CHAPTER 11

STUDIES ON CERTAIN ASPECTS OF METABOLISM AND ITS REGULATION IN THE LIVER OF GROWING AND ADULT PIGEONS: A GENERAL CONSIDERATION

PART I. METABOLIC ACTIVITIES OF DEVELOPING AVIAN LIVER

During development of an animal, the tissues and organs have to observe an absolute economy in utilizing available energy so as to maximize processes of cell proliferation, growth, differentiation and functional maturity (O'Connor, 1977). Since these processes not only call for high energy requirements but also for specific metabolic activities, while establishing complete metabolic machinery in the liver, certain properties have to be fixed for auspicating each set of enzyme complexes, taking into account, the need of the organ or organism as well as the availability and type of nutrients supplied to the embryo or the young ones.

In birds, the embryo gets, within the egg, a limited but sufficient amount of nutrients. The metabolic machinery,

apart from providing for cell division, growth and differentiation, should also deal with gluconeogenic, lipolytic and protein anabolic activities. The packed food in the egg is also conducive for such metabolic activities, as it is rich in protein and fat.

Upon hatching, the nestlings get a selected diet, and in pigeon a highly nutritive 'crop milk' is given for the first few days and thereafter, pulverized grains. Since the development is still continuing in post hatching period, protein anabolic activities still persist but as the diet undergoes a change, the metabolic machinery is readjusted to bring about glycogenic and lipogenic activities. As the growth rate subsides, the metabolic machinery becomes adjusted more and more to resemble the adult condition. Although such shifts in the metabolic activities are found to be directly correlated with the process of growth as well as the food, some sort of anticipatory triggering of the onset of laying down the machinery (enzyme synthesis) is observed. Such manifestation, well in advance of actual demand, needless to say, is brought by an interplay of hormones.

The rate of growth of liver during post-hatching development of a bird depends upon the nestling period. A nestling period is found only in altricial birds

whereas precocial birds upon hatching, actively move around and are not confined to the nest. In altricial birds the nestling period varies from species to species. The pigeon young one leaves the nest by 25th day but could fly only by 30th day. A sparrow chick ceases to be a nestling and flies out of the nest by 14th day. The house swifts take nearly 30 to 40 days to fly out of nest. Thus according to the time available, the growth rate of the nestlings are hastened or spaced out; at the same time priorities are also fixed as to which organ or system should develop earlier and faster. O'Connor (1977) states that components associated with the ingestion and assimilation of food, such as mouth, gizzard, intestine and liver develop early in the nestling period whilst locomotory components, such as wings and pectoral muscles, develop late in altricial species of birds.

The bird, during post-hatching development, regulates the growth of each organ by distributing the available energy differentially to various organs; the organs too economises on the energy thus made available, to bring about structural and functional maturity. From the present studies carried out on the developing pigeon liver it could be seen that there are two distinct phases, one concerned with structural aspects and the other concerned with

functional aspects of the liver. Thus there are (1) a period of rapid growth and (2) a period of development of functional maturity. The first phase falls between the day of hatching and 10th day and the second between 10th and 20th day. In each period certain metabolic activities are given operational upperhand in conjuction with the morphophysiological processes that are initiated then.

GROWTH PHASE

During the growth phase the mass (non-fat dry tissue) of the liver increases about 4 times from the level at hatching (Chapter 1). This increase could be through both hyperplasia and hypertrophy. Since the growth processes are given priority during this period the metabolic machinery is predominently geared for anabolic activities. In fact such building up processes are a continuation of what was in in ovo stage, ~~state~~. During the in ovo development, the nutrients available are mostly protein and fat. Since the growth phase is continued immediately after hatching the metabolic machinery in the liver does not undergo any drastic change. At this stage as the diet (crop milk) also more or less resembles the nutrients that were available in the egg as far as organic constituents are concerned (protein and fat), the same enzyme complex that was active in the embryonic liver is maintained without much change. In the

growth phase the most characteristic metabolic activities in the liver are gluconeogenesis, lipolysis and protein anabolism. Such preferential reactions take care of both growth and diet of the nestlings during this post-hatching growth phase lasting about 10 days.

FUNCTIONAL MATURITY PHASE

Soon after the rapid growth period, the liver undergoes several changes that endow structural and functional maturity to the organ. Structurally the liver acquires a certain mass, connective tissue arrangements, proper nuclear-cytoplasmic ratio and even perhaps a dinuleate condition. Along with such changes, the liver also gains the enzyme complements that are necessary in adult life.

The functional maturity of the liver as far as metabolic activity is concerned, means the development of adult metabolic machinery or in other words the synthesis and activation of enzymes which are characteristic of adult liver. Although there are no such enzymes that appear only in adult condition, there are several enzymes and metabolic pathways that become highly operational in the adult liver.

In the case of pigeon liver, the first 10 days after hatching are mainly meant for growth. Then onwards the liver

metabolic activities become more and more similar to that of the adult, with increased synthesis of specific enzymes.

As the liver function is closely related to the diet and digested food, the metabolic changes in the liver during this 'functional maturity (FM) phase' is also to a certain extend manipulated to cope up with the type of food (grains) the adult consumes. The complete switch over to a carbohydrate diet by 15th day exerts a tremendous influence over the liver metabolism.

Many of the metabolic enzymes that become more active in the liver during FM phase are also correlated to the food. The non-specific acid phosphatase activity, which is low in the beginning, reaches a high level in this phase, while the diet provided to the squab is gradually switched over to grains (Chapter 5). In fact, the graminivorous birds have a high concentration of acid phosphatase while carnivorous and insectivorous birds have a high alkaline phosphatase activity (Shah et al., 1972a). It is suggested that increased acid phosphatase activity, facilitates both glucose utilization and conversion (Chapter 5). The increased carbohydrate food also burdens the liver with a glucose load. Although the liver has the capacity to take up glucose, it is advantageous to increase the rate of

uptake by some mechanisms. It is possible that avian liver takes up glucose by the flow coupled transport mediated by acetylcholine (ACh). It was observed that at the time of maximum glycogen deposition, acetylcholinesterase (AChE) activity also increased (Chapter 2), a fact which is indicative of acetylcholine's participation in the glucose transport in the liver. The function of ACh is to induce permeability changes in the membrane so as to facilitate ionic transport. When the AChE activity increased in the FM phase the ionic concentrations were also found to increase (Chapter 2). By sharing the energy expended for the ionic transport, the glucose also enters the liver cells at a rapid rate. When confronted with a high influx of glucose, the liver must convert a large portion of it to fat. This can happen only if the liver also establishes an active lipid synthesizing machinery. In fact, while the liver is undergoing the growth process, the lipogenic machinery too is added to an already established metabolic machinery. This is evident from the studies on lipogenic enzymes such as 'malic' enzyme (ME) (Chapter 4). The $NADPH_2$ generating enzymes such as G-6-PDH and ME become maximally active only towards the end of FM phase i.e., 20th day, coinciding with maximum availability of food. A hyperlipogenic capacity is definitely an adult metabolic pattern and this was achieved by 20th day.

