis in ~~the~~ birds.

An active response of malic enzyme observed in the liver during starvation and in the liver of glucose injected pigeon indicates that 'malic' enzyme plays an active role in lipogenesis as in most other avian species. A reduction in the activity of 'malic' enzyme by half in the liver of 48-hr starved pigeon suggests that lipogenic activity was at a slower rate in the absence of enough glucose supply from gut. A longer lasting response of 'malic' enzyme activity after glucose injection suggests that in pigeon liver too, like in other avian species, the lipogenic activity ensues glucose loading. And it is also interesting that, when the effects of glucose load on activities of glycogen synthetase and 'malic' enzyme were compared, the response of the former one is short lived while that of the latter one is more prolonged.

LACTATE DEHYDROGENASE

The activity of lactate dehydrogenase was found to be higher (88.88 units) in the liver of 48-hr starved pigeon than that of normal pigeon (53.35 units). This increased activity of LDH in the liver of starved pigeon might be probably due to either conversion of lactate (from muscle) to pyruvate and finally to glucose for maintaining high blood glucose level which is the characteristic feature of birds, or for the utilization of lactate for slow fatty acid synthesis. The glucose load increased the activity of LDH, however, the response was very slow during first 60 minutes. But it increased significantly by 90 minutes to 141.63 units. This elevation in lactate dehydrogenase after glucose administration is probably due to the rise in blood lactate which normally occurs in humans infused with glucose or fructose. Fitch and Chaikoff (1960) have also reported an increase in the LDH activity in the liver of rats fed with a high glucose diet. Another possibility for the maximum rise in LDH activity 90 minutes after glucose administration is that, that there might be some amount of lactate supply from skin. In fact, temporary increase of glucose concentration in skin without resulting in any increase in glycogen content at 30 minutes has been reported (Chapter 8a). At

the same time there was also a high LDH activity in the skin at 90 minutes after glucose administration (unpublished data). So it could be considered that the glucose taken up by skin is partly converted into lactate when blood glucose level is very high, stored temporarily there and released into the blood stream, which then is converted into pyruvate in the liver by this enzyme.

In conclusion it could be stated that a glucose challenge to liver resulted in an increased glycogen synthetase activity at 30 minutes after administration, which coincided with maximum AChE activity and glycogen deposition in the pigeon liver (Chapter 8a). The glycogen synthetase activity and glycogen deposition, both of which showed peak levels at 30 minutes after glucose injection, ~~then~~ showed a decrement trend thereafter. However, the 'malic' enzyme that showed a sudden spurt in the activity at 30 minutes continued to increase and so did G-6-PDH, probably due to the lipid synthesis that lasted for a longer duration. In fact, Goodridge (1975) observed that ~~the~~ lipogenesis lasts several hours in birds. The LDH activity started increasing by 60 minutes and peaked at 90 minutes, perhaps, due to the fact that skin lactate (formation of which is a temporary phenomenon during hyperglycaemia) might be getting released at this period.