Thus in the FM phase a shift in the enzyme activity pattern that resembles more or less that of adult liver takes place. Such shift is more pronounced in the case of those enzymes that exist in polymorphic forms. Several isoenzymes which are active in the embryonic condition as well as in the post-hatching growth phase, probably give way to more efficient and compatible isoenzymic forms that are characteristic of the adult liver. It has been shown (Chapter 5) that part of the alkaline phosphatase present in the liver at the time of hatching as well as during growth phase ... more or less belongs to embryonic intestinal type (I-type), understandably so, as the liver primordium itself develops as a diverticulum of the alimentary canal. The liver type (L-type) of alkaline phosphatase replaces the I-type totally in the FM phase. Similarly, it is possible that the isoenzyme of LDH present in the embryonic liver is more of M-type which is functional in anaerobic condition generating NAD while converting pyruvate to lactate. Even in the growth phase, when anaerobic glycolysis is more prevelant (Chapter 6), the LDH in the liver must be of M-type. In the FM phase it is possible that M-type is replaced by H-type of LDH (Chapter 6). Although this contention needs corroboration,

Fine et al. (1963) have already shown that adult domestic pigeon liver contains 72% of H-subunits in the total enzyme molecules.

With such a shift in the enzyme pattern the whole metabolic profile of the liver undergoes certain changes. In fact, oxidative metabolism (both oxidative phosphorylation and ATP utilization) is progressively increased in the FM phase, judging from the activity of the enzymes such as SDH, LDH and ATPase (Chapter 6). It is also possible that during this phase the respiratory system also becomes more efficient, thereby providing sufficient amount of oxygen, reducing possible oxygen debt incurred during growth phase. At the same time because of increased muscular activity more lactate will be released from the muscles and the liver LDH effectively converts it into pyruvate (Chapter 6). This could also be the possible reason why, H-type of LDH is synthesized more during FM phase. Although at hatching, serum exhibited a high LDH activity, later there was about 50% reduction. Perhaps in in ovo stage serum too actively converts lactate to pyruvate, a function which in the later stages of development as well as in the adult condition, is perhaps competitively shared by hepatic and other extra-hepatic tissues. The oxidative metabolism also calls for

stabilization of oxido-reductive enzymes, which is provided by competitive hydrogen ion release and acceptance by complementary molcules. Ascorbic acid which provides such facilities is found to increase in the FM phase (Chapter 3). Moreover, ascorbic acid could also aid the hydroxylation of procollagen that brings about extrusion of collagen from the fibroblasts. Connective tissue formation is also found to increase soon after growth phase.

The timing of the acquisition of functional maturity by the liver is important for ~~the~~ survival. The pigeon squab remains in the nest till 20th day. Thereafter they are nudged out of the nest by the parents and are forced to lead an independent life. It may take a few days for the young one to learn the trick to procure sufficient amount of food. Till then the young one has to survive on the stored metabolites. Hence it is absolutely necessary that the nestling converts all the available metabolites into lipid and stores. Thus lipogenic machinery is driven at a full speed towards 20th day, making use of the best opportunity available due to an inactive life in the nest, affluent food supply as well as due to congenial conditions prevailing in the liver (Chapters 1 and 4). Thus the maximum synthesis and deposition of lipid in the liver by 20th day is an adaptive mechanism that forsees the ensuing lean period

when the pigeon nestlings, all of a sudden, become ~~an~~ evacuees from the nests. Because of this, initial failure to gather sufficient food will not have any adverse effect. Meanwhile, as the growth is almost over, the balance of energy is ~~now~~ utilized for development of feathers required for flight.

Although the sequential manifestations of preferential metabolic machinery is in a way related to the need and food of the pigeon nestlings, some sort of anticipatory triggering, probably initiated by hormonal interplay is found in the pigeon liver. Since the metabolic aspects have been taken into account here, it will be interesting to know how far the endocrine glands exert their influence in adjusting the reactions in the liver during post-hatching development. A very elementary study was undertaken to obtain a shadow of knowledge of endocrine activities, especially those that regulate intermediary metabolism, such as adrenal, thyroid and pancreatic islets.

The adrenal gland on the day of hatching mostly contained interrenal tissue and the chromaffin cells were very few. By twentieth day the adrenal reached more or less an adult size and histological pattern (Chapter 7). The glucocorticoids released by the interrenal tissue during growth phase could facilitate gluconeogenesis.

The thyroid follicles were found to be very active (judged from the height of the cells) around 20th day. Since thyroxine is known to induce the 'malic' enzyme synthesis (Goodridge et al., 1974) it is possible that thyroxine is involved in the elevation of lipogenic activities (Chapters 4 and 7).

Similarly, an increase in the number of small islets in the splenic lobe of the pancreas where B-islets are more, was also found to occur around 20th day (Chapter 7). This ~~is~~ of course coincided with the heavy intake of carbohydrate food.

Although, from the preliminary studies carried out here some type of relationship could be established between metabolic activities of the developing liver with the endocrine development and activities, the precise mechanism and knowledge can be had only if hormonal assays are carried out.

However, it could be surmised that the structural and functional development of any organ in a growing altricial bird has to be modulated and synchronized for which hormones are necessary. It is difficult, although, to specify which hormone is exclusively meant for each such regulation. Apparently an integrated action of several hormones is required to bring about adaptive changes in

an organ. Such actions of a medley of hormones need not necessarily mean that all should show parallel increase in concentration in the circulating blood. On the contrary a drop in the concentration of one and an increase of another may be required for bringing about changes. In rat liver, the gluconeogenic enzyme, phosphoenol pyruvate carboxykinase is synthesized soon after birth when a drop in the insulin and an increase in glucagon concentrations take place (Hanson et al., 1975). Such considerations should carry a lot of weightage as in a living system there is no condition when one hormone is totally removed from circulation. The differences in the ratio between hormones are all that could be expected to occur and tissues respond to such altered situations. The responses of the tissues also depend upon their sensitivity as well as cytoarchitectonic characteristics. Incidently, functional maturity of a tissue also requires the development of sensitivity (by synthesis of receptor molecules etc.) to hormones. Hommes and Beere (1971) report that during last 7 days of mammalian development, the activity of adenyl cyclase steadily increases so that the liver becomes "competent" to respond to glucagon. Hence, the hormones have to establish first a sensitivity in the tissues and then should influence the cellular processes, whether metabolic or

morphogenic. The transition of cells and tissues from growth to differentiation and then to maturation, thus is also a story of when they become sensitive to different hormones.

PART II. METABOLIC ACTIVITIES OF ADULT AVIAN LIVER

The present state of understanding regarding the role of insulin in birds is that this hormone is less significant in the control of carbohydrate metabolism as compared to ~~that~~ ~~of~~ glucagon (Hazelwood, 1973). Although, the primary effect of insulin is similar to that found in mammals, because when injected, avian insulin did lower blood sugar level in chicken (Hazelwood *et al.*, 1968), it is puzzling as to why insulin plays only a second fiddle to glucagon in birds. It is then natural to believe that insulin lost its initiative due to evolutionary changes that affected either the molecule itself or the sensitivity of target organs. Perhaps, the carbohydrate metabolism is more effectively controlled by other neuroendocrinological mechanisms that dispensed off a greater need of insulin. In the liver the metabolic activities concerned with

carbohydrates are set in motion with the uptake of glucose. The modes of glucose transport into the cells are found to differ in different tissues. Some show active transport while others do not. Some tissues have a transport system sensitive to insulin, as not requiring energy expenditure. While a sugar transport system sensitive to insulin is present in the skeletal muscle, heart muscle and adipose tissue (in mammals but not in birds), such a system is reported to be doubtful in the liver. The liver, does have a specific hexokinase (glucokinase) that may be partially sensitive to insulin. The liver, like brain and kidney is believed to take up glucose along a concentration gradient. But when faced with a glucose load, unless the glucose that enters the cell is phosphorylated, a feed back retardation due to counterflow may limit the entry of glucose. Hence, activation of glucokinase should also take place for the steady influx of glucose into hepatocytes. The liver glucokinase is adjusted to the level of portal blood sugar and is not inhibited by an increase in the glucose-6-phosphate within the cell. In mammals this enzyme is greatly reduced in diabetes and starvation. Aparently, the insulin has no direct role in the uptake of glucose by liver cells, but can increase the rate by activating glucokinase. Perhaps, in the

liver, as Randle and Smith (1958) suggested, the insulin counteracts the factors that inhibit the uptake of glucose thereby facilitating glucose entry. Thus a major effect of insulin could be to prevent the release of glucose from the liver by counteracting the hormones such as glucagon, norepinephrine and adrenocorticosteroids.

In mammals, the glucose uptake and the metabolism of G-6-P in liver are also found to be under the influence of autonomic nervous system (Shimazu and Amakawa, 1968a and b). The stimulation of sympathetic nerves increased the glycogenolytic enzymes like phosphorylase and glucose-6- phosphatase, while the stimulation of vagus nerve increased the glycogen synthetase activity in the liver (Shimazu, 1967). In fact, in mammals, the circulating level of adrenaline is too low to stimulate glycogenolysis in the liver and hence the adrenergic effect on mobilization of glucose from liver must be mediated through sympathetic stimulation (Bentley, 1976). The idea of participation of cholinergic system in the assimilation of metabolites has taken roots quite early. Bertrand (1954) observed cyclic variations in the intralobular localization of hepatic cholinesterase during feeding and fasting. Gerebtzoff (1959) indicated a relationship of diet with the concentration and distribution pattern of cholinesterase in the rat liver. Being endowed with both cholinergic and adrenergic fibres,

the avian liver too must be under their influence as far as assimilation is concerned. In 1969, Pilo, through his studies on hepatic cholinesterases in a migratory and some non-migratory birds, has suggested that ACh-AChE system in some way or the other is involved in the metabolism of carbohydrates and lipids in the liver. Mondon and Burton (1971) clearly demonstrated that the acetylcholine or choline in the presence of insulin significantly enhanced the uptake of glucose and deposition of glycogen by the liver of rats. Stimulation of cholinergic fibres in the presence of insulin also showed similar results.

It is possible then that in the avian liver too, this cholinergic (ACh-AChE) system is playing a prominent role in the uptake of glucose. Since the level of activity of AChE could be taken as an index of ACh secretion, AChE could be used as a yardstick to judge the degree of ACh secretion. In fact, the birds consuming a carbohydrate rich diet (Pigeon, Dove and Parakeet) showed a very high histochemical reactivity of AChE, especially in the periportal region of the lobules, than that in the birds which are omnivorous (Mynas, Babbler, Robin, Bulbul, Koel, Crow, Sparrow, Barbet, Fowl and Duck), insectivorous (Drongo, Martin, Tailor bird, Bee-eater and Swift) or carnivorous (raptorials-kite and carrion feeders-Vulture)

(Shah et al., 1972b). The observations that, during post-hatching development, when the diet provided to the pigeon nestlings changed from crop milk to grains, there occurred in the liver an increase in the AChE activity together with elevated levels of glycogen, fat and cations, have denoted a relationship of ACh-$AChE^{E}$ system with carbohydrate diet and increased glycogen, fat and ionic concentrations (Chapter 2).

If ACh facilitates glucose transport, there is sufficient data to suggest, that this must be taking place through flow coupled transport, which is one of the mechanisms by which cells accumulate glucose (see Wilbrandt, 1975). The ACh could initiate permeability changes that bring about movements of ions across the plasma membrane of hepatocytes probably through the release of membrane bound Ca ions. During this to and fro transport of Na and K ions, the sugar molecules move into the cell utilizing perhaps the same carrier and energy (Fig. 2). Similar Na^{+}/K^{+} dependent sugar transport is also seen in the intestine (Riklis and Quastel, 1958; Crane, 1962; Schultz and Curran, 1970). It is pertinent to mention here that insulin could counteract the action of epinephrine and glucagon on plasma membrane bound Na^{+}/K^{+}-ATPase mediated

by c-AMP dependent negative modulation of a plasma membrane located protein kinase (Luly et al., 1972; Barnabei et al., 1973; Tria et al., 1974).

A natural sequel to this concept was the origin of a belief that if ACh facilitates the uptake of glucose, then a glucose load in in vivo conditions, should increase the secretion of ACh and a corollary increase in the activity of AChE in the liver. This was easy to prove. When glucose was administered in a dose that resulted in a physiological hyperglycaemia, the liver of both pigeon (Chapter 8a) and rat (Chapter 8b) showed an increased AChE activity. In the pigeon liver this response was more rapid (at 30 minutes after glucose administration) while in rat liver it was at 60 minutes. As such, the normal pigeon liver has twice as much AChE activity as rat liver. In the pigeon liver, the cholinergic vascular nerve plexus runs along the blood vessels and continues along sinusoidal linings right upto the central collecting vein (Pilo, 1969; Shah et al., 1972b; Chapter 8a) (Fig. 1). However, it is possible that in the rat liver, the cholinergic fibres are very few and reach only upto the blood vessels around periportal areas and hence the AChE is found to be more or less localized around blood vessels in the periportal areas (Chapter 8b). Obviously, this could be the reason

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Fig. 1 (A, B, C and D) Schematic diagram of a liver lobule showing sites of ACh secretion and AChE activity at the sinusoidal linings.

A:- A schematic representation of liver lobule.

B:- Magnified portion of liver lobule showing portal area and sinusoids.

C:- Magnified sinusoid with hepatocytes.

D:- Hepatocytes and adjoining sinusoid.

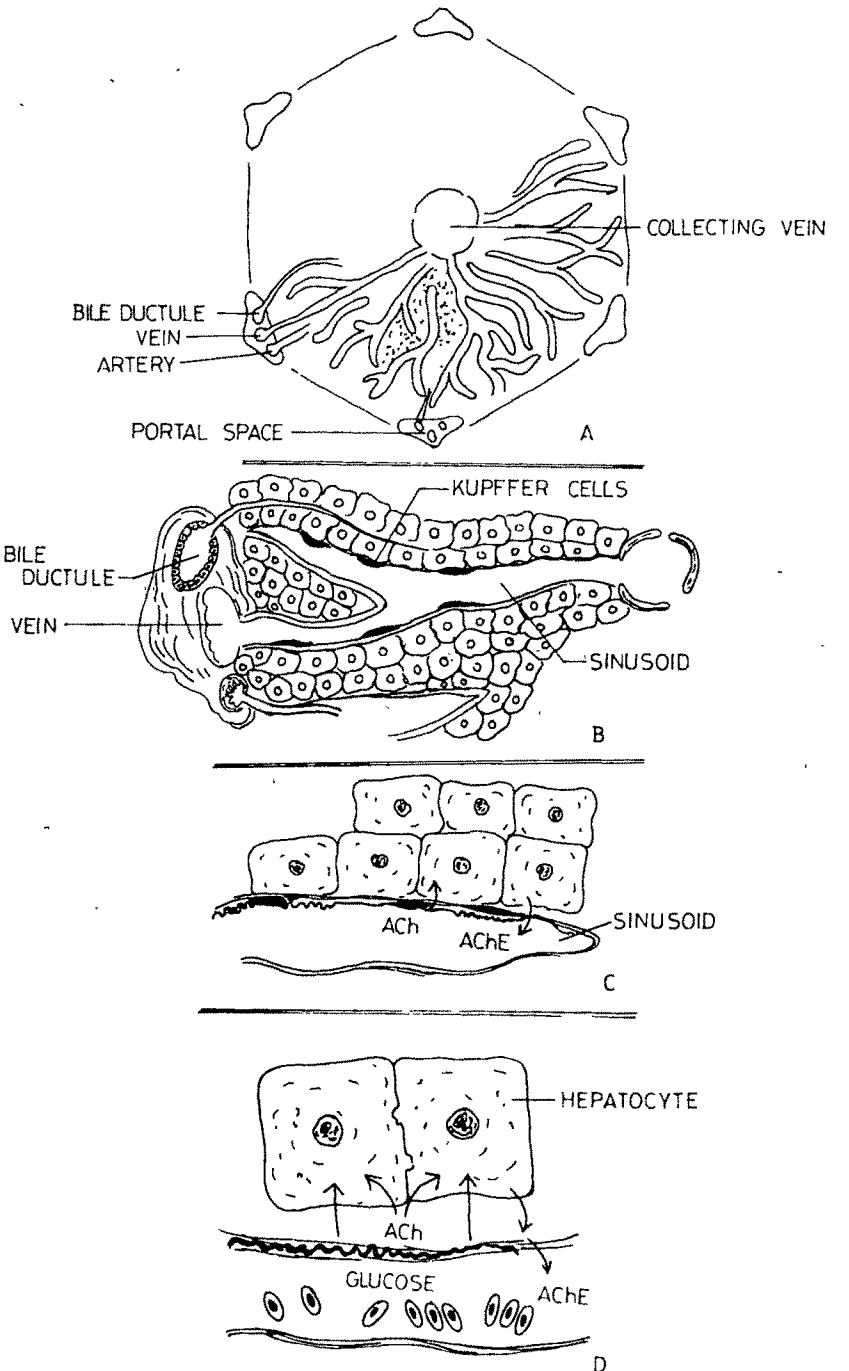
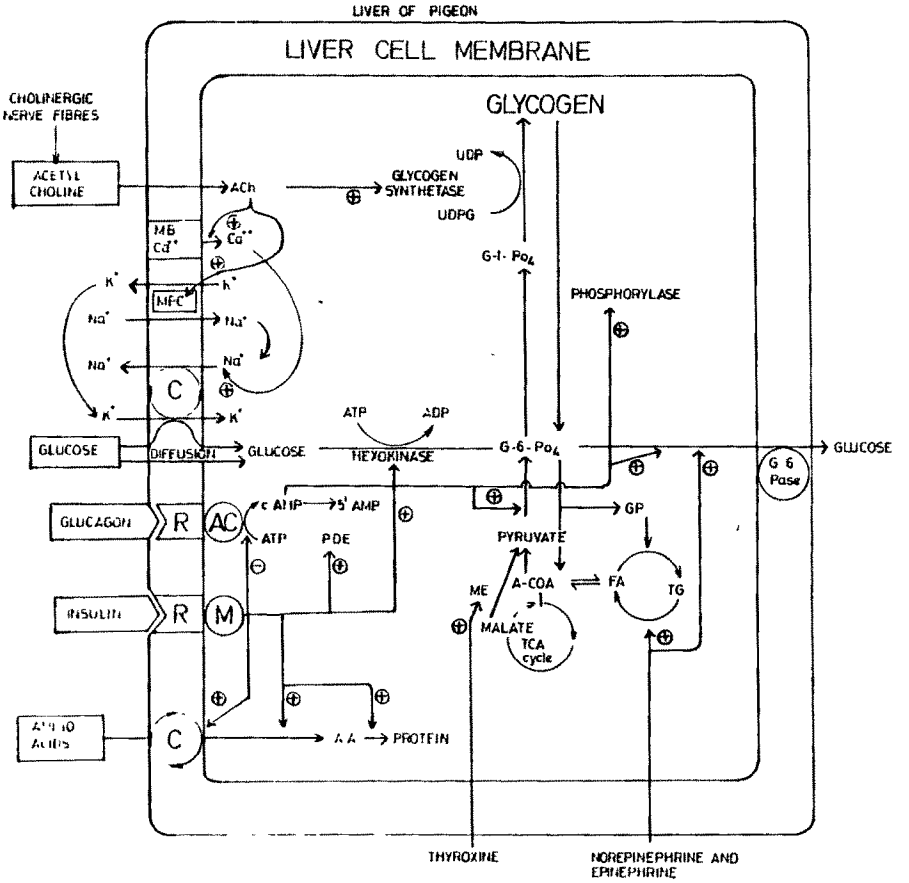

COLLECTING VEIN
BILE DUCTULE
VEIN
ARTERY
PORTAL SPACE
A
KUPFFER CELLS
BILE DUCTULE
VEIN
SINUSOID
B
ACh
AChE
SINUSOID
C
HEPATOCYTE
ACh
GLUCOSE
AChE
D

FIG 1

FIG 2 EFFECT OF ACh AND INSULIN AND OTHER HORMONES ON THE GLUCOSE UPTAKE AND RELEASE IN THE LIVER OF PIGEON



ABBREVIATIONS

MPC	MEMBRANE PERMEABILITY CHANGES	M	MESSENGER
C	CARRIER MOLECULE	PDE	PHOSPHODIESTERASE
MBCa++	MEMBRANE BOUND Ca++	G6Pase	GLUCOSE - 6 - PHOSPHATASE
R	RECEPTOR PROTEINS	AC	ADENYL CYCLASE
⊕	ACTIVATION	⊖	INHIBITION

why the rat liver showed far less AChE activity compared to that of pigeon liver.

An increase in the activity of AChE in the liver and pancreas of pigeon and rat following glucose load apparently denote an enhanced secretion of ACh by the cholinergic fibres present in these organs. This increased cholinergic activity could be due to the influence of glucose load on the glucoregulator centre in the brain as reported by Chieri et al. (1975) in dogs. In mammals the stimulation of ventrolateral hypothalamic nuclei (VLH) produced a hypoglycaemia (Kuzuya, 1962; Shimazu et al., 1966; Szabo and Szabo, 1975a and b). VLH stimulation could also increase plasma insulin (Kuzuya, 1962; Steffens et al., 1972) and pancreatic insulin secretion (Idahl and Martin, 1971). Vagal stimulation also elicited the insulin release from islets (Findlay et al., 1969; Porte et al., 1973), which was more pronounced in the presence of elevated glucose level in blood (Bergman and Miller, 1973). In mammals, in fact, the release of hormones from the islet cells is also under the control of autonomous nervous system (see Woods and Porte, 1974), and because of this reason, insulin could be released reflexly in response not only to changes in blood sugar level but also to stimuli directly connected ~~to~~ with food (taste and smell) or conditioned through

the cholinergic system. But the cholinergic stimulation of insulin is doubtful in birds, as most of the studies have failed to show intrainsular nerve plexus in avian pancreatic tissue (Kobayashi and Fujita, 1969; Sims et al., 1971; Kern and Grube, 1972). However, the possibility of general secretion of ACh into the circulating blood in the pancreas in response to glucose load can not be ruled out as both pancreas and serum showed increased AChE activity (Chapter 8a). In fact ACh secretion into the blood stream must be of a higher degree in pigeon than in rats; the AChE activity being 6 times more in the serum of the former than that of the latter. The ACh secreted in the peripheral regions of islets could pass through the insular sinusoids and could stimulate insulin secretion. The presence of AChE in the sinusoids of islets of Langerhans (Chapter 8a) points to this possible mechanism of stimulation of insulin release. However, this mechanism of release of insulin may not be as efficient as that in the rat where the ACh is supplied directly to the B-cells through insular plexus. The cholinergic nerve supply is also more in rats as evident from the fact that rat pancreas showed twice the amount of AChE than pigeon pancreas (Chapter 8b). The reason for the 'sluggish' response of insulin to glucose load (Hazelwood,

1973) also may probably rest on this fact. The relegation of reflexly mediated acute insulin response to a lesser status in birds may be also due to the fact that smell and taste have only a limited role to play in their aerial life. Because of this less sensitive mechanism of insulin release from B-cells, the liver of birds might have become more dependent on flow coupled transport of glucose facilitated by ACh, a high concentration of which is ensured by the copious supply of cholinergic plexus. This could be one of the reasons why the insulin has only a secondary role in the regulation of carbohydrate metabolism in birds. However, the necessity of insulin is not completely dispensed with, in birds. Insulin is needed to counteract glucagon's glucose releasing action, as well as for activating glucokinase enzyme. Tria et al. (1976) have conclusively proved that adenosine 3,5'-cyclic monophosphate phosphodiesterase of hepatocyte plasma membrane is activated by insulin. The glucose that enters the hepatic cells has to be readily phosphorylated and deposited as glycogen or converted to fat to prevent feed back retardation of glucose influx across the membrane. In all probability, the glucokinase enzyme is in the cytosol rather than in a membrane bound state and hence in avian liver, glucose might be getting phosphorylated only as it enters, or after its entry into, the cells, unlike

in mammalian liver where phosphorylation is the major means by which glucose is drawn into the cell (chemiosmotic transport). The poor sensitivity of hepatic cells to insulin could be also due to this architectonic disposition of enzyme glucokinase. Insulin also has other useful effects on other tissues which are also part of physiological response to control carbohydrate metabolism. Insulin could release glucagon from A_2-cells, stimulate (β-adrenergic) epinephrine release from adrenal medulla, counteract α-adrenergic action and also accentuate the metabolic impact of glucagon. All these actions are necessary for glucostatic control of hepatic influx and outflux of glucose. However, insulin may have only a minor role to play directly in the mechanism of glucose transport across the membrane of cells, for which ACh has an upper hand.

The in vitro studies using pigeon and rat liver slices provided conclusive evidence as to the role of ACh in the glucose transport (Chapter 9). The pigeon liver slices when incubated in a medium containing glucose deposited more glycogen in the presence of ACh than in the presence of insulin. Both insulin and acetylcholine together induced only a slightly further increase in the amount of glycogen deposition than that was observed in slices incubated with ACh alone. The rat liver slices on the other hand showed

a higher sensitivity to insulin than ACh in the deposition of glycogen, however, together insulin and ACh tremendously boosted up the glucose uptake. From these experiments, it was realized that not only the ACh mediated transport of glucose is more efficient in the pigeon liver slices but also that the concentration gradient differences also influence the entry of glucose into the hepatocytes. When incubated in a medium containing no glucose, pigeon liver slices depleted more glycogen than rat liver slices. It was suggested that in pigeon liver a major part of glucose enters the cells through ACh mediated flow coupled transport and a small part by simple diffusion, (could this be a reason for maintaining a very high concentration of glucose in the plasma in birds?). In rat liver, ACh, along with insulin accentuated the rate of uptake of glucose. In the presence of insulin, ACh by activating glycogen synthetase (Mondon and Burton, 1971) and by reducing the concentration of c-AMP through the activation of phosphodiesterase, might be facilitating elevated deposition of glycogen. Moreover, it was observed that rat liver slices deposited more glycogen than pigeon liver slices. It suggested (Chapter 9) that this difference in the rate of glycogen deposition (the amount of glycogen deposited in a specified time) could be due to the fact that (1) rat liver diverted more glucose for glycogen synthesis,

while in pigeon liver the major part is utilized for lipid synthesis (2) the chemiosmotic (phosphorylation) method of transport of glucose also operates in the rat liver and hence the glucose transport is more efficient than that in the pigeon liver and (3) the type of glucokinase present in the rat liver is more sensitive to insulin than that is present in the pigeon liver.

The ability of avian liver to divert a large amount of assimilated carbohydrate for lipid synthesis is well known. In fact the liver is the major lipogenic centre in birds while adipose tissue assumes only a lipid storage function (see Langslow and Hales, 1971). The lipogenic enzymes such as G-6-PDH and 'malic' enzyme are highly active in the pigeon liver (Chapter 4) as well as in the liver of migratory birds (Patel, 1976). These two enzymes are designated as lipogenic enzymes since these enzymes generate $NADPH_2$ required for lipid synthesis. Of these two, the involvement of 'malic' enzyme in lipogenesis in birds is much more than that of G-6-PDH as the avian liver exhibits very high 'malic' enzyme activity compared to the other (Chapter 4 ; Patel, 1976). Although administration of glucose did enhance the activities of both these enzymes, the response of 'malic' enzyme was much more than that of G-6-PDH. However, both these enzymes remained very active for a longer duration after glucose injection (Chapter 10). This prolonged activation could be obviously for converting excess of G-6-P to fat. The glycogenesis was found to be in

operation only in the initial period (around 30 minutes after glucose injection) which was evident from the fact that the peak activity of glycogen synthetase was also observed to be at this time. Since this short lived spurt in the activity of glycogen synthetase in the liver of pigeon following glucose loading also coincided with maximum AChE activity, it could be reasoned that the enzyme activation was due to the influence of ACh. Apparently even in the activation of 'malic' enzyme, the insulin has only a small role in birds unlike in mammals as insulin failed to increase the activity of induction of this enzyme or fatty acid synthesis in the chicken liver cells (Goodridge et al., 1974). However, induction of 'malic' enzyme was observed when treated with thyroxine, and insulin was found to accentuate the action of thyroxine not only in the induction of ME but also in the incorporation of carbon moieties from glucose to fatty acids (Goodridge, 1975). The increase of 'malic' enzyme activity and induction as well as lipogenesis in the liver ~~are~~ operate only on a cause-effect basis and thyroxine plays a permissive role by influencing the induction of enzymes (Goodridge, 1975). In other words, an increased influx of glucose automatically induces the lipid synthesis in birds, provided the hormonal and intracellular 'climate' is favourable (Fig. 3). Hazelwood (1971)

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Fig. 3. Schematic presentation of various factors that control glucose uptake, glycogenesis, lipogenesis and lipolysis in the liver of birds.

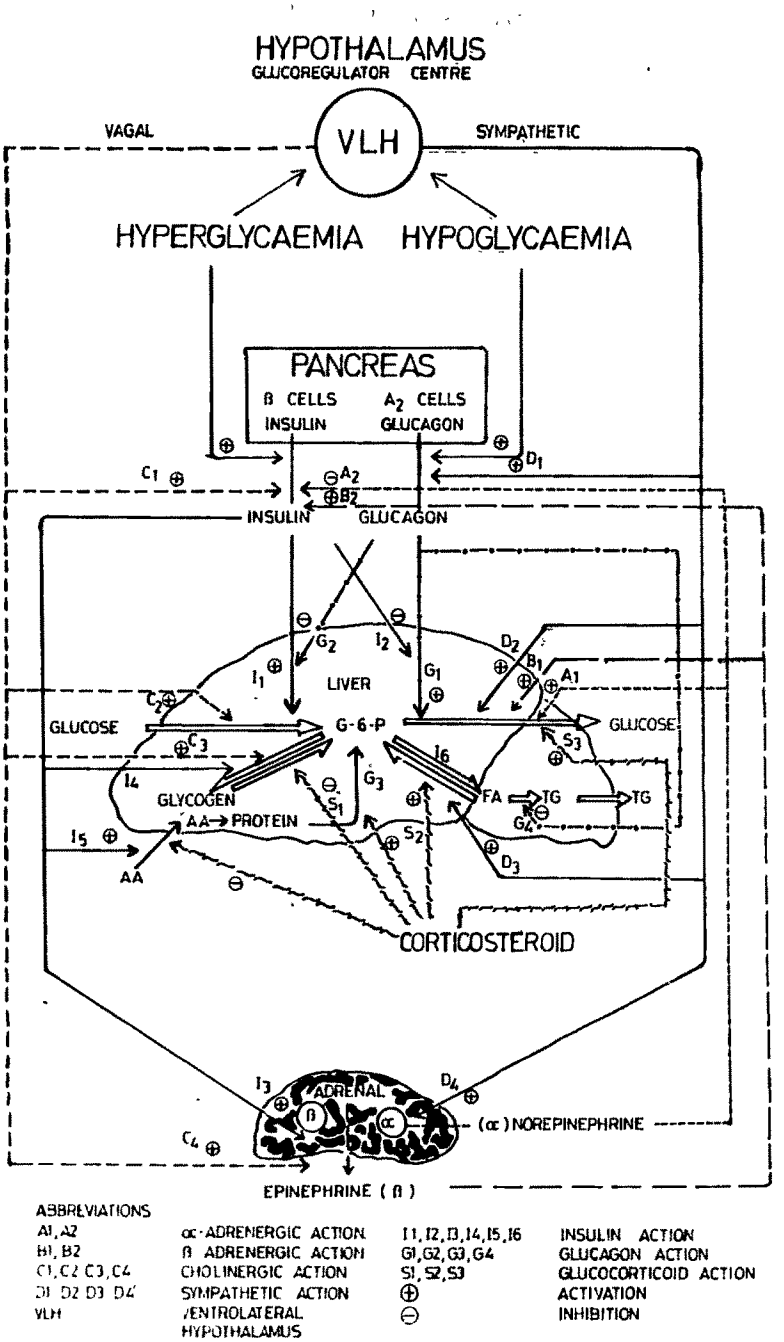
HYPOTHALAMUS
GLUCOREGULATOR CENTRE
VAGAL
VLH
SYMPATHETIC
HYPERGLYCAEMIA
HYPOGLYCAEMIA
PANCREAS
β CELLS
INSULIN
A2 CELLS
GLUCAGON
INSULIN
GLUCAGON
LIVER
GLUCOSE
G-6-P
GLUCOSE
GLYCOGEN
AA→PROTEIN
AA
FA
TG
TG
CORTICOSTEROID
ADRENAL
(α) NOREPINEPHRINE
EPINEPHRINE (β)
ABBREVIATIONS
A1, A2 α-ADRENERGIC ACTION
B1, B2 β ADRENERGIC ACTION
C1, C2 C3, C4 CHOLINERGIC ACTION
D1 D2 D3 D4 SYMPATHETIC ACTION
VLH VENTROLATERAL HYPOTHALAMUS
I1, I2, I3, I4, I5, I6 INSULIN ACTION
G1, G2, G3, G4 GLUCAGON ACTION
S1, S2, S3 GLUCOCORTICOID ACTION
⊕ ACTIVATION
⊖ INHIBITION

Fig. 3

opined that the role of insulin may be only in the uptake of glucose by the cells rather than on metabolic events that take place after glucose has entered the cells. But at the same time, Hazelwood (1973) cautions that such assumption does not preclude the possibility of insulin's role to 'adjust' the influence of other endocrine secretions at the cellular level. One such role could be to counteract the effect of glucagon by reducing c-AMP which prevents ultimately the activation of glucose-6-phosphatase (Goodridge, 1975). Insulin may also accentuate the action of thyroid hormones in lipogenesis (Goodridge, 1975). To this list of hormones, the actions of which are 'adjusted' by insulin, neurohumor substances such as acetylcholine and norepinephrine should also be now added. In the in vitro conditions ACh alone increased the uptake of glucose by liver slices much more than by insulin alone, and both together showed only a slight increase in the rate of glucose uptake over that observed with ACh alone (Chapter 9). Perhaps in in vivo conditions insulin could accentuate the action of ACh further by counteracting the action of glucagon and norepinephrine. In rat liver slices, insulin together with acetylcholine showed an accentuated glucose uptake which probably means that in rats insulin is also directly involved in the uptake of glucose and not just by counteracting the

action of antagonistic endocrine secretions.

The lipogenesis in avian liver being a cause-effective response, the insulin's role is confined to the preparation of a suitable cellular environment required for lipogenic reactions. As suggested in Chapter 9, there is reason to believe that major amount of glucose that enters the avian liver is quickly converted to fat. The migratory bird, Rosy Pastor (Sturnus roseus) changes its diet from a mixed one consisting of insects and grains to a carbohydrate diet (fruits and grains) during premigratory period and this ensures an elevated supply of glucose for lipogenesis (Pilo, 1967). Diurnal observations on the fat and glycogen contents in the liver of Rosy Pastor during premigratory period also revealed that fat synthesis in the liver was more in the day time when feeding takes place (Pilo, 1967). In fact a speedy conversion of carbohydrates to fat must be an evolutionary adaptation of the avian liver. The avian alimentary canal is primarily evolved for carbohydrate rich diet consisting of fruits, seeds and grains (the presence of gizzard bespeaks of that), although secondarily many may have developed adaptations for flesh, insects or fish (Shah and Panicker, 1975). Accessary organs like liver too showed several enzymic adaptations according to the diet in each species (Shah et al., 1972a, 1972b; 1975;

Pilo et al, 1973a; 1973b; Asnani et al., 1973; 1974; Yadav et al., 1975). These studies have shown that primary metabolic adaptations of the liver of birds are also oriented towards dealing with heavy influx of carbohydrates. Again, the carbohydrate rich diet of birds could be the reason why the liver in birds is the major site of lipogenesis and not adipose tissue. On a similar note, the avian tissues have also become adapted to an almost constant influx of glucose as most of the birds consuming grains and fruits are voracious and continuous feeders. The sensitivity of tissues to insulin in such a condition is bound to be less. This phenomenon is also observed in mammals, the carnivores are more sensitive to insulin than herbivores (see Bentley, 1976). Even among birds the degree of sensitivity of tissues to insulin should vary according to diet. For example birds of prey, carrion feeders, piscivores and insectivores may preferably have a sensitive acute insulin response as well as insulin sensitivity. Many such birds (Falcon, Buzzard, Owl and Raven) show hyperglycaemia following pancreatectomy (see Hazelwood, 1965). Most of the birds that consume carbohydrate rich diet, irrespective of the fact that they are stenophagus (Pigeon) or facultatively euryphagous (Fowl) do not show adverse reaction to pancreatectomy, while obligatory euryphagous birds like Goose could develop

hyperglycaemia upon pancreatectomy (see Hazelwood, 1965). Perhaps one has to be cautious in generalizing a concept that evolved from the study of one or few avian species. Total pancreatectomy in duck and chicken resulted in the development of hypoglycaemia which led to convulsions and death (see Langslow and Hales, 1971). This could be corrected with injection of glucose or glucagon. On the basis of such observations, it is concluded that glucagon and not insulin that is essential to birds in the control of carbohydrate metabolism (Hazelwood, 1973).

The avian species are not only tolerant of high glucose levels in the blood but most probably are also tolerant of high levels of insulin. The apparent tolerance to large doses of insulin by birds appears to be due to certain plasma factors (insulinolytic action) as well as due to the compensatory adrenomedullary release of epinephrine (Pittman and Hazelwood, 1973). The release of epinephrine, like the glucagon release, influenced by insulin is obviously for having a glucostatic control that maintains the blood sugar level within an acceptable range. Thus, it seems logical to venture an assumption here that in most birds, the glucostatic control is turned high; i.e., the hyperglycaemia checking and controlling mechanism becomes sensitive or becomes operative only at a higher range

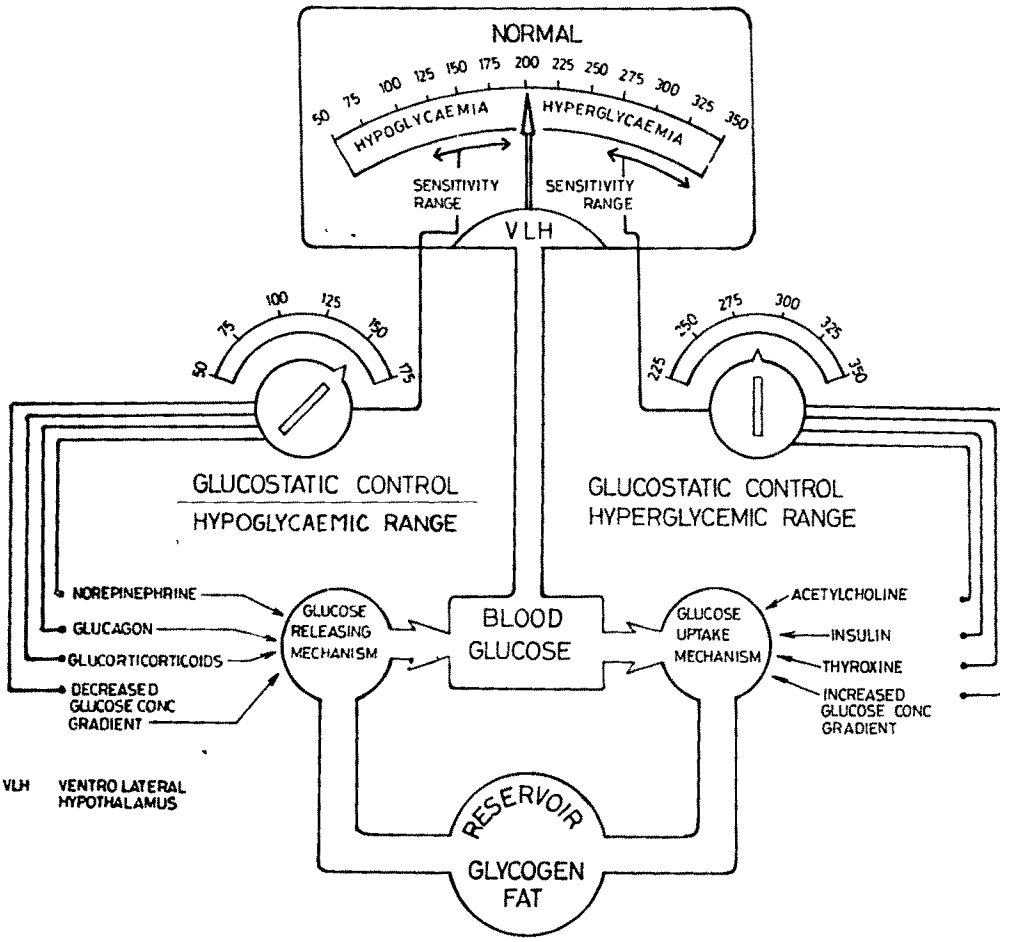

FIG 4 SCHEMATIC PRESENTATION OF BLOOD GLUCOSE REGULATION IN BIRDS

of blood sugar level while the hypoglycaemia controlling mechanism is very sensitive to minute changes. These mechanisms involve not only endocrine mediation but also neural participation (Fig. 4). So far, the discussion is centered around the mechanism of regulation of blood glucose by the liver, and the role played by the insulin in birds. It would not be out of place to add here the reasons for why ~~the~~ insulin has only a secondary role in ~~the~~ birds. Adelman (1975) suggests that several modifications in the control of liver function by insulin could account for delayed enzyme adaptation during ageing: (1) changes in the hormonereceptor sensitivity (2) impairment of the ability to initiate adaptive changes in the concentration of hormones in the blood and (3) changes in the molecular architecture of the hormone that reduce the biological effectiveness. If such changes could take place during ageing, it is conceivable that similar changes might have also taken place evolutionarily in birds resulting in the reduced effectiveness of insulin. However, homeostatic mechanisms are still operative in birds as in mammals but the range and sensitivity could be different